STUDIES ON TWO NEW ENTOMOGENOUS FUNGI (STILBACEAE : *Hymenostilbe*) AND THE BIOLOGY AND DISTRIBUTION OF THEIR HOST

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C O N T E N T S

INTRODUCTION

Species of the sand weevil Cecyropa, occur on the foreshore all around the New Zealand coast, and extend for several miles inland in pastures of consolidated sand country. The adults vary widely in size and in the pattern of the mottled grey and brown cryptic colouration of the elytra and pronotum. Larvae of Cecyropa are external feeders on plant roots at depths of up to eighteen inches. In the Manawatu there are two species of Cecyropa: a larger species (C. maritima) confined to the unstablized sand dune area, and a smaller species (C. setigera) occurring in both the unstabilized dunes and in pastures of the consolidated sand country.

The stimulus for the present study was provided by reports indicating that adults of Cecyropa (presumably C. setigera) had caused damage, sometimes severe, to the seedling stages of crops grown in the Manawatu sand country (Graham and Hopkins 1965, May 1966). Consequently a study was initiated into the life history and ecology of Cecyropa setigera in pastures of this area. The study initially took the form of a sampling programme designed to recover larvae from the field and was supported by breeding studies in the laboratory. However, due to difficulties associated with the recovery of larvae and adults in sufficient numbers, and the distance of the study area from the University, the emphasis of the study was swung to an investigation of two previously undescribed natural enemies of C. setigera. These were two entomogenous fungi, host specific on the immature stages of the sand weevil *C. setigera* in pastures of the Manawatu sand country.

Thus the areas of study can be defined as follows:

PART 1. The Host.

Aspects of the bionomics of *C. setigera* and a consideration of factors affecting spatial distribution.

PART 2. The Two Entomogenous Fungi.

(a) the morphdogy and taxonomy of two entomogenous fungi (*Hymenostilbe sp.*) pathogenic to the immature stages of *C. setigera*.

(b) the two fungi in artificial culture.

(c) the infectivity and etiology of the two fungi.

PART ONE

THE HOST

CHAPTER 1.

BIONOMICS AND DISTRIBUTION OF CECYROPA SETIGERA

INTRODUCTION

The genus *Cecyropa* Pascoe (1875) is an adelognathous member of the Otiorrhynchinae, one of 15 sub-families that comprise the family Curculionidae. The genus is endemic to New Zealand and comprises 18 species one of which was described by Pascoe, one by Sharp and the remaining 16 by Broun (1880-1921). There is scant reference in the literature to species of *Cecyropa* apart from the original descriptions. Hudson (1934, 1950) refers briefly to *Cecyropa* and illustrates *Cecyropa lineifera* Broun in the former work. The most recent references were those by Somerfield (1966) who dealt briefly with the distribution of an adult *Cecyropa* in the sand dunes at Piha, and May (1966) who briefly described aspects of the biology of *Cecyropa discors* Broun and provided a valuable key to the larvae of *C. discors* and other common soil inhabiting weevils.

Damage caused by *Cecyropa* species has only been recorded for the adults and the effect of larvae on pasture growth is unknown. Adults of *Cecyropa* have damaged foliage of onion and radish at Wanganui, and turnip seedlings at Foxton (May 1966). May also stated that adults caused damage to lucerne crops at Palmerston North but as *Cecyropa* species are restricted to sand country areas she was probably referring to crops of lucerne at Himatangi, 20 miles towards the coast from Palmerston North, where Dale (pers. comm.) witnessed severe damage to a crop of seedling lucerne probably caused by adults of *Cecyropa*.

The identity of *Cecyropa* species in the Manawatu has caused some confusion. At the beginning of this study a comprehensive series of adults of *Cecyropa* taken from both dunes and pasture was sent to Dr. G. Kuschel (D.S.I.R., Nelson) for identification. Dr. Kuschel identified the smaller species as *C. discors* and the larger as *Cecyropa maritima* Broun and remarked that these were probably the only species of *Cecyropa* in this area. This study on the smaller weevil was thus carried out under the impression that the correct identificat- ionwas *C. discors*. Larvae taken in pasture were readily identified and separated from the other weevil species using May's description and key for *C. discors* and the morphology of larvae corresponded closely to that described and figured in her paper. However, shortly before this thesis was completed, Dr. Kuschel informed the author that following a study of the New Zealand species of *Cecyropa* he had ~~concluded that he had~~ concluded that he had mistakenly identified the smaller species and that *C. discors* was correctly identified as *Cecyropa setigera* Broun. He further mentioned that *C. setigera* occurred on both coasts south of Gisborne, C. *discors* north of Gisborne, while *C. maritima* was found right around the New Zealand coasts. This amendment thus casts doubt on the identity of the larvae described by May. Unfortunately the author has no knowledge of the source, or correct identification, of the adults from which May obtained larvae for her description. In view of the close similarity of larvae of *C. setigera* to those described as

C. discors by May it is probable that either her specimens were incorrectly identified and were actually *C. setigera*, or that larvae of *C. setigera* are morphologically very similar to those of *C. discors*.

This chapter firstly presents data accumulated on the bionomics of *Cecyropa setigera* and then considers factors affecting the spatial distribution of larvae within sand country pastures. Brief descriptive notes are included to expand May's description and to point out differences between larvae of *C. setigera* and *C. discors* where they occur.

A. MATERIALS AND METHODS.

The data in this chapter are obtained from three sources:

1. A sampling programme in a paddock 1½ miles inland from Himatangi Beach.

The sampling programme provided data on larval bionomics and distribution. A full account of the sampling sites, sampling technique and extraction processes is contained in Appendix 1. Briefly, the method consisted of taking a number of four inch cores to a depth of 14 inches. These cores were transferred to the laboratory where they were wet sieved and larvae extracted from the residue by a combined process of flotation and differential wetting. Head capsule widths of all larvae were measured using an eyepiece scale in a stereoscopic microscope.

2. Collection of adults in the field.

Adults were difficult to detect amongst sand and debris because of their small size and cryptic colouration. After several methods were unsuccessfully attempted (Berlese funnel, wet sieving, and dry sieving beneath pasture) two methods of collection were adopted.

(a) Using bait

Carrots were cut in half and placed on the ground amongst a mature crop of lucerne. *Cecyropa* weevils emerged after dusk and climbed onto the carrots. Two hours after dusk, four or five weevils of *C. setigera* could generally be picked off each carrot slice.

(b) Dry sieving in the sand dunes

In the dunes adults of both *C. setigera* and *C. maritima* congregate under the spreading leaves of the flatweed 'catsear' (*Hypocheoris radicata*) and are also found in considerable numbers beneath sowthistle (*Sonchus oleraceus*). Spade sized samples taken to a depth of three inches and incorporating these plants were sieved through a 12 gauge garden sieve. The residue of leaves, plant roots and other debris was transferred to plastic bags and carefully hand sorted in the laboratory. (Searching for adult weevils in the field proved too time consuming).

3. Breeding methods in the laboratory.

The methods of this section are presented in the text.

B. THE EGG

1. Descriptive notes and egg development.

May (1966) described the eggs of *C. discors* as sub-spherical, pearly-white turning grey as they mature and easily desiccated. Egg dimensions were given as 0.8 x 0.6 mm while hatching took eleven days in February, 21 days in April and 32 days in June.

Observations on the eggs of *C. setigera* conform closely to May's description but a difference in egg width was noted.

The mean egg dimensions of C. setigera were 0.78 x 0.51 mm with a range of 0.69 - 0.87 x 0.46 - 0.55 mm. The distribution of egg lengths and widths is shown in Table 1.

TABLE 1

Frequency distribution of egg length and width.

Width			Length		
Class (mm)		Frequency	Class (mm)		Frequency
0.449	-	0	0.692	-	1
0.462	-	3	0.718	-	5
0.475	-	3	0.744	-	9
0.487	-	16	0.770	-	20
0.500	-	15	0.796	-	18
0.513	-	22	0.820	-	13
0.526	-	7	0.846	-	2
0.538	-	3	0.872	-	2
0.551	-	1	0.897	-	0

The eggs of C. setigera were thus of the same length as those of C. discors but narrower. As May gave no indication of size range it is difficult to assess the significance of this size difference since such a difference could be a manifestation of a population difference within a species or could reflect a more fundamental difference between two species. ~~or could reflect a more fundamental difference between two species~~.

Eclosion from the egg in C. setigera was witnessed on one occasion. The mature embryo was clearly visible through the chorion and began moving on exposure to the heat of the microscopelight. After ten minutes the larva was very active, arching the head back and forth within the egg and scraping the mandibles against the interior upper surface of the egg. The mandibles broke through the chorion after 15 - 20 minutes and by further wriggling and tearing, the larva managed to create an irregular rent and escape from the egg. There was no evidence of an egg burster being employed and thus the mode of hatching in Cecyropa corresponds to that of other Otiorrhynchid larvae (Van Emden, 1952).

The effect of incubation temperature on the egg was not investigated but it can be noted that for three eggs in which the date of laying was accurately known, hatching took place at 11, 11, and 12 days at temperatures ranging from 66 - 72°F.

C. THE LARVA

No difference could be found between May's description of larvae of *C. discors* and the morphology of *C. setigera*. Accordingly her descriptive notes are presented below.

> "The larva has a pale yellow head and is lightly sclerotized on the lobes of the last three segments giving it a reddish tinge and making detection extremely difficult in the light sand. It is distinguished by the unusual type of setae on these segments and by having three or four epipleural setae instead of the more usual two."

It was found that the most useful characters for the separation of *Cecyropa* from the other weevil larvae were the three or four epipleural setae and the thick blunt-ended setae of the last three segments. The use of May's key presented no problems in the separation of *Cecyropa* from all other weevil larvae encountered in the soils of the sand country.

1. Rearing individual larvae in the laboratory.

Although a number of attempts were made and several methods tried, only one larva was reared through from an egg to an adult in the laboratory. The following account describes the technique employed.

Discs of carrot approximately 3/16 inch thick were placed on damp filter paper in a petri dish. Single larvae were placed in small holes made in the tissue of the carrot. (Unless a hole was made the larvae could not obtain sufficient purchase to penetrate the carrot). After twenty four hours the larvae burrowed further into the tissue of the carrot and could be left unattended for five to six days. Following this period larvae were dissected from the carrot, measured and replaced in a fresh carrot disc. This method proved satisfactory for studies on the growth of larvae but because of the labour involved and the mortality on dissection, it was not suitable for rearing large numbers of larvae in the laboratory.

2. <u>Number of instars</u>.

In an ecological study it is desirable to have some method of determining the age of the insect. Such an assessment is usually made in soft-bodied insects by measuring the changes in head capsule width that accompany each moult. As there was no information on this aspect for any species of *Cecyropa*, an investigation was undertaken to determine the number of instars in *Cecyropa setigera*.

Live larvae were measured after they had been anaesthetized with CO_2 and all measurements were taken at the widest point across the head capsule. Measurements were made using a stereoscopic microscope with an eyepiece scale and all head capsule widths are expressed in as eyepiece divisions (100 divs. = 2.56 mm). An assessment of the number of instars, and the head capsule widths characteristic of each, was made from measurements of larvae recovered from the sampling programme and also from larvae reared in the laboratory.

The head capsule widths of larvae collected in the field during the sampling programme and extracted from soil cores in the laboratory, were measured immediately after death. The frequency distribution of head capsule widths for 493 larvae is illustrated in Fig. 1. On the basis of these results the measurements can be grouped into six classes that may be regarded as corresponding to six instars.

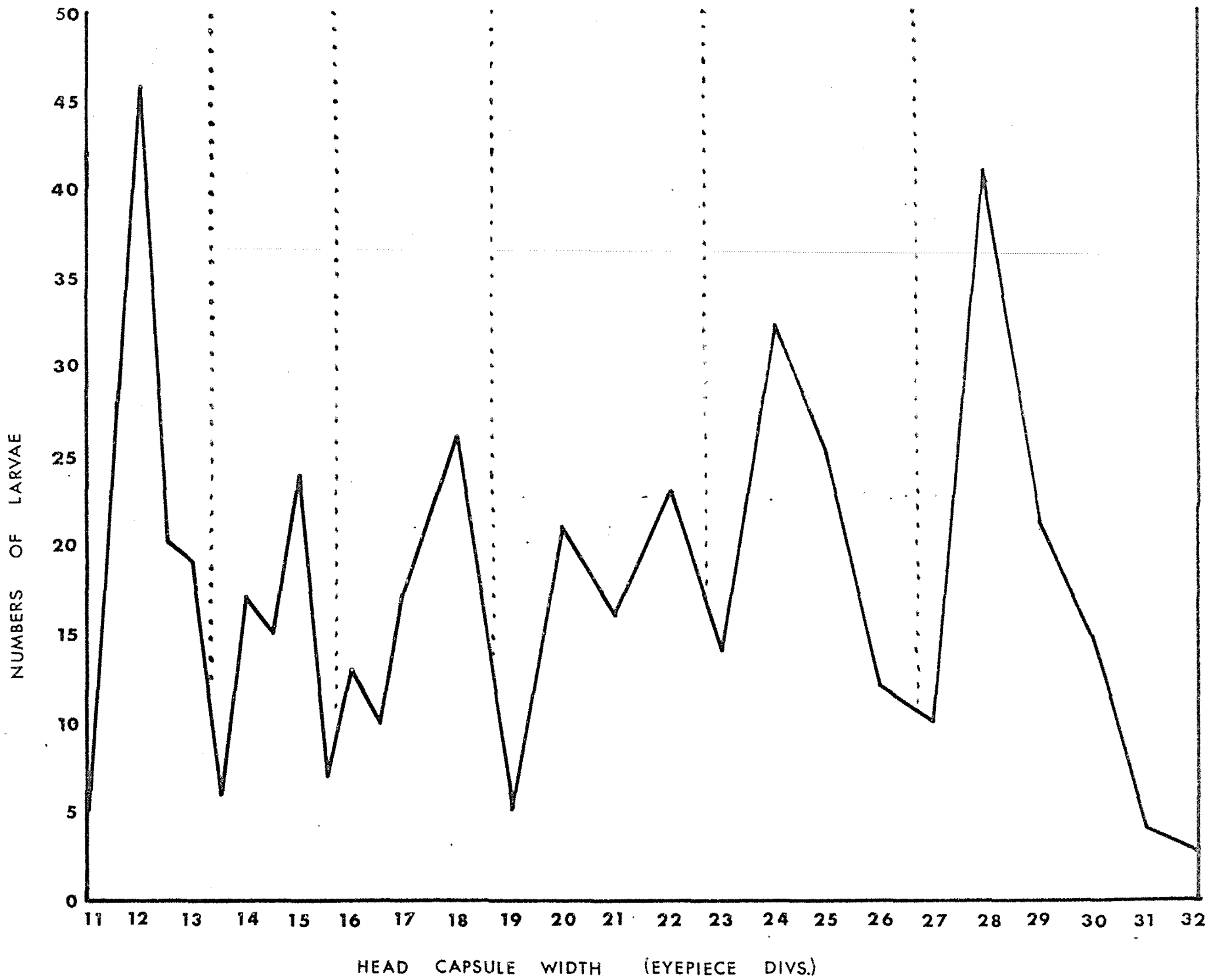
Instar	Class Interval			Mean
one	11.0	to	13.25	12.08
two	13.5	to	15.75	14.57
three	16.0	to	18.75	17.14
four	19.0	to	22.75	20.88
five	23.0	to	26.75	24.44
six	27.0	to	32.25	28.69

The division into six instars is supported by changes that took place in the head capsule widths of larvae reared in the laboratory. Larvae recovered alive from the field were reared on carrot slivers in the laboratory until one ecdysis had taken place. The following changes in individual head capsule widths occurred.

Before ecdysis		after ecdysis	Before ecdysis		After ecdysis
12	-	15	25	-	28.5
12	-	15.5	25	-	pupa
16	-	21	26	-	28.5
16	-	20.5	28	-	pupa
22	-	26	29	-	pupa
22	-	25	31	-	pupa

In each case the increase in size of the head capsule was sufficient to elevate the larva from its original instar class to the one immediately above. In no instance did the increase in size accompanying an ecdysis elevate a larva through more than one of the proposed instar classes.

Figure 1



Distribution of head capsule widths of 493 larvae recovered from the field

...... proposed instar divisions

Further evidence substantiating the validity of the proposed instar classes was provided by the changes in head capsule widths of a larva reared from an egg to an adult. The egg was laid between the 1st and 5th of April and hatched between the 13th and 17th of April. The egg dimensions were 0.75 x 0.49 mm. After hatching the larva was reared on carrot slivers and displayed the following changes in head capsule widths.

17th April	-	11.5	-	1st instar
24th April	-	14.0	-	2nd instar
1st May	-	17.5	-	3rd instar
8th May	-	22.0	-	4th instar
17th May-7th June	-	25.0	-	5th instar
12th June		pupated		

On the basis of these measurements it is evident that the larva passed through five instars before pupation and that the changes in head capsule widths fell within the first five age classes proposed above.

From a consideration of all results it is suggested that larvae of C. setigera generally pass through six instars in the field before pupation. Pupation can however, occur after five instars as evidenced by the pupation of laboatory reared larvae but the large number of sixth instar larvae encountered in the field suggests that such an occurrence is not the norm. It is also apparent from the variability in the head capsule widths of various instars that there is some degree of overlapping in the various instars. However, it is suggested that the six instar classes proposed above are ~~the most~~ suitable divisions for determining the age structure of larval populations of C. setigera as they occur in the field.

3. Duration of larval development.

The only evidence relating to the duration of larval development was obtained from the single larva reared from egg to adult in the laboratory. In this case the first four instars occupied approximately seven days each but the development of the final instar took 28 days before pupation occurred. Thus larval development in the laboratory in this one example occupied eight weeks at 66 to 72°F. As such temperatures occur in the soil only during the summer period, it is likely that larval development may be somewhat slower in the field at other seasons.

D. THE PUPA

Although the ecdysis of the prepupa has not been observed it was noted that one or two weeks before pupation the final instar larvae assumed an opaque white appearance and became slightly compressed dorsally and ventrally. In the laboratory prepupae also ceased feeding during this period. The pupa of *C. setigera* could not be distinguished from that of *C. discors* and accordingly May's (1966) descriptive notes are presented below.

> "The pupa, like the adult weevil, is proportionately broad. It is clothed with fine, rather long, pale bristles which, on the terminal segments, are longer than the horn-like pseudocerci. Secondary pterothecae are lacking and thecae of the mandibular cusps are inconspicuous. In the teneral adult, these deciduous cusps are small and straight and the scar, resulting from their loss can be easily overlooked."

May also mentioned that pupation took 28days in October. The pupal durations of three individuals of *Cecyropa setigera* were found to be 13, 15, and 16 days at 66 - 72°F.

The periods of occurrence of pupae in the field are not known with any accuracy since this stage, being delicate, was easily broken during sieving. Only eight pupae were recovered from the 412 cores of sampling programme. Pupae were observed in the field, however, during spade searches and it was noted that numbers peaked during early November to early December. Numbers then fell off until March when they were again more frequently encountered with numbers apparently decreasing in April and early May. Pupae were not found from May to late October. It must be emphasized here that these are subjective observations and consequently only a limited weight can be placed on their significance.

E. THE ADULT

Members of the Otiorrhynchinae are charaterized by deciduous mandibular cusps that break off shortly after emergence to leave an oval scar on each mandible. In *Cecyropa setigera* the mandibular cusps of the teneral adult are small and straight and the scar resulting from their loss is difficult to detect. May (1966) describes similar mandibular cusps for *C. discors*.

In *Cecyropa setigera* the adult male can be distinguished from the female by the concavity of the first and second ventrites and the presence of two widely separated tubercules. In the female the tubercules are absent and the ventrites are convex.

1. Adult emergence

Although an adult emergence was not observed the following events were noted. Four days before emergence the sclerotization of mandibles and cusps, compound eyes and tarsal tips was visible through the pupal cuticle. Twenty four hours before emergence the sclerotization had spread to other parts of the body which now appeared light brown while the mandibles, cusps and tarsi were a dark brownish-black. Twenty four hours after emergence the teneral adult was a golden brown colour which gradually darkened over the next 10 days to the mottled grey, brown and black colouration of the typical adult. The mandibular cusps broke off on the first feeding, which took place at seven days.

2. Oviposition

(a) Obtaining eggs in the laboratory

Van Emden (1952) states that generally adelognathous weevils readily lay eggs in a petri dish lined with damp filter paper, but May (1966) found that females of C. discors would not deposit eggs in such a container until a small quantity of sand had been sprinkled beneath the filter paper. In this study it was found that although May's method was satisfactory for up to two pairs of weevils, the confinement of more than this number to a petri dish inhibited oviposition.

The most satisfactory method for obtaining large numbers of eggs in the laboratory was as follows. Two inches of sieved (52 mesh) damp sand was placed in a clear plastic lunch box (approx. 6x4x4") and 20 males and 20 females added. Carrot pieces were added as food and the perforated lid replaced Fresh carrot was added as needed. After four weeks' incubation at room temperatures, the contents of the lunch box were wet sieved through 52 mesh gauze. Eggs and adults were washed into a petri dish, removed and counted using a stereoscopic microscope.

(b) Rate of oviposition and fecundity

No records were made of oviposition rates under field conditions. In the laboratory the average oviposition rate, as established from breeding experiments above, varied from one egg per female every three days to one egg every ten days. However, results from a study (see below) on the oviposition rates of two individual females indicate that the figures above may be considerably lower than normal.

Two weeks after emergence, two females reared in the laboratory from field collected pupae (collected, 30th November 1967) were paired with two field collected adult males. The two pairs were confined to two petri dishes lined with damp filter paper with a sprinkle of sand beneath. The two cultures were examined weekly by washing the contents of the two petri dishes onto a 52 mesh sieve. Following this process fresh filter paper and sand were added, adults replaced and fresh carrot given as food supply. Copulation was first noted ten days after the addition of males (i.e. approximately 24 days after emergence). One female began laying eggs at five weeks after emergence and laid the following number of eggs weekly (beginning at fifth week): 4, 7, 8, 4, 6, 4, 3, 4, 2. Eggs were deposited singly amongst the sand grains. Both male and female died from a fungus infection in the 16th week after female emergence. The second female began laying at the seventh week and laid the following number of eggs weekly: 4, 7, 7, 6, 2, 1, 2, 0, 1, 0, 0, 0. This female died, also from a fungus infection, 21 weeks after emergence. The first female thus layed 42 eggs and the second female 30 eggs.

These figures indicate that, under laboratory conditions, there is a peak in oviposition rates about eight to ten weeks after female emergence. This peak is followed by a gradual decline.

(c) Depth of Oviposition.

During attempts to obtain eggs in the laboratory an opportunity was taken to observe the depth at which females laid their eggs.

Twenty males and twenty females were placed in a narrow container with sliding glass walls (2" apart and 10" long and 10" deep). The container was filled with damp sand, 52 mesh sieved, to a depth of eight inches. Carrot was supplied as food and the container topped with gauze. At three weeks, when the contents of the container were examined, the top inch of the soil had dried out in a sharply defined layer, but the remainder of the sand was still damp. The soil was removed in three layers and passed through a 52 mesh sieve. The top inch of the soil contained two eggs and 24 adults. At the one to three inch depth there were 29 eggs, two first instar larvae and seven adults while in the bottom five inches there were no eggs and only one adult.

These results thus support May's (1966) contention that in the field females burrow down to the damp sand to deposit their eggs.

(d) Oviposition periods in the field.

Oviposition periods in the field were assessed indirectly by counting the number of first instar larvae present at various times of the year. Unfortunately, due to a change in the extraction technique, results from the first four samples from December 1966 till 3rd March 1967 are invalid for assessing proportions of first instar larvae. In addition, samples five, six and seven are based on 16 cores, a number which gives only a crude estimate of population composition over that period of the year. Results are presented in Table 2 below.. (Table 1, Appendix 1 presents total numbers of larvae in different instars recovered from samples 1-11)

TABLE 2.

Percentage of first instar larvae present in samples five to eleven.

	5/4/67 s.5 16cores	6/5/67 s.6 16cores	17/6/67 s.7 16cores	10/8/67 s.8 80cores	24/9/67 s.9 80cores	14/12/67 s.10 80cores	4/5/69 s.11 60cores
Percentage of 1st instar larvae	25	3	0	0	50	17	33

A peak in numbers of first instar larvae occurred in late october. This is probably a reflection of the increase in soil temperatures that occured in early September (Table 5). This increase in temperature probably provided the stimulus for the arousal of adults from their winter quiescence to begin oviposition. Oviposition diminished in late November as evidenced by the smaller proportion of first instar larvae, and from subjective observations it is thought that it was also low during January to March when the soil was very dry. During late March and early April, oviposition appeared to increase again, probably due to the increasing rainfall of that period, and finally tailed off into May and June. It is probable that oviposition was either absent or occured very infrequently in the months late June to mid-August, a period during which the adults were in a quiescent state.

It must be emphasized that this is an indirect method of assessing oviposition periods and that it is subject to the possibility that the early spring peak of first instar larvae may originate from eggs that had undergone a winter diapause which was broken by the rise in spring temperatures. However, since adults collected from the field during winter began laying eggs after being exposed to the warm temperatures of the laboratory, it is probable that at least part of this early peak of first instar larvae is due to a spring oviposition.

3. Adult Nutrition

It was found that adults were polyphagous in the laboratory. They consumed the following foods in rough order of preference: 'catsear', carrot, cabbage, lettuce, 'Hawksbeard', spinach, radish seedlings, tomato, lucerne seedlings, white clover stems and leaves, bread, ryegrass, young and mature lucerne leaves. The main preference appeared to be one for succulence rather than any one particular food plant. The white clover, lucerne leaves and ryegrass were eaten only with reluctance. As adults in the field are found clustered beneath the flatweeds 'catsear', 'hawkbit', and 'hawksbeard', it is probable that these plants are the main adult food supply, especially in summer when they are the only common succulent plants which survive the summer dryness.

4. Adult Behaviour

Otiorrhynchid adults are typically nocturnal insects, being most active just after dusk (Van Emden, 1952). In the laboratory adults of *C. setigera* crawled about foliage and up the walls of the container if they were kept in a dark cupboard. On being exposed to bright light however, the majority immediately climbed down and burrowed into the sand, leaving only an occasional individual on the surface. The few weevils remaining on the surface were generally actively feeding on carrot.

During the day adults in the field typically bury themselves just beneath the surface of the sand, usually beneath or among the spreading leaves of flatweeds. They emerge to feed at night. The nocturnal habits of Cecyropa setigera were the subject of a brief investigation in the field on the 14th April, 1967.

This investigation consisted of recording weevil activity in a lucerne crop over one night. Activity was gauged in two ways: (i) 17 carrots were split longitudinally in half and placed on the ground at the base of lucerne plants. The number of weevils visible on the flat surfaces of the carrots were counted at intervals during the night. (ii) Two hundred sweeps with a net were made through the foliage of the lucerne crop at each of the observation periods. Different transects of the paddock (which was uniformly flat) were taken at each period. The contents of the net were placed in plastic bags and weevils removed by hand sorting in the laboratory. Air temperatures were recorded during the night. Dusk began falling at 6 p.m. and it was fully dark by 7 p.m. First light was noted at 5.15 a.m. and it was fully light at 6.15 a.m.

Results are summarized in Table 3 below.

TABLE 3. Number of weevils taken at intervals over one night

Time	Nos. on carrots	Nos. from sweeps	Air temp. °F.
5pm.	22	0	50
6.15	38	7	49
7.15	6	44	45
8.30	8	79	43
9.30	12	58	42
10.30	22	59	41
11.30pm.	18	54	40
12.30am.	21	36	39
1.45	18	20	39
2.30	22	12	38
4.30	33	3	41
5.30	37	2	33
5.45	33	not taken	34
6.15	11	0	32
6.45	2	not taken	31
7.00am.	1	0	31

From a study of this table and from observations during the night, the following course of events is suggested. Firstly, the weevils emerged at dusk, possibly on the stimulus of a decrease in soil temperatures. The emergence was reflected in the build up of numbers on the carrot at ground level. The weevils then climbed on the lucerne plants and migrated upwards. This is reflected by the decrease in the numbers of the carrot and an increase in the number of weevils taken from the sweeps of the lucerne foliage. This situation apparently remained more or less static until 12.30 a.m. when a number of weevils began to descend. This descent continued through the night as evidenced by the decrease in numbers on the foliage. Finally with the stimulus of the brightening sky the adults burrowed down into the surface sand or hid beneath flatweeds. This is reflected in the decrease in numbers on carrots over the period 4.30 to 7 a.m. The sudden drop in temperature from 4.30 to 5.30 am. apparently had little to do with the regulation of weevil activity since the weevil numbers on carrots did not drop appreciable until the period 5.45 - 6.15 a period at which the light intensity was rapidly increasing. It is of interest to note that whereas copulation was observed at intervals all through the night, most couples broke their association at the onset of dawn, although one couple was observed in copulo at 6.45 am. when temperatures were one degree below freezing!

From these observations it is evident that the major period of activity for adults of Cecyropa setigera occurs during the night. It is regretable that, despite an assiduous search by torchlight, no adults were actually observed on the foliage of the lucerne plants. It is possible that the adults ascended to feed on the young lucerne shoots but this is difficult to reconcile with the reluctance of adults to feed on either lucerne shoots or mature leaves in the laboratory, and it should be noted that more observations are needed to verify the suggested pattern of nocturnal behaviour.

5. Natural enemies of Cecyropa setigera.

(a) Eggs

Eggs were attacked in the laboratory by two fungi, Fusarium sp, and Metarrhizium anisopliae.

(b) Larvae

(i) Fungi. In the field large numbers of dead larvae were found that had been attacked by the two Hymenostible fungi discussed in Part II of this thesis. Metarrhizium anisopliae was observed on dead larvae in the field.

(ii) Nematodes. The following nematodes were recovered from field collected dead larvae of C. setigera and three other insect larvae.

TABLE 4. Species of Nematodes recovered from dead larvae of C. setigera and three other insects.

Date	Sample	Larval instar	Nematodes
10th Aug.	8	4th	Mermithid & Neoaplectana sp. (juveniles)
24th Oct.	9	6th	Neoaplectana sp. (females)
"	9	6th	Juvenile Rhabditid*
"	9	6th	Neoaplectana sp. (females)
"	9	4th	Juvenile Rhabditid*
"	9	6th	Rhabditid (female)*
"	9	5th	Alloionema ? juveniles*
"	9	Larva Costelytra zealandica.	Rhabditid females*
"	9	Larva Phlyctinus callosus.	Mermithid
"	"	Larva Graphoghathus leucoloma.	Rhabditid*

* these were probably saprophytic upon hosts already dead.

(c) Pupae.

(i) Fungi. Pupae attacked by Metarrhizium anisopliae and the two Hymenostilbe fungi were recovered from the field.

(d) Adults.

(i) Fungi. Adults in the laboratory were attacked by both Metarrhizium anisopliae and Beauvaria sp.

(ii) An hymenopterous parasite. Of 41 adults collected on the night of March 25th, 1968, 30 were subject to parasitism from an hymenopterous parasite. The first hymenopteran adults emerged on the 30th March and emergence continued till 11th April. Specimens were sent to Mr. E. Valentine, D.S.I.R. for identification but were damaged and Mr. Valentine could only suggest that they appeared to be in the subfamily Euporinae (Braconidae) and very near, if not in fact, the genus *Perilitus*.

F. SPATIAL DISTRIBUTION OF THE LARVAL POPULATION

Both vertical and horizontal distributions of larvae were investigated during the course of the sampling series in pasture near Himatangi.

1. Description of the Habitat.

The map reference of the field sampling site is given in Appendix 1 together with the materials and methods employed in sampling and extraction of larvae. The sampling site was situated on a pasture-covered sand plain which was characterized by small undulations. The difference between the highest and lowest elevation of these undulations was about four feet. In the winter of 1967 the water table rose to flood the low portions of the paddock for several days, but the sandy soils of the hummocks remained comparatively dry. 1/

The pasture cover varied with the elevation and the season. In the low elevations, subject to winter saturation, Yorkshire fog and cocksfoot predominated but at intermediate elevations this gave way to crested dogstail, ryegrass, subclover, some danthonia, and the flatweeds, catsear (*Hypochoeris radicata*) hawksbeard (*Crepis capillaris*) and hawkbit (*Leontodon taraxicoides*).

1/ Where subsequent mentions are made of low elevations they refer to the lowest portions of the paddock that become very wet in winter; medium elevations are those approximately two feet above the low elevations, while high elevations refer to the dry upper levels of hummocks approximately two feet above those of the intermediate levels.

At the highest and driest elevations, the plant cover was predominately a mixture of moss, Danthonia, crested dogstail, some ryegrass and a large number of flatweeds. Over the summer period the sandy soil dried out severely, particularly at the intermediate and upper levels where the flatweeds which possessed a very deep rooting system, were the only common green plants.

The soils of the sampling site are classifed as Hokio strongly mottled sand (Cowie and Smith 1958) and are part of the Hokio - Waitarere association consisting of Hokio soils on the sand plains and Waitarere sand in the dunes. (Cowie, Fitzgerald and Owens, 1967). Cowie and Smith describe the Hokio strongly mottled sand as a "weakly gleyed soil developed on low rises on the sand plains of the younger dune complex. The water table is lower than Hokio sand and during summer there is insufficient soil moisture to maintain high quality pasture growth." A typical profile of this soil is (after Cowie and Smith 1958):

0 - 2 inches - black to very dark brown sand, very friable, weakly developed fine granular structure, boundary abrupt.

2 - 5 inches - brown to light brown sand, extremely friable, loose.

5 - 10 inches - light grey, loose, single-grained sand with abundant, distinct, medium yellowish-brown mottles.

10 inches and below - grey, compact sand with a few distinct dark red mottles.

2. Changes in the soil environment over the year.

Both soil moisture and soil temperature were measured over the year. Soil temperatures were taken at the surface and at three, and ten inch depths using an ordinary laboratory mercury thermometer pushed several inches into the wall of a hole dug in the sand. Soil temperature changes at monthly intervals are presented in Table 5 . Soil moisture levels were measured at five depths: $\frac{1}{2}$, 3, 5, 10 and 14 inches. Samples for soil moisture determinations were taken with glass tubes (3 x $\frac{3}{4}$") that were scraped against the wall of the hole at the required depth until they were full of sand. Moisture levels were assessed in the laboratory by measuring the weight loss of these samples after drying for 24 hours at 110°C. Soil moisture levels are expressed as a percentage loss of weight resulting from the water loss on drying. Soil moisture levels over ten samples, taken at various times of the year and at each of the three elevations; low, medium and high, are presented in Table 6 .

3. Vertical Distribution of Larvae in the Soil

The vertical distribution of larvae in the soil was assessed from the combined results of the first ten samples. In each of the ten samples 16 cores were removed in five layers at depths of 0 - 2, 2 - 4, 6 - 10, and 10 - 14 inches. These were sieved separately and results noted for individual depths. Table 7 presents the combined results for the distribution of different larval instars over ten samples.

TABLE. 5 Soil temperatures of Hokio strongly mottled sand at four depths over twelve months (taken in the first week of each month)

Months (1967)	Depth (inches) 0	3	10
January	28°C.	22°C.	21°C.
February	24	22	22
March	20	19	19
April	18	17	16.5
May	18	15	14
June	11	9	9
July	8	7	7
August	8	7.5	8
September	14	13	13
October	17	15	14
November	21	16	15
December	33	21	20

TABLE 6 Moisture content (% loss in weight on drying) of Hokio strongly mottled sand at low, medium and high elevations over ten sampling periods at five depths.

Sample 1 23/12/66	Low.	Med.	High
A	21.1	2.7	1.2
B	9.7	1.5	1.3
C	7.1	1.4	1.0
D	11.7	2.5	0.9
E	15.3	3.2	0.9

Sample 2 15/1/67	Low.	Med.	High
A	21.0	8.5	4.8
B	6.6	3.4	1.4
C	6.3	3.9	0.8
D	14.6	3.6	3.3
E	13.0	10.3	2.8

Sample 3 6/2/67	Low.	Med.	High
A	27.1	9.9	2.1
B	9.3	7.4	0.5
C	17.7	5.9	1.3
D	19.0	6.8	1.9
E	17.0	12.5	3.5

Sample 4 2/3/67	Low.	Med.	High
A	29.2	11.9	3.2
B	24.6	2.4	1.1
C	11.3	4.7	1.1
D	11.6	4.6	2.4
E	14.4	5.8	3.7

Sample 5 5/4/67	Low.	Med.	High
A	14.3	6.4	3.3
B	7.3	1.4	1.2
C	5.7	1.7	0.8
D	7.7	2.1	2.6
E	9.6	5.0	2.9

Sample 6 6/5/67	Low.	Med.	High
A	10.6	10.2	1.9
B	4.4	5.1	1.9
C	4.0	4.7	1.8
D	4.2	4.2	1.8
E	4.9	4.1	2.5

Sample 7 17/6/67	Low.	Med.	High
A	19.2	6.9	8.1
B	11.4	3.7	2.8
C	5.2	6.3	2.1
D	5.6	4.2	3.5
E	7.8	4.5	3.5

Sample 8 16/8/67	Low.	Med.	High
A	25.6	18.4	7.1
B	20.2	9.2	1.7
C	8.9	8.9	2.3
D	7.3	6.8	6.3
E	10.7	8.1	7.5

Sample 9 24/10/67	Low.	Med.	High
A	26.3	7.4	1.8
B	14.5	5.5	1.0
C	11.4	6.8	0.7
D	14.1	6.2	1.0
E	16.4	8.0	3.3

Sample 10 4/4/68	Low.	Med.	High
A	27.6	6.2	2.3
B	23.6	2.5	0.5
C	14.2	2.7	0.6
D	7.0	2.2	0.9
E	8.3	4.1	1.7

Level A - 0 to 2"
Level B - 2 to 4"
Level C - 4 to 6"
Level D - 6 to 10"
Level E - 10 to 14"

TABLE 7 The distribution of larval instars at five successive depths.

Depth (inches)	Instars 1	2	3	4	5	6	Total no. of larvae.
0 - 2	11	9	2	2	3	0	27
2 - 4	1	3	11	10	13	2	40
4 - 6	0	2	8	13	9	21	53
6 - 10	0	0	1	2	13	7	23
10 - 14	0	0	1	0	5	6	12

Two features are evident from a study of this table. Firstly, most larvae are found in the two to six inch zone. Secondly, first instar larvae are found almost exclusively in the 0 - 2 inch level and there is a general trend for larvae to move downwards with increasing age.

From an examination of the soil moisture and temperature characteristics of the various depths (Tables 5.6) it can be seen that the four to six inch level, where most larvae occur, is characterized, at all periods tested, by generally having the lowest soil moisture level of any of the other depths from 0 - 10 inches. Larvae at this level would thus have the least chance of dying from asphyxiation due to saturation of the soil with surface moisture from heavy rain or in the medium and high elevations from the rising water table of winter. Also soil temperatures in this region do not display the seasonal variability characteristic of the upper level, while plant roots occur commonly down to the ten inch depth. Also the soil is generally of a loose single grained structure at the 4 - 6 inch depth permitting easy larval movement.

The restriction of first instar larvae to the upper soil horizon further supports the laboratory observations that females actively seek out damp sand to deposit their eggs, since over all periods of the year the soil moisture content in the 0 - 2 inch zone is higher than that of all other levels down to ten inches. This is due to the high organic matter content of this horizon which retains a large amount of moisture. Thus in being laid in the upper horizon, the eggs stand the least chance of being subject to desiccation while in addition they are in a zone where there is a plentiful supply of roots and rootlets as a food source. The tendency for the final instar larvae to burrow deeper into the soil probably represents the fact that there is generally some moisture (from the water table) at this level over all periods of the year. Thus the immobile pupae would stand the least chance of being subject to dessication if they inhabited the lower levels.

It is thus suggested that the general preference shown for this level is a reflection of the relatively stable soil environment in this zone connected with adequate food supplies and a soil structure that permits easy larval movement to find new food sources or to avoid unfavourable conditions.

4. Horizontal distribution of larvae in the field.

The stimulus for this study of the major factors affecting the horizontal distribution of larvae in the sand plain pasture was provided firstly by the realization that the small numbers of larvae collected in samples five, six and seven were inadequate to reflect larval population changes and secondly by an examination of larval distribution characteristics, which revealed that larvae were exhibiting a clumped or contagious distribution. Clumped distributions are of the negative binomial type where the intensity of clumping can be expressed in terms of the dispersion parameter 'K' (Southwood, 1966). A usual value of K, indicating moderate clumping is two. Values above eight indicate a distribution approaching randomness while the smaller the value of K, the greater the amount of clumping. The K values of samples one to seven, as estimated by $k = \frac{\bar{x}^2}{s^2 - \bar{x}}$ were as follows (in order): 1.74, 0.36, 0.31, 0.71, 0.23, 0.54, 0.67. It was obvious that there was a considerable degree of clumping in the larval populations of C. setigera. Accordingly, in sample eight the number of cores was increased from 16 to 80 to increase the numbers of larvae taken and as it had been noted that the larval population appeared to be at its greatest density in the higher elevations of the paddock, each of the 64 additional cores was subjectively scored for elevation; either low, medium or high, as defined earlier. The method of sampling for the 64 additional cores is explained in Appendix 1, but briefly it consisted of taking a series of transects across the paddock along which samples were taken every 15 yards.

The mean numbers of larvae per core in sample eight, taken at each of the three elevations, low, medium and high were (in order): 0, 0.36, 1.32. A single factor analysis of variance on the population density at three levels is presented below in Table 8. A log x + 1 transformation was used.

TABLE 8 Analysis of variance on the number of larvae at three elevations (Log x + 1 transformation)

Analysis of Variance

Source	d.f.	S.S.	M.S.	F test
Elevations	2	0.69	0.345	7.97**
Error	61	2.64	0.043	
Total	63	3.33		

There was a highly significant difference ($P<0.01$) between the larval population densities at the three elevation categories. On a comparison of the means it was found that the population at the high elevation was significantly greater ($P<0.05$) than that at either the medium or the low elevations but that the density at the medium elevation was not significantly different from that at the low elevation.

It is suggested that the complete absence of larvae in samples recovered from the low elevation was directly due to the effects of winter flooding as at the time of this sample the soil in this level was saturated and two weeks previously had been under several inches of water. Survival of larvae under such conditions is unlikely. High moisture contents also probably had a similar effect on the populations at the intermediate levels but it must be noted that there is the possibility that the lower larval density at this elevation could be an indirect reflection of the effect of water content on the distribution of preferred host plants.

These observations on the preference of larvae for regions of higher elevation were further strengthened by samples nine and ten. In sample nine the mean larval densities/core (in order of increasing elevations) were 0, 0.77 and 2.19 while in sample ten the mean larval densities/core of the intermediate and higher elevations were 0.37, and 0.77. (In sample ten, cores were not taken from the low elevation because of the previously demonstrated absence of larvae.)

Although these results cast light on the gross distribution characteristics of the larvae they did not satisfactorily explain the high degree of clumping exhibited. In samples one to ten, all cores had been scored for percentage plant cover. However on analysis this was found to be an inadequate method for assessing the preference of larvae for various plant species. No correlations were evident and Whittaker and Fairbanks' Index of Association (Southwood, 1966) failed to reveal any clear associations between larvae of <u>C.setigera</u> and any particular plant. However from observational data it was noted that larvae and adults appeared to be grouped under the flatweeds: catsear, hawkbit and hawksbeard. Accordingly on the 15th of April samples were taken from a different sampling site (Appendix 1) 6½ miles inland from Himatangi beach. This site was chosen because from previous spade observations it was thought that larval populations were high in this particular paddock.

The method of investigation involved taking 30 cores in which one edge of the four inch diameter soil corer was placed over the centre of a large flatweed or group of flatweeds and a core taken to a depth of 12 inches. The remaining 30 cores were taken in the same area but at sites which were at least 18 inches distant from the nearest flatweed. A comparison of the numbers of larvae of C.setigera and other insects under flatweeds and not under flatweeds is presented in Table 9 below.

TABLE 9 A comparison of the numbers of larvae of C.setigera and larvae of other insects under flatweeds and not under flatweeds.

Species	Under flatweeds		Not under flatweeds	
	No.	No./core	No.	No./core
C. setigera	126	4.2	29	0.97
Graphognath us leucoloma Boheman	280	9.3	123	4.1
Phlyctinus callosus Boheman	173	5.8	9	0.3
Listroderes sp.	0	0	4	0.13
Grassgrubs	17	0.56	6	0.2
Phycocus lobatus Broun (adults)	122	4.07	21	0.7
Desiantha maculata Boheman	11	0.33	3	0.08

(A complete list of Coleoptera recovered in all of the eleven samples is presented in Appendix 1B)

From this table it is evident that larval populations of Cecyropa setigera display a marked clumping under flatweeds. (It was also observed that the distribution of dead larvae infected with the Hymenostilbe fungi followed a very similar pattern of clumping.)

The association of the larvae of Cecyropa setigera with flatweeds illuminates the observation of an increase in the density of larvae at the higher elevations of the sand plain. It has been shown previously that larvae do not inhabit the lower elevations of the sand plain pasture. This is probably due to the saturated and sometimes flooded conditions over winter and early spring. Thus the larvae are confined to the medium and high elevations. However, these elevations dry out severely over summer and larvae and adults must find some source of food and moisture to survive. Flatweeds, the most common of which is catsear, provide these requirements. The extensive and deep root systems of these plants enable them to penetrate the sand to sufficient depths to reach the moisture. Thus larvae, in being clumped under these plants, are supplied not only with food in the form of roots but also with moisture absorbed through the roots from the water below. In addition, flatweeds supply shelter to adults of C.setigera which burrow into the sand beneath the spreading leaves, and from the laboratory observations, it is suggested that they also serve as a source of food for the adult. The close association of larvae with the flatweeds of the sand plain of pastures is thus the basis for the strong degree of clumping evident in the distribution of the larval populations of C.setigera.

G. GENERAL DISCUSSION

In habits and biology Cecyropa setigera has proved similar to other Otiorrhynchid weevils. From a comparison of the biology of C. discors with that of C.setigera it is evident that the two weevils display a close similarity and that the correct identification of the larvae described by May could in fact be C.setigera.

The area of major interest in this study was found in the investigation of factors affecting the spatial distribution of Cecyropa setigera in pastures of the sand plain. The vertical and horizontal distributions characteristic of the larval population of C.setigera can be regarded as a response to the dominating environmental influence of changing soil moisture levels at various times of the year.

The vertical distribution of C.setigera in the soil reflects the varying capacities of different soil horizons to provide conditions most suitable for the survival of the different stages in the life cycle. The adults of C.setigera deposit their eggs in the top two inches of soil, a region in which moisture retention is greater than at any other depth, because of the large quantities of raw organic matter present in this horizon. In view of the clumped distribution of both adults and larvae and the necessity for an easily available food source for first instar larvae, it is probable that oviposition takes place beneath the flatweeds. After hatching the larvae exhibit a downward movement through the soil with increasing age, to the more stable but drier environment of the four to six inch zone. Both food and moisture are available from the deep and extensive root systems of the flatweeds. The greater proportion of final instar larvae at the deep levels probably represents a selection of this environmentally stable yet moist zone for pupation.

The clumped horizontal distribution of C.setigera is a reflection of the changing soil moisture conditions of the sand plain, which by winter saturation of the low lying areas and summer drought of the higher elevations, favour the close association of larvae with the deep rooted flatweeds. It is also evident that the distribution of other soil inhabiting Coleoptera is similarly affected.

As weevil species are the most numerous of the injurious Coleoptera in pastures of the sand plains the demonstration of this association with pasture flatweeds is probably the most important single point discovered in the sampling programme. Large areas of the Manawatu sand country are characterized by open pastures containing large numbers of flatweeds. These pastures thus supply a multiplicity of suitable sites for the maintenance of high weevil populations and as farmers generally select the poorer weedy pastures for cropping the chances of damage to the seedling stages of crops becomes correspondingly high. The association demonstrated thus opens the possibility of reducing weevil numbers in pastures by controlling the plant host. This control could be effected by improved pasture management techniques or by the employment of weedicides and in being an indirect cultural method would diminish the need for the application of insecticides with their attendant residue problems.