MAKING USE OF A PARASITE TO CONTROL RED SCALE IN SOUTH AUSTRALIA.

A study of methods of disseminating *Aphytis melinus* DeBach, and its establishment on populations of *Aonidiella aurantii* Mask.

By

M.M. CAMPBELL B. Ag. Sc. (Adel).

A thesis submitted for the degree of Master of Agricultural Science in the University of Adelaide.

Horticulture Branch
South Australian Department of Agriculture
Loxton Research Centre
Loxton.

Department of Entomology
Waite Agricultural Research Institute
University of Adelaide

June 1971.

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SUMMARY.

An ectoparasite, Aphytis melinus DeBach, of red scale, Aonidiella aurantii Mask., was imported to the Riverland and released in a few citrus orchards. Variable degrees of success in colonisation were recorded. Experiments showed that, as the number of adults of A. melinus in the initial release into any one tree was increased from 10 to 100 and then 1,000, the number of times that colonisation succeeded showed an approximately two-fold increase for each ten-fold increase in the number of adults. It was shown that the number of hosts present had no effect on the number of times that successes were recorded, and that if releases were made in the third tree in each third row before March, A. melinus would most probably be present in all trees in the orchard by July.

It is suggested that there is an upper limit to the number of adults in any release, above which no increase in the ratio of successes to failures (in colonisation) will be achieved and that this limit is reached with 100 adults released from December to April inclusive but that in October, November and May, the limit is 1,000 adults. It is suggested that in any programme to release A. melinus the above numbers be released in the nominated months and that it is not commercially feasible to make releases during winter.

Experiment showed that there was no effect of the size of the initial population of red scales on the time required to achieve control. Populations ranging from 264 to 1,253 on 150 leaves were reduced to less than ten living red scales in a period of 14 months. It is suggested that the time at which control is achieved is best described as the end of the summer after the one during which A. melinus was released. Despite control being achieved in the same time, it is suggested that the initial population of red scales should be as small as possible to reduce to a minimum the commercial losses in the two harvests immediately after release.

The degree of control achieved is shown to be adequate for an indirect but not a direct pest and it is suggested that red scale can be classified into either type. Control in less than three years suggests, but does not promise, that permanent control will be achieved.

The time of weathering required, before a residue of a Maldison, Oil or Omethoate spray killed less than 50% of adults confined above it for four hours, was determined. It was shown that the value for Maldison decreased from 48 days in late winter to 41 days in October, and 27 days in mid-summer. It was shown that the period for oil was 2½ days and suggested that this would vary from three days (32 to 35°C) to three weeks (16 to 21°C) but that as soon as the visible traces of oil on leaves were lost, it was safe to release A. melinus. These results suggest that it is possible to reduce the size of a population of red scales before A. melinus is released, provided that the appropriate time - lapse is observed between spray and release.

Experiments showed that the most commonly used nutrient spray had no effect on adults of A. melinus.

The number of adults which could be reared from the red scales on one orange was shown to be greater when that orange came from a basket of 50 oranges under a tree (25.6 adults) than when it was free hanging on the tree (9.4 adults). A technique was described to use such a basket to spread A. melinus around an orchard.

Experiences gained in handling and rearing A. melinus showed that, if the source of A. melinus for release was the insectary, adults were the most economic stage, but that if A. melinus was collected from the field, immature stages (in red scales on oranges) were more easily collected.

DECLARATION.

The work presented in this thesis is my own, unless otherwise acknowledged in the appropriate place; it has not been previously published or submitted to this or any other University for the award of any degree.

(Malcolm M. Campbell)

ACKNOWLEDGEMENTS.

This work was carried out while I was employed by the South Australian Department of Agriculture. I am grateful for their permission to present these results as a thesis for the degree of Master of Agricultural Science, and for the encouragement of Mr. M.B. Spurling.

I am grateful to field assistants at Loxton, particularly Miss C. Broad and Mr. C. Wilmshurst for their help with breeding parasites and field work. I am grateful to the many orchardists whose trees have been used to conduct some of the experiments described.

Helpful advice and criticism has been received throughout this study from Professor T.O. Browning, and Dr. D.A. Maelzer, Department of Entomology, Waite Agricultural Research Institute.

INTRODUCTION.

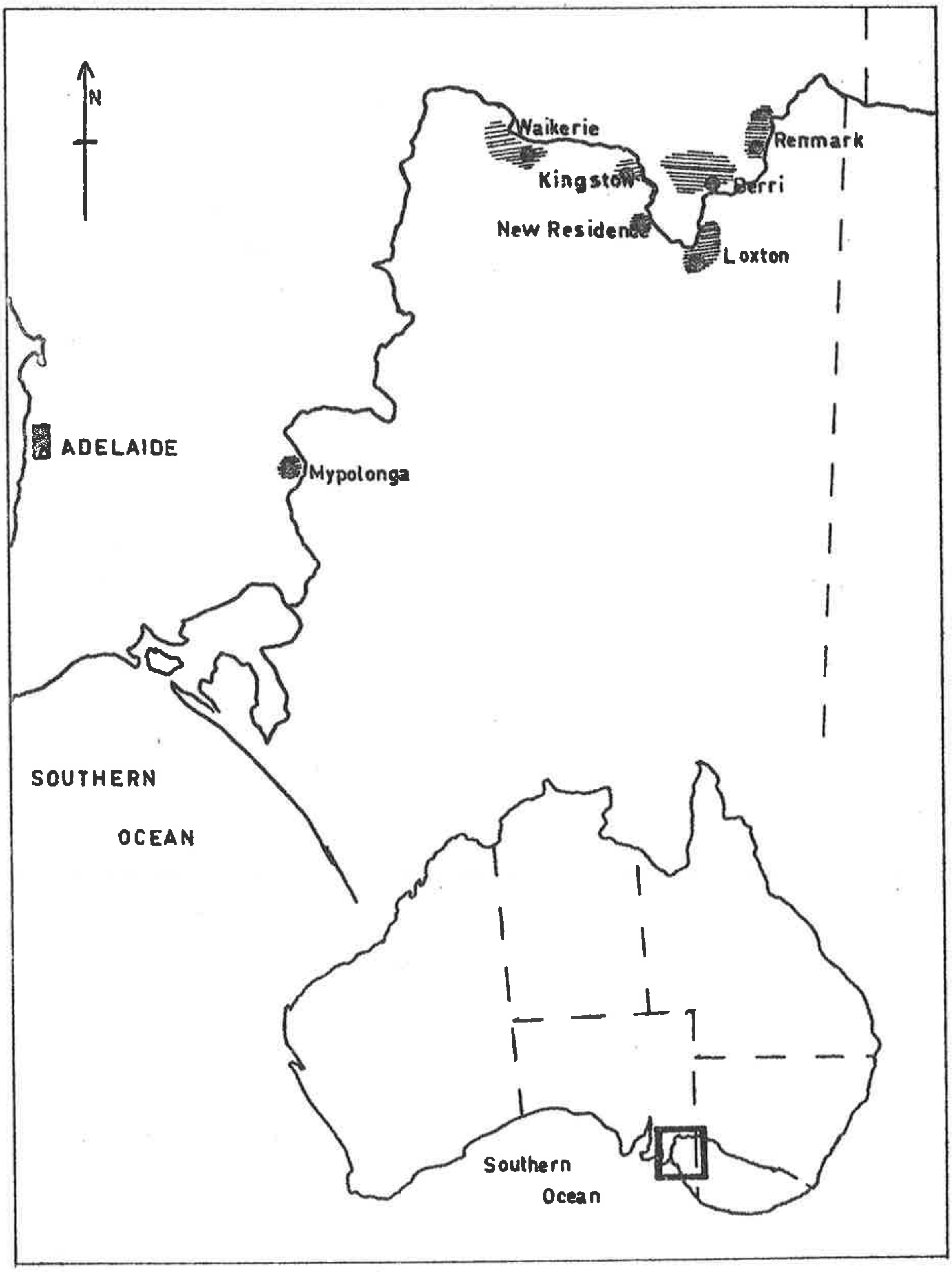
Along the banks of the River Murray there are a number of areas in which fruit and other crops are grown, irrigated by water from the river. Each area is located around a town and has its own irrigation system. The areas within South Australia are known collectively as the "Riverland", which is a belt of land, on either side of the river, ranging from five to twenty miles wide and four hundred miles long. The rainfall is low (277 mm at Loxton) and distributed almost evenly throughout the year. Summer is hot with a mean maximum temperature in January of 31^{o}C., while winter is cool with a mean minimum temperature of 4.75^{o}C. in July. Both rainfall and summer temperatures are characteristic of inland, semi-desert areas. The dominant land form is a long series of aeolian sandridges with their long axes running East to West. Orchards are planted between and over them.

The main crop in the irrigated areas is citrus. There were 1.642 million citrus trees in the Riverland in June 1966 (C.O.C.,[1.] 1967) of which almost 90% were growing in the areas including and upstream from Waikerie (see Figure 1), which has the largest number of trees of all the areas (C.O.C., 1967). The Riverland produced 2.6 million bushels of citrus fruits in 1969 (C.O.C., 1970).

1. Citrus Organisation Committee of South Australia.

FIGURE 1.

Map of part of South Australia showing the River Murray, the main towns of the Riverland (their irrigation areas are crosshatched) and places mentioned in the text.



N
Waikerie
Renmark
Kingston
Berri
New Residence
Loxton
ADELAIDE
Mypolonga
SOUTHERN
OCEAN
Southern
Ocean

In the Riverland, red scale, Aonidiella aurantii Mask. is the only insect which consistently causes loss of income to citrus growers and poses the threat that it will cause damage to citrus trees. At least 90% of the trees in the Waikerie district, and in all districts upstream from Waikerie, require insecticidal treatment for the control of red scale (Personal communication, District Horticultural Advisers, Department of Agriculture, South Australia).

Red scale can infest all parts of a citrus tree; leaves, fruit and bark. One red scale on a fruit will make it unfit for sale to most export markets (C.O.C., 1969). The export regulations enacted by the Commonwealth of Australia demand that fruit shall be sound and free from disease (Regulations under the Customs Act, 1901-68). Disease, by definition (The Commerce (Trade Descriptions) Act of 1905-6, and amendments to 16th March, 1970), includes red scale. Many of the countries which import citrus from Australia prohibit the entry of red scale for quarantine reasons. Frequently this is not quarantine against red scale, which is present in virtually every citrus growing area of the world, but rather a policy designed to exclude any pest not already present by excluding all pests (personal communication, Mr. H.W. King, Secretary, Australian Citrus Growers' Federation). Inspectors of the Commonwealth Department of Primary Industry have been known to reject from export whole shipments of fruit because a single red scale has been found (personal communication, Mr. H.W. King). It is not possible to ensure that sorting, either during picking or packing, will exclude all oranges with one or more red scales from export packs, as reports of red scale on fruits sent to New Zealand testify (A.C.G.F. [2], 1970). A policy of minimising the populations in the field has therefore been accepted as a necessary part of the overall programme to enable the export of large quantities of oranges free from red scale. A heavy infestation of red scale can kill part or all of a tree (Rimes, 1966), thus reinforcing the need to obtain adequate control in the field.

Red scale has been described as probably the most important citrus pest in the world (Ebeling, 1959) and is known as a serious pest in countries with semi-arid climates such as California (DeBach, 1969); South Africa (Bedford, 1968); Israel (Cohen, 1969); and Australia (Rimes, 1966). It is probably indigenous to China but has been known in Australia for seventy years (Rimes, 1966).

Red scale (Aonidiella aurantii, Mask; Hemiptera; Coccoidea; Diaspididae (Mackerass, 1970)) is one of the armoured scales. At most stages of

2. Australian Citrus Growers' Federation.

development it is a soft-bodied, immobile insect tightly adpressed to the surface of its food plant. The adult female is nearly two millimetres in diameter and gives birth to living and motile young, or "crawlers". That motility is lost after no more than 24 hours of life as soon as the rostralis is inserted into the plant tissues, a phase known as "settling". The legs, eyes and antennae of the crawlers are lost during the first moult (Baranyovits, 1953). Soon after settling, the crawler starts to form its scale with waxy threads extruded from glands in the pygidium as it oscillates around its rostralis (Baranyovits, 1953). The female moults twice but the male four times before becoming adult (Baranyovits, 1953). The male is a weak flyer and lives (at 22 to 24°C) for a mean of six to seven hours (Tashiro & Beavers, 1968).

The length of the life-cycle is 36 days at 28°C (Flanders, 1951) but varies from three months (summer) to five months (winter) in the field in the Riverland (Botham, 1961).

In the Riverland areas, crawlers are produced from mid-October to April (Botham, 1961) during which period two or three generations may be produced and a very rapid increase may occur (Botham, 1961; Rimes, 1966).

The South Australian Department of Agriculture recommends a programme of two sprays for the control of red scale (Campbell, 1970a). The first is of Maldison (an organo-phosphate insecticide; 5- (1,2 - di (ethoxy-carbonyl) ethyl) dimethyl phosphorothiolo-thionate) applied at 0.15% concentration in mid-October and the second is an oil spray, at 2 to 2½% concentration, to be applied in January or February. Both are usually applied by an "oscillating boom" machine with which adequate coverage can be achieved using eight to sixteen gallons of material on each tree. Hand spraying, a more efficient but slower method, is used by some growers. The first treatment costs 33.8 cents and the second 32.2 cents for each tree (appèndix 1.). Application of the recommended treatments to all of the infested trees in Waikerie and upstream of that town would cost the industry nearly $1,000,000 a year (Appendix 1).

The above is an oversimplified estimate as it accounts for neither those growers who do not spray nor losses from fruit infested with red scale in sprayed orchards. It indicates the magnitude of the costs involved. At least 2% of all the oranges that were delivered to packing sheds at Renmark in the 1965-66 season were downgraded in quality because of infestation by red scale (Wishart, 1966).

Red scale was present at Mypolonga in the south and Renmark in the east (Figure 1) in the 1930's but is a relatively new pest to other areas. It has been slowly spreading west from Renmark into other areas, some of which, particularly parts of Waikerie, have only just reported their first serious infestations. As late as 1963, it was estimated that red scale was infesting less than 1% of all trees in the Riverland (Spurling, 1963).

The policy of the Department of Agriculture has been to try to prevent the spread of red scale by a programme of insecticidal treatment aimed at eradication. Initially, infested trees were fumigated with hydrogen cyanide (Botham, 1961), but high costs, high labour requirements and an inability to fumigate sufficient trees to keep up with the spread of red scale forced changes in this practice. The present programme is listed above. The attempt to prevent the spread of red scale has, unfortunately, failed. There arose, therefore, a need to review the control measures with the aim of learning to live with the pest and using those techniques of control which minimised the economic loss caused, rather than those which attempted to reduce the population of red scale to zero.

The returns from the sale of citrus fruits have decreased in the last ten to fifteen years while production costs have increased to such an extent that growers must attempt to reduce costs in order to maintain profitability. There have been increasing demands for methods to reduce the cost incurred in controlling red scale. Many growers now omit one of the treatments in some years simply to reduce costs. As they have looked for cheaper methods of control, a small, but increasing group of growers have become interested in the use of parasites of red scale. This interest was fostered by the observations of a few people that a parasite, *Aphytis melinus* DeBach, was giving excellent control of red scale at Boundary Bend in Victoria.

A parasite of red scale, *Comperiella bifasciata* (How.), has been present in Riverland areas for some years. It has been an effective agent in the control of yellow (*Aonidiella citrina* (Coq.)) but not red scale (Brewer, 1971). This same result has been observed in other areas (Flanders, 1944b).

Aphytis melinus DeBach (Hymenoptera : Aphelinidae), a parasite which has achieved a valuable degree of control over red scale in California (DeBach, 1969), was introduced to South Australia in November 1968. It was bred in the insectary at Loxton and released throughout the summers of 1968-69 and 1969-70. Most of these parasites were released as part of the experimental work for this thesis.

A. melinus is a small yellow wasp, 0.5 to 1.0 mm long, the immature stages of which develop as an ectoparasite on red scale. The adult female lays its egg underneath the scale covering and on the surface of the red scale. During its larval development the parasite consumes the body of the red scale and then pupates within the space thus created. Development from egg to adult requires 13 to 16 days (egg 3-4 days; larva 4-5 days; prepupa 1½ days; pupa 5-6 days) at 26.7 °C but much longer at lower temperatures (DeBach and White, 1960). The female usually mates within four hours of emergence as an adult but, whether virgin or mated, begins to oviposit between four and twelve hours after emergence (DeBach and White, 1960). One female may produce a mean of 24 parasites in the next generation with the eggs being laid uniformly throughout her life. The mean time, at 26.7 °C, to 75% mortality of adults of *A. melinus* is 26 days (DeBach and Sundby, 1963). The only stages of red scale attacked by *A. melinus* are second and early third instar females and prepupal males, which are stages when the scale covering is not glued to the scale body (DeBach and White, 1960).

Approximately 70,000 adults of *A. melinus* have been released in the field from the insectary, where *Aspidiotis hederae* Vallot, the oleander scale, is used as the host.

In the two years since its first release, *A. melinus* has achieved good control over red scale in one area (Campbell, 1970b). Throughout the summer of 1968-69 small numbers of *A. melinus* (both adults and pupae) were released at frequent intervals. The pupae were in a late stage of development in red scales on oranges when they were put out into the field. Colonisation [3] succeeded on each occasion that more than two hundred adults were released into one tree (Appendix 2) but was successful on only 51% of the total number of occasions that adults were released. The numbers of pupae released into one tree ranged from 20 (15 trees) to 200 (11 trees) but a success was recorded at each release (Appendix 2).

Within four months of colonisation, significant decreases in the numbers of red scales per tree were recorded (Campbell, 1970b).

With this apparent success soon after release there was a need to determine the effect of different initial populations of parasites and of hosts on the probability that an attempt at colonisation would be successful, in order to maximise the efficiency of colonisation. Another aim of the experimental work was

3. Colonisation - the attempted establishment of a community of organisms in a new locality (DeBach and Bartlett, 1964 p. 402).

to assess the effect of the time of the year on the probability of success in attempts at colonisation and to develop techniques whereby A. melinus could be colonised throughout the Riverland as cheaply and as quickly as possible. The time required for A. melinus to reduce the size of the population of red scales to a low level was determined. The technique required was one which would allow the release of as few parasites as possible in one place but one which maximised the chance of success in colonisation.

Parasites can be produced continuously in an insectary, but in the field it is unlikely that colonisations will be equally successful in all seasons (DeBach and Bartlett, 1964). It was necessary to determine that period of the year when colonisation was most likely to succeed.

Californian workers (DeBach, Landi and White, 1955) showed that 4,000 adults of Aphytis sp. should be released into each tree if large populations of red scale were to be reduced to low numbers within a year. Fewer parasites could, however, be successful :-

> "Indications were that fewer numbers of parasites (than 400,000 per acre) would be sufficient in some groves or in certain years" (DeBach, Landi and White, 1955).

DeBach and White (1960), while accepting the above numbers, preferred to release equal numbers into each tree on nine occasions, at monthly intervals. The cost of this schedule was found to be excessive, forcing a modification (DeBach and White, 1960) to the release of 4,000 adults on each of nine occasions into the third tree in each third row. The parasites were released into the same tree each month. They considered this method to be both economical and efficient. Even with such large numbers being released, breeding of the parasite in the field was necessary to ensure effective control of red scale, of which there were up to 2 million per tree (De Bach and White, 1960). They considered that colonisation in the field could be successful during a nine month period of each year in California; the spring, summer and autumn months (DeBach and White, 1960).

These workers (DeBach and White, 1960; DeBach, et al, 1950) released only adults of the parasite. They thought this to be the most economical way of releasing a parasite because immature stages are frequently more subject to destruction, either by predators or during periods when the meteorological conditions are less favourable (DeBach and Bartlett, 1964).

The cost of rearing and colonising A. melinus has not been calculated for Riverland conditions. DeBach and White (1960) estimated the total costs to be

$ (U.S.) 16,946 a year which included running costs and a fifth of the capital costs. At this cost they produced 176 million females a year, and this was sufficient to colonise 441 acres or 44,100 trees.

At the above rate all of the trees in the Riverland could be colonised in four years at a cost of at least $70,000. A sum of this magnitude is not available even though it is only 10% of the estimate of the annual cost of insecticidal control. This emphasises the need to develop a system by which much smaller numbers of the parasite can be released in the field with a good chance of success.

METHODS AND RESULTS.

1. EXPERIMENT 1.

1.1 INTRODUCTION.

Examination of the effects of different numbers of *A. melinus*, densities of red scale and time of year of release on the success of attempts to establish *A. melinus* on red scale in the field.

1.1.1 Adults or Pupae as the release material.

In the first releases of *A. melinus* a greater degree of success was achieved when pupae rather than adults were put into the field (Appendix 2 and Introduction). If, however, more than 200 were released then success followed whichever were used. It became obvious during the mass-rearing work of the summer of 1969 that the problems of handling oranges in the insectary were too great to allow them to be used as a medium for rearing the red scales to be used as hosts (the pupae put out in the field were in red scales on oranges). Large proportions of the oranges used were discarded before red scale could develop (Table 1).

TABLE 1.

The condition of oranges held in the insectary at 26-27 ^{o}C and 50% R.H. for 20 days. The percentage of oranges (total number examined = 154) which fell into each of the nominated classes.

Condition of oranges	Percentage of total oranges examined.
Firm	0
Soft	51
Early stage of drying	20
Dry	23
Rotten	6

A minimum of 33 days in the insectary is required of oranges to be used in such a programme.

The transport to the field of oranges, rather than adults of A. melinus, is far more costly and the distribution in the field requires much more time. A glass tube, 9.7 cm long x 2.2 cm diameter, is adequate space for at least 400 adults while 16 to 40 oranges would be needed for 400 pupae.

These two disadvantages make the use of pupae of A. melinus in red scales on oranges, as the material to be moved from the insectary to the field, unattractive relative to the movement of adults, and so all further experimental work was confined to releasing adults.

1.1.2 Interactions between the numbers released and the successfulness of colonisation.

There has been virtually no attempt in the past history of biological control projects to measure the effects of different numbers of parasites on the degree of success in colonisation nor on any of a number of other variables, such as the number of releases, the time of day and the best type of weather for releases. All of these could be expected to influence the success of any one release (Turnbull & Chant, 1961). These authors claim, and there are no published reports to dispute them, that releases have not been made on an experimental basis and that experience can give us neither answers nor guides to the solutions of the above problems.

"What then are the limited objectives we should strive for? In essence, applied biological control, and this is our ultimate purpose, hinges on being able to select species that will fulfil our needs, and to import, rear and release these species with maximum of success and minimum of expense, time and effort". (Turnbull & Chant, 1961).

The same authors suggested that a dichotomy in approach to biological control, which they considered to be a separate discipline within Entomology, was needed. That all research workers needed to be looking at both ad hoc projects (the rapid implementation of biological control in the field) and fundamental problems in ecology and they further suggested that this would be of value to both the workers and the discipline.

A similar plea to carry out more experimental work so that biological control could be placed on a scientific basis was made by Thompson (1954) in an address to the 6th Commonwealth Entomology Conference.

1.2 AIMS.

This experiment was designed to provide some answers, in relation to one host and one parasite, to the questions posed by Turnbull & Chant (1961). It was designed to give information of value to fundamental studies and to the *ad hoc* project of which it was a part (the implementation of biological control of red scale in the Riverland areas).

1.2.1 The relationship between the number of adults of *A. melinus* released and the success of establishment was examined. An estimate was made of the number of adults needed in a release to give a high probability that colonisation would be successful.

1.2.2 The influence of the number of hosts into which the parasite was released was examined, and this allowed an assessment of the influence of the number of hosts on the probability that any release would succeed. The effect of the size of the population of red scales on the tree, on the costs of introducing biological control, was assessed, and the smallest number of scales needed, on a tree, to give a high probability that an attempt at colonisation would succeed was determined.

1.2.3 The relationship between the time of the year and the probability that a release of the parasite would be successful was examined, and the limits of the period when adults of A. melinus could be released in the field in the Riverland was defined.

1.2.4 A final aim, relating entirely to the ad hoc project, was to examine the distance of migration of A. melinus away from a release tree to discover whether the suggested release plan of DeBach & White (1960) was commercially feasible with the numbers of A. melinus to be released.

1.3 TREATMENTS.

1.3.1 The number of adults of A. melinus to be released.

Experience (Appendix 2) has already indicated that numbers in the range of 50 to 200 would be well worth study, so the numbers chosen ranged around these values.

The numbers released were :-

0 (equivalent to control)

10 ; 100 ; 1000.

1.3.2 The size of the population of red scales was measured using a rating system (Appendix 6). When this trial was being designed, A. melinus had been colonised in trees for 13 months and had reduced populations of red scales (see later) to an index range of 30 to 32. Three ranges of index were chosen to span this observed value and they were :-

Low	15 - 25	Red scales difficult to find on trees.
Medium	40 - 60	
Heavy	80 - 150	Very heavy population of red scale.

1.3.3 The time of the year when *A. melinus* was released.

The number of times, 9, at which releases were made was determined by two factors, the number of trial sites and the number of adults of *A. melinus* available. It was planned to make releases at weekly intervals into one block a week for 9 weeks starting in mid-February, but the supply of adults of *A. melinus* was limiting. Releases were made, a whole block at a time, when sufficient (3330) adults were available and warm weather prevailed.

The date of release into each block is shown below.

Block	Date	No. days after 11.2.70
1	12.2.70	1
2	20.2.70	9
3	6.4.70	54
4	14.4.70	62
5	20.4.70	68
6	21.4.70	69
7	1.5.70	79
8	4.5.70	82
9	17.6.70	126

1.4 DESIGN.

The trial was set out as a series of blocks, each containing the complete range of treatments of number of parasites and number of hosts. There were twelve treatments in each block with each treatment located on a single tree.

The correct number of parasites was released into the appropriate tree but the correct index of red scale was achieved by preselecting the trees from the orchard (i.e. four trees preselected for each range of index). The parasite treatment was allocated at random to the four trees selected in each index range.

During the selection, an attempt was made to have a barrier of at least one tree between trial trees. All barrier and surrounding trees were sprayed with an emulsion of 2½% oil in early February.

1.5 REPLICATION.

The number of trial sites and the number of adults of *A. melinus* available precluded any replication. If the initial plan had been followed regarding the time of release, each block would have been much more nearly a replicate.

1.6 SELECTION OF TRIAL SITES (BLOCKS).

At least one trial site (block) was found in each of the citrus areas of the Riverland with a preponderance of sites in Kingston, Moorook and Berri. Orchards heavily-infested with red scale and unsprayed for a year were sought and provided that four trees in each index-range could be found and the grower was willing not to use insecticide, the orchard was acceptable. In every case the 12 trees selected were spread over at least one acre of orchard.

1.7 ASSESSMENT METHODS.

1.7.1 Colonisation.

Samples of the red scale on each treatment tree were searched for signs (either immature stages or meconium) of parasitism by *A. melinus* on two occasions. In the blocks into which parasites were released before May, the samples (of thirty leaves and five fruits) were collected one and three months after release. These were lengthened to six and 16 weeks on the later samples to compensate, in part, for the slower development of immature parasites at lower temperatures (DeBach & White, 1960).

Leaves and fruit heavily infested with red scale were selected haphazardly from all round the tree.

Every red scale in the sample was examined for signs of parasitism until 20 parasitised scales or 100 scales suitable for parasitisation (Flanders, 1951) were recorded. The former, expressed as a percentage of the latter, was called the percentage parasitisation. The samples of leaves and fruits were treated separately. Most of the samples were exhausted before either criterion was satisfied. The examination of a complete set of samples from each block required two man weeks of work, which precluded any chance of increase in sample size.

The numbers recorded, in many cases, were so small that they could not be used to estimate percentage parasitisation even though 300 to 1000 red scales were examined in each sample. The data ~~is~~ are, therefore, presented as success or failure in the attempt, with a success being recorded if one sign of _A. melinus_ was found in ~~either~~ any sample. The percentage data ~~is~~ are shown in Appendix 3.

1.7.2 Migration Assessment.

From July 1970, five fruits or 30 leaves (if fruits were not available) heavily infested with red scale were collected from each third tree in each third row and, in addition, from three trees in succession away from two sides of each treatment tree. The red scales from each tree were held until either an adult of *A. melinus* emerged or 14 days elapsed (the time it should take for an egg to develop to, and emerge as an adult, at 26°C to 27°C). If the latter occurred, another sample was taken and treated as before. If *A. melinus* emerged from neither sample it was assumed that it had not migrated to that tree.

In late December 1970, a sample of 30 leaves, each heavily infested with red scale, was collected from every tree on the first orchard and every scale was examined for a sign of *A. melinus*. A second and third sample were collected if necessary, and if no sign was found in the third sample, *A. melinus* was assumed not to have migrated to the tree.

1.8 RESULTS.

1.8.1 Colonisation.

The result, measured as the success of colonisation and as percentage parasitisation in each sample, is shown in Appendix 3. The combined result as number of successes in colonisation is shown in Table 4 below.

TABLE 2.

The successes achieved in colonising *A. melinus* with different numbers released into different sized populations of red scale.

+ indicates a success

– indicates a failure

BLOCK		1	2	3	4	5	6	7	8	9
	No. days after 1.2.70	1	9	54	62	68	69	79	82	126
Scale index	No. parasites									
LOW	0	–	–	–	–	–	–	–	–	–
	10	–	–	–	+	–	–	–	–	–
	100	+	+	+	+	–	–	–	–	–
	1000	+	+	+	+	+	+	+	+	–
MEDIUM	0	–	–	–	–	–	–	–	–	–
	10	–	+	–	–	–	+	–	–	–
	100	+	+	–	+	–	–	–	–	–
	1000	+	+	+	+	+	+	–	–	–
HIGH	0	–	–	–	–	–	–	–	–	–
	10	+	+	–	–	–	+	–	–	–
	100	+	+	+	+	+	–	+	–	–
	1000	+	+	+	+	+	+	+	+	–
Date released		12-2	20-2	6-4	14-4	20-4	21-4	1-5	4-5	17-6
No. of successes		7	8	5	7	4	5	3	2	0

1.8.1.1 The effect of the number of adults of *A. melinus* released on the success of colonisation.

The number of successes recorded (out of a total of 27 attempts) for each number of parasites released is shown in Table 3.

TABLE 3.

Number of successes with different numbers of *A. melinus* in each block and for the whole experiment. Three attempts in each block.

Number of *A. melinus* released	BLOCK NUMBER 1	2	3	4	5	6	7	8	9	Total
0	0	0	0	0	0	0	0	0	0	0
10	1	2	0	1	2	0	0	0	0	6
100	3	2	3	3	1	0	1	0	0	13
1000	3	3	3	3	3	3	2	2	0	22

The overall result has been subject to χ^2 analysis as below. Expected frequencies have been nominated and then tested against the data.

ASSUMPTION. That no success would be recorded where no parasites were released and that there would be no difference between number of successes with other treatments.

TABLE 4.

Expected and actual number of successes in colonisation and χ^2 value computed.

No. released	Expected Successes	Actual success
0	0	0
10	13.7	6
100	13.7	13
1000	13.7	22

$$\chi^2_3 = 9.4 \ast \quad 5\% > P > 2.5\%$$

On this test the assumption of no effect of the number released on the number of successes is not valid.

ASSUMPTION. That no success would occur where no parasites were released and that the number of successes would be directly proportional to the number of parasites released.

TABLE 5.

Expected number of successes on the above assumption and assuming that the number of successes with 1000 adults was the limit. Actual successes and computed χ^2 value shown.

Number released	Expected successes	Actual success
0	0.0	0
10	0.2	6
100	2.2	13
1000	22.0	22

$$\chi^2_2 = 221.2 \ast\ast\ast$$

$$P < 0.5\%$$

On this test the assumption of a directly proportional increase (i.e. 10 x as many successes if 10 x as many parasites) in the number of successes is not valid. This indicates that, while there is an increase in the number of successes (Table 3), the rate of increase is much less than the rate of increase that was required to cause it.

1.8.1.2 The effect of the number of hosts (host density) on the number of successes.

TABLE 6.

The number of successes out of a total of 27 attempts at each density of red scale, excluding the attempts where no adults of *A. melinus* were released.

Density of red scale	Number of successes
Low	13
Medium	11
High	17

A χ^2 analysis was carried out on the assumption of no difference between the number of successes at different densities of scale.

$$\chi^2_2 = 1.4 \quad \text{Not significant.}$$

This indicates that the variations in the actual numbers observed are not greater than could be caused by chance alone and, therefore, the hypothesis is acceptable i.e. there was no measurable effect of the density of the population of red scale on the number of successes recorded when *A. melinus* was released.

1.8.1.3 The influence of the time of the year on the number of successes.

The number of successes recorded in each block (out of nine attempts when parasites were released) is shown together with time measured as number of days, in Table 7. For convenience, the independent variate has been measured as the number of days after the 11th February, 1970.

TABLE 7.

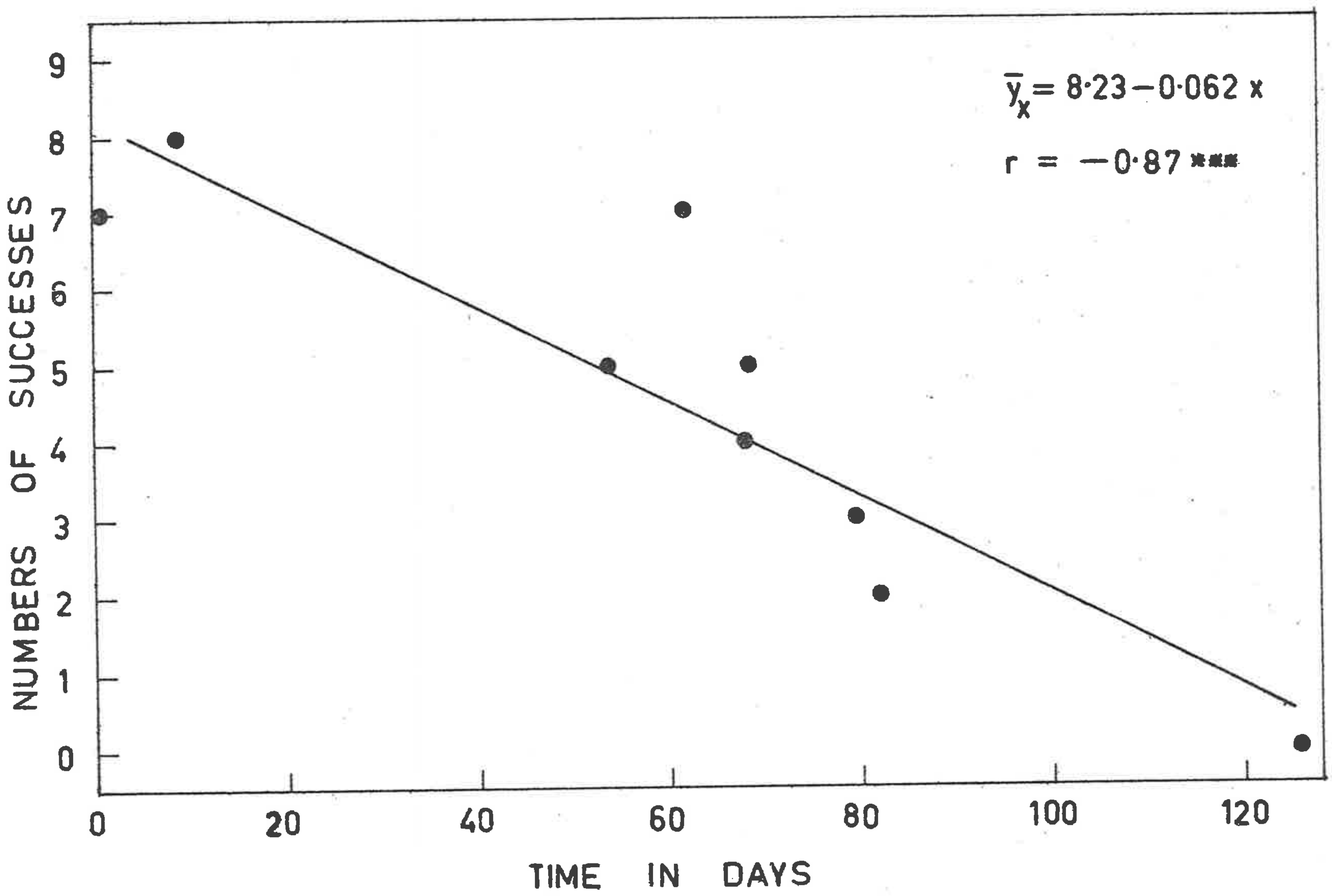
Number of days after 11.2.70 that *A. melinus* was released, and the number of successes achieved, in each block

Number of Days (x)	Number of successes (y)
1	7
9	8
54	5
62	7
68	4
69	5
79	3
82	2
126	0

The determination of a correlation coefficient between these two variates showed :-

$$r = -0.87 \quad ***$$

$$\bar{y}_x = 8.23 - 0.062 \; X$$



$\bar{y}_x = 8{\cdot}23 - 0{\cdot}062\ x$
$r = -0{\cdot}87^{***}$
NUMBERS OF SUCCESSES
0
1
2
3
4
5
6
7
8
9
0
20
40
60
80
100
120
TIME IN DAYS

There was a significant decrease in the number of successes as the number of days, between 11.2.71 and the release increased; the probability of success is decreased later in the year.

The values recorded and the regression line generated by the above analysis are shown in Figure 2.

1.8.2 MIGRATION.

1.8.2.1 Orchard Number One.

As can be seen by examining Figure 3, *A. melinus* was recovered from virtually every one of the three trees in sequence away from treated trees and from two trees at least five away from the nearest treated tree. In the July sample, of the 81 trees searched for *A. melinus*, the latter was recovered from red scale on 56; recovery from six trees for each one into which a release was made.

The result of the December sample can be seen in Figure 4 which shows the trees into which *A. melinus* was released and those from which it was recovered.

FIGURE 3.

Ground plan of Orchard Number 1 showing extent of migration of *A. melinus* 5 months after colonisation. Only the section of orchard above channel was examined.

O *A. melinus* present

X *A. melinus* absent

● *A. melinus* released.

. Tree not examined

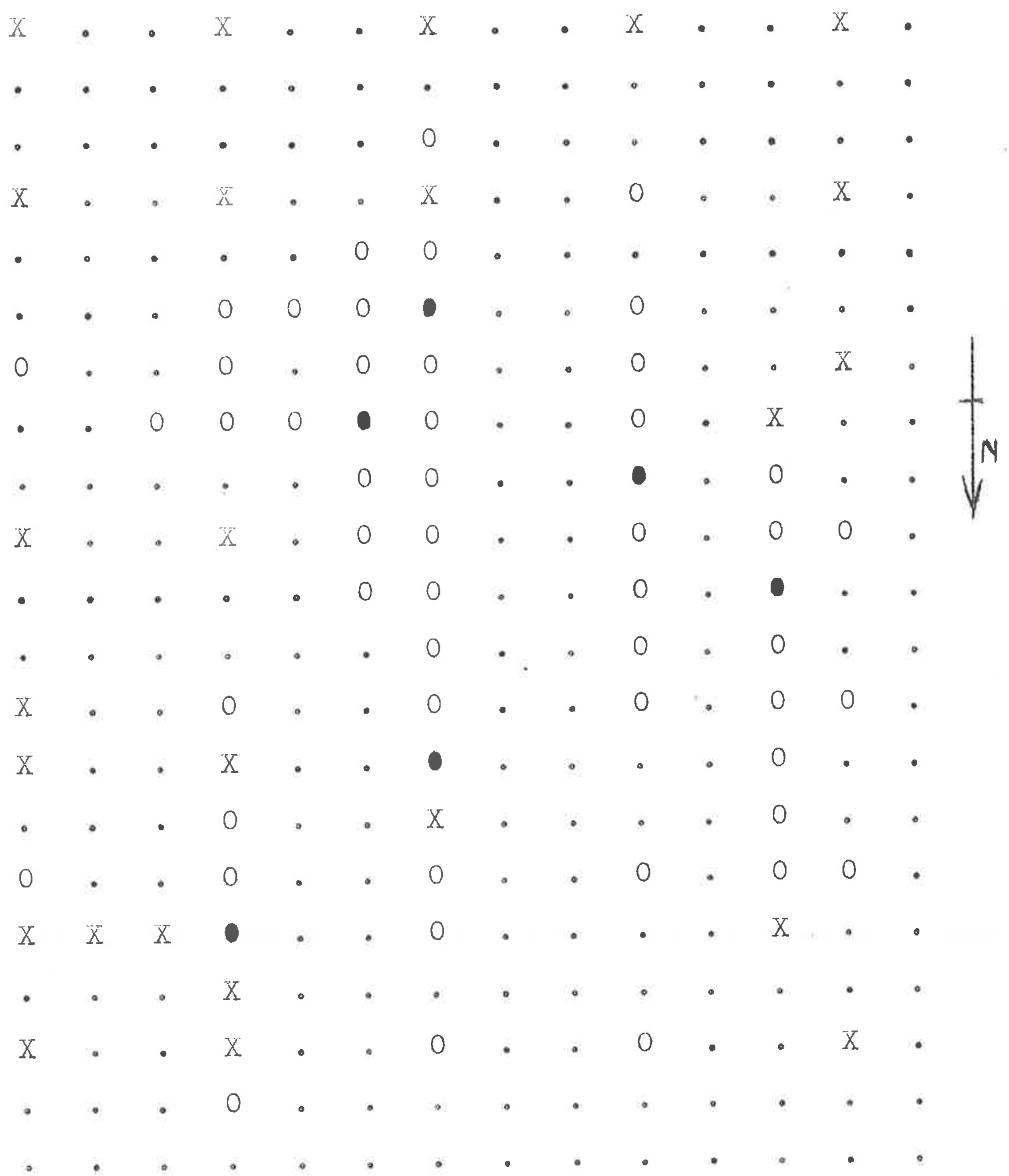

N

FIGURE 4.

Ground plan of Orchard Number 1 showing the extent of the spread of _A. melinus_, 10 months after colonisation.

O _A. melinus_ present

X _A. melinus_ absent

● _A. melinus_ released

```
O X O O O O O X X O O O O
O O O O O O O O O O O O O
O O X O O O O O O X X X O O
O O O X O X O O O O O X X O O
O O O O O O O O O O X O O O O
O O O O O O ● O O O O X O X O
O O O O O O O O X O O O X O O
X O O O O ● O O O O O O O O O
O O O O O O O O O ● O O O O O O
O X O O O O O O O O O O O X X O
X X X O O O O O O O O ● O X X O
X X X O O O O O O O O O O O O O
X X O O O O O O O O O O O O O X
X X X X O O ● O O O O O O O O O
X X O O X O O O O O O O O O O O
O O X O O O O O O O O O O O O O
O X X ● O O O O X O O O O O O O
X O X O O O X O O O O O O O O O
O O X X O O O O O O O O O O X X
O O O O O O O O O                 Channel
O O O O O O
  O O O O
O X O O O
O X X ● O ● O O
X X O O O O O O X X X
X
X O X X X O X O O O
O O X X X X X X
● O X O O O O O
```

TABLE 8.

A resumé of the trees searched for *A. melinus* and the result of the search, combining results of both the July and December samples.

Category	Number of trees	% of total number
(a) *A. melinus* recovered	286	81.0
(b) *A. melinus* Not found	62	17.5
(c) No red scale found	6	1.7
TOTAL	354	100.2

The greatest number of leaves collected from any tree of category (b) (Table 8) was 41 (mean 11.5, standard deviation 8.8) indicating that only a very small population of red scales was present on them. (An attempt was made to collect 3 samples of 30 leaves from each tree from which *A. melinus* was not recovered).

1.8.2.2 Orchard Number Two.

The ground plan of this orchard was less useful than that of No. 1 for estimating migration but it can be seen (Figure 5) that *A. melinus* was recovered from nearly three trees around each treated one with a maximum distance of four trees (only in one instance). Recoveries were made from 2.5 trees for each treated one.

FIGURE 5.

Ground plan of Orchard Number 2 showing the extent of the spread of *A. melinus*, 5 months after colonisation.

O *A. melinus* present

X *A. melinus* absent

● *A. melinus* released

. Tree not examined

X1 *A. melinus* released but not recovered

MAIN PATCH

0	0	●	0	X	0	.	.
.	.	.	.	.	.	.	.
●	0	.	.	0	.	.	.
0	.	.	.	.	.	.	.
X	0	0	●	0	X	0	.
.	.	.	.	.	.	.	.
0	X	●	0	0	X	.	.

HOUSE ROW

●
0
0
X
0
X
X
X
X
X
X[1]
X
X

TOP PATCH

.	X	.	●	.	
.	.	.	.	.	
.	.	.	.	.	
.	.	.	.	.	
.	X	X	●	0	●

A. melinus was discovered in a sample of red scales from the trees next to those into which 1000 adults were released one month after release regardless of the density of the red scales. This suggests that either some of the adults released or some of the first field generation migrated from the treated tree.

1.8.2.3 Orchard Number Three.

Migration was confined to short distances (Figure 6) with *A. melinus* recovered from only 1.6 trees for each treated tree. Recoveries were made from widely separated trees.

1.8.2.4 Orchards Number Four to Nine Inclusive.

Migration did not seem to have occurred on these orchards.

1.8.3 Weather Observations at Loxton Research Centre.

On each day, a number of parameters of the weather are measured at Loxton Research Centre. Weekly mean values for some of these have been calculated for the weeks when releases were made.

FIGURE 6.

Ground plan of Orchard Number 3 showing the extent of the spread of *A. melinus*, 5 months after release.

O *A. melinus* present

X *A. melinus* absent

● *A. melinus* released

. Tree not examined

X1 *A. melinus* released but not recovered

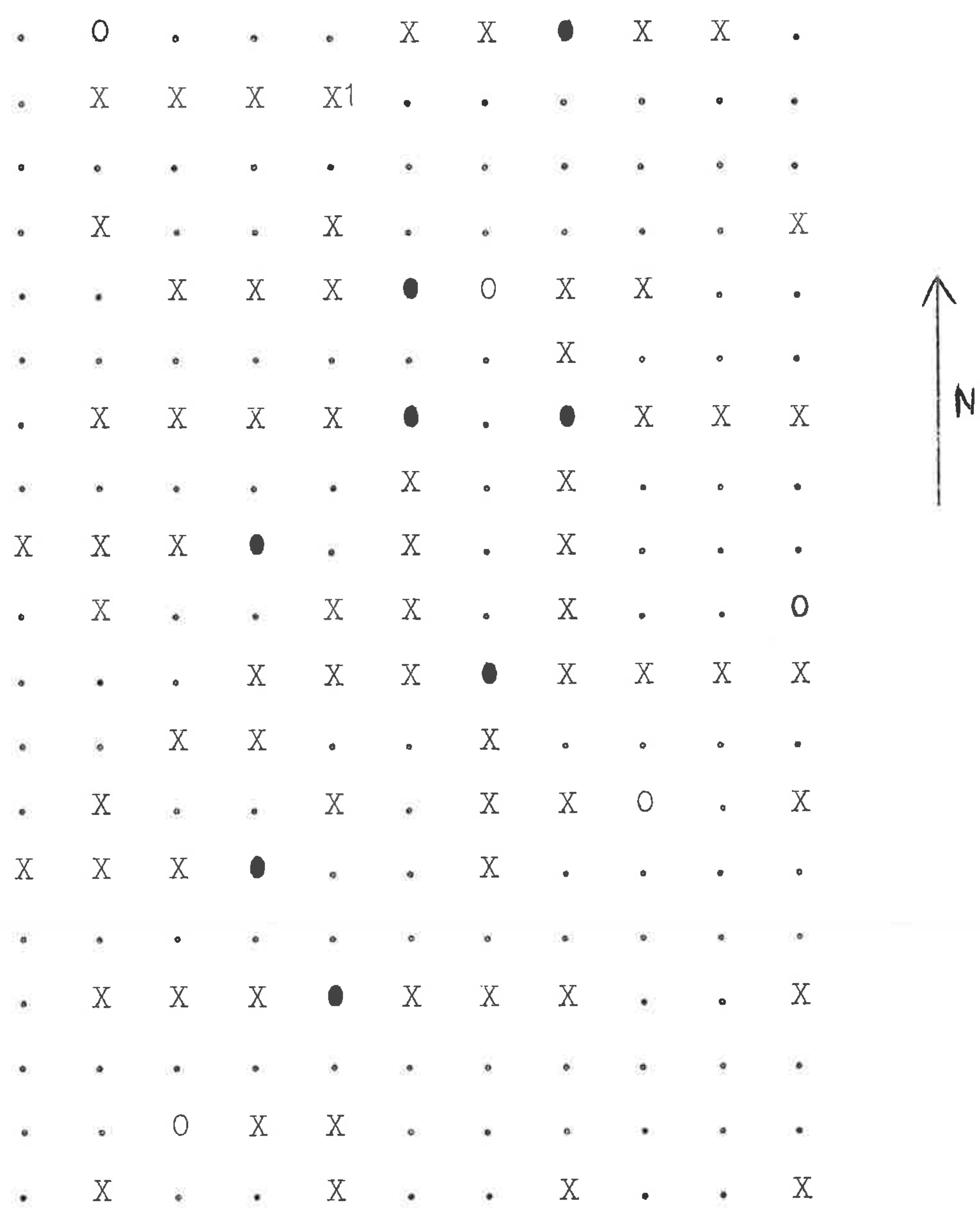

X1
N

TABLE 9.

Mean values of temperature as recorded at Loxton Research Centre for the week during which *A. melinus* was released into each block.

BLOCK Number	MEAN Maximum Temperature (°C)	MEAN Minimum Temperature (°C)	AVERAGE (estimated) Temperature (°C)
1	33.9	17.8	25.8
2	26.7	10.9	18.7
3	24.0	11.7	17.8
4	27.9	11.1	19.5
5	27.9	11.1	19.5
6	22.0	11.7	16.9
7	25.2	9.3	17.2
8	17.0	6.7	11.8
9	16.2	7.3	11.7

Migration only occurred in Blocks One, Two and Three when the average temperature exceeded 19.5°C; colonisation failed only in Block nine when the average temperature was 11.7°C.

1.9 DISCUSSION.

1.9.1 Number of *A. melinus* released.

1.9.1.1 Increase in successes with larger numbers of *A. melinus*.

The analysis carried out on the observed results showed that there was a significant increase in the number of successes as the number of adults of A. melinus was increased but that a 10-fold increase in the latter only gave about a two-fold increase in the number of successes.

In an examination of the number of hosts (Musca domestica L.) found by different numbers of a parasite (Mormoniella vitripennis (Walter)) DeBach and Smith (1947) found a similar response. Their results showed a four-fold increase in the number of hosts found, with a ten-fold increase in the number of parasites at low numbers, but no increase in the number of hosts found with increases in the number of parasites at high numbers.

They counted the number of hosts found in one "universe" while I have counted the number of times that no hosts were found (no success) when parasites were released into a number of "universes". The ~~situations~~ results are sufficiently similar to suggest that there could be an upper limit to the number of parasites released, above which, no increase in numbers of successes would occur. It will be suggested later (1.9.3.2) that this limit was passed in Experiment One.

1.9.1.2 Large Numbers Usually Released.

Examination of the written reports of many projects aimed at implementing biological control show that in most cases very large numbers of a parasite were released on the assumption that this was necessary.

Turnbull & Chant (1961) in summarising projects in Canada quote a case where 900 million adults of Dahlbominus fuscipennis (Zett.) were released against Diprion hercyniae (Htg) (they did not become established!); Conradie (1968) released from 5,600 to 8,600 adults of A. melinus into each tree; DeBach and White (1960) recommended the release of 400,000 adults of A. melinus into each acre of trees; Tooke (1955) released 500 to 1,500 adults or eggs of Anaphoidea nitens Gerault; Muggeridge (1943) released more than 10,000 adults of Apanteles glomeratus L. in a number of places. Several authors (Flanders, 1959; Sweetman, 1958; Boettger, 1956) advocate the release of as many as possible of the parasite at each release site :-

1. To ensure a sufficiently large number of adults in the first field generation so that mating (if necessary) will occur.
2. Because severe selection is imposed upon the first generation in the field, the larger the number released the greater will be the chance that a successful genotype will be present.

The assessments of colonisation in this experiment were made after at least one, and again after at least three, complete field generations. It is possible to obtain mating in the field when only 10 adults of A. melinus are released and to have mating occur on almost every occasion when 100 adults are released.

A third field generation, after the release of mated females of A. melinus, cannot occur unless mating in the field occurs (4.5). In situations where biological control has been successful there is frequently an extremely low density of parasites and they are at all stages of development. Mating must occur in these cases (when mating is necessary to ensure the continued existence of the parasite species) when numbers are far less than should be produced in the first generation after thousands of adults have been released.

In many instances the number of individuals initially collected from the field for importation to a new area is small. It is very likely that this is the time when the greatest selection of genotypes is made and that any selection at time of release in the new area is consequently relatively unimportant.

1.9.1.3 Small Numbers have been successful.

There are at least two reported instances where very small numbers of adults have been sufficient to establish a parasite or predator on its host.

DeBach & Bartlett (1964) reported that ten mated females of Rodolia cardinalis (Muls.) were sufficient to obtain colonisation on Icerya purchasi Mask. in California.

An escape of an estimated ten individuals (on one occasion) of *Opius ilicis* Nixon was sufficient to establish it onto *Phytomyza ilicis* Curt on Vancouver Island (Downes & Andison, 1940; Turnbull & Chant, 1961).

1.9.1.4 Conclusion.

Despite these successes there has been no real attempt to determine the minimum number of adults needed in a release. It has been claimed (DeBach and Bartlett, 1964) that such a value could not be determined. If they mean that one value for all different parasites in all localities is not possible, then I must concur. However, I would suggest that it is possible to determine such a value for a given parasite at least in a given area and that if we accept the defined objectives of biological control (Turnbull & Chant, 1961, see 1.1.2) one of which is to minimise time and cost of projects, workers in biological control are obligated to examine this question.

There is no experimental evidence upon which to examine this claim of DeBach and Bartlett (1964). Determination of the smallest number of adults needed to effect colonisation in future biological control projects would allow an examination of this statement and at the same time provide a valuable piece of information for the *ad hoc* project being investigated.

The savings to be gained by determining the number of parasites needed in a release can be evaluated in the comment by Taylor (1937) that the size of each colony and the number of colonies (he released a total of 126,000 adults of _Pleurotropis parvullus_ Ferr.) was excessive and that 2,000 adults would have been sufficient.

1.9.2 _Number of Hosts present._

1.9.2.1 Because there was no increase in the likelihood of a successful establishment of _A. melinus_ as the density of hosts increased (1.9.1.2) it seems likely that the searching capacity (Smith, 1939) of _A. melinus_ was adequate to find hosts at the lowest density examined.

Once the treatment trees had been selected, all others in each orchard were sprayed with a 2½% emulsion of oil. At the first assessment for colonisation in Orchards one and two (the oil spray was applied one and two weeks respectively before adults of _A. melinus_ were released) signs of _A. melinus_ were found on trees which had a "low" density of scale and which had been sprayed. Colonisation by _A. melinus_ can, therefore, be expected to succeed in much smaller populations of red scale than those examined. The observation (Appendix 4), that only 11.6% (S.D. = 5.9) of the red scales on leaves were suitable for parasitisation at any one time, highlights the excellent searching ability of _A. melinus_.

1.9.2.2 High host density.

Probably because many of the biological control projects of the past have been conducted to control serious outbreaks of pests, colonisation has usually been attempted into large populations of the host (Tooke, 1955; DeBach and White, 1960; Muggeridge, 1942; Williams et al, 1951; and many others). Sweetman (1958) recommends that the host population be as dense as possible. I suggest that the results of Experiment One and the work of Bedford (1968) where parasites, principally *Aphytis africanus* Quednau, migrated into orchards previously sprayed with Parathion[R] cast doubt upon the validity of this recommendation.

The value of small populations of the host when biological control is being implemented will be discussed later (2.4).

1.9.3 Time of Release.

1.9.3.1 It has been shown (1.9.1.3) that there is a decrease in the number of successes to be expected as summer progresses to winter. From the equation of the regression line ($\bar{y}_x = 8.23 - 0.06x$) it can be calculated that at least 67% of attempts will be successful if *A. melinus* is released before 20th March and that at least 50% of attempts will be successful if released before the 14th April. The date for any level of probability can be calculated.

R = Registered Trade Mark.

1.9.3.2 Such an examination, however, hides a lot of information. The data in Table 3 shows that with releases of ten adults, complete success (i.e. three successes out of three attempts) was not obtained; with releases of 100 adults, complete success was achieved up to and including the 14th April and with 1,000 adults to the 1st May. I have already suggested (1. 9.1.1) that there might be an upper limit to the number of successes and that this limit will not be surpassed by increasing the number of adults released. The above results suggest that this limit (in this case complete success) has been reached when 100 adults are released during the period February 12 to April 14. However, during cooler weather the number of adults required increases to 1,000. Even more are required after May 1.

1.9.3.3 The time of the year when *A. melinus* can be released.

One of the requirements of the *ad hoc* project was information on when *A. melinus* could be released with a reasonable chance of success.

The above results suggest that a high probability of success in colonisation exists if 100 adults are released into each tree before the middle of April (April 14, 1970) and that if releases are to be continued after this, 1,000 adults of *A. melinus* are needed. Any release of adults after the beginning of winter would appear to have little chance of success.

It was assumed early in the project that any release Before January would have little chance of success. This was based on the observation that there is no reproduction of red scale during winter and, as a result, very few red scale were in a suitable stage for parasitisation during spring and early summer. The first week of January, by which time red scales of the first summer generation are suitable for parasitisation, was accepted as the time to begin releases. The observation that A. melinus could find suitable hosts even when they were widely separated (1. 9.2.1) suggests that there is a good chance for success in early summer. I will argue (1. 9.3.4) that weather conditions are responsible for failures during winter. On this basis, I suggest that a high probability of success exists during October and November if 1,000 adults of A. melinus are released and after the end of November (to mid-April) if 100 adults are released.

1.9.3.4 Failures.

Flanders (1947) suggested seven attributes of the hymenopterous female, all of which influence searching capacity and hence ability to find and parasitise a host. Of these, two can vary in time and place with the same female; the power of locomotion and the capacity for survival.

I suggest that both of these attributes of A. melinus were reduced during cooler weather and that this was responsible for the decrease in the observed number of successes.

Field observations show that, at temperatures below 18°C (after mid April - Table 8), adults of <u>A. melinus</u> are normally found quite still on the underside of leaves. Adults have been observed to remain perfectly still for at least half an hour, even though the leaf was moved. Any movement is confined to short distances by walking rather than flying. Despite many hours of observation, I have not seen <u>A. melinus</u> parasitising a red scale at these temperatures. These observations are in direct contrast to rapid and almost continuous motion and frequent oviposition at temperatures in excess of 27°C. It appears that the power of locomotion is so seriously reduced during cool weather, thatany one female may never find a host. Flanders (1944c) suggested that, if a parasite spent too much time in useless searching, ovarian degeneration might occur and with it, the loss of urge to oviposit. If this response occurs when no host is found because the parasite does not move, the effects of cold temperatures as suggested above would be compounded.

Adults (and immatures) of <u>Aphytis</u> species are killed by cold weather (DeBach, 1965; DeBach & White, 1960). The longevity of adults can therefore be expected to be shorter in winter than summer.

Very small numbers could also have little chance of establishing themselves. Ten adults could fail if all were either males, or virgin females. Only adults at least 24 hours old were used in this experiment, and since most females mate within four hours of emergence (DeBach & White, 1960), it is probable that most, if not all, would have mated before being collected from the rearing cage (collections were made only from cages containing large numbers of adults).

When A. melinus is reared from Oleander scale, about six females are produced for each four males (DeBach & Sundby, 1963). The probability that ten males would be collected in any one tube is extremely low ($p = 0.0001$) and can be ignored as a possible cause of failures.

I suggest that inclement weather was responsible for the observed reduction in the number of successes as the time of the year changed.

1.9.4 Migration.

1.9.4.1 Time of year.

Migration from the treated trees ceased much earlier in the year than colonisation failed. It was probably a response to decreasing temperatures as no migration was observed after mid-April, the time when the adults of A. melinus were almost still (1. 9.3.4).

1.9.4.2 These observations showed that A. melinus, if released on each third tree in each third row before March, would be present in every tree by mid-winter. Where later releases are made, migration to all trees should occur in early summer.

1.9.5 Number of release sites.

Turnbull & Chant (1961) suggested that "as a rule of thumb, however, it is probably better to release larger numbers of individuals at fewer points".

Since I have been able to show that small numbers can be successfully colonised and that the distance migrated by *A. melinus* is but a few trees a year (personal communication; Dr. J. Cullen, C.S.I.R.O., Canberra, Australia), I suggest that the converse is true in this case. Many small colonisations should spread *A. melinus* over a larger area than a few larger releases.

I suggest that Turnbull & Chant's rule of thumb applies where the parasite is capable of migrating long distances in relation to the area of distribution of its host, but that the converse applies if the parasite can migrate only small distances in relation to the distribution of its host.

1.9.6 The answer to the ad hoc problem.

The greatest efficiency can be obtained if adults of *A. melinus* are released on each third tree in each third row, with 1,000 adults being released in October, November and May, and 100 adults from December to April inclusive. Releases should not be made outside this period.

2. EXPERIMENT 2.

2.1 INTRODUCTION AND AIM.

The aim of this experiment was to assess, in detail, the influence of *A. melinus* on a population of red scale in the field. Adults were released in an orchard at Waikerie where the size of the population of red scale was increasing, and had reached a serious level. Adults from the insectary were colonised into some trees and ~~then~~ the progeny were moved around the orchard as pupae in red scales on oranges (Experiment 5) until *A. melinus* was present in most trees.

In April 1969 five trees, of typical health and infested with moderate to very large populations of red scale, were selected. The size of the populations, of both red scales and immature stages of *A. melinus*, were assessed on each tree at monthly intervals (two monthly when the population of red scales was low).

2.2 METHODS USED TO MEASURE THE SIZE OF THE POPULATIONS.

Two methods were used to estimate the total number of red scales on a standard sample of 150 leaves. The second replaced the first after December 1969.

In the first method, 150 leaves, selected at random, were tagged on each tree. The number of the second and third instars of the female and the number of male scales were counted on each leaf at each sample. As new growth flushes occurred, the tagged leaves became more difficult to find and began to fall from the tree, making accurate counting nearly impossible. A rating system (Appendix 6) replaced the above with assessments made on 520 leaves selected haphazardly each time. The estimated counts (number of leaves x mean number of red scales in each rating) of red scales on 520 leaves were reduced to give an estimate appropriate to 150 leaves.

At each assessment a sample of thirty leaves heavily infested with red scale, was collected from each tree. Every red scale on them was examined and classified according to its stage of development, sex and condition (dead, alive or parasitised by A. melinus).

From these two estimates (number and condition of scales) an estimate of the number of living and parasitised red scales on 150 leaves was obtained.

2.3 RESULTS.

2.3.1 Observed changes.

There were large differences between each of the five trees, partly because the size of their initial populations of red scales were different, and partly because the red scale on them was colonised at different times. The estimated numbers of living and parasitised red scale (each on 150 leaves) is shown for each sample date and each replicate in Tables 10 and 11.

An analysis of variance was carried out on the data in Table 10 (except for assessment dated 10.11.70 which was not available at the time of analysis). A resumé of that analysis is presented in Table 12.

TABLE 10(a).

The number of living red scales (estimate) on 150 leaves at each assessment.

ASSESSMENT Date	No.	REPLICATE 1	2	3	4	5	Mean
16.4.69	1	574	264	1253	555	284	586
28.5.69	2	316	276	852	309	276	406
9.7.69	3	121	199	1228	159	190	359
14.8.69	4	138	150	890	33	149	272
1.9.69	5	177	85	666	107	190	245
8.10.69	6	106	60	561	100	172	200
11.11.69	7	46	17	340	22	19	89
17.12.69	8	22	30	446	16	126	128
8. 4.70	9	13	66	6	9	39	27
15. 5.70	10	13	9	11	8	18	12
14. 7.70	11	6	4	9	6	10	7
12. 8.70	12	9	9	25	12	21	15
17. 9.70	13	17	10	15	7	7	12
10.11.70	14	4	1	4	6	3	4
MEAN		119.8	90.7	484.8	103.3	115.8	

TABLE 10 (b)

Number of living red scales on 150 leaves on a tree in which *A. melinus* was not present.

DATE	NUMBER OF RED SCALES
18.2.70	555
8.4.70	980
15.5.70	1126
14.7.70	1027
12.8.70	1351
17.9.70	1493

A. melinus was first recorded in these red scales on 14.7.70 and then only in small numbers.

TABLE 11.

The number of parasitised red scales estimated to be on 150 leaves at each assessment.

ASSESSMENT Date	No.	REPLICATE 1	2	3	4	5	Mean
16. 4.69	1	103	4	0	63	0	34
28. 5.69	2	87	47	60	36	15	49
9. 7.69	3	22	35	11	38	0	21
14. 8.69	4	23	8	15	3	8	11
1. 9.69	5	31	22	2	23	1	16
8.10.69	6	16	8	10	11	6	10
11.11.69	7	7	2	8	5	3	5
17.12.69	8	5	3	12	4	2	5
8. 4.70	9	3	15	2	3	0	5
15. 5.70	10	4	4	2	2	4	3
14. 7.70	11	3	4	3	1	10	4
12. 8.70	12	2	4	6	0	6	4
17. 9.70	13	1	2	3	1	3	2
10.11.70	14	0	0	1	2	3	1
MEAN		21.9	11.3	9.6	13.7	4.3	

TABLE 12.

Resumé of the analysis of variance of the data in Table 10(a)

(a)

Variation due to	D.F.	S.S	M.S.	V.R.
Replicates	4	1488560	372140	12.6 xxx
Assessment times	12	2085595	173799	5.9 xxx
Residual	48	1418958	29561	
TOTAL	64	4993113		

(b) Duncan's New Multiple Range test was used to rank the means for each Assessment time because their order of sequence could be predicted before analysis (Li, 1964).

S.E. (of means of assessment times) = 76.9

The significant studentised range (SSR) for the number of assessments (g) in sequence between any two means being tested is as follows :-

g.	SSR	g.	SSR
2	219.9	8	253.7
3	231.4	9	256.0
4	238.4	10	257.6
5	243.7	11	259.1
6	247.6	12	260.7
7	251.4	13	262.2

The results of the test in terms of values significantly different from each other was as follows (each vertical line indicates those values which are not different from each other) :-

ASSESSMENT NO.	No. OF DAYS AFTER 1.4.69	MEAN	SIGNIFICANCE
1	17	586	
2	57	406	
3	99	359	
4	135	272	
5	154	245	
6	190	200	
7	224	89	
8	260	128	
9	372	27	
10	409	12	
11	469	7	
12	498	15	
13	534	12	

(c) Least significant Differences (L.S.D.) were calculated for the means of each replicate.

L.S.D. (p = 0.05) = 244.7

REPLICATE	MEAN	SIGNIFICANCE
1	119.8	A
2	90.7	A
3	484.8	B
4	103.3	A
5	115.8	A

Values followed by the same letter are not significantly different from each other.

The difference between the mean of replicate three and the others was expected because of the larger size of the initial population of red scales on that tree. It is of no importance to the results and conclusions. From the mean and the S.S.R. values it can be seen that the means of assessments one and two are greatest, assessments four and five are intermediate and assessments seven to twelve inclusive are significantly lower than the others. There were significantly fewer living red scales on the leaves after A. melinus had been present for more than four months.

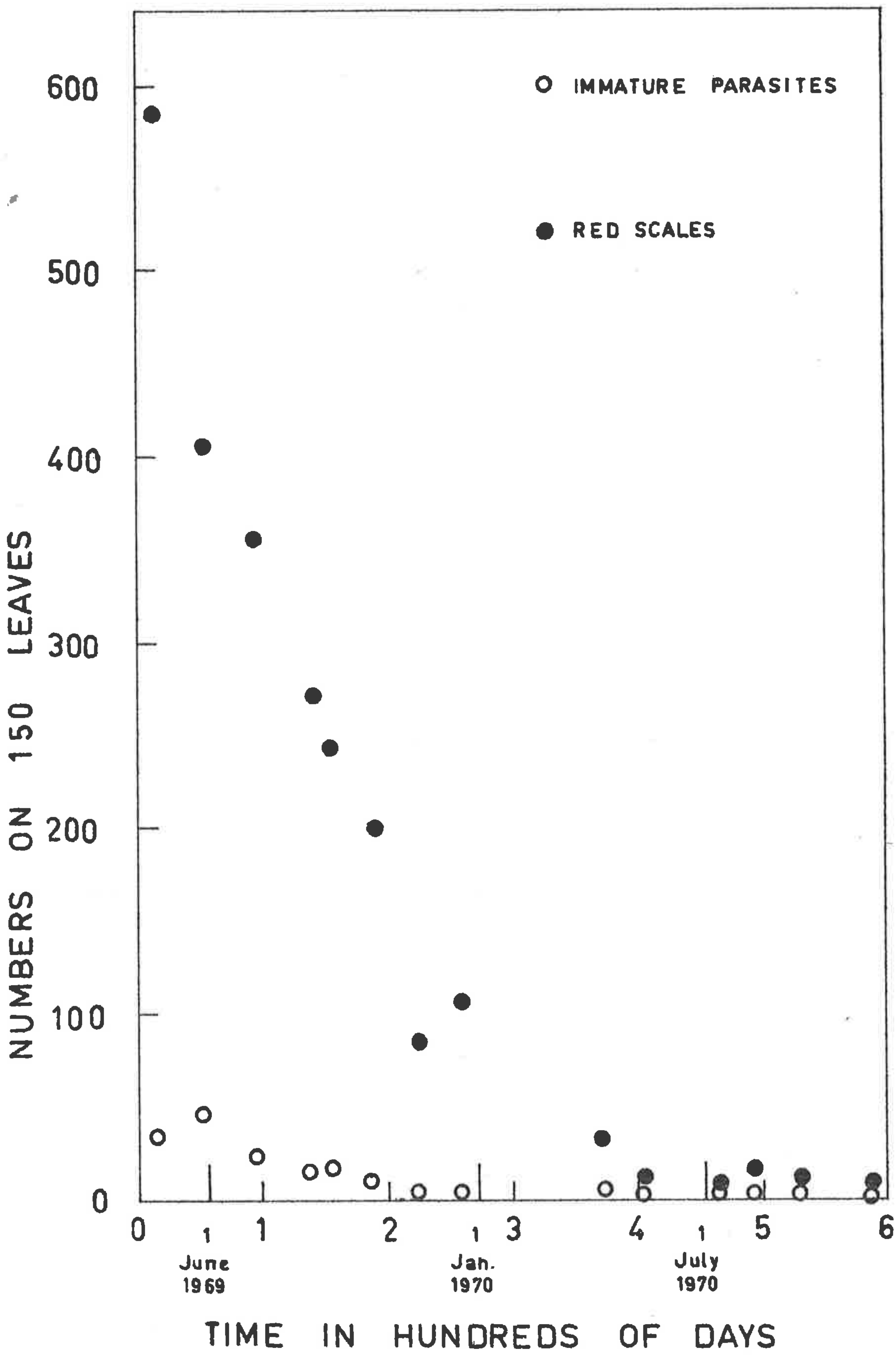
2.3.2 Changes in the Population of red scales.

The number of living red scales decreased very rapidly (Figure 7) soon after A. melinus was established, but the rate of decrease became less as the number of red scales decreased.

Very large fluctuations in numbers were observed particularly until August 1969 in replicate 3. These are a result, I believe, of the small size of the sample collected in relation to the very large number of red scales on the tree, and hence can be classified as sampling error.

FIGURE 7.

Mean number of living red scales, and mean number of immature stages of *A. melinus*, on 150 leaves at each assessment. Time recorded as the number of days after April 1, 1969.



O IMMATURE PARASITES
● RED SCALES
NUMBERS ON 150 LEAVES
600
500
400
300
200
100
0
0
1
1
2
1
3
4
1
5
6
June
1969
Jan.
1970
July
1970
TIME IN HUNDREDS OF DAYS

On trees 1, 2 and 4, a mean value of eight living red scales on 150 leaves was recorded after April 1970. A mean of seven living red scales was recorded on tree 3 after May 1970 while a value of five living red scales was recorded in September 1970 on tree 5.

2.3.3 Changes in the population of *A. melinus*.

With five exceptions, no more than forty immature stages of *A. melinus* were recorded (Table 11). At no time, until good control was achieved, did the numbers of *A. melinus* approach the numbers of red scale and yet the spectacular decrease in the number of red scale occurred. One contributing factor to this apparently small number of *A. melinus* could be that this parasite was migrating throughout the orchard at the same time that the observations were being made. Migration of adults away from trees to ones in which *A. melinus* was not present could account for the apparently low numbers observed.

2.3.4 Percentage clean fruit.

In September 1970, when oranges are often harvested, a sample, selected haphazardly, of 125 oranges from each tree was examined for the presence of red scale.

TABLE 13.

The number of fruit completely free from infestation by red scale as a percentage of the total number examined on 17th September, 1970.

TREE	COMPLETELY CLEAN %
1	63
2	64
3	34
4	60
5	49

On the assumption of no difference between the values recorded

$$\chi^2_4 = 11.9 \ ^*$$

It can be assumed that there is a real difference between the values observed, specifically that the yield of clean fruit is significantly less on tree three.

2.4 DISCUSSION.

The values recorded in Table 10(b) are from a tree in the same orchard as those in Table 10(a) and are an indication of the changes in numbers of red scales which would have been expected if *A. melinus* was not present. Nearly a three-fold increase was recorded between the 18th February and the 17th September on this tree at the same time that the mean number on the sample trees was decreasing. This suggests that it was A. melinus which was responsible for the low level of red scale achieved, not weather or other coincidental conditions. If it is true that adults of A. melinus were migrating away from the sample trees (2.3.3), then in a situation where adults were evenly distributed, (i.e. nett migration to and from any one tree is zero), A. melinus should be even more efficient than was recorded in Experiment 2.

In each replicate (except 5) a mean value of less than ten living red scales on 150 leaves was recorded after April-May 1970, some 14 months after A. melinus was released and found in the trees. (Tree 5 is atypical in this way, and also in that it carried a much larger population of the long-tailed mealy bug, Pseudococcus adonidum L.). This control in the same time is independent of the very large differences in the numbers of red scales present on each tree when A. melinus was colonised. This lack of response was predicted as a possibility by W.R. Thompson (1951). Using an appropriate set of values in the equation

$$t = \log n/p \Big/ \log s$$

n = host population

p = initial parasite population

s = reproductive rate of parasite

t = number of generations to control

he showed that at fixed values of s and p, a tenfold increase in the number of hosts would require only one more generation of the parasite to achieve the same degree of control. The largest difference in the number of hosts in this experiment was five times, and as no effect of this was observed on the time required to achieve low numbers of host this is an indication that Thompson's assumptions may approximate the real situation.

While low numbers may be achieved in the same time, there is a commercial disadvantage in starting with large numbers of red scales. The greater the number present, the fewer will be the oranges completely free from red scale (greater commercial value). Therefore, there will be a commercial loss in the year in which A. melinus is released and, if the initial numbers were very great, in the year in which low numbers are achieved (Table 13 ~~nb.~~ compare tree 3 with trees 1,2 & 4 +5) if the initial population of red scale was greater than necessary.

In past projects in biological control, the parasite or predator has been released to control large numbers of hosts (see 1. 9.2.2). It has not been the practice to reduce the size of populations of the host before introduction. Bedford (1968) found that five species of pests were under biological control within a year of cessation of spraying with Parathion®, indicating that it is possible to establish a parasite shortly after the use of an insecticide. Experiment 3 was designed to examine this possibility.

3. EXPERIMENT 3.

The time lapse needed after a spray of an insecticide and before adults of *A. melinus* can be released.

3.1 AIM.

The aim of this series of experiments was to assess the length of time, after the application of an insecticide capable of killing red scale, before adults of *A. melinus* would survive exposure to its residue, and so to assess the feasibility of reducing the size of the population of red scales before *A. melinus* is released. The most commonly used insecticide, Maldison, was examined in more detail than the less common ones, Omethoate and oil. The effects of the nutrient spray used in citrus orchards was examined.

3.2 METHOD.

Ten limbs, each with at least 30 leaves, were sprayed with the insecticide at the nominated strength and ten with water. All limbs were selected on a ten year old citrus tree (a different tree for each experiment) in an orchard. The leaves were sprayed and left *in situ*. One leaf was collected from each of the treated and control limbs for each assessment, with three assessments made during each week. These were continued until the residue of the insecticide killed less than 25 to 30% of the adults of *A. melinus* confined on it for four hours.

From each leaf a strip 2.2 cm wide x 7.8 cm long was cut and inserted into a 7.8 cm long x 1 cm (internal) diameter, glass tube. There were ten replicates of each treatment at each assessment. Ten adult A. melinus were collected from the breeding cages and enclosed in each tube, with a piece of gauze cloth at each end.

The number of adult A. melinus which died in each tube was recorded four hours later. The tubes were not as sophisticated as those used by other workers (Bartlett, 1951 ; Rosen, 1967), but the very small mortality of adults in the controls indicates that they were adequate for this experiment.

For ease of reference, each residue tested is referred to as a different experiment :-

EXPERIMENT	INSECTICIDE	DATE APPLIED
3a	0.15% Maldison	18. 8.70
3b	0.15% Maldison	4.10.70
3c	0.15% Maldison	4. 1.71
3d	2½% Spraying Oil	4. 1.71
3e	0.075% Omethoate	3.12.70
3f	All combinations of 1, 2, or 3 of the following :- 0.2% Zinc oxide; 0.4% Manganese sulphate, 0.7% Urea.	

The mean number (with its standard deviation) of dead adults above the treated leaves after four hours was calculated for each assessment. The percentage of the adults killed by the residue was calculated from the following formula which I developed, but which matches Abbot's correction for natural mortality (Finney, 1952).

$$K = \frac{d_t - d_c}{n - d_c}$$

where K = % kill; d_t = number of dead adults over treated leaves; d_c = number of dead adults over control leaves; n = number of adults included in one tube.

From the estimates of % kill, probit analysis was used to calculate the number of days weathering before the residue of the insecticide killed 50% or less of the adults of A. melinus.

3.3 RESULTS.

The actual values recorded are in Appendix 5.

3.3.1 EXPERIMENT 3a.

Leaves sprayed with 0.15% Maldison, 18.8.70.

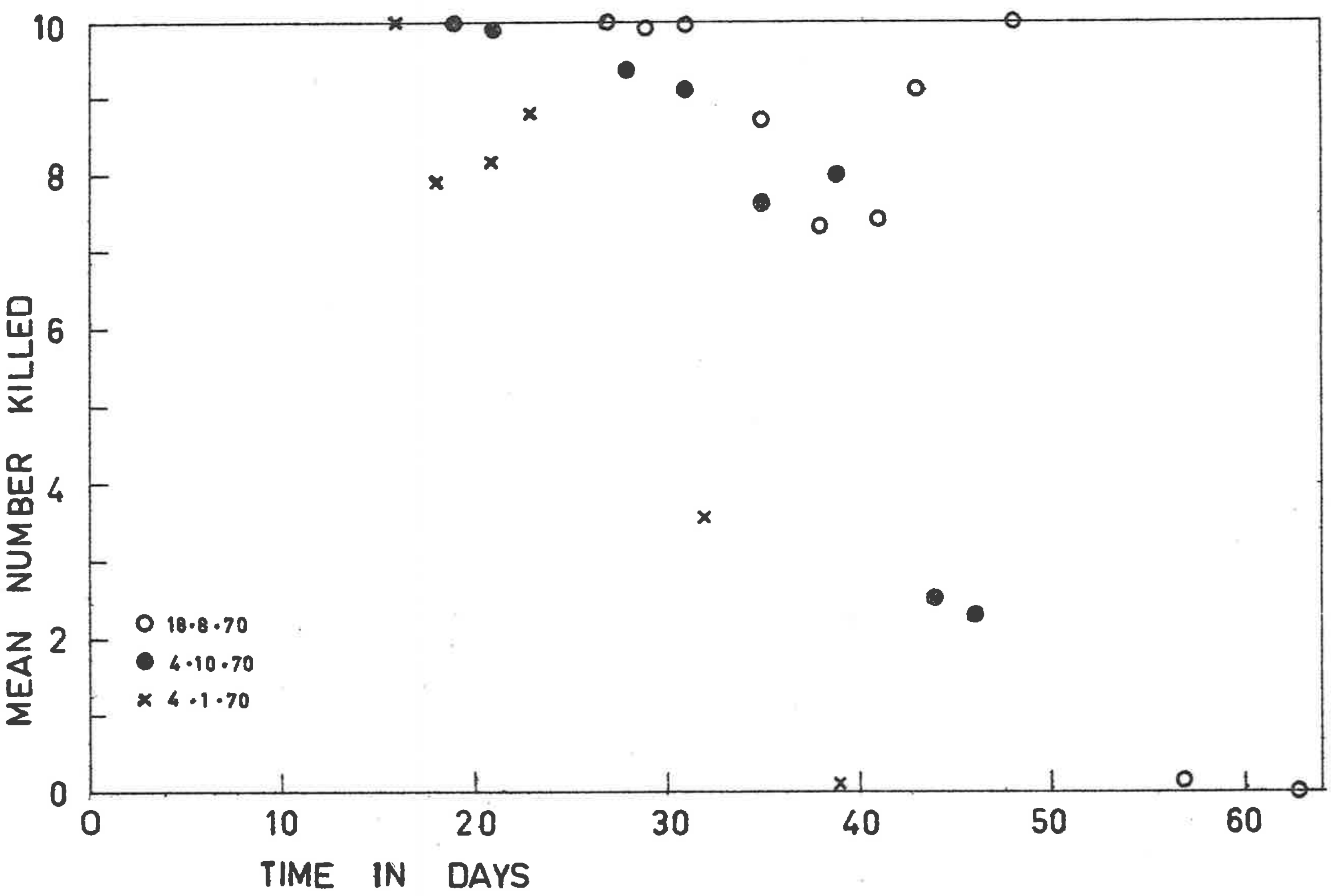
TABLE 14.

The number of days of weathering of the residue of 0.15% Maldison, the mean number (and standard deviation) of dead adults of A. melinus after enclosure over the residue for four hours, the mean number dead in the control and the estimated kill for each assessment. Ten adults were enclosed in each of ten replicates for each assessment.

ASSESSMENT NUMBER	NUMBER OF DAYS OF WEATHERING	MEAN NUMBER DEAD	S.D OF MEAN DEAD	MEAN NUMBER DEAD IN CONTROL	KILL %
1	1	10.0	0.0	0.1	100
2	3	10.0	0.0	0.1	100
3	7	10.0	0.0	1.7	100
4	8	10.0	0.0	0.0	100
5	10	10.0	0.0	0.1	100
6	13	10.0	0.0	0.0	100
7	15	10.0	0.0	0.0	100
8	19	10.0	0.0	0.1	100
9	22	10.0	0.0	0.1	100
10	24	10.0	0.0	0.0	100
11	27	10.0	0.0	0.0	100
12	29	9.9	0.3	0.0	99
13	31	10.0	0.0	0.0	100
14	35	8.7	2.2	0.0	87
15	38	7.3	3.3	0.0	73
16	41	7.4	4.1	0.0	74
17	43	9.2	1.1	0.0	92

FIGURE 8.

Mean number of adults of A. melinus killed by the residue of 0.15% Maldison applied at different times. The decrease in the kill with time of weathering (days) is shown.



MEAN NUMBER KILLED
TIME IN DAYS
0
2
4
6
8
10
0
10
20
30
40
50
60
18·8·70
4·10·70
4·1·70

ASSESSMENT NUMBER	NUMBER OF DAYS OF WEATHERING	MEAN NUMBER DEAD	S.D OF MEAN DEAD	MEAN NUMBER DEAD IN CONTROL	KILL (%)
18	48	10.0	0.0	0.0	100
19	57	0.1	0.3	0.0	1
20	63	0.0	0.0	0.0	0

Estimated time for weathering to 50% kill of adults ($W.T._{50}$) after four hours confinement :-

$W.T._{50}$ = 48.3 days : ∴ 48.3 $\pm$ 4.4 days

$S.D._{W.T_{50}}$ = 2.2 days

$W.T._{50}$ was calculated (Appendix 5) by probit analysis (Mather, 1943) without using the correction for special accuracy. The percentage kill values were transformed to probits and correlated against the number of days of weathering.

The result shown in Table 14 and Figure 8 was unexpected in that an insecticide, which has a withholding period of seven days (Anon), killed adults of *A. melinus* for 48 days ($W.T._{50}$) after application. The frequency of assessments was reduced after the 43rd day because the large number of assessments used more leaves than was expected.

3.3.2 EXPERIMENT 3 (b).

Leaves sprayed with 0.15% Maldison, 4.10.70.

TABLE 15.

The number of days of weathering of the residue of 0.15% Maldison, the mean number (and standard deviation) of dead adults of *A. melinus* after enclosure over the residue for four hours, the mean number dead in the control and the estimated kill for each assessment. Ten adults were enclosed in each of ten replicates for each assessment.

ASSESSMENT NUMBER	NUMBER OF DAYS OF WEATHERING	MEAN NUMBER DEAD	S.D OF MEAN NO. DEAD	MEAN NO DEAD IN CONTROL	KILL (%)
1	1	10.0	0.0	0.0	100
2	3	10.0	0.0	0.0	100
3	5	10.0	0.0	0.0	100
4	8	10.0	0.0	0.0	100
5	12	10.0	0.0	0.0	100
6	16	10.0	0.0	0.0	100
7	19	10.0	0.0	0.0	100
8	21	9.8	0.6	0.1	98
9	28	9.3	1.1	0.0	93
10	31	9.1	1.5	0.3	91
11	35	7.6	4.2	0.1	76
12	39	8.1	2.2	0.5	80
13	44	2.8	3.4	0.4	25
14	46	2.5	2.0	0.3	23

WT_{50} = 40.7 days $\therefore$ 40.7 $\pm$ 4.8 days

$S.D._{WT_{50}}$ = 2.4 days

This treatment was applied at the time recommended by the South Australian Department of Agriculture for the first insecticide spray of the season. The time, 41 days, required to achieve W.T.$_{50}$ is lower, but not more than 2 x S.D. lower than the range of values determined for the application in August.

It is still far in excess of the withholding period of seven days.

3.3.3 EXPERIMENT 3(c).

Leaves sprayed with 0.15% Maldison, 4.1.71.

TABLE 16.

The number of days of weathering of the residue of ~~0.015%~~ 0·15% Maldison, the mean number (and standard deviation) of dead adults of *A. melinus* after enclosure over the residue for four hours, the mean number dead in the control and the estimated kill for each assessment. Ten adults were enclosed in each of ten replicates for each assessment.

ASSESSMENT NUMBER	NUMBER OF DAYS OF WEATHERING	MEAN NUMBER DEAD	S.D OF MEAN NO. DEAD	MEAN NO. DEAD IN CONTROL	KILL (%)
1	2	10.0	0.0	0.0	100
2	4	10.0	0.0	0.0	100
3	9	10.0	0.0	0.4	100
4	11	10.0	0.0	0.5	100
5	16	10.0	0.0	0.8	100
6	18	7.9	2.1	0.1	79
7	21	8.2	1.9	0.0	82
8	23	8.8	1.3	0.0	88
9	32	3.9	2.5	0.6	36
10	39	0.2	0.4	0.0	2

$W.T_{50}$ = 27.2 days ∴ 27.2 ± 0.4 days

$S.D._{W.T._{50}}$ = 0.2 days

The $W.T._{50}$ was achieved in 27 days which is significantly less than was recorded for a spray in October, but still more than the withholding period.

3.3.4 EXPERIMENT 3(d).

Leaves sprayed with 2½% Spraying oil; 4.1.71.

TABLE 17.

The number of days of weathering of the oil residue, the mean number (and standard deviation) of dead adults of *A. melinus* after enclosure over the residue for four hours, the mean number dead in the control and the estimated percent kill for each assessment. Ten adults were enclosed in each of ten replicates for each assessment.

ASSESSMENT NUMBER	NUMBER OF DAYS OF WEATHERING	MEAN NUMBER DEAD	S.D OF MEAN NO. DEAD	MEAN NO. DEAD IN CONTROL	KILL (%)
1	1	10.0	0.0	0.2	100
2	3	5.1	2.0	0.7	47
3	8	0.9	1.1	0.7	2

A layer of oil was visible on the surfaces of the leaves one day after spraying but all had disappeared by the eighth day. This suggests that, if the adults of *A. melinus* were released after the visible traces of oil on the surfaces of the leaves had been lost, they would survive.

Field observations show that, depending on the air temperature on the days immediately after the oil is applied, a residue can be observed for anything from three days (32°C - 35°C) to three weeks or more (16°C - 21°C). It is reasonable to assume that the $W.T._{50}$ value will fluctuate with the time needed for this oil layer to be lost, i.e. $W.T._{50}$ will be shorter in hotter weather.

Oil sprays are applied when the ambient temperature ranges between 29°C and 35°C. The $W.T._{50}$ value for an oil spray can, therefore, be expected to range from 3 to 6 days.

3.3.5 EXPERIMENT 3(e).

Leaves sprayed with 0.075% Omethoate, 3.12.70.

TABLE 18.

The number of days of weathering of the residue, the mean number and standard deviation of dead adults of *A. melinus* after enclosure over the residue for four hours, the mean number dead in the control and the estimated percent kill for each assessment. Ten adults were enclosed in each of ten replicates for each assessment.

ASSESSMENT NUMBER	NUMBER OF DAYS OF WEATHERING	MEAN NUMBER DEAD	S.D OF MEAN NO. DEAD	MEAN NO. DEAD IN CONTROL	KILL (%)
1	1	10.0	0.0	0.1	100
2	5	9.7	0.7	0.4	97
3	8	10.0	0.0	0.0	100
4	14	8.0	1.5	0.3	79
5	20	4.0	3.1	0.6	36

$W.T_{50}$ = 18.9 days ∴ 18.9 ± 5.0 days

$S.D._{W.T_{50}}$ = 2.5 days

The period of time before $W.T_{50}$ is achieved was less than would have been predicted for a 0.15% Maldison spray applied about the same time.

3.4 DISCUSSION.

Experiments 3(a), 3(b) and 3(c) showed that the time required to achieve $W.T._{50}$ after leaves were sprayed with 0.15% Maldison decreased as the season progressed from winter to summer. This result is not unreasonable because the main processes involved in the reduction of the toxicity of the residue are first order chemical reactions (Gunther and Jeppson, 1960) which can be expected to progress faster at the higher temperatures of summer. The toxicity of the residue was not reduced by occasional washing. A total of 168 points of rain distributed over 15 days (maximum fall, 48 points) fell on the leaves used in Experiment 3(a), and an irrigation (300 points in 18 hours) from over-tree sprinklers fell on the leaves used in Experiment 3(b) without affecting the toxicity of either residue. This suggests that it would not be possible to apply a spray of 0.15% Maldison, wait for a few days for it to kill most of the red scales (12 hours is sufficient - Richardson, 1968), and then wash off any toxic residue.

A large and unexpected disparity was found between the withholding period, the time that must elapse after spraying and before harvesting, and the $W.T._{50}$ values for Maldison. The withholding period is seven days but the estimates of $W.T._{50}$ ranged from 50 days in winter, to 30 days in summer. This warns that the withholding period cannot be used as a rough measure of the time needed to weather an insecticide residue.

The W.T.$_{50}$ values measured do show a similarity to the half life of a residue of Maldison (17 and 32 days on different varieties of citrus (Gunther and Jeppson, 1960)). This comparison makes the estimated values of W.T.$_{50}$ appear less of an aberrant result.

A similar weathering time, one to three months, was shown to be necessary after an application of Parathion^{R} to citrus leaves before Aphytis chrysomphali, Mercet. could survive for four hours (Bartlett, 1953).

The film of oil on the surface of a leaf is broken and removed by mechanical means such as dust and wind (personal communication, Mr. A. Opey, Technical Manager for South Australia, Golden Fleece Pty. Ltd.), but temperature obviously plays some part. With this type of breakdown it is not unreasonable to expect that, soon after the layer of oil on the surface is lost, its lethal effect is lost. The result observed is similar to that determined for adults of Aphytis holoxanthus DeBach (Rosen, 1967). The results of these experiments show that it is possible to apply an insecticide such as Maldison or spraying oil to kill a large percentage of the red scales on a tree and later in the same season to release adults of A. melinus. The release of the adults would need to be delayed 30 (summer) to 50 (winter) days but safer periods would be six and ten weeks respectively.

The period when _A. melinus_ can most efficiently be colonised extends from December to mid-April (Experiment 1). A spray of 0.15% Maldison in October would allow the release of _A. melinus_ during most of this season. A spray of 2½% oil can be used on citrus trees without risk of damage during January and February, making a week (temperatures greater than 29°C) all that is needed before adults of _A. melinus_ can be released.

An insecticide used to kill its host can reduce the size of the population of a parasite in two ways. Firstly, by direct poisoning and, secondly, by reducing the size of the population of the host to such low levels that many parasites die before they are able to find hosts. It has been shown that, if the suggested time lapses are observed, there should be negligible deaths by direct poisoning. An application of either 0.15% Maldison or 2½% oil kills between 85 and 95% of all the red scales on a tree, leaving few living, and even fewer scales suitable for parasitisation. An attempt to colonise _A. melinus_ could fail simply because these adults could not find red scales suitable as hosts. It has been shown (1.9.2.1) that colonisation succeeded on trees soon after they had been sprayed with 2½% oil, indicating that the population of living red scales was not reduced to a level below which colonisation would fail.

At 26.7°C, the generation time of *A. melinus* is twelve days (DeBach and White, 1960). In the field in January when temperatures are similar, a generation can be expected to take 14-20 days to complete, so that 1½ to 2 generations of *A. melinus* should be completed before the residue of the insecticide lost its toxicity. Any immature parasites which were not killed by Maldison would be likely to die when, as adults, they contacted the lethal residue. An application of 0.15% Maldison should severely reduce the size of the population of *A. melinus*.

An oil spray, by contrast, will kill those adults and immature stages contacted, but any adults emerging more than three days after spraying should survive.

A spray of oil is likely to cause a less severe reduction in the size of the population of *A. melinus*, than one of Maldison.

3.5 EXPERIMENT 3 (f). Nutrient Sprays.

The only other residues commonly found on citrus trees are those of the nutrient sprays. These are applied annually using a mixture of 0.2% Zinc Oxide, 0.4% Manganese sulphate and 0.7% Urea in one spray. The effects of the combination (and all combinations of one, two or three of the nutrients) on adults of *A. melinus* was assessed using the same technique.

The number of dead parasites was counted after four and twenty hours' confinement. An analysis of variance was carried out on the 20 hour counts ~~counts at 20 hours~~ (Table 19 and 20).

Seven replicates of each treatment were used and leaves were collected for assessment as soon as the spray had dried.

TABLE 19.

Mean number of dead adults confined for (a) four hours and (b) twenty hours ~~after four and twenty hours' confinement~~ over residues of nutrients.

TREATMENT (U = Urea; Zn = Zinc oxide; Mn = Manganese sulphate).	Mean No. Dead Adults - 4 hours		Mean No. dead Adults - 20 hours	
	MEAN	S.D.	MEAN	S.D.
0.2% Zn	1.1	0.9	4.3	2.1
0.4% Mn	0.3	0.5	3.6	0.8
0.7% U	1.1	1.1	4.3	0.8
0.2% Zn + 0.4% Mn	0.3	0.5	3.6	1.0
0.2% Zn + 0.7% U	0.4	0.3	1.9	0.9
0.4% Mn + 0.7% U	0.0	0.0	3.0	1.5
0.2% Zn + 0.4% Mn + 0.7% U	0.9	1.1	2.6	2.0
Control	0.9	1.1	2.4	0.8

TABLE 20.

Resumé of Analysis of Variance on 20 hours' confinement data.

Variation due to	D.F.	S.S.	M.S.	V.R.	
REPLICATES	6	8.464	1.411	1.18	N.S.
TREATMENTS	7	38.268	5.467	4.59	**
RESIDUAL	42	50.107	1.193		
TOTAL	55	96.839			

L.S.D. 5% (treatment means) = 1.2

The residue of the 0.2% Zinc oxide spray and the 0.7% urea spray killed more adults than died in the control. No more adults died on leaves with a residue of the full combination than on the control leaves, therefore it can be assumed that such a residue will not reduce the number of adults of *A. melinus*.

No attempt was made to examine the effect of these sprays on long term changes in populations of red scale in the field. Griffiths (1951) suggested that zinc sprays prevent the growth of entomogenous fungi on Florida red scale (*Chrysomphalus ficus* Ashmead), and thereby, cause large increases in populations of this pest. Entomogenous fungi are present in the Riverland but are thought to be of little or no value in the control of red scale (personal communication, Mr. N.L. Richardson, Senior Research Officer, South Australian Department of Agriculture). I suggest that any such effect will be of no consequence within the Riverland.

4. EXPERIMENT 4.

4.1 INTRODUCTION.

Although not a common practice, parasites and predators have been collected from the field in one area for use against a host in another (Flanders, 1944a).

If large numbers of A. melinus could be collected from the field, this would increase the number of sites colonised in one season. Adults, however, are so small and delicate that any attempt to collect them in large numbers would be hazardous. Immature stages, in contrast, are sessile and can easily be collected in large numbers by picking oranges infested with red scales that are parasitised by A. melinus. Early experiences suggested that colonisation could be achieved by distributing such oranges in trees (Appendix 2).

Adults of A. melinus were colonised in two trees, both heavily infested with red scale, at the southern end of an orchard 17 trees wide and 28 trees long. A wire basket (30.5 cm x 20.4 cm x 25.4 cm), filled with oranges (about 50) infested with red scale, was placed underneath each tree. At approximately two weekly intervals, half of the oranges in these baskets were replaced. Those removed were distributed around the orchard, putting 15 into a small wire container, one of which was hung in each third tree in each third row.

This was started in February, and each candidate tree had received such a basket by July 1969.

4.2 AIM.

The aim of this experiment was to examine the relative merits of taking red scales from a breeding basket or directly from trees for the distribution technique listed above.

4.3 METHOD.

The oranges from five breeding baskets were taken to the insectary after all the adults of *A. melinus* present had been counted and removed. On each of the next 13 days, those adults which emerged were counted and removed. By the thirteenth day, any eggs laid while the oranges were in the field should have developed to adults.

One group of 57, and 20 groups of 15 oranges, were collected haphazardly, one group per tree, from trees in which *A. melinus* had been observed. These were treated in the same way as those recovered from the breeding baskets. In both cases only very heavily infested oranges were collected and an attempt was made to have approximately the same number of red scales on each orange.

4.4 RESULTS.

The mean number of adults of A. melinus emerging from the red scales on one orange from each source, is shown in Table 21.

TABLE 21.

Source of oranges used, number examined and mean number (and standard error) of adults which emerged from red scales.

SOURCE OF ORANGES	TOTAL NUMBER OF ORANGES	MEAN NUMBER OF ADULTS FROM SCALES ON ONE ORANGE	S.E. OF MEAN
Breeding Basket	239	25.6	8.0
Tree	357	9.4	2.1

The number of adults collected, each day, from the oranges from the first breeding basket is shown in Figure 9 to illustrate the daily variation in the number of adults emerging.

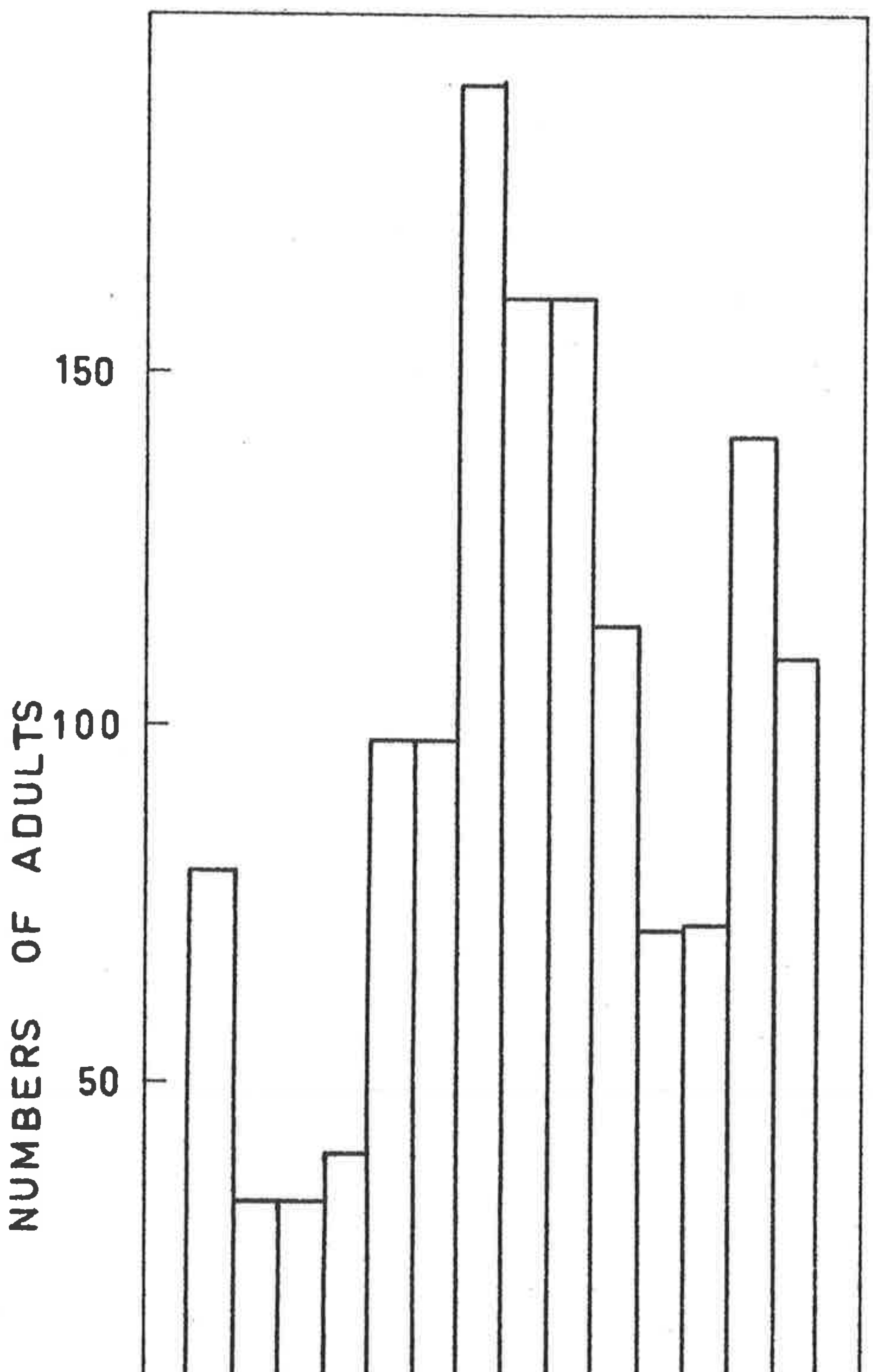
4.5 DISCUSSION.

The results showed that many more adults of A. melinus were produced from the scales on an orange out of a breeding basket than one on the tree, even though the number of scales on each orange was similar. This indicates that, as a source of A. melinus for spreading around an orchard, breeding baskets are of considerable value. The breeding basket, is, virtually,

FIGURE 9.

Histogram showing the number of adults of *A. melinus* produced from breeding basket No. 1 each day for 13 days after removal from the field.

Day 0; is the number of adults counted on the oranges before the basket was removed from the field.


150
100
50
NUMBERS OF ADULTS
0 1 2 3 4 5 6 7 8 9 10 11 12 13
DAYS

a miniature field insectary.

The greater number could be a result of one or both of the following effects.

1. Red scales are closer together when the oranges are in a basket rather than hanging on the tree. This should reduce the amount of time spent by the adult female in searching for hosts and, thereby, allow her to lay more eggs (DeBach & Smith, 1941).
2. Adults of A. melinus will be closer together, increasing the probability that any one female will contact a male and be mated. This should increase the ratio of female to male progeny in the next generation (mated females produce female and male off-spring, but virgin females produce only male off-spring, DeBach & White (1960)) and, therefore, the total number of adults in the second generation.

This field distribution system could be integrated into the overall programme for the distribution of A. melinus around an orchard by preparing one or more such baskets and moving them around an orchard, on a three by three tree grid for example, leaving them under any one tree for long enough for 100 adults to emerge. This would have been achieved if a breeding basket producing at the rate that the mean of the five produced (Table 18) was kept under a tree for a little over one day (94.1 adults each day). Changing every two days would have been adequate.

5. DISCUSSION.

5.1 THE PROGRESS OF BIOLOGICAL CONTROL OF RED SCALE IN THE RIVERLAND.

A rapid decrease in the number of living red scales has been observed (2.3.2) but, despite this decrease, 40 to 50% of the fruits examined during the second harvest after A. melinus was released (2.3.4), were still infested with red scale. This degree of control could improve in subsequent harvests as either or both of the following factors operate :-

(1) Experience has shown (DeBach, 1951) that a true idea of the effectiveness of a project cannot be obtained until two to four years after release. The above observations were made less than two years after A. melinus was released.

(2) The trees examined were in an orchard surrounded by areas treated with insecticide. Any drift of insecticide into the "biological control" orchard and any migration out of this area by A. melinus will reduce the number and, therefore, effectiveness of this parasite. As more contiguous orchards and, therefore larger areas, cease to use insecticides, the degree of control should improve in each.

Neither ants (DeBach *et al*, 1950) nor dust (Bartlett, 1951) interfered with A. melinus in this orchard.

Turnbull and Chant (1961) defined complete control as being achieved when economic damage is not significant, and described any measurable reduction of damage as partial control. Such partial control has been achieved over red scale by the use of A. melinus and is of value to the orchardist and to the overall programme to implement biological control. The introduction of any parasite or predator that reduces the size of the population of the pest is a step forward (Simmonds, 1956).

Insect pests were split into two ~~major~~ main categories (Turnbull & Chant, 1961) depending on the relation of the numbers of the pest to the damage they cause.

A direct pest is one where a small population, by directly attacking produce, immediately destroys a significant part of its value. Indirect pests attack produce and cause economically significant damage only by intensive or extended feeding.

In listing all attempts at biological control in Canada they found that seven out of nine attempts to control direct pests but only eleven out of twenty four attempts to control indirect pests, failed. They suggested that direct pests were not suitable subjects for biological control. This conclusion was supported by Oosthuizen (1964) when he claimed that only on land of low monetary value could biological control of weeds be contemplated. The failure to save the Bermudan Cedars (Thompson, 1954) even though the scale insects killing them were reduced to low numbers by parasites and predators is an excellent example of the failure to control a direct pest.

Red scale is presently treated as a direct pest in Riverland areas with this status being conferred by legislative action, i.e. oranges will not be exported if they are infested by red scale (c.f. Introduction). Its immediate effect, however, upon fruits or trees is that of an indirect pest, damage to either only occurring when great numbers are present. If fruit is to be sold to a factory (i.e. for juice), some infestation can be tolerated and again red scale is an indirect pest. The degree of control achieved at present by A. melinus is adequate only if red scale can be treated as an indirect pest. If, however, control improves to the degree achieved in South Africa (Bedford, 1968) where less than one percent of oranges are culled because of red scale, then control will be good enough even if red scale continues to be classified as a direct pest.

5.2 ADVANTAGES OF BIOLOGICAL CONTROL.

The immediate advantages, cheaper and more permanent control, and reduced chance of insecticide poisoning of spray operators, are obvious.

In some seasons and some areas the mealy-bug (Pseudococcus adonidum L.) achieves the status of a pest but a number of its parasites and predators are present in the Riverland (Browning, 1959). There are some indications that the numbers of mealy-bugs may increase where Maldison is used to control red scale. It is believed that the numbers in the Loxton area are slowly increasing from a situation five years ago when they were virtually unknown. (Personal communication, Mr. W.J. Basket, Farm Club Advisor, Loxton). At the same time, red scale has migrated into Loxton and Maldison has been used. The orchard with the worst problem in Loxton has been sprayed two and sometimes three times in each of the last five years with Maldison (personal communication, Mr. W.J. Basket).

This is a hint, not proof, that mealy-bugs may no longer be a problem if A. melinus is controlling red scale.

5.3 THE LENGTH OF TIME TO ACHIEVE CONTROL.

Adequate control, if red scale is classed as an indirect pest, was achieved in the vicinity of the release site by A. melinus within 14 months. In each replicate (2.4) control (defined in this case as less than ten living red scales on every 150 leaves) was achieved 14 months after colonisation. Both red scale (Botham, 1961) and A. melinus (1.9.3.4) are much less active during winter than summer. Rather than say that control will be achieved in any 14 months, which may include variable numbers of months of summer, I suggest that it will be achieved at the end of the summer after the season in which A. melinus was colonised.

Clausen (1951, 1958) by examining recorded projects in biological control concluded that if a parasite could control its host within three years (which may be only three generations) it might be a parasite which will give complete control. Not all parasites that do this necessarily give good control in the long term (Doutt and DeBach, 1964).

The achieving of control by A. melinus within three years in the Riverland is cause to hope, but no guarantee, that permanent control will ensue.

5.4 THE RATE OF REDUCTION IN THE NUMBER OF RED SCALES.

While the observed rate of reduction of red scale was spectacular (Figure 8) it is in fact no greater than that recorded in Pseudococcus adonidum L. against which two parasites were released (Flanders, 1944a) or in Saissetia oleae (Bern.) against which Metaphycus helvolus (Compere) was released (Argyriou and DeBach, 1968).

Control of Pieris rapae L. was achieved by Apanteles glomeratus L. within 12 months (Muggeridge, 1943). Three months was all that was required by Pleurotropis paruulus Ferr. to control Promecotheca reichei Baly. in Fiji (Taylor, 1937). None of these are as fast as the epizootic created by application of a polyhedrosis virus which reduced a field population of 100 larvae of Colias philodice eurytheme Boisduval to zero in seven days (Thompson, C.G., 1951). In the light of these examples the rate of reduction of the number of red scales seems rather slow.

5.5 LOSS OF PRODUCTION BEFORE CONTROL ACHIEVED.

Both Simmonds (1960) and Ullyett (1946) warned that there could be considerable economic losses to be faced during a transition from control of red scale by insecticides to parasites. Observations taken during such changes (Bedford, 1968), when the use of parathion was stopped and indigenous parasites and predators allowed to migrate into the area, showed that complete control was regained within three years and that while losses did occur in the first two years, they were not significant.

The situation in the Riverland is different to the extent that, when the use of Maldison is stopped, A. melinus can be introduced and spread over each orchard and this can be done no more than three months after the last spray. The results of Experiment 2 (2.4) and the experiences of Bedford (1968) suggest that, while there may be an increase in numbers of red scales after cessation of Maldison treatments, this should not cause serious losses.

Oil sprays (3.4) would have a minimal effect on the population of A. melinus and could, if needed, be integrated (Inserra, 1968) into the colonisation procedures to reduce the numbers of any outbreak of red scale.

5.6 THE RELATIVE MERITS OF ENDO- AND ECTO-PARASITES.

Brewer (1971), after examining the value of Comperiella bifasciata How. as a parasite of red scale, suggested that ecto-parasites may be of more value in controlling pests than endo-parasites because the former are not subject to immunity responses by the host.

The effectiveness of A. melinus as reported in this thesis has certainly justified his suggestion about the relative merits of the ectoparasite A. melinus and the endo-parasite C. bifasciata. No conclusion of a more general nature is possible.

5.7 THE RELATIVE MERITS OF ADULTS OR IMMATURE STAGES OF A PARASITE FOR COLONISATION.

It has been claimed that colonisation is usually made using adults since "immature stages are more difficult to colonise and may be more subject to destruction by unfavourable meteorological conditions and by predators" (DeBach & Bartlett, 1964). I suggest that this statement is too general. Booth Tooke (1955) and Dodd (1940) released eggs and Flanders (1959) released larvae or pupae because these were the most efficient. A number of factors need to be examined in each case :-

1. The comparative success in colonisation achieved using different stages of development.
2. The ease of handling different stages.
3. The costs involved.

I suggest that these factors will vary with each situation and that this thesis illustrates how two different stages of the same parasite could be needed in the same project. Only adults can be released from the insectary (1.1) because with them the costs of production and distribution are least, but immature stages should be used if material for release is to be collected from the field because this minimises the cost of collection (4.1).

6. APPENDICES.

APPENDIX 1.

An estimate of the costs involved in applying the two insecticide treatments currently recommended by the South Australian Department of Agriculture for the control of red scale.

APPENDIX 1.

October Spray with 0.15% Maldison

Maldison estimated (1970) to cost \$1.88 for a hundred gallons of spray strength solution. This is the minimum cost - some brands are more expensive.

January Spray with 2% Oil ($2\frac{1}{2}$% is recommended - 2% is commonly used).

Oil estimated to cost \$1.72 for 100 gallons of Spray strength solution.

At least 10 gallons of material is required to cover a 15 year old tree in good health if sprayed by an oscillating boom machine.

∴ Spray material alone would cost for each tree -

1st spray	18.8	cents
2nd spray	17.2	cents

An oscillating boom on contract can cost \$15 to \$18 an acre. Assuming \$15 and 100 trees an acre, this will cost 15 cents per tree.

The number of trees in an orchard ranges from 75 to 300 per acre but 100 is a common figure. This cost will be additional to spray material.

APPENDIX 1.

The cost for one tree will be as follows (cents).

	MATERIAL	APPLICATION	TOTAL
1st Spray	18.8	15.0	33.8
2nd Spray	17.2	15.0	32.2
TOTAL	36.0	30.0	66.0

These are the minimum costs and are frequently exceeded.

The estimated number of trees at and upstream from Waikerie which require spraying (90% of total) each year for red scale is estimated to be 1,467,358. At 66.0 cents per tree the total cost is $968,456.28.

APPENDIX 2.

Successes and failures in colonisation by release of adults or pupae of *A. melinus* from December 1968 to March 1969 in the field in the Riverland.

APPENDIX 2.

Orchard at Waikerie.

DATE OF RELEASE	ADULTS OR PUPAE	TOTAL NO. OF PARASITES RELEASED	NO. OF TREES	NO. OF PARASITES PER TREE	NO. OF SUCCESSES IN COLONISATION	NO. OF FAILURES IN COLONISATION
18.11.68	Adults	600	4	150	-	4
10.12.68	Pupae	2000	10	200	10	-
28. 1.69	Adults	80	1	80	-	1
10. 2.69	Adults	500	5	100	3	2
10. 2.69	Pupae	200	1	200	1	-
27. 2.69	Pupae	1000	10	100	10	-
5. 3.69	Adults	400	1	400	1	-
5. 3.69	Pupae	3000	15	200	15	-

Other orchards.

31.10.68	Adults	640	5	125	1	4
28. 2.69	Adults	1450	8	180	5	3
1.11.68	Adults	1200	6	200	6	-
7.11.68	Adults	80	2	40	2	-
7.11.68	Adults	140	2	70	-	2
7.11.68	Adults	360	4	90	4	-
14.11.68	Adults	100	2	50	-	2
14.11.68	Adults	140	2	70	-	2

APPENDIX 2.

Summary of successes and failures.

Release of adults.

Number released per tree	Number trees	Successes	Failures	% success
0 - 49	2	2	0	100
50 - 99	11	4	7	36
100 -199	22	9	13	41
200 -	7	7	0	100

Weighted average % success = 51.

Release of Pupae.

100- 200	36	36	0	100

APPENDIX 3.

The detail of results for Experiment 1 including (a) Dates of release into each block.

(b) Percentage of parasitisation data recorded for each sample from each block.

Appendix 3(a).

The dates when *A. melinus* was released into each block.

BLOCK	DATE
1	12. 2.70
2	20. 2.70
3	6. 4.70
4	14. 4.70
5	20. 4.70
6	21. 4.70
7	1. 5.70
8	4. 5.70
9	17. 6.70

APPENDIX 3(b).

1st Sample after release of *A. melinus* (four weeks).

ESTIMATES OF % PARASITISATION   L = low   M = medium
H = high density of red scale.

Block	Scale Density	Leaf samples Number of parasites released 0	10	100	1000	Fruit samples Number of parasites released. 0	10	100	1000
1	L	0.0	0.0	50.0	50.0	0.0	0.0	58.8	0.0
	M	0.0	0.0	42.9	30.8	0.0	0.0	-	35.7
	H	0.0	16.7	1.9	29.4	0.0	0.0	0.0	54.3
2	L	0.0	0.0	0.0	0.0	0.0	0.0	14.4	27.3
	M	0.0	0.0	5.1	9.6	0.0	23.0	47.1	5.5
	H	0.0	1.0	0.0	4.8	0.0	0.0	2.3	47.2
3	L	0.0	0.0	13.3	39.0	0.0	0.0	25.0	100.0
	M	0.0	0.0	0.0	32.3	0.0	0.0	0.0	42.3
	H	0.0	0.0	0.0	80.0	0.0	0.0	20.0	6.3
4	L	0.0	0.0	28.3	37.5	0.0	3.6	0.0	38.5
	M	0.0	0.0	17.1	28.4	0.0	0.0	3.3	15.5
	H	0.0	0.0	12.9	3.7	0.0	0.0	1.6	16.3
5	L	0.0	0.0	0.0	10.8	0.0	0.0	0.0	5.6
	M	0.0	0.0	0.0	3.4	0.0	0.0	0.0	20.7
	H	0.0	0.0	0.0	5.6	0.0	0.0	5.6	1.3
6	L	0.0	0.0	0.0	5.4	0.0	0.0	0.0	1.1
	M	0.0	1.0	0.0	3.8	0.0	0.0	0.0	0.0
	H	0.0	0.0	0.0	4.1	0.0	16.7	0.0	4.2

APPENDIX 3 (b).

7	L	0.0	0.0	0.0	9.9	0.0	0.0	0.0	9.1
	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	H	0.0	0.0	2.0	0.9	0.0	0.0	0.0	1.3
8	L	0.0	0.0	0.0	14.3	0.0	0.0	0.0	0.0
	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	H	0.0	0.0	0.0	4.8	0.0	0.0	0.0	4.9
9	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	H	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

2nd Sample After Release (eight weeks).

* Meconium found

1	L	0.0	0.0*	32.1	22.9	0.0	0.0	60.0	33.3
	M	0.0	1.4	2.2	27.4	0.0	44.1	0.0	50.0
	H	0.0	0.0	20.9	26.2	0.0	0.0*	31.5	57.5
2	L	0.0	0.0	22.4	17.2	0.0	0.0	23.8	21.1
	M	0.0	21.7	37.0	19.2	0.0	16.7	54.5	22.0
	H	0.0	15.0	26.7	27.1	0.0	16.7	42.9	22.5
3	L	0.0	0.0	7.3	50.0	0.0	0.0	18.2	80.0
	M	0.0	0.0	0.0	50.0	0.0	0.0	0.0	60.0
	H	0.0	0.0	0.0	80.0	0.0	0.0	25.0	16.6
4	L	0.0	0.0	66.7	45.5	0.0	0.0	33.3	0.0*
	M	0.0	0.0	50.0	36.4	0.0	0.0	0.0	5.9
	H	0.0	0.0	50.0	12.5	0.0	0.0	0.0	5.0

APPENDIX 3 (b).

5	L	0.0	0.0	10.0	25.7	0.0	0.0	0.0	42.8
	M	0.0	0.0	0.0	15.3	0.0	0.0	0.0	0.0
	H	0.0	0.0	0.0	6.9	0.0	0.0	3.5	3.6
6	L	0.0	0.0	0.0	30.0	0.0	0.0	0.0	20.0
	M	0.0	0.0	0.0	24.1	0.0	0.0	0.0*	0.0
	H	0.0	0.0	0.0	33.3	0.0	0.0	0.0	0.0
7	L	0.0	0.0	0.0	3.3	0.0	0.0	0.0	0.0
	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	H	0.0	0.0	4.9	21.0	0.0	0.0	0.0	9.9
8	L	0.0	0.0	0.0	5.9	0.0	0.0	0.0	0.0
	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	H	0.0	0.0	0.0	11.1	0.0	0.0	0.0	0.0
9	L	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	M	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	H	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

APPENDIX 3 (b)

Mean values for percentage of red scales parasitised.

1st SAMPLE.

L = Red scale on leaves.

F = Red scale on fruits.

	Scale Density	BLOCK 1	2	3	4	5	6	7	8	9
L	Low	33.0	0.0	17.4	21.9	3.6	1.8	3.3	4.8	0.0
L	Medium	24.6	4.9	10.8	15.2	1.1	1.6	0.0	0.0	0.0
L	High	16.0	1.9	26.7	5.5	1.9	1.4	1.0	1.6	0.0
F	Low	19.6	13.9	41.7	14.0	1.9	0.4	3.0	0.0	0.0
F	Medium	11.9	25.2	14.1	6.3	6.9	0.0	0.0	0.0	0.0
F	High	18.1	16.5	8.8	6.1	2.3	7.0	0.4	1.6	0.0

2nd SAMPLE.

	Scale Density	BLOCK 1	2	3	4	5	6	7	8	9
L	Low	18.3	13.2	9.1	37.4	11.9	10.0	1.1	2.0	0.0
L	Medium	10.3	26.0	16.7	28.8	5.1	8.0	0.0	0.0	0.0
L	High	15.7	22.9	26.7	20.8	2.3	11.1	8.6	3.7	0.0
F	Low	31.1	15.0	32.7	11.1	14.3	6.7	0.0	0.0	0.0
F	Medium	31.4	31.1	20.0	2.0	0.0	0.0	0.0	0.0	0.0
F	High	29.7	27.4	13.9	1.7	2.7	0.0	3.3	0.0	0.0

APPENDIX 3 (c).

Mean values for percentage of red scales parasitised.

1st Sample after release. % parasitisation.

No. parasites released		1	2	3	4	5	6	7	8	9
Leaves	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10	5.6	0.3	0.0	0.0	0.0	0.3	0.0	0.0	0.0
	100	31.6	1.7	4.4	19.4	0.0	0.0	0.7	0.0	0.0
	1000	36.7	4.1	50.4	23.2	6.6	4.4	3.6	6.4	0.0
Fruit	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10	0.0	7.7	0.0	1.2	0.0	5.6	0.0	0.0	0.0
	100	19.6	21.3	15.0	1.6	1.9	0.0	0.0	0.0	0.0
	1000	30.0	26.7	49.5	23.4	9.2	1.8	3.5	1.6	0.0

2nd Sample after release. % parasitisation.

No. parasites released		1	2	3	4	5	6	7	8	9
Leaves	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10	0.5	12.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	100	18.4	28.7	2.4	55.6	3.3	0.0	1.6	0.0	0.0
	1000	25.5	21.2	60.0	31.5	16.0	29.1	8.1	5.7	0.0
Fruit	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10	14.7	11.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	100	30.5	40.4	14.4	11.1	1.2	0.0	0.0	0.0	0.0
	1000	46.9	21.9	52.2	3.6	15.5	6.7	3.3	0.0	0.0

APPENDIX 4.

An estimate of the percentage of red scales which were suitable for parasitisation by *A. melinus*.

Counts of number of suitable scales and total number of scales on leaves from ten trees in Orchards 1, 2 and 3.

APPENDIX 4.

ORCHARD 1.			ORCHARD 2.			ORCHARD 3.		
Suit-able	Total	%	Suit-able	Total	%	Suit-able	Total	%
9	235	3.8	207	743	27.8	106	530	20.0
4	528	0.8	111	636	17.4	9	80	11.2
54	450	12.0	91	567	16.1	2	101	2.0
24	213	11.2	64	423	14.9	14	99	14.1
42	281	15.0	65	540	12.0	45	249	18.1
41	434	9.5	83	711	11.7	26	267	9.8
10	271	3.7	108	645	16.7	13	166	7.8
23	228	10.0	88	619	14.2	47	546	8.6
28	217	9.2	63	675	9.3	12	141	8.5
96	753	12.8	96	537	17.9	38	302	12.6

Mean (% suitable) = 11.6

S.D. of mean = 5.9

APPENDIX 5.

Detailed results of Experiment 4.

(a)	0.15%	Maldison	18.8.70
(b)	0.15%	Maldison	4.10.70
(c)	0.15%	Maldison	4. 1.71
(d)	2½%	Oil	4. 1.71
(e)	0.075%	Omethoate	8.12.70
(f)	Nutrients.		

The great majority of the counts of the number dead in the controls were 0. They have not been included.

NUMBER OF DEAD ADULTS AFTER 4 HOURS CONFINED OVER LEAF CARRYING WEATHERED RESIDUE OF 0.15% MALDISON - APPLIED 18/8/70.

	Assessment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	No. days weather	1	3	7	8	10	13	15	19	22	24	27	29	31	35	38	41	43	48	57	63
Treated leaves	Replicate 1	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	1	0
	2	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	0	10	10	0	0
	3	10	10	10	10	10	10	10	10	10	10	10	10	10	4	9	10	9	10	0	0
	4	10	10	10	10	10	10	10	10	10	10	10	10	10	10	2	10	10	10	0	0
	5	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	6	10	10	0	0
	6	10	10	10	10	10	10	10	10	10	10	10	10	10	6	2	10	10	10	0	0
	7	10	10	10	10	10	10	10	10	10	10	10	10	10	10	9	10	7	10	0	0
	8	10	10	10	10	10	10	10	10	10	10	10	10	10	10	6	8	8	10	0	0
	9	10	10	10	10	10	10	10	10	10	10	10	9	10	7	10	0	10	10	0	0
	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	5	10	8	10	0	0
Total		100	100	100	100	100	100	100	100	100	100	100	99	100	87	73	74	92	100	1	0
Average		10.0	10	10	10	10	10	10	10	10	10	10	9.9	10	8.7	7.3	7.4	9.2	10.0	0.1	0

APPENDIX 5 (a).

Rearrangement of data and calculations to determine WT_{50} Value.

Probit transformation from Table 9 Fisher & Yates (1943).

Mean values of number dead less than 10.0 only used.

Correlation calculated between d and probit.

No. days weathering (d)	Mean No. dead	Mean dead in control	% kill	log. No. days (d - 33)	Probit of % kill
35	8.7	0.0	87	0.301	6.126
38	7.3	0.0	73	0.699	5.613
41	7.4	0.0	74	0.903	5.643
43	9.2	0.0	92	1.0~~41~~00	6.405
48	9.9	0.0	99	1.176	7.326
57	0.1	0.0	1	1.380	2.674

Regression coefficient = -0.12

Regression equation = $\bar{y}_x = 10.80 - 0.12x$

Correlation coefficient = -0.59 N.S.

WT_{50} = 48.3 days

Variance of WT_{50} = 5.00

S.D. of WT_{50} = 2.2 days

NUMBER OF DEAD ADULTS AFTER FOUR HOURS CONFINED OVER LEAF CARRYING WEATHERED RESIDUE OF 0.15% MALDISON SPRAYED 4.10.70.

Assessment	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Days of weathering	1	3	5	8	12	16	19	21	28	31	35	39	44	46
Treated leaves Replicate 1	10	10	10	10	10	10	10	10	10	10	1	9	7	4
2	10	10	10	10	10	10	10	10	10	10	10	9	0	3
3	10	10	10	10	10	10	10	10	10	10	9	10	1	5
4	10	10	10	10	10	10	10	10	10	7	8	9	8	1
5	10	10	10	10	10	10	10	10	8	10	9	6	0	2
6	10	10	10	10	10	10	10	10	8	10	9	10	0	6
7	10	10	10	10	10	10	10	8	10	8	10	10	5	0
8	10	10	10	10	10	10	10	10	7	10	10	9	2	2
9	10	10	10	10	10	10	10	10	10	10	2	4	3	0
10	10	10	10	10	10	10	10	10	10	6	8	5	2	2
Total	100	100	100	100	100	100	100	98	93	91	76	81	28	25
Average	10	10	10	10	10	10	10	9.8	9.3	9.1	7.6	8.1	2.8	2.5

APPENDIX
~~APPDNEIX~~ 5 (b).

Rearrangement of data and calculations to determine

WT_{50} Values.

No. days weathering (d)	Mean No. dead	Mean No. dead in control	% kill	log. No. days (d - 19)	Probit % kill
21	9.8	0.1	97	0.301	6.880
28	9.3	0.0	93	0.954	6.476
31	9.1	0.3	88	1.079	6.175
35	7.6	0.1	75	1.204	5.675
39	8.1	0.5	76	1.301	5.706
44	2.8	0.4	24	1.400	4.294
46	2.5	0.3	23	1.431	4.261

Regression coefficient = - 0.11

Regression equation $\bar{y}_x$ = 9.44 - 0.11 x

Correlation coefficient = - 0.96 ***

WT_{50} = 40.7 days

Variance of WT_{50} = 5.9

S.D. of WT_{50} = 2.4 days

APPENDIX 5 (c).

NUMBER OF DEAD ADULTS AFTER FOUR HOURS CONFINED OVER A LEAF CARRYING WEATHERED RESIDUE OF 0.15% MALDISON SPRAYED 4.1.71.

ASSESSMENT		1	2	3	4	5	6	7	8	9	10
No. days of weathering		2	4	9	11	16	18	21	23	32	39
Treated leaves	Replicate 1	10	10	10	10	10	10	7	10	6	0
	2	10	10	10	10	10	6	6	10	6	0
	3	10	10	10	10	10	10	9	10	3	0
	4	10	10	10	10	10	8	10	10	4	1
	5	10	10	10	10	10	5	7	7	0	0
	6	10	10	10	10	10	8	8	10	8	0
	7	10	10	10	10	10	10	10	8	2	1
	8	10	10	10	10	10	10	5	7	2	0
	9	10	10	10	10	10	5	10	8	6	0
	10	10	10	10	10	10	7	10	8	2	0
Total		100	100	100	100	100	79	82	88	39	2
Average		10	10	10	10	10	7.9	8.2	8.8	3.9	0.2

APPENDIX 5 (c).

Rearrangement of data and calculations to determine WT_{50} values.

No. days weathering	Mean No. dead	Mean No. dead in control	% kill	log. no. days	Probit % kill
18	7.9	0.1	79	1.255	5.806
21	8.2	0.0	82	1.322	5.915
23	8.8	0.0	88	1.362	6.175
32	3.9	0.6	35	1.505	4.615
39	0.2	0.0	2	1.591	2.946

Regression coefficient = - 0.15

Regression equation $\bar{y}_x$ = 8.95 - 0.15 x

Correlation coefficient = - 0.94 ж ж

WT_{50} = 27.2 days

Variance of WT_{50} = 0.06

S.D. of WT_{50} = 0.2 days

APPENDIX 5 (d).

Number dead adults after four hours' confinement over a leaf carrying a weathered residue of 2½% oil - sprayed 4.1.71.

ASSESSMENT	1	2	3
Days of weathering	1	3	8
Replicate 1	10	3	2
2	10	6	0
3	10	9	1
4	10	6	0
5	10	2	0
6	10	5	2
7	10	6	3
8	10	4	1
9	10	6	0
10	10	4	0
TOTAL	100	51	9
MEAN	10	5.1	0.9
S.D. of mean	0	2.0	1.1
Mean dead control	0.2	0.7	0.7
% kill	100	47	2

APPENDIX 5 (e).

Number dead adults after four hours' confinement over a leaf carrying weathered residue of 0.075% Omethoate sprayed 3.12.70.

ASSESSMENT		1	2	3	4	5
No. days weathering		1	5	8	14	20
Replicate	1	10	8	10	6	6
	2	10	9	10	8	3
	3	10	10	10	9	10
	4	10	10	10	9	1
	5	10	10	10	10	3
	6	10	10	10	5	4
	7	10	10	10	8	5
	8	10	10	10	7	1
	9	10	10	10	9	7
	10	10	10	10	9	0
TOTAL		100	97	100	80	40
Average		10.0	9.7	10.0	8.0	4.0

APPENDIX 5 (e).

Rearrangement of data and calculations to determine WT_{50}.

No. days weathering	Mean No. dead	Mean dead in control	% kill	Log. no. days	Probit % kill
5	9.7	0.4	97	0.699	6.881
8	10.0	0.0	100	0.903	10.000
14	8.0	0.3	79	1.146	5.806
20	4.0	0.6	36	1.301	4.642

Regression coefficient = - 0.25

Regression equation $\bar{y}_x$ = 9.72 - 0.25 x

Correlation coefficient = - 0.71 N.S.

$W.T_{50}$ = 18.9 days

Variance of $W.T_{50}$ = 6.38

S.D. of $W.T_{50}$ = 2.5 days

APPENDIX 5 (f).

Counts of number of dead adults after four hours' confinement over residues of Nutrient Sprays.

Zn = Zinc Oxide   Mn = Manganese Sulphate   U = Urea

REPLICATE	1	2	3	4	5	6	7	Total	Averages
Zn	0	1	2	1	2	2	0	8	1.14
Mn	0	0	1	0	0	1	0	2	0.29
Urea	3	1	0	1	0	2	1	8	1.14
Zn + Mn	1	0	0	0	0	1	0	2	0.29
Zn + Urea	0	0	1	1	0	0	1	3	0.43
Mn + U	0	0	0	0	0	0	0	0	0.00
Zn + Mn + U	3	1	0	0	1	0	1	6	0.86
Control	0	0	0	2	2	2	0	6	0.86

After twenty hours' confinement.

Replicate	1	2	3	4	5	6	7	Total	Averages
Zn	3	3	4	4	7	6	3	30	4.3
Mn	4	3	4	3	5	3	3	25	3.6
U	5	4	5	5	3	4	4	30	4.3
Zn + Mn	5	3	5	3	3	3	3	25	3.6
Zn + U	2	1	2	2	2	2	2	13	1.9
Zn + Mn + U	5	3	3	1	1	3	2	18	2.6
Mn + U	1	3	5	5	3	2	2	21	3.0
Control	4	2	2	2	3	2	2	17	2.4

APPENDIX 6.

Rating system used to estimate the size of the population of red scales on a tree.

APPENDIX 6.

520 leaves selected haphazardly from a tree (not removed) with the proviso that 100 are sampled in each of the cardinal quadrats of the tree, 100 from the top of the tree and 20 from the centre.

Each leaf was given one of the following ratings :-

Rating	Number of Red Scales Present
0	0
1	1 - 4
2	5 -12
3	13 -25
4	More than 25

The number of leaves in each rating was recorded.

The index was determined by multiplying the number of leaves in each rating by the number of the rating and then adding the 4 numbers together. This was reduced to a value appropriate to 100 leaves (estimated on 520 leaves).

The number of red scales was estimated by summing over all ratings the number of leaves multiplied by the mean number of red scales in each rating.

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