AN ECOLOGICAL STUDY

OF

APHODIUS HOWITTI HOPE : (COLEOPTERA : SCARABAEIDAE)

IN

THE SOUTH - EAST OF SOUTH AUSTRALIA

BY

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SUMMARY

The pasture cockchafer Aphodius howitti Hope has been recognized as a pest of improved pastures in the lower South-East of South Australia for the last 25-30 years. The larvae live in vertical burrows in the soil and feed first on organic matter on the surface of the soil and then on surface herbage.

Field and laboratory experiments done to study the influence of the environment on the distribution and abundance of A. howitti in the lower South-East are discussed. These experiments confirmed that food was a major factor limiting the numbers of A. howitti in much of the area over which the species was distributed. Larvae of A. howitti survive in largest numbers in improved pastures which have been topdressed heavily with superphosphate, are dominated by subterranean clover and have a high stocking rate.

The experiments suggested further that there are three critical periods in the life-cycle of the species during which weather influences the rate of increase. The first critical period is in summer (January-February) when the adults might be exposed to dry conditions, the second critical period is in the autumn (March-May) when the young larvae might similarly be exposed to dry conditions, and the third period is in winter (June-September) when the mature larvae might be exposed to extreme wetness.

It was shown that females lay eggs in moist places, and that the survival-rate of the adult and the number of eggs laid by the female are related to the water in soil. Adults usually have a better chance of surviving and laying eggs when the pasture has a bare surface, so pastures usually infested are those which have been heavily grazed in summer, have been cut for hay or simply consist of annual grasses and clovers.

The young larvae hatching from the eggs in autumn only migrate to the surface of the soil and start feeding when the soil is saturated with rain. If drought conditions prevail after the eggs hatch the larvae die from starvation and water loss.

Mature larvae and prepupae may be exposed to excess wetness if the place in which they are living becomes water-logged. Larvae may be drowned on the poorly-drained soils or forced from their burrows and exposed to predators, of which birds are the most important. On the well-drained soils excess wetness promotes infection with the entomophagous fungus _Cordeyceps aphodii_ Mathieson.

Predators have little influence on the numbers of _A. howitti_ except when assisted by unfavourable weather, and no significant parasites other than _Cordyceps_ were found.

Crowding was shown not to affect the survival-rate of young larvae; older larvae may kill each other when searching for food but crowding is considered, on the whole, not to have much influence on the survival-rate.

Major changes in the environment of _A. howitti_ have occurred as land has been developed for more intensive agriculture in the lower South-East. These changes have resulted in a great increase in the area favourable for the survival of the species; consequently its numbers have increased. In years when A. howitti has reached peak numbers, however, not all of the suitable places have been occupied by the species, nor has all the food been eaten except in restricted areas. With food available the numbers reached have depended mainly on the weather.

The evidence suggests that a year optimal for an increase in numbers of A. howitti is one in which (a) the summer is moist (b) the autumn drought is short and (c) the winter is relatively dry. It appears that wetness of winter has never caused a catastrophic reduction in numbers on the well-drained soils and that, if weather is favourable during summer and autumn, the numbers of A. howitti can increase on well-drained soils despite a wet winter. Economic damage to pastures might be expected when there is a succession of years in which weather, on the whole, is favourable for the species to increase in numbers.

The influence of weather on the distribution and abundance of A. howitti during 1927-1956 is discussed.

It is concluded that the problem of A. howitti in pastures is largely one of grassland management and methods are discussed for keeping the numbers of A. howitti to a level at which economic damage to pastures will not occur.

I. INTRODUCTION

Aphodius howitti Hope is an indigenous insect which has been recognized as an economic pest of improved pastures in southern Australia for about 25 years. It appears to have first reached large numbers and damaged pasture in South Australia about 1925. Swan (1934) records that by 1930 the matter was of sufficient importance for the secretary of the Stockowners' Association of South Australia to draw the attention of the Australian Pastoral Research Trust Ltd. to the desirability of the problem being investigated.

The earliest observations on A.howitti were made in South Australia by Swan (1934) and our knowledge of the species in this State was later extended by Andrewartha (1945) and Madge (1952). Carne (1956) has recently published the results of an ecological study of the same species in New South Wales and the Australian Commonwealth Territory.

In 1934 A.howitti was known only from a restricted area in the south-east of South Australia but by 1952 the species had been recorded from a much wider area, including Kangaroo Island, Eyre Peninsula and Fleurieu Peninsula (Fig. 1,(Swan 1934, Madge 1952)). It is considered that the species is widely distributed within approximately the 18" isohyet and that the observed increase in its distribution is really an increase in abundance in places in which it had been very rare. The species mainly damages pastures in the lower South East, which is the largest area in the State with an assured rainfall, but is reported to damage cereals as well as pastures in other parts of the State.

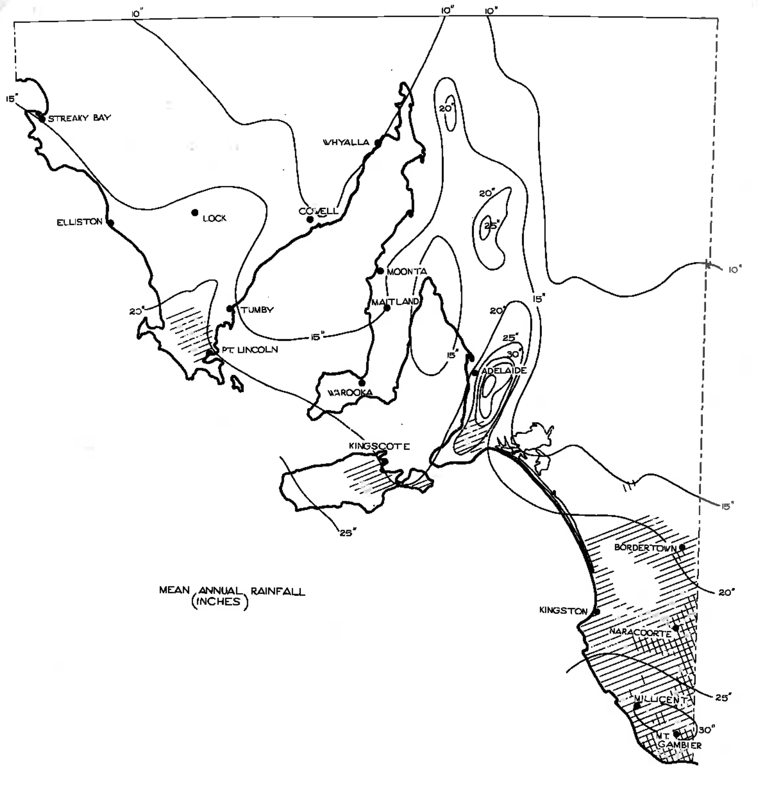

Fig. 1. The distribution of Aphodius howitti in South Australia in relation to mean annual rainfall in inches -
(a) Double hatching - after Swan 1934.
(b) Single hatching - after Madge 1952.

This study of the ecology of A. howitti was restricted to Counties Grey and Robe (Fig. 2-4) because of the extended distribution of the species in the State and its prominence as a pasture pest in the lower South East.

There is only one generation of A. howitti each year in South Australia, as in other parts of southern Australia. The adults fly, mate, and lay their eggs in the soil during the summer period January-March. Larvae hatch at varying periods in late summer to early autumn but remain in the soil until the soil is saturated by the first substantial winter rains. They then migrate to the surface of the soil, make individual vertical burrows, and begin feeding on plant debris on the surface. After a while they increase in size, extend their burrows, and commence feeding on the foliage of pasture plants. Their presence is easily detected at this stage for the ground is strewn with soil cast up from their burrows. By May, the pasture may have been seriously damaged. Larvae stop feeding about September, retreat to the bottom of their burrows, construct cells in the soil, and enter diapause. Pupation occurs about January and the adults emerge 2-3 weeks later.

It appears that the numbers of <u>A.howitti</u> in the lower South East of South Australia attained pest proportions before 1930, but that in the ensuing years unfavourable weather caused a decrease in numbers. A period of favourable weather again appears to have permitted a build up in numbers in 1940-44 but a dramatic drop in numbers occurred in 1945-47. In 1952 the largest flights of beetles since 1945 were reported and Madge (1952) records enormous numbers of eggs being laid. However, damage to pastures was not particularly extensive this year, or in 1953, but increased in 1954 and reached a peak in 1955. Since then the death-rate has been high and the numbers have decreased, with a corresponding reduction in damage to pastures.

The survival-rate of <u>A.howitti</u> and its chance of maintaining large numbers seems to depend on the interaction of weather and certain soil and pasture types. Field observations were made between 1953 and 1956, and field and laboratory experiments were conducted during this period to determine the factors which controlled the distribution and abundance of the species in South Australia.

II. GENERAL DESCRIPTION OF AREA OF STUDY

Counties Grey and Robe are principally areas of primary production. Cereals, particularly barley, were grown widely in the earlier days but in recent years more stock have been carried on pastures which have been greatly improved by the use of superphosphate, subterranean clover (*Trifolium subterraneum*) and exotic perennial grasses. The discovery of legumes for soil types unsuitable for subterranean clover and a knowledge of the role played by trace elements in pasture growth have also aided in the development of permanent pastures which have revolutionized the stock-carrying capacity of the area. The produce now consists mainly of dairy products - wool, fat lambs and beef cattle. Flax, potatoes and onions are grown around Mt. Gambier and Kalangadoo, and vines and other fruit are cultivated at Coonawarra. *Pinus radiata* is grown on much of the poorer soil.

(i) Climate

The lower South East has a Mediterranean type of climate with an effective rainfall season of 8 to 9.5 months (Trumble 1948). The period of effective rain, according to Trumble (op. cit.), is the period during which rainfall exceeds one third of the evaporation from a 36" evaporimeter. Effective rain enables the surface four inches or so of soil to be maintained above the wilting coefficient for plants. This is a useful concept but, as pointed out by Tiver and Crocker (1951), the actual growing season can be modified by a large number of factors including temperature, soil drainage and the ability of the species concerned to exploit sub-surface moisture.

The main check to the growth of pasture plants during the summer is lack of moisture because during these months evaporation exceeds precipitation. After the topsoil has dried, growth can only continue if the root system is capable of extending down to further supplies of moisture. Annual herbage plants make little or no growth during this period for they cannot exploit any great depth of soil. Perennial herbage plants, such as lucerne, Phalaris, etc. can make substantial growth after the winter rains have ceased.

Mean air temperatures for the coldest month (July) vary between 49°F and 51°F and retard, but rarely inhibit, pasture growth (Trumble 1948).

Most of the area receives more than 25 inches of rain annually and a portion of County Grey receives more than 30 inches annually; none of it receives less than 20 inches (Fig. 2). Normally, the opening seasonal rains do not commence until autumn. This is known as the "break" of the season and usually occurs in March or April, but occasionally does not occur until May.

The largest proportion of pastures are built around subterranean clover, most strains of which thrive where the period of effective rainfall is 7.5 months or more.

(ii) Physiography and soils

The chief physiographic feature of the lower south-east is a series of "stranded sand-dune" ranges which run more or less parallel to the existing coastline and mainly transverse to the prevailing winds. These ranges are usually not more than 100 feet high and not more than a few miles wide, and

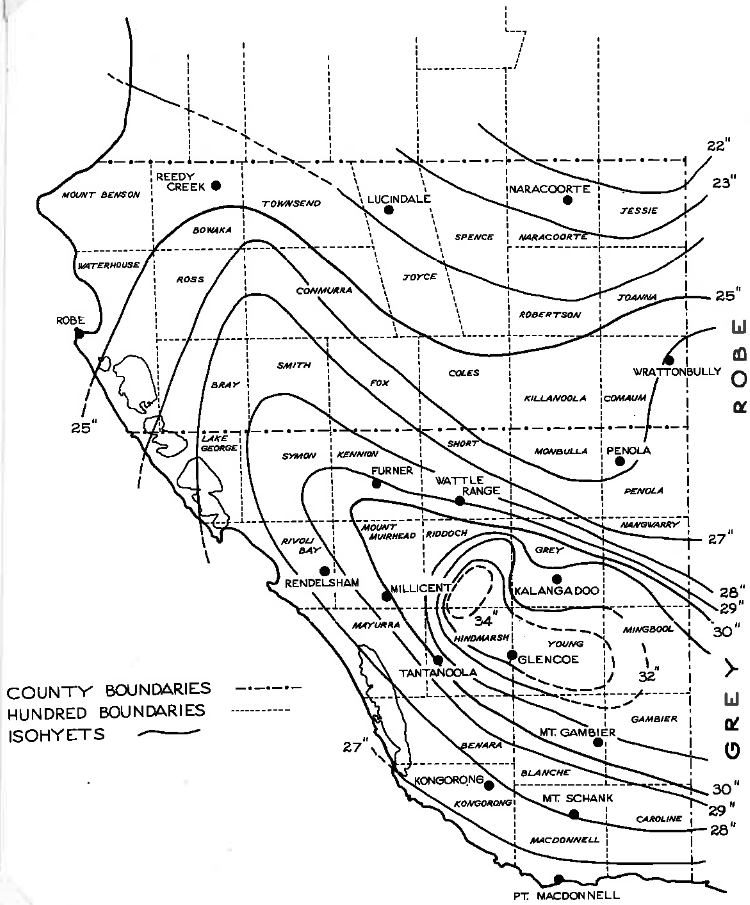

Fig. 2. Rainfall isohyets in Counties Grey and Robe. The larger towns and hundred boundaries are also shown (after Sprigg 1952).

are separated by a series of plains or flats (Fig. 3).

The land only rises gradually from the coast to the Victorian border, and there are no active surface streams. The sand-dune ranges further impede the drainage of water with the result that swamps, lagoons,

Fig. 3. The distribution of stranded coastal dunes in Counties Grey and Robe (after Sprigg 1952).

and marshes are prominent features of the flats. A relatively high winter rainfall coupled with the drainage of surface and underground water into this area from western Victoria produces extensive seasonal flooding of the land between the ranges. Artificial drainage has now made much of the country suitable for agriculture.

Much of the area is underlain by very porous and cavernous limestone, and sub-surface drainage in these limestones transports immense volumes of water to the ocean. Abnormal rains have a marked effect on the water-table, but generally the water-table is highest in September (end of winter) and lowest in April (end of summer), (Sprigg 1952).

The physiographic features have influenced the distribution of the major soil groups which consist of rendzinas, terra rossas, meadow podsols, podsolized sands, soils derived from volcanic material, and peat. The distribution of these soils is shown in Fig. 4.

The plains between the ranges are composed mainly of rendzinas - black or dark grey clays usually overlying limestone. These soils are markedly alkaline and are frequently water-logged in winter and early spring. They are never colonized by A.howitti.

Alongside the rendzinas, and flanking the ranges, occur meadow podsols which are light-textured sands or sandy loams overlying clay at a depth of 1-3 feet. They are acid in reaction and are subject to seasonal water-logging. A.howitti has severely damaged pasture on these soils in some years, particularly around Wrattonbully where there is a major occurrence of this soil on higher land along the oldest of the stranded dunes.

Terra rossa soils occur on well-drained sites and are usually developed over limestone. They are sands, sandy loams and loams and when of sufficient depth constitute the most favourable sites for the occurrence of A.howitti.

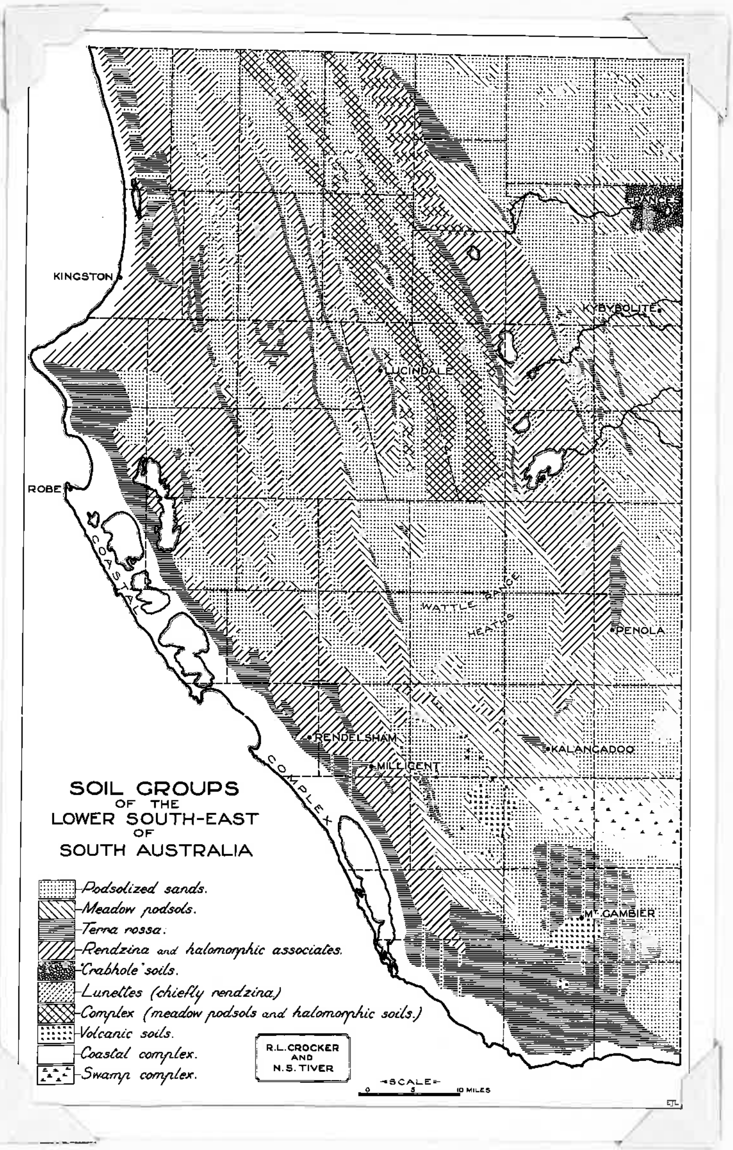

Fig. 4. Distribution of soil groups in Counties Grey and Robe. See text for a description of the soils (after Tiver and Crocker 1951).

Volcanic soils are found to a limited extent around Mt. Gambier (630 feet), Mt. Schank (500 feet) and Mt. Burr Range (up to 802 feet) all of which are the remnants of volcanic activity. The soils are chiefly loams and clay loams of high fertility on which A. howitti occurs, but is not often abundant.

The ranges are composed mainly of podsolized sands overlying limestone, but in restricted areas they may overlie volcanic material. Superficial deposits of sand may also occur in the inner-range regions where they are shallower. These sands are aeolian, are low in fertility, and do not at present support large numbers of A. howitti.

The area studied has been the subject of numerous investigations. Crocker (1944) and Tiver and Crocker (1951) studied soil and vegetational relationships, Ward (1941) has studied the problem of underground water, and the geology of the area has been studied by a number of authors of whom Sprigg (1952) is the most recent. Stephens et al. (1941) have published a detailed soil survey of part of County Grey.

III. FACTORS WHICH INFLUENCE THE SURVIVAL-RATE OF A.HOWITTI

Andrewartha (1945) concluded that weather was the most important factor affecting the abundance of A.howitti. There were, he thought, two critical stages in the life-cycle of the insect -

(a) the young larva which may suffer if the period March/April is too dry, and

(b) the older larva which may suffer during autumn, winter or spring, if a prolonged or excessively wet spell makes the soil water-logged.

During the course of this study it was confirmed that a prolonged dry spell during the early larval stage was a major factor influencing the numbers of A.howitti. First instar larvae do not feed in dry soil, and a considerable proportion of them may die of starvation when there is a drought. The relationship between the length of the drought and the ensuing percentage mortality of larvae is treated in section VI, and in section X estimates are made of the mortality of first instar larvae over a number of years.

It was also confirmed that excess water limited the numbers of older larvae surviving by

(a) drowning them, and

(b) promoting infection with the entomophagous fungus Cordyceps aphodii Mathieson.

It is clear that food, in addition, is a major factor limiting the abundance of A.howitti throughout its range. Larvae do not survive in large numbers except in pastures which have been sown with annual grasses and clovers for 3-4 years.

But even in the most favourable pastures food is sometimes in short supply locally. This is usually because a lack of rain during the period in which eggs are laid results in eggs being laid only in particular sites. Sometimes the shortage of food seems not to be influenced by the weather.

Carne (1956) has suggested that larval combat due to shortage of food is the most common cause of larval mortality. Larvae of A. howitti forage for food at night and injure each other whenever they meet, but in South Australia, at least, death due to larval combat does not occur with the frequency with which Carne implies it occurs in Canberra. This subject is discussed in section X.

Finally, it was thought that the survival-rates of diapausing larvae, adults and eggs might be affected by drought. The diapausing larvae remain immobile in the soil during the early summer months, the adults often emerge when weather is not suitable for egg-laying, and eggs could well lose water and die if laid in certain places; so all these stages were studied.

During the period of study many predators were seen to eat various stages of A. howitti. Some adults are eaten by birds; but the eggs are not exposed to predators, and the larvae, which forage by night and shelter by day in burrows in the soil, are similarly protected from their natural enemies, though not to the same extent. No specific predators are known and the total mortality caused by the recorded predators must be very small.

On the other hand, there are a number of fungal parasites of A.howitti and at least one of these (Cordyceps aphodii) is capable of killing high percentages of the population of larvae in the field. High mortalities due to fungal infections are only recorded, however, in conjunction with excess moisture. The part which Cordyceps plays in the ecology of A.howitti is discussed in section VI.

Andrewartha and Birch (1954), when discussing the concept of "environment" which would help ecologists understand and explain the observed distribution of animals in nature, concluded "The components of the environment must be defined in such a way that they can be studied individually by observation and experimentation especially their respective influences on the longevity, speed of development and fecundity of the animal. These determine the rate of increase or decrease of the population and this, in the long run, is at the root of all studies of distribution and abundance". They then proceeded to divide the environment of an animal into four components (1) weather, (2) food, (3) other animals and organisms causing disease, and (4) a place in which to live.

I have accepted this analysis of environment and my aims have been (1) to discover which components of the environment have an important influence on the birth-rate and death-rate of A.howitti, and (2) to explain the occasional outbreaks of A.howitti in terms of these components of environment.

IV. THE BEHAVIOUR OF THE ADULT

The adults lie quiescent in the cells constructed by the diapausing larvae some 6-8 inches below the surface of the soil, and emerge from their cells in large numbers during summer in response to rain. They fly only on particular nights during the "flight season" which, at Mt. Gambier, usually extends from January to the middle of April. Usually beetles fly on a number of nights following one or two peak periods of emergence from the soil. For convenience a "flight" is said to have taken place when beetles have been observed or have been reported flying on one particular night; and the period of time over which a number of flights may occur, after a peak period of emergence, has been designated a "flight period".

The incidence of larval damage depends largely on where the females lay their eggs. Local patches of pasture a few feet square often contain 400-500 first instar larvae per square foot, whilst the general body of the pasture may contain 0-20 larvae per square foot. The suggestion is that adults often aggregate in certain areas to lay their eggs. The main purpose of studying the adult was to determine the factors which control aggregation and oviposition in the field.

Much of the behaviour of the adult cannot be observed and has to be inferred from the stage of development of gonads and the presence or absence of fat body and food residues in the alimentary canal, so these criteria are discussed first.

The gonads of the males are probably mature when the insects emerge from their cells, but there is considerable variation in the stage of development of the ovaries during the female's cycle of activity, as is shown in Fig. 5 a-c. This is largely because the female can lay one batch of eggs without feeding and can lay a second smaller batch of eggs after having fed on dung.

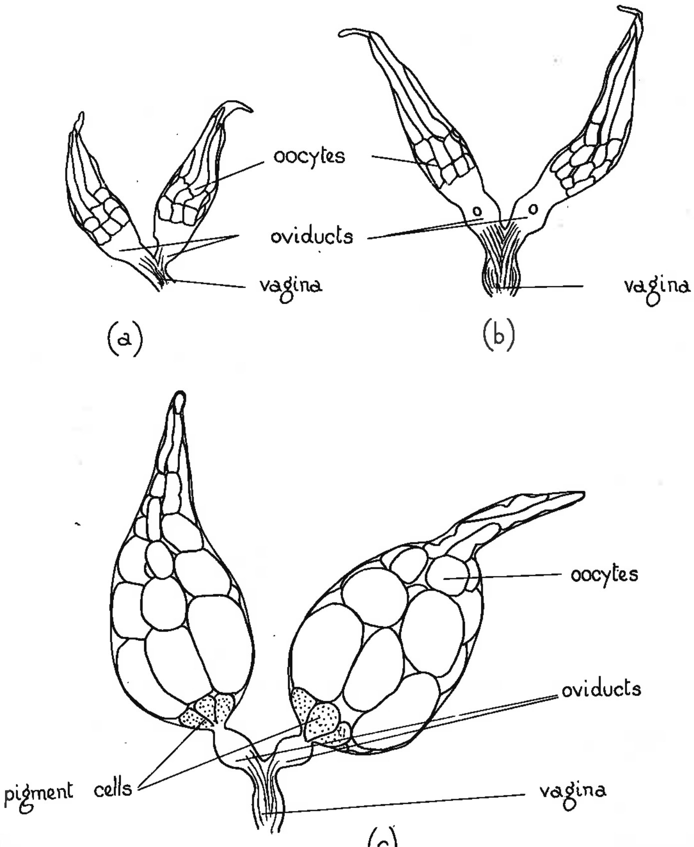

Fig. 5. Stages in the development of the ovary. (x 32).
See text for explanation.

Shortly after metamorphosis the ovaries are immature (Fig. 5a) but the female lays eggs 10-14 days later if kept in moist soil; about two days before oviposition the size of the ovaries approximates the stage shown in Fig. 5c. Laboratory and field observations suggest that, in nature, the ovaries do not develop beyond about stage (b) (Fig. 5) until the females have emerged from their cells. The ovaries then develop rapidly after the beetles emerge and eggs are laid 5-7 days later.

After the first batch of eggs has been laid the ovaries appear again like those in Fig. 5a, but the first batch of eggs is developed at the expense of the fat body (even if the beetles feed on dung), and whether or not the first batch of eggs has been laid can be determined by the presence or absence of the fat body.

(i) The influence of weather on emergence

The mass flights of adults which have often been observed by earlier writers (i.e. Andrewartha 1945) suggested that adults emerged from the soil in response to a particular stimulus; this was thought to be high humidity (Andrewartha, private communication). In March 1953 initial experiments were carried out at Wrattonbully to test this hypothesis by watering plots with the equivalent of 10 points of rain. More beetles emerged in the wet plots than in the dry plots, but no general emergence of beetles occurred. It was therefore considered, as an alternative, that rain might affect the emergence of A. howitti as it affects the emergence of adults of Lepidiota caudata Blackburn (Smith 1936).

Unfortunately no further experiments were possible in this year, but before the next flight season Carne (private communication) revealed that the analysis of four years' flights of A. howitti at Canberra, A.C.T., in relation to meteorological data, had indicated a significant correlation between flight and rainfall above a certain number of points.

A total lack of "effective" rain in January, 1955 at Mt. Gambier, and the build up of a large number of beetles awaiting the stimulus to emerge, presented ideal conditions for testing experimentally whether an increase in the water content of the soil did stimulate emergence of the adults in South Australia.

A strip of pasture 12 feet by 2 feet, known to contain large numbers of beetles, was encircled with lengths of metal sheeting dug into the soil and watered on January 25th, 1955, with 190 gallons of water (equivalent to about 1½ inches of rain). The area within the strip and two feet all around it were then covered with fly-wire. There were no beetles on the surface of the soil that night, but sampling on the next day (January 26th, 1955) revealed that a number of adults had left their cells and were within an inch or two of the surface. Nevertheless, the beetles did not appear on the surface until the following night (January 27th, 1955). A small section of the strip was uncovered on this night and beetles took off in flight. Others, under the fly-wire, crawled on the surface or copulated. No beetles were caught in a light trap this night and diligent searching confirmed that no beetles had emerged from the rest of the paddock. There is thus no doubt that soaking the soil with water stimulated the emergence of the adults in the experimental strip.

A second strip was similarly watered on January 28th, 1955, to study the behaviour of the adults after emergence. Since rain threatened to fall on January 29th, 1955, another strip of the same size was covered to keep the soil dry, and comparisons of emergence were made between the two. Beetles emerged from the wet strip only.

Observation has since confirmed that the adults appear "en masse" on the surface of the soil at dusk, two or three days after rain has fallen. It appears that the number of points of rain required in summer to stimulate emergence of adults varies with the soil type, its original water content, the topography of the area etc. These are factors which control the penetration of rain into soil. In addition there is a suggestion that the threshold of response may be lowered if rain does not fall for some time. Laboratory observations have even suggested that adults may emerge if the stimulus is withheld long enough; it is probable, however, that such emergence rarely, if ever, occurs in the field.

(ii) Behaviour before aggregation and oviposition

(a) Mating

I have never seen A. howitti copulating in flight but I have seen copulating pairs on the surface of the soil and on a variety of objects above the soil surface such as grass stalks, dung pads etc. The beetles may also copulate in the soil where they shelter during the day.

If weather is not suitable for flight on any night after emergence from their cells, the adults crawl on the surface of the soil, and appear to mate only when males and females meet by chance. Several copulating pairs may be observed if a number of adults have emerged in close proximity to each other. If, however, emergence is scattered over a large area many beetles may be on the surface but copulating pairs are seen only infrequently.

Males are more active than females and, particularly after flight, will mount any object about the size, shape or colour of a female, extrude the genitalia and attempt to copulate.

It appears that chemical, tactile and optical stimuli control mating behaviour of the adults but that these stimuli are only effective at a range of 0-3 inches.

The lack of attraction between the sexes over greater distances is compensated by the tendency of the adults to aggregate in particular places i.e. around trees, in soil which has the optimum water content for oviposition, dung pads, etc.

Beetles will fly quite readily before they have mated. Of 60 females caught in flight on February 11th, 1955 and kept singly in tubes of moist soil for 1 week, only two laid batches of fertile eggs. Another 40 females, which were caught in flight on the same night and were kept with males for one day, laid 28 batches of fertile eggs. It is unlikely that the viability of sperm is markedly affected by one day, so it is probable that the majority of the females did not mate before flying. But if bad weather delays flight for a number of days after the beetles have emerged from the soil, increasing numbers of beetles copulate before flying. Beetles caught in other flights had mated and laid their first batch of eggs before flying.

(b) Feeding

Swan (1934) first noticed that the mouthparts of the adults are only slightly sclerotized and thus restrict the adults to a liquid or a semi-liquid diet.

Adults aggregate in pads of fresh cow and horse manure in the field but sheep droppings, even when fresh, attract comparatively few. Cow pads, when present in any area, are the main source of food. Horse manure dries out rapidly, as do sheep pellets, but cow pads commonly form a dry crust on the surface whilst remaining semi-fluid underneath. They consequently not only provide food but also a moist environment in which the adults, if necessary, can survive for the few days needed for further ovarion development. A few likewise can survive in sheep pellets if the latter adhere to each other.

The number of eggs which females can produce after feeding on dung is highly variable. A number of large females which laid a mean number of 37.5 eggs in the first batch in February 1955 laid a second batch of 22.1 eggs; and a number of females caught in flight in March 1956 after having laid their first batches of eggs laid a mean number of 16.7 eggs. It seems that the number of eggs laid in the second batch is related to the size of the female; small females, for instance, often do not lay more than 2-3 eggs. In addition, females die if they have been starved for 5-6 days after laying their first batch of eggs, and it is probable that the number of eggs a female subsequently lays is influenced by the time it takes to find food.

(c) <u>Flight</u>

Adults fly on the first night on which the weather is suitable after emergence from the soil. If they emerge in an area which has a bare surface, they spread their wings and rise from the ground; but if there is pasture stubble in the vicinity they usually crawl to the tips of pieces of straw before launching into the air. When the air is still they rise to a height of about 10 feet, level off, and fly in all directions until they are lost from sight. If a wind is blowing they take off with the wind at an angle to the ground and continue flying downwind.

Observations on behaviour in flight can rarely be made for more than a few minutes after emergence from the soil as flight usually commences about 15 minutes after sunset. On the night of February 4th, 1955, however, a fairly large flight occurred and beetles were flying until 10 minutes before sunrise the following morning. Between dawn and sunrise a gentle wind was blowing from the north and about 80 per cent. of the beetles in flight were flying south with the wind. The remainder were flying south-south-west to south-west. Most of the beetles in flight remained at about the same level and flew for 400 or 500 yards before being lost from sight. There were, however, a number of bare areas in the paddock (which was otherwise covered with stubble) and occasionally adults, when flying over a bare area, would drop to a height of 8-12 inches from the ground in a spiralling descent. Sometimes the beetles would circle the bare area several times, then fly up to about 10 feet again in a spiralling ascent and continue flying in a straight line in the same direction as before; sometimes they would alight on the ground near some straw and burrow under it into the soil. Adults did not drop down to inspect every bare area in their line of flight and they did not make these circling movements over stubble.

These observations, meagre as they are, support the general hypothesis based on other evidence that beetles are attracted to bare areas in which they subsequently lay eggs.

The incidence of flight has mostly been recorded with a light trap. Some flights have been large ones and beetles have been seen flying in the paddocks before dark, but many flights have been small and would not have been recorded without the aid of the light trap. Adults are attracted to lights, particularly blue ones, but do not start flying towards a light source until after dark. A mercury-vapour light which was worked off a vibrator and a 6-volt battery was used for recording the incidence of flight and the duration of flight after dark. It was noticed that many beetles landed a few feet from the trap and then flew into it, so a large white sheet was usually placed under the trap to give some idea of the numbers flying in from the various directions. Flight was essentially at random when it was calm, or when a very gentle breeze was blowing, and beetles flew into the trap from all points of the compass. The majority of insects flew downwind to the trap, however, when the wind speed increased. Thus the orientation of flight to wind direction was similar to that observed before it was dark.

Swarming flights <u>against</u> the wind have been observed in Canberra (Carne 1956). These flights, which usually ceased before it was dark, were to dung pads, and the females participating in them had laid their first batches of eggs.(Carne op.cit.). Such flights have not been observed in South Australia but it is suspected that they do occur because (a) dung pads which swarm with beetles may often be found only a few feet from seemingly identical pads which are totally devoid of beetles, and (b) large numbers of beetles are never observed in dung pads unless weather has been suitable for flight at least once since the mass emergence of the adults from their cells. The females which participate in these flights in South Australia are expected to be those which have laid their first batch of eggs.

(1) The influence of weather on flight

Carne (1956) noted the time when flight began on a number of occasions in a flight season at Canberra and found a close correlation between sunset time and time of flight. Flight began 14-15 minutes after sunset when the light intensity was within the range of 5-2 foot-lamberts with a mean value of 3.5 foot-lamberts. This enabled him to calculate the time when flight would have commenced on every day during the flight season for each of four years if weather had been suitable. He consulted the meteorological records for these periods of time and noted the weather prevailing at the theoretical time of flight each day. These data enabled him to find a significant correlation between flight and rainfall above a certain number of points. He was also able to deduce that flight was inhibited by wind speeds above about 10 m.p.h., that it was not affected by humidity or pressure, and that it occurred only when the temperature at the critical time was above a threshold which appeared to be about 60°F. Air temperature appeared to be of greater significance than soil temperature at a depth of 3 inches in determining the level of flight activity, (Carne, op. cit.).

All these measurements were made according to standard meteorological practice and, whilst it may be argued that they may differ from those of the micro-environment of the adult, their use can be justified because (a) in this instance they are adequate for ecological purposes, and (b) they are the only record of any sort available.

A number of scattered observations on the time of initiation of flight of A. howitti in South Australia in relation to sunset time agree well with those of Carne (1956). The time of initiation of flight has therefore been taken as 15 minutes after sunset, and the meteorological data at Mt. Gambier Aerodrome at the required time each day have been plotted to determine the influence of weather on the occurrence of flights in South Australia (Fig. 6, a-c).

As Mt. Gambier is 300 miles from Adelaide a complete record of the occurrence of flights has not been made but those flights which have been observed, or have been recorded by responsible persons, are plotted in Fig. 6, a-c. Minor flights which have not been recorded may have occurred, but those recorded mark at least the main flight periods. Due to the paucity of the data statistical analysis is not attempted, but the plotted data do bring out clearly the correlation of the flight periods with rainfall above a certain number of points.

A flight period follows a period of peak emergence of beetles from the soil, may extend up to 3 weeks, and is marked by the occurrence of flights whenever the weather is suitable. There may thus be a number of flights on successive nights or at irregular intervals during the flight period. The first flight usually involves the largest number of beetles and the numbers participating in successive flights gradually decrease as the beetles die. Eventually, despite suitable weather, no beetles can be observed in flight and this situation prevails until a further shower of rain stimulates a further number of beetles to emerge and ushers in another flight period. A number of flights involving large numbers of beetles may then occur again. This is particularly well shown by Carne's (1956) light trap data and may also be deduced from Fig. 6, a-c.

The variation in the number and spacing of flight periods in South Australia depends on the distribution of rain, but generally two or three flight periods can be distinguished, as in 1954 and 1956. The bulk of the population of adults appears to be ready to emerge from the soil about the second week of February every year, but the proportion of adults participating in flights during the various flight periods also depends on the quantity of rain and the time at which it falls. This is illustrated in Fig. 6 by recording visual estimates of the numbers of beetles involved in the observed and recorded flights which are classified as small, large and very large. The data for 1954 and 1956 indicate that when rain stimulates emergence in the third or fourth week of January, a number of adults are not ready to emerge, and, if February is dry, large flights can occur in March. If, on the other hand, a substantial quantity of rain falls in the second and third week of February only one big flight period in the season occurs as in 1955 (Fig. 6b).

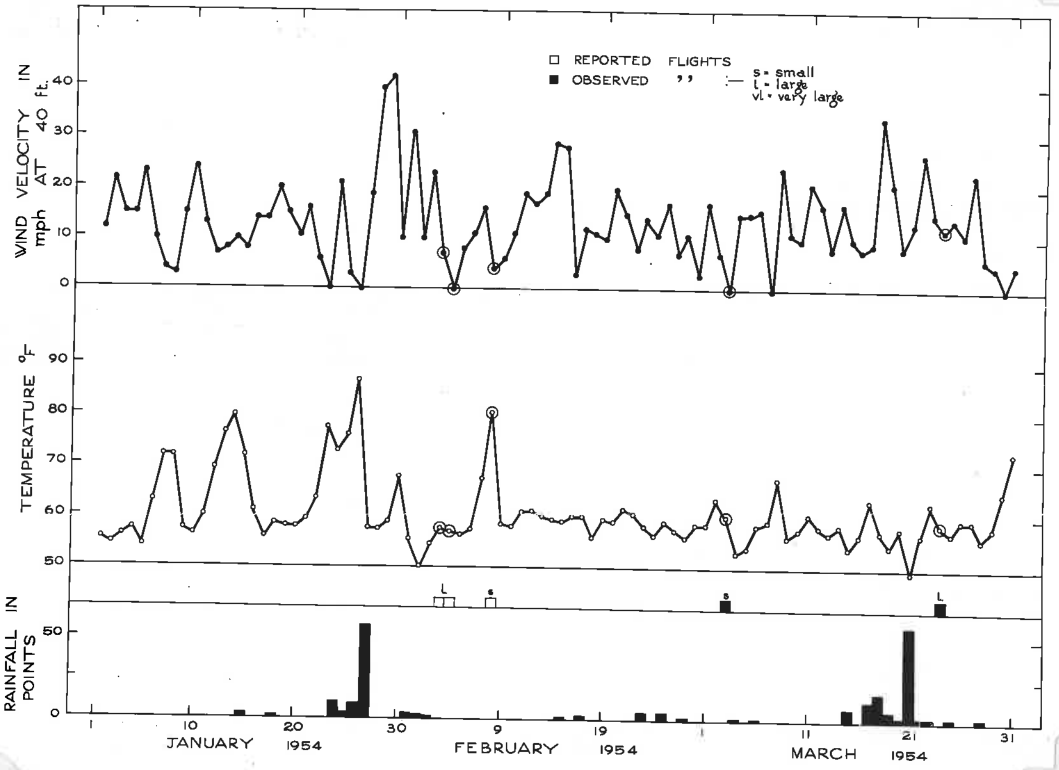

Fig. 6 (a). The occurrence of flights at Mt. Gambier in 1954 in relation to weather.
The temperatures and wind speed recordings at the expected times of flight are marked by circles for the nights on which flight occurred.
See text for further explanation.

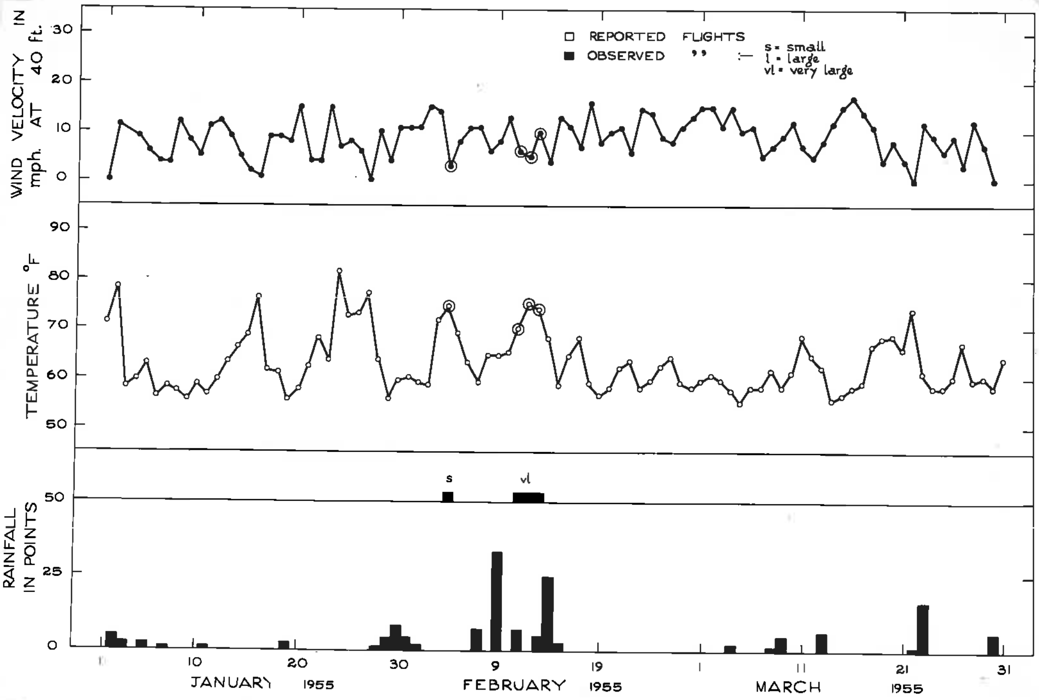

Fig. 6 (b). The occurrence of flights at Mt. Gambier in 1955
in relation to weather.
See Fig. 6(a) and text for further explanation.

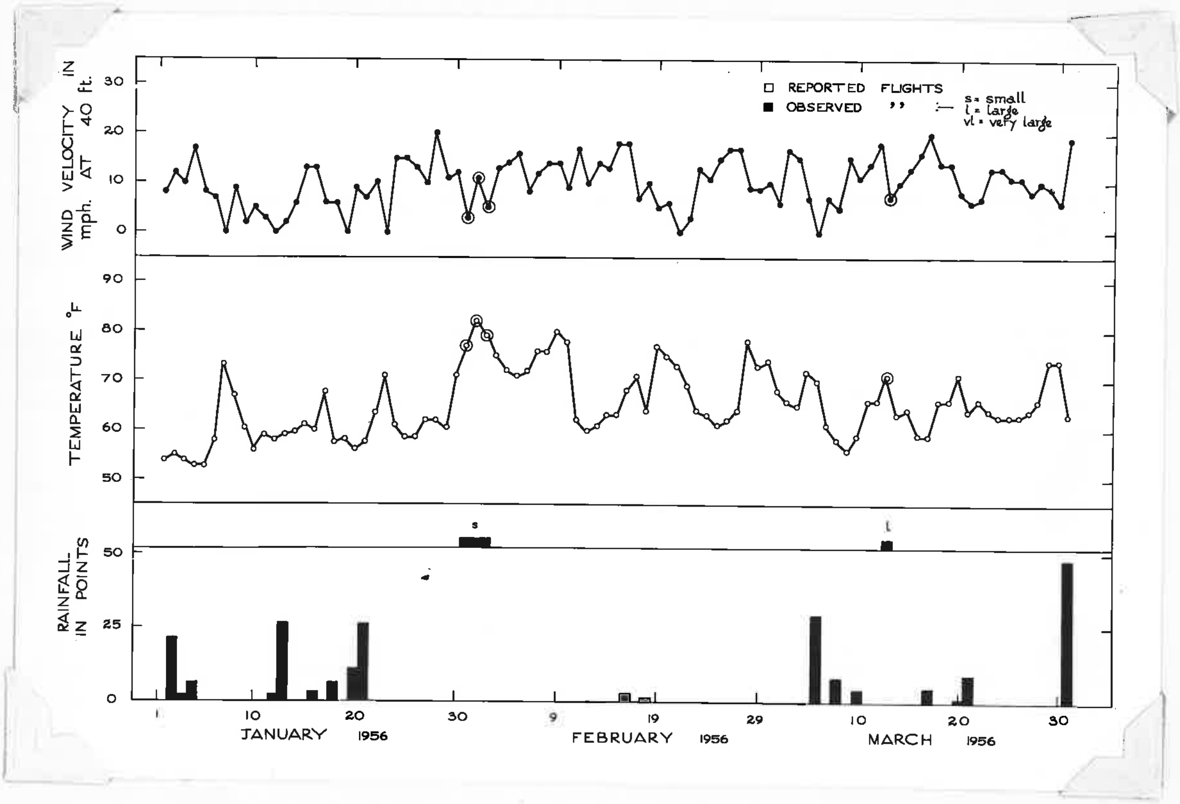

Fig. 6 (c). The occurrence of flights at Mt. Gambier in 1956 in relation to weather.
See Fig. 6(a) and text for further explanation.

It follows that other components of the weather such as temperature, wind speed etc. only influence the incidence of flights during the flight periods, i.e. after beetles have emerged from their cells. Adults generally emerge from the soil some 2-3 days after rain has fallen, but may first fly some days later. In South Australia pressure and humidity have been observed to have no effect on the occurrence of flight of A.howitti, but low temperatures and high wind velocities may inhibit flight for many days (Fig. 6, a-c).

The orientation of adults with the wind has been observed to increase as the wind velocity at 40 feet increases but flight is inhibited when the wind velocity increases above 12-13 miles per hour. This corresponds to a surface speed of 6-7 miles per hour. Similarly, beetles do not fly unless the air temperature at the time of initiation of flight is above about 58°F. These figures agree with those of Carne (1956).

Apart from totally inhibiting flight on any one day, temperature and wind velocity may inhibit flight if the critical values are reached any time after flight commences. Thus flight is often cut short by low temperature; the flights reported on the 3rd and 4th of February, 1954 were reported to have ceased some 20-30 minutes after they commenced, and the flight on 24th March was similarly of short duration - about one hour. On the other hand, beetles flew all night on 4th February, 1955 when the temperature did not drop below 66°F, though they were not flying in as large numbers at dawn as at dusk the previous evening. The inhibition of flight by high wind velocity may cause adults to settle and may account for some aspects of the distribution of the larvae (IV, (iv), (g):).

It is suspected that humidity also influences the duration of flight or the numbers of beetles participating in flight, but more evidence is required to distinguish its effect from that of temperature.

(2) <u>The relation between weather and oviposition in situ</u>

It has previously been thought that beetles laid a batch of eggs near their cells before flying. This conclusion was based partly on dissections of females caught at traps but mainly on the observation that areas of *A. howitti* damage tended to be recolonized year after year.

However, females taken from flights at Wrattonbully and Mt. Gambier during 1953 and 1954 were found to contain entire fat bodies indicating that they had not yet laid any eggs. Since females were known to lay their eggs 5-8 days after emergence it was suspected that they laid their eggs before flying only if weather was not suitable for flight for a sufficient length of time after emergence; on the other hand, females which had not laid their first batch of eggs would be caught in flight if flight was possible soon after emergence.

To test this hypothesis it was decided to water plots at Mt. Gambier at successive intervals during the flight season in 1955. Theoretically, the water would stimulate emergence and it was hoped that the weather after emergence at these different times would be sufficiently diverse to prove the hypothesis one way or the other.

As recorded in section IV, (i) water was applied to a strip of pasture on January 15th, 1955 and the beetles first emerged to the surface on January 27th, 1955. Weather was ideal for flight on this night and a number of beetles flew from the plot soon after they came to the surface.

Many females were caught in a net as they flew and were found not to have laid their first brood of eggs.

Another area was watered on January 28th, 1955 and adults emerged to the surface on the night of January 30th, 1955. None flew, however, on this evening or on any night before February 4th, 1955, low temperature and/or high wind speeds successively inhibiting flight each night. This plot, unfortunately, did not have many beetles and no female was seen flying from the plot on February 4th, 1955 when a general flight from the paddock occurred. Sampling the next day did reveal, however, that some of the females had laid eggs. So it is probable that if any females from this plot had been caught in flight the previous evening they would have been found to contain immature ovaries and no fat body.

The general flight from the paddock which occurred on February 4th, 1955 followed 16 points of rain which fell between January 29th and 31st (see Fig. 6, b). The adults first appeared on the surface on February 3rd 1955, but flight was inhibited by high wind velocity. Females which were on the surface this night and in flight the following night had not laid their first batch of eggs.

Similarly, 33 points of rain fell on February 9th and stimulated the emergence of masses of beetles which first appeared on the surface on the 11th and flew the same night (Fig. 6,b). By the following night, February 12th, 1955, the bulk of the beetles had emerged from the soil and the flight of beetles which occurred was probably the biggest ever recorded in the south-east. Beetles flew again on the 13th, but in much smaller numbers. None of the females in these flights had laid eggs before flying.

It is of interest to note here that weather was ideal for flight on February 14th, but few beetles were flying.

On the other hand, a flight of beetles took place on March 13th, 1956, seven days after 28 points of rain had fallen on the 6th. The adults first appeared above the soil on March 9th, but were inhibited from flying on 9th and 10th by a combination of low temperature and high wind velocity, and on the 11th and 12th by high wind velocity (Fig. 6,c). All the females caught at a light trap on the 13th had laid eggs before flying.

It can be concluded that the occurrence of oviposition in situ in South Australia depends on the number of days elapsing between the time of emergence of the adults and the night when flight is first possible. The females do not lay eggs before flying if they fly 4-5 days after rain has fallen.

(d) Hypothesis of adult behaviour

The females caught in light traps in South Australia have either

(1) had ovaries in the stages of development shown by Fig. 5a and 5b and a considerable amount of fat body, or

(2) had ovaries in the stage of development depicted by Fig. 5a and no fat body.

It is clear that these females had or had not laid their first batch of eggs. The feature they had in common was that they had never fed on dung.

Samples of females collected from the surface of the soil on nights when flight was inhibited and net samples of females in flight have also consisted mainly of the two types described above. Occasionally, females which have fed on dung have been recorded in such samples.

Males and females which have fed on dung seem not to be attracted to lights. Females with ovaries at the developmental stage depicted by Fig. 5c also apparently do not fly or visit the surface of the soil and probably stay in the soil until they have laid their eggs. This probably explains why very few beetles flew on February 14th, 1955.

The sequence of events leading up to oviposition can therefore be summarised as follows.

Rain stimulates the emergence of adults from their cells to the surface of the soil, where they appear 2-3 days later. Mating and oviposition may take place before flight, but are not prerequisites for flight, which occurs on the first night on which weather is suitable after the beetles have emerged.

The ovaries mature rapidly after emergence and, if flight is inhibited for more than 3-4 nights after the adults appear on the surface of the soil, the females lay their eggs close to the place from which the adults emerged. If, however, flight is possible soon after the adults appear on the surface, the beetles fly and the females lay their eggs elsewhere.

After having laid their first batch of eggs, but before having fed on dung, females fly down wind to a light trap. They must at some time, however, fly up wind to dung pads; it would seem that the physiological stimulus which controls this change in orientation to wind, and the subsequent behaviour of the adults, operates some time after the first batches of eggs are laid or after the initial flight. Similarly males have been observed flying downwind in South Australia, but must later fly upwind to dung pads.

The behaviour of the adult after it has found dung has been adequately documented by Carne (1956). The eggs which females develop, after feeding on dung, are not always laid near the feeding sites, because the females, when gravid, apparently change their responses to stimuli again and, like females which are ready to lay their first brood of eggs, tend to aggregate in particular areas in which they subsequently lay their eggs. The aggregation of the adult in these particular areas is treated in more detail in the following section.

Martyn (1950) observed that adults of A.pseudotasmaniae flew with the wind during flight and that the females either had some mature eggs or immature ovaries. These observations agree with those on A.howitti in South Australia and it seems that the behaviour of the two species is very similar. Both sets of observations are, however, at variance with those of Carne (1956) who records A.howitti flying upwind in swarms to dung pads but not ever flying downwind. Carne's data appears to diverge even more from those obtained in South Australia for he goes on to describe the flying population as consisting of (1) unfed or partly fed individuals which are attracted to dung and mostly cease flying after dark, and (2) fully-fed gravid females, searching for oviposition sites, which fly for a longer period and constitute the bulk of the female population attracted to light (Carne op.cit.).

These observations suggest either that females always lay their first batch of eggs before flight, or that females which have not laid their first batch of eggs are not attracted to lights or to dung. Carne (1956) does record, however, "under more favourable conditions most of the females fly before oviposition", and (private communication) "females in the pre-primary oviposition stage are only found in net samples of flying insects.". So females before laying their first batch of eggs are not attracted to lights or to dung. But this raises a contradiction; if Carne's account is true one would not expect the beetles to reach the light trap in less than 8-12 days after rain had brought them to the surface because, in order to become "a fully fed gravid female" a beetle must have time to lay the first batch of eggs, and then to find dung and feed on it. This would take at least 8-12 days. Reference to Carne's (1956) light-trap data reveals, however, that large numbers of beetles are often caught in a light-trap very shortly after rain has presumably stimulated their emergence (see particularly Carne 1956 Fig. 2).

It is possible that real differences in behaviour exist between adults of <u>A.howitti</u> in South Australia and in Canberra, but such differences cannot be considered until the contradiction in Carne's data is resolved.

(iii) <u>The aggregation of adults and oviposition</u>

It is difficult to study the behaviour of <u>A.howitti</u> when it is laying eggs because the females caught in flight have often laid their first batch of eggs and they are not ready to lay eggs again until they have eaten dung.

The flights of beetles which occurred in February, 1955, were therefore of especial interest because the females participating in the flights had not laid their first batch of eggs and were well suited for experiments to discover how Aphodius chooses a place to lay its eggs.

About 6,000 beetles were caught at lights on 4th, 11th and 12th February, 1955 and were kept in cages until required for experimental purposes. The period of time after flight during which the experiments could be conducted was short, because the females were expected to lay eggs in 4-5 days. The shortage of time and the labour involved in the preparation of the experiments restricted the experiments to a series of simple ones which were conducted, for the most part, in succession as time and material allowed.

A . Laboratory experiments

(a) The effect of water in the soil

Andrewartha (private communication) suggested that females of Aphodius howitti tended to lay their eggs in soil which was moist. Davidson (1932a) had shown that the number of eggs laid by Smynthurus viridus was influenced by the water content of the soil and Travis (1939) obtained similar results with Phyllophaga lanceolata. Evans (1943, 1944) had shown, however, that the rate of loss of water from wireworms in soils of various water contents was a function of the force with which the water was held rather than the amount of water present in the soil; the energy with which the soil held water was suitably expressed by Schofield's (1935) pF scale. Evans and McGuild (1948) then demonstrated that the rate of production and size of cocoons of earthworms could similarly be correlated with the pF value of the soil rather than its water content. As females of Aphodius howitti lay eggs in a variety of soils it was considered possible that if they did react to water in the soil it would be to the "available" water in the soil, and that this "available" water could be conveniently expressed in terms of the energy with which the water was held, i.e. on Schofield's pF scale.

The influence of the water content of soil on the ovipositional behaviour of A. howitti was therefore investigated with two soils - a sand denoted by a soil survey map as Wandilo sand and a light clay loam found in the vicinity of Mt. Schanck.

Quantities of dry sand and clay loam were obtained from the field on January 29th, 1955. Each soil type was kept in a separate container fitted with a tight lid and kept in a cool place to preserve as far as possible its water content, which was determined by weighing samples and drying them to constant weight. A sample of each soil type was forwarded to Dr. Quirk of the C.S.I.R.O. Division of Soils, who kindly worked out the pF-water content curves. At the time of the experiments these curves were not available, so the experiments were conducted by adding convenient quantities of water to standard weights of dry loam or sand. The results were expressed later in terms of water contents and their equivalent pF values.

The pF-water content curves for the two soils are reproduced in Fig. 7. The pF values used in the results have not been derived without error because the points on the pF curve for loam are not as numerous as could be desired, and Dr. Quirk pointed out that, as the loam was of

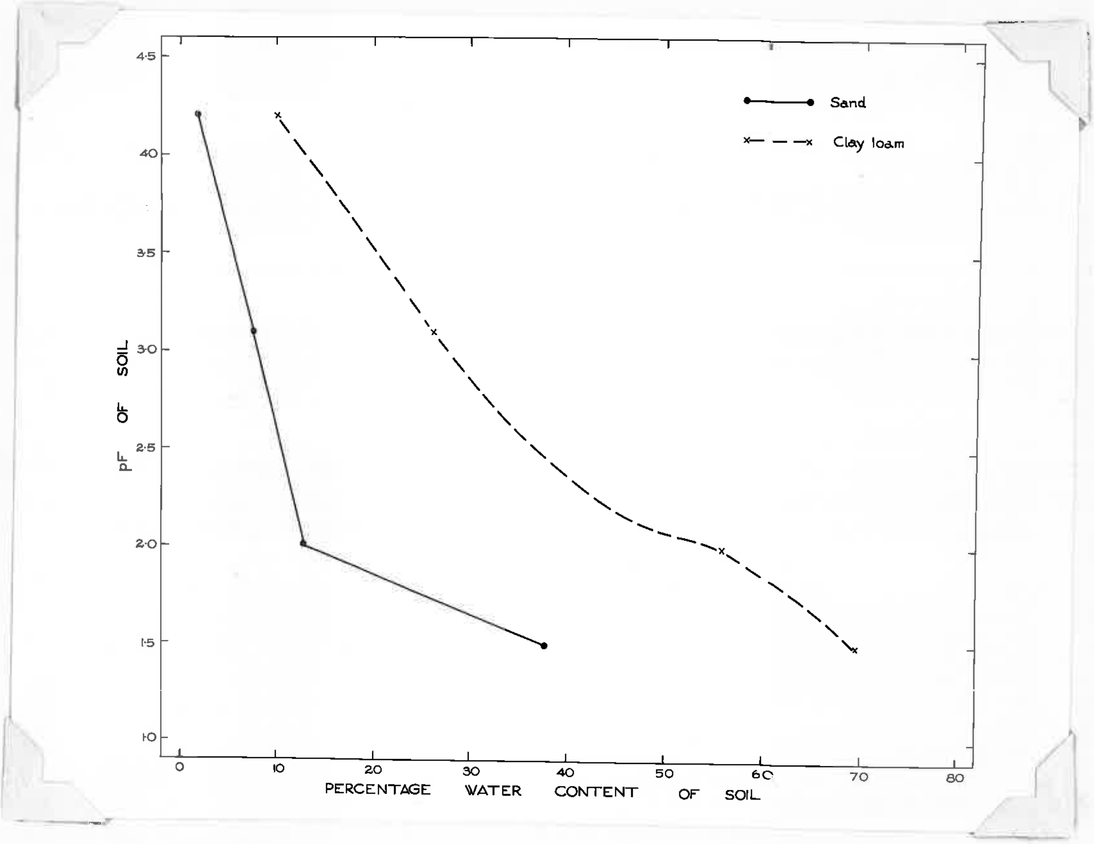

Fig. 7. The pF-water content curves for Wandilo sand and Mt. Schanck loam.

disturbed structure, the value at pF 2.0 for this soil would not be related to the field capacity in situ. In addition the curves are "drying" curves, and the pF values relating to the water contents employed should be derived from "wetting" curves. Further it was impossible to prevent water loss completely from the soils. Nevertheless it is considered that the values derived from the pF-water-content curves are sufficiently accurate for a comparison to be made of the "availability" of water in the two soils.

(1) The effect of water in the soil on the fecundity of females and viability of adults

A series of plastic boxes were filled with soil of various water contents, two boxes (replicates) being filled with soil of each water content. Adults were caught at a light trap on the same night and 20 copulating pairs were placed in each box. Plastic coverings were then placed on all the boxes and they were stored in a cool room in which the mean temperature was about 60°F and the daily fluctuation in temperature was small.

Due to an initial shortage of beetles a number of water contents of clay loam were prepared and seeded with adults on February 4th, 1955, and a number of water contents of sand were similarly treated on February 11th, 1955. After nine days the soil in each box was carefully sieved and the numbers of egg batches and the numbers of eggs in each batch were recorded. The number of egg batches and the total number of eggs laid in each box are recorded in Table 1a and Table 1b. After examination on the ninth day the beetles alive in the sand were replaced in their boxes and the boxes were re-examined on the fourteenth day. The number of egg batches and the total number of eggs laid up to the fourteenth day (in the sand) are recorded in Table 1c. It is clear that the beetles laid many more eggs at the moderate pF values than at the extremes. The values for χ^2 reflect the departures from the hypothesis that the eggs were distributed equally through all the treatments.

The mean number of eggs laid per female were calculated for the treatments and are included in Table 1, a-c. They are plotted against the

Table 1

The numbers of eggs laid by *Aphodius* females
in relation to water in soil

(a) Mt. Schanck terra rossa (clay loam) after 9 days

Water added (ccs.) to 1 lb of dry soil	% water content (calculated)	pF	No. of egg batches		Total No. of eggs		Mean No. of egg batches	Mean total No. of eggs	Mean No. of eggs per female
			A	B	A	B			
0	8.2	4.30	0	0	0	0	0	0	0
25	13.0	4.00	6	3	162	114	4.5	138.0	6.9
50	17.3	3.70	6	5	185	168	5.5	176.5	8.8
100	24.8	3.20	12	14	350	454	13.0	402.0	20.1
150	31.0	2.80	13	12	394	384	12.5	389.0	19.5
200	36.3	2.50	10	12	309	357	11.0	333.0	16.7

Comparing total number of eggs laid at different pF values:-

	χ^2	p
3.70 and 3.20	175.8	$<<0.1\%$
3.20 and 2.80	0.4	N.S.
2.80 and 2.50	8.7	$<1\%$
3.2 and 2.5	13.0	$<0.1\%$

(b) Wandilo sand - after 9 days

0	1.84	4.20	0	0	0	0	0	0	0
6	2.70	4.00	5	7	162	220	6	191.0	9.6
12	3.54	3.85	9	10	265	282	9.5	273.5	13.7
25	5.32	3.50	13	14	391	421	13.5	406.0	20.3
50	8.55	2.80	13	14	405	518	13.5	461.5	23.1
75	11.58	2.15	9	11	299	355	10.0	327.0	16.4
100	14.40	1.95	0	0	0	0	0	0	0

Comparing total number of eggs laid at different pF values:-

	χ^2	p
3.85 and 3.5	51.7	$<0.1\%$
3.5 and 2.8	7.10	$< 1\%$
2.8 and 2.15	20.5	$<0.1\%$

(c) Wandilo sand - after 14 days

0	1.84	4.20	0	0	0	0	0	0	0
6	2.70	4.00	5	7	162	220	6.0	191.0	9.6
12	3.54	3.85	9	10	265	282	9.5	273.5	13.7
25	5.32	3.50	13	14	391	421	13.5	406.0	20.3
50	8.55	2.80	19	17	619	597	18.0	608.0	30.4
75	11.58	2.15	10	11	331	355	10.5	343.0	17.2
100	14.40	1.95	1	2	41	77	1.5	59.0	3.0

Comparing total number of eggs laid at different pF values:-

	χ^2	p
3.5 and 2.8	80.5	$<<0.1\%$
2.8 and 2.15	147.7	$<<0.1\%$

percentage water contents of the two soils in Fig. 8a and against the equivalent pF values in Fig. 8b. The numbers of egg batches laid in each treatment are similarly plotted against the pF value of the soils in Fig. 8c.

It can be seen from these figures that the mean numbers of eggs per female or the numbers of egg batches laid in the treatments give closely corresponding curves for both soils when the water content of the soil is expressed on Schofield's pF scale. It appears that the beetles respond to the force with which the water is held rather than the amount of water present in the soil.

The numbers of eggs in each batch laid at the various water contents of both soils after nine days are given in Tables 1a and 1b Appendix. Analyses of variances indicated that the differences in the mean numbers of eggs in a batch could be attributed to chance. Dissection of dead beetles confirmed that the larger number of eggs laid in the range of optimum pF was due to more beetles laying all their eggs rather than all the beetles laying more eggs.

The numbers of adults which had died at the various treatments up to the times of examination are recorded in Table 2 Appendix. The percentage mortality is plotted against the water-contents of the soils in Fig. 9a and the percentage mortality of the females is plotted against the pF values of the soils in Fig. 9b. The striking resemblance of the mortality curves for the two soils in Fig. 9b adds considerable support to the hypothesis that Schofield's pF scale is a most convenient basis for comparing the availability of water to adults in the two soils. It is interesting to notice that the optimum pF value for survival in sand was 2.8; this was the pF value in

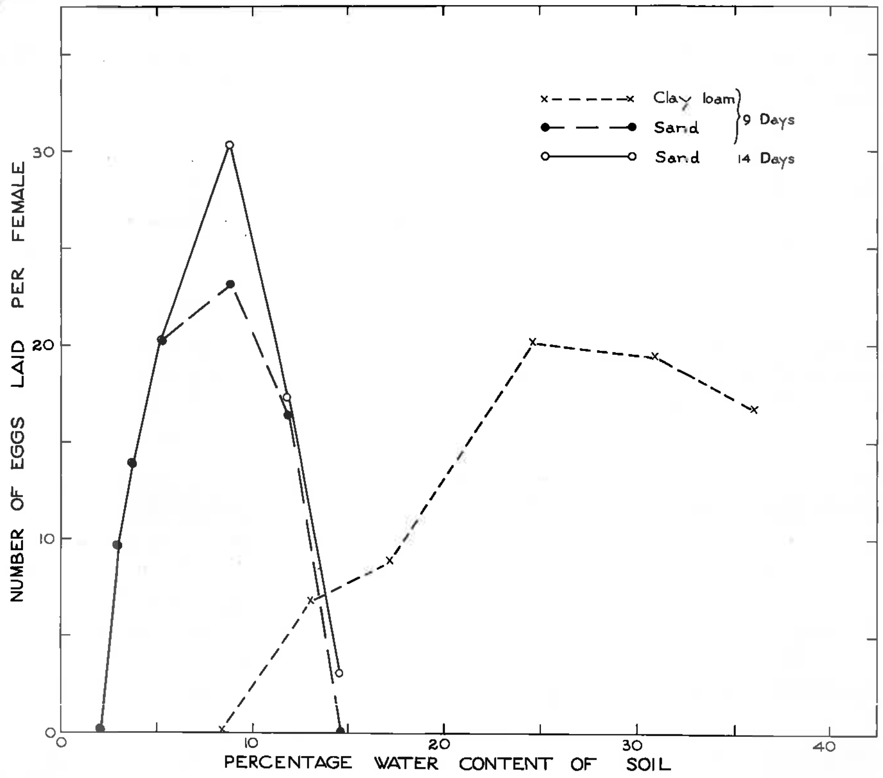

Fig. 8(a). The number of eggs laid by **A. howitti** females in relation to the water content of two soils.

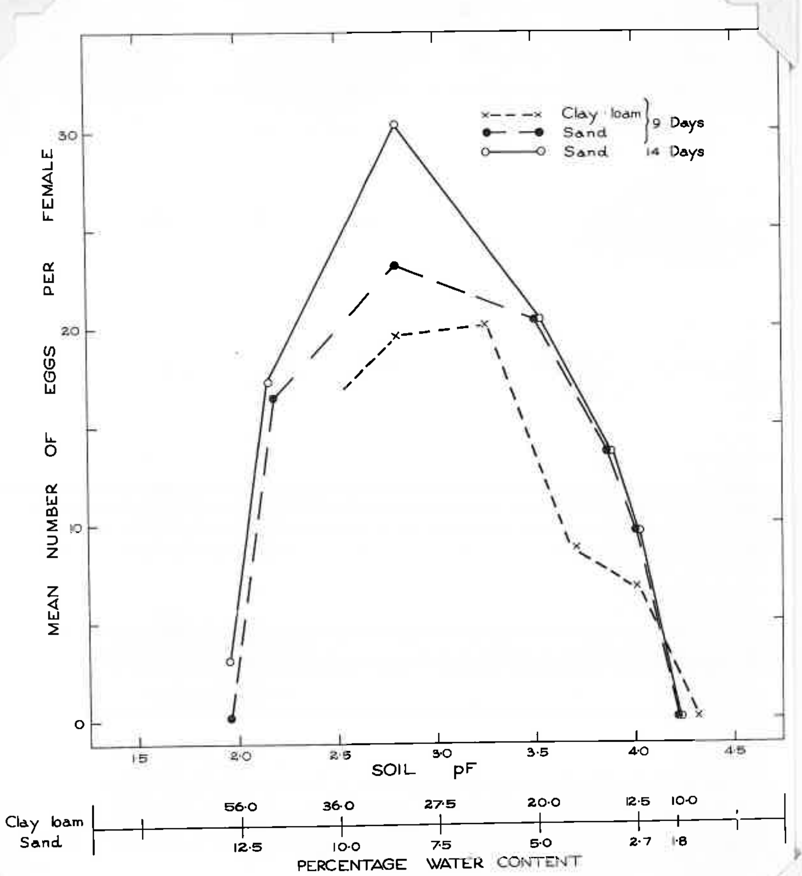

Fig. 8(b). The number of eggs laid by <u>A. howitti</u> females in relation to the pF value of two soils. The water contents of the two soils over the range of pF are added for reference.

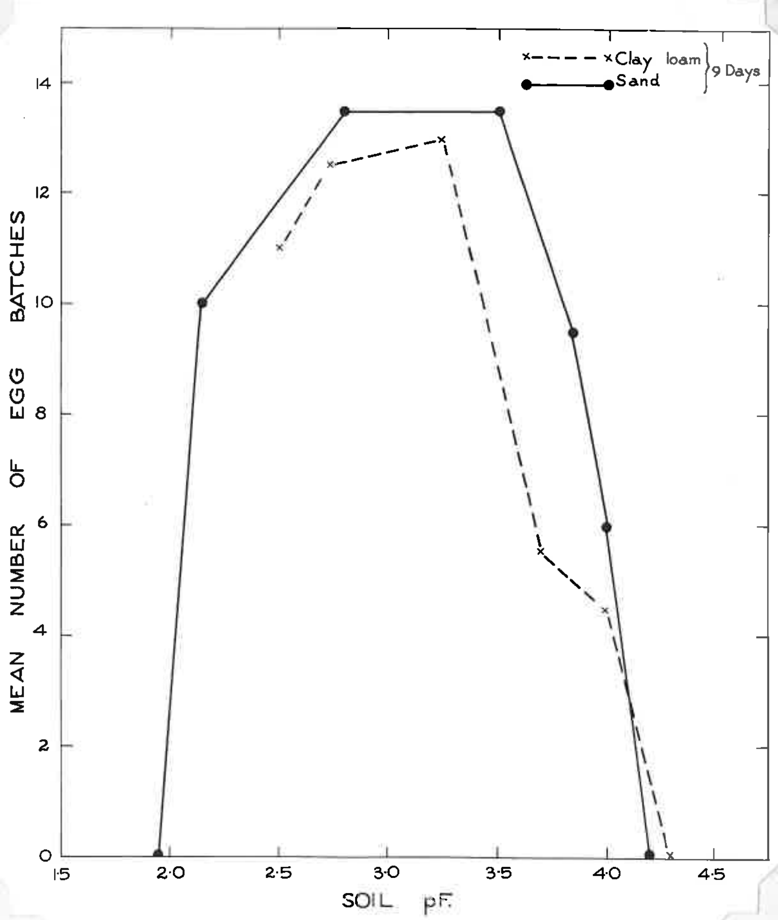

Fig. 8(c). The number of egg batches laid by A. howitti females after 9 days in relation to the pF value of two soils.

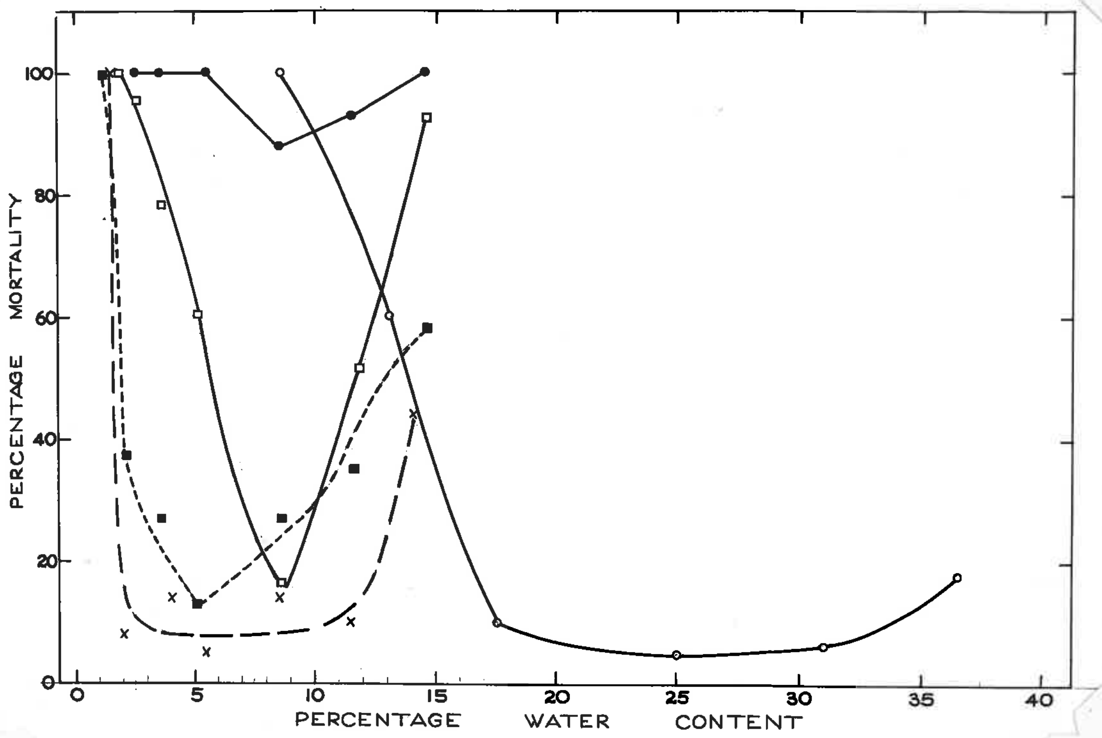

Fig. 9 (a). The percentage mortality of adults of A. howitti at various water-contents in two soils.

(1) In a sand

■- - -■ The percentage mortality of males after 9 days

×— —× The percentage mortality of females after 9 days

●——● The percentage mortality of males after 14 days

■——■ The percentage mortality of females after 14 days

(2) In a clay loam

○——○ The percentage mortality of females after 9 days

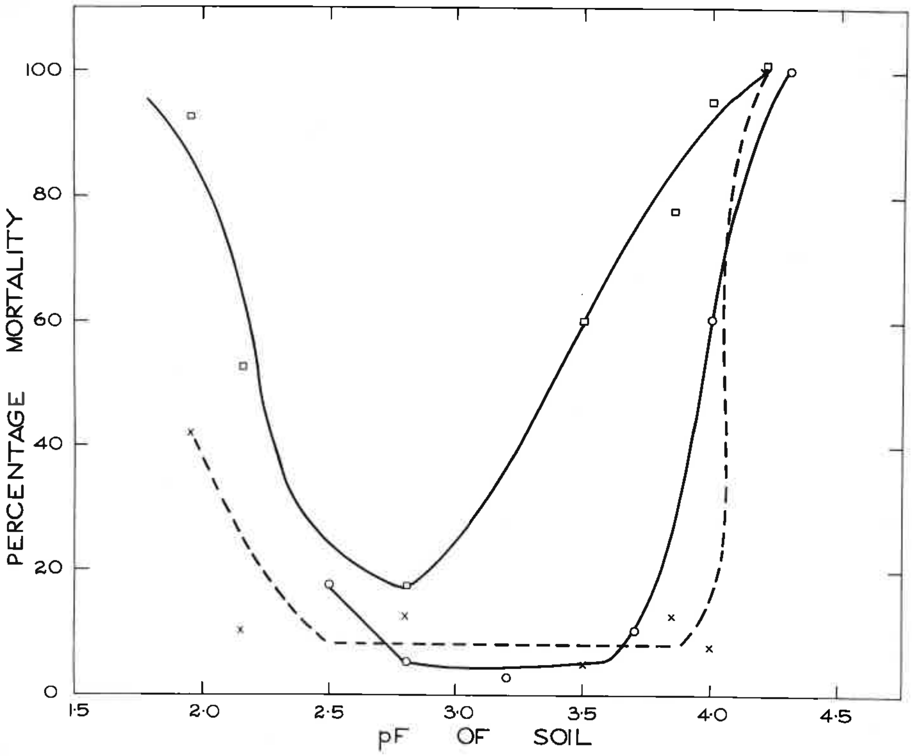

Fig. 9 (b). The percentage mortality of females of A. howitti in two soils in relation to the pF value of the soils.

sand at which the largest number of eggs were laid. The data for the clay loam were not as extensive and suggested that there was an optimum range of pF for survival somewhere in the vicinity of 2.8 to 3.2.

It is clear that the larger number of eggs laid in the optimum range of pF was partly a function of differential survival, but it is also apparent that many females lived but did not lay eggs in soil containing more or less water than the optimum.

The data suggested that soil with more or less water than the optimum might provoke an "avoiding" response, and that if beetles were given a choice between a number of water contents they might aggregate in the one which was optimal for oviposition. Such aggregation would be expected to occur in the range of pF 2.8 to 3.2.

(2) The aggregation of adults in relation to water in soil

The experiments in this section were designed to determine whether beetles, if given a choice between a number of water contents, would aggregate in the one which was optimal for oviposition, and also, to confirm whether or not the range of optimum water content for oviposition in both soils was indeed within the pF range 2.8 to 3.2.

Experiment 1

This experiment was conducted primarily to determine whether behaviour experiments could be conducted satisfactorily in the laboratory.

Two wooden boxes, each a square foot in diameter, were filled with Wandilo sand to a depth of 6 inches. The sand was moistened with water to give four water contents, and each quarter of each box was filled with sand of a different water content. One hundred and twenty beetles of each sex were introduced on to the surface of each box on the night of February 12th, 1955 and were dug up the following morning. The numbers recorded in the various treatments were as follows:-

% water content of soil	pF	Replicate 1		Replicate 2		Total		Total of both sexes
		F	M	F	M	F	M	
2.7	4.0	28	29	36	34	64	63	127
5.3	3.5	42	38	21	18	63	56	119
8.6	2.8	24	29	59	51	83	80	163
11.6	2.15	26	24	14	17	40	41	81

Features of this experiment were:-

(1) A greater amount of side seepage of water had taken place than had been expected. This nullified the results but, since the wettest treatment could not have become any wetter, the data did suggest that the beetles moved away from this treatment and that if the technique could be improved this type of experiment would work satisfactorily.

(2) The insects tended to congregate in groups except in the lowest water content in which they were present singly or in pairs.

(3) The surface of the soil had only been broken in a few places and it was obvious that most of the beetles had entered the soil at these points, and had then crawled along the bottoms or along the sides of the boxes. Consequently, they could not be said to have originally burrowed down in the treatment area in which they were found.

From this experiment it was apparent that -

(a) it was essential to keep the areas of different water contents completely separated from each other by water-proof partitions.

(b) the partitions between the blocks of soil would have to extend down to the bottom of the soil and terminate close to the surface to prevent beetles which had burrowed down into the soil in one treatment area from crawling through the soil into another treatment area.

(c) each treatment area should have an equal area, an equal number of corners and equal perimeter.

In all other experiments, therefore, there were four treatments to each box, each quarter being as nearly as possible identical with the others, and the quarters of soil with different water contents (or other treatments) were separated by means of a bituminous paper (sisalcraft). The sisalcraft strips were turned over along the bottom and along the sides of the box where necessary and held in position by packing the soil firmly against them; they terminated $\frac{1}{4}$ of an inch from the surface. In addition, the table on which the boxes were placed was tested with a spirit level to eliminate any possible bias due to slope, and the room in which the boxes were kept during the course of the experiment was darkened so that the adults would not orientate to any source of light.

The soil was moistened to the required water contents and the apparatus set up just before an experiment was started. The surface of the soil in each treatment was tamped down with the same force, as nearly as could be judged. Except in experiments dealing with different types of surface the top $\frac{1}{4}$ inch of soil in each treatment area was then lightly stirred up with a needle to facilitate burrowing.

The beetles were placed in the boxes in the dark room using the least amount of light possible, and the boxes were covered with a sheet of plastic material to prevent water loss from the soil and to keep the beetles in. Beetles which were found dead on the surface at the termination of any such experiment were not included in the results.

Experiment 2

Adults caught at a light the previous night and placed in moist soil were caught as they emerged to the surface at dusk on February 14th, 1955. Two boxes were prepared as described above, each box consisting of Wandilo sand having water contents of 2.7, 5.3, 8.6 and 11.6 per cent. Two hundred females were placed in each box, 50 in the centre of each treatment area. The soil in the boxes was dug up on the morning of February 16th, 1955 and the numbers of females recorded in each treatment are given in Table 2a.

The only successive treatments which are not significantly different at the 0.1% level are treatments 2 and 3, a comparison of which gives a x^2 of 6.25 with a probability only slightly larger than 1 per cent.

? ?. 95% is the usual level for judging significance)

The pF values equivalent to the water contents are also given in Table 2a and it is interesting to see that the pF value 2.8 was again the optimum.

Experiment 3

It was assumed in the previous experiment that if beetles chose a particular water content of soil in which to burrow they would stay there to lay their eggs. To confirm this an experiment was conducted with Wandilo sand of water content 1.8, 3.5, 5.3 and 8.6 per cent. This range of water content is probably closer than that of the preceding experiment to the water content in the field at the time of flight. The experiment was started on February 16th, 1955 and was left for three days and three nights to enable the beetles to lay their eggs. Only females were used in this experiment, 200 being used in each replicate. The females had been in a large cage with many males since February 11th, 1955 and had probably mated.

The numbers of females and the numbers of egg batches recorded in the different treatments are given in Table 2b. The differences between successive treatments are all significant at 0.1 per cent level and the pF value 2.8 stands out clearly as the optimum level for oviposition.

It can also be noted that the number of egg batches recorded in any treatment was roughly proportional to the number of females which was found there.

Experiment 4

Having determined that adults will aggregate in response to water in sand, it was left to determine whether beetles would similarly aggregate in clay loam within the pF range 2.8-3.2.

The water contents of the clay loam in the experiment were 7.3, 24.8, 31.0 and 36.3 per cent. Two hundred females were placed in each of two boxes on February 16th, 1955 and were dug up on February 19th, 1955, the experiment again being extended to give the females a chance to lay their eggs. The results are given in Table 2c. All successive

Table 2 (a)

The numbers of females recovered from the different treatments in Experiment 2

Treatment No.	% water content	pF	Replicate 1	Replicate 2	Total	Mean
1	2.7	4.0	4	20	24.0	12.0
2	5.3	3.5	70	63	133	66.5
3	8.6	2.8	101	76	177	88.5
4	11.6	2.15	24	36	60	30.0
Σ			199	195	394	

		χ^2	p.
Comparing treatment	1 and 2	75.7	<< 0.1%
	2 and 3	6.25	2.0-1.0%
	3 and 4	57.8	<< 0.1%
	1 and 4	15.4	< 0.1%

Table 2 (b)

The numbers of females and egg batches recovered from the different treatments in Experiment 3

Treatment No.	% water content of sand	pF	No. of beetles		Total	Mean	No. of egg batches		Total	Mean
			Rep. 1	Rep. 2			Rep. 1	Rep. 2		
1	1.8	4.2	0	0	0	0	0	0	0	0
2	3.5	3.85	28	10	38	19	2	4	6	3
3	5.3	3.5	50	56	106	53	7	10	17	8.5
4	8.6	2.8	114	124	238	119	19	13	32	16
Σ			192	190	382		28	27	55	

		χ^2	p.
Comparing treatments	1 and 2	38.0	< 0.1%
	2 and 3	32.1	< 0.1%
	3 and 4	50.7	< 0.1%

Table 2 (c)

The numbers of females and egg batches recovered from the different treatments in Experiment 4

Treatment No.	% water content of loam	pF	No. of beetles		Total	Mean	No. of egg batches		Total	Mean
			Rep. 1	Rep. 2			Rep. 1	Rep. 2		
1	17.3	3.7	30	19	49	24.5	4	3	7	3.5
2	24.8	3.2	46	67	113	56.5	6	7	13	6.5
3	31.0	2.8	93	60	153	76.5	12	8	20	10.0
4	36.3	2.5	31	54	85	42.5	8	9	17	8.5
Σ			200	200			30	27	57	

		χ^2	p.
Comparing treatments	1 and 2	25.3	< 0.1%
	2 and 3	6.0	2.0-1.0%
	3 and 4	19.4	< 0.1%
	1 and 4	9.67	1-0.1%
	2 and 4	3.96	2-5%

treatments are significantly different at 0.1 per cent. level, except 2 and 3 which are significant at the 5 per cent. level but not at the 1 per cent. level. Again the number of egg batches laid in any treatment was roughly proportional to the number of beetles aggregated there.

The experiments described above demonstrated that adults, if given a choice of soil with many water contents, would aggregate in largest numbers at one particular water content or within a range of water contents. This optimum water content was clearly 8.4 per cent. for sand, and for the clay loam was between 24.8 and 31 per cent.

(3) Conclusions - the "available" water in the soil

The evidence from these and other experiments suggests that males and females alike respond to water in soil. It is conceivable though that the males were attracted to females and consequently were recorded in similar numbers in experiments in which both sexes were used. The only conclusive way of demonstrating that males are capable of responding to the water content of the soil themselves is to conduct an experiment with males only. This has not been done. However, females chose the optimum range of water content some days before they laid their eggs (experiment 2) and the survival-rate of the adults was a function of water in the soil so the response of the adults to the water content of soil is probably a survival mechanism rather than a specific response for oviposition. This is confirmed by the results of experiments 3 and 4, which indicate that the number of egg batches laid in any treatment was roughly proportional to the numbers of females which aggregated there. Males are therefore likely to respond independently to water in soil.

Sweetman (1931) recorded that there was a range of water content in soil in which adults of Phyllophaga sp. laid most eggs and survived for the longest time. Similarly the results of experiments described thus far indicate that there was an optimum of water content in sand in which adults lived longest, aggregated in largest numbers and laid most eggs. This optimum re-occurred in a number of experiments and was clearly 8.4 per cent. water content with an equivalent pF value of 2.8.

The data only demonstrate, however, that pF 2.8 was the optimum of the values used in the experiments, and it is probable that the clarity with which pF 2.8 stood out as the optimum in sand was due to the fortuitous spacing of the water levels tested. The response to water in soil is likely to be normally distributed and a further series of tests with a larger number of water contents at closer intervals would probably reveal, not a single optimum water content, but a range of optimum water content such as Sweetman (1931) recorded for Phyllophaga sp.

The results of the experiments with clay loam are instructive therefore, because the water contents, when expressed on an energy basis, were spaced closer together on the pF scale than those of the sand. A single optimum water content could not be clearly distinguished; the results rather indicated that there was an optimum range of water content between 24.8 and 31.0 per cent. or pF 3.2 to 2.8.

The combined data are only intelligible if the response of the adult is related to the pF value of water in the soil, and indicate that there is an optimum range of water content probably in the vicinity of pF 2.8 to 3.2 for both soils. The pF scale thus provides a convenient basis for comparing the behaviour of adults over a range of water contents in different soils in terms of "available" water.

It is tempting to speculate on the physiological basis for the reaction of adults to pF rather than water content. Evans (1943, 1944) demonstrated that a 0.33M sucrose solution was approximately isotonic for larvae of Agriotes sp., and that, in hypotonic solutions, wireworms were constantly imbibing through the cuticle water which was subsequently excreted. Since a 0.33M solution of sucrose has a pF value of 3.94 wireworms are expected to imbibe water in soils which are wetter than pF 3.94, and the amount which they imbibe is probably related to the pF value of the soil. Lees (1943) confirmed that wireworms migrate rapidly out of dry soils and aggregate in wet soils, and concluded that the results could be explained by the differential effect of moisture on the rate of burrowing. Lees (1943) suggested that the intake of water by wireworms in wet soils results in akinesis. Clearly, if larvae of Agriotes were given a "choice" of one of two soils they would aggregate in relation to the pF value of the soils rather than their water contents.

Now it is clear that the adult of A.howitti which moves away from soil drier than pF 2.8-3.0 is reacting to a lack of water in the soil, but observation suggests that aggregation at pF 2.8-3.0 is not a result of akinesis, as it may be in wireworms. Adults of A.howitti appear to react to moisture on the surface of the soil or in the top inch or so, and will not attempt to burrow in soil which is very dry (pF 4.0) or very wet (pF 2.0).

In soil which is wetter than pF 2.8 they might react to excess water or to a lack of oxygen. The pore-space distribution of the two soils used in the experiment is expected to be different, but the responses of adults at pF values lower than 2.8 in both soils appear not to differ widely; so it appears that the adults respond to excess water in very wet soils. If this is true it can only mean that adults of A.howitti have a specialized receptor(s) for detecting "available water" in soil.

(b) The effect of "shelter" and type of surface on the choice of a place in which to lay eggs

Andrewartha (1945) observed that A.howitti females tend to lay their eggs in paddocks which have a very thin cover of stubble or else are completely bare (see Plate 1); these observations have been confirmed by Madge (1952) and by myself. Andrewartha (personal communication) was of the opinion that the moisture status of the bare paddocks attracted the beetles. Swan (1934) relayed the opinion of farmers that beetles were attracted to the accumulation of dung so often present in the areas in which the eggs are laid. Carne (private communication and 1956) on the other hand, suggested that bare paddocks with a mass of newly germinated clover were attractive to adults because the clover facilitated burrowing; and many farmers interviewed in the course of these investigations maintained that adults seemed to specially favour fallow paddocks for laying eggs. It had similarly been noted in Experiment 1 that the beetles had burrowed into the soil at relatively few points; these points were at the corners of the boxes where the adults could get a certain amount of purchase and break the surface of the soil.

Therefore, though it had been demonstrated that the water content of the soil had a marked influence on the choice of a place in which to lay eggs, there were obviously other factors which might similarly influence the behaviour of the adults. It was decided that a laboratory study of some of these factors and their interaction might profitably be conducted along the lines of the behaviour experiments previously described.

An attempt was made to reproduce characteristics of some areas attacked in the field by using straw or accumulations of dung and straw debris from sheep camps as "shelter". Other experiments were designed to test the attractiveness of places in which adults could burrow easily.

(1) The attraction of "shelter" to adults.

Experiment 5

The boxes used in this experiment were filled to a depth of six inches with Wandilo sand of water content 8.4 per cent. On one quarter in each replicate dry dung fragments were scattered lightly to act as a possible attractant. Another quarter was covered with a thick layer of dry dung fragments to simulate "shelter", a third was covered with straw to simulate another aspect of "shelter" and the fourth quarter in each replicate was left bare. The surface of the soil was not broken in any of the treatments.

One hundred beetles of each sex were introduced into each box at the start of the experiment on February 14th, 1955, and were dug up on February 16th, 1955. The results are given in Table 3a. No significant difference was found between the sexes at the different treatments, so the numbers of each sex were used as further replicates in each box.

The analysis of variance indicated that the numbers of beetles found in the different treatments differed significantly ($p < 0.1\%$). The difference between the treatments with "shelter" was not significant at 5 per cent. level, and similarly the difference in the numbers found in the treatments with a bare surface could be attributed to chance. Of the treatment sums of squares however, 88.5 per cent. could be ascribed to the difference between the bare surfaces on the one hand and the treatments with "shelter" on the other; this difference was highly significant ($p < 0.1\%$). Dry dung thus had no value as an attractant, but the data suggested that it could promote the aggregation of adults by acting as "shelter" if it was in sufficient quantity.

(2) The attraction of a surface which is easier to burrow in.

Experiment 6

This experiment was set up on 16.2.55 to determine whether the adults would aggregate in areas in which burrowing was relatively easy. The boxes were treated as above, with water content constant, but the treatments at the surface were different. One quarter of each replicate was left bare but with a compact surface; one quarter had a bare, compact surface on which 20 dead beetles were placed as a possible attractant and the top ½ inch of soil was stirred up with a needle on the third quarter. A 2 inch wire mesh cage was placed on the bare compact surface of the last quarter of each replicate and 50 females which were placed in the cage were allowed to burrow into the soil two hours before dusk. This they did along the periphery of the cage. At dusk 50 females were placed in the centre of each of the other treatment areas so that a total of 200 females were used in each replicate. The numbers of beetles which were recorded in the different treatments three days later are tabulated in Table 3b.

The difference between the treatments with compact surfaces was not significant, and there was no significant difference between the treatment in which the soil was artificially loosened at the surface and the treatment in which the same result was achieved by compelling adults to break the surface before the experiment started. The difference between the treatments with loose soil at the surface and the treatments with compact soil on the surface was, however, strikingly large and was significant at 0.1 per cent. level.

Thus it appears that the ease with which burrowing can take place at the soil surface is another factor capable of bringing about aggregations of adults.

(c) The relative influence of water and shelter on the choice of a place in which to lay eggs

Experiment 7

This experiment was started on February 16th, 1955 and was designed to determine the relative attraction of shelter to that of the water content of the soil. The treatments were:-

(1) Fresh dung and dry soil - loose surface

(2) Cover and dry soil - loose surface

(3) Cover and wet soil - compact surface

(4) Bare wet soil - compact surface.

Fresh dung was introduced into this experiment to make the bare, dry soil more attractive to the beetles because beetles are strongly attracted to fresh dung. The dung was placed on the dry soil in small heaps, however, so that it would not moisten the soil beneath and would not be in sufficient quantities to constitute shelter. Two hundred females were used in each of two replicates.

The dry soil was Wandilo sand with a water content of 1.8 per cent. and, being dry, could not be compacted and necessarily had a loose surface; the wet sand had the optimum water content for oviposition (8.4 per cent.). The surfaces of the treatments with wet soil were compact, and the "shelter" used was a ½ inch layer of dung and straw debris obtained from the field. The numbers of beetles recorded in the different treatments after three days are given in Table 3c.

The outstanding feature of this experiment was the aggregation of all the living beetles in the treatment with wet soil despite the attractiveness in other circumstances of cover, loose surface and fresh dung on the dry soil surfaces. The other interesting feature of the experiment was that the difference between the two treatments with wet soil was statistically significant at the 0.1 per cent. level, despite the previously demonstrated attractiveness of cover compared to unbroken surface. This was probably because the layer of "shelter" was deeper than that used in experiment 5 and consisted of a larger number of smaller particles of dung etc. which were relatively dry. A number of beetles thus apparently preferred to expend their energy in burrowing into a wet compact surface than burrowing through a relatively dry layer of cover only ½ inch deep.

The experiment demonstrates the outstanding importance of water in soil for oviposition, and also suggests that the top ½ inch of the soil surface is all important in promoting or inhibiting aggregations of adults.

Table 3(a)

The attraction of shelter to adults; the numbers of beetles recovered from different treatments

Treatment	Replicate 1		Replicate 2		Total	Mean
	M.	F.	M.	F.		
Bare-dung attractant	19	21	22	16	78	19.5
Bare	12	20	15	13	60	15.0
Shelter, straw	36	27	37	42	142	35.5
Shelter, dung	33	32	22	30	117	29.3

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	V.R.	p
Total	15	1,284.4			
Treatments	3	1,033.7	344.57	16.49	<0.1%
Bare v. shelter	1	915.0	915.0	43.8	<<0.1%
Others	2	118.7	-	-	N.S.
Residual	12	250.7	20.89		

S.E. of difference of any two treatment means = 3.24
Difference for significance 5% = 7.0
Difference for significance 1% = 9.9

Table 3(b)

The attraction of a surface which is easier to burrow in; the numbers of beetles recovered from different treatments

Treatment	Replicate 1	Replicate 2	Total	Mean
1. Loose soil at surface	80	68	148	74.0
2. "worked" soil at surface	76	91	167	83.5
3. Compact soil	22	13	35	17.5
4. Compact soil with dead beetles	16	28	44	22.0

Comparing treatments-
3 and 4 $\chi^2 = 1.03$ (1 d.f.) $p > 5\%$
1 and 2 $\chi^2 = 1.03$ (1 d.f.) $p > 5\%$
1,2 and 3,4 $\chi^2 = 141.4$ (1 d.f.) $p < 0.1\%$

Table 3 (c)

The relative influence of water and shelter on the choice of a place in which to lay eggs; the numbers of adults recovered from soil with different treatments

No.	Treatment	Replicate 1	Replicate 2	Total	Mean
1.	Fresh dung - dry soil	0	0	0	0
2.	Shelter - dry soil	0	0	0	0
3.	Shelter - wet soil	76	86	162	81.0
4.	Bare - wet soil	123	104	227	113.5

Comparison of treatments 3 and 4, $\chi^2 = 10.86$ with 1 d.f.
$p = <0.1\%$

(a) The effect of gregariousness on the choice of a place in which to lay eggs

Larvae of A. howitti may be found in very restricted areas in pastures for a variety of reasons, but sometimes they occur in restricted areas in pastures which "appear to be uniform". Their patchy distribution in such pastures may be the result of more eggs being laid or more eggs and larvae surviving in certain places - but in this section we are concerned only with the possibility of more eggs being laid.

It is difficult to conceive how aggregations of active larvae in "uniform" pastures can result from more eggs being laid in restricted areas because it is difficult to conceive how any local area could differ so markedly from the rest of the paddock as to be selected almost exclusively by the adults for oviposition. Females could lay eggs in exclusive places in a uniform pasture only if swarms of females settled by chance, but there is no evidence for the occurrence of such swarms. To explain the necessary aggregation of adults Carne (1956) suggested that females may possess some olfactory or auditory mechanism whereby they can detect the presence of other females, or possibly larvae, in the soil.

It was necessary therefore to determine whether females were, in fact, attracted to others of their own kind, and whether such attraction could bring about aggregations of adults in sufficient numbers to explain the distribution of larvae in apparently uniform pastures.

It would seem, on a priori grounds, that adults could not be attracted to dead animals of their own kind, but have been seen attempting to copulate with dead beetles of their own species; so it was just conceivable that dead beetles on the surface would delay a few live males long enough to allow females to alight in the same place and mating to take place. A series of such happenings could conceivably result in an aggregation of adults. This is the reason why dead beetles were placed on the surface in experiment 6. It was seen from the results of that experiment (Table 3(b)) that it was most unlikely that there was a preference for compact soil and dead beetles to compact soil alone. However, it was considered that it would be worth determining whether beetles were attracted to other dead beetles on a loose surface. On such a surface the sequence of events which could result in mating would lead to aggregation more easily than on a compact surface on which the beetles were inhibited from burrowing anyway. An experiment was set up to test this hypothesis but unfortunately was ruined and no more experiments were possible in 1955.

In 1956 a small flight of beetles was observed on February 1st, and a number of the beetles were caught at a light. The females had already laid their first batch of eggs so the beetles were kept in moist soil in a cage and fed on dung. There were only a few adults available and it was unlikely that they would live for more than a few days, so it was decided to concentrate on the attraction of beetles to other living beetles in the soil.

Experiment 8

If adults in flight are attracted to others burrowing in the soil it is reasonable to assume that adults crawling on the surface should similarly be attracted to others burrowing in the soil. In one of the loose soil treatments in experiment 6, 50 beetles were allowed to burrow down into the soil before dusk and 50 beetles were subsequently placed on the other treatment areas. It was not possible, however, to decide how many beetles were attracted to the loose soil at the surface and how many were attracted to the beetles already in the soil.

In this experiment beetles were enclosed in cages under the soil surface and the soil surface was loosened artificially; the relative attractiveness of the loose soil at the surface and of the adults actually in the soil could then be estimated separately. The treatments were as follows:-

(1) Compact surface - beetles in cage under the surface
(2) Loose surface - beetles in cage under the surface.
(3) Compact surface - no beetles but a similar cage under the surface.
(4) Loose surface - no beetles but a similar cage under the surface.

Boxes were filled with Wandilo sand having a water content of 8.6 per cent. and the treatment areas were partitioned off as before. Wire mesh cages, 2 x 1 x 1 inch were half filled with soil and 20 females and 10 males enclosed in each. The cages were placed in the centre of the necessary treatment areas so that their surfaces were about ½ inch under the surface of the soil. Similar cages were placed at similar depths in the other treatment areas. Twentyfive males and 25 females were then placed in the centre of each treatment area so that 100 beetles of each sex were used in each replicate; four replicates were used to give a larger number of degrees of freedom in the analysis of variance. The boxes had to be kept in the open, so they were randomized, placed in the shade of a tree and covered with a sheet of plastic and a sheet of canvas.

After two days the soil in the various treatment areas was dug up and the numbers of live beetles (excluding those in the cages) in each area recorded. The results are given in Table 3 Appendix. Different numbers of males and females had died in the course of the experiment so that numbers of each sex recorded were corrected to the numbers expected if all the beetles had survived. These are tabulated in Table 4.

The numbers of males and females recorded in the treatments were kept separate because it was possible that one sex and not the other would be attracted to the adults under the surface. The introduction of sex into the analysis therefore increased the number of "treatments" to 8. The analysis of variance (Table 4) revealed that the differences between the treatments were highly significant ($p < 0.1\%$). The treatment sum of squares was divided into (a) a component for sex which was zero because the same number of males as females were used in the experiment, (b) a component for the four original treatments of the experiment which are here termed types of surface, and (c) a component for the interaction of sex and types of surface which, instead of (a), can be attributed to the differences in distribution of the two sexes. The variance ratios of the three components indicated that the differences between the types of surface were highly significant ($p < 0.1\%$), and that the difference in the distribution of the sexes in the types of surface could be attributed to chance.

The sum of squares for types of surface could further be divided into its components to test (a) the relative attraction of beetles under the surface and no beetles under the surface (b) the relative attraction

Table 4

The attraction of beetles to others of their own kind; the numbers recovered from different treatments in Experiment 8 converted to numbers expected out of 400

Treatment	Replicate 1		Σ	Replicate 2		Σ	Replicate 3		Σ	Replicate 4		Σ
	M	F		M	F		M	F		M	F	
Compact surface, beetles	11.9	11.3	23.2	7.1	6.2	13.3	12.5	7.1	19.6	8.5	12.1	20.6
Compact surface, no beetles	3.6	3.1	6.7	14.3	16.5	30.8	6.3	6.1	12.4	4.3	3.0	7.3
Loose surface, no beetles	36.9	34.0	70.9	40.5	38.1	78.6	49.0	50.0	99.0	48.8	45.5	92.3
Loose surface, beetles	47.6	51.5	99.1	38.1	39.2	77.3	32.3	36.7	69.0	40.4	39.4	79.8

Table of treatment totals

Treatment	Male	Female	Σ
Compact surface, plus beetles	40.0	36.7	76.7
Compact surface, no beetles	28.5	28.7	57.2
Loose surface, no beetles	173.2	167.6	340.8
Loose surface, plus beetles	158.4	166.8	325.2
Σ	400.1	399.8	799.9

Analysis of variance of numbers recorded in treatments

Source of variation	D.F.	S.S.	M.S.	V.R.	p
Total	31	9,644.1			
Treatments	7	8,900.9	1,271.56	41.07	<0.1%
Sexes	1	0	0	-	-
Types of surface	3	8,886.8	2,962.27	95.65	<0.1%
Sex x type of surface	3	14.1	4.70	-	N.S.
Error	24	743.2	30.97		

Partitioning sums of squares for type of surface

Source of variation	D.F.	S.S.	M.S.	V.R.	p
Beetles v. no beetles	1	0.5	0.5	-	N.S.
Compact v. loose surface	1	8,847.8	8,847.80	285.69	<<0.1%
Interaction	1	38.5	38.5	1.24	N.S.

of a compact as opposed to a loose surface and (c) the interaction of (a) and (b). This final analysis demonstrated that (1) beetles of either sex placed on the surface were not attracted to other beetles under the surface; (2) aggregation of adults occurred wherever the surface soil was loose and burrowing was easy.

The results of experiment 6 also indicated that beetles aggregated in areas in which the surface soil was loose. The magnitude of the differences between loose and compact surfaces in both the experiments provides convincing evidence that the ability to burrow into a surface rapidly is an important factor in the choice of a place in which to lay eggs. It is highly probable that the type of surface encountered often controls the aggregation of adults in the field.

The data do not conclusively prove, of course, that beetles are not attracted to other beetles. The possibilities are nevertheless limited because any attraction of beetles to other beetles must, if it exists, be due to an attribute which was not present or was masked in both the experiments.

B. Field experiments:

In conjunction with laboratory experiments an experiment was set up in the field to determine whether females would aggregate and lay eggs in response to the water content of soil, but the experiment was not successful because most of the beetles escaped from the cages placed on the treated plots. The laboratory data were so clear and are supported by so much other evidence that it was not worth repeating the experiment. Attempts were made instead to get beetles to aggregate in response to other factors which induced the aggregation of adults in the laboratory.

(a) The effect of "shelter" and type of surface on the choice of a place in which to lay eggs

An area was specially chosen so that the surface of the soil in some of the plots would be hard and would make burrowing difficult for adults. It was located near a gate where the trampling of sheep and cattle had removed the pasture coverage and had compacted the soil within a radius of 10 feet or so from the gate (Plate 7). Beyond this circle pasture was growing normally; at this time of the year it consisted of a few perennial rye plants with a smattering of stubble and loose straw on the surface.

Since a portion of the pasture was included in the experiment it was not possible to randomise the treatments, and the plan of the experiment was as follows:-

pasture	bare ground and shelter	bare ground
pasture	bare ground	bare ground and shelter

Each plot was 2 feet square so the total experimental area was 6 feet x 4 feet. Strips of metal were placed in the soil along the periphery of the experimental area and a half inch layer of dung and straw from a sheep "camp" was placed on the plots which required "shelter". On the night of 16.2.55, 600 females, which had all probably mated by this time, were placed on the experimental area, 100 to each plot. The whole area was then covered with fly-wire and the beetles were allowed to roam over the entire area and lay their eggs where they chose to do so.

It had been intended to dig up the plots after three days to record the number of beetles and the numbers of eggs laid in each plot, but an experiment which had been designed to obtain an estimate of 1st instar mortality had to be omitted and the plots in the experiment above were left for this purpose. The surfaces of all the plots were consequently covered on 6.4.55 with dung and straw to give the larvae something to eat when they migrated to the surface, and so prevent movement of larvae away from the places in which the eggs were laid.

The larvae migrated to the surface in response to rain on 12.4.55 and the plots were sampled on 20-24.4.55 when it was considered that all the larvae were at the surface. A 3 inch guard area was left around each plot and the number of larvae and number of dead adults in each plot were recorded; these are given in Table 5a.

The similarity of the ratios of larvae/adults recorded in the plots gives considerable confidence to the estimate of the relative attractiveness of the different treatments to the adults, which can be based either on the number of larvae or the number of dead adults recorded.

A test of homogeneity suggested that there was a significantly greater number of adults in the soil under "shelter" than under pasture ($\chi^2 = 13.1$ p $>0.1\%$) but the non-randomized positions of the relevant plots might have been sufficient to account for the difference. The difference between bare ground and bare ground with shelter is not invalidated by the experimental design ; the magnitude of the difference, as well as that between bare ground and pasture, demonstrates how unsuitable the bare ground was for burrowing. In fact, the few larvae which were found in the plots with a bare surface were along the strips of metal where the soil was probably not so compact and permitted a few beetles to burrow and lay their eggs.

The soil under the pasture was not compacted as was the bare soil and the soil with "shelter" so it is not known to what extent the pasture plants themselves facilitated burrowing. But the results generally agree with those obtained in the laboratory and demonstrate that

(1) the readiness with which adults can burrow into soil is an important factor in promoting aggregations of adults

(2) "shelter" can transform a particularly unsuitable surface for burrowing into a suitable place in which to lay eggs.

(b) The necessity of water for oviposition

This experiment was designed initially to test the relative attractiveness of shelter and a loose surface to adults in the field.

Strips of metal were placed around an area of ground 6 feet x 3 feet which was divided into two replicated plots of 3 feet x 3 feet, and each replicate was divided into 18 inch squares which were given one of the following treatments:-

(1) Bare ground - soil loosened at surface

(2) As above and with small pieces of dry dung as attractant but not in sufficient quantity to act as shelter

(3) Bare ground - layer of dry dung as shelter

(4) Bare ground - cover of straw as shelter

Fifty females were placed on each replicate and the plots were covered with fly-wire. The plots were to have been dug up 3-4 days later but, like those in the previous experiment, were dug up later to get an estimate of larval mortality. The number of dead beetles and the number of larvae were recorded when replicate 1 was dug up on March 22-24, 1955

Table 5(a)

The effect of shelter and type of surface on the choice of a place in which to lay eggs. The numbers of adults and larvae recovered from soil with different treatments

Treatment No.	Treatment	No. of adults		No. of larvae		Totals		
	Plot:	1	2	1	2	Adults	Larvae	Larvae/adult
1	Bare ground	12	4	73	13	16	86	5.4
2	Bare ground with dung and straw(shelter)	124	136	731	889	260	1620	6.2
3	Pasture	89	95	547	602	184	1149	6.4
					Σ	460	2855	$\bar{x}$ = 6.2

Comparing treatments:

2 and 3, $\chi^2 = 13.1$ $p > 0.1\%$

Table 5(b)

The necessity of moisture for oviposition

The numbers of adults and larvae recorded in plots with different treatments

Treatment	Replicate 1 Adults	Replicate 1 Larvae	Larvae/ adults	Replicate 2 Adults	Replicate 2 Larvae	Larvae/ adults	Total adults
Bare, loose surface	62	757	12.2	11	49	4.1	73
As above with dung as attractant	38	548	14.4	70	262	3.7	108
Layer of dung as shelter	63	720	11.4	53	157	3.0	116
Layer of straw as shelter	14	159	11.4	51	270	5.3	65
Total	177	2184	12.3	185	738	4.0	362

and replicate 2 on March 22, 1955. The results are given in Table 5b.

It was noticed whilst sampling the first replicate that particular patches of soil were wetter than others and that most of the first instar larvae occurred in aggregations in these particular patches. The young larvae were still in the cells in which the eggs were laid so most of the eggs had either been laid or had survived in the wetter patches of soil. Many beetles were found in the wet patches too, so the relative scarcity of larvae outside the wetter areas was probably due to few eggs being laid rather than only a few eggs surviving.

There was a rough correlation between the number of adults and the number of larvae found in any treatment area, but the numbers found in different replicates of the same treatments differed considerably. It is likely that none of the treatments influenced the aggregation of the adults and that the results were due instead to the erratic distribution of patches of soil with a favourable water content for survival and egg-laying.

The results confirm that water is the most important component of the soil environment and that, when water is limiting, other factors have no influence on the choice of a place in which to lay eggs.

(iv) The relation between the behaviour of the adults and the distribution and abundance of larvae in the next generation

The readiness with which adults aggregated in the experiments suggests that aggregation of adults in the field normally occurs in response to similar stimuli. It is likely that other stimuli remain to be investigated before a comprehensive account can be given of the behaviour of the adults in the field, but it is considered that a tentative hypothesis can be presented to account for the kinds of places in which A. howitti lays its eggs. This hypothesis is mainly based on the responses of adults to stimuli which have been investigated, the pertinent facts of which can be summarized as follows:-

(1) The survival of adults and the numbers of eggs laid by females were related to the "availability" of water in soil. There was a range of optimum pF in two soils in which adults aggregated in largest numbers, lived longest and laid most eggs.

(2) Water in the soil was the primary factor controlling the aggregation of adults and the subsequent deposition of eggs.

(3) When water was not limiting, "shelter" was capable of promoting aggregations even if the surface of the soil underneath ordinarily inhibited burrowing.

(4) Similarly, when water was not limiting, aggregations occurred in areas in which burrowing was relatively easy.

(5) Aggregations of beetles occurred in areas in which other beetles had previously burrowed. This was probably due not to any attraction of beetles to other beetles but to the loosening of the surface soil by the original beetles which helped later arrivals to burrow rapidly.

(6) The number of egg batches laid in any place was roughly proportional to the number of females aggregated there.

Ideally, the kinds of places in which A. howitti lays its eggs should be determined by plotting the distribution of eggs in the field. But the eggs cannot be separated from soil by any of the mechanical sifting devices employed for soil-inhabiting insects and sampling by manual method is unsatisfactory because the eggs are difficult to find in dry soil, though they are easily seen in moist soil. The kind of places in which A.howitti lays its eggs have, therefore, been ascertained largely by observing the kind of places in which the larvae occur and determining the characteristics which the infested areas had in common at the time of flight. This method has the disadvantage of only being able to distinguish areas in which eggs were laid and survived and not being able to distinguish areas in which eggs were laid and did not survive. On the other hand observation, and a knowledge of the requirements of the eggs and the young instars, permits the discussion of certain kinds of places in which eggs are laid but do not survive.

The distribution of A.howitti, like that of many other animals, tends to be patchy. This patchiness is determined partly, perhaps, by differential survival of eggs and larvae, but mainly by the tendency of the adult to lay its eggs in certain kinds of places which, in turn, are unevenly distributed.

It is probable that a number of beetles normally lay their eggs in unsuitable areas and none or few of the eggs survive; indeed it is not uncommon to find scattered larvae in "atypical" places. This variability in response to stimuli is a characteristic of biological populations and does not affect the discussion which deals with the kinds of places in which the majority of the populations are usually found.

(a) Pasture:

The pastures damaged by larvae of A.howitti in South Australia have been mainly those which (a) were cut for hay the previous spring, (b) were heavily grazed in summer, or (c) simply consisted of a dominant cover of subterranean clover (*Trifolium subterraneum*). These pastures have a relatively bare surface which is the chief visual characteristic of sites in which eggs are laid in the field. Similarly, there is some evidence that females may lay eggs in large numbers in fallow paddocks and in paddocks in which are grown summer crops such as rape, turnips etc. and in which, consequently, there are usually high proportions of bare ground.

Observation has consistently indicated that females do not lay many eggs in areas which are covered by dense stands of grass stubble at the time of flight. In pastures cut for hay, for example, the mower misses circles of pasture around tree stumps, and there may be patches of pasture which for some other reason are not cut, i.e. inferior pasture growth or dominance of weeds such as thistles. These local areas are covered with stubble which is dense and taller than that in the rest of the paddock, and they invariably are not infested to the same extent as the rest of the paddock which does not make much more growth in the summer and is relatively bare when the adults fly (Plate 1).

Similarly, pastures which are heavily grazed in summer may contain patches in which the pasture is not particularly palatable to stock, with the result that these patches are left with relatively more stubble and are rarely infested with larvae. A typical example was seen on the property of Mr. Furness, Pareen, in a paddock which had had a thick cover of thistle (*Silybum marianum*). Mr. Furness sprayed most of the thistles in the paddock but did not have sufficient weedicide to finish the task and left a patch of thistles in the centre of the paddock. The paddock was particularly close to his house so he heavily grazed it in summer to eliminate the fire risk. In February, 1955, A. howitti females laid their eggs in this paddock by the thousands; in the patch of thistles the cover of other grasses was much better than in the rest of the paddock, because the stock did not favour entering the thistles, and virtually no eggs were laid there.

Pastures which are dominated by subterranean clover are particularly favoured as places in which to lay eggs, because the clover dies off in summer and leaves the ground bare (Plate 1). Improved pastures which contain a high proportion of clover are particularly vulnerable to infestation with A. howitti, and significant damage is also recorded in natural pastures in which volunteer clovers have come in, as a stage in succession, due to the application to such pastures of superphosphate.

With continued application of superphosphate pastures which are clover dominant are often invaded by annual grasses and weeds such as Cryptostemma calendulaceum (capeweed) and Erodium moschatum. Large numbers of eggs are frequently laid in pastures which are thus infested with weeds. Eggs are also frequently laid around limestone outcroppings (Plate 2) in pastures on terra rossa soils; the soil is shallow around such outcroppings and supports annual grasses only. Indeed, it may be generally said that any agency which promotes the establishment of annual grasses or legumes in a pasture ameliorates the environment of A. howitti.

(b) The water content of soil

The bare surface which attracts adults is probably a "token" stimulus, for it is difficult to conceive how the adults could benefit from the bareness of the soil. The soil under a bare surface, however, commonly retains more water than soil under perennial grass (Table 4 Appendix) and moreover is usually more easily wetted by light to medium showers of rain.

It is not surprising, therefore, that adults should aggregate in areas with bare surfaces, for it is in such places that they are able to survive in largest numbers and lay the largest number of eggs.

But apart from bare areas, there is a wealth of information which indicates that adults lay large numbers of eggs in areas in which the soil moisture must be optimal for survival and oviposition. Such areas are often highly localized and, naturally, tend to be most apparent in dry summers. For example, Andrewartha (private communication) conducted an experiment at Kybybolite in 1944 to determine where females laid their eggs. He treated plots in various ways but records that most of the eggs were laid along a furrow running right through the experimental area. Similarly, cockchafer patches are common at Pareen alongside flinty outcrops where the soil receives more than its fair share of run-off, and depressions in paddocks are often heavily infested by cockchafers (Plates 3 and 4).

After wet summers larvae still tend to be more numerous in areas which had bare surfaces in summer, but are more widely distributed within such areas than after dry summers. In undulating country they tend to move out and colonize the slopes and the tops of the rises, and are generally more numerous in places in which eggs are laid in dry summers. There can be little doubt that the extended distribution of larvae after wet summers is mainly the result of larger numbers of females surviving to lay a larger number of eggs.

Further comments on the water content of soil on places in which eggs are laid are given in the following pages.

(c) Soil Type:

Soil type has an important influence on the choice by A.howitti females of places in which to lay eggs, because the soils on which the species is found differ widely in their water-retaining capacities. The soils not only lose water at different rates but need varying amounts of water to increase their water contents from wilting point (about pF 4.2) to the water contents which are optimal for oviposition, i.e. equivalent to a pF value of 2.8-3.2. Reference to Fig. 7 will make this clear. Wandilo sand at the pF values 4.2 and 2.8 contains 1.8 per cent and 8.6 per cent. of water respectively, but the light clay loam from Mt. Schanck contains 8.4 per cent and 31 per cent. of water at these two pF values. Consequently, if both soils are at wilting point in the field 3-4 times as much rain must fall on the loam to make it as "wet" as the sand.

The penetration of water into soils is a complex problem, but it is safe to say that the same amount of rain falling on the sand and clay loam discussed above would produce totally different conditions for beetles in the two soils. Firstly, if the rain was sufficient to saturate the sand and make its water content optimal for the adults, widespread oviposition would occur. On the loam, however, this amount of rain would only suffice to wet the depressions and beetles would only lay their eggs in the depressions. On the other hand, if sufficient rain fell on the clay loam to make its water content optimal over a wide area the sand would be very wet indeed and most of the eggs would probably be laid on well-drained slopes.

The observed variation in the distribution of A. howitti on sands and clay loams tends to follow the pattern described above.

(d) Obstacles and obstructions

Circles of larval damage are often seen around haystacks, sheds, trees or other conspicuous objects in pasture paddocks (Plate 5). Their occurrence could indicate that adults of A. howitti orientate visually to objects on the horizon as do adults of Lepidiota caudata Blackburn (Smith 1936), Melolontha vulgaris L and M. hippocastani F (Schneider 1952).

It is considered that visual stimuli may play some part in the choice of ovipositional sites because adults in flight have been observed to inspect bare areas in paddocks. There is no evidence, however, for the orientation of adults to objects on the horizon. M. vulgaris and M. hippocastani fly directly to objects on the horizon (Schneider 1952), and it is recorded of L. caudata "adults . . . . . fly directly to buildings, trees, charred stumps and fenceposts and cling to the western side of any such obstacles" (Smith 1936). A. howitti could only possibly orientate to light (a) in the very short period between the time of commencement of flight at dusk and the time when it becomes too dark for orientation to light to be possible, or (b) at dawn. But adults which have been observed flying at these times fly with the wind if there is a wind or fly apparently at random if it is calm. Their observed lack of orientation to light at these critical times is a serious objection to the hypothesis of visual orientation suggested by Carne (1956).

The flight season in the south-east of South Australia in 1955 was a particularly interesting one and threw some light on the subject above because, for all practical purposes, only one flight occurred. Consequently, the occurrence of larval damage in the winter of 1955 could be correlated with the known direction of flight and the subsequent behaviour of adults participating in this one flight. The occurrence of larval damage in this year with respect to prominent objects can be summarized as follows:-

(1) There were a few trees which were completely surrounded by extensive pasture damage. These trees had apparently sheltered sheep or were surrounded by soil of an optimal water content, and had attracted a particularly large number of beetles. Nevertheless, the bulk of the larval damage was usually found on the north or north-eastern side of the trees.

(2) Under all trees which were not completely ringed with larval damage the intensity of larval damage could be correlated roughly with the height and spread of the trees (i.e. their surface area), and with their density. Ring-barked trees or stumps usually had no more damage in their immediate vicinities than in the pasture generally (Plate 5). Similarly, sheds generally had comparatively few larvae around them.

(3) Under the trees referred to in (2) the precise location of larval damage was usually under the outer edge of the canopy of leaves, and often a belt of damage followed the outline of the canopy. In the red-gum country (Plate 5) such belts of damage were invariably on the north or north-eastern

sides of the trees.

At Keith, however, the gums (E. diversifolia) are more spindly and are inclined to lean one way or the other. In this district the circles or belts of damaged pasture varied in position in relation to the trunk of the tree but almost invariably were under the canopy of leaves, again with a tendency towards the northern side.

As a result of these observations it is suggested that adults in flight collide with objects by chance, probably after dark. It is not difficult to imagine how such chance collisions with objects may lead to aggregation in the field.

If a beetle is allowed to fly in a room with a dim light it is seen to fly until it collides with a wall; it rebounds from the wall and strikes it again 2-3 feet from where it hit the previous time. This may occur a number of times but usually the beetle falls to the ground after 2-5 collisions. A tree has a very large number of surfaces with which a beetle can collide and if a beetle flies into one it is likely to fall to the ground before it flies out again. But if a beetle hits a brick wall of a width and height similar to that of the tree it could very well be free to carry on flying in its initial direction after two or three collisions with the surface of the wall. The number of beetles which may accumulate around an object could thus be dependent on the total effective surface which the object intrudes into the path of flight of the adults. Once a beetle has fallen to the ground it is probable that soil moisture, shelter etc. determine whether it burrows into the soil or takes off in flight again.

The tendency of larval damage to be on the north or north-eastern side of trees in the winter of 1955 is particularly relevant to the hypothesis of chance collisions because during the flight of 1955 the wind was from the north to north-east and the beetles were flying with the wind from this direction. Similarly, most northern or north-eastern slopes were heavily infested with larvae this year.

In other years patches of larval damage have been seen on eastern and western sides of trees, but further detailed observation of the relation of larval damage around trees to the direction of flight of adults is necessary to confirm the hypothesis.

Larvae are also commonly found around fallen logs of wood, bales of hay, etc. The soil under such objects is usually wetter than soil in the open, so if beetles crawled under the objects by chance they would survive and lay eggs which would also survive.

(e) Animals of different kinds

Any animal which grazes pasture enters the environment of A.howitti and those animals which graze it heavily play a significant part in the ecology of A.howitti. There are many degrees of interaction, of course, between the pasture and the grazing animal, but the particular interaction of the two which directly favours oviposition by adults of A.howitti is the phenomenon known as "overstocking". This has already been discussed under "pasture".

Sheep and cattle are the most obvious herbivores which are concerned in "overstocking" pastures. A.howitti infestations have also been known to follow the ravages of rabbits, and it is a common occurrence for them to follow infestations of Oncopera fasciculata in parts of County Grey. The larvae of Oncopera simulate the action of larvae of A.howitti by damaging areas of pasture which subsequently are favourable sites for oviposition by A.howitti.

In pastures which are not overstocked there are often, however, specific areas which are made favourable for oviposition by reason of the grazing habits of the herbivores. Sheep in particular thus ameliorate the environment because in any paddock they tend (a) to use particular tracks and (b) to form "camps" (Plates 6 and 7). The characteristics of the tracks and the camps are much alike - they are bare and are covered with an accumulation of sheep droppings, and both are often used extensively as ovipositional foci. The camps are situated along fences, beneath trees, or on slopes on which the animals can be exposed to wind, and are particularly favoured as places in which to lay eggs. It is true that there is an accumulation of dung in such areas and certainly a number of beetles may be attracted to the sites because of the dung; but dung is not a necessary component of a place in which to lay eggs. It is more probable that the accumulation of dung acts as shelter, and when coupled with the higher moisture content of the sub-surface soil and the looseness of the surface, produces a place in which adults readily aggregate.

(f) Other animals of the same kind

Other animals of the same kind may influence the choice of places in which to lay eggs, in two ways:- (1) as larvae and (2) as adults.

(1) As larvae:

Pastures which are severely damaged by larvae of A.howitti rarely recover in the spring, so that when the adults fly the following summer the damaged areas are relatively bare and many eggs are laid in them. The eggs are most frequently laid along the edges of the bared areas and the ensuing larvae migrate out further in search of food. The area of damage may thus increase in size from year to year as the larvae each year destroy the pasture and again make the site attractive for adults the following summer.

Damage to pastures by larvae of A.howitti is probably also a major cause of infestation by annual weeds such as capeweed (Cryptostemma calendulaceum). These weeds die off in summer in much the same manner as T.subterraneum and similarly perpetuate the favourability of the pastures as places in which to lay eggs (Plate 6). The weeds then increase as the larvae destroy more pasture.

(2) As adults:

Larvae in a very heavily infested area are often of different ages, so it is clear that adults from a number of flights have laid their eggs in the same area. But sometimes, as in 1955, very heavy infestations may result from the one flight. Nevertheless, there is no evidence for the settling of a swarm of adults, and we have to look elsewhere for the causes of the aggregations which occur.

No evidence could be obtained in the laboratory for the attraction of adults to other adults beneath the soil surface. The data suggested, though, that adults might aggregate in areas in which other adults had burrowed previously because the original beetles had made the surface soil more favourable for burrowing. A certain amount of snowballing may thus occur as Carne (1956) has suggested, particularly in years in which a number of flights occur; but it is problematical how significant is the amelioration of the surface in this way in the field because the surface must have been favourable initially for the original beetles to burrow in.

Observation does suggest, however, that adults may be stimulated, in the presence of others, to form loose aggregations on the surface. In the laboratory, when adults were extremely active, they sometimes formed a tight bunch of milling beetles around an individual which had started to burrow, and then all the beetles disappeared into the soil down the same hole. Loose aggregations of adults have been observed by Carne (1956) before flight and they probably form in places in which adults accumulate by chance, i.e. under trees. In such situations it is quite likely that larger numbers of adults will burrow into the soil where only a few beetles would have burrowed individually. Their behaviour may thus make the penetration of a type of surface easier, but whether or not the beetles stayed and laid eggs would probably still depend on the individual's choice of water in soil.

It is difficult to see how, in apparently uniform pastures, adults could accumulate to form aggregations which result in highly localized patches of larval damage. The alternative, that the pastures only appear to be uniform, is nearer the truth and leads to a more satisfactory solution. It was seen from Field Experiment 2 that adults responded to the water content of the soil in a plot 6 feet x 3 feet which was, to all intents and purposes, highly uniform. So in 40-50 acres of apparently uniform pasture it is highly probable that considerable variations exist in the water content of the soil. The distribution of larvae in such pastures is explicable in terms of the responses of adults to water in the soil and does not necessitate considerations of the attraction of adults to others in the soil.

(g) Topography

In 1955 larval damage frequently occurred on slopes and the tops of rises in undulating country, particularly on the windward side. This suggests that adults in flight might settle on elevated ground if the wind velocity, increasing with elevation, exceeds the critical value for flight activity. Alternatively, they could well collide with rising ground as they appear to collide with other obstacles in the path of flight. As has been mentioned before, such elevated ground is often the site of a sheep camp but, in addition, adults could sometimes accumulate in these places as postulated above.

(V) THE INFLUENCE OF TEMPERATURE AND MOISTURE ON THE SURVIVAL-RATE AND DEVELOPMENT OF EGGS

The eggs of *Aphodius howitti*, when newly laid, are ovoid and yellowish and they are about 1.4 mm long and about 0.9 mm wide. They are found in batches in the soil, each female laying all her eggs in one batch. The depth at which they are laid is influenced by the depth of the soil, its compactness and its water content, but when none of these factors are limiting they are found at a depth of 6-8 inches under the surface of the soil.

The eggs are laid at a time of the year when lack of moisture is probably the major hazard limiting their chance of survival. Some information was therefore required of the ability of eggs to tolerate water loss and their ability to absorb water from soil. It was desired to know, in addition, the rate of development of eggs at constant temperatures.

(a) Speed of development at constant temperatures

Davidson (1944) used the logistic curve to express the relationship between temperature and the rate of development of insects, but Browning (1952) showed that, although this curve closely followed the observed points, deviations of the points from the curve were significant. Therefore eggs which were 0-24 hours old were placed on moist filter paper in petri dishes at various temperatures and the numbers of larvae which hatched each day were recorded. The mean duration of the incubation period was then calculated for each temperature. These were plotted against temperature (Fig. 10) and a freehand sigmoid curve was drawn through the observed points. The mean percentage development per day was likewise calculated and a freehand curve drawn through the points, (Fig. 10). These computations were considered sufficiently accurate for ecological purposes.

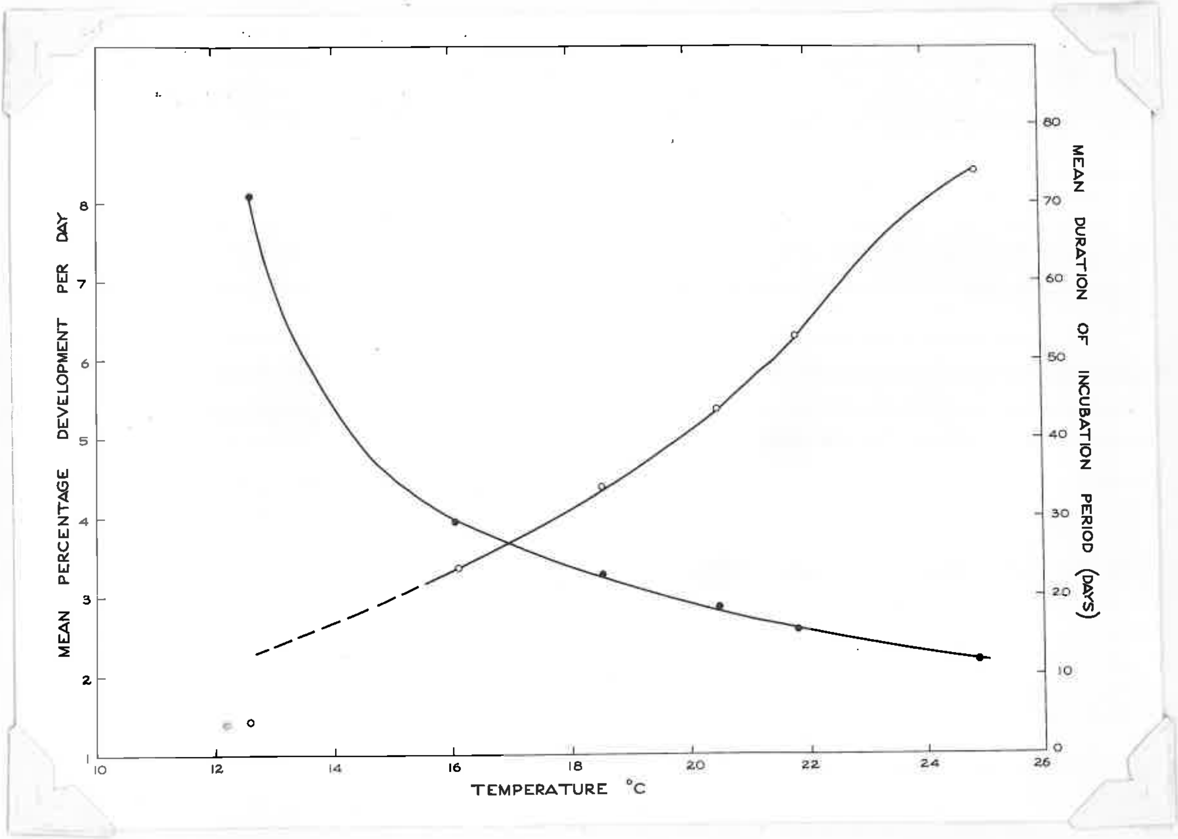

Fig. 10. The development of eggs of A. howitti at constant temperatures.

(b) <u>The absorption of water by eggs</u>

The eggs of A. howitti, in common with those of other insects which lay eggs in soil, require contact water for normal development.

In addition to determining the water content of soil from which eggs can absorb water, it was desirable to know at what stage of development the uptake of water occurred.

In almost all studies on the uptake of water by insect eggs estimated changes in water content have been based on changes in the wet weight of the egg as a whole. This procedure is admissible because the error introduced by the weight of dry matter used in oxidative processes is always small compared with the changes in water content that occur. Moreover, insect eggs require no substances other than water from the environment so that no error is introduced in this way (Needham 1942).

Eggs were weighed individually on a torsion balance to 0.01 mgm. They were obtained, when 0-24 hours old, from beetles which were kept in soil containing the optimum water content for oviposition. Fifty eggs were drawn at random from 17 egg-batches and were kept, during the course of the experiment, on small numbered squares of filter paper in petri dishes over water. The filter papers were saturated with water and the petri dishes were kept in air-tight jars over water at 20.4 ± 0.1°C. Preliminary observations had indicated that eggs did not absorb water at all until they had been at 20°C for 5-6 days. The eggs were therefore first weighed on the fifth day and were weighed on every successive day until the first larva emerged on the sixteenth day. They were then dried in an oven at 105°C, the dry weight of each egg was recorded, and the percentage water content of each egg was calculated from the fifth day onwards.

Ten eggs did not absorb water and turned black towards the end of the incubation period. This had previously been recorded for whole batches of eggs which apparently had not been fertilized. There was no way of distinguishing these sterile eggs until about ten days after incubation when it became apparent that they were not simply eggs which were commencing water uptake abnormally late. Seven eggs were injured in the process of weighing and, with the sterile ones, have been omitted from the results.

The mean, standard error and the increment of the per cent. increase in wet weight per day are given in Table 5 Appendix. The mean per cent. water content per day was calculated and is plotted against time of incubation in days in Fig. 11.

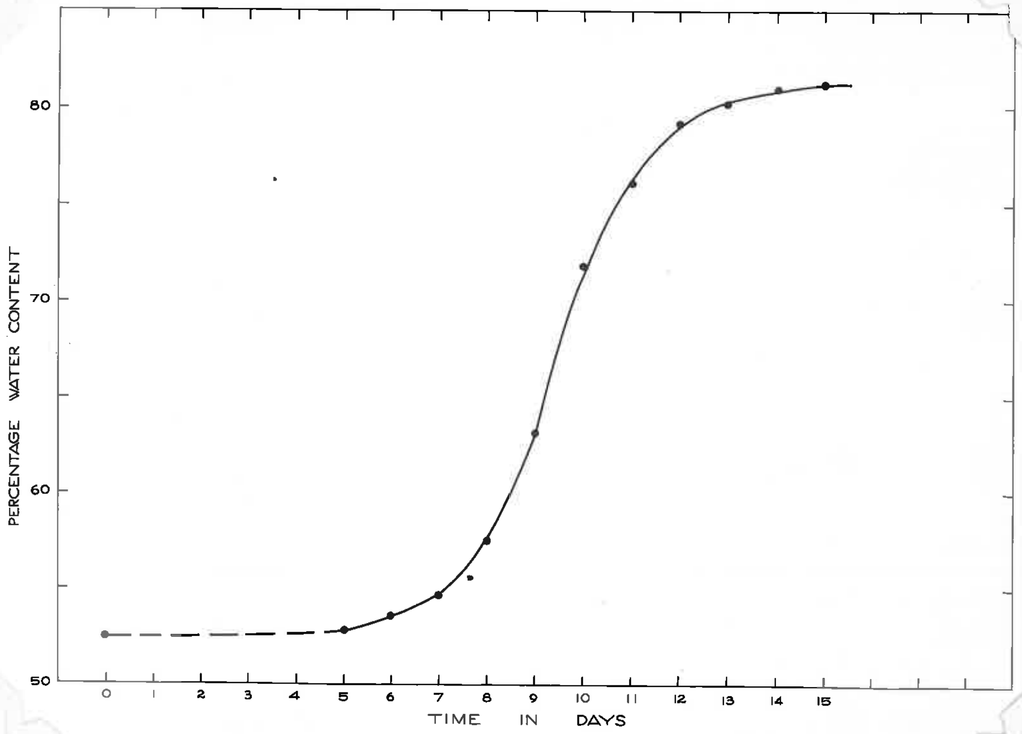

Fig. 11. The mean daily percentage water content of eggs during development at 20°C.

The wet weight and water content of eggs on the fifth day are taken as the original wet weight and water content. An increase in wet weight of 154.6 per cent. therefore resulted in an increase in water content of 28.5 per cent. No significant correlation ($r = 0.269$ with 31 d.f. $p<10\%$) was found between the original wet weight of eggs and their water contents, or between the original water content of the eggs and their subsequent increases in water content ($r = 0.221$ with 31 d.f. $<p$ 10%).

The variation in the uptake of water between individual eggs was considerable, but the data indicated that when eggs were kept at 20°C the greater proportion of water uptake had occurred by the eleventh or twelfth day.

The eggs of Scarabaeidae do not appear to possess a hydropyle. Kerenski (1930) demonstrated that water is absorbed through the general surface of the membranes of eggs of *Anisoplia.* He also showed that in *Anisoplia* eggs the gain in weight was not simply due to osmosis. Laughlin (1953), on the other hand, has suggested that the egg of *Phyllopertha horticola* L. absorbs water passively due to its high osmotic pressure, but no measurements of the osmotic pressure are given.

Lower (1957) has made a study of the cuticular membranes of eggs of *Aphodius howitti* and has suggested that the initial restriction of entry of water into the eggs is due to a specialized lipoid layer, about 0.5µ in thickness, located immediately inside the vitelline membrane at the time of oviposition. About the fifth day at 20°C the blastoderm begins to form and by the time it is complete the water resistant layer has disappeared. These changes coincide with the commencement of absorption of water by the eggs.

Preliminary observations have suggested that the absorption of water by eggs of *Aphodius howitti* is not a passive process. Further investigations are planned.

(c) The effect of the water content of soil on the absorption of water by eggs and their viability

(A) Laboratory experiment

Most, if not all, insect eggs which are laid in soil require liquid water for normal development, and the water content of the soil has often been demonstrated to markedly affect their viability. The eggs of a number of *Phyllophaga* sp. for example, when placed in soil with different water contents, hatched in largest numbers between water contents denoted as 20 and 73 per cent. of soil saturation (Sweetman 1931). Similarly, the development of eggs of *Smythurus viridis* was retarded when the water content of the surface soil fell below about 40 per cent. of saturation and could be completely arrested if low values were maintained (Davidson 1932b), and the literature on the absorption of water by locust eggs is extensive (Roonwal 1936; Husain *et al.* 1940; Birch and Andrewartha 1942; Matthee 1951).

Matthee (1951) demonstrated that the eggs of *Locusta pardalina* were in equilibrium in a 0.65M solution of sucrose; in hypotonic solutions they absorbed water and in hypertonic solutions they lost water. When their changes in weight are plotted against the osmotic pressure of the sucrose solutions two rough linear relationships exist - one above and one below the equilibrium point. The egg of L.pardalina can, like Evan's wireworm, be regarded as an osmotic system and its ability to absorb water is clearly related to the energy with which water is held. Its ability to absorb water from soil would thus be related to the pF value of soil rather than its water content. It was probable that the ability of the eggs of A.howitti to absorb water was, similarly, related to the available water in the soil. An experiment was therefore carried out with two soils to determine whether the pF scale could be used directly as a criterion of the ability of eggs to absorb water and survive in the two soils.

The clay loam and sand used in the experiments with adults were used again, and water contents of the two soils were read from the pF-water-content curves (Fig. 7) to correspond to pF values of 2.5, 3.0, 3.25, 3.5, 3.75 and 4.0.

Quantities of both soils were obtained from the field and were moistened with water to obtain water contents 5-10 per cent. or so above the required ones. The soils were then spread out on trays and allowed to dry slowly to the required water contents. Quantities of soil of the required water contents were placed in bottles with air-tight lids and stored at 20.2°C for two weeks, during which time they were shaken 5-6 times a day. Plastic tubes with air-tight lids were then filled with the soils of required water contents and stored at 20.2°C for 3-4 days before the experiment was started. Weighing of the tubes of soil after the experiment indicated that a negligible amount of water had been lost. As a result, the soil-water-energy conditions in this experiment were more closely defined than those used in the experiments on adults.

In addition to the above twelve treatments (2 soils x 6 pF's) eggs were placed on moist filter paper over water (treatment 13). The eggs were obtained when they were 0-24 hours old. Those in any one batch were allotted singly, and at random, to all the treatments in the first replicate until the number of eggs remaining was less than the number of treatments. These were then discarded and the procedure repeated with another batch until the required number in replicate 1 was attained. Eggs were then similarly randomized in replicate 2 and later in replicate 3. This procedure, which was necessary because of the relatively high proportion of sterile eggs, increased the risk of random variation between replicates but ensured that the random variation between treatments was at a minimum. Replicate 1 consisted of 17 eggs per treatment and there were 10 eggs per treatment in replicates 2 and 3.

Eggs were kept on moist filter paper at 20.2 ± 0.1°C after being laid. They were first weighed on the fifth day in groups of five so that each group in any one treatment was directly comparable to the other groups of the same number in the other treatments. There were three groups of five and one of two eggs in replicate 1, and two groups of five in replicates 2 and 3. No significant difference was found between the original weights of groups of eggs in the different treatments.

The eggs in replicate 1 were weighed before treatment but could not be weighed again before the eggs had hatched, and so only the number of eggs which hatched were recorded. Eggs in replicates 2 and 3 were washed on the fifteenth day in solutions of sodium chloride, the osmotic pressures of which were equivalent to the suction forces of water in the respective treatments. The eggs were then weighed; most of the absorption of water had occurred by this time.

The estimated absorption of water in the different treatments is therefore based on four groups of five at each treatment. A number of eggs which did not absorb water in each group of five were considered to be sterile and were rejected. The mean weights on the fifteenth day were based on the weights of the survivors only. The mean weights before and after treatment were used to calculate the mean percentage increases in weight for each group, which are recorded in Table 6.

The main point of interest in the results was in the testing of the hypothesis that the absorption of water by eggs was influenced by the energy with which the soil held water rather than by its water content. The analysis of variance showed that there was no significant difference between soils at the same pF values (V.R. <1.0 with 5 and 36 d.f.), and so did not furnish any evidence against the hypothesis that the absorption of water by eggs was related to the "available water" in the soil. The increases in weight of eggs in soils at different energy levels could not, however, be attributed to chance (V.R. = 190.3 with 5 and 36 d.f., $p < 0.1\%$). The minimum differences for significance indicated that -

(a) eggs in soil at a pF value of 2.5 absorbed more water than eggs in soil at a pF value of 3.5, but the difference was not significant at the 1 per cent. level.

(b) the difference between the percentage increase in weight of eggs at the pF values of 3.75 and 3.5 was significant at the 0.1 per cent. level.

(c) the difference between the uptake of water by eggs at the pF values 3.75 and 4.0 was also significant at the 0.1 per cent. level. In fact, the eggs in soil at a pF value of 4.0 lost a considerable part of their original weight and obviously differed from eggs of all other treatments.

In addition, the mean percentage increase in weight (152.2) of eggs kept on moist filter paper did not differ significantly from that of eggs in soil at any of the pF values 2.8, 3.0, 3.25 and 3.5, but did differ significantly from that of eggs in soil at a pF value of 3.75 ($t = 6.22$ with 10 d.f., $p < 0.1\%$).

The data therefore suggests that eggs -

(1) absorb water from soil in relation to the energy with which the water is held in the soil.

(2) can absorb the necessary water for normal development from soils at a pF value of 3.5 or less.

(3) can absorb only a portion of the necessary water for development or absorb it at a slower rate, from soil at a pF value of 3.75.

(4) cannot absorb water from soil at a pF value of 4.0 and cannot retain their original water at this energy level.

The percentage emergences of larvae from eggs at the different treatments after 25 days are given in Table 7. The eggs which had been in soil at a pF value of 4.0, and which had lost 29.8 per cent. of their original weight, were placed on moist filter paper for a few days, but they did not develop and were considered to be dead. The survival of eggs in soil at this pF value was so obviously different from that in all other treatments that they were omitted from the subsequent statistical analysis.

Table 6

The percentage increase in weight of eggs at different treatments

Type of soil	Group No.	Treatment pF of soil 2.5	3.0	3.25	3.50	3.75	4.0	on moist paper*
Sand	1	163.8	183.0	167.1	156.3	109.2	-35.6	160.1
	2	167.8	155.2	151.3	115.9	117.6	-36.0	145.5
	3.	150.2	145.8	158.9	127.1	90.7	-29.4	140.0
	4	185.9	154.5	139.3	158.5	91.1	-34.2	163.2
Clay Loam	1	199.6	145.9	141.7	172.5	114.7	-19.2	
	2	147.1	161.4	156.5	121.2	85.9	-27.4	
	3	151.0	157.6	165.5	166.8	110.3	-31.4	
	4	151.1	156.8	168.0	154.1	116.8	-25.2	

* Not included in analysis of variance

Table of treatment totals for analysis of variance

Soil	pF of soil 2.5	3.0	3.25	3.50	3.75	4.0	Σ	$\bar{x}$
Sand	667.7	638.5	616.6	557.8	408.6	-135.2	2754.0	114.8
Loam	648.8	621.7	631.7	614.6	427.7	-103.2	2841.3	118.4
Σ	1316.5	1260.2	1248.3	1172.4	836.3	-238.4	5595.3	
$\bar{x}$	164.6	157.5	156.0	146.6	104.5	- 29.8		

Analysis of variance

Source of variation	D.F.	S.S.	M.S.	V.R.	p.
Total	47	233,212.69			
Treatments	11	224,733.61	20,430.33		<<0.1%
Soils	1	158.80	158.80	-	N.S.
pF's	5	224,048.29	44,809.65	190.3	<<0.1%
pF x soil	5	526.52	105.30	-	N.S.
Error	36	8,479.08	235.53		

S.E. of Δ of two pF means = 7.67

Minimum difference for significance 5% = 15.6
1% = 20.9
0.1% = 27.7

The remainder of the results were transformed to degrees and subjected to an analysis of variance (Table 6, Appendix). No significant difference was found between the treatments (V.R. = 1.47 with 10 and 22 d.f., p >20%). The percentage emergence of larvae from eggs kept on the moist filter paper was then omitted, and the transformed data for the percentage emergences of larvae from the two soils at the different pF values were subjected to an analysis of variance (Table 6, Appendix). No significant difference was found between soils or between pF values (V.R. for soils = 2.15 with 1 and 20 d.f., p >20%; V.R. for pF values = 0.5 with 4 and 20 d.f. N.S.).

The finding of no significant difference between soils, coupled with the total lack of survivors in both soils at the pF value 4.0 adds support to the hypothesis that eggs of <u>Aphodius howitti</u> are influenced by the energy with which water is held by the soil rather than by its water content

The lack of significance between pF values was interesting in view of the conclusion arrived at earlier that eggs absorbed more water at pF 3.5 than at pF 3.75. The mean incubation period was not noted, so it is not known whether the eggs at pF 3.75 hatched without their full complement of water, or whether development was slower and the eggs hatched later after having absorbed the same amount of water as those at lower pF's.

(B) Field observations:

It remains to discuss whether these results can be applied to the field. In the experiments described above all the eggs were in direct contact with the soil, but in the field the eggs are laid in batches of 25-50 and not more than 10 may be in direct contact with the soil. The

Table 7

The percentage emergence of larvae from eggs in various treatments

Replicate No.	No. used in replicate	Treatment							
		Soil Type	pF of soil						on moist paper
			2.5	3.0	3.25	3.50	3.75	4.0	
1	17	Sand	29	41	53	47	41	0	59
2	10	"	40	50	30	20	30	0	50
3	10	"	50	70	60	40	30	0	50
1	17	Loam	47	47	41	53	59	0	
2	10	"	40	40	30	50	50	0	
3	10	"	60	50	50	60	40	0	

eggs in a batch, when first laid, are held together by a thin film of mucilage around each egg but the individual eggs can be separated if the batch is immersed in water for a few hours. All the eggs in a batch absorb water, irrespective of position, if the batch is placed on a moist surface, and it is likely that water is held as a thin film on the surface of the eggs and so passes from one to another. Usually the eggs fall apart after a certain amount of water has been absorbed. When the soil has been very wet the eggs in contact with the soil are often dead, but differential survival of eggs due to their position in the batch has never been observed in dry soil. The application of the laboratory data to the field is therefore not invalidated by differences in the behaviour of eggs in the experiments and in the field.

The eggs in the field are exposed to fluctuating temperatures whilst eggs in the laboratory experiment were at constant temperature. Movements of water in the soil due to temperature gradients are not, however, likely to influence the ability of eggs to absorb water from surrounding soil because of the small quantities of water involved and the periodic reversal of the temperature gradient.

Estimates of the differential survival of eggs in relation to water content in the field are difficult to obtain because of the tendency of the adult to lay eggs at a water content which is well within the optimum range for the development of eggs. The water contents of soil at the depth at which the eggs are normally laid should, however, give reasonable estimates of the probability of survival of the eggs at sampling sites in the field. Four soil samples were therefore taken at a depth of 8 inches from each of three stations which had a bare surface and from each of three stations which had a cover of stubble. The samples were taken on March 24th, 1956 within a radius of 50 feet of each other in a paddock at Mt. Schanck. The percentage water contents of the soil (clay loam) were determined in Adelaide and are presented in Table 4 Appendix. The difference between the mean water contents under the two types of surface was significant at 0.1 per cent. level. The equivalent pF values for the means are 3.70 under a bare surface and 4.05 under stubble. Eggs laid in the soil under the bare surface would, therefore, on the average, have been able to absorb water and develop whilst those laid under stubble would have died.

(d) <u>The advantages of measuring water in soil on an energy basis</u>

The water available to insects and other animals in the soil is not dependent solely on the water content of the soil. The eggs of

Schistocerca gregaria, for example, hatched at different water contents in different soils (Husain et al. 1940) and the threshold water content for the hatching of eggs of two species of Limonius varied according to soil type (Jones 1951). Attempts have been made in the past to measure the "available water" in soil by using the saturation value of soil (Lees 1943; Davidson 1932a, b; Fidler 1936) or the concept of maximum hygroscopic moisture (Husain et al. 1940), but they have been unsuccessful because the so-called equilibrium points only express one relationship which may exist between soil and water (Baver 1948).

Despite the demonstration by Evans (1943, 1944) and by Evans and McGuild (1948) that Schofield's pF scale was a convenient measure of "available water" in soils to animals and its widespread use by plant physiologists, the measurement of water in soil on a free-energy basis has been virtually neglected by animal ecologists. The pF scale has several advantages over the employment of capillary potential as such as a measure of water in soil (Baver 1948). It may also be of utility in ecology because (a) it relates the suction forces in the soil to the percentage R.H. of the soil atmosphere through the equation $pF = 6.5$ plus $\log_{10} (2 - \log R.H.)$ and (b) it is related to the suction force of solutions of varying osmotic pressure through the equation $pF = 3$ plus $\log_{10}$ O.P. (Evans 1944).

The results of the last experiment indicated that the egg of A.howitti, like that of Locusta pardalina, absorbed water in relation to the energy with which it was held in the soil. Similarly, pressure deficiency influenced the hatching of eggs of Heterodera schachtii within the cysts; larval emergence ceased in solution of 0.65 M sucrose and 0.42 M urea (Wallace 1956b). The osmotic pressure exerted by these solutions is approximately 16 atmospheres and corresponds to the free energy value in soil at wilting point, i.e. approximately pF 4.2. Locusta and Heterodera should thus absorb water from soil at the same pF value. Extrapolation suggests that eggs of A.howitti cannot absorb water from soil which has a pF value higher than 3.95, and Davidson's (1932b) figures suggest that retardation of development of eggs of Smynthurus viridis commences at a pF value of about 3.5.

The ability of insect eggs to survive in soil is dependent on (a) the rate at which they lose water in "unsaturated" atmospheres and their ability to tolerate this loss of water, and (b) their ability to absorb water from soil. But the amounts of water which eggs lose in soil may be influenced by their relative abilities to absorb water from the soil. For example, in soil at pF value of 4.0 eggs of Locusta would absorb water, whereas eggs of A.howitti would lose water. The pressure deficiency against which eggs can absorb water can be determined by immersing eggs in chemical solutions when the eggs are in the developmental stage in which they absorb water. Eggs can only develop, however, at some pF smaller than that at the equilibrium point, and the range of pF values in which they can develop must be determined in soil.

The outstanding advantage of measuring water in soil on an energy basis is that it permits the examination of other factors, such as pore-space distribution and aeration, which also influence the distribution and abundance of animals in soil.

Parry (1954), for example, demonstrated that two species of lycosid spiders drank water from carborundum powders at a rate which depended on the suction to which the capillary water was subjected. With increasing suction spiders could not drink water from any particular powder, either when most of the capillaries had emptied or when the suction exceeded a certain value - whichever happened first. When the particle size of the soil was large, the spiders could not drink water from the soil above a critical tension because the capillaries had emptied and no water was available; when the particle size was small, spiders could not drink water from above a critical tension because they could not overcome the suction. The results indicated that the limiting suction in sandy soils from which spiders could drink water was lower than that in clay soils. Consequently, if water limits the distribution of the spiders they should be found in clay rather than in sandy soils, i.e. those with higher "water - retaining capacities".

It has been postulated that the distribution of Diabrotica 11-punctata in the foothills of California and of Acheta commodus in South Australia are similarly explicable in terms of the water retaining capacity of soils (Smith and Michelbacher 1949; Browning 1954). The availability of water in soil to these animals should be capable of analysis along similar lines to Parry's.

It has often been thought that the aeration of soil was an important factor limiting the distribution of animals in soil. That it may be so has been recently shown by Wallace for Heterodera schachtii in an interesting series of papers.

The rate of emergence of larvae from cysts of H. schachtii in sand was related to the moisture characteristic of the sand. The pressure deficiency at which the maximum rate of larval emergence occurred was, however, dependent on sand particle size, and subsequent investigations confirmed that larval emergence was related to the pore-space distribution rather than the pressure deficiency. Maximum emergence occurred approximately at the points of inflexion of the moisture characteristics (Wallace 1955a, b).

The addition of clay to sand reduced the porosity of the sand, and Wallace suggested that the rate of diffusion of oxygen probably influenced the rate of larval emergance which was drastically reduced as the clay content increased (Wallace 1956a).

Wallace (1956c) further demonstrated that there was an optimum pressure deficiency for eelworm migration and suggested that there was a similar relation between the distribution of water in sand and migration. The ecological implications of this work have been discussed by Wallace (1955a, b; 1956a).

(VI) THE ACTIVE LARVA - THE INFLUENCE OF THE ENVIRONMENT ON THE SURVIVAL RATE AND RATE OF DEVELOPMENT

The aphodiid larvae which damage pastures in south-eastern Australia have been ascribed by Given (1950) to the three species A.howitti Hope, A.yorkensis Blackburn and A.pseudotasmaniae Given. The larvae of these species are much larger than most aphodiid species, native or exotic, and have the peculiar habit, for scarabs, of feeding on the foliage of pasture rather than its roots.

There are three larval instars of A.howitti, which is the only species found in the South-east of South Australia. The first and second instar larvae subsist mainly on humus and leaf debris on the surface of the soil. Most of the damage to pasture is done by third instar larvae.

Estimates of mortality, especially of the 3rd instar, are difficult to obtain in the field because, when food is in short supply, larvae have the disconcerting habit of migrating over the surface of the soil in search of it. Consequently, there is usually a movement of larvae outwards from the foci of infestation. When the densities of larvae in the foci are high the pasture may be completely destroyed, and the movements of larvae outwards in search of food result in the formation of "fronts". As has been pointed out by Carne (1956), the density of larvae/sq. foot in advance of a "front" is relatively low; it is highest on the "front" and drops off considerably behind the "front" over a distance of 2-3 feet. Plate (8) depicts the clear-cut demarcation of pasture damage which may occur as the result of concentrated feeding along a "front".

The behaviour of the larvae renders sampling futile under these conditions, unless an allowance is made for the extended area of ground colonized by the larvae, or the rate of movement of larvae out of areas which are sampled.

When larval density is relatively low food is rarely in short supply, so there is little larval movement and sampling estimates are more reliable. Consequently, estimates of larval mortality in the field have been derived by (a) sampling plots in which the movements of larvae have been stopped by the addition of a suitable quantity of food - this method has been used particularly for the first instars -, and (b) sampling plots which have been seeded with larvae so that the density of larvae/sq. foot was relatively low.

(i) The effect of temperature and water

The mean monthly soil temperatures at 9 a.m. at a depth of 6 inches under pasture at Mt. Burr are shown in Table 7 (Appendix). These temperatures have been taken as approximating to the mean soil temperature at Mt. Gambier at a depth of 6 inches under pasture - which is about the depth at which larvae of A. howitti are found. Larvae in the field are seldom exposed to lethal temperatures, so temperature is chiefly important for its influence on the speed of development.

The maximum damage to pastures occurs when the temperature of the soil is 9-13°C, and it is probable that larval development proceeds most rapidly in this range of temperature.

A deficiency of water, on the other hand, may affect the survival-rate of young larvae; an excess of water may influence the survival-rate of any stage, but principally that of the 3rd instar. Water may also affect the rate of development, since larvae only feed when the soil is wet.

(1) <u>Laboratory experiments</u>

(a) The migration of 1st instar larvae to the surface.

During early attempts to rear young larvae it was noticed that they (a) migrated to the surface without feeding and then fed on dung, leaf debris or humus on the surface of the soil, and (b) seemed to migrate to the surface only if the soil was wet. An experiment was therefore conducted to determine (1) at what water content of soil migration occurred, and (2) whether the activity of the young larvae in soil was related to the pF value of the water in soil.

Eggs which were about to hatch were placed in Wandilo sand and Mt. Schanck clay loam having pF values 2.5, 3.0 and 3.5. The soils were initially made wetter than was required because (1) dry soil is too crumbly for experiments with young larvae, and (2) the pF-water content curves for the soils (Fig. 7) were "drying curves". Quantities of wet soil were then placed in 3½ inch waxed paper cups, holes a quarter inch in diameter were made in the wall of the cups near the bottoms and "cells" were made in the wet soil with a piece of glass rod. The holes were then covered with waxed paper, the cups were weighed and the soils were allowed to dry to the water contents with the required pF values. The waxed papers over the holes were then removed, the eggs were randomized amongst the treatments and were placed in the "cells" and the holes were covered again. Fragments of dung were placed on the surface of the soil to feed the larvae after they migrated to the surface. The cups were then covered with plastic material and stored in an incubator at 22^{o}C.

Ten eggs were used in each of three replicates for each treatment. The number of larvae found at the surface one week later are recorded in Table 8. Not all the larvae at pF 2.5 had migrated by the time the cups were examined, and several larvae at the surface had not fed or had fed very little.

Table 8

The proportions of larvae which migrated to the surface of the soil in response to the water content of soil. The denominators are the total number of larvae recorded (alive) in each cup of soil

pF value of soil	Replicate No.	Type of soil	
		Sand	Loam
2.5	1	6/10	6/9
	2	7/9	8/10
	3	7/10	5/8
	Σ	20/29	20/27
3.0	1	2/9	1/8
	2	0/9	0/8
	3	1/10	0/9
	Σ	3/28	1/27
3.5	1	0/7	0/9
	2	0/10	0/10
	3	0/9	0/8
	Σ	0/26	0/27

The data indicated that (a) larvae migrated in response to the water content of soil, (b) larvae did not feed below the surface, (c) the response to water was, within the limits of the experiment, a response to the available water in the soil; migration occurred at pF value 2.5 in both soils but was inhibited below pF 3.0, (d) migration to the surface occurred a few days after the soil had been moistened.

(b) The tolerance of first instar larvae to drought before migration to the surface

It seems that, in nature, larvae come to the surface only after rain has penetrated the soil to the depth at which the eggs were laid. The occurrence of suitably heavy falls of rain varies considerably from year to year, and in some years larvae apparently do not migrate to the surface until some considerable time after hatching. Sampling in the field in 1955 indicated that up to 50 per cent. of young larvae might die if they had not come to the surface by 4-5 weeks after emergence from the eggs, and a laboratory experiment confirmed that the survival rate of young larvae was influenced by the length of time that they spend in dry soil before moving to the surface. It was possible to get an estimate of when the eggs hatched and when the larvae migrated to the surface in the field each year; so it was considered that an estimate could be made of the percentage mortality of the young larvae during this period from year to year. It was necessary, for this purpose, to determine how long larvae could survive in soil before migrating to the surface.

It was thought that young larvae in dry soil died either from starvation or from water loss, so the effects of both factors on the survival-rate of larvae were examined. Larvae were kept in soil having pF values of 3.30, 3.80 and 4.10 - all of which were above the critical value for migration to the surface. The numbers of larvae alive and their weights were recorded after 1, 2, 3, and 5 weeks. A control was kept in soil which had a pF value of 2.5.

The required pF values of soil were obtained by the method used in the previous experiment. Unfortunately, the clay which was to be used became mouldy and sand was used instead.

Six hundred and fifty newly-hatched larvae were randomized into 13 petri dishes, and the 50 larvae in each petri dish were allotted at random to one of the 12 treatments or the control. The larvae to be placed at each treatment were then randomly sorted into five lots of 10, and each lot of 10 was weighed and placed in a cell in a cup of soil. There were thus five replicates for each treatment. Dung was placed on the surface of the soil in the cups containing the larvae of the control group so that they could commence feeding immediately. The cups were then stored at random in an incubator at 18 ± 0.1°C and rolls of blotting paper standing in beakers of water were placed in the incubator to raise the humidity and reduce the loss of water from the soil in the cups.

After the required time each cup was weighed to obtain an estimate of the amount of water lost by the soil and hence its resultant water content. The number of larvae alive in each cup was recorded and they were weighed in a group.

The cups containing the larvae of the control group were examined after the same intervals of time. The cups were weighed after the dung had been removed from the surface, water was added to restore the water content of the soil to a pF value of 2.5, and the number of larvae alive in each cup was recorded. The larvae were weighed in a group and were then replaced in the soil in artificial tunnels. The same larvae served as controls during the course of the experiment.

The numbers of larvae alive in each replicate after treatment are given in Table 9. The control data were not included in the analysis of variance which was conducted on the remaining untransformed data, and are presented in Table 9. The variance ratio for treatments was significant at the 0.1 per cent. level, and when the treatment sums of squares was partitioned it was seen that the largest proportion was due to differences in the numbers of larvae alive at the different times. The interaction of pF x time was not significant and the differences between pF values could likewise be attributed to chance, but the variance ratio for times was significant at 0.1 per cent. level. The minimum differences for significance between the means of the times indicated that (a) the difference in survival at 1 and 2 weeks was significant at 0.1 per cent. level, (b) the difference between 2 and 3 weeks was significant at 5 per cent. level but not at 1 per cent. level, (c) the difference between 3 and 5 weeks was significant at 1 per cent. level but just not significant at 0.1 per cent. level, and (d) the differences between 1 and 3, 2 and 5, and 1 and 5 weeks were all significant at 0.1 per cent. level.

The control mortality is also given in Table 9. A χ^2 test indicated that the mortality of larvae within the treatments did not differ significantly from that of the control after 1 week; the differences in survival between the control and the treatments after 2, 3 and 5 weeks were however all significant at the 0.1 per cent. level. The mortality

Table 9

The numbers of young larvae alive (out of 10) in soil at 3 pF values after 1, 2, 3 and 5 weeks

		pF value of soil			
Time	Group	3.30	3.80	4.10	Control
1 week	1	6	9	10	7
	2	9	3	8	6
	3	6	7	8	6
	4	8	10	7	9
	5	5	8	6	10
2 weeks	1	4	4	5	7
	2	6	5	3	6
	3	2	8	6	6
	4	6	5	5	9
	5	7	4	3	10
3 weeks	1	6	1	3	7
	2	3	4	7	6
	3	1	3	6	6
	4	5	2	4	9
	5	2	0	4	10
5 weeks	1	1	1	2	7
	2	0	1	0	6
	3	1	1	5	6
	4	0	0	1	8
	5	4	1	0	10

Table of treatment totals

	pF value of soil					Control*	
Time	3.30	3.80	4.10	Σ	$\bar{\bar{x}}$		$\bar{\bar{x}}$
1 week	34	37	39	110	7.33	38	7.60
2 weeks	25	26	22	73	4.87	38	7.60
3 weeks	17	10	24	51	3.40	38	7.60
5 weeks	6	4	8	18	1.20	37	7.40
Σ	82	77	93	252			
$\bar{\bar{x}}$	4.10	3.85	4.65				

* not included in analysis of variance.

Minimum difference for significance between means of pF values: 5% level = 1.30

Minimum difference for significance between means of times: 5% level = 1.30

1% level = 1.73

0.1 % level = 2.26

The analysis of variance

Source of variation	d.f.	S.S.	M.S.	V.R.	p
Treatments	11	324.0	29.45	9.44	<0.1%
pF's	2	6.7	3.35	-	N.S.
Times	3	298.5	99.50	31.89	<0.1%
pF's x times	6	18.8	3.13	-	N.S.
Error	48	149.6	3.12		
Total	59	473.6			

of larvae after 1 week could thus have been due to chance or experimental error, but the mortality of larvae in the treatments between 1 and 5 weeks could not be attributed to either of these sources of error. The data suggest that the larvae died of starvation rather than water loss after the first week.

Similar results were obtained with the weights of larvae. The differences in the weights of groups of larvae before the experiment were not significant (V.R. = 1.6 with 11 and 48 d.f.). The % gains in weight after treatment are given in Table 10.

The large differences in the mean weight gained by larvae in the control group and in the treatment groups stress the importance of water for optimal development. An analysis of variance indicated that the increases of weight of larvae in the treatments were significantly different from each other ($p < 0.1\%$). Partitioning of the treatment sums of squares revealed that the differences between pF values could be attributed to chance, but the differences between times were significant at the 0.1 per cent. level. The standard errors of the means of the times, which are also given in Table 10, indicated that the significance of the times in the analysis of variance was probably due to the difference in weight between 1 week and the others; this difference was highly significant ($t = 6.68$ with 28 d.f., $p < 0.1\%$).

The gross difference in weight between the larvae in the control and in the treatments, the smaller increase in weight of larvae in dry soil after 1 week compared to that of the controls, and the subsequent decreases in weight with time indicate that larvae do not feed to any extent in dry soil after the first few days and support the hypothesis that the larvae

Table 10

The percentage gain in weight of young larvae in soil at 3 pF values after 1, 2, 3 and 5 weeks

		pF value of soil			
Time	Group	3.30	3.80	4.10	Control
1 week	1	104.3	80.6	81.1	174.5
	2	56.2	63.6	86.2	209.6
	3	85.4	90.4	153.2	210.3
	4	117.1	112.6	77.9	227.4
	5	80.8	125.4	89.4	267.0
2 weeks	1	50.7	24.2	76.9	393.9
	2	43.9	18.3	48.9	437.6
	3	39.1	41.7	28.0	427.2
	4	66.9	25.4	0.8	417.3
	5	44.4	60.8	38.8	501.6
3 weeks	1	17.5	20.5	14.4	
	2	28.7	63.4	50.7	
	3	72.1	19.7	77.2	
	4	28.9	45.2	42.5	
	5	15.6	*	18.9	
5 weeks	1	22.1	9.2	17.5	
	2	*	36.9	*	
	3	67.4	26.6	66.7	
	4	*	*	15.8	
	5	20.6	52.1	*	

* No larvae surviving.

Table of treatment totals

	pF value of soil					
Time	3.30	3.80	4.10	Σ	$\bar{x}$	S.E.
1 week	443.8	472.6	487.8	1404.2	93.6	6.46
2 weeks	245.0	170.4	193.4	608.8	40.6	5.05
3 weeks	162.8	148.8	203.7	515.3	36.8	5.84
5 weeks	110.1	124.8	100.0	334.9	33.7	6.76
Σ	961.7	916.6	984.9	2863.2		
$\bar{x}$	53.4	50.9	54.7			

Comparing 1 week and 2 weeks, $t = 6.68$ with 28 d.f. $p < 0.1\%$

The analysis of variance

Source of variation	d.f.	S.S.	M.S.	V.R.	p.
Total	53	58991.18			
Treatments	11	35534.01	3230.36	5.78	< 0.1%
pF's	2	134.08	67.04	-	N.S.
Times	2	34530.36	17265.18	30.9	< 0.1%
pF x time	4	869.57	217.40	-	N.S.
Error	42	23457.17	558.50		

Table of treatment means

	pF of soil		
Time	3.30	3.80	4.10
1 week	88.7	94.5	97.6
2 weeks	49.0	34.1	38.7
3 weeks	32.6	37.2	40.7
5 weeks	36.7	31.2	33.3

subsequently died of starvation. But water loss can be ruled out only if the larvae were exposed to different degrees of dryness in the soils supposedly at different pF values, and a disturbing feature of the experiment was that the sand in the cups lost water during the course of the experiment. The estimated percentage of water contents of the soils at the start and at the end of the experiment are given in Table 8a (Appendix), from which it can be seen that there was not much difference in the water contents of the soils after the second week. This data and the water-content curve of the sand in Fig. 7 point out the impracticability of using sand in an experiment unless the system is completely sealed, for the range of water content between pF 2.0 and pF 4.2 is small in a sand and the loss of a relatively small amount of water results in a large change in the pF value of the soil. In addition, it is difficult to obtain pF values with any accuracy.

The determination of the water content of sand in numerous cups and their comparison with the estimated percentage of water contents revealed further that the sand was invariably drier than was estimated (Table 8b Appendix). The two estimates were, however, significantly correlated ($r = 0.94$, 16 d.f. $p < 0.1\%$) so that differences in the estimated water contents of the sand, provided they are reasonably large, can be taken as reflecting real differences in the water contents. It is probable therefore that larvae were exposed to different degrees of dryness, for at least 2 weeks, and that the larvae did die of starvation.

In conclusion, it is legitimate to combine the numbers of larvae alive at each interval of time and estimate the effect of drought on the survival-rate of the larvae.

A summary of the probit analysis conducted on the combined figures is given in Table 9 (Appendix) and the calculated probit line is drawn through the observed points in Fig. 12. The calculated LD50 was 19.4 days with 95 per cent. limits 17.4-21.6 days.

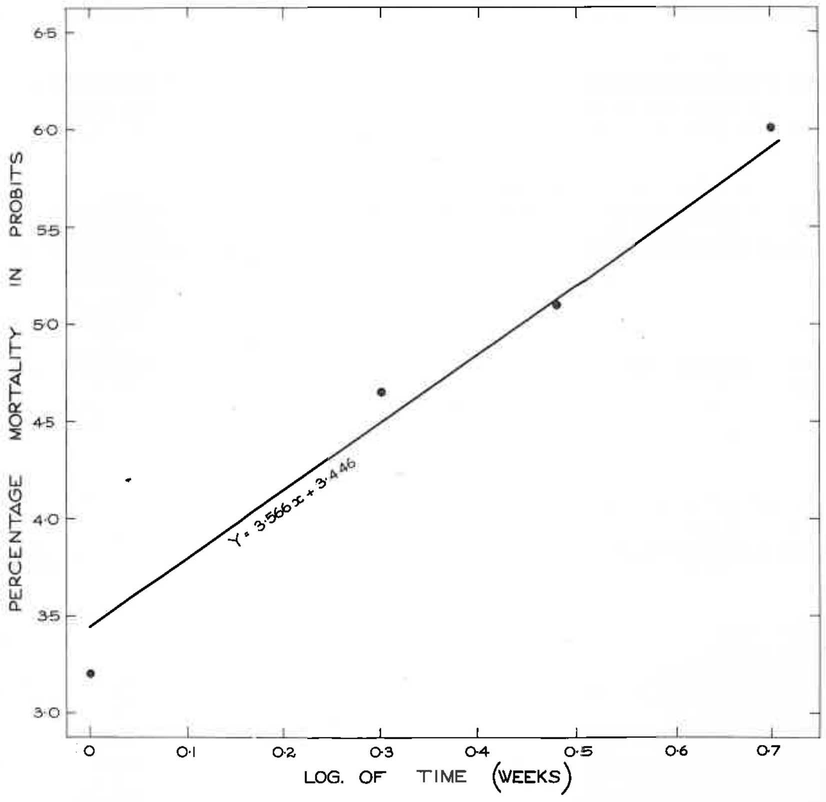

Fig. 12. The effect of "drought" on the mortality of first instar larvae before migration to the surface.

The accuracy of the LD50 is governed largely by the estimated control mortality, and the design of the experiment was poor insofar as no provision was made to estimate the control mortality with greater accuracy -

particularly the mortality after 1 week. But the numbers of larvae alive in the treatments and in the control after 1 week were not significantly different, so it is probable that the deaths were due mainly to the experimental technique and that the recorded control mortality was a fairly good estimate. It is also likely that, because of the artificial conditions, the survival-rate of larvae was lower than it would be in the field, and that the number of days of drought required to kill 50 per cent. of the larvae in the field was underestimated in this experiment.

(c) The survival of unfed first instar larvae in a saturated atmosphere at different temperatures

If starvation is the only important cause of deaths among first instar larvae that are living in soil that is too dry to bring them to the surface it should be relevant to measure the death-rate among first instar larvae that are kept on moist filter paper without food. An experiment was done to determine how long young larvae could survive without food at different temperatures.

Thirty newly-hatched larvae were placed in a petri dish at each of five temperatures, 13.3, 15.8, 18.0, 20.3 and 21.8°C, each larva being placed in a piece of straw standing on filter paper in the petri dishes. The petri dishes were covered with black paper to reduce any irritation to larvae caused by light, and the pieces of filter paper were moistened each day. The number of larvae alive were recorded every four days; the percentage mortality was as follows:-

Temperature in degrees C.	Number of days						
	4	8	12	16	20	24	28
13.3	3	10	20	27	30	33	43
15.8	7	17	27	37	43	47	57
18.0	3	13	27	30	43	47	67
20.3	3	13	20	27	37	57	90
21.8	7	27	37	43	50	73	100

The percentage mortality at 18.0°C was plotted in probits against the logarithm of time. A regression line drawn by eye through the points gave an LD50 of log. 1.35, or 22.5 days. The similarity of the estimated LD50 to that of the previous experiment indicates that though the larvae in the previous experiment may have lost water whilst in the dry soil it is likely that death was due mainly to starvation. The data also indicate that the length of time which larvae can survive without feeding depends on the temperature.

(d) The tolerance of first instar larvae to "drought" after migration to the surface

After larvae migrate to the surface and commence feeding, their rate of development and rate of survival are likely to depend on the quality and quantity of food. Even when very young the larvae construct tunnels and forage at night, but they only forage after rain so that the consideration of quantity of food involves two factors (a) the statistical probability of larvae being stimulated to forage for food, and (b) the statistical probability of food being found.

This experiment was conducted to determine how long larvae could survive in periods of "drought" during which time they would be inhibited from foraging for food on the surface.

First instar larvae, which had migrated to the surface and had commenced feeding, were placed in artificial tunnels in soil in waxed paper cups. The pF value of the soil was initially 2.5. The top of the soil in each cup was allowed to dry out and the cups were left in this condition for 10, 14, 18, 22, 26 and 30 days. After the larvae had been inhibited from visiting the surface for any required period of time, the soil was remoistened and food was placed on the surface. A control group was supplied with food and kept in moist soil during the course of the experiment.

Three cups of soil each holding 10 larvae were used for each treatment. The numbers of larvae alive in each cup after 36 days are recorded in Table 10 (Appendix).

The experiment was designed to be analysed with probits so the total percentage mortality at each treatment was corrected for mortality among the controls and the resultant percentage mortality was converted to probits and plotted against the logarithm of the duration of drought in days (Fig. 13). The estimated log LD50 was 13.9 days, with 95 per cent. limits 12.1 and 16.1 days.

Besides the differences in mortality there were big differences in the rates of development of larvae in the different groups. The control group contained mainly third instar larvae and there were a few second instars in the groups which had been starved for 10 days; the rest were first instars. The data confirm that the rate of development of larvae is controlled by the frequency with which they can eat. This is controlled by the weather.

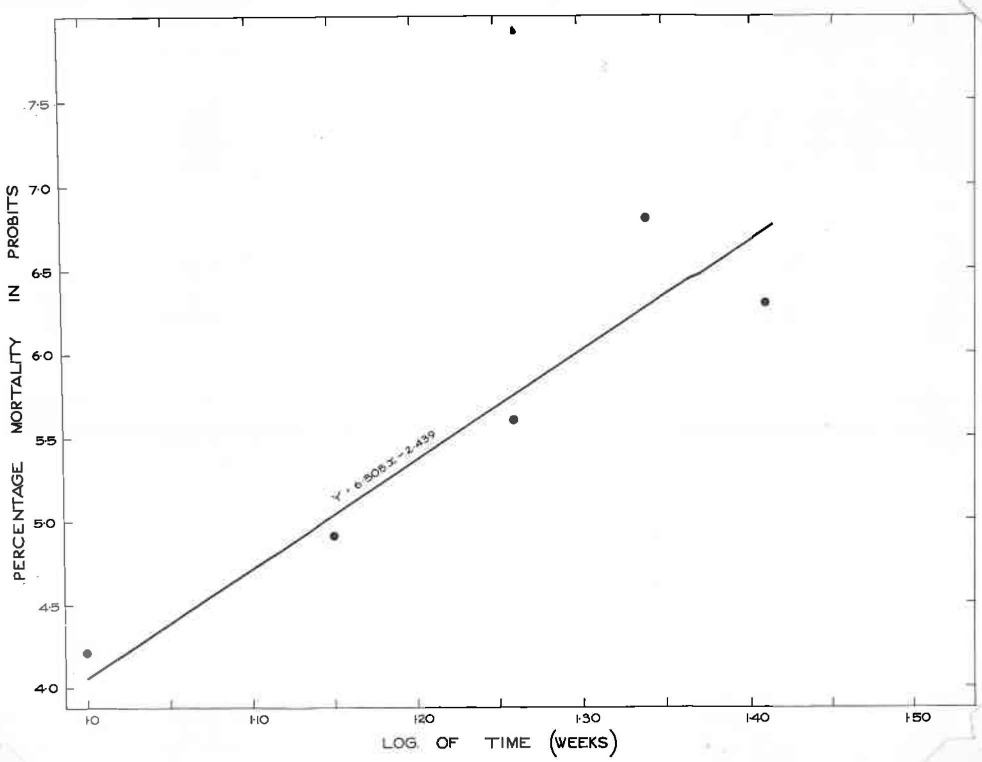

Fig. 13. The effect of a prolonged dry spell on the survival rate of first instar larvae after migration to the surface of the soil.

Larvae in the field commonly take food into their tunnels and subsist on this stored food for 6–8 days. A period of "drought" would therefore start to affect larvae 6–8 days after the last rain fell. It is therefore likely that more than 20 consecutive days without "effective" rain are required to kill 50 per cent. of larvae in the field.

(e) The lethal influence of excess water

Excess water affects larvae of A.howitti in a number of ways:-

(a) It simply drowns or asphyxiates them

(b) It promotes infection with pathogenic organisms

(c) It exposes those which do not drown to predators and other unfavourable environmental influences.

First instar larvae, particularly before feeding, have often died in the laboratory when the soil has been too wet and the larvae have been held by the surface tension of water. Observation suggests that young larvae may similarly die in the field if unseasonal rains occur. However, the probability of water accumulating is highest in the period June-September. It is in this period that larvae are most likely to be drowned or infested by Cordyceps. The following discussion, therefore, pertains mainly to the third instar larva.

Larvae of A.howitti do not leave their tunnels immediately the tunnels are filled with water. Many of them crawl to the top of their burrows within 24 hours and, if the ground is covered with water, they may crawl on the surface for a while or float to the top of the water. Most of them, however, remain with their heads out of their burrows and their posterior ends in the burrows. A rising water-table may eventually displace all the air from the soil but often flooding does not do this and bubbles of air are trapped in the soil. Examination of localities which have been covered with water after heavy rain have revealed that a certain number of larvae have remained in their burrows even later than 24 hours after flooding; many of these, curled up in their burrows, had trapped a bubble of air beneath them.

Similar results were obtained from an experiment which was conducted to determine what larvae would do when their tunnels were flooded. One hundred third instar larvae were placed individually in tubes of soil, were given food, and allowed to settle down for a few days. Water was then added to each tube till an inch of water lay above the soil. Four-hourly readings were then taken of the numbers that emerged to the surface. The experiment was conducted at 8°C and 16°C. Twentyfour hours later 81 per cent. of the larvae at 8°C and 80 per cent. of the larvae at 16°C had come to the surface; fortyeight hours later the figures were 83 per cent. and 82 per cent. respectively. Seven larvae at 8°C and five larvae at 16°C floated to the surface. The remainder crawled on the surface for a while before passing into a comatose condition or stayed with their heads just showing above the surface of the soil. When some of these larvae were taken out of the water they recovered rapidly and burrowed into soil without showing any obvious signs of distress or injury resulting from their immersion. Further experiments showed that larvae may become comatose within seconds of being immersed in water.

Many of the areas which are flooded in the South-east are due to the rise of the water-table and it was of interest to know how long larvae could survive when water accumulated after rain and slowly drained away. Larvae of A. howitti were therefore immersed in water at 8°C and 16°C for 4, 8, 16, 20, 24, and 48 hours. They were then placed at the tops of artificial tunnels in moist soil and the numbers alive were recorded 22 days later when larvae began entering diapause. Eighty larvae were used for each treatment but those infected with Cordyceps were omitted from the results which are given in Table 11 (Appendix); the percentage mortality in probits is plotted against time of immersion in Fig. 14.

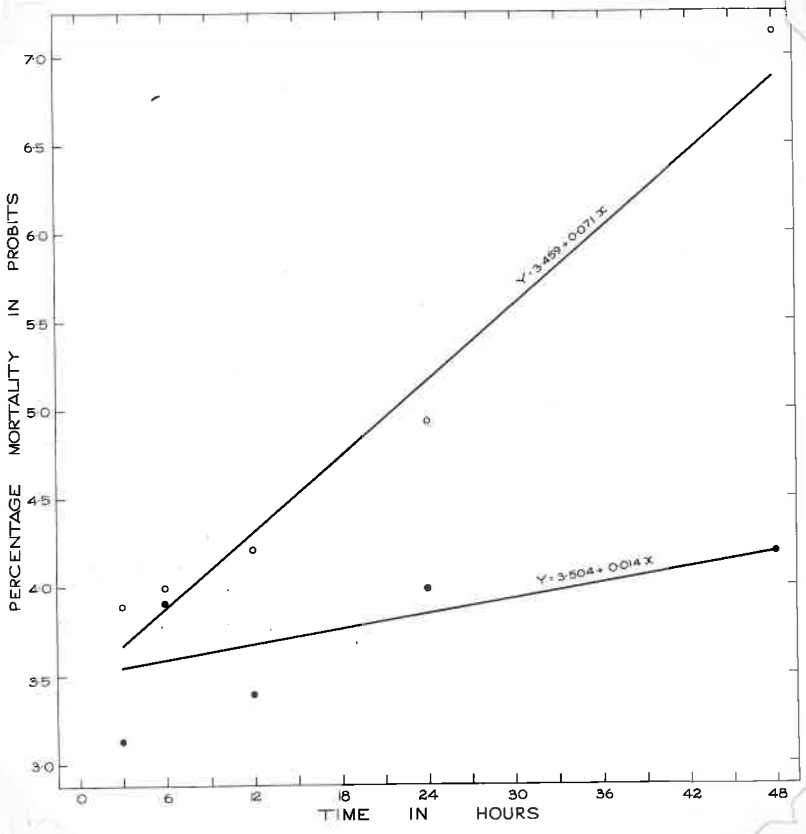

Fig. 14. The percentage mortality of third instar larvae after immersion in water at two temperatures

It is evident that temperature had a marked effect on the survival-rate. The LD50 at 16°C was calculated to be 21.7 hours, with 95 per cent. limits ± 4.4 hours, but more than 50 per cent. of larvae survived being immersed in water at 8°C for 48 hours. Temperature similarly affected the survival-rate of larvae of Heliothis armigera (Barber and Dicke 1939).

The mean temperature of flood water at Mt. Gambier would not be far from 8°C in winter, so larvae in the field would be able to tolerate immersion for more than 48 hours.

When flooding is due to the rise of the water-table the excess water drains away very slowly and larvae are drowned. Flooding was particularly extensive on the meadow podsols in the winter of 1955 and larvae were killed in thousands at Pleasant Park, Kalangadoo, Eight Mile Creek and Tarpeena. Larvae were also drowned in large numbers at Wrattonbully, but many also survived on the better-drained slopes. On the terra rossa soils around Mt. Gambier and Millicent and on the sandy ridges flooding may occur but is not due to the rise of a water-table, and the water does not lie on the surface more than a few hours. Consequently, A. howitti is rarely, if ever, drowned in these localities.

An interesting situation was seen at Kalangadoo. Flood water spread up to the base of a tree, completely submerging a large area which was colonized by larvae of A. howitti. When it had retreated a number of larvae were observed to have survived in dung pads which were not covered by water (Plate 9).

Generally, however, few larvae survive in an area if it remains flooded for more than 3-4 days. A few larvae may float to the surface and finally reach dry land, but larvae have never been seen climbing up pieces of vegetation as larvae of Oncopera fasciculata do under similar conditions

(Madge Ph.D. thesis). When larvae leave their tunnels they are exposed to water loss and to the action of predators - particularly birds such as seagulls, white ibis and magpies, which have been observed feeding on larvae on a number of occasions.

Excess water also influences the survival-rate of larvae of A. howitti by promoting infection with the entomophagous fungus Cordyceps aphodii Mathieson whose part in the ecology of A. howitti is described in section VI, (iii).

There were indications that bacterial infections of A. howitti increased with excess water in the soil, and it is also probable that excess water generally weakens larvae so that they succumb more readily to diseases and other components of their environment.

2. Field experiment

(a) The effect of a prolonged dry spell on the survival-rate of larvae

The effect of a prolonged dry spell on the survival of larvae can best be assessed in the field by "seeding" plots with adults, and then estimating the number of eggs laid and the percentage mortality of the young larva at the termination of the dry spell. It was intended to seed a number of plots with females in 1955 for this purpose, but the supply of adults ran out. Two field experiments, one consisting of 8 plots and one consisting of 6 plots, had been commenced, however, to determine where females aggregated and laid eggs. So, instead of sampling the plots for adults and eggs, the plots were left intact for 1-2 months and were sampled at appropriate intervals to estimate larval mortality. The details are given on pages 69-72 and in Tables 5a and 5b.

The recording of adults as well as larvae in the plots enabled estimates to be made of the number of larvae per adult which were alive at any time. The porportions of larvae/adults recorded in the plots at any one time were roughly constant and lend confidence to the estimates.

The females were placed in the plots on February 16, 1955 and would have laid their eggs by February 19-20, 1955. Working from this date it can be calculated that 50 per cent. of the eggs were due to hatch by March 13, 1955 (section X). When the first plots were examined on March 22-24, 1955 all but three of the eggs had hatched and the first instar larvae were all within the cells in which the eggs were laid. The agreement between laboratory and field data was, therefore, good. The numbers of larvae/adults recorded from the four plots examined were 12.2, 14.4, 11.4 and 11.4 (Table 5b) with a mean of 12.3. Migration to the surface was inhibited by a lack of "effective" rain until more than an inch fell on April 12, 1955. Another lot of plots were dug up on April 22 after the larvae had migrated to the surface; the numbers of larvae/adults recorded were 6.1, 3.3, 6.0, 6.5, 6.15, 6.3 with a mean of 6.2 (Table 5a).

It is unlikely that many eggs or young larvae died between March 13, 1955 when 50 per cent. of the eggs hatched (theoretically) and March 22, 1955 when the first plots were examined. The reduction in number of larvae per adult observed between March 22, 1955 and April 22, 1955 thus roughly measured the percentage mortality of the first instar larvae from the time of hatching (March 13, 1955) to the time when the soil was wetted and the larvae migrated to the surface (April 12, 1955). It is estimated that approximately 50 per cent. of larvae died in the 30 days of "drought".

It was estimated in the laboratory that a "drought" of 17.4-21.6 days caused 50 per cent mortality of first instar larvae before migration to the surface (Fig. 12), but it was concluded that the number of days of drought necessary to cause 50 per cent. mortality in the field was probably greater. It was also seen that the time necessary to cause 50 per cent. mortality was dependent on temperature and the temperature in the field was lower than the temperature in the laboratory experiment for a portion, at least, of the time. Allowing for these sources of error the laboratory and field estimates of the time taken to cause 50 per cent. mortality are not widely different. The field estimate is probably a better one and has been used for phenological purposes.

A field experiment was set up on March 13, 1956 at Wrattonbully to get further quantitative data. Adults were placed on 40 plots some of which were to be dug up every week for 10 weeks, but more than 6 inches of rain fell in April, the area was flooded, and the experiment was abandoned.

The "break" of the season varies from year to year in South Australia and there have been some years in which there was no "effective" rain for more than 30 days after the eggs hatched. The percentage mortality of first instar larvae in these years must have been higher than it was in 1955.

(ii) The effect of food

1. Laboratory experiments and observations

Larvae in all three instars are stimulated to feed only when the soil is wet, and they develop best in situations that allow them to feed continuously. Laboratory experiments are complicated by the necessity of maintaining soil at an optimum water content, particularly if different soils are used. In a laboratory experiment, for instance, first instar larvae seemed to develop equally well in a number of soils except in a rendzina from Millicent. It is possible, however, that the water content of the rendzina was unfavourable for development, rather than that food was lacking. Similarly, Carne (1956) records that the infestation of soil types by A. howitti seems to depend more on the texture of the soil than on its chemical nature. Neither pH nor total nitrogen was correlated with the occurrence of the species (op. cit.).

First instar larvae will develop faster in the laboratory if a layer of leaf debris or dung is supplied on the surface of the soil; second instar larvae die if such food is absent. The alimentary canals of larvae are normally packed with soil but larvae do not ingest soil in the absence of other food.

Since the greater portion of the damage to pastures is done by the third instar larvae it was of interest to determine what type of food they thrived on and which food they would not eat. Two hundred and forty third instar larvae were divided into twelve groups and each larva was weighed and placed in a glass tube filled with moist soil. The larvae were supplied with 12 types of food, most of which were pasture plants of different species, and were weighed again six weeks later. The types of food and the percentage increases in weight of the larvae in descending order of magnitude are given in Table 11.

Table 11

The percentage gain in weight by third instar larvae when fed different types of food

Treatment No.	Type of food	No. used	$\bar{x}$	S.E. of $\bar{x}$
1	Musky crowfoot (*Erodium moschatum*)	20	69.3	5.36
2	Capeweed (*Cryptostemma calendula*)	18	58.7	4.94
3	Cocksfoot (*Dactylis glomerata*)	16	48.1	3.93
4	*Danthonia* sp.	14	46.7	6.05
5.	Cattle dung	15	44.5	5.96
6	Subterranean clover (*Trifolium subterraneum*)	13	42.8	6.04
7	Silver grass (*Vulpia myurus*)	14	31.5	5.38
8	Perennial rye grass (*Lolium perenne*)	14	31.3	3.49
9	White clover (*Trifolium repens*)	16	17.7	3.97
10	*Eucalyptus* humus	15	11.7	1.75
11	Oats	14	7.0	2.87
12	Soil	17	2.9	0.86

Comparing treatments:-	t	d.f.	p
1 and 3	2.99	34	1-0.1%
1 and 4	2.77	32	5-1%
3 and 8	3.37	30	1-0.1%
7 and 9	2.10	28	5-1%
8 and 9	2.54	28	5-1%

The standard errors in Table 11 indicate that most consecutive treatments were not significantly different from each other. It is clear that larvae do not develop on soil alone and develop very little on oats. Surprisingly, too, they did not develop to any extent on white clover and only gained 11.7 per cent. weight when fed on Eucalyptus humus. The gain in weight of larvae fed on white clover was lower than that on silver grass or perennial rye grass, but the gain in weight on perennial rye grass was significantly lower than that on cocksfoot. Larvae gained most weight when fed on musky crowsfoot or capeweed, the gain in weight being significantly higher than that on cocksfoot and the other types of food.

A number of third instar larvae from the above experiment were maintained in the laboratory on subterranean clover and were weighed every week until they had entered diapause. The weights of the prepupae in diapause were correlated with the maximum weights attained by the feeding larvae ($r = 0.79$ with 24 d.f., $p < 0.1\%$).

2. Field Experiments and Observations

First instar larvae construct burrows ½-1 inch long and feed mainly on plant debris on the surface; occasionally they eat the thinnest shoots of pasture grasses.

At first the second instar larvae continue to feed like the first instar larvae. They dig deeper burrows and occasionally feed on silver grass (Vulpia myurus) and subterranean clover, but even in the presence of abundant silver grass and clover the majority will be found feeding on plant debris on the surface. Towards the end of the second stadium, the bodies of the larvae become noticeably larger and the larvae commence feeding extensively on subterranean clover and other grasses which may be present.

Third instar larvae dig burrows 6-10 inches deep in the soil and feed mainly on the foliage of the pasture plants. The structure of their mouthparts restricts their feeding habits to plants which lie close to the ground or which have slender petioles. The larvae do not climb up stalks of grass like other phytophagous insects; and they rarely eat whilst on the surface, but take whole clover leaves and portions of other grasses down into their burrows and feed on this store of food for 7-10 days. They forage for food only when hungry and only when the soil is wet; so they do not come to the surface every night. When rain falls after a number of dry days all the larvae visit the surface for food and the next day all their tunnels are partially filled with fresh pasture foliage. The presence of feeding larvae is characterised by the large quantity of loose soil which is cast upon the surface by the larvae as they enlarge their tunnels or make new ones (Plate 9). As Carne (1956) has pointed out this soil is simply pushed out of the tunnels by the larvae; it often adds considerably to the damage done to the pasture by smothering plants which have not been eaten.

Larvae of <u>A.howitti</u> apparently have no difficulty in eating newly germinated perennial plants but perennial plants, once they are established, are not eaten to the same extent as the annual grasses. Larvae only forage for food within a distance of one inch from their burrows and since they have difficulty in burrowing amidst the rooting systems of perennial grasses they usually only tear off pieces of foliage from the periphery of a perennial plant. The few larvae which have been found burrowing in the rooting systems of perennial grasses have been smaller than those burrowing under clover. The distinction between perennial and annual grasses is best seen in a well established pasture when the larvae are early third instars. Tiny circles of damage may then be seen around each perennial plant, particularly those of perennial rye (Lolium perenne), and, in any locality, the perennials are usually eaten last.

A.howitti larvae are found in greatest numbers in subterranean clover pastures. Damage to such a pasture is rarely extensive until 3-4 years after the paddock was ploughed and the pasture was sown. Similarly, crops which are sown in July-August are sometimes attacked heavily because of the presence of older larvae, but crops sown in March-April are virtually immune to attack. These observations can be explained by the presence or absence of food for the young larvae. To test this hypothesis the following experiments were done in the field.

(a) The effect of food on the survival-rate of 1st instar larvae

Experiment 1

Four hundred larvae in their first instar were distributed, 25 at a time, among 16 plots that were treated as follows. There were 4 plots of each sort.

(1) Ungrazed, untopdressed pasture with a bare surface

(2) Ungrazed, untopdressed pasture with grass debris on the surface

(3) Second year pasture of perennial ryegrass, cocksfoot and subterranean clover

(4) Sixth year pasture of perennial ryegrass, cocksfoot and subterranean clover, with subterranean clover dominant.

The larvae were derived from eggs laid in moist soil in the laboratory and had started to ingest particles of food and soil after migration to the surface. They were randomised into 16 groups of 25 and the groups were then randomly allotted to the pastures, each group of 25 being placed in a separate plot.

Each plot was enclosed with a strip of metal sheeting and was 1 foot square. The larvae were placed at random within the plots, each larva being placed in an artificial hole in the soil. The pastures were all within a radius of ½ a mile of each other so that it is probable that they received the same amount of rain during the course of the experiment. The larvae were placed in the soil on April 8, 1955 and the numbers alive were recorded on May 25, 1955 when the soil in the plots was dug up.

The results are given in Table 12a. A χ^2 of 52.4 with 3 d.f. ($p < 0.1\%$) indicated that the differences between pastures were highly significant. The number of larvae surviving in the different treatments can be summarised as 6th year > 2nd year = natural pasture with debris on the surface > bare ground.

Because of the low numbers surviving the larvae were not weighed to estimate their rate of development, but those from the 6th year pasture were larger than the others.

Table 12a

The numbers of 1st instar larvae surviving in different pastures

Pasture	Replicate No. 1	2	3	4	Σ	$\bar{x}$
1	0	0	0	0	0	0
2	3	2	6	3	14	3.5
3	3	2	7	4	16	4.0
4	11	6	10	11	38	9.5
Σ	17	10	23	18		

Table of numbers alive and dead

Pasture	Alive	Dead	Total
1	0	100	100
2	14	86	100
3	16	84	100
4	38	62	100
Σ	68	332	

χ^2 with 3 d.f. = 52.4, $p \ll 0.1\%$

Testing 1 v. 2, $\chi^2 = 14.1$ $p < 0.1\%$

3 v. 4, $\chi^2 = 12.3$ $p < 0.1\%$

(b) The effect of food on the survival-rate of 2nd instar larvae

Experiment 2

The similarity in the feeding habits of first and second instar larvae suggested that the low survival rate of larvae in certain types of pastures was due to a lack of suitable food for both the early instars. To assess the effect of food on the development and survival rate of second instar, apart from its effect on the first instar, a number of well developed first instar larvae were collected from a 6th year pasture and were kept in the laboratory until they had moulted to the second instar. Four hundred of them were then randomised into 16 groups of 25 each and were placed in artificial tunnels in soil within plots so that there were 4 replicates to each of the following treatments:

(1) Natural, untopdressed pasture

(2) Natural, topdressed pasture

(3) Second year pasture of perennial ryegrass, cocksfoot and subterranean clover.

(4) Sixth year pasture of perennial ryegrass, cocksfoot and subterranean clover in which the clover was dominant.

The larvae were placed in the soil on April 24 and 25, 1955 and the soil in the plots was dug up on June 25, 1955 when it was considered that the larvae had all moulted to the third instar. The numbers alive in each plot are recorded in Table 12b.

Differences between pastures were analysed by means of χ^2 calculated from a R x 2 table. The value of χ^2 (25.4) with 3 d.f. ($p < 0.1\%$) indicated that the numbers of larvae surviving in the different pastures differed significantly. This difference was due to fewer larvae surviving in the natural, untopdressed pasture than in the other pastures. There was no difference in the survival of larvae in the other pastures.

Table 12b

The numbers of 2nd instar larvae surviving in different pastures

Treatment No.	Type of pasture	Plot 1	Plot 2	Plot 3	Plot 4	Σ
1	Natural untopdressed	11	9	11	4	35
2	Natural topdressed	18	15	13	16	62
3	2nd year	14	20	17	15	66
4	6th year	13	16	19	15	63
	Σ	56	60	60	50	226

Table of numbers alive and dead

Treatment No.	Alive	Dead	Σ
1	35	65	100
2	62	38	100
3	66	34	100
4	63	37	100
Σ	226	174	400

χ^2 with 3 d.f. = 25.4 $p < 0.1\%$

Testing 1 v. 2. χ^2 = 14.6, $p < 0.1\%$

The mean weight in mg of 3rd instar larvae recovered from plots

Treatment No.	Type of pasture	Plot 1	Plot 2	Plot 3	Plot 4
1	Natural untopdressed	84.4	82.3	72.3	72.0
2	Natural topdressed	77.3	84.2	73.9	84.5
3	2nd year	63.0	61.5	61.3	62.2
4	6th year	93.7	104.1	101.9	100.0

Analysis of variance

Source of variation	d.f.	S.S.	M.S.	V.R.	p
Total	212	169,081.8			
Treatments	15	47,307.0	3,153.8	5.10	< 0.1%
Pastures	3	44,293.9	14,764.6	23.9	< 0.1%
Plots	3	296.6	98.9	-	N.S.
Interaction	9	2,716.5	301.8	-	N.S.
Error	197	12,174.8	618.2		

Table of treatment totals

Treatment	mg	No. used	$\bar{x}$	S.E. of $\bar{x}$
1	2752	35	78.63	4.202
2	4716	59	79.93	3.237
3	3776	61	61.90	3.183
4	5826	58	100.4	3.265

S.E. of Δ between 1 and 3 = 5.717

Min. difference for significance
5% level = 11.3
1% level = 14.9
0.1% level = 19.1

S.E. of Δ between 2 and 4 = 4.598

Min. difference for significance
5% level = 9.1
1% level = 12.0
0.1% level = 15.4

The larvae from each plot were, in addition, weighed individually on a chemical balance to the nearest mg ; their mean weights are given in Table 12 (Appendix). An analysis of variance indicated that the larvae from the different pastures differed significantly in weight, so the standard errors were calculated.

The mean weight of larvae in the 2nd year pasture (61.90 mg) was lowest, and was significantly lower (at 1 per cent. level) than the mean weights of larvae from both the natural pastures. The weights of larvae in the natural pastures were similar (78.63 and 79.93 mg) but both were significantly lower (at 0.1 per cent. level) than the mean weight of larvae from the 6th year pasture (100.4 mg).

The rate of development and survival rate of second instar larvae, like those of the first instar, are thus influenced by the kinds of places in which the larvae may live, and are probably determined by the quality and/or quantity of food available to the larvae. The data suggest that the same type of food was available to the larvae in the natural pastures but was more abundant in the one which was topdressed. The high survival rate of larvae in the 2nd year pasture was surprising because the small size of the larvae indicated that food was not abundant or was of poor quality. This pasture, which was sown 13 months before the experiment, was sown with a cover crop of oats and barley. It was grazed more heavily the previous year than are most newly sown pastures, and also had a considerable growth of silver grass. It is possible, therefore, that the larvae did have access to a dense but poor supply of food. The 6th year pasture was again the most favourable place for survival and development.

No attempt has been made to estimate the survival rate of larvae in different pastures over the combined first and second instars because the low survival rate expected in most pastures would have necessitated the use of a much larger number of larvae than those used in the above two experiments. The survival-rate and rate of development of both the first and the second instars is, however, highest in well established clover pastures. When it is remembered that the second instar larvae in other than clover pastures normally do not commence the second stadium as healthy, well-developed larvae (as they did in Experiment 2) but have had to survive the hazards of life as first instars with an inferior food supply, it is not surprising that few survive to moult to the third instar.

(c) The effect of food on the survival-rate of 3rd instar larvae

Experiment 3

Since it was known that the rate of development of third instar larvae was influenced by the type of food available it was of interest to determine/whether the size of the prepupa, and hence the fecundity of the adult, was influenced by the type of food which third instar larvae ate in different pastures. Four hundred newly-moulted third instar larvae were collected from the field on June 24, 1955 and were randomised into 80 batches of 5. Each batch of 5 larvae was weighed to the nearest mg. and was allotted at random to one of the following pastures:-

(1) Natural untopdressed pasture

(2) Natural topdressed pasture

(3) Second year pasture

(4) Sixth year pasture.

The mean weight of the larvae was 86.6 mg. The differences in the weights of larvae allotted to different treatments were not significant (V.R. < 1.0 with 76 and 3 d.f., $p > 5\%$).

As in the previous experiments there were four replicates of 25 larvae in each pasture and the larvae were placed in artificial holes in the soil within 1 foot square plots. On October 6, 1955 when it was considered that the larvae in the various pastures had entered diapause the soil in the various plots was dug up and the surviving larvae were recovered and weighed individually.

The numbers of larvae which survived in the different pastures and their mean weights are given in Table 12c. The statistical analyses, which are included in the tables, indicated that there was no difference in the survival-rate or in the weight of prepupae from the different pastures.

The extremely low survival-rate was due mainly to the depredations of Cordyceps aphodii, which killed 50-60 per cent. of the larvae in each plot.

(iii) The effect of other factors

1. Crowding

(a) The effect of crowding on the survival-rate of first instar larvae before migration to the surface

If drought prevents young larvae from migrating to the surface the larvae may have to stay close together for a number of days in the soil, so an experiment was designed to determine whether crowding affected the survival-rate of larvae during periods of drought.

The method was essentially similar to that described in the previous laboratory experiments. Clay loam was moistened and then dried until it

Table 12c

The number of prepupae which were recovered from different pastures

Type of pasture	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Σ
Natural untopdressed	6	6	9	4	25
Natural topdressed	10	5	7	7	29
First year	7	4	5	4	20
Sixth year	6	8	6	4	24

The mean weights of prepupae recovered from different pastures

Type of pasture	No. of prepupae weighed	Mean weight of prepupae (mg)
Natural untopdressed	23	106.9
Natural topdressed	27	111.6
First year	20	113.8
Sixth year	23	110.5

Analysis of variance

Source of variation	d.f.	S.S.	M.S.	V.R.	P
Total	92	31723.93			
Treatments	3	547.60	182.5	0.53	N.S.
Error	89	31176.33	350.3		

reached the water content with a pF value of 3.0. Cups were filled with the soil of the desired water content 2-3 days before the experiment started. They were then covered with plastic material and stored at 18°C.

The total number of larvae required for the experiment could not be obtained in one day so 450 larvae were randomised into nine petri dishes and 50 larvae from each petri dish were allocated at random to each of nine treatments consisting of all the combination of three levels of crowding (5, 25, 50) and three periods of time. The larvae were placed in cells in the cups of soil as in previous experiments, and the cups still with the plastic covers on, were stored at 18 ± 0.2°C. The procedure was repeated three times to obtain a total of four replicates.

Time was included as a variable because (a) it was not known how long young larvae would live in the cells before making individual tunnels and, therefore, an interaction between density and time was possible, and (b) it was necessary for a later experiment to get a rough estimate of the number of days larvae could survive in dry soil before migration to the surface.

The cups were to have been examined after 2, 4 and 6 weeks and the numbers of larvae alive recorded, but a large number of larvae had died after four weeks. To get a better estimate of the effect of density on the survival-rate the six weeks series was also examined after four weeks and is designated as four weeks B. The number of larvae surviving in each treatment is recorded in Table 13. The percentage survivals were transformed to degrees and subjected to an analysis of variance (Table 13).

Four conclusions may be drawn from the results:-

Table 13

The effect of crowding on survival of 1st instar larvae

The numbers surviving in each treatment:-

Replicate No.	Treatment								
	2 weeks			4 weeks A			4 weeks B		
Larvae per cell	5	25	50	5	25	50	5	25	50
1	34	26	21	12	13	12	13	19	10
2	13	22	12	14	6	7	6	2	4
3	8	12	9	5	4	3	12	3	7
4	41	38	37	16	22	18	12	18	24

Percentage surviving transformed to degrees:-

Time	Replicate No.	Levels of crowding (larvae per cell) 5	25	50
2 weeks	1	55.6	46.1	40.4
	2	30.7	41.6	29.3
	3	23.6	29.3	25.1
	4	64.9	60.7	59.3
4 weeks A	1	29.3	30.7	29.3
	2	31.9	20.3	22.0
	3	18.4	16.4	14.2
	4	34.4	41.6	36.9
4 weeks B	1	30.7	38.1	26.6
	2	20.3	11.5	16.4
	3	29.3	14.2	22.0

Table of treatment totals

Time	Levels of crowding 5	25	50	Σ	$\bar{\bar{x}}$
2 weeks	174.8	177.7	154.1	506.6	42.2
4 weeks A	114.0	109.0	102.4	325.4	27.1
4 weeks B	109.6	100.7	108.9	319.2	26.6
Σ	398.4	387.4	365.4	1151.2	
$\bar{\bar{x}}$	33.20	32.28	30.45		

} Combined mean = 26.9

The analysis of variance

Source of variation	d.f.	S.S.	M.S.	V.R.	p
Total	35	6290.17			
Blocks (replicates)	3	3233.06	1077.69	24.48	<< 0.1%
Treatments	8	2000.63	250.08	5.68	< 0.1%
Times	2	1888.64	944.32	21.45	<< 0.1%
Crowding	2	47.06	23.53	-	N.S.
Time x Crowding	4	64.93	16.23	-	N.S.
Error	24	1056.48	44.02		

Minimum difference for significance between means of levels of crowding
5% level = 5.6

Minimum difference for significance between means of 2 weeks and 4 weeks
5% level = 4.8
1% level = 6.6
0.1% level = 8.8

(1) There was no significant effect of crowding on the numbers of larvae which survived (V.R. < 1.0 with 2 and 27 d.f. $p > 5\%$) Since the number of eggs laid by any one female rarely exceeds 50 the level of crowding in the field is not likely to be any higher than those in the experiment. Density therefore is not expected to influence the survival-rate before larvae migrate to the surface.

(2) The interaction of crowding and time was not significant (V.R. < 1.0 with 2 and 27 d.f.; $p > 5\%$). If larvae hatch in dry soil they may, after a few days, leave the cell in which the eggs were laid and construct individual, vertical tunnels within a radius of about one inch. They construct small cells at the top of these tunnels in which they apparently wait for the stimulus to migrate to the surface. When the larvae in the 2 weeks series were examined they had already made individual tunnels, so the interaction of crowding and time should test the effect of density on the survival-rate whilst the larvae were in the ovipositional cell and within the period between movement away from the cell and migration to the surface. Density did not affect the survival-rate during either period.

(3) The mean percentage survival of larvae after 2 weeks was 45.5 (transformed value 42.2) and after 4 weeks 21.8 (transformed value 26.9). The difference was significant at the 0.1 per cent. level, so it appears that desiccation and/or starvation influenced the numbers of larvae surviving between the two periods. A control in wet soil was unfortunately not included in the experiment, so it is not possible to say what proportion of the mortality between 0 and 2 weeks was due to experimental technique and what proportion was due to other factors. Some of the deaths were probably

due to desiccation and/or starvation, but a considerable proportion of the high mortality in replicates 2 and 3 was due to infection with the fungus *Metarrhizium* sp.

(4) The experiment was set out as four randomised blocks (replicates). The variation in the survival of larvae between blocks was considerable and the differences were significant at 0.1 per cent. level. The sum of squares for replicates, in fact, accounted for 51.4 per cent. of the total sum of squares

The use of randomised blocks enabled the variation in lots of larvae randomised each day to be separated and enabled estimates to be made of the effect of crowding and time on the survival of larvae. If, however, the larvae available in any one day had been allotted at random to the 36 combinations of 9 treatments x 4 replicates it is probable that the additional variation included in the treatments would have observed the issue. ?

Much of the variation in the replicates was due to the infection of larvae with *Metarrhizium*, but in the absence of any precise knowledge of the mode of infection of the fungus no good reason can be given for its occurrence in replicates 2 and 3 and not in replicates 1 and 4.

(b) The effect of crowding on the survival-rate of older larvae

The results of the previous experiment indicated that crowding does not have any effect on survival immediately after hatching or, in fact, until larvae migrate to the surface. Observation has confirmed that first instar larvae may fight but their fighting rarely results in death even after larvae have migrated to the surface, and that larval death due to combat is confined mainly to the older larvae.

Second and third instar larvae often injure each other whilst foraging for food on the surface of the soil. Death invariably follows such injury because injured larvae are usually not able to burrow into the soil and are exposed to desiccation and predators - particularly birds.

Larvae in diapause are rarely found in numbers higher than 10-20 per square link though they may be in excess of 200 per square link when they are first instars. Competition for food therefore does occur, but observation in South Australia suggests that mortality resulting from such competition does not occur to the extent which has been described by Carne (1956) for populations of A. howitti in Canberra.

No critical experiments have been conducted on the effects of crowding on the survival-rate of late instars because of the following considerations:-

(1) An analysis of competition for food involves a consideration of the total stock of food available to the animals feeding on it; and, as pointed out by Andrewartha and Birch (1954) when animals such as A. howitti are feeding on growing plants the total stock of food available at any time varies with the number of feeding animals.

(2) The dispersal of larvae outward from centres of high density presents a complicated sampling problem, both with regard to the numbers of larvae present at any one time in an area and the total stock of food available to the larvae.

(3) Even if the sampling problems above were solved, it would be necessary to determine what proportion of the population occurs at the high density

levels at which competition would induce high mortality.

The importance of larval combat as a factor controlling the abundance of A. howitti in South Australia is discussed in section X.

2. Parasites and predators:

Only the parasites of A. howitti which kill the insects are considered in this section so no distinction is made between parasites and predators.

(a) Cordyceps aphodii Mathieson. The infection of larvae of A. howitti by the fungus has been described by Mathieson (1949) and by Carne (1956). Little is known of its biology but, like those of other entomophagous fungi, the hyphae probably enter the integument of the insects.

Carne (1956) records that the incidence of Cordyceps is high only in places which have been occupied by large numbers of A. howitti larvae for a number of years. This seems to be usually true in South Australia. In 1955 however Cordyceps infested large numbers of larvae in places which previously had only been sparsely occupied by A. howitti.

The winter of 1955 was unusually wet. Ten samples taken along a "front" in a pasture of terra rossa indicated that 64.1 per cent. (95% limits 55.6-72.1%) of larvae had been killed by Cordyceps. Like other estimates of larval mortality this estimate is influenced by many sources of error, but in 1953 and 1954 similar estimates of infection with Cordyceps were not higher than 20 per cent.

Cordyceps is more prevalent in wet years than in dry years, and in dry years occurs mainly in the wetter situations in which A. howitti lives. In wet years, however, larvae are drowned in these wetter places and

Cordyceps is more prevalent amongst larvae on the well drained soils. It is possible that the occurrence of the fungus in places which are repeatedly colonised by larvae in dry years is due to the higher moisture content in these places which permits the survival of fungal spores as well as larvae. In wet years spores may be widespread and infection of larvae may then be possible over a much wider area, irrespective of the density of larvae in the preceding years. In the absence of any experimental data it is therefore necessary to discuss with caution the relationship between the fungus, its host and the weather.

(b) Metarrhizium sp. First instar larvae, before migrating to the surface, are sometimes infested with Metarrhizium sp. The fungus never seems to infest larvae after they have commenced feeding, so that the longer the larvae are in the soil before migrating to the surface the greater are their chances of being parasitised by this species.

Pupae are sometimes infested with a Metarrhizium sp., but it is not known if it is the same species which attacks the young larvae.

Species of Metarrhizium parasitise many insect species (Steinhaus 1949). They are probably to be regarded as true parasites; wireworms, for example, can be infected with spores in the laboratory (Rockwood 1951).

Laboratory cultures of A.howitti have often been infested with the fungus but observation suggests that it does not kill many larvae in the field.

(c) Others: The eggs, prepupae and pupae of A.howitti are well hidden in the soil from all predators except entomophagous fungi. Carabid beetles and other miscellaneous insect predators kill some larvae in the soil, particularly when the larvae are in the first instar. But even the larvae have some protection in the soil and are killed in largest numbers when they leave their burrows, i.e. when the soil gets very wet.

The third instar larva and prepupa is also attacked by Thynnid wasp *Tachynomyia* sp., the adult males of which feed on tea-tree, *Melaleuca*, and *Eucalyptus* blossom. The males fly back from the blossom to feed the apterous females which are found in the soil. Parasitism rarely occurs more than 200 yards from blossom so that the total number of larvae killed in this way is small.

VII. THE LARVA IN DIAPAUSE

(i) The termination of diapause

The larvae of A. howitti stop feeding about the end of September, discard the contents of their guts and construct cells in the soil in which they live for 3-4 months. They have by this stage accumulated much fat which gives them a creamy-yellow appearance, are morphologically prepupae (Lower 1957) and seem simply to be waiting for the right stimulus to pupate. The length of this prepupal stage and its annual occurrence suggested that the insects entered an obligative diapause.

Prepupae kept in moist soil did not emerge any earlier than they did in the field so the prepupae did not simply suspend development because of a lack of water in the soil. Attempts to break diapause by using xylol and by injuring the insects were unsuccessful and suggested that the diapause was a "firm" one.

Temperature had a marked effect on the termination of diapause. In the first experiment prepupae which had recently entered diapause were randomised into four groups. Three groups were placed at 10.0, 15.1 and 25.0°C; the fourth was left for three weeks at 10.0°C and was then placed at 25.0°C. Prepupae were examined every week and the numbers which pupated were recorded. The mean number of weeks to pupation were calculated for the treatments and were as follows:-

Temperature	Mean period to pupation in weeks
10°C	> 30
15.1°C	14.5
25°C	9.8
25°C *	11.5

* 3 weeks at 10.0°C and remainder at 25.0°C.

No prepupae had pupated after 30 weeks at 10°C but the group which had been at 10°C for 3 weeks, and had then been placed at 25°C, pupated after a further 8.5 weeks. The data indicated (1) that 10°C was below or near the threshold for morphogenesis but that diapause development was possible at this temperature, (2) that diapause development occurred at 25°C.

In a later experiment prepupae were randomised into ten groups of which 6 were placed at 13.5, 15.8, 18.0, 20.3, 21.8 and 26.0°C. The other 4 groups were kept at 13.5°C for 10, 20, 30, and 40 days and were then placed at 18°C. The prepupae in this experiment were placed in moist soil and examined 13 weeks later. Fifty prepupae were used in each treatment but fungal infections drastically reduced the numbers of survivors. The percentage pupation is given in Table 14.

The numbers of survivors were small but the results follow a general trend which can be summarised as follows:

(1) Morphogenesis did not proceed or proceeded very slowly at 13.5°C.

(2) Prepupae which had been at 13.5°C for 10 and 20 days and had then been kept at 18.0°C developed more rapidly than those which had been at 18.0°C continuously. Diapause development thus proceeded more rapidly at 13.5°C than at 18.0°C.

Table 14

The effect of temperature on the termination of diapause

Temp. °C	No. alive	No. pupae	% pupation
13.5	31	0	0
15.8	23	1	5
18.0	20	11	55
20.3	24	22	91
24.8	18	14	78
26.0	17	8	48
* 13.5 (10 days)	24	20	83
13.5 (20 ")	18	11	61
13.5 (30 ")	16	8	50
13.5 (40 ")	17	1	6

* Insects at 13.5°C for 10, 20, 30 or 40 days and then transferred to 18.0°C.

(3) The continuous temperature at which diapause development and morphogenesis proceeded most rapidly was 20.3°C.

(4) The percentage pupation decreased at temperatures higher than 20.3°C. Since morphogenesis is likely to be more rapid at these higher temperatures, this suggests that the rate of diapause development decreased with increasing temperature.

The results of the first experiment indicated that diapause development did not proceed much more rapidly at 10°C than at 25°C. So it is probable that diapause development occurs more rapidly at 13.5°C than at 10°C.

As in other insects (Andrewartha 1952) diapause development and morphogenesis in A. howitti proceed most rapidly at different temperatures, and the temperature at which diapause development is most rapid (13.5°C) appears to be near the lowest temperature at which morphogenesis is possible. Diapause in A. howitti is atypical in that some diapause development seems to be possible over much of the range of temperature favourable for morphogenesis.

(ii) The effect of drought on the survival-rate of prepupae

Prepupae are in diapause in their earthen cells during the early part of the summer. A considerable amount of water is lost from the soil at this time of the year and the water content of the soil is at or below the wilting coefficient (pF 4.2) for many days or weeks.

Prepupae, similarly, were expected to lose water during the early part of summer and it was suspected that long periods of dry weather would kill many of them. Experiments were therefore conducted to determine (a) the rate at which prepupae lost water in unsaturated atmospheres, (b) the effect of loss of water on the survival-rate, and (c) the influence of the cell on the rate of loss of water from the prepupae.

1. The rate of loss of water from prepupae in unsaturated atmospheres

The rate of loss of water from prepupae was determined by placing them in Fowler jars in which humidities were controlled by using sulphuric acid-water mixtures (Solomon 1951). Weighing the insects before and after treatment enabled the percentage loss in weight to be calculated. The loss in weight was assumed to be all water because the insects (a) were in diapause and did not feed, and (b) were left in the treatments for short periods of time. The results are expressed as percentage weight lost.

Experiment 1:

This was a preliminary experiment in which prepupae from Wrattonbully were placed for 24 hours within glass tubes in Fowler jars in which were maintained saturation deficits of 3.25, 6.10, 9.39, 12.54 and 15.44 mm of Hg at 20.1°C.

A control group was kept for checking mortality, but the insects in this group were not kept over water for 24 hours to determine whether they lost water. In later experiments the percentage weight loss of a control group over water was found to be considerable.

In later experiments too prepupae were placed individually in pieces of $\frac{1}{4}$ inch plastic tubing which were open at both ends and rested on a platform of wire gauze half an inch or so above the acid-water mixture in the Fowler jars.

Experiment 2:

This experiment was designed to determine the effect of temperature on the rate of loss of water from prepupae and their subsequent survival-rate. Prepupae were exposed for 24 hours to a number of saturation deficits at 16.9 and 26.8°C and a control group was kept over water for the same period of time.

The mean percentage weight lost and the variance at each treatment are given in Table 15. The variances in the different treatments are roughly proportional to the means and Bartlett's test of homogeneity of variances (Snedecor 1946) indicates that the variances are markedly heteroscedastic. If the mean percentage weights lost are to be used in the regression analysis the variances must, therefore, be

Table 15

The % weight lost by prepupae in relation to saturation deficit at two temperatures - Expt. 2

Temperature	Sat. def. in mm of Hg x time	No. of prepupae used	Mean % weight lost	s^2	Range
16.9°C	0	19	5.0	1.35	3.8
	0.60	19	8.5	1.52	4.5
	1.19	20	9.4	9.18	10.2
	1.73	20	11.5	8.68	11.3
	2.27	20	11.6	5.84	8.7
	3.97	20	14.8	13.21	12.9
	7.80	20	22.1	17.50	17.4
	9.78	19	29.2	40.68	23.7
	11.62	19	32.1	49.63	24.3
	13.50	20	34.6	38.20	21.7
26.8°C	0	20	6.9	4.54	9.7
	0.65	20	8.4	3.26	6.2
	1.22	18	10.0	6.05	8.5
	1.92	20	11.0	6.41	9.6
	2.11	20	12.7	7.90	10.6
	3.44	20	15.4	13.47	16.8
	6.41	20	26.2	24.60	18.3
	9.12	20	29.3	33.36	21.5
	12.16	20	36.6	47.46	30.7
	15.46	20	46.2	67.33	29.8

made approximately equal by using a homoscedastic transformation on the data. A regression analysis on the untransformed data can be done however, if the total sum of squares is obtained from the deviation of the percentage weight loss of each individual insect from the general mean (Fisher 1936).

The analyses of variance (Table 12, Appendix) indicated that the percentage weight lost was a linear function of saturation deficit at each temperature; the slope of the regression line at 26.8°C when compared to that at 16.8°C, was found to be significantly higher at the 1 per cent.

level. These results are in agreement with those on other insects (Andrewartha and Birch 1954).

For comparison with the results of later experiments the mean percentage weights lost are plotted against the products of saturation deficit x time in days in Fig. 15, and the positions of the regression lines are demonstrated.

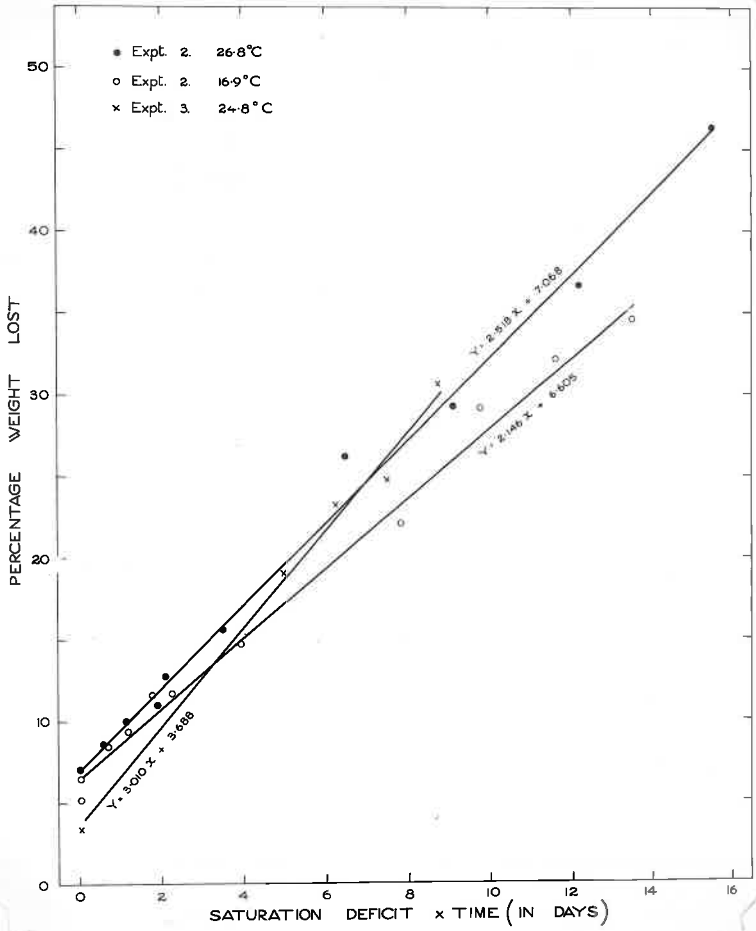

Fig. 15. The % weight lost by prepupae in relation to the product of saturation deficit and time; Experiment 2 and Experiment 3.

Experiment 3

This experiment was primarily designed to determine the effect of water loss on the survival-rate of prepupae, but also supplied data on weight lost by prepupae in relation to saturation deficit at 24.8°C. The losses in weight of the prepupae were obtained after 20 hours of treatment.

The analysis of variance indicated that there was a significant regression of percentage weight lost on saturation deficit; so, to compare the rate of weight loss with that in other experiments, the saturation deficit was expressed as saturation deficit x time and the regression equation was calculated. Again, for convenience, the mean percentage weight lost at each saturation deficit is tabulated in Table 16 and the data are plotted in Fig. 15.

The slope of the regression line, 3.011 was significantly higher than the slope of the regression line calculated for the data in experiment 2 at 26.9°C. The data therefore suggested that the insects did not lose the same amount of water when exposed to the same product of saturation deficit x time but data from different years are not comparable because (a) there is considerable variation in the mean wet weight of prepupae, and of their percentage water content from year to year, and (b) the percentage weight lost by prepupae tends to be correlated with their wet weight, and their wet weight in turn tends to be correlated with their percentage water content.

Before using laboratory data for forecasting it was desirable to know if prepupae did in fact lose the same amount of weight if exposed to equal products of saturation deficit x time at the same temperature. This was the purpose of experiment 4.

Table 16

The mean % weight lost by prepupae in relation to (sat. def. x time) at 24.8°C, Experiment 3

Sat. def. x time (days)	No. used	mean % wt. lost	s^2
0	40	3.5	1.82
5.0	81	19.0	38.80
6.25	80	23.2	43.41
7.50	80	24.8	55.83
8.75	78	30.7	61.17

Analysis of variance

Source of variation	d.f.	S.S.	M.S.	V.R.	p
Total	358	36,761.02			
Regression	1	15,976.42	15,976.42	>2.00	< 0.1%
Error	357	20,784.60	58.22		

Calculated regression equation $Y = 3.01x + 3.688$

$$\left.\begin{aligned} Sy^2 &= 36{,}761.02 \\ Sxy &= 6{,}903.18 \\ Sx^2 &= 2{,}292.75 \end{aligned}\right\}$$

<u>Experiment 4</u>:

This experiment was designed so that there were 12 treatments in two series of 6 at 13°C. In one series prepupae were exposed to saturation deficits of 0, 1, 2, 3, 4, and 4.5 mm. of Hg for 5 days, and in the other series prepupae were exposed to saturation deficits of 0, 2, 4, 6, 8 and 9 mm of Hg for 2½ days. The insects in each series were thus exposed to 0, 5, 10, 15, 20 and 22.5 mm/days of evaporation and any treatment in one series was directly comparable with a treatment in the other series.

The mean percentage weights lost at each treatment and the variances are given in Table 17, and the mean percentage weight lost at each treatment is plotted against the (sat. def. x time) in Fig. 16. The results of the

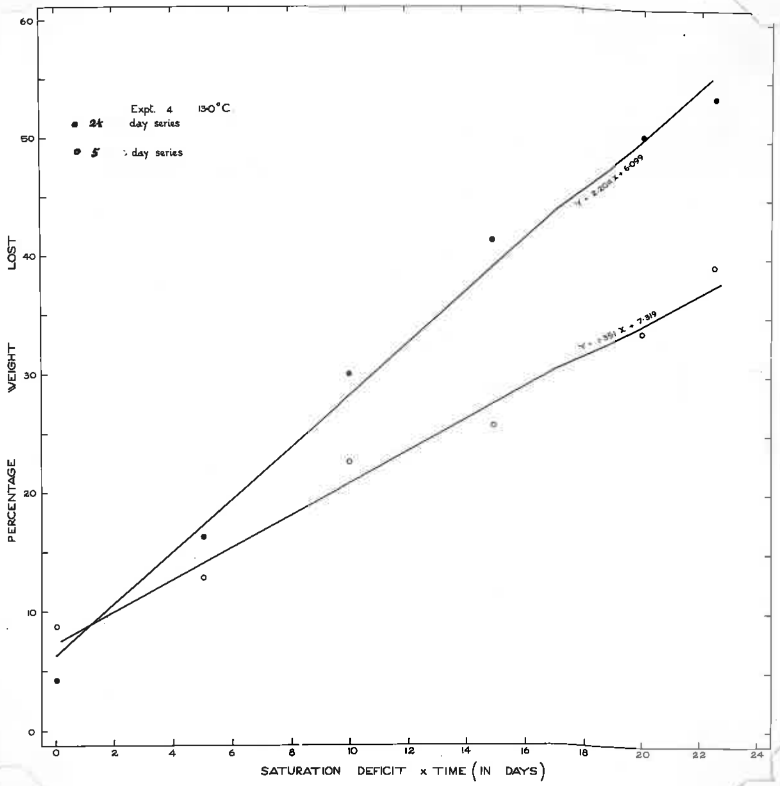

Fig. 16. The mean % weight lost in relation to the product of saturation deficit and time at 13.0°C; Experiment 4. Linear regression equations have been fitted to the data. Note the tendency of the points of the 2½ day series to lie along a curve.

Table 17

The mean % weight lost in relation to (sat.def. x time) at 13°C - Experiment 4

Series	2½ days			5 days						
Sat. def. x time	No. used	Mean % wt. lost	s^2	No. used	Mean % wt. lost	s^2	S.E. of of means	Total d.f.	t	p
0	50	4.2	9.04	50	8.6	6.10	0.550	98	8.00	<0.1%
5	71	16.3	20.66	76	12.8	14.28	0.688	145	5.09	<0.1%
10	79	30.0	57.20	72	22.5	98.57	1.429	149	5.25	<0.1%
15	79	41.3	52.95	71	25.6	107.94	1.597	148	9.83	<0.1%
20	77	50.2	91.06	84	33.7	117.02	1.614	159	10.22	<0.1%
22.5	73	53.4	84.20	71	39.3	104.96	1.620	142	8.70	<0.1%

two series can be compared by a regression analysis, but this assumes that the percentage weight lost in each series is a linear function of the product of saturation deficit x time. It is therefore preferable to directly compare the mean percentage weight losses of the groups in the two series by using their standard errors. The values of t indicated that the groups lost different amounts of water at each treatment, and that the prepupae in the $2\frac{1}{2}$ day series lost more weight than the prepupae in the 5 day series at each level of evaporation, except over water. The differences are all significant at the 0.1 per cent. level of probability. It is probable that excretion took up a larger proportion of the percentage weight lost in the 5 day series than in the $2\frac{1}{2}$ day series and that the differences between the two series in the water lost by prepupae by transpiration were even greater than are apparent from the data.

When the data are now analysed by a regression analysis (Table 13, Appendix) it is seen that not only was the percentage weight lost greater in the $2\frac{1}{2}$ day series but the rate of weight loss was greater in the 5 day series. The data are, however, complicated by the fact that the percentage weight lost in relation to saturation deficit in the $2\frac{1}{2}$ day series is best fitted, not by a linear, but by a polynomial regression; the quadratic term in the equation was significant at $p < 0.1\%$ (Table 13, Appendix). A similar test for the data of the 5 day series indicated that the quadratic term in the equation was not significant, but since much of the weight lost at the lower levels of evaporation was probably due to excretion it is possible that the loss of weight excluding excretion would be best fitted by a polynomial. Similarly, a polynomial regression would fit Salt's (1946) data for Cephus cinctus and Ludwig and Landsman's (1937) data for Popillia japonica larvae. A polynomial regression is indeed expected because insects have not got an inexhaustible supply of water and the weight lost must sometime approach a limit.

2. The effect of loss of water on the survival-rate

After having been desiccated in the experiments described in the previous section the prepupae were placed in individual burrows in moist soil and the numbers of prepupae alive were recorded at the required times. In Experiment 1 more than 40 per cent. of the control group died, so the results were not analysed but were used as a rough guide for Experiment 2.

Experiment 2:

After prepupae had been desiccated at different saturation deficits at 16.9 and 26.8°C and had lost the amounts of weight shown in Table 15, they were placed in moist soil. The percentage mortality after 22 days is recorded in Table 18. Details of the probit analyses of the data for the two temperatures are also given in Table 18; a test of parallelism of the two probit lines indicated that the slopes did not differ significantly, and the LD50's were not significantly different.

A disturbing feature of the experiment was the wide range in percentage weight lost by insects within the groups, particularly at the higher saturation deficits. This variability can be neglected statistically, - even if the mean percentage weight lost is used - because the probit analysis

Table 18.

Percentage mortality of prepupae in relation to saturation deficit

(a) 16.9°C

Sat. def.	% mortality	Corrected % mortality	Probit
13.50	85	75	5.7
11.62	63	38	4.7
9.78	83	72	5.6
7.80	60	33	4.6
3.97	50	17	4.0
2.27	40	0	-
1.73	37	0	-
1.19	37	0	-
0.65	37	0	-
0.0	40	0	-

Y = 0.201x + 3.021
b = 0.201
S.E. of b = 0.024
L.D.50 = 9.85
Sm = 1.06

(b) 26.8°C

Sat. def.	% mortality	Corrected % mortality	Probit
15.46	90	83	6.0
12.16	95	91	6.3
9.12	58	28	4.4
6.41	58	28	4.4
3.44	50	14	3.9
2.11	42	0	-
1.92	47	9	3.7
1.22	82	69	-
0.60	42	0	-
0.0	42	0	-

Y = 0.202x + 3.115
b = 0.202
S.E. of b = 0.058
L.D.50 = 9.33
Sm = 1.163

Test of parallelism of probit lines

Source of variation	d.f.	S.S.	M.S.	V.R.	p
Parallelism of regressions	1	0.389	0.389	< 1.0	N.S.
Residual heterogeneity	7	9.210	1.1315		
Total	8	9.599			

is not complicated by the inequalities of the variances of the points along the horizontal axis. But any such analysis is biologically unsound unless the range of weight (water) lost by insects at the different treatments is small and is constant.

For use in later experiments the results of this experiment were combined and it was estimated that 50 per cent. of prepupae died when they had lost 29.6 per cent. of their weight; 95 per cent. limits of the LD50 were ± 5.0 per cent. The details are given in Table 14, Appendix.

Experiment 3:

In this experiment and in Experiment 4 two new features were introduced:- (1) only prepupae whose weight losses fell within the prescribed limits were used to estimate the survival rate, and (2) after having lost the required amount of weight prepupae were placed on moist blotting paper and allowed to absorb water before being transferred to soil. It was thought that prepupae treated in this manner would have a chance to recover, at least partially, before expending energy in constructing a cell in the soil. The effect of water loss on the survival rate might then be more akin to natural conditions in which a prepupa does not have to construct a new cell after being desiccated.

The procedure in this experiment was as follows. Insects were randomised into groups and each group was allotted at random to one of five saturation deficits which were chosen from the results of Experiment 2 to give a good spread of mortality. The expected percentage weight loss in 24 hours for each group was calculated and it was decided that only those insects whose weight losses fell within the range of the expected mean weight loss ± 1.5 per cent. would be used to estimate the survival-rate.

Since the percentage weight loss of a group of insects at any saturation deficit was approximately normally distributed there would be some insects which would lose too much weight if left in the treatment for 24 hours. So the prepupae were weighed 20 hours after treatment. Those whose weight losses fell within the prescribed limits were considered to have completed treatment and were then placed on moist filter paper and allowed to absorb water for 24 hours. The prepupae which had lost more weight than required were discarded, and those which had not lost as much weight as was required were replaced over the acid-water mixture and weighed again 4 hours later. This procedure was repeated every 4 hours, if necessary, until many prepupae had each lost an amount of weight falling within the prescribed limits.

The procedure was repeated with each group at each saturation deficit so that the variation in percentage weight lost at all the treatments was ± 1.5 per cent. and the variance of the percentage weight lost in each group was small and constant. The distribution of the percentage weight lost by each group after 20 hours and the distribution, after 20 hours,of the weights lost by the prepupae selected finally to estimate the survival-rate are shown in Fig. 17.

The mean percentage weight lost by the selected insects at each saturation deficit and the percentage mortality of larvae 2 and 4 weeks after treatment are given in Table 19. The slopes of the probit lines were much smaller than that calculated for the data of Experiment 2, so the prepupae died in much smaller numbers than expected. An LD50 could not be calculated

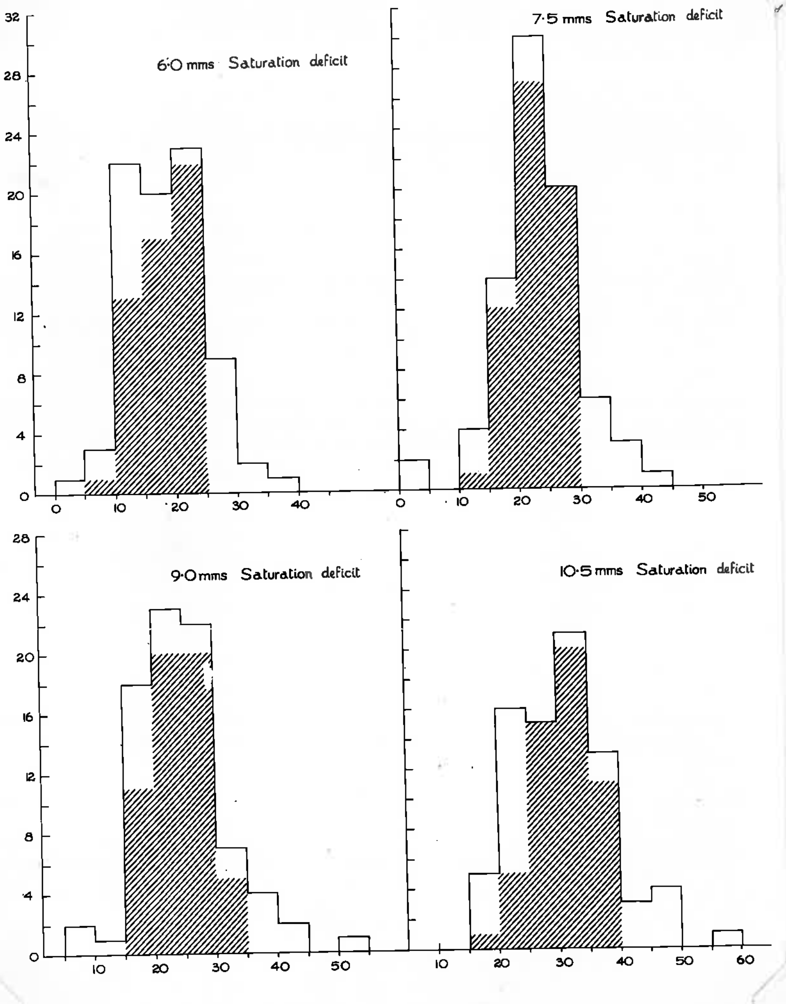

Fig. 17. **The distribution of percentage weight lost in relation to saturation deficits, Experiment 3.**

The shaded portion in each group represents those insects whose weight losses were adjusted to fall within the range of the mean ± 1.5 per cent.

Frequency along the ordinate; percentage weight lost along the abscissa.

Table 19

The effect of percentage weight lost by prepupae on the mortality rate - Experiment 3

Time	Sat. def.	% wt. lost	No. used	No. dead	% mortality	Correc. % mort.	Probit
2 weeks	0	3.5	40	2	5	-	
	6	23.1	53	6	11	6	3.49
	7.5	26.9	60	8	13	8	3.64
	9.0	30.5	56	12	21	17	4.06
	10.5	34.8	52	12	23	19	4.13
4 weeks	0	3.5	35	3	9	-	
	6	23.1	50	15	30	23	4.27
	7.5	26.9	55	18	33	26	4.37
	9.0	30.5	54	21	39	33	4.57
	10.5	34.8	50	21	42	36	4.66

2 weeks

b = 0.035

S.E. of b = 0.032

L.D.50 = 66.6 (?)

4 weeks

b = 0.034

S.E. of b = 0.025

L.D.50 = 45.2 (?)

for the data and the experiment had to be repeated as follows:-

Experiment 4:

This experiment was designed for two purposes (1) to determine in more detail the effect of percentage weight lost on the survival-rate

(2) to determine whether the loss of equivalent amounts of water at different rates affected the survival-rate.

The experimental procedure was the same as that in the previous experiment. The mean percentage weight loss of the selected prepupae from each treatment, and the respective percentage mortalities of larvae 10, 20, 30, and 40 days after treatment are given in Table 20. The relation between the mean percentage weight lost and percentage mortality is given in Fig. 18.

Initially, it was planned to obtain six lots of prepupae which had lost six different percentages of their original weight over a period of three days and to compare directly their survival-rate with another six lots of prepupae which had lost the same weights over a period of six days. It was also essential to get a good spread of mortality to estimate the effect of percentage weight lost on the survival-rate of prepupae; so the first treatment commenced was that at which prepupae were expected to lose most weight - 22.5 mm saturation deficit for three days. It was soon apparent that no prepupa would survive after having lost 60 per cent. of its weight and that the percentage weight losses in the three day series were too widely spaced for a good estimate of mortality. So when it was discovered that the rate of loss of water of prepupae in the six day series was lower than that from prepupae in the three day series (Fig. 16) the prepupae in the six day series were selected so that their mean percentage weight losses filled some of the gaps which existed in the three day series.

There were consequently only two lots of prepupae in each of

Table 20

The effect of percentage weight lost on percentage mortality of prepupae - Experiment 4

Series	Mean % wt. lost	No. of days after treatment							
		10		20		30		40	
		No. used	% mort.	No. used	% mort.	No. used	% mort.	No. used	% mort.
3 days	4.2	29	0	29	0	28	4	28	4
	18.2	50	6	50	6	49	6	48	8
	33.0	39	3	39	3	38	3	38	5
	48.1	32	63	32	66	31	68	31	71
	55.0	41	93	41	93	41	95	41	95
	59.6	34	100	34	100	34	100	34	100
6 days	8.6	50	2	50	2	50	4	50	4
	17.8	48	4	48	4	46	7	46	7
	32.7	40	3	39	5	39	8	37	11
	39.0	29	7	28	7	28	7	26	8
	45.4	39	21	38	24	38	32	36	39
	51.0	32	38	31	52	29	66	29	76

the two series which lost similar amounts of water - 18.2 and 33.0 in the three day series, and 17.8 and 32.7 per cent. in the six day series. The numbers of prepupae which died in these groups were not affected by the rate at which the prepupae lost their water.

The outstanding feature of the results, as shown in Fig. 18, was that one dosage-mortality curve did not give as good a fit as a combination of two curves to cover different parts of the range. The death-rate of prepupae was not influenced to any extent by weight loss up to about 40 per cent., but rapidly increased after this point with relatively small increments of weight loss. Using combined figures for the two series it was estimated

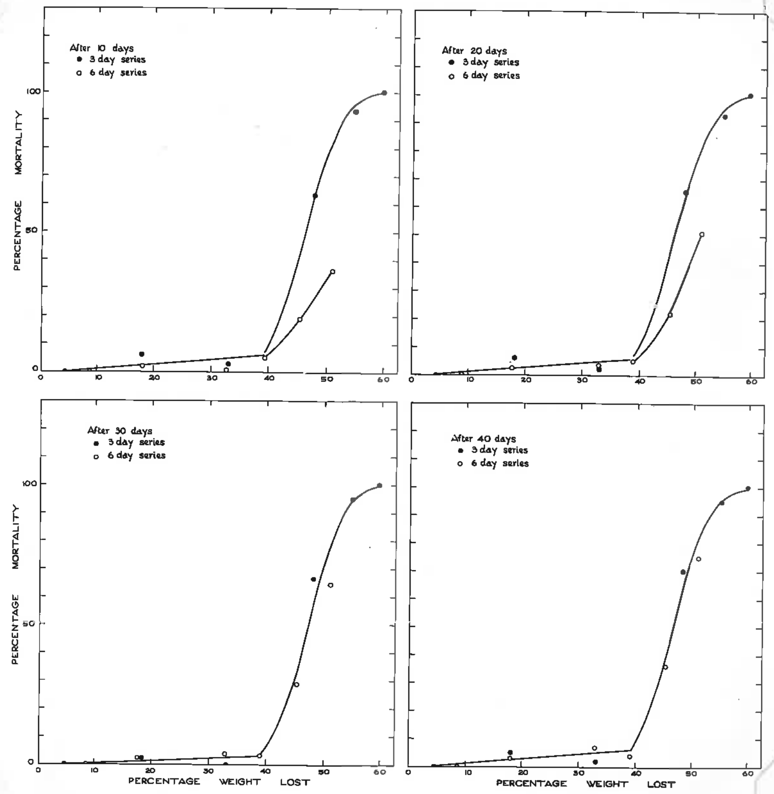

Fig. 18. The effect of percentage weight loss on the mortality rate of prepupae. Experiment 4.

that a weight loss of 46.6 per cent. (95 per cent. limits = ± 1.9 per cent.) would be necessary to kill 50 per cent. of the prepupae after 40 days (Table 15, Appendix).

The interpretation of the data needs a certain amount of judgment. Seven to 8 per cent. of prepupae died after losing 39.0 per cent. of their weight in six days. There are no comparable figures for the three day series, but the similarity of the data in the two series, particularly after 40 days, does suggest that if a group of prepupae had lost 39.0 per cent. of their weight in three days they would not have died in any larger numbers than in the six day series. A weight loss of 39 per cent. has therefore been taken as the point at which the death-rate of prepupae increased in both series. The water content of the prepupae averaged about 77 per cent. at the start of the experiment so the death-rate increased when the percentage water content of the prepupae fell below 64 per cent.

The data suggest that the rate of loss of water after the 39.0 per cent. weight limit may have had some affect on the survival-rate initially. The results of both series above this limit could be represented by one mortality curve at each interval of time, but the fit became progressively better as time increased. To emphasise this point the data for the two series are represented by different mortality curves after 10 and 20 days, but the scarcity of points does not permit the comparison of their slopes with any confidence. After 40 days there was no difference in the survival-rate of prepupae in the two series.

Pupation was retarded when prepupae lost more than about 25 per cent. of their weight and some prepupae which died later died when pupating. The increase in mortality with time emphasises the warning by Andrewartha and Birch (1954) that the influence of water loss on the death-rate may not be immediate and that time is an integral part of any mortality data.

3. The influence of the cell on the rate of water loss from the diapausing prepupae.

The cell of the diapausing prepupae is not lined with wax, but it was considered possible that a biological membrane of some kind - perhaps proteinaceous - was secreted on its inner surface and enhanced the chances of survival of the larva by reducing the permeability of the cell water to water vapour.

wall ?

surface ?

To test this hypothesis prepupae were dug out of the soil and portions of their cells were used to construct simple evaporimeters. The permeability of the inner surfaces of the cells to water vapour was then measured. It was found that Dekatinsky's cement would give a satisfactory initial seal between glass and the soil of which the cell was composed, if the soil was dry; so lengths of $\frac{1}{4}$ inch glass tubing were attached to the cells as shown in Fig. 19 and the seal between soil and glass was completed with hot beeswax. The glass tubes (with cells attached) were then immersed vertically in water until they were filled with water, corks were inserted into the free ends of the tubes under water and the corks were covered with wax.

The following "evaporimeters" were also constructed to operate as controls:-

(a) Evaporimeters made from artificial cells (i.e. cells carved out of dry soil to the size, shape etc. of the natural cells).

(b) Evaporimeters consisting of glass tubing open at the top end.

(c) Evaporimeters which incorporated natural cells but which were filled with a saturated solution of sodium chloride.

Four of each type of evaporimeter were assembled and were allocated at random to positions on a stand. A sheet of graph paper placed behind

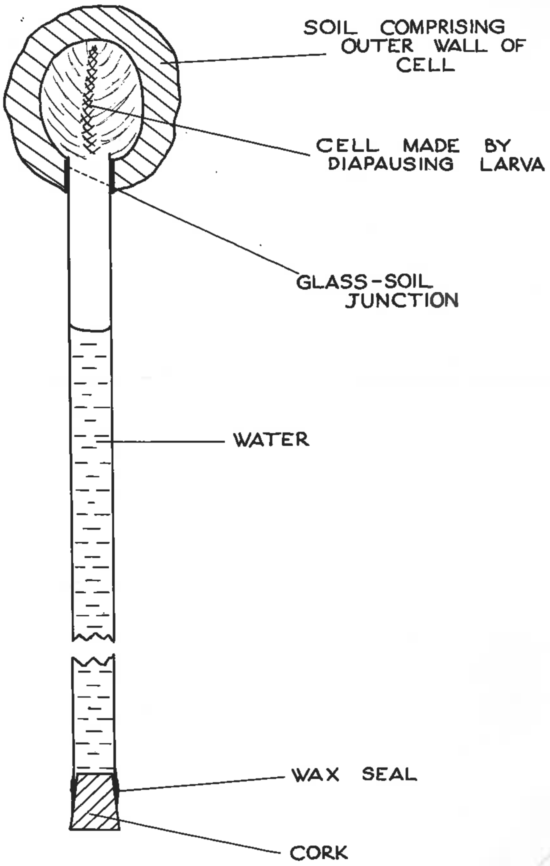

Fig. 19. A schematic diagram of the system used to measure the permeability to water vapour of the wall of the cell constructed by the diapausing prepupa.

them was used to determine the levels of liquid at convenient intervals of time.

At the completion of the experiment each cell was carefully cut away from the top of its glass tubing and was filled with mercury up to the mark of its attachment. The mercury was weighed and the internal surface area was calculated on the assumption (a) that the portion of the cell used was half a sphere, and (b) that the diameter of the sphere was equal to the external diameter of the glass tubing. The internal surface area of the tubes was taken as the evaporating surface of the tubes which were open at the top end. The increments of water loss, expressed as ccs/sq. cm. of the evaporative surface, are given for each evaporimeter in Table 16, Appendix.

Powdered potassium permanganate placed at the bottom of each evaporimeter indicated from time to time that certain evaporimeters were leaking. The bottoms of these evaporimeters were resealed and the resultant excess water losses, marked with asterisks in Table 16, Appendix, were not included in the analysis of the results.

The loss of water from the open tubes was clearly much greater than that of water from the other "evaporimeters". The losses of water from the other evaporimeters were subjected to an analysis of variance. The main point of interest in the analysis was the lack of evidence for a significant difference in water loss from the evaporimeters which incorporated both artificial and natural cells.

However, the evaporimeters which contained brine (Type 2) were expected to lose less water than those with water. This they did, but the differences were not significant. The method, therefore, was fairly crude. Nevertheless, if there was a difference between artificial and natural cells it should have been sufficiently large to have been detected in the experiment.

It is probable that the lining of the prepupal cell does not influence the rate at which water passes out of the cell and therefore does not enhance the chances of survival of the prepupa.

4. Conclusions:

Since the cell which the prepupa makes seems not to influence the rate of water loss, the tolerance of prepupae to dry weather is dependent on (a) the rate at which they lose water, and (b) their ability to tolerate the loss of water.

The ability of the prepupa to tolerate water loss has been demonstrated to fall off rapidly after a critical amount of water is lost. Similar data have been recorded for larvae of *Cephus cinctus* (Salt 1946); larvae, prepupae and pupae of *Popillia japonica* (Ludwig and Landsman 1937) and eggs of *Calandra oryzae* (Birch 1944). The rate at which *Cephus cinctus* and *Popillia japonica* lost water did not affect the survival-rate (Salt, Ludwig and Landsman op. cit.). Similarly the rate at which *A. howitti* lost water did not affect the mortality recorded after 40 days.

Prepupae of *A. howitti* absorbed water rapidly after desiccation. The variances of the percentage absorbed by any group were large and tended to be a function of the mean percentage absorbed (Table 17, Appendix), so to compare the effect of water absorption on the survival-rate the data were subjected to a logarithmic transformation. The analysis of variance of the data such as that of Experiment 3, (Table 17, Appendix), indicated that the amount of water which prepupae absorbed after desiccation did not affect the survival-rate.

Prepupae in the field would absorb water when rain penetrated to the depth at which the insects were in the soil, so the termination of drought can be estimated from rainfall data. But prepupae do not lose equivalent amounts of water when exposed to equal products of saturation deficit x time and no reasonable estimate can be made of the amount of water they would lose during any given period of drought. However, the insects in the field are expected to lose water more slowly than they did in the six day series in the laboratory. An estimate can thus be made of the minimum amount of dryness required to kill 50 per cent. of them; this is 30 mm of saturation deficit x days. Soil at wilting point at 20°C has a saturation deficit of roughly 0.35 mm of Hg, so 50 per cent. of larvae would be killed if they lived in soil at wilting point for about 86 days at this temperature.

It can be concluded that the prepupae are remarkably resistant to desiccation and it is unlikely that very many die of water loss.

What about drowning in
the cell? i.e. does cell wall
keep out water?

VIII. THE PUPA

Pupae lie in the cells constructed by the larvae before entering diapause. They are found in the soil from the end of December onwards, i.e. at the height of the southern summer. Pupae rarely, however, die from water loss because the pupal period is relatively short, and the change from the prepupa occurs with a negligible amount of water loss; in fact the loss of dry matter at pupation results in the water content of the pupa (79.6%, 95% limits ± 1.3%) being significantly higher than that of the larva. Further, the cuticle of the pupa is not abraded as is that of the prepupa (Lower 1957), and so the pupa would be expected to lose its water more slowly.

It was of interest to determine the rate of loss of water from pupae so prepupae left over from Experiment 2, Section VII were allowed to pupate and their weight losses were measured at a number of saturation deficits at 16.8 and 26.9°C. The results are expressed in Table 18, Appendix. The excessive variability within treatments was probably due to the fact that the pupae actively excrete water before metamorphosis. It was estimated roughly from this data that the pupae lost 0.7 per cent. of their weight per mm sat. def., so that they lost water only one-half or one-third as fast as prepupae. The difference in the rates of water loss in the two stages is probably correlated with cuticular structure (Lower op. cit.), but it is possible that differences in metabolic rate are also involved.

IX. THE DISTRIBUTION AND ABUNDANCE OF A.HOWITTI IN COUNTIES GREY AND ROBE

The first examination of A.howitti in the lower South-east was made in 1933 by Davidson (Swan 1934). Comments by Swan (op. cit.) and Davidson (1935) indicate that A.howitti was fairly abundant during the years 1931-1934. The distribution of the species at this time is that which was plotted by Swan (1934) and is reproduced in Fig. 1. The numbers of A.howitti then apparently decreased for Swan (1937) records that the species was not widely troublesome between July 1934 and June 1936. By 1940 reports of damage were again being received and in 1944 many thousands of acres of feed were denuded by the larvae (Andrewartha 1945). The numbers then decreased again and did not build up till 1952. In this year Madge (1952) recorded the largest flights of beetles since 1945 and plotted the distribution of the species in South Australia.

Mr. Pearson, formerly District Agricultural Adviser at Mt. Gambier, observed that A.howitti was more prevalent in 1951 than in 1950 and did considerable damage in scattered patches all over the district in 1951. In 1952 the insects damaged paddocks on individual properties but the damage done to pastures in the district as a whole was not serious.

I commenced to make observations on the abundance of A.howitti in 1953. In this year, following a dry summer, cockchafers were widely distributed and thinned out pastures over a wide area, but only did serious damage in some areas on light soils.

In 1954 cockchafers were baring some of the sandy loams of the red-gum country and some of the pastures in terra rossas by early May. By June considerable damage had been done in the lower South East, and damaged pastures were seen on some of the higher banks of podsolised sands in the hundred of Mingbool, on some of the podsolised sands and sands of the ranges in the Glencoe district, on some of the sandy ridges near Tarpeena, and in the hundred of Caroline and Gambier. Larvae were numerous on many of the meadow podsols such as those around Kalangadoo, Penola and Pleasant Park and those adjacent to the heath country in the Hundred of Fox. Larvae were particularly abundant, however, on the meadow podsols of Wrattonbully, on the terra rossas around Mt. Schanck and Mt. Gambier and on the meadow podsols of the Burrungule district in the hundreds of Benara and Hindmarsh.

In 1955 larvae were found in all the places mentioned above but were more numerous. They were most numerous, however, around Mt. Schanck and Allendale and at Wrattonbully.

In 1956 larvae were generally rather scarce. They were perhaps most numerous around Wrattonbully and in the Burrungule district and their numbers were relatively low on the terra rossas.

It would seem that A. howitti is found more or less in the same places every year. The numbers in these places may vary from year to year in relation to rainfall, but there are two districts - Mt. Schanck-Allendale and Wrattonbully which, of recent years, have appeared to be especially favourable for the survival of the species and the largest numbers of larvae have been found in these areas each year.

X. ANALYSIS OF THE ENVIRONMENT OF A.HOWITTI.

(i) Influence of the weather on the rate of increase

There is an optimum water content of soil (between about pF 3.0 and pF 2.6) at which A.howitti develops most rapidly and has the best chance of surviving. These limits are about the same for all stages of the life-cycle. But only those stages which are present during January-March are likely to be harmed by excessive dryness and usually only those stages which are present during June-September are likely to be harmed by excessive wetness. Unless sufficient rain falls during January-March the beetles may not emerge from their cells and lay eggs. If there is not sufficient rain during March-May the eggs in the soil may die from desiccation and the young larvae in the soil may die from desiccation and starvation. Finally if an excess of rain falls in the period June-September larvae may drown or be infested with Cordyceps aphodii. The chance of the insect surviving in each of these three periods is discussed below and an attempt is made to estimate the effect of the weather on the survival-rate of the species between 1927 and 1956 on the terra-rossa soils in the vicinity of Mt.Gambier.

In dry summers, such as that of 1953, light showers of rain permit sufficient water to percolate down burrows or other passages in the soil to stimulate the emergence of adults in small numbers. The soil is not generally wetted, however, and the adults can only survive in moister pockets of soil. It is probable that in such weather many adults die before laying their eggs. Even if sufficient rain falls to stimulate the mass emergence of adults many females may die before laying eggs. Sampling of experimental plots at Mt. Schanck in 1955, for example, indicated that there were only 12 larvae per female (Table 5a) in the soil shortly after the eggs had hatched. As it was almost certain that every female had mated before being placed in the experimental plots it would appear that 50 per cent. of the females had not laid eggs or that 50 per cent. of the eggs had died. If 50 per cent. of the eggs had died because of a lack of water in the soil all the larvae should have been found in the wetter patches. Some larvae were found in the drier patches, however, so it is more likely that 50 per cent. of the females did not lay eggs and that, whilst most of the adults had aggregated and laid eggs in the wetter patches, a few eggs had been laid in the drier soil.

If soaking rain falls between January and March the adults emerge en masse, the survival-rate is almost certainly higher, and many more females lay eggs in suitable places before dying. Weather, during January-March, therefore, primarily influences the birth-rate by controlling the number of places in which adults can survive and lay their eggs.

It is interesting to note that there was a great reduction in numbers of Popillia japonica larvae in the U.S.A. in 1933; this was attributed to the deficient rainfall of the preceding summer at a time when egg-laying and hatching were normally at a maximum (Fox 1934). Similarly, the numbers of Lepidiota caudata in Queensland decreased after dry summers (Smith 1936).

The survival-rate of adults of A. howitti in South Australia is probably also influenced by the weather which prevails after they have emerged from the soil. Changes in weather which bring rain in summer can be divided into northerly and southerly. Southerly changes bring cold air over the continent from the Antarctic Ocean so that the days following the rain are cool and the nights are cold; northerly changes involve the movement of moist tropical air southwards and usually produce hot, humid days and warm thundery nights. Beetles frequently cannot fly for a number of days after a southerly change and usually lay their eggs before flying, but beetles which emerge after thunder storms fly before laying their eggs.

The soil from which the adults emerge is usually relatively bare at the surface and, because it is wetter than soil under stubble (Table 4, Appendix), is more likely to be a suitable place for the female to lay her eggs. In dry summers, however, even bare areas are not wet enough to be suitable places unless they are in hollows. If the soil from which the adults emerge is, therefore, not a suitable place in which to live and the rain is brought by a southerly change, many adults would die before laying their eggs or would lay their eggs in unsuitable places; if the rain is brought by a northerly change, the adults fly and so have a better chance of finding a suitable place to lay their eggs.

Weather might also influence the birth-rate in wet summers by stimulating the germination of clover seedlings because, as Carne (1956) has suggested, the clover seedlings may provide innumerable points of entry into the soil. In addition, where the clover germinates the water content of the soil is likely to be most favourable for survival and oviposition. These observations of Carne's are interesting because I have never seen clover germinating before a major flight of A.howitti in South Australia. Canberra, however, has a wetter summer than South Australia and clover usually germinates in Canberra in the middle of the flight season. In South Australia the rain which may stimulate the clover to germinate usually stimulates the emergence of the adults from the soil and only adults in late flights would benefit from the presence of clover. When January is wet, however, as it sometimes is, clover germination may indeed stimulate adults to burrow into clover-dominant areas in very large numbers. This is another means by which a wet summer may provide a larger number of favourable places in which to live.

Most of the large flights of adults of A.howitti in South Australia take place between February 1 and March 1 but rain which falls before February 1 may stimulate beetles to emerge in late January. On the other hand large flights of beetles may occur in March, particularly in dry summers, but even in such summers probably half of the beetles emerge before March 1. The amount of rain which falls between January 15 and March 1 can probably therefore be used to obtain an estimate of the "wetness" of the summer in any year. The total amount of rain cannot be used for this purpose because 35-40 points of rain seem to be required to stimulate a general emergence of adults from the soil and therefore falls of rain of less than 35-40 points are not "effective" rain.

An examination of the rainfall data for Mt. Gambier indicated that rain in summer tended to fall within clearly defined "wet spells", and that small amounts of rain falling on successive days could add up to more than the 40 points required to stimulate adult emergence and classify as "effective" rain. All the rain falling within a wet spell does not, however, penetrate the soil, because the surface of the soil dries out rapidly after very light showers. An arbitrary figure of 10 points has therefore been taken as the smallest quantity of rain falling on any one day which contributes towards wetting the soil, and all showers heavier than 10 points per day have been totalled for each wet spell. If the total rain so obtained for any wet spell was more than 40 points it has been considered as "effective" rain. The total "effective" rain for the period January 15 to March 1 for each of the years 1927 to 1956 is plotted in Fig. 20 and is discussed below in relation to the numbers of A.howitti.

Unfavourable dry weather in March-May may kill first instar larvae before they migrate to the surface. The data of section VI ((i),(2)) indicate that 50 per cent. of the young larvae at Mt. Schanck in 1955 were killed because sufficient rain did not fall until 30 days after the eggs had hatched. Consultation of the rainfall data for Mt. Gambier suggested that in certain other years the period of drought to which young larvae were exposed was more than 30 days and that in these years more than 50 per cent. of the young larvae were killed. Estimates for each year from 1927 to 1956 have therefore been made from the rainfall data of (a) the peak period between January 15 and March 1 when beetles emerged from the soil, (b) the date when the young larvae migrated to the surface. It has been assumed that the bulk of the eggs were laid 7 days after rain stimulated the adults to emerge from the soil, and soil temperature data taken at a depth of 6 inches under pasture at Mt. Burr have been used, in conjunction with the temperature-development curve (Fig. 10), to estimate (c) the day by which 50 per cent. of the eggs hatched after each flight. The details are given in Table 19, Appendix.

The period of drought - the period between the day on which the eggs hatched and the day the larvae migrated to the surface - has been calculated from the above estimates for each year and is given in Table 19, Appendix. No temperature records are available before 1940. The number of days which the eggs required to hatch during the period 1927-1940 have been calculated on the mean monthly temperatures for the years 1940-56. The estimates obtained in this way of the numbers of days of drought to which young larvae were exposed between 1927 and 1940 may thus be out by 4-5 days. This error is small compared to the number of days of drought required to kill 50 per cent. of the population.

The estimated number of days of drought after the eggs hatched in each year after 1927 has been plotted in Fig. 20. Its relation to the numbers of A. howitti in the Mt. Gambier district is also discussed below.

It has been recorded earlier that excess water affects the survival-rate of A. howitti on the heavier soils as well as on the sands of the flats. Though flooding does not occur on the heavier soil many larvae may be killed by Cordyceps in very wet winters.

The wetness of the winter in any year depends on the total amount of rain which falls and the distribution of rain. It is probable that the total amount of rain in winter is a better criterion of the risk of A. howitti being infested with Cordyceps. In the absence of any experimental data, however, the total rain in the months June-September and also the total number of days on which rain fell in the same period are plotted in Fig. 20 for the years 1927-1956.

Fig. 20 therefore shows estimates of the wetness or dryness of the environment at Mt. Gambier during the three critical stages of the life-cycle of A. howitti for the years 1927 to 1956. The dotted horizontal lines indicate the means of wetness or dryness for 1927-1956 and the arrows mark the years in which the species was most abundant. An examination of Fig. 20 together with the observations and experimental results accumulated during the period of study suggest that weather affects the three critical stages of A. howitti in the following ways:-

(1) Notwithstanding direct evidence that Cordyceps kills many larvae in some years the data over the years suggests that at no time within the period 1927-1956 has wetness in winter caused a widespread and severe decline in numbers of A. howitti. The numbers of A. howitti increased over the years 1940-1944, for example, despite the wet winters of 1942 and 1943. These winters, by either criteria, were extremely wet and rank with those of 1946, 1947 and 1955 as some of the wettest on record.

(2) The winter of 1944 was drier than average so that few larvae would have been killed by Cordyceps, and the summer of 1945 was wetter than average so was probably favourable for the survival of adults and eggs. Nevertheless larvae of A. howitti were difficult to find in the winter of 1945. Reference to Fig. 20 suggests that the only possible

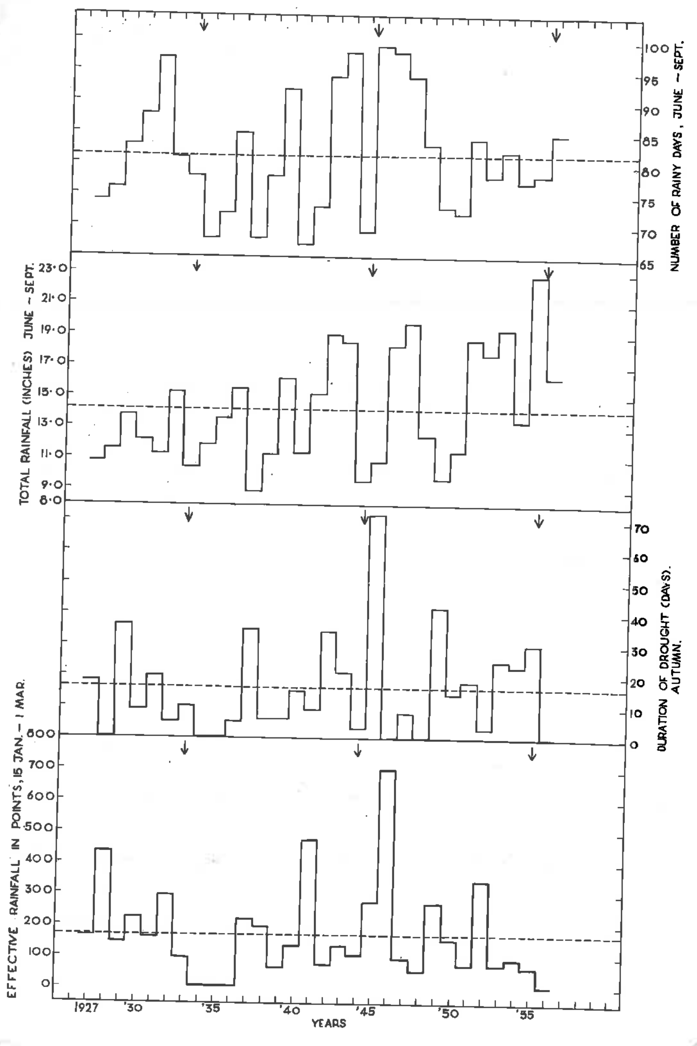

Fig. 20. Estimates of the wetness of summer and winter and the dryness of autumn as they have affected populations of *A. howitti* at Mt. Gambier during the period 1927-1956.

cause of this sudden decline in numbers was the excessively long drought to which young larvae were exposed in the autumn of 1945.

(3) The "effective" rain in summer is not an estimate of the probability of the adults surviving to lay eggs. Nevertheless it was estimated that 50 per cent. of adults died before laying eggs in 1955. The "effective" rain in this summer was only 58 points which is well below the mean figure for the period. The inference is that in years which were drier than 1955 fewer adults survived to lay eggs. In particular it seems from Fig. 20, in the absence of other obvious causes of catastrophe, that the decline in numbers of A. howitti after 1933 was due to the abnormally dry, successive summers of 1934, 1935 and 1936.

The results of experiments indicate that a year optimal for survival of A. howitti is one in which (a) the summer is reasonably wet, with 150 points or so of "effective" rain falling between January 15 and March 1, (b) the season "breaks" early and young larvae do not have to tolerate many days of drought, and (c) the winter is relatively dry and not many larvae are killed by Cordyceps. Fig. 20 suggests that the outbreak of A. howitti in the early 1930's followed a succession of years in which weather was near to optimal. Similarly weather was optimal in all but a couple of critical stages during the years 1936-1941 and it is known that during this period A. howitti built up to plague proportions.

The numbers of A. howitti reached one of the maxima in 1944. Though the wet winters of 1942 and 1943 did not cause a severe decline in numbers of larvae on terra rossa soils they must have checked the rate of increase of the species and, if they had not been so wet, the damage done to pastures in 1942, 1943 and 1944 would have been much more severe.

It is surprising that the autumn droughts of 1942 and 1943 together with the wet winters did not reduce the numbers of A. howitti in 1944. The autumn drought of 1942 was probably, however, shorter than that plotted in Fig. 20. In 1942, 66 points of rain fell on January 20 and 23 points of rain fell on January 25. To be consistent with the other rainfall data it has been estimated that a major flight of beetles laid eggs by February 1. It is more probable that the rain which fell in January stimulated adults to emerge as they metamorphosed, that the majority of eggs were laid by February 10 or 11, and that the young larvae were in the soil for about 24 days before sufficient rain fell to wet the soil. In addition the wet winter of 1943 followed a wet summer so it is probable that not as many larvae were killed by Cordyceps as in 1955 in which year excess water in winter followed a dry summer.

The data of Fig. 20 indicate that the years 1947-1952 were on the whole favourable for A. howitti. Following the decline of 1945 the species increased during this period and was fairly numerous by 1952. The dry summer of 1953 checked the rate of increase so that larvae were less numerous in 1953 than in 1952, but this check was not sufficient and especially favourable weather in 1954 led to a maximum build-up in numbers by 1955. In February 1955 the potential damage to pastures was probably as high as it has ever been in the South-east, but, as recorded earlier, it was estimated that about 50 per cent. of the adults failed to lay eggs and that about 50 per cent. of the young larvae died before migrating to the surface.

The numbers of larvae in 1955 were further reduced by a heavy (55-70 per cent.) infestation of Cordyceps in winter. The dry summer of 1956 further restricted the rate of increase of the species and A.howitti was relatively scarce in 1956. The decline in numbers in 1956 therefore was due to weather being unfavourable in four critical stages in succession. Apparently, however, no catastrophic reduction in numbers had occurred because, with the aid of a wet summer and an early break to the season, larvae of A.howitti have been fairly numerous in 1957.

It does, in fact, seem that once A.howitti has built up to a certain level of abundance, such as it attained in 1941 and 1954 and appears to be maintaining now, one favourable year, like that of 1954, will suffice for its numbers to rise to plague proportions.

Weather also kills A.howitti in other than the critical periods and occasionally may help to reduce numbers significantly. The exceedingly wet summer of 1946 for instance probably helped the decline in numbers of A.howitti after the catastrophic autumn drought of 1945. Similarly an abnormal wet April-May in 1956 may have affected the survival-rate of young larvae at the surface, for young larvae are often trapped by the surface tension of water in excessively wet soils.

First instar larvae may also die after migrating to the surface if dry weather prevails and it was estimated that 50 per cent. of them would die if 20 or more days without effective rain were recorded (section VI, (i)(d)). Young larvae in the field extend their burrows into moist soil as the soil dries on the surface and probably die of starvation rather than desiccation during spells of dry weather, though they may also leave their burrows to find food and then succumb to water loss. It is possible that desiccation helped to kill the larvae in the laboratory experiment, and that the number of dry days required to kill 50 per cent. of larvae in the field is more than 20. An attempt to estimate the effect of drought on mortality in the field was not successful because, to prevent larvae from feeding, it is necessary to prevent rain from falling on the experimental area and also to let the soil in the area dry out. The larvae usually migrate to the surface with the rain which constitutes the break of the season and do not have to tolerate long periods of drought because, once the season breaks, rain usually falls frequently enough, and in sufficient quantities, to keep the soil wet. In 1950, however, virtually no rain fell between April 10 and May 26, and it is probable that many larvae died during these 46 consecutive dry days.

Finally, dry summers have been assumed to affect the survival-rate of the adult, but it is possible that the reduced numbers after dry summers is partly due to the low survival-rate of eggs laid in unsuitable places. No data have been obtained to confirm this, although it has been suggested that eggs survive in higher numbers in soil with a bare surface than in soil under stubble.

Observation suggests that weather similarly controls the numbers of A. howitti on the sands in which it lives; so it is worthwhile discussing to what extent weather affects the death-rate and birth-rate of the species on a sand during the three critical periods postulated for the terra rossa soils around Mt. Gambier. (1) The wetness of summer. Sands require a smaller quantity of water (rain) to wet them to a particular pF value than do terra rossas (Fig. 7), so beetles in a sand would emerge in response to lighter showers of rain in summer. Similarly, the amounts of rain required to make a sand optimal for survival of adults and eggs over a wide area would probably be about 50 points. It is interesting, therefore, to notice that after the dry summers of 1953 and 1956 larvae were more abundant and did more damage at Wrattonbully than at Mt. Schanck. (2) The availability of rain after the eggs hatch. Since sands are more easily wetted than heavier soils, it is expected that larvae migrate to the surface sooner after the eggs hatch in a sand than in a terra rossa. Observation has confirmed that in some years rain falls on sands in autumn and enables young larvae to mirgrate to the surface and also enables clover to germinate, but is not of sufficient quantity to stimulate either of these activities on heavier soils. However, though the duration of drought may be shorter in a sand, as a sand would dry out more rapidly than a soil with a higher clay content, it is probable that the young larvae in a sand would lose water as well as starve. In most years, therefore, drought may kill as many larvae in a sand as in a terra rossa. (3) Wetness of winter. Cordyceps aphodii seems to be as abundant in well-drained sands as on terra rossas. In wet years when Cordyceps kills many larvae on the heavier soils, A. howitti survives on the sands if the sands are well-drained, as some of them are at Wrattonbully. On low-lying sands, such as those around Kalangadoo, however, excess water eliminates entire populations of A. howitti (Plate 9), and is one of the major factors influencing the numbers of the species.

It is possible that weather during these three critical periods may not affect larvae on some sands to the same extent as it affects larvae on terra rossas, and that drought after larvae migrate to the surface plays a more important part in limiting the abundance of the species.

Some pupae and adults were observed to have died in their cells at Wrattonbully in 1953 and it is possible that in dry summers some prepupae, despite their tolerance to desiccation, also die of water loss towards the northern extremity of County Robe. As we proceed north from County Robe evaporation increases and the frequency of summer rain decreases. A. howitti becomes less numerous as the summer drought is prolonged and towards the limits of its distribution it is most abundant after wet summers. It is likely that, as the rainfall decreases, the ability of the prepupa and adult to tolerate drought also plays a more important part in determining whether or not the species will survive, and the abundance of the species is restricted more and more by the shortness of the time in which the rate of increase is positive.

The weather also controls the rate of development of the larvae and hence the fecundity of the adult by determining the frequency with which the larvae feed. Larvae from lawns and other places which are watered during dry spells are usually bigger and more advanced than larvae in pastures generally, and they generally grow into bigger adults. Similarly, too, the larvae which complete their development early (by August) are large and become the biggest adults which emerge first. Larvae which do not complete their development till October feed less frequently because the number of rainy days per month decreases towards spring and evaporation increases. These larvae are usually small and become adults which emerge relatively late.

(ii) Influence of food on fecundity and the survival-rate

Food is one of the major factors controlling the abundance of *A. howitti* for it affects the death-rate and the birth-rate of the species both in the larval and the adult stages.

No estimates have been made of the proportion of adults which survives to lay a second batch of eggs, but there are large tracts of country through which the species is distributed where scattered sheep droppings are the only source of food. In these areas the contribution which the second batch of eggs makes towards the numbers in the population must be very small, if not negligible. In districts in which the stocking rate is higher and, in particular, in which mixed farming is practised, the numbers of beetles surviving to lay a second batch of eggs must be higher. The contribution which their additional eggs make to the total numbers in the population might well be considerable, especially in dry summers. Food sources also influence the survival-rate of adults, again probably more so in dry summers than in wet summers, because they constitute favourable places in which the adults can survive long enough to develop their eggs.

The number of eggs laid by a female before feeding on dung was found to be significantly correlated with the length of the elytra ($r = 0.856$ with 20 d.f. $p < 0.1\%$); the weight of the adult was significantly correlated with the weight of the prepupa, and the weight of the prepupa was correlated with the maximum weight attained by the feeding larva. The fecundity and size of the adults are thus directly related to the food reserves of the larva, as they are in Phyllopertha horticola (Raw 1951).

(1) Pastures

The adults of A. howitti tend, as was seen, to aggregate in certain kinds of places. In these places they survive and lay their eggs and the eggs are able to develop normally. In such places, too, the right type of food is often available and larvae are able to survive, but eggs may be laid in places in which food for the young larva is not available. The lack of food is clearly the reason for the low survival of first and second larvae in recently ploughed paddocks and is also the reason why larvae of A. howitti are never abundant in natural unstocked grasslands even when weather is favourable.

The abundance of A. howitti seems generally to be related to the stocking rate. The larvae can survive in relatively large numbers in recently ploughed paddocks if the stocking rate of the paddock during summer is very high and the rains break early. Mr. Wicks, at Wrattonbully for example, stocked a paddock of rape with 10 sheep to the acre over summer. He then ran a disc harrow over the paddock and sowed it to pasture. The pasture was subsequently destroyed by A.howitti. Similarly, at King's property, Mt. Schanck, larvae survived in large numbers, but particularly in sheep camps, in a paddock of rape which was subsequently sown to pasture. In natural pastures damage may occur around sheep camps.

Larvae survive in highest numbers in well-established clover grass pastures because, in such pastures, heavy applications of superphosphate and a heavy stocking rate produce a layer of organic matter on the surface on which the young larvae can feed. After the break of the season these pastures then produce a lush growth of annuals which serve as an admirable stock of food for the older larvae. Larvae in these pastures also develop into larger adults which lay more eggs.

The abundance of A.howitti seems generally, in fact, to be related to the abundance of subterranean clover. This self-regenerating annual pasture plant is estimated to be sown in some 18 million acres of southern Australia (Willoughby 1954). It is sown alone or with grasses such as Phalaris tuberosa, Wimmera rye-grass (Lolium rigidum Gaud.), Dactylis glomerata (cocksfoot) and perennial rye-grass (Lolium perenne L.).

Wimmera rye-grass and subterranean clover are commonly sown together on the meadow podsols and it is in paddocks sown with these two annuals that A.howitti reaches its highest numbers because, in addition to the feeding value, these pastures are often relatively bare in summer. The rye-grass usually only contributes towards the sward when the pasture is first sown. After some time the pasture becomes increasingly "clover-dominant" and the rye-grass virtually disappears.

Permanent pastures, i.e. pastures consisting of sown perennial grasses and subterranean clover, are usually not infested to the same extent as annual grass-clover pastures if the balance is maintained between the clover and the perennial grass. It is suggested that this is because (a) there would usually be some stubble on the surface when the adults of A.howitti were flying and so few eggs would be laid there, (b) the perennial plants may extract water from the soil so that the eggs die from desiccation, (c) after larvae migrate to the surface there is not much suitable food for them to eat.

The amount of food and the dominance or otherwise of clover in clover-grass pastures is influenced largely by the weather. Early rains whilst soil temperatures are still high lead to "clover-dominance" and dry weather in spring brings a rapid response from clover and many poorer type of annual weeds, in contrast to the better pasture grass species. Some factors affecting clover-grass relationships are discussed by Willoughby (1954) and Tiver and Crocker (1951).

A.howitti has been particularly abundant since the war in natural grasslands which have been prepared for close settlement by soldier settlers. The following information was obtained from Mr. F.B.Pearson, District Agricultural Adviser at Mt. Gambier. Most of the land for closer settlement was obtained from large properties south of Mt. Schanck, south east of Tantanoola, north-east of Mt. Gambier around Kalangadoo, and around Wrattonbully. Pastures on this land were of poor quality because before World War II it was not a common practice to use superphosphate except in particular paddocks (lambing paddocks etc.) and this fertilizer was very scarce during the war. It is very likely that most of the land taken over for soldier settlement received its first liberal application of superphosphate in 1947. At the same time subterranean clover was sown.

This treatment together with a number of favourable seasons and the almost complete absence of stock, accounted for the plague of O. fasciculata in 1950 (Madge 1957). The aftermath of the O. fasciculata outbreak was that much of this area was clover-dominant and covered with thistles and other weeds. Since then overgrazing by soldier settlers has eliminated most of the natural grasses and produced paddocks which are dominated by subterranean clover, capeweed and musky crowfoot. Such pastures, like clover-dominant ones, are ideal places for the survival and rapid build up of numbers of A. howitti and remain today the areas most heavily infested with A. howitti.

(iii) Influence of crowding on the survival-rate

The distribution of A. howitti populations in South Australia, as in New South Wales (Carne 1956), is characterised by (a) comparatively small areas in which the population density is very high, and (b) much more extensive areas in which the population density is much lower. Carne (op. cit.) suggested that (1) mortality was low in the areas of low density except where a previous heavy infestation had resulted in a concentration of fungal spores, (2) few or no eggs are laid in large parts of the distribution area which appear to be favourable for larval development, (3) a reasonable account of the limitation of numbers of the species can therefore be given in terms of the factors operating in areas of high density, and so (4) density induced combat is the most common cause of death in A. howitti populations.

If the behaviour of A. howitti in the two states is similar I do not agree with Carne (op. cit.), because observations in South Australia indicate that (1) a reasonable account of the limitation of numbers of the species cannot be given in terms of the factors operating in areas of high density, and (2) larval combat is not always the most common cause of death in areas of high density.

Firstly, Carne's assumption that few or no eggs are laid in areas which appear to be favourable for larval development is a repetition of his statement that adults often lay eggs only in particular places in pastures which "appear quite uniform to the observer". It has been pointed out in section IV that if eggs are laid in particular places it is probable that the water content of the soil in these places is more favourable for the survival of the adults, and it was concluded that weather influenced the number of favourable places in which eggs could be laid.

Similarly in South Australia the occurrence of larvae in high densities around trees, objects in paddocks, on slopes of hills, etc. is considered to depend on the vagaries of the weather, the topography of the landscape, etc. (section IV). If, for example, the wind blew from the north when the adults were flying so that the adults flew south, a paddock to the north of the previous infested one would not be colonised by the species. There are thus always some favourable places in which to live which are unavailable to the adults. The statistical availability of these places varies from year to year in relation to weather and other components of the environment.

The number of places in which adults can survive and lay eggs is maximal after wet summers, and I think Carne has not considered what proportion of the larvae may be found in areas of low density in such years. At Mt. Schanck in 1954 after a reasonably wet summer, larvae were widely distributed over the property of Mr. King. Many scores of samples indicated that there were 1-25 larvae per sq. link over his entire 900 acres of pasture, and that there was a portion of about 50 acres in which larvae averaged betweeb 50-100 per sq. link. In the areas of low density (1-25 per sq. foot) the pasture was often severely thinned; in the areas of high density it was completely destroyed. Even if it is conservatively estimated that there were only five larvae per sq. foot in the areas of low density, there were as many larvae in these areas as a whole as there were in the areas of high density. Similarly, in other parts of Counties Grey and Robe, for every acre of pasture which was completely destroyed there were many acres which had obviously been thinned out or supported many billions of larvae at densities too low to cause any obvious damage to the pasture.

Not only are there at least as many larvae distributed at low densities as there are distributed at high densities after wet summers, but the death-rate of the larvae in the areas of low density is unlikely to be influenced by larval combat. After wet summers, therefore, it is presumptive to consider the areas of high density as representative of the population as a whole. In dry summers, however, there are only a few places in which adults can survive and lay eggs, and the larvae are found almost exclusively in these areas in high densities. Paradoxically, it is in such unfavourable years that Carne's assumption comes nearest the truth, i.e. in years when the numbers of A.howitti are decreasing.

Secondly, it is true that competition does occur among larvae of A.howitti because they injure one another when searching for food. However, it was estimated that 50 per cent. of young larvae died before migrating to the surface at Mt. Gambier in 1955, and that in 1945 most of the larvae were killed by drought (section X). These survival-rates would apply to larvae in areas of high as well as low density because density does not affect the number of larvae surviving during a period of drought (section VI). Similarly, very high mortalities occur in wet winters when larvae are either drowned or are killed by Cordyceps aphodii. There is little doubt, therefore, that death due to larval combat is not always the most significant cause of death in areas of high density.

Carne (1956) placed a cage over a 8 ft. x 8 ft. square of pasture and recorded that the number of larvae dropped from 90 to 17 per cent. per sq. link. He quotes this as an example of combat as a mortality factor, but by enclosing the larvae in the cage he artificially limited the amount of food. The results of his other 8 ft. x 8 ft. square are more instructive because larvae were trapped in troughs as they moved out from the area. These larvae were all healthy specimens and Carne postulates "their periodic removal from the area kept the density below that level at which conflict would become a serious mortality factor". If the larvae had not been trapped, would they not have moved away from the area anyway and thus, in precisely the same way, kept the density below that at which conflict would have become a "serious mortality factor"? If, in fact, the larvae had only moved three feet out on all sides they would have finally occupied an area three times the size of the original area and very few larvae would have been killed or suffered from a shortage of food. The fall in density to 14 or 17 larvae per sq. link in the plots only tells us, therefore, that pasture cannot support more than this number of larvae per sq. link; it tells us nothing of the proportion of larvae which may have been killed.

Carne (op. cit.) observes that dispersal of larvae from patches of high density was of the order of 3-6 feet over the whole season, irrespective of the size of the patch. In South Australia, however, observations suggest that the larger the size of the patch or the greater the initial density the further is the movement of larvae outwards. If food is in short supply even first instar larvae may move outwards in search of it. Larvae may thus start to move out from areas of high density long before they commence to feed on pasture and form the characteristic "fronts". Carne allows that such movement may introduce errors into estimates of density changes due to mortality, but we apparently do not agree on the magnitude of the error of the sampling estimates involved in this way.

Larvae are sometimes found at the rate of 400-500 per sq. link when larvae are in the first instar, but the mean density over any extensive area is usually much less. Indeed, the numbers of A.howitti often fluctuate violently within feet, and a characteristic of the distribution of A.howitti is the magnitude of the standard deviations obtained when estimating the number of larvae per unit area. For example, when I conducted an insecticidal trial at Mt. Schanck in 1955 I divided a 30 acre paddock into four blocks and each block into twelve strips so that each strip was about five-eighths of an acre in area. A station 6 ft. x 6 ft. was then selected on each strip where it was thought that larvae of A.howitti were most abundant. Block 4 was particularly infested with A.howitti, so a second station was set up on each strip in this block. Three square link samples were then taken at random from each station in the middle of May whilst most of the larvae were still in the second instar. On six strips in Block 4 the two stations were not more than 12 feet from each other, and the area including both stations appeared to be fairly uniformly infested. It was expected that the numbers of larvae would be similar at the two stations but the numbers of larvae which were found in each sample were as follows:-

Strip No.	Station 1 Sample No.			Station 2			Mean	S.E. of mean	95% limits of mean
	1	2	3	1	2	3			
5	112	110	78	42	61	154	92.8	16.6	± 42.7
6	37	17	47	38	49	39	37.8	4.6	± 11.9
8	73	97	68	30	47	26	56.8	11.2	± 28.8
9	27	38	41	201	161	241	118.2	38.6	± 99.2
11	31	6	17	16	14	11	15.8	3.4	± 8.8
12	9	5	7	51	27	43	23.7	6.6	± 20.9

The means, S.E's and 95 per cent. fiducial limits of the means of the combined samples are shown above. It can be seen that the S.E's are often very large and the fiducial limits of the means are very wide.

Unfortunately Carne does not give the S.E's of his samples and in their absence I view with mistrust his estimates of population densities taken at random over one acre lots of pasture "selected for their uniformity of infestation". I think, in fact, that Carne's sampling estimates may be subject to such gross errors that it has still to be demonstrated that larval combat is ever a major cause of death in populations of *A.howitti.* (I should say "not often" rather than "ever")

(iv) Influence of parasites, predators and other animals on the survival-rate

It has already been described how sheep, cattle, rabbits and larvae of *Oncopera fasciculata* may, by eating pastures, produce places in which *A.howitti* chooses to lay eggs. They also enter into the environment of *A.howitti* by eating the same food. None of them, however, deplete the food supply to such an extent that the cockchafer larvae starve.

The larvae of *Oncopera fasciculata* live in silk-lined burrows in the soil in very similar pastures to those in which larvae of *A.howitti* are found and they eat the pasture at the same time of the year. The adults of *O.fasciculata,* however, lay their eggs in tall grass so that where one insect species is abundant the other is scarce. The *Oncopera* larvae are already half-grown when larvae of *A.howitti* appear and develop rapidly as soon as the season breaks. They would probably have an advantage over larvae of *A.howitti,* and if *A.howitti* larvae were present where Oncopera larvae are numerous it is possible that they would die of starvation or would be exposed to predators and other hazards whilst searching for food. The numbers of A.howitti which may be affected in this way would, however, be very small because when the species are found living together the places in which they live are not very favourable for either species, so the total numbers of insects are small and food is not limited.

Oncopera fasciculata moreover is not found north of County Grey and even in County Grey it inhabits some areas in which A.howitti is never found, i.e. the black rendzina soils of the Millicent flats. Its effect on the abundance of A.howitti is perhaps most marked in pastures on the terra rossa soils around Mt. Schanck, for Oncopera like A.howitti is responsible for the invasion of pastures by annual weeds. However Oncopera never inhabits the same area in successive years and is probably responsible for the introduction of weeds over a wider area than A.howitti. These well drained terra rossa soils normally constitute one of the most favourable places for the survival of A.howitti, and there seems little doubt that the depredations of O.fasciculata in the same area further enhances the chances of survival of A.howitti.

Predators other than Cordyceps aphodii are considered to have little influence on the numbers and distribution of A.howitti. It is possible, though, that in earlier days when Leptospermum was more abundant the Thynnid wasp Tachynomyia sp. exercised more control over the numbers in the population than it does today.

Cordyceps aphodii is prevalent in wet winters and is considered, in conjunction with excess water, as being one of the major factors influencing the distribution and abundance of A. howitti.

(v) Influence of vegetation and soil on the distribution and abundance of A. howitti

The utilisation of grasslands in this State commenced about 1840, and because of the grazing history of the region it is not possible today to find grasslands in the virgin condition. Similarly, most of the original vertebrate fauna has been exterminated and, though A. howitti is an indigenous species, it has never been found associated with the dung of a native animal. One can only speculate therefore on the types of places in which the insect lived in the lower South-East before exogenous herbivores were introduced.

A. howitti is found today mainly on the terra rossa soils and the meadow podsols and it is probable that it was restricted mainly to these soils before agricultural development. It is also found on some of the podsolised sands of the ranges and on some of the sands of the ranges which have been sown with better pastures.

Kalangadoo-Penola is a typical area of meadow podsols. In general the natural vegetation on these soils is a typical savannah woodland dominated by Eucalyptus camaldulensis (red gum) with a ground flora consisting chiefly of grasses and Cyperaceae and Juncaceae. The grasses are found mainly on the higher banks and include such perennials as Danthonia sp., Stipa setacea (spear grass), Themeda australis (kangaroo grass) and Microlaena stipoides (weeping rice grass); the wetter portions of the land are covered with sedges and rushes.

Molineux (1882) says of the county around Penola .."swampy country in wet weather with good grass wherever the land is slightly elevated. There is a good deal of coarse vegetation over it consisting of rough grass, rushes and sedges principally, with plenty of gums...". Before artificial drainage most of this meadow podsol country was under water for at least part of the winter-spring period.

A. howitti larvae were found by Dr. Madge in relatively unchanged red-gum country near Kalangadoo. The area had been lightly grazed by sheep and a few clovers and annual grasses had been introduced, but there were also a variety of moss-like annual plants covering the higher banks and it is probable that such annuals constituted the only source of food when the land was in its natural state. If that was so, the total amount of food for A. howitti on these soils must have been small before the introduction of exotic annuals, and the amount of water which gathered in winter permitted the species to survive only on the higher land.

The meadow podsols of Wrattonbully do not get as wet as the flats of Kalangadoo and it is probable that they constituted a more favourable area for the survival of A. howitti. E. leucoxylon is sometimes found as an associate species in this area but the undergrowth is the same as above.

It is difficult to determine the original vegetation of the well-drained lands - the volcanic soils and terra rossas - because these areas were cleared first for agricultural purposes. The shallower terra rossas however have not been developed much and Woods says of such places

... "The ridges with limestone croppings .... are always well grassed and not thickly timbered. In summer the grass is then dry and withered, leaving the red soil and dry rock disagreeably bare and parched". The trees consisted of Acacia sp., Banksia (honeysuckle) and native box; of the natural grasses Danthonia sp. were probably most abundant.

The soil was probably covered in parts with a variety of small annual plants. Larvae of A.howitti were found feeding on such moss-like plants on a limestone ridge near Millicent. Sheep and rabbits had been grazing on the ridge and there were a few introduced annuals, but the majority of the plants eaten by the larvae were much like those found at Kalangadoo. It is probable that it was in such places that A.howitti lived before South Australia was colonised by Europeans and that the total amount of available food was small.

Similarly a lack of food or excess water in winter would probably have made the podsolised sands, the sandy ridges and the rendzinas most unfavourable places in which to live.

The podsolised sands are associated with sclerophyllous heath vegetation characterised by low and stunted shrubs such as Banksia ornata (honeysuckle), B.marginata, Xanthorrhoea australis (yacca), Hakea rostrata (kidney bush), Casuarina sp. and Leptospermum scaporium (tea tree). Woods (1869) said of this country .... "immense level sandy tracts, heavy and dusty in summer, and boggy in winter, supporting no grass, nor any trees but those of a stunted and worthless character, run through, here and there, with belts of short and crooked 'stringy bark', and in all places covered with tangled brushwood, about two feet high..."

The stranded dune sands, like the heath sands, are very infertile. They are deeper, however, and are better drained. The natural vegetation is a dry sclerophyll forest dominated by stringy bark (E. obliqua and/or E. Baxteri). Woods said these sandy dunes .... "are nearly destitute of grass, but are very shrubby, rarely supporting any other tree but a stunted and irregular growth of E. Fabrorum, or Stringy Bark,...". Much of this country still remains under natural forest, but in the higher rainfall area the dunes have been cleared and planted with Pinus radiata.

A characteristic feature of the natural vegetation on the rendzinas is the almost complete absence of trees. The native flora today is represented by a savannah dominated by Poa australis (white tussock), Gahnia trifida (cutting grass) and Cladium filum (thatching grass). A very large proportion of these soils is still liable to complete flooding during the winter months.

We know that a feature of the native grasslands wherever they occurred was the high percentage of bare ground. In addition the native grasses, which were mainly perennial grasses of a tussocky nature, must have been high in fibre and low in protein because there were no native clovers or medics in South Australia. It is probable that such grasses were not suitable as food for larvae of A. howitti.

The native grasses developed under three important environmental conditions (1) soils low in readily available phosphate (2) climate characterised by summer drought and (3) mild influence of grazing animals. When sheep were introduced many of the native species were eliminated by the higher grazing intensity. At the same time, the bare surfaces resulting from the removal of the native perennial cover favoured the rapid colonisation of the land by annual plants, the seeds of which were carried in the fleeces of the sheep or were distributed in their excreta. These annual plants included species of Hordeum, Bromus, Vulpia, Medicago, Trifolium, Erodium and Cryptostemma, some of which forms evolved with the merino and accompanied it throughout the Mediterranean to California, South Africa and southern Australia, (Trumble 1949). By 1880 the Mt. Gambier district produced large crops of these "artificial" grasses, but the practice of sowing improved pastures did not become widespread until the 1920's when it was discovered that superphosphate had to be applied to the poorer soils before introduced clovers and grasses would grow well.

The abundance of A. howitti in any one year depends to a large extent on the number and distribution of favourable situations. Before the advent of superphosphate and subterranean clover in 1923, A. howitti must have been a rare species, for it would seem that the larvae could survive only in places in which annual plants grew and would probably have been restricted to highly localised areas on well-drained lands and to the higher banks of the poorly-drained soils.

As the native grasses were replaced by introduced clovers more situations became favourable for A. howitti because in these places there was more food for the larvae to eat. The artificial drainage of the poorly-drained soils also meant that less of this land would be flooded and so the insect would be able to survive over a wider area.

XI. CONCLUSIONS

Before sheep were introduced into South Australia, A.howitti must have been a very rare species because there would have been very little suitable food and very few places in which larvae could survive. Since then major changes in its environment have occurred as land has been developed for more intensive agriculture. These changes have resulted in a great increase in the area favourable for the survival of the species; consequently its numbers have increased.

A.howitti is a troublesome pest of improved pastures in South Australia because the larvae eat pasture grasses and may also severely retard the development of a pasture during the critical winter months. At this time of the year plant growth is slowest and the carrying-capacity of pastures is at a minimum, so when the larvae are abundant and pasture damage is severe there is a serious shortage of food for stock and farmers have to hand-feed or sell their stock. Light infestations of A.howitti are reputed to be beneficial to pasture, but even light infestations remove a considerable amount of pasture and ultimately do more harm than good.

There are wide tracts of country in the South-East in which pasture improvement has lagged considerably behind that on the meadow podsols and, consequently, in which food is still a major factor limiting the abundance of A.howitti. There are also extensive areas which are still too wet for the survival of the larvae, but as this land is developed the numbers of A.howitti will probably increase. In the areas in which man has provided a more favourable environment for the species, and food is not a limiting factor, the relative numbers of larvae found each year are determined by the weather.

Food is not in short supply in heavily stocked pastures of introduced clovers and grasses, and it is in such pastures, particularly when subterranean clover or annual weeds are dominant, that the largest number of larvae are found. In these favourable places A. howitti may increase to very large numbers if the weather is favourable for a succession of years. A favourable year is one in which (a) the summer (January-February) is moist and permits the adults to lay eggs and the eggs to survive, (b) the season breaks early (March-April) and the larvae can migrate to the surface of the soil soon after the eggs hatch and (c) excess water in winter (June-September) does not drown many larvae or promote infection with Cordyceps.

It appears that the presence of A. howitti and the weeds which follow its ravages are indicative of too high a level of nitrogen in a pasture, and that the problem of A. howitti is therefore largely a problem of grassland management. This study has suggested that it should be possible to reduce the numbers of A. howitti to a level at which they do not cause any economic damage to pastures by:-

(1) Maintaining a balance between perennial grasses and clover. The association of grass with legume is basic to pasture improvement, and pastures consisting of perennial grasses and clovers are rarely infested to any extent by larvae of A. howitti as long as the balance is maintained between the associated pasture species. The maintenance of perennial grass-clover pastures in areas now mainly dominated by subterranean clover and annual

weeds would therefore reduce the number of favourable places in which *A. howitti* could live. This applies particularly to the annual grass - subterranean clover type of pasture which is representative of the great majority of improved pasture sown on the meadow podsols within the 8-9 months' effective rainfall zone. The instability of these annual pastures is a distinct disadvantage and the introduction of a perennial grass which confers greater stability on the pasture is the next logical step in pasture improvement. (Tiver and Crocker 1951).

The range of perennial grass suitable for the particular soil and climatic conditions (including 3-4 months of summer drought) and capable of persisting under the type of uncontrolled grazing management practised is, however, a very restricted one. The only perennial grass which has shown considerable promise is *Phalaris tuberosa*, but unfortunately this has been overlooked by the great majority of landholders (Tiver and Crocker 1951).

(2) Introducing a ley system of farming in which paddocks are systematically ploughed every few years, sown to a cereal crop and then resown to pasture. The pasture can be sown to a summer crop instead of a cereal crop, but it would then be necessary to plough the land again before resowing the pasture. This practice would limit the number of favourable places in which *A. howitti* could live, because a pasture usually cannot support many larvae until it has been grazed for 3-4 years and a layer of organic matter has been developed at the surface. In essence, this type of farming is carried out on much of the "developed" land where *A. howitti*

is rarely a problem and its value is reflected in the opinion held by older farmers that A. howitti only infests land at a particular stage of "development".

(3) Grazing management.- The numbers of A. howitti can be minimised in any pasture by maintaining a cover of stubble on the pasture when the adults fly in summer. This can be done by selective grazing. Further, if a paddock has to be cut for hay, the farmer could select the paddock which is to be ploughed next in a rotational scheme.

Grazing management also covers the general problem of overstocking. Many of the soldier settlers in the area of study have been particularly troubled with infestations of A. howitti. The majority of these farmers confessed that their properties were overstocked.

(4) Failing control by any of the above methods the insects can be killed cheaply with D.D.T. or lindane applied in superphosphate or as a spray to the pasture.

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XIII. REFERENCES

ANDREWARTHA, H.G. (1945).- Cockchafer grubs as pest of pastures in the South-East.
J. Dept. Agric. S.Australia 49: 11.

——————— (1952).- Diapause in relation to the ecology of insects.
Biol. Rev. 27: 50-107.

ANDREWARTHA, H.G. and BIRCH, L.C. (1954).- "The Distribution and Abundance of Animals".
(Uni. of Chicago Press).

BARBER, G.W. and DICKE, F.F. (1939).- Effect of temperature and moisture on overwintering pupae of the corn earworm in the north-eastern states.
J. Agr. Research 59: 711- 23.

BAVER, L.D. (1948).- "Soil Physics"
(John Wiley & Sons, Inc., New York).

BIRCH, L.C. (1944).- The effect of temperature and dryness on the survival of the eggs of *Calandra oryzae* L.(small strain) and *Rhizopertha dominica* Fab. (Coleoptera).
Australian J. Exper. Biol. & Med. Sci. 22: 265-269.

BIRCH, L.C. and ANDREWARTHA, H.G. (1942).- The influence of moisture on the eggs of *Austroicetes cruciata* Saus, with reference to their ability to survive desiccation.
Australian J. Exper. Biol. & Med. Sci. 20: 1-8.

BROWNING, T.O. (1952).- The influence of temperature on the rate of development of insects with special reference to the eggs of *Gryllulus commodus*.
Australian J. Res., Series B, 5: 96-111.

——————— (1954).- Observations on the ecology of the Australian Field Cricket, *Gryllulus commodus* Walker, in the field.
Australian J. Zool. 2: 205-222.

CARNE, P.B. (1956).- An ecological study of the pasture scarab *Aphodius howitti* Hope.
Australian J. Zool. 4: 259-315.

CROCKER, R.L. (1944).- Soil and vegetation relationships in the lower South-East of South Australia.
Trans. Soc. S.Australia 68: 144-172.

DAVIDSON, J. (1932a).- Factors affecting oviposition of *Sminthurus viridis* L. (Collembola).
Australian J. Exper. Biol. & Med. Sci. 10: 1-16.

DAVIDSON, J. (1932b).- On the viability of the eggs of *Sminthurus viridis* L. in relation to their environment. Australian J. of Exper. Biol. & Med. Sci. *10*: 65-68.

______________ (1944).- On the relationship between temperature and rate of development of insects at constant temperatures. J. Anim. Ecol. *13*: 26-38.

EVANS, A.C. (1943).- Value of the pF scale of soil moisture for expressing the soil moisture relationships of wireworms. Nature, *152*: 21-22.

______________ (1944).- Observations on the biology and physiology of wireworms of the genus *Agriotes Esch.* Ann. Appl. Biol. *31*: 235- 50.

EVANS, A.C. and McGUILD, W.J. (1948).- On the life cycles of some British Lumbricidae. Ann. Appl. Biol. *35*: 471-484.

FISHER, R.A. (1936).- "Statistical methods for research workers". (Oliver and Boyd, London).

FINNEY, D.J. (1947).- "Probit Analysis". (Cambridge Uni. Press).

FOX, H. (1934).- The known distribution of the Japanese beetle in 1932 and 1933. J. Econ. Ent. *27*: 461.

GIVEN, B.B. (1950).- Notes on the Aphodiinae of Australia (Coleoptera, Scarabaeidae). The *Aphodius tasmaniae, howitti, yorkensis, andersoni* complex. Proc. Linn. Soc. N.S.W. *75*: 153.

HUSAIN, M.A. *et al.* (1940).- Studies on *Schistocerca gregaria* Forsk. X. Role of water in the bionomics of the desert Locust. Indian J. Agric. Sci. *10* (6): 927- 44.

IMM, A.D. (1946).- "A general text-book of Entomology" (Methuen & Co. Ltd., London).

JONES, E.W. (1951).- Laboratory studies on the moisture relation of *Limonius* (Elateridae). Ecology 32: 284-293.

KERENSKI, J. (1930).- Beobachtungen über die Entwicklung der Eier von *Anisoplia austriaca* Reitt. Z. Angew Ent. *16*: 178-188.

LAUGHLIN, R. (1953).- Absorption of water by the egg of the garden chafer *Phyllopertha horticola* L. Nature, *171*: 577.

LEES, A.D. (1943).- On the behaviour of wireworms of the genus *Agriotes Esch.* (Col. Elateridae). II. Reactions to moisture.
J. Expt. Biol. 20: 54-60.

LOWER, H. (1957).- J. Morph. (In press).

LUDWIG, D. and LANDSMAN, H.M. (1937).- The effect of different relative humidities on survival and metamorphosis of the Japanese beetle (*Popillia japonica* Newman).
Physiol. Zool. 10: 171.

MADGE, P.E. (1952).- The pasture Cockchafer in South Australia.
J. Agric. S. Aust. 55: 463-467.

———— (1956).- Ph. D. Thesis (University of Adelaide).

———— (1957).- The ecology of *Oncopera fasciculata* (Walker) (Lepidoptera : Hepialidae) in South Australia.
Australian J. Zool. (In Press).

MARTYN, E.J. (1950).- Pasture insect investigation. Report on the occurrence of pasture cockchafers in Tasmania, 1949-50.
Tasmania J. Agric. 21: 216.

MATHIESON, J. (1949).- *Cordyceps aphodii*, a new species, on pasture cockchafer grubs.
Trans. Brit. Mycol. Soc. 32: 862.

MATTHEE, J. J. (1951).- The structure and physiology of the egg of *Locusta pardalina* (Walker).
Dept. Agric. South Africa Scientific Bull. No. 316: 83.

MOLINEUX, A. (1883).- Both sides of the border.
The Adelaide Observer 60 (1): 574.

NEEDHAM, J. (1942).- "Biochemistry and Morphogenesis".
(Cambridge University Press).

PARRY, D.A. (1954).- On the drinking of soil capillary water by spiders.
J. Exp. Biol. 31: 218-228.

RAW, F. (1951).- The ecology of the garden chafer, *Phyllopertha horticola* (L) with preliminary observations on control measures.
Bull. Ent. Res. 42: 605- 46.

ROCKWOOD, L.P. (1951).- Entomogenous fungi of the genus *Metarrhizium* on wireworms in the Pacific North-West.
Ann. Ent. Soc. Amer. 43: 495- 98.

ROONWAL, M.L. (1936).- Growth changes and structure of the eggs of the African migratory locust *Locusta migratoria migratoroides.*
Bull. Ent. Res. 27: 1-14.

SALT, R.W. (1946).- Moisture relationships of the wheat stem sawfly (*Cephus cinctus* Nort.) I. Some effects of desiccation.
Sci. Agric. 26: 622-630.

SCHNEIDER, F. (1952).- Untersuchungen über die optische Orientierung der Maikäfer (*Melolontha vulgaris* F. und *M. hippocastani* F.) sowie über die Entstehung von Schwärmbahnen und Befallskonzentrationen.
Mitt. Schweiz ent. Ges. 25: 269-340.

SCHOFIELD, R.K. (1935).- The pF of water in soil.
Trans. 3rd International Congress Soil Sci. 2: 37-48.

SMITH, J.H. (1936).- White grub damage to pasture on the Atherton tableland.
Queensland Agric. Jour. 46: 446-467.

SMITH, R. and MICHELBACKER, A.E. (1949).- The development and behaviour of populations of *Diabrotica 11-punctata* in foothill areas of California.
Annals. Ent. Soc. Amer. 42: 497-508.

SNEDECOR, G.W. (1946).- Statistical methods, 4th edition.
(Iowa State College Press).

SOLOMON, M.E. (1951).- Control of humidity with potassium hydroxide, sulphuric acid or other solutions.
Bull. Ent. Res. 42: 543-554.

SPRIGG, R.C. (1952).- The geology of the South-East province, South Australia, with special reference to quaternary coast-line migrations and modern beach developments.
Bull. S.Aust. Dep. Mines No. 29. 120 pp.

STEINHAUS, E.A. (1949).- "Principles of Insect Pathology".
(McGraw-Hill, New York).

STEPHENS, C.G. *et al.* (1941).- A soil and land use survey of the hundreds of Hindmarsh, Young, Riddock, Grey and Nangwarry, South Aust.
C.S.I.R. Australia Bull. No. 142.

SWAN, D.C. (1934).- A scarab beetle (*Aphodius tasmaniae* Hope) destructive to pastures in the South-East of South Australia.
J. Dept. Agric. S.Aust. 37: 1149-1156.

__________ (1937).- Insects and other invertebrates of economic importance in South Australia during the period July, 1934, to June 1936.
J. Agric. S.Aust. 40: 717-731.

SWEETMAN, H.L. (1931).- Preliminary report on the physical ecology of certain *Phyllophaga*.
Ecology **12** (2) : 401-422.

TIVER, N.S. and CROCKER, R.L. (1951).- The grasslands of south-east South Australia in relation to climate, soils and development history.
J. Brit. Grassland Soc. **6** : 29-80.

TRAVIS, B.V. (1939).- Habits of the June beetle, *Phyllophaga lanceolata* (Say), in Iowa.
J. Econ. Ent. **32**: 690.

TRUMBLE, H.C. (1948).- Rainfall, evaporation and drought-frequency in South Australia.
J. Dept. Agr. S. Aust. **52**: 55-64.

———— (1949).- The ecological relations of pasture in South Australia.
J. Brit. Grassland Soc. **4**: 135-160.

WALLACE, H.R. (1955a).- Factors influencing the emergence of larvae from cysts of the beet eelworm *Heterodera schachtii* Schmidt.
J. Helminth. **29** : 3-16.

———— (1955b).- The influence of soil moisture on the emergence of larvae from cysts of the beet eelworm *Heterodera schachtii* Schmidt.
Ann. Appl. Biol. **43**: 477-485.

———— (1956a).- Soil aeration and the emergence of larvae from cysts of the beet eelworm *Heterodera schachtii* Schmidt.
Ann. Appl. Biol. **44**(1) : 57-66.

———— (1956b).- The emergence of larvae from cysts of the beet eelworm, *Heterodera schachtii* Schmidt, in aqueous solutions of organic and inorganic substances.
Ann. Appl. Biol. **44**(2) : 274-282.

———— (1956c).- Migration of nematodes.
Nature **177**: 287-288.

WARD, E. (1869).- The South-East district of South Australia. 95 pp. (South Australian Press; Adelaide).

WARD, L.K. (1941).- The underground water of the South-Eastern part of South Australia.
Dept. Mines Geo. Survey of S. Australia Bull. 19.

WILLOUGHBY, W.M. (1954).- Some factors affecting grass-clover relationships.
Australian J. Agr. Res. **2**: 157-180.

WOODS, J.E. (1862).- "Geological observations in South Australia".
(Dwight, Melbourne), 404 pp.

APPENDIX

Table 1a - Appendix

The number of eggs in batches laid in Wandilo sand after 9 days

No. of egg batch	pF of soil 4.0		3.85		3.50		2.80		2.15	
Replicate	A	B	A	B	A	B	A	B	A	B
1	17	50	20	41	39	39	54	29	25	42
2	26	31	22	26	38	26	48	41	37	25
3	40	21	42	16	44	24	22	24	35	49
4	27	31	41	21	24	24	39	24	29	27
5	52	39	35	31	20	23	49	36	31	39
6		31	44	22	35	41	23	44	46	29
7		17	24	25	12	38	22	27	30	29
8			19	22	24	36	18	36	30	22
9			18	35	41	20	16	54	36	26
10				43	36	21	26	44		33
11					24	22	24	41		34
12					24	24	21	37		
13					30	41	43	49		
14						42		32		
Total per treatments	382		547		812		923		654	
$\bar{x}$	31.8		28.8		30.1		34.2		32.7	
S.E. of $\bar{x}$	2.84		2.26		1.89		1.89		2.20	

The Analysis of variance of treatment means

Source of variation	d.f.	S.S.	M.S.	V.R.	p
Total	104	10,091.2			
Treatments	4	418.1	104.5	1.04	20%
Error	100	9,673.1	96.7		

Greatest difference between means = 5.4
S.E. of difference = 2.94
t = 1.84 with 44 d.f. (p >5%)

Table 1b - Appendix

The number of eggs in batches laid in terra rossa (clay loam) after 9 days

No. of egg batch	4.0		3.70		3.20		2.80		2.85	
	A	B	A	B	A	B	A	B	A	B
1	31	40	46	23	25	27	23	22	38	24
2	28	27	24	34	23	48	17	30	32	23
3	40	47	25	36	25	26	36	28	40	21
4	19		47	31	33	37	44	30	33	32
5	25		24	44	32	29	24	36	18	26
6	19		19		34	35	21	28	38	39
7					31	27	34	37	31	37
8					23	31	39	44	28	36
9					31	28	28	29	30	38
10					30	18	32	36	21	20
11					36	33	44	41		30
12					26	36	25	23		31
13						44	27			
14						35				
Total per treatment	276		353		804		778		666	
$\bar{x}$	30.7		32.1		30.9		31.1		30.3	
S.E. of $\bar{x}$	2.57		2.32		1.51		1.54		1.64	

The Analysis of variance of treatment means

Source of variation	d.f.	S.S.	M.S.	V.R.	p
Total	92	5,265.9			
Treatments	4	25.9	6.5	-	N.S.
Individuals	88	5,240.0	59.5		

Greatest difference between means = 1.8
S.E. of difference = 2.8
t = 0.64 with 31 d.f. (p >80%)

Table 2 - Appendix

Survival of adults in relation to water in the soil

(a) Females in clay loam after 9 days

% water content	pF	No. dead R_1	R_2	Total No. dead	% mort.
8.2	4.3	20	20	40	100
13.2	4.0	14	10	24	60
17.3	3.7	3	1	4	10
24.8	3.20	0	1	1	2.5
31.1	2.8	1	1	2	5.0
36.4	2.5	3	4	7	17.5

(b) Adults in sand after 9 days

		Females				Males			
% water content	pF	No. dead R_1	R_2	Total No. dead	% mort.	No. dead R_1	R_2	Total No. dead	% mort
1.8	4.2	20	20	40	100	20	20	40	100
2.5	4.0	0	3	3	7.5	8	7	15	37.5
3.5	3.85	3	2	5	12.5	6	5	11	27.5
5.1	3.5	1	1	2	5.0	2	3	5	12.5
8.4	2.8	2	3	5	12.5	8	7	15	37.5
11.4	2.15	2	2	4	10.0	6	8	14	35.0
14.3	1.95	11	6	17	42.5	9	14	23	57.5

(c) Adults in sand after 14 days

		Females				Males			
% water content	pF	No. dead R_1	R_2	Total No. dead	% mort.	No. dead R_1	R_2	Total No. dead	% mort
1.8	4.2	20	20	40	100	20	20	40	100
2.5	4.0	20	18	38	95	20	20	40	100
3.5	3.85	15	16	31	77.5	20	20	40	100
5.1	3.5	11	13	24	60	20	20	40	100
8.4	2.8	3	4	7	17.5	18	17	35	87.5
11.4	2.15	9	12	21	52.5	19	18	37	92.5
14.3	1.95	19	18	37	92.5	20	20	40	100

Table 3 - Appendix

The numbers of adults recorded in treatments. Experiment 8

	Replicate 1			Replicate 2			Replicate 3			Replicate 4		
	Male	F/mle		Male	F/mle		Male	F/mle		Male	F/mle	
Compact surface plus beetles	10	11	21	6	6	12	12	7	19	8	12	20
Compact surface - no beetles	3	3	6	12	16	28	6	6	12	4	3	7
Loose surface - no beetles	31	33	64	34	37	71	47	49	96	44	45	89
Loose surface plus beetles	40	50	90	32	38	70	31	36	67	38	39	77
Σ	84	97	181	84	97	181	96	98	194	94	99	193

<u>Table of treatment totals</u>

	Male	Female	Σ
Compact surface plus beetles	36	36	72
Compact surface - no beetles	25	28	53
Loose surface - no beetles	156	164	320
Loose surface plus beetles	141	163	304
Σ	358	391	749

Table 4 - Appendix

The % water content of soil samples in bare areas and under grass stubble

Type of surface	% water content of individual samples												Σ	$\bar{x}$
Bare	17.4	17.8	17.1	16.4	15.0	17.6	18.0	17.8	16.5	15.9	15.0	15.0	199.5	16.6
Stubble	10.9	10.6	11.5	11.6	10.9	11.6	11.7	12.2	12.2	13.3	14.4	13.4	144.2	12.0

Summary of data for comparison of the two groups

Type of surface	No. of samples	Degrees of freedom	Mean % water content	Sum of squares
Bare	12	11	16.6	14.94
Stubble	12	11	12.0	14.26
		Σ = 22	Δ = 4.6	Σ = 29.20

s^2 = 1.3273

S.E. of difference = 0.47

t = 9.81 (p = <.1%)

Table 5 - Appendix

The absorption of water by eggs

Time in days	Mean % wet weight (mg)	Mean % increase in wet weight per day	S.E. of % wet weight
6	2.6	2.6	0.83
7	7.5	4.9	1.54
8	17.9	10.4	3.46
9	38.5	20.6	4.92
10	79.3	40.8	6.19
11	108.9	29.6	5.91
12	131.9	23.0	4.72
13	142.8	10.9	4.10
14	151.6	8.8	4.06
15	154.6	3.0	4.12

Table 6 - Appendix

The % emergence of larvae from eggs in pF's 2.5 - 3.75 transformed to degrees

Soil Type	Replicate No.	pF of soil 2.5	3.0	3.25	3.5	3.75	on moist paper
Sand	1	32.6	39.8	46.7	43.3	39.8	50.2
"	2	39.2	45.0	33.2	26.6	33.2	45.0
"	3	45.0	56.8	50.8	39.2	33.2	45.0
Loam	1	43.3	43.3	39.8	46.7	50.2	
"	2	39.2	39.2	33.2	45.0	45.0	
"	3	50.8	45.0	45.0	50.8	39.2	

The analysis of variance of % emergence of larvae from all treatments

Source of variation	D.F.	S.S.	M.S.	V.R.	p
Total	32	1389.9			
Treatments	10	557.7	55.77	1.47	20%
Error	22	832.2	37.83		

Table of treatment totals of larvae hatching in soil

Soils	2.5	3.0	3.25	3.50	3.75	Σ	$\bar{\bar{x}}$
Sand	116.8	141.6	130.7	109.1	106.2	604.4	40.3
Loam	133.3	127.5	118.0	142.5	134.4	655.7	43.7
Σ	250.1	269.1	248.7	251.6	240.6	1260.1	
$\bar{\bar{x}}$	41.7	44.9	41.5	41.9	40.1		

The analysis of variance

Source of variation	D.F.	S.S.	M.S.	V.R.	p
Treatments	9	496.7	55.2	1.36	20%
Soils	1	87.7	87.7	2.15	20%
pF's	4	72.8	18.2	-	N.S.
pF x soil	4	336.2	84.1	2.07	10%
Error	20	814.2	40.7		
Total	29	1310.9			

Minimum difference for significance of pF means 5% = 7.7
Minimum difference for significance of soil means 5% = 4.9

Table 7 - Appendix

Mean monthly temperatures in Centigrade - Mt. Gambier

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Soil Temperature at Mt. Burr (Mean of 18 years)	19.4	19.2	18.0	15.1	12.5	10.3	9.2	9.4	11.1	13.2	15.4	17.9
Air Temperature at Mt. Gambier (Mean of 85 years)	18.0	18.5	17.0	14.4	12.1	10.3	9.5	10.3	11.6	13.3	14.9	16.6

Table 8a - Appendix

The mean % water content, and its pF value, of the sand before and after treatment

Time of treatment	Treatment											
	pF 3.3.				pF 3.8				pF 4.1			
	% water content		pF value		% water content		pF value		% water content		pF value	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1 week	6.31	4.59	3.30	3.70	3.87	2.44	3.85	4.05	1.54	2.06	4.05	4.15
2 weeks	6.55	3.10	3.30	3.95	3.90	2.19	3.85	4.10	2.15	1.94	4.10	4.20
3 weeks	6.62	2.75	3.30	4.00	4.03	2.03	3.80	4.15	2.48	1.97	4.05	4.20
5 weeks	6.31	2.13	3.30	4.10	4.20	1.72	3.80	4.20	2.38	1.74	4.10	4.20

Table 8b - Appendix

The determination of water contents of samples of soil

Sample No.	% water content	
	Calculated	Observed
1	2.18	1.52
2	2.35	1.94
3	1.62	1.51
4	2.09	1.67
5	2.07	1.72
6	1.86	1.85
7	1.74	1.52
8	1.70	1.59
9	2.47	2.14
10	1.87	1.66
11	1.67	1.60
12	1.85	1.74
13	3.61	3.11
14	1.60	1.48
15	2.02	1.71
16	1.61	1.51
17	1.97	1.79
Σ 17	34.28	30.06

$Sxx = 3.7596$

$Sxy = 2.8309$

$Syy = 2.4028$

$r = 0.94$ with 16 d.f., $p < 0.1\%$

Table 9 - Appendix

The combined mortality of 1st instar larvae before migration to the surface

Summary of probit analysis:

Time	No. Used	No. Dead	% mortality	Corrected % mortality	Probit	Log of time
1 week	150	40	26.7	3.6	3.20	0
2 weeks	150	77	51.3	35.9	4.64	0.30
3 weeks	150	99	66.0	55.3	5.13	0.48
5 weeks	150	132	88.0	83.8	5.99	0.70

Control mortality 1 - 3 weeks = 12/50 = 24%

5 weeks = 13/50 = 26%

b = 3.566, S.E. of b = 0.436

L.D.50 = 2.77 weeks = 19.4 days

95% limits=2.49 and 3.09 weeks = 17.4 and 21.6 days

Table 10 - Appendix

The effect of drought on the survival rate of 1st instar larvae after migration to the surface

Duration of drought in days	Group No. 1	Group No. 2	Group No. 3	Total used	Total alive	Total dead	% mortality	Corrected % mortality
0	6	9	8	30	23	7	23	0
10	4	6	8	30	18	12	40	22
14	3	6	4	30	13	17	57	44
18	1	3	2	30	6	24	80	74
22	0	1	0	30	1	29	97	96
26	1	1	0	30	2	28	93	91
30	0	0	0	30	0	30	100	100

b = 6.505, S.E. of b = 1.099

L.D.50 = antilog 1.144 = 13.9 days

95% limits = antilog 1.144 ± 0.063 = 12.1-16.1 days

Table 11 - Appendix

The effect of immersion in water on the survival-rate of larvae

8°C:

Time in hours	No. used	% mort.	Corrected % mort.	Probit
0	61	29.5	0	-
3	57	31.6	3.0	3.1
6	64	39.1	13.6	3.9
12	60	33.3	5.4	3.4
24	60	40.0	14.9	4.0
48	68	44.1	20.7	4.2
16°C:				
0	50	28.0	0	-
3	56	37.5	13.2	3.9
6	64	39.1	15.4	4.0
12	60	43.3	21.3	4.2
24	68	61.8	46.9	4.9
48	80	98.8	98.3	7.1

<u>16°C</u>

$y = 3.459 + 0.071x$

L.D.50 = 21.7 ± 4.4 (95% limits)

S.E. of b = 0.009

<u>8°C</u>

$y = 3.504 + 0.014x$

S.E. of b = 0.005

Table 12 - Appendix

Analysis of variance of % weight lost in relation to saturation deficit at 16.9°C

Source of variation	d.f.	S.S.	M.S.	V.R.	p
Total	195	23,725.82			
Regression	1	20,098.63	20,098.63	>1000	<0.1%
Error	194	3,627.19	18.70		

$Sy^2 = 23,725.82$
$Sxy = 9,367.25$
$Sx^2 = 4,365.74$

$b = 2.146 \pm 0.129$ (99% limits)

Analysis of variance of % weight lost in relation to saturation deficit at 26.8°C

Source of variation	d.f.	S.S.	M.S.	V.R.	p
Total	197	36,812.79			
Regression	1	32,516.15	32,516.15	>1000	<0.1%
Error	196	4,296.64	21.92		

$Sy^2 = 36,812.79$
$Sxy = 12,911.12$
$Sx^2 = 5,126.59$

$b = 2.518 \pm 0.129$ (99% limits)

The regression of % weight lost on saturation deficit at 16.9 and 26.8°C

	d.f.	S.S.	M.S.	V.R.	p
Differences in regression coefficients	1	327.67	327.67	16.13	<0.1%
Distances between regression lines	1	577.95	577.28	28.41	<0.1%
Total difference between fegressions	2	904.95	452.48	22.27	<0.1%
Residual about regression lines	390	7,923.83	20.32		

Table 13 - Appendix

The analysis of variance of % weight lost in relation to equivalent product of (sat. def. x time) - Experiment 4

Source of variation	d.f.	S.S.	M.S.	V.R.	p
Difference in regression coefficients	1	8,931.2	8,931.2	-	≪0.1%
Distance between regression lines	1	20,213.6	20,213.6	-	0.1%
Total difference between regressions	2	29,144.8	14,572.4	-	0.1%
Residual about regression lines	849	61,050.8	71.91		

Test of significance of departure from linear regression

(a) 2½ day series

Source of variation	d.f.	S.S.	M.S.	V.R.	p
Deviations from linear regression	427	27,170.39			
Deviations from curvilinear regression	426	26,100.75	61.27		
Curvilinearity of regression	1	1,069.64	1,069.64	17.46	<0.1%

$$y = 0.0329x^2 + 2.9861x + 3.3655$$

(b) 5 day series

Source of variation	d.f.	S.S.	M.S.	V.R.	p
Deviations from linear regression	422	33,883.8			
Deviations from curvilinear regression	421	33,726.4	80.11		
Curvilinearity of regression	1	157.4	157.4	1.97	>5%

$$y = 0.0131x^2 + 1.0406x + 8.3794$$

Table 14 - Appendix

The effect of % weight lost by prepupae on the mortality rate

	% wt. loss	Corrected % mort.	Empirical probit
26.8°C	46.2	83	6.5
	36.6	91	5.7
	29.4	28	5.1
	26.2	28	4.8
	15.4	14	4.0
16.9°C	34.6	75	5.6
	32.1	38	5.3
	28.3	72	5.0
	22.1	33	4.5
	14.8	17	3.9

$y = 0.073x + 2.836$

S.E. of b = 0.021

L.D. 50 = 29.6, 95% limits = ± 5.0

$\chi^2(8) = 9.1$

Table 15 - Appendix

The effect of the weight lost by prepupae on their survival-rate

Experiment 4:

Mean % wt. lost	% mortality	Probit
39.0	4	3.5
45.2	36	4.7
48.1	70	5.3
51.0	75	5.9
55.0	95	6.7
59.6	100	7.7

y = 0.205 - 4.550

S.E. of b = 0.031

L.D.50 = 46.6, 95% limits = ± 1.9

χ^2 (5) = 2.1

Table 16 - Appendix

Increments of water loss from evaporimeters expressed as ccs/sq. cm of evaporative surface

Treatment No.	Type of system	No. of evaporimeter	No. of days 4	10	17	23	27	35
1	Natural cells	1 †	0.44	1.48*	0.41	0.26	0.18	0.74
		5	0.21	0.28	0.42	0.28	0.14	0.42
		16	0.26	0.32	0.41	0.38	0.20	0.50
		Σ	0.91	0.60	1.24	0.92	0.52	1.66
2	Natural cells - saturated solution of salt	2	0.13	0.22	0.31	0.88*	0.22	0.31
		7	0.81*	1.01*	0.19	0.12	0.04	0.70
		11	0.39	0.39	0.23	0.10	0.03	0.39
		13	0.12	0.48	0.45	0.15	0.21	2.59*
		Σ	0.64	1.09	1.18	0.37	0.50	1.40
3	Artificial cells - water	3	0.39	0.48	0.70	0.26	0.18	0.35
		8	0.20	0.20	0.37	0.20	0.10	0.44
		9	0.28	0.25	0.35	0.19	0.13	0.25
		14	0.21	0.27	0.34	0.21	0.07	0.62
		Σ	1.08	1.20	1.76	0.86	0.48	1.66
4	Open tubes - water	4	1.98	3.17	12.30*	31.3*	17.1*	38.1*
		6	2.38	3.17	3.97	2.38	0.79	3.57
		10	1.98	2.38	3.57	1.59	0.79	1.98
		15	2.78	3.97	4.37	3.17	1.19	5.16
		Σ	9.12	12.69	11.91	7.14	2.77	10.71

† One cell - no. 12 - was broken.

* Evaporimeter leaking

Table of means of treatments 1-3

Treatment No.	4	10	17	23	27	35	Σ	$\bar{x}$
1	0.30	0.30	0.41	0.31	0.17	0.55	2.04	0.34
2	0.21	0.36	0.30	0.12	0.13	0.47	1.59	0.27
3	0.27	0.30	0.44	0.22	0.12	0.42	1.77	0.30
Σ	0.78	0.96	1.15	0.65	0.42	1.44	5.40	

Analysis of variance

Source of variation	d.f.	S.S.	M.S.	V.R.	p
Total	17	0.2672			
Treatments	2	0.0171	0.0086	3.07	5-10%
Times	5	0.2217	0.0443	15.82	< 0.1%
Error	10	0.0284	0.0028		

Table 17 - Appendix

The mean percentage absorption of water by prepupae after desiccation

Experiment 3:

	Sat. def.	Mean % wt. lost by larvae	Alive		Dead		Total	
			No.	Mean % absorp. ($\bar{x}$)	No.	Mean % absorp. ($\bar{x}$)	No.	Mean % absorp ($\bar{x}$)
	0	3.5	32	14.6	3	10.2	35	14.2
	6	23.1	35	8.3	15	11.0	50	9.1
	7.5	26.9	37	6.0	18	7.3	55	6.3
	9.0	30.5	33	9.0	21	9.4	54	9.2
	10.5	34.8	30	9.8	20	15.1	50	11.9
Experiment 4:								
3 day series	0	4.2	27	8.4	1	11.6	28	12.3
	2	18.2	44	12.6	4	10.2	48	12.4
	4	33.0	36	11.6	2	11.7	38	11.6
	6	48.1	9	16.7	22	13.5	31	14.4
	8	55.0	2	4.9	39	19.5	41	18.8
	9	59.6	0	-	34	27.6	34	27.6
6 day series	0	8.6	48	13.8	2	12.3	50	13.7
	1	17.8	43	11.0	3	29.0	46	12.2
	2	32.7	33	18.8	4	12.4	37	18.1
	3	39.0	24	21.7	2	12.3	26	20.9
	4	45.4	22	13.5	14	23.7	36	17.4
	4.5	51.0	7	17.0	22	18.7	29	18.3

Analysis of Experiment 3:

Table of treatment means - logarithmic scale

Sat. def.	% wt. lost	No. used	Mean % wt. water absorbed			
			Alive	Dead	Total	total
0	3.5	35	1.157	1.067	1.114	0.079
6	23.1	50	0.784	0.893	0.817	0.066
7.5	26.9	55	0.667	0.728	0.687	0.063
9.0	30.5	54	0.711	0.709	0.710	0.063
10.5	34.8	50	0.690	0.896	0.772	0.066
	Σ	244	$\bar{x}$ 0.798	0.810	0.802	

Analysis of variance

Source of variation	d.f.	S.S.	M. S	V.R.	p
Total	243	56.877			
Treatments	9	6.067	0.663	3.06	
Dead vs. alive	1	0.070	0.070	-	N.S.
Saturation deficits	4	5.325	1.331	6.13	
IA.	4	0.672	0.168	-	N.S.
Error	234	50.810	0.217		

Table 18 - Appendix

The loss of weight from pupae at different saturation deficits

Temperature	Sat. def.	No. used	Mean % wt. loss	s^2	95% limits (±) of mean
16.8°C	0	20	0	-	-
	0.65	20	1.5	7.81	1.3
	1.19	20	1.8	19.71	2.1
	1.73	20	4.9	34.70	2.8
	2.27	19*	2.2	15.44	1.9
	3.97	20	5.0	27.81	2.5
	7.80	20	4.7	43.93	3.1
	9.78	20	3.7	15.91	1.9
	11.62	20	10.3	58.77	3.6
	13.50	20	13.4	155.57	5.8
22.9	0	20	0	-	-
	0.60	20	1.5	7.86	1.3
	1.32	20	3.3	20.62	2.1
	1.67	19*	5.2	28.42	2.6
	2.41	20	2.3	4.60	1.0
	3.51	20	2.6	6.31	1.2
	6.58	20	5.7	14.92	1.8
	9.65	20	9.6	47.03	3.2
	13.10	20	11.7	100.03	4.7
	16.45	20	12.4	104.97	4.8

* One injured

Table 19 - Appendix

Estimates of the dates by which (a) eggs were laid (b) eggs hatched and (c) larvae migrated to the surface in the years 1927-1956. From these are derived the number of days of drought to which the young larvae were exposed in each year

Year	Date by which eggs were laid	Date by which 50% of eggs hatched	Date young larvae migrated to surface	Duration of drought (days)
1927	February 12	March 4	March 23	18
1928	January 27	February 19	February 19	0
1929	February 7	February 28	April 5	36
1930	February 28	March 25	April 3	9
1931	January 31	February 21	March 13	20
1932	February 12	March 4	March 9	5
1933	February 1	February 22	March 4	10
1934	April 1 (?)	May 5	May 5	0
1935	March 23 (?)	April 22	April 22	0
1936	March 19 (?)	April 17	April 22	5
1937	February 2	February 23	March 30	35
1938	February 3	February 24	March 2	6
1939	March 3	March 28	April 6	6
1940	January 28	February 15	March 2	15
1941	February 1	February 22	March 3	9
1942	February 1	February 22	March 26	34
1943	February 25	March 18	April 8	21
1944	February 15	March 9	March 12	3
1945	February 7	March 3	May 14	72
1946	February 1	February 23	February 23	0
1947	February 23	March 21	March 29	8
1948	February 9	March 12	March 12	0
1949	February 27	March 25	May 6	42
1950	February 24	March 21	April 4	14
1951	February 26	March 19	April 6	18
1952	February 17	March 17	March 20	3
1953	February 16	March 10	April 4	25
1954	February 5	February 27	March 22	23
1955	February 18	March 13	April 12	30
1956	March 13	April 4	April 4	0

<u>NOTE:</u> (a) When the larvae migrated to the surface after 0 days of dought sufficient rain to stimulate their movement had fallen before the eggs hatched. The amount and the date on which this rain fell in these years were as follows:-

Year	Points of rain	Date
1928	176	Feb. 12-13
1934	129	April 3-4
1935	101	March 14-16
1936	66	April 18-22
1946	444	March 18-19
1948	71	Feb. 19 - March 3
1956	135	April 2

<u>NOTE:</u> (b) it is difficult to estimate flights in dry summers.

Plate 1. The contrast between a pasture with a bare surface (background) and a pasture covered with dense stubble (foreground). The pasture in the background has been cut for grass hay and has the type of surface to which adults of A. howitti are attracted to lay eggs.
Photograph by K.P. Phillips, Mil-lel, October, 1949.

Plate 2. Pasture damage on shallow terra rossa soil overlying limestone. In such places perennial plants do not thrive and the ground is bare in summer. Mt. Schanck, 1955.

Plate 3. Typical depression in pasture on terra rossa soils infested with A. howitti. Mt. Schank, 1954.

Plate 4. A meadow podsol pasture at Wrattonbully showing a depression and some well-drained slopes. Adults of A. howitti laid many eggs in the depression and along the bottom parts of the slopes but the larvae were all drowned by excess water which accumulated in the depression in winter. September, 1955.

Plate 5. Pasture on a meadow podsol in typical "red gum country" at Kalangadoo. In the foreground is an infestation of A. howitti on the northern side of a red gum (E. cameludensis). A number of ring-barked trees and stumps can be seen in the background. July, 1955.

Plate 6. A pasture on terra rossa soil near Mt. Schanck showing the weeds which have invaded a site previously eaten bare by larvae of *A. howitti*; the weeds in the picture are mainly *Cryptostemma calendulaceum.* Note the sheep tracks leading to the infested site which was once a sheep "camp". Limestone outcroppings are visible in the background. September, 1955.

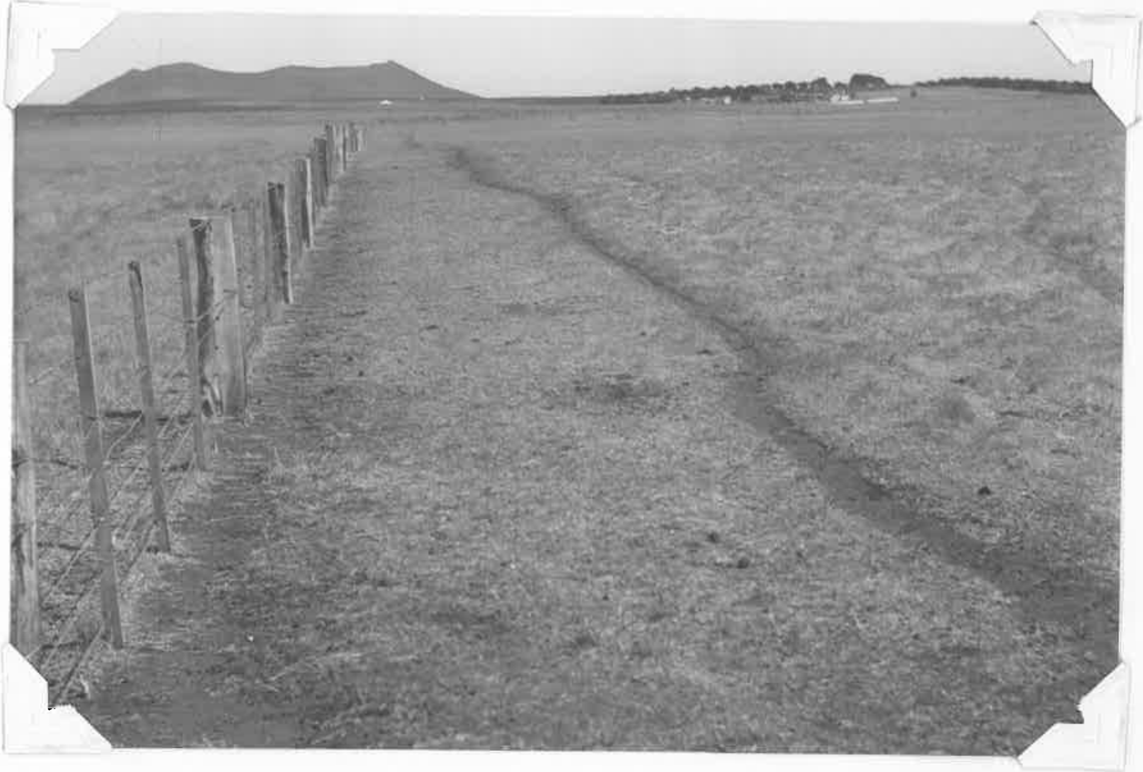

Plate 7. A sheep "camp" near a gate and along a fence showing the type of surface to which adults of <u>A. howitti</u> are attracted to lay their eggs. Mt. Schanck is in the background.

Mr. J. King's property, Mt. Schanck; March, 1955.

Plate 8. Typical damage to a pasture resulting from the feeding of larvae of A. howitti along a concentrated "front"; the contrast between the damaged and the undamaged pasture has been exaggerated by taking the photograph in October by which time the undamaged pasture had grown considerably.

The photograph also shows the regrowth of perennial rye plants in the damaged area, and illustrates how the feeding of the larvae provides a site which is attractive to adults for egg-laying the following summer.

Plate 9. Close up of an area at Kalangadoo in which some larvae of *A. howitti* survived in dung heaps when the ground was flooded. The soil cast upon the surface by larvae can be seen clearly. July, 1955.