THE ESTABLISHMENT OF IBALIA LEUCOSPOIDES IN NEW ZEALAND.

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Introduction:-

The purpose of this research note is to record the progress made towards the establishment of Ibalia leucospoides, Hochenw, in New Zealand and to give details of the preliminary experimental work carried out.

Only work done by the Forest Research Institute is recorded and no attempt has been made to cover work done by Dr.Miller of the Cawthron Institute, in securing shipments of the parasite through the Commonwealth Bureau of Biological Control; or of Dr.H.S.Hanson who has undertaken the task of collection in England and despatch to New Zealand.

1. Ibalia and its Host:-

The life history of Ibalia leucospoides, Hochenw, has been worked out in detail by Dr.R.N.Chrystal (1930). Briefly, it may be stated as follows:-

Ibalia lays its eggs within the eggs of Sirex and the larvae live as internal parasites of the Sirex larvae. The third stage larva leaves the interior of its host and completes its feeding from the outside. The fourth stage larva pupates and the adult emerges by boring its way out of the wood. The life cycle occupies three years.

Sirex noctilio, F., is a forest insect which is usually of secondary importance. The eggs are laid in the wood of suppressed trees, usually pine. The trees are killed by a fungus inoculated into the wood with the eggs, and the larvae tunnel in the wood of the dead trees.

Following the drought of 1946, Sirex noctilio became epidemic in stands of Pinus radiata and caused considerable mortality. Accounts of this epidemic have been given in two recent papers (1948), (1949).

It is to reduce the severity of such epidemics in the future, that the introduction of the parasite is being attempted.

2. Methods of Establishment:-

Since the life cycle of Ibalia occupies three complete years it is necessary either to import them in their first, second and third year stages, or to secure establishment on three successive years.

Ibalia may be introduced as first, second, or early third stage larvae within the host, in which case the Sirex larvae would need to be placed in logs to continue feeding. This is not easy and involves complications owing to the importance of the presence of specific symbiotic fungi. Alternatively Ibalia may be introduced as late third or fourth stage larvae, as pupae, or as adults.

Once the parasites have been imported there are two possible methods of securing establishment. The first methods involves regulating the development of the Ibalia so that the adults emerge at a time when Sirex eggs are available, that is, in January or February. The second method necessitates the rearing of Sirex, in order to provide a supply of eggs in which the Ibalia may oviposit whenever they emerge, or whenever the adults are received from England.

All these methods are being tried, but the importation of adults, which takes only seven days, promises to be the most satisfactory.

3. Rearing of Sirex noctilio:-

Rearing experiments were started in June 1949 and, although the adults secured had to be sacrificed for urgent insecticide tests, sufficient information was obtained to indicate that the securing of out-of-season adults was possible and that females so secured would oviposit.

Experiments were continued in the winter of 1950 and a technique has now been evolved for obtaining adults at any predetermined date.

From the 1949 experiments, in which wood blocks were used, it was found that the time taken from pupation to the emergence of the adult was about twenty-one days at 22° C.

In August 1950 twenty-five adults were secured from second year larvae, they were reared in single glass tubes, lined with protruding strips of blotting paper and plugged with cotton wool, these were incubated at 26° C. and 75 per cent relative humidity.

The time taken to pupate varied from fifteen to twenty-five days, except for six specimens which took from thirty-six to forty-two days. These six were probably either not mature or had suffered damage when being removed from the log.

The time spent in the pupal stage was fifteen to nineteen days, the majority emerged on the sixteenth day. At 21° C. the time was eighteen to nineteen days.

About a week is required by the adults before they commence boring their way out and, when in logs, another week may be occupied in reaching the exterior.

Some pupae were kept at 75 per cent humidity and others at 100 per cent humidity, no difference was noted in the time taken to complete their development, nor in the condition of the adults produced. There was no difference between the time taken by males and females.

More larvae were collected in October and some of these pupated in eleven days, indicating that they were already preparing to pupate before being collected.

Many methods of rearing <u>Sirex</u> have been tried and the following may be worth recording.

A. In wood blocks over salt solutions:-

Blocks of *P. radiata* timber were cut, measuring 5 in. x $2\frac{1}{4}$ in. x $1\frac{3}{4}$ in, and three lines of $\frac{3}{8}$ in. holes were bored through the broad face; twenty-one holes were secured in each block. One end of each hole was sealed with wood pulp and the blocks were placed in Agee jars, in which they were supported on glass stands over saturated solutions of various salts. A perforated celluloid disc was placed between the block and the stand to prevent the *Sirex* falling into the solutions. The jars were then sealed and placed in an incubator to allow the humidity to become constant. When required a block was removed and a fully developed *Sirex* larva was placed, head first in each hole. The holes were blocked with cotton wool and the block was replaced in the jar and incubated at 22° C.

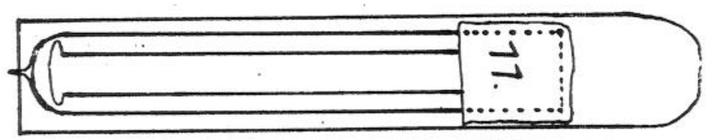
The results were good but not entirely satisfactory and this method was not carried beyond the experimental stage.

Salts were used which gave humidities ranging from 36 per cent to 89 per cent and it was found that the best results were obtained using sodium chloride, which gives a relative humidity of about 75 per cent.

B. In glass tubes:-

Larvae were placed in glass tubes plugged with cotton wool and stacked in Agee jars as with the wood blocks. At first the results were unsatisfactory but finally the following method was evolved which gave excellent results.

Blotting paper was cut in strips, 4 in. x 1 in, a strip was placed inside a 12 x 75 mm. test tube. A larva was then placed in the tube on the blotting paper, with its head outwards. The end of the blotting paper was then folded back over the end of the tube and numbered.



Test-tube for rearing Sirex.

The small tube was then pushed inside a 16 x 100 mm. test-tube. A small strip of adhesive plaster was fastened to the end of the smaller tube to form a tag for withdrawing the tube if necessary. Tubes were stacked over sodium chloride in dessicators and incubated.

With this method the progress of pupation and emergence could be followed without damage to the insect and, when ready to bore out, the adult could chew through the blotting paper into the larger tube.

C. In logs:-

Short lengths of logs containing second year Sirex larvae were taken and the bark removed. The logs were then heavily coated with boiling 'Texwax' and placed in a room with controlled temperature; excellent results were obtained but the number of Sirex secured could not be predicted.

One log, 6 in. diameter and 21 in. long, put in the hot room on August 29th, and incubated at 26° C. gave the following results.

Days from Aug. 29th.	Male adults.	Female adults.
47	1	-
49	2	-
52	1	-
53	1	-
55	1	1
56	1	-
57	1	-
62	1	2
63	-	3
65	2	-
67	1	-
69	-	1
	12	7

The Sirex obtained were all large and of nearly the same size.

An unwaxed log put in at the same time produced only one male in fifty days. The log was then cut up, as it was considerably dried out, and found to contain one larva, one male pupa, and one male and two female adults.

The adults had emerged badly from the pupae and one female was remarkable in that the antennae were completely undeveloped. When put on a pine log, however, she commenced to oviposit and continued to do so up to the time of death three weeks later. This example is interesting as indicating that no stimulus is required from the antennae to initiate oviposition.

Rearing in logs would have been necessary if test-tube reared adults had been found unsatisfactory.

4. <u>The fungus symbiont</u>:-

The female Sirex carries a fungus which she inoculates into the tree with the eggs. The fungus serves to kill the tree and is almost certainly necessary for the development of the larvae after the death of the tree.

There was considerable doubt as to whether the fungus could be carried through the pupal stage by females reared in test-tubes. It has now been demonstrated that the fungus is carried through, and cultures have been obtained from the neighbourhood of oviposition punctures, made, in fresh P.radiata logs, by virgin test-tube reared Sirex. This point was of the utmost importance, because the Sirex eggs would have been useless had the fungus been absent.

5. <u>Parthenogenesis</u>:-

It was suspected that the preponderance of male over female Sirex might indicate that the males were derived from unfertilised eggs. In order to test this possibility, unfertilised test-tube reared females were allowed to oviposit in fresh pine logs. Young larvae were later recovered from the oviposition punctures, proving that the eggs were viable.

It has not yet been established whether or not the larvae will continue to develop or give rise to male adults.

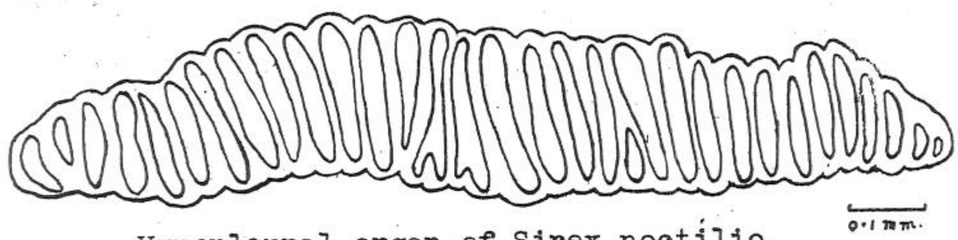
As copulation is difficult to secure under confined conditions, this point is of considerable importance and investigations are in progress to determine whether or not male larvae are haploid. Should this be the case it may be taken that *Sirex noctilio* is facultatively parthenogenetic and that males are normally produced from unfertilised eggs. Such eggs will therefore be suitable for *Ibalia*.

6. Sexing of larvae:-

Parkin (1942) has described a structure which he terms the hypopleural organ. This organ is situated on the first abdominal segment of some, but not all, *Sirex* larvae, and, from the proportion of larvae having this structure, he deduced that it was present in females only. The hypopleural organ contains hyphae of the fungus symbiont and appears to have some connection with the carrying-over of the fungus from the larva to the adult.

In order to test the relationship of the hypopleural organ to sex, fifty larvae with and fifty larvae without this structure, were placed in tubes and reared. When the larvae pupated the sex was found to have been correctly determined in every case. As Parkin had surmised, it is present only in the females.

The ratio of length to width of the hypopleural organ appears to be specific.



Hypopleural organ of Sirex noctilio.

Parkin gives measurements for S. gigas and S. cyaneus.

	Length.	Width.	Ratio.
Sirex gigas.	0.94 mm.	0.31 mm.	3.03
Sirex cyaneus.	1.22 mm.	0.25 mm.	4.88
Sirex noctilio.	1.24 mm.	0.18 mm.	6.89

Since 60-75 per cent of the Sirex normally reared are males, the sexing of larvae, so that only females are reared, (in conjunction with the parthenogenetic production of eggs, laid with the fungus symbiont), promises to simplify enormously the work of establishing Ibalia.

7. Oviposition:-

At 26° C. Sirex were observed to bore oviposition punctures almost ceaselessly, and were found to continue oviposition in the dark. At 21° C. the process took longer and the females rested for longer periods. Probably better results would be obtained at the lower temperature. Ovipositing females were studied under a binocular microscope and it was interesting to observe the motion of the ovipositor which was rotated through 360° to bring the saw into contact with all parts of the bore hole. Another point of interest observed was that the ovipositor was withdrawn immediately if a resin duct was encountered. This may account, in part, for the large number of bore holes in which no egg is laid. Eggs may be kept for two weeks at normal temperatures without hatching.

8. Ibalia imported in 1950:-

Dr.H.S.Hanson, of the British Forestry Commission, undertook the work of collection in England and the first consignment arrived at the Cawthron Institute on March 12th, 1950.

This consignment contained ten live and five dead Ibalia larvae, with one hundred and eighty five live and sixty eight dead Sirex larvae, thought to be parasitised by Ibalia. These were held at the Cawthron Institute pending the completion of an insectary at the Forest Research Institute, Rotorua.

On June 15th, the Forest Research Institute received from Dr.Miller the ten Ibalia and ninety of the Sirex larvae and on August 1st, the thirty four surviving Sirex larvae were forwarded.

The Ibalia and Sirex were kept in a cool store at 7° C. but, as mortality was heavy, attempts were made to rear some in logs. From eighteen Sirex larvae put in logs, two adult Sirex emerged, but no Ibalia. Of six Ibalia larvae put in tubes over sodium chloride, one male emerged after two months.

The remaining Sirex larvae were removed from the cool store to the hot room on September 18th. Under these conditions the third stage larvae emerged from the Sirex larvae and completed their feeding from the outside; they were then removed to the cool store.

Twenty-five of the Sirex larvae pupated, and the remainder, about thirty, died. Only ten Ibalia larvae were still alive by the end of October.

The heavy losses may be attributed to dessication, due to leaving the larvae too long in the gelatine capsules, and also to the growth of fungi and attack by mites, both of which flourished at 7° C., once the humidity was increased. It may be worth recording that only one female and six male Sirex emerged successfully from the pupae.

. On October 25th, two adult female *Ibalia* were received from England, one died but the other oviposited readily in *Sirex* eggs in the hot room.

On November 2nd, one male and one female *Ibalia* were received, the female died without being observed to oviposit and the male was placed in the cool store.

On November 4th, one female and three male adults were received, the female began ovipositing immediately. The temperature was reduced to 21° C. at this stage and the female continued ovipositing until November 7th.

On November 9th, one male and three females were received.

These females were allowed to oviposit in eggs laid by *Sirex* which emerged from the waxed log already mentioned, and from test-tubes.

On November 16th, three female adults were received and, since no *Sirex* eggs were available, they were placed in the cool store. With this shipment there were eleven live larvae, and also a number of pupae, none of which survived the journey.

On November 20th, two small female *Sirex* started ovipositing and one female *Ibalia* was removed from the cool store and commenced oviposition.

On November 21st, one male adult, nineteen larvae and eleven pupae were received; the pupae appeared to be dead; the adult was put in the cool store.

On November 23rd, one male and four female adults were received; these were also put in the cool store.

On November 28th, four female and eleven male adults, together with thirty-one larvae and twenty-two pupae, were received

On December 6th, two female and one male adult, and three larvae were received.

On December 8th, one male adult and four larvae were received.

On December 10th, fresh supplies of *Sirex* eggs were available and all surviving *Ibalia* were placed in the hotroom, at 22° C., where the females commenced ovipositing immediately.

What degree of success has been achieved will not be known until adult *Ibalia* emerge from the logs in 1952 or 1953, but it may not be over-optomistic to expect that several hundred will be obtained.

Success in obtaining pupation of the *Ibalia* larvae has not yet been secured and they are being held at various temperatures and humidities, in an attempt to discover the optimum conditions for their development.

9. Programme for 1951:-

Rearing of *Sirex* will be continued until wild adults can be obtained in January in order that eggs may be available for any *Ibalia* that emerge. *Sirex* larvae will be kept in the cool store to provide adults from March to August should they be required.

Rearing of *Sirex* at different temperatures and humidities and at fluctuating temperatures, will be continued, but, owing to the excellent results already achieved, this is no longer of great importance.

Optimum growth of the symbiotic fungus in culture is 21° - 22° C. This temperature may prove to be optimum for *Sirex* also, at least in the larval stages.

Next winter it is proposed to concentrate on securing adult *Ibalia* from England. A considerable amount of preparatory work is required for their reception. Assuming that only ten female *Ibalia* are received each week from about the middle of August to the end of November, this will necessitate the rearing of fifty female *Sirex* each week from August 1st onwards. A supply of fresh logs equivalent to ten twelve-year-old trees of

P.radiata will be required each week to provide material in which the *Sirex* can oviposit. Rooms maintained at 21° C. to 26° C. will be required for oviposition and rearing.

A number of additional *Sirex* must be reared in case larger numbers of *Ibalia* are received.

This programme is dependent upon proof that male *Sirex* are haploid; should this prove not to be the case, males will have to be reared and methods of securing copulation studied.

In estimating the number of *Sirex* required it is assumed that the *Sirex* will lay 400 eggs each and the *Ibalia* 700.

Fifty *Sirex* a week will be needed to produce 20,000 eggs a week and ten *Ibalia* will produce 7,000 eggs in the same time. It is considered that to attempt to secure a greater degree of parasitism that one in three would result in wastage of *Ibalia* eggs, owing to two or more being placed in the same *Sirex* egg.

Another problem with *Ibalia* is to secure copulation. It has been noted that mating appears to be stimulated when the insects are brought from the cool store into the hot room; cold night and hot day temperatures may therefore be required in the oviposition room. It has been recorded that males wait on the trunks of trees where females are due to emerge. It may be possible to put males in the ends of the large tubes ready for the females when they emerge, through the blotting paper, from the small ones.

O. Concluding remarks:-

For the successful establishment of *Ibalia*, *Sirex* eggs must be available and the eggs must develop into larvae.

Under existing conditions the *Sirex* population fluctuates enormously and eggs are deposited in trees which may be capable of resisting the attack, so that either the eggs do not hatch or the larvae fail to develop. *Ibalia* eggs laid in these eggs would be entirely wasted.

The site for the first liberations must be carefully selected, because there must be Sirex killed trees available every year.

Liberations must be spread over a wide area as quickly as possible, so that, during times when Sirex becomes scarce, the Ibalia may be able to survive in a number of small pockets. Such sites may be found in smaller forests having a variety of pine species of many age classes.

Unfortunately most of the forests with these requirements already have Rhyssa persuasoria established, and it may not be desirable to have both species of parasite established in the same area.

Large areas of even-aged stands of a single species are less suited for initial establishment, since it is in these areas that the Sirex population fluctuates most widely.

If parthenogenesis is proved to be of normal occurrence, it will be possible to offer explanations for many observed phenomena.

For example, the first females to emerge will be less likely to be fertilised than those emerging later. Hence their first eggs will give rise to males which may have a greater chance of emerging first the following year. This may explain why the first males to emerge usually do so before the first females.

Females in a dense population are more likely to be fertilized that those in a sparse population, and fertilization is also more likely to occur when calm hot days are frequent. These factors will tend to hasten the development of an epidemic during a series of drought.

No such relationship between density of population and sex ratio has been noted but such speculations open up interesting lines for research.

Summary:-

1. A brief account is given of Sirex noctilio, F., and its parasite Ibalia leucospoides, Hochenw.

2. Possible methods which may be adopted to establish the parasite in New Zealand are discussed.

3. Observations on Sirex which have a direct bearing on the successful establishment of Ibalia are given. These include rearing techniques, the carrying over of the symbiotic fungus, parthenogenesis, sex determination in larvae, and notes on oviposition.

4. The Ibalia received from England in 1950 are enumerated and the results so far achieved are noted.

5. The programme for 1951 is set out.

References:-

1930	Chrystal, R.N.	Studies in Sirex Parasites. Oxf. For. Mem. 11.
1942	Parkin, E.A.	Symbiosis and Siricid Woodwasps. Ann.Appl.Biol. 29 (3).
1948	Rawlings, G.B.	Recent observations on Sirex noctilio.N.Z.J.For. 5 (5).
1949	Rawlings, G.B. and Wilson, Nancy M.	Sirex noctilio as a beneficial and destructive insect to Pinus radiata in New Zealand. N.Z.J.For.6 (1)

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