PHYTOSTEROL REQUIREMENT FOR PROPAGATION OF *RHINOCYLLUS CONICUS* (COLEOPTERA: CURCULIONIDAE) ON ARTIFICIAL DIETS

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ABSTRACT

The development of *Rhinocyllus conicus* Froelich was evaluated on 52 artificial diets. Selected diet constituents were varied to determine their effects on weevil development. High mortality without initial feeding on diets with cholesterol suggested that phytosterol is essential as a phagostimulant. Only nine of the diets supported development of first instar larvae to the adult stage; sitosterol was present in each of the successful diets. The low yields of adult weevils indicate inadequacies in the diets.

Key Words: *Rhinocyllus conicus, Carduus thoermeri,* musk thistle, artificial diets, phytosterol requirement.

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INTRODUCTION

A biological control program for thistles in the genus *Carduus* L. was initiated in Virginia in 1969 with the release of *Rhinocyllus conicus* Froelich (Surles et al. 1974). Establishment of this weevil on *Carduus thoermeri* Weinmann (musk thistle) was achieved in 1972 and successful biological control was documented in 1975 (Kok and Surles). After this first success, attempts were made to enhance the spread and distribution of *R. conicus* in the United States. The method most often used to augment the natural spread of *R. conicus* is to collect adults in the field and release them in other locations. The ability to rear *R. conicus* in large numbers within the laboratory would have many advantages over field collection of weevils; the principal advantage is the time and effort that could be saved. Currently, adults of *R. conicus* can be maintained in the laboratory with leaves of the host plant as food. However, completion of its life cycle requires flowering plants, the maintenance of which involves a careful manipulation of the life cycle of the thistle (Rowe 1981). Research on development of an artificial diet for this weevil was undertaken to facilitate production in the laboratory.

MATERIALS AND METHODS

All dietary ingredients, except musk thistle, were purchased commercially. The following procedures were followed in the preparation of diets. Plant material was field-collected, washed and lyophilized for at least 72 h in a Virtis USM-15® freeze dryer. It was then powdered in a Waring® blender before incorporation into

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diets. Dry ingredients (except vitamins and sterols) were mixed with distilled water in a Waring® blender until homogeneous (ca. 2 - 3 min.); pH was maintained at 6.2 - 6.4 with potassium hydroxide. The mixture was autoclaved at 121°C and 21 psi for 17 min. and allowed to cool to ca. 60°C. The vitamins and sterols were then added and the entire mixture was reblended. The diet was immediately transferred to sterile 15 ml plastic dishes (4.1 diam. × 2 cm. deep) within a sterile plexiglass sleeve cabinet (Trumble 1977). Dishes were then covered and allowed to cool.

Diets were initially formulated to induce larval feeding. Ingredients were modified in a "trial and error" fashion (Davis 1972). Seventy-five newly hatched larvae (from field-collected eggs) were inoculated per diet (5 larvae in each of 15 dishes). The surface of each diet was roughened with a scalpel just prior to inoculation to facilitate movement and penetration of the larvae. Dishes containing larvae were held at 21 ± 1°C in constant darkness. The condition of both the diet and the larvae were briefly checked at 3-day intervals. Larvae alive after 30 days were transferred to an identical, fresh diet. Duration of the larval stage is reported for those that completed larval development.

Based on initial results which indicated the need of phytosterol, the primary sterols within musk thistle were also determined. Sterols were extracted and identified from the leaves, buds and flower heads of the plant using the method of Orcutt and Patterson (1975). Emphasis was placed on the sterols because of the apparent inability of the larvae to feed on diets augmented with cholesterol.

RESULTS AND DISCUSSION

High mortality of first instar larvae without any feeding on many of the initial diets augmented with casein, vitamins and cholesterol indicated that some other chemical(s) may be essential as a phagostimulant. Because of the lack of response to increased levels of vitamins and casein (protein), we next focussed on lipids. We decided to examine the sterols present in musk thistle since insects need a sterol source. Analysis of the sterol fraction of musk thistle revealed sitosterol to be the dominant sterol throughout the plant, while cholesterol comprised less than 1% in all parts (Table 1). Cholesterol was incorporated in diets 1 - 18. Of the 1425 larvae inoculated onto these diets, 87.2% (1243) died within 48 hours of inoculation. There was little evidence of larval feeding and only 1 larva (0.07%) molted

Table 1. Quantities of sterols found in the leaves, buds and blooms of musk thistle.

	Plant part					
	Leaves		Buds		Blooms	
Sterol	a*	b†	a*	b†	a*	b†
Sitosterol	244.72	42.30	757.80	63.20	413.30	59.30
Stigmasterol	133.80	23.10	174.30	14.60	118.90	17.10
Unknown	114.66	19.80	190.60	15.90	95.38	13.70
Campesterol	85.80	14.80	75.60	6.30	64.48	9.30
Cholesterol‡	trace	–	trace	–	trace	–
Total	578.98		1198.30		697.40	

* Micrograms per gram of plant material.
† Percent of total sterol fraction.
‡ Less than 1% of total sterol fraction.

successfully to the second stage. Beginning with diet 19, sitosterol was incorporated into all diets. Larval feeding increased substantially and many successfully reached later instars.

Adults were obtained from 9 of the 52 diets; ingredients of the 9 successful diets are presented in Table 2. All 9 of the diets contained sitosterol; however, 6 also contained an additional phytosterol, stigmasterol. No conclusions could be drawn when other dietary ingredients were manipulated.

In these 9 successful diets, 26.5% (179) of the 675 larvae inoculated reached the second larval stage. Diet 28 was the most promising for initial feeding, with at least 3 times as many larvae attaining the second larval stage than any of the other diets. It was not markedly different from the next best diet (29) except for a lower yeast extract. Both contained the phytosterols, sitosterol and stigmasterol. However, mortality during the second stage was high; 4.4% (30 individuals) subsequently reached the third stage. Survival to the third larval stage was too low for any meaningful conclusions about the effects of diet on subsequent development. Nineteen individuals (63.4% of those reaching the third stage) molted to the fourth stage. Twelve larvae subsequently pupated, and 9 emerged as adults (one per diet) (Table 3). These yields are low compared to larvae developing naturally on thistle blooms in the field which had mean survival of 32% (Surles et al. 1975) and 12 to 42% (Dowd and Kok 1981).

The average length of larval development for those pupating successfully was 38.4 days. This is longer than that reported from the field, 25 - 30 days (Zwölfer 1967). This may reflect an inadequacy of the diet, but may also be a function of cool, constant temperatures ($21 \pm 1°C$) inside the laboratory as compared to a range of field temperatures fluctuating widely on a day to day basis during the summer months. The shorter durations of larval stages, 30, 30, 32 and 33 days, were from diets 49 and 51, both containing a combination of sterols (Table 2). Only three of the diets produced more than one pupa, 2 from each, and each of these diets (#29, 49 and 51) also contained a combination of sterols.

All phytophagous insects can utilize phytosterols and several studies (Levinson 1962; Clayton 1964; Robbins et al. 1971; Dad 1973; Svoboda et al. 1977) have indicated that a number of phytophagous and omnivorous insects can modify Δ^5-C_{28} and -C_{29} phytosterols from their diet to provide a structure more appropriate to their physiological needs. Conversion of the phytosterols to cholesterol may be a prerequisite to the utilization of these compounds as structural components and as precursors for synthesis of ecdysones. What is unusual is the inability of *R. conicus* to develop on diets containing only cholesterol. It seems to require sitosterol as a feeding stimulant and thus is among the few insects that cannot utilize cholesterol. However, this study indicates that *R. conicus* can complete development on artificial diets. Even though the yields obtained were low, these results demonstrate an important first step towards the development of an adequate artificial diet for *R. conicus.* Exploitation of combinations of sitosterol with other nutrient groups may lead to more successful diets. No documentation exists indicating even limited success in the development of artificial diets for *R. conicus.* Our experiences suggest that development of satisfactory diets for this weevil may be as difficult as that for the thistle rosette weevil *Trichosirocalus horridus* (Trumble 1977). We feel that our results could serve as a starting point for other researchers who might be interested in the development of a better diet for *R. conicus* since we are no longer pursuing this.

Table 2. Composition of nine diets which yielded adults of *Rhinocyllus conicus.*

	Diet #								
Ingredients*	21	28	29	32	36	44	49	50	51
Distilled water (ml)	300	315	315	315	315	315	315	315	315
Wheat germ	25	25	25	25	15	25	25	25	25
Yeast extract	4	1	4	4	4	4	4	4	4
Casein	5	5	5	2.5	5	2.5	5	5	5
Tryptophan	–	–	–	–	–	0.25	–	–	–
Sitosterol	1	0.75	0.75	0.75	0.75	1	1.25	1.25	1.25
Stigmasterol	–	0.25	0.25	0.25	0.25	–	0.25	–	0.25
Cholesterol	–	–	–	–	–	–	–	0.1	0.1
Aureomycin	–	–	–	0.03	0.03	0.03	0.03	0.03	0.03
methyl p-hydroxybenzoate	1	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5

* Grams unless specified; all diets included: musk thistle head 12 g; agar 8 g; sucrose 6 g; ascorbic acid 1.75 g; Vanderzant's Vitamin supplement 8 g; Wesson Salt Mixture 0.5 g; sorbic acid 0.5 g; potassium hydroxide 1 g; Celufil (cellulose fiber) 2 g.

Table 3. Number of *Rhinocyllus conicus* successfully completing each developmental stage on nine artificial diets.

	Developmental stage*				
Diet #†	2nd	3rd	4th	P	A
21	14	2	1	1	1
28	71	4	2	1	1
29	24	5	3	2	1
32	23	3	2	1	1
36	17	3	2	1	1
44	21	4	3	1	1
49	21	3	3	2	1
50	19	2	1	1	1
51	23	4	2	2	1
Total	179	30	19	12	9

* 2nd, 3rd and 4th are larval stages, P = pupa and A = Adult.

† 75 larvae/diet; survival of field-reared larvae on musk thistle was 32% (Surles et al. 1975), and 12 to 42% (Dowd and Kok 1981).

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