LABORATORY PROPAGATION OF *CEUTHORRHYNCHIDIUS HORRIDUS* (COLEOPTERA: CURCULIONIDAE),[1] AN INTRODUCED WEEVIL FOR BIOCONTROL OF *CARDUUS* THISTLES[2]

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The development of *Ceuthorrhynchidius horridus* (Panzer) was observed on five diets consisting of less than 15% dry weight of host-plant material. Adult yields per inoculated larvae were low (less than 1%), although yields from uncontaminated last instars reached as high as 16.6%. Comparisons of diet versus plant-reared weevils showed that (1) larval weight gain at 28 days was significantly greater for plant-reared larvae, (2) plant-reared adults were significantly larger than diet-grown weevils, but there was no difference in fertility, and (3) egg size, viability, and first instar head capsule widths were not significantly different for progeny from diet and plant-reared weevils.

Ceuthorrhynchidius horridus was imported from Italy to the United States because of its potential to control *Carduus nutans* (nodding thistle)[3] and *Carduus acanthoides* (plumeless thistle) (Frick 1969). It was officially approved for field release in Virginia in 1974 following host specificity testing (Ward *et al.* 1974; Kok 1975; Kok *et al.* 1975). The importance of determination of the safety of biological agents was discussed by Zwölfer and Harris (1971), and the selection of effective agents by Harris (1973). As part of a continuing biocontrol program for *Carduus* thistles in Virginia, the propagation and systematic release of this weevil was undertaken to expedite field establishment. This report summarizes our research on the weevil's dietary requirements during the past 3 years. It was conducted to facilitate weevil production in the laboratory.

Methods and Materials

The insects used in this study were from a laboratory colony of *C. horridus* maintained using procedures by Trumble and Kok (1976). Experiments were conducted for: (1) development of an artificial diet acceptable to *C. horridus* larvae, and (2) comparison of diet and plant-reared weevils.

Development of artificial diets for *C. horridus* larvae. Diets were initially formulated to induce larval feeding. Once feeding occurred, refining the test diets for larval development was based on a two part strategy of systematic and intuitive modification propounded by Davis (1972). Of 49 diets tested, only five supported development to the adult stage. Ingredients for these diets are listed in Table I. For the components of the other diets see Trumble (1977). All diet ingredients, except plant material, were available commercially. Plant materials were grown in the greenhouse without the use of pesticides. Plant components were lyophilized for at least 48 h in a Virtis USM-15 freeze dryer before incorporation into test diets. Upon removal from the lyophilizer, the plant material was powdered in a Waring® blendor and stored at −10°C. This technique produced a plant powder of consistent moisture and texture.

Standard procedures for diet production were as follows: Constituents to be added after sterilization were stored in beakers until needed. Other ingredients were blended for 2 min with distilled water (as specified in Table I). pH of the diets was maintained at 6.2–6.4. This mixture was autoclaved at 121°C and 21 psi for 17 min. After removal, the diet was allowed to cool to 60°C before any additional ingredients were added. The

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[3] Nodding thistle = musk thistle.

diet was reblended and transferred to 15 ml sterile plastic containers. Diets were allowed to cool for 2 h in a sterile plexiglass sleeve cabinet, then capped and stored at 4°C; those not used within 30 days were discarded.

In order to determine the usefulness of artificial diets for *C. horridus*, 600 larvae were placed on each of five diets selected on the basis of performance in earlier tests. Larvae (10 per container) were allowed 6 weeks for development before being placed in 50 dram snap-cap vials filled to ca. 5 cm with sterilized Weblite® (commercial potting soil). Because pilot studies indicated that LD:0–24 and 21°(±1°C) were optimum for weevil development on diets, those conditions were maintained throughout this experiment. Distilled water was added periodically to keep the potting soil moist. Daily observations were made for evidence of pupation or adult emergence. After 60 days the soil was examined for adults, pupae, or larval remains.

Comparison of diet and plant-reared weevils. Adults were reared from nodding thistle rosettes as well as from the five diets listed in Table I. Egg measurements and first instar head capsule widths were based on observations of eggs and newly eclosed larvae resulting from plant-reared adults, diet-reared adults, and from a diet-reared male mated with a plant-reared female. Adult lengths for diet-reared *C. horridus* were compared with data presented by Kok *et al.* (1975). Measurements were made using an ocular micrometer and a dissecting microscope.

To compare larval weight gain, newly eclosed larvae were inoculated into artificial diets (10 per container) and nodding thistle rosettes (10 per plant). Thistle rosettes were ca. 45 cm in diameter. Diets and thistle plants were maintained at 21°C and photoperiods of LD:0–24 and LD:9–15 respectively. Based on previous experiments and established rearing routines, these represented optimum growing conditions for the larvae (Ward *et al.* 1974; Trumble and Kok 1976). After 28 days, larvae were dissected from both the diets and the rosettes. To prevent errors due to desiccation, larvae were weighed on a Mettler® balance within 5 min of removal from the host substrate. A students *t*-test ($P < 0.01$) was used to test for significance.

Results and Discussion

Based on data from the 49 diets tested, wheat germ, ascorbic acid and *C. nutans* were essential for development to the third instar. Addition of vitamins, sterols, and casein hydrolysate or soy hydrolysate yielded more robust larvae, and only diets incorporating these ingredients permitted development to the adult stage. With only five diets producing adults, and most differing in at least two components, the relationship

Table I. Composition of five selected diets used in *C. horridus* feeding tests

	Diet number				
Ingredients[1]	13	19	23	28	34
Ascorbic acid	1.63	1.75[2]	1.75[2]	1.75[2]	1.75[2]
Casein hydrolysate	5.0	0	5.0	5.0[2]	5.0
Distilled water (ml)	340	350	360	360	360
Cholesterol	2.0[2]	1.0[2]	1.0[2]	2.0[3]	1.0[2]
Soy hydrolysate	0	7.5	0	5.0[2]	0
Vanderzant-Adkisson vitamin mixture	2.0[2]	1.0[2]	1.0[2]	2.0[3]	4.0[2]

[1]Grams unless specified; all diets included agar 6.4 g, *C. nutans* L. 10 g, methyl-*p*-hydroxybenzoate 1.0 g, potassium hydroxide 1.0 g, sorbic acid 0.5 g, torula yeast 16 g, Wesson salt mixture 'w' 0.5 g, and wheat germ 25 g.
[2]Added after autoclaving.
[3]Includes 1.0 added after autoclaving.

between individual diet ingredients and either percentage of larvae surviving to third instar or percent adult emergence was not well defined. Although diet 34 had a higher concentration of vitamins than diet 23, there was no significant difference between these two diets (t-test: $P < 0.05$) for the development and emergence of *C. horridus*.

However, the following trends were evident: (1) the addition of ascorbic acid and vitamins after sterilization improved insect development and emergence; (2) sorbic acid, methyl-*p*-hydroxybenzoate and formaldehyde inhibited larval development when constituting over 3% (by weight) of the dry diet; (3) larval development did not occur when less than 320 ml or more than 400 ml of distilled water were incorporated in the diets; and (4) use of unsterilized diet components increased contamination. A pH of 6.2–6.4 was optimum; increasing or decreasing pH levels adversely affected larval development.

The percentages of inoculated larvae surviving to third instar were generally low (Table II), but only diet 19 yielded less than 10%. Although diet 23 produced the greatest percentage of last instars (21.7%), no living adults were recovered from this diet. In spite of sterilizing procedures, contamination was a problem. Losses of third instars due to the effects of *Penicillium* ranged from 10% (diet 28) to 38.7% (diet 23).

The results presented in Table II indicate that laboratory colonies of *C. horridus* could be maintained on diets number 13, 19, 28, 34, if fungal contamination were controlled. Based on average fecundity determined for laboratory reared females by Kok *et al.* (1975), a 1:1 sex ratio of emerging adults, and data on diets 28 and 34, a colony of *C. horridus* would increase by at least 50% each generation. However, this assumes that diet-reared females are as productive as plant-reared females. Although the proportion of diet-reared larvae reaching the adult stage was generally low, results indicate that excess laboratory populations of *C. horridus* larvae could be maintained on diets until plant material became available. This is a significant advantage when egg production exceeds host plant supply, especially in the winter months.

Comparisons between diet and plant-reared weevils showed that egg size and viability (Table III) were not significant for offspring from plant-reared adults versus progeny from either diet-grown adults or from a diet-reared male mated with a plant-reared female. This indicates that the fertility of diet-reared weevils is not impaired.

First instar head capsule widths (Table II) differed only slightly, with progeny from diet-reared weevils averaging within .01 mm of the offspring from plant-reared adults. Head-capsule widths of larvae from a diet-reared male mated with a plant-reared female averaged slightly larger (.03 mm) than those of plant-reared weevils, but because these data were from a single pair of weevils, statistical conclusions were considered invalid.

Table II. Propagation of *C. horridus* on five selected diets

Observation	Diet number				
	13	19	23	28	34
% diets contaminated[1]	3.3	18.3	30.0	50.0	31.7
% of larvae surviving to third instar[2]	10.5	4.7	21.7	12.0	12.9
% of third instars contaminated in soil	12.9	25.0	38.7	10.0	22.2
% uncontaminated third instars producing adults	3.7	16.6	5.3[3]	11.1	7.1

[1]Based on 60 diet containers per diet, 10 larvae per container.
[2]Does not include larvae from contaminated diets.
[3]Failed to reach soil surface—died in soil.

Table III. Comparison of eggs, larvae, and adults of plant-reared versus diet-reared *C. horridus*

Developmental stage	Plant-reared $\bar{x} \pm$ S.D.	Diet & plant $\bar{x} \pm$ S.D.	Diet-reared $\bar{x} \pm$ S.D.
Eggs[1]			
Length × width (mm)	.54±.01×.33±.01	.54±.01×.33±.02	.54±.01×.33±.01
Viability (%)	69.0±24.4	70.0±9.5	79.7±15.2
Larvae[2]			
First instar head capsule width (mm)	.26±.02	.29±.01	.27±.02
Weight at 4 weeks (mg)	10.5±0.3[3]	–	7.3±0.8[4]
Adults[5]			
Length of female (mm)	4.3±0.3	–	3.8±0.1
Length of male (mm)	3.9±0.2	–	3.5±0.3

[1] Plant-reared means based on 80 eggs, others on 50 eggs each.
[2] Plant-reared and mean from diet-reared male mated with a plant-reared female based on 20 larvae, diet-reared mean based on 19 larvae.
[3,4] Based on 15 and 23 larvae, respectively.
[5] Plant-reared data from Kok *et al.* (1975), diet-reared values based on five weevils of each sex.

Larval weight gain at 28 days was significantly greater on plants than on artificial diets. However, larval weights at 30 days for plant-grown larvae (Kok *et al.* 1975) were not significantly different from weights of diet-reared larvae produced in this experiment. Temperature and photoperiod were the same for both tests. This suggests a deviation in larval capabilities or in host suitability between larvae or rosettes used for this test and those utilized by Kok *et al.* (1975).

Differences between diet-reared and plant-reared *C. horridus* were confirmed when adult measurements were compared (Table III). Adults reared from plants were significantly larger than diet-reared weevils. Although the eggs from diet-reared weevils were as viable as eggs from weevils reared on plants, the smaller diet-reared females may not produce as many eggs as the plant-grown females. The smaller sizes of adults developing from diets implies nutritional defects in these artificial diets. These apparent defects remain the foremost obstacles to a "mass" production program designed for large scale field releases.

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