Evaluation of Two Formulations of A Laboratory Diet for the Orchid Weevil *Orchidophilus aterrimus* (Waterhouse)[1,2]

RONALD F. L. MAU[3] and PO-YUNG LAI[4]

ABSTRACT

A diet for laboratory rearing of the orchid weevil, *Orchidophilus aterrimus* (Waterhouse), was developed and evaluated. Evaluation of 2 formulations of the diet showed that both formulations were suitable for orchid weevil development. Evaluation of one formulation of the diet showed that it was suitable as a food for adult weevils.

The orchid weevil, *Orchidophilus aterrimus* (Waterhouse), is a serious pest of orchids in Hawaii (Champion 1913, Fullaway 1938, Swezey 1945, Waterhouse 1874). Adults feed on exposed plant parts while larvae feed within pseudostems. Weevil development in *Dendrobium* is completed in an average of 144 days (Mau 1983).

Since orchid growers apply pesticides regularly to kill the adult weevils, it is difficult to obtain adults in sufficient quantity for laboratory studies on insecticidal efficacy and residual activity and to conduct other research to develop better pest management methods. Although weevil cultures can be established and maintained on orchid plants in the greenhouse, this method is not very practical because of the cost of maintaining the stock plants. The development of a laboratory diet was viewed as an easier method of obtaining the necessary weevils for research purposes.

Many diets have been developed for rearing other species of weevils (Singh 1977). A review of the ingredients used in four of these diets was helpful in the formulation of our diet (Kovitavadhi and Kerr 1968, Power and Singh 1974, Shanks and Finnigan 1973, Toba et al. 1969).

We report here the results of and evaluation of two formulations of a diet in which we were able to rear larval and adult stages of the orchid weevil.

MATERIALS AND METHODS

Stock Cultures. Adults were collected from *Dendrobium* and *Vanda* orchids on Kauai and Hawaii. Since there are two species of *Orchidophilus*

[1]COLEOPTERA: Curculionidae

[2]Journal Series No. 3132 of the Hawaii Institute of Tropical Agriculture and Human Resources.

[3]Mailing Address. 3050 Maile Way, Honolulu, HI 96822.

[4]Chief, Plant Pest Control Branch, Hawaii Department of Agriculture. P.O. Box 22159, Honolulu, HI 96822.

in Hawaii, the collected weevils were identified using descriptions provided by Buchanan (1935). Only *O. aterrimus* adults were used in the study.

Stock cultures of the adults were reared in groups of 40-50 in unwaxed 235 cc paper containers covered with plastic lids. Fresh *Dendrobium* leaves were added at 2-3 day intervals. Eggs were collected from the leaves using a watchmaker's forceps and incubated on moist filter paper in covered petri dishes.

Preliminary Experiments. We first tested the pepper weevil diet described by Toba et al. (1969). Two batches were prepared. The first was the original pepper weevil diet and the second contained blended dendrobium leaves and pseudostems. Ten eggs were added to each rearing unit.

Chang and Jensen (1972) determined that sorbic acid and methyl-p-hydroxybenzoate (methyl paraben) inhibited larval growth of the sugarcane weevil, *Rhabdoscelus obscurus* (Boisduval). They speculated that the preservatives were either toxic to the grubs or affected the nutritive substances in the diet. We tested sorbic acid, methyl paraben, and formaldehyde separately to determine effects on larval survival. Ten eggs were placed on the surfaces of agar plates (6%) containing sorbic acid (0.25 g/100 ml agar), methyl paraben (0.4 g/100 ml agar), and 10% formalin (1 ml/100 ml agar).

Orchid Weevil Diet. The ingredients used in preparing the orchid weevil diets are provided in Table 1. They are listed in groups (A-E) in the order of their addition during preparation of the diet. Diet formulations were prepared as follows. Group A ingredients were blended and poured into an Erlenmeyer flask. For formulations 1 and 2 which included dendrobium, the Group B ingredient (succulent dendrobium pseudostems) were cut into 3-4 mm pieces, blended with distilled water, and added to the flask. The combined ingredients of groups A and B were mixed and sterilized in an autoclave at 121°C and 30 psi for 15 minutes. Dissolved ingredients of groups C and D were added after the sterilized formulation had cooled to 60-62°C and the mixture was mixed thoroughly. Formulation 2 contained an additional quantity of wheat germ and it became necessary to add 200 ml sterile distilled water after autoclaving to facilitate mixing and pouring. The liquid diet was poured into one ounce plastic containers (ca. 20 ml per container) (Dixie^{R} P01-10), covered with plastic lids and allowed to solidify. The rearing containers were stored in a refrigerator at 2°C until they were used.

Diet Evaluation. The surface of the diet in each container (rearing unit) was scarified with a clean, sharp instrument (ie. dissecting needle or forceps), and 5 eggs were placed within the grooves formed by the scarification. Benzyl benzoate was swabbed on the lip of the container and on the bottom of the lid to discourage mite entry and colonization. The rearing units were held in an environmental chamber at 26.7 ± 1°C and 12-hr-photoperiod.

Ninety-three rearing units containing diet formulation-1 and 106 rearing units containing diet formulation-2 were evaluated. The rearing

TABLE 1. Diet formulations for rearing *Orchidophilus aterrimus* larvae and adults.

Ingredients	Diet Formulations Basic	1	2
Group A			
Vanderzandt's wheatgerm diet	12.5 g	12.5 g	15.0 g
Agar	6.0 g	6.0 g	6.0 g
Cholesterol	0.05 g	0.05 g	0.5 g
Distilled water	100.0 ml	100.0 ml	80.0 ml
Group B			
Dendrobium pseudobulbs (Without leaves)	—	25.0 g	25.0 g
Distilled water	—	50.0 ml	50.0 ml
Group C			
Vanderzandt's vitamin mixture	3.2 g	3.2 g	3.2 g
Cysteine hydrochloride	0.1 g	0.1 g	0.1 g
Chlorotetracycline HCl (Aureomycin)	0.13 g	0.13 g	0.13 g
Streptomycin sulfate	0.15 g	0.25 g	0.15 g
Distilled water	25.0 ml	20.0 ml	20.0 ml
Group D			
Methyl-parahydroxybenzoate	0.15 g	0.15 g	0.15 g
Sorbic acid	0.15 g	0.15 g	0.15 g
Ethyl alcohol, 95%	—	5.0 ml	4.0 ml
Group E			
Sterile distilled water (added when autoclaved medium is too thick)	—	—	200.0 ml

units were observed at weekly intervals for larval feeding, adult emergence, and mite infestation. Mite infested units were discarded. Commencing with the first observation of adult emergence, the diet in remaining containers was dissected. Pupae were collected and larvae were placed back into tunnels in the diet. Observations for pupation and adult eclosion were made daily.

Emerging adults were collected and held in age groups according to the week of emergence. Pieces of dendrobium leaves were provided for food and oviposition at 3-4 day intervals. The leaves were examined for eggs upon removal from the adult rearing cages. The eggs were dissected from the leaves, placed on moistened filter paper in enclosed petri plates, and observed for eclosion.

Adult Diet Evaluation. Standard adult rearing practices required fresh dendrobium leaves for food and egg collection. Because dendrobium plants were slow-growing, many plants were needed to main tain laboratory colonies of the weevil, and this required extensive greenhouse space. Formulation B was evaluated to determine if it could be used in place of fresh leaves. Four field collected adults (2 of each sex) were placed in each of 5 rearing containers. About 20 pin holes were made in the lids for ventilation. The adults were transferred to fresh rearing units once every 2 months.

The formulation was also evaluated to determine the oviposition rate of weevils reared on it. Two pairs of field collected, gravid weevils were placed in each of 20 rearing containers containing ca. 5 ml of diet. The weevils were transferred to fresh rearing units at weekly intervals for 6 weeks. Containers of the used diet containing eggs were stored in a refrigerator until the experiment was completed. The diet was liquified by heating, and eggs were collected by washing the liquified diet through a 70 mesh sieve.

RESULTS

Preliminary Evaluation. There was high mortality of first instar larvae in both the standard and modified pepper weevil diets. There was no larval development beyond the second instar, and it was obvious that these diets were unsuitable for rearing orchid weevil larvae.

All larvae on agar plates containing sorbic acid and methyl paraben died soon after hatching. There was no tunneling in the agar. Grubs in the formalin and check treatments lived for 2-3 days and tunneled extensively during this time. Although the sorbic acid and methyl paraben were found to be toxic at the rates tested, they were used because of possible health hazards associated with use of formalin. The rates of sorbic acid and methyl-p-hydroxybenzoate were arbitrarily reduced to 0.15 g/100 ml in later diets which were tested.

Preliminary testing of the orchid weevil diet (Table 1) was conducted to determine whether orchid tissue was required. The major difference between the basic diet and diet formulations -1 and -2 was the addition of dendrobium tissue in the latter two formulations. Five eggs were placed in each of 12 basic formulation and 26 formulation-1 rearing units. The units were examined one month after egg placement, and although there were no discernable differences in feeding activity (eg. tunneling), the larvae in the formulation-1 group were visibly larger. Adults were reared from 67% and 83% of the basic and Diet A rearing units, respectively. The sex ratios (male:female) were 1:2.5 from the basic diet formulation and 1:1 from formulation-1.

Orchid Weevil Larval Diet. Since preliminary testing of the orchid weevil diet showed that addition of dendrobium tissue was desirable, another diet formulation (formulation-2) was evaluated to determine whether additional wheat germ would enhance larval development.

Ninety-three rearing units containing formulation-1 and 106 units containing formulation-2 were evaluated. One hundred and fifty-eight days after egg placement, 6 and 10 adults were found in the formulation-1 and formulation-2 diets, respectively. Although the actual dates of eclosion of these 16 adults could not be determined, we were able to determine larval to adult periods for all remaining adults reared from both diet formulations.

There were no significant differences in the developmental periods which could be attributed to the diets. The mean larva to adult period of

the remaining adults was 182 days (n=51; SD=24) for diet formulation-1, and 185 days (n=61; SD=26) for formulation-2. Adult production among the two diets was similar. Adults were reared from 61% of the formulation-1 rearing units and from 58% of the formulation-2 rearing units. The sex ratios (male:female) were 1:0.97 and 1:0.91 from formulations -1 and -2, respectively.

Another attribute which could be used to evaluate diet suitability is pupal weight. We found no differences in the weights of the pupae. The mean weights of male pupae from diet formulations -1 and -2 were 25.9 (n=13; SD=4.8) and 22.6 (n=7; SD=3.5) mg, respectively. Mean weights of female pupae from the same groups were 19.8 (n=11; SD=3.3) and 19.0 (n=4; SD=3.2) mg, respectively.

As could be expected from the pupal weight results, the adults reared from the two diet formulations did not differ significantly in size. Measurements (L=length; W=width across pronotum) of males were: diet formulation-1, L=6.1 ± 0.3 and W=2.5 ± 0.2 mm (n=15); diet formulation-2, L=5.8 ± 0.4 and W=2.4 ± 0.2 mm (n=9). Female measurements were: formulation-1, L=5.4 ± 0.3 and W=2.1 ± 0.1 mm (n=13); formulation-2, L=5.2 ± 0.2 and W=2.1 ± 0.2 mm (n=13).

Other biological attributes which are useful in evaluating suitability of artificial diets are fecundity and egg viability. We determined the fecundity of females reared from the two diets and the results are presented in Fig. 1. Between the 4th and 14th week, each female reared from Diets 1 and 2 laid an average of 4.7 ± 3.2 (range 0.8 to 9.7) and 4.3 ± 2.3 (range 0.3 to 6.6) eggs per week, respectively. An average of 97% of the eggs laid by formulation-1 females and 96% of eggs laid by formulation-2 females hatched.

Adult Diet Evaluation. The experiment showed that Diet 2 could be utilized as a general laboratory diet for orchid weevil adults. Eighty percent of the females and all of the males reared on the laboratory diet were living at the end of the six month test. A separate 6-week study showed that females readily oviposited in the diet. These females laid an average of 1.7 ± 0.7 eggs per week. In comparison, the egg laying average was ca. 1 egg per week less than reported by Mau (1983) for females reared on a diet of dendrobium leaves.

DISCUSSION

Larval Diets. Our results also showed that there were no obvious differences in suitability of diet formulation-1 and -2. In general, adults reared from the diets were slightly larger than those collected in the field. Field collected males and females averaged 4.8 and 4.4 mm in length, respectively (Mau 1983). Furthermore, females that were reared from the diets laid ca. one egg per week more than the field collected females studied by Mau (1983).

It was interesting to note that the sex ratio (male:female) of adults reared from the orchid weevil laboratory diet was similar to that reported

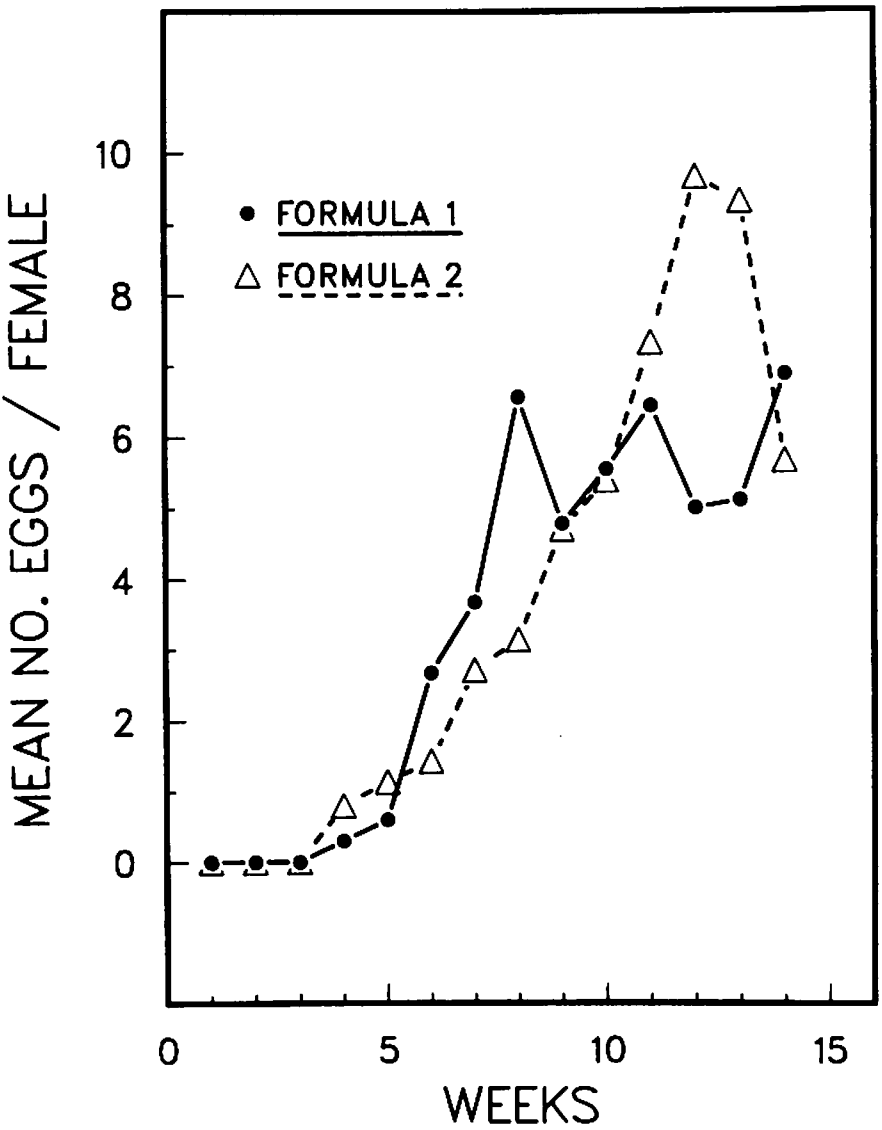

FIGURE 1. Fecundity of *Orchidophilus aterrimus* females reared on 2 formulations of semi-artificial laboratory diets.

by Mau (1983) for field collected adults (1 male: 1 female), but differed with the 1:0.4 sex ratio obtained by Mau (1983) for weevils reared from dendrobium plants. Nonetheless, the adult sex ratio obtained using the laboratory diet is preferred to that obtained in rearing the weevil in dendrobium plants.

Although the biological attributes of weevils reared from the laboratory diet compared favorably with those of weevils reared on dendrobium plants by Mau (1983), the diets were less than adequate in two respects. Larval to adult development in the laboratory diet was approximately 1 month longer than in dendrobium. In addition, many of the larvae did not complete their development. There was only a 60 percent success rate in rearing larvae to adults using the diet. Further improvement of the diet is obviously needed to make laboratory rearing of the orchid weevil more efficient.

Adult Diets. One of the problems encountered in studying orchid weevils was the necessity for having a stock of orchid plants available for rearing the adult weevils. Since dendrobium leaves were used to rear adults and for oviposition, it is possible to completely defoliate the plants in a short period of time.

Our results showed that diet formulation-2 was suitable for maintaining laboratory cultures of orchid weevil adults. Although adult females oviposited readily into the surface of the diet, but it was a little difficult to find the eggs. An easy method for extracting eggs from the diet is needed if collection of the eggs is required.

REFERENCES CITED

Buchanan, L. L. 1935. A new genus and species of orchid weevils (Coleoptera: Barinae). Proc. Hawaii. Entomol. Soc. 9: 45-48.

Champion, G. C. 1913. *Acyrthopeus* (*Baridius*) *aterrimus*, D. Waterh., in the orchid-house at Kew. Entomol. Mon. Mag. 24: 33.

Chang, V. C. S. and L. Jensen. 1972. A diet for studying clonal resistance of sugarcane to the New Guinea sugarcane weevil. J. Econ. Entomol. 65:1197-99.

Fullaway, D. T. 1938. Orchid insects. Proc. Hawaii. Entomol. Soc. 10: 45-9.

Kovitvadhi, K. and S. H. Kerr. 1968. Artificial diet for the zoysia grass billbug, *Sphenophorus venatus vestitus* (Coleoptera: Curculionidae), and notes on its biology. The Florida Entomol. 51: 247-49.

Mau, R. F. L. 1983. Development of the orchid weevil, *Orchidophilus aterrimus* (Waterhouse). Proc. Hawaii. Entomol. Soc. 24: 293-97.

Power, R. J. B. and P. Singh. 1974. Laboratory rearing method for the stem weevil, *Hyperodes bonariensis* (Coleoptera: Curculionidae). New Zealand J. Zool. 1: 531-36.

Shanks, C. H. and B. Finnigan. 1973. An artifical diet for *Otiorhynchus sulcatus* larvae. Ann. Entomol. Soc. Am. 66: 1164-66.

Singh, P. 1977. Artificial diets for insects, mites and spiders. Plenum Publishing Corporation. New York. 594 pp.

Swezey, O. H. 1945. Insects associated with orchids. Proc. Hawaii. Entomol. Soc. 12: 343-403.

Toba, H. H., A. N. Kishiba, R. Pangaldan, and S. Riggs. 1969. Laboratory rearing of pepper weevils on artificial diets. J. Econ. Entomol. 62: 257-58.

Waterhouse, C. O. 1874. Description of a new species of *Baridius* Coleoptera: Rhynchophora from Singapore, which destroys orchids. Entomol. Mon. Mag. 10: 226-27.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of Janice Nagata and Randall Hamasaki.