THE CONSERVATION OF WATER IN STARVING *TENEBRIO MOLITOR* LINNAEUS

by

STEPHANUS SEBASTIAAN WALTERS M.Sc. (Stellenbosch)

DEPARTMENT OF ZOOLOGY

THE UNIVERSITY OF ADELAIDE

A thesis submitted to the University of Adelaide in part fulfilment of the requirements for the degree of Doctor of Philosophy

January 1966

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and, to the best of my knowledge, contains no material previously published or written by another person, except when due reference is made in the text of the thesis.

CONTENTS

PART 3

MATERIALS, METHODS AND GLOSSARY OF TERMS

PART 4

WATER CONSERVATION IN STARVING TENEBRIO MOLITOR L. ADULTS

PART 6

APPENDICES, ACKNOWLEDGEMENTS AND REFERENCES

INDEX OF FIGURES IN THE TEXT

INDEX OF TABLES IN THE APPENDIX

SUMMARY

(i) A study was made of water conservation in starving T. molitor adults and larvae that were kept at various combinations of temperature and humidity. Particular attention was paid to the way in which environmental humidity and temperature influence the internal condition of these insects and how these insects respond to changes in their internal condition.

(ii) The relationship between the length of life of starving T. molitor adults and atmospheric humidity and temperature has been defined.

(iii) Causes of death were found to be; desiccation in the lower humidity range, starvation in the intermediate range and "water-logging" of the tissues in the upper humidity range. Death as a result of desiccation occurred after the initial water content of the beetles had been reduced to a certain minimum. To be able to make this statement it was found necessary to show that some water, an amount increasing with humidity, is "bound" and thus unavailable to the insect. Water incorporated in eggs was found to constitute a form of "bound" water in starving virgin T. molitor females. Death as a result of "water-logging" of the tissues was found to occur when the wet/dry ratio has increased to a certain high level and that this condition occurs after the initial water content was reduced to below the level considered normal for newly emerged beetles. This suggested that the water content must be reduced in response to a reduction in dry matter content and at a certain minimum rate.

(iv) It was concluded that the malpighian tube rectal system acts as a water regulatory mechanism by excreting water with, or re-absorbing water from, the faeces.

(v) Evidence obtained, indicated that the rate of dry material consumption in starved T. molitor adults is independent of atmospheric humidity, suggesting that no additional energy is used to relieve internal conditions caused by either very low or very high humidities. To arrive at this conclusion it was necessary to (a) demonstrate a change in the rate of dry material consumption with time (b) to identify two populations within the experimental population. A change in the metabolic rate with time was found to be associated with egg maturation in females and possibly with sperm production in males. It is further associated with a change in the rate of reduction of the water content, but only in the lower humidity range.

Two populations within the experimental population were identified as females and males of the species. Differences in the response of the sexes to temperature and humidity were found to be in magnitude only and not in principle.

(vi) It was finally concluded that for maximum use of their energy resources starving T. molitor beetles have to exploit atmospheric humidity to either reduce excessive evaporation or to facilitate water loss.

(vii) The metabolic rate of starving T. molitor larvae in contrast to the adults decreased, at first independently of atmospheric humidity.

and then increases with time in all humidities. The increase in metabolic rate could be associated with either (a) the production of metabolic water which allow larvae to maintain an adequate ratio of water to dry material in very low humidities or (b) with the continuation of development in higher humidities.

(viii) The ability of T. molitor larvae to absorb water from unsaturated air was confirmed. In connection with this phenomenon, evidence is presented which indicates (a) that larvae need to be deprived of food to acquire the ability to absorb water from unsaturated air (b) that in larvae which were simultaneously deprived of food and desiccated a greater proportion absorbed water and at a lower relative humidity than larvae which were merely deprived of food (c) that water absorption stimulates metamorphosis in immature larvae, and also in larvae in which metamorphosis was prevented by desiccation.

PART I

1. INTRODUCTION : A BRIEF DISCUSSION OF THE MECHANISMS INVOLVED IN WATER CONSERVATION IN TERRESTRIAL INSECTS.

Tenebrio molitor (Linnaeus, 1758), is a member of the Family Tenebrionidae: Order Coleoptera.

The family Tenebrionidae is described by Imms (1957) as one of the largest families of the Order Coleoptera - it comprises more than 10,000 known species, a large number of which is found in desert habitats all over the world. Buxton (1923) attributed the success of these insects in colonizing desert habitats all over the world to their remarkable ability to economise water and resist drying.

Buxton (1933) classified terrestrial insects into what he called "spenders" e.g. (Lucillia, Calliphora, Glossina and Musca), and "Savers" e.g. (Adult Cimex, Rhodnius, clothesmoths and T. molitor larvae). The former living in environments where water losses are easily made good by oral intake. These forms have only but a slight resistance to water loss and starvation. Savers on the other hand live in dry places, eat dry foods and are resistant to water loss and starvation. In terrestrial insects living in humidities substantially below saturation, water conservation is a more urgent problem because of the high surface area/ volume ratio of the insect's body and their active metabolism (Buck, 1953). Nevertheless, insects are, as Mellanby (1939) said, "the only really small animals able to live in dry conditions", because they are remarkably well adapted to conserve water and resist desiccation. Adaptations

for water conservation are conveniently discussed under the headings morphological, physiological and behavioural.

1.1 Morphological adaptations.

Water loss from the external body surface of the terrestrial insect occurs both through the spiracles and the general body surface. Gaseous respiratory exchange between the air and the tissues depends on diffusion. Because of the low diffusion constant of oxygen in chitin relative to that in water, the supply of oxygen to the tissues would be insufficient unless the respiratory surfaces are wet (Edney, 1957), thus making these surfaces a great potential source of water loss.

Excessive loss of water from these surfaces is prevented in terrestrial insects by means of a closing mechanism or the development of a felt-chamber which reduces the diffusion of water vapour across the spiracular openings (Lees, 1957). Xerophilous species show strongly developed mechanisms of this sort (Bergold, 1935).

The effectiveness of the closing mechanism of the respiratory openings in reducing water loss was clearly demonstrated by a number of workers. (Mellanby,(1934 a) working with a number of different insect species, found that the rate of evaporation from insects with spiracles kept open by 5% carbon dioxide, to be as much as seven times higher than that of the controls, kept in the same humidity but with the closing mechanism still functioning normally. In the larvae of the flea Xenopsylla cheopis where the spiracles lack closing mechanisms, however, carbon dioxide made little difference to the rate of evaporation. (Ramsay (1935 a)

found that the rate of evaporation increased from 3.9 - 6.00 mg/hr. in stimulated Blatta orientalis. Similarly Rhodnius which is normally resistant to desiccation, died in three days if spiracles were kept open (Wigglesworth & Gillet, 1936).

Diffusion is the only factor involved in oxygen transport in all pupae, almost all terrestrial larvae and a large number of small imagines. In larger adults, diffusion is probably the mode of transport in the terminal parts of the tracheal system, but is supplemented in other parts by mechanical ventilation (Imms, 1957). According to the same author the control of respiratory activity results from 'diffusion control' due to the opening and closing of the spiracles and 'ventilation control' caused by the variation in the frequency and intensity of the respiratory movements. Co-ordination of these movements makes a directed flow of air through the spiracles possible. Miller (1960) showed that hyperventilation can bring about abrogation of this co-ordinated rhythm of inspiratory and expiratory spiracles, changing this directed flow to a tidal flow. The latter suggested by Buck (1962) to be more efficient in supplying oxygen and removing carbon dioxide, while the former normally used in resting respiration might reduce water loss.

The primary stimulus for both ventilation and spiracular activity is taken to be either oxygen lack or carbon dioxide excess or both (Buck, 1962). Recent results however indicate that other factors can also have an indirect or direct influence on the spiracular closing mechanism. Miller (1961) working with adult dragon flies, showed that the spiracles

of partially desiccated insects do not open in an atmosphere containing less than 5% carbon dioxide, and that they close immediately after flight. In contrast to this the spiracles of well hydrated insects open in carbon dioxide concentrations as low as 1 - 2%, and remained open for some time after flight. Bursell (1957) demonstrated a greater spiracular control in desiccated individuals of the tsetse fly Glossina morsitans. In addition to this Bursell also showed that spiracular control in young G. morsitans flies to be directly affected by atmospheric humidity.

The outward movement of carbon dioxide is effected in the same way as the inward movement of oxygen. Imms (1957) however, points out that because the cuticle and body tissues are more permeable to carbon dioxide than oxygen, an appreciable portion of the former can escape through the tracheal walls and the general surface of the body. This would allow for a more efficient utilization of oxygen and a less frequent opening of the spiracles.

Cyclic release of carbon dioxide has been observed in several insect species (Buck, 1962). This phenomenon is most extreme in forms spending long periods without water such as diapausing lepidopterous larvae and pupae. It consists of a rhythmic alternation of long periods of spiracular restriction with brief periods of wide opening. Buck (1958) has shown theoretically that more water can be conserved this way than when spiracles are permanently set at the minimum apperture that will suffice for diffusion respiration.

Despite the various mechanisms reducing water loss through the

spiracles a large proportion of water lost still passes through the spiracles: 60% in the pupae of Bombyx mori and 70% in Gastrimargus adults (Koidsumi, 1935) after Edney (1957).

Ramsay (1935 a), by blocking the spiracles was able to show that transpiration from the cockroach Periplaneta americana took place through both the spiracles and the cuticle. He also showed that the loss through the cuticle increased at temperatures above 30°C. This observation switched the attention from the belief that the spiracles are practically the only site of water loss from the external surface of the insect (Buxton, 1932; Mellanby, 1934 a) to the importance of the cuticle in reducing water loss from the external body surface. This observation started a line of investigation which lead to a better understanding of structure and permeability characteristics of the insect cuticle and the important part it plays in the ability of the insects to regulate their water balance, enabling them to exploit the terrestrial environment in full.

The structure and permeability characteristics of the insect cuticle have been fully discussed in recent reviews of the subject, e.g. (Edney, 1957; Wigglesworth, 1957; Beament, 1961; Barton-Browne, 1964).

The insect cuticle, secreted by a single-layered epidermis, consists of two major sub-divisions - a relative thick inner layer the endocuticle and a thinner outer layer the epicuticle. The endocuticle consists essentially of a protein and chitin matrix, the outer regions which might be hardened and darkened by tanning to give an outer exocuticle.

The waterproofing properties of the insect cuticle largely resides in the lipids disposed superficially in one or more discrete layers. The lipid epicuticle when present ranges from about 0.1 - 0.4 μ thick (Richards, 1953), rendering resistance to water transpiration through the entire thickness. Disproportionate water loss with removal of grease from the cockroach cuticle, gave Beament (1960) direct evidence for the hypothesis that the excessive impermeability to water, conferred by the lipid layer on the cuticle, resides in a monolayer of orientated polar molecules of wax next to the tanned epicuticle, the surface of the latter being important in the arrangement of the monolayer (Beament, 1961). The same author further postulates that this special impermeable state must extend through a much larger thickness of wax in more waterproof insects. Some insects e.g. Rhodnius and pupae of Tenebric and Pieres acquired additional waterproofing from a secondary wax layer formed over the cement layer (Beament, 1959).

The structure of the insect cuticle is by no means fully understood, less is known about specialised membranes which enter into the physiology of the cuticle. The role it plays in reducing water loss however is obvious. Waterloss through the cuticle has to a large extent been eliminated by waterproofing with wax. The degree of impermeability depends a great deal on the composition and structure of the lipid epicuticle, and seems to be closely correlated with the dryness of the habitat of the particular insect.

Although water loss from the respiratory surfaces cannot entirely

be eliminated, it has been reduced considerably by the invagination of these surfaces into the body cavity, and the development of an effective closing mechanism. An internal respiratory system with an effective closing mechanism evolving together with a practically impermeable cuticle account for most of the success of insects in exploiting the terrestrial environment.

These mechanisms can be regarded as long term investments in a terrestrial life. Because of the limited control of insects over these mechanisms a situation can easily be visualized where these same mechanisms can be an embarrassment to the insect, i.e. it could be too efficient in extreme wet conditions and insufficient at the other extreme. The necessity for an active control system coping with extreme or unusual short term fluctuations together with the long term investments can easily be seen.

1.2 Physiological Mechanisms

The amount of water present in an insect at any particular time will primarily depend on the amount of water present in their food and the moistness of the place in which they live.

The degree of hydration or dehydration that can be tolerated before death occurs or before the general physiology is adversely affected, varies considerably amongst insects (Wigglesworth, 1950; Barton-Browne, 1964).

Apart from mere tolerance, insects show a remarkable ability to counteract or regulate internal osmotic changes. As regards the mechanisms of internal regulation, there are two main systems operating

in insects. (a) The amino acid/serum protein balance which is capable of counteracting large variations in osmotic pressure (Edney, 1957; Barton-Browne, 1964). (b) The malpighian tube and rectal system which regulates the total osmotic pressure and ionic balance (Edney, 1957; Craig, 1960).

1.21 Excretion

The physiology of excretion and the role it plays in the maintenance of water balance has been discussed by Craig (1960) and Edney (1957) respectively.

Although many organs and tissues contribute to the complex of homoistatic mechanisms known collectively as excretion, the malpighian tubes and rectum plays the most important part in insects.

Urine is formed in the malpighian tubes and together with food residues from the stomach form the faeces. The urine ranges in appearance from a clear copious liquid in aquatic insects, blood- and sap-suckers, to a bone dry powder in the mealworm T. molitor (Wigglesworth, 1950).

Insects excrete nitrogen mainly as ammonia, urea in the amino form or as uric acid. The latter being by far the most important nitrogenous constituent in the urine (Wigglesworth, 1950).

Excretion of nitrogen in the form of uric acid is considered to be a remarkable adaptation for the conservation of water. Uric acid containing less hydrogen than any other nitrogenous compound, is almost insoluble, thus non-toxic and requires very little water for its elimination.

Wigglesworth (1932) showed with a number of different insect species,

that in spite of the dry state of the excrement, the urine is a clear fluid when discharged from the tubes into the gut. The gut contents become progressively drier as it passes along the hind-gut and rectum, suggesting absorption of water in this region. Dehydration was particularly marked in the rectum where the rectal glands are believed to play an important part in the re-absorption of water. Water or salt or both may thus be re-absorbed in the rectum according to need and the final urine may be hypo-, iso- or hyper-tonic to the blood.

Evidence was produced by a number of workers, suggesting hormonal control of the activity of the malpighian tubes. Altman (1956) studied the effect of corpus allatum and corpus cardiacum extracts on the water balance of the honey bee. His experiments showed that extracts of the corpus allatum increase the rate of urine production by the malpighian tubes, while extracts from the corpus cardiacum has the opposite effect. Nunez (1956) demonstrated with the subterranean larva of Anisotarsus cupripennis (Germar) that ligation of the neck, severing of the nerve cord connectives and removal of the dorsal part of the brain, inhibit excretion of excess water absorbed through the general body surface, causing the larvae to inflate. Nunez (1962) showed a decrease in the rate of excretion and an increase in haemolymph volume in Rhodnius prolixus, on being decapitated after a blood meal.

Up till now only mechanisms involved in reducing and controlling water loss have been discussed. Water loss from any insect for a given time depends on the effectiveness of these water loss reducing mechanisms

and the degree of control exercised over them in relation to the drying power of the air. Recognising the fact that most insects need to keep the proportion of water in their bodies constant within narrow limits, the overall water loss must be made good by overall water intake.

1.22 Intake of water from the surroundings

Water taken in with food, is the primary source of water in all insects. This can be supplemented by drinking, water absorption through the general body surface, absorption of water vapour and by retaining water formed as a by product of metabolism.

The food of insects varies from liquids to dry materials such as cereal products containing from 1 - 20% water. Thus Euphestia kuehniella will grow in flour containing 1% of water, Tribolium grows fairly well in food with 6% water content, while Lasioderma, Sitodrepa and Ptinus require at least 10% of water in their food (Wigglesworth, 1950).

Insects feeding on liquids, usually get rid of excess water by excretion. In the case of insects living on cereals and its products without the option of drinking, the water content of the food might be the determing factor in their distribution.

1.221 Absorption of water vapour from unsaturated air

The ability to absorb water vapour from unsaturated air has been demonstrated in insects, ticks and certain isopods. Occurrence of this phenomena and possible mechanisms have been discussed by Andrewartha and Birch (1954), Beament (1954, 1961), and Edney (1957).

It seems incontrovertable that some arthropods can at certain times

during their life cycle extract water from unsaturated air, which can be as low as 50% relative humidity in the case of the prepupae of the rat flea Xenopsylla brasiliensis (Edney, 1947, 1957). The mechanism of this process is however not yet well understood. The difficulty in providing a satisfactory explanation lies in the fact that water is being moved in the vapour phase against a considerable osmotic gradient.

While uptake of water vapour is apparently correlated with relative humidity rather than with saturation deficiency (Edney, 1947, 1957) and (Mellanby, 1932 a), desiccation seems to be a prerequisite (Lees 1946, 1947; Browning, 1954; Beament, 1954. Mellanby (1932 a) suggested that in the larvae of Tenebrio molitor the site of water absorption is in the tracheoles which are known to have water-absorbing abilities (Buck, 1962). Lees (1946, 1947) and Browning (1954) working with ticks showed however that blocking of the spiracles does not necessarily interfere with uptake, but that death, anoxia, anaesthesia and wounding of the cuticle as well as commencement of feeding will interfere. This suggests that the process can occur over the whole body surface, and that it is not a purely physical one. Active uptake must involve the expenditure of energy, but it is not known how this is done (Beament, 1961).

The ability to absorb water vapour from unsaturated air enables the animals to replenish water without necessarily finding a place where the air is saturated and this enhances their ability to live in dry places.

1.222 The utilization of metabolic water

It has long been suspected that insects living permanently in dry environments manage to survive by retaining the water derived from the complete oxidation of their food. This method of regulation has been reviewed and discussed by Andrewartha and Birch (1954), Buck (1953) and Edney (1957).

Although there seems to be some disagreement as regards the presence and the usefulness of this regulating mechanism, three interesting features arise from the literature:-

(i) Some starved insects are able to maintain a constant proportion of water to dry material in their bodies, in a dry atmosphere, e.g. larvae of T. molitor (Berger, 1907), (Buxton, 1930), adults of Phyllophaga implicata (Sweetman, 1931), Tribolium confusum and T. castaneum (Roth and Willis, 1951).

(ii) Some insects starved in different humidities used additional dry material in lower humidities, presumably to make extra water to compensate for that lost by evaporation, e.g. Tenebrio molitor larvae (Buxton, 1930, 1932), starving Glossina (Buxton and Lewis, 1934) and the adults of T. confusum (Nelson and Winston, 1964).

(iii) Some insects require more food in low humidities to produce a given weight of pupa than in higher humidities, e.g. Tribolium, Euphestia and Dermestis (Fraenkel and Blewett, 1944). Here it is claimed that more food is consumed in the lower humidities because part of the food is utilized as water.

Starving insects, even when in a resting stage require energy for maintenance. This energy can only be obtained by oxidising reserve food materials. If we assume complete oxidation of these food materials, then water and carbon dioxide will be two of the main end products. The amount of water derived from complete oxidation of reserve food material will depend on the type of food and the rate of metabolism (Mellanby, 1942). The availability of this water for regulating purposes can only depend on the ability of the insect to retain this water.

Maintaining a constant proportion of water in the body does not necessarily signify an active regulatory mechanism. This could be brought about by reducing water loss to such an extent, that dry matter and water is lost in the same proportion as it was present at the start. However because dry matter is being used some water must be formed, and this could well be incorporated in the balance sheet.

The metabolism of additional reserved foods on the other hand signifies active regulation by this means. Buxton (1930) showed that T. molitor larvae when starved at different relative humidities below 60%, lost more weight in lower humidities than in the high, but the proportion of dry to wet weight remained constant. This he explained by suggesting that in dry air additional dry material was oxidised and the water of metabolism retained to compensate for that lost by evaporation. Mellanby (1932 a, 1932 b, 1934 a, 1936) failed to confirm these results in T. molitor or in other species starving at low humidities. Mellanby (1936) argued that such an increase, even if it did occur, would be of no

advantage to the insect because it would entail an increase in the rate of air flow through the spiracles with a consequent increase in water loss. Mellanby (1942) added that in dry places (deserts), starvation is as great a danger as desiccation. He believes that fats for instance are stored as a food and not a water reserve, and that desiccation can be avoided by other means, e.g. living in burrows, where a microclimate different to the outside air exists. Gunn and Cosway (1942) working on the respiration rate of Periplaneta americana in different relative humidities, came to the same conclusion. According to them, "there is no reason to believe that at a given body temperature, air humidity influences basal metabolic rate, or that the desiccated condition is relieved by extra production of water."

Now the absence of a mechanism in some animals does not necessarily mean the absence of such a mechanism in all animals. Andrewartha and Birch (1954) proposed that Buxton's hypothesis (1930) implies that the insect by doing work can dry out the air before it is expired from the spiracles and thereby retain in their bodies some of the water that is produced as a by product of respiration. This seems to be the only way in which water of metabolism can be retained and at the same time satisfy Mellanby's argument that an increased metabolic rate will cause an increase in water loss.

Kalmus (1936), found that air in a small airtight chamber containing T. molitor larvae, reached a relative humidity of 90% and remained in equilibrium at this level until the animals died; the air then became saturated. Edney (1947) found that when a number of Xenopsylla brasiliensis

pre-pupae were enclosed in a small space together with moist air, the humidity fell to 50%, remained there until the fleas pupated, and then rose again as the pupae lost water. This surely indicates that certain insects can extract water vapour from unsaturated air and lend strong support to Andrewartha and Birch's suggestion that a mechanism of some sort for drying out air does exist.

Fraenkel and Blewett (1944), claimed to have provided conclusive evidence for the conservation of metabolic water. Working with Tribolium confusum, Ephestia kuehniella and Dermestes vulpinus, they came to the conclusion that these insects, which normally live on dry food "acquire a substantial, or, at extremely low humidities, the greater part of the water ultimately found in the body from the oxidation of food." Now it is obvious from their results that more water is present in the pupae than could have been obtained from the food eaten. However as has been pointed out by Andrewartha and Birch (1954), there is a possible alternative explanation, which is not answered by these experiments, i.e. that this water might have been obtained by dehydration of undigested food.

Water derived from the complete oxidation of food, must play some part in the insects ability to maintain a constant dry/wet weight ratio. The question as to whether it can be regarded as an active regulatory mechanism in dry conditions, is not completely answered. The problems seems to be to distinguish between water of metabolism and the water

normally present in the insects body; also the problem is complicated by the fact that loss by excretion, and loss through the spiracles may vary directly or indirectly as a result of the ambient humidity.

1.3 Behaviour that helps to conserve water

Behavioural responses of insects to water and atmospheric humidity, according to the wealth of information available, contribute significantly to the ability of insects to maintain a necessary degree of water balance. The ability to recognize and respond to water and atmospheric humidity can be recognized as adaptations helping the insect to avoid extremes of wetness or dryness, leading them to places where the moisture is favourable.

A puzzling reversal of the response to a moisture gradient has been reported for a number of species, namely Blatta orientalis (Gunn and Cosway, 1938), Ptinus tectus (Bentley, 1944), T. castaneum, T. confusum (Roth and Willis, 1951), adults of T. molitor (Dodds and Ewer, 1952) and the larvae of T. molitor (Perttunen and Lehrman, 1962). The reversal in the behaviour pattern is explained in terms of the water relation and degree of starvation of the insects. The advantage of such a behaviour is fairly obvious, however little is known about the mechanisms, physiology or sensory perception that underlie this behaviour.

The ability of insects to recognise small differences in a humidity gradient, has frequently been demonstrated. Larvae of the wireworm (Agriotes spp.), tend to move towards the moist end of the gradient, providing the relative humidity exceeds 70%. In this range a difference

of 7% in relative humidity is enough to produce a reaction in almost every individual in the sample (Lees, 1943). Roth and Willis (1950) showed that T. castaneum preferred 95% relative humidity to 100%, and Perttunnen and Lahermaa (1962, 1963) claimed to have shown that both the larvae and adults of T. molitor can distinguish between 100% and 99.5% relative humidity.

Pielou and Gunn (1940) were successful in identifying hygroreceptors on the antennae of adults of T. molitor. The same has also been found on the antennae of T. confusum, T. castaneum and T. destructor (Roth and Willis, 1951) and on the antennae of Blatella germanica and Aedes aegypti (Roth and Willis, 1952). The presence of hygroreceptors poses the question as to their nature and mode of action. Moisture in the air at a constant temperature may be measured in units of absolute humidity, relative humidity, saturation deficit or rate of evaporation. The latter under certain circumstances, e.g. in a constant high wind from an open water surface might be nearly proportional to the saturation deficit of the air (Andrewartha and Birch, 1954). According to these authors hygroreceptors may work on the principle of a hygrometer or an evaporimeter, which might in some circumstances, though not necessarily always, be simply related to the saturation deficit. The behaviour of larvae of Choristoneura fumiferana (Wellington, 1949) could better be explained in terms of evaporation than in terms of either saturation deficit or relative humidity. Lees (1943) found a better correlation between intensity of reaction and saturation deficity than between

intensity of reaction and relative humidity in Agriotes larvae, suggesting that the receptors may be like an evaporimeter. In contrast to this, the responses of the mosquito Culex fatigans (Thomson, 1938) and that of the beetle T. molitor (Pielou, 1940) were more closely correlated with humidity, suggesting a receptor working on the principle of a hygrometer. Barton-Browne (1964) suggested a third type of hygro-receptor, a chemoreceptor specifically sensitive to water vapour, measuring the absolute water content of the air, the existence of which, however, is yet to be demonstrated.

The survival value of responses to differences in humidity as well as its usefulness as a mechanism for internal water regulation is obvious. The ability of insects to recognise and respond to differences in humidity, enable them to avoid extremes of dryness, to search out and stay in the most favourable microclimates within their habitats, and may enable them to search out such places where water can be taken in from the surroundings. The ability to and the necessity for recognising and utilising suitable habitats can best be illustrated by the statement of Cloudsley - Thompson (1962), that insects are perhaps the best indicators for the identification and classification of microclimates.

From the material discussed it is clear that water regulation in terrestrial insects is an extremely complex phenomena. The general rule seems to be that terrestrial insects cannot survive severe desiccation of their tissues. Exceptions to this relate chiefly to some insects in diapause (Andrewartha and Birch, 1954; Andrewartha, 1961).

Because of their large surface area/volume ratio, insects start off at a disadvantage in a terrestrial life. Despite this disadvantage, insects became remarkably adapted to life on land. Most adaptations for life in dry places comprise either behavioral patterns allowing them to avoid extremes of dryness and to utilise free water and water in the atmosphere, and morphological and physiological mechanisms reducing the amount of water lost by evaporation, respiration and excretion.

It is further obvious that some terrestrial insects are better equipped to live in very dry places than others in that they have additional and more effective mechanisms for the conservation of water. Some terrestrial insects however are able to survive in areas where the humidity is likely to fluctuate widely by virtue of the fact that some stage in the life cycle is more resistant to extremes in dryness. This fact was very well illustrated by Andrewartha and Birch (1948) in the diapausing eggs of the grasshopper Austroicetes cruciata.

2.0 WATER CONSERVATION IN TENEBRIO MOLITOR L.

A large number of species of the family Tenebrionidae is found in desert habitats (Imms, 1957).

The members of this family, Tenebrio molitor L. and the closely related species Tenebrio obscures are recognised pests of grain and grain products (Cotton and St George, 1929; Van Emden, 1947; Cotton, 1950; Rivnay, 1962).

Although it is believed that these species are of European or Asiatic origin (Cotton and St. George, 1929), they are today nearly

cosmopolitan in their distribution (Imms, 1957; Rivnay, 1962). They are found in mills, warehouses and storehouses, feeding on meals, flours of all kinds, bran, refuse grain, course cereals, mill sweepings, as also food of animal origin, e.g. meat scraps, bodies of dead insects, feathers, etc. (Cotton and St.George, 1929). T. molitor has also been reported found in mills, rotting roof timber and oak mould (van Emden, 1947).

By virtue of the food they eat and the places in which they live, T. molitor can be classified as an insect able to live in dry places. Their distribution signifies their adaptability to variations in climate, and their ability to live in dry places suggests that they are well adapted to con-serve water or utilise water in whatever form present.

T. molitor a very useful laboratory animal in that it is easily reared, has frequently been used for scientific studies. Because of this quite a lot of knowledge is available regarding its water relations. The water saving mechanisms known to operate in T. molitor, and gaps in our knowledge of these mechanisms can best be discussed in light of the general discussion on water saving mechanisms operating in terrestrial insects (Section 1).

2.1 Morphology of the cuticle

The cuticle of T. molitor shows a high degree of impermeability compared to some other insects, This has been demonstrated by Wigglesworth (1945), Beament (1945, 1959) and Edney (1957).

The epicuticle is responsible for most of the impermeability to

water. Wigglesworth (1948) studying the structure of the epicuticle in adult T. molitor suggested four different layers: the cuticulin, a layer of polyphenols and a wax layer covered by a cement layer protecting the delicate wax layer. Very little is known about the structure of the epicuticle of the larvae, but evidence suggests the presence of a cement layer over the wax layer (Beament, 1959). As far as the epicuticle of the pupae is concerned, evidence produced by Beament (1959) suggests the presence of two special waterproof layers of wax with different transition temperatures. The one with the higher transition temperature occurring on the outer surface of the cement layer. If one of the differences between the structure of the epicuticle of the larvae and the pupae could be shown to be the occurrence of a double wax layer in the latter, then this can partly explain the difference in permeability of the cuticle of larvae and pupae as have been demonstrated by Edney (1957) and Beament (1959).

The waxes extracted from the cuticle of T. molitor has been shown by Beament (1945) to be hard, white, with a crystalline structure and a relatively high melting point, compared to the waxes from more permeable cuticles of some other insects, which were softer, showing a lesser crystalline structure and a lower melting point.

Abrasions of the epicuticle in T. molitor, which must be a common occurrence with the insect moving through cultures, has been shown by Wigglesworth (1945) to increase the permeability of the cuticle tremendously The same author however demonstrated the ability of the larvae to restore

the waterproofing of the cuticle. This is presumably achieved by pouring fresh wax, secreted by the epidermal cells, by way of the pore canals over the wounded areas.

2.2 Excretion

Like in most insects, the malpighian tube/rectal system is the main excretory sytem operating in T. molitor. In these insects there are six malpighian tubes individually attached to the midgut. The distal ends of the tubes are cabled into a common trunk, leading them under a membrane covering the rectum, from which point they separate into U-shaped structures covering the entire organ (Patton and Craig, 1939). According to these authors the free portion and to a lesser extent the portion of the malpighian tubes surrounding the rectum, absorb non-selectively, solution of electrolytes and other compounds, e.g. amino acids, uric acid and urea. This filtrate when discharged into the gut contains no suspended matter (Wigglesworth, 1932). Presumably this filtrate acts as a lubricant for the food pellets, and/or functions as a solvent to remove digested materials from these pellets and thus facilitates absorption. Absorption of such food materials and re-absorption of salts and water occurs in the rectum where the distal portion of the malpighian tubes surrounding the rectum is supposed to play an important part (Patton and Craig, 1939). The contents of the hindgut are semi-fluid at the anterior end but in the rectum it becomes quite dry and the amorphous uric acid separates out. Re-absorption of water in this region is so efficient that the urine and food residues of both the larvae and adults are reduced to a bone dry powder (Wigglesworth, 1932, 1950), the

latter containing over 50% uric acid in the fasting insect.

The excretory system in T. molitor thus seems to be very efficient in eliminating waste products, making use of the minimum amount of fluid and reducing waterloss by excretion to a minimum. It also provides a means of internal regulation by re-absorbing or not of water and electrolytes in the rectum, according to the need of the moment.

2.3 Water Intake

2.31 (a) Drinking:- Mellanby (1958) reported the ability of T. molitor larvae to drink water when given a chance. Water taken in this way is conserved as thoroughly as the water in normal larvae. Both these groups under favourable conditions, completed their development, producing normal adults still showing comparable differences in water content. Drinking in adults were reported by Dodds and Ewer (1952) and born out by my own observations.

2.32 (b) Absorption of water vapour from unsaturated air:- The ability of T. molitor larvae to absorb water vapour from unsaturated air was first reported by Buxton (1930) and confirmed by Mellanby (1932 a). Starved larvae were able to regulate their water content in air with a relative humidity of 60% and below, in 88% R.H. and above however they gained weight in excess to what could be explained by retention of water of metabolism. Both Buxton and Mellanby's work suggested that this process is dependent on the relative humidity of the air and not saturation-deficit.

Gain of weight by starved Tenebrio larvae has also been reported

by Lefon and Tessier (1939). These authors found the process to continue for 12-15 days in saturated air, after which it remained constant for a long time, having increased 10-15% of their initial weights irrespective of size. This ability was also reported by Sidney (1957) and unpublished observations) who found the process to be irregular and intermittent.

Burton (1930) working in 90% relative humidity at two different temperatures, showed an initial loss of weight during the first two days, after which the weight increased, remaining between 100-105% of the initial weight from the ninth day till the experiment was terminated after a month. Beament (1954) referring to unpublished observations by Locke(1953), found that following partial desiccation, T. molitor larvae will take up water from air with a relative humidity of 93%, occasionally reaching an equilibrium weight below the starting point. Following this, a brief period of desiccation (2 hours) which does not lead to a measurable loss of weight, promotes a further uptake of water on being returned to 90% relative humidity.

Mellanby (1932 a) suggested the tracheal endings as the site of water uptake. Hinton (personal communication) found that only a small proportion of total water intake occurs through the general body surface.

The ability to absorb water from an unsaturated atmosphere is apparently restricted to the larvae. The adults of T. molitor is unable to gain water by absorbing it, even from air nearly saturated with water vapour (Andrewartha and Birch, 1954).

The occurrence of this phenomena is well established in the larvae

of T. molitor, the mechanism of the process however is not well understood. As has been pointed out by Andrewartha and Birch (1954) this ability must have considerable survival value for the insect. The advantage of this ability has however to my knowledge not been demonstrated in T. molitor.

2.33 (c) The utilisation of metabolic water:- Berger (1907) found that T. molitor larvae, although they lost weight, maintained a constant proportion of water to drymatter in their bodies, when kept in absolute dryness. These early experiments were confirmed by Buxton (1930). Working with starved T. molitor larvae, he found that larvae kept in relative humidities of 0-60% for as long as one month, maintained a constant proportion of water to drymaterial in their bodies, in the face of a greater waterloss at the lower humidities. Buxton explained his findings by suggesting that in dry air additional food reserves were metabolised and the water of metabolism used to compensate for the loss by evaporation.

Andrewartha and Birch (1954) suggested that Buxton's hypothesis implies that the larvae of T. molitor, by doing work, can dry out the air before it is expired from the spiracles and thereby retain in their bodies some of the water produced as a by-product of respiration. Mellanby (1932 a) and Kalmus (1936) showed that the air in a small closed chamber, containing T. molitor larvae, reached a relative humidity of 88-90% and remained in equilibrium at this level till the insects died, when the air became saturated. This suggests the presence of a mechanism

in T. molitor larvae capable of drying out air.

Buxton's hypothesis also implies that the metabolic rate of starved T. molitor larvae is to some extent determined by the humidity of the atmosphere surrounding the insects. Mellanby (1932 a, 1936) did not confirm this hypothesis and came to the conclusion that in T. molitor larvae the rate of metabolism is governed by temperature alone, and is unaffected by a change in atmospheric humidity.

Both authors showed that starved T. molitor larvae can stay alive for a considerable length of time. Maintenance energy is derived from metabolising reserve food materials. Buxton (1930) and Mellanby (1934) showed a decrease in carbon dioxide production and weight loss respectively, with time. It is thus clear that starved T. molitor larvae can metabolise and use reserve foods to stay alive. The rate at which reserved foods are metabolised, apparently decreases with time. If we assume complete oxidation, then water must be one of the end products. The ability of T. molitor larvae to dry out air, possibly signifies their ability to dry out the air before it is expired, thus allowing them to retain some of the water which will otherwise be lost through respiration. The presence of a completely different mechanism, i.e. that T. molitor larvae are able to increase their metabolic rate in order to produce extra water to counteract excessive evaporation, although generally accepted, must be considered to be still doubtful.

2.4 Behaviour in response to atmospheric humidity and water

Both Buxton (1930) and Mellanby (1934) showed with the larvae of

T. molitor, that the rate of water loss into the air decreases as starvation proceeds. This was linked with a decrease in the rate of metabolism, allowing the spiracles to remain closed for longer periods. The presence of a very effective spiracle closing mechanism was clearly demonstrated by Mellanby (1934), who demonstrated that waterloss, even in the seventeenth week of starvation, increased more than seven times when the spiracles were kept open with 5% carbon dioxide. Nichal (1931) demonstrated a daily oscillation in the oxygen consumption of mealworm larvae which reaches a maximum during the night when the insects are most active. This was confirmed by Cloudsley-Thompson (1953) who demonstrated a composite 24 hour rhythm of activity, correlated with light and darkness and independant of fluctuating temperatures. The greatest activity of T. molitor thus occurs during the night when the temperatures are lowest and the humidities highest, assuring the minimum of waterloss through the spiracles.

The ability of T. molitor to recognise and respond to a gradient in atmospheric humidity has been well established, in both the adults and the larvae, by a number of workers, e.g. Pielou and Gunn (1940), Pielou (1940), Gunn and Pielou (1940), Dodds and Ewer (1952), Howard (1955) and Perttunen and Lehrman (1958, 1962, 1963).

The advantage of the ability to respond to a humidity stimulus, may lie in avoiding extremes of humidity and/or leading the insect to a favourable zone where evaporation is at an optimum.

Normal un-desiccated individuals of both adult and larval T. molitor,

when tested in a humidity gradient, show a well-definedddry preference. Perttunen and Lahermaa (1962, 1963) claimed to have demonstrated the ability of both larvae and adults to distinguish between a relative humidity of 100% on the wet side and 99.5% on the drier side. In both cases this marked hygro-negative reaction was shown throughout the humidity range. The intensity of the reaction however seem to be determined by the specific value of the highest humidity (Pielou and Gunn, 1940; Perttunen and Laherman, 1963). The last mentioned authors also pointed out the importance of the steepness of the gradient, when the highest humidity is kept constant at saturation level.

The nett result of the hygro-negative reaction is the congregation of the insects in the drier section of the humidity gradient. This may be brought about, either by differences in the rate of movement when they are active and/or by some other mechanism tending to keep them in the drier half of the test chamber. Gunn and Pielou (1940) studying the mechanism of the reaction of T. molitor adults in a humidity gradient, separated the reaction into its component behaviour elements. They found that the bulk of the reaction is accounted for by a marked differential activity or ortho-kinesis which is intensified by frequent turning movements on the dry side, which they termed "virtual inactivity." In addition to this they observed turning movements of the insects on approaching regions of high humidity. These turning movements showed both an undirected component (klino-kinesis) and a directed component (klino-taxis).

Interference with this normal behaviour pattern in response to differences in humidity was demonstrated in both the adults and larvae:-

(a) due to desiccation by Dodds and Ewer (1952) and Perttunen and Lahermaa (1962, 1963).

(b) due to light by Perttunen and Lahermaa (1958, 1963).

Normal un-desiccated adults and larvae show a marked hygro-negative and photo-negative reaction. Dodds and Ewer (1952) demonstrated a decrease in the intensity of the hygro-negative reaction in adult T. politor on being desiccated. This hygro-negative reaction is completely reversed when desiccation proceeds beyond forty hours, but restored after these adults were allowed to drink. A similar reversal in the humidity reaction was demonstrated by Perttunen and Lahermaa (1962) in the larvae, after they have been desiccated for fourteen days.

The photo-negative reaction was shown by Perttunnen and Lahermaa (1962) to deminate the hygro-negative reaction both in normal undesiccated adults and larvae. High humidities however somewhat reduce the intensity of the photo-negative reaction. Desiccation for a time long enough to cause a reversal in the humidity response, also caused a reversal in the photo-reaction of adults. Desiccation of larvae for an equivalent time however only results in a reduced intensity of the photo-negative reaction.

Pielou (1940) identified the humidity receptors in adult T. politor as the pit peg organs and the peg organs situated on the antennae. It was suggested by this author that these hygro-receptors function

hygroscopically, and that the adults react to relative humidity rather than to saturation deficiency. Possible hygro-receptors in Tenebrio larvae are the placoid sensillae and/or the basiconic sensillae found on the apex of the second segment of the antennae (Roth and Willis, 1951 b).

It is completely clear from the literature cited that both larvae and adults of T. molitor can recognise and respond to differences in humidity. The initial hygro-negative response in adult T. molitor, increasing rapidly in intensity till the maximum is reached when the highest humidity available is 100% (Pielou and Gunn, 1940), suggests that these insects have to avoid humidities in the higher range. The reversal of the hygro-negative reaction due to desiccation, and the fact that drinking restores the original response, suggests a disturbance in the water balance. The puzzling question however remains, why this hygro-negative reaction, significant through the whole humidity range, should lead the insects to a humidity which is obviously disadvantageous.

Perttunen and Lahermaa (1962) suggest that the reversal in the hygro-negative reaction of T. molitor larvae, after being desiccated for fourteen days, probably indicates a severely disturbed water balance and a considerable loss of water. Buxton (1930) however stated that T. molitor larvae can maintain their normal dry/wet ratio even when starved for a month in a completely dry atmosphere, thus eliminating the possibility of a severely disturbed water balance.

Dodds and Ewer (1952) observed that T. molitor adults will accumulate

in, and drink from cotton wool soaked in water, when the latter is placed in the cultures. This suggests that the adults at least can seek out and utilise free water. T. molitor larvae are known to be able to absorb water vapour from unsaturated air, and also to drink. If the reversal of the initial hygro-negative reaction should lead it to seek out such places where water can be taken in, it will be an obvious advantage. The reason for the reversal as also the advantage of water absorption however has to my knowledge not yet been demonstrated.

PART 2

3.0 THE AIM OF THIS PROJECT.

From the literature cited and discussed in Section 2 it is obvious that a lot of information exists on the ways in which T. molitor can conserve water and/or counteract and overcome excessive evaporation. At the same time quite a few aspects need confirmation, clarification and coordination to get a better overall picture of water regulation in T. molitor. Although a lot is known about the physiology of water balance in T. molitor, a lot more information is needed to fully appreciate how this fits in with the general physiology and ecology of this insect.

It was the aim of this investigation to re-investigate and correlate some of the studies on water relations in T. molitor previously reported, to examine in detail certain aspects not studied before, giving particular attention to the influence on the insects and its response to changes in its internal environment in relation to differences in environmental humidity and temperature.

The choice of working on starved T. molitor was primarily based on two reasons:-

(a) According to Leoleroq (1948) and Edney (1957), T. molitor can complete its life cycle in relative humidities of 1-10%, when food is present, Berger (1907) and Buxton (1930), showed that larvae of T. molitor can maintain a constant dry/wet ratio for a considerable time in complete dryness. These observations suggest that water can be obtained by metabolising both raw and reserved food material. I realised thus, that

by including food, an additional variable would be introduced, which would be difficult to evaluate and separate, thus complicating the study immensely.

(b) Andrewartha and Birch (1954) saw diapause as an important adaptation in insects, enabling them to persist in regions from which they might otherwise be killed out by extremes of climate, or to maintain high numbers in an area which might otherwise support only a few. This suggested to me that the ability of an insect to colonize a certain habitat permanently or to maintain high numbers in other areas, might to a large extent depend on their successful survival of the most severe part or parts of the annual climatic cycle. Although to my knowledge no evidence exists suggesting the presence of diapause in any stage of the life cycle of T. moliter, Buxton's (1930) observation on the time T. moliter larvae can stay alive, and the reduction in the production of carbon dioxide when starved, suggests at least a state of quiescence.

My argument at this stage was that an investigation into what happens to and inside T. moliter when subjected to different combinations of humidity and temperature in the absence of their usual source of energy and water (food), might give a better insight as to the ways and means by which these insects conserve their water and energy, and by what means they stay alive during adverse conditions. This in turn may allow us to explain more fully the ability of the insects to colonize and live in dry places.

The way in which I proposed to carry out this investigation, was to

subject both adults and larvae to different combinations of temperature and humidity - then to measure the changes in their total body water, total dry material and total weight loss with time in relation to these atmospheric conditions. This data would allow me to make inferences about the relation between atmospheric conditions and changes in the internal condition of these insects as also the insect's ability to maintain a constant water balance. Knowledge about the possible changes in the internal condition of the insects might also reveal the reasons for the changes in behavioural response to humidity as a stimulus.

Changes in the rate at which the measured parameters alter with time could pin-point and identify mechanisms for conservation of water coming into operation or being relaxed in response to atmospheric conditions and/or changes in the internal environment.

Carrying the study through till the insects die, would reveal the cause of death which in turn would throw light on the insect's ability to utilise available resources, and the efficiency or inefficiency of its water saving devices in relation to environmental conditions.

During starvation, metabolic water and water in the atmosphere are the only two water sources available to the insects. Water regulation in starved T. molitor will thus depend on their ability to conserve original body water. Their ability to obtain and utilise water from the sources mentioned above, or to escape these conditions and search out more favourable conditions.

Metabolic water and water in the atmosphere being the only water

sources available to starved T. molitor, special attention was given to metabolism in relation to atmospheric humidity and temperature, and the phenomena of water absorption.

Results obtained from a preliminary investigation revealed that pupation occurs in starved larvae of T. molitor, even after a moult with no additional food. Pupation seemed to be prevented in lower humidities and promoted in the humidity range where water can be absorbed. The possibility of a relation between water absorption and pupation, as also the prevention of pupation in lower humidities, seemed very relevant to this study. Since the larvae can stay alive for a considerably longer period than the adult (Buxton, 1930; Pielou and Gunn, 1940), this might be a way in which the insect escapes unfavourable conditions, in that it is confirmed to a drought resistant stage in the life cycle. The incidence of pupation in relation to atmoshperic humidity and temperature was therefore included in this investigation.

PART 3

MATERIALS, METHODS AND GLOSSARY OF TERMS

4.0 MATERIALS AND METHODS

4.1 Rearing methods

T. molitor cultures were reared in the laboratory in wooden boxes 15" x 24" x 8", with tight fitting sliding lids. The food mixture used consisted of equal parts of bran, pollard and wheat germ (after Andrewartha, 1961). This food mixture was put into the boxes in a single layer 3 - 4" thick with a double layer of thinly woven jute sackcloth on top. With the food mixture, a medium sized carrot sliced in half was supplied at least twice a week. One hundred-and-fifty newly emerged adults were placed in each culture box. These were removed and discarded after three weeks. Cultures were kept in a room, where the temperature varied between 23 - 25°C.

4.2 Problems considered and encountered in rearing T. molitor

Both Burton (1930) and Mellanby (1932) pointed out that the chief disadvantage in T. molitor as material for the study of water relations was the extreme variability of the water content of their bodies. It is desirable to retain in the stock cultures as much genetic variability as possible while reducing as much as possible the variability caused by the environment. Consequently the following method was adopted.

Breeding stock for my first series of cultures was obtained from a series of cultures kept in the Department of Zoology, of the University of Adelaide, for feeding marsupial mice and possums. In the last mentioned

cultures, started in sequence, the insects were allowed to breed continuously in the same containers. They thus contained T. molitor in all stages of development. In order not to select a particular type of insect, breeding stock were randomly picked from all of these cultures, using only newly hatched adults. A series of nine cultures were built up in this way, starting one every three weeks. The number one container was again used to start the tenth culture.

A number of authors, e.g. (Ludwig, 1956; Tracey, 1958; Ludwig and Fiori, 1960, 1961) demonstrated a relationship between the rate of larval growth, length of larval stage, number of moults, length of life of the resulting adults and the parental age of T. molitor. These relationships were evident only after the parents had aged at least 4 - 6 weeks. Not to introduce this possible source of variation into this investigation, adults were removed and discarded from the cultures three weeks after starting a new culture.

Cotton and St George (1929) showed T. molitor to moult 10 - 20 times during the larval stage. Mellanby (1932) pointed out that there seems to be no hard and fast rules as to what size a mealworm should reach before it pupates. These observations suggest the possibility of some genetic factor or factors operating in a natural populations determining the size of the adults and therefore also the size of the larvae. The importance of the size of the experimental animal in water relation studies was recognised by Kennedy (1927) who pointed out that, all other parameters remaining the same, the water content is a function of the volume of the

animal (cube of the linear dimention), whereas evaporation is a function of surface area (square of the linear dimention).

In a single culture, started as described, pupae first appeared after eight weeks, while the last pupae from the same culture were collected after six months. It was further obvious from these observations that the average size of pupae collected, increased with time. The possibility of selecting for size by taking beetles at any particular time was then obvious, as was also the possibility of obtaining results relevant only to a portion of the population. To prevent selection in this way, breeding stock for new cultures started every three weeks, were randomly selected from cultures ranging from nine weeks to six months old.

The relevance of this precaution is obvious in view of the findings of Leclercq (1963). He showed that selection for weight brought about strains fitted to various ecological hazards. These strains were shown to differ in the size of their offspring, rate of development, length of larval life, resistance to low temperatures, nutritional requirements, fecundity and response to water (drinking).

The variability amongst individuals of T. molitor due to their genetical composition does not make the insect the "ideal" experimental animal. I realised that this fact might make it difficult to demonstrate small differences in experimental results. Selection in any way however will have the disadvantage of giving information about only a portion of the population without the assurance of making this portion more homogeneous

for any specific characteristic one wishes to study.

In rearing T. molitor, most workers supplied water to their cultures in one or other form. Ludwig (1956) added water in cotton wool, as also directly into the food of the larvae, claiming that by doing so the rate of larval development increasee. Andrewartha (1961) suggested pieces of carrot or banana for adults are likely to result in more eggs being laid. These facts were borne out by my own observations. Difficulties however arose. Frequently the food mixture turned mouldy and as has been pointed out by Howard (1955) , mouldy culture mediums adversely effect the growth rate of the larvae. A high humidity in the container also seems to be a pre-requisite for the building up in numbers of the mite Tyroglyphus farinae. These mites, living on the culture medium became so plentiful at times that they covered the whole culture to a depth of a $\frac{1}{4}$ inch, badly effecting the culture, probably through mere suffocation of the insects.

These problems were overcome by supplying water in the form of sliced carrots put on top of the layer of sackcloth, thus preventing the carrot from being "worked" into the culture medium by the movement of the larvae. The moist carrot in the medium as also water applied directly into the medium, assist the moulds to grow. Mouldy patches being confined to the spots where water was introduced.

In addition to the normal ventilators in the lid of the container, three holes ($1\frac{1}{2}$" in diameter) were drilled in all four sides of the container just above the level of the culture medium. These holes allowed

cross ventilation and prevented an increase in humidity in these containers, The latter and not mouldiness seemed to be the main reason for the increase in the number of mites. In addition to this, culture boxes were sterilised with boiling water before starting a new culture.

Apart from the mites, some other insect species living on cereals and their products, very often found their way into the cultures. The most common accidentally introduced species were the flour beetles, Tribolium confusum Duval., Tribolium castaneum Herbst., the flour moths, Ephestia kuhniella Zeller and Plodia interpunctella Hubner. Distinct from the crowding out effect (Howard, 1955) some other interference by these insects was observed. Flour moth larvae especially can cause deterioration of cultures as a result of their habit to spin a great deal of silk webbing over the entire surface of the medium. - the larvae living in little tunnels made of silk and bits of food. Positioned vertically in the top layers of the medium, it can form a thick continuous layer of silk and frass over the whole surface, trapping especially the young larvae and possibly suffocating them. These insects were found to be introduced with the food medium. Storing the food mixture in a low temperature ($-5^{\circ}C$) for 1 - 2 months before using, together with sterilization of culture boxes and cleanliness in the culture room, did away with the interference by all of these insects.

Migration of larvae away from the culture medium was observed when (a) larvae were in the final stage ready for pupation (b) when the culture medium have deteriorated to a great extent. This together with

a general wandering about of larvae especially during the night can lead them to escape from the cultures. Hence the close fitting sliding lid, which also prevented infestation of cultures by other cereal pests that might be around. Larvae often eat there way through the sides of the wooden culture boxes, especially in the corners or at the joints. To prevent larvae escaping this way, joints and corners were lined with strips of copper gauze. This same material was also used to cover ventilator holes in the sides of the culture boxes.

During the pre-pupal instar, larvae leave the culture medium and gather in between the two layers of sackcloth on top of the culture medium, where they pupate, and from where they can easily be collected without disturbing the rest of the culture. This double layered sackcloth placed on top of the culture, was used in preference to the alternate food, sackcloth layer method proposed by Andrewartha (1961), for the following reasons:- (a) Larvae ready to pupate will move upwards through the medium till they reach the first layer of sackcloth. They then may pupate either just below this layer or on top of it. The mere weight of the food and sackcloth of the top layer on the pupae may result in both the pupae and the adults resulting from them, to be misformed and thus useless for experimental pupposes. (b) The continuous movement of larvae within the culture medium, especially from the time they are 3 - 4 weeks old, tend to sieve the finer faecal material down to the bottom of the container, the larvae only occuppying the top layer of clean food. Collecting of any particular stadium could thus be done without

necessarily mixing food and faecal material. This was almost impossible to avoid with the multi-layered method. Mixing of food and faecal material when selecting experimental material was thought not advisable. Faecal material which can be in a fine powdered form, mixed up with food, seemed to cause some distress to the larvae. Also Karlson and Schmialek (1959) demonstrated the presence of a substance in Tenebrio faecal material, with the characteristics of a juvenile hormone. Mixing of faecal material with food will result in a forced feed on this material with a possible effect on the general physiology of experimental material.

Cultures were cleaned out regularly. New food was supplied 4 - 5 weeks after starting a new culture. This was then repeated every 2 - 3 weeks as seemed necessary with larvae growing bigger and consuming more food. Cultures were also cleaned out and food replaced immediately before selection of experimental material commenced. The procedure for cleaning was as follows. Coarser food stuff and bigger larvae were removed by sieving the culture through a standard soil sieve (7 mesh to the inch), into a sieve (16 mesh to the inch) which retained everything but dust and faecal material. Pieces of food and larval or pupal skins retained by the sieves were then fanned out by a blower or in a light breeze. Culture boxes were then either sterilized if necessary or just washed out, dried and used immediately.

Fresh carrots were supplied once or twice a week, depending on the degree of drying and the amount eaten.

4.3 Selection of Experimental Material

The purpose of standardising the breeding technique was to minimise differences amongst individuals caused by differences in the environment

before introduction to the experimental environment, at the same time retaining the original genetical composition of the population throughout the investigation. The next step then was to standardise the technique of selecting experimental material, in order to reduce the gariability in an experimental sample, or else to systematise it so that it could be allowed for.

The work of Leclercq (1963) clearly demonstrated the variability in certain physiological characteristics of individuals of T. molitor in natural populations, as controlled by their genetical composition. The difference in length of larval life, growth rate as well as certain other physiological characteristics, genetically linked with parental size, posed a problem in selecting physiological homogeneous experimental animals, representative of the population. None of the parameters normally used for selecting homogeneous exp erimental material, e.g. same physiological age, same chronological age, same weight, same size, could be used without introducing a considerable amount of variability within the experimental population regarding the other parameters. The latter had to be accepted and was allowed for by using large experimental samples.

Material for any particular experiment was selected from the whole series of cultures in order to get a sample representative of the population. Size of the experimental animal, having a possible bearing on its water relations had to be considered. Weight as a measure of size was adopted to select experimental material. Variability in weight amongst individuals of the population was taken into account, by either (1) including the

whole weight range in an experiment (ii) by conducting separate experiments to test possible differences because of size, and (iii) by using experimental animals in a weight range round the mean of the distribution of the population.

Weight as a measure of size, and total weight consisting of two components, total dry weight and total weight of water were the parameters adopted and measured to describe the body composition of the experimental animals in relation to atmospheric temperature and humidity.

Both larvae and adults were used for experimental purposes within twenty-four hours after a moult or after emergence from the pupae respectively. During this period the insects are very delicate and extremely prone to damage and need to be handled very carefully. Using such animals had the advantage of starting an experiment with animals probably as physiologically similar as they can ever be. Because they had not had a chance to feed their guts should theoretically be free of food particles. The latter is a source of error when determining the drymaterial of individuals at the start of the experiment, or could be in subsequent weight loss studies due to the cleaning-out of the gut. Wigglesworth (1945) demonstrated that T. molitor larvae can heal abrassive wounds of the epicuticle by wax excretions, he came to the conclusion however that healing is never as efficient as the unharmed cuticle. Water loss could thus be different amongst individuals because of a difference in the degree of damage done to the waterproof layer of the cuticle, which should to a large extent depend on the time passed since the last moult. Using

newly emerged adults and newly moulted larvae have the advantage that the cuticle will be practically speaking in the same state, in all experimental animals.

4.4 Handling of Experimental Material

Adults: To get adults of the same age, pupae were collected daily. These were carefully examined for any superficial damage, which would be caused by larvae feeding on them. The incidence of pupal damage seems to increase with deterioration of the food and in the absence of the water source (carrot) in the medium. Daily collection of pupae assures the exact age of the pupae and prevents loss through larval wounding. These pupae were stored at constant temperature and humidity.

Larvae: Newly moulted larvae used in the experiments, were obtained directly from the cultures. Larvae within six hours after a moult can easily be recognised from amongst other larvae in the culture by their colour. They have a shiny yellowish white colour compared with the older larvae who are a dull yellowish brown. These larvae were collected daily by working through the culture, removing all newly moulted larvae as they became exposed.

The utmost care was exercised in handling young adults and larvae, because especially the adults are very fragile shortly after emergence. Specimens were picked up with the aid of a broad point forceps (which responded to a very light touch), and transferred to a glass tube (2" x 1"). From the tubes they were slid directly onto the pan of the balance, weighed and transferred back to the tubes with the help of a camel hair brush. This assured the minimum handling and the minimum possible

damage to the delicate cuticle.

On being weighed, all the animals falling in a previously determined weight group, were separated and then randomly allotted to (n) different groups (n being the number of treatments in a particular experiment). These groups were in turn randomly allotted to the different treatments in the experiments. Because only a specific type of animal was used for experiments, and also because of large samples used, experiments had to be "built up". This meant that equal numbers of insects were added to all treatments for a number of consecutive days until the required number of replicates were reached. Randomisation of replicates were done making use of a table of random numbers (Fisher and Yates, 1957).

4.5 Standard Apparatus and Techniques used

Weights of experimental animals were measured on a Mettler Type H. 16 balance, weighing correct to 0.05 of a milligram.

Specimens were kept individually in glass tubes (2" x 1"), marked with a "tech-pen". This type of marking lasted for any length of time, and was not spoiled or rendered unreadable when subjected to a temperature of 100°C, used for drying experimental animals to a constant weight.

All experiments were conducted in stationary air, using a constant humidity reference. I realised that this technique had serious disadvantages (Ramsay, 1935 b; Beament, 1961), but an attempt was made to keep experimental conditions as constant as possible in experiments running for several months in some cases, the main idea being to study the changes in the animal caused by its being present in certain atmospheric conditions

and not the absolute water loss.

Experiments were done in desiccators with a diameter of six inches. The relative humidity of the air in these desiccators, was controlled with saturated salt solutions (Winston and Bates, 1960). Saturated salt solutions were preferred to various concentrations of sulphuric acid. The latter being very unpleasant to work with, and considered time consuming and wasteful to maintain at a constant concentration over a long period of time. "Silica Gel" impregnated with cobalt thiocyanate was used to obtain dry air. This is an extremely useful chemical that can be used repeatedly.

Only reagent grade chemicals were used. Salts were chosen for their stability, low temperature coefficients and regular behaviour. Care was taken not to use any salts prone to hydrolysis, or salts likely to release some or other gas that might be harmful or effect the experimental animals in some other way, or salts which might have a transition point close to the experimental temperature.

Saturated salt solutions were prepared according to the method described by Winston and Bates (1960). These were allowed to stand for 3 - 7 days in the desiccators in the relevant temperatures to assure saturation, before being used for experimental purposes.

Relative humidity values over saturated salt solutions at various temperatures as listed by Winston and Bates (1960), were accepted as such. Cobalt thiocyanate-impregnated papers were always kept in the desiccators and compared (at least twice a week), and after the colour

was fixed in liquid paraffin, with standard cobalt thiocyanate-impregnated papers, prepared in the same way at the onset of the experiment, as also with colour standards in a "Lovibond comparator". This enabled me to see whether any changes occurred in the relative humidity within the desiccators compared to that at the onset of the experiment. Although this technique requires judgement of small colour changes which can be rather difficult, especially in the lower range of the humidity scale, one does with experience acquire a skill enabling one to pick out small colour differences relatively easily.

Cobalt thiocyanate papers were kept in similar glass tubes and at the same level as experimental animals, assuring in this way that the humidity measured was the same as that experienced by the animals.

Air leakage between the lid and the desiccator was prevented by sealing with petroleum jelly in temperatures below 25°C. For temperatures beyond 25°C a high vacuum silicone grease was used instead, because these temperatures caused the petroleum jelly to run and made air leakage possible. A ring of grease was applied to the inside of the desiccator just above the salt solution, to prevent the salt from crystallising out and moving up the sides of the desiccator.

An airtight container is essential to maintain a constant humidity. This constituted the problem of fresh air supply to the experimental animals. To overcome this problem, desiccators were aired by moving the lids up and down over the open desiccator for a constant number of times. This was done within the incubator thus keeping the air temperature

constant within the desiccator. Airing was repeated every two days so as to coincide with the necessary opening of desiccators for weighing purposes. I realised the short-comings in this technique, but a compromise had to be reached between upsetting the humidity and airing the desiccator. According to measurements made of the oxygen consumption, half the oxygen supply was used between airings. Although Mellanby (1932) using different concentrations of KOH for controlling the humidity, found no difference in weight loss of T. molitor as compared with Buxton's (1930) findings, using different concentrations of sulphuric acid, the carbon dioxide tension in the experimental chamber must increase with time with some effect on the spiracles and consequently on the water loss of the animals. This was recognised as a possible source of error, but being common to all treatments should not influence general conclusions to any great extent.

Experiments were conducted in temperature cabinets, set at the appropriate temperatures, controlled to within $\pm$ 0.5°C by contact thermometers operating a cooling and heating unit. Brine tanks inside the cabinets assured the stability of the pre-set temperatures, preventing large fluctuations in temperature when the doors are opened, and facilitate speedy return to the set temperature on closing the doors.

5.0 GLOSSARY OF TERMS USED DURING THIS INVESTIGATION

(a) **Initial live weight:-** The weight in milligrams of adults and larvae at the onset of the experiment. This will mean the weight of adults and larvae within 24 hours of emerging from the pupae or after a moult, respectively.

(b) **Initial dry weight:-** Total dry material, measured in milligrams, obtained by drying the whole insect (described under 'a') to a constant weight in a temperature of 100°C.

(c) **Final live weight:-** The weight in milligrams of insects at the termination of an experiment or in the cases where the experiment lasted till the insects die - the weight of the insect on the day prior to death.

(d) **Final dry weight:-** The weight in milligrams, of insects under 'c' after being dried to constant weight in a temperature of 100°C.

(e) **Water:-** The difference between the live weight and the dry weight, i.e. water is taken as the total weight of material evaporated on the insects being dried to constant weight.

(f) **Drymatter:-** The residue of the material left after the insect has been dried to constant weight at 100°C.

(g) Water drymatter ratio:- The amount of water, measured in milligrams, expressed as a ratio of the live weight of the insect. For convenience this was multiplied by one hundred. A water drymatter ratio of 60% would thus mean that an insect weighing 100 milligrams, contains 60 milligrams of water and 40 milligrams of dry material. The reverse will also be true, i.e. a drymatter water ratio of 40% will mean that an insect weighing 100 milligrams, contains 40 milligrams of drymaterial and 60 milligrams of water.

(h) Normal water drymatter ratio:- The water (in milligrams) per 100 milligrams of live insect, at the beginning of the experiment.

(i) Internal condition:- A general term used - implying the relation between total body fluids and total solids in the body. A constant internal condition will mean no change in the relation or ratio of water to drymatter.

PART 4

WATER CONSERVATION IN GRAZING TERRITO

MELVIN L. ADAMS

6.0 PRELIMINARY STUDIES ON THE WATER RELATIONS OF STARVED ADULTS OF T. MOLITOR.

In the experiments reported in section 12.16 some larvae of *T. molitor* that had been without food for 40 days and more, moulted to pupae and subsequently moulted to adults which seemed quite normal, even those that had been kept as larvae and as pupae in a relative humidity of 0%. Because both larvae and pupae had proved to be so hardy it was considered desirable to investigate the ability of adults to contribute to the next generation when deprived of food and water, and kept at various levels of temperature and humidity.

In the absence of food the only source of energy will be the "food-reserves" stored in the insect's tissues. The duration of life for a starving beetle will then depend on the amount of food-reserves that are stored in its body, the proportion of these reserves that it can use before it dies and the rate at which the reserves are used up. The rate is likely to be a function of the ambient temperature. The proportion that is used may depend on whether the animal dies ultimately from starvation or prematurely from some other cause. In my experiments in which I varied temperature and relative humidity the most important causes for premature death were likely to be desiccation at the low humidities and "flooding" (or "water-logging") of the tissues at the high humidities.

This argument raises several questions. Is the rate of metabolism of starving beetles, at any one temperature, independent of relative

humidity? or does the rate of metabolism vary with humidity? If it varies, does it increase with low humidity making available more metabolic water to offset water lost by evaporation? or does it decrease at low humidities thus reducing the amount of water lost by evaporation through the spiracles? These general questions can be analysed into a number of more particular ones. In order to make myself familiar with the experimental animal and in order to eliminate a number of the questions raised in the present discussion I planned a number of experiments designed to test the following series of null hypotheses:

(a) Atmospheric humidity has no influence on the length of adult life.
(b) " " " " " on the amount of dry material used.
(c) " " " " " on the metabolic rate.
(d) " " " " " on the internal environment.
(e) " " " " " on the total amount of water lost during life.
(f) The rate of water loss is not a linear function of relative humidity.

The design of the experiments had to take into account three technical difficulties.

(1) The cultures did not yield enough beetles emerging at one time to set up a large experiment which might require numbers of the order of 700 or 800. Such numbers were accumulated over a number of days, each daily yield being distributed evenly but

at random through all treatments.

(2) The treatments consisted in keeping beetles for prolonged periods without food or drink at various temperatures and humidities. It was necessary to measure or to estimate the amount of water and of dry matter in each beetle at the beginning of the experiment and at intervals during the experiment. The only direct measurement that was possible while the beetle remained alive was to weigh the living beetle. When it died it could be dried and the dry matter weighed. As the beetles for the experimental treatments were being accumulated some were randomly set aside to be weighed, dried and the dry matter weighed. The statistics obtained from this sample were used, in conjunction with the equation that is described in section 6.1, to estimate the dry matter (and therefore the water) in the beetles that were used in the experiments.

(3) The beetles in the stock cultures were reared, not excessively crowded, in circumstances that seemed highly favourable to them yet the weight of the newly emerged adults varied widely, between 85-140 mg. Because my results were obtained by weighing the beetles it was necessary for the experiments to be designed either to eliminate the influence of initial weight or to permit it to be measured and allowed for.

6.1 RELATION OF TOTAL DRY MATERIAL TO INITIAL LIVE WEIGHT IN NEWLY EMERGED ADULTS OF T. MOLITOR L.

To test the hypothesis that initial dry weight is a linear function of the initial live weight in newly emerged adults of T. molitor, a sample containing 406 newly emerged adults (mixed sexes) in the weight range 70-180 milligrams was divided into nine weight-groups. Starting at 70 milligrams, the first group contained all the insects weighing 70-79.95 mg, the second group, those weighing from 80-89.95 mg etc. The ninth group, because of small numbers, contained all the insects weighing from 160-180 mg.

The insects in the different weight groups were then individually dried to a constant weight in a temperature of 100°C and the initial dry weight determined by weighing.

Regressions of initial dry weight on initial live weight were calculated for each weight-group. A summary of the data obtained is given in Table A1. The hypothesis will be accepted if these regression lines can be shown to be identical.

The slopes (regression coefficients) and intercepts on the Y-axis (displacement) of the individual regression lines were compared in the following analysis of variance (Table 1).

TABLE 1. ANALYSIS OF VARIANCE OF DATA APPENDIX 1

Source	DF	SS	MS	VR
Slopes	8	66.7394	8.342425	0.817 N.sig.
Displacements	8	140.2340	17.529250	1.718 N.sig.
ERROR	388	3958.7606	10.202991	

The analysis of variance shows clearly no significant differences between either the slopes of the individual regression lines or their intercepts on the Y-axis. The hypothesis may therefore be accepted; initial dry is a linear function of initial wet weight.

Identical regression lines for weight-groups permit all the observations to be pooled to calculate a single regression of dry material on initial weight involving all the observations. Since water is defined as the difference between initial live and initial dry weight, a linear relationship between water and initial live weight will also be true.

The relation between initial dry weight (y) and initial live weight (x) can be expressed by the regression equation

$$= 49.788152 + 0.37848\ (x - 126.94322) \quad \ldots\ldots (1)$$

obtained from the "pooled" line (see Table A1).

To test the hypothesis that the slope of the "pooled" line is zero we have the quantity

$$\frac{\text{Slope}}{\text{S.E. of Slope}} = C$$ distributed as t with 404 DF (obtained from "pooled" line Table A1).

The hypothesis to be rejected at the 5% level if $C > 1.96$

$$C = \frac{.37848}{.006599} = 57.35414$$

95% confidence limits for the slope (B) are

$$\pm 1.96 \times \text{S.E. Slope}$$

i.e. $0.37848 - 1.96 \times .006599$

$$= 0.37848 \pm 0.01293$$

Equation (1) was used to estimate the initial dry weight of insects used in the next experiment so it was necessary to construct confidence limits for this estimate. At the same time this will illustrate the variability amongst experimental insects.

(i) 95% Limits for the Value of y for an Individual whose x value is known.

For a single insect of given live weight (x), the estimated dry weight (y) is given by the equation

$$y = 49.788152 + 0.37848\ (x - 126.94322)$$

The estimated variance (V) of this estimate is given by the equation

$$V(y) = s^2\left[1 + \frac{1}{N} + \frac{(x - \overline{X})^2}{(X - \overline{X})^2}\right] = A^2 \qquad \ldots\ldots (2)$$

where s^2 = the calculated sample variance (allowing for the regression), N = total number of pairs of observation, $\overline{X}$ = mean of all x values, $(X - \overline{X})^2$ = sum of squares for all values of X.

In this particular case the equation can be written as

$$V(y) = 10.311227\left[1 + \frac{1}{406} + \frac{(x - 126.94322)^2}{236,757.70}\right] = A^2$$

Then 95% limits for $y = 49.788152 + 0.37848\ (x - 126.94322) \pm t_{404} \cdot A$

(ii) 95% Confidence Limits for Mean Values of Y for any given X Value.

The estimated mean dry weight of insects with given live weight (x) is

$$Y = 49.788152 + 0.37848\ (x - 126.94322)$$

Its estimated variance is

$$V(Y) = s^2\left[\frac{1}{N} + \frac{(x - \bar{X})^2}{\Sigma(X - \bar{X})^2}\right] = B^2 \qquad \ldots\ldots (3)$$

$$= 10.311227\left[\frac{1}{406} + \left(\frac{x - 126.94322}{236,757.70}\right)^2\right] = B^2$$

Then 95% confidence limits for populations mean dry weight (Y).

$$= Y \pm t_{404} \cdot B$$

This information is best summarised and illustrated in Fig. 1.

The following inferences can be made from the data presented in the text and illustrated in Fig. 1.

(i) The weight of adults taken from a culture at a specific time may vary over a considerable range.

(ii) The size of the interval expected to contain 95% of the values of (y) (initial dry material) for a given value of (x) (initial weight) is relatively large. Initial water content, being by definition the difference between x and y, will be similarly variable. The fact that the adults came from the same culture, taken during a short time interval, suggests that the variability might be characteristic of the population studied.

(iii) In this experiment the class sizes of the independent variate (weight-groups) ranged from 25-61 but most exceeded 45 (Table A1). Fig. 1 shows that the means of the dependent variate (initial dry weight) for each weight

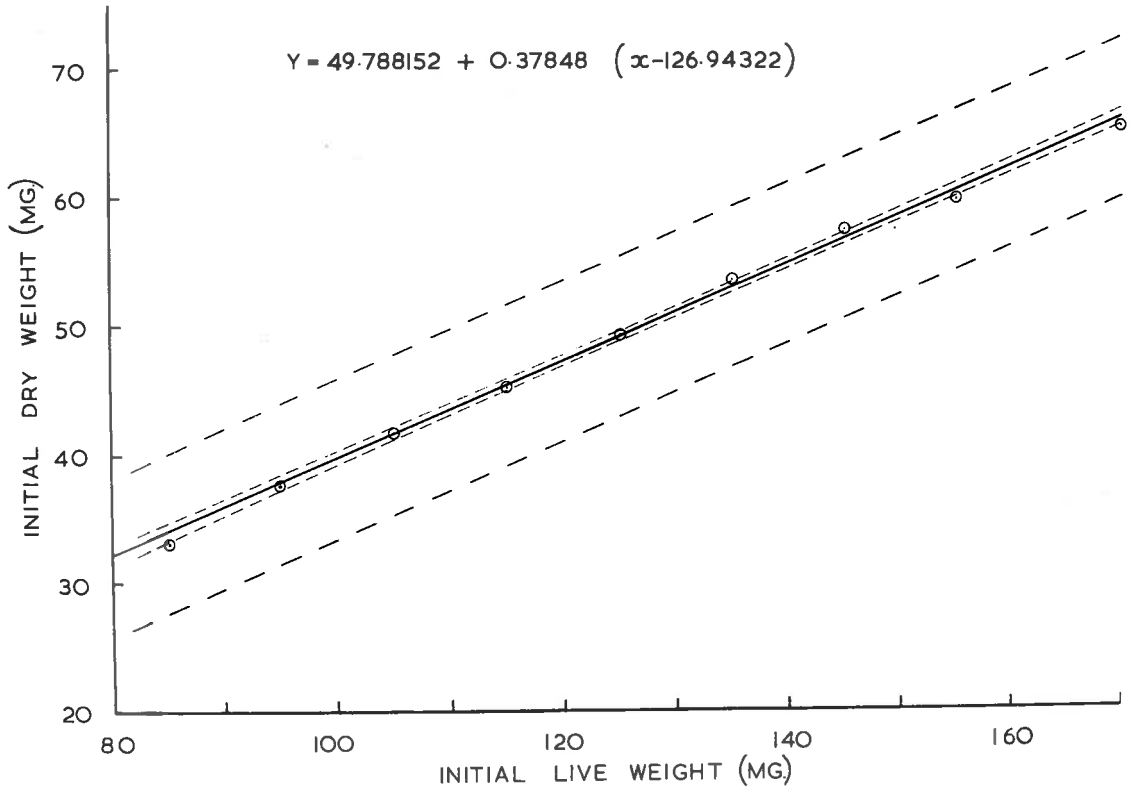


FIG. 1: The relationship between dry weight and live weight of newly emerged adults of T. molitor L.

..... 95% confidence limits for mean values of (y) for any given value of x.

– – – – 95% limits for a value of (y) for a given value of (x).

class fall within or close to the 95% confidence limits for the estimated population mean dry weight. This suggests that the sizes of the samples used in this experiment adequately over-comes the problem of variability between individual observations.

6.2 PRELIMINARY INVESTIGATION REGARDING THE INFLUENCE OF ATMOSPHERIC HUMIDITY ON STARVED ADULTS OF T. MOLITOR.

A sample of 140 newly emerged adults in the weight range 85-140 mg were randomly allotted to seven groups. These seven groups in turn were randomly allotted to seven different humidities, 0%, 20%, 40%, 60%, 80%, 90%, 95%, in a constant temperature of 25°C.

Confined in individual glass tubes in these humidities the insects were weighed daily until death. From the daily weights the following quantities were calculated for each individual beetle: the daily rate of change in weight, the total dry material at death, the daily rate of dry material consumption, the total amount of water lost and the daily rate of water loss; the length of life in days was also recorded.

For estimating the initial dry weight of individual insects used in this experiment, the equation $y = 49.788152 + 0.37848\ (x - 126.94322)$ obtained from the previous experiment was used. The difference between the estimated initial dry weight and the measured final dry weight, divided by the number of days a particular insect stayed alive, gave an estimate of the daily rate of consumption of dry material.

The total amount of water lost by an insect during its life was obtained by subtracting from the difference between the measured initial live weight and the estimated initial dry weight, the difference between the measured final live weight and the final dry weight. Dividing this quantity by the number of days a particular insect stayed alive gave an estimate of the daily rate at which the insects lost water.

6.21 INFLUENCE OF ATMOSPHERIC HUMIDITY ON LENGTH OF LIFE OF T. MOLITOR ADULTS STARVED IN A CONSTANT TEMPERATURE.

(A) Before testing for the possible influence of humidity on length of life (because of the large range in initial weight of the population sample) I thought it necessary to test the hypothesis that initial weight has no influence on the length of life, irrespective of humidity.

To test this hypothesis, regressions of length of life (y) on initial weight (x) were calculated, one for each humidity. The hypothesis is to be accepted if the slopes of these regression lines can be shown to be identical and not different from zero.

A summary of the data and results are given in Table A2. The slopes of the different regression lines are compared in Table 2.

TABLE 2.

Source	DF	SS	MS	VR
Slopes	6	113.9794	18.996566	1.097 N.sig.
Displacements	6	3573.9118	595.651960	34.426
ERROR	119	2058.9488	17.302090	

The variance ratio associated with the slopes of the regression lines (y_i) on (x_i) being non-significant at the 5% level we accept the hypothesis that the slopes of these regression lines are identical. For testing the hypothesis that these slopes are zero we have the estimate of the common slope, viz., .03440495 and its standard error, viz., .26163774 obtained from "parallel regression" Table A2.

The quantity .03440495/.26162744 = 1.315035991 = A is distributed as t with 125 degrees of freedom. Now for 125 DF $P(t > 1.96) = .05$. We therefore accept the hypothesis that these slopes are not different from zero, and therefore also the hypothesis that size has no influence on the mean length of life of adult T. molitor, irrespective of humidity.

(B) Table 2A contains a summary of the information on the influence of humidity on length of life of starved adults of T. molitor in a constant temperature (25°C).

The hypothesis that humidity has no influence on the length of life of starved adults of T. molitor was tested by means of an analysis of variance. This being a single factor experiment and, because of a difference in the size of the samples, the method - analysis of variance, single classification for samples of unequal size - described

TABLE 2A.

Relative Humidity %	0	20	40	60	80	90	95
n	18	19	19	19	19	20	19
Σx	309	372	459	540	567	625	577
$\bar{x}$	17.1667	19.5790	24.1579	28.4211	29.8421	31.2500	30.3684
Σx^2	5393.000	7536.0000	11341.0000	15820.0000	17277.0000	19801.0000	18025.0000
s^2	5.2058	8.4795	14.0294	26.7018	19.8069	14.1974	27.9123

TABLE 2B.

Humidity	0%	20%	40%	60%	80%	90%	95%
n	18	19	19	19	19	20	19
Σx	22.1580	24.3993	26.1869	27.4928	27.9311	29.8395	28.0527
$\bar{x}$	1.2310	1.2842	1.3783	1.4470	1.4701	1.4920	1.4765
Σx^2	27.3345	31.4604	36.1707	39.8905	41.1406	44.5688.	41.5153
s^2	0.0034	0.0070	0.0042	0.0060	0.0044	0.0024	0.0053

by Snedecor (1956), was adopted.

Observing the sample variances, bottom row of Table 2A, it is obvious that they vary through a relatively wide range, the largest being more than five times the smallest. In addition to this they seem to be correlated with the means. Under these circumstances it is not permissible to pool the variances. The transformation of the data to logarithms got over this difficulty. This is demonstrated in Table 2B containing a summary of the data after the logarithmic transformation was performed.

From the data summarised in Table 2B an analysis of variance (Table 2C) was constructed.

TABLE 2C. ANALYSIS OF VARIANCE

Source	DF	SS	MS	VR
Between humidities	6	1.1932	0.198867	41.8139 F*** (6.126)
Within humidities	126	0.5992	0.004756	
Total	132	1.7924		

The analysis of variance shows the differences between humidities to be highly significant and the null hypothesis is rejected at the 0.1% level.

Plotting length of life (days) against humidity (Fig. 2) showed an asymmetrical sigmoid trend with the curve flattening out in the upper half of the humidity range. This posed the question as to the significance of differences amongst humidities, especially between humidities in the upper and lower part of the range.

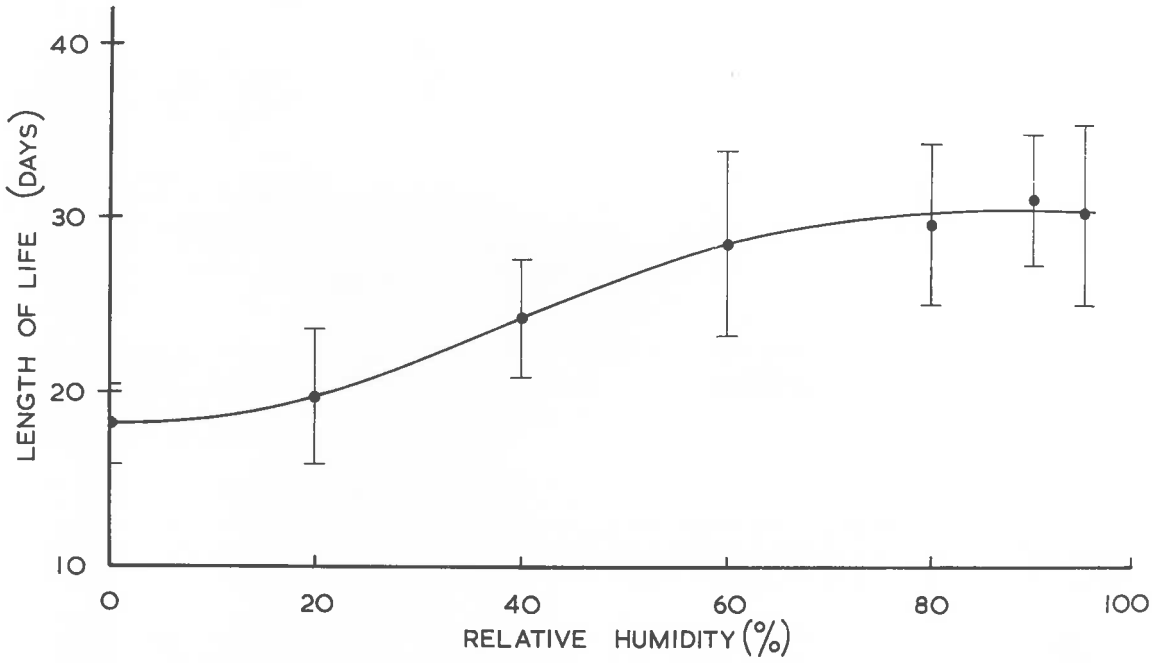


FIG. 2: The influence of atmospheric humidity on the life span of starved adults of T. molitor kept at 25°C.

represents the means and standard deviations of sample populations and not the standard error of the means.

Comparing the means by a method of multiple comparisons, described by Snedecor (1956) pp. 252, lead to the construction of Table 2D.

Tests were made by computing differences D_1 and D_2 which is significant at the 5% and the 1% level respectively. The difference between two means is considered significant at the 5% or 1% level, if it exceeds the value of D_1 and D_2 respectively, associated with this specific difference. D is the product of S_x and a factor Q (one for each range of the treatment means), taken from a table of upper 5% and 1% points in the Studentized range. S_x is the square root of the residual mean square divided by the number of observations in each class taken in this case as n = 19. The possible number of comparisons are given by the formula a(a - 1)/2 where a is the number of treatments.

This test being based on the assumption of equal class numbers does not, strictly speaking, apply in this case. However, although the class numbers are not equal they are so close together that it should not measurably bias the comparisons.

From Table 2D it is clear that differences between the means in the upper humidity range (60 - 95% RH) are not significant, even at the lower 5% level. On the other hand, in the lower range (0 - 40% RH) every humidity is significantly different from every other; and every humidity in the range 0 - 40% is significantly different from every humidity in the range 60 - 95%.

There is thus an increase in the life span of starved T. molitor

TABLE 2D. MULTIPLE COMPARISONS OF MEANS FROM TABLE 2B.

Humidities			0%	20%	40%	60%	80%	95%
	Differences $\bar{x}$	D	$\bar{x}$ = 1.2310	$\bar{x}$ = 1.2842	$\bar{x}$ = 1.3783	$\bar{x}$ = 1.4470	$\bar{x}$ = 1.4701	$\bar{x}$ = 1.4765
90%	1.4920		0.2610	0.2078	0.1137	0.0450	0.0219	0.0155
		D_1	0.0671*	0.0649*	0.0620**	0.0584	0.0532	0.0443
		D_2	0.0793**	0.0771**	0.0745**	0.0712	0.0665	0.0585
95%	1.4765		0.2455	0.1923	0.0982	0.0295	0.0064	
		D_1	0.0649*	0.0620**	0.0584*	0.0532	0.0443	
		D_2	0.0793**	0.0745**	0.0712**	0.0665	0.0585	
80%	1.4701		0.2391	0.1859	0.0913	0.0231		
		D_1	0.0620**	0.0584*	0.0532*	0.0443		
		D_2	0.0745**	0.0712**	0.0665**	0.0585		
60%	1.4470		0.2160	0.1628	0.0687			
		D_1	0.0584*	0.0532*	0.0443*			
		D_2	0.0712**	0.0665**	0.0585**			
40%	1.3783		0.1473	0.0941				
		D_1	0.0532*	0.0443*				
		D_2	0.0665**	0.0585**				
20%	1.2842		0.0532					
		D_1	0.0443*					
		D_2	0.0585					
0%	1.2310							

* Indicate differences between means significant at the 5% level.
** " " " " " " " " 1% level.

adults with an increase in humidity up to the 60% level, beyond which no additional increase occurs. This suggests that death in these insects might be caused by desiccation in the lower humidity range and by starvation in the upper humidity range.

When considering the sample variances in Table 2A it is obvious that they are relatively large, and also vary through a wide range. In addition to this the size of the variances tends to be positively correlated with the size of the means. Since the mean length of life tends to increase with humidity it follows that the higher the humidity the more variable is the response of the beetles.

The cause for this is not obvious. Weight is the only obvious variable within a sample (85 - 140 mg); but I have already shown that the length of life of T. molitor beetles in any particular humidity is not a function of the weight of the insect. It seems that there must be some factor (or factors) other than humidity influencing the length of life of individual insects, the effect of which is enhanced by an increase in humidity. Assuming that death is caused by starvation in the upper humidity range and by desiccation in the lower range, then there should be at least two factors operating, one in each of the two humidity ranges. Knowledge of these factors, and also of the way in which they influence or determine length of life, will be extremely interesting, and perhaps most revealing. Their presence on the other hand will contribute to the variability of all measurements on any sample population in this particular study.

If we now consider the variability in the relationship between initial dry material and initial weight amongst individuals as demonstrated in Fig. 1, and we assume that the variability is due to differences in the amount of reserved food materials, then this, together with a metabolic rate negatively correlated with humidity might help to explain the observed phenomena, providing insects in all humidities use up all (or the same proportion) of their food reserves before they die.

These hypotheses will be tested later.

6.22 THE RELATIONSHIP BETWEEN WEIGHT LOSS AND ATMOSPHERIC HUMIDITY.

If we assume that the adults of T. molitor metabolise reserve food materials when starved, then the total weight loss in these insects will have two components:-

(a) loss of reserved food materials,

(b) loss of water.

Assuming complete oxidation of reserved food material, we must also assume water of metabolism to be formed. Total weight loss will then consist of (a) loss of dry material, defined as the difference between initial and final weight of dry matter plus (b) water loss, defined as total water lost less the water of metabolism and given by the difference between initial and final water content.

The possibility exists that humidity might influence:-

(a) the metabolic rate, Buxton (1930), and therefore the amount

of metabolic water formed

(b) the rate of evaporation of both initial body water and water added as a by-product of metabolism

(c) directly or indirectly the control over water regulating and water saving mechanisms, Edney (1957), Barton - Browne (1964). Any relationship between humidity and total weight loss might thus be brought about in a number of different ways.

At this stage I was merely interested to determine (1) how the daily rate of loss of weight is related to humidity (2) whether there is a change in time in this relationship (3) whether humidity influences the total amount of weight lost before death occurs and I also wanted to consider the usefulness of making such measurements for studying the conservation of water in T. molitor.

Daily weight changes measured as described in section 6.1 were used in this study. The insects ranged in weight from 85 - 140 mg. To correct for initial weight, which might influence total weight loss, regressions of final on initial weight were calculated for each humidity group, both for the 10th day after starting and on the day prior to death. The main reason for using two different times was to see whether any changes in the relationship occur with time. Because the information for different times was not independently obtained it is not strictly comparable. Each relationship however is legitimate in its own rights, and can thus serve as a test for what happens at

that particular time.

A summary of the data obtained for both day ten and the day prior to death are given in Table A3 and Table A4 respectively.

(1) Relationship between Weight Loss and Atmospheric Humidity after Starvation for Ten Days.

Slopes (regression coefficients) and intercepts on the y-axis (displacements) of the individual regressions of final weight at day ten on initial weight, one for each humidity (Table A3), were compared in the following analysis of variance, Table 3A.

TABLE 3A. ANALYSIS OF VARIANCE OF WEIGHT LOSS DATA FOR DAY TEN (TABLE A 3)

Source	DF	SS	MS	VR
Slopes	6	63.0670	10.511166	0.785 N.sig.
Displacements	6	5166.8850	861.14750	64.337 ***
Error	119	1592.8050	13.384915	

Since the part of the total sum of squares associated with slopes are non-significant at the 5% level, the hypothesis is accepted that the seven regression lines of final weight (y) on initial weight (x) one for each humidity, have the same slope. This, plus the fact that the variance ratio for displacements is highly significant, suggest that the individual regression lines are parallel but displaced.

To test the hypothesis that these slopes are zero, we have the estimate of the common slope and its standard error obtained from Table A3 (Parallel regression), viz., .08464219 and .022838837 respectively.

$$\frac{.88464219}{.022838837} = C \text{ is distributed as t with 125 DF.}$$

This hypothesis is rejected at the .001% probability level because

$$C > 3.373$$

This suggests that initial weight has an influence on total weight loss independent of humidity; and the slopes being identical suggests that the regression of final on initial weight adequately accounted for differences in final weight due to differences in initial weight.

I have now to test the hypothesis that the mean final weight at day ten, for a given initial weight, depends linearly on humidity.

For a given initial weight, x = 107.32593 say, the estimated mean final weight (day ten) at the i^{th} humidity is given by the equation $Y = y_i + b(107.32593 - x_i)$, where b is the estimate of the common slope viz., .88464219 (Table A3). These calculations give;

TABLE 3B.

Humidity	0	20	40	60	80	90	95
Y	77.7999	81.9229	56.4415	88.7451	93.8156	94.6045	95.4662

I wish to test whether or not these can be accepted as estimates of true means that depends linearly on the humidity.

For testing linearity of regression of mean final weight on humidity, the sums of squares associated with displacements (Table 3A)

were divided into two parts.

(a) Sums of squares due to regression

(b) Sums of squares due to deviation from regression

This was tested against error sum of squares (Table 3A).

Division of displacements sums of squares was done making use of the method of least squares (described in Appendix 2) and this lead to the construction of Table 3C.

TABLE 3C. ANALYSIS OF VARIANCE FOR REGRESSION

Source	DF	SS	MS	VR
Regression	1	5129.6579	5129.6579	383.2417 ***
Deviation from Regression	5	37.2271	7.4454	0.55625 N.sig.
Error	119	1592.8050	13.384915	

Since the variance ratio associated with deviation from regression is non-significant at the 5% level the hypothesis is accepted that the mean final weight of T. molitor adults after ten days starvation, depends linearly on humidity. For example, the estimated mean final weight (at day ten) of adults with mean initial weight of 107.32593 mg at 25°C, in any particular humidity (x_i) is given by the equation:-

$$Y = 78.20058 + 0.185448\ x_i \quad \text{(Appendix 2B)}$$

(2) Relationship between Weight Loss and Atmospheric Humidity after Starvation till Death (Table A4).

The data for weight loss on the day prior to death was handled in the same manner as the data for day ten.

Slopes and displacement for different regressions (one for each humidity) of final on initial weight were compared in the analysis of variance (Table 4A).

TABLE 4A. ANALYSIS OF VARIANCE OF WEIGHT LOSS DATA (TABLE A4) ON DAY PRIOR TO DEATH.

Source	DF	SS	MS	VR
Slopes	6	196.7268	32.787800	2.038 N.sig.
Displacements	6	4373.4590	728.909830	45.307 ***
Error	119	1914.4962	16.088203	

This analysis shows slopes to be non-significant at the 5% level. We then accept the hypothesis that the seven regression lines of final weight (y_1) on the day prior to death on initial weight (x) have the same slope.

We now test the hypothesis that the mean final weight on the day prior to death, for given initial weight, depends linearly on humidity.

For a given initial weight, x = 107.32593 mg say, the estimated mean final weight at death at the i^{th} humidity is given by the equation

$$Y = \bar{y}_i + b(107.32593 - \bar{x}_i)$$

where b in this case is taken as the estimate of the common slope, viz., 0.5987947. These calculations give:-

TABLE 4B.

Humidity	0	20	40	60	80	90	95
Y	60.0036	62.0806	62.6595	63.9874	71.5845	74.4118	74.2923

We wish to test whether or not these can be accepted as estimates of true means that depend linearly on humidity.

Testing for linearity by separating displacement sums of squares into two parts, i.e. sums of squares due to regression and sums of squares due to deviation from regression, by making use of the method of least squares described in Appendix 2, lead to the construction of the analysis of variance for regression (Table 4C).

TABLE 4C. ANALYSIS OF VARIANCE FOR REGRESSION

Source	DF	SS	MS	VR
Regression	1	3874.1040	3874.1040	240.8040 ***
Deviation from Regression	5	501.3550	100.27	6.23 *** F(5, 119)
Error	119	1914.4962	16.088203	

From Table 4C it is clear that although the coefficient for linear regression is significant, the deviations from linear regression are also significant, $P < .001$.

The relationship between weight loss and atmospheric humidity is best summarised and illustrated in Fig. 3,

(a) where Line A represents the mean initial weight of insects used in this experiment, viz. 107.32593 mg

(b) points on graph B are estimates of the mean final weight on day ten at the i^{th} humidity given by the equation

$$\bar{y}_i + 0.88464219\ (107.32593 - \bar{x}_i)$$

(c) points on graph C are estimates of the mean final weight on the day prior to death at the i^{th} humidity given by the equation

$$\bar{y}_i + 0.5987947\ (107.32593 - \bar{x}_i)$$

The following inferences can be made from the evidence presented in the text and in Fig. 3.

(i) The amount of weight lost by an insect in air at any specific humidity depends on the initial weight and the length of life of the insect.

(ii) The weight lost by the insect in ten days (when corrected for the initial weight) is linearly related to humidity, and so will be the rate of weight loss.

(iii) Insects die in different humidities after losing different amounts of weight (total weight loss being measured at the day prior to death and corrected for initial weight) thus total weight loss in say humidity A could not be used to predict the length of life in humidity B even if the rate of weight loss in both humidities were known. Such predictions could be made only when the mean total weight loss in humidity B was also known.

(iv) When the relationship between total weight loss and humidity

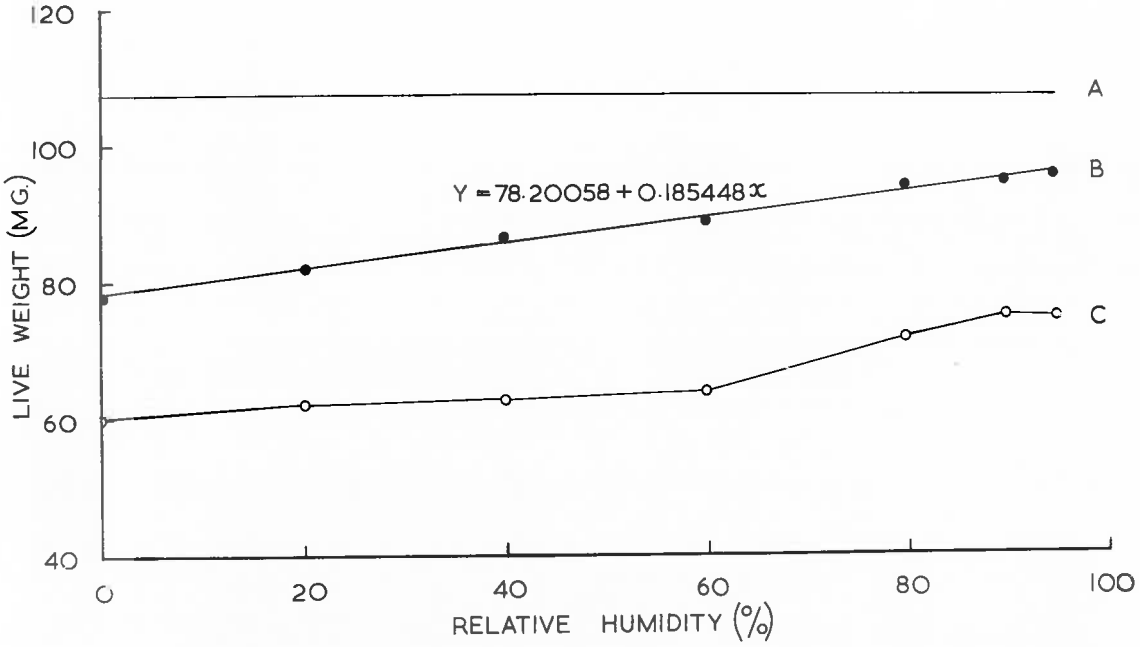


FIG. 3: The influence of atmospheric humidity on the change in the live weight of starved adults of T. molitor L. kept at 25°C.

(A) Mean initial live weight for all the insects used in the experiment.

(B) Relationship between atmospheric humidity and live weight at day 10 (corrected for initial live weight).

(C) Final live weight (corrected for initial live weight) on the day prior to death.

is known, measurements of the weight lost during any particular period will be useful in predicting length of life only if no unforeseen changes in the measured rate occur.

To test the hypothesis, implicit in '(iv)', that there is no change in the rate of loss of weight with time during the life of an adult T. molitor starving to death at any particular humidity, we have the equation

$$Y = 78.20058 + 0.185448\ x_i$$

where Y is the live weight at day ten of a beetle with the arbitrary initial weight of 107.32593 mg starving at humidity $_i$. And, assuming the above hypothesis to be true we also have the alternative estimate of the live-weight of a beetle at day ten

$$Q_1 = \frac{10(W_o - W_t)}{t}$$

where Q_1 is the live-weight on day ten

W_o is the initial live-weight (on day zero)

W_t is the final live-weight (on the day before death)

t is the length of life in days

Having determined the quantity (Q_1) for each individual insect in all the humidities then, in order to correct for initial weight, regressions of Q_1 on initial weight were calculated, one for each humidity. A summary of these calculations is given in Table A5.

Slopes and intercepts of these seven regression lines were compared in the analysis of variance (Table 5).

TABLE 5. ANALYSIS OF VARIANCE OF DATA IN TABLE A5

Source	DF	SS	MS	VR
Slopes	6	16.2660	2.7110	.411 N.sig.
Displacements	6	4952.8940	825.48233	125.214*** F (6, 119)
Error	119	784.5150	6.592563	

Since the variance ratio associated with slopes is not significant at the 5% level, we accept the hypothesis that the seven regression lines of Q_1 (y) on initial weight (x), one for each humidity, have the same slope. I then wish to test the hypothesis that the mean Q_1 for a given initial weight depends linearly on humidity.

For a given initial weight, $\bar{x}$ = 107.32593 mg say, the estimated mean Q_1 at the i^{th} humidity (Q) is given by the equation;

$$Q = \bar{Y} + b(107.32593 - x_i)$$

where b is the estimate of the common slope obtained from "parallel regression" viz., .89035406 (Table A5). These calculations give:-

TABLE 5A.

Humidity	0	20	48	60	80	90	95
Q	79.6922	83.5289	88.4232	91.8635	95.1381	96.7304	96.2556

To test whether these means can be accepted as true means depending linearly on the humidity, the displacement sums of squares (Table A5) was separated into two components, i.e. (a) sums of

squares due to regression and (b) sums of squares due to deviation from regression. This was done making use of the method of least squares, as described in Appendix 2, and led to the construction of the analysis of variance (Table 5B).

TABLE 5B. ANALYSIS OF VARIANCE FOR REGRESSION

Source	DF	SS	MS	VR
Regression	1	4888.0753	4888.073	741.4530 ***
Deviation from Regression	5	64.8187	12.9637	1.9664 N.sig
Error	119	784.5150	6.5926	

The variance ratio associated with "deviation from regression" being non-significant at the 5% level, we accept the hypothesis that for given initial weight (107.32593 mg) the mean calculated weight at day ten depends linearly on humidity.

At this stage we have determined that for a given initial weight of 107.32593 mg, the estimated mean weight at day ten, both when estimated from the actual weights on day ten or when calculated by the alternative method described on p. 79 is a linear function of humidity. The last step then was to test the hypothesis that the two sets of regression lines are identical.

To test this hypothesis two regressions were calculated:-

(a) The regression of the estimated mean measured weight (y) at day ten, for given initial weight (107.32593), on humidity (x).

(b) The regression of the estimated mean calculated weight (y_1, calculated from the estimate of θ_1) at day ten, for given initial weight (107.32593), on humidity (x).

The regression equations describing these two lines are:-

(a) $y = 88.3993 + .185660\ (x - 55.00)$

(b) $y_1 = 90.2331 + .181211\ (x - 55.00)$

The calculations involved are summarised in Table A6, and the slopes and intercepts of the two regression lines are compared in Table 5C.

TABLE 5C.

Source	DF	SS	MS	VR
Slopes	1	0.07870	0.07870	0.144 N.sig.
Displacements	1	11.76637	11.76637	21.549 $F^{***}(1, 10)$
Error	10	5.46007	0.546007	

The variance ratio associated with slopes is non-significant at the 5% level. We therefore accept the hypothesis that the two regression lines are parallel.

The variance ratio associated with displacements being significant at the 0.1% level, we reject the hypothesis that the two regression lines are identical, and therefore also the hypothesis that there is no change in the rate of weight loss during the life of the starved insect.

The fact that the estimate for mean live weight on day ten obtained from the actual measurements on day ten (equation of y, table A6A)

was lower than the estimate obtained from the alternative method (equation of y, Table A6B) suggests that the rate of weight loss measured over the entire life is less than that measured over the first ten days of adult life. This indicates then that the rate of weight loss decreases with time.

This also points to the fact that no major breakdown occurred in the mechanimms controlling weight loss or any one of its components while the insects were still alive.

The regression lines being parallel suggests that the decrease in rate of weight loss with time does not depend on humidity.

6.23 INFLUENCE OF HUMIDITY ON THE AMOUNT OF DRY MATERIAL LEFT AT DEATH AND THE RATE OF DRY MATERIAL CONSUMPTION

In this section I discuss the causes of death when adult T. molitor are dept without food at different humidities, and in particular I ask in what circumstances do they die from desiccation or from shortage of energy producing reserves.

Total dry material in newly emerged adults can be considered to consist of two components (1) usable dry material, e.g. reserve food materials (2) non+usable dry material, e.g. some body tissues and the exo-skeleton.

The first question asked was whether starved adults are able to use reserve food materials as a source of energy. Assuming this, then, if they die of starvation, it can be expected that they will

use all of the dry material of the first component.

Turning back to section 6.21 we notice that beyond 60% relative humidity, there was no measurable increase in the time they stay alive. Assuming that these insects died of starvation in humidities of 60% and beyond and of desiccation in lower humidities, we will expect to find differences between the amounts of dry material left in the lower and upper humidity range. Insects in the upper humidity range should contain only dry material of the second component, while in the lower range the insect should contain dry material of the second component plus part of that of the first component which they could not use before death as a result of desiccation intervened.

A possible alternative, however, exists. All the dry material of the second component may be used up in all humidities, but at a faster rate in the lower humidity range, in which case the insects in both humidity ranges would die of starvation.

To get answers to these various questions I started off by testing the null hypothesis that humidity has no influence on the total amount of dry material left at death. For testing this hypothesis, the same insects used in the previous section were dried at 100°C to a constant weight immediately after death in humidities 0%, 20%, 40%, 60%, 80%, 90% and 95%.

Taking initial weight as a standard reference, and also to take into account differences in initial weight, regressions of total dry material at death (y) on initial live weight (x) were calculated

for each humidity. A summary of the data of this experiment is given in Table A7, p. 402

Slopes and displacements for different regressions of final dry weight on initial live weight were compared in the analysis of variance (Table 6A).

TABLE 6A. ANALYSIS OF VARIANCE OF DATA IN TABLE A7

Source	DF	SS	MS	VR
Slopes	6	14.12376	2.35396	1.517 N.sig.
Displacements	6	277.73070	46.28845	28.847 ***
Error	119	184.54974	1.550838	

Because the variance ratio associated with slopes is non-significant at the 5% level we accept the hypothesis that the seven regression lines of final dry weight (y) on initial live weight (x), one for each humidity, have the same slope.

To test the hypothesis that these slopes are zero, we have the estimate of the common slope and its standard error obtained from "Parallel regression". Table A7, viz., .19551732 and .0079109855.

.19551732/.0079109855 = C distributed as t with 125 DF.

The hypothesis is rejected at the .001% probability level since

C > 3.373

The common slope being significantly different from zero indicates that initial weight has an influence on the total amount of dry material left at death. The slopes being identical however, suggests that there is no interaction between initial weight and

humidity on the final dry weight. The regression of final dry weight on initial live weight thus adequately takes into account differences in final dry weight due to differences in initial live weight. Possible differences in the mean final dry weight in the different classes could then be attributed to the influence of humidity.

Since the variance ratio associated with displacement is significant at the 0.1% level, we reject the hypothesis that humidity has no influence on the amount of dry material left at death.

For a given initial weight, x = 107.32593 say, the estimated mean final dry weight (y) at the i^{th} humidity is given by the equation

$$Y = \bar{y} + 0.19551732\ (107.32593 - \bar{x}_i)$$

where b is the estimate of the common slope.

These differences were compared in Table 6B by the method of multiple comparisons described in Section 6.21.

The total amount of dry material used during adult life in different humidities in relation to the overall weight lost in these humidities is best illustrated in Fig. 4 where:-

(A) represents the mean initial weight of all insects used in this experiment, viz., 107.32593 mg.

(B) points on graph B are estimates of the mean final weight on the day prior to death at the i^{th} humidity given by the equation $Y = \bar{y}_i + 0.5987947\ (107.32593 - \bar{x}_i)$ (Table A4).

(C) represents the estimated mean initial dry weight of insects with a given initial weight of 107.32593, obtained from the

TABLE 6B. MULTIPLE COMPARISONS OF CORRECTED MEAN WEIGHTS OF DRY MATERIAL LEFT AT DEATH.

Humidities			95%	90%	80%	60%	40%	20%
	Differences $\bar{x}$	D	$\bar{x}$ - 20.7071	$\bar{x}$ - 21.0437	$\bar{x}$ - 21.4495	$\bar{x}$ - 21.6461	$\bar{x}$ - 22.4794	$\bar{x}$ - 23.6500
0%	25.0989		4.3918	4.0552	3.6494	3.4528	2.6195	1.4489
		D_1	1.2263*	1.1858*	1.1338*	1.0672*	.9718*	.8098*
		D_2	1.4490**	1.4085**	1.3623**	1.3015**	1.2147**	1.0701**
20%	23.6500		2.9429	2.6063	2.2005	2.0039	1.1706	
		D_1	1.1858*	1.1338*	1.0672*	.9718*	.8098*	
		D_2	1.4085**	1.3623**	1.3015**	1.2147**	1.0701**	
40%	22.4794		1.7723	1.4357	1.0299	0.8333		
		D_1	1.1338*	1.0672*	.9718*	.8098*		
		D_2	1.3623**	1.3015**	1.2147	1.0701		
60%	21.6461		.9390	0.6024	0.1966			
		D_1	1.0672	.9718	.8098			
		D_2	1.3015	1.2147	1.0701			
80%	21.4495		.7424	.4058				
		D_1	.9718	.8098				
		D_2	1.2147	1.0701				
90%	21.0437		.3366					
95%	20.7071							

* Indicates differences between two means significant at the 5% level.
** Indicates differences between " " " " " 1% level.

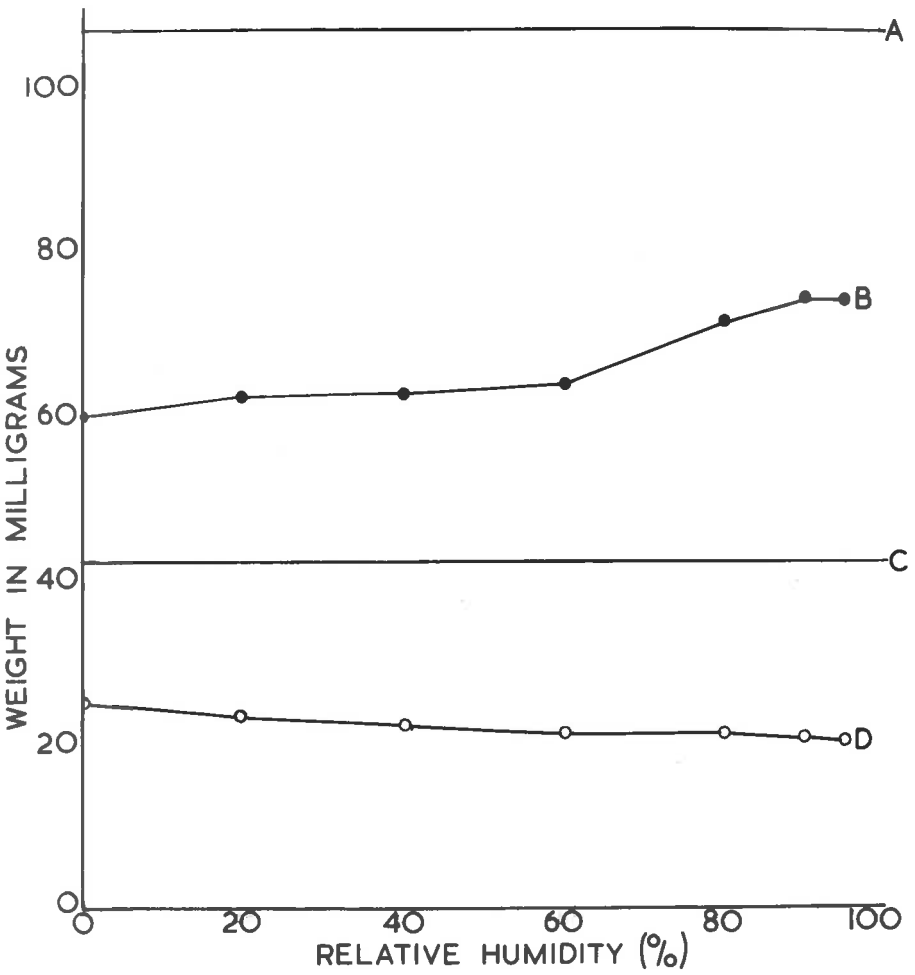


FIG. 4: The total change in live weight and dry weight of T. molitor L. adults starved to death at different relative humidities and kept at 25°C.

(A) Mean initial live weight of all insects used in the experiment.

(B) Final live weight (measured on the day prior to death) corrected for initial weight.

(C) Initial dry weight calculated from mean initial live weight.

(D) Weight of dry material measured at death and corrected for initial weight.

equation $Y = 49.788152 + 0.37848\ (107.32593 - \bar{x})$ (From Section 6.1).

(D) points of graph D are estimates of the mean final dry weight at death in the i^{th} humidity, given by the equation $Y = \bar{y}_i + 0.19551732\ (107.32593 - \bar{x}_i)$ (Table A7).

From the evidence presented in the text and illustrated in Fig. 4 the following conclusions can be drawn.

(i) T. molitor adults do utilize part of their total dry material when starved.

(ii) More dry material is left at death when these insects are starved in humidities below 60% RH, than when they are being starved in higher humidities. While differences between means in the lower humidity range is significantly different from each other, those in the upper humidity range are not, suggesting that all available reserves have been used within the latter range.

(iii) Judging by the position of corresponding points on Graphs B and D the water/dry matter ratio measured at death is not constant through the humidity range.

(iv) Since the amounts of dry material left at death vary amongst humidities, total weight lost cannot be used to predict total water lost over a range of humidities. By itself, total weight loss therefore seems not to be a very useful quantity to measure.

(v) Comparing the amounts of dry materials used in different humidities with the length of life (Fig. 2), it seems that for the additional amount of dry material used in higher humidities there is a disproportionate increase in the length of life. Where in 0% relative humidity the mean amount of dry material used is 17.2 milligrams and the mean length of life is 17.2 days, at 90% relative humidity the corresponding figures are 21.2 milligrams and 31.25 days respectively.

6.24 INFLUENCE OF ATMOSPHERIC HUMIDITY ON THE RATE OF METABOLISM.

To test the null hypothesis that atmospheric humidity has no influence on the rate of metabolism, I used the daily consumption of dry material as a measure of the rate of metabolism in different humidities. The ratio was obtained by dividing the total amount of dry material in mg each insect used by the number of days this insect stayed alive. The total amount of dry material used was obtained by estimating the initial dry weight of each insect, making use of the equation

$$Y = 49.788152 + 0.37848 (x - 126.94322)$$ (From Section 6.1)

and subtracting from this the total amount of dry material left at death for that particular insect.

A summary of the data concerning the influence of humidity on the metabolic rate (x) is given in Table 7A (all figures in milligrams).

Because the size of the insect might have an influence on the

TABLE 7A.

Humidity %	0	20	40	60	80	90	95
n	18	19	19	19	19	20	19
Σx	17.8795	19.0859	16.3512	14.1231	13.0934	13.6308	14.1173
$\bar{x}$	0.9933	1.0045	0.8606	0.7433	0.6891	0.6815	0.7430
Σx^2	18.2518	20.0952	14.4464	10.9100	9.3462	9.5005	10.9016
s^2	0.0289	0.0513	0.0208	0.0229	0.0180	0.0111	0.0229

rate of metabolism, a linear regression analysis was performed on this data, to test simultaneously the following two hypotheses;-

(a) that humidity has no influence on the metabolic rate

(b) that size, as measured by initial weight, has no influence on the rate of metabolism.

For these tests regressions of rate of dry material consumption (y) on initial live weight (x) were calculated, one for each humidity. The data is summarised in Table A8. Slopes and intercepts for different regressions were then compared in the following Analysis of Variance, Table 7B.

TABLE 7B. ANALYSIS OF VARIANCE OF DATA IN TABLE A8.

Source	DF	SS	MS	VR
Slopes	6	.010008	.001668	.076 N.sig.
Displacements	6	2.035301	.339216	15.644 ($F_{6,\ 119}^{**}$)
Error	119	2.580298	.021683	

The variance ratio associated with displacement being significant at the 0.1% level, we reject the hypothesis that humidity has no influence of the metabolic rate, as measured in this experiment.

The variance ratio associated with slopes, being non-significant at the 5% level, we accept the hypothesis that the slopes of the regression lines of rate of dry material consumption (y) on initial live weight (x), one for each humidity, are identical. This will mean that the relation between size and metabolic rate, if any, will

be independent of relative humidity.

The hypothesis that size as measured by initial weight has no influence on the rate of metabolism will then be accepted if the common slope for these regression lines can be shown to be zero.

For testing the hypothesis that these slopes are zero we have the estimate of the common slope and its standard error obtained from Parallel regression, Table A8, viz., .0046872681 and .00090330888 respectively.

.0046872681/.00090330888 = C, is distributed as t with 125 DF.

This hypothesis is rejected at the .001% level of probability since C > 3.733, and therefore also the hypothesis that size has no influence on the rate of metabolism.

I then tested the hypothesis that the mean metabolic rate for a given initial weight depends linearly on humidity. For a given initial weight x = 107.32593 say, the estimated mean metabolic rate at the i^{th} humidity is given by the equation

$$Y = \bar{y}_i + b(107.32593 - \bar{x}_i),$$

where b is the estimate of the common slope viz., .0046872681. These calculations give:-

TABLE 7C.

Humidity	0	20	40	60	80	90	95
Y (mg/day)	1.003401	.995233	.842556	.748022	.708925	.683784	.733912

I wish to test whether or not these can be accepted as estimates of true means that depends linearly on humidity.

For testing linearity of the regression of mean rate of dry material consumption on humidity, the sums of squares associated with displacements (Table 7E) were divided into its two components:-

(a) due to regression

(b) due to deviation from regression.

This was done as described in Appendix 2, and led to the construction of Table 7D.

TABLE 7D. ANALYSIS OF VARIANCE FOR REGRESSION.

Source of Variance	DF	SS	MS	VR
Regression	1	.194671	.194671	9.196345 **
Deviations from Regression	5	1.84063	.368126	16.977632 ***
Error	119	2.580298	.021683	

We reject the hypothesis that the mean rate of dry material consumption depends linearly on humidity, on the basis of the variance ratio associated with deviation from linear regression being significant at the 0.1% level.

The difference between mean rates of dry material consumption corrected for initial weight were compared in Table 7E by the method of multiple comparisons described in section 6.21.

It is obvious from Table 7E that significant differences between mean rates of dry material consumption occur mainly between the lower and upper humidity range. Differences within either of these ranges are not significant.

TABLE 7E. MULTIPLE COMPARISONS OF MEANS FROM TABLE 6C.

Humidity		90%	80%	95%	60%	40%	20%
	Differences $\bar{x}$	$\bar{x}$ = .6838	$\bar{x}$ = .7089	$\bar{x}$ = .7339	$\bar{x}$ = .7480	$\bar{x}$ = .8426	$\bar{x}$ = .9952
		D					
0%	1.0034	0.3696**	0.2945**	0.2695**	0.2554*	0.1608	0.0082
		D_1 0.1400*	0.1354**	0.1295**	0.1219*	0.1110	0.0925
		D_2 0.1655**	0.1608**	0.1555**	0.1486**	0.1387	0.1222
20%	.9952	0.3114**	0.2863**	0.2613**	0.2472**	0.1526**	
		D_1 0.1354**	0.1295**	0.1219**	0.1110**	0.0925**	
		D_2 0.1608**	0.1555**	0.1486**	0.1387**	0.1222**	
40%	.8426	0.1588**	0.1327**	0.1087	0.0946*		
		D_1 0.1295**	0.1219**	0.1110	0.0925**		
		D_2 0.1555**	0.1486	0.1387	0.1222		
60%	.7480	0.0642	0.0391	0.0141			
		D_1 0.1219	0.1110	0.0925			
		D_2 0.1486	0.1387	0.1222			
95%	.7339	0.0501	0.0250				
		D_1 0.1110	0.0925				
		D_2 0.1387	0.1222				
80%	.7089	0.0251					
		D_1 0.0925					
		D_2 0.1222					
90%	.6838						

* Indicates differences between means significant at the 5% level.
** " " " " " " " " 1% level.

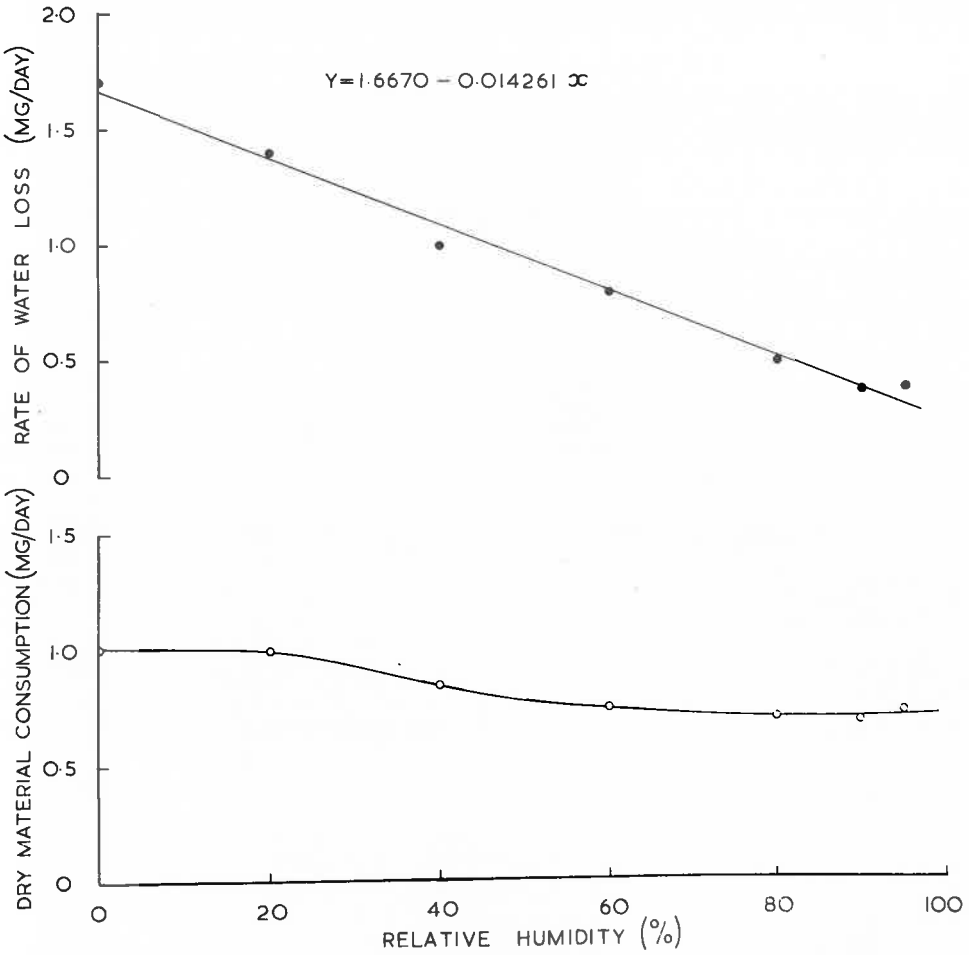


FIG. 5: (A) The relationship between rate of water loss and atmospheric humidity in starved adults of T. molitor L.

(B) The relationship between rate of dry material consumption and atmospheric humidity in starved adults of T. molitor.

From the evidence put forward in the section, it is clear that humidity influences the rate of metabolism, measured as rate of consumption of dry matter. Metabolic rate and humidity, however, are not linearly related (see also Fig. 5).

The conclusion is open to the criticism that, because the beetles lived for different periods at different humidities (Table 2A), the influence of humidity in these experiments is confounded with the influence of time (i.e. of duration of life).

The difference in the mean metabolic rate between humidities, as has been demonstrated by this method, should however hold true even when measured for uniform times unless there happened to be a change in the metabolic rate with time, independent of humidity, and if death occurred at a progressively later stage after this change in metabolic rate with increase in humidity.

This hypothesis will be tested at a later stage.

6.25 THE INFLUENCE OF ATMOSPHERIC HUMIDITY ON TOTAL AMOUNT OF WATER LOST AND THE RATE AT WHICH IT IS LOST WHEN T. MOLITOR ADULTS ARE STARVED TO DEATH.

(A) As a measure for testing the hypothesis that atmospheric humidity has no influence on the amount of water lost during the time the insect stayed alive in a particular humidity, I used the difference between water present in the insect at death and that present in the insect at the start of the experiment.

Water initially present in the insect was estimated by subtracting the estimated initial dry material (making use of the equation

$y = 49.788152 + 0.37848 (\quad - 126.94322)$ from section 6.1)

from the initial live weight, while the water at death was determined by subtracting the final dry material (measured) from the final live weight.

The total amount of water lost during the life of the insect at a certain humidity estimated in the way, will under-estimate the absolute amount of water lost by a quantity equal to the amount of water of metabolism added during this time.

A study of this measure in relation to humidity might give an indication of the cause of death and of the amount of desiccation that can be tolerated.

Correcting for initial weight, and at the same time testing the hypothesis that size as measured by initial weight has no influence on the amount of water lost, a linear regression analysis was performed on this data. Regressions of amount of water lost (y) on initial weight (x) were calculated, one for each humidity. The data and results are summarised in Table A9. Slopes and intercepts for different regression lines were then compared in the following analysis of variance, Table 8.

TABLE 8. ANALYSIS OF VARIANCE OF DATA IN TABLE A9

Source	DF	SS	MS	VR
Slopes	6	191.7144	31.9524	2.056 N.sig.
Displacements	6	6368.7500	1061.4583	68.328 *** F (6, 119)
Error	119	1848.6307	15.534711	

The variance ratio associated with slopes being non-significant at the 5% level, we accept the hypothesis that the seven regression lines of total water lost (y) on initial weight (x) have the same slopes. The hypothesis that size has no influence on the total amount of water lost will then be accepted if these slopes can be shown to be zero. For testing this hypothesis we have the estimate of the common slope, viz., .21942349, and its standard error, viz., .025351522 obtained from 'Parallel regression, Table A9.

Now .21942349/.025351522 = C is distributed as t with 125 DF.

P (t > C) = .001

We therefore reject both these hypotheses.

The variance ratio associated with displacements being significant at the 0.1% level we also reject the hypothesis that atmospheric humidity has no influence on the amount by which the initial water content is reduced up till the time of death.

For a given initial weight, x = 107.3259 say, the estimated mean weight of water loss (y) at the i^{th} humidity is given by the equation $Y = \bar{y} + .21942349\ (107.3259 - \bar{x}_i)$

The differences between these means are compared in Table 8B, by the method of multiple comparisons described in section 6.21.

If we consider the relationship between humidity and length of adult life as well as the amount of dry material left at death (Section 6.23), this does suggest that death in the lower half of the humidity range might be caused by desiccation, while the cause

of death in the upper half of the range might be starvation.

Death as a result of desiccation can occur under two possible conditions (a) after the initial water content is reduced by a certain amount, or (b) after the ratio of water to dry material has reached a certain minimum value.

If either of these conditions occurred in the lower half of the humidity range we would expect under condition (a) that a similar amount of water has been lost in all humidities in the lower range, say from 60% down to 0%, (b) that the lines describing the relationship between total weight lost and relative humidity should form a curve similar to the curve illustrating the relationship between dry material left at death and the relative humidity of the air (Fig. 4, p. 29) with the curves converging on each other as the humidity increases within this range.

Comparisons of differences between mean weights of water lost in different humidities (Table 8B) should give an indication as to whether the data satisfy either of these conditions. To satisfy condition (a) the mean amount of water lost in humidities 0-60% (Column 2) should be similar. To satisfy condition (b) the means should increase with increase in humidity. This is so because the amount of dry material left at death increases with a decrease in humidity.

Considering the differences in the mean weights of water lost in humidities in the 0-60% range then, if we observe the relative

TABLE 8B. MULTIPLE COMPARISONS OF CORRECTED MEAN WEIGHTS OF WATER LOST TILL DEATH IN DIFFERENT HUMIDITIES.

Humidities			95%	90%	80%	60%	40%	20%
	Differences $\bar{x}$	D	$\bar{x}$ = 11.3293	$\bar{x}$ = 11.5861	$\bar{x}$ = 14.8341	$\bar{x}$ = 22.6236	$\bar{x}$ = 24.7868	$\bar{x}$ = 26.5309
0%	29.5620		18.2327	17.9759	14.7279	6.9384	4.7752	3.0311
		D_1	3.9299*	3.8002*	3.6333*	3.4202*	3.1143*	2.5952*
		D_2	4.6436**	4.5139**	4.3656**	4.1709**	3.8929**	3.4294
20%	26.5309		15.2016	14.9448	11.6968	3.9073	1.7441	
		D_1	3.8002*	3.6333*	3.4202*	3.1143*	2.5952	
		D_2	4.5139**	4.3656**	4.1709**	3.8929	3.4294	
40%	24.7868		13.4575	13.2007	9.9527	2.1632		
		D_1	3.6333*	3.4202*	3.1143*	2.5952		
		D_2	4.3656**	4.1709**	3.8929**	3.4294		
60%	22.6236		11.2943	11.0373	7.7895			
		D_1	3.4202*	3.1143*	2.5952*			
		D_2	4.1709**	3.8929**	3.4294**			
80%	14.8341		3.5048	3.2480				
		D_1	3.1143*	2.5952*				
		D_2	3.8929	3.4294				
90%	11.5861		0.2568					
		D_1	2.5952					
		D_2	3.4294					
95%	11.3293							

* Indicates differences between two means significant at the 5% level.
** Indicates " " " " " " " 1% level.

positions of the different humidities in Table 8B, as well as the presence and absence of asterisks, it is obvious that the data do not satisfy either of the conditions specified under (a) or (b). This in itself will make it very difficult to say with certainty whether these insects do die of desiccation in the lower humidity range or not.

The consequences of the two hypothetical conditions of the beetles at the time of their death as set out on page 100 are compared with the actual observations in Fig. 6.

(A) The actual observed final weight in different humidities.

(B) The calculated final weight showing what the final weight should have been had equal amounts of water been lost in all humidities concerned.

(C) The calculated final weight showing what the final weight should have been had the dry material/water ratio reached the same level in all humidities.

(D) The observed amount of dry material left at death in the different humidities.

Although the data do not satisfy either conditions (a) or (b), they come closer to satisfying (a) than (b). Now if we observe the difference between means, there is an almost regular increase from 0-60% R.H. Condition (a) can then still be satisfied if some of the water, an amount directly or indirectly determined by humidity, is bound in some form or another (e.g. in eggs). As such

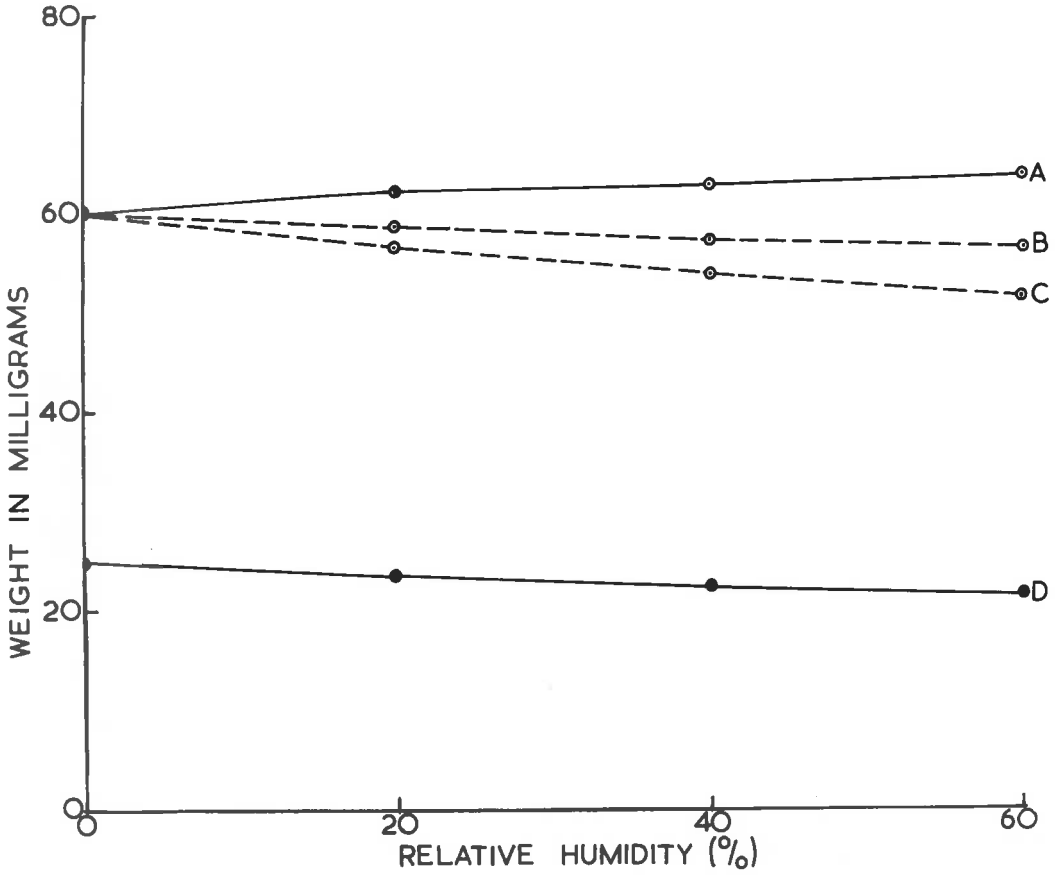


FIG. 6: Observed and expected live weight (A, B, C) relative to the weight of dry material (D) at the time of death of *T. molitor* adults starved to death at different humidities at 25°C.

(A) Observed weights at death.

(B) Expected weights, given that a similar amount of water has been lost in all humidities concerned, taking 0% R.H. as a reference point.

(C) Expected weights, given that a similar dry material/water ratio has been reached in all humidities concerned, taking 0% R.H. as a reference point.

(D) Observed weight of dry material.

it will be unavailable to the insect, yet it will be measured when this insect is dried to constant weight. An alternative possibility is that the insects can withstand a higher degree of desiccation with more reserved foods present in their bodies.

This evidence then makes it clear that we cannot simply attribute the obvious adverse effect of humidities in the lower range on the length of life to an unbalance between total water and total dry material at the point where death occurs.

The estimated mean dry weight of newly emerged adults of T. molitor with a given live weight of 107.3259 mg. is 42.3634 mg (Fig. 4). The initial water content, obtained by subtraction is then 64.9625 mg. It was shown in section 6.21 that humidity in the range 60-95% has no effect on the length of life. At 60% R.H. the total amount of water lost was 22.6236 mg while at 90% R.H. for instance, the amount lost in a comparable period was 11.5861 mg. This means that during the life of the adult in the upper humidity range, the total water content can be reduced by between 17.83 to 34.83 per cent of the original without any adverse effect on the mean length of life.

On the other hand, there was not a significant difference in the amount of water lost at 40% and 60% R.H. However, the difference between the mean lengths of life in these two humidities was significant. It seems as if at 60% relative humidity the maximum amount of water than can be lost, was lost during a period of time

that coincided with the time required to metabolise all the available reserved foods. It follows that beyond 60% relative humidity, all the food reserves will be used before the initial water content is reduced to a critical point, while below 60% the opposite will be true, the reserves of water will be reduced to the critical level before all the reserves of food have been used up.

On the assumption that adults of T. molitor die in the lower humidity range after the original water content is reduced to a certain minimum, then the rate at which it is reduced must determine the length of adult life. This brings us to the question as to what the relationship is between the water content of the air and the rate at which the initial water content is reduced.

Where the difference between the initial water content (a) and the final water content (b) measures the amount by which the original water content is reduced in a particular humidity, the ratio a - b/the time (days) the insect stayed alive, measures the daily rate, by which it is reduced in different humidities.

Since the amount of water lost is a function of the size of the insect (measured as the initial weight) while the length of life is not, the daily rate of water loss should also be a function of initial weight. To compare the rate of water loss in different humidities, taking into account differences in initial weight, regressions of the daily rate of water loss (y) on initial weight (x) were calculated, one for each humidity, i.e., 0, 20, 40, 60, 80, 90

and 95% R.H.

A summary of the data and calculations is given in Table A10. Slopes and intercepts of the different regression lines are compared in Table 8C.

TABLE 8C. ANALYSIS OF VARIANCE OF DATA FROM TABLE A10.

Source	DF	SS	MS	VR
Slopes	6	.251876	.041979	1.707 N.sig.
Displacements	6	30.319349	5.053224	205.517*** F(6, 119)
Error	119	2.925949	.024587	

The variance ratio associated with displacements, being significant at the 0.1% level of probability - confirms the expected, that is that atmospheric humidity does influence the rate of water loss.

Plotting the mean rate of water loss against humidity, suggested a linear relationship and this hypothesis was subsequently tested.

Since the slopes sums of squares is non-significant at the 5% level, we accept the hypothesis that the seven regression lines of rate of water loss (y) on initial weight(x), one for each humidity, have the same slope. I then tested the hypothesis that the mean rate of water loss, for a given initial weight, depends linearly on humidity.

For a given initial weight, $x = 107.32593$ say, the estimated mean rate of water loss at the i^{th} humidity is given by the equation:

$$Y = \bar{y}_i + b(107.32593 - \bar{x}_i)$$

where b is the estimate of the common slope obtained from "Parallel regression (Table A10), viz., .0061995337; these calculation give:-

Relative Humidity %	0	20	40	60	80	90	95
Y (mg/day)	1.7072	1.3777	1.0451	.8031	.4929	.3705	.3864

To test whether these means can be accepted as true means that depend linearly on humidity, displacements sums of squares were separated in the two components (a) sums of squares due to regression (b) sums of squares due to deviation from regression (method described in Appendix 2). This led to the construction of Table 8D.

TABLE 8D. ANALYSIS OF VARIANCE FOR REGRESSION.

Source	DF	SS	MS	VR
Regression	1	30.1081	30.1081	1224.5536 ***
Deviation from Regression	5	.211249	.0422498	1.7184 N.sig.
Error	119	2.925949	.024587	

The variance ratio associated with deviation from regression being non-significant at the 5% level, we accept the hypothesis that the mean rate at which the initial water content is reduced, depends linearly on humidity. The relationship between the rate of water loss (y) and humidity (x_i) can be described by the equation

$$y = 1.6670 - .014261\, x_i \qquad \text{(Fig. 5).}$$

The way in which the daily rate of water loss was determined is open to criticism because the beetles lived for varying times

at different humidities. The actual rates as measured, as well as their relation to humidity should however be true, unless a change in the rate of water loss took place with time.

On considering this possibility, we know that water loss is by definition a component of total weight loss. We know of the latter that:-

(a) total weight loss when measured after a uniform time (eg.10 days) is linearly related to humidity. (Fig. 3).

(b) the rate of weight loss decreases with time but the decrease is the same in all humidities. (sec 6.22).

We might therefore expect a possible change in the rate of water loss with time.

If on the other hand we consider weight loss together with its two components, i.e. (a) water loss and (b) dry material loss, then the picture becomes more complex.

In Fig. 5 and section 6.24 it was shown that the rate of dry material consumption is not linearly related to humidity. From the way in which it is related, it was postulated that the metabolic rate must either (a) change with time over the whole humidity range, or (b) change with humidity in the lower humidity range.

We know that the rate of loss of weight (which is merely the sum of dry matter plus water) is a linear function of humidity and that it decreases with time. Now this is consistent with hypothesis (a), if the metabolic rate change only with time, and the rate of

water loss change with it or stay the same all the way through. But hypothesis (b) requires that the rate of water loss has to change in such a way as to compensate for the increased rate of metabolism in the lower humidity range.

From the discussion above it is obvious that the rate of water loss as measured, although it promoted this argument, is in itself not a very useful statistic, as it does not give us a sufficiently detailed picture of the rate at which water is lost during the life of a starved T. molitor beetle.

6.3 A SUMMARY AND DISCUSSION OF DATA OBTAINED DURING A PRELIMINARY INVESTIGATION OF THE INFLUENCE OF ATMOSPHERIC HUMIDITY ON THE ABILITY OF STARVING ADULTS OF T. MOLITOR TO STAY ALIVE.

From the evidence collected during this preliminary investigation it became evident that atmospheric humidity must influence, in a rather complex way, the ability of starved adults of T. molitor to stay alive.

In an attempt to analyse the relationship between the atmospheric humidity and the ability of starved T. molitor adults to stay alive, the following points emerged, which to my mind need further consideration and investigation.

6.31 Variability in the dry material and water content of newly emerged T. molitor adults.

Both Buxton (1930) and Mellanby (1932) pointed out the extreme variability in the water content of T. molitor larvae, which they

rightly considered a serious disadvantage in studying water relations.

Variability in the quantities measured is a disadvantage in that larger samples need to be used to get reliable estimates of the population parameters. If the variability, however, is due to different strains or types within the population, which might respond differently to the same environmental stimuli, then an analysis of, and an investigation into the matter must lead to a better evaluation of the importance, and explanation of how various factors contribute to the species' ability to stay alive.

A high degree of variability in the initial dry material content for a given initial weight is demonstrated for adults of T. molitor in Fig. I. Since the initial water content is by definition the difference between initial live weight and initial dry weight, the same variability can be demonstrated for the initial water content. Knowing that my material was highly variable I took the precaution to use only newly emerged beetles and to collect them all from the same stock culture over a relatively short period. They were distributed rigorously at random through all the treatments in the experiment. So systematic differences between treatments are not likely to have been caused by faulty sampling.

A high degree of variability was found in the length of life of individual adults in any one humidity; and the variability seemed to increase with an increase in humidity. Since size was shown in section 6.71 not to influence length of life, the variability cannot

be attributed to a possible advantage of bigger insects over smaller ones. If we assume that the variability in the initial dry material content, demonstrated in Fig. 1, is due to varying amounts of usable dry material then this could explain the variability in length of life in the higher humidity range where the insects die of starvation. At the same time, providing all other factors remain the same, the difference in initial water content might explain the variability in the lower humidity range, where death is a result of desiccation.

On the basis of this argument it will be the insect with the highest water content which will live longest in the lower humidity and for the shortest period in the higher humidity range. Under these circumstances we would expect the variance to decrease with increase in humidity to a point in the vicinity of the 60% R.H. level, and then to increase again. Since this does not happen, there should be at least a different explanation for the variability in the lower humidity range.

It would seem desirable to investigate the nature of the high variability of T. molitor and in this connection the following questions seem to be important.

(1) Whether the variability in the initial weight and composition of the body is caused by

(a) differences between sexes, or

(b) differences between two or more inter-breeding strains in the population, as was demonstrated by Leclercq (1963), with

possible differences in body composition.

(ii) Whether or not the difference in inital dry material is due to differences in the amount of usable dry material.

(iii) Whether insects with the greater initial water content lose water at a different rate than those with less.

(iv) Whether insects with a greater initial water content can tolerate a greater reduction in the original water content when subjected to humidities in the lower range.

6.32 Possible causes for death of T. molitor adults starved in different relative humidities.

The curve describing the relationship between length of life and relative humidity, being of an asymmetrical sigmoid type (Fig. 2), shows that this is no simple direct relationship. Length of life increases only until the relative humidity reaches the 60% level, which suggests that the insects die of desiccation in the lower humidity range and of starvation in the upper humidity range. This was substantiated by the fact that compared to the higher humidity range, significantly more dry material was left in the insects when they died in the lower humidity range.

Although these observations point to desiccation as the cause of death in the lower humidity range, the amount of water present in the bodies of the insects when they died, relative to the dry material left at death, or to the initial water content does not comply with either of the two predictions which seem to be the only two plausible

ones for death due to desiccation. These are that the insect dies (i) when the water/dry material ratio has reached a certain minimum, or (ii) after the initial water content was reduced to a certain minimum (see also Fig. 6).

The observed condition of the beetles when they die however, more closely resembles the condition described under (ii). To my mind this can be accepted if one can show that an amount of water increasing with humidity (0-60% R.H.), to the order of 0 - 7 mg in insects with mean initial weight of 107.3295 mg (Table 7B) is bound in some form or another and thus unavailable to the insect. The only suggestion I could put forward at this stage that would seem to fit the facts is that the number of eggs matured is directly or indirectly determined by humidity, and thus with an increase in humidity an increasing amount of water is bound in eggs and unavailable to the insects.

6.33 The relationship between relative humidity and the length of life of starved T. molitor adults.

On the assumption that death occurs in the lower humidity range after the initial water content is reduced to a certain minimum, and since the rate at which the initial water content is reduced depends linearly on humidity (section 6.25), I would have expected the length of life in this range also to be linearly related to humidity. Referring to Fig. 2 this is obviously not so. The way in which length of life is related to humidity suggests that the rate of water loss

at any humidity may have been reduced towards the latter part of the beetle's life. At 0% R.H. the beetle may have died about the time that this change in rate of water loss was due to occur. At the higher humidities, with the beetles living longer, the reduced rate of water loss would operate for a longer time and thus have a bigger influence on the overall rate of water loss.

6.34 The influence of relative humidity on the metabolic rate of starved T. molitor adults.

Buxton (1930) showed that the larvae of T. molitor can maintain a nearly constant water content in their bodies when starved for a month in humidities 0-60%. He argued that more water was lost in the lower humidities compared to that in the higher, but that this loss was made good by the oxidation of additional reserves in the lower humidities, while the water of metabolism was retained in the body.

If we consider the mean weight of dry material used by adults of T. molitor (Fig. 4) we found (a) that when they were confined to 0% R.H. they used 17.26 mg of reserves during a mean length of life of 17.2 days while (b) when confined to 90% R.H. they used 21.20 mg during a mean length of life of 31.25 days. These figures by themselves suggest that reserved foods were used at a higher rate in the lower humidity (section 6.24) which will be in accordance with Buxton's hypothesis for T. molitor larvae.

Since there is no difference in the amount of dry material left

at death in insects starved in humidities (60-95%) and also no difference in the time they stayed alive, we must conclude that in the higher humidity range reserved foods were metabolised at the same rate in all humidities.

In the lower humidity range the overall rate of dry material consumption is higher than in the higher range. Since the rate at which the initial water content is reduced depends linearly on humidity (section 6.25) then if the metabolic rate was increased merely to produce water to compensate for that lost by evaporation I would have expected the rate of dry material consumption also to be linearly related to humidity. This was shown not to be so (section 6.24). Another unexpected observation in relation to the same argument is that some of the available food reserves are left when the insects die in the lower humidity range. This suggests that the explanation of the situation might be different or more complex in the adults than has been suggested by Buxton for the larvae.

If we consider only the lower humidity range, then the results obtained during the preliminary investigation for the adults of T. molitor concerning the inter-relationship between dry material consumption, lengthof life and atmospheric humidity can be more profitably discussed in relation to the two opposing hypotheses put forward by Buxton (1930) and Mellanby (1936 b) for the larvae of T. molitor.

(a) Buxton's hypothesis suggests that at constant temperature the

metabolic rate of T. molitor larvae is determined by the humidity of the atmosphere surrounding the insect.

(b) Mellanby on the other hand stated that the rate of metabolism is governed by temperature alone and is uneffected by a change in atmospheric humidity.

If either of these hypotheses hold true for adults of T. molitor we should expect from Buxton's hypothesis

(i) The rate of dry material consumption to decrease with increase in humidity. This was demonstrated in section 6.24 to be so, but the technique used to determine the rate of dry material consumption did not allow for a possible change in rate with time.

(ii) That the length of life should increase with decrease in metabolic rate and therefore with increase in humidity. This was demonstrated in section 6.21 to be so.

(iii) Should the increase in the metabolic rate be an adaptation to compensate for water lost by evaporation I would have expected:-

(a) Length of life to be determined by the rate of metabolism and the amount of available reserves, i.e. that the insects should die of starvation and notas a result of desiccation as was indicated by the increasingly greater amount of dry material left at death as humidity decreased.

(b) That since the rate at which the initial water content is reduced depends linearly on humidity for length of life also to be a

linear function of humidity instead of the sigmoid type of relation as indicated in Fig. 2.

Mellanby's hypothesis that the metabolic rate is unaffected by atmospheric humidity should be rejected for adults of T. molitor on grounds of the evidence produced in section 6.24. If we however consider the possibility that the metabolic rate was unaffected by atmospheric humidity, but that a change in the rate occurred with time, then the evidence produced could be circumstantial. The disproportionate increase in life span for the increase amount of reserves used, shown at the beginning of this section in 0% and 90% R.H., could equally well indicate either a change in the rate of metabolism with time or a difference in the rate of metabolism due to humidity, or both.

Let us then assume that there is a change in the rate of dry material consumption at a certain point in the life of the starved insect, and that this in itself caused a decrease in the rate of water loss. Now the rate at which the initial water content is reduced was shown to depend linearly on humidity. It was also postulated that these insects die in the lower humidity range after the initial water content is reduced to a certain minimum. If the latter point is reached in 0% R.H. shortly after the change in the rate of dry material consumption occurred, then the slower rate of water loss as a result of this, plus the slower rate of water loss as a result of increased humidity could explain the sigmoid type of

increase in the length of life. The higher rate of dry material consumption, demonstrated in the lower humidity range (in section 6.24), could then be a coincidence caused by differences in length of life and the change in the rate of dry material consumption.

Knowledge of whether or not a change in the metabolic rate occurs with time could swing the argument in favour of one of the hypotheses put forward and would clear up quite a few uncertainties in connection with the inter-relation between metabolic rate, length of life and atmospheric humidity.

6.35 The relationship between relative humidity and weight loss in starved T. molitor adults.

For given initial weight, the weight change in T. molitor adults in relative humidities, e.g. 0, 20, 40, 60, 80, 90, and 95%, when measured at unit time (day 10) was shown in section 6.22 to depend linearly on humidity. Similarly the calculated weight at day ten, making use of the ratio, total weight lost/length of life, also depends linearly on humidity.

In addition to this the two regression lines describing the relationship between (a) measured weight (y) and (b) calculated weight (y_1) and (c) humidity (x_1) are parallel but displaced. From this I concluded that total weight loss measured at uniform time depends linearly on humidity as also does the rate of weight loss. Moreover, a change in the rate of weight loss occur with time which is the same in all humidities.

Total weight lost consists of two components, i.e. (a) water lost and (b) dry material lost. Of these it was demonstrated (section 6.25) that the ratio, total amount of water lost/length of life, depends linearly on humidity while the ratio, total amount of dry material consumed/length of life, is not linearly related to humidity (Fig. 5). Now whatever the cause of the latter relationship the sum total of the rates at which these two components decrease cannot add up to a statistic linearly related to humidity unless a compensatory action took place in the rate at which water is lost over time in different humidities.

The situation as pictured at this stage, suggests that the compensatory action might either be an increase in the rate of water loss with time in the higher humidity range, or a decrease in the lower range, or both. This in turn makes it obviously necessary that in order to get a true picture of the inter-relationship between metabolic rate, water loss, length of life and atmospheric humidity in starved adults of T. molitor, the possible change of the dependent variates with time and in relation to each other must be investigated. This investigation will be described in the next section.

7.0 Change In The Dry Material And Water Content of Starving Adults of T. molitor L. With Respect To Time and Humidity.

Information obtained during the preliminary investigation (Section 6.34) suggested that in order to explain the relationship between atmospheric humidity and the ability of starving *T. molitor* adults to stay alive, it is necessary to determine whether or not the metabolic rate and rate of water loss change with respect to time and each other, and differently in different humidities. Therefore in an attempt to test these various hypotheses, the following experiment was conducted :

Newly emerged adults from the same cultures were subjected to relative humidities 0, 30, 60 and 90%, in a constant temperature of 25°C for various lengths of time. Fifty adults were used for each of twenty days in humidities 30, 60 and 90% and for fourteen days in 0% humidity. Starting at day zero, this involved 1050 beetles in each of the first three humidities and 750 in 0% R.H.

Because controlled temperature space was limited, and also because the cultures used yielded each day only 20-60 beetles fit for experimental purposes, it was decided to do the experiment in four parts, one humidity at a time. The sequence 30, 0, 90 and 60% R.H. was decided on by chance. Individual experiments were "built" up in the following way: Groups of twenty-one beetles were randomly picked from one day's supply, until this was exhausted. Beetles in any one group were then randomly allotted to twenty-one treatments. Each treatment represented a certain number of days (0-20) in a particular humidity. This procedure was repeated daily until there were fifty

replicates for each day. Large samples were necessary because of the high variability in initial body composition demonstrated in Section 6.1 and also because an increasing death rate was expected towards the end of the experiment especially in the lower humidities. For the same reasons the experiment was continued for only fourteen days in 0% R.H. where the mean length of life was shown to be 17.2 days (Section 6.21).

Insects used were less than 24 hours old. These were weighed, put in the humidity chamber for the appropriate number of days, weighed again and then dried to constant weight in 100°C to determine the amount of dry material left. These measurements gave the initial live weight, final live weight and final dry weight, while the difference between the last two measurements gave the amount of water in the insect's body at the end of the treatment.

In order to allow for the differences in initial weight of inidividuals in the same humidity and in that initial weight could be used as a standard reference, the crude measurements of dry matter and of water made for each insect in each humidity on each day were corrected by multiplying by 100/initial live weight mg. The corrected values were what would have been expected for beetles with an initial live weight of 100 mg. Similarly the ratio of dry matter to live weight (calculated for each beetle in each humidity on each day) was multiplied by 100 so that the resulting figures could be thought of as milligrams of dry material per 100 mg of final live weight.

Initial dry material as well as initial water content was shown to be linearly related to size as measured by initial weight (Section 6.1). Measurements of dry matter and water corrected in this

way will therefore give an unbiased estimate of the mean initial body composition of the insects in any particular humidity, and thus a measure to test for possible differences in this statistic in insects in the different humidities.

At the same time, if we assume a proportional decrease in water and dry material content with respect to size, the differences between the successive daily means of these statistics should give an unbiased estimate of the mean rate by which they decrease with time and humidity.

For the sake of clarity, these three factors (dry matter, water and the ratio of dry matter to live weight) involved in the explanation of the relationship between atmospheric humidity and the ability of starved T. molitor adults to stay alive will be treated separately.

7.1 The Change Of Dry Material Content In Starved T. molitor Beetles With Respect To Time And Humidity.

The ability of T. molitor adults to utilise reserved foods when starved in different humidities was demonstrated in Section 6.23.

The main interest in the utilisation of food reserves at this stage was to determine the rate at which it is being metabolised with respect to time and atmospheric humidity. I proposed to do this by measuring the change in total dry material content with time and in different humidities and then to test the following null hypotheses:

(a) There is no change in the rate of dry material consumption with time, irrespective of the humidity of the air surrounding the insects.

(b) The rate of dry material consumption is not influenced by atmospheric humidity.

The data for testing these hypotheses, obtained and corrected as described in Section 7.0 is summarised in Tables A 11 A-D.

7.11 Change In Dry Material Content With Time

The simplest way in which the dry material content (y) can change with time (x), is b units decrease in y per unit increase in x.

The hypothesis that there is no change in the rate of dry material consumption with time will then be accepted if the regression of dry material (y) on time (days) (x) can be shown to be linear.

To test the hypothesis that dry material content (y) depends linearly on time (x), the following procedure was followed: An analysis of variance, one for each of the humidities 0, 30, 60 and 90% R.H. was performed on the dry material data summarised in Tables A 11 A-D. In these analyses the different classes were dry material (milligrams per 100 milligrams of initial weight) as measured on consecutive days after starting the experiment. A summary of these analyses is given in Tables 9 A-D.

Table 9. Analyses of Variance of Dry Material Data (Tables A 11 A-D)

(A) 0% R.H.

Source of Variation	D.F.	S.S.	M.S.	V.R.
Between classes	14	13,120.1836	937.1560	123.4500 HIGHLY SIGNIFICANT
Within classes	678	5,146.9688	7.5914	
Total	692	18,267.1524		

(B) 50% R.H.

Source of Variation	D.F.	S.S.	M.S.	V.R.
Between classes	20	20,391.0339	1019.5517	134.8791***
Within classes	889	6719.9813	7.5590	
Total	909	27,111.0152		

(C) 60% R.H.

Source of Variation	D.F.	S.S.	M.S.	V.R.
Between classes	20	24,272.7070	1213.6353	153.4972***
Within classes	946	7,479.6067	7.9066	
Total	966	31,752.3137		

(D) 90% R.H.

Source of Variation	D.F.	S.S.	M.S.	V.R.
Between classes	20	29,176.7227	1458.8361	147.6480***
Within classes	966	9,544.5747	9.8805	
Total	986	38,721.2974		

Using the method described by Snedecor (1956) (p.455) the Between classes sums of squares $\leq (y-\bar{y})^2$, obtained from the analyses of Variance Tables 9 A-D were separated into two parts :

(i) Sum of Squares attributable to regression $[\leq (x-\bar{x})(y-\bar{y})]^2 / \leq (x-\bar{x})^2$

and

(ii) Sum of Squares attributable to deviation from regression.

These were tested against the appropriate Within classes sums of squares (Table 9 A-D) in the analyses of Regression Tables 10 A-D.

Table 10. Analyses of Regression of Mean Dry Material Content on Time. (Data from Tables A 11 A-D)

(A) 0% R.H.

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	1	12,911.3666	12,911.3666	1700.7986***
Deviation	13	208.8170	16.0628	2.1159*
Within classes	678	5,146.9688	7.5914	

(B) 30% R.H.

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	1	19,554.8968	19,554.8968	2586.9680***
Deviation	19	836.1371	44.0072	5.821828***
Within classes	869	6,719.9613	7.5590	

(C) 60% R.H.

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	1	23,470.7267	23,470.7267	2968.5131***
Deviation	19	801.9803	42.2095	5.3385***
Within classes	946	7,479.6067	7.9066	

(D) 90% R.H.

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	1	28,303.5138	28,303.5138	2864.5851***
Deviation	19	873.2089	873.2089	4.65142***
Within classes	966	9,544.5747	9.8805	

Tables 10 A-D make it clear that the bulk of the Between classes sums of squares are attributable to regression of dry material content on time. That part of the sums of squares attributable to deviation from regression is significant however in all the humidities

concerned. We therefore reject the hypothesis that there is no change in the rate of dry material consumption with time.

Having established that the rate of dry material consumption does not depend linearly on time, the question remains as to the way in which it does deviate from linearity. To conform to the model suggested by the proposed hypothesis (Section 6.34) the relationship between dry material content (y) and time (x) should best be described by two straight lines the slopes (rate of metabolism) of which should be different. The slope of the line describing the relation during the first part of the insect's life should be steeper than the one describing the relationship during the latter part. At the same time the slopes of both these lines should be the same in all humidities.

In figure 7 dry material content is plotted against time for humidities 0, 30, 60 and 90%. It also contains the straight line best fitting the data for the first fourteen days in all the humidities concerned. (For the reason for fitting this particular line see also Section 7.12).

Visual inspection of figure 7 shows that the points representing the mean dry material content over the first 12-14 days in all humidities, adhere fairly close to the fitted line but for a few minor oscillations. From between day 12-14 and beyond however a general deviation upward from the fitted line seems to be indicated in humidities 30, 60 and 90%. This picture then seems to fit in with the hypothesis that the rate of dry material consumption in starved adults of T. molitor decreases after a certain period in their lives, irrespective of the humidity of the air surrounding them.

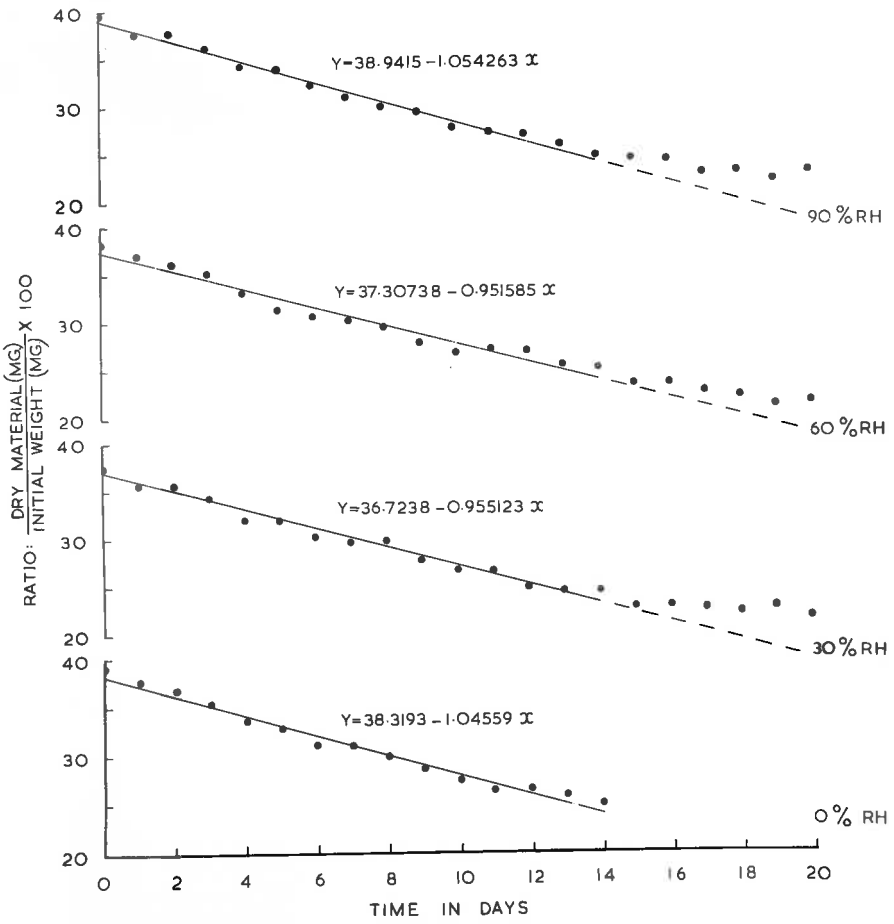


FIG. 7: The change in dry material content of T. molitor adults starved in different relative humidities at a temperature of 25°C.

During the course of the experiment it was noticed that no faecal material was excreted in any humidity until the fourth day after starting the experiment. The faecal material ranged from a dry powder in 0 and 30% R.H. to a wet smear in 90% R.H. Retained faecal material will be measured as dry material when insects are dried to constant weight. Irregular retention and passing of faecal material within and between classes could therefore be responsible for the minor wide oscillation of points round the fitted line during the first 12-14 days of the experiment. In addition to this some eggs were deposited from day twelve onwards. Since eggs contain dry material not used by the insect this will cause an over estimation of the rate of dry material consumption from day twelve onwards. As these points became apparent only some time after the start of the experiment no corrective measures could be applied. This, together with the fact that I had no legitimate reason for picking any particular day for the change in the rate of dry material consumption, made me decide not to determine and compare the rate of dry material consumption during different parts of the life of starved T. molitor beetles at this stage of the investigation.

Since it fits in with the proposed hypothesis (Section 6.34) my first inclination was to ascribe the observed change in the regular decrease in dry material content after day twelve (figure 7) to an "inbuilt" decrease in the rate of dry material consumption. However if we inspect the within class variances (Tables A 11 A-D) the following becomes apparent :

(i) The within class variances are relatively large.

(ii) As can be expected, these variances increase slightly with time.

However towards the end of the experiment in all the humidities an - to me - unexpected decrease occurs in the size of the within class variances. This decrease in the size of the variances also seems to coincide with a decrease in the class size, the latter being caused by insects dying presumably of natural causes.
(iii) The decrease in class size and with it the decrease in variance seems to start rather suddenly at day 17 in 90% R.H. and earlier as the humidity decreases.

The overall picture suggests that we might be dealing with two separate populations within the experimental population responding differently to the same experimental conditions. The initial body composition (dry material/water) of the two populations might have different, but overlapping distributions, causing the relatively large variance to start off with. Should the population with the lower mean dry material content die sooner than the second population, then their removal could cause the reduction in the size of the variance. Chance selection of more of any one particular population for any particular class could then also explain the fluctuation in the size of the class variances during the early part of the experiment.

To test the hypothesis that removal of part of the experimental population will cause a change in size of the within class variances, I applied "Bartlett's Test" for homogeneity of variances (after Snedecor (1956) p. 287), to the data in Tables All A-D.

The calculated (uncorrected) χ^2 values for testing for homogeneity of variances were :

0% R.H. : χ^2 = 19.4878 n. sig. at the 5% level with 14 DF

30% R.H. : χ^2 = 42.5587*** with 20 DF

60% R.H. : χ^2 = 49.7101*** with 20 DF

90% R.H. : χ^2 = 28.7945 n. sig. at the 5% level with 20 DF

Following this I did the same test but removed all the classes with n smaller than 40. This meant removing the last few classes in each humidity. It also means that, if we have two evenly distributed populations with the experimental population, one of which dies off sooner than the other, at least 40% of this population will be removed.

In this new test the values for χ^2 were in all cases considerably reduced, e.g. the χ^2 value for 30% R.H. was reduced from 42.5587*** with 20 degrees of freedom to 11.7205 n. sig. with 15 degrees of freedom. Similarly the χ^2 value for 60% R.H. was reduced from 49.7101*** with 20 D.F. to 26.2922 n. sig. with 16 D.F.

This seems to lend support to the idea of two populations within the experimental population.

Let us then assume that there are two populations within the experimental population, members of one dying sooner than the other. Under these circumstances the deviation from a linear decrease in mean dry material content with time, demonstrated in figure 7 could equally well be explained in at least two different ways other than the proposed "inbuilt" reduction in rate of dry material consumption with time. These are :

(a) If members of population (A) metabolise food reserves at a higher rate than those of population (B) the former will also exhaust their supply of reserved food sooner than the latter. Removal of members of

population (A) from the experimental population by death as a result of starvation, will leave increasingly larger proportions of population (B) which metabolise at a slower rate. Hence it might appear as if the metabolic rate of the experimental population decreases with time.

(b) If both populations (A) and (B) metabolise at the same rate but population (A) has a lower mean initial dry material content (reserved foods), then again this population will exhaust its food supplies sooner. The removal of increasingly larger numbers of population (A) could then also produce the same effect as was observed in figure 7. The situation as pictured here could also explain the initial large within class variances which were drastically reduced by the removal of increasingly larger numbers of the experimental population.

Until such time as this possibility of two populations within the experimental population can be verified, we cannot consider an "inbuilt" reduction in metabolic rate with time as the ultimate cause of the observed change in the reduction of dry material content demonstrated in figure 7.

7.12 The Influence of Atmospheric Humidity On The Rate of Dry Material Consumption

To explain the relationship between atmospheric humidity and length of life of starved adults of T. molitor it was suggested in Section 6.34 that the rate of dry material consumption might either :

(i) Not be influenced by atmospheric humidity, but change with time,

(ii) Be influenced by atmospheric humidity in the lower humidity range, but not change with time, or

(iii) Both, be influenced by atmospheric humidity and change with time.

To investigate further the possible influence of atmospheric humidity on the rate of dry material consumption, I set out to test the null hypothesis that in a constant temperature, atmospheric humidity has no influence on the metabolic rate of starved T. molitor beetles.

Using the original data summarised in Tables A 11 A-D, linear regressions of dry material content (y) on time in days (x) were calculated for each humidity. Since the experiment was terminated after 14 days in 0% R.H. two groups of regressions were calculated :

(i) Regressions involving only days 0-14 in humidities 0, 30, 60 and 90%.

(ii) Regressions involving days 0-20 only in humidities 30, 60 and 90%.

The data and results are summarised in Table A 12A and B respectively, while the slopes and intercepts within these two groups of regressions are compared in Tables 11A and B respectively.

The null hypothesis will be accepted if the regression lines for the different humidities can be shown to be identical.

These tests were done despite the fact that there were a few points on which they can be criticised, e.g.

(a) Since the experiments in different humidities were done in sequence, the data is, strictly speaking, not comparable.

(b) A straight line was shown in Section 7.11 not adequately describing the rate of reduction in dry material content over time.

The only way in which I thought that beetles from the same cultures could change with age of the culture, is in their mean size as measured by initial weight. In regard to size it was argued in Section 7.0 that, since initial dry material content is linearly related to size

the way in which the data was processed should give an unbiased estimate of the mean initial dry material content of beetles in all humidities concerned.

As far as the rate of dry material consumption is concerned, it was established (Section 6.24) that the larger beetles metabolise food reserves at a faster rate than smaller beetles. However at the same time no interaction with humidity could be shown, therefore these tests should be unbiased should we assume a proportionate decrease in dry material content over time with respect to mean size of the beetles.

Considering the second point of criticism, it was shown in figure 7 that a change occurred with time in the rate at which the dry material content was reduced. Fitting the best fit straight line to this data would therefore give a biased estimate of the overall rate of dry material consumption. However, since these regressions were calculated over comparable times in the different humidities, this should not introduce any bias with respect to a particular humidity.

Table 11 A. Analysis of Variance of Rate of Dry Material Consumption Data Summarised in Table A 12A.

Source	D.F.	S.S.	M.S.	V.R.
Slopes	3	124.1320	41.3773	4.603**
Displacements	3	915.4210	305.1403	33.948***
Error	2905	26,110.8440	8.9882	

Table 11 B. Analysis of Variance of Rate of Dry Material Consumption Data Summarised in Table A 12B.

Source	D.F.	S.S.	M.S.	V.R.
Slopes	2	93.2510	46.6255	5.075***
Displacements	2	942.1670	471.0835	51.279***
Error	2857	26,246.2250	9.1866	

From Tables 11A and B it is obvious that in both tests, regressions of dry material content on time in different humidities differ both in slopes and intercepts, i.e. the regression lines are not identical. On the strength of this, the null hypothesis should be rejected and we should accept that humidity has an influence on the metabolic rate.

On inspection of the results summarised in Tables A 12A and B, contrary to what I would have expected, there seems to be no relation between metabolic rate (regression coefficients) and humidity. This suggests that factor/s other than humidity might be responsible for the results obtained.

For these tests I worked on the assumption of a proportional decrease in dry material content over time with respect to size of the experimental animal. If the opposite is true, and the mean size of beetles used in different humidities are different, then this could explain the differences in slopes as observed. As far as the significant differences in intercepts are concerned, this might indicate differences in initial body compositions of beetles used in the different humidities. A further point that could have had a bearing on the results is the fact that, although the beetles used were of the same age, since the experi-

ments were done in sequence, the larvae they originated from were twelve weeks older in the last experiment compared to the first experiment done. I might have been dealing with different strains re-acting differently, an idea not impossible in the light of Leclercq's (1963) findings.

To gain some knowledge for future reference regarding these points I decided to use the available data to test the following hypotheses :

(a) The initial body composition of beetles used in the different humidities were the same.

(b) The mean initial live weight of beetles used in the different humidities were the same.

(i) To test whether the initial body composition of beetles used in the different humidities was the same, an analysis of variance Table 10C was constructed from the data summarised in Tables A 11A-D (using only the data for day zero).

Table 11 C. Analysis of Variance For Testing Similarity of Initial Body Compositions of Beetles used in Different Humidities.

Source	D.F.	S.S.	M.S.	V.R.
Between classes	3	138.2501	46.0834	7.2737***
Within classes	197	1,248.1190	6.3356	
Total	200	1,386.3691		

We accept that the mean initial body composition of beetles used in the different humidities were not the same, as the variance ratio associated with between classes sums of squares was significant at the 0.1% level.

(ii) The test for possible differences in mean initial live weights of beetles used in different humidities, was done in two stages :
(a) Testing for possible differences in mean initial live weight of beetles used on consecutive days within a particular humidity. This was done mainly to determine whether or not a particular size of beetle die first. Data concerning the initial live weight of beetles used in different humidities is summarised in Tables A 13 A-D. From this data analyses of variance Tables 12 A-D were constructed to test for differences in mean initial weight within humidities. (b) Testing for differences in mean initial weight of beetles used in different humidities. Data to test for differences in mean initial weight between humidities is summarised in Table 13 A (this constitutes a further summary of Tables A 13 A-D and led to the construction of the analysis of variance Table 13 B.

Table 12 A. Analysis of Variance For Testing Differences in Mean Initial Weight Within Humidities (0% R.H.)

Source	D.F.	S.S.	M.S.	V.R.
Between classes	14	4,341.96	310.1400	0.9068 n. sig.
Within classes	678	231,899.04	342.0340	
Total	692	236,241.00		

Table 12 B. Analysis of Variance For Testing Differences in Mean Initial Weight Within Humidities (30% R.H.)

Source	D.F.	S.S.	M.S.	V.R.
Between classes	20	3,717.73	185.8865	0.9981 n. sig.
Within classes	889	165,564.27	186.2365	
Total	909	169,282.00		

Table 12 C. Analysis of Variance For Testing Differences in Mean Initial Weight Within Humidities (60% R.H.)

Source	D.F.	S.S.	M.S.	V.R.
Between classes	20	3,864.02	193.2010	0.8584 n. sig.
Within classes	945	212,680.98	225.0592	
Total	965	216,545.00		

Table 12 D. Analysis of Variance For Testing Differences in Mean Initial Weight Within Humidities (90% R.H.)

Source	D.F.	S.S.	M.S.	V.R.
Between classes	20	7,655.63	382.7815	1.3861 n. sig.
Within classes	966	266,772.37	276.1619	
Total	986	274,428.00		

Table 13 A. A Summary of Data Concerning Mean Initial Weight of Beetles Used In Different Humidities

Humidities	0%	30%	60%	90%
n	693	910	966	987
$\bar{x}$	133.8967	120.9857	151.6055	144.7997
s^2	341.3987	186.2288	224.3990	278.3245

Table 13 B. Analysis of Variance For Testing Differences In Mean Initial Weight Between Humidities

Source	D.F.	S.S.	M.S.	V.R.
Between classes	3	498,898.13	166,299.3766	658.8935**
Within classes	3552	896,496.00	252.3919	
Total	3555	1,395,394.13		

The variance ratios associated with between classes sums of squares in Tables 12 A-D all being non-significant at the 5% level, we accept the hypothesis that the mean initial weight of beetles used on different days in any particular humidity were the same.

If we consider the wide range in initial weights of beetles used in these experiments (indicated by the size of the within class variances Table A 13 A-D) plus the fact that a fair number of beetles in each class die towards the end of the experiment in all humidities (indicated by size of R), then similar mean initial weights of beetles used within any particular humidity suggests that death occurs randomly with respect to size. This also confirms the hypothesis regarding size and length of life (Section 6.21).

Since the variance ratio (Table 13B) associated with between classes sums of squares is significant at the 0.1% level, we accept that the mean initial weights of beetles used in the different humidities were different.

To demonstrate a possible relationship between initial size and rate of metabolism, the mean initial weight of beetles used in different humidities is tabulated in Table 13C together with regression coefficients (dry material content on time), in the sequence in which the experiments were done.

Table 13 C. Metabolic Rate (b) With Respect To Size of Beetles

Relative Humidity %	30	0	90	60
Regression Coefficients (b)	-0.955123	-1.04559	-1.054263	-0.951585
Mean Initial Weight (mg)	120.9857	133.8967	144.7997	151.8055

From Table 13 C it is obvious that the mean initial weight of newly emerged beetles from the same cultures increases with the age of the cultures.

Contrary to what was suggested there seems to be no correlation between mean initial weight and the regression coefficients calculated for the different humidities. The last humidity seems to be the odd one out. This in turn suggests a possible additional influence of culture age on the rate of metabolism of the resulting adults.

All these factors together suggest that this experiment and the way in which it was laid out, does not supply us with a reliable test for comparing the influence of various atmospheric humidities on the rate of metabolism of starving T. molitor adults. This problem is re-investigated in Section 10 eliminating differences in culture age, initial body weight and initial body composition.

7.2 The Change In the Water Content of Starved T. molitor Adults With Respect To Time and Humidity

On the assumption that T. molitor adults when starved in the lower humidity range (0-60%) die after their initial water content is reduced to a certain minimum, it was suggested (in Section 6.33) that a change in the rate of water loss with time could explain the relationship between length of life and atmospheric humidity (figure 2), in this range.

It was demonstrated in Section 6.22 that the overall rate of weight loss depends linearly on humidity. At the same time it was shown for the two components of total weight loss, i.e. (a) Total water loss and (b) Total dry material loss, in Section 6.25 and

Section 6.24 respectively, that the overall rate of water loss depends linearly on humidity, while the overall rate of dry material loss does not. To explain this discrepancy it was argued in Section 6.35 that the rate of water loss must either increase with time in the upper humidity range or decrease with time in the lower humidity range, or both.

Apart from merely explaining certain phenomena a change in the rate of water loss and knowledge about the way in which it is brought about will also help us to understand how T. molitor adults regulate and conserve their water supplies.

The most urgent questions to answer regarding the rate of reduction in initial water content, seems to be :

(a) Whether there is a change in the rate at which the initial water content is reduced with time, and if so

(b) Whether this change is the same in all humidities or not.

I proposed to find an answer to this by measuring the change in initial water content with time and in different humidities, and then to test the null hypothesis: "There is no change in the rate at which the initial water content is reduced with time, irrespective of the humidity of the air surrounding the insects."

The data for testing this hypothesis, obtained and corrected as described in Section 7.0 is summarised in Tables A 14 A-D.

If the reduction in initial water content is simply a function of the humidity of the air surrounding the insects, then the relationship between water content (y) and time (x) should be a straight line in all humidities, the regression coefficients of which should depend linearly on humidity. On assumption of an "inbuilt" reduction in the metabolic rate with time, I would however expect

a change in the rate at which the water content is reduced after day 12-14 (Section 7.11).

It was shown in the previous Section 7.12 that both the initial body composition and the mean initial weight of beetles used in different humidities were different. Since size does influence the rate of water loss (Section 6.25) no comparisons of rate of water loss between humidities were attempted. On the other hand, since size has no influence on length of life (Section 6.21) I took it that size would not influence the way or time the rate of water loss might change in any particular humidity.

The hypothesis that there is no change in the rate at which the initial water content is reduced with time will then be accepted if the regression of water content (y) on time (days) (x) can be shown to be linear for all the humidities concerned.

The test for this hypothesis was done in two stages :

(i) An analysis of variance, one for each of the humidities 0, 30, 60 and 90%, was performed on the water content data summarised in Tables A 14 A-D. In these analyses the different classes were water content (milligrams per 100 milligrams of initial weight) as measured on consecutive days after starting the experiment. A summary of these analyses is given in Tables 14 A-D.

Table 14. Analyses of Variance On Water Content Data (Tables A 14 A-D)

(A) 0% R.H.

Source of Variation	D.F.	S.S.	M.S.	V.R.
Between classes	14	48,779.3552	3,198.5254	244.4304***
Within classes	678	8,871.7783	13.0852	
Total	692	57,651.1335		

(B) 30% R.H.

Source of Variation	D.F.	S.S.	M.S.	V.R.
Between classes	20	56,897.3472	2,844.8673	288.8630***
Within classes	889	8,755.3288	9.8485	
Total	909	65,652.6760		

(C) 60% R.H.

Source of Variation	D.F.	S.S.	M.S.	V.R.
Between classes	20	23,672.3024	1,183.6151	69.2516***
Within classes	946	16,204.4785	17.1476	
Total	966	39,876.7809		

(D) 90% R.H.

Source of Variation	D.F.	S.S.	M.S.	V.R.
Between classes	20	8,534.2955	426.7148	25.2566***
Within classes	966	16,320.8060	16.8952	
Total	986	24,855.1015		

(ii) Using the method described by Snedecor (1956) (p. 455), the between classes sums of squares $\leq(y-\bar{y})^2$, obtained from the analyses of Variance Tables 14 A-D were separated into two components :

(i) Sums of squares due to regression $\left[\leq(x-\bar{x})(y-\bar{y})\right]^2/\leq(x-\bar{x})^2$

and

(ii) Sums of squares due to deviation from regression.

These were tested against the appropriate within classes sums of squares (Table 14 A-D) in the analyses of variance for regression Tables 15 A-D.

Table 15. Analyses of Variance for Regression of Mean Water Content On Time. (Data from Tables A 14 A-D)

(A) 0% R.H.

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	1	48,595.6268	48,595.6268	3,713.7855***
Deviation	13	183.7284	14.1330	1.0801 n. sig.
Within classes	678	8,871.7793	13.0852	

(B) 30% R.H.

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	1	56,430.7774	56,430.7774	5729.8855***
Deviation	19	466.5698	24.5563	2.4934***
Within classes	889	8,755	9.8485	

(C) 60% R.H.

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	1	23,406.1863	23,406.1863	1,364.9832***
Deviation	19	266.1161	14.0061	0.8168 n. sig.
Within classes	945	16,204.4785	17.1476	

(D) 90% R.H.

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	1	8,087.7401	8,087.7401	478.7005***
Deviation	19	446.5554	23.5029	1.3911 n. sig.
Within classes	966	16,320.8060		

From Tables 15 A-D it is obvious that the bulk of the between classes sums of squares are attributable to regression of water content on time. Only in 30% R.H. a significant portion of the between classes sums of squares are attributable to deviations from regression.

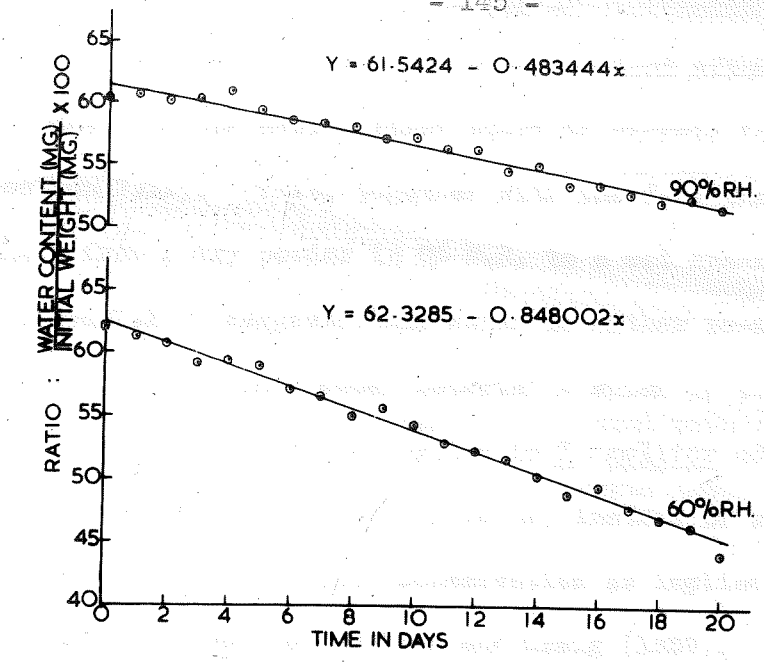
The information from Tables 15 A-D can best be discussed together with Figure 8 where the mean water content of starved T. molitor beetles is plotted against time for humidities 0, 30, 60 and 90%.

Figure 8 makes it clear that the mean water content is reduced with time in all humidities concerned but at different rates. Contrary to what I first expected however, only 30% R.H. shows a reduced rate of reduction which coincides with the proposed decrease in metabolic rate commencing from between day 12-14 (Section 7.11).

Discussing one humidity at a time, then :

(a) On closer inspection of the relationship between mean water content and time in 30% R.H. (Figure 8) it becomes obvious that between days 0-4 no change occurred in the mean water content of the beetles. It is true that no deviation from regression was demonstrated in Table 15D. However, the shallow slope of the regression of water content on time, plus the fact that relatively few days show this unchanged water content, might be responsible for the fact that no deviation from regression was registered.

No change in the water content during the first four days suggests either that no water was lost through evaporation or that an amount equivalent to that lost through evaporation was replenished by water obtained as a by product of metabolism.



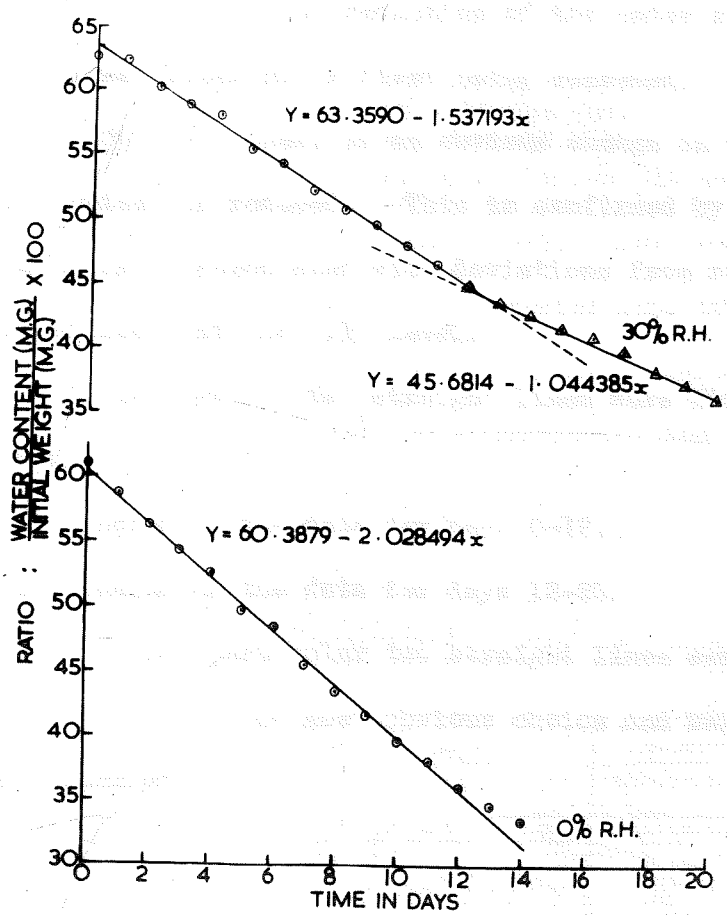


FIG. 8: The change in water content of T. molitor adults starved in different relative humidities at a temperature of 25°C.

The decrease in mean water content after day four coincides with the time the adults first start to excrete faecal material (Section 7.11). This, together with the fact that faecal material ranges from a dry powder in 0% R.H. to a wet smear in 90% R.H. (Section 7.11), suggests that water is either re-absorbed from, or let through with the faecal material - which in turn suggests that the Malpighian tube and rectal system in T. molitor adults might act as an internal regulatory system for water, instead of merely being an efficient system for water conservation as implied by the works of Wigglesworth (1932) and Patton and Craig (1959).

(b) In 60% R.H. there seems to be only but very faint traces of an increase in the rate of reduction of the water content at the point where faecal material is first being excreted.

(c) In 30% R.H. there is an obvious change in the rate at which the water content is reduced. This is confirmed by the fact that the variance ratio associated with deviations from regression (Table 15D) is significant at the 0.1% level.

In Figure 8 two straight lines were fitted to the data for 30% R.H. :

(i) Including the data for days 0-12.

(ii) Including the data for days 12-20.

These particular two straight lines were fitted because :

(a) They look the most obvious choice and way of describing the data concerned.

(b) I expected a change in the rate at which the mean water content is reduced with time, which should coincide with the decrease in metabolic rate, which in turn was shown in Section 7.11 to occur between days 12-14.

It was pointed out in Section 7.11, that the way in which the dry material content was observed to change with time (Figure 7), could be explained in a number of different ways other than by an "inbuilt" change in metabolic rate. These however were eliminated as explanations. See Section 10·31 and Section 10·32.

The hypothesis that the two fitted lines best fit and describe the data will be accepted if it can be shown that the variance ratio associated with deviation from regression is non-significant in both cases.

To test this hypothesis the data in Table A 14B was used to construct two analyses of Variance Tables 16A and B involving data for days 0-12 and days 12-20 respectively. The between classes sums of squares obtained this way were separated into two components (i) due to regression, (ii) due to deviations from regression, using the same method described earlier in this section. The tests for significance of these two components were performed in the Analyses of Variance for Regression Tables 17A and B.

Table 16. Analyses of Variance of Data in Table A 14B.

(A) Days 0-12.

Source of Variation	D.F.	S.S.	M.S.	V.R.
Between classes	12	21,377.9956	1,781.4996	190.0602***
Within classes	633	6,123.2265	9.6733	
Total	645	27,501.2221		

(B) Days 12-20

Source of Variation	D.F.	S.S.	M.S.	V.R.
Between classes	8	2,028.8304	253.6038	25.2369***
Within classes	303	3,038.8083	10.0291	
Total	311	5,067.6387		

Table 17. Analyses of Variance For Regression (Data from Table A 14B)

(A) Days 0-12

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	1	21,262.9722	21,262.9722	2,196.1095***
Deviation From Regression	11	115.0234	10.4567	1.0810 n. sig.
Within classes	638	6,123.2265	9.6753	

(B) Days 12-20

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	1	2,015.3398	2,015.3398	200.9426***
Deviation From Regression	7	13.4906	1.9272	0.1922 n. sig.
Within classes	303	3,038.8083	10.0291	

From Tables 17A and B and Figure 8 it is obvious that the fitted lines adequately describe the relationship between water content and time. From this I deduced that there is a genuine reduction in the rate at which the water content is reduced with time in 30% R.H. and that this goes together with a reduced metabolic rate. By comparing the regression coefficients of the two lines Figure 8 it appears that the reduced metabolic rate causes an approximate 1/3 reduction in the rate at which the water content is reduced.

The reduced metabolic rate was also demonstrated for humidities 60 and 90% (Section 7.11). Figure 8 however suggests no decrease in the rate of water loss in these humidities. For the rate of water loss to stay the same in the face of a drop in metabolic rate, the beetles should either get rid of proportionally more water with the faeces or exercise less rigorous control over the spiracles or both. The latter was demonstrated in Glossina morsitans by Buxsell (1957).

(d) Table 15D shows no deviation from regression in the relationship between water content on time for 0% R.H. This experiment was terminated at day 14 for reasons specified in Section 7.0. It is therefore impossible to say whether there would have been a decrease in the rate of water loss should the experiment have been continued. This seems to be indicated if we consider the last two points on the graph for 0% R.H. in figure 8.

The overall picture suggests to me that the rate at which the water content in starved T. molitor adults is reduced, is primarily determined by the water content of the atmosphere, the rate at which they metabolise reserved food material and the degree of regulation exercised through the excretory system.

The mechanical resistance of the cuticle of these insects to the evaporation of water is insufficient in the lower humidity range and over-efficient in the higher humidity range. This situation can partly be relieved by the Malpighian tube and rectal system acting as an internal water regulatory system, excreting water in the higher humidity range only with the faecal material, and re-absorbing water from the faeces in the lower humidity range. The efficiency of this system in

re-absorbing water from the faecal material was pointed out by Wigglesworth (1932) and borne out by my own observations in that the faecal material is reduced to a dry powder in the lower humidities. That this is not sufficient is borne out by the fact that these insects die of desiccation in the lower humidity range (Section 6.25).

An "inbuilt" change in the rate of metabolism causes a reduction in the rate of water loss in the lower humidity range. This will delay the time in which the body water is reduced to a critical level. In contrast to this, in the higher humidity range a similar reduction in the metabolic rate causes no change in the rate of water loss. This either suggests a deliberate upkeep of a higher rate of water loss, or signifies no need for water conservation in the higher range.

The change in the rate of water loss which is different in the lower and upper humidity range can then explain the relationship between length of life and atmospheric humidity (Section 6.21) as well as the discrepancy referred to in Section 6.35 regarding the relationship between total weight loss and atmospheric humidity and that of its components.

7.3 The Change In The Dry Material/Water Ratio With Respect To Time And Humidity In Starving Adults of T. molitor L.

In the literature on water relations of T. molitor and closely related species, especially in relation to their behaviour in a humidity gradient, a great emphasis seems to have been placed on either the necessity for or the ability of these insects to maintain a constant proportion of water to dry matter in their bodies. Other phrases

frequently used to describe the same idea is "a constant proportion of water" or "water balance".

Thus, Berger (1907) found that larvae of T. molitor maintain a constant proportion of water to dry matter in their bodies when starved in unsaturated air. These findings were confirmed by Buxton (1930), suggesting that larvae under these conditions maintain a due proportion of water in their bodies by making inroads on some reserves. In order to explain a reversal in the behavioural response of desiccated T. molitor larvae, Perttunen and Lahermaa (1962) suggested that since the direction of the humidity reaction is usually a good indicator of the state of the water balance in the normally hygro-negative terrestrial arthropods, the hygro-positive reaction observed in these larvae after desiccation for two weeks probably indicates a severely disturbed water balance and a considerable loss of water.

Perttunen and Lahermaa (1958, 1963) found that the sign of the light reaction for adults of T. molitor depends on the degree of desiccation, the latter phrase being explained as the water balance of the insect's body.

In the closely related species, Tribolium confusum and T. castaneum, Willis and Roth (1950) showed that the preference for a low or high humidity and the intensity of the humidity reaction could be related to the degree of starvation and to the water balance of the insect. Roth and Willis (1951) found for the same two species that the loss of water rather than a change in the proportion of water to solids is the more important factor in the reversal of the dry reaction.

Some of these phrases especially "water balance" can be misleading and confusing, since it is not always used in the context right

through the literature on water relations in terrestrial arthropods and then also because it is not always clearly defined.

Taking "water balance" in the case of work done on T. molitor to mean a constant proportion of water to dry material in the insect's body and since this is considered to be important (Andrewartha and Birch, 1954) I thought it necessary to determine:

(a) Whether adults of T. molitor maintain a constant proportion of water to dry material in their bodies when starved in different humidities.

(b) Whether a constant ratio of water to dry material is physiologically important or essential to these insects.

It was shown in Section 6.25 and Section 7.2 that the rate at which the water content is reduced depends on humidity. This rate however changes with respect to time and also differently in different humidities. A constant ratio of dry material to water implies that the rates of loss of dry matter and water are closely linked by a relationship that is simply predicted by the original ratio of dry matter to water. In Section 6.24 and Figure 5 it was shown that the rate of dry material consumption changes with humidity in the lower range but not in the higher range. In Section 7.11 and Figure 7 it was shown that the rate of dry material consumption changes with time after the same period in all humidities. We can therefore expect a difference in the dry material/water ratio with respect to humidity and possibly a change with time.

We know, at least as far as length of life is concerned, which humidities are favourable to starved T. molitor adults, and which are not. This study should then tell what the optimum ratio should be.

While a change in the ratio with respect to time might tell us whether the change in the rate of dry material consumption will cause a change in the ratio of water to dry material.

Working on the assumption that starved T. molitor adults die in the lower humidity range after a certain amount of water is lost (Section 6.25), I would not expect the dry/wet ratio to be of any importance in the physiology of these insects in this part of the humidity range. This point however needs confirmation and this study was therefore continued.

The data for testing the hypotheses in connection with the dry/wet ratio, obtained and corrected as described in Section 7.0 is summarised in Tables A 15 A-D.

To test the hypothesis that T. molitor adults when starved in different humidities, maintain a constant dry matter/water ratio, an analysis of variance, one for each humidity, was performed on the data summarised in Tables A 15 A-D. The different classes in each humidity constitute the dry material/water ratio as measured on consecutive days after starting the experiment. The hypothesis will be accepted if the variance ratio associated with between classes can be shown to be non-significant at the 5% level.

A summary of the analyses is given in Tables 18 A-D.

Table 18. Analyses of Variance of Data Summarised in Table A 15 A-D.

(A) 0% Relative Humidity

Source of Variation	D.F.	S.S.	M.S.	V.R.
Between classes	14	949.9439	67.8531	5.9334***
Within classes	678	7,753.4151	11.4357	
Total	692	8,703.3590		

(B) 30% Relative Humidity

Source of Variation	D.F.	S.S.	M.S.	V.R.
Between classes	20	383.3633	19.1722	2.0721**
Within classes	889	8,228.1625	9.2555	
Total	909	8,611.7258		

(C) 60% Relative Humidity

Source of Variation	D.F.	S.S.	M.S.	V.R.
Between classes	20	3,596.7050	179.8353	15.2825***
Within classes	946	11,131.9486	11.7674	
Total	966	14,728.6536		

(D) 90% Relative Humidity

Source of Variation	D.F.	S.S.	M.S.	V.R.
Between classes	20	8,958.2076	447.9104	35.5445***
Within classes	966	12,172.9165	12.6014	
Total	986	21,131.1242		

The variance ratios associated with between classes sums of squares are significant for all humidities. We therefore reject the hypothesis that T. molitor adults maintain a constant dry material/water ratio in their bodies when starved in different humidities.

To determine (a) whether the change in dry matter/water ratio depends on time, (b) whether the change is different in the different humidities, the between classes sums of squares obtained from Table 18 A-D were divided in the two components (i) due to regression of dry matter/water ratio on time (ii) due to deviation from regression.

Tests for regression of dry matter/water ratio on time (days), one for each humidity, are summarised in Table 19 A-D.

Table 19. Analyses of Variance For Regression of Dry Matter/Water Ratio on Time

(A) 0% Relative Humidity

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	1	797.5437	797.5437	69.7416***
Deviation From Regression	13	152.4002	11.7231	1.0251 n. sig.
Within classes	678	7,753.4151	11.4357	

(B) 50% Relative Humidity

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	1	2.8066	2.8066	0.3032 n. sig.
Deviation From Regression	19	380.7567	20.0398	2.1652**
Within classes	899	8,228.1625	9.2555	

(C) 80% Relative Humidity

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	1	3,123.1605	3,123.1605	265.4080***
Deviation From Regression	19	473.5445	24.9234	2.1180***
Within classes	946	3,596.7050	11.7674	

(D) 90% Relative Humidity

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	1	8,411.7137	8,411.7137	667.5222***
Deviation From Regression	19	546.4939	28.7628	2.2825***
Within classes	966	12,172.9166	12.6014	

The findings in Table 19 can best be discussed, together with Figure 9. In Figure 9 the mean dry material/water ratio is plotted against time (days) for relative humidities 0, 30, 60 and 90% R.H., together with the straight line best fitting the data in all cases.

Discussing one humidity at a time we have :

(A) 0% Relative Humidity

From Table 19A it is obvious that there is a relationship between the dry material/water ratio (y) and time (x). This relationship can best be described by a straight line. The regression coefficient being positive (Figure 9), then suggests that the dry material content of the insect steadily increases with time relative to the water content.

(B) 30% Relative Humidity

Table 19B indicates no relationship between the dry material/water ratio and time in 30% R.H. This suggests that the dry material/water ratio remains constant with time. From Table 16B, however, we know that significant differences between classes do occur. If we observe the graph for 30% R.H. in Figure 9, it does suggest that the significant differences might have been caused by the slightly larger means in the first and last few classes. Although significant these differences are so small, that I think for all practical purposes we might consider the dry material/water ratio to remain constant with time in this particular humidity.

From Section 7.2 and Section 7.1 we know that a decrease does occur with time, in the rate of water loss and the rate of dry material consumption respectively. For the dry material/water ratio

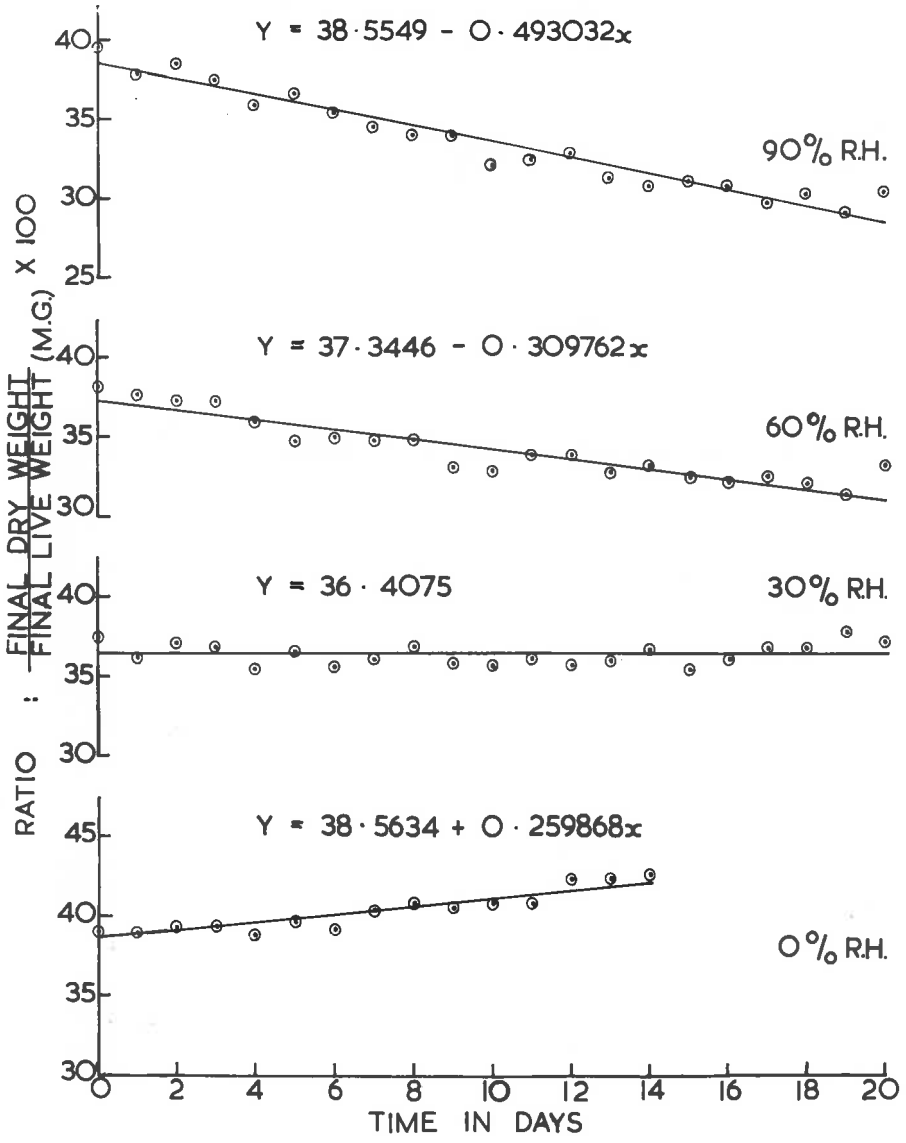


FIG. 9: The change in the dry material/water ratio of T. molitor adults starved in different relative humidities at a temperature of 25°C.

to stay constant, the change in rate of loss of both water and dry material had to coincide, while the rate of reduction of both water and dry material content had to be of the same relative magnitude.

(C) 60% Relative Humidity

Table 19C indicates a relationship between the dry material/water ratio (y) and time (x) in 60% R.H. The regression coefficient being negative suggests that the water content increases with time relative to the dry material content. This is just the opposite to what happened in 0% R.H.

Table 19C indicates that a straight line does not adequately describe the relationship between y and x. Visual inspection of Figure 9 suggests that this relationship might be curvilinear. This hypothesis was subsequently tested by calculating a multiple regression of mean y on x_1 introducing x^2 as a second variate. A summary of the data, calculations and results is given in Table 20 A-C. Where Table 20A gives the A-matrix (corrected sums of squares and cross products), Table 20B information regarding the regression coefficients of y on x and x^2 and Table 20C the analysis of Variance for regression.

Table 20A. A. MATRIX

	x_1	x^2	y
x_1	770.0000	15,399.999	-228.59964
x^2	15,399.999	330,422.590	-4,215.46390
y			78.656861

$\bar{x}_1 = 10.00$, $\bar{x}^2 = 136.66$, $\bar{y} = 34.295$, $n = 21$

Table 20B. Information Regarding Regression Coefficients

Variates	Regression Coefficients	S.E.	T.	P.
x_1	-.614749 (b_0)	.07378	8.33_{18}	< .001
x^2	.015633 (b_1)	.00356	4.46_{18}	< .001

$R\ y\ x\ x^2$ = .9668

Table 20C. Analysis of Variance For Regression

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	2	73.533373	36.766665	129.17***
Residual	18	5.123151	.284619	
Total	20	78.656881		

The regression coefficients b_0 and b_1 (Table 20B), both being significant, I accept the hypothesis that the relationship between y and x is curved. The regression coefficients having different signs and b_1 being positive then indicates that the curviture is away from the x axis.

On inspection of Table A 15C there seems to be a gradual decrease in the dry material/water ratio reaching a minimum value of (32-33) which is then maintained from day ten onwards.

By itself the data in this sub-section suggests that the dry material/water ratio is reduced to a certain minimum value which is then maintained. From the previous sub-section we know that in 30% R.H. the dry material/water ratio stays constant at a level similar to that when the beetles first emerge. From Section 6.21 we know that in 30% R.H. T. molitor beetles when starved, die prematurely of desiccation but in 60% R.H. they die of starvation. This in turn suggests that only if the atmospheric humidity is high enough to ensure

that so little water is lost that the ratio of dry material to water is reduced to a level below what it was when the beetles first emerged, will the beetles be able to stay alive long enough to consume all their reserves.

(D) 90% Relative Humidity

Table 19D indicates a relationship between dry material/water ratio (y) and time (x). The regression coefficient (Figure 9) being negative, suggests that the water content relative to the dry material content increases with time. Table 19C also suggests that a straight line does not adequately describe the relationship between x and y, a curvilinear relationship is suggested in Figure 9.

To test this hypothesis again as in (C) above a multiple regression of mean y on x were calculated, introducing x^2 as a second variate. The hypothesis will be accepted if the regression coefficient of x^2 can be shown to be significantly different from zero.

A summary of the data, calculations and results is given in Table 21 A-C.

Table 21A. A-Matrix

	x_1	x^2	y
x_1	770.000	15,399.999	-272.93240
x^2	15,399.999	330,432.590	-7087.3441
y			192.8971

$\bar{x}_1 = 10.00$, $\bar{x}^2 = 136.66$, $\bar{y} = 33.651$, $n = 21$

Table 21B. Information Regarding Regression Coefficients

Variates	Regression Coefficients	S.D.	T.	P.
x_1	-.815367 (b_0)	.08044	10.13_{18}	<.001
x^2	.016552 (b_1)	.003883	4.26_{18}	<.001

$R\ y\ x_1\ x^2$ = .9840

Table 21C. Analysis of Variance For Regression

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	2	186.76733	93.383665	276.02***
Residual	18	6.08977	.33832	
Total	20	192.85710		

The regression coefficients b_0 and b_1 (Table 21B) are both significantly different from zero. The hypothesis is therefore accepted that the relationship between y and x is curved. The size and signs of the coefficients suggest a relationship very similar to what was observed for 60% R.H. If Tables A 15C and D are compared, we see that the dry material/water ratio is reduced to a value (32-33) by day ten which is similar to that for 60% R.H.

The similarity between the values of the dry material/water ratio, reached after ten days in two humidities which are very different from each other (60-90% R.H.) and also since the values of this ratio in either humidities hardly changes after this point suggests that the minimum value of this ratio could have some importance. On the other hand the way in which the dry material content (Figure 7) and the water content (Figure 8) change with time in these two humidities suggests that the relationship between the dry material/water ratio and time might just be the result of the way in which the two components change with time; relative to each other.

7.4 Summary and Discussion

(i) T. molitor adults when starved in a constant temperature in different humidities do not maintain a constant dry material/water ratio similar to that when they first emerge. This ratio increases with time in 0% R.H., stays constant in 30% R.H. and decreases in almost identical manner in 60% and 90% R.H.

(ii) Starved T. molitor adults which maintain their initial dry material/water ratio (Figure 9 30% R.H.), will die after a relatively short time (Section 6.21) and while it still contains some food reserves (Section 6.23). On the other hand animals which decrease their dry material/water ratio (Figure 9 - 60%, 90% R.H.) survive for a longer time (Section 6.21) and die containing no food reserves. Therefore animals in which the dry material/water ratio decreases seems to be more successful.

(iii) The terms "maintaining" and "reducing" the dry material/water ratio suggest at least an active process or even a purpose. They should be used cautiously because they could easily lead into a circular argument and confusion. The problem here is that both components of the ratio change with time under the influence of various factors which are not the same for both. This change ends with the death of the insect which could be caused by exhaustion of the supplies of either of these components (resources), at a time which might coincide but not necessarily. The significance or importance of a specific ratio of these components can only be judged by something like length of life, which could be caused by a short supply of either component, but then always in the presence of the other.

It is indicated by information obtained in Section 7.1 and Section 10 that the dry material content changes according to an "inbuilt" pattern, independent of humidity. On the other hand the rate of change of the water content (Section 7.2) is basically determined by atmospheric humidity. In the lower humidity range the rate of water loss is influenced by the metabolic rate. While in the upper humidity range the rate of water loss is governed by the rectal system by re-absorption of water from, or excretion of water with the faecal material. Therefore although at first glance these two components seem to change independently of each other, they are really very closely interrelated. This, plus the fact that both are resources, makes it difficult and perhaps confusing to talk about the importance of one component relative to the other.

From Section 6.25 we know that T. molitor adults when starved in the lower humidity range, die prematurely of desiccation and after they have lost a certain amount of water. This means that the insects die as a result of a short supply in one of the resources (water).

From Section 7.2 and Figure 8 we know that in 90% R.H. the water content of the insects stays constant for the first four days after which it is reduced by excretion of water with the faeces. From Section 10 we know that T. molitor adults when starved in the higher humidity range die prematurely of "water-logging" of the tissues when the atmospheric humidity reaches saturation level. Death as a result of "water-logging" occurs despite the fact that water is excreted with the faeces and at a time when the amount of water present in the insect's body is less than what it was when they

first emerged. In the light of this it seems reasonable to accept that these insects not only do, but also have to reduce their water content to below the level which is considered "normal" for newly emerged beetles.

During the first four days and on subsequent days the dry material content is reduced according to an "inbuilt" pattern, thus broadly speaking not under the control of the insect. If we should argue that the insects have to reduce their water content because the dry material is reduced (which seems reasonable) then it follows that the amount of water relative to the dry material must be important, at least in the higher humidity range. It also follows that since the dry material content continuously decreases, that the rate at which the water content is decreased relative to the rate of dry material consumption must be important.

The opposite of this argument does not follow for the lower end of the humidity range. Since the rate of dry material consumption cannot be "controlled" or regulated it seems to serve no useful purpose talking about, or thinking in terms of a "surplus" of dry material or the amount of dry material relative to the amount of water. To cover the whole humidity range, it seems more useful and less confusing to think in terms of the importance of, or the ability to maintain a certain rate of water loss, rather than in terms of the importance of, or the ability to maintain a certain ratio of water to dry material.

The whole picture suggests to me that for these insects to stay alive for the maximum length of time their energy resources will allow them to, their initial water content has to be reduced and at a

certain rate. The main factors, other than physical reduction of evaporation, which determine or influence the rate of water loss is, atmospheric humidity and the regulatory system of the insect. The regulatory system, although functional throughout the humidity range, is only effective between 60-90% R.H. Both beyond and below this range the influence of atmospheric humidity overrides the usefulness of the regulatory system and this results in the premature death of the insect. Thus only between 60-90% R.H. can these insects maintain an optimum rate of water loss. Both beyond and below this range, the inability of the insect to efficiently control water loss results in a disadvantageous rate of water loss which will ultimately result in the premature death of the insect, by desiccation or "water-logging".

(iv) By its own means then, T. molitor beetles when starved, are able to maintain a required rate of water loss in the humidity range 60-90% R.H. only. In other words T. molitor adults when starved have to exploit and use atmospheric humidity together with its own water saving devices to maintain a required rate of water loss. This is substantiated by the fact that T. molitor adults acquired the ability to recognise and respond to small differences in humidity gradients as was shown by, e.g. Pielou and Gunn (1940), Pielou (1940), Gunn and Pielou (1940), Dodds and Ewer (1952), Howard (1955) and Perttunen and Laherrana (1956, 1962, 1963).

This entire picture also tenders a possible reason for the reversal of the response to a moisture gradient observed in T. molitor adults, changing from an initial hygro-negative to a hygro-positive reaction (Leclercq (1947), Dodds and Ewer (1952). Beetles when first

subjected to a higher humidity, with the regulatory mechanism not in operation, seeks out a lower humidity which acts as a substitute for this mechanism, but which in itself cannot support the water requirements of the insect. With the regulatory mechanism in operation the insect on return to the higher humidity can regulate its rate of water loss.

8.0 Size and Its Importance In The Water Relations Of T. molitor

The importance of the size of the experimental animal in water relation studies was recognised by Kennedy (1927) who pointed out that all other parameters remaining the same, the water content is a function of the volume of the animal (cube of the linear dimensions), whereas evaporation is a function of the surface area (square of the linear dimensions).

The fact that adults of T. molitor may vary considerably in size as measured by weight, has long been recognised (Hein, 1920; Mellanby, 1932). By selecting for weight, Leclercq (1963) was able to separate two strains of T. molitor, which he found were "fitted to various ecological hazards". Little work however has been done to evaluate the advantage, if any, of larger beetles over smaller ones as far as the conservation of water is concerned.

A wide range in size (as measured by weight in milligrams) of newly emerged adults was illustrated in Figure 1. These adults were obtained during a relatively short period of time from cultures of the same age, which had been producing adults for periods ranging from 4 to 6 weeks. I wish to distinguish this range in size from that demonstrated in Section 7.12 and Tables A 13 A-J, for newly emerged beetles taken from the same cultures but at progressively later stages in the productive life of the cultures. We can thus get a wide range in the weight of newly emerged adults taken from cultures of any age, the mean weight of which increases with the age of the cultures.

Beetles used in this study were taken from cultures of the same age (12-13 weeks), which had been producing adults for periods ranging from 2-4 weeks.

The main purpose of this study was :

(i) To confirm the hypothesis that larger beetles lose water faster than smaller ones (see also Section 6.25), and if so

(ii) To find whether larger beetles lose proportionally less water than smaller ones, and if so

(iii) To determine whether this can be considered an advantage.

To my mind, only those characteristics which put bigger insects as compared to smaller ones in a more favourable position to contribute to the next generation can be considered as advantageous. They should either :

(a) Allow them to survive for a longer period in order to, either

(i) Give them a better chance of outlasting adverse conditions of food and water, or

(ii) Give them a greater chance to search for more favourable conditions, and/or

(b) Allow them to produce proportionally more eggs under adverse conditions of food and water.

The production of eggs by beetles of different sizes starved in a hot dry place will be discussed in Section 9. In this section the influence of size on the rate of water loss and the length of life of beetles placed under a severe water stress was investigated in the following experiments.

Two groups of thirty newly emerged beetles in each of the

weight ranges, i.e. 90-100, 110-120, 130-140, 150-160 and 170-180 mg. were randomly selected from cultures of the same age. Beetles of group one were weighed and immediately dried to constant weight in a temperature of 100°C, in order to provide an estimate of the initial dry weight of beetles in group two. Beetles in group two were individually confined in glass tubes (2" x 1") in an atmosphere of 0% R.H. at a temperature of 25°C. The weight of these beetles were recorded daily until they died, when they too were dried to constant weight. These measurements thus provided the following data :

(a) The initial live and the initial dry weight of individual insects in the control group.

(b) Daily weight changes, until death, of individual insects in the experimental group.

(c) The length of life (days) of insects in the experimental group.

(d) The final dry weight of each beetle in the experimental group, measured at death.

These data have been analysed in Sections 8.1 to 8.4 to discover the influence of size on (i) the longevity of the beetles, and (ii) the rate of water loss from the beetles.

8.1 The Influence of Size on the Rate of Change in Total Weight of T. molitor Adults, Starved in 25°C and 0% R.H.

In a constant temperature it was shown that (i) the rate of weight loss (Section 6.22), (ii) the rate of dry material consumption (Section 6.24) and (iii) the rate of water loss (Section 6.25) are all

functions of the relative humidity of the atmosphere and of the size of the beetles. It was further shown that the regressions of these statistics on initial weight, although displaced, were parallel, which indicates that the influence of size on these statistics is proportionally the same in all humidities, but it does not necessarily indicate a linear relationship between humidity and the rate of water loss, allowing for the size of the beetles.

From Section 6.22 we know that there is a positive correlation between rate of weight loss (y) and the weight of beetles (x). Should bigger insects lose weight proportionally more slowly than smaller ones, then the relationship between x and y must be curved, with y increasing progressively more slowly with unit increase in x.

To test this hypothesis I needed to get an estimate of the rate of weight loss for individual animals for the time they stayed alive in this experiment. The best estimate of this rate is the coefficient of linear regression of live weight on time, the line being arbitrarily drawn through W_o (the weight at day zero). An estimate of the weight (W_t) of any particular insect at any particular time (t) can then be obtained from the equation $W_t = W_o - b_i t$

From Section 6.22 we know that the rate of weight loss decreases with time. The coefficient b therefore might under-estimate the rate of weight loss during the first part of the life span, and over-estimate it during the latter parts. However, if we assume a decrease proportional to size, this estimate should give an unbiased comparison between animals of different sizes.

A listing of initial weights (W_o) together with the appropriate regression coefficients (B) (daily rate of weight loss) and the length of life (days) for any particular insect used in this experiment is given in Table A 16.

When plotting B against W_o, the graph shows a curved trend. To test the hypothesis that the relationship between B and W_o is curved, a multiple regression was calculated, adding a third variate W_o^2 (method described by Snedecor (1956) p. 452). This hypothesis will be accepted if the regression coefficient for W_o^2 can be shown to be significantly different from zero. Then if the regression coefficient for W_o and W_o^2 have different signs and that for W_o^2 is negative, the hypothesis that bigger beetles lose weight at a relatively slower rate than smaller ones, will be accepted.

To make calculations easier W_o was reduced by a factor of ten i.e. $W_o/10 = W_1$.

A summary of the calculations of the regression of B on W_1 and W_1^2, and of the results, are given in Tables 22 A-C.

Table 22A. A-Matrix

	W_1	W_1^2	B
W_1	1,069.8850	29,375.834	327.3527
W_1^2	29,375.834	798,185.87	8,708.7496
y			127.56474

$\bar{W}_1 = 13.412$, $\bar{W}_1^2 = 186.17$, $\bar{B} = 4.0699$, $N = 132$

Table 22B. Information Regarding Regression Coefficients

Variates	Regression Coefficients	S.D.	t	P
W_1	.7812301 (b_0)	.1551	5.03_{129}	$< .001$
W_1^2	-.0178402 (b_1)	.005733	5.11_{129}	$< .01$

Table 22C. Analysis of Variance For Regression of B on W_1 and W_1^2

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	2	100.36843	50.181215	237.97***
Residual	129	27.20831	.21087	
Total	131	127.56674		

Multiple Correlation Coefficient = R B W_1 W_1^2 = .8669

The regression coefficient for W_1^2 (b_1) is significantly different from zero, at the .01% level of probability. I therefore accepted the hypothesis that the relationship between B on W_0 is curved. Since b_1 also assumed a negative value compared to the positive value of b_0, I also accepted the hypothesis that when adults of T. molitor are starved in 0% R.H., bigger beetles lose weight relatively more slowly than smaller ones.

The regression equation describing the relationship between the rate of weight loss and the initial weight of the beetles, under the conditions specified for this experiment, can be written as $B = .0781201\ W_0 - .00178402\ W_0^2 - 3.030521$ while the weight (Wt_i) of any particular sized beetle at any particular time can be estimated by the equation

$Wt_i = W_0 - (.0781201\ W_0 - .00178402\ W_0^2 - 3.030521)t$.

8.2 The Influence of Size on The Amount of Dry Material Used Till Death.

Having accepted the hypothesis that bigger beetles lose weight relatively more slowly than smaller ones, then, if it can be shown that dry material is consumed either

(i) at a rate proportional to size, or

(ii) at a proportionally faster rate in bigger animals,

then it can be inferred directly from this, that water is lost relatively more slowly in bigger animals.

To be able to make inferences regarding the rate of dry material consumption relative to size, I needed to estimate the amount of dry material consumed during a comparable stage in the life of the beetle. Assuming, at this stage, that beetles of different sizes have the same mean life span under identical atmospheric conditions (see also Section 8.3), I decided to estimate the amount of dry material used during the time that they stayed alive under the conditions of this experiment, i.e. 0% R.H. at 25°C.

The amount of dry material consumed is the difference between the initial dry weight and the dry weight at the day of death. The latter was measured in beetles from group two, as described in Section 8.0. The initial dry weight of these beetles had to be estimated, and for this purpose insects from group one, selected and treated as described in Section 8.0, were used.

To test the hypothesis that *T. molitor* beetles when starved to death in an atmosphere with 0% R.H., consume an amount of dry material proportional to its initial weight, it was argued as follows :

If both initial dry weight and final dry weight have a linear relationship with initial live weight, then this hypothesis can be accepted, if it can be shown that the constants (intercepts of the two regression lines) $a_1 = a_2$. From Section 6.1 we already know that initial dry weight has a linear relationship with initial live weight. This needs confirmation for this set of data, and then it only remains to be shown that a similar relationship exists between final dry weight and initial live weight.

This test might also tender an answer to the question raised in Section 7.1 as to whether or not differences in initial weight could have been partly responsible for differences in the rate of dry material consumption, shown between different humidities.

For these reasons two groups of regressions were calculated. These were :

(a) Regressions of initial dry weight (y_0) on initial live weight (x), one for each of the different weight groups using the data obtained from beetles in group one (Section 6.0). A summary of the data and the calculations is given in Table A 17, while the slopes and intercepts of the different regression lines are compared in the Analysis of Variance Table 23A.

(b) Regressions of final dry weight (y_1) on initial live weight (x), one for each of the different weight groups, using the data obtained from beetles in group two (Section 6.0). A summary of the data and calculations is given in Table A 18, while the slopes and intercepts of the different regression lines are compared in the Analysis of Variance Table 23B.

Table 23A. Analysis of Variance of Data Summarised in Table A 17

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	4	6.9415	1.735375	0.175 n. sig.
Displacements	4	89.6462	22.41205	2.267 n. sig.
Error	149	1,473.5203	9.892287	

Table 23B. Analysis of Variance of Data Summarised in Table A 18

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	4	59.8418	14.96045	1.289 n. sig.
Displacements	4	30.0444	7.5111	0.647 n. sig.
Error	122	1,415.7732	11.604698	

The variance ratio associated with both slopes and displacements in both Tables 23A and B being non-significant at the 5% level of probability, I accept that the regression lines within either of the groups are identical. This also means that both initial dry weight and final dry weight, as measured, holds a linear relationship with initial live weight. These relationships which can be described by the regression equations obtainable from "Pooled Regressions" Tables A 17 and A 18 are illustrated in Figure 10. The regression equations describing these relationships are :

(i) $y_0 = 51.204338 + 0.351372\ (x - 134.45) = 3.982338 + 0.351372\ x$

(ii) $y_1 = 30.474242 + 0.229257\ (x - 134.12719) = -0.275389 + 0.229257\ x$

and the mean dry weight for given initial weight, as illustrated in Figure 10 were calculated making use of the "common slope" obtained from "Parallel regression" in Tables A 17 and A 18 respectively.

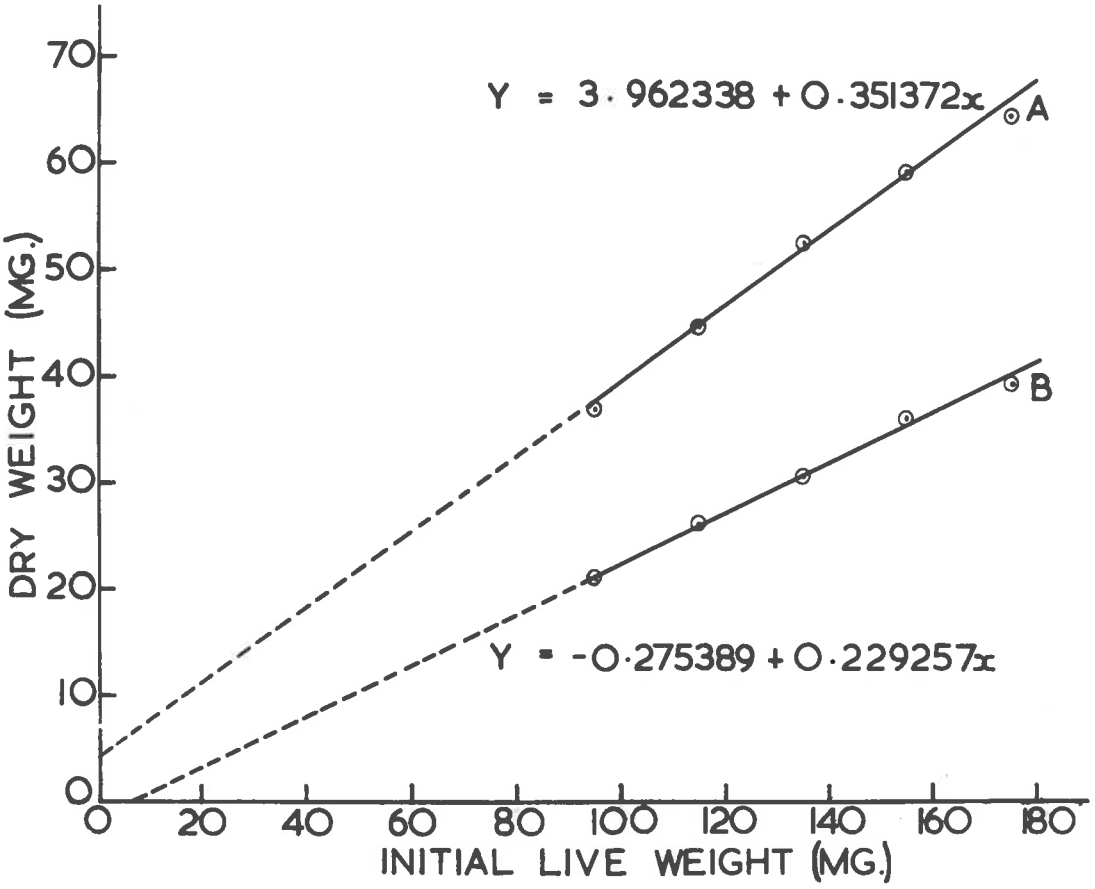


Fig. 10: The change in the dry material content of T. molitor adults with different initial weights starved to death in 0% R.H. and a temperature of 25°C.

(A) Linear Regression of Initial dry (Y) on initial live weight (x).

(B) Linear Regression of Final dry (Y) on initial live weight (x).

The hypothesis that T. molitor beetles, when starved to death, consume an amount of dry material proportional to its initial live weight will then be accepted if the difference between the intercepts of the regression lines describing the relationship between (a) initial dry weight (y_0) (b) final dry weight (y_1), and initial live weight (x) is shown to be not significantly different from zero. To test this hypothesis, the data in each group of regressions (Tables A 17 and A 18) were treated as two single regressions. The calculations are summarised in Table A 19 and the slopes and intercepts of these two regressions compared in the Analysis of Variance Table 24.

Table 24. Analysis of Variance of Data Summarised in Table A 19.

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	1	847.0394	847.0394	78.868***
Displacements	1	29,697.5210	29,697.5210	2,765.765***
Error	276	2,965.6886	10.739387	

The displacements (intercepts) of the two regression lines are significantly different from each other at the 0.1% level of probability. I therefore rejected the hypothesis that T. molitor beetles when starved in 0% R.H., use amounts of dry material proportional to their initial weight before they die.

To measure the amount of dry material used relative to the initial weight (x) of the insect, we have :

(a) Initial dry weight = y_0 = 3.962338 + 0.351372 x

(b) Final dry weight = y_1 = -0.275389 + 0.229257 x

i.e. Dry material consumed = $y_0 - y_1$ = $4.237727 + 0.122115\,x$

i.e. Ratio = $\frac{\text{Dry material consumed}}{\text{Initial live weight}} = \frac{4.237727 + 0.122115\,x}{x}$

$= 0.122115 + 4.237727/x$

i.e. The ratio Dry material consumed/initial live weight decreases with an increase in x (initial live weight). In other words bigger beetles used proportionally less dry material than smaller ones during the time they stayed alive, under the conditions of this experiment. On the assumption that beetles of different sizes live on the average for the same length of time, I accepted that bigger beetles consume reserved foods at a relatively slower rate than smaller beetles. This being so makes it virtually impossible to make a definite statement as regards the rate of water loss relative to size. However, it does suggest that bigger beetles might have an advantage over smaller ones, as regards water loss, for a further reason.

(i) A more favourable surface area/volume ratio

(ii) A relatively less active metabolism (see also Section 7.2, rate of water loss in relation to metabolic rate).

It will be extremely difficult to separate and describe quantitatively the amount each of these characteristics contribute towards the advantage bigger insects hold over smaller ones, as regards rate of water loss.

Although no definite statement could be made as regards the advantage of bigger over smaller insects, to my mind it will not be unreasonable to accept (even only because of the relatively slower metabolic rate) that for different sizes observed in T. molitor adults, bigger insects are at an advantage as far as the rate of water loss

is concerned.

Since the ratio dry material consumed/initial weight (x), decreases with increase in x, instead of the otherway round, differences in the rate of dry material consumption, as was observed between humidities (Section 7.12) cannot be explained by differences in mean initial weight.

8.3 The Relationship Between Size And The Life Span of T. molitor Adults Starved to Death in an Atmosphere of 0% R.H. in 25°C.

Having accepted that bigger beetles lose water relatively more slowly than smaller ones (Section 6.2), it remains to be shown that this can be considered an advantage to the species. The latter will be accepted, as was stated in Section 8.0, if it can be shown that bigger beetles, either stay alive for a longer time, or are able to mature proportionally more eggs under adverse conditions. The latter point came more prominently to my attention during the course of this experiment, when it was observed that, although no beetle laid many eggs, more were deposited by bigger than smaller insects. This will be discussed in more detail in Section 9.

It was postulated in Section 6.32 that T. molitor adults when starved in the lower humidity range, die after their initial water content is reduced by a certain amount. It is thus conceivable that bigger insects losing water at a proportionally slower rate might be able to stay alive for a longer time.

Having measured the life span (days) of beetles with different

initial weights (listed in Table A16) I used this data to test the null hypothesis that size has no influence on the length of life of *T. molitor* adults starved in a low relative humidity.

Table 25A contains a summary of the data :

Table 25A Summary of Data Concerning the Life Span (days) of *T. molitor* Beetles with Different Initial Weights, Starved to Death in 0% R.H. at 25°C

Weight Range (mg)	90-100	110-120	130-140	150-160	170-180
n	27	28	25	26	26
$\bar{x}$	16.00	15.39	15.48	14.88	16.42
s^2	4.77	2.56	2.92	4.16	

The data summarised in Table 25A was used to construct the analysis of Variance Table 25B as also the analysis of variance for Regression Table 25C.

Table 25B Analysis of Variance of Data Summarised in Table 25A

Source of Variation	D.F.	S.S.	M.S.	V.R.
Between classes	4	36.6269	9.1567	2.6196*
Within classes	127	443.9186	3.495422	
Total	131	480.5455		

Table 25C Analysis of Variance for Regression of Life Span on Initial Weight

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	1	.3857	.3857	0.104 n.sig.
Deviation from regression	3	36.2412	12.0804	3.456*
Within classes	127	443.9186	3.4954	
Total	131			

From the result presented in Tables 25B and C, I made the following inferences.

(a) The variance ratio associated with between classes (weight groups) sums of squares is just significant at the 5% level of probability (Table 25B). The absence of a regression factor in the relationship between life span and initial weight (Table 25C) indicates that there is no systematic change in life span with change in initial weight. Since the mean life span for different weight groups (Table 25A), suggests no definite trend, I accepted the hypothesis that size has no influence on the length of life of starved T. molitor beetles, and that the differences recorded are due to other factor/s not associated with size.

(b) These results confirm the findings, as regards the relationship between life span and initial weight in Section 6.21 and Section 7.12.

(c) The differences between the mean life span of different weight groups (Table 25A), although significant, being relatively very small and non-systematic, should not in any way bias any conclusion drawn (Section 8.2) on the assumption of equal mean life span for different weight groups.

(d) Any advantage of bigger over smaller insects is not reflected in an increased life span.

8.4 The Change in Total Weight, With Respect to Time And Size, of T. molitor Beetles Starved in an Atmosphere of 0% R.H. in 25°C.

It was shown in Section 8.1 that bigger beetles lose weight more slowly than smaller ones relative to their initial weight

while in Section 8.3 it was shown that beetles of different weights stay alive for the same length of time. This will mean that bigger beetles will have to end up having lost a smaller proportion of their initial live weight than smaller ones. This should be so unless at some time during the life of the beetles an increase in the rate of weight loss occurs which should be more prominent in bigger beetles.

In Section 8.3 it was mentioned that bigger beetles were observed to deposit more eggs than smaller ones. In Section 7.11 it was mentioned that eggs are being deposited from day twelve onwards. Working on the assumption that bigger beetles put their advantage regarding loss of water towards maturing and depositing more eggs, I would then expect a relatively sharper drop in weight in bigger beetles around day twelve. To get information on this point for future reference, and also to illustrate the change in weight with respect to size and time, I used the data on rate of change in live weight obtained as described in Section 8.0, to construct Figure 11.

Five groups of regressions, each containing five regressions of final live weight (y_i) on initial live weight (x) were calculated, one for each of the weight groups, e.g. 90-100, 110-120, 130-140, 150-160 and 170-180 mg, on each of days 3, 6, 9, 12 and on the day prior to death (overall mean length of life 15.39 days).

A summary of the data and calculations for different weight groups, on each specified day, is given in Tables A 20-24 respectively. Information from individual regressions together with the "common slope" for each individual group of regressions were used to estimate

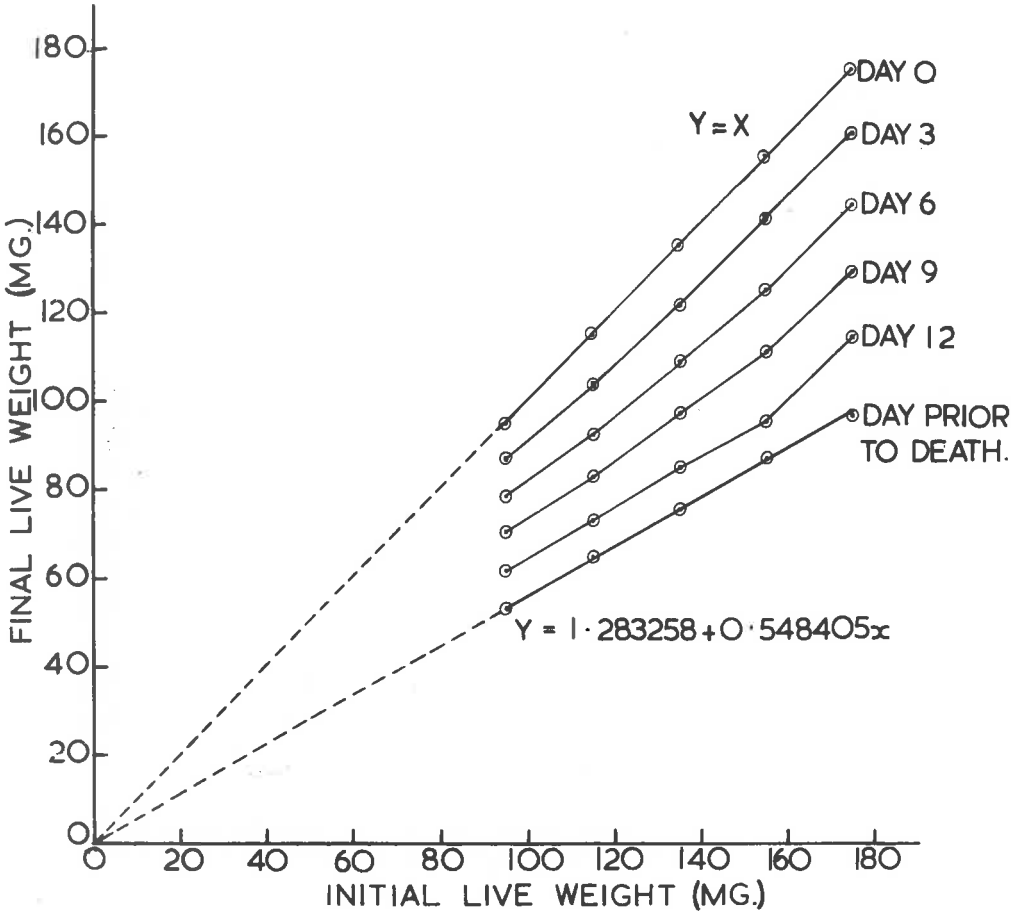


Fig. 11: The change in total weight of T. molitor adults with different initial weights starved to death in 0% R.H. and a temperature of 25°C.

the weight on any particular day, of a beetle with initial weight lying on the mid-point of each weight group. These estimates were used to construct Figure 11.

Slopes and intercepts of the regression for different weight groups are compared for days 3, 6, 9, 12 and on the day prior to death in the analysis of variance tables incorporated in Tables A 20-24 respectively.

Neither the slopes nor the intercepts of the different regression lines describing the relationships between final live weight (on the day prior to death) and initial live weight, for different weight groups (Table A 24) are significantly different from each other, even at the 5% level of probability. I therefore accepted, that these regression lines are identical, and therefore that the "pooled regression" adequately describes the relationship for the experimental populations. The equation for this line is $y = 1.283258 + .949405\ x$.

Testing the hypothesis that this regression line goes through the origin (or similarly, the intercept = a = 0) we have the intercept (constant) viz. 1.283258 and its standard error viz. 1.530829. The quantity 1.283258/1.530829 = C is distributed as t with 122 degrees of freedom. Since C is less than unity we accept the hypothesis that this regression line goes through the origin. The regression line $y = x$ (Figure 11) by definition goes through the origin. The intercepts of these regression lines, describing (a) the live weight at day 0, and (b) the live weight at day prior to death relative to the initial live weight, are similar. I therefore accept that beetles of different initial weights lose the same proportion of their original weight before they die.

Since bigger beetles lost proportionally less of their dry material than smaller ones (Section 8.2) it follows that they must have lost proportionally more water. There is then a discrepancy between these findings and that in Section 8.2 where it was postulated that bigger beetles lose water at a relatively slower rate than smaller ones. On inspection of Figure 11 a relatively sharper drop in weight is indicated after day nine, which is more obvious in the higher weight ranges. This observation seems to fit the picture sketched in the first two paragraphs of this section. Assuming then that the drop in weight is due to eggs being deposited, then this could also explain the discrepancy mentioned above.

Although this is not considered as evidence that bigger beetles put their advantage towards maturing more eggs, it was considered enough evidence to motivate an investigation into this possibility.

9.0 The Ability of Starved Females of T. molitor to Mature Eggs And How This is Influenced by Atmospheric Humidity And The Size of The Insect

The physiological mechanisms that control the maturation of eggs in insects in general were reviewed and discussed by, e.g. Wigglesworth (1950), Bonhag (1958) and Telfer (1965). The neuro-endocrine control of egg production was more specifically described for T. molitor by Mordue (1965). The literature on the influence of ecological factors, such as temperature, food and water on the fecundity and fertility of insects were reviewed and discussed by Andrewartha and Birch (1954).

In this section I wish only to consider the influence of water, measured in terms of the relative humidity of the atmosphere, on the ability of starved T. molitor adults to mature eggs, and then only in so far as this could help me to explain certain phenomena in the water relations of these insects that had been observed in the earlier part of this investigation.

(A) In Section 6.32 two hypothetical conditions were considered under which T. molitor adults might die of desiccation when starved in the lower humidity range. These were : (i) after they have lost a certain amount of water, and (ii) after the water/dry material ratio has reached a certain minimum level. Although the amount of water actually measured in the bodies of the insects, at the time of death, did not satisfy the requirements of either of these hypothetical

conditions, it more closely resembled that of condition (i). It was further suggested that death due to desiccation could still be explained in these terms if it can be shown that some water, an amount increasing with humidity, is bound in some form or another and is thus unavailable to the insects. I thought of water incorporated in the eggs as one of the most likely ways in which water could be present in the body, yet not available to the insect.

(B) Since no relationship could be demonstrated between size of the insect and length of life, it was suggested in Section 8.3 that the advantage of bigger over smaller insects regarding the loss of water, might have been put towards maturing more eggs. In Section 8.4 evidence was brought forward which suggested that bigger beetles might deposit more eggs under adverse conditions of food and atmospheric humidity but it was necessary to verify this inference or at least discover whether bigger beetles are potentially able to produce relatively more eggs than smaller ones, when kept at 25°C and 0% R.H.

Before considering the ability of starved T. molitor females to mature eggs, and how this is influenced by atmospheric humidity and the size of the beetles, a few preliminary factors had to be dealt with. These were :

(i) Females and males had to be separated. A method described by Hein (1920) by which females can be separated from males during the pupal stage, was found useful and convenient and was therefore adopted.

(ii) Having used virgin males and females in the previous experiments which lead me to consider (A) and (B) above, virgin females also had to be used for these experiments. It is known that impregnation might

influence both egg maturation and ovulation (Wigglesworth 1950). The former was more specifically demonstrated for T. molitor by Mordue (1965). The number of eggs matured by virgin females could therefore not be considered as a measure of even the potential fecundity of starved females. Since impregnation might also influence ovulation, the possibility exists that matured eggs might be stored within the reproductive system. In these experiments therefore eggs both within the reproductive system, and those already laid, were considered where appropriate.

(iii) I proposed to measure the ability of virgin T. molitor females to mature eggs, quantitatively, i.e. counting the maximum number of eggs matured under different experimental conditions.

T. molitor females having a telotrophic type of ovariole (Bonhag 1958), all eggs counted during this investigation as being matured can arbitrarily be defined as all eggs detached from their nutritive cords and which are more or less similar in size form and general appearance to those deposited by, or present in the oviducts of virgin females. Eggs were dissected from the reproductive system immediately after termination of an experiment. Matured eggs were sorted and counted with the help of a microscope.

(iv) Since eggs had to be dissected from the reproductive system, the time at which an experiment should be terminated, constituted a problem.

It was shown in Section 7.11 that a decrease in the metabolic rate (measured as the rate of dry material consumption) of starved T. molitor adults occur after day twelve at 25°C. This decrease in metabolic rate occurred after the same period in all humidities. It

was further pointed out in Section 7.11 that eggs were first observed being laid on day twelve. Mordue (1965) found that oocyte resorption in virgin females of T. molitor began 11-12 days after emergence from the pupae. This suggested to me:

(a) That the initial higher metabolic rate demonstrated for starved T. molitor adults (Figure 7) might be associated with development to sexual maturity.

(b) That the maximum number of eggs might be matured around day twelve.

To investigate this idea a group of sixty female pupae, weighing from 125-135 mg. were randomly allotted to five groups. On emergence, the adults weighing from 120-130 mg were individually confined in glass tubes in an atmosphere with a relative humidity of 80% and a temperature of 25°C. Individual groups of beetles were removed at five day intervals starting five days after the beginning of the experiment. The sequence was decided on by chance. Matured eggs were removed by dissection and counted. Daily inspections of all insects within the experiment also revealed the number of eggs deposited by individual insects.

A summary of the data is given in Table 26A. Line three contains the total number of eggs for each treatment with the number deposited during the course of the experiment in brackets. The total number of eggs in column six includes the eggs from five beetles that died, two that died after 24 days and three that were dead on day 25. This data summarised in Table 26A was used to construct the analysis of variance Table 26B.

Table 26A. A Summary of the Data Concerning the Number of Eggs (x) Matured by Virgin T. molitor Females, Starved for Varying Lengths of Time in 80% R.H. at 25°C.

No. of Days Starved	5	10	15	20	25
No. of Beetles	12	12	12	12	12
Σx	47	169	168(9)	144(12)	117(11)
$\bar{x}$	3.9167	14.0833	14.0000	12.0000	9.7500
s^2	8.6288	25.5379	19.6364	52.7273	29.4773

Table 26B. Analysis of Variance of Data From Table 26A

Source of Variation	D.F.	S.S.	M.S.	V.R.
Between classes	4	851.1666	212.7916	7.8228***
Within classes	55	1496.0834	27.2015	
Total	59	2346.2500		

Although the variance ratio associated with between classes sums of squares is significant at the .1% level of probability, only the mean number of eggs matured after five days could be shown to be different from that of the rest of the treatments. The fact that differences between the means of the latter classes could not be shown significant is not surprising in view of the large size of the individual class variances relative to the class means. This indicates the great variability within classes, a fact which could not be accounted for in this series of experiments.

Although not conclusive, the way in which the mean number of eggs change with time, indicated to me

(a) that all the eggs were matured after the emergence of the adults from the pupae.

(b) that the maximum number to be matured was reached 10-15 days after emergence, a time which coincides with the change in the metabolic rate demonstrated in Figure 7.

(c) that some eggs were re-absorbed after day fifteen. Mordue (1965) observed resorption of oocytes in virgin T. molitor females to start 11-12 days after emergence. This discrepancy might be explained by the fact that this experiment was conducted at a lower ambient temperature.

9.1 The Influence of Atmospheric Humidity on the Ability of Virgin T. molitor Females to Mature Eggs When Starved in Different Relative Humidities

It was shown in Section 9.0 that virgin females do mature eggs when starved. It was further indicated that the maximum number to be matured is reached 10-15 days after emergence. This time coincides with the change in the metabolic rate demonstrated in Figure 7, which was the same for all humidities. It seems reasonable therefore to assume that the initial higher metabolic rate is associated with the maturation of eggs, and that humidity will not influence the time when the maximum number is matured.

To determine the influence of atmospheric humidity on the ability of virgin T. molitor females to mature eggs when starved, the following experiment was conducted. A group of 60 female pupae weighing from 125-135 mg were randomly allotted to six groups. These groups, on emergence of the beetles, were transferred to relative humidities,

0, 20, 40, 60, 80 and 100% respectively, in a temperature of 25°C where they were kept for twelve days. At the end of this period all the matured eggs were dissected out and counted for each individual beetle. A summary of the data is given in Table 26C.

Table 26C. A Summary of Data Concerning the Number of Eggs (x) Matured by Virgin T. molitor Females Starved for Twelve Days in Different Relative Humidities at 25°C.

Relative Humidity %	0	20	40	60	80	100
n	10	10	10	10	10	10
$\leq x$	66	76	120	146	174	94
$\bar{x}$	6.6	7.6	12.0	14.6	17.4	9.4
s^2	8.49	10.27	12.67	5.82	26.04	9.82

The data summarised in Table 26C was used to construct the analysis of variance Table 26D.

Table 26D. Analysis of Variance of Data Summarised in Table 26C.

Source of Variation	D.F.	S.S.	M.S.	V.R.
Between classes	5	879.7333	175.9467	14.4394***
Within classes	54	658.0000	12.1852	
Total	59	1537.7333		

The variance ratio associated with between classes sums of squares being significant at the .1% level of probability, I accepted the hypothesis that atmospheric humidity has an influence on the ability of virgin T. molitor females to mature eggs.

The mean number of eggs matured in different humidities are compared in Table 26E, making use of the method of multiple comparisons described in Section 6.21.

Table 26E. Multiple Comparisons of Means From Table 26C.

Humidities %			0	20	100	40	60	80
	Differences		$\bar{x}$ - 6.6	$\bar{x}$ - 7.6	$\bar{x}$ - 9.4	$\bar{x}$ - 12.0	$\bar{x}$ - 14.6	$\bar{x}$ - 17.4
	$\bar{x}$	D						
80	17.4		10.8	9.8	8.0	5.4	2.8	
		D_1	5.9711**	5.6576**	5.3441**	4.8595**	4.0472	
		D_2	7.1539**	6.9116**	6.6124**	6.1278	5.3868	
60	14.6		8.0	7.0	5.2	2.6		
		D_1	5.6576**	5.3441**	4.8595**	4.0472		
		D_2	6.9116**	6.6124**	6.1278	5.3868		
40	12.0		5.4	4.4.	2.8			
		D_1	5.3441**	4.8595	4.0472			
		D_2	6.6124	6.1278	5.3868			
100	9.4		2.8	1.8				
		D_1	4.8595	4.0472				
		D_2	6.1278	5.3868				
20	7.6		1.00					
		D_1	4.0472					
		D_2	5.3868					
0	6.6							

x Indicates differences between two means significant at the 5% level

xx Indicates differences between two means significant at the 1% level.

Referring to the relative positions of different humidities and the presence and absence of asterisks in Table 26E indicates that humidities at both extremes of the range adversely affects the ability of virgin T. molitor females to mature eggs. The mean number of eggs increased with an increase in humidity, and rather sharply between 30-40%, reached a peak between 60-80% R.H., and dropped considerably as the relative humidity reached saturation level.

9.11 Amount of Water Incorporated in Eggs

From Section 9.0 and 9.1 we know that virgin females do mature eggs, when starved and that atmospheric humidity does influence the numbers. It was also indicated in Section 9.0 that although some eggs might be reabsorbed, the insectswill die with some matured eggs still in their bodies. From this I deduced that at least some of the discrepancy between observed and expected amounts of water present in the insects, when they presumably died of desiccation (this was discussed in Section 6.32 and demonstrated in Figure 6) can be explained in terms of water incorporated in eggs.

This leads to the question as to the amount of water that can be incorporated in eggs and thus unavailable to the insects. To get an estimate of this amount, 10 samples each containing 10 newly laid eggs (less than 12 hours old), were weighed on an Electrobalance (accuracy 0.005 mg), then dried to constant weight in a temperature of 80°C and the dry weight measured. The amount of water in the eggs was calculated as the difference between the fresh weight and the dry weight. Table 26F contains a summary of the results.

Table 26F. The Mean Fresh Weight, Dry Weight and Water Content (plus Their Standard Errors) of Newly Laid Eggs of T. molitor (All Figures are in mg).

Live Weight	Dry Weight	Water Content
0.73644 ± .004344	0.29904 ± .002060	0.43740 ± .002698

From Tables 26C and Table 26E it is obvious that an appreciable amount of water can be bound in eggs. Some of this water will not be available to the insect because

(a) Some is lost as eggs are being deposited.

(b) Some is retained in eggs which are not re-absorbed.

Since I had no estimate of the number of eggs left within the insects at the time of death, no estimate was made of the amount of water present in the insect but not available to them.

Although eggs alone were considered as a form in which water can be present, yet not available to the insect, the possibility also exists that since sperm is known to be transferred in an enclosed sac or "spermatophore" in some Coleoptera (Wigglesworth, 1950) that there might yet be another form of bound water in T. molitor adults.

9.2 Egg Maturation And The Size of The Beetle

Both Dick (1937) and Leclercq (1949) demonstrated the adverse affect of low atmospheric humidities on the fecundity of T. molitor females, while Andrewartha (1961) suggested water in the form of carrots or bananas for an increase in egg production. In Section 9.1

it was shown that even the potential fecundity of starved virgin females is adversely affected by low atmospheric humidity.

This suggested to me that both free and atmospheric water will influence the fecundity of T. molitor females and also that beetles which are at an advantage as far as water, in any form, is concerned, might produce more eggs.

It was shown in Section 6.2 that bigger beetles have an advantage over smaller ones as regard water loss. It was also postulated that bigger beetles might put this advantage towards maturing more eggs. To demonstrate this it was necessary to subject female beetles of different sizes to both favourable (a) and unfavourable (b) humidity conditions and then to determine the number of eggs matured by different sized beetles under both these conditions. The hypothesis that bigger beetles put their advantage to maturing relatively more will then be accepted if it can be shown that the ratio : eggs matured under condition (b)/eggs matured under condition (a), is greater for the bigger beetles.

For this purpose 24 newly emerged female beetles in each of the weight ranges 80-90, 100-110, 120-130, and 140-150 mg were selected from cultures of the same age. The 24 beetles in each weight range were randomly divided into two lots of 12 each. One lot was subjected to 80% R.H., the other to 30% R.H. both at a temperature of 25°C. These particular humidities were used, because we know from Section 6.21 at least as far as length of life is concerned that 80% is a favourable, and 30% R.H. an unfavourable humidity.

The insects were left in these conditions for 12 days, at the end of which the matured eggs were dissected out and counted. Table 26C contains a summary of the data where :

Line A = Total number of beetles used per treatment.

Line B = Number of beetles which matured eggs.

Line C = Total number of eggs matured.

Line D = Total number of eggs matured in 30% R.H. expressed as a percentage of those in the corresponding cells in 60% R.H.

Table 26C. A Summary of Data Concerning the Number of Eggs Matured by T. molitor Females of Different Sizes in 60% and 30% R.H. at 25°C

Weight Range (mg)	80-90	100-110	120-130	140-150
60% Relative Humidity				
A	12	12	12	12
B	6	11	12	12
C	33	94	166	211
30% Relative Humidity				
A	12	12	12	12
B	1	6	11	12
C	5	24	66	127
D	15.1515	25.5319	39.7590	60.1596

From line D (Table 26C) it is obvious that bigger beetles do mature proportionally more eggs than smaller ones when subjected to conditions where they are under a water stress.

10.0 Reasons for Discriminating Between the Sexes in Studying The Water Relations of T. molitor

From evidence obtained during a preliminary investigation into the water relations of adult T. molitor (Section 6), it was argued in Section 6.34, that a change in the metabolic rate with time which in itself causes a reduction in the rate of water loss, could explain the sigmoid type of relationship between atmospheric humidity and the length of life of starved T. molitor adults (Figure 2). It was further argued that the relationship between atmospheric humidity and the metabolic rate of starved T. molitor adults, demonstrated in Section 6.24 and Figure 6, could be an artifact brought about by the differences in length of life, associated with relative humidity, and a change in the metabolic rate with time, independent of humidity.

In Figure 7 a change was demonstrated in the rate at which the dry material content of starved T. molitor adults is reduced with time. This reduction in the rate of change which occurred after the same period in all humidities, has associated with it a change in the rate of water loss in the lower humidity range (Figure 8). Assuming that the reduction in the rate of change of dry material content is brought about by a reduced metabolic rate, then this seemed to fit the requirements proposed for explaining the sigmoid type of relationship between atmospheric humidity and the length of life of starved T. molitor adults (Section 6.34).

Information obtained and discussed in Section 7.11, however, suggested an alternative explanation for the observed change in the rate

at which the dry material content of starved T. molitor adults is reduced with time. The alternative explanation considered includes the presence of two hypothetical populations within the experimental population, one of which could either (a) metabolise at a higher rate and/or (b) have a different initial body composition. In both cases an earlier exhaustion of the food reserves in the one population could result in increasing numbers of this particular population being removed from the experimental population. This will produce the same effect as was illustrated in Figure 7.

Up till now various arguments centred round the assumption of an "inbuilt" reduction in the metabolic rate of T. molitor adults, with time. I therefore considered it essential to determine whether the reduction in the rate of change in the dry material content of T. molitor adults illustrated in Figure 7, was a result of a genuine reduction in the metabolic rate with time, or whether it was an artifact brought about by the removal through death, of increasing numbers of certain members of the experimental population, which were different from the rest.

Information obtained and discussed in Section 7.11 indicated the presence of two populations within the experimental population, one of which died sooner than the other. This information is not in accordance with the assumption of an "inbuilt" reduction in the metabolic rate. However the possibility still exists that, although the above might be true, both these populations could still have an "inbuilt" reduction in their metabolic rate with time. In order to determine this it was

necessary to separate the two populations. Early identification of these populations constitutes a problem since length of life was the only known measure of difference.

In connection with the idea of two populations within the experimental population, I considered two possibilities :

(i) Since Leclercq (1963), by selecting for size, succeeded in developing two strains, "fitted to various ecological hazards", that sizes, on the extremes of the range observed for T. molitor adults, might constitute two different populations. This was also considered because it was shown in Section 8.2 that bigger beetles consume their reserved foods at a relatively slower rate than smaller ones. The latter condition is one of the requirements stipulated as a difference between the two hypothetical populations considered (Section 7.11) which could lead to explain the proposed change in metabolic rate with time, illustrated in Figure 7, differently.

In Section 8.3 it was shown that size of the beetles has no influence on the length of life of starved T. molitor adults. Since another of the required differences between the two hypothetical populations was for them to differ in length of life, the idea of different sized beetles constituting the two hypothetical populations was therefore rejected.

(ii) Since males and females fulfil two distinct functions in the life of the species, I also considered the possibility that the two sexes might constitute two distinct populations within the experimental population. To verify this I set out by testing the null hypothesis

that there is no difference in the initial body composition of males and females.

To test this hypothesis a number of pupae were selected from cultures of the same age. The pupae were sexed, using the method described by Hein (1928). On emergence of the beetles, 48 of each sex in the weight range 100-120 mg were selected and dried to constant weight. To compare the initial dry weight of the two sexes, but taking into account the range in initial weight, regressions of initial dry weight (y) on initial live weight (x) were calculated, one for each sex. A summary of the data and calculations is given in Table A25, while the slopes and intercepts of the two regressions are compared in the analysis of variance Table 27A.

TABLE 27A. Analysis of Variance of Data Summarised in Table A25

Source of Variance	D.F.	S.S.	M.S.	V.R.
Slopes	1	9.7712	9.7712	1.312 n. sig.
Displacements	1	204.7395	204.7395	27.501***
Error	92	684.2962	7.4445	

The variance ratio associated with slopes being non-significant at the 5% level, and that associated with displacements being significant at the .01% level, I accepted that the regression lines describing the relationship between initial dry weight (y) and initial live weight (x) for female and male T. molitor adults in the weight range 100-120 mg, are parallel but displaced. I therefore rejected the null hypothesis, and accepted that the initial body composition of male and female T. molitor adults is different.

The equations (obtained from Table A25) for estimating the initial dry weight of the two sexes in this particular experimental population are :

Female = $y = 42.2437 + .405199\ (x - 110.509)$

Males = $y = 39.1966 + .259725\ (x - 110.153)$

These equations indicate that for a given initial weight, females have a higher initial dry material content and therefore a lower initial water content.

In addition to the differences in initial body composition indicated here, it was also found that females stay alive for a longer period of time than males, when starved at the same temperature and relative humidity. (This will be discussed in more detail in Section 10.2).

This then suggested that the two sexes might constitute the two populations within the experimental population which was indicated in Section 7.11.

The presence of two populations within the experimental population introduces a new complexity in the water relation studies of T. molitor. The questions immediately arise as to the consequence of these differences, and to whether or not sexes are different in aspects other than the observed difference in their initial body composition. Based on the assumption that all differences between sexes are only quantitative and not qualitative, interpretations of results previously made should not be jeopardised by the presence of these two populations within the experimental population.

The differences between males and females T. molitor in their initial body composition is extremely relevant to this particular study, because I originally selected to measure the dry material and water content of beetles and how these change with respect to humidity, temperature and time. The ability to separate the sexes should lead to more homogeneous experimental material and consequently to more sensitive tests and accurate estimates of population parameters. That it is essential to consider sexes separately in future experimental work, is obvious in view of the fact that it was these differences between males and females which primarily lead me to consider an alternative explanation for a phenomenon believed to be an "inbuilt" reduction in metabolic rate, Section 7.11.

In the sections to follow I shall compare the responses of males and females to the same treatments to verify the assumption made regarding the differences between them.

10.1 Differences in The Initial Body Composition of Adult Males And Female T. molitor

It was shown in Section 6.1 that initial dry weight of T. molitor adults (mixed sexes) has a linear relationship with initial live weight over the entire weight range observed for this species. Since it was shown in Section 10.0 that males differ from females in their initial dry material content, the question arises as to whether this particular type of relationship will hold when the different sexes are separated and also whether the difference in the dry material content of males and females is constant over the entire weight range.

To get answers to these questions the following experiment was conducted. Newly emerged T. molitor adults were selected from cultures of the same age : twenty-four males and 24 females in each of the weight groups, 70-79, 80-89, 90-99, 100-109, 110-119, 120-129, 130-139, 140-149 and 150-159 mg. These beetles were individually weighed and then dried to constant weight in a temperature of 100°C to determine the initial dry weight. Regressions of initial dry weight (y) on initial live weight (x) were calculated for each weight group and separately for males and females. A summary of the data and calculations is given in Table A26 for the females and in Table A27 for the males.

The hypothesis that initial dry weight has a linear relationship with initial live weight over the whole weight range for both males and females may be accepted, if, for both males and females, the regression lines are similar.

The slopes and intercepts of the different regression lines for females, are compared in the analysis of variance Table 28A, while the slopes and intercepts of the different regression lines for males are compared in Table 28B.

TABLE 28A. Analysis of Variance of Data From Table A26 (Females)

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	8	57.2317	7.1165	1.025 n. sig.
Displacements	8	85.3007	10.6376	1.521 n. sig.
Error	198	1385.6698	6.9983	

TABLE 28B. Analysis of Variance of Data From Table A27 (Males)

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	6	24.0969	3.1124	0.457 n. sig.
Displacements	6	26.5420	3.3178	0.465 n. sig.
Error	198	1409.7151	7.1199	

The analyses of variance show clearly no significant differences between either the slopes or intercepts of the individual regression lines for either females or males. The hypothesis may therefore be accepted that initial dry weight is a linear function of initial live weight in the case of both males and females.

Identical regression lines for different weight groups permit all the observations to be pooled to calculate a single regression of dry weight (y) on initial live weight (x). This was done for both females and males in Table A28. Providing that these two new regression lines are parallel but displaced the hypotheses may be accepted that males and females differ in their initial dry material content and therefore in their initial water content and also that these differences are constant over the observed weight range. To test these hypotheses the slopes and intercepts of the regression lines calculated in Table A28 were compared in the analysis of variance Table 28C.

TABLE 28C. Analysis of Variance of Data From Table A29 (Females vs. Males)

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	1	78.6720	78.6720	11.271***
Displacements	1	947.0340	947.0340	135.673***
Error	428	2987.3190	6.9797	

The variance ratios associated with sums of squares of both slopes and intercepts are significant at the .01% level. I therefore accepted that the way in which the initial dry material content and therefore also the initial water content change with a change in initial live weight is different in males and females. Because the slopes are significantly different the significant difference for intercepts has little bearing on the problem that is being investigated. However from inspection of the two lines in Figure 12 it is obvious that males have consistently less dry material than females for any initial live weight.

In Table 28C it was shown that the slopes of the regressions of initial dry weight on initial live weight for females are different from that of the males. This is different to what was shown in Table 27A. This discrepancy might be explained by the fact that in the latter case only a relatively small section of the observed weight range was considered.

The differences in the relationship between initial dry material content (y) and initial live weight (x) of females and males are illustrated in Figure 12. The regression of initial dry on initial live weight (Table A29) in the case of the females is given by the equation $y = 2.36383 + .36025\ x$ and in the case of the males $y = 3.28120 + .326536\ x$.

Observed points on the graphs are means obtained from the original regressions, of initial dry on initial live weight for different weight groups of females and males (Tables A27 and A28 respectively) and adjusted for differences in initial weight, making use of the common

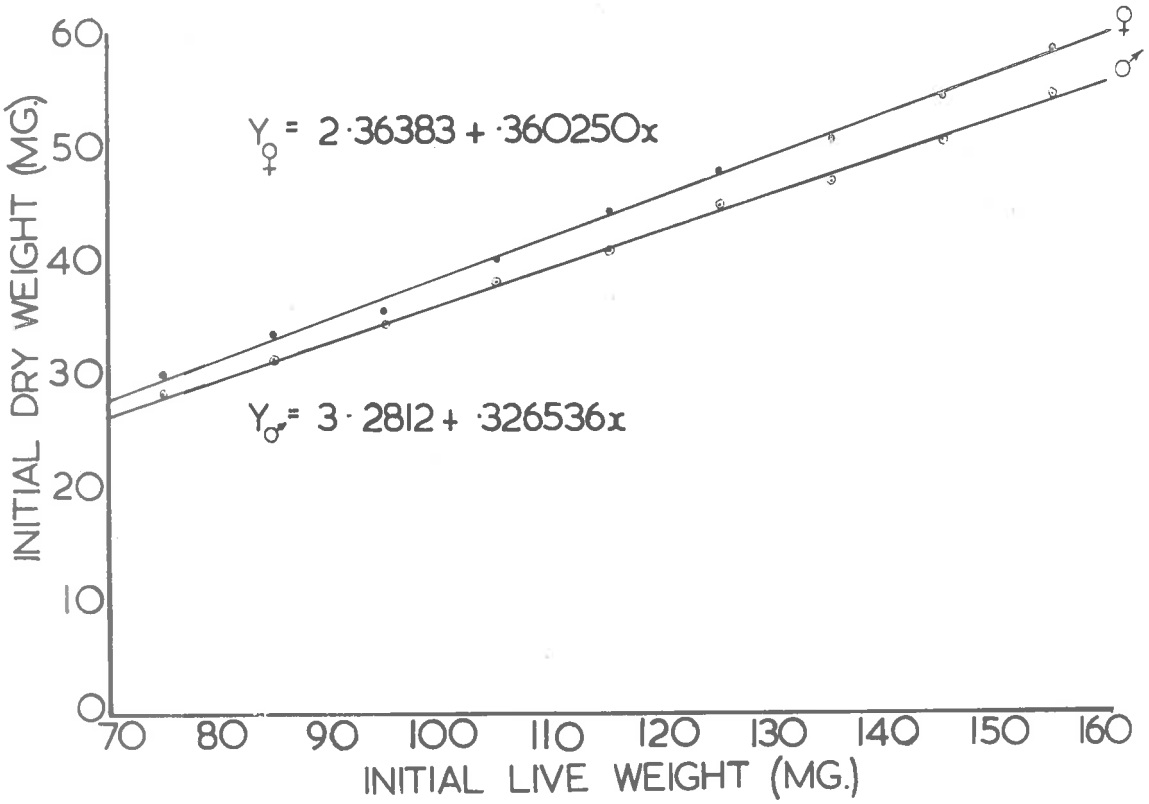


Fig. 12: The difference in the relationship of initial dry weight (Y) and initial live weight (X) in females and males of T. molitor.

slopes from the appropriate parallel regressions.

If we consider Table 28C together with Figure 12 the following inferences can be made :

(i) *S. politor* females differ from males in their initial dry material content and this difference is apparent over the observed weight range.

(ii) For a given initial weight the dry material content of females are higher than that of the males, and it follows that the initial water content of females are lower than that of males for any given initial weight.

(iii) The increase in initial dry weight for unit increase in live weight is slower in males as compared to females.

Referring back to the regressions of initial dry weight (y) on initial live weight (x) (Table A28) if we then consider the values of S.D. (Standard deviation of points from fitted lines) for females and males and together with S.D. for pooled regression viz. 2.670651, 2.612884 3.054353 respectively, it is obvious that variability in initial dry weight can be reduced by separating sexes. The differences between these standard deviations (when sexes are considered separately or together) suggest that a considerable amount of overlapping of y values for a given value of x occur between sexes and further that a considerable amount of variability still exists, even with sexes separated.

Assuming that the differences in dry material content of males and females are due to a difference in usable reserved foods, then providing all other factors remain the same, males will die of starvation before

females. These differences could then at least partly explain the variability in length of life of T. molitor adults (mixed sexes) demonstrated in the upper humidity range (Figure 2.) In the lower humidity range where the beetles die of desiccation, the variability might be caused by the differences in the water content of males and females or perhaps some other difference associated with males and females. This will be discussed in Section 10.4.

Should males with the lower initial dry material content die before the females then the change in the rate of change of the dry material content demonstrated for T. molitor (mixed sexes) (Figure 7) could be an artifact, brought about by the removal, through death, of increasing numbers of males from the experimental population. However the possibility also exists that both males and females have an "inbuilt" reduction in their metabolic rate with time. This possibility is discussed in Section 10.3.

10.2 The Influence of Humidity And Temperature on The Length of Life of Starved T. molitor Males And Females

During a preliminary investigation into the water relations of starved T. molitor adults it was demonstrated in Section 6.32 and Figure 2 that, at a temperature of 25°C, the mean length of life of these insects (mixed sexes) has a sigmoid type of relationship with atmospheric humidity. At that stage I was confronted with the problem of explaining (a) this particular type of relationship and also (b) the variability in length of life of these insects at any particular humidity.

The explanation for the sigmoid type of relationship between length of life of starved *T. molitor* adults and atmospheric humidity may be explained : if one assumes that

(a) The beetles die of desiccation in humidities below 60% and after their water content is reduced to a certain minimum.

(b) The metabolic rate of starved *T. molitor* adults decreases with time and this in itself causes a decrease in the rate of water loss.

(c) The beetles die of starvation in the humidities 60-95%.

From arguments put forward and evidence produced in Section 6.22, Section 6.25, Section 6.32 and Section 9.12 it seems reasonable to accept that starved *T. molitor* adults die of desiccation in the lower humidity range (below 60%) and of starvation in the higher humidity range (60-95%). I have further reason to believe that beetles die prematurely (without using all available reserves) when kept at humidities greater than 95% (observations not reported, which need verification). This leaves us with a possible third cause of death, i.e. waterlogging of the tissues.

It was postulated in Section 7.4, that the three conditions, desiccation, starvation and water-logging of the tissues could be brought about by the inability of these insects to maintain a required rate of water loss outside the humidity range (60-90%). In the humidity range 60-90% the insects, by their own means, are able to maintain a rate of water loss which will allow them to use all their available reserves according to an "inbuilt" pattern (Section 10.3) and hence the flat part or upper asymtote of the sigmoid curve illustrated in Figure 2. In the lower humidity range a critical amount of water is lost before all

available reserved foods are metabolised. The time at which this critical low level is reached depends firstly on the humidity and secondly on the benefit derived from a reduced rate of water loss (Figure 8) associated with an "inbuilt" reduction in the metabolic rate. The combined effect of humidity and the reduction in metabolic rate with time can explain the lower part of the sigmoid curve illustrated in Figure 2. With the means at their disposal the insects are unable to reduce the water loss to the required rate when subjected to an atmosphere with a relative humidity near saturation level. This results in the amount of water relative to dry material reaching a critical high level and the insects die prematurely of "water-logging" of the tissues. Under these circumstances I would expect the curve illustrated in Figure 2 to change its shape beyond 95% R.H. (See also Figure 16).

It was suggested in Section 6.31 that the variability in the length of life of starved T. molitor adults (illustrated in Figure 2) might be a result of the variability in the initial body composition of the beetles. In Section 10.1 it was shown that a substantial part of the variability in the initial body composition can be explained by differences between males and females. Assuming that differences in initial dry weight of males and females constitute differences in reserved food materials, then providing (a) sexes metabolise at the same rate (b) the metabolic rate changes similarly with time in both sexes this could result in females living longer than males. Possible differences in the length of life of males and females brought about in this way could account for some of the variability in length of life illustrated for mixed sexes.

Figure 2 but then only in that part of the humidity range where the insects die of starvation.

Possible differences in the length of life of males and females in those parts of the humidity range where the insects die of desiccation or "water-logging" of the tissues might be explained by (i) differences in the initial water content of males and females and/or (ii) differences in the ability of the sexes to retain water in the lower humidity range or rid themselves of excess water when the humidity reaches saturation level and/or (iii) differences in the metabolic rate of sexes and/or differences in the way in which the metabolic rate changes with time.

Not only do I want to know whether the cause of death (i.e. desiccation at low humidities, starvation at moderate humidities and "water-logging" at high humidities) remains the same at any humidity for both sexes, but I also want to know whether for either or both sexes the cause of death remains the same at any humidity independent of temperature.

With an increase in temperature I would expect an increase in the metabolic rate and therefore an increase in the airflow through the spiracles of the insects. If the relative humidity of the air expired by the insects remains constant at different temperatures, an increase in temperature could cause more water to be lost for two different reasons (a) Hotter inspired air will require a greater amount of water to raise its relative humidity to any particular level, (b) An increase in metabolic rate will increase the airflow through the spiracles or the amount of air the humidity of which needs to be raised to this certain level. Should the relative humidity of the expired air change with temperature

in a compensatory way, then the water loss will only increase for reason (b).

In Section 7.4, in a discussion on whether the ratio of dry material to water is important or not, it was argued that a starved T. molitor beetle, in order to stay alive until all its reserves of dry material have been used, requires to reduce its water content to below the level considered normal for newly emerged beetles, and at a certain "required rate", the latter which is basically determined by the rate of metabolism. By their own means starved beetles can regulate their water loss and therefore maintain this "required rate", only in the humidity range 60-90%. Beyond 90% R.H. in 25°C these insects cannot reduce their water content fast enough by excretion of water with the faeces to keep up with the continuously decreasing dry material content (the latter phenomena which is independent of atmospheric water Section 10.3); below 60% R.H. at 25°C the initial water content is reduced by water loss through evaporation, at a rate higher than the "required rate", despite the fact that faeces are dried to capacity, thus the beetles die before they have used all their reserves of dry material.

At other temperatures for the reasons given above, the rate of loss of water at any humidity, may differ from the rate at 25°C. So will the rate of consumption of dry matter and therefore also the "required rate" at which the water content should be reduced. If these changes in rates move in a compensatory way with temperature then the beetles should die at the same physiological age (i.e. from the same causes and in the same condition) at all temperatures in the normal range. That this

could be the case in T. molitor adults was indicated in the work of Pielou (1949) who suggested that these insects react to relative humidity rather than to saturation deficiency.

The change in length of life of starved T. molitor adults with a change in temperature at the same levels of relative humidity, should give a fair indication of whether the response of these insects to various levels of atmospheric humidity compensates for the response to temperatures as described above.

To investigate the influence of temperature and humidity on the length of life of T. molitor males and females the following experiment was conducted :

Two groups (males and females) of newly emerged T. molitor adults were selected from cultures of the same age. From these groups, 288 each of males and females were randomly selected and divided into twenty-four lots of twelve each. One lot each of males and females were randomly allotted to each of the relative humidities 0, 20, 40, 60, 80 and 100% in each of the temperatures 20°, 25°, 30° and 35°C, giving us a 2 x 4 x 6 factorial lay-out. Beetles were inspected daily and the length of life (days) recorded for individual beetles in the various combinations of temperature and humidity.

A summary of the data is given in Table A29 while the mean length of life for different sexes, plotted against humidity for different levels of temperature is illustrated in Figure 13 and the mean length of life for the different sexes plotted against temperature for the different levels of humidity is illustrated in Figure 14.

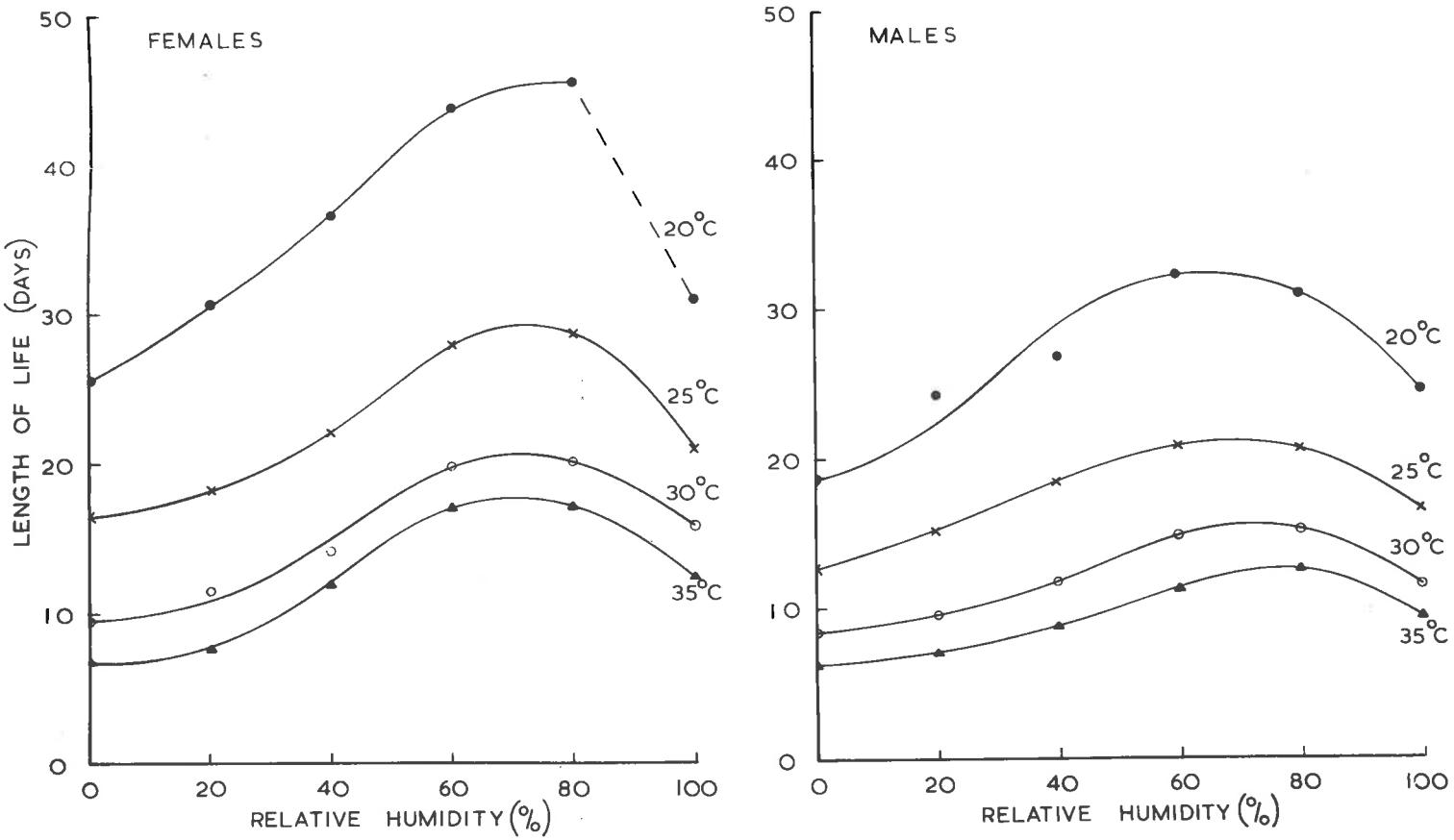


Fig. 13: The influence of atmospheric humidity on the length of life (days) of starved *T. molitor* females and males at different temperatures.

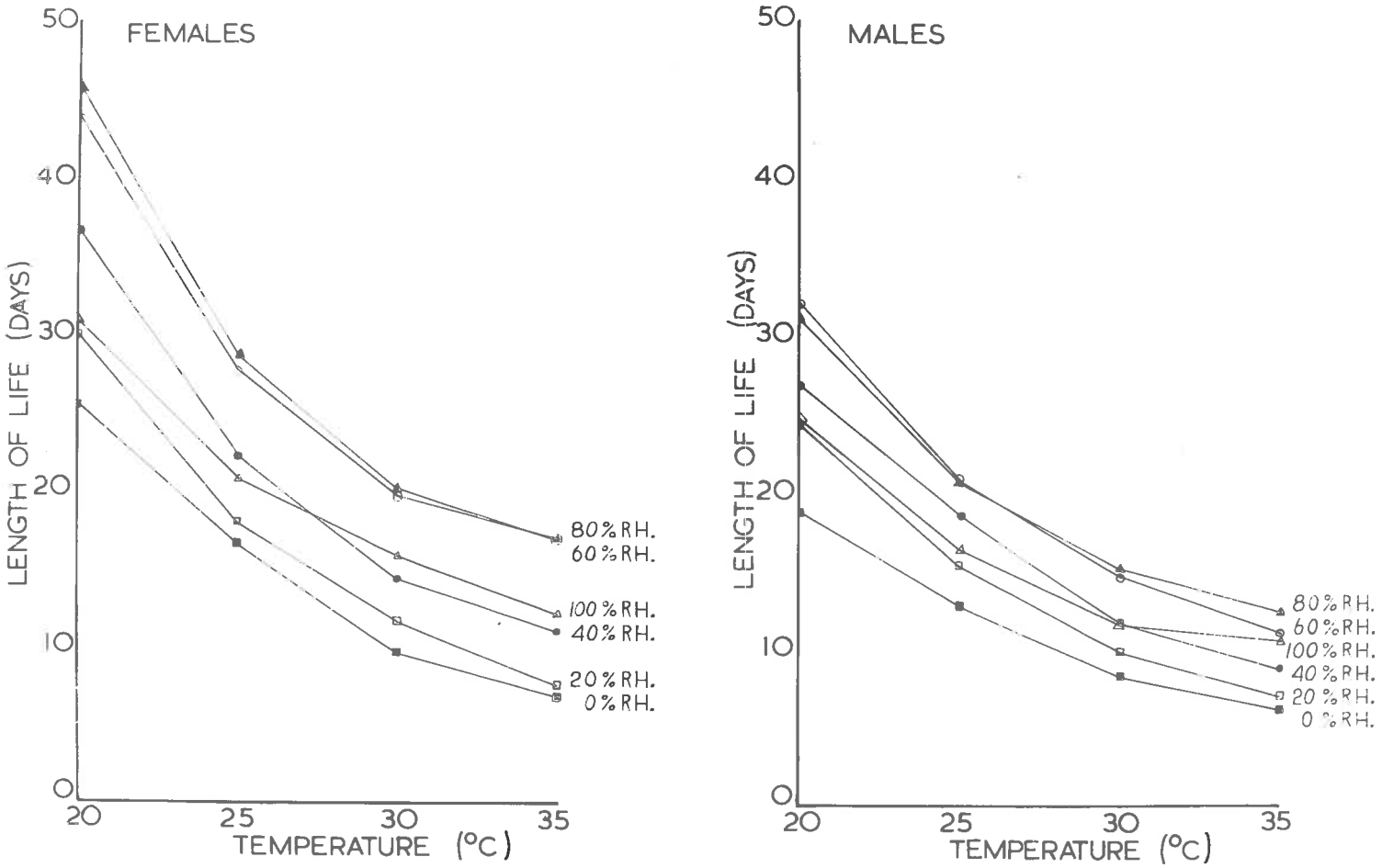


Fig. 14: The influence of temperature on the length of life (days) of starved T. molitor females and males at different humidities.

To investigate how the life span of starved T. molitor adults changes with a change in humidity and temperature and to test for possible differences in the length of life of males and females; and then if there are differences to discover if and how these differences change with respect to humidity and temperature I started off by performing a three-way classification analysis of variance on the data summarised in Table A29. This led to the construction of the analysis of variance Table 29A.

TABLE 29A. Analysis of Variance of Data Summarised in Table A29.

Source of Variation	D.F.	S.S.	M.S.	V.R.
Between Temperatures (T)	3	34,304.5529	11,434.8843	1136.4046***
Between Humidities (H)	5	8,652.7378	1,730.5476	171.9833***
Between Sexes (S)	1	3,665.2934	3,665.2934	364.2600***
Interactions				
T x H	15	935.7275	62.3818	6.1996***
T x S	3	892.3366	297.4462	29.5605***
H x S	5	564.6543	112.9309	11.2232***
T x H x S	15	137.2652	9.1509	.9094 n. sig.
Within classes	528	5,312.9167		
Total	573	54,465.4566		

Examination of Table 29A reveals highly significant variance ratios associated with the sums of squares of the interactions between any two of the main factor effects investigated i.e. temperature, humidity and sex. In the presence of an interaction it is not permissible to test the main factor effects related to the particular interaction against the

residual mean square (Moroney 1951). However the size of the variance ratios associated with main factors leaves little doubt that all of them do affect the length of life of starved T. molitor adults. A significant interaction between, e.g. temperature and humidity effects, indicates that these main factor effects are not independent in their effect (Moroney 1951) on length of life of starved T. molitor adults and suggests that no useful overall statement can be made about either main effect.

To describe the meaning of a significant interaction between two main effects (e.g. temperature x humidity) on the mean length of life of starved T. molitor, I prefer to think of it in the following way, i.e. that the mean length of life of starved T. molitor adults changes with a change in humidity, but that the amount by which it changes for a given change in humidity, is different at different levels of temperature. Similarly, a significant interaction between sex and temperature will mean that sexes differ in their mean length of life but that these differences are different at different levels of temperature, while a significant interaction between sex and humidity will mean that sexes differ in their mean length of life but that these differences are different at different levels of humidity.

Apart from the fact that the analysis of variance Table 29A demonstrates the presence of interaction amongst the main treatments, i.e. that any treatment causes a change in length of life which in every instance depends on the level of the other treatments, the information it contains is not very instructive. The presence of interaction amongst main effects makes it difficult and perhaps confusing to talk or even to

think in terms of changes in length of life or differences in mean length of life.

Since it was necessary to make more definite statements regarding the trends of length of life on humidity and temperature as also regarding the differences between sexes, I decided to pursue this investigation further, adopting a method similar to the "break-down analysis" method suggested by Mourney (1951), i.e. considering the influence of one main factor on the length of life of starved T. molitor adults, and separately at all levels of the second main factor, including all levels of the third main factor.

I was basically interested to know :

(A) (i) How the length of life changed with humidity, and whether, for each sex the trend was the same at all temperatures.

(ii) How the length of life changed with temperature and whether, for each sex, the trend was the same at all humidities.

(iii) How the responses of males differed from the responses of females.

(B) (i) To what extent the length of life of males differ from that of females.

(ii) If this difference in length of life is consistent at all levels of humidity and temperature.

For clarity these two main points are investigated in two separate sub-sections, 10.21 and 10.22 respectively.

10.21 Trends of Length of Life of Starved T. molitor Males and Females on Temperature and Humidity

On inspection of Figures 13 and 14 the following becomes apparent:

(a) As can be expected, the length of life of starved T. molitor adults decreases with an increase in temperature, in other words there is a regression of length of life on temperature. The striking feature of this regression is that it seems to be quadratic in form and that this basic pattern is maintained at all levels of humidity in both sexes (to be tested). This suggests that this particular type of relationship describes the basic way in which temperature affects length of life, and that this basic pattern does not change with either humidity or sex, in other words no qualitative changes occur in the response to temperature at any level of humidity in either sex.

(b) As was the case with temperature effects, a similarity in the trend of length of life on humidity is apparent in both sexes at all levels of temperature considered (to be tested), which then also suggests that no qualitative changes occur in the response to humidity at any level of temperature in either sex.

The general shape of the response curves of length of life on humidity (Figure 13) differs to some extent from the response curve illustrated in Figure 2. The difference lies in a sharp drop in the length of life beyond 80% R.H. in the former, which is apparent at all levels of temperature in both sexes. In Figure 2 the 96% R.H. level was included, but the 100% R.H. was omitted. This suggests that the drop in length of life must occur between 95-100% R.H., and also indicates as

was suggested in Section 10.2, that these beetles might die prematurely of "water-logging" of the tissues as the humidity reaches saturation level. This particular point is being investigated further.

(A) To examine the trends of length of life on temperature and on humidity, and to discover whether the trends are similar at different levels of humidity and different levels of temperature respectively, then since the various levels of each variate were equally spaced, the method of orthogonal comparisons in regression was adopted (after Snedecor 1956 p. 348).

For this particular part of the problem an a x b Table (29B) (Temperature x Humidity) was constructed from Table A29, containing total length of life (days) summed over all original observations of both sexes in various combinations of temperature and humidity.

TABLE 29B Total length of Life (days) of 24 Starved T. molitor Beetles (12 males + 12 females) In Different Combinations of Temperature and Humidity (Constructed From Table A29)

	Relative Humidity						
Temperature	0%	21%	43%	60%	80%	100%	Total
20°C	634	653	760	912	918	666	4443
25°C	392	432	487	561	589	448	2859
30°C	234	256	313	414	423	328	1948
35°C	159	176	251	359	354	276	1575
Total	1419	1467	1811	2246	2284	1718	10805

To examine the trends of length of life on temperature the appropriate number of orthogonal temperature comparisons were calculated at each level of humidity. This led to the construction of Table 29C.

TABLE 29C Orthogonal Comparisons in Regression of Length of Life on Temperature at Different Humidity Levels

	Relative Humidity						
Comparisons	0%	20%	40%	60%	80%	100%	Total
Linear (L)	-1263	-1577	-1707	-1838	-1850	-1290	-9575
Quadratic (Q)	+127	+171	+211	+256	+260	+166	+1191
Cubic (C)	+39	-39	+13	-72	-66	-30	-155

The comparisons in Table 29C were converted to sums of squares figured in Table 29D, where at the same time the sums of squares contributed by each temperature regression component at each humidity level (with 1 degree of freedom) was tested for significance against the appropriate within classes mean squares obtained from Table A29.

TABLE 29D Sums of Squares For Each Temperature Regression Component At Each Level of Humidity

	Relative Humidity						
Comparisons	0%	20%	40%	60%	80%	100%	Total
Linear	5525.3556	5181.1021	6067.9158	7410.4083	7192.5063	3464.5750	32801.5813
Quadratic	166.0104	304.5938	463.7604	682.6667	704.1667	287.0417	2610.2397
Cubic	3.1668 ns	3.1693ns	.3521ns	10.8000ns	9.0750ns	1.8750ns	28.4397

From Table 29D it is obvious that a quadratic regression adequately describes the relationship between the length of life of starved T. molitor adults and temperature at all levels of humidity. The regressions describing this relationship at different levels of humidity are similar in that (a) Both the linear and quadratic regression components are significant (Table 29D). (b) All the coefficients for linear regressions are negative, while all the coefficients for quadratic regression are positive (Table 29C). Therefore, since the significant interaction between humidity and temperature (Table 29A) indicates that the curves describing the relationship between length of life and temperature at different levels of humidity are not identical, it seems reasonable to accept that the differences are only in detail, i.e. that no qualitative changes occur in the relationship of length of life on temperature with a change in humidity.

(B) To examine the trends of length of life on humidity at different levels of temperature the same procedure was followed as in (A) above. From Table 29B, by using the appropriate coefficient and divisors for sets of orthogonal comparisons, Tables 30A and 30B were constructed, which are the equivalents of Tables 29C and 29D respectively.

TABLE 30A Orthogonal Comparisons in Regression of Length of Life on Humidity at Different Levels of Temperature

	Temperature				
Comparisons	20°C	25°C	30°C	35°C	Total
Linear (L)	+1807	+1135	+1172	+1307	+5121
Quadratic (Q)	-2259	-1265	-877	-715	-5114
Cubic (C)	-1503	-1206	-1033	-1015	-5024
Quartic (Q_1)	-169	-37	-41	+25	-222
Quintic (Q_2)	+327	+101	+239	+107	+824

TABLE 30B Sums of Squares For Each Humidity Regression Component At Each Level of Humidity

	Temperature				
Comparisons	20°C	25°C	30°C	35°C	Total
Linear	1537.1720***	766.9106***	617.6095***	867.6095***	3898.7541
Quadratic	2531.2902***	791.2545***	381.5124***	253.5636***	3957.6409
Cubic	752.5021***	326.1169***	232.6725***	257.5591***	1559.0506
Quartic Q_1	42.5015n.sig	2.6572n.sig	2.5015n.sig	.9301n.sig	47.9703
Quintic Q_2	17.6901n.sig	1.6567n.sig	13.9037 ns	1.8230 ns	35.0695

From Table 30B we learn that a third degree or cubic regression adequately describes the relationship between length of life and humidity at all levels of temperature. The regressions describing these relationships at the different temperature levels are similar in that (a) All linear, quadratic and cubic regression components are significant (Table 30B) (b) All the coefficients for linear regression are positive, while

all the coefficients for quadratic and cubic regression are negative (Table 30A). There, and since the significant interaction between humidity and temperature (Table 29A) indicates that the curves describing these relationships are not identical, it seems reasonable to accept that these differences are only in detail; i.e. that no qualitative change occurs in the relationship between length of life and humidity with a change in temperature, within the limits of this experiment.

(C) (i) To examine the trends of length of life of male and female T. molitor adults on temperature an a x b Table (31A) (Sex x Temperature) was constructed from Table A29, containing total length of life (days) for each sex, summed over all individual observations at all levels of humidity, at each level of temperature.

TABLE 31A. Total Length of Life of Starved Male (72) and Female (72) T. molitor adults at Different Levels of Temperature

	Temperature				
Sex	20°C	25°C	30°C	35°C	Total
Females	2280	1606	1094	677	6129
Males	1905	1251	834	676	4676

From Table 31A the appropriate number of orthogonal temperature comparisons were calculated for each sex (Table 31B). These were converted into sums of squares (Table 31C) and tested against the appropriate within classes mean squares, obtained from Table A29.

TABLE 31B Orthogonal Comparison in Regression of Length of Life on Temperature for Different Sexes

	Comparisons		
Sex	Linear	Quadratic	Cubic
Females	-.5555	+.725	-.151
Males	-.4042	+.466	-.24
Total	-.9575	+1.191	-.155

TABLE 31C Sums of Squares For Each Temperature Regression Component At Each Level of Sex

	Regression Components			
Sex	Linear	Quadratic	Cubic	Total
Females	21,259.7840**	1025.8068**	11.3174 n.sig.	
Males	11,345.6624**	754.0153**	0.4000 n. sig	
Total				33,196.8725

The regressions describing the relationship between length of life and temperature for the two sexes of T. molitor are similar in that :

(a) Both the linear and quadratic components are significant (Table 30C).

(b) The coefficients for linear regressions are negative, while the coefficients for quadratic regressions are positive.

Thus in both sexes, the change of length of life with increase in temperature follows a similar pattern. Length of life decreases with a decelerated rapidity as temperature increases (within the limits of this data). It seems reasonable therefore to accept that there are no basic or qualitative differences in the response of the two sexes to temperature.

I then considered the sums of squares associated with the interaction between temperature and sex (calculated from Table 31B), these are :

(a) $T_L S$ = 772.9081***

(b) $T_Q S$ = 116.4601***

(c) $T_C S$ = 3.9754 n. sig.

This indicates that the curves describing the relationship between length of life and temperature, for the two sexes, are not identical and that differences (quantitative) occur both in the linear and quadratic regression components.

(ii) To examine the trends of length of life on humidity and separately for males and females, the same procedure was followed as in (i) above. Three Tables 32A-C were constructed which are the equivalents of Tables 31A-C respectively.

TABLE 32A Total Length of Life of Starved Female (48) and Male (48) T. molitor Adults at Different Levels of Humidity

	Relative Humidity						
Sex	0%	20%	40%	60%	80%	100%	Total
Females	705	809	1021	1301	1332	961	6129
Males	554	673	790	945	952	757	4676

TABLE 32B Orthogonal Comparisons in Regression of Length of Life On Humidity For Different Sexes

	Comparisons				
Sex	Linear	Quadratic	Cubic	Quartic	Quintic
Females	+3129	-3099	-3501	-113	+441
Males	+1592	-2015	-1523	-109	+383
Total	+4721	-5114	-5024	-222	+824

TABLE 32C Sums of Squares For Each Humidity Regression Component At Each Level of Sex

	Regression Components					
Sex	Linear	Quadratic	Cubic	Quartic	Quintic	Total
Females	2813.[illegible]	2501.[illegible]	1418.[illegible]	9.5007ms	16.0781ms	
Males	1149.[illegible]	1007.[illegible]	289.[illegible]	0.3400ms	12.1271ms	
Total						9217.3825

The regressions describing the relationships of length of life on humidity for males and females of *T. molitor* are similar in that :

(a) In both cases the linear, quadratic and cubic components are significant.

(b) The coefficients for linear regression are positive, while the coefficients for quadratic and cubic regressions are negative in both cases.

Thus, as in the case of temperature, it seems reasonable to accept that there are no basic or qualitative differences in the response of the two sexes to humidity.

I also considered the sums of squares associated with the interaction between the humidity and sex (Calculated from Table 32B), these are :

(a) $H_L S$ = 132.3764***

(b) $H_Q S$ = 145.7152***

(c) $H_C S$ = 226.4169***

(d) $H_{Q_1} S$ = .0059 n. sig

(e) $H_{Q_2} 3$ = .1391 n. sig

This indicates that the curves describing the relationship between length of life and humidity, for the two sexes of T. molitor which are both cubic in form, are not identical. Differences (quantitative) occur in the linear, quadratic and cubic regression components.

By way of summary, Figure 15 was constructed from Tables 31A and 32A, illustrating the general change in length of life in response to temperature and humidity both for males and females of T. molitor and for the sexes considered together (Length of life taken as the mean of all observations at all levels of humidity and all levels of temperature, respectively).

To summarise the findings : it is learned that the regression of length of life on temperature is parabolic in form; length of life decreases with decelerated rapidity as temperature increases. The relationship between length of life and temperature retain this basic pattern at all levels of humidity in both sexes, suggesting that no qualitative changes occur in the response of starved T. molitor adults to temperature at any level of humidity (within the levels of this data).

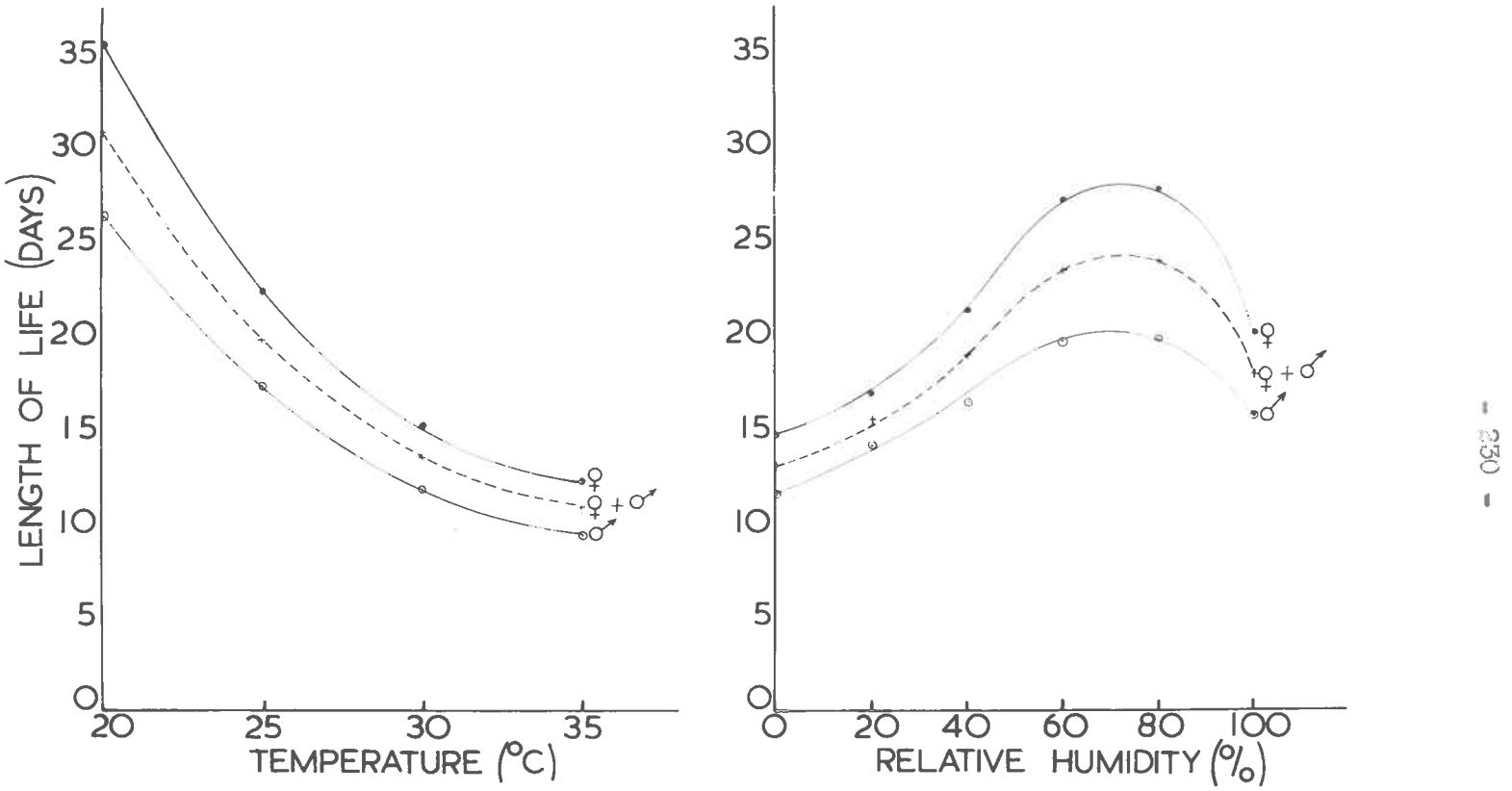


Fig. 15: General change in length of life of T. molitor adults in response to changes in temperature and atmospheric humidity.

The regression of length of life on humidity is cubic in form; length of life increases with accelerated rapidity as humidity increases, but only up to a point, after which the rate of increase slows down and eventually becomes negative. The relationship between length of life and humidity retains this basic pattern at all levels of temperature in both sexes. This suggests that no qualitative changes occur in the response of starved T. molitor adults to humidity at any level of temperature (within the limits of this data).

In Section 10.2 it was argued that starved T. molitor adults die of desiccation in the lower humidity range and of starvation in moderate high humidities. Because of a sharp drop in length of life after the peak is reached at 80% R.H. (Figures 13 and 15) it seems possible that there could be a third cause of death as the humidity reaches saturation level, (presumably water-logging of the tissues). Whatever the causes of death the similarity in the basic shape of the curves illustrating the relationship between length of life of starved T. molitor adults and humidity at different levels of temperature, suggests that these beetles die of the same causes at the different levels of humidity in all the temperatures considered.

The regression of length of life on temperature being quadratic in form in that part of the humidity range where the insects die of starvation, suggests that this is the basic way in which temperature influences the metabolic rate of starved T. molitor adults. That the shape of the curves illustrating this relationship stays basically the same at all levels of humidity, plus the fact that the coefficients

for quadratic regression are positive, suggests that the length of life of starved T. molitor adults is largely determined by their metabolic rate which depends on temperature but which is independent of humidity.

The differences in the elevation of the curves describing the relationship between length of life of males and females on both temperature and humidity (Figure 15), suggest that there are differences in the response of the sexes to temperature and humidity. The similarity in the shape of the curves describing the relationship of length of life, for both males and females, on both temperature and humidity, suggests that the overall response of the sexes are basically the same, i.e. the differences in response of males and females to temperature are in magnitude not in principle.

With respect to humidity the differences in length of life of sexes increase with an increase in humidity, reach a peak in that part of the humidity range where the beetles die of starvation and then decrease again. This pattern is apparent at all levels of temperature (Figure 16).

With respect to temperature the differences in length of life of T. molitor males and females decrease with an increase in temperature. This is apparent at all levels of humidity. The way in which these differences decrease with an increase in temperature (i.e. with a decrease in length of life), suggests that the differences in length of life of the sexes might be proportional to the overall length of life at any particular level of temperature for each level of humidity. It will thus mean that temperature does not add anything (over and above its influence on metabolism) to the existing differences in length of life of males and

females at different levels of humidity. This possibility is being investigated in the section to follow.

10.22 Differences in The Length of Life of Male And Female T. molitor Adults Starved to Death in Different Combinations of Temperature and Humidity

In this section I wish to test the hypotheses implied in Section 10.21, regarding the differences in length of life of male and female T. molitor adults starved to death in different combinations of temperature and humidity. These are :

(a) That males live for a shorter time than females at all levels of humidity and that these differences are real at all levels of temperature.

(b) That with a change in temperature the differences in length of life of males and females change in the same proportions as would length of life.

(c) That both males and females live for a shorter time at 100% R.H. than at 80% R.H. irrespective of temperature. The data summarised in Table A29 were used to test these hypotheses.

(A) To test hypothesis (a) I set out by constructing a table containing the differences (days) between the mean length of life of males and females at all combinations of temperature and humidity (Table 33A).

TABLE 33A Differences in Length of Life (days) of Starved Female and Male T. molitor Adults (Females, Males)

	Relative Humidities						
Temperature	0%	20%	40%	60%	80%	100%	Total
20°C	6.84	5.75	9.67	11.65	14.50	6.34	54.76
25°C	3.83	2.66	3.75	7.08	7.92	4.50	29.74
30°C	1.33	1.83	2.75	5.00	4.75	4.33	19.99
35°C	0.59	1.67	3.08	3.91	4.50	1.84	17.59
Total	12.59	11.91	19.25	29.65	31.67	17.01	

The differences all being positive, suggests that the lifespan of males are generally shorter than that of females. To test for significance, individual t. tests (one-tailed) were performed on mean length of life of females and males in all the corresponding sub-classes. The results are given in Table 33B, where :

a = calculated t - values with 22 degrees of freedom.

b = the probability that a given t - value could have been derived at by chance.

TABLE 33B t. tests For Comparing Mean Length of Life of Females and Males in Different Combinations of Temperature and Humidity

Temperature		Relative Humidities					
		0%	20%	40%	60%	80%	100%
20°C	a	5.9180	3.0166	3.5192	7.6216	8.1000	2.5470
	b	<.0005	<.005	<.005	<.0005	<.0005	<.01
25°C	a	7.0810	3.0286	2.5906	4.3600	5.3257	3.0908
	b	<.0005	<.005	<.01	<.0005	<.0005	<.0005
30°C	a	11.6626	3.3455	3.7875	6.4836	4.0414	4.5928
	b	<.0005	<.005	<.005	<.0005	<.0005	<.0005
35°C	a	4.6456	3.0681	4.2209	5.7879	5.3044	1.7156
	b	<.0005	<.0005	<.0005	<.0005	<.0005	<.1

From the results in Table 33B I concluded that starved T. molitor males stayed alive for a shorter period of time than females at any combination of temperature and humidity, within the limits of this experiment.

To illustrate the differences in the length of life of starved male and female T. molitor adults at different levels of temperature a three dimensional graph (Figure 16) was constructed from Table A29. Together with the differences between males and females Figure 16 also illustrates the complexity of the inter-relationship between length of life and temperature, humidity and sex.

(B) In the analysis of variance Table 29A, constructed from the original length of life data (Table A29) it was shown that there is a significant interaction between sex and temperature. This can be translated into

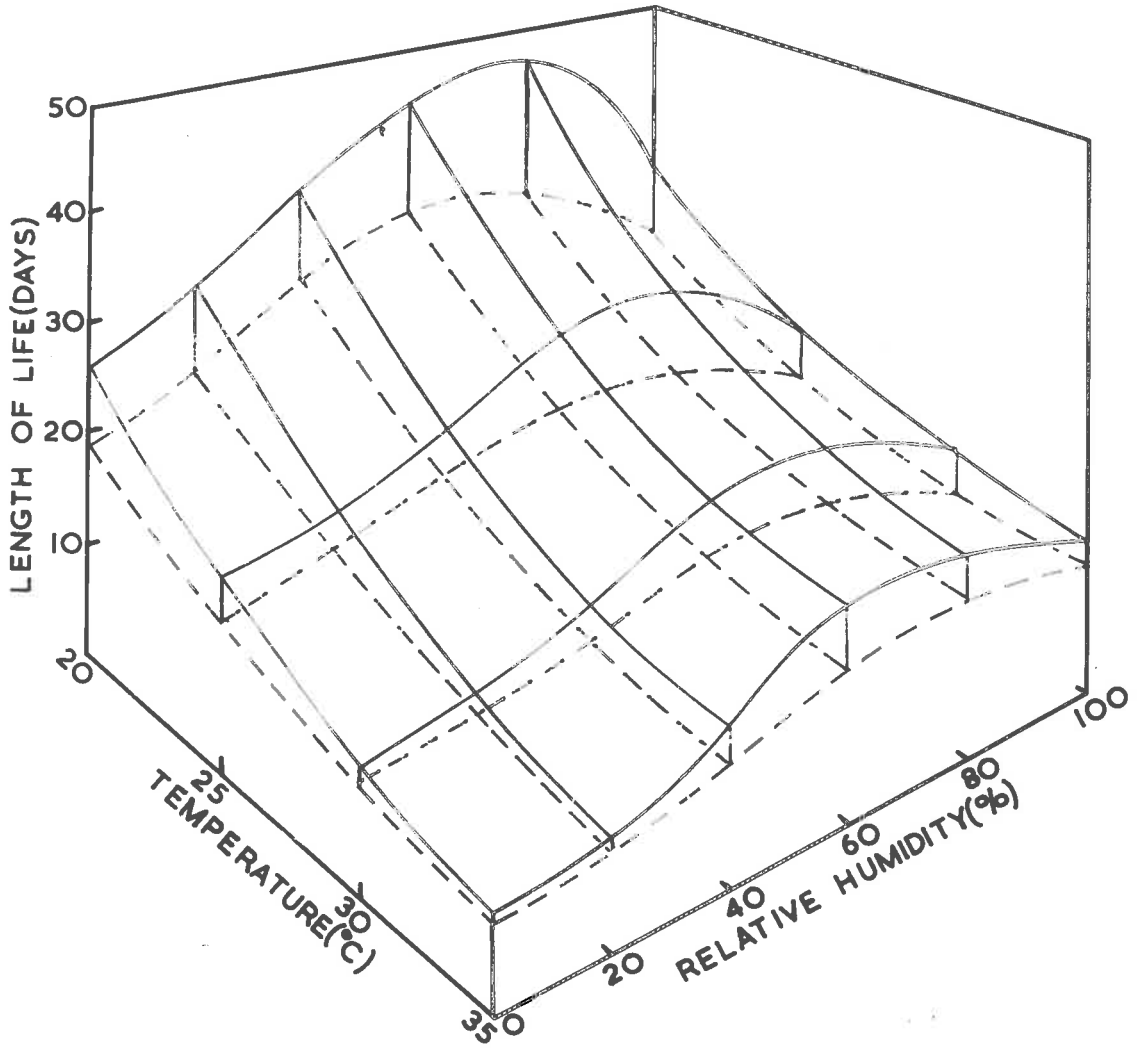


Fig. 16: The length of life of starved T. molitor males and females at various combinations of temperatures and relative humidity. The graph illustrates the differences in length of life of T. molitor males and females.

(Solid lines, T. molitor females)
(Broken lines, T. molitor males)

saying that the differential response of sexes is different at different levels of temperature. In connection with this I argued that if it can be shown that the differential response of sexes at different levels of temperature is not different on the relative scale, that is if the interaction between sex and temperature can be shown to be non-significant, when the length of life data are transformed to logarithms, then it may be accepted that, with a change in temperature the differences in length of life of males and females at different levels of humidity change in the same proportions as would length of life.

To test this hypothesis, the original length of life data summarised in Table A29 were transformed to logarithms. This transformed data is summarised in Table A30, and led to the construction of the analysis of variance Table 33C.

TABLE 33C Analysis of Variance of Data Summarised in Table A31

Source of Variance	D.F.	S.S.	M.S.	V.R.
Between Temperatures (T)	3	95.2224	31.7408	1303.7502***
Between Humidities (H)	5	28.8802	5.7776	237.3176***
Between Sexes (S)	1	9.0499	9.0499	371.7285***
T x H	15	2.0087	.1339	55.0050***
H x S	5	.5947	.1189	4.8855***
T x S	3	.0625	.0208	.8556 n.sig.
H x T x S	15	.4652	.0310	1.2739 n.sig.
Within classes	528	12.8345	.0243	
Total	575	149.1461		

The variance ratio associated with temperature x sex interaction is non-significant at the 5% level. I therefore accepted the hypothesis that, with a change in temperature, the differences in length of life of males and females at different levels of humidity change in the same proportion as the length of life. From this I concluded that temperature did not contribute significantly to the differences in the length of life of starved male and female T. molitor adults, at different levels of humidity that were found in this experiment.

This left me to find reasons for the differences in length of life of males and females at the different levels of humidity. Possible reasons for these differences are considered in Section 10.3.

(C) In Section 10.21 it was argued that the way in which the length of life of starved T. molitor adults changes with respect to humidity, especially when the humidity reaches saturation level, suggests that there might be a possible third cause of death, presumably "water-logging" of the tissues. This is in addition to two causes previously suggested namely desiccation and starvation (Section 6.32). The assumption of desiccation and starvation as causes of death in the humidity ranges 0-60% R.H. and 60-95% R.H. respectively was based mainly on two pieces of evidence.

(a) There were no differences in the length of life of T. molitor beetles starved to death in humidities 60-95%. In this range beetles lived longer than those starved in humidities below 60% R.H. (Section 6.21).

(b) Significantly more dry material was present at death in beetles dying in humidities below 60% R.H. than in beetles dying in the 60-95% R.H. range. In the latter range no differences could be detected between classes (Section 6.23).

By the same token, I argued that differences in the length of life of beetles starved at 60% R.H. and 100% R.H. might be a step towards establishing the presence of a third cause of death in humidities near saturation level.

To test the hypothesis that both male and female T. molitor adults live for a shorter time at 100% R.H. than at 60% R.H. irrespective of temperature, individual t-tests (one-tailed) were performed on the appropriate sub-class means (data summarised in Table A20). This led to the construction of Table 33D where :

a = The number of days by which the mean length of life of beetles starved in 100% R.H. is shorter than that of the beetles starved in 60% R.H.

b = Calculated t- values with 22 degrees of freedom.

c = The probability that a given t- value could have been derived at by chance.

TABLE 35D Information Regarding Differences in Mean Length of Life of T. molitor Adults at 80% R.H. and 100% R.H.

	Females			Males		
Temperature	a	b	c	a	b	c
20°C	14.58	5.3455	< .0005	6.42	4.3641	< .0005
25°C	7.58	5.3008	< .0005	4.16	3.4689	< .005
30°C	4.17	3.8903	< .0005	3.75	4.1937	< .0005
35°C	4.53	4.5400	< .0005	1.92	2.0489	< .05

From Table 35D I concluded both males and female T. molitor adults live for a shorter time at 100% R.H. than at 80% R.H. at all temperatures considered in this experiment.

This conclusion led me to ask the question as to whether the shorter life span in 100% R.H. results in less dry material being used than in 80% R.H. To answer this question the mean dry material content of beetles which died in 100% R.H. was compared with that of beetles which died in 80% R.H. by means of t-tests (one-tailed). This was done for both males and females at the 30°C level. This particular temperature level was decided on by chance. Information regarding the mean dry material contents and the comparisons is summarised in Table 35E.

TABLE 33E Information Regarding the Mean Dry Material Content (mg) of Starved T. molitor Adults When They Died in 80% and 100% R.H. At a Temperature of 30°C.

Sexes	Females		Males	
Humidities	80%	100%	80%	100%
No. of Observations	12	12	12	12
Mean Dry material content at death (mg)	21.4667(A)	23.2569(B)	21.7792(C)	23.2567(D)
Standard deviation of sample population	±1.5482	±2.8103	±1.2879	±1.1125
t. tests	P (B-A) ≤ 0 is < .01 P (A-C) ≤ 0 is > .35		P (D-C) ≤ 0 is < .005 P (B-D) ≤ 0 is > .45	

From Table 33E I concluded that both males and females of T. molitor when starved died in 100% R.H. containing more dry material than similar beetles which died in 80% R.H. Furthermore that there is no differences between the final dry material content of sexes at either humidity.

Assuming that the beetles have used all their reserved foods at 80% R.H. then it follows that beetles die prematurely in 100% R.H. i.e. still containing usable dry material. Since I had no reason to believe these beetles died prematurely in 100% R.H. because they were diseased I concluded that they died because of "drowning" or "water-logging" of the tissues.

In Section 7.4 it was argued that for T. molitor beetles the water/dry material ratio might be important in the higher humidity range. To get some estimate of the value this ratio could reach before death occurs the ratio 100 x final dry weight/final live weight was calculated for all the beetles used to construct Table 33E. The information concerning these ratios is summarised in Table 33F.

TABLE 33F Information Regarding the Ratio (100 x final dry weight/final live weight (mg)) of Starved T. molitor Adults When They Died in 80% and 100% R.H. at a Temperature of 30°C.

Sexes	Females		Males	
Humidities	80%	100%	80%	100%
No. of observations	12	12	12	12
Mean dry/live weight ratio	33.3795(A)	28.8175(B)	31.2608(C)	28.8325(D)
Standard deviation of sample population	±1.0592	±2.3000	±1.5143	±1.4577
t. tests (one-tailed)	P (A-B) $\leq$ 0 is $<$ .0005 P (C-A) $\leq$ 0 is $<$ .05		P (C-D) $\leq$ 0 is $<$ .0005 P (D-B) $\leq$ 0 is $>$ .30	

From Table 33F I concluded that the final dry/wet weight ratio at death of beetles starved in 100% R.H. is lower than that of beetles starved in 80% R.H. Although males have a lower dry/live weight ratio than females at 80% R.H., there is no difference between sexes at 100% R.H.

The mean final dry/wet weight ratio when the beetles died at 100% R.H., taking males and females together, is 28.7500 ± S.D. 1.9645, i.e. a beetle weighing 100 mg at death contains on the average 28.75 mg

of dry material and 71.25 mg of water. Assuming that these beetles die in 100% R.H. because their tissues get "water-logged" then it follows that the tissues will become "water-logged" when the proportion of water to body tissues appear in the ratio 71.25/25.75 (or 2.48:1) this is as against the ratio 1.59:1 in newly emerged beetles (calculated from observations at day 0, Table A15a-3).

10.3 Dry Material Consumption in Starved Male And Female T. molitor Adults as Influenced by Atmospheric Humidity And Time

Evidence obtained during a preliminary investigation into the water relations of starved T. molitor adults suggested that their rate of dry material consumption is influenced by atmospheric humidity (Section 6.24 and Figure 5). In Section 6.34 it was argued that this proposed relationship between dry material consumption and atmospheric humidity could be explained in two possible ways : (a) There is a genuine relationship between atmospheric humidity and the metabolic rate of starved T. molitor adults. (b) There is no relationship between metabolic rate and atmospheric humidity and the proposed relationship illustrated in Figure 5 is an artifact brought about by differences in length of life at different levels of humidity together with a metabolic rate independent of humidity but which changes with time.

Condition (b) was preferred as an explanation for the phenomena illustrated in Figure 5 because it also tendered a possible explanation for the sigmoid type of relationship between length of life and atmospheric humidity illustrated in Figure 2. (See also Section 6.34).

Investigating the change in dry material content of starved *T. molitor* adults with respect to time and humidity (Section 7.1) I was able to demonstrate what I thought at the time to be a change in the rate of dry material consumption with time (Section 7.12 and Figure 7). However, additional information obtained at the same time suggested that this proposed change in the rate of dry material consumption with time could be an artifact brought about by the presence of two populations within the experimental population which should differ in either their metabolic rate and/or their initial body composition, resulting in members of one population dying sooner than that of the other. The alternative hypothesis, i.e. that atmospheric humidity has an influence on the rate of dry material consumption, could not be accepted on evidence produced in Section 7.12, because it was found that the influence of humidity on dry material consumption in that particular experiment was confounded with the influence of initial body weight and initial body composition, and the age of the culture from which the beetles had been taken.

Two populations within the experimental population were subsequently identified - being the males and females of the species (Section 10.0 and 10.1). Males were found to differ from females in that they contain less dry material and therefore more water than females when they first emerge from the pupae. When starved, males also live for a shorter period than females at all levels of humidity in temperatures ranging from 20-25°C (Figure 16). Although the presence of two populations is evidence against accepting the hypothesis of a reduction in the rate of dry material consumption with time, the possibility still

exists, as was argued in Section 10.1 that both males and females might have an "inbuilt" reduction in their metabolic rate with time.

Having identified and thus been able to eliminate a number of factors which might bias the evidence towards accepting either hypothesis i.e. (a) That the metabolic rate of starved T. molitor adults depends on atmospheric humidity, or, the alternative (b) That the metabolic rate of starved T. molitor adults is independent of atmospheric humidity but changes with time, I wish in this section to re-investigate the relationship between atmospheric humidity and the rate of dry material consumption. I particularly wanted to get answers to the following questions :

(a) Does the rate of dry material consumption depend on humidity?

(b) Does the rate of dry material consumption change with time?

The null hypotheses implied in the questions above are tested in the sub-section to follow.

10.31 The Influence of Atmospheric Humidity on The Dry Material Consumption of Starved T. molitor Adults

To test the null hypothesis that (a) atmospheric humidity has no influence on the rate of dry material consumption of either starved male and female T. molitor adults, and if so (b) that there is no difference in the metabolic rate of the sexes, the following experiment was conducted.

Groups of newly emerged males and females in the weight range 100-120 mg were selected from cultures of the same age. Lots of sixteen males and of sixteen females were randomly selected from these groups, weighed and then allotted (randomly) to relative humidities 0, 20, 40, 60,

90 and 100% in each of the temperatures 20°C, 25°C and 30°C. Insects were individually confined in glass tubes in the respective treatments, remained there for eight days, were then removed, weighed, dried to constant weight at 100°C and their dry weight determined. This experiment was planned to last eight days, since this is near the point where beetles starved in 30°C at 0% R.H. will start to die (See Table A29). Using different temperatures, will then allow me to test the influence of atmospheric humidity on dry material consumption at various stages after emergence up till the time of death in the lowest humidity.

Together with the experimental animals control groups both of males and females were selected weighed and immediately dried to constant weight to determine their initial dry weight. Regressions of initial dry on initial live weight were then calculated for each control group. The data are summarised and the regression lines describing the relationship between initial dry and initial live weight for the two sexes compared in Table A31.

(A) Taking into account differences in initial weight, and in order to test the null hypothesis that atmospheric humidity has no influence on dry material consumption, regressions of final dry weight (day 8) (y) on initial live weight (x) were calculated for each experimental lot at each level of humidity, temperature and sex.

A summary of the data and calculations are given in Tables A32-34 and Tables A35-37 for females and males respectively.

The null hypothesis will be accepted if it can be shown that the regression lines describing the relationship between final dry

weight (y) and initial weight (x) at different levels of humidity are similar for both males and females and then also at each level of temperature.

The slopes and intercepts of the regression lines at each level of humidity, in the case of the females are compared in Tables 34A-C for temperature levels 20°C, 25°C and 30°C respectively, and similarly for males in Tables 35A-C.

TABLES 34A-C Analyses of Variance of Data in Tables A32-34 (Females)

(A) 20°C

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	5	38.7081	7.74162	2.156 n. sig.
Displacements	5	14.47967	2.895934	.806 n. sig.
Error	84	301.51602	3.589453	

(B) 25°C

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	5	42.46127	8.492254	1.300 n. sig.
Displacements	5	69.64673	13.929346	2.132 n. sig.
Error	84	548.60777	6.531044	

(C) 30°C

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	5	7.2816	1.45632	.331 n. sig.
Displacements	5	47.2904	9.45808	2.154 n. sig.
Error	84	368.71913	4.389513	

TABLES 35A-C Analyses of Variance of Data in Tables A35-37 (Males)

(A) 30°C

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	5	30.83451	6.167902	0.939 n. sig.
Displacements	5	10.62080	2.124160	0.323 n. sig.
Error	84	551.21239	6.562052	

(B) 25°C

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	5	17.573620	3.514724	0.439 n. sig.
Displacements	5	43.2812	8.656240	1.082 n. sig.
Error	84	671.97908	7.999750	

(C) 30°C

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	5	30.65699	6.131798	0.759 n. sig.
Displacements	5	94.77160	18.954320	2.204 n. sig.
Error	84	696.88281	8.296229	

The variance ratios associated with slopes and displacements being non-significant at the 5% level, at all levels of temperature both in the case of the males and the females, I accepted the hypothesis that the rates of dry material consumption in starved T. molitor adults are independent of atmospheric humidity, at least within the limits of this experiment.

Although not conclusive, the acceptance of the hypotheses that "dry material consumption is independent of atmospheric humidity" points towards the possibility that the change in the rate of change of dry material content with time illustrated in Figure 7 might be a genuine change in the metabolic rate of starved T. molitor adults with time. However other possible explanations that have to be considered include

(a) possible differences in the metabolic rate of males and females and/or

(b) similar metabolic rates in sexes but less usable dry material in males.

(B) Setting out to test the null hypothesis that "there is no difference in the rate of dry material consumption of males and females" I was confronted with the following problem :

Since the data concerning the final dry material content (day eight) of males and females summarised in Tables A32-37 are confounded with differences in initial dry material content (Section 10.0) comparing final dry material content of males and females will give a biased test for differences in the rate of dry material consumption of males and females. To overcome this problem the actual amount of dry material consumed by males and females over eight days at different levels of temperature were calculated in the following way :

The amount of dry material initially present in the bodies of individual insects used in the experiment was estimated making use of the regressions of initial dry (y) on initial live weight (x) calculated from data obtained from the control group (Table A31). The regression equations are :

(a) For females, $y = .36527x - .529795$

(b) For males, $y = .360877x - 1.628602$

From the estimated initial dry weight, the measured final dry weight was subtracted to give the amount (mg) of dry material used by individual insects over eight days in different experimental treatments. Since it was shown in (A) above that dry material consumption is independent of atmospheric humidity, for the purpose of this test insects at different levels of humidity for any particular level of temperature and sex were considered together as a single treatment.

Taking into account differences in initial weight, regressions of amount of dry material (mg) (y) used on initial live weight (x) were calculated for both females and males at temperatures 20°C, 25°C and 30°C. A summary of the data and calculations are given in Tables A38-40.

Slopes and intercepts of the regression lines describing the relationship between dry material (y) consumed and initial weight (x) for females and males, are compared in Tables 36A-C at temperatures 20°C, 25°C and 30°C respectively. The null hypothesis, "There is no difference in the rate of dry material consumption of starved males and females of *T. molitor*" may be accepted, if it can be shown that the regression lines describing the relationship between dry matter consumed and initial live weight for females and males are identical at all temperatures considered.

TABLES 36A-C Analyses of Variance of Data Summarised in Tables A38-40

(A) 20°C

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	1	7.52261	7.52261	1.492 n.sig.
Displacement	1	8.34561	8.34561	1.655 n.sig.
Error	188	947.37477	5.040291	

(B) 25°C

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	1	1.0411	1.0411	0.140 n.sig.
Displacement	1	7.7259	7.7259	1.042 n.sig.
Error	188	1393.5851	7.412676	

(C) 30°C

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	1	1.1726	1.1726	0.177 n.sig.
Displacement	1	0.0684	0.0684	0.010 n.sig.
Error	109	1245.0717	6.622721	

The variance ratios associated with slopes and displacements being non-significant at the 5% level in temperatures 20°C, 25°C and 30°C I accepted that the regression lines describing the relationship between dry material consumed and initial live weight, in males and females, are identical at each level of temperature considered and therefore also that there is no difference in the metabolic rate (as measured by rate of dry material consumption) of males and females.

The mean amount of dry material (mg) used by a single insect (taking over all levels of humidity and sex) during a period of eight days

is 6.6564 mg, 9.4288 mg and 12.2084 mg in temperatures 20°, 25° and 30°C (Tables A38-40) which give us an average daily rate of .6580, 1.1786 and 1.5261 mg respectively. The difference between the mean dry material content of females and males is 3.2403 mg (Table A31). If we consider the differences in initial dry material content of males and females (above) and the daily rate of dry material consumption during the first 8 days together with the differences in length of life of females and males (Table XXA) especially in that part of the humidity range where beetles die of starvation, it seems obvious that the difference in initial dry material content cannot solely be responsible for the differences in length of life of the sexes. Having accepted that dry material consumption is independent of atmospheric humidity and also that there is no difference in the rate of dry material consumption of the sexes, it seems extremely likely that not only might the metabolic rate of starved T. molitor adults change with respect to time, but also that the metabolic rate of males might change to a slower rate some time after that of the females. This idea is to be investigated in the section to follow.

10.32 The Change in Rate of Dry Material Consumption of Starved T. molitor Adults With Respect to Time

In this section I investigate the possibility of a change in the rate of dry material consumption of starved T. molitor adults with respect to time. In particular I ask (a) whether the rate of dry material consumption change with respect to time, and if so, (b) whether the point at which the change occurs, is the same for females and males.

To get possible answers to these questions, the following experiment was conducted : Two groups (Females and males) of newly emerged T. molitor adults in the weight range 100-120 mg. were selected from cultures of the same age. From each of these groups, six lots of 25 beetles each were randomly selected. These beetles were weighed and then individually confined in glass tubes in a relative humidity of 80% at a temperature of 25°C. Starting on day zero one lot each of males and of females were removed at four day intervals. The sequence being decided on by chance. On removal from the experimental treatment, beetles were again weighed and then dried to a constant weight at 100°C to determine their dry weights.

The null hypothesis implied in the first question, i.e. "there is no change in the rate of dry material consumption with time" will be accepted if it can be shown that the mean dry material content after consecutive four day intervals can be shown to be linearly related to time.

To correct for differences in initial weight regressions of dry weight (day_t) (y) on initial weight were calculated, one for each four day period and separately for the two sexes. A summary of the data and calculations are given in Tables A41 and A42 for females and males respectively.

Slopes and intercepts of the six regression lines were compared in the analyses of variance Tables 37A and 37B for females and males respectively.

TABLE 37A Analysis of Variance of Data in Table A41 (Females)

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	5	13.859804	2.771768	0.584 n. sig.
Displacements	5	5519.129450	1103.825900	232.669***
Error	130	654.857750	4.744185	

TABLE 37B Analysis of Variance of Data in Table A42 (Males)

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	5	27.720560	5.544112	1.565 n.sig.
Displacements	5	6851.762450	1370.352490	382.127***
Error	130	464.092556	3.586112	

Since the variance ratios associated with slopes are not significant at the 5% level, I accepted the hypothesis that the six regression lines of final dry weight (y) and initial weight (x), one for each time interval, have the same slope both for males and for females.

For a given initial weight, x = 110 mg say, the estimated mean final dry weight (y) at the i^{th} time interval is given, in the case of females, by the equation $y = \bar{y}_i + b\,(110 - \bar{x}_i)$ where b is the estimate of the common slope, viz. .21905898 (Table A41) and in the case of the males, by the equation $y = \bar{y}_i + b\,(110 - \bar{x}_i)$ where b is the estimate of the common slope, viz. .20018652 (Table A42).

The estimated mean final dry material content of starved T. molitor females and males, with a given initial weight of 110 mg starved for various periods of time in 60% R.H. at 25°C, is given in Table 37C.

TABLE 37C Estimated Mean Dry Material Content of T. molitor Males and Females Starved in 80% R.H. at 25°C for Varying Periods of Time

Time (Days)	0	4	8	12	16	20
Females	44.4509	39.7163	34.6349	32.1441	29.3005	26.6303
Males	40.2000	35.4752	31.0755	25.9996	22.8655	21.2006

I have now to test whether or not these means can be accepted as estimates of true means that depends linearly on time.

To test for linearity of regression of mean dry material content on time, the sums of squares associated with displacements (Tables 37A and 37B) (Females and males) were divided into two components.

(a) Sums of squares due to regression.

(b) Sums of squares due to deviation from regression.

These components of displacements sums of squares were then tested against the appropriate error mean squares.

Division of displacements sums of squares were done making use of the method of least squares (described in appendix 2A) and led to the construction of Tables 37D and 37E for females and males respectively.

TABLE 37D Analysis of Variance for Regression of Dry Material Content On Time (days). (Females)

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	1	5387.6555	5387.6555	1135.6335***
Deviations from Regression	4	131.4694	32.8674	6.9279***
Error	132	654.69787	4.7443	

TABLE 37E Analysis of Variance for Regression of Dry Material Content On Time (days). (Males)

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	1	6445.0891	6445.0891	1797.2259***
Deviations from Regression	4	406.6754	101.6688	28.3507***
Error	138	494.8836	3.5861	

On the basis of the variance ratios associated with deviations from linear regression being significant at the 0.1% level, I rejected the hypothesis that the mean dry material content depends linearly on time both for males and females. Therefore I also rejected the null hypothesis that there is no change in the rate of dry material consumption with time.

When plotting mean dry material content (Table 37C) against time (Figure 17) the positions of the points on the graph suggested that for both sexes the relationship might best be described by two straight lines with the lines describing the relationship during the early part of the experiment having steeper slopes than the lines describing the relationship during the latter part. The points on the graph further suggested that the change over from a higher to a lower rate of dry material consumption occurs between days 8-12 in the females and between days 12-16 in the males.

Using the change over times suggested above as a basis then to test whether the relationship between mean dry material content and time can best be described by two straight lines, the following procedure was followed :

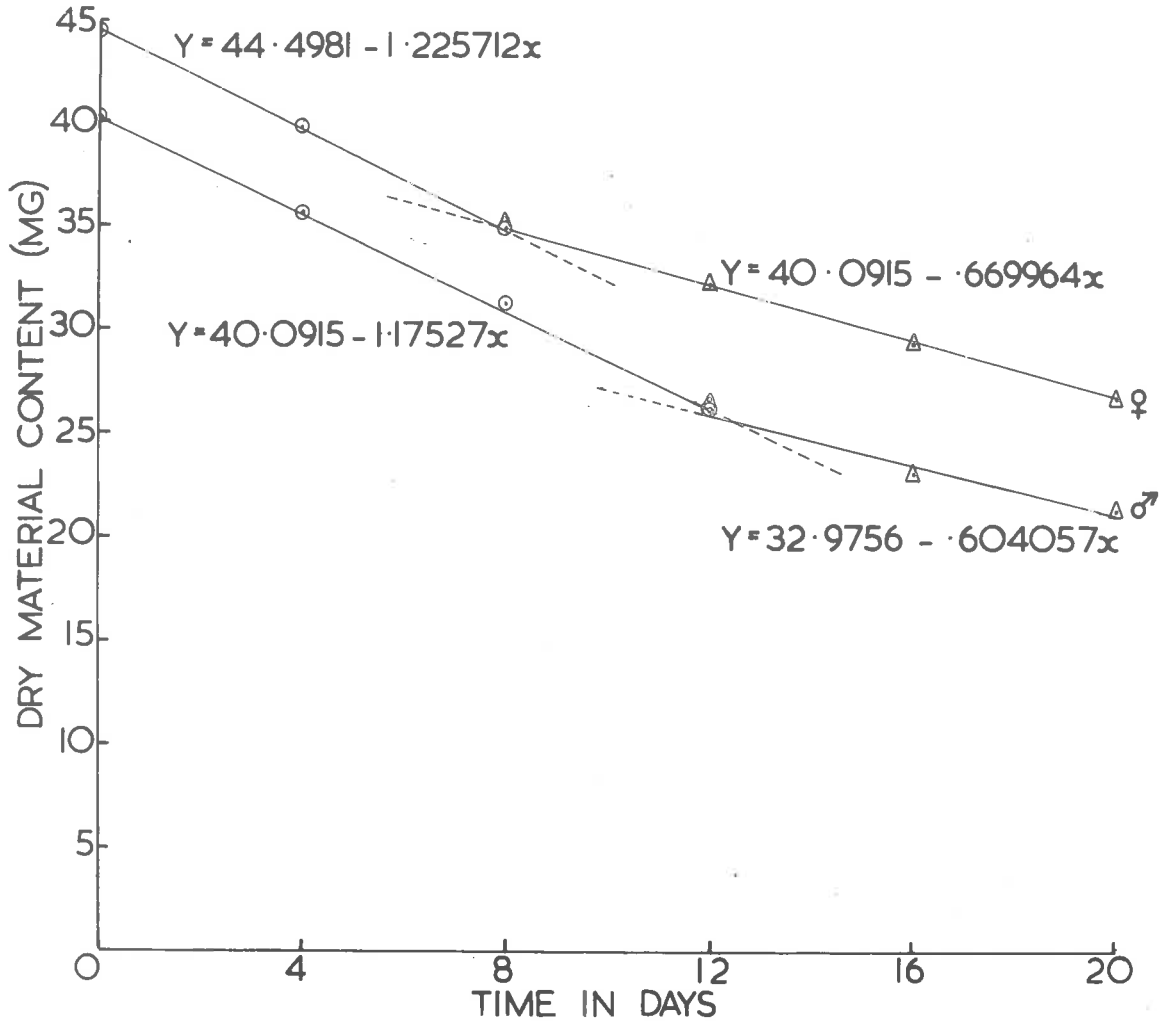


Fig. 17: The change in the dry material content of T. molitor males and females starved in 80% R.H. at a temperature of 25°C.

(A) Females Regressions of final dry weight on initial live weight (Table A41) were split up into two groups

(i) Those calculated for days 0, 4 and 8.

(ii) Those calculated for days 8, 12, 16 and 20.

Regressions within these groups were compared in Tables A43A and A43B respectively.

(B) Males Regressions of final dry weight on initial live weight (Table A42) were split up into two groups

(i) Those calculated for days 0, 4, 8 and 12.

(ii) Those calculated for days 12, 16 and 20.

Regressions within these groups were compared in Tables A44A and A44B respectively.

To test for linearity of regression of mean dry material content on time in each of the cases (A) (i) and (ii), (B) (i) and (ii), the sums of squares associated with displacements (Tables A43A and B, Tables A44A and B respectively) were in each case divided into its two components.

(a) Sums of squares due to regression.

(b) Sums of squares due to deviation of regression.

These were then tested against the appropriate error mean squares.

Division of displacements sums of squares were done making use of the method of least squares (described in Appendix 2) and led to the construction of Tables 38A and B and Tables 39A and B for females and males respectively.

TABLES 38A and B Analyses of Variance for Regression of Dry Material Content on Time, Females. Data from Tables A43A and B

(A) Days 0, 4, 8

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	1	1197.3097	1197.3097	199.6401***
Deviation from Regression	1	.6358	.6358	0.1060 n.sig.
Error	69	413.81670	6.9973	

(B) Days 8, 12, 16, 20

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	1	897.6981	897.6981	201.3059***
Deviation from Regression	2	1.3138	.6569	0.1473 n.sig.
Error	92	410.2624	4.4594	

TABLES 39A and B Analyses of Variance for Regression of Dry Material Content on Time, Males. Data From Tables A44A and B

(A) Days 0, 4, 8, 12

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	1	2760.3652	2760.3652	649.9927***
Deviation from Regression	2	2.1232	1.0616	0.2500 n.sig.
Error	92	390.70195	4.2468	

(B) Days 12, 16, 20

Source of Variation	D.F.	S.S.	M.S.	V.R.
Regression	1	278.6982	278.6982	85.0256***
Deviation from Regression	1	5.7951	5.7951	1.7680 n.sig.
Error	69	226.16383	3.2778	

The variance ratios associated with deviations from linear regression being non-significant at the 5% level (Tables 38 and 39, a and b), I accepted that the regression of dry material content on time is linear for each of the periods considered both for males and females and therefore that the change in dry material content with time can be described by two straight lines. The regression equations describing these two lines for females and males are :

(A) Females

(i) Day 0-8 ; $y = 44.4981 - 1.225712x$

(ii) Day 8-20 ; $y = 40.0915 - .669964x$

(B) Males

(i) Day 0-12 ; $y = 40.1836 - 1.17527x$

(ii) Day 12-20; $y = 32.9756 - .604057x$

The constants in these equations were calculated making use of the method described in Appendix 2B. The equations were used to construct Figure 17 where the observed points are the calculated mean dry material contents given in Table 37C.

From the evidence presented in the text and illustrated in Figure 17 the following conclusions can be drawn.

(i) The rate at which starved T. molitor adults consume their reserved food materials changes with time.

(ii) Both male and female T. molitor adults when starved use their food reserves at a higher rate during the early part of their starvation period, than during the later part.

(iii) The rate of dry material consumption during the early part is almost twice as high as that during the later part.

(iv) The change in the rate of dry material consumption occurs between days 8-12 in females and between days 12-16 in males.

Because the rate of dry material consumption of starved T. molitor adults is independent of atmospheric humidity (Section 10.3) but changes with time, it would seem that the relationship between rate of dry material consumption and atmospheric humidity proposed in Section 6.34 and illustrated in Figure 5 is an artifact brought about by the technique used to determine the rate of dry material consumption.

In females the time of change from a higher to a lower rate of dry material consumption coincides with the time when the maximum number of eggs were found to be matured (Section 9.0). It therefore seems reasonable to accept that the higher initial rate of dry material consumption in females is associated with the maturation of eggs. Although I have no evidence in support the possibility exists that in males the initial higher rate of dry material consumption might similarly be associated with the production of sperm. Assuming the latter, then it seems to follow that T. molitor adults, when deprived of food, use whatever reserves they have to produce offspring and not to stay alive for as long as possible.

A metabolic rate changing with time from a higher to a lower rate which has associated with it a similar change in the rate of water loss in the lower humidity range (Section 7.2 and Figure 8) could explain the sigmoid type of relationship between length of life and atmospheric humidity (illustrated in Figure 2) in the manner proposed in Section 6.34.

10.4 Possible reasons for the Difference in Length of Life of *S. molitor* Males and Females Starved to Death in Different Combinations of Temperature and Humidity

It was shown in Section 10.22 and Figure 16 that the length of life of starved *S. molitor* males is shorter than that of females at all combinations of temperature and humidity considered. It was further proposed in the same Section that temperature does not add significantly to existing differences in the length of life of sexes at different levels of humanity.

In Section 10.21 it was proposed that differences between sexes are quantitative and not qualitative, i.e. males die sooner than females but for the same reasons at the same levels of humidity for all temperatures considered. The reasons for death at different levels of humidity, proposed in Section 10.22, are : desiccation in the humidities below 60%, starvation in humidities 60-95% and "water-logging" of the tissues in humidities beyond 95%.

Differences known to exist between males and females, are :

(i) Differences in their initial body composition.

Males have an initial lower dry material content and therefore a higher initial water content than females (Section 10.0 and 10.1).

(ii) Differences in the pattern of dry material consumption.

Males and females consume their reserved foods at the same rate during the first 8 days at 25°C (Section 10.31B). The rate of dry material consumption changes with time from an initial high to relatively low rate in both sexes, however in males the initial higher rate is maintained for a longer period of time than in females (Section 10.32).

These differences could be responsible for the differences in the length of life of males and females in the following way :

Taking one humidity range at a time, we have :

(A) 95-100% R.H. Where T. molitor Adults die of "water-logging" of their tissues

Since males have an initial higher water content than females they could reach the point where their tissues become "water-logged" sooner than females providing this point is the same for both sexes. That this could be the case was indicated in Section 10.22 where it was shown that males and females die in 100% R.H. having the same amount of dry material left in their bodies and also that the ratio of dry material to water in their bodies at death, is the same in both sexes.

(B) 60-85% R.H. Where T. molitor adults die of Starvation

It was argued in Section 10.31 that even if the difference in initial dry material content of males and females constitutes differences in reserved food material this by itself could not explain the differences in length of life of the sexes in the humidity range where they die of starvation.

It was shown in Section 10.22 that males and females when starved in 80% R.H. die having the same amount of dry material left in their bodies. This indicates that females have initially more reserved foods stored in their bodies than the males.

In Section 10.32 it was shown that in both sexes the rate of dry material consumption changes with time from an initial high to a relative low rate, but sooner in females than in males. In Section

10.31 it was shown that the rate of dry material consumption during the first eight days at least are similar in the two sexes. But males maintain the initial high metabolic rate for a longer time than females; also females start off with more reserves to their disposal. These two facts should then at least partly explain why males live for a shorter time than females in the humidity range where both sexes die of starvation.

(C) 0-60% R.H. Where T. molitor adults die of desiccation

It was shown in Section 7.2 that with time a reduction occurs in the rate of water loss of starved T. molitor adults. This reduction in the rate of water loss was only observed in the lower humidity range and was found to be associated with a reduction in the rate of dry material consumption. Since this change in the metabolic rate occurs sooner in females than in males, the former will have the advantage of a lower rate of water loss for a longer time.

In Section 6.32 it was postulated that T. molitor beetles when starved die in the lower humidity range of desiccation and after their initial water content is reduced to a certain minimum. Assuming that males and females lose water at the same rate during periods of high and low metabolism then the advantage of females over males (described above) could at least partly explain why females live longer than males providing both males and females die after their initial water content is reduced by the same amount.

To find out whether males and females die in the lower humidity range after their initial water content is reduced by the same amount the following experiment was conducted.

Two groups (males and females) of newly emerged T. molitor adults in the weight range 100-115 mg were selected from cultures of the same age. From each of these groups eight lots of 25 beetles each were randomly selected. Beetles were then weighed individually and confined in glass tubes. Four lots, again randomly selected, were then subjected to an atmosphere of 0% R.H. in temperatures 20°C, 25°C, 30°C and 35°C respectively. These beetles were weighed daily and the amount of water present in their bodies on the day prior to death determined. This was done by subtracting the dry weight at death from the live weight on the day prior to death.

The rest of the beetles used as controls were immediately dried to constant weight and the initial water content determined by subtraction. From this data regressions of initial water content mg (y) on initial live weight (x) were calculated one for each sex. A summary of the data and calculations are given in Table A45. Regression equations describing the relationship between initial water content (y) and initial live weight (x) are :

(i) Females : $y = 64.0345 + .497870\ (x - 107.5780)$

(ii) Males : $y = 67.4563 + .571459\ (x - 107.4165)$

These regression equations were used to estimate the initial water content of individual beetles in the experimental lots. Subtracting from these quantities the measured final water contents, gave me an estimate of the amount by which the initial water content could be reduced before death occurs. This amount which for convenience I will refer to as "amount of water lost" will under-estimate the total amount of water lost by an amount equal to the amount of water of metabolism formed

during this period.

To correct for differences in initial weight regressions of amount of water lost (y) on initial weight (x) were calculated, one for each temperature and separately for the two sexes. A summary of the data and calculations are given in Tables A46 and A47 for females and males respectively.

Slopes and intercepts of the four regression lines, one for each temperature were compared in the analyses of variance Tables 40A and 40B for females and males respectively.

TABLE 40A Analysis of Variance of Data in Table A46 (Females)

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	3	13.78622	4.52541	.702 n.sig.
Displacements	3	6.18638	2.062126	.315 n.sig.
Error	92	601.67004	6.539891	

TABLE 40B Analysis of Variance of Data in Table A47 (Males)

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	3	24.53292	8.17764	.933 n.sig.
Displacements	3	2.74519	.95065	.102 n.sig.
Error	92	823.44836	8.95049	

Since the variance ratios associated with slopes and displacements are non-significant at the 5% level in both Tables 40A and B, I accept the hypothesis that the regression lines describing the relationship between amount of water lost till death and initial weight at different temperatures are identical both in the case of the females and the males. Thus I also accept that temperature has no influence on

the amount by which the water content is to be reduced before death occurs.

Identical regression lines for temperatures for each sex permit all the observations for one sex to be pooled to calculate a single regression of water lost on initial live weight. A summary of these calculations is given in Table A45, while the slopes and intercepts of the two regressions are compared in Table 40C.

TABLE 40C Analysis of Variance of Data in Table A45 (Females vs Males)

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	1	8.3566	8.3566	1.109 n.s.
Displacements	1	12.2393	12.2393	1.626 n.s.
Error	198	1492.6365	7.5135	

The variance ratios associated with slopes and displacements both being non-significant at the 5% level, I accept the hypothesis that the regression lines describing the relationship between amount of water lost and initial weight for males and females are identical and therefore that males and females die after their initial water content is reduced by the same amount.

Since the initial water content of males is higher than that of females (Table A45) it follows that the final water content of males must be higher than that of males. Since the higher initial water content of males seems to carry no advantage the reason for it is difficult to explain.

Since males and females die after their initial water content is reduced by the same amount and since males live for a shorter time than females it follows that males must lose their water at a higher

overall rate. As was postulated in the beginning of this section, this difference might be brought about by males metabolising at a high rate for a longer time than females. The possibility however also exists that males might lose water faster than females under the same environmental conditions. To verify this, I used total weight loss data measured in the experiment described in Section 10.5%.

From Section 10.5% we know that :

(a) Humidity has no influence on the rate of dry material consumption.

(b) There is no difference in the rate of dry material consumption of males and females during the first 8 days).

Since these measurements were taken over eight days it will not be confounded with a change in metabolic rate at 35°C (See Figure 17). Since the amount of dry material used during this time, is the same for males and females, differences in total weight loss of the sexes will constitute differences in the amount by which the initial water content is reduced.

To correct for initial weight regressions of total weight lost over eight days (y) on initial live weight were calculated one for each of the humidities 0, 20, 40, 60, 80 and 100% R.H. both for females and males. Summaries of the data and calculations are given in Tables A49 and A50 for females and males respectively.

The variance ratios associated with slopes being non-significant at the 5% level, both for females and males (Tables A49 and A50 respectively) I accepted that the regression lines describing the

relationship between total weight lost over eight days and initial weight at different humidities at 25°C have the same slopes.

Using estimates of the common slopes (obtained from parallel regression tables A49 and A50) I then calculated the total weight (mg) lost over eight days by beetles with given initial weight of 110 milligrams at each level of humidity. These amounts together with the amount of dry material used during the same period (which is the same in females and males at all levels of humidity (Section 10.31)) calculated for a given initial weight of 110 mg from pooled regression (Table A39) are listed in Table 40D and illustrated in Figure 18.

TABLE 40D Total weight lost and Dry Material Used by T. molitor Males And Females Starved for Eight Days in Different Relative Humidities at 25°C

Humidities %	0	20	40	60	80	100
Weight Lost (mg)						
(a) Females	27.6416	25.5401	19.2806	16.2356	13.3268	10.9493
(b) Males	29.5291	25.9360	21.0954	18.2275	14.5456	10.5253
Dry matter used (mg)	9.2964	9.2964	9.2964	9.2964	9.2964	9.2964

From Table 40D and Figure 18 it is obvious that compared to females the initial water content of males is reduced by a greater amount at all levels of humidity except at saturation level. The reasons for this difference are difficult to see and the complexity of the phenomena seems to be increased by the fact that the difference also occurs in that part of the humidity range where it was suggested (Section 7.4) these

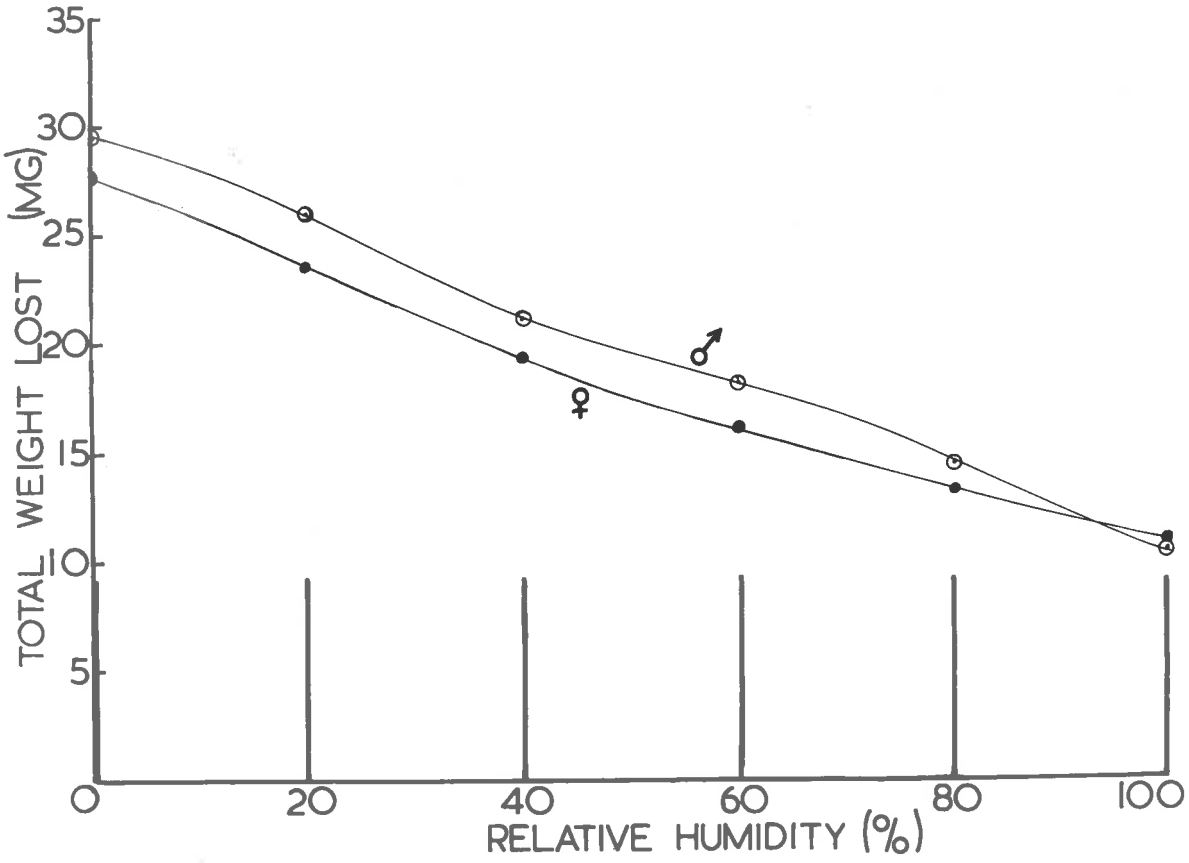


Fig. 18: Differences in the total weight lost by T. molitor males and females when starved for eight days in various humidities at 25°C.

(Vertical columns represent the amount of dry matter metabolised over eight days in the various humidities.)

beetles are able to regulate their rate of water loss by excretion or reabsorption of water from the faeces.

It is interesting to notice that even at saturation level where these insects are known to eventually die from "water-logging" of their tissues, the total amount of weight lost after 8 days exceeds the amount by which the dry material content is reduced. This means that, since no water can be lost through evaporation, that the insects have excreted water exceeding the amount of water of metabolism formed during this period.

Since males and females die after their initial water content is reduced by the same amount, the fact that the rate of reduction is higher in males than in females plus the fact that females have the advantage of a slower rate of water loss (which is associated with a reduced metabolic rate) for a longer period of time, should then at least partly explain the difference in length of life of males and females in that part of the humidity range where both sexes die of desiccation.

10.3 The Influence of Saturation deficiency on the Physiological Age at Which Death Occurs in Starved T. molitor Adults

In Section 10.21 the similarity in the basic shape of the curves describing the relationship between length of life of starved T. molitor adults and (a) relative humidity (b) temperature, at different levels of temperature and of humidity, respectively (Figure 16) led me to believe that both males and females die of the same causes at any particular level of humidity and at all levels of temperature considered.

It was further suggested in Section 10.21 that the quadratic curve describing the relationship between length of life and temperature in that part of the humidity range where beetles die of starvation indicates the basic way in which temperature influences the metabolic rate of starved T. molitor adults. The similarity in the basic shape of this particular curve and the curve describing the relationship between length of life and temperature at e.g. 0% R.H. where insects die of desiccation, led me to believe that metabolic rate plays the major part in determining length of life of starved T. molitor adults and that the influence of saturation deficiency if any, is negligible.

In this sub-section I wish to test the null hypothesis that saturation deficiency has no influence on the physiological age at which death occurs in starved T. molitor adults (see also Section 10.21). Since T. molitor adults can regulate water loss in the higher humidity range (Section 7.3) this particular hypothesis could be tested only in the lower humidity range. For this reason, and for the purpose of testing this hypothesis, I subjected experimental animals to conditions in which saturation deficiency was varied by keeping relative humidity constant at 0% while temperature was increased from 20 - 35°C.

From evidence produced and discussed in Sections 6.23, 6.25, 6.32 and 9.12 I concluded that T. molitor adults when starved at 25°C die prematurely of desiccation in the lower humidity range. Death in the lower humidity range occurs with beetles still containing some reserved foods in their bodies but after their initial water content was reduced by the same amount. The amount of dry material at death however

decreases with an increase in humidity, suggesting that the relative rate in reduction of the water content determines the amount of dry matter that will be left at death.

From Section 10.4 we know that both males and females die at 0% R.H. in temperatures 20°, 25°, 30° and 35°C after their water content was reduced by the same amount. Should the insects also contain the same amount of dry material at death, when starved at 0% R.H. in temperatures 20 - 35°C, then to my mind the null hypothesis may be accepted, i.e. that saturation deficiency has no influence on the physiological age at which death occurs. This situation could also be described as follows: that starved T. molitor adults are successful in counteracting the influence of the increased drying power of the air, brought about by increase in temperature even in that part of the humidity range where they cannot regulate water loss by means of the excretory system.

To test the null hypothesis stated above, I used additional data obtained in experiment (i) in Section 10.4(c). In this experiment it was shown that both T. molitor males and females when starved at 0% R.H. in temperatures 20°, 25°, 30° and 35°C die after their water content is reduced by the same amount. Should these insects have died at the same physiological age I would expect the dry material/water ratio at death to be the same under all experimental conditions. To test this hypothesis, the ratio : 100 x final dry weight (mg)/final live weight (mg) were calculated for both females and males starved to death at 0% R.H. in temperatures 20°C, 25°, 30° and 35°C. A summary of the data is given in Tables 41A and B for females and males respectively.

TABLE 41A A Summary of Data Concerning the Final Dry Material/Water Ratio (x) of T. molitor Females Starved to Death at 0% R.H. at Different Temperatures

Temperatures °C	20°	25°	30°	35°
N	25	25	25	25
$\bar{x}$	44.7136	44.7348	45.7760	46.7140
Σx	1117.84	1118.37	1144.45	1167.85
s^2	7.7387	13.3093	5.2429	4.2384

TABLE 41B A Summary of Data Concerning the Final Dry Material/Water Ratio (x) of T. molitor Males Starved to Death at 0% R.H. at Different Temperatures

Temperatures °C	20°	25°	30°	35°
N	25	25	25	25
$\bar{x}$	40.1104	40.5712	41.5312	41.9696
Σx	1002.76	1014.30	1038.20	1049.24
s^2	7.5133	8.3819	12.0542	11.7165

Data summarised in Tables 41A and B were used to construct the analysis of variance Table 41C.

TABLE 41C Analysis of Variance of Data Summarised in Tables 41A and B

Source of Variance	D.F.	S.S.	M.S.	V.R.
Sex (S)	1	1029.8084	1029.8084	121.9979***
Temperature (T)	(3)	(100.3129)		
(i) Linear Regression	1	95.7469	95.7469	10.1614**
(ii) Deviations	2	4.7660	2.3830	.2823 n.sig.
Interaction S x T	3	7.5062	2.5021	.2964 n.sig.
Residual	192	1620.7044	8.4412	
Total	199	2758.3309		

Temperature sums of squares were separated in the two components :

(a) Sums of squares due to linear regression of final dry matter/water ratio on temperature.

(b) Sums of squares due to deviation from linear regression.

This was done making use of the method "Orthogonal comparison in regression" after Snedecor (1956).

Since the sums of squares associated with linear regression of final dry matter/water ratio on temperature is significant at the 1% level, and sums of squares associated with deviation from linear regression is nonsignificant, I accept the hypothesis that the final dry matter/water ratio changes with temperature in a linear fashion. Considering the means in Tables 41A and B the regression coefficient is obviously positive, I therefore also accept that the final dry matter/water ratio increases with increase in temperature. Interaction sums of squares being nonsignificant suggests that the same happens in both sexes. I therefore reject the null hypothesis and accept that T. molitor adults

when subjected to 0% R.H. at different temperatures die at an earlier physiological age in the higher temperatures.

T. molitor adults die in 0% R.H. at temperatures 20 - 35°C after their water content is reduced by the same amount (Section 10.4). The fact that the final dry matter/water ratio increases with temperature then seems to be evidence in support of the hypothesis that starved T. molitor adults die in the lower humidity range after their initial water content is reduced by a certain amount and not when the dry material/water ratio has reached a certain minimum.

11.0 A General Summary and Discussion of Work Done on the Water Relations of Starved T. molitor Adults

During a preliminary investigation into the water relations of starved T. molitor adults it was found in Section 6.25 that at a given temperature of 25°C, the rate at which the water content of these insects is reduced depends linearly on atmospheric humidity. At the time this suggested a possible causal relationship between the drying power of the air and the rate of water loss of starved T. molitor adults. The technique for determining the rate of water loss in this particular case was open to criticism, since it was confounded with differences in length of life of beetles at different levels of humidity. The proposed relationship however, was accepted in principle on the assumption that no change should occur in the rate of water loss with time under the influence of factor/s independent of atmospheric humidity.

In Section 7.2 it was shown that the rate at which the water content is reduced does change with time and differently in different sections of the humidity range. This suggested that factors other than the drying power of the air also influence the rate of water loss in starved T. molitor adults and that the relationship described above was a coincidence.

A change in the rate of reduction of the water content of starved T. molitor beetles in the lower humidity range was found to be associated with a change in the rate of dry material consumption (Section 7.2 and 10.32). In the lower humidity range, the rate of reduction of the water content as well as the rate of dry material consumption changes from an initial high to a final lower rate.

With a reduction in the metabolic rate (measured as rate of dry material consumption) it seems reasonable to expect a reduction in the airflow through the spiracles of the insects. A reduction in the rate of water loss associated with a reduced air flow through the spiracles to my mind is to be expected and I therefore consider this particular situation as normal.

Experimental evidence produced in Section 10.3 indicated that the metabolic rate of starved T. molitor adults is independent of atmospheric humidity, suggesting that no additional energy is used to relieve internal conditions caused by exposure to either very low or very high humidities. In Section 10.3 it was indicated that the initial high metabolic rate in female T. molitor is associated with the maturation of eggs. It was further suggested that the initial high

metabolic rate in males might similarly be associated with the production of sperm. Assuming the latter, then it seems to follow that the lower rate of water loss of starved T. molitor adults during the later part of their lives in the lower humidity range is just a consequence of a reduced metabolic rate associated with the termination of certain physiological processes and does not signify an active conservation of water.

In the higher humidity range the rate of dry material consumption changes with time in a manner identical to that in the lower humidity range, but the water content changes differently. Data concerning the rate of change of the water content illustrated in Figure 8 indicated a steady rate of reduction of the water content in 60% R.H. past the point of change in the metabolic rate. In the light of the discussion above, a steady rate of reduction of the water content in 60% R.H. despite the reduction in the metabolic rate, suggested a deliberate or active excretion of water. In 90% R.H. (Figure 8) no change was observed to occur in the water content of beetles during the first four days of starvation. After this initial period of no change however, the water content was reduced and again at a steady rate past the point of change in the metabolic rate. The point where the water content started to change in 90% R.H. coincided with the time when faecal material was first observed to be excreted, suggesting active excretion of water mainly with the faecal material.

Faecal material excreted by starved T. molitor adults was observed to range from a bone dry powder in the lower humidity range to

a wet smear in the higher humidity range (Section 7.11); suggesting that the rectal system in these insects acts as a water regulatory mechanism by excreting water with faecal material or re-absorbing water from the faecal material according to need.

During this investigation I found no indication which even vaguely suggests that T. molitor beetles can absorb water from the atmosphere. Since these beetles had no chance to drink, the only water available to them was water present in their bodies and water formed as a by-product of metabolism. Because the metabolic rate is independent of atmospheric humidity, the same amount of metabolic water should be available to the insect irrespective of the humidity it is subjected to. Never-the-less at low humidities the total amount of water will be inadequate and at high humidities it will be excessive as is indicated by the following :

At a given temperature T. molitor adults were found to live for a shorter time when starved in humidities below 60% and beyond 95% compared to when they were starved in the humidity range 60-95% (Section 6.21 and 10.22). At the same time they die having more dry material left in their bodies when starved in the lower and upper humidity ranges than when starved in the intermediate ranges (Section 6.23, 10.22).

This suggested to me :-

(a) That T. molitor adults when starved in different humidities die of starvation in the intermediate humidity range and prematurely of desiccation and "water-logging" of their tissues in the lower and upper humidity ranges respectively.

(b) That whatever water saving devices are operating these are insufficient to counteract the influence of the drying power of the air in the lower humidity range to such an extent as to allow the beetles to make full use of their energy resources.

(c) That the excretory system is insufficient to cope with excess water accumulating in the bodies of these insects when starved in the upper humidity range, causing the body tissues to become "water-logged" and the insects to die before they could have made full use of their energy resources.

Investigating internal conditions which could result in death due to desiccation in the lower humidity range (Section 6.25, 6.32), it was found that the observed conditions did not comply with either of the two predictions (Figure 6) which seemed to be the only two plausible ones for death due to desiccation.

These are that these insects die:

(i) When their water/dry material ratio has reached a certain minimum.

(ii) After their initial water content is reduced to a certain minimum.

Since the observed internal condition of beetles when they die more closely resembled the condition described under (ii), it was accepted that T. molitor beetles die of desiccation in the lower humidity range and after their initial water content is reduced to a certain minimum. This was accepted on the assumption that some water, an amount increasing with humidity, is bound in some form or another and

thus unavailable to the insects. Additional evidence for accepting this hypothesis was obtained in Section 10.5 where it was shown that T. molitor beetles when starved to death in 0% R.H. in different temperatures died after their water content was reduced by the same amount, yet their dry material/water ratio differed significantly.

Investigating the idea of bound water it was shown in Sections 9.0 and 9.1, that water incorporated in eggs matured by virgin females, but not deposited or re-absorbed could constitute a form of bound water in starved T. molitor females. The number of eggs matured and therefore the amount of water bound increases with an increase in humidity. It was further suggested that water incorporated in sperm sacs or "spermatophores" might constitute a form of bound water in males.

Assuming that active excretion of water at the higher humidities occurs only with the faeces, then the fact that the water content of beetles starved at 90% R.H. (Figure 8) remained constant over the first 4 days, suggests that at this particular humidity an amount of water equal to that formed as a by-product of metabolism over this period has been evaporated. Should we consider the subsequent reduction in the water content as absolutely necessary for the welfare of the insect, then it seems to follow that these insects have to reduce their water content to below what is considered "normal" for newly emerged beetles. Since the dry material content seems to be the only other factor changing with time and independent of humidity, it further seems to follow that the initial water content has to be reduced in response to a reduction in the dry material content and at a rate determined by the rate of dry material consumption. This in turn suggests that the water/dry material

ratio might be important at least in the higher humidity ranges.

Evidence produced in Section 10.22 plus evidence illustrated in Figure 16, indicate that males and females of T. molitor die (a) containing more dry material in their bodies (b) containing a greater proportion of water relative to dry material (c) after a shorter period of time, when starved in 100% R.H. compared to when they were starved in 80% R.H. The cause of death in 100% R.H. was stated as "water-logging" of the tissues. From Figure 17 it is obvious that T. molitor adults even when starved in 100% R.H. are able to reduce their water content to below what is considered normal for newly emerged beetles. These points suggested to me, that when starved in the upper humidity range:

(a) Water of metabolism does not merely accumulate on top of water already present in the insect's body.

(b) That T. molitor beetles have to reduce their water content to below what is considered "normal" for newly emerged beetles.

(c) That the water content has to be reduced at a certain minimum overall rate.

(d) That T. molitor adults by their own means are not able to maintain this required rate when very little or no evaporation of water takes place.

(e) That the so termed "water-logging" of tissues occurs at a point where reserved foods are reduced considerably and also when the beetles contain a total amount of water which is less than what was present in their bodies when they first emerged.

In Section 10.22 it was calculated that "water-logging" of tissues occurs when the average ratio of 2.40 mg of water to 1.0 mg of dry body tissue is reached. This is as against 1.59 mg of water to 1.0 mg of dry body tissue in newly emerged beetles. Taking these figures at their face value suggest that T. molitor adults can tolerate a considerable reduction in osmotic pressure of the haemolymph. However a point is reached, in that part of the humidity range where little or no evaporation takes place, where a further reduction cannot be tolerated. This situation arises presumably because water is not excreted fast enough. However, there seems to be an alternative explanation for this situation to have arisen.

Taking that beetles when they first emerge have sufficient solutes present in their haemolymph to sustain osmotic balance, and since the total water content at death in 100% R.H. is lower than when beetles first emerge, it seems to follow that T. molitor adults are unable to re-absorb salts in the rectum (as was claimed for larvae, Patton and Craig, 1959) when forced to excrete water. Thus that the haemolymph gets too diluted not because water is not excreted fast enough, but because salts are depleted with water excreted. More water is lost in 80% R.H. than in 100% but in the former more water is lost through evaporation which should not cause salt depletion. This might mean that beetles stay alive in 80% R.H. for the maximum time their energy resources would allow them not because they get rid of enough water through excretion with the faecal material, but because enough water is lost through evaporation to prevent salt depletion with water

excretion.

While investigating the variability in (a) the body composition of newly emerged T. molitor beetles (b) the length of life of beetles starved in different combinations of humidity and temperature (Section 6.1, 6.21, 10.1, 10.2, 10.3, 10.4) it was found that a substantial part of the variability in both cases could be explained in terms of differences which exists between males and females of the species.

Differences between sexes were found to include the following:

(a) Differences in the initial body composition.

(b) Differences in the rate at which the water content is reduced at any particular level of humidity.

(c) Differences in the time when the metabolic rate changes from an initial high to a lower rate.

(d) Differences in the length of life.

Despite these differences between males and females, their general response to changes in atmospheric humidity and temperature were found (Section 10.2) to differ in detail only and not in principle.

Knowledge about water regulation in starved T. molitor adults could broadly be summarized as follows:

Compared to some insects the cuticle of T. molitor adults shows a high degree of impermeability (Wigglesworth, 1945). The fact that T. molitor adults die prematurely in both the lower and upper humidity range indicates that the physical resistance to evaporation is insufficient in the lower humidity range and over-efficient in the upper humidity range. This situation can partly be relieved by the malpighian-tube and rectal system acting as an internal water regulatory mechanism by excreting water with the faeces in the higher humidity range and

re-absorbing water from the faeces in the lower humidities. The water regulatory system although functional throughout the humidity range is only effective between 60-98% R.H. Both below and beyond this the influence or the lack of influence of the drying power of the air overrides the usefulness of the regulatory system, resulting in the premature death of the insect by desiccation or "water-logging" of the tissues respectively.

For maximum use of their energy resources, starved T. molitor adults therefore have to exploit and use atmospheric humidity to either reduce excessive evaporation or to facilitate water loss. This is substantiated by the fact that T. molitor adults have acquired the ability to recognise and respond to small differences in atmospheric humidity as was shown by the work of Pielou and Gunn (1940), Pielou (1940), Gunn and Pielou (1940), Dodds & Ewer (1952), Howard (1955), Perttunen and Lehrman (1959, 1962, 1963).

PART V

A STUDY OF SOME ASPECTS IN THE WATER RELATIONS OF STARVED TENEBRIO molitor LARVAE

12.0 Introduction

Burton (1953) reported that starved T. molitor larvae remained alive for periods exceeding two hundred days in dry air at 25°C. If we compare this with the life span of starved T. molitor adults (Figure 16) it seems obvious that larvae must be better equipped to save water and energy than the adults and could therefore be considered the stage in the life cycle of T. molitor better suited to bridge periods when conditions are not suitable for normal development.

From the literature on the water relations of starved T. molitor larvae, cited and discussed in Section 2, it is obvious that these insects are remarkably adapted to prevent excessive water loss and at the same time are able to use water from almost any available source to replenish water lost through evaporation.

The following adaptations for prevention of excessive water loss in T. molitor larvae have been reported in the literature :

(a) A cuticle showing a comparatively high degree of impermeability (Wigglesworth, 1945).

(b) The ability to re-absorb water from faecal material in the rectum (Patton and Craig, 1939; Wigglesworth, 1932, 1950).

(c) A mechanism for drying air before it is expired (Mellanby, 1939a, Kalmus, 1936).

(d) Reduction in the metabolic rate and with it the rate of water loss (Buxton, 1930; Mellanby, 1934a).

(e) The presence of a very effective spiracle closing mechanism (Mellanby, 1934a).

(f) The ability to recognise and respond to differences in atmospheric humidity (Perttunen and Lehrman, 1962).

(g) Restricting major activities to periods of natural darkness when humidity is more favourable (Michal, 1931; Cloudsley - Thompson, 1953).

Water resources known to be utilised by starved T. molitor larvae, to replenish their water supplies include :

(a) Free water, used for drinking (Mellanby, 1932), this was borne out by my own observations.

(b) Absorption of water from the atmospher (Buxton, 1930; Mellanby 1932a; Lefon and Teissier, 1938; Locke, 1953; Edney, 1957).

(c) Metabolism of additional food reserves thereby making available more water of metabolism to offset water lost through evaporation (Buxton, 1930, 1932).

From the literature cited it is obvious that starved T. molitor larvae have at their disposal a fair number of mechanisms which could be used to prevent excessive water loss. At the same time however, the ability of these insects to draw on water supplies other than that present within themselves, suggests that conditions might arise under which the available mechanisms might fail to limit water loss to such an extent as to keep the internal conditions of these insects within tolerable limits.

In subsequent sections I propose to investigate the circumstances which stimulate the insects to replenish their water supplies from the following sources :

(a) Water derived from the metabolism of food reserves.

(b) Water absorbed from the atmosphere.

I was particularly interested to get answers to the following general questions.

(i) (a) Whether desiccation of starved T. molitor larvae stimulates metabolism?

(b) Whether starved T. molitor larvae can absorb water from the atmosphere?

and if so

(ii) What stimuli, arising either from the environment or from the condition of the insect, will cause them to replenish water in this way.

(iii) Whether it can be considered an advantage having the ability to replenish water in these ways.

These questions arose in relation to the following considerations :

(A) Although the capacity of starved T. molitor larvae to metabolise more dry material in dry than in moist air has been widely quoted in the literature on water relations of terrestrial insects, I was not convinced that this was true. Buxton (1930, 1932) proposed that these insects by just these means are able to maintain a constant dry/wet ratio in humidities below 60%. Mellanby (1932a, 1932b, 1934b) failed to confirm these results in T. molitor or in many other insect species he studied. Mellanby (1936) came to the conclusion that the rate at which

starved T. molitor larvae utilise their reserves is governed by temperature alone and is unaffected by atmospheric humidity (See also Section 2.3).

(b) Although the ability of starved T. molitor larvae to absorb water from the atmosphere is well established (Buxton, 1930; Mellanby, 1932a; Lefon and Teissier, 1939; Locke, 1953; Edney, 1957), very little further information as regards this phenomena in these insects, is available.

Experiments designed to test hypotheses implicit in the questions outlined above, had to take into account certain technical difficulties, as was the case with adults, Section 6.0.

(i) According to Buxton (1930) pupation could occur in starved T. molitor larvae which have reached a weight of 130 mg and above. Assuming that larvae require a certain minimum feeding period after a larval moult before they will proceed to moult again or to pupate, only newly moulted larvae were used in all experiments.

(ii) Cultures did not yield enough newly moulted larvae at any one time to set up large experiments which might require numbers of the order of 500-600. Such numbers were accumulated over a number of days, each daily yield being distributed evenly, but at random, through all treatments.

(iii) Larvae in stock cultures were reared in circumstances that seemed highly favourable, yet the weight of newly moulted larvae of approximately the same age varied considerably. Because results were obtained by weighing the larvae, it was necessary for the experiments to be designed either to eliminate the influence of initial weight or to permit it to be measured

and allowed for.

(iv) Treatments consisted of keeping larvae for prolonged periods without food and water at various temperatures and humidities. It was necessary to measure or to estimate the amount of both water and dry material in each larva at the beginning of the experiment and at intervals during the experiment. The only direct measurement which was possible while the larvae remained alive was to weigh the living larvae. As larvae for the experimental treatments were being accumulated, some were randomly set aside to be weighed, dried and the dry matter weighed. The statistics obtained from these samples were used to estimate the dry matter (and therefore the water) in the larvae that were used in the experiments.

12.1 The Influence of Atmospheric Humidity on Starved *T. molitor* Larvae

According to Buxton (1930), *T. molitor* larvae starved at 25°C in different relative humidities below 60% lost more weight in lower humidities than in high humidities, yet the ratio of water to dry material in their bodies remained constant. He explained this by proposing that in dry air additional reserved foods were oxidised and the water of metabolism retained to compensate for that lost by evaporation; in other words that the metabolic rate of starved *T. molitor* larvae is influenced by atmospheric humidity.

Mellanby (1932a) working at a slightly higher temperature could not confirm Buxton's (1930) results for starved *T. molitor* larvae and came to the conclusion (Mellanby, 1936) that the rate at which these

insects utilise their reserves is governed by temperature alone.

Mellanby (1934a) showed that the rate of water loss of starved T. molitor decreases with time in dry air. This finding could be associated with that of Buxton (1930) who showed that carbon dioxide output in starved T. molitor larvae also decreases with time in dry air. The information as regards the influence of atmospheric humidity and time on the metabolism of starved T. molitor larvae as outlined above, although somewhat contradictory, can give rise to a number of questions. Assuming that starved T. molitor larvae do lose more weight in low than high humidities, the fact that they maintain a constant dry/wet ratio indicates that water and dry matter is lost in the same proportion as present in the insects body. It further suggests that this condition might be of importance in starved larvae. Assuming that starved T. molitor larvae do maintain a constant dry/wet ratio irrespective of humidity then the question still remains as to whether this is achieved by :

(a) Increasing their metabolism in the lower humidity range, making available more water of metabolism to offset water lost by evaporation, or

(b) Decreasing their metabolism in the lower humidities thus reducing the amount of water lost by evaporation through the spiracles.

These general questions can be analysed into a number of more specific ones. To make myself familiar with the experimental animal and to eliminate some of the questions raised, I planned an experiment designed to test the following series of null hypotheses :

(a) The rate of weight loss of starved T. molitor larvae is not a linear function of atmospheric humidity.

(b) The rate of weight loss of starved T. molitor larvae does not change with time.

(c) The water/dry material ratio of starved T. molitor larvae is not a function of atmospheric humidity.

(d) Dry material consumption in starved T. molitor larvae is not a function of atmospheric humidity.

For the purpose of testing these null hypotheses the following experiment was conducted :

A number of newly moulted T. molitor larvae in the weight range 110-130 mg was selected from cultures of the same age. From these larvae, seven groups each containing forty larvae were randomly selected, weighed and individually confined to glass tubes. Six of these groups were then randomly allotted, one to each of the relative humidities 0, 20, 40, 60, 80 and 100% in a temperature of 25°C.

Daily weight changes were recorded for each individual larva up to and including day 35, when larvae were dried to constant weight in 100°C and the final dry weight recorded. Larvae in the seventh group were weighed and immediately dried to constant weight in 100°C and the initial dry weight recorded. Data obtained from this experiment were used to test the null hypotheses mentioned above, in the sub-sections to follow.

During the course of this experiment an unexpected observation was made : although the larvae used had no chance to feed since their previous moult, some pupated. Also it was evident that some sort of

relationship exists between numbers pupated and atmospheric humidity (Table 41).

TABLE 41 Number of Newly Moulted Larvae which Pupated from a total of 40, during a 35 day Period of Starvation in Different Relative Humidities at 25°C

Humidities % :	0	20	40	60	80	100
No. of Pupae :	2	1	1	10	11	14

Of the larvae which did not pupate a number moulted in all the humidities considered, while a few died at 100% R.H. Data regarding these individuals were omitted from the experimental result, which accounts for the difference in class sizes (Table 42).

12.11 The Influence of Atmospheric Humidity on Total Weight Lost By Starved T. molitor Larvae and how the Rate of Weight Loss Changes with Time

Assuming that T. molitor larvae use reserved food materials when starved, then total weight loss will consist of two components (a) dry material lost (b) water lost.

To get an idea of the general response in time of starved T. molitor larvae to different atmospheric humidities the following procedure was followed. The mean weight changes (corrected for differences in mean initial weight) of T. molitor larvae starved in relative humidities 0, 20, 40, 60, 80 and 100% (listed in Table 43A) were plotted against time in Figure 19.

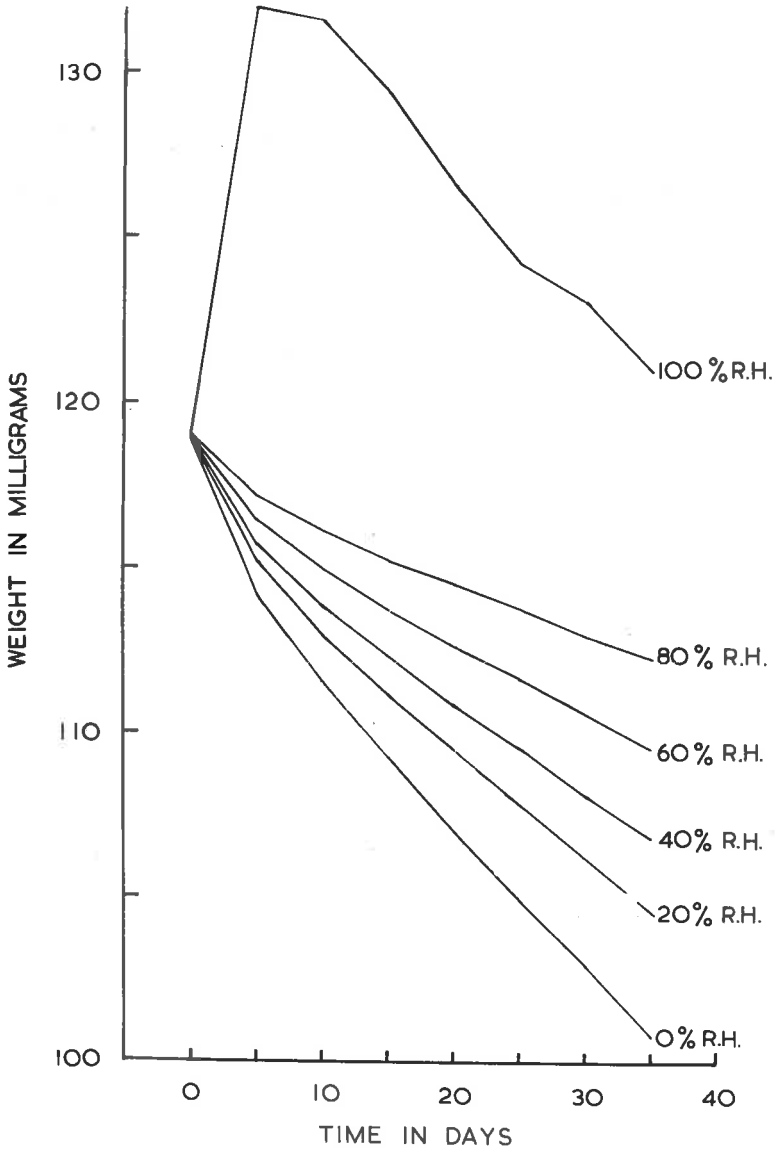


Fig. 19: The mean weight change (corrected for differences in initial weight) of T. molitor larvae starved in different humidities at 25°C.

TABLE 42A Mean Weight Changes (corrected for differences in mean initial weight) of T. molitor Larvae Starved in Different Relative Humidities at 25°C (All figures in milligrams)

Relative Humidity	N	Time in Days							
		0	5	10	15	20	25	30	35
0%	35	110.000	114.172	111.651	109.228	107.055	104.986	102.926	100.858
20%	30	110.000	115.302	112.987	111.179	109.380	107.877	106.290	104.671
40%	30	110.000	115.766	113.638	112.328	110.925	109.547	108.154	106.638
60%	30	110.000	116.440	114.973	113.759	112.600	111.617	110.397	109.585
80%	25	110.000	117.348	116.105	115.387	114.499	113.725	112.972	112.282
100%	15	110.000	121.901	121.913	122.365	123.467	124.175	122.903	120.922

From the data listed in Table 42A and illustrated in Figure 19 the following is apparent :

(a) T. molitor larvae when starved in different relative humidities lose weight faster in the lower than in the high humidities.

(b) During the initial period of starvation weight is lost at a faster rate than subsequently. This phenomena is apparent at all levels of humidity up to and including 80%.

(c) At 100% R.H. the weight of starved T. molitor larvae increases over the first ten days and then decreases at a relatively rapid rate.

12.111 Weight Loss as a Function of Atmospheric Humidity

To test the null hypothesis that weight loss in starved T. molitor larvae is not a linear function of atmospheric humidity, the following procedure was followed : The total weight lost by individual larvae starved for 35 days in humidities 0, 20, 40, 60 and 80% at 25°C were

calculated by subtracting initial live weight from final live weight (day 35). This data is summarised in Table 425.

TABLE 425 Weight Lost (mg) by T. molitor Larvae Starved for 35 days in Different Relative Humidities at 25°C.

Humidities	0	20	40	60	80
N	35	36	36	30	25
$\bar{x}$	18.0700	14.4361	12.1625	9.5150	6.7520
Σx	632.45	519.70	437.85	285.45	168.80
Σx^2	11,662.8025	7555.6900	5460.0525	2940.0075	1220.0350
s^2	7.7774	5.1773	3.9481	4.2743	2.5132

Weight loss data summarised in Table 425 were used to construct the analysis of variance for linear regression Table 426. The within classes variances being obviously heterogenous and correlated with treatment means, sums of squares when calculated were weighted according to a factor $w_i = s_i^2/n_i/s_i^2$ using a method similar to that described by Snedecor (1956) p. 288.

TABLE 426 Analysis of Variance For Regression of Total Weight Lost (35 days) on Humidity (Weighted Sums of Squares)

Source of Variation	D.F.	S.S.	M.S.	V.R.
Linear Regression	1	54.2091	54.2091	54.2091***
Deviation from Regression	3	0.3661	0.12205	0.12205 n.sig.
Between classes	(4)	(54.5752)		
Within classes	150	159.6000	1.0640	

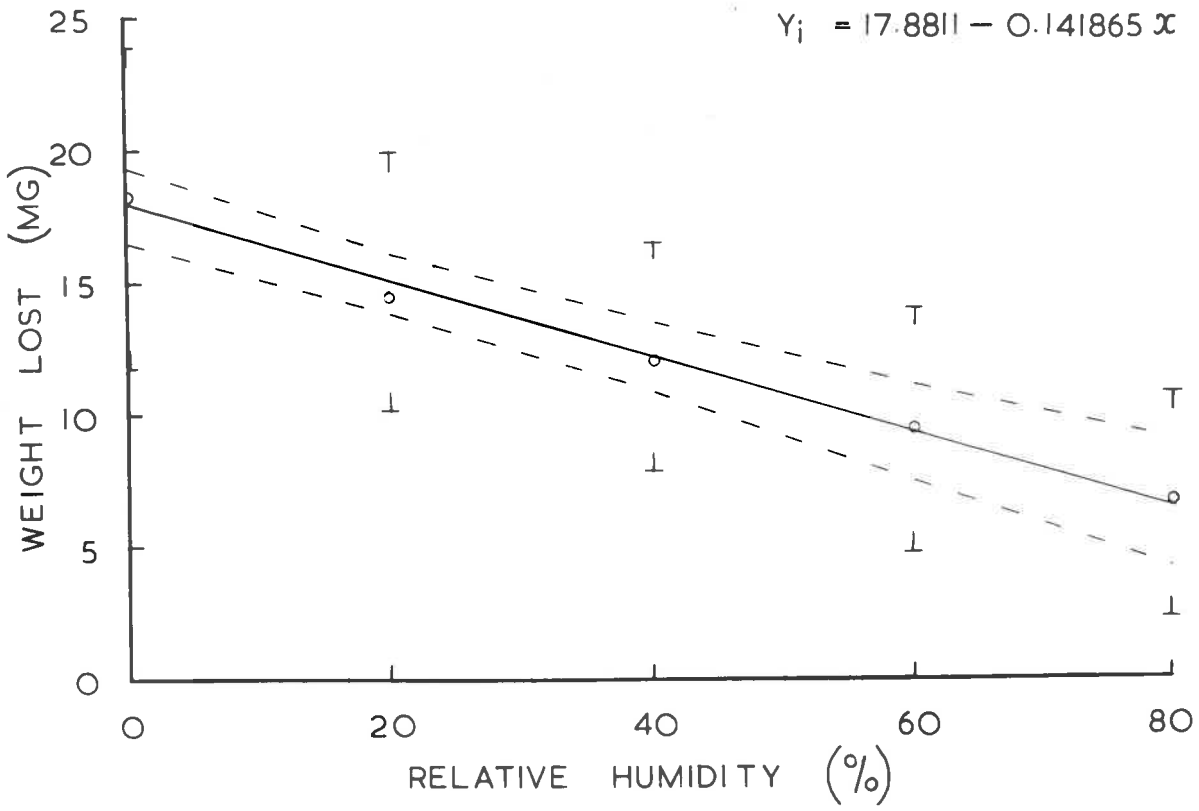


Fig. 20: The relationship between total weight lost and atmospheric humidity in T. molitor larvae starved for 35 days in different humidities at 25°C.

- - - 95% confidence limits for mean values of (y) for any given value of (x)

T
⊥ 95% limits for a value of (y) for a given value of (x)

The variance ratio associated with linear regression of total weight loss on humidity is significant at the 0.1% level, while the variance ratio associated with deviations from regression is non-significant at the 5% level. Therefore I rejected the null hypothesis and accepted that in T. molitor larvae after 35 days of starvation, total weight lost (y) is a linear function of atmospheric humidity (x) in the range 0-93%.

This relationship which can be described by the equation

$$y = 17.0311 - .141065x$$

is illustrated in Figure 20 together with :

(a) 95% confidence limits for mean values of (y) for any given value of (x).

(b) 95% limits for values of (y) for a given value of (x).

The size of the interval expected to contain 95% of the values of (y) (Total weight lost mg) for a given value of (x) (relative humidity) is relatively large and gives an indication of the variability to be expected in measurements taken from insects selected to be physiologically as similar as possible.

12.112 Change in the Rate of Weight Loss with Respect to Time

To investigate the possibility of a change in the rate of weight loss in starved T. molitor larvae with respect to time and if so to find whether this change is independent of atmospheric humidity, the following procedure was followed :

The total weight lost by individual beetles during seven successive five day periods of starvation in relative humidities 0, 20, 40, 60 and 80% was calculated making use of data obtained in the experiment described in Section 12.0.

Data concerning the mean amount of weight (mg) lost during successive five day periods of starvation are given in Table 42D together with the standard errors of the means.

TABLE 42D Mean Weight Lost (mg) by T. molitor Larvae During Successive Five Day Periods of Starvation in Different Humidities at 25°C Together with Standard Errors (S.E.) of the Means

Humidities	Details	Successive Five Day Periods						
		1	2	3	4	5	6	7
0%	$\bar{x}$	4.0000	2.7000	2.2134	2.1000	2.0500	2.0429	2.0714
	S.E.	.1011	.0205	.0070	.0320	.0094	.0419	.0040
20%	$\bar{x}$	3.0004	2.2208	1.0520	1.0405	1.0000	1.0057	1.0007
	S.E.	.1061	.0078	.0155	.0050	.0174	.0152	.0020
40%	$\bar{x}$	3.0547	1.0006	1.0523	1.4058	1.0100	1.0051	1.0155
	S.E.	.0092	.0075	.0055	.0130	.0121	.0212	.0150
60%	$\bar{x}$	2.5000	1.4017	1.2007	1.0100	1.0750	1.0000	1.0050
	S.E.	.0570	.0427	.0512	.0150	.0117	.0155	.0154
80%	$\bar{x}$	1.0500	1.0040	.0020	.7720	.7000	.7540	.0040
	S.E.	.0772	.0105	.0171	.0001	.0102	.0145	.0141

Using the method of least squares, the best fit line was fitted to these means, at each level of humidity. The curves describing the relationship between weight loss and time at different levels of humidity (illustrated in Figure 21) all assume a similar basic shape which can be described by an equation of the form $y = a + be^{-cx}$ where the constants

a = asymptote

b = intercept on the y-axis in excess of a

c = the relative rate of approach to the asymptote

The values of the constants, giving the line of best fit, are listed in Table 42E, together with the standard errors of the constants.

TABLE 42E The Values and Standard Errors (S.E.) of the Constants in the Regression Equation ($y = a + be^{-cx}$) Describing the Relationship Between Weight Loss and Time in T. molitor Larvae Starved at Different Relative Humidities at 25°C

Relative Humidities		Constants		
		a	b	c
0%		2.07321	11.31603	-1.46386
	S.E.	.03165	.36137	.07375
20%		1.62371	7.34837	-1.28039
	S.E.	.03049	.36107	.08915
40%		1.38361	6.35297	-1.22226
	S.E.	.02892	.71603	.12125
60%		1.04480	5.09712	-1.19342
	S.E.	.01677	.37966	.07289
80%		0.74259	4.05126	-1.37938
	S.E.	.01455	.45761	.10477

Fig 21:

$$Y_o = 2.073214 + 11.816083e^{-1.463565x}$$

$$Y_{so} = 1.628714 + 7.948368e^{-1.286393x}$$

$$Y_{ao} = 1.358609 + 6.352972e^{-1.222264x}$$

$$Y_{eo} = 1.044800 + 5.097118e^{-1.198421x}$$

$$Y_{so} = 0.742590 + 4.031856e^{-1.279375x}$$

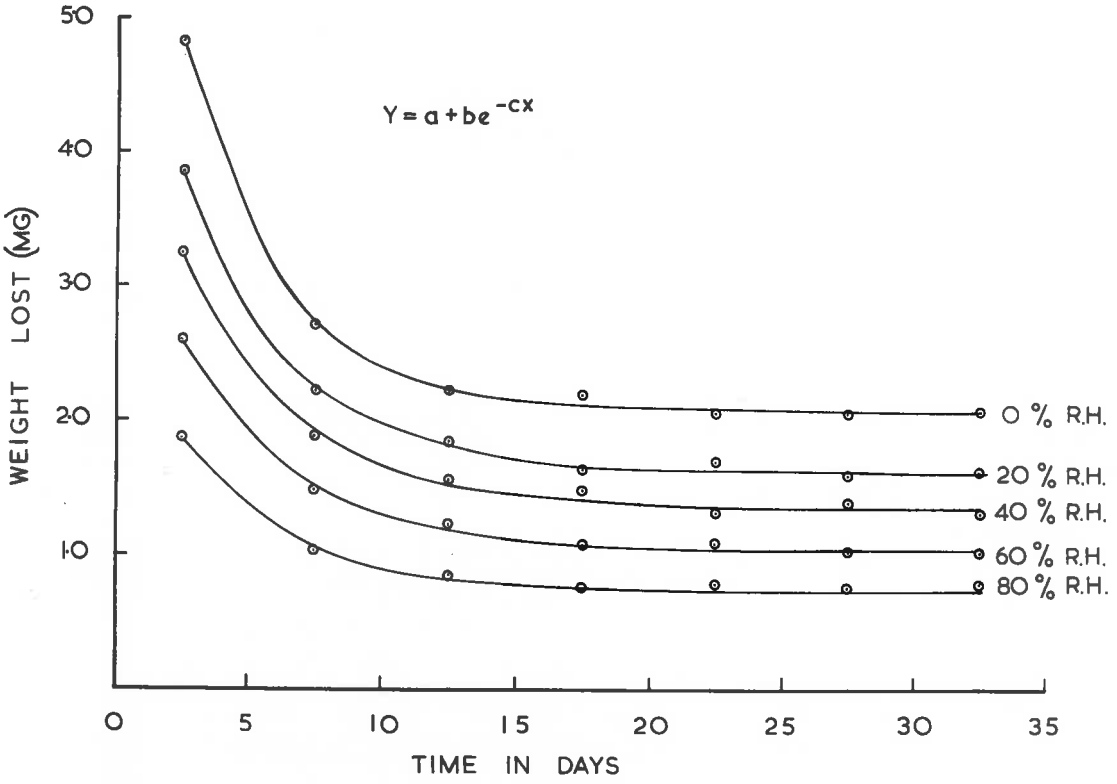


Fig. 21: The change in the mean rate of weight loss (mg/5 days) of starved T. molitor larvae, with respect to time and humidity, at 25°C.

Constants given in Table 42E were used to construct Figure 21 where observed points are the mean amounts of weight (mg) lost by larvae during successive five day periods of starvation at different humidities (listed in Table 42D).

Visual inspection of Figure 21 indicates that all the observed points at each level of humidity lie closely along the calculated curve. Since the curves describing the relationship between weight loss (y) and time (x) at different humidities, all surround a similar basic shape which can be described by an equation of the form $y = a + be^{-cx}$. I accepted :

(i) That there is a change in the rate of weight loss of starved T. molitor larvae with respect to time.

(ii) That the influence of atmospheric humidity on this change with time, is only in magnitude and not in principle, in other words the change in the rate of weight loss with time is independent of humidity and is possibly associated with starvation.

12.12 The Change in the Water/Dry Material Ratio of Starved T. molitor Larvae with Respect to Humidity, Time and Temperature

Buxton (1930) claimed that T. molitor larvae when starved in humidities below 65% can maintain a constant dry/wet ratio. This author suggested that this is achieved by additional dry material being consumed in the lower humidities and the water of metabolism retained. However, should Mellanby's (1936) claim be true that the rate of dry material consumption of starved T. molitor larvae is determined by temperature alone, then since it was shown (Section 12.11) that

larvae
total weight loss in starved T. molitor decreases with an increase in humidity, I would expect the water/dry material ratio to increase with an increase in humidity.

(A) To test the null hypothesis that the water/dry material ratio is not a function of atmospheric humidity, I calculated the ratio 100 x final water content (mg)/final live weight for individual insects starved for 35 days in humidities 0, 20, 40, 60 and 80% at a temperature of 25°C (Experiment described in Section 12.0). A summary of this data is given in Table 43A. For comparison data concerning the wet/dry ratio of larvae starved for 35 days at 100% R.H. as well as data concerning this ratio in the control group at day zero were included in Table 43A but were not used in testing the null hypothesis.

TABLE 43A Summary of Data Concerning the Water/Dry Material Ratio of T. molitor Larvae Starved for 28 days in Different Relative Humidities at 25°C

Details	Controls	Relative Humidities (%)					
		0	20	40	60	80	100
N	40	36	36	36	30	25	13
$\bar{x}$	58.0780	56.1497	58.8078	59.8833	60.8000	61.7804	69.3531
Σx	2323.12	2035.24	2117.08	2155.80	1824.00	1545.01	901.59
Σx^2	135,406.7672	118,818.4170	124,609.8582	129,197.0578	111,162.4686	95,556.4861	62,620.6802
s^2	12.4770	13.0174	6.8047	9.7154	9.0783	12.3878	7.7190

Inspection of Table 43A indicates an apparent increase in the mean water/dry material ratio with an increase in humidity (range 0-60%). This data was therefore used to construct an analysis of variance for regression Table 43B, using the method described by Snedecor (1956) p. 426.

TABLE 43B Analysis of Variance of Regression of Water/dry Material Ratio on Humidity for Data Summarised in Table 43A

Source of Variation	D.F.	S.S.	M.S.	V.R.
Linear Regression	1	350.4477	350.4477	33.9428 ***
Deviations from regression	3	1.2466	0.4162	< 1 n. sig.
Between classes	(4)	(351.6943)		
Within classes	156	1702.4500	10.7753	

Since the variance ratio associated with linear regression of water/dry material ratio on humidity is significant at the 0.1% level, while the variance ratio associated with deviations from linear regression is non-significant at the 5% level, I reject the null hypothesis and instead accept that, in T. molitor larvae after 35 days of starvation, their water/dry material ratio (y) is linearly related to atmospheric humidity (x) in the range 0-60%. This relationship which can be described by the equation : $y = 14.00535 + .05566x$ (constants calculated from data in Table 43A) is illustrated in Figure 22, together with :

(a) 95% confidence limits for mean values of (y) for given values of (x).

(b) 95% limits for values of (y) for given values of (x).

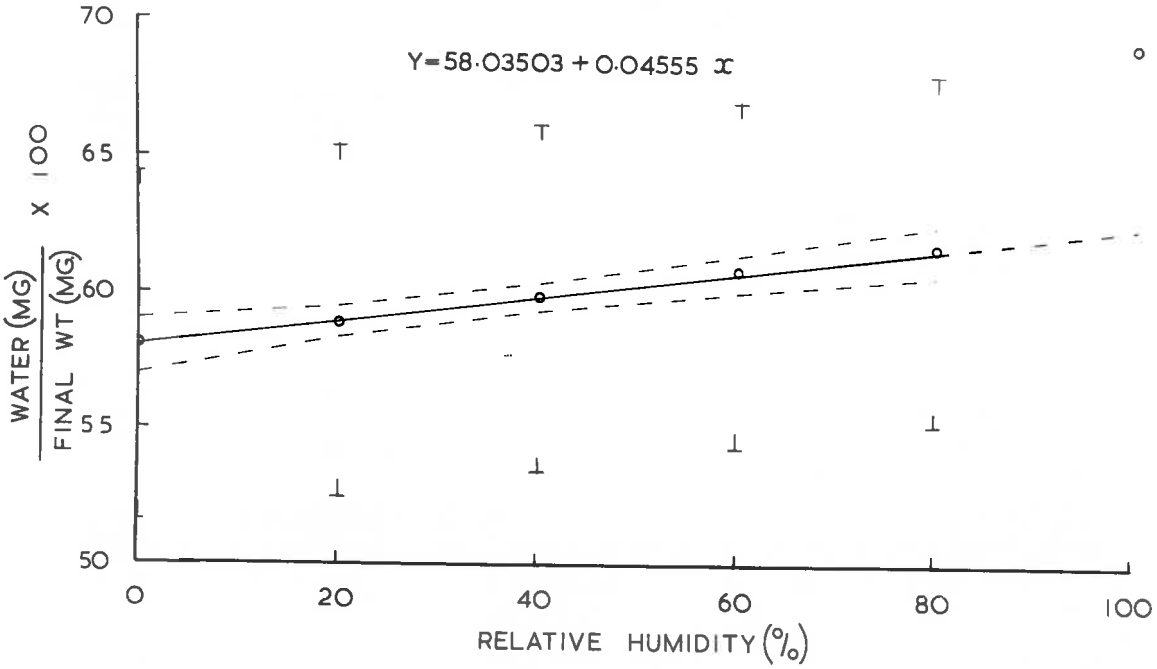


Fig. 22: The relationship between the water/dry material ratio of T. molitor larvae, after being starved for 35 days in different humidities at a temperature of 25°C.

- - - 95% confidence limits for mean values of (y) for a given value of (x)

⊤ ⊥ 95% limits for a value of (y) for a given value of (x)

The size of the interval expected to contain 95% of the values of (y) for a given value of (x) is relatively large. This substantiates the statement made by Mellanby (1936) as regards the variability in the body composition of T. molitor larvae.

A positive linear regression of water/dry material ratio on atmospheric humidity is not in accordance with the findings of Buxton (1930). It seems more to favour the hypothesis put forward by Mellanby (1936) that the rate of dry material consumption of starved T. molitor larvae is determined by temperature alone.

If we compare the mean water/dry material ratio of larvae starved for 35 days in 0% R.H. with the mean ratio of the control group (Table 45A), they appear to be very similar. To find at what humidity level the wet/dry ratio of larvae starved for 35 days is higher than that of newly moulted larvae, t-tests (one-tailed) were performed between the mean wet/dry ratio of the control group and that of larvae starved at different humidities, working from 0% R.H. upwards.

TABLE 45C Differences between the mean wet/dry Ratio of Non-Starved T. molitor Larvae and Larvae Starved for 35 days in Different Humidities at 25°C

Humidity	Differences between means	t-values	P
0%	.0717	t_{78} = .[illegible]	< .45
25%	.7293	t_{78} = .[illegible]	< .15
40%	1.7439	t_{78} = 1.[illegible]	> .025

Information in Tables 43A and 43C suggest that after 35 days of starvation at 0% R.H. at 25°C the wet/dry ratio of *T. molitor* larvae is the same as that of normal newly moulted larvae, and that only in relative humidities of 40% and above does the ratio exceed that of newly moulted larvae.

(B) The fact that *T. molitor* larvae can maintain a constant wet/dry ratio even when starved for 35 days at 0% or 20% R.H. at 25°C suggested to me that these insects whatever the mechanism, might be so adapted, to maintain this ratio in dry air independent of temperature and time. To investigate this idea the following experiment was conducted :

A group of newly moulted larvae in the weight range 130-140 mg was selected from cultures of the same age. From these larvae, 30 groups of 30 larvae each were randomly selected. From these groups, six, again randomly selected were allotted to each of the temperatures 15°, 20°, 25°, 30° and 35°C. Starting at day 0, one group from each temperature was removed at 30 day intervals. The live weight of individual insects in these groups was then recorded upon which the insects were dried to constant weight and the dry weight recorded.

From this data the ratio : 100 x final water content (mg)/ final live weight for each insect was calculated. A summary of the data is given in Table 43B.

TABLE 43D <u>A Summary of Data Concerning the Wet/dry Ratio of T. molitor Larvae Starved in dry air for Different Periods of Time in Different Temperatures</u>

Temp.	Details	Number of Days Starved					
		0	20	40	60	80	100
15°C	N	30	30	30	29	30	28
	$\bar{x}$	57.3407	57.4537	56.9655	56.0041	58.0045	58.2296
	s^2	8.1825	10.4791	15.3054	6.9941	11.6909	7.0515
20°C	N	30	30	27	23	17	15
	$\bar{x}$	56.0155	57.0950	57.4178	58.7765	56.6018	57.8565
	s^2	16.0796	20.1221	11.3072	6.3643	9.6610	12.0926
25°C	N	30	27	20	11	16	17
	$\bar{x}$	58.1170	57.0985	55.5955	58.8132	55.7456	57.0200
	s^2	20.4535	12.1129	12.6733	16.5371	12.1697	14.0232
30°C	N	30	27	25	17	24	21
	$\bar{x}$	57.4170	55.3619	57.2061	55.9094	55.6300	56.4014
	s^2	10.1086	13.2645	10.1810	9.0214	4.4605	11.3023
35°C	N	30	30	21	14		
	$\bar{x}$	56.2266	54.7577	56.0089	58.0350		
	s^2	14.0360	14.3385	27.3605	12.9461		

After studying the contents of Table 43D, I decided that it would be more useful to discuss the data concerning the wet/dry ratio of <u>T. molitor</u> larvae starved for different numbers of days in dry air at different temperatures than to do extensive statistical calculations to test the null hypothesis concerning the consistency of the wet/dry ratio.

The size of the within sub-class variances at day zero, indicates the variability in the wet/dry ratio of newly moulted larvae. The actual limits observed between which the wet/dry ratio varies were found to be 50.39-65.66. An explanation for this variability is difficult to make since these larvae were bred in the same cultures under conditions which seemed very favourable to them. However, the possibility of chance selection of samples with different means is obvious.

On inspection of the individual sub-class sizes the following is apparent. Although each sub-class started with 30 experimental animals the recorded numbers differ considerably. The way in which class sizes decreases, is especially noticeable.

(i) As time of starvation increases.

(ii) As the temperature increases.

The differences in sub-class numbers was brought about by larvae that pupated during the course of the experiment and had to be discarded. This was so in all temperatures except at 35°C where larvae neither moulted nor pupated. Differences in class numbers in this temperature was a result of larvae dying.

On inspection of sub-class means, it becomes apparent that means do differ. Although it is possible to demonstrate differences between means significant at the 5% level, in temperatures 15°, 30° and 35°C, this knowledge does not seem very relevant because no trend with either time or temperature seems obvious.

It is conceivable that significant differences could have been brought about by removal of a certain type of experimental animal (e.g.

those that pupated). In Table 41 a relationship was shown between the number of larvae which pupated and atmospheric humidity. In Section 12.124 a relationship was shown between the wet/dry ratio of larvae and humidity. This seems to suggest a possibility that those larvae with the higher wet/dry ratio might pupate first, in which case the mean dry/wet ratio should drop on removal of pupae from the experiment. That this could be the case, is vaguely suggested in Table 45b.

At 35°C where larvae in some sub-classes were discarded because they died, the situation could be different. Should these larvae have died of desiccation, then a more or less constant wet/dry ratio could have been maintained artificially, if those larvae with a low wet/dry ratio died first. However, since Buxton (1930) suggested that T. molitor can regulate their metabolism so as to maintain a constant dry/wet ratio, I would expect them to have died of starvation, in which case a lower wet/dry ratio, noticeable after 20 days of starvation in 35°C, might have been a stimulus to increase the metabolic rate. Assuming that larvae in 35°C died of starvation, then it follows that since all larvae were dead within 80 days, while the wet/dry ratio after 60 days is higher if anything, than that of newly moulted larvae, that T. molitor can maintain a constant wet/dry ratio when starved in dry air, until they die.

By means of a summary we can say that, although significant differences exist between sample mean wet/dry ratios of T. molitor larvae starved for different periods of time, at some temperatures, these differences are relatively small and since the means show no trend with either time or temperature it seems reasonable to accept that T. molitor

larvae maintain a constant wet/dry ratio until death in dry air, and independent of temperature.

12.13 The Influence of Atmospheric Humidity on Dry Material Consumption in Starved T. molitor Larvae

Buxton (1930) claimed that T. molitor larvae, when starved in humidities below 60%, can maintain a constant dry/wet ratio. This, he suggested, is achieved by additional dry material being consumed in lower humidities and the water of metabolism retained to compensate for that lost by evaporation.

Since my findings as regards the relationship between the wet/dry ratio of starved T. molitor larvae and atmospheric humidity (Section 12.12) do not substantiate that of Buxton (1930), doubt was also raised in my mind as to the presence of the mechanism by which these insects maintain a constant dry/wet ratio as proposed by this author.

To test the null hypothesis that dry material consumption is not a function of atmospheric humidity, I compared the final dry weight of larvae starved for 33 days at 25°C in relative humidities 0, 20, 40, 60 and 80% (data obtained in experiment described in Section 12.0).

Because the size of the larvae might have an influence on the final dry weight, a linear regression analysis was performed on the data. Regressions of final dry weight (mg) (y) on initial live weight (mg) (x) were calculated, one for each humidity. The data and calculations are summarised in Table A11A. To determine the amount of dry material used during starvation, the regression of initial dry weight (y) on

initial live weight (x) for the control group was calculated and is summarised in Table A51D.

Slopes and intercepts of the regressions (Table A52A) were compared in the analysis of variance Table 44A

TABLE 44A Analysis of Variance of Data Summarised in Table A51A

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	4	14.4578	3.60945	0.236 n.sig.
Displacements	4	11.2532	2.81330	0.183 n.sig.
Error	182	2782.8860	15.28509	

Since the variance ratio associated with both slopes and displacements are non-significant at the 5% level, I accept the hypothesis that the five regression lines of final dry weight (y) on initial live weight (x), one for each of the humidities 0, 20, 40, 60 and 80% are identical. Therefore I also accept that atmospheric humidity in the range 0-80%, has no influence on the amount of dry material left in the bodies of T. molitor larvae starved for 35 days in 25°C. Again, these results are not in accordance with that of Buxton (1930) and seem more to favour the idea put forward by Mellanby (1936), who proposed that the lowering of the humidity does not increase the metabolic rate of starved T. molitor larvae. This idea is to be further investigated in Section 12.14.

The relationship between initial dry weight (y) and initial live weight (x) in newly moulted larvae (Control group, Table A51B) can be described by the equation :

$$y_{(1)} = 49.9309 + .513079\ (x - 119.025)$$

After 35 days of starvation in 25°C the relationship between final dry weight (y) and initial live weight (x) can be described by the equation

$$y_{(2)} = 43.2280 + .469807\ (x - 119.4105)$$ (Pooled regression Table A51A)

Since the mean live weight of beetles in the control group is practically the same as that of the experimental group, the difference between $\bar{y}_1 - \bar{y}_2$ i.e. 49.9309 - 43.2280 = 6.7029 mg. should give a fair estimate of the amount of dry material used by the average larva during a 35 day period of starvation in 25°C. This amount represents an average rate of dry material consumption of 0.2 mg/day. Since Buxton showed a reduction in the carbon dioxide output of starved T. molitor larvae with time, 0.2 mg/day might over-estimate the actual rate of consumption during the latter part of starvation.

If we compare the dry weight lost during 35 days of starvation (6.7029 mg) with the total weight lost at 80% R.H. during the same period of starvation (6.7020 mg) (Table 42B), the similarity of the two quantities suggests that the weight lost in 80% R.H. comprises dry material lost. Assuming that no change occurred in the water content with time then the basic shape of the curves describing the change in weight loss with time (Figure 21) must also describe the basic way in which dry material consumption changes with time.

In testing the null hypothesis that dry material consumption is not a function of humidity (above), the data obtained at 100% R.H. was omitted. This was done mainly because :

(a) 100% was the only humidity in which the weight of starved larvae increased (Figure 19), and

(b) Because of deaths and pupation only 13 of the original experimental animals were left at day 35 which could have represented a certain type.

Keeping this in mind, but trying to get a general indication of whether the response of larvae starved in 100% R.H. is similar to those in lower humidities, a linear regression analysis was performed using the same data summarised in Table A51A but including the regression of final dry weight on initial live weight of larvae starved in 100% R.H. The data and calculations are summarised in Table A52 while slopes and intercepts of the different regression lines were compared in Table 44B.

TABLE 44B Analysis of Variance of Data Summarised in Table A52

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	5	14.8724	2.97448	0.185 n.sig.
Displacements	5	369.2031	73.84062	4.615***
Error	165	2640.6958	16.00421	

If we compare Table 44A and B it is obvious that the inclusion of data obtained from larvae starved in 100% R.H. cause a displacement in the regression lines.

Comparing the mean final dry weight of larvae starved in different humidities (Table A52), then those starved in 100% R.H. show a lower mean final dry weight. These results, although not considered conclusive evidence for, do suggest that the overall rate of dry material consumption of larvae starved in 100% R.H. where water was taken in from

the surroundings might be higher than that of larvae starved in humidities of 80% and below.

12.14 The Change in Oxygen Intake of Starved T. molitor Larvae With Respect to Time and Humidity

The metabolic rate of starved T. molitor larvae decreases with time in dry air (Buxton, 1930; Mellanby, 1934a) and to a greater extent in higher humidities (Buxton, 1930).

Because :

(a) The average daily rate of dry material consumption in starved T. molitor larvae is relatively low (Section 12.13).

(b) The initial dry material content of larvae is very variable (Buxton, 1930; Mellanby, 1936 ; also Table A5B). I realised that it might be extremely difficult to demonstrate differences in dry material consumption of larvae starved in different humidities over relatively short periods of time, either by :

(i) measuring differences in the final dry material content over 28-35 days (Buxton, 1930; also Section 12.13), or by

(ii) analytic measures, over similar periods of time (Mellanby, 1932a).

In order to : (a) get further evidence for or against accepting the hypothesis that atmospheric humidity has no influence on the metabolic rate of starved T. molitor larvae, as well as

(b) to investigate the possibility suggested in Section 12.13 that water intake from the surroundings might influence the metabolic rate of T. molitor larvae; I decided to measure the metabolic rate of T. molitor larvae starved in different humidities.

Oxygen intake (microliters/hour) was used as a measure of metabolic rate. Oxygen intake was measured experimentally, using "Warburg" constant volume respirometers, and the technique described by Umbreit, Burris, Stauffer (1957).

Since the apparatus used could accommodate only fifteen manometers at a time, the numbers of insects involved in the experiment required that a number of successive runs should be completed daily. Since this could result in, taking into account time required for equilibration purposes, that some measurement need to be taken after natural light has faded, a technical difficulty had to be overcome. Michal (1961) demonstrated a daily oscillation in the oxygen consumption of mealworm larvae which reached a maximum during the night when the insects were most active. This was confirmed by Cloudsley-Thompson (1955), who demonstrated a composite 24 hour rhythm of activity correlated with light and darkness.

In order not to confound the experimental results with the effects of greater activity during natural darkness, the temperature control mechanism (2 darkened electric bulbs) in the apparatus used was altered by replacing one darkened bulb with a clear one, (40 watts) thus providing a continuous source of light.

To test the effectiveness of this system in preventing larvae from becoming active during periods of natural darkness, as well as to get some preliminary knowledge on the change in oxygen intake of starved *T. molitor* larvae with time, the following experiment was conducted :

Twelve newly moulted larvae in the weight range 110-130 mg were selected from the same culture, weighed and the oxygen intake measured. Oxygen intake was measured in microliters/hour at 25°C ± 0.1°C and the relative humidity within the manometer flasks was ± 85%. Measurements were taken at hourly intervals over a period of twenty-four hours, after which larvae were weighed again and placed in dry air at 25°C. This procedure was repeated five times at four day intervals.

Although minor fluctuations occurred in hourly measurements of the oxygen intake of individual insects, readings were remarkably similar. Thus, for the purpose of this experiment the mean oxygen intake (microliters/hour) was calculated from the total volume of oxygen taken in between 7am - 6pm (day time) and from 7pm - 6am (night time). Data as regards oxygen intake of starved T. molitor larvae during day time and night time and how this changes with time are summarised in Table 45A and illustrated in Figure 23.

TABLE 45A: Summary of Data Concerning the Mean Oxygen Intake (microliters/hour) of T. molitor Larvae After Consecutive 4 day Periods of Starvation in dry air at 25°C; measured (a) 7am - 6pm (day time), (b) 7pm - 6am (night time)

Details	Days Starved									
	0		4		8		12		16	
	Day	Night	Day	Night	Day	Night	Day	Night	Day	Night
N	14	14	14	14	14	14	14	14	14	14
Σx	680.69	752.37	470.73	527.60	394.34	445.72	432.12	423.85	410.71	410.89
$\bar{x}$	48.62	53.74	33.62	37.69	28.17	31.84	30.87	30.28	29.34	29.35
Σx^2	33081.7537	42060.6535	16590.2295	20913.0644	12060.1442	14468.9960	14344.0874	13979.3863	13121.7129	13211.4507
s^2	60.5061	186.6069	58.7913	79.0631	73.1990	175.1749	77.2725	68.0929	82.4217	98.6022
S.E.	2.0769	3.6528	1.5147	2.3764	2.2866	3.5373	2.3493	2.5085	2.4264	2.5257

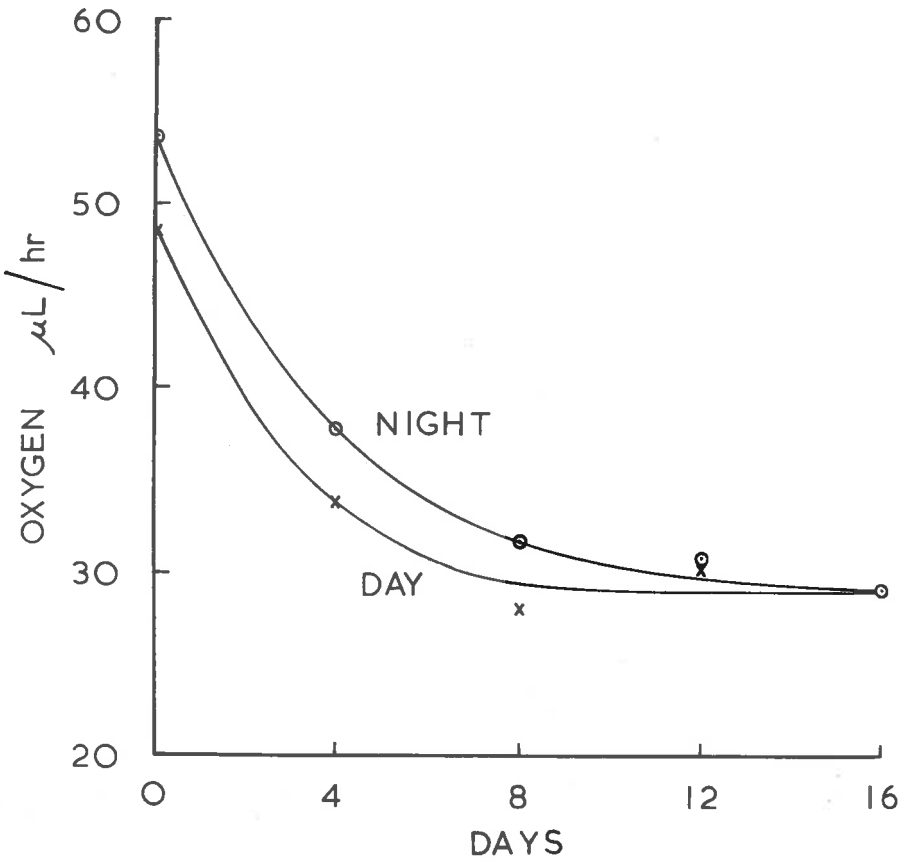


Fig. 23: The change with time in the mean rate of oxygen intake of T. molitor larvae starved in dry air at 25°C.

Night: Mean rate of oxygen intake during the hours 7 p.m. - 6 a.m.

Day: Mean rate of oxygen intake during the hours 7 a.m. - 6 p.m.

From the data summarised in Table 45A and illustrated in Figure 23 the following general conclusions were drawn :

(i) Despite the presence of a continuous source of light, the mean oxygen intake of starved T. molitor larvae is higher during the hours of natural darkness than during daylight hours. This is especially noticeable during the early stages of starvation.

(ii) The within classes variances of night measurements are generally higher and do fluctuate more than day measurements. This was the result of only some larvae, showing only on some nights a relatively large increase in their oxygen intake over that during the day time, the latter being continued at a steady rate.

(iii) The size of the within class variances can partly be explained by differences in the initial size of the larvae used in this experiment.

(iv) The mean oxygen intake of T. molitor larvae starved in dry air decreases with time in a manner very similar to the way in which total weight loss decreases with time, Figure 24.

Based on the knowledge obtained from this experiment, a further experiment was designed to find whether or not atmospheric humidity has an influence on the O_2 intake of starved T. molitor larvae.

For the purpose of this experiment, the oxygen intake of newly moulted larvae in the weight range 100-150 mg, starved at 25°C in humidities 0, 40, 60, 80, 90 and 95% was measured after 0, 5, 10, 15, 20, 25 and 30 days. Oxygen intake was measured in microlitres/hour at 25°C ± 0.1°C and the relative humidity within the manometer flasks was ± 3%. Measurements were taken at hourly intervals over a period of four hours

after allowing two hours for equilibration in the constant temperature bath. The latter was found necessary especially during the early part of the experiment to allow larvae to settle down after being weighed and transferred to the manometer flasks.

Starting at 6 am, two runs were completed daily and during natural day light hours. Using 12 manometers for each run only four replicates could be handled each day. To get a reasonable number of replicates the experiment was started in four waves. Twenty four larvae collected on day zero were randomly distributed with respect to humidity, time of the day and manometers and the oxygen intake measured. This procedure was repeated on days 1 and 2 and on day 3 only half the number of larvae were used, giving a total of fourteen replicates per treatment. This completed the measurement of oxygen intake after zero days starvation in the various humidities. This whole procedure was repeated at five day intervals, using the same manometers for the same larvae, and measuring their oxygen intake during the same time of the day as was done previously.

To allow for the possible influence of size on oxygen intake; the oxygen intake (microliters/hour), the mean of four hourly measurements, of individual larvae after consecutive five day periods of starvation in the various humidities were expressed as a ratio of their mean oxygen intake on day zero, i.e. (100 x oxygen intake, microliters/hour (day x_1)/ oxygen intake, microliter/hour (day zero). These data are summarised in Table 45B.

TABLE 45B Summary of Data Concerning the Oxygen Intake (expressed as a ratio (100 x Oxygen-intake, microliters/hour (on day x_1)/Oxygen-intake, microliters/hour (on day zero)), of T. molitor larvae after Successive 5 day Periods of Starvation in Different Humidities at 26°C

Humidities	Details	Period of Starvation (days)						
		0	5	10	15	20	25	30
0%	N	13	13	13	13	13	13	13
	$\bar{x}$	100.000	68.2736	58.0015	66.1162	72.4569	79.2658	78.3851
	s^2		140.9307	132.2439	664.5466	981.1358	1097.8087	1514.0180
	S.E.		3.2926	3.1524	7.1497	8.8535	9.1896	10.0556
40%	N	14	14	14	14	14	14	14
	$\bar{x}$	100.00	52.1921	46.1921	59.1950	75.4143	72.3707	74.3564
	s^2		29.5396	95.4290	629.1309	1005.0469	672.7887	772.1503
	S.E.		1.4277	2.8107	6.7035	8.4720	6.3321	7.4265
60%	N	14	14	14	14	14	14	14
	$\bar{x}$	100.00	50.1929	39.1714	45.2949	53.2935	56.1607	55.0550
	s^2		62.3732	75.0975	149.7206	552.3800	490.9825	966.7235
	S.E.		2.1107	2.3160	3.2732	6.1806	5.9091	6.4736
80%	N	12	12	12	12	12	12	12
	$\bar{x}$	100.00	45.3092	39.0442	45.2685	45.2833	49.2258	53.0475
	s^2		56.8830	78.5540	307.2802	233.2724	631.9690	754.5859
	S.E.		2.1706	2.5405	5.0801	4.4279	6.5575	7.0514
90%	N	12	12	12	12	12	12	12
	$\bar{x}$	100.00	50.3017	42.1492	54.8558	55.0858	65.0242	77.2592
	s^2		59.2821	70.2969	439.4523	309.3142	995.2725	2159.0269
	S.E.		2.2218	2.4305	5.9796	5.0765	9.1076	15.3536
95%	N	9	9	9	9	9	9	9
	$\bar{x}$	100.00	46.2044	42.0833	52.0222	51.2478	64.8733	75.7144
	s^2		127.4974	92.1922	428.0130	420.9750	2101.7143	3031.6285
	S.E.		3.9828	3.5747	7.3214	7.2542	16.5142	18.7379

When studying the data summarised in Table 45B, I decided that it might be more useful and relevant to the problem at hand to discuss the data rather than to make elaborate statistical tests to demonstrate differences between sub-class means.

If we consider the mean relative rates of oxygen intake at different levels of humidity and time, the following becomes apparent :

Having expressed oxygen intake on day (x_t) as a (percent) ratio of the oxygen intake at day zero, the relative rate of intake for all humidities at day zero is 100%. During the first five days a sharp drop in the relative rate of oxygen intake, down to round the 50% mark occurred in all the humidities considered. After day five a further decrease took place, but at a slower rate, and the all time minimum rate of oxygen uptake is reached after ten days of starvation in all the humidities considered. At this stage the minimum rate of oxygen uptake reached after ten days' starvation in 0% R.H. for example, is higher (statistically significant) than the minimum value reached in humidities 60 and 80%. This seems to lend some support to Buxton's (1930) hypothesis that carbon-dioxide output in starved *T. molitor* larvae is reduced to a greater extent in higher humidities.

Having reached the minimum value after ten days of starvation, the mean relative rate of oxygen intake does increase beyond this minimum value in all humidities concerned, as starvation proceeds. It is further obvious that the mean relative rate of oxygen intake increases at a faster rate and reaches a higher value in humidities 40% and below also in humidities 90% and above, as compared to that in humidities 60

and 80%. The fact that the differences between the mean relative rate of oxygen intake after 30 days starvation, in the intermediate humidity range (60-80%) on the one side and that in the lower and upper humidity range on the other side, just fail to reach the 5% level of significance, is not surprising in view of the extremely large within sub-class variances.

The within sub-class variances although large are relatively small during the period when the mean relative rate of oxygen intake decreases. As starvation proceeds after day ten and the mean relative rate of oxygen intake increases, so do the within sub-class variances increase to reach a maximum by day 30.

The large within sub-class variances, which are indications of the variability in the measurements of individual insects, up to and including day ten are a result of the relative rate of oxygen intakes of individual larvae being reduced at different rates. The limits between which the rate of oxygen intake of individual larvae varied after ten days starvation, taken over all humidities, were observed to be 28.77 - 92.0% of the oxygen intake at day zero.

The within sub-class variances increasing in size after day ten were brought about by an increase in the rate of oxygen intake of some larvae, the numbers of which increased with time, while in some larvae the rate of oxygen intake remained at the level reached at day ten or was even reduced to below this level. The larvae, increasing their rate of oxygen intake, were not necessarily those with a comparatively high rate of intake at day ten.

The differences in the mean relative rate of oxygen intake observed between humidities after 30 days were brought about by the fact that loss larvae have increased their oxygen intake in humidities 80-86% than in either of the lower and the higher humidities. The limits between which the rate of oxygen intake of individual larvae varied, taken over all humidities after 30 days starvation, were observed to be 19.53 - 184.0% of the oxygen intake at day zero.

From the data summarised in Table 45B and discussed above, it is obvious that the inter-relationship between the metabolic rate of starved T. molitor larvae, and atmospheric humidity and time, is more complex than implied in the work of either Buxton (1930) or Mellanby (1932a, 1934a, 1936). It will seem that the metabolic rate which decreases with starvation does eventually increase in all humidities, but possibly for different reasons and that the comparatively low mean relative rate of oxygen intake shown in humidities 80 and 86% after 30 days starvation (Table 45B) might just be a delayed action and these treatments might come more closely into line with the others after a more prolonged period of starvation.

The picture, as sketched above, can to my mind be explained, if we assume :

(a) That starved T. molitor larvae, after an initial reduction (Table 45B) increase their metabolic rate but in response to different stimuli in different parts of the humidity range.

(b) That these stimuli are :

(i) A low ratio of water to dry matter in the lower humidities stimulates metabolism which results merely in the accumulation of more metabolic water.

(ii) A high ratio of water to dry matter in the higher humidities stimulates metabolism which results in a moult or metamorphosis.

It was shown in Section 12.12 that starved larvae maintain a constant wet/dry ratio when starved in dry air. It was further shown that the initial wet/dry ratio varies considerably round the mean. The larvae with the lower initial wet/dry ratio could be expected to reach the critical lower limit sooner than those with the higher initial wet/dry ratio. The fact that some larvae even after 30 days' starvation in 0% R.H. have not increased their oxygen intake and hence the large within sub-class variance (Table 45B) seems to tie in with this explanation.

In Section 12.12 and Figure 22 it was shown that the mean wet/dry ratio of larvae after being starved for 35 days in different humidities, increases linearly with an increase in humidity. Again, because of the variability in the wet/dry ratio of individual larvae, those with the initial higher wet/dry ratio could reach the critical higher limit sooner than those with an initial low wet/dry ratio. Some larvae were observed to increase their weight when starved in humidities of 90% and above, presumably by absorbing water from the atmosphere (To be discussed in more detail in Section 12.2). This will result in the critical high limit being reached much sooner in R.H. 90% and above, than in, e.g. humidities 80 and 85%. The fact that the mean relative rate of oxygen intake of larvae after 30 days starvation in 90 and 95% R.H. is higher than those starved in 60 and 80% R.H. could tie in with this explanation if we

assume that in 60 and 80% R.H. relatively few larvae have reached the critical high wet/dry limit after 30 days of starvation. The relationship between number of larvae pupated and atmospheric humidity (Table 41) also seems to lend some support to the proposed explanation of the interrelationship between metabolic rates and atmospheric humidity and time as indicated by data summarised in Table 45B.

The hypothesis that the oxygen intake of starved T. molitor larvae, after the initial decrease, increases again in all humidities, but for different reasons and in response to different stimuli in different parts of the humidity range, to my mind may be accepted if it can be shown :

(a) That larvae live for a shorter period of time in the lower and upper humidity range than in the intermediate range, but that they die in all cases after the same amount of dry material has been used.

(b) That more larvae will moult and/or pupate in higher humidities as compared to lower humidities. Further if moulting and/or pupation occurs sooner as humidity increases.

Because : (a) The initial body composition of individual larvae, and therefore presumably also the amount of available reserves varies considerably (Table 45A) (b) The total amount of weight loss and therefore presumably also the amount of water lost over a comparable period of time, varies considerably among individual larvae (Table 45B).

I realised that it will be extremely difficult to test any of these proposed hypotheses. It is attempted however in the sections to follow.

12.15 The Influence of Atmospheric Humidity, Temperature and Size on the Length of Life of Starved T. molitor Larvae

In this section I wish to investigate the influence of temperature and humidity on the length of life of starved T. molitor larvae. I particularly wanted to get answers to the following questions :

(a) Whether or not humidity has any influence on the length of life of starved T. molitor larvae, and if so

(b) Whether length of life changes with a change in humidity in the way suggested in Section 12.14 i.e. whether length of life increases with an increase in humidity up to a point after which it decreases with a further increase in humidity.

(c) If the way in which the length of life changes with humidity is independent of temperature within the normal range.

(d) Whether T. molitor larvae die after having lost the same amount of dry material irrespective of environmental humidity and temperature.

From Section 6 we know that newly emerged T. molitor adults coming from cultures of the same age, may vary in weight ranging from 70-160 mg. These adults originated from larvae, which can be expected to have varied in weight, approximately within the same limits. In order not to confound the influence of size with that of temperature and humidity on length of life a preliminary experiment was designed to test the null hypothesis that the size of the larvae has no influence on the time they would stay alive when starved.

For the purpose of testing this null hypothesis newly moulted larvae were selected from cultures of the same age. These larvae were weighed and divided into the following weight groups containing 50 larvae each : 60-70, 80-90, 100-110, 120-130 and 140-150 mg. These larvae were individually confined in glass tubes in dry air at 25°C. Larvae were inspected every two days until they died and the length of life (days) recorded. At the same time to find whether different sized larvae use proportionately the same amount of dry material before they die a control group was randomly selected together with the experimental larvae, 50 in each weight group. These larvae were weighed and immediately dried to constant weight and the initial dry weight recorded.

(A) To test the null hypothesis that size has no influence on the length of life of starved T. molitor larvae, at the same time taking into account differences in initial weight within weight groups, regressions of length of life in days (y) on initial live weight (x) were calculated one for each weight-group.

A summary of the data and calculations is given in Table A53 while slopes and intercepts of the different regression lines are compared in the analysis of variance Table 46A.

TABLE 46A Analysis of Variance of Data Summarised in Table A53

Source of Variance	D.F.	S.S.	M.S.	V.R.
Slopes	4	6,963.04	1740.760	2.300 n.sig.
Displacements	4	2,701.76	675.425	.892 n.sig.
Error	207	156,650.72	756.7067	

Variance ratios associated with both slopes and displacements being non-significant at the 5% level I accept that the five regression lines, of length of life (y) on initial weight (x) one for each weight group, are identical.

Identical regression lines permit all the observations to be pooled to calculate a single regression of length of life (y) on initial weight (x), involving all the observations. This relationship illustrated in Figure 24 can be described by the equation :

$y = 154.2258 + .75009\ (x - 105.6182)$ (from "Pooled" regression Table A53).

To test the hypothesis that the slope of the pooled regression line is zero, we have the quantity :

Slope/S.E. of Slope = C, distributed as t with 215 D.F.

This hypothesis is rejected at the .001% level of probability because $C > 3.291$.

The hypothesis may therefore be accepted that the life span of T. molitor larvae starved in dry air is a positive linear function of initial wet weight. The standard deviation of points round the fitted line being 27.8129 (Table A53) indicates that the length of life of starved T. molitor larvae, even with the influence of size removed is still very variable and therefore tests based on length of life cannot be expected to be very sensitive.

(3) To determine whether different sized larvae use proportionately the same amount of dry material before they die, the following procedure was followed. Two groups of regressions were calculated;

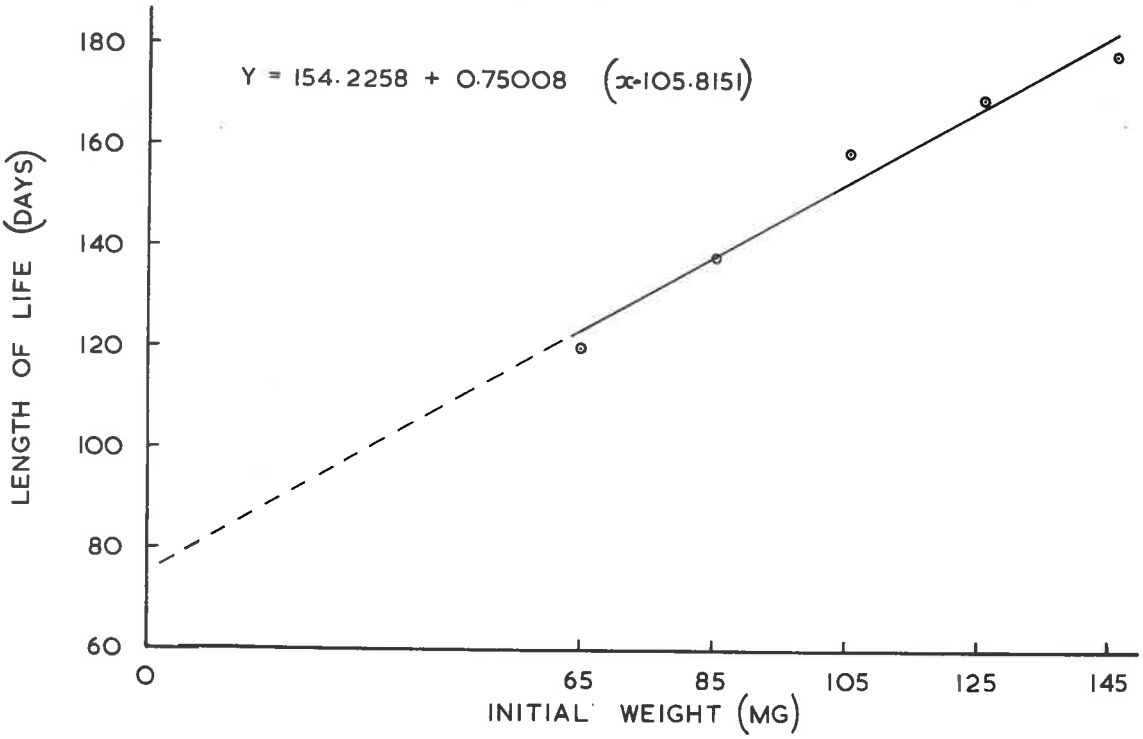


Fig. 24: The relationship between length of life and live weight of T. molitor larvae starved in dry air at 25°C.

(a) Regressions of initial dry weight (y) on initial live weight (x) one for each weight group, using data obtained from larvae in the control group.

(b) Regressions of final dry weight (y) at death, on initial live weight (x), one for each weight group, using data obtained from larvae in the experimental group.

Data and calculations for the control group are summarised in Table A54 and those for the experimental group in Table A55. Slopes and intercepts of the different regressions in these two groups are compared in the analysis of variance Tables 46B and C respectively.

TABLE 46B Analysis of Variance of Data Summarised in Table A54

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	4	31.5544	7.8886	.734 n.sig.
Displacements	4	72.6416	18.1604	1.689 n.sig.
Error	240	2582.2989	9.9268	

TABLE 46C Analysis of Variance of Data Summarised in Table A55

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	4	42.3237	10.580925	1.094 n.sig.
Displacements	4	54.0479	13.511975	1.398 n.sig.
Error	237	2290.3189	9.665376	

The variance ratios associated with both slopes and displacements in both Tables 46B and C being non-significant at the 5% level of probability I accept that the regression lines within both the control and experimental groups are identical. This means that both initial dry weight and dry weight at death are linearly related to initial live weight. These relationships are illustrated in Figure 25, where

observed points were calculated making use of the "common slope" obtained from "Parallel regression" in Tables A54 and A55 respectively. The regression equations describing these relationships are

$$Y_{(I)} = -4.033732 + .475007x$$

$$Y_{(F)} = -0.328724 + .138514x$$

The hypothesis that different weight groups used proportionally the same amount of food reserves before they die may be accepted, if it can be shown that both regression lines go through the origin. To test the latter we have the quantities :

Constant/Standard error of constant = C_1 and C_2 distributed as t with 248 and 215 D.F. respectively.

$C_1 = t_{248} = -4.033732/.770661$: $C_2 = t_{215} = -0.328724/.824454$

$t_{248} = 5.3055$: $t_{215} = 0.3987$

$p < .001$ $p < .7 > .6$

Thus, only the regression line describing the relationship between final dry weight and initial live weight goes through the origin. I therefore reject the hypothesis that different weight groups used proportionally the same amount of reserved foods before death occurred.

To estimate the proportion of dry matter used by larvae with different initial weights we have :

(a) The mean initial dry weight, estimated by

$a_1 + b_1x$ or $-4.033732 + .475007x$ (Table A54)

(b) The mean final dry weight, estimated by

b_2x or $.138514x$ (Line goes through origin).

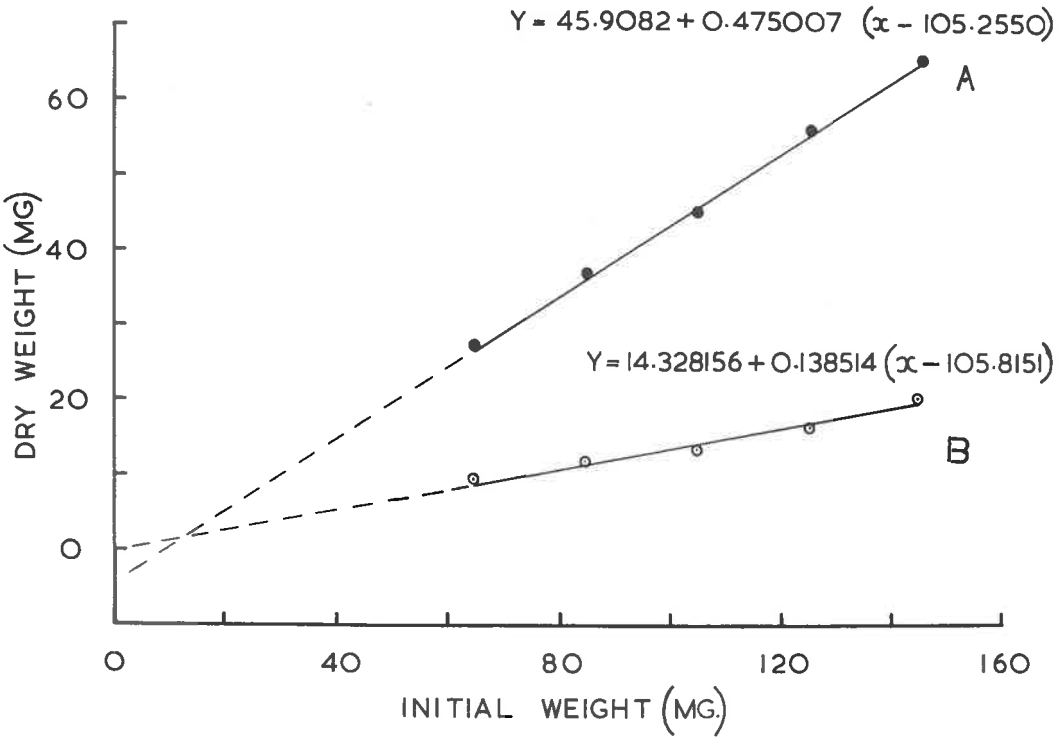


Fig. 25: The change in the dry material content of T. molitor larvae with different initial weights starved to death in 0% R.H. and a temperature of 25°C.

(A) Linear regression of initial dry weight (y) on initial live weight (x)

(B) Linear regression of final dry weight (y) on initial live weight (x)

Therefore mean amount of dry matter used while alive

$$= a_1 + (b_1 - b_2)x$$

and,

Proportion (P) used = mean amount used/mean initial dry weight

$$= \frac{a_1 + (b_1 - b_2)x}{a_1 + b_1 x}$$

$$= 1 - \frac{b_2 x}{a_1 + b_1 x}$$

$$= 1 - \frac{1}{a_1/b_2 + a_1/b_1 x}$$

$$= 1 - \frac{1}{5.4295 - 29.5165/x}$$

i.e. The proportion of initial dry weight used until death occurred, varied between 66.39% for larvae with mean initial weight of 65 mg to 80.00 mg for larvae with mean initial weight of 145 mg.

(C) To investigate the influence of humidity and temperature on the length of life of starved T. molitor larvae, the following experiment was conducted :

A group of newly moulted larvae in the weight range 80-90 mg were selected from cultures of the same age. These larvae were randomly divided into 24 groups of 25 larvae each. These 24 groups were randomly allotted to humidities 0, 20, 40, 60, 80 and 95% in each of the temperatures 20°, 25°, 30° and 35°C where larvae were individually confined in glass tubes. Larvae were regularly inspected and their weights recorded at five day intervals. In addition, the numbers and time of moults and pupations which occurred during the course of the experiment were

recorded as well as the length of life of individual larvae. At death, larvae were weighed and immediately dried to constant weight and the final dry weight recorded.

Data concerning the length of life of T. molitor larvae starved to death in different humidities and temperatures is summarised in Table 46D, while the change in the mean length of life of these larvae with a change in humidity at different temperatures is illustrated in Figure 26.

TABLE 46D A Summary of Data Concerning the Length of Life (days) of T. molitor Larvae Starved in Different Combinations of Temperature and Humidity

Temp.	Details	Relative Humidity %					
		0	20	40	60	80	95
20°	N	24	25	20	22	22	14
	$\bar{x}$	177.5000	174.3696	170.5000	212.3182	205.6739	185.0000
	s^2	1360.8695	1051.2240	759.4736	2498.9137	1614.9602	606.6667
	S.E.	7.5501	6.6362	6.1623	10.6577	8.3703	6.1577
25°	N	24	23	20	24	18	6
	$\bar{x}$	137.9167	138.3043	152.6000	162.2917	151.0000	130.1667
	s^2	534.5906	636.8646	1314.6421	1246.6881	1259.3235	561.4614
	S.E.	4.7136	5.2621	8.1011	7.2073	8.3644	9.6735
30°	N	22	23	20	20	20	11
	$\bar{x}$	95.4545	97.2826	106.7500	118.0000	112.5000	98.2273
	s^2	111.0976	206.6245	436.5132	519.7368	352.6316	934.8182
	S.E.	2.2472	2.9901	4.6718	5.0978	4.1990	9.2286
35°	N	24	24	25	24	25	21
	$\bar{x}$	43.1250	43.7500	57.0000	60.4167	56.9000	50.3571
	s^2	22.4155	32.6739	79.3333	65.0841	67.7500	81.4524
	S.E.	.9665	1.2059	1.7813	1.6461	1.6462	1.9692

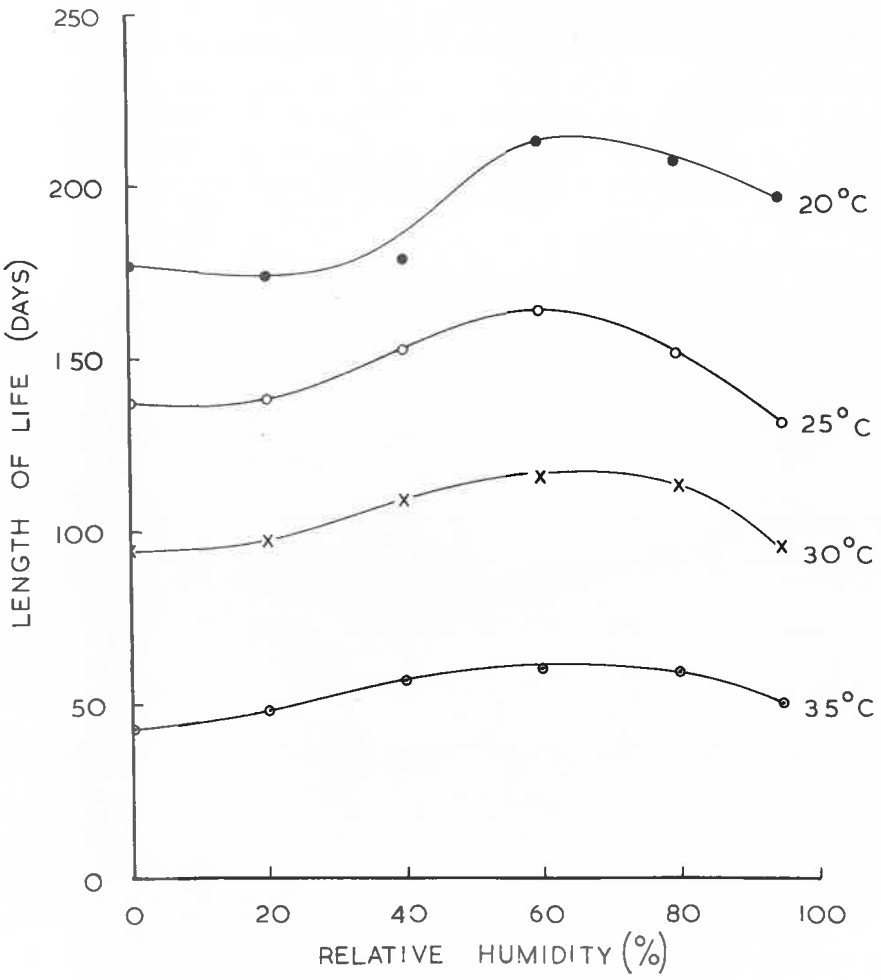


Fig. 26: The influence of temperature and humidity on the length of life of starved T. molitor larvae with initial weight ranging from 80 - 90 mg.

From data summarised in Table 46D and illustrated in Figure 26 it is obvious that temperature plays the major role in determining the length of life of starved T. molitor larvae and that atmospheric humidity contributes but very little. The way in which the mean length of life changes with an increase in humidity (which follow the same trend in all temperatures) is in accordance with the proposed explanation put forward in Section 12.14, for the inter-relationship between oxygen intake, time and atmospheric humidity demonstrated in Table 45B.

The magnitude of the differences between the mean length of life (Table 46B) of larvae starved at 60 and 80% R.H. (at any level of temperature) and those starved at lower and higher humidities do suggest that the comparative lower rate of oxygen intake demonstrated in the humidities 60 and 80% after 30 days starvation (Table 45B) was only a temporary phenomena. This was suggested in Section 12.14.

(D) To determine whether T. molitor larvae died in different combinations of temperature and humidity after having used the same amount of food reserves, regressions of final dry weight at death (y) on initial live weight (x) were calculated, one for each humidity and separately for temperatures 20°, 25°, 30° and 35°C.

Summarises of the data and calculations are given in Tables A56, A57, A58 and A59 respectively, and slopes and intercepts of the different regression lines are compared in the analyses of variance Tables 47A-D respectively.

TABLE 47 Analyses of Variance for Data Summarised in Tables A56-A59.

(a) 20°C

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	5	6.30386	1.260776	.768 n.sig.
Displacements	5	61.56164	12.312328	7.503***
Error	116	190.35381	1.640981	

(b) 25°C

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	4	24.79176	6.19794	2.559*
Displacements	4	75.37062	18.844905	7.782***
Error	99	239.71326	2.421346	

(c) 30°C

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	5	9.08939	1.817878	.450 n.sig.
Displacements	5	103.30589	20.661178	5.115***
Error	104	420.07095	4.038183	

(d) 35°C

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	5	6.41578	1.283156	.322 n.sig.
Displacements	5	139.90811	27.981622	8.594***
Error	131	426.50703	3.255779	

From Tables 47A-D it is obvious that differences do occur in the mean final dry weight of larvae starved to death in different humidities and this is so at all levels of temperature considered.

If we compare the mean final dry weight at the different humidities in Tables A56-A59, the means seem to decrease with an increase in humidity suggesting a relationship between final dry weight and atmospheric humidity. It further suggests that more reserved foods were used by larvae starved in higher humidities than those starved in lower humidities. This is not in accordance with the hypothesis that T. molitor larvae starved in lower humidities increase their metabolic rate to make available more water of metabolism in which case I would have expected equal amounts of food reserves being used in all humdidities (Section 12.14). However, during the course of the experiment observations were made which might prove this proposed relationship between final dry weight and atmospheric humidity to be an artefact. These were

(a) That more larvae moulted in higher than in lower humidities (This information is given in Table 49A in Section 12.16).

(b) That larvae which moulted during the course of the experiment seemed to have a lower final dry weight than those who did not moult.

(c) During starvation, faecal material, dark in colour, was passed by all larvae at irregular intervals in very small quantities. Shortly before a moult however large numbers of very small yellow crystals were passed, presumably uric acid. These crystals were also observed to be passed by all larvae shortly before death occurred. This suggested to me that

T. molitor larvae might retain nitrogenous wastes within their bodies in the form of uric acid crystals and only rid themselves of it, either before a moult or during the latter part of starvation.

Differences in the final dry weight of larvae which moulted and those which did not moult, might thus be a result of the former losing a skin and having less uric acid stored in their bodies when they die. Then, since the number of larvae which moulted increases with an increase in humidity (Table 49A), the relationship between final dry weight and atmospheric humidity (Proposed above), could be an artefact.

To test the hypothesis that larvae which moulted have a lower mean final dry weight than larvae which did not moult, regressions of final dry weight (y) on initial live weight (x), one for larvae which moulted and one for larvae who did not, were calculated for each of the temperatures 20°, 25° and 30°C.

A summary of the data and calculations is given in Tables A60-62 for temperatures 20°, 25° and 30° respectively and slopes and intercepts of different regression lines are compared in the analyses of variance Tables 48A-C respectively.

TABLE 48 Analyses of Variance of Data From Tables A60-A62

(A) 20°C

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	1	.000699	.000699	.000 n.sig.
Displacements	1	153.62600	153.62600	209.643***
Error	123	90.048121	.732342	

(B) 25°C

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	1	.281593	.281593	.317 n.sig.
Displacements	1	249.56271	249.56271	281.415***
Error	105	93.115997	.886819	

(C) 30°C

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	1	.232729	.232729	.166 n.sig.
Displacements	1	375.42306	375.42306	267.845***
Error	112	156.96471	1.401649	

From Table 48 it is obvious that significant differences do exist between the mean dry weight of larvae which moulted and those who did not moult. Since the latter shows the higher mean final dry weight (Tables A60-A62) and since less larvae moulted in the lower humidities (Table 49A) to my mind it may be accepted that the differences (which are small) shown in the mean final dry weight of larvae starved in different humidities (Tables A56-A58) are just a result of a change in the ratio of moulted to non-moulted larvae and does not indicate that larvae use more dry material in higher humidities.

Since no larvae moulted at 35°C the explanation (given above) does not hold here. In this particular case I can only suggest that since the mean final dry weight Table A59 change in the same way as does length of life (Table 46D, 35°C), that more uric acid was excreted in the intermediate humidity range where larvae lived for a longer period than in either the lower or upper humidity range.

12.16 The Influence of Atmospheric Humidity and Temperature On Moulting and Pupation in Newly Moulted T. molitor Larvae Deprived of Food

Buxton (1930) suggested that starved T. molitor larvae decrease their metabolic rate with time, but to a greater extent in higher humidities. Work done by Mellanby (1932a, 1934a, 1936) led him to conclude that atmospheric humidity has no influence on the metabolic rate of starved T. molitor larvae but that the metabolic rate decreases with time.

In Section 12.14 it was suggested that the influence of atmospheric humidity and time on the metabolic rate in starved T. molitor larvae might be more complex than was implied in the work of either Buxton or Mellanby. Measurements of oxygen intake by T. molitor larvae starved for 30 days in different humidities (Section 12.14) led me to believe that these larvae after an initial decrease in their metabolic rate (irrespective of humidity), increase their metabolic rate both in low and high humidities, but with different consequences and in response to different stimuli. These were postulated to be :

(a) That a low ratio of water to dry matter which results from desiccation stimulates the increase in metabolism at low humidities and the additional water of metabolism serves to maintain an adequate ratio of water to dry matter and

(b) At the higher humidities the accumulation of water of metabolism stimulates development and metamorphosis which causes an increase in the rate of metabolism.

One of the conditions stipulated in Section 12.14 for accepting that the metabolic rate of starved T. molitor larvae changes with humidity and time, in the way proposed and with the consequences given above, was that more larvae should moult and/or pupate at the higher humidities and those that do moult or pupate should do so sooner at the higher humidities. With the purpose in mind of getting answers to these questions, the influence of humidity and temperature on moulting and pupation in starved T. molitor larvae were investigated.

Data summarised in Table 41, indicates that after 35 days' starvation, more larvae have pupated the higher the humidity. This data suggested to me:

(a) A possible relationship between the number of starved T. molitor larvae which will pupate and atmospheric humidity, and/or

(b) That the time taken for newly moulted larvae to pupate might decrease with an increase in atmospheric humidity.

The possibility that factors other than humidity might also influence pupation in starved T. molitor larvae was indicated in the work of Lafon and Teissier (1939). These authors working with starved T. molitor larvae in saturated air at 27°C suggested that starvation stimulates pupation but also that the size of the larvae has an influence on the proportion that will pupate. Thus for instance they found pupation stimulated this way to shorten larval life by as much as 2 months at 27°C, and to occur in 100% of the cases where larvae weighed over 90mg, in 80% of the cases of larvae weighing between 70-90mg, but no pupation occurred in larvae weighing 50 mg and less.

If the hypothesis stated above is correct namely that the increase in the rate of metabolism in starving larvae of T. molitor results merely in the conservation of water at the lower humidities but in continued development at the higher humidities then it follows that the stimulus to pupation provided by starvation should depend on humidity. However, since Lafon and Teissier (1939) experimented in a humidity where T. molitor larvae will absorb water (Mellanby, 1932a), it occurred to me that, in their experiments, the accumulation of water and not starvation might have stimulated pupation.

Cotton and St. George (1929) observed 10-21 moults during the larval stage of T. molitor. The wide range in the initial weights of adults from cultures of the same age, illustrated in Figure 1, also suggests that there is no definite recognisable size T. molitor should reach before they pupate. This, I realised would make it extremely difficult to demonstrate stimulation or prevention of pupation or moulting by atmospheric humidity, or differences in time taken to moult or pupate; and to add to the complexity could be the variability in the body composition of larvae, assuming that the latter is the primary stimulus for moulting and pupation.

In connection with this problem, since larvae pupate after reaching weights ranging from 70-180 mg, and on the assumption that a larva will pupate at a pre-determined weight, it was argued that a group of larvae weighing, e.g. 80-90 mg, randomly selected from a culture, will include less larvae which have reached maturity and will moult rather than pupate, than in a group of larvae weighing 140-150 mg for example.

Keeping this in mind, the influence of humidity and temperature on the incidence of moulting and pupation was studied in two groups of larvae (a) one group weighing 80-90 mg, (b) another group weighing 140-150 mg and the results for the two groups compared.

(A) Information as regards the influence of humidity and temperature on the incidence of moulting and pupation in newly moulted larvae in the weight range 80-90 mg were obtained from the experiment described in Section 12.15 which was also designed to study the influence of humidity and temperature on the life span of starved T. molitor larvae.

Data as regards the number of larvae which moulted (from a possible 25) in different combinations of temperature and humidity is listed in Table 49A together with the number of larvae which pupated (in brackets).

TABLE 49A Number of newly moulted larvae in the weight range 80-90 mg which moulted or pupated (in brackets) when being starved in Different combinations of humidity and temperature (from a possible 25)

Temperature	Relative Humidity						Totals
	0%	20%	40%	60%	80%	98%	
20°C	4 (1)	6 (2)	16 (4)	15 (5)	18 (2)	14 (6)	87 (20)
25°C	3 (1)	6 (2)	9 (4)	14 (1)	14 (5)	5 (19)	51 (32)
30°	1 (2)	7 (2)	7 (3)	7 (4)	11 (4)	5 (13)	38 (28)
35°	0	0	0	0	0	0 (2)	0 (2)
Totals	8 (4)	19 (6)	32 (11)	34 (6)	43 (11)	24 (42)	

From Table 49A, the following is apparent :

(a) Moulting

(i) The numbers of larvae which moulted (excluding those in 95% R.H.) from a possible 25, show a general increase with an increase in humidity.

(ii) The number of larvae which moulted decrease with an increase in temperature until in 35°C no moults were observed.

(b) Pupation

(i) Taken over all temperatures, more larvae pupated in 95% R.H. than in all the other humidities taken together.

(ii) Excluding 95% R.H. the number of pupae in each cell is fairly similar except in 35°C where no larvae pupated.

(iii) Compared to other humidities, the larger number of larvae which pupated in 95% R.H. where larvae absorbed water suggests that water absorption might stimulate pupation in starved T. molitor larvae. Temperature however also seems to influence pupation in starved larvae. The proportion of larvae which pupated seemed to have reached a maximum at 25°C and to decrease both when temperature increases or decreases.

Other observations made during the course of the experiment include :

(a) A number of larvae listed as having moulted in 95% R.H. have actually moulted twice during the course of the experiment. This was observed only at temperatures 20° and 25°C.

(b) In the treatment (95% R.H. at 25°C), a few larvae were observed first to moult and then to pupate (these are listed as having pupated).

(c) Although the sum total of larvae which either moulted or pupated increased with an increase in humidity (Table 49A), each cell contained larvae which at death had neither moulted nor pupated.

(d) After 180 days' starvation larvae in 20°C were used to conduct a mobility test. Although all larvae were able to move, e.g. their heads and limbs, only those starved in 95% R.H. could move away from the point of release.

(B) Together with (A) above, another experiment was conducted, more specifically to test :

(a) Whether or not atmospheric humidity and temperature have any influence on pupation in starved T. molitor larvae, and

(b) Whether pupation occurs sooner in higher humidities than in low humidities.

For the purpose of testing these hypotheses, a group of newly moulted larvae in the weight range 140 - 150 mg were selected from cultures of the same age. These larvae were randomly divided into 80 groups, containing 25 larvae each. Four groups again randomly selected were allotted to each of the relative humidities 0, 33, 55, 75 and 95% in each of the temperatures 20°, 25°, 30° and 35°C, giving 20 treatments each of which were replicated three times.

Assuming that no more larvae will pupate after being starved for four months (120 days) in any combination of temperature and humidity, larvae were observed for a period of four months or till death

intervened, and the incidence and time of pupation recorded. Information as regards the number of larvae which pupated, expressed as a percentage of the total number of larvae in each sub-class, is given in Table A63; while the overall mean change in percent pupation with change in humidity and temperature is illustrated in Figure 27.

Because the variable consists of the proportion of larvae which pupated, data in Table A63 were transformed to angles (Table A64). Then to test for possible differences between temperatures and between humidities the transformed data were used to construct the analysis of variance Table 49B.

TABLE 49B Analysis of Variance of Data from Table A64

Source of Variation	D.F.	S.S.	M.S.	V.R.
Humidities (H)	4	26,490.0317	6622.5079	106.0963***
Temperature (T)	3	20,613.6654	6871.2219	110.0508***
T x H	12	3,115.2654	259.6054	4.1580***
Residual	60	3,745.1070	62.4185	
Total	79	53,964.1495		

If we consider Table 49B together with Figure 27, then the following becomes apparent.

(i) There is a significant interaction between temperature and humidity effects on pupation in starved *T. molitor* larvae. From Figure 27 it seems obvious that 35°C contributed the most, if not solely, to the significant interaction between main effects.

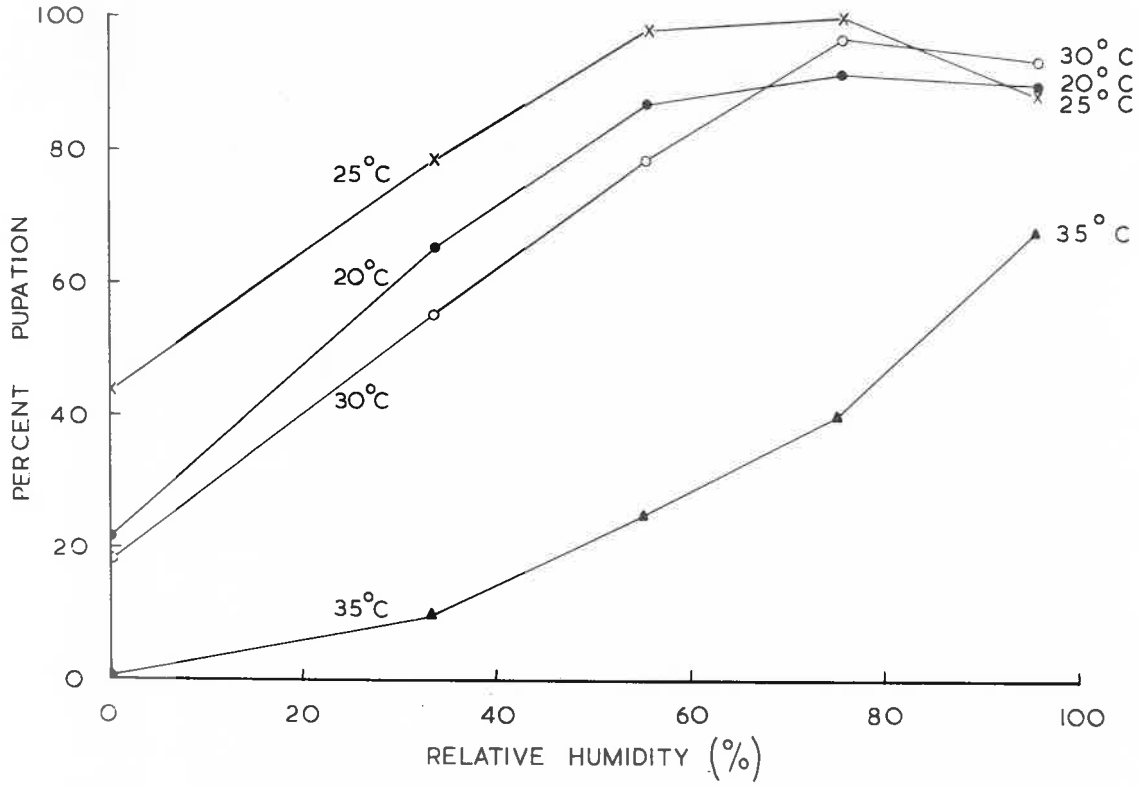


Fig. 27: The influence of temperature and humidity on pupation in starved T. molitor larvae with initial weight ranging from 140-150 mg.

(ii) Although it is not valid to test main effects against residual mean squares in the presence of an interaction (Moroney, 1951) the size of the variance ratios associated with main effects leaves little doubt that both main factors has a significant influence on pupation in starved *T. molitor* larvae.

(iii) The proportion of larvae which pupated increased with an increase in humidity in all temperatures considered (Figure 27). Excluding 35°C then the proportion of larvae which pupated still increased with an increase in humidity but reached an asymptote at 55% R.H. This is different to what happened to smaller larvae, which were assumed not mature enough to pupate, (Table 49A), where obvious differences exist between the number of larvae which pupated in 95% R.H. and for example in the next lower humidity 80%. Since starved *T. molitor* larvae are known to absorb water in 95% R.H. but not in 80% R.H. (Section 12.2), the difference in the proportion of larvae which pupated in 95% as compared to 80% or 75% in small and bigger larvae respectively (Table 49A, Table AC3) to my mind can be regarded as evidence for accepting that water absorption stimulates pupation in starved *T. molitor* larvae which would otherwise not have pupated.

(iv) Although the proportion of larvae which pupated decreased with a decrease in humidity some larvae did pupate in dry air in all temperatures except 35°C. The possibility then exists that the larvae which pupated in dry air might be of a certain type.

(v) Although the proportion of larvae which pupated increased with an increase in humidity at 35°C, the general trend in the relationship differs markably from that in other temperatures considered. A similar difference was observed between moulting in younger larvae at 35°C and other temperatures considered, suggesting that 35°C is outside the temperature range which can be considered normal for T. solitor.

(C) Influence of Temperature and Humidity on the time taken by Starved T. solitor larvae to pupate

To test the hypothesis that pupation occurs sooner the higher the humidity, I used data obtained in the experiment described in (B) above.

Time taken for pupation can be defined as the time lapse (days) between the last larval moult (start of the experiment) and the date on which a particular larva was observed to pupate.

Since 35°C was so obviously different from other temperatures considered (Figure 27), data obtained in this temperature were not included in the test.

The time (days) taken by individual larvae to pupate in the different combinations of temperature and humidity is listed in Table AG5. Considering the data listed in Table AG5 then the following becomes apparent :

(i) The time taken for pupation by individual larvae (taken over all treatments) ranges from 10-120 days. In Section 12.12 and Figure 22 it was shown that during starvation the water/dry matter ratio of larvae increases at all humidities but relatively more at the higher humidities.

If we assume that a certain minimum high ratio of water to dry matter stimulates metabolism which will result in metamorphoses, then the variability in the initial water/dry matter ratio of newly moulted larvae (Table 45A), could explain why the time taken by larvae to pupate varies between such wide limits.

(ii) The larvae which pupated in the lower humidities in all the temperatures considered did so during the early part of the experiment.

The latter observation makes it obvious that any test based on the mean length of time taken for larvae to pupate will be biased, unless variation in sub-class sizes are taken into consideration. The only way which seemed open to me to overcome this difficulty and still test the hypothesis that pupation occur sooner in higher humidities was to compare the mean length of time taken to pupate but to consider only those combinations of temperature and humidity where the numbers of larvae which pupated are about the same. This condition holds for humidities 55, 75 and 95% at temperatures 20°, 25° and 30°C (see in Figure 27 and Table 49C).

For these reasons and in order to test the hypothesis that pupation occurs sooner in higher humidities Table 49C was constructed from Table A65. Table 49C contains the mean length of time (days) taken to pupate, considering all the larvae in each replicate, at humidities 55%, 75%, 95% in temperatures 20°C, 25°C and 30°C. Table 49C also includes the number of larvae which pupated from a possible fifteen in each replicate (in brackets).

TABLE 49C Mean length of time (days) taken to moult by a group of newly moulted T. molitor larvae (weight range 140-150mg), starved in different combinations of temperature and humidity. Total number which moulted from a possible fifteen (in brackets)

Temperature	Replicates	Relative Humidity		
		50%	75%	95%
20°C	1	64.93 (14)	50.00 (14)	54.20 (15)
	2	47.00 (14)	49.54 (13)	42.73 (11)
	3	68.62 (13)	59.29 (14)	4[illegible].64 (14)
	4	75.00 (11)	46.00 (14)	51.21 (14)
	Total	255.55 (52)	204.83 (55)	196.78 (54)
	$\bar{x}$	63.8875	51.2075	49.1950
25°C	1	33.93 (15)	31.00 (15)	21.15 (13)
	2	26.00 (14)	28.53 (15)	19.79 (14)
	3	31.60 (15)	28.20 (15)	26.23 (13)
	4	28.80 (15)	27.73 (15)	20.77 (13)
	Total	120.33 (59)	115.26 (60)	87.94 (53)
	$\bar{x}$	30.0825	28.8150	21.9850
30°	1	26.08 (12)	16.40 (15)	14.71 (14)
	2	35.18 (11)	29.85 (13)	15.08 (13)
	3	33.79 (14)	34.40 (15)	20.73 (15)
	4	29.70 (10)	21.36 (15)	21.21 (14)
	Total	124.75 (47)	101.18 (58)	74.73 (56)
	$\bar{x}$	31.1875	25.2950	18.6825

Data from Table 49C were used to construct the analysis of variance Table 49D.

TABLE 49D Analysis of Variance of Data from Table 49C

Source of Variation	D.F.	S.S.	M.S.	V.R.
Temperature (T)	2	6636.7851	3318.3925	99.4034***
Humidities (H)	(2)	(834.7645)	(417.3822)	(12.5020***)
(A) Linear regression	1	830.4913	830.4913	24.8776***
(B) Deviation from regression	1	4.2730	4.2730	.1280 n.sig.
T x H	4	137.7007	34.4252	1.0312 n.sig.
Residual	27	901.5427	33.3905	
Total	35	8510.5928		

Since the variance ratio associated with the interaction of temperature and humidity effects is non-significant at the 10% level I accepted that temperature and humidity are independent in their effect on the mean length of time (days) taken by starved H. zea larvae to pupate. A non-significant interaction also allowed me to test the main effects against residual mean squares, both which are significant at the .01% level.

To test whether or not there is a regression factor in the relationship for mean time (days) taken to pupate and atmospheric humidity, the humidity sums of squares were separated into the two components :

(a) Sums of squares due to linear regression of mean length of time (days) taken to pupate on humidity.

(b) Sums of squares due to deviations from linear regression. This was done using the method "Orthogonal comparisons in regression", after Snedecor (1956).

Since the variance ratio associated with linear regression is significant at the .01% level (Table 49D) and the variance ratio associated with deviation from linear regression is non-significant, I accepted the hypothesis that the mean time (days) taken by starved *T. molitor* larvae (weight range 140-150) to pupate, changes with humidity in a linear fashion. If we consider the means in Table 49C, it becomes obvious that the regression coefficient is negative. I therefore also accept that the time taken to pupate decrease with an increase in humidity. The variance ratio associated with interaction between temperature and humidity effects being non-significant, suggest that the influence of humidity is the same at all temperatures considered.

On completion of this experiment after 120 days, 47 larvae were still alive in the treatment (0% R.H. at 20°C). For the purpose of finding :

(a) Whether still more larvae will pupate given more time

(b) Whether these larvae could be stimulated to pupate either :

(i) By placing them in a high humidity e.g. 75%, or

(ii) By placing them in a humidity e.g. 95% where they can absorb water.

Forty five of the larvae still alive after 120 days' starvation in dry air at 20°C were randomly divided into three groups. One group was subjected to each of the humidities 0, 75 and 95% at 20°C. These larvae were observed till they died or till they pupated and the incidence of moulting was also recorded. The findings are listed in Table 49E where :

D = number of larvae which died without moulting or pupating

M = number of larvae which moulted but did not pupate

P = number of larvae which pupated

TABLE 49E Changes observed in larvae after being starved in dry air for 120 days and then transferred to different humidities at 20°C

Changes	Humidities		
	0%	75%	95%
D	11	9	2
M	4	6	6
P	0	0	7

Data presented in Table 49E suggests that some of the larvae in which pupation was prevented by starvation in dry air at 20°C can be stimulated to pupate even after 120 days, but then only if they are transferred to a humidity in which they can absorb water.

(D) Summary and Conclusions

The inter-relationship between temperature, humidity and moulting and pupation in starved *T. molitor* larvae was shown in this section to be rather complex, and is by no means considered fully explained.

The determination of the exact nature of this inter-relationship is complicated by the fact that factors not related to either temperature or humidity, also exercise some influence on moulting or pupation, and make it difficult to isolate the influence of temperature and humidity. Thus for instance is it virtually impossible to predict or identify the stage of development reached by any larvae at any particular time. This is brought about by the fact that larvae from the same cultures may go through anything from 10-21 larval instars before they are matured and ready for pupation (Cotton and St. George, 1929).

That the size or rather the stage of development reached by larvae is important in that in a sense this determines whether on starving larvae will respond to humidity by moulting or pupating is apparent when we compare Table 49A and Table A63 where larvae in the weight range 80-90 mg and 140-150 mg respectively were starved in humidities covering the entire range.

In the case of the smaller larvae the numbers moulted increased with an increase in humidity, until a humidity is reached, e.g. 95% where larvae absorbed water and this seems to stimulate larvae, apparently immature to form pupae which metamorphosed to normal healthy adults.

In the case of the bigger larvae which I assume have on the average reached a more advanced stage of development, the tendency is more towards pupation. The proportion of larvae which pupated increased with an increase in humidity, reached a maximum at 85% R.H. and stayed at this level with further increase in humidity. However the average length of time (days) taken for larvae to pupate, measured in the humidity range

55-65% decrease with an increase in humidity. Atmospheric humidity therefore seems to influence pupation in starved E. politor larvae in at least three ways :

(a) In lower humidities, pupation is prevented in larvae which would otherwise have pupated.

(b) In higher humidities the time necessary for pupation is reduced.

(c) In the humidities where water can be absorbed pupation is stimulated in larvae which would otherwise not have pupated.

The fact that both the number of larvae which moulted and the numbers which pupated increased with an increase in humidity while in the latter case also the time necessary to pupate decreased with an increase in humidity, is in accordance with one of the conditions stipulated as a pre-requisite for accepting the hypothesis that starved E. politor larvae after an initial decrease in their metabolic rate (independent of humidity) increase their metabolic rate in all humidities, but for different reasons in different parts of the range and in response to different stimuli (Section 12.14).

Possible advantages suggested to explain the increase in the metabolic rate were that the production of more metabolic water serves (a) in the lower humidities to offset water lost by evaporation, and (b) in the higher humidities allows for the continuation of development making use of their reserved foods.

It was suggested that

(a) a low ratio of water to dry matter stimulated the increase in metabolism at low humidities, and

(b) a high ratio of water to dry matter stimulated pupation (and a consequent increase in the rate of metabolism) at the higher humidities.

A second condition stipulated as a pre-requisite for accepting that the metabolic rate of starved T. molitor larvae changes with time and under the influence of humidity, in the way, and for the reasons given above, was that

(a) T. molitor larvae should live for a longer time in the intermediate humidity range, than in either the lower or upper range

(b) that T. molitor larvae should die after having used all available food reserves in all the humidities. That this is so was concluded in Section 12.15. Therefore, to my mind it seems reasonable to conclude that the metabolic rate of starving T. molitor larvae, after an initial decrease (which is independent of humidity), increases again in both low and high humidities, but with different consequences and in response to different stimuli, and that these are :

(a) That a low ratio of water to dry matter which results from desiccation, stimulates the increase in metabolism at low humidities and the additional water of metabolism allows an adequate ratio of water to dry matter to be maintained.

(b) That in higher humidities the accumulation of water of metabolism, allows the ratio of water to dry matter to build up to a high level which permits continuation of development and a consequent increase in metabolism.

12.2 Water Absorption in Starved T. molitor larvae

The literature on water absorption in terrestrial insects and ticks was reviewed and discussed by Andrewartha and Birch, 1954; Beament, 1954; Edney, 1957.

The ability of starved T. molitor larvae to absorb water from the atmosphere was first reported by Buxton (1930), who found that larvae could by this means increase their weight by as much as 18% in humidities of 90% and above. Mellanby (1932a) confirmed this ability in T. molitor larvae and reported that equilibrium was established at 68% R.H. at several temperatures, suggesting that the process is dependent upon relative humidity and not saturation deficit.

Locke (1953) (after Beament, 1954) has shown, that if following partial desiccation mealworms take up water from 93% R.H., they may occasionally reach an equilibrium weight below their starting point. Following this, a two hour period of desiccation, which does not lead to a measurable loss of weight, promotes further uptake, on the insects being returned to 90% R.H. This suggests that desiccation might be a stimulus for water absorption. The process was found to be irregular and intermittent by both Mellanby (1932a) and Edney (1957).

The fact that T. molitor larvae after absorbing water reach an equilibrium weight which is then maintained up till the time of death, was also reported by Buxton (1930) and Lafon and Teissier (1939). Buxton (1930) quoted this equilibrium weight as just over 90% of the initial weight and that is reached after 17 days in a saturated atmosphere. The results of my experiments concerning the weight change

of T. molitor larvae starved in 100% R.H. (illustrated in Figure 19), are not in agreement with Burton's findings.

From the literature cited it is obvious that the ability of starved T. molitor larvae to absorb water from unsaturated air is well established but it is also clear that little further information is available about the nature of this phenomena in T. molitor.

In this section I wish to investigate, more quantitatively, the influence of desiccation and starvation on the ability of T. molitor larvae to absorb water from unsaturated air at different relative humidities and then to discuss the advantages to these insects of having this ability.

I was particularly interested to get answers to the following general questions:

(a) Whether T. molitor larvae in any condition are able to absorb water from the atmosphere providing atmospheric humidity is sufficiently high, or

(b) Whether desiccation and/or starvation are pre-requisites for T. molitor larvae to have this ability, and if so

(c) Whether the degree of desiccation and/or starvation determine

(i) The amount and rate of water absorption

(ii) The minimum level of atmospheric humidity where T. molitor larvae will still be able to absorb water.

(d) Whether the ability to absorb water can be considered an advantage to these insects.

To get answers to these general questions, a series of experiments were conducted and will be discussed in the sub-sections to follow.

12.21 The Influence of Desiccation and Starvation on Water Absorption in T. molitor larvae

Portmann and Labarrere (1962) explained a reversal in the hygro-negative reaction of T. molitor larvae, after being desiccated, by suggesting a possible severely disturbed water balance. In Section 12.12 however, it was shown that although T. molitor larvae lose water, they can maintain a constant dry/wet ratio in dry air, until they die of starvation (Section 12.15). In Section 12.13 it was shown that the water content of T. molitor larvae even after 35 days starvation in 80% R.H., remained unchanged.

(i) Should we define desiccation as a "disturbed water balance" or a reduction in the dry/wet ratio, then it may be argued that T. molitor larvae are not desiccated when starved in dry air.

(ii) Should we define desiccation merely as a reduction in the water content to below the level initially present, then we must conclude that T. molitor larvae are desiccated only when starved in humidities below 80% and that they are "starved" but not desiccated when deprived of food in humidities of 80% and beyond.

Thus, to prevent confusion I will use the terms desiccation and starvation in the sense, outlined in (ii) above.

To familiarise myself with the phenomena of water absorption in T. molitor larvae and also to discover whether the severity of desiccation has an influence :

(a) On the number of larvae which will absorb water on return to a suitable humidity,

(b) On the amount of water that will be absorbed in a given time, the following experiment was conducted :

A sample of 200 newly moulted larvae in the weight range 120-140 mg were selected from cultures of the same age and randomly divided into 5 lots of 40 larvae. One lot of 40 larvae was placed immediately in 95% R.H. at 25°C. The remaining 4 lots were placed in dry air at 25°C. Those 4 lots were randomly assigned to treatments in which the larvae were destined to lose 5%, 10%, 15% and 20% of their original weight before being transferred to 95% R.H. at 25°C. The larvae were weighed daily and removed to 95% R.H. at 25°C when each larvae had lost the amount of weight appropriate to its treatment. While exposed to 95% R.H. at 25°C weight increases (of individual larvae) in excess of that on the previous day (water absorbed) were recorded daily for five days.

The data obtained from this experiment is summarised in Tables 50A and B, and discussed in sub-sections A and B to follow.

(A) To get an indication of whether the severity of desiccation has an influence on the numbers of larvae which will absorb water on return to a suitable humidity, data obtained from the experiment described above, were summarised in Table 50A where :

A = Total number of larvae observed in a treatment.

B = The number of larvae which absorbed water on any particular day, expressed as a percentage of the total number observed.

C = The percentage of the total number of larvae observed, which absorbed water on a particular day and had not done so on any previous day.

D = Total number of occasions on which water was absorbed, expressed as a percentage of the total possible number of occasions.

E = Proportion of larvae which absorbed water some time during the 5 day period in 95% R.H.

TABLE 50A A summary of data concerning the numbers of *P. solitus* larvae which absorbed water on return to 95% R.H., after being desiccated in dry air at 25°C till they have lost 0, 5, 10, 15 and 20% of their initial weight

Degree of desiccation % Loss of initial weight	Details	Days in 95% R.H.					D	E
		1	2	3	4	5		
0%	A	40	40	40	40	40		
	B	0	17.5	50.0	47.5	40.0		
	C	0	17.5	32.5	15.0	10.0	31.0%	75.0%
5%	A	40	40	40	40	40		
	B	95.0	87.5	57.5	45.0	40.0		
	C	95.0	2.5	0.0	0.0	0.0	65.0%	97.5%
10%	A	35	35	33	33	35		
	B	93.9	33.9	72.7	60.6	54.5		
	C	93.9	6.1	0.0	0.0	0.0	75.13%	100%
15%	A	22	22	22	22	22		
	B	100	100	90.9	59.1	40.9		
	C	100	0.0	0.0	0.0	0.0	78.18%	100%
20%	A	22	22	22	22	22		
	B	100	90.9	81.8	54.5	40.9		
	C	100	0.0	0.0	0.0	0.0	72.8%	100%

From the data summarised in Table 50A we have the following:

(i) 75% of the total number of newly moulted larvae starved in 95% R.H. for five days acquired the ability to absorb water. None of these larvae did absorb water on the first day and 10% only absorbed water only on the fifth day.

(ii) With increasing desiccation:

(a) The proportion (B) of larvae, which absorbed water on the first day in 95% R.H. increased with increasing desiccation reaching an assymptote with the group which had lost 15% of their weight by desiccation.

(b) The proportion (E) of larvae which absorbed water during the 5 day period in 95% increased with increasing desiccation reaching an assymptote with the group which had lost 10% of their weight by desiccation.

(c) The proportion (D) of the occasions on which larvae were observed to absorb water during a five day period increased with increasing desiccation.

(iii) If we exclude the first treatment, then the proportion (B) of larvae which absorb water decreases as the time spent in the higher humidity increases.

This data then seems to suggest:

(a) That *C. salitor* larvae can also acquire the ability to absorb water from unsaturated air merely by being starved.

(b) That this ability in starved T. molitor larvae is acquired with time and sooner in some larvae than in others.

(c) That since the proportion (B) of desiccated larvae which absorb water on being exposed to 95% R.H. decreases with time, that the ability to absorb water is gradually lost as larvae absorb water.

(d) That the more severe the desiccation the more water might be absorbed and at a faster rate.

(B) Data concerning the total amount (mg) of water absorbed during a five day period in 95% R.H. by larvae used in (A) above, is summarised in Table 50B.

TABLE 50B A summary of data concerning the amount (mg) of water absorbed by T. molitor larvae during a five day period in 95% R.H. after being desiccated in dry air at 25°C to lose different proportions of their initial weight

Details	Proportion of weight lost in dry air				
	0%	5%	10%	15%	20%
N	30	39	33	22	22
Σx	173.70	426.10	487.40	370.80	334.00
$\bar{x}$	5.7900	10.9256	14.7697	16.8545	15.1818
s^2	16.0112	22.4486	30.0796	16.3046	45.5835
S.E.	.7323	.7587	.9547	.8609	1.4396

Data summarised in Table 50B seem to suggest that on the average T. molitor larvae absorb water faster the more they were desiccated prior to being introduced to a higher humidity where they can absorb water. If we however consider these data together with those in column (B) Table 50A then since these larvae were observed to have absorbed water on more occasions the longer they were desiccated, this might be an artefact, and the explanation might be that a longer period of desiccation might only cause the process to be more regular and less intermittent.

The size of the within class variances, indicates that the amount of water absorbed by individual larvae in a given time, varies between relatively wide limits. The only suggestions I could put forward at this stage to explain this variability, is that :

(a) It might be the result of different sized larvae absorbing different amounts of water.

(b) Since the wet/dry ratio of newly moulted larvae also vary between relatively wide limits (Table A5lB), larvae with a low wet/dry ratio might require to absorb more water than those with a high wet/dry ratio to stimulate metabolism and continue development (See also Sections 12.15 and 12.16).

To test the null hypotheses implicit in (a) and (b) above, the following experiment was done.

200 newly moulted larvae in the weight range 120-140 mg were selected from cultures of the same age. These larvae were subjected to

a relative humidity of 100% for a period of seven days and the amount of water absorbed (mg) (difference between weight on day seven - weight on day zero), calculated for individual larvae.

In Figure 19 it was shown that the mean weight of a group of larvae starved in 100% R.H. increases and reaches a maximum by day five, then stays constant till day ten after which it decreases relatively rapidly. I therefore argued that by starving larvae in 100% R.H. for seven days and then calculating the total amount of water absorbed I may get a fair estimate of (a) the maximum amount of water that starved larvae will absorb (b) the rate of water absorption in 100% R.H.

To test the null hypotheses (a) that initial weight has no influence on the amount and rate of water absorption and (b) that the dry/wet ratio has no influence on the amount and rate of water absorption the following procedure was followed:

The 200 larvae were grouped in two ways:

(a) Into four weight groups containing larvae weighing 120-124.95, 125-129.95, 130-134.95 and 135-139.95 mg respectively.

(b) Into seven groups containing larvae with a dry/wet ratio of below 42.95%, 42-42.95, 43-43.95, 44-44.95, 45-45.95, 46-46.95 and above 47% respectively.

Following this two groups of regressions were calculated :

(i) Regressions of amount (mg) of water absorbed during 7 days in 100% R.H. (y) on initial live weight (x) one for each of the weight groups.

(ii) Regressions of amount (mg) of water absorbed (y) on initial dry/wet ratio, one for each of the seven groups.

A summary of the data and calculations is given in Tables A66 and A67 respectively, while slopes and displacements of the regression lines in the different groups are compared in Tables 50C and D respectively.

TABLE 50C Analysis of Variance of Data Summarised in Table A66

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	3	40.4495	13.4832	.372 n.sig.
Displacements	3	18.6782	6.2261	.171 n.sig.
Error	192	6956.0419	36.2294	

Since the variance ratios associated with both slopes and displacements are non-significant, I accepted that the regression lines describing the relationship between amount of water absorbed (y) and initial weight (x), are identical. This allowed me to pool all observations to calculate a single regression of amount of water absorbed (y) on initial live weight (x).

To test whether the slope of this new regression line is zero, we have the quantity :

Slope/S.E. of slope, i.e. .070694/.07273 = C, distributed as t_{192}

(Pooled Regression, Table A66)

Since $C < 1.96$, I accepted the null hypothesis that initial weight has no influence on the amount of water absorbed by *S. solitor* larvae in the weight range 120-140 mg.

TABLE 50D Analysis of Variance of Data Summarised in Table A67

Source of Variation	D.F.	S.S.	M.S.	V.R.
Slopes	6	111.2410	18.5402	.531 n.sig.
Displacements	6	519.9052	86.4842	2.480*
Error	186	6485.2304	34.8668	

Since the variance ratio associated with displacements is significant at the 5% level, I accepted that there are significant differences between the mean amounts of water absorbed by larvae with different initial dry/wet ratios. If we consider the mean amount (mg) of water absorbed together with the mean dry/wet ratio of different groups (Table A67) then no systematic relationship is suggested. I therefore concluded that the differences in the amounts of water absorbed shown in Table 50D are not associated with differences in the original water/dry matter ratio but must have been caused by some influence that was not taken account of in the experiment.

The size of the standard deviation (which is strikingly similar) of points round the fitted lines (Tables A66 and A67) however confirm the variability in the amounts of water absorbed by individual larvae. A further possible explanation for this phenomenn might be that since males were shown to have a higher initial wet/dry ratio than females (Section 10.) that larvae which will eventually develop into adult males, might absorb more water and at a faster rate than those which will eventually develop into adult females.

this hypothesis could be tested by an experiment designed in the same way as the one discussed above, but to leave larvae in a high humidity until they pupate, upon which males and females can be separated by the method described by Hein (1920).

12.22 The Influence of Humidity on Water Absorption on starved and Desiccated T. molitor larvae.

Hellemby (1932a) reported that mealworm larvae come into equilibrium at 83% R.H. i.e. in lower humidities they lose water while in higher humidities they absorb water.

To verify his finding for the population studied and also to determine whether or not desiccation has an influence on the humidity at which equilibrium is reached, the following experiment was conducted.

A group of newly moulted larvae in the weight range 110-125 mg were selected from cultures of the same age. These larvae were randomly divided into sixteen groups of 25 larvae each. Eight of these groups again randomly selected were subjected to relative humidities 80, 85, 88.5, 90, 92.5, 96, 97.5 and 100% R.H. at 25°C where larvae were observed for 24 days, and the numbers which absorbed water as well as the amount (mg) of water absorbed daily (individual larvae), recorded. The other eight groups were desiccated in dry air at 25°C for a period of 12 days, upon which they were randomly allotted to the humidities listed above. These larvae were then observed for a further 12 days in the higher humidities and numbers which absorbed water and the amount (mg) absorbed daily, recorded.

The total numbers of larvae from a possible 25 in each treatment, which have absorbed water by day 24 are listed in Table 51A.

TABLE 51A Numbers of larvae which absorbed water in different humidities

Condition of Larvae	Relative Humidities %							
	80%	85%	88.5%	90%	92.5%	95%	97.5%	100%
Starved	0	0	1	5	16	24	25	24
Desiccated	0	5	21	25	24	24	24	24

In desiccated larvae the numbers listed as having absorbed water did so during the first 24 hours on being subjected to the higher humidities. The numbers which absorbed water on subsequent days decreased, till only ± 10% of the total were observed to absorb water on day 24. This phenomena was also demonstrated in Table 50A.

To demonstrate when during starvation T. molitor larvae acquire the ability to absorb water and how this is influenced by humidity, Table 51B was constructed. This table contains the accumulative totals of larvae having acquired the ability to absorb water on consecutive days in different humidities.

TABLE 51D : Accumulative numbers of T. molitor larvae (from a possible 26) which acquired the ability to absorb water when starved in different humidities at 25°C

Relative Humidity	Number of days starved in different humidities																							
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
100%	9	14	18	20	21	23	23	23	23	24														
97.5%	1	13	16	16	19	20	20	23	23	23	25													
95.0%	-	5	7	9	11	12	12	15	14	15	16	17	19	20	21	23	24							
92.5%	-	-	1	3	3	5	4	6	9	10	11	11	12	12	12	12	12	13	13	14	15	15	15	16
90.0%	-	-	1					2							3					4				5
88.5%																				1				

From the data summarised in Tables 51A and B the following deductions were made:

(i) Previous desiccation, as compared to starvation conditions *T. molitor* larvae in such a way that they are able to absorb water from unsaturated air with a lower relative humidity.

(ii) Desiccation decreases the time necessary to acquire the ability to absorb water, this is apparent in the lower humidities used in this experiment.

(iii) As far as starved *T. molitor* larvae are concerned more larvae acquired the ability to absorb water and sooner the higher the humidity, beyond a minimum of 80.5%.

To demonstrate how irregular and intermittent water absorption is in *T. molitor* larvae, yet that there is a trend with time Table 51C was constructed. This Table contains the amount (mg) of water absorbed by individual larvae during consecutive 48 hour periods in 98% R.H. at 25°C after previously being desiccated for twelve days in dry air at 25°C.

TABLE 51C The amount (mc) of water absorbed by T. molitor larvae during consecutive 48 hour periods in 95% R.H. at 25°C after previously being desiccated for 12 days in dry air at 25°C

Larval Number	Days in 95% R.H. at 25°C					
	2	4	6	8	10	12
1	15.65	1.05	-	-	-	-
2	14.80	-	-	-	-	-
3	13.00	5.00	-	-	2.20	-
4	14.10	9.10	-	-	-	-
5	14.85	7.05	-	-	-	-
6	15.75	7.75	-	-	-	-
7	15.70	-	-	3.90	3.60	-
8	15.20	4.80	-	-	-	-
9	16.10	6.55	-	-	-	-
10	10.15	-	10.40	2.80	2.60	-
11	10.60	2.80	2.60	11.00	-	-
12	10.55	4.15	4.55	1.00	5.20	3.80
13	11.15	-	-	2.65	2.15	-
14	11.45	1.15	6.30	3.55	-	-
15	8.70	2.10	2.25	2.65	1.55	-
16	11.85	4.30	8.90	-	-	-
17	10.80	9.40	-	10.10	-	-
18	10.60	8.85	-	-	-	-
19	11.45	11.00	1.45	-	-	-
20	9.20	9.45	8.55	-	-	-
21	10.85	11.20	11.15	-	-	-
22	12.20	11.50	-	-	1.45	-
23	10.15	4.50	2.80	-	-	-
24	9.15	4.10	2.65	1.80	-	1.80
N	24	20	11	9	7	2
$\bar{x}$	12.2042	6.2800	5.6000	4.3833	2.6785	2.7000
s^2	6.1465	11.2164	12.9015	15.0033	1.7428	1.4200
S.E. of mean	.5061	.7489	1.0650	1.2260	.4990	1.1916

From Table 51C which demonstrates the typical way in which water is absorbed by desiccated larvae on return to a suitable high humidity, the following is apparent

(i) Desiccated larvae absorb water immediately when subjected to a suitable high humidity.

(ii) The mean amount (mg) of water absorbed during a given time (and thus the rate of water absorption) decreases with time.

(iii) The numbers of larvae absorbing water, decrease with time.

Since the maximum number of desiccated larvae which absorbed water when subjected to higher humidities, did so during the first 48 hours, I used this data to test the null hypothesis that atmospheric humidity has no influence on the rate of water absorption.

A summary of the data, concerning the amount (mg) of water absorbed, during the first 48 hours on subjection to humidities ranging from 88.5-100% by *T. molitor* larvae after being desiccated for 12 days in dry air at 25°C is given in Table 51D.

TABLE 51D A summary of data concerning the amount (mg) of water absorbed by previously desiccated *T. molitor* larvae during the first 48 hours starvation in different humidities at 25°C

Details	Relative Humidities						Total
	88.5%	90%	92.5%	95%	97.5%	100%	
N	31	25	24	24	24	24	142
Σx	60.45	135.75	176.10	292.90	394.30	531.90	1261.40
$\bar{x}$	2.0786	5.4300	7.3375	12.2042	11.8458	13.8292	
Σx^2	135.1175	931.4575	1446.8350	3715.9600	5825.1150	4763.6550	14,456.1400
s^2	1.0002	5.0965	6.7261	6.1455	6.8415	7.3541	

Data summarised in Table 51D were used to construct the analysis of variance Table 51E.

TABLE 51E Analysis of Variance of Data Summarised in Table 51D

Source of Variation	D.F.	S.S.	M.S.	V.R.
Between classes	(5)	(2172.2557)		
(i) Linear Regression	1	2054.2659	2054.2659	382.0829***
(ii) Deviation from regression	4	137.9898	34.4975	6.4127***
Within classes	136	722.6029	5.3133	
Total	141	2894.8586		

From Tables 51D and E it is obvious that humidity has an influence on the rate of water absorption in previously desiccated T. molitor larvae.

Also the variance ratio associated with deviations from linear regression was significant. It was difficult to see the reason for this but, because of the fact that the relationship contains a highly significant linear regression factor, which accounts for the largest portion of the between classes sums of squares, I concluded that in general the rate of water absorption in previously desiccated T. molitor larvae increases (Table 51D) with an increase in humidity beyond 88.5% R.H.

12.23 Water Absorption in T. molitor larvae which have previously been fed

In Section 12.21 it was shown that T. molitor larvae also acquire the ability to absorb water when they are starved without being desiccated and was it concluded that desiccation is not necessarily a pre-requisite for acquiring this ability.

In this Section I wish to investigate whether T. molitor do acquire the ability to absorb water when subjected to a suitable high humidity after being desiccated in the presence of food, or whether they need to be deprived of food to acquire this ability.

To get answers to these questions, the following experiment was conducted:

Newly moulted larvae in the weight range 110-130 mg were selected from cultures of the same age. These larvae were randomly divided into ten lots of 30 larvae each. Five lots again randomly selected were deprived of food in a relative humidity of 40% at 25°C for a period of 15 days. The rest were allowed to feed for a period of 15 days on the usual food mixture (equal portions of bran, pollard and wheat germ) which had been allowed to equilibrate in 40% R.H. at 25°C, for a period of 10 days before starting the experiment. On day 16 the larvae were removed from the food and one lot from each of these groups were randomly allotted to each of the humidities 88.5%, 90%, 92.5%, 95% and 100% at a temperature of 25°C. The proportion (percent of total) of larvae which absorbed water within 24 hours of being introduced to the higher humidities were recorded. This data is listed in Table 52 where:

A = larvae which were desiccated at 40% R.H. for 15 days prior to being exposed to higher humidities.

D = larvae which were allowed to feed at 40% R.H. for 15 days prior to being exposed to higher humidities.

TABLE 52 The proportion of larvae absorbing water in different humidities after previously being desiccated (A) or allowed to feed (B)

Condition of larvae	Relative Humidities				
	80.5%	90%	92.5%	98%	100%
A	13.33	20.00	80.33	100.00	100.00
B	0.0	0.0	0.0	6.67	13.33

Interesting observations made during the course of this experiment include :

(a) Larvae deprived of food were virtually immobile after 15 days while those which had a chance to feed were normally active.

(b) The larvae listed as having absorbed water in line B (Table 52) pupated within ten days after termination of the experiment.

Data listed in Table 52 suggests that T. molitor larvae acquire the ability to absorb water when deprived of food, but not when they were allowed to feed.

12.24 The advantages to T. molitor of larvae having the ability to absorb water from unsaturated air

In Section 12.23 it was indicated that T. molitor larvae acquire the ability to absorb water only when deprived of food.

In Section 12.15 it was shown that T. molitor larvae deprived of food stay alive for a longer period of time in medium high humidities than in either the low or the very high humidities. It was further shown

(a) (Section 12.15) that at death the same amount of reserved foods have been used in all humidities considered and (b) (Section 12.12) that larvae can maintain a constant dry/wet ratio in dry air.

This situation arises as was concluded in Section 12.16, because "the metabolic rate of T. molitor larvae when deprived of food, after an initial decrease (which is independent of humidity), increases again, in response to internal conditions brought about by the drying power of the air surrounding them, and for the purpose of making available more water of metabolism in the lower humidities and because they continue their development in higher humidities."

In Section 12.16 it was shown that more T. molitor larvae moulted and pupated and sooner at the higher humidities. It was further shown in Section 12.16 that low humidities prevent pupation and that water absorption stimulate T. molitor larvae which otherwise would not have pupated, to metamorphose into pupae which develop into normal adults.

To my mind then, the species can benefit from the ability of the larvae to absorb water from unsaturated air in the following ways.

(a) Bellamy (1956) reported that T. molitor larvae given a chance to drink conserve water obtained this way as thoroughly as the water in normal larvae. Assuming that this is the case with water absorbed from the atmosphere, then by absorbing atmospheric water after being desiccated the larvae could avoid the stimulus to increase the metabolic rate which arises from a low ratio of water to dry matter. The result would be to save reserved foods and thus allow larvae to stay alive for a longer period of time.

(b) When ready to pupate or moult *T. molitor* larvae were observed to leave the cultures (Section 4.2) and to hide in cracks or under any available object. Since (a) pupation is prevented in lower humidities (b) the proportion of the larvae which pupate increases, and the time necessary for pupation decreases as humidity increases (c) pupation is stimulated in immature larvae by water absorption -

then water absorption could :

(i) speed up the process of pupation which will save maintenance energy and what seems more important

(ii) synchronise pupation and thus the time of emergence of the adults.

(c) In Section 12.16 it was stated that larvae starved and desiccated in different humidities at 20°C for 150 days, were observed to be able to move about only in 98% R.H. where water was absorbed. It was further shown in Section 12.16 that some larvae which were prevented to pupate by being desiccated in dry air at 20°C for 120 days will pupate but only when subjected to 98% R.H. where they could absorb water.

This suggests that water absorption might allow larvae previously in a state of "quiescence" primarily as a result of starvation and/or desiccation to resume normal activities. Cases parallel to this in other insect species were reviewed and discussed by Andrewartha (1952) and Lees (1955).

PART 6

APPENDICES, ACKNOWLEDGMENTS, REFERENCES

APPENDIX I

IDENTIFICATION OF ABBREVIATIONS USED IN THE APPENDICES

Consider k sample regressions in each Linear Regression analysis. Then

(A) FIRST SAMPLE REGRESSION

ID = Identification.

N = n_i = number of pairs of observations $(x_{i1}, y_{i1}) \ldots\ldots (x_{in_i}, y_{in_i})$.

X MEAN = $\bar{x}_i = \Sigma x_i / n_i$,

Y MEAN = $\bar{y}_i = \Sigma y_i / n_i$.

XCSS = Corrected sums of squares for $x_i = \Sigma(x_{ij} - \bar{x}_i)^2$

XYCSP = Corrected sums of products for x_i and $y_i = \Sigma(x_{ij} - \bar{x}_i)(y_{ij} - \bar{y}_i)$

YCSS = Corrected sums of squares for $y_i = \Sigma(y_{ij} - \bar{y})^2$

R = r_i = correlation coefficient

$$= \frac{\Sigma(x_{ij} - \bar{x}_i)(y_{ij} - \bar{y}_i)}{\sqrt{\Sigma(x_{ij} - \bar{x}_i)^2 \Sigma(y_{ij} - \bar{y}_i)^2}}$$

SLOPE = b_i = Regression coefficient of y_i on x_i

$$\frac{\Sigma(x_{ij} - \bar{x}_i)(y_{ij} - \bar{y}_i)}{\Sigma(x_{ij} - \bar{x}_i)^2}$$

CONST. = a_i = Constant = $\bar{y}_i - b_i \bar{x}_i$

S.E. SLOPE = Standard Error of Slope = $s_i / \sqrt{\Sigma(x_{ij} - \bar{x}_i)^2}$

S.E. CONST. = Standard error of constant =

$$= s_i \sqrt{\frac{1}{n_i} + \bar{x}_i^2 / \Sigma(x_{ij} - \bar{x}_i)^2}$$

SD = Standard Deviation =

$$s_i = \sqrt{(\Sigma(y_{ij} - \bar{y}_i)^2 - b_i^2 \Sigma(x_{ij} - \bar{x}_i)^2)/(n_i - 2)}$$

with $n_i - 2$ degrees of freedom.

(B) i^{th} SAMPLE REGRESSION

Similarly for the i^{th} sample regression we will have n_i, $\bar{x}_i$, $\bar{y}_i$, b_i, a_i, s_i, with XCSS $= \Sigma(x_{ij} - \bar{x}_i)^2$ etc.

(C) PARALLEL REGRESSION = PARLL.REG.

FROM LEFT TO RIGHT.

(i) $\sum_i \sum_j (x_{ij} - \bar{x}_i)^2 = \sum_i$ (XCSS)

(ii) $\sum_i \sum_j (x_{ij} - \bar{x}_i)(y_{ij} - \bar{y}_i) = \sum_i$ (XYCSP)

(iii) $\sum_i \sum_j (y_{ij} - \bar{y}_i) = \sum_i$ (YCSS)

(iv) $\dfrac{\sum_i \sum_j (x_{ij} - \bar{x}_i)(y_{ij} - \bar{y}_i)}{\sum_i \sum_j (x_{ij} - \bar{x}_i)^2} = B_c$ = Common slope

(v) Standard deviation of $B_c = s_c / \sqrt{\sum_i \sum_j (x_{ij} - \bar{x}_i)^2}$

where $s_c^2 = \dfrac{\text{ERROR SS + SLOPES SS}}{\text{ERROR DF + SLOPES DF}}$.

(D) POOLED REGRESSION = POOL.

N = Total number of pairs of observations in all regressions $= \sum_i n_i = N_p$.

X MEAN $= \bar{x}_p = \sum_i \sum_j x_{ij} / N_p$

Y MEAN $= \bar{y}_p = \sum_i \sum_j y_{ij} / N_p$

XCSS = Corrected sums of squares for all values of x = $\sum_i \sum_j (x_{ij} - \bar{x}_p)^2$

XYCSP = Corrected sums of products for all values of x and y = $\sum_i \sum_j (x_{ij} - \bar{x}_p)(y_{ij} - \bar{y}_p)$

YCSS = Corrected sums of squares for all values of y = $\sum_i \sum_j (y_{ij} - \bar{y}_p)$

R = r_p = Correlation coefficient

$$= \frac{\sum_i \sum_j (x_{ij} - \bar{x}_p)(y_{ij} - \bar{y}_p)}{\sqrt{\sum_i \sum_j (x_{ij} - \bar{x}_p)^2 \sum_i \sum_j (y_{ij} - \bar{y}_p)^2}}$$

SLOPE = b_p = Regression coefficient

$$= \frac{\sum_i \sum_j (x_{ij} - \bar{x}_p)(y_{ij} - \bar{y}_p)}{\sum_i \sum_j (x_{ij} - \bar{x}_p)^2}$$

CONST. = a_p = Constant = $\bar{y}_p - b_p\bar{x}_p$

S.E. SLOPE = Standard Error of Slope = $s_p / \sqrt{\sum_i \sum_j (x_{ij} - \bar{x}_p)^2}$

S.E. CONST. = Standard Error of Constant =

$$s_p \sqrt{\frac{1}{N_p} + \bar{x}_p^2 / \sum_i \sum_j (x_{ij} - \bar{x}_p)^2}$$

S.D. = Standard Deviation =

$$s_p = \sqrt{(\sum_i \sum_j (y_{ij} - \bar{y}_p)^2 - b_p^2 \sum_i \sum_j (x_{ij} - \bar{x}_p)^2)/(N_p - 2)}$$

with $N_p - 2$ degrees of freedom.

(E) ANALYSIS OF VARIANCE

Source = Source of Variation

D.F. = Degrees of Freedom

S.S. = Sums of Squares

M.S. = Mean Squares = S.S./D.F.

V.R. = Variance Ratio = F

The analysis of variance shows the sum of squares and their degrees of freedom derived from various sources i.e.

(i) SLOPES: This row shows the sums of squares attributable to differences between the slopes $b_1, b_2, \ldots b_K$. It is given by:

$$\sum_{i=1}^{k}[(b_i - B_c)^2 \sum_{j=1}^{n_i}(x_{ij} - \bar{x}_i)^2],$$ and has (k-1) D.F.

Its mean square, found by dividing the S.S. by D.F. is used to test whether the Slopes (b_i) are significantly different from each other.

(ii) DISPLACEMENTS: This row shows the sums of squares attributable to differences between constants $a_1, a_2 \ldots a_K$. It is given by

$(N_p - 2)s_p^2 - \sum_{i=1}^{k}(n_i - 2)s_i^2$ - Slopes Sums of squares,

and has (k-1) D.F.

Its mean square obtained as above, is used to test whether the constants (a_i) are significantly different from each other.

(iii) ERROR: This row shows the sums of squares attributable to random deviations from each individual regression line. It is given by

$$\sum_{1}^{k}(n_i - 2)s_i^2,$$ and has $(N_p - 2k)$ D.F.

Its mean square is found as above and provides an estimate of variance with which the mean squares in (i) and (ii) can be compared to make the tests mentioned in (i) and (ii).

Thus, e.g. the test in (i) consists of calculating the ratio, Slopes M.S./Error M.S., shown in the column headed V.R. (Variance ratio). This ratio is read together with tables of the F-distribution with (k-1) D.F. in the numerator and $(N_p - 2k)$ D.F. in the denominator. If the value in the tables of the F-distribution, at the required level of probability, is < or = the ratio (Slopes M.S./Error M.S.), it is then accepted that differences between slopes exist.

Note:- All x means, y means, XCSS, XYCSP, YCSS are written in E-type format

i.e. (a) 105172E + 03 = 105·172

(b) 105172E - 01 = 0·0105172 .

APPENDIX 2

(A) TESTING FOR LINEARITY

Having established that the seven regression lines of final weight at day ten (y) on initial weight (x), one for each humidity, have the same slopes, (Table A2), the data was then used to test the hypothesis that the mean final weight, for given initial weight, depends linearly on humidity.

This was done by dividing the "displacements" sums of squares viz. 5166·8850 with 6 degrees of freedom into its components (a) Sums of squares attributable to regression on humidity with 1 D.F.

(b) Sums of squares attributable to deviation from regression with 5 D.F.

Using the method of least squares, lead to the solution for the coefficients, $\bar{b}$ and $\bar{c}$ say, in the following equations:-

$$\bar{c}\,\Sigma\, n_i\,(t_i-\bar{t})^2 + \bar{b}\,\Sigma\, n_i\,(t_i-\bar{t})\bar{x}_i = \Sigma\, n_i\,(t_i-\bar{t})\bar{y}_i = h_1$$

$$\bar{c}\,\Sigma\, n_i\,(t_i-\bar{t})\bar{x}_i + \bar{b}\,\sum_i\sum_j\,(x_{ij}-\bar{x}_p)^2 = \sum_i\sum_j\,(x_{ij}-\bar{x}_p)(y_{ij}-\bar{y}_p) = h_2$$

where:-

$$\bar{t} = \Sigma\, n_i t_i/\Sigma\, n_i\,, \quad \bar{x}_p = \sum_i\sum_j x_{ij}/\Sigma\, n_i\,, \quad \bar{y}_p = \sum_i\sum_j y_{ij}/\Sigma\, n_i$$

$t_i = (\text{humidity})_i/s$.

Having solved these we form $\bar{c}h_1 + \bar{b}h_2$ and subtract from it

$$\frac{[\sum_i\sum_j\,(x_{ij}-\bar{x}_p)(y_{ij}-\bar{y}_p)]^2}{\Sigma\,\Sigma\,(x_{ij}-\bar{x}_p)^2} = k \text{ say}$$

(obtained from "pooled Regression").

The quantity $\bar{c}h_1 + \bar{b}h_2 - k$ = sums of squares attributable to regression with 1 D.F., and subtracted from "displacements" sums of squares, give sums of squares attributable to deviation from regression with 5 D.F., which is to be tested against "Error" sums of squares. (Table A2 viz. 1592·8050 with 119 D.F.)

In this particular case the equations are:-

$\bar{c}$ 5967·564 - $\bar{b}$ 248·8323 = 5314·0412 = h_1

$\bar{c}$ 248·8323 + $\bar{b}$ 26,269·5 = 23023·700 = h_2

$\bar{b}$ = 0·885,226,858

$\bar{c}$ = 0·927,242,296.

Thus $\bar{c}h_1 + \bar{b}h_2$ = 25,308·6015

$k = (23023·700)^2/26,269·500 = 20,178·9437$

i.e. $\bar{c}h_1 + \bar{b}h_2 - k$ = 5129·6579 = Sums of squares attributable to regression with 1 D.F.

and "Displacement" sums of squares viz. 5166·8850-5129·6579 = 37·2271 = Sums of squares due to deviation from regression with 5 D.F.

Analysis of Variance for Regression

Source	D.F.	S.S.	M.S.	V.R.
Regression	1	5129·6579	5129·6579	383·2417*** ($F_{1,119}$)
Deviation from Regression	5	37·2271	7·4454	0·55625 N.Sig.
Error	119	1592·8050	13·384915	

(B) CALCULATION OF CONSTANTS

The variance ratio associated with deviations from linear regression is non-significant at the 5% level of probability. I therefore accept the hypothesis that for a given initial weight, the mean final weight at day ten depends linearly on humidity.

Constants in the regression equation describing the relationship between mean final weight (day ten) and humidity were calculated in the following way:

(i) Regression coefficient (b) = $\bar{c}$

Since t_i = (humidity)$_i$/5, b in this case will be $\bar{c}/5$ = 0·927,242/5 = ·185448.

(ii) Constant = a = $\bar{y}_p - \bar{c}\,\bar{t}$.

In this case a = 78·20058.

APPENDIX 3

LINEAR REGRESSION ANALYSIS : DIFFERENT WEIGHT GROUPS OF T. MOLITOR ADULTS

LINEAR REGRESSION OF INITIAL DRY WEIGHT (Y) ON INITIAL LIVE WEIGHT (X)

ID / ID	N / R	X MEAN / SLOPE	Y MEAN / CONST	XCSS / SE OF SLOPE,CONST	XYCSP	YCSS / SD
Group 1	25	.843220E+02	.327740E+02	.77423000E+03	.31897000E+03	.18398300E+03
	.8451	.411983	-1.965274	.054335	4.591615	1.511874
Group 2	34	.952779E+02	.375308E+02	.22816000E+03	.35830000E+02	.15961500E+03
	.1877	.157038	22.568537	.145227	13.842098	2.193657
Group 3	52	.104289E+03	.414000E+02	.53275000E+03	.27695000E+03	.34270400E+03
	.6481	.519849	-12.814837	.086374	9.012211	1.993647
Group 4	55	.115102E+03	.451052E+02	.47776000E+03	.20820000E+03	.56075000E+03
	.4022	.435783	-5.054611	.136243	15.687118	2.977968
Group 5	55	.125794E+03	.494972E+02	.41693000E+03	.82570000E+02	.63524000E+03
	.1604	.198042	24.584565	.167354	21.057274	3.417180
Group 6	61	.135304E+03	.536180E+02	.54290000E+03	.13169000E+03	.81525000E+03
	.1979	.242567	20.797631	.156379	21.163930	3.643676
Group 7	44	.144146E+03	.571954E+02	.37816000E+03	.61330000E+02	.59033000E+03
	.1298	.162180	33.817757	.191159	27.560636	3.717345
Group 8	45	.154634E+03	.595777E+02	.36980000E+03	.16343000E+03	.70296000E+03
	.3205	.441941	-8.761613	.199161	30.802509	3.829911
Group 9	35	.170414E+03	.655528E+02	.11290000E+04	.30975000E+03	.55512000E+03
	.3912	.274357	18.798365	.112333	19.153828	3.774466
* PARLL REG		.48496900E+04	.15887200E+04	.45459520E+04	.32759207E-00	.45783113E-01
POOL	406	.12694322E+03	.49788152E+02	.23675770E+06	.89608200E+05	.38080700E+05
	.9437	.378480	1.742605	.006599	.852769	3.211109

* See Appendix 10 for identification

TABLE 41

LINEAR REGRESSION ANALYSIS: T. MOLITOR ADULTS IN DIFFERENT RELATIVE HUMIDITIES

LINEAR REGRESSION OF LENGHT OF LIFE (DAYS) (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
0% R.H.	18	.105172E+03	.171666E+02	.29224200E+04	.11843400E+03	.88500000E+02
	.2328	.040526	12.904457	.042308	4.482264	2.287197
20% R.H.	19	.109305E+03	.195789E+02	.26723600E+04	-.36507000E+02	.25263160E+03
	-.0444	-.013660	21.072161	.074497	8.190779	3.851148
40% R.H.	19	.111173E+03	.241578E+02	.46810000E+04	.56137900E+03	.25252700E+03
	.5163	.119927	10.825151	.048242	5.416483	3.300645
60% R.H.	19	.106323E+03	.284210E+02	.36835300E+04	.83811000E+02	.48063200E+03
	.0629	.022752	26.001880	.087435	9.375811	5.306627
80% R.H.	19	.103102E+03	.298421E+02	.34464100E+04	.29306000E+02	.35652700E+03
	.0264	.008503	28.965389	.077980	8.108308	4.577938
90% R.H.	20	.106847E+03	.312500E+02	.39532800E+04	-.30924000E+03	.26975000E+03
	-.2994	-.078223	39.608001	.058744	6.330758	3.693538
95% R.H.	19	.109268E+03	.303684E+02	.40372000E+04	.42657200E+03	.50242200E+03
	.2995	.105660	18.823081	.081631	8.998808	5.186805
*PARLL REG		.25396200E+05	.87375500E+03	.22029896E+04	.34404950E-01	.26162744E-01
POOL	133	.10732593E+03	.25932330E+02	.26269500E+05	.59664000E+03	.57603910E+04
	.0485	.022712	23.494715	.040865	4.423332	6.623368

SOURCE	DF	SS	MS	VR
SLOPES	6.	113.979400	18.996566	1.097
DISPLACEMENTS	6.	3573.911800	595.651960	34.426
ERROR	119.	2058.948800	17.302090	

* See Appendix 10 for identification

LINEAR REGRESSION ANALYSIS : T. MOLITOR ADULTS IN DIFFERENT RELATIVE HUMIDITIES

LINEAR REGRESSION OF FINAL LIVE WEIGHT (DAY 10) (Y) ON INITIAL LIVE WEIGHT (X)

ID ID	N R	X MEAN SLOPE	Y MEAN CONST	XCSS SE OF SLOPE,CONST	XYCSP YCSS	YCSS SD
0% R.H.	18	.105172E+03	.758944E+02	.29224200E+04	.24369700E+04	.22231500E+04
	.9560	.833887	-11.807372	.063910	6.770801	3.454985
20% R.H.	19	.109305E+03	.836736E+02	.26723600E+04	.22245500E+04	.21516300E+04
	.9277	.832429	-7.315185	.081241	8.932284	4.199790
40% R.H.	19	.111173E+03	.898447E+02	.46810000E+04	.42354300E+04	.40373500E+04
	.9742	.904813	-10.746670	.050765	5.699716	3.473239
60% R.H.	19	.106323E+03	.878578E+02	.36835300E+04	.34054300E+04	.33338600E+04
	.9717	.924501	-10.438535	.054432	5.836838	3.303599
80% R.H.	19	.103102E+03	.900789E+02	.34464100E+04	.33667000E+04	.36639600E+04
	.9474	.976871	-10.639090	.080016	8.320026	4.697474
90% R.H.	20	.106847E+03	.941825E+02	.39532800E+04	.33002600E+04	.29419600E+04
	.9677	.834815	4.984536	.051242	5.522363	3.221898
95% R.H.	19	.109268E+03	.971842E+02	.40372000E+04	.34972100E+04	.31788200E+04
	.9762	.866246	2.530835	.046652	5.142788	2.964241
* PARLL REG		.25396200E+05	.22466550E+05	.21530730E+05	.88464219E-00	.22838837E-01
POOL	133	.10732593E+03	.88525563E+02	.26269500E+05	.23023700E+05	.27001700E+05
	.8644	.876442	-5.539418	.044526	4.819643	7.216793

SOURCE	DF	SS	MS	VR
SLOPES	6.	63.067000	10.511166	.785
DISPLACEMENTS	6.	5166.885000	861.147500	64.337
ERROR	119.	1592.805000	13.384915	

* See Appendix 10 for identification

LINEAR REGRESSION ANALYSIS : I. MOLITOR ADULTS IN DIFFERENT RELATIVE HUMIDITIES

LINEAR REGRESSION OF FINAL LIVE WEIGHT (AT DEATH)(Y) ON INITIAL LIVE WEIGHT (X)

ID / ID	N / R	X MEAN / SLOPE	Y MEAN / CONST	XCSS / SE OF SLOPE,CONST	XYCSP	YCSS / SD
0% R.H.	18	.105172E+03	.587138E+02	.29224200E+04	.15225200E+04	.93603200E+03
	.9205	.520979	3.921351	.055268	5.855240	2.987795
20% R.H.	19	.109305E+03	.632657E+02	.26723600E+04	.14535500E+04	.10515250E+04
	.8671	.543919	3.812474	.075783	8.332129	3.917608
40% R.H.	19	.111173E+03	.649631E+02	.46810000E+04	.23267500E+04	.13262550E+04
	.9338	.497062	9.702880	.046181	5.185060	3.159623
60% R.H.	19	.106323E+03	.633868E+02	.36835300E+04	.22689600E+04	.16462560E+04
	.9213	.615974	-2.105818	.063012	6.756889	3.824340
80% R.H.	19	.103102E+03	.690552E+02	.34464100E+04	.26835500E+04	.24253210E+04
	.9282	.778650	-11.225684	.075703	7.871517	4.444246
90% R.H.	20	.106847E+03	.741250E+02	.39532800E+04	.23362100E+04	.18740100E+04
	.8583	.590954	10.982952	.083270	8.973939	5.235643
95% R.H.	19	.109268E+03	.754552E+02	.40372000E+04	.26155700E+04	.19577600E+04
	.9303	.647867	4.663823	.061928	6.826814	3.934894
PARLL REG		.25396200E+05	.15207110E+05	.11217159E+05	.59879470E-00	.25788594E-01
POOL	133	.10732593E+03	.67110902E+02	.26269500E+05	.15386730E+05	.15497090E+05
	.7625	.585726	4.247311	.043409	4.698717	7.035722

SOURCE	DF	SS	MS	VR
SLOPES	6.	196.726800	32.787800	2.038
DISPLACEMENTS	6.	4373.459000	728.909830	45.307
ERROR	119.	1914.496200	16.088203	

TABLE A1

LINEAR REGRESSION ANALYSIS: T. MOLITOR ADULTS IN DIFFERENT RELATIVE HUMIDITIES

LINEAR REGRESSION OF CALCULATED WEIGHT (DAY 10) (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
0% R.H.	18	.105172E+03	.777744E+02	.29224200E+04	.24643300E+04	.22664800E+04
	.9575	.843249	-10.912006	.063481	6.725335	3.431785
20% R.H.	19	.109305E+03	.852910E+02	.26723600E+04	.22976800E+04	.21810100E+04
	.9517	.859794	-8.688991	.067252	7.394229	3.476626
40% R.H.	19	.111173E+03	.918484E+02	.46810000E+04	.41102400E+04	.37433100E+04
	.9819	.878068	-5.769717	.041071	4.611365	2.810030
60% R.H.	19	.106323E+03	.909705E+02	.36835300E+04	.32910600E+04	.30376600E+04
	.9838	.893452	-4.024657	.039409	4.225890	2.391816
80% R.H.	19	.103102E+03	.913773E+02	.34464100E+04	.31705800E+04	.29532200E+04
	.9938	.919965	-3.473545	.024923	2.591500	1.463157
90% R.H.	20	.106847E+03	.963040E+02	.39532800E+04	.35815600E+04	.33103700E+04
	.9900	.905971	-.496817	.030357	3.271565	1.908721
95% R.H.	19	.109268E+03	.979847E+02	.40372000E+04	.36961600E+04	.34410700E+04
	.9916	.915525	-2.053310	.028854	3.180780	1.833363
PARLL REG		.25396200E+05	.22611610E+05	.20933120E+05	.89035406E-00	.15882450E-01
POOL	133	.10732593E+03	.90360827E+02	.26269500E+05	.23148800E+05	.26152500E+05
	.8831	.881204	-4.215258	.040889	4.425961	6.627305

SOURCE	DF	SS	MS	VR
SLOPES	6.	16.266000	2.711000	.411
DISPLACEMENTS	6.	4952.894000	825.482330	125.214
ERROR	119.	784.515000	6.592563	

TABLE A2

LINEAR REGRESSION ANALYSIS : T. MOLITOR ADULTS IN DIFFERENT RELATIVE HUMIDITIES

(A) LINEAR REGRESSION OF MEAN MEASURED WEIGHT (DAY 10) (Y) ON HUMIDITY (X)

(B) LINEAR REGRESSION OF MEAN CALCULATED WEIGHT (DAY 10) (Y) ON HUMIDITY (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
(A)	7	.550000E+02	.883993E+02	.79500000E+04	.14760040E+04	.27602100E+03
	.9963	.185660	78.188037	.007066	.455799	.630047
ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
(B)	7	.550000E+02	.902331E+02	.79500000E+04	.14406280E+04	.26453300E+03
	.9934	.181211	80.266520	.009350	.603128	.833698
PARLL REG	.15900000E+05	.29166320E+04	.54055400E+03	.18343597E-00	.56274514E-02	
POOL	14	.55000000E+02	.89316257E+02	.15900000E+05	.29166310E+04	.55232000E+03
	.9842	.183435	79.227282	.009523	.614302	1.200872

SOURCE	DF	SS	MS	VR
SLOPES	1.	.078700	.078700	.144
DISPLACEMENTS	1.	11.766370	11.766370	21.549
ERROR	10.	5.460070	.546007	

LINEAR REGRESSION ANALYSIS: *T. MOLITOR* ADULTS IN DIFFERENT RELATIVE HUMIDITIES

LINEAR REGRESSION OF FINAL DRY WEIGHT (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
0% R.H.	18	.105172E+03	.246777E+02	.29224200E+04	.66421600E+03	.19719600E+03
	.8749	.227282	.773934	.031443	3.331201	1.699836
20% R.H.	19	.109305E+03	.240368E+02	.26723600E+04	.50441000E+03	.13318000E+03
	.8455	.188750	3.405391	.028910	3.178655	1.494543
40% R.H.	19	.111173E+03	.232315E+02	.46810000E+04	.82957000E+03	.17770700E+03
	.9095	.177220	3.529304	.019638	2.204920	1.343613
60% R.H.	19	.106323E+03	.214500E+02	.36835300E+04	.84536200E+03	.20750020E+03
	.9669	.229497	-2.951050	.014678	1.573967	.890851
80% R.H.	19	.103102E+03	.206236E+02	.34464100E+04	.73558800E+03	.17533700E+03
	.9462	.213436	-1.382130	.017690	1.839452	1.038552
90% R.H.	20	.106847E+03	.209500E+02	.39532800E+04	.66359400E+03	.12943500E+03
	.9276	.167859	3.014676	.015924	1.716136	1.001241
95% R.H.	19	.109268E+03	.210868E+02	.40372000E+04	.72265700E+03	.14913940E+03
	.9313	.178999	1.527844	.016978	1.871625	1.078782
PARLL REG		.25396200E+05	.49653970E+04	.11694946E+04	.19551732E-00	.79109855E-02
POOL	133	.10732593E+03	.22265789E+02	.26269500E+05	.51254700E+04	.14764400E+04
	.8230	.195111	1.325313	.011765	1.273569	1.907006

SOURCE	DF	SS	MS	VR
SLOPES	6.	14.123760	2.353960	1.517
DISPLACEMENTS	6.	277.730700	46.288450	29.847
ERROR	119.	184.549740	1.550838	

TABLE A7

LINEAR REGRESSION ANALYSIS: T. MOLITOR ADULTS IN DIFFERENT RELATIVE HUMIDITIES

LINEAR REGRESSION OF RATE OF UTILISING RESERVES MG/DAY (Y) ON INITIAL WEIGHT (X)

ID / ID	N / X MEAN / Y MEAN / XCSS / XYCSP / YCSS
0% R.H.	18 / .105172E+03 / .993305E-00 / .29224200E+04 / .13473900E+02 / .49198500E-00
	R / SLOPE / CONST / SE OF SLOPE,CONST / SD
	.3553 / .004610 / .508406 / .003032 / .321217 / .163909
20% R.H.	19 / .109305E+03 / .100451E+01 / .26723600E+04 / .17045100E+02 / .92344400E-00
	.3431 / .006378 / .307329 / .004234 / .465603 / .218917
40% R.H.	19 / .111173E+03 / .860589E-00 / .46810000E+04 / .19891000E+02 / .37474200E-00
	.4749 / .004249 / .388178 / .001909 / .214415 / .130658
60% R.H.	19 / .106323E+03 / .743321E-00 / .36835300E+04 / .15843900E+02 / .41203100E-00
	.4066 / .004301 / .285992 / .002343 / .251287 / .142226
80% R.H.	19 / .103102E+03 / .689126E-00 / .34464100E+04 / .17330100E+02 / .32322270E-00
	.5192 / .005028 / .170679 / .002007 / .208720 / .117843
90% R.H.	20 / .106847E+03 / .681540E-00 / .39532800E+04 / .17597500E+02 / .21058650E-00
	.6098 / .004451 / .205922 / .001363 / .146919 / .085717
95% R.H.	19 / .109268E+03 / .743015E-00 / .40372000E+04 / .17857300E+02 / .41226300E-00
	.4377 / .004423 / .259700 / .002203 / .242919 / .140016
PARLL REG	.25396200E+05 / .11903880E+03 / .31482742E+01 / .46872681E-02 / .90330888E-03
POOL	133 / .10732593E+03 / .81414285E-00 / .26269500E+05 / .13267100E+03 / .52956480E+01
	.3557 / .005050 / .272105 / .001159 / .125492 / .187909

SOURCE	DF	SS	MS	VR
SLOPES	6.	.010008	.001668	.076
DISPLACEMENTS	6.	2.035301	.339216	15.644
ERROR	119.	2.580298	.021683	

TABLE A6

LINEAR REGRESSION ANALYSIS: T. MOLITOR ADULTS IN DIFFERENT RELATIVE HUMIDITIES

LINEAR REGRESSION OF TOTAL AMOUNT OF WATER LOST (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
0% R.H.	18	.105172E+03	.290894E+02	.29224200E+04	.98868000E+03	.61676700E+03
	.7364	.338308	-6.491229	.077698	8.231520	4.200357
20% R.H.	19	.109305E+03	.269652E+02	.26723600E+04	.71198500E+03	.45104400E+03
	.6485	.266425	-2.156451	.075847	8.339196	3.920931
40% R.H.	19	.111171E+03	.256305E+02	.46819800E+04	.14146020E+04	.57313700E+03
	.8635	.302137	-7.958423	.042789	4.804163	2.927884
60% R.H.	19	.106323E+03	.224036E+02	.36835300E+04	.86558600E+03	.46480900E+03
	.6615	.234988	-2.581123	.064610	6.928257	3.921333
80% R.H.	19	.103102E+03	.139073E+02	.34464100E+04	.19419600E+03	.28314540E+03
	.1965	.056347	8.097811	.068161	7.087324	4.001492
90% R.H.	20	.106847E+03	.114810E+02	.39532800E+04	.78202000E+03	.56953420E+03
	.5211	.197815	-9.655089	.076352	8.228418	4.800685
95% R.H.	19	.109268E+03	.117557E+02	.40372000E+04	.61566900E+03	.30469810E+03
	.5551	.152499	-4.907536	.055421	6.109494	3.521440
PARLL REG		.25397180E+05	.55727380E+04	.32631347E+04	.21942349E-00	.25351522E-01
POOL	133	.10732556E+03	.20043759E+02	.26270200E+05	.61140000E+04	.98320380E+04
	.3804	.232735	-4.934676	.049431	5.350599	8.011963

SOURCE	DF	SS	MS	VR
SLOPES	6.	191.714400	31.952400	2.056
DISPLACEMENTS	6.	6368.750000	1061.458300	68.328
ERROR	119.	1848.630700	15.534711	

TABLE A9

LINEAR REGRESSION ANALYSIS: T. MOLITOR ADULTS IN DIFFERENT RELATIVE HUMIDITIES

LINEAR REGRESSION OF RATE OF WATER LOSS MG/DAY (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
0% R.H.	18	.105172E+03	.169388E+01	.29224200E+04	.36517500E+02	.13050280E+01
	.5913	.012495	.379694	.004260	.451352	.230314
20% R.H.	19	.109305E+03	.139000E+01	.26723600E+04	.19746500E+02	.63680000E-00
	.4786	.007389	.582325	.003287	.361411	.169929
40% R.H.	19	.111173E+03	.106894E+01	.46810000E+04	.35741000E+02	.81017900E-00
	.5803	.007635	.220099	.002598	.291740	.177777
60% R.H.	19	.106323E+03	.796842E-00	.36835300E+04	.27547000E+02	.51161100E-00
	.6345	.007478	.001708	.002209	.236888	.134076
80% R.H.	19	.103102E+03	.465789E-00	.34464100E+04	.62481900E+01	.20946320E-00
	.2325	.001812	.278868	.001838	.191212	.107958
90% R.H.	20	.106847E+03	.367500E-00	.39532800E+04	.20167350E+02	.53377500E-00
	.4390	.005101	-.177574	.002460	.265192	.154720
95% R.H.	19	.109268E+03	.398421E-00	.40372000E+04	.11477060E+02	.14705270E-00
	.4710	.002842	.087789	.001291	.142338	.082042
PARLL REG		.25396200E+05	.15744460E+03	.41539089E+01	.61995337E-02	.10005197E-02
POOL	133	.10732593E+03	.87308270E-00	.26269500E+05	.18083700E+03	.34742040E+02
	.1892	.006883	.134260	.003119	.337706	.505671

SOURCE	DF	SS	MS	VR
SLOPES	6.	.251876	.041979	1.707
DISPLACEMENTS	6.	30.319349	5.053224	205.517
ERROR	119.	2.925949	.024587	

TABLE A10

Table All A-D. RATIO = 100 X DRY MATERIAL (mg)/INITIAL LIVE WEIGHT (mg)

	(A) 0% RELATIVE HUMIDITY			(B) 30% RELATIVE HUMIDITY		
DAYS	n	$\bar{x}$	s^2	n	$\bar{x}$	s^2
0	51	39.0925	4.9937	50	37.4512	7.9478
1	51	37.6255	9.7117	50	35.6344	6.2145
2	51	36.5851	5.1406	50	35.5192	8.4917
3	51	35.2841	6.5952	50	34.2554	8.5106
4	51	33.5312	6.2452	50	31.9545	7.4156
5	50	32.6752	6.0962	50	31.8552	10.4917
6	51	31.2580	10.8734	50	30.0876	8.4706
7	48	30.0363	8.5225	50	29.6068	8.0076
8	50	29.8388	9.1287	49	29.6229	5.0359
9	49	28.5524	8.8692	50	27.7944	9.6703
10	45	27.4369	8.9377	50	26.5918	9.0939
11	44	26.2666	7.7901	49	26.4741	10.2054
12	41	26.4083	8.2927	48	24.8777	8.3173
13	34	25.6532	5.7049	48	24.4158	7.7069
14	26	24.7627	5.9389	46	24.5259	8.2835
15				42	22.8786	6.6691
16				33	23.0303	4.5766
17				29	22.7290	4.9662
18				24	22.2679	3.1833
19				25	22.8672	2.6453
20				17	21.7047	2.5227

n = number of beetles in each class

s^2 = individual class variances

FOR STARVED T. MOLITOR ADULTS AT DIFFERENT TIMES AND IN DIFFERENT HUMIDITIES

	(C) 60% RELATIVE HUMIDITY			(D) 90% RELATIVE HUMIDITY		
DAYS	n	$\bar{x}$	s^2	n	$\bar{x}$	s^2
0	50	38.1456	6.0693	50	39.5970	6.4590
1	50	37.0268	8.0422	50	37.4754	8.0880
2	50	36.0298	4.3751	50	37.6872	9.8094
3	50	35.0358	7.2513	50	36.1256	11.9700
4	50	33.0650	7.7373	48	34.0240	9.8057
5	50	31.2406	9.3230	50	33.8554	9.0286
6	49	30.6557	8.3522	49	32.2492	14.3263
7	50	30.1392	8.1842	48	30.8971	9.5213
8	49	29.4608	10.2057	50	29.9874	9.7928
9	49	27.7498	7.4719	50	29.3402	11.8621
10	50	26.7056	9.7715	50	27.6200	7.4548
11	49	27.1259	11.2644	48	27.1938	13.7413
12	48	26.8494	9.5818	49	26.8945	13.9343
13	50	25.4340	9.2064	50	25.7904	8.4417
14	48	25.0560	12.3151	47	24.6598	12.8124
15	48	23.4450	6.8767	45	24.2798	10.8727
16	45	23.4700	7.0707	48	24.0100	9.4504
17	37	23.2305	6.3463	36	22.6275	6.8416
18	34	22.1615	4.7925	43	22.7951	7.0721
19	33	21.2930	3.3519	41	21.8163	7.7249
20	28	21.5204	3.2445	35	22.6026	6.2950

$\bar{x}$ = mean number of mgs dry material per 100 mgs of initial live weight.

LINEAR REGRESSION ANALYSIS: T. MOLITOR ADULTS IN DIFFERENT RELATIVE HUMIDITIES

LINEAR REGRESSION OF THE RATIO 100 X DRY MATERIAL / INITIAL LIVE WEIGHT (Y)

ON TIME (DAYS) (X)

ID / ID	N / R	X MEAN / SLOPE	Y MEAN / CONST	XCSS / SE OF SLOPE	XYCSP / ,CONST	YCSS / SD
0% R.H.	693	.645743E+01	.315679E+02	.11809995E+05	-.12347950E+05	.18263210E+05
	-.8407	-1.045550	38.319508	.025611	.196288	2.783245
30% R.H.	740	.692567E+01	.301089E+02	.13660913E+05	-.13047820E+05	.18710580E+05
	-.8161	-.955120	36.723787	.024895	.202900	2.909739
60% R.H.	741	.695681E+01	.306873E+02	.13824619E+05	-.13155290E+05	.19250110E+05
	-.8064	-.951584	37.307376	.025669	.210197	3.018155
90% R.H.	739	.696211E+01	.316001E+02	.13788940E+05	-.14528900E+05	.23086530E+05
	-.8143	-1.053663	38.935855	.027665	.226668	3.248621
PARLL REG		.53084467E+05	-.53079960E+05	.79310430E+05	-.99991509E-00	.13036451E-01
POOL	2913	.68314452E+01	.30981476E+02	.53212240E+05	-.53260400E+05	.80459000E+05
	.8139	-1.000905	37.819103	.013239	.106684	3.053985

SOURCE	DF	SS	MS	VR
SLOPES	3.	124.132000	41.377333	4.603
DISPLACEMENTS	3.	915.421000	305.140330	33.948
ERROR	2905.	26110.844000	8.988242	

LINEAR REGRESSION ANALYSIS: T. MOLITOR ADULTS IN DIFFERENT RELATIVE HUMIDITIES

LINEAR REGRESSION OF THE RATIO 100 X DRY MATERIAL / INITIAL LIVE WEIGHT (Y)

ON TIME (DAYS) (X)

ID / ID	N / X MEAN / Y MEAN / XCSS / XYCSP / YCSS
30% R.H.	910 / .881648E+01 / .287205E+02 / .28300353E+05 / -.23524710E+05 / .27107920E+05
	-.8493 / -.831251 / 36.049307 / .017144 / .178851 / 2.884137
60% R.H.	966 / .934057E+01 / .288189E+02 / .32548950E+05 / -.27639590E+05 / .31748640E+05
	-.8598 / -.849169 / 36.750703 / .016242 / .178624 / 2.930369
90% R.H.	987 / .957750E+01 / .294609E+02 / .34622830E+05 / -.31303400E+05 / .38717540E+05
	-.8549 / -.904125 / 38.120174 / .017475 / .196792 / 3.251758
PARLL REG	.95472133E+05 / -.82467700E+05 / .97574100E+05 / -.86378817E-00 / .98233173E-02
POOL	2863 / .92556758E+01 / .29009001E+02 / .95756850E+05 / -.82224510E+05 / .97886200E+05
	.8492 / -.858680 / 36.956666 / .009979 / .108911 / 3.087993

SOURCE	DF	SS	MS	VR
SLOPES	2.	93.251000	46.625500	5.075
DISPLACEMENTS	2.	942.167000	471.083500	51.279
ERROR	2857.	26246.225000	9.186638	

TABLE A12

TABLE A13 A-D. A SUMMARY OF DATA CONCERNING THE INITIAL LIVE WEIGHT EXPERIMENT DESCRIBED IN SEC. 7.0

	(A) 0% RELATIVE HUMIDITY			(B) 30% RELATIVE HUMIDITY		
DAYS	n	$\bar{x}$	s^2	n	$\bar{x}$	s^2
0	51	129.672	379.1772	50	118.184	136.9347
1	51	130.724	364.3070	50	116.579	119.2706
2	51	133.604	251.8089	50	118.706	212.7320
3	51	131.631	364.9568	50	117.646	91.3031
4	51	135.966	422.9382	50	121.982	194.5163
5	50	133.058	307.0055	50	122.800	195.9296
6	51	132.611	295.5842	50	119.105	164.3300
7	48	133.690	379.2336	50	122.089	214.3120
8	50	136.159	352.6447	49	121.254	204.1225
9	49	131.923	328.8256	50	123.257	255.8759
10	45	134.541	278.3089	50	122.203	303.4047
11	44	137.197	473.1600	49	122.021	184.4142
12	41	136.346	311.7208	48	120.318	130.3551
13	34	135.893	248.9206	48	122.581	164.3872
14	26	140.059	352.0912	46	122.035	144.1642
15				42	123.016	207.1888
16				33	123.592	189.8100
17				29	121.550	217.4818
18				24	119.735	186.2130
19				25	122.348	200.4425
20				17	121.964	267.5188

n = number of beetles in each class

s^2 = individual class variances

(mg) OF BEETLES USED ON DIFFERENT DAYS AND IN DIFFERENT HUMIDITIES IN

	(C) 60% RELATIVE HUMIDITY			(D) 90% RELATIVE HUMIDITY		
DAYS	n	$\bar{x}$	s^2	n	$\bar{x}$	s^2
0	50	152.270	249.1980	50	141.900	229.2061
1	50	149.090	230.8878	50	141.380	269.9592
2	50	148.730	269.3469	50	140.306	350.8837
3	50	150.041	295.0592	50	144.869	326.4714
4	50	150.527	172.6531	48	147.885	360.0787
5	50	154.552	243.3918	50	141.515	271.2714
6	49	151.705	190.9083	49	140.353	254.5290
7	50	151.249	256.4386	48	145.367	238.2085
8	49	148.156	183.2771	50	141.544	261.7898
9	48	152.355	139.7681	50	148.037	222.3592
10	50	151.522	255.3163	50	146.236	206.2694
11	49	151.058	159.4625	48	147.353	298.5936
12	48	154.220	310.2340	49	144.645	317.5271
13	50	154.209	214.7510	50	144.812	277.1327
14	48	151.270	213.7872	47	143.372	277.2113
15	48	148.642	251.2489	45	146.442	270.9032
16	45	152.023	215.3977	48	146.640	318.8383
17	37	153.081	171.9564	36	146.003	246.8843
18	34	154.770	256.4370	43	146.524	332.0155
19	33	151.721	242.0484	41	150.229	246.9610
20	28	154.883	278.9926	35	148.286	269.5465

$\bar{x}$ = mean initial live weight (mg)

TABLE 214 A-D. RATIO : 100 X TOTAL WATER (mg)/INITIAL LIVE WEIGHT (mg) FOR

	(A) 0% RELATIVE HUMIDITY			(B) 30% RELATIVE HUMIDITY		
DAYS	n	$\bar{x}$	s^2	n	$\bar{x}$	s^2
0	51	60.9075	4.9937	50	62.5542	7.8478
1	51	58.6533	16.1597	50	62.4184	6.1204
2	51	56.2949	7.3444	50	60.2002	8.9018
3	51	54.3982	16.3405	50	58.8594	9.5255
4	51	52.6461	8.9443	50	58.0412	7.8443
5	50	49.6628	15.6390	50	55.2558	9.3384
6	51	48.3518	9.8245	50	54.3556	8.4210
7	48	45.5483	15.1525	50	52.1722	13.9466
8	50	43.4906	15.6782	49	50.8351	8.4917
9	49	41.6669	16.2819	50	49.6794	7.2304
10	45	39.6542	18.0441	50	48.1372	17.5400
11	44	38.1311	11.0395	49	46.5945	11.8712
12	41	36.0251	12.9411	48	44.6510	8.6533
13	34	34.7415	7.0662	48	43.5163	10.1905
14	26	33.5138	10.5903	46	42.4922	11.4818
15				42	41.5600	11.0613
16				33	40.9194	11.4964
17				29	38.9793	11.2485
18				24	38.3371	8.1033
19				25	37.4372	10.1913
20				17	36.2059	4.8280

n = number of beetles in each class

s^2 = individual class variances

STARVED T. MOLITOR ADULTS AT DIFFERENT TIMES AND IN DIFFERENT HUMIDITIES

	(C) 60% RELATIVE HUMIDITY			(D) 90% RELATIVE HUMIDITY		
DAYS	n	$\bar{x}$	s^2	n	$\bar{x}$	s^2
0	50	61.8544	6.0693	50	60.4030	6.4590
1	50	61.2288	10.7849	50	60.6382	10.8959
2	50	60.6466	11.4872	50	60.1056	21.2391
3	50	59.1158	24.1714	50	60.2702	20.9580
4	50	59.2578	15.5915	48	60.8885	16.7387
5	50	58.8646	19.5526	50	59.4156	16.3056
6	49	57.1267	23.2610	49	58.6063	20.1314
7	50	56.5404	22.1328	48	58.4413	12.7409
8	49	54.9733	21.0854	50	58.0976	23.4102
9	48	53.7448	7.7769	50	57.2094	20.8864
10	50	54.2872	17.3156	50	57.2746	13.1751
11	49	52.6823	14.2306	48	56.4081	13.1836
12	48	52.2502	11.0298	49	56.3072	19.7413
13	50	51.7410	15.2115	50	54.7055	11.2166
14	48	50.3306	21.0782	47	55.1440	15.0172
15	48	48.8565	24.3965	45	53.5282	21.8460
16	45	49.5356	22.1751	48	53.5331	19.2007
17	37	47.7659	20.3759	36	52.9294	13.4124
18	34	46.9918	16.5627	43	52.3028	19.6852
19	33	46.3615	12.7868	41	52.6456	20.3394
20	28	44.1607	14.9299	35	51.9046	19.2577

$\bar{x}$ = mean number of mgs of water per 100 mgs of initial live weight.

TABLE A15 A-D. RATIO : 100 X DRY MATERIAL (mg) ON (DAY)$_1$/FINAL LIVE AND IN DIFFERENT HUMIDITIES

	(A) 0% RELATIVE HUMIDITY			(B) 30% RELATIVE HUMIDITY		
DAYS	n	$\bar{x}$	s^2	n	$\bar{x}$	s^2
0	51	39.0925	4.9937	50	37.4512	7.8478
1	51	39.1116	12.5068	50	36.3404	6.3977
2	51	39.3963	5.6774	50	37.0944	8.9702
3	51	39.3967	11.3246	50	36.7924	9.7656
4	51	38.9233	8.4209	50	35.4546	8.4495
5	50	39.7428	10.8411	50	36.5516	11.9929
6	51	39.2541	13.1519	50	35.6192	10.1386
7	48	40.4035	13.1274	50	36.1754	10.9912
8	50	40.7910	15.9514	49	36.8231	6.1784
9	49	40.6982	14.8436	50	35.8480	9.3315
10	45	40.9462	14.9807	50	35.6032	11.5212
11	44	40.7852	10.4272	49	36.1949	11.2333
12	41	42.5017	9.3441	48	35.7225	7.5930
13	34	42.4706	9.7149	48	35.9200	10.0505
14	26	42.6712	10.2179	46	36.5752	11.5865
15				42	35.4245	8.7688
16				33	36.0518	10.3239
17				29	36.8548	8.7759
18				24	36.7700	7.0938
19				25	37.9828	7.5237
20				17	37.3082	5.4704

n = number of beetles in each class

s^2 = individual class variances

WEIGHT (mg) ON $(DAY)_i$ FOR STARVED T. MOLITOR ADULTS AT DIFFERENT TIMES

	(C) 60% RELATIVE HUMIDITY			(D) 90% RELATIVE HUMIDITY		
DAYS	n	$\bar{x}$	s^2	n	$\bar{x}$	s^2
0	50	38.1456	6.0693	50	39.5970	6.4590
1	50	37.6986	9.2328	50	37.8332	9.1541
2	50	37.3008	6.3300	50	38.5984	12.5167
3	50	37.2964	13.1878	50	37.5308	17.0799
4	50	35.9478	11.1273	48	35.6852	13.0845
5	50	34.7124	13.6595	50	36.6046	11.9963
6	49	34.9898	13.6598	49	35.5196	18.5064
7	50	34.8344	13.6679	48	34.5844	10.7009
8	49	34.9414	16.4341	50	34.0998	14.6634
9	48	33.2133	7.6591	50	34.0364	18.6756
10	50	32.9828	12.5264	50	32.1552	7.9433
11	49	33.9608	15.1009	48	32.4798	14.2444
12	48	33.9288	12.3333	49	32.9920	18.8558
13	50	32.9536	12.0853	50	31.3936	8.8593
14	48	33.2548	18.3213	47	30.8409	12.0629
15	48	32.4910	10.2639	45	31.2036	12.2145
16	45	32.2273	14.7283	48	30.9471	14.0361
17	37	32.7778	11.0004	36	29.9561	9.8948
18	34	32.2074	16.0798	43	30.4258	13.5873
19	33	31.5203	7.4139	41	29.3049	9.4276
20	28	32.8129	6.1883	35	30.6889	8.4114

$\bar{x}$ = mean number of mgs dry material per 100 mgs of final live weight.

Table A16. A Listing Of Initial Weights (A), Rate of Weight Loss (B), and Length of Life (Days) (C) of T. molitor Adults With Different Initial Weights (mg) Starved to Death in 0% R.H. at 25°C (Sec.8.1

A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
91.00	2.8291	14	110.00	4.2188	13	130.55	3.6015	16	150.20	5.1971	14	170.00	4.0111	19
91.25	2.8065	15	110.00	3.5694	15	131.10	3.6404	17	150.75	4.3740	15	170.10	4.3060	20
91.30	2.3737	18	110.40	3.1886	19	131.90	4.1495	16	151.45	5.6289	13	170.60	5.1291	15
92.10	2.9240	14	111.10	3.3006	16	132.15	3.1663	20	151.55	4.7465	14	170.75	5.6291	14
92.35	2.8801	14	111.15	3.7947	15	132.45	4.4166	13	152.15	3.5078	14	171.00	4.8182	17
92.45	2.6464	15	111.35	3.3170	14	132.60	4.4240	14	152.25	4.9380	14	171.00	4.4933	17
92.50	2.9816	14	111.45	3.3039	16	132.90	4.7129	14	153.00	4.6327	15	171.45	5.4564	14
93.25	2.6959	14	111.50	3.4073	15	133.00	4.3229	15	153.00	4.5128	15	172.25	5.4400	16
93.30	2.8201	17	111.60	4.0104	14	133.20	3.3406	16	153.30	4.5581	15	172.25	5.0242	15
93.35	2.4240	20	112.20	3.3420	15	133.30	4.1209	14	153.50	5.2851	14	172.60	4.9690	16
93.55	3.2472	15	112.70	4.2699	13	133.90	3.8854	16	154.35	4.9770	15	173.15	5.3766	16
93.60	2.3199	19	113.00	3.1224	16	134.00	4.7564	14	156.00	4.9586	15	173.90	6.4340	13
93.65	2.6136	19	113.10	3.4429	16	134.20	4.6076	14	156.15	5.9325	19	174.60	5.1430	17
94.00	2.3266	17	113.80	3.5667	15	135.10	3.6616	19	156.20	4.3375	18	174.85	5.9369	14
94.10	3.3304	13	114.20	3.4260	16	135.30	4.2683	15	156.70	5.6310	14	175.15	4.6698	16
94.25	2.5628	19	114.40	2.9287	16	135.60	4.1062	15	157.00	4.9387	14	175.20	4.5672	18
94.90	2.7857	16	114.90	3.4472	15	135.60	4.6607	15	157.00	5.2995	14	177.10	4.7969	16
95.40	3.1101	15	115.15	3.2869	17	136.40	5.0188	13	157.10	5.7916	13	177.30	6.4239	13
95.90	2.5972	16	115.90	3.0503	17	136.50	3.5142	17	157.15	4.6864	17	178.40	4.1089	21
96.00	2.9651	14	116.00	3.6974	15	137.00	4.2873	15	157.20	4.5695	18	178.45	5.5442	15
96.00	2.9312	16	116.20	3.6548	16	138.00	4.1348	16	157.45	5.3532	13	178.90	5.4545	15
96.10	2.6834	17	116.95	3.6529	15	138.55	4.1161	17	157.85	5.6351	13	178.90	4.9745	17
96.90	2.7671	15	117.35	3.8908	14	138.60	4.0292	15	158.00	4.9201	15	179.00	5.0939	16
97.10	2.4053	19	118.00	3.7897	16	138.70	4.3445	15	158.25	5.1914	14	179.50	4.4110	18
98.40	2.6419	19	118.85	3.0085	19	139.00	4.7001	14	159.00	4.9936	14	179.85	5.1659	17
98.80	2.7153	17	119.60	3.7710	14				159.15	4.4453	18	180.00	5.1255	16
100.00	3.5300	13	120.00	4.0272	14									
			120.00	4.0566	13									

LINEAR REGRESSION ANALYSIS: DIFFERENT WEIGHT GROUPS OF T. MOLITOR ADULTS IN 0% RH

LINEAR REGRESSION OF DRY WEIGHT (DAY 0)(Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
GROUP 1	30	.947200E+02	.364950E+02	.26410000E+03	.12887000E+03	.20611800E+03
	.5523	.487959	-9.724486	.139174	13.189112	2.261752
GROUP 2	32	.115917E+03	.448703E+02	.24383000E+03	.81620000E+02	.46861800E+03
	.2414	.334741	6.068032	.245618	28.479470	3.835346
GROUP 3	31	.135811E+03	.525774E+02	.27016000E+03	.73630000E+02	.42940000E+03
	.2161	.272542	15.563113	.228574	31.050395	3.756982
GROUP 4	28	.154971E+03	.591250E+02	.23438000E+03	.86910000E+02	.27408600E+03
	.3428	.370808	1.660345	.199220	30.878877	3.049962
GROUP 5	29	.174731E+03	.642948E+02	.18514000E+03	.58370000E+02	.16620000E+03
	.3327	.315274	9.206515	.171949	30.048087	2.339653
PARLL REG	.11976100E+04	.42940000E+03	.15444220E+04	.35854744E-00	.89792606E-01	
POOL	150	.13445000E+03	.51204333E+02	.11844590E+06	.41618600E+05	.16103730E+05
	.9529	.351372	3.962338	.009188	1.262121	3.162395

SOURCE	DF	SS	MS	VR
SLOPES	4.	6.941500	1.735375	.175
DISPLACEMENTS	4.	89.648200	22.412050	2.267
ERROR	140.	1383.520300	9.882287	

TABLE A17

LINEAR REGRESSION ANALYSIS: DIFFERENT WEIGHT GROUPS OF I. MOLITOR ADULTS IN 0 % RH

LINEAR REGRESSION OF DRY WEIGHT (AT DEATH)(Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
GROUP 1	27	.945000E+02	.209851E+02	.14532000E+03	.34789000E+02	.86885000E+02
	.3096	.239395	-1.637719	.147047	13.900213	1.772643
GROUP 2	28	.114344E+03	.262214E+02	.27508000E+03	-.37922000E+02	.16858600E+03
	-.1760	-.137858	41.984710	.151131	17.287482	2.506592
GROUP 3	25	.134780E+03	.306260E+02	.15466000E+03	.55490000E+02	.73380000E+02
	.5208	.358787	-17.731313	.122604	16.527426	1.524736
GROUP 4	26	.155219E+03	.360192E+02	.18903000E+03	.16340000E+02	.12929800E+03
	.1045	.086441	22.601877	.167895	26.064581	2.308368
GROUP 5	26	.174863E+03	.392173E+02	.30371000E+03	.14227000E+03	.10591470E+04
	.2508	.468440	-42.695781	.369003	64.537554	6.430727
PARLL REG	.10678000E+04	.21096700E+03	.15172960E+04	.19757164E-00	.10472642E-00	
POOL	132	.13412719E+03	.30474242E+02	.10914730E+06	.25022810E+05	.72423200E+04
	.8900	.229257	-.275389	.010301	1.413058	3.403233

SOURCE	DF	SS	MS	VR
SLOPES	4.	59.841800	14.960450	1.289
DISPLACEMENTS	4.	30.044400	7.511100	.647
ERROR	122.	1415.773200	11.604698	

TABLE A18

LINEAR REGRESSION ANALYSIS: DIFFERENT WEIGHT GROUPS OF T. MOLITOR ADULTS IN 0% RH

(A) LINEAR REGRESSION OF DRY WEIGHT (DAY 0)(Y) ON INITIAL LIVE WEIGHT (X)

(B) LINEAR REGRESSION OF DRY WEIGHT (AT DEATH) (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE,CONST		SD
(A)	150	.134450E+03	.512043E+02	.11844400E+06	.41618200E+05	.16103670E+05
	.9529	.351374	3.962034	.009188	1.262125	3.162380
ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE,CONST		SD
(B)	132	.134127E+03	.304742E+02	.10914580E+06	.25022740E+05	.72423000E+04
	.8900	.229259	-.275725	.010300	1.413036	3.403157
PARLL REG		.22758980E+06	.66640940E+05	.23345970E+05	.29281162E-00	.77691855E-02
POOL	282	.13429890E+03	.41500886E+02	.22759730E+06	.67110800E+05	.53518970E+05
	.6080	.294866	1.900651	.023006	3.158096	10.975662

SOURCE	DF	SS	MS	VR
SLOPES	1.	847.039400	847.039400	78.868
DISPLACEMENTS	1.	29897.521000	29897.521000	2783.783
ERROR	278.	2985.688600	10.739887	

TABLE A19

LINEAR REGRESSION ANALYSIS: DIFFERENT WEIGHT GROUPS OF *T. MOLITOR* ADULTS IN 0% RH

LINEAR REGRESSION OF LIVE WEIGHT (DAY 3) (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE,CONST		SD
GROUP 1	27	.945000E+02	.868037E+02	.14532000E+03	.12693000E+03	.14390000E+03
	.8777	.873451	4.262518	.095354	9.013687	1.149483
GROUP 2	28	.114344E+03	.103128E+03	.27508000E+03	.29903000E+03	.45359000E+03
	.8465	1.087065	-21.171150	.134053	15.333975	2.223344
GROUP 3	25	.134820E+03	.121772E+03	.16004000E+03	.11494000E+03	.16638000E+03
	.7043	.718195	24.944890	.150911	20.349506	1.909138
GROUP 4	26	.155219E+03	.140740E+03	.18903000E+03	.15672000E+03	.21902000E+03
	.7702	.829074	12.052040	.140131	21.754453	1.926648
GROUP 5	26	.174863E+03	.160069E+03	.30371000E+03	.31384000E+03	.44096000E+03
	.8575	1.033354	-20.626640	.126506	22.125502	2.204655
PARLL REG	.10731800E+04	.10114600E+04	.14238500E+04	.94248867E-00	.58991056E-01	
POOL	132	.13413477E+03	.12194431E+03	.10915400E+06	.99716500E+05	.91655500E+05
	.9969	.913539	-.593110	.006285	.862197	2.076480

SOURCE	DF	SS	MS	VR
SLOPES	4.	19.432780	4.858195	1.313
DISPLACEMENTS	4.	89.969500	22.492375	6.082
ERROR	122.	451.127720	3.697768	

TABLE A20

LINEAR REGRESSION ANALYSIS: DIFFERENT WEIGHT GROUPS OF T. MOLITOR ADULTS IN 0% RH

LINEAR REGRESSION OF LIVE WEIGHT (DAY 6) (Y) ON INITIAL LIVE WEIGHT (X)

ID ID	N R	X MEAN SLOPE	Y MEAN CONST	XCSS SE OF SLOPE, CONST	XYCSP	YCSS SD
GROUP 1	27 .7506	.945000E+02 .828860	.778944E+02 -.432868	.14532000E+03 .145927	.12045000E+03 13.794270	.17720000E+03 1.759133
GROUP 2	28 .7354	.114344E+03 .927257	.915553E+02 -14.471240	.27508000E+03 .167526	.25507000E+03 19.162910	.43724000E+03 2.778519
GROUP 3	25 .4590	.134820E+03 .546863	.109176E+03 35.447900	.16004000E+03 .220660	.87520000E+02 29.754736	.22709000E+03 2.791512
GROUP 4	26 .5111	.155219E+03 .609056	.125226E+03 30.689600	.18903000E+03 .209063	.11513000E+03 32.455601	.26841000E+03 2.874378
GROUP 5	26 .6621	.174863E+03 .918409	.144071E+03 -16.525030	.30371000E+03 .212190	.27893000E+03 37.111479	.58436000E+03 3.697905
PARLL REG	.10731800E+04	.85710000E+03	.16943000E+04	.79865446E-00	.86415248E-01	
POOL	132 .9919	.13413477E+03 .823130	.10907462E+03 -1.335820	.10915400E+06 .009227	.89848000E+05 1.265813	.75164800E+05 3.048530

SOURCE	DF	SS	MS	VR
SLOPES	4.	25.979100	6.494775	.805
DISPLACEMENTS	4.	198.386700	49.596675	6.150
ERROR	122.	983.794200	8.063886	

TABLE A21

LINEAR REGRESSION ANALYSIS: DIFFERENT WEIGHT GROUPS OF I. MOLITOR ADULTS IN 0% RH

LINEAR REGRESSION OF LIVE WEIGHT (DAY 9) (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
GROUP 1	27	.945000E+02	.698685E+02	.14531000E+03	.11999000E+03	.27470000E+03
	.6005	.825751	-8.165031	.219870	20.784014	2.650419
GROUP 2	28	.114344E+03	.820250E+02	.27508000E+03	.22201000E+03	.50813000E+03
	.5938	.807074	-10.259330	.214461	24.531677	3.556961
GROUP 3	25	.134820E+03	.974060E+02	.16004000E+03	.60960000E+02	.38285000E+03
	.2462	.380904	46.052419	.312571	42.148344	3.954249
GROUP 4	26	.155219E+03	.111130E+03	.18903000E+03	.11441000E+03	.57507000E+03
	.3470	.605247	17.184660	.333909	51.836964	4.590858
GROUP 5	26	.174863E+03	.129253E+03	.30371000E+03	.26185000E+03	.10298600E+04
	.4682	.862171	-21.508390	.332139	58.090079	5.788280
PARLL REG		.10731700E+04	.77922000E+03	.27706100E+04	.72609185E-00	.12769312E-00
POOL	132	.13413477E+03	.97487121E+02	.10915400E+06	.80020200E+05	.61137400E+05
	.9795	.733094	-.846344	.013206	1.811747	4.363334

SOURCE	DF	SS	MS	VR
SLOPES	4.	30.701200	7.675300	.430
DISPLACEMENTS	4.	270.205200	67.551300	3.790
ERROR	122.	2174.123600	17.820685	

TABLE A22

LINEAR REGRESSION ANALYSIS: DIFFERENT WEIGHT GROUPS OF T. MOLITOR ADULTS IN 0% RH

LINEAR REGRESSION OF LIVE WEIGHT (DAY 12) (Y) ON INITIAL LIVE WEIGHT (X)

ID ID	N R	X MEAN SLOPE	Y MEAN CONST	XCSS SE OF SLOPE,CONST	XYCSP	YCSS SD
GROUP 1	27 .3864	.945000E+02 .656596	.614518E+02 -.596494	.14531000E+03 .313403	.95410000E+02 29.625515	.41946000E+03 3.777904
GROUP 2	28 .4915	.114344E+03 .738849	.723250E+02 -12.158168	.27509000E+03 .256696	.20325000E+03 29.362772	.62146000E+03 4.257522
GROUP 3	25 .1903	.134792E+03 .428775	.851960E+02 27.400433	.15367000E+03 .461169	.65890000E+02 62.172435	.77994000E+03 5.716824
GROUP 4	26 .3939	.155219E+03 .897741	.957615E+02 -43.585150	.18903000E+03 .427584	.16970000E+03 66.379409	.98179000E+03 5.878787
GROUP 5	26 .3110	.174863E+03 .753877	.114071E+03 -17.754400	.30371000E+03 .470171	.22896000E+03 82.231465	.17839300E+04 8.193804
PARLL REG	.10668100E+04	.76321000E+03	.45865800E+04	.71541324E-00	.17337739E-00	
POOL	132 .9524	.13412946E+03 .638353	.85377651E+02 -.244386	.10914670E+06 .017900	.69674200E+05 2.455578	.49023500E+05 5.913945

SOURCE	DF	SS	MS	VR
SLOPES	4.	20.012800	5.003200	.151
DISPLACEMENTS	4.	506.147500	126.536870	3.839
ERROR	122.	4020.556700	32.955382	

TABLE A23

LINEAR REGRESSION ANALYSIS: DIFFERENT WEIGHT GROUPS OF T. MOLITOR ADULTS IN 0% RH

LINEAR REGRESSION OF LIVE WEIGHT (PRIOR TO DEATH) (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
GROUP 1	27	.945000E+02	.529037E+02	.14532000E+03	.38320000E+02	.33296000E+03
	.1742	.263693	27.984629	.298106	28.179537	3.593634
GROUP 2	28	.114344E+03	.640357E+02	.27508000E+03	.21841000E+03	.40641000E+03
	.6532	.793987	-26.752181	.180491	20.645951	2.993552
GROUP 3	25	.134820E+03	.756000E+02	.16004000E+03	.92000000E+02	.38114000E+03
	.3725	.574856	-1.902124	.298625	40.267719	3.777814
GROUP 4	26	.155219E+03	.864634E+02	.18903000E+03	.12457000E+03	.55554000E+03
	.3844	.658995	-15.825380	.323046	50.150649	4.441512
GROUP 5	26	.174863E+03	.969192E+02	.30371000E+03	.15980000E+03	.45602000E+03
	.4293	.526159	4.913103	.225892	39.507819	3.936684
PARLL REG	.10731800E+04	.63310000E+03	.21320700E+04	.58992899E-00	.11404079E-00	
POOL	132	.13413477E+03	.74843560E+02	.10915400E+06	.59860700E+05	.34594970E+05
	.9741	.548405	1.283258	.011159	1.530829	3.686783

SOURCE	DF	SS	MS	VR
SLOPES	4.	29.093800	7.273450	.513
DISPLACEMENTS	4.	8.422000	2.105500	.148
ERROR	122.	1729.492200	14.176165	

TABLE A21

LINEAR REGRESSION ANALYSIS: I. MOLITOR FEMALES AND MALES INITIAL WEIGHT 100-120MG

LINEAR REGRESSION OF INITIAL DRY WEIGHT (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE,CONST		SD
Females	48	.110509E+03	.422437E+02	.15003700E+04	.60945000E+03	.65775900E+03
	.6134	.406199	-2.645134	.077093	8.530494	2.986202
ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE,CONST		SD
Males	48	.110153E+03	.391966E+02	.13852100E+04	.40133000E+03	.39097300E+03
	.5453	.289725	7.282551	.065658	7.241080	2.443703
PARLL REG	.28855800E+04	.10107800E+04	.10487320E+04	.35028659E-00	.50878119E-01	
POOL	96	.11033125E+03	.40720208E+02	.28886000E+04	.10368200E+04	.12715600E+04
	.5409	.358935	1.118448	.057553	6.357781	3.093247

SOURCE	DF	SS	MS	VR
SLOPES	1.	9.771200	9.771200	1.312
DISPLACEMENTS	1.	204.739500	204.739500	27.501
ERROR	92.	684.898200	7.444545	

TABLE A25

T25

LINEAR REGRESSION ANALYSIS: T. MOLITOR FEMALES DIFFERENT WEIGHT GROUPS

LINEAR REGRESSION OF INITIAL DRY WEIGHT (Y) ON INITIAL LIVE WEIGHT (X)

ID ID	N R	X MEAN SLOPE	Y MEAN CONST	XCSS SE OF SLOPE,	XYCSP CONST	YCSS SD
GROUP 1	24 .3393	.761000E+02 .336994	.302958E+02 4.650574	.17819000E+03 .199166	.60049000E+02 15.166278	.17573900E+03 2.658629
GROUP 2	24 .2427	.846958E+02 .213839	.332812E+02 15.169941	.11684000E+03 .182214	.24985000E+02 15.438028	.90688000E+02 1.969601
GROUP 3	24 -.0221	.964000E+02 -.019948	.357000E+02 37.623002	.10417000E+03 .191946	-.20780000E+01 18.507954	.84477000E+02 1.959076
GROUP 4	24 .4344	.105558E+03 .543178	.401354E+02 -17.201616	.20172000E+03 .240112	.10957000E+03 25.355412	.31537500E+03 3.410271
GROUP 5	24 -.0092	.115460E+03 -.009505	.443520E+02 45.449543	.12204000E+03 .219204	-.11600000E+01 25.314250	.12902100E+03 2.421588
GROUP 6	24 .1398	.125462E+03 .165658	.479979E+02 27.213974	.17856000E+03 .250055	.29580000E+02 31.380051	.25053000E+03 3.341405
GROUP 7	24 .0871	.135014E+03 .100369	.504479E+02 36.896627	.18153000E+03 .244696	.18220000E+02 33.044402	.24095400E+03 3.296866
GROUP 8	24 .2532	.142645E+03 .244683	.539645E+02 19.061548	.91220000E+02 .199241	.22320000E+02 28.423583	.85127000E+02 1.902936
GROUP 9	24 .6762	.154760E+03 .463536	.583537E+02 -13.383414	.42879000E+03 .107648	.19876000E+03 16.665977	.20144900E+03 2.229109
PARLL REG		.16030600E+04	.46024600E+03	.15733600E+04	.28710466E-00	.66062778E-01
POOL	216 .9601	.11512199E+03 .360245	.43836527E+02 2.364355	.13893000E+06 .007165	.50048900E+05 .844631	.19556210E+05 2.670645

TABLE A26

LINEAR REGRESSION ANALYSIS: I. MOLITOR MALES DIFFERENT WEIGHT GROUPS

LINEAR REGRESSION OF INITIAL DRY WEIGHT (Y) ON INITIAL LIVE WEIGHT (X)

ID / ID	N / R	X MEAN / SLOPE	Y MEAN / CONST	XCSS / SE OF SLOPE, CONST	XYCSP	YCSS / SD
GROUP 1	24	.763187E+02	.285291E+02	.21980000E+03	.19280000E+02	.14574900E+03
	.1077	.087716	21.834783	.172600	13.183043	2.558922
GROUP 2	24	.854625E+02	.310020E+02	.16927000E+03	.67735000E+02	.14184700E+03
	.4371	.400159	-3.196548	.175533	15.008781	2.283758
GROUP 3	24	.958875E+02	.344229E+02	.19028000E+03	.75344000E+02	.16223000E+03
	.4288	.395963	-3.545067	.177840	17.060019	2.453165
GROUP 4	24	.105554E+03	.378479E+02	.16460000E+03	.35718000E+02	.12326600E+03
	.2507	.216998	14.942792	.178604	18.858292	2.291439
GROUP 5	24	.114752E+03	.405454E+02	.20539000E+03	.67910000E+02	.18039000E+03
	.3528	.330639	2.603872	.186956	21.460587	2.679351
GROUP 6	24	.123704E+03	.441291E+02	.16529000E+03	.59750000E+02	.16301400E+03
	.3640	.361485	-.588140	.197202	24.400315	2.535343
GROUP 7	24	.134545E+03	.466666E+02	.23526000E+03	.86310000E+02	.32636000E+03
	.3114	.366870	-2.694255	.238616	32.113606	3.659951
GROUP 8	24	.144295E+03	.500958E+02	.21516000E+03	.51880000E+02	.22247200E+03
	.2371	.241122	15.302807	.210609	30.396673	3.089296
GROUP 9	24	.154170E+03	.541562E+02	.21270000E+03	.10650000E+03	.15231900E+03
	.5916	.500705	-23.037888	.145448	22.428087	2.121254
PARLL REG	.17777500E+04	.57042700E+03	.16176470E+04	.32087020E-00	.62589081E-01	
POOL	216	.11496574E+03	.40821712E+02	.13794560E+06	.45043500E+05	.16169250E+05
	.9537	.326530	3.281847	.007035	.828137	2.613011

TABLE A27

LINEAR REGRESSION ANALYSIS; FEMALE AND MALE T. MOLITOR ADULTS

LINEAR REGRESSION OF INITIAL DRY WEIGHT (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
Females	216	.115121E+03	.438365E+02	.13892630E+06	.50048200E+05	.19556170E+05
	.9601	.360250	2.363830	.007165	.844637	2.670631
ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
Males	216	.114965E+03	.408217E+02	.13794200E+06	.45043100E+05	.16169230E+05
	.9537	.326536	3.281200	.007035	.828107	2.612884
PARLL REG	.27686830E+06	.95091300E+05	.35725400E+05	.34345318E-00	.50806603E-02	
POOL	432	.11504386E+03	.42329120E+02	.27687100E+06	.95142100E+05	.36707020E+05
	.9437	.343633	2.796218	.005805	.683903	3.054933

SOURCE	DF	SS	MS	VR
SLOPES	1.	78.672000	78.672000	11.271
DISPLACEMENTS	1.	947.034000	947.034000	135.683
ERROR	428.	2987.319000	6.979717	

TABLE A28

T28

TABLE A29 Summary of Data Concerning the Life Span (Days) of T. molitor Females and Males Starved to Death in Different Combinations of Temperature and Relative Humidity (Section 10.2)

Females

Temp.		Relative Humidity						
		0%	20%	40%	60%	80%	100%	Totals
20°C	N	12	12	12	12	12	12	72
	$\bar{x}$	25.67	30.08	36.50	45.83	45.50	30.92	35.4167
	Σx	308	361	438	526	546	371	2550
	Σx^2	7918	11265	16604	23268	25094	12201	96418
	$\Sigma (x-\bar{x})^2$	82.6667	402.9167	617.0000	211.6667	251.0000	730.9917	2296.2418
25°C	N	12	12	12	12	12	12	72
	$\bar{x}$	16.58	18.08	22.17	27.75	28.50	20.92	22.3333
	Σx	199	217	266	333	342	251	1608
	Σx^2	3311	3993	6040	9455	9918	5549	38266
	$\Sigma (x-\bar{x})^2$	10.9167	68.9167	143.6667	214.2500	171.0000	98.9167	707.6668
30°C	N	12	12	12	12	12	12	72
	$\bar{x}$	9.58	11.58	14.42	19.75	20.00	15.83	15.1944
	Σx	115	139	173	237	240	190	1094
	Σx^2	1111	1651	2533	4711	4882	3078	17966
	$\Sigma (x-\bar{x})^2$	8.9167	30.9167	38.9167	30.2500	82.0000	69.6667	260.6668
35°C	N	12	12	12	12	12	12	72
	$\bar{x}$	6.92	7.67	12.00	17.08	17.00	12.42	12.1944
	Σx	83	92	144	205	204	149	877
	Σx^2	585	718	1740	3563	3502	1947	12055
	$\Sigma (x-\bar{x})^2$	10.9167	12.6667	12.0000	40.9167	34.0000	96.9167	227.4168

TABLE A29 (Continued)

Males

Temp.		Relative Humidity 0%	20%	40%	60%	80%	100%	Totals
20°C	N	12	12	12	12	12	12	72
	$\bar{x}$	18.83	24.33	26.83	32.17	31.00	24.58	26.3917
	Σx	226	292	322	386	372	295	1593
	Σx^2	4350	7132	9020	12498	11704	7357	52061
	$\Sigma (x-\bar{x})^2$	33.6667	76.6667	379.6667	61.6667	172.0000	66.9167	890.5835
25°C	N	12	12	12	12	12	12	72
	$\bar{x}$	12.75	15.42	18.42	20.67	20.58	16.42	17.3750
	Σx	153	185	221	248	247	197	1251
	Σx^2	1971	2685	4203	5256	5205	3303	22625
	$\Sigma (x-\bar{x})^2$	20.2500	32.9167	132.9167	130.6667	120.9167	66.9167	506.5835
30°C	N	12	12	12	12	12	12	72
	$\bar{x}$	8.25	9.75	11.67	14.75	15.25	11.50	11.8611
	Σx	99	117	140	177	183	138	854
	Σx^2	825	1155	1664	2659	2847	1636	10786
	$\Sigma (x-\bar{x})^2$	8.2500	14.2500	30.6667	48.2500	56.2500	49.0000	206.6667
35°C	N	12	12	12	12	12	12	72
	$\bar{x}$	6.33	7.00	8.92	11.17	12.50	10.58	9.4167
	Σx	76	84	107	134	150	127	678
	Σx^2	480	600	959	1574	1956	1399	6968
	$\Sigma (x-\bar{x})^2$	8.6667	12.0000	14.9167	77.6667	81.0000	54.9167	253.1668
Totals	ΣN	96	96	96	96	96	96	576
	Σx	1280	1487	1811	2246	2304	1713	10835
	Σx^2	20631	29427	42773	62964	65088	36250	257133
	$\Sigma (x-\bar{x})^2$	244.2502	641.2502	1360.7502	655.2505	949.1667	1256.2419	5314.9027

TABLE A30 Summary of Data Concerning the Life Span of T. molitor Adults Starved to Death in Different Combinations of Temperature and Relative Humidity (Raw Data Transformed to Logarithms)

Females

Temp.		Relative Humidity 0%	20%	40%	80%	65%	100%	Totals
20°C	N	12	12	12	12	12	12	72
	$\bar{x}$	3.2400	3.3824	3.5766	3.7758	3.8125	3.5906	3.5299
	Σx	38.8810	40.6032	42.9139	45.3101	45.7504	40.6873	254.1519
	Σx^2	126.0991	137.3017	154.0255	171.1929	174.5510	139.0625	902.0325
	$\Sigma (x-\bar{x})^2$	.1212	.5162	.5151	.1036	.1260	1.1073	2.4944
25°C	N	12	12	12	12	12	12	72
	$\bar{x}$	2.8067	2.8849	3.0851	3.3117	3.3407	3.0810	3.0768
	Σx	33.6806	34.6197	37.0223	39.7415	40.0893	36.9731	221.5265
	Σx^2	94.5727	100.1355	114.3592	131.6995	134.1537	110.4786	685.7992
	$\Sigma (x-\bar{x})^2$	.0404	.2576	.3382	.2738	.2240	.2279	1.3618
30°C	N	12	12	12	12	12	12	72
	$\bar{x}$	2.2589	2.4429	2.6605	2.9800	2.9875	2.7502	2.6798
	Σx	27.0716	29.3150	31.9271	35.7601	35.8506	33.0032	192.9276
	Σx^2	61.1704	71.7761	85.1322	106.6401	107.2564	91.0557	525.0729
	$\Sigma (x-\bar{x})^2$	.0574	.1617	.1871	.0742	.1924	.2879	1.0007
35°C	N	12	12	12	12	12	12	72
	$\bar{x}$	1.8238	2.0275	2.4815	2.8299	2.8283	2.4918	2.4505
	Σx	23.0861	24.3306	29.7780	33.9586	33.9404	29.9017	174.9563
	Σx^2	44.6840	49.5801	73.9753	96.2936	96.1117	75.1791	435.7838
	$\Sigma (x-\bar{x})^2$	.2459	.2293	.0807	.1892	.1154	.6695	1.5300

TABLE A30 (Continued)

Males

Temp.		Relative Humidity 0%	20%	40%	60%	80%	100%	Totals
20°C	N	12	12	12	12	12	12	72
	$\bar{x}$	2.9242	3.1862	3.3653	3.4676	3.4256	3.1964	3.2609
	$\sum x$	35.0915	38.2355	39.1838	41.6114	41.1100	38.3578	233.6900
	$\sum x^2$	102.8938	121.9649	128.5626	144.3725	141.0408	122.7412	761.5758
	$\sum(x-\bar{x})^2$	.2787	.1515	.6149	.0790	.2047	.1311	1.4577
25°C	N	12	12	12	12	12	12	72
	$\bar{x}$	2.5406	2.7297	2.8970	3.0155	3.0120	2.7858	2.8301
	$\sum x$	30.4879	32.7570	34.7641	36.1864	36.1441	33.4300	203.7695
	$\sum x^2$	77.5728	89.5544	101.1011	109.4369	109.1732	93.4544	580.2918
	$\sum(x-\bar{x})^2$	.1153	.1368	.3869	.3142	.3065	.3256	1.5853
30°C	N	12	12	12	12	12	12	72
	$\bar{x}$	2.1045	2.2708	2.4467	2.6814	2.7148	2.4256	2.4407
	$\sum x$	25.2545	27.2496	29.3614	32.1770	32.5777	29.1079	175.7285
	$\sum x^2$	53.2858	62.0568	72.0872	86.5330	88.6733	71.0239	433.6580
	$\sum(x-\bar{x})^2$	.1445	.1571	.2458	.2428	.2308	.4180	1.4390
35°C	N	12	12	12	12	12	12	72
	$\bar{x}$	1.8367	1.9349	2.1800	2.3897	2.5120	2.3438	2.1995
	$\sum x$	22.0407	23.2196	26.1609	28.6771	30.1446	28.0724	158.3153
	$\sum x^2$	40.7029	45.2015	57.2218	69.0631	76.0268	65.1451	354.3612
	$\sum(x-\bar{x})^2$	.2800	.2722	.1897	.5315	.3016	.4735	1.9075
Totals	N	96	96	96	96	96	96	576
	$\sum x$	235.5893	250.3304	271.1175	288.4351	285.6071	266.0534	1615.0054
	$\sum x^2$	600.9695	678.1306	766.6849	915.4104	927.0289	769.1403	4677.3448
	$\sum(x-\bar{x})^2$	1.2624	1.0606	2.5534	1.8139	1.7014	3.6336	12.0254

LINEAR REGRESSION ANALYSIS: FEMALE AND MALE I. MOLITOR ADULTS

LINEAR REGRESSION OF INITIAL DRY WEIGHT (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
Females	57	.109522E+03	.416666E+02	.16906600E+04	.65137000E+03	.68549800E+03
	.6050	.385275	-.529793	.068360	7.496296	2.810826
ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
Males	57	.110992E+03	.384263E+02	.28721900E+04	.10365100E+04	.74428200E+03
	.7089	.360877	-1.628602	.048411	5.384294	2.594499
PARLL REG		.45628500E+04	.16878800E+04	.14297800E+04	.36991792E-00	.39877417E-01
POOL	114	.11025789E+03	.40046491E+02	.46244000E+04	.15521000E+04	.17290300E+04
	.5488	.335632	3.040334	.048296	5.333923	3.284289

SOURCE	DF	SS	MS	VR
SLOPES	1.	.633540	.633540	.086
DISPLACEMENTS	1.	402.691500	402.691500	55.041
ERROR	110.	804.769460	7.316086	

TABLE A31

LINEAR REGRESSION ANALYSIS : T. MOLITOR FEMALES IN DIFFERENT RELATIVE HUMIDITIES

AT A TEMPERATURE OF 20° C

LINEAR REGRESSION OF FINAL DRY WEIGH (DAY 8) (Y) ON INITIAL LIVE WEIGHT (X)

ID / ID	N / R	X MEAN / SLOPE	Y MEAN / CONST	XCSS / SE OF SLOPE,CONST	XYCSP	YCSS / SD
0% R.H.	16	.112021E+03	.361687E+02	.58247000E+03	.86509000E+02	.48520000E+02
	.5145	.148520	19.531155	.066139	7.419801	1.596236
20% R.H.	16	.112484E+03	.355843E+02	.71162000E+03	.26191400E+03	.18546300E+03
	.7209	.368053	-5.815854	.094550	10.654152	2.522254
40% R.H.	16	.106556E+03	.339500E+02	.58340000E+03	.22883200E+03	.13817900E+03
	.8059	.392238	-7.845474	.076997	8.217702	1.859766
60% R.H.	16	.110693E+03	.361125E+02	.55942000E+03	.24515400E+03	.13417700E+03
	.8948	.438228	-12.396697	.058435	6.477649	1.382116
80% R.H.	16	.107409E+03	.345031E+02	.41511000E+03	.19022400E+03	.12044600E+03
	.8507	.458249	-14.717178	.075669	8.136720	1.541704
100% R.H.	16	.111225E+03	.362093E+02	.85925000E+03	.23301900E+03	.13153100E+03
	.6931	.271188	6.046398	.075372	8.401427	2.209377
PARLL REG		.37112700E+04	.12456520E+04	.75831600E+03	.33564036E-00	.32094215E-01
POOL	96	.11006510E+03	.35421354E+02	.42038000E+04	.14185400E+04	.83338000E+03
	.7578	.337442	-1.719268	.029960	3.303555	1.942537

SOURCE	DF	SS	MS	VR
SLOPES	5.	38.708100	7.741620	2.156
DISPLACEMENTS	5.	14.479670	2.895934	.806
ERROR	84.	301.516820	3.589485	

T32

LINEAR REGRESSION ANALYSIS : I. MOLITOR FEMALES IN DIFFERENT RELATIVE HUMIDITIES AT A TEMPERATURE OF 25°C

LINEAR REGRESSION OF FINAL DRY WEIGH (DAY 8) (Y) ON INITIAL LIVE WEIGHT (X)

ID / ID	N / R	X MEAN / SLOPE	Y MEAN / CONST	XCSS / SE OF SLOPE	XYCSP / CONST	YCSS / SD
0% R.H.	16	.111184E+03	.316718E+02	.89354000E+03	.17562400E+03	.14713000E+03
	.4843	.196548	9.818748	.094878	10.572862	2.836136
20% R.H.	16	.110965E+03	.323943E+02	.56609000E+03	.25536800E+03	.18612900E+03
	.7867	.451108	-17.663157	.094603	10.512857	2.250878
40% R.H.	16	.109340E+03	.337500E+02	.49773000E+03	.15123800E+03	.11476900E+03
	.6327	.303855	.526251	.099375	10.879899	2.217052
60% R.H.	16	.112190E+03	.338125E+02	.51082000E+03	.13893700E+03	.14205800E+03
	.5157	.271988	3.297979	.120747	13.563938	2.729060
80% R.H.	16	.112575E+03	.340250E+02	.72341000E+03	.25401700E+03	.17821900E+03
	.7074	.351138	-5.504400	.093755	10.573342	2.521675
100% R.H.	16	.108653E+03	.321156E+02	.75406000E+03	.10169200E+03	.11667300E+03
	.3428	.134859	17.462742	.098756	10.751580	2.711864
PARLL REG	.39456500E+04	.10768760E+04	.88497800E+03	.27292740E-00	.41026532E-01	
POOL	96	.11081822E+03	.32961562E+02	.41375000E+04	.11272100E+04	.96781000E+03
	.5633	.272437	2.770528	.041216	4.575581	2.651205

SOURCE	DF	SS	MS	VR
SLOPES	5.	42.461270	8.492254	1.300
DISPLACEMENTS	5.	69.646730	13.929346	2.132
ERROR	84.	548.607770	6.531044	

T33

TABLE A33

LINEAR REGRESSION ANALYSIS: T. MOLITOR FEMALES IN DIFFERENT RELATIVE HUMIDITIES AT A TEMPERATURE OF 30°C

LINEAR REGRESSION OF FINAL DRY WEIGH (DAY 8) (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
0% R.H.	16	.111893E+03	.288906E+02	.96509000E+03	.37445400E+03	.19057100E+03
	.8731	.387999	-14.524043	.057892	6.493369	1.798475
20% R.H.	16	.107565E+03	.291656E+02	.33704000E+03	.13692200E+03	.12686400E+03
	.6621	.406248	-14.532748	.122872	13.228926	2.255780
40% R.H.	16	.110034E+03	.305687E+02	.97884000E+03	.28132400E+03	.14695000E+03
	.7417	.287405	-1.055733	.069449	7.661085	2.172818
60% R.H.	16	.109234E+03	.292343E+02	.34516000E+03	.10608700E+03	.92189000E+02
	.5947	.307356	-4.339464	.111041	12.140504	2.062981
80% R.H.	16	.110075E+03	.299687E+02	.58140000E+03	.19327300E+03	.10105400E+03
	.7973	.332426	-6.623141	.067243	7.412931	1.621393
100% R.H.	16	.110112E+03	.295843E+02	.61634000E+03	.19124400E+03	.14905400E+03
	.6309	.310289	-4.582407	.101965	11.245500	2.531416
PARLL REG		.38238700E+04	.12833040E+04	.80668200E+03	.33560345E-00	.33238986E-01
POOL	96	.10981927E+03	.29568750E+02	.39826000E+04	.12836000E+04	.83698800E+03
	.7030	.322302	-5.826221	.033625	3.699060	2.122025

SOURCE	DF	SS	MS	VR
SLOPES	5.	7.281600	1.456320	.331
DISPLACEMENTS	5.	47.280400	9.456080	2.154
ERROR	84.	368.719130	4.389513	

T34

TABLE A34

LINEAR REGRESSION ANALYSIS : T. MOLITOR MALES IN DIFFERENT RELATIVE HUMIDITIES

AT A TEMPERATURE OF 20°C

LINEAR REGRESSION OF FINAL DRY WEIGH (DAY 8) (Y) ON INITIAL LIVE WEIGHT (X)

ID ID	N R	X MEAN SLOPE	Y MEAN CONST	XCSS SE OF SLOPE,CONST	XYCSP YCSS	YCSS SD
0% R.H.	16 .7955	.110000E+03 .479471	.312875E+02 -21.454352	.79036000E+03 .097605	.37895500E+03 10.758454	.28711200E+03 2.744005
20% R.H.	16 .7793	.112206E+03 .405744	.314937E+02 -14.033295	.86747000E+03 .087175	.35197100E+03 9.802686	.23510400E+03 2.567569
40% R.H.	16 .7066	.110775E+03 .325050	.314156E+02 -4.591821	.84502000E+03 .086974	.27467400E+03 9.655323	.17877400E+03 2.528284
60% R.H.	16 .8328	.107871E+03 .398656	.302500E+02 -12.753819	.89243000E+03 .070805	.35577300E+03 7.656207	.20446900E+03 2.115213
80% R.H.	16 .7386	.110503E+03 .419866	.314843E+02 -14.912160	.66117000E+03 .102398	.27760300E+03 11.334514	.21361400E+03 2.633002
100% R.H.	16 .4762	.108165E+03 .213602	.310937E+02 7.989254	.67081000E+03 .105394	.14328700E+03 11.420439	.13492500E+03 2.729710
PARLL REG		.47272600E+04	.17822630E+04	.12539980E+04	.37701818E-00	.37194722E-01
POOL	96 .7308	.10992031E+03 .370686	.31170833E+02 -9.575089	.49444000E+04 .035709	.18328200E+04 3.933585	.12720740E+04 2.510982

SOURCE	DF	SS	MS	VR
SLOPES	5.	30.839510	6.167902	.939
DISPLACEMENTS	5.	10.620800	2.124160	.323
ERROR	84.	551.212990	6.562059	

T35

TABLE A35

LINEAR REGRESSION ANALYSIS : T. MOLITOR MALES IN DIFFERENT RELATIVE HUMIDITIES

AT A TEMPERATURE OF 25°C

LINEAR REGRESSION OF FINAL DRY WEIGH (DAY 8) (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
0% R.H.	16	.112431E+03	.283687E+02	.11930700E+04	.26793600E+03	.20938400E+03
	.5360	.224576	3.119285	.094515	10.657828	3.264656
20% R.H.	16	.108750E+03	.288312E+02	.85494000E+03	.19588500E+03	.18171000E+03
	.4969	.229121	3.914304	.106919	11.653723	3.126254
40% R.H.	16	.111206E+03	.291562E+02	.78798000E+03	.25620000E+03	.19568900E+03
	.6524	.325135	-7.000811	.100934	11.246911	2.833339
60% R.H.	16	.112284E+03	.294750E+02	.12291000E+04	.27356100E+03	.12486900E+03
	.6982	.222570	4.483849	.060977	6.867699	2.137797
80% R.H.	16	.112709E+03	.298218E+02	.12469600E+04	.30231200E+03	.19846500E+03
	.6076	.242439	2.496704	.084676	9.573101	2.990135
100% R.H.	16	.110309E+03	.275500E+02	.79234000E+03	.92402000E+02	.95170000E+02
	.3364	.116619	14.685818	.087224	9.641191	2.455229
PARLL REG		.61043900E+04	.13882960E+04	.10052870E+04	.22742583E-00	.35626042E-01
POOL	96	.11128177E+03	.28867187E+02	.62920000E+04	.14322800E+04	.10588710E+04
	.5548	.227635	3.535551	.035200	3.927486	2.792150

SOURCE	DF	SS	MS	VR
SLOPES	5.	17.573620	3.514724	.439
DISPLACEMENTS	5.	43.281200	8.656240	1.082
ERROR	84.	671.979080	7.999750	

T36

LINEAR REGRESSION ANALYSIS: I. MOLITOR MALES IN DIFFERENT RELATIVE HUMIDITIES

AT A TEMPERATURE OF 30°C

LINEAR REGRESSION OF FINAL DRY WEIGH (DAY 8) (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE,	CONST	SD
0% R.H.	16	.108800E+03	.239906E+02	.67943000E+03	.16830700E+03	.10403630E+03
	.6330	.247717	-2.961087	.080957	8.824013	2.110241
20% R.H.	16	.111912E+03	.266812E+02	.82859000E+03	.24856100E+03	.25186900E+03
	.5440	.299980	-6.890339	.123630	13.864424	3.558746
40% R.H.	16	.109287E+03	.256156E+02	.62312000E+03	.12226600E+03	.16084400E+03
	.3862	.196215	4.171690	.125250	13.710568	3.126539
60% R.H.	16	.110275E+03	.254625E+02	.88298000E+03	.37139200E+03	.32403800E+03
	.6943	.420612	-20.920489	.116517	12.878061	3.462307
80% R.H.	16	.113759E+03	.270062E+02	.82606000E+03	.15103400E+03	.11828500E+03
	.4831	.182836	6.206875	.088544	10.092874	2.544889
100% R.H.	16	.108065E+03	.271250E+02	.57890000E+03	.16506400E+03	.10895000E+03
	.6572	.285133	-3.688168	.087382	9.457688	2.102458
PARLL REG		.44190800E+04	.12266240E+04	.10680223E+04	.27757451E-00	.43009889E-01
POOL	96	.11035000E+03	.25980208E+02	.47842000E+04	.13144300E+04	.11834460E+04
	.5524	.274743	-4.337786	.042761	4.728349	2.957706

SOURCE	DF	SS	MS	VR
SLOPES	5.	30.658990	6.131798	.739
DISPLACEMENTS	5.	94.771600	18.954320	2.284
ERROR	84.	696.883810	8.296235	

T37

LINEAR REGRESSION ANALYSIS: T. MOLITOR FEMALES AND MALES IN A TEMPERATURE OF 20°C

LINEAR REGRESSION OF DRY MATTER(MG) USED IN 8 DAYS(Y) ON INITIAL LIVE WEIGHT(X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE,CONST		SD
Females	96	.110065E+03	.644906E+01	.42034000E+04	.20073000E+03	.36442430E+03
	.1621	.047754	1.192991	.029967	3.304335	1.942904
ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE,CONST		SD
Males	96	.109920E+03	.686364E+01	.49441000E+04	-.48396000E+02	.59320990E+03
	+.0282	-.009788	7.939615	.035712	3.933912	2.511116
PARLL REG		.91475000E+04	.15233400E+03	.95763420E+03	.16653074E-01	.23503996E-01
POOL	192	.10999270E+03	.66563541E+01	.91485000E+04	.14945000E+03	.96588440E+03
	.0502	.016336	4.859512	.023542	2.594649	2.251833

SOURCE	DF	SS	MS	VR
SLOPES	1.	7.522610	7.522610	1.492
DISPLACEMENTS	1.	8.345610	8.345610	1.655
ERROR	188.	947.574770	5.040291	

TABLE A38

LINEAR REGRESSION ANALYSIS: T. MOLITOR FEMALES AND MALES IN A TEMPERATURE OF 25°C

LINEAR REGRESSION OF DRY MATTER(MG) USED IN 8 DAYS(Y) ON INITIAL LIVE WEIGHT(X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
Females	96	.110818E+03	.919906E+01	.41370000E+04	.46690800E+03	.71333410E+03
	.2717	.112861	-3.308047	.041216	4.575588	2.651049
ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
Males	96	.111281E+03	.965843E+01	.62914000E+04	.83855000E+03	.84471120E+03
	.3637	.133285	-5.173766	.035204	3.927970	2.792362
PARLL REG		.10428400E+05	.13054580E+04	.15580453E+04	.12518296E-00	.26600442E-01
POOL	192	.11105000E+03	.94287500E+01	.10438800E+05	.13156800E+04	.15681750E+04
	.3251	.126037	-4.567711	.026590	2.959374	2.716760

SOURCE	DF	SS	MS	VR
SLOPES	1.	1.041100	1.041100	.140
DISPLACEMENTS	1.	7.725900	7.725900	1.042
ERROR	188.	1393.583100	7.412676	

TABLE A39

LINEAR REGRESSION ANALYSIS: T. MOLITOR FEMALES AND MALES IN A TEMPERATURE OF 30°C

LINEAR REGRESSION OF DRY MATTER(MG) USED IN 8 DAYS(Y) ON INITIAL LIVE WEIGHT(X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE,CONST		SD
Females	96	.109819E+03	.122076E+02	.39819000E+04	.25033000E+03	.43872000E+03
	.1893	.062866	5.303599	.033616	3.698078	2.121276
ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE,CONST		SD
Males	96	.110350E+03	.122091E+02	.47834000E+04	.41183000E+03	.85754600E+03
	.2033	.086095	2.708510	.042758	4.728095	2.957301
PARLL REG		.87653000E+04	.66216000E+03	.12962660E+04	.75543335E-01	.27427567E-01
POOL	192	.11008463E+03	.12208385E+02	.87788000E+04	.66221000E+03	.12962650E+04
	.1963	.075432	3.904387	.027335	3.014835	2.561159

SOURCE	DF	SS	MS	VR
SLOPES	1.	1.172600	1.172600	.177
DISPLACEMENTS	1.	.068400	.068400	.010
ERROR	188.	1245.071700	6.622721	

TABLE A10

T40

LINEAR REGRESSION ANALYSIS : T. MOLITOR FEMALES STARVED IN 80%RH AT 25°C

LINEAR REGRESSION OF FINAL DRY WEIGHT DAY (0,4,8,12,16,20) (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE,	CONST	SD
Day 0	25	.110270E+03	.445100E+02	.14965000E+03	.45150000E+02	.14737100E+03
	.3040	.301703	11.241103	.197125	21.742393	2.411467
Day 4	25	.109890E+03	.396940E+02	.19232000E+03	.59130000E+02	.12886600E+03
	.3756	.307456	5.907625	.158186	17.388690	2.193727
Day 8	25	.109852E+03	.345820E+02	.22531000E+03	.62271000E+02	.18659200E+03
	.3037	.276379	4.221191	.180791	19.867765	2.713745
Day 12	25	.110666E+03	.322900E+02	.20915000E+03	.51836000E+02	.85349000E+02
	.3879	.247841	4.862399	.122767	13.590779	1.775459
Day 16	25	.110518E+03	.294140E+02	.17661000E+03	-.39550000E+01	.95037000E+02
	-.0305	-.022393	31.888937	.152887	16.901719	2.031795
Day 20	25	.109686E+03	.265620E+02	.25820000E+03	.50901000E+02	.83465000E+02
	.3467	.197137	4.938736	.111197	12.202075	1.786793
PARLL REG	.12112400E+04	.26533300E+03	.72668000E+03	.21905898E-00	.62127790E-01	
POOL	150	.11014700E+03	.34508666E+02	.12308000E+04	.27775000E+03	.62503600E+04
	.1001	.225666	9.652208	.184305	20.307611	6.465961

SOURCE	DF	SS	MS	VR
SLOPES	5.	13.858840	2.771768	.584
DISPLACEMENTS	5.	5519.124900	1103.824900	232.669
ERROR	138.	654.697590	4.744185	

T41

TABLE A4.1

LINEAR REGRESSION ANALYSIS: T. MOLITOR MALES STARVED IN 80%RH AT 25°C

LINEAR REGRESSION OF FINAL DRY WEIGHT DAY (0,4,8,12,16,20) (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE	CONST	SD
Day 0	25	.110076E+03	.402300E+02	.20605000E+03	.49620000E+02	.12658200E+03
	.3072	.240815	13.722012	.155526	17.125546	2.232494
Day 4	25	.109726E+03	.353960E+02	.22117000E+03	.13111400E+03	.15059200E+03
	.7184	.592820	-29.651767	.119682	13.137160	1.779899
Day 8	25	.109470E+03	.309220E+02	.16551000E+03	.19173000E+02	.83438000E+02
	.1631	.115841	18.240783	.146065	15.994194	1.879141
Day 12	25	.109932E+03	.259800E+02	.15915000E+03	.35639000E+02	.12996800E+03
	.2478	.223933	1.362555	.182553	20.073747	2.302996
Day 16	25	.110480E+03	.231040E+02	.17942000E+03	.47484000E+02	.91065000E+02
	.3714	.264652	-6.134838	.137921	15.241997	1.847421
Day 20	25	.108504E+03	.208480E+02	.17260000E+03	.36203000E+02	.33277000E+02
	.4776	.209750	-1.910807	.080434	8.730012	1.056725
PARLL REG		.11039000E+04	.31923300E+03	.61492200E+03	.28918652E-00	.57537835E-01
POOL	150	.10969800E+03	.29413333E+02	.11610000E+04	.52926000E+03	.76156400E+04
	.1779	.455865	-20.594215	.207164	22.732842	7.058811

SOURCE	DF	SS	MS	VR
SLOPES	5.	27.720560	5.544112	1.545
DISPLACEMENTS	5.	6851.764500	1370.352900	382.127
ERROR	138.	494.883560	3.586112	

T42

TABLE A4.2

LINEAR REGRESSION ANALYSIS : T. MOLITOR FEMALES STARVED IN 80%RH. AT 25°C

(A) LINEAR REGRESSION OF FINAL DRY WEIGHT DAY (0,4,8) (Y) ON INITIAL LIVE WEIGHT (X)

PARLL REG	.56728000E+03	.16655100E+03	.46282900E+03	.29359575E-00	.10137613E-00

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE,CONST		SD
POOL	75	.11000400E+03	.39595333E+02	.56994000E+03	.21800000E+03	.16952600E+04
	.2217	.382496	-2.480801	.196829	21.658809	4.698988

SOURCE	DF	SS	MS	VR
SLOPES	2.	.113560	.056780	.009
DISPLACEMENTS	2.	1197.945500	598.972750	99.872
ERROR	69.	413.816780	5.997344	

(B) LINEAR REGRESSION OF FINAL DRY WEIGHT DAY (8,12,16,20) (Y) ON INITIAL LIVE WEIGHT (X)

PARLL REG	.86927000E+03	.16105300E+03	.45044300E+03	.18527385E-00	.71367025E-01

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE,CONST		SD
POOL	100	.11018050E+03	.30712000E+02	.88670000E+03	.18876000E+03	.13597990E+04
	.1719	.212879	7.256862	.123231	13.582686	3.669532

SOURCE	DF	SS	MS	VR
SLOPES	3.	10.341690	3.447230	.773
DISPLACEMENTS	3.	899.011900	299.670630	67.200
ERROR	92.	410.262410	4.459374	

T43

TABLE A.3 A-B

LINEAR REGRESSION ANALYSIS: T. MOLITOR MALES STARVED IN 80%RH AT 25° C

(A) LINEAR REGRESSION OF FINAL DRY WEIGHT DAY (0,4,8,12) (Y) ON INITIAL LIVE WEIGHT (X)

PARLL REG	.75188000E+03	.23554600E+03	.49058000E+03	.31327605E-00	.76387463E-01

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE,CONST		SD
POOL	100	.10980100E+03	.33132000E+02	.75700000E+03	.27495000E+03	.32791400E+04
	.1745	.363210	-6.748825	.207015	22.737655	5.695751

SOURCE	DF	SS	MS	VR
SLOPES	3.	26.087130	8.695710	2.047
DISPLACEMENTS	3.	2762.486400	920.828800	216.830
ERROR	92.	390.701950	4.246760	

(B) LINEAR REGRESSION OF FINAL DRY WEIGHT DAY (12,16,20) (Y) ON INITIAL LIVE WEIGHT (X)

PARLL REG	.51117000E+03	.11932600E+03	.25431000E+03	.23343701E-00	.78991253E-01

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE,CONST		SD
POOL	75	.10963866E+03	.23310666E+02	.56321000E+03	.20440000E+03	.58512900E+03
	.3560	.362919	-16.479362	.111478	12.226190	2.645617

SOURCE	DF	SS	MS	VR
SLOPES	2.	.286050	.143025	.043
DISPLACEMENTS	2.	284.493320	142.246660	43.396
ERROR	69.	226.168850	3.277809	

T44

TABLE A.1, A-B

LINEAR REGRESSION ANALYSIS: NEWLY EMERGED I. MOLITOR FEMALES AND MALES

LINEAR REGRESSION OF INITIAL WATER CONTENT (MG) (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE,CONST		SD
Females	100	.107678E+03	.640245E+02	.17888000E+04	.89059000E+03	.87405000E+03
	.7122	.497870	10.414846	.049564	5.341103	2.096284
ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE,CONST		SD
Males	100	.107155E+03	.674565E+02	.20494000E+04	.11711500E+04	.11735100E+04
	.7551	.571459	6.221711	.050106	5.373954	2.268337
PARLL REG	.38382000E+04	.20617400E+04	.20475600E+04	.53716325E-00	.35260053E-01	
POOL	200	.10741650E+03	.65740500E+02	.38519000E+04	.19719000E+04	.26365100E+04
	.6187	.511929	10.750860	.046187	4.965485	2.866593

SOURCE	DF	SS	MS	VR
SLOPES	1.	5.172410	5.172410	1.084
DISPLACEMENTS	1.	686.967800	686.967800	144.021
ERROR	196.	934.896690	4.769881	

TABLE A45

T45

LINEAR REGRESSION ANALYSIS: I. MOLITOR FEMALES STARVED TO DEATH AT 0%RH. IN DIFFERENT TEMPERATURES

LINEAR REGRESSION OF WATER LOST (MG) (Y) ON INITIAL LIVE WEIGHT (X)

ID / ID	N / R	X MEAN / SLOPE	Y MEAN / CONST	XCSS / SE OF SLOPE	XYCSP / SE OF CONST	YCSS / SD
20°C	25	.107738E+03	.315652E+02	.51500000E+03	.29709000E+02	.12308000E+03
	.1180	.057687	25.350078	.101223	10.915289	2.297126
25°C	25	.107528E+03	.315952E+02	.64082000E+03	.16055700E+03	.28335400E+03
	.3767	.250549	4.654136	.128435	13.825685	3.251264
30°C	25	.107946E+03	.322232E+02	.25048000E+03	.18170000E+02	.93672000E+02
	.1186	.072540	24.392720	.126612	13.673208	2.003843
35°C	25	.106686E+03	.314928E+02	.41836000E+03	.89775000E+02	.16408800E+03
	.3426	.214587	8.599274	.122681	13.098055	2.509316
PARLL REG		.18246600E+04	.29821100E+03	.66419400E+03	.16343373E-00	.59586217E-01
POOL	100	.10747450E+03	.31719100E+02	.18475000E+04	.30743000E+03	.67280000E+03
	.2757	.166403	13.834995	.058595	6.302569	2.518589

SOURCE	DF	SS	MS	VR
SLOPES	3.	13.786230	4.595410	.702
DISPLACEMENTS	3.	6.186380	2.062126	.315
ERROR	92.	601.670040	6.539891	

TABLE A46

LINEAR REGRESSION ANALYSIS: I. MOLITOR MALES STARVED TO DEATH AT 0%RH IN DIFFERENT TEMPERATURES

LINEAR REGRESSION OF WATER LOST (MG) (Y) ON INITIAL LIVE WEIGHT (X)

ID / ID	N / R	X MEAN / SLOPE	Y MEAN / CONST	XCSS / SE OF SLOPE	XYCSP / CONST	YCSS / SD
20°C	25	.107654E+03	.313376E+02	.63516000E+03	.17221700E+03	.25694000E+03
	.4263	.271139	2.148343	.119965	12.928948	3.023424
25°C	25	.107736E+03	.313940E+02	.44190000E+03	.19505900E+03	.21097400E+03
	.6388	.441409	-16.161728	.110843	11.950875	2.330077
30°C	25	.108528E+03	.315656E+02	.42328000E+03	.68450000E+02	.21167800E+03
	.2286	.161713	14.015181	.143547	15.590170	2.953323
35°C	25	.107430E+03	.309104E+02	.41020000E+03	.57758000E+02	.29585100E+03
	.1657	.140804	15.783775	.174631	18.773970	3.536876
PARLL REG	.19105400E+04	.49348400E+03	.97544300E+03	.25829556E-00	.68352198E-01	
POOL	100	.10783700E+03	.31301900E+02	.19276000E+04	.50161000E+03	.98125500E+03
	.3647	.260225	3.240000	.067107	7.242694	2.946328

SOURCE	DF	SS	MS	VR
SLOPES	3.	24.532920	8.177640	.913
DISPLACEMENTS	3.	2.745190	.915063	.102
ERROR	92.	823.445360	8.950493	

TABLE A47

T47

LINEAR REGRESSION ANALYSIS; T. MOLITOR FEMALES AND MALES STARVED TO DEATH AT 0%RH. IN DIFFERENT TEMPERATURES

LINEAR REGRESSION OF WATER LOST (MG) (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
Females	100	.107472E+03	.317191E+02	.18458000E+04	.30665000E+03	.67278000E+03
	.2751	.166133	13.864355	.058631	6.306295	2.518979
ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
Males	100	.107837E+03	.313019E+02	.19271000E+04	.50137000E+03	.98124200E+03
	.3646	.260168	3.246150	.067119	7.243964	2.946463
PARLL REG		.37729000E+04	.80802000E+03	.16540220E+04	.21416417E-00	.44637803E-01
POOL	200	.10765450E+03	.31510500E+02	.37797000E+04	.80043000E+03	.16627200E+04
	.3192	.211770	8.712424	.044668	4.812663	2.746174

SOURCE	DF	SS	MS	VR
SLOPES	1.	8.336600	8.336600	1.109
DISPLACEMENTS	1.	12.239300	12.239300	1.628
ERROR	196.	1472.636500	7.513451	

TABLE A.48

LINEAR REGRESSION ANALYSIS: T. MOLITOR FEMALES STARVED IN DIFFERENT RELATIVE HUMIDITIES AT 25°C

LINEAR REGRESSION OF TOTAL WEIGHT LOST OVER 8 DAYS (Y) ON INITIAL LIVE WEIGHT (x)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE, CONST		SD
0% R.H.	16	.111184E+03	.278031E+02	.89354000E+03	.19191300E+03	.13078700E+03
	.5613	.214778	3.923136	.084616	9.429279	2.529373
20% R.H.	16	.110965E+03	.236718E+02	.56609000E+03	.12901000E+02	.80775000E+02
	.0603	.022789	21.143006	.100772	11.198281	2.397632
40% R.H.	16	.109340E+03	.190906E+02	.49773000E+03	.96024000E+02	.14213110E+03
	.3610	.192923	-2.003791	.133185	14.581569	2.971360
60% R.H.	16	.112190E+03	.165343E+02	.51082000E+03	.97347000E+02	.12495860E+03
	.3853	.190570	-4.845798	.121979	13.702321	2.756902
80% R.H.	16	.112575E+03	.135781E+02	.72341000E+03	-.12351000E+02	.11005990E+03
	-.0437	-.017073	15.500152	.104145	11.745110	2.801135
100% R.H.	16	.108653E+03	.107656E+02	.75406000E+03	.15238200E+03	.28750360E+03
	.3272	.202082	-11.191221	.155938	16.977029	4.282105
PARLL REG	.39456500E+04	.53821600E+03	.87621520E+03	.13640743E-00	.47813330E-01	
POOL	96	.11081822E+03	.18573958E+02	.41375000E+04	.67736000E+03	.41005570E+04
	.1644	.163712	.431643	.101282	11.243646	6.514847

SOURCE	DF	SS	MS	VR
SLOPES	5.	36.177360	7.235472	.792
DISPLACEMENTS	5.	3186.866300	637.373260	69.838
ERROR	84.	766.621180	9.126442	

T49

TABLE A4.9

LINEAR REGRESSION ANALYSIS: T. MOLITOR MALES STARVED IN DIFFERENT RELATIVE HUMIDITIES AT 25° C

LINEAR REGRESSION OF TOTAL WEIGHT LOST OVER 8 DAYS (Y) ON INITIAL LIVE WEIGHT (x)

ID / ID	N / R	X MEAN / SLOPE	Y MEAN / CONST	XCSS / SE OF SLOPE, CONST	XYCSP	YCSS / SD
0% R.H.	16	.112431E+03	.300187E+02	.11930700E+04	.40911000E+03	.25542900E+03
	.7410	.342905	-8.534518	.083027	9.362374	2.867839
20% R.H.	16	.108750E+03	.257343E+02	.85494000E+03	.21972300E+03	.13976400E+03
	.6356	.257004	-2.214810	.083421	9.092513	2.439178
40% R.H.	16	.111206E+03	.214906E+02	.78798000E+03	.31348000E+02	.21110630E+03
	.0768	.039782	17.066537	.137924	15.368610	3.871684
60% R.H.	16	.112284E+03	.186875E+02	.12291000E+04	.24279600E+03	.26350750E+03
	.4266	.197539	-3.493116	.111921	12.605218	3.923788
80% R.H.	16	.112709E+03	.152093E+02	.12469600E+04	.13412600E+03	.88871100E+02
	.4029	.107562	3.086086	.065301	7.382666	2.305957
100% R.H.	16	.110309E+03	.105875E+02	.79234000E+03	.19222700E+03	.14223250E+03
	.5726	.242606	-16.174292	.092832	10.261161	2.613111
PARLL REG		.61043900E+04	.12293300E+04	.11009104E+04	.20138457E-00	.39631956E-01
POOL	96	.11128177E+03	.20288020E+02	.62920000E+04	.11954600E+04	.50729170E+04
	.2115	.189996	-.855162	.090515	10.099362	7.179894

SOURCE	DF	SS	MS	VR
SLOPES	5.	59.459080	11.891816	1.258
DISPLACEMENTS	5.	3992.441100	798.488220	84.487
ERROR	84.	793.883320	9.450991	

T50

TABLE A50

LINEAR REGRESSION ANALYSIS: T. MOLITOR LARVAE IN DIFFERENT RELATIVE HUMIDITIES AT A TEMPERATURE OF 25°C

(A) LINEAR REGRESSION OF FINAL DRY WEIGHT (DAY 35) (Y) ON INITIAL LIVE WEIGHT(X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE,CONST		SD
0% R.H.	35	.118528E+03	.421328E+02	.15240100E+04	.74627000E+03	.98595300E+03
	.6087	.489675	-15.907651	.111078	13.186304	4.336326
20% R.H.	36	.119879E+03	.435000E+02	.12948800E+04	.70186000E+03	.75566400E+03
	.7095	.542027	-21.477749	.092320	11.081155	3.322104
40% R.H.	36	.119009E+03	.429361E+02	.13082100E+04	.54050000E+03	.62716200E+03
	.5967	.413159	-6.233940	.095286	11.354555	3.446434
60% R.H.	30	.120233E+03	.434483E+02	.74447000E+03	.33096000E+03	.57277300E+03
	.5068	.444557	-10.002340	.142895	17.195586	3.898910
80% R.H.	25	.119560E+03	.432280E+02	.10068600E+04	.42315000E+03	.67539300E+03
	.5131	.420266	-7.019118	.146579	17.549704	4.651119
PARLL REG	.58784300E+04	.27427400E+04	.36169450E+04	.46657696E-00	.50484633E-01	
POOL	162	.11941049E+03	.43027777E+02	.59402000E+04	.27907500E+04	.36596000E+04
	.5985	.469807	-13.072156	.049708	5.943380	3.831191

SOURCE	DF	SS	MS	VR
SLOPES	4.	14.437800	3.609450	.236
DISPLACEMENTS	4.	11.239200	2.809800	.183
ERROR	152.	2322.808000	15.281631	

(B) LINEAR REGRESSION OF INITIAL DRY WEIGHT (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE,CONST		SD
CONTROLS	42	.119028E+03	.499309E+02	.15174700E+04	.78617000E+03	.10821800E+04
	.6134	.518079	-11.735302	.105444	12.566894	4.107558

TABLE A51 A-B

LINEAR REGRESSION ANALYSIS: T. MOLITOR LARVAE IN DIFFERENT RELATIVE HUMIDITIES

AT A TEMPERATURE OF 25°C

LINEAR REGRESSION OF FINAL DRY WEIGHT (DAY 35) (Y) ON INITIAL LIVE WEIGHT (X)

ID / ID	N / R	X MEAN / SLOPE	Y MEAN / CONST	XCSS / SE OF SLOPE	XYCSP / CONST	YCSS / SD
0% R.H.	35	.118528E+03	.421328E+02	.15240100E+04	.74627000E+03	.98595300E+03
	.6087	.489675	-15.907651	.111078	13.186304	4.336326
20% R.H.	36	.119879E+03	.435000E+02	.12948800E+04	.70186000E+03	.75566400E+03
	.7095	.542027	-21.477749	.092320	11.081155	3.322104
40% R.H.	36	.119009E+03	.429361E+02	.13082100E+04	.54050000E+03	.62716200E+03
	.5967	.413159	-6.233940	.095286	11.354555	3.446434
60% R.H.	30	.120233E+03	.434483E+02	.74447000E+03	.33096000E+03	.57277300E+03
	.5068	.444557	-10.002340	.142895	17.195586	3.898910
80% R.H.	25	.119560E+03	.432280E+02	.10068600E+04	.42315000E+03	.67539300E+03
	.5131	.420266	-7.019118	.146579	17.549704	4.651119
95% R.H.	13	.117761E+03	.367807E+02	.57470000E+03	.28470200E+03	.42691700E+03
	.5747	.495392	-21.557394	.212653	25.082326	5.097929
PARLL REG		.64531300E+04	.30274420E+04	.40438620E+04	.46914319E-00	.49193246E-01
POOL	175	.11928800E+03	.42563714E+02	.65475000E+04	.31994200E+04	.45561500E+04
	.5857	.488647	-15.726077	.051401	6.139629	4.159230

SOURCE	DF	SS	MS	VR
SLOPES	5.	14.872400	2.974480	.185
DISPLACEMENTS	5.	369.203100	73.840620	4.613
ERROR	163.	2608.685800	16.004207	

T52

TABLE A52

LINEAR REGRESSION ANALYSIS: T. MOLITOR LARVAE DIFFERENT WEIGHT GROUPS IN DRY AIR

AT A TEMPERATURE OF 25° C

LINEAR REGRESSION OF LENGTH OF LIFE (DAYS) (Y) ON INITIAL LIVE WEIGHT (X)

ID / ID	N / R	X MEAN / SLOPE	Y MEAN / CONST	XCSS / SE OF SLOPE	XYCSP / SE OF CONST	YCSS / SD
GROUP 1	42	.653035E+02	.120714E+03	.45477000E+03	.30359000E+03	.22512580E+05
	.0948	.667568	77.119700	1.107447	72.412011	23.616685
GROUP 2	42	.853500E+02	.138476E+03	.37665000E+03	.87886000E+03	.27372480E+05
	.2737	2.333359	-60.676070	1.296427	110.718150	25.160377
GROUP 3	43	.105200E+03	.159000E+03	.46422000E+03	.52466000E+03	.31982000E+05
	.1361	1.130196	40.103300	1.284207	135.164470	27.669226
GROUP 4	44	.124247E+03	.169931E+03	.28703000E+03	.72750000E+02	.35348800E+05
	.0228	.253457	138.440260	1.711929	212.748210	29.003432
GROUP 5	46	.144433E+03	.179717E+03	.34208000E+03	-.12083000E+04	.46567400E+05
	-.3027	-3.532214	689.888180	1.676397	242.171450	31.005653
PARLL REG		.19247500E+04	.57156000E+03	.16378326E+06	.29695285E-00	.63471859E-00
POOL	217	.10581520E+03	.15422580E+03	.17201490E+06	.12902470E+06	.26309390E+06
	.6065	.750078	74.856090	.067060	7.342859	27.812933

SOURCE	DF	SS	MS	VR
SLOPES	4.	6963.040000	1740.760000	2.300
DISPLACEMENTS	4.	2701.700000	675.425000	.892
ERROR	207.	156650.500000	756.765700	

T53

TABLE A53

LINEAR REGRESSION ANALYSIS: I MOLITOR LARVAE DIFFERENT WEIGHT GROUPS

LINEAR REGRESSION OF INITIAL DRY WEIGHT (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE	CONST	SD
GROUP 1	50	.652300E+02	.270380E+02	.45403000E+03	.14705800E+03	.21831400E+03
	.4670	.323894	5.910336	.088497	5.778858	1.385706
GROUP 2	50	.856370E+02	.367830E+02	.50659000E+03	.15852000E+03	.22598800E+03
	.4685	.312915	9.985832	.085168	7.298651	1.916945
GROUP 3	50	.105328E+03	.450000E+02	.50154000E+03	.28627000E+03	.51218000E+03
	.5648	.570781	-15.119325	.120365	12.683638	2.695606
GROUP 4	50	.125037E+03	.558940E+02	.56736000E+03	.19788000E+03	.79307000E+03
	.2949	.348773	12.284438	.163055	20.395379	3.883873
GROUP 5	50	.145043E+03	.648260E+02	.47190000E+03	.25680000E+03	.11021400E+04
	.3560	.544183	-14.103946	.206125	29.903702	4.477708
PARLL REG	.25014200E+04	.10465280E+04	.28516920E+04	.41837356E-00	.62887905E-01	
POOL	250	.10525500E+03	.45908200E+02	.20056560E+06	.95270200E+05	.47740570E+05
	.9736	.475007	-4.088732	.007070	.770661	3.166415

SOURCE	DF	SS	MS	VR
SLOPES	4.	31.554400	7.888600	.794
DISPLACEMENTS	4.	72.641600	18.160400	1.829
ERROR	240.	2382.298000	9.926241	

TABLE A54

T 54

LINEAR REGRESSION ANALYSIS: I. MOLITOR LARVAE DIFFERENT WEIGHT GROUPS

LINEAR REGRESSION OF FINAL DRY WEIGHT (Y) ON INITIAL LIVE WEIGHT (X)

ID ID	N R	X MEAN SLOPE	Y MEAN CONST	XCSS SE OF SLOPE, CONST	XYCSP 	YCSS SD
GROUP 1	42 .2113	.653035E+02 .128442	.904285E+01 .655074	.45477000E+03 .093903	.58412000E+02 6.140037	.16790790E+03 2.002531
GROUP 2	42 .1075	.853500E+02 .082418	.117300E+02 4.695565	.37665000E+03 .120489	.31043000E+02 10.290135	.22128370E+03 2.338403
GROUP 3	43 -.0319	.105200E+03 -.030901	.134604E+02 16.711281	.46422000E+03 .151119	-.14345000E+02 15.905531	.43510280E+03 3.255986
GROUP 4	44 .2716	.124247E+03 .370352	.165181E+02 -29.497050	.28717000E+03 .202481	.10635400E+03 25.163060	.53387900E+03 3.431265
GROUP 5	46 .2270	.144433E+03 .331559	.202423E+02 -27.646023	.34208000E+03 .214424	.11342000E+03 30.975678	.72964400E+03 3.965872
PARLL REG	.19248900E+04	.29488400E+03	.20878174E+04	.15319524E-00	.70917269E-01	
POOL	217 .7819	.10581511E+03 .138514	.14328156E+02 -.328724	.17201430E+06 .007529	.23826400E+05 .824454	.53969820E+04 3.122827

SOURCE	DF	SS	MS	VR
SLOPES	4.	42.323700	10.580925	1.094
DISPLACEMENTS	4.	54.047900	13.511975	1.398
ERROR	207.	2000.318900	9.663376	

TABLE A55

T55

LINEAR REGRESSION ANALYSIS: T. MOLITOR LARVAE IN DIFFERENT RELATIVE HUMIDITIES

AT A TEMPERATURE OF 20°C

LINEAR REGRESSION OF FINAL DRY WEIGHT (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE	CONST	SD
0% R.H.	24	.865687E+02	.110416E+02	.12645000E+03	-.60930000E+01	.38393400E+02
	-.0874	-.048185	15.212985	.117028	10.134548	1.315982
20% R.H.	23	.860956E+02	.104304E+02	.16507000E+03	.44000000E-01	.38808700E+02
	.0005	.000266	10.407485	.105808	9.114065	1.359423
40% R.H.	20	.856450E+02	.921000E+01	.13142000E+03	.14561000E+02	.16338000E+02
	.3142	.110797	-.279246	.078896	6.760088	.904454
60% R.H.	22	.850613E+02	.963863E+01	.19219000E+03	.33863000E+02	.30239700E+02
	.4441	.176195	-5.348787	.079466	6.763592	1.101662
80% R.H.	23	.849608E+02	.908260E+01	.23269000E+03	-.13300000E+01	.37258100E+02
	-.0142	-.005715	9.568224	.087310	7.423189	1.331853
95% R.H.	16	.847937E+02	.925000E+01	.14092000E+03	.15030000E+02	.38800000E+02
	.2032	.106656	.206216	.137310	11.650191	1.630007
PARLL REG		.98874000E+03	.56075000E+02	.19983790E+03	.56713595E-01	.40543544E-01
POOL	128	.85569531E+02	.98285156E+01	.10429800E+04	.11126000E+03	.27008800E+03
	.2096	.106675	.700377	.044327	3.795174	1.431558

SOURCE	DF	SS	MS	VR
SLOPES	5.	6.303880	1.260776	.768
DISPLACEMENTS	5.	61.561640	12.312328	7.503
ERROR	116.	190.353810	1.640981	

T56

TABLE 456

LINEAR REGRESSION ANALYSIS: I. MOLITOR LARVAE IN DIFFERENT RELATIVE HUMIDITIES

AT A TEMPERATURE OF 25° C

LINEAR REGRESSION OF FINAL DRY WEIGHT (Y) ON INITIAL LIVE WEIGHT (X)

ID ID	N R	X MEAN SLOPE	Y MEAN CONST	XCSS SE OF SLOPE, CONST	XYCSP SE OF CONST	YCSS SD
0% R.H.	24 .2281	.846000E+02 .112047	.112250E+02 1.745797	.23665000E+03 .101955	.26516000E+02 8.631363	.57090000E+02 1.568423
20% R.H.	23 -.0406	.845847E+02 -.020687	.103195E+02 12.069393	.20225000E+03 .110869	-.41840000E+01 9.383611	.52293700E+02 1.576722
40% R.H.	20 .6327	.858500E+02 .397740	.977500E+01 -24.371040	.16067000E+03 .114740	.63905000E+02 9.855813	.63492500E+02 1.454397
60% R.H.	24 .5044	.860729E+02 .315708	.932083E+01 -17.853150	.23248000E+03 .115214	.73396000E+02 9.923344	.91064600E+02 1.756712
80% R.H.	18 .0289	.860305E+02 .012928	.925277E+01 8.140562	.13722000E+03 .111753	.17740000E+01 9.619157	.27442400E+02 1.309089
PARLL REG	.96927000E+03	.16140700E+03	.29138320E+03	.16652429E-00	.51472582E-01	
POOL	109 .1842	.85386697E+02 .108082	.10022935E+02 .794128	.10219600E+04 .055751	.11045600E+03 4.763500	.35182300E+03 1.782271

SOURCE	DF	SS	MS	VR
SLOPES	4.	24.791760	6.197940	2.559
DISPLACEMENTS	4.	75.379620	18.844905	7.782
ERROR	99.	239.713260	2.421346	

T57

LINEAR REGRESSION ANALYSIS: T. MOLITOR LARVAE IN DIFFERENT RELATIVE HUMIDITIES

AT A TEMPERATURE OF 30°C

LINEAR REGRESSION OF FINAL DRY WEIGHT (Y) ON INITIAL LIVE WEIGHT (X)

ID	N	X MEAN	Y MEAN	XCSS	XYCSP	YCSS
ID	R	SLOPE	CONST	SE OF SLOPE	CONST	SD
0% R.H.	22	.842340E+02	.121454E+02	.17693000E+03	.14860000E+01	.50689600E+02
	.0156	.008398	11.437989	.119671	10.086119	1.591809
20% R.H.	23	.853152E+02	.115152E+02	.11127000E+03	-.40250000E+01	.11296720E+03
	-.0359	-.036173	14.601347	.219734	18.752891	2.317856
40% R.H.	20	.857150E+02	.106225E+02	.16109000E+03	.40084000E+02	.10688740E+03
	.3054	.248829	-10.705950	.182818	15.678911	2.320360
60% R.H.	20	.847950E+02	.106100E+02	.18159000E+03	.31405000E+02	.93508000E+02
	.2410	.172944	-4.054832	.164152	13.928120	2.212046
80% R.H.	20	.848675E+02	.942000E+01	.15456000E+03	.30673000E+02	.54642000E+02
	.3337	.198453	-7.422267	.132108	11.217748	1.642403
95% R.H.	11	.849500E+02	.993181E+01	.10916900E+03	.16892800E+02	.25641400E+02
	.3192	.154739	-3.213336	.153091	13.014078	1.599562
PARLL REG		.89460900E+03	.11651580E+03	.44433560E+03	.13024215E-00	.66340678E-01
POOL	116	.84977586E+02	.10813362E+02	.92116000E+03	.10144000E+03	.54363700E+03
	.1433	.110122	1.455459	.071207	6.054372	2.161193

SOURCE	DF	SS	MS	VR
SLOPES	5.	9.089390	1.817878	.450
DISPLACEMENTS	5.	103.305890	20.661178	5.115
ERROR	104.	420.070950	4.039143	

T58

TABLE 458

LINEAR REGRESSION ANALYSIS; T. MOLITOR LARVAE IN DIFFERENT RELATIVE HUMIDITIES

AT A TEMPERATURE OF 35°C

LINEAR REGRESSION OF FINAL DRY WEIGHT (Y) ON INITIAL LIVE WEIGHT (X)

ID ID	N R	X MEAN SLOPE	Y MEAN CONST	XCSS SE OF SLOPE,	XYCSP CONST	YCSS SD
0% R.H.	24 .5339	.848333E+02 .288597	.151500E+02 -9.332689	.2322300E+03 .097424	.67021000E+02 8.270406	.67835000E+02 1.484662
20% R.H.	24 .4519	.839270E+02 .369881	.138395E+02 -17.203498	.1232800E+03 .155651	.45599000E+02 13.068159	.82575000E+02 1.728225
40% R.H.	25 .5020	.845920E+02 .273740	.122600E+02 -10.896217	.2083400E+03 .098327	.57031000E+02 8.322524	.61940000E+02 1.419251
60% R.H.	24 .5000	.852354E+02 .409724	.126937E+02 -22.229330	.1803600E+03 .151271	.73898000E+02 12.900396	.12107660E+03 2.031555
80% R.H.	25 .5398	.852700E+02 .407089	.141120E+02 -20.600559	.2767300E+03 .132369	.11265400E+03 11.295697	.15738140E+03 2.201986
95% R.H.	21 .3811	.844261E+02 .243133	.140880E+02 -6.438740	.1829900E+03 .135311	.44491000E+02 11.430782	.74474500E+02 1.830404
PARLL REG		.12039300E+04	.40069400E+03	.56528250E+03	.33282167E-00	.51360946E-01
POOL	143 .4274	.84723076E+02 .321667	.13675174E+02 -13.577472	.1235500E+04 .057293	.39742000E+03 4.856974	.69966800E+03 2.013836

SOURCE	DF	SS	MS	VR
SLOPES	5.	5.415780	1.083156	.332
DISPLACEMENTS	5.	139.908110	27.981622	8.594
ERROR	131.	426.507080	3.255779	

T59

TABLE 459

LINEAR REGRESSION ANALYSIS : T. MOLITOR LARVAE STARVED TO DEATH IN DIFFERENT RELATIVE HUMIDITIES AT A TEMPERATURE OF 20°C

LINEAR REGRESSION OF FINAL DRY WEIGHT (Y) ON INITIAL LIVE WEIGHT (X)

(A) LARVAE WHICH DID NOT MOULT DURING THE COURSE OF THE EXPERIMENT

ID ID	N R	X MEAN SLOPE	Y MEAN CONST	XCSS SE OF SLOPE,CONST	XYCSP	YCSS SD
	56	.856491E+02	.110455E+02	.45753000E+03	.47360000E+02	.56996500E+02
	.2932	.103512	2.179797	.045918	3.935069	.982194

(B) LARVAE WHICH DID MOULT DURING THE COURSE OF THE EXPERIMENT

ID ID	N R	X MEAN SLOPE	Y MEAN CONST	XCSS SE OF SLOPE,CONST	XYCSP	YCSS SD
	71	.855232E+02	.881690E+01	.58358000E+03	.59448000E+02	.44009800E+02
	.3709	.101867	.104838	.030701	2.627131	.741658
PARLL REG		.10411100E+04	.10680800E+03	.10100630E+03	.10259050E-00	.26410696E-01
POOL	127	.85578740E+02	.97996062E+01	.10416200E+04	.11559000E+03	.25650200E+03
	.2236	.110971	.302816	.043260	3.704286	1.396208

SOURCE	DF	SS	MS	VR
SLOPES	1.	.000699	.000699	.000
DISPLACEMENTS	1.	153.626000	153.626000	209.843
ERROR	123.	90.048121	.732098	

TABLE A60

T60

LINEAR REGRESSION ANALYSIS: T. MOLITOR LARVAE STARVED TO DEATH IN DIFFERENT RELATIVE HUMIDITIES AT A TEMPERATURE OF 25°C

LINEAR REGRESSION OF FINAL DRY WEIGHT (Y) ON INITIAL LIVE WEIGHT (X)

(A) LARVAE WHICH DID NOT MOULT DURING THE COURSE OF THE EXPERIMENT

ID ID	N R	X MEAN SLOPE	Y MEAN CONST	XCSS SE OF SLOPE	XYCSP ,CONST	YCSS SD
	63	.854063E+02	.113103E+02	.59876000E+03	.69797000E+02	.72920900E+02
	.3340	.116569	1.354564	.042115	3.599298	1.030555

(B) LARVAE WHICH DID MOULT DURING THE COURSE OF THE EXPERIMENT

ID ID	N R	X MEAN SLOPE	Y MEAN CONST	XCSS SE OF SLOPE	XYCSP ,CONST	YCSS SD
	46	.853663E+02	.824239E+01	.42465000E+03	.35205000E+02	.31249900E+02
	.3056	.082903	1.165220	.038939	3.326232	.802429
PARLL REG		.10234100E+04	.10500200E+03	.10417080E+03	.10260013E-00	.29342024E-01
POOL	109	.85389449E+02	.10015596E+02	.10234400E+04	.10826900E+03	.35441400E+03
	.1797	.105789	.982307	.055962	4.781701	1.790317

SOURCE	DF	SS	MS	VR
SLOPES	1.	.281593	.281593	.317
DISPLACEMENTS	1.	249.562710	249.562710	281.413
ERROR	105.	93.115997	.886819	

TABLE A61

T61

LINEAR REGRESSION ANALYSIS: T. MOLITOR LARVAE STARVED TO DEATH IN DIFFERENT RELATIVE HUMIDITIES AT A TEMPERATURE OF 30°C

LINEAR REGRESSION OF FINAL DRY WEIGHT (Y) ON INITIAL LIVE WEIGHT (X)

(A) LARVAE WHICH DID NOT MOULT DURING THE COURSE OF THE EXPERIMENT

ID / ID	N / R	X MEAN / SLOPE	Y MEAN / CONST	XCSS / SE OF SLOPE	XYCSP / SE OF CONST	YCSS / SD
	79	.849829E+02	.120455E+02	.67345000E+03	.64872000E+02	.15240500E+03
	.2024	.096327	3.859347	.053089	4.514379	1.377726

(B) LARVAE WHICH DID MOULT DURING THE COURSE OF THE EXPERIMENT

ID / ID	N / R	X MEAN / SLOPE	Y MEAN / CONST	XCSS / SE OF SLOPE	XYCSP / SE OF CONST	YCSS / SD
	37	.849527E+02	.818243E+01	.24913000E+03	.32910000E+02	.15176100E+02
	.5352	.132099	-3.039794	.035240	2.995165	.556229
PARLL REG		.92258000E+03	.97782000E+02	.16758110E+03	.10598755E-00	.38833727E-01
POOL	116	.84973275E+02	.10813362E+02	.92259000E+03	.10071000E+03	.54363600E+03
	.1422	.109160	1.537673	.071164	6.050378	2.161550

SOURCE	DF	SS	MS	VR
SLOPES	1.	.232720	.232720	.166
DISPLACEMENTS	1.	375.425060	375.425060	267.845
ERROR	112.	156.984710	1.401649	

TABLE A63 The number of larvae which pupated (expressed as a percentage of the total) when T. molitor larvae in the weight range 140-150 mg were starved in different combinations of temperature and humidity (Section 12.16 P)

Temp.	Replicates	Humidities				
		0%	33%	53%	75%	95%
20°C	1	13.33	73.33	93.33	93.33	100.00
	2	33.33	66.67	93.33	86.67	73.33
	3	6.67	53.33	86.67	93.33	93.33
	4	33.33	66.67	73.33	93.33	93.33
	Totals	86.66	260.00	346.66	366.66	359.99
	$\bar{x}$	21.6650	65.00	86.6650	91.665	89.9975
25°C	1	40.00	73.33	100.00	100.00	86.67
	2	33.33	73.33	93.33	100.00	93.33
	3	60.00	86.67	100.00	100.00	86.67
	4	40.00	80.00	100.00	100.00	86.67
	Totals	173.33	313.33	393.33	400.00	353.34
	$\bar{x}$	43.3325	78.3325	98.3325	100.00	88.3350
30°C	1	6.67	53.33	80.00	100.00	93.33
	2	6.67	46.67	73.33	86.67	86.67
	3	26.67	60.00	93.33	100.00	100.00
	4	33.33	60.00	66.67	100.00	93.33
	Totals	73.34	220.00	313.33	386.67	373.33
	$\bar{x}$	18.335	55.00	78.3325	96.6675	93.3325
35°C	1	0.00	6.67	13.33	53.33	53.33
	2	0.00	26.67	26.67	40.00	66.67
	3	0.00	0.00	33.33	33.33	86.67
	4	0.00	6.67	26.67	25.33	66.67
	Totals	0.000	40.01	100.00	152.99	273.34
	$\bar{x}$	0.00	10.0025	25.00	38.2475	68.3350

TABLE A64 Transformation of Percentages in Table A63 to Angles

Temp.	Replicates	Humidities					Totals
		0%	35%	55%	75%	95%	
20°C	1	21.39	58.00	75.00	75.00	90.00	320.28
	2	35.24	54.76	75.00	68.61	58.69	292.50
	3	15.00	46.89	68.61	75.00	75.00	280.50
	4	36.24	54.76	58.00	75.00	75.00	298.89
	Totals	106.87	215.30	277.50	293.61	298.89	1192.17
25°C	1	39.23	58.89	90.00	90.00	68.61	346.73
	2	35.24	58.89	75.00	90.00	75.00	334.13
	3	50.77	68.61	90.00	90.00	66.61	367.99
	4	39.23	63.44	90.00	90.00	68.61	351.28
	Totals	164.47	249.83	345.00	360.00	280.83	1400.13
30°C	1	15.00	46.89	63.44	90.00	75.00	290.33
	2	15.00	45.11	58.89	68.61	68.61	254.22
	3	31.11	50.77	75.00	90.00	90.00	336.88
	4	35.24	50.77	54.76	90.00	75.00	305.77
	Totals	96.35	193.54	252.09	338.61	308.61	1187.20
35°C	1	0.00	15.00	21.39	46.89	46.89	130.17
	2	0.00	31.11	31.11	39.23	54.76	156.21
	3	0.00	0.00	35.24	35.24	68.61	139.09
	4	0.00	15.00	15.00	35.24	54.76	120.00
	Totals	0.00	61.11	102.74	156.60	225.02	545.47

TABLE A65 Time (days) before pupation occurred in newly moulted larvae weighing 140-150 mg. stayed in different combinations of temperature and humidity

Temp.	Humidities	Replicates			
		1	2	3	4
20°C	0%	20, 36	20, 40, 42, 51, 56	37	22, 32, 35, 50, 55
	33%	18, 21, 24, 46, 75, 80, 85, 87 100, 111, 112	24, 26, 27, 27 39, 52, 71, 77 86, 106	31, 36, 60, 72 86, 90, 112, 117	20, 21, 26, 31 46, 71, 72, 74 82, 109
	58%	31, 38, 38, 49 54, 55, 57, 61 75, 77, 87, 90 104, 114	20, 25, 25, 27 28, 29, 31, 33 38, 47, 69, 77 97, 112	20, 25, 34, 35 39, 70, 78, 80 94, 96, 100, 106, 116	25, 32, 33, 49 59, 87, 89, 96 105, 110, 120
	75%	18, 19, 28, 29 29, 31, 34, 36 37, 58, 63, 69 109, 118	19, 21, 24, 26 34, 38, 59, 54 72, 75, 76, 85 85	17, 20, 21, 22 33, 37, 48, 71 72, 80, 95, 101 102, 111	20, 20, 25, 26 27, 28, 32, 35 45, 50, 75, 85 87, 91
	95%	20, 24, 24, 28 46, 46, 52, 57 60, 67, 68, 68 72, 80, 101	22, 23, 26, 28 28, 28, 32, 38 61, 79, 105	20, 24, 25, 25 29, 30, 34, 35 36, 62, 73, 86 95, 107	19, 22, 23, 24 36, 46, 47, 48 49, 50, 51, 76 104, 120
25°C	0%	12, 13, 14, 15 21, 32	13, 16, 19, 22 30	13, 14, 15, 18 21, 23, 33, 34 41	14, 16, 17, 25 27, 29
	58%	13, 13, 16, 16 23, 28, 40, 41 47, 48, 68	13, 14, 16, 20 20, 36, 39, 45 48, 52, 75	15, 19, 24, 29 31, 34, 40, 43 56, 61, 64, 66 94	14, 20, 20, 27 29, 29, 32, 41 42, 42, 66, 120

TABLE A65 Continued

Temp.	Humidities	Replicates			
		1	2	3	4
25°C	55%	13, 13, 16, 16 17, 18, 19, 19 24, 26, 35, 42 69, 93, 99	12, 15, 15, 16 16, 17, 18, 20 22, 30, 30, 30 33, 70	15, 16, 17, 17 18, 19, 22, 27 34, 39, 39, 42 44, 51, 73	10, 13, 13, 19 24, 24, 25, 28 29, 29, 30, 35 44, 48, 61
	75%	14, 16, 19, 19 21, 22, 24, 25 29, 31, 32, 35 35, 40, 103	12, 14, 14, 20 23, 24, 27, 28 29, 31, 33, 34 35, 39, 61	12, 15, 17, 17 17, 17, 25, 28 28, 33, 34, 41 44, 47, 48	13, 13, 15, 17 17, 18, 19, 20 21, 22, 25, 27 40, 72, 77
	95%	11, 14, 15, 16 17, 17, 18, 21 25, 27, 28, 29 37	12, 12, 14, 17 17, 18, 18, 18 19, 20, 21, 22 34, 35	13, 15, 19, 20 23, 23, 23, 25 27, 29, 37, 42 45	13, 14, 14, 14 15, 17, 20, 20 22, 26, 26, 30 39
30°C	0%	16	12	10, 11, 13, 14	11, 12, 20, 21 25
	33%	14, 16, 23, 24 28, 35, 36, 52	11, 12, 13, 17 19, 29, 36	11, 11, 15, 20 21, 28, 30, 30 43	16, 18, 19, 19 21, 23, 33, 34 56
	53%	15, 16, 17, 17 19, 23, 26, 27 31, 35, 37, 62	18, 23, 24, 33 34, 37, 37, 39 45, 48, 49	11, 12, 13, 24 24, 26, 27, 30 31, 39, 46, 55 59, 76	11, 12, 14, 19 30, 31, 39, 42 42, 57
	75%	11, 12, 12, 13 13, 14, 14, 15 15, 16, 17, 18 20, 22, 35	10, 13, 13, 15 19, 23, 23, 26 30, 36, 44, 49 78	11, 11, 12, 13 19, 24, 31, 32 36, 40, 41, 52 58, 59, 77	9, 10, 11, 14, 15 17, 17, 18, 19 27, 27, 28, 31 33, 47

TABLE A65 Continued

Temp.	Humidities	Replicates			
		1	2	3	4
30°C	95%	10, 11, 12, 12 12, 13, 14, 14 16, 16, 17, 19 19, 21	10, 11, 11, 12 12, 13, 15, 15 18, 25, 26, 30 37	12, 13, 14, 14 14, 14, 16, 17 18, 18, 26, 28 32, 34, 41	11, 12, 13, 13 14, 15, 16, 21 22, 22, 26, 30 37, 46
35°C	0%	No Pupae			
	33%	18	10, 11, 12, 20	-	16
	55%	34, 50	16, 23, 49, 64	12, 12, 15, 17 18	20, 36, 38, 42
	75%	22, 24, 28, 35 36, 40, 41, 53	25, 25, 37, 43 58, 58	15, 19, 20, 45 52	20, 28, 28, 31 33
	95%	17, 19, 24, 25 30, 30, 32, 43	19, 25, 27, 32 34, 37, 39, 40 46, 49	10, 16, 17, 19 28, 29, 31, 32 37, 37, 41, 53 55	10, 12, 16, 25 28, 28, 29, 33 45, 57

LINEAR REGRESSION ANALYSIS : T. MOLITOR LARVAE STARVED AT SATURATION LEVEL IN 25° C

LINEAR REGRESSION OF AMOUNT OF WATER ABSORBED (MG)(Y) ON INITIAL LIVE WEIGHT (X)

ID ID	N R	X MEAN SLOPE	Y MEAN CONST	XCSS SE OF SLOPE	XYCSP SE OF CONST	YCSS SD
GROUP 1	42 -.0529	.122354E+03 -.216927	.177428E+02 44.284920	.11071000E+03 .647096	-.24016000E+02 79.182282	.18595300E+04 6.808671
GROUP 2	51 -.1002	.127608E+03 -.328251	.181450E+02 60.032928	.11086000E+03 .465507	-.36390000E+02 59.406882	.11890770E+04 4.901336
GROUP 3	55 -.0107	.132497E+03 -.041044	.171538E+02 22.592104	.11987000E+03 .525062	-.49200000E+01 69.573737	.17516940E+04 5.748653
GROUP 4	52 .1036	.137863E+03 .472579	.167625E+02 -48.388934	.10576000E+03 .641185	.49980000E+02 88.400802	.21976170E+04 6.593932
PARLL REG		.44720000E+03	-.15346000E+02	.69979180E+04	-.34315742E-01	.28326953E-00
POOL	200 .0747	.13051605E+03 -.076694	.17428550E+02 27.438391	.66988000E+04 .072730	-.51376000E+03 9.501809	.70554720E+04 5.952704

SOURCE	DF	SS	MS	VR
SLOPES	3.	40.449500	13.483166	.372
DISPLACEMENTS	3.	18.678200	6.226066	.171
ERROR	192.	6956.941900	36.234072	

TABLE A66

LINEAR REGRESSION ANALYSIS T MOLITOR LARVAE STARVED AT SATURATION LEVEL IN 25°C

LINEAR REGRESSION OF AMOUNT OF WATER ABSORBED (MG) (Y) ON DRY/WET RATIO (X)

ID ID	N R	X MEAN SLOPE	Y MEAN CONST	XCSS SE OF SLOPE	XYCSP SE OF CONST	YCSS SD
GROUP 1	29 .0557	.406624E+02 .375972	.145258E+02 -.762085	.22366000E+02 1.295557	.84090000E+01 52.692760	.10167582E+04 6.127039
GROUP 2	26 .1198	.425453E+02 2.266521	.194942E+02 -76.935806	.23000000E+01 3.832585	.52130000E+01 163.062770	.82263200E+03 5.812402
GROUP 3	42 -.1601	.435790E+02 -3.780236	.190226E+02 183.761710	.26210000E+01 3.684096	-.99080000E+01 160.552020	.14604020E+04 5.964369
GROUP 4	25 -.1228	.445704E+02 -2.536808	.173520E+02 130.418550	.18610000E+01 4.274643	-.47210000E+01 190.526140	.79409740E+03 5.831403
GROUP 5	25 .0217	.454944E+02 .439098	.172980E+02 -2.678518	.23070000E+01 4.201237	.10130000E+01 191.137040	.93699250E+03 6.381180
GROUP 6	23 .1150	.464817E+02 2.346634	.174708E+02 -91.604810	.16790000E+01 4.422141	.39400000E+01 205.552300	.69874740E+03 5.730044
GROUP 7	30 -.2694	.481886E+02 -1.373294	.164533E+02 82.630549	.34442000E+02 .927549	-.47299000E+02 44.708426	.89465470E+03 5.443538
PARLL REG	.67576000E+02	-.43353000E+02	.66242842E+04	-.64154433E-00		.71303193E-00
POOL	200 .0013	.44410350E+02 .003413	.17443650E+02 17.292060	.11513400E+04 .176670	.39300000E+01 7.857450	.71153900E+04 5.994684

SOURCE	DF	SS	MS	VR
SLOPES	6.	111.241000	18.540166	.531
DISPLACEMENTS	6.	518.905200	86.484200	2.480
ERROR	186.	6485.230400	34.866830	

TABLE A67

ACKNOWLEDGEMENTS

My sincere thanks are due to Professor H.G. Andrewartha, Head of the Department of Zoology, University of Adelaide, for accepting me as a student in his department and for his guidance and encouragement while supervising this study as also for reading and criticizing this manuscript. I am also grateful to Professor T.O. Browning, Head of the Department of Entomology, Waite Agricultural Institute, University of Adelaide, for helpful discussions on certain aspects of the work.

I am grateful to my wife Joanna for reading the proof copy and also for her encouragement and patience throughout this study, and to my mother for the opportunities to continue my studies.

I would like to thank Mr. W.A.K. Morris of the Department of Mathematics, University of Adelaide, and Mr. N. Steinhouse of the C.S.I.R.O. Division of Mathematical Statistics for their advice on some of the statistical problems encountered during this study. I also wish to thank Miss B. Denton, Mrs. J. Kempster, Mr. L. Bennett and Mr. S. Harris for their assistance in preparing the graphs and Mrs. A. Briedis and Miss S. Lawson for typing the manuscript.

This investigation was conducted under a grant from the Wheat Control Board of the Republic of South Africa while on study leave from the Department of Agricultural Technical Services of the Republic of South Africa, to whom I am greatly indebted.

REFERENCES

Altmann, G. (1956). Die regulation des Wasserhaltes der Honigbiene. Insects Sociaux, 3, 33-40.

Andrewartha, H.G. (1952). Diapause in relation to the ecology of insects. Biol. Revs. Cambridge Phil. Soc., 27, 50-107.

Andrewartha, H.G. (1961). Introduction to the study of animal populations. Methuen and Co. Ltd., London.

Andrewartha, H.G., and Birch, L.C. (1948). Measurement of "environmental resistance" in the Australian plague grasshopper. Nature, 161, 447-448.

Andrewartha, H.G., and Birch, L.C. (1954). The distribution and abundance of animals. The university of Chicago Press, Chicago.

Barton-Browne, L.B. (1964). Water regulation in insects. Ann. Rev. Entomol., 9, 63-82.

Beament, J.W.L. (1945). The cuticular lipoids of insects. J. Exptl. Biol., 21, 115-31.

Beament, J.W.L. (1954). Water transport in insects. Symp. Soc. Exptl. Biol., 8, 94-117.

Beament, J.W.L. (1959). The waterproofing mechanism of arthropods. i. The effect of temperature on cuticle permeability in terrestrial insects and ticks. J. Exptl. Biol., 36, 391-422.

Beament, J.W.L. (1960). The wetting properties of insect cuticle. Nature, Lond., 186, 408-9.

Beament, J.W.L. (1961). The water relations of insect cuticle. Biol. Revs. Cambridge Phil. Soc., 36, 281-320.

Bentley, E.W. (1944). The biology and behaviour of Ptinus tectus Boie. (Coleoptera: Ptinidae) a pest of stored products. v. Humidity reactions. J. Exptl. Biol., 20, 152-158.

Berger, B. (1907). Uber die Widerstands fahigkeit der Tenebrio larven gegen Austrocknung. Arch. ges. Physiol., 118, 607-612.

Bergold, G. (1935). Die Ausbildung der Stigmen bei Coleopteran verschiedener Biotipe. Z. Morph. Oekol. Tiere, 29, 511-526.

Bonhag, P.F. (1956). Ovarian structure and vitellogenesis in insects. Ann. Rev. Entomol., 1, 137-160.

Buck, J. (1958). Possible mechanism and rationale of cyclic CO_2 retention by insects. Proc. 10th Intern. Congr. Entomol., 2, 539-42.

Buck, J. (1962). Some physical aspects of insect respiration. Ann. Rev. Entomol., 7, 27-56.

Buck, J.B. (1953). The internal environment in regulation and metamorphosis. Insect Physiology, Chap. 7, 191-208. (Roeder, K.D., Ed.) John Wiley and Sons, New York.

Bursell, E. (1957). Spiracular control of water loss in the tsetse fly. Proc. Roy. Entomol. Soc. Lond., A, 32, 21-29.

Buxton, P.A. (1923). Animal Life in Deserts. Edward Arnold and Co., London.

Buxton, P.A. (1930). Evaporation from the mealworm (Tenebrio-Coleoptera) and atmospheric humidity. Proc. Roy. Soc. London, B, 106, 560-577.

Buxton, P.A. (1932). Terrestrial insects and the humidity of the environment. Biol. Rev., 7, 275-320.

Buxton, P.A. (1933). The effect of climatic conditions upon populations of insects. Trans. Roy. Soc. Trop. Med. and Hyg., 26, 325-364.

Buxton, P.A. and Lewis, D.J. (1934). Climate and tsetse flies: laboratory studies upon Glossina submorsitans and tachinoides. Phil. Trans. B, 224, 175-240.

Browning, T.O. (1954). Water balance in the tick Ornithodorus moubata Murray, with particular reference to the influence of carbon dioxide on the uptake and loss of water. J. Exptl. Biol. 31, 331-40.

Cloudsley-Thompson, J.L. (1953). Studies in diurnal rhythms. iv. Photoperiodism and geotaxis in Tenebrio molitor L. (Coleoptera: Tenebrionidae). Proc. Roy. Ent. Soc. Lond., A, 28, 117-132.

Cloudsley-Thompson, J.L. (1962). Microclimates and the distribution of terrestrial arthropods. Ann. Rev. Entomol., 7, 199-222.

Cotton, R.T. (1950). Insect pests of stored grain and grain products. (Rev. Ed.), Burgess Pub. Co. Minneapolis, Minnesota.

Cotton, R.T., and St. George, R.A. (1929). The mealworms. Tech. Bull. U.S. Dept. Agric., 95, 1-37.

Craig, R. (1960). The physiology of excretion in the insect. Ann. Rev. Entomol., 5, 53-68.

Dick, J. (1937). Oviposition in certain Coleoptera. Ann. appl. Biol., 24, 762-796.

Dodds, S.E., and Ewer, D.W. (1952). Effect of desiccation on the humidity response of Tenebrio. Nature, London, 170, 758.

Edney, E.B. (1947). Laboratory studies on the bionomics of the rat fleas, Xenopsylla brasiliensis Baker and X. cheopsis. ii. Water relations during the cocoon period. Bull. Entomol. Res., 38, 263-80.

Edney, E.B. (1957). The water relations of terrestrial arthropods. Cambridge University Press, Cambridge.

Emden, van, F.I. (1947). Larvae of British beetles. vi. Tenebrionidae. Entomologists' Monthly Mag., 83, 154-171.

Fisher, R.A., and Yates, F. (1957). Statistical tables for biological, agricultural and medical research workers. Oliver and Boyd, London.

Fraenkel, G. and Blewett, M. (1944). The utilisation of metabolic water in insects. Bull. ent. Res., 35, 127-139.

Gunn, D.L., and Conway, C.A. (1938). The temperature and humidity relations of the cockroach. v. Humidity preference. J. Exptl. Biol., 15, 555-63.

Gunn, D.L., and Conway, C.A. (1942). The temperature and humidity relations of the cockroach. vi. Oxygen consumption. J. Exptl. Biol., 19, 124-32.

Gunn, D.L., and Pielou, D.P. (1940). The humidity behaviour of the mealworm beetle Tenebrio molitor (iii) The mechanism of the reaction. J. Exptl. Biol., 17, 306-316.

Hein, Arendsen C.A. (1920). Studies on variation in the mealworm, T. molitor. J. Genetics, 10, 227-264.

Hinton, H.E. (1964). Personal communication.

Howard, S.T. (1955). The biology of the grain beetle Tenebrio molitor with particular reference to its behaviour. Ecology, 36, 262-269.

Imms, A.D. (1957). The respiratory system. A general Textbook of Entomology, revised, 133-150. (Richards, O.W., and Davies, R.G., Eds.) Methuen and Co. Ltd., London.

Kalmus, H. (1936). Die Verwendung der Tracheenblasen der Corethra-larve als Mikrohygrometer. Z. wiss. Mikr., 53, 215-19.

Karlson, P., and Schmialek, P. (1959). Z. Naturforschg., 14b, 821.

Kennedy, C.H. (1927). Some non-nervous factors that condition the sensitivity of insects to moisture, temperature, light and odors. Ann. Ent. Soc. Amer., 20, 87-106.

Koidsumi, K. (1935). Experimentelle Studien uber die Transpiration und den Warmehaushalt bei Insekten. Mem. Fac. Sci, Agric. Taihoku. 12, 1-380.

Lafon, M., and Teissier, G. (1939). Inanition et metamorphose chez Tenebrio molitor. C.R. Soc. Biol., 2, 417-420.

Leclercq, J. (1947). Mise en evidence de reactions au gradient d'humidite chez plusiers insectes. Arch. Int. Physiol., 55, 93-116.

Leclercq, J. (1948). Influence des conditions hygrometriques sur les larves, les nymphes et les adultes de Tenebrio molitor L. Arch. Int. Physiol. 55, 366-376.

Leclercq, J. (1963). Artificial selection for weight and its consequences in Tenebrio molitor L. Nature (London), 198, 106-107.

Lees, A.D. (1943). On the behaviour of wireworms of the genus Agriotes Esch. (Coleoptera, Elateridae). J. Exptl. Biol., 20, 43-60.

Lees, A.D. (1946). The water balance in Ixodes ricinus L. and certain other species of ticks. Parasitology, 37, 1-20.

Lees, A.D. (1947). Transpiration and the structure of the epicuticle in ticks. J. Exptl. Biol. 23, 379-410.

Lees, A.D. (1955). The physiology of diapause in arthropods. University Press, Cambridge.

Locke, M. (1953). Unpublished work, after Beament, J.W.L. (1954).

Ludwig, D. (1956). Effects of temperature and parental age on the life cycle of the mealworm, Tenebrio molitor L. (Coleoptera, Tenebrionidae). Ann. Ent. Soc. Amer., 49, 12-15.

Ludwig, D., and Fiore, C. (1960). Further studies on the relationship between parental age and the life cycle of the mealworm T. molitor. Ann. Ent. Soc. Amer., 53, 595-600.

Ludwig, D., and Fiore, C. (1961). Effects of parental age on offspring from isolated pairs of the mealworm T. molitor. Ann. Ent. Soc. Amer., 54, 463-64.

Mellanby, K. (1932a). The effect of atmospheric humidity on the metabolism of the fasting mealworm (Tenebrio molitor L., Coleoptera). Proc. Roy. Soc. B, 111, 376-90.

Mellanby, K. (1932b). Effects of temperature and humidity on the metabolism of the fasting bed-bug (Cimex lectularius), Hemiptera. Parasitology, 24, 419-28.

Mellanby, K. (1934a). The site of water loss from insects. Proc. Roy. Soc. B, 116, 139-149.

Mellanby, K. (1934b). Effects of temperature and humidity on the clothes moth larva, Tinecla bisselliella Hum. (Lepidoptera). Ann. appl. Biol., 21, 476-482.

Mellanby, K. (1936). Humidity and insect metabolism. Nature, London, 138, 124-5.

Mellanby, K. (1939). The functions of insect blood. Biol. Rev., 14, 243-260.

Mellanby, K. (1942). Metabolic water and desiccation. Nature, London, 150, 21.

Mellanby, K. (1958). Water content and insect metabolism. Nature, London, 181, 1403.

Michal, K. (1931). Oszillation im Saerstoffverbrauch der Mehlwurmlarven (Tenebrio molitor). Zool. Anz., 95, 65-75.

Miller, P.L. (1960). Respiration in the desert locust. iii. Ventilation and the spiracles during flight. J. Exptl. Biol., 37, 264-78.

Miller, P.L. (1961). Spiracle control in dragon-flies. Nature, 191, 621-22.

Mordue, W. (1965). 1. Studies on oocyte production and associated histological changes in the neuro-endocrine system in T. molitor L. J. Insect. Physiol., 11, 493-503.

Mordue, W. (1965). 2. The neuro-endocrine control of oocyte development in T. molitor L. J. Insect. Physiol., 11, 505-11.

Mordue, W. (1965). 3. Neuro-endocrine factors in the control of oocyte production in T. molitor L. J. Insect. Physiol., 11, 617-629.

Moroney, M.J. (1951). Fact from figures. Penguin Books Ltd. Middlesex, England.

Nelson, V.E., and Winston, P.W. (1964). Water content and the production of metabolic water in the flour beetle T. confusum. Amer. Zool., 4, 143.

Nunez, J.A. (1956). Untersuchungen uber die Regelung des Wasserhaushaltes bei Antsotarsus cupripennis Germ. Z. Vergleich. Physiol., 38, 341-54.

Nunez, J.A. (1962). Regulation of water economy in Rhodnius prolixus. Nature, 197, 312.

Patton, R.L., and Craig, R. (1939). The rate of excretion of certain substances by the larvae of the mealworm, Tenebrio molitor L. J. Exptl. Zool., 81, 437-51.

Rerttunen, V., and Lahermaa, M. (1958). Reversal of negative phototaxis by desiccation in Tenebrio molitor L. (Col., Tenebrionidae). Ann. Ent. Fenn., 24, 69-73.

Perttunen, V., and Lahermaa, M. (1962). Humidity reactions of the larvae of Tenebrio molitor L. (Col., Tenebrionidae) and the effect of cannibalism on these reactions. Ann. Ent. Fenn., 28, 71-80.

Perttunen, V. and Lahermaa, M. (1963). The light reactions of the larvae and adults of Tenebrio molitor L. (Col., Tenebrionidae) and their interference with the humidity reactions. Ann. Ent. Fenn., 29, 83-106.

Pielou, D.P. (1940). The humidity behaviour of the mealworm beetle Tenebrio molitor. (ii) The humidity receptors. J. Exptl. Biol., 17, 295-305.

Pielou, D.P., and Crump, D.L. (1940). The humidity behaviour of the mealworm beetle Tenebrio molitor. (1) The reactions to differences in humidity. J. Exptl. Biol., 17, 286-294.

Ramsay, J.A. (1935a). The evaporation of water from the cockroach. J. Exptl. Biol., 12, 373-383.

Ramsay, J.A. (1935b). Methods of measuring the evaporation of water from animals. J. Exptl. Biol., 12, 355-372.

Richards, A.G. (1953). The insect cuticle. Insect physiology Chapt. 1-3 (Roeder, K.D., Ed.). John Wiley and Sons, Inc.

Rivnay, E (1962). Field crop pests in the near East. W. Junk, Den Haag.

Roth, L.M., and Willis, E.R. (1951). Hygroreceptors in adults of Tribolium (Coleoptera, Tenebrionidae). J. Exptl. Zool., 116, 527-70.

Roth, L.M., and Willis, E.R. (1951). Hygroreceptors in Coleoptera. J. Exptl. Zool., 117, 451-87.

Roth, L.M., and Willis, E.R. (1951). The effects of desiccation and starvation on the humidity behaviour and water balance of T. confusum and T. castaneum. J. Exptl. Zool., 118, 337-361.

Roth, L.M., and Willis, E.R. (1952). Possible hygroreceptors in Aedes aegypti (L.) and Blatella germanica L. J. Morphol., 91, 1-14.

Snedecor, G.W. (1956). Statistical methods. (5th Edition). The Iowa State University Press, Iowa.

Sweetman, H.L. (1934). Preliminary report on the physical ecology of certain Phyllophaga (Scarabaeidae). Ecology 15, 401-22.

Telfer, W.H. (1965). The mechanism and control of yolk formation. Ann. Rev. Entomol., 10, 161-184.

Thomson, R.C.K. (1938). The reactions of mosquitoes to temperature and humidity. Bull. Entomol. Res., 29, 135-140.

Tracey, K.M. (1958). Effects of parental age on the life cycle of the mealworm Tenebrio molitor L. Ann. Ent. Soc. Amer., 51, 429-432.

Umbreit, W.W., Burris, R.H., Stauffer, J.F. (1957). Manometric techniques. Burgess, Pub. Co.

Wellington, W.G. (1949). The effects of temperature and moisture upon the behaviour of the spruce budworm, Choristoneura fumiferana (Clemens) (Lepidoptera: Tortricidae). Scient. Agric., 29, 201-29.

Wigglesworth, V.B. (1932). On the function of the so-called 'rectal glands' of insects. Quart. J. micr. Sci., 75, 131-50.

Wigglesworth, V.B. (1941). The sensory physiology of the human louse, Pediculus humanus corporis, de Geer. (Anoplura), Parasitology, 33, 67-109.

Wigglesworth, V.B. (1945). Transpiration through the cuticle of insects. J. Exptl. Biol., 21, 97-114.

Wigglesworth, V.B. (1948). The structure and deposition of the cuticle in the adult mealworm Tenebrio molitor L. Quart. J. micr. Sci., 89, 197-217.

Wigglesworth, V.B. (1950). The principles of insect physiology, 4th ed. Methuen and Co., London.

Wigglesworth, V.B. (1957). The physiology of insect cuticle. Ann. Rev. Entomol., 2, 37-54.

Wigglesworth, V.B., and Gillet, J.D. (1936). The loss of water during ecdysis in Rhodnius prolixus Stahl. (Hemiptera). Proc. R. ent. Soc. Lond. A, 11, 104-7.

Willis, E.R., and Rother, L.M. (1950). Humidity reactions of Tribolium castaneum (Herbst.) J. Exptl. Zool., 115, 561-87.

Winston, P.W., and Bates, D.H. (1960). Saturated solutions for the control of humidity in biological research. Ecology, 41, 232-237.