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THE ALAN FLETCHER RESEARCH STATION BULLETIN No. 1

DIET-REARING THE LANTANA INSECT  
PLAGIOHAMMUS SPINIPENNIS (THOMS.)  
(COL. CERAMBYCIDAE)

by B. W. WILLSON, B.Sc.

SUMMARY

Difficulties in cage rearing *Plagiohammus spinipennis* on the weed *Lantana camara* made it necessary to develop a diet-rearing technique.

Observations in determining the quantity of food required, optimum frequency of medium changes, and the effects of continuous diet-rearing on the insect are discussed. Studies on larval development showed that two overlapping generations occurred, each with diapausing and non-diapausing phases. Diapause could be broken by holding the larvae at 10°C for 8 weeks. After diet-rearing for 6 years, no differences in fecundity, egg viability or adult longevity were found.

I. INTRODUCTION

The stem-boring beetle *Plagiohammus spinipennis* was collected in Mexico and shipped to Hawaii in 1954 as a potential biological control agent of the weed *Lantana camara* (Krauss 1962).

An unidentified insect which, from its description, appears to have been *P. spinipennis* (Harley 1969) was collected by Koebele in Mexico in 1902 and forwarded to Hawaii, but failed to become established (Perkins and Swezey 1924).

Harley and Kunimoto (1969) carried out supplementary host specificity testing in Hawaii during 1965-66. After this, it was shipped to Australia (Willson 1968).

Cage rearing of *P. spinipennis* on potted *L. camara* plants in Queensland was unsatisfactory because of high larval mortality caused by callus tissue formation at the site of attack. This tissue growth killed larvae before they were able to penetrate the xylem tissue. This reaction apparently does not occur in Mexico where Koebele reported that *L. camara* plants were usually very soft and knotty as a result of fungoid disease and were inhabited by numerous Cerambycid larvae (Perkins and Swezey *op. cit.*).

Because of the difficulties associated with rearing the insect in living host tissue, an artificial diet was developed (Harley and Willson 1968).

The purpose of the study reported here was to devise a suitable mass rearing technique for *P. spinipennis* by investigating its larval growth rate and development in this diet under varying conditions. The effects of continuous diet-rearing were also examined.

## II. MATERIALS AND METHODS

Eggs were excised from *L. camara* stems 6 days after oviposition and held on moist filter paper in petri dishes until larvae hatched 1 to 2 days later. These larvae were placed individually into 3.2 cm ID screw-capped glass jars containing approximately 3 ml of prepared diet as described by Harley and Willson (*op. cit.*). The diet was allowed to dehydrate at room temperature for 24 hours before use when small holes were cut in the medium to enable the larvae to penetrate. Larvae were transferred to increased quantities of diet at regular intervals with first and subsequent changes being made into 4 cm ID jars.

Unless otherwise stated, larvae were cultured at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ .

The optimum frequency of medium changes was determined by measuring the growth rate of three groups of larvae whose medium was changed at 2-week (Group A), 3-week (Group B) and 4-week (Group C) intervals respectively. All groups consisted of 20 randomly selected larvae, each given 12 ml of medium at the first and subsequent changes. The trial was replicated with larvae from the next generation and the means for each treatment calculated.

To test the hypothesis that food quantity and not quality was the limiting factor in growth rate of Group C, another group of 20 larvae (Group D), also changed at intervals of 4 weeks, was given 12 ml medium at the first change and 18 ml at subsequent changes. This test was run concurrently with the other replicates.

Larval development was observed using 80 randomly selected larvae each held in 12 ml of medium changed at 4-week intervals. Each ecdysis was noted and the widths of all cast head capsules measured by ocular micrometer.

The incidence of diapause was determined in 3 913 larvae throughout the course of the rearing programme from 1967 to 1973 under uncontrolled laboratory conditions. The duration of diapause was measured from the last larval weight gain until pupation.

To determine the effect on *P. spinipennis* of continuous rearing in an artificial diet for 5 years, six pairs of adults were studied at the beginning of the project in December–January 1967–68, and of 25 pairs at the conclusion in December–January 1972–73. Paired adults were held on potted *L. camara* plants under wire mesh cages in an insectary. Any males that died during the trial were replaced with males of similar age. All oviposition sites were marked and the date of oviposition noted, so that the larvae could be removed and recorded shortly after hatching.

## III. RESULTS

**OPTIMUM FREQUENCY OF MEDIUM CHANGES.** Mean weights of Groups A, B and C were similar until the eleventh week, after which the mean growth rate of Group C fell below that of the other two. Data subsequent to the ninth week are plotted in figure 1. Mean maximum weight of Group A occurred at the sixteenth week (992 mg), of Group B at the eighteenth week (1 104 mg) and of Group C at the seventeenth week (870 mg). The result of medium changes after the twelfth week is shown in figure 2.

In Group A, 50% diapaused before the eighteenth week and 50% of Groups B and C before the nineteenth week. Analysis of the data was not undertaken after this stage was reached because of the low numbers of insects still feeding. Larval feeding became sporadic before diapause and the mean weight declined.

This decline continued after the onset of diapause for the remainder of larval life so that on pupation 9 to 16 weeks later 23% of maximum body-weight had been lost. Further weight loss occurred between pupation and adult emergence. On emergence, males averaged 506 mg and females 688 mg.

QUANTITY OF MEDIUM REQUIRED. The mean growth rate of Group D was similar to that of Groups A and B and data obtained from this trial are plotted in figure 1.

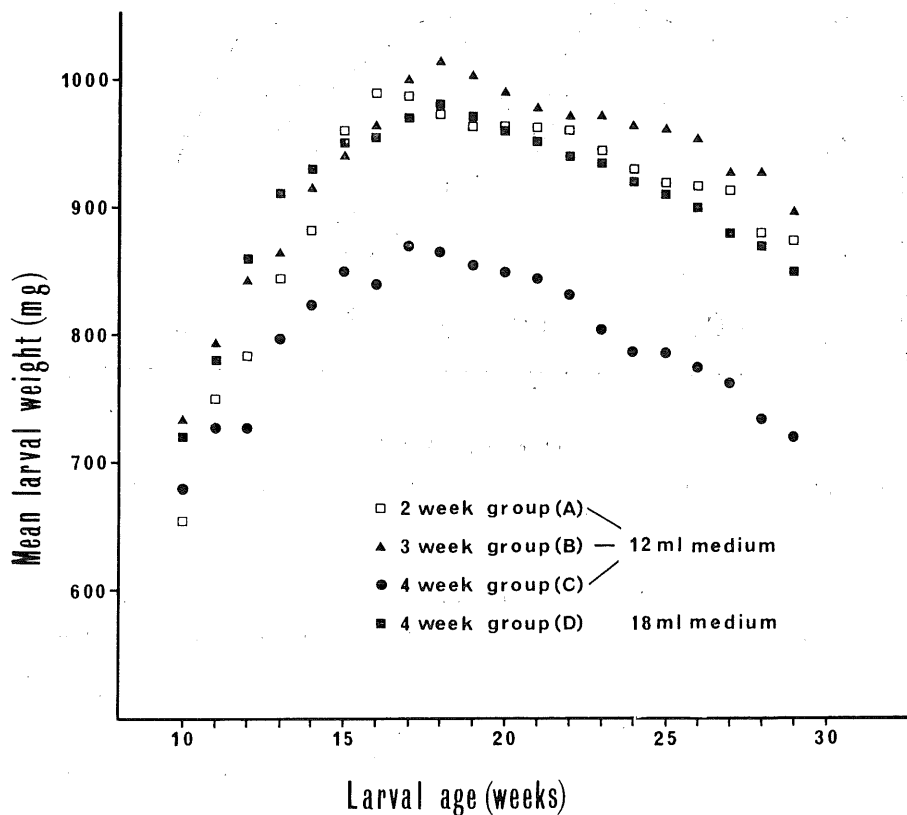


Figure 1.—Growth curves (from 10 weeks) of groups of *P. spinipennis* larvae given fresh medium at intervals of 2, 3 and 4 weeks respectively.

LARVAL DEVELOPMENT. Of the 80 larvae, 50 pupated after six instars, 24 after seven instars and 6 after eight instars. Seventy-two larvae diapaused during the sixth, seventh or eighth instar, the remainder pupated without diapause after the sixth or seventh instar. The duration of each of the instars 1 to 7 is given in table 1. Where diapause occurred in instars 6 to 8, instar duration was not measured.

A head capsule width frequency distribution histogram (figure 3) was constructed. The measurements of the first three instars were within discrete ranges, those of the fourth overlapped slightly with those of the fifth which, in turn, had a larger overlap with the sixth. The seventh and eighth instars overlapped completely.

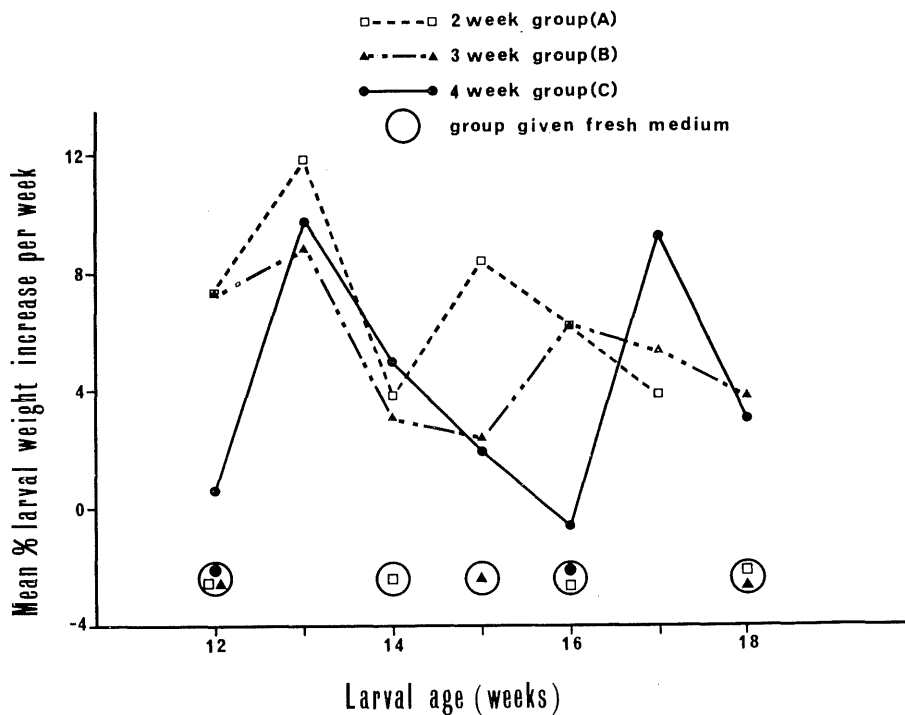


Figure 2.—The relation between frequency of medium-change and percentage weight increase per week in *P. spinipennis* larvae.

TABLE 1

INSTAR DURATION OF *P. spinipennis* HELD IN THE MERIDIC DIET OF HARLEY AND WILLSON (1968) AT 25°C ± 2°C

		INSTAR							
		1	2	3	4	5	6	7	8
Instar Duration (Days)	Mean	3.4	6.0	7.6	8.1	19.4	32.7*	38.1	†
	Range	2-4	5-8	6-10	7-11	11-27	28-40	31-49	
Age (Days)	.. ..	2-4	7-12	13-22	20-33	31-60	59-100	90-149	

\* Data from non diapausing larvae.

† No data as all larvae diapaused in this instar.

The mean head-capsule measurements for each instar were plotted (figure 4) and the line drawn through the first five points was the straight line of best fit calculated by the method of least squares. The equation so obtained  $\log_{10} y = 0.13851x - 0.24433$  gives a growth-ratio of 1.376 which is in agreement with Dyar (1890) who found that the widths of the head-capsules in 28 species of Lepidoptera increased in a regular geometrical progression in successive instars by a ratio of approximately 1.4. The sixth, seventh and eighth instars failed to follow this progression.

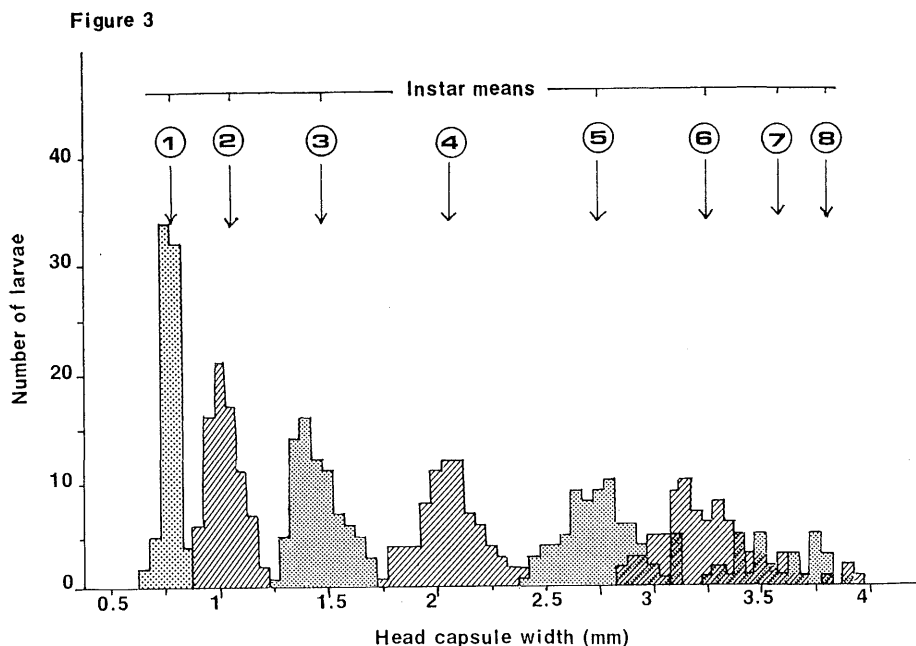


Figure 3.—Frequency distribution of head-capsule widths of successive instars of *P. spinipennis*.

Under uncontrolled laboratory conditions, diapause varied from 9 to 17 weeks with a mean of 13 weeks in the autumn generation and up to 28 weeks in the spring generation. All diapausing larvae pupated in spring.

Of the 3 913 larvae observed, 25% of the spring generation did not diapause and these provided the parents for an autumn generation of which 95% diapaused. It was found that cooling for 8 weeks at 10°C followed by a return to 25°C was sufficient to break diapause; pupation occurred 2 to 3 weeks later and all adults emerged by the end of the sixth week.

Gardiner (1970) found that a small percentage of the population of the Cerambycid *Graphisurus fasciatus* (De Geer) did not diapause and that diapausing larvae could be made to pupate much faster if held at just above 0°C for two months.

TABLE 2

EFFECT OF DIET-REARING ON THE FECUNDITY, EGG VIABILITY AND ADULT LONGEVITY OF *P. spinipennis*

	Mean No. eggs per female	% non-viable eggs	Adult longevity mean no. days	
			Male	Female
1967-68 .. .. .	84	<1%	63	77
1972-73 .. .. .	78	<1%	65	76

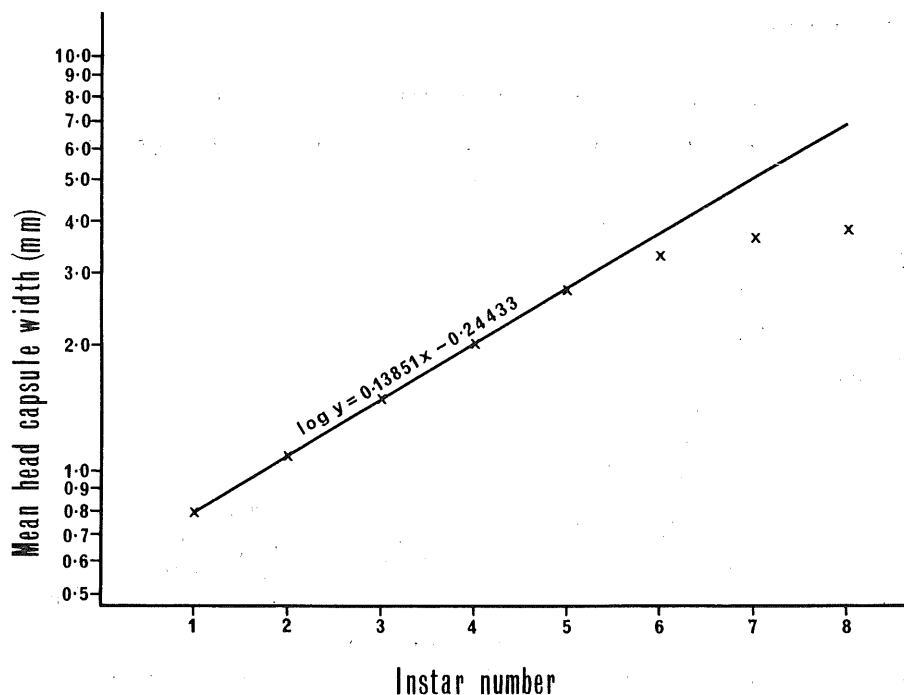


Figure 4.—The relation between instars and head-capsule widths of *P. spinipennis*. The regular geometrical progression between instars 1 to 5 is represented by the equation as shown and gives the growth ratio between these instars.

EFFECT OF DIET REARING ON ADULT LONGEVITY, FECUNDITY AND EGG VIABILITY. Fecundity, egg viability and adult longevity were similar in material from both 1972–73 and 1967–68. This is shown in table 2. Because of the small number of beetles available for rearing and field liberation, the number of insects observed in the earlier period was low, and statistical analysis of the figures was not practicable. In both test periods it was found that up to 35% of oviposition sites did not contain eggs. This preparation of sites but failure to oviposit occurred more frequently as insects aged.

#### IV. DISCUSSION

In considering any rearing technique, labour costs are usually a limiting factor. This was particularly so with an insect such as *P. spinipennis* which required individual handling. The longer the insect can be left undisturbed with an adequate food supply, the lower the food and labour costs. The need to provide a larger quantity of diet per larva before the twelfth week and at all subsequent changes was shown by the mean growth rate of Group D (larger quantity, less frequency) being very similar to that of Groups A and B (smaller quantity frequently) (figure 1). The growth rates of these groups were superior to that of Group C (smaller quantity, less frequently).

The importance of the frequency of medium change is shown in figure 2. In the week before the transfer of all groups at 12 weeks, Group C was experiencing a food shortage as the insects had consumed the medium around the margins of the jar but could not utilize the central core. During this period, Group C increased weight at a mean rate of 0.63% and the other two groups at a mean rate of 7.3%. This food shortage was again noted during the fifteenth week.

When given freshly prepared medium at 12 weeks, there was a rapid increase in mean percentage weight gain of all groups, and particularly of Group C. After 13 weeks, it again fell for all groups and did not rise until fresh medium was provided. As sufficient food was always available for Groups A and B, this indicates a nutritional loss by the food after 1 week. However, as both Group A and B were similar in weight after 30 weeks of larval life, it appears that, in this instance, any decrease of growth rate due to this nutritional loss was compensated by an increased growth rate when fresh medium was given.

This study indicated that medium changes every 4 weeks were satisfactory for larvae up to 11 weeks of age, after which they should either be changed every 3 weeks or given extra medium. The technique of Hadlington and Johnston (1974) whereby strips of prepared medium were cut and placed in 10 x 2.5 cm specimen tubes presumably avoids waste of the central core and could thus be more economical.

Leaving the insects in medium longer than 4 weeks increased the chances of its bacterial decomposition, unless time-consuming sterile techniques were employed. To avoid this, the technique adopted in the rearing programme was to change the medium every 4 weeks using a quantity of 3 ml initially then 12 ml and subsequently 18 ml.

The ability to break diapause enabled field-releases of adult beetles to be planned. With the spring generation, it was possible to obtain late summer releases and, in spring, instead of beetles emerging over a period of 8 weeks, all could be made to emerge within any 2- or 3-week period. By combining data from table 1 and figure 3, it was possible to identify larvae in their sixth to eighth instars. Such larvae were those over 60 days old with a head capsule width greater than 2.7 mm. By holding such larvae, both diapausing and non-diapausing, at 10°C for up to 6 months, releases were able to be planned to suit field requirements. Normally, however, larvae younger than 12 weeks were not forced to pupate.

The inability to identify the seventh and eighth instars by head-capsule measurements, and their failure to follow Dyar's 'Law', was probably the result of differences in growth rates between males and females. Adult males were found to weigh approximately 25% less than adult females on emergence.

Over 5 years of mass-rearing, the use of this artificial diet had no obvious effect on the fecundity, longevity or egg viability of the insect.

## V. ACKNOWLEDGEMENT

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The author is an officer of the Biological Section, Queensland Department of Lands, stationed at The Alan Fletcher Research Station, Sherwood, Brisbane, Q. 4075.