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## DIETS FOR REARING *SCYPHOPHORUS ACUPUNCTATUS* (COLEOPTERA: CURCULIONIDAE)

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The weevil, *Scyphophorus acupunctatus* Gyllenhal, is a pest of several species of economically important century plants, *Agave* spp. (Asparagales: Asparagaceae), and of Mexican tuberose, *Polygonum tuberosum* L. (Asparagales: Asparagaceae). Larvae feed in the tuberose bulb, and damage 35 to 69% of the crop (Camino et al. 2002). In the field, Hernández (2003) observed that *S. acupunctatus* larvae feeding on tuberose bulbs form a series of galleries or tunnels and complete their development within a cocoon from which the adult emerges. Copulation is carried out among the bulbs, where the female lays her eggs.

Due to the economic importance of *S. acupunctatus*, the scarcity of information on its biology, and the urgent need to accelerate both basic and applied research, it is essential to develop meridic diets for immature stages as alternatives to the natural diet.

Meridic diets for insects confer a basic advantage over the seasonally available natural nourishment, since they make possible continuous rearing to support laboratory bioassays for the study of their ecology, physiology, and behavior (Singh 1983). Contamination of rearing media by microorganisms is a major issue that must be resolved when attempting to develop an artificial diet adequate for rearing an insect culture under laboratory conditions. To this end, antimicrobial agents are used, such as formaldehyde, methyl P-hydroxybenzoate, sorbic acid, and certain antibiotics such as penicillin, streptomycin, and aureomycin (Singh 1976). These constituents are not always innocuous, because they can be directly toxic to insects, and because they can affect essential microbial symbionts (Dunkel et al. 1982). A compound's safety level can be defined as the concentration that does not reduce the yield of pupae and that does not prolong the period of larval development more than 25% of the control (Singh & House 1970). Above this safety level, antimicrobial agents produce toxic effects in proportion to the concentration utilized; and the most frequent toxic effects are reduction of the insect's size, prolongation of the larval period, and the increase in mortality of the larval and pupal stages (Bass & Barnes 1969; Singh & House 1970; Dunkel & Read 1991).

The purpose of this study was to develop a meridic diet for mass rearing of *S. acupunctatus* in

the laboratory that would supply the nutritional requirements of development, fecundity, and fertility of the insect; and that could facilitate the reliable production of larval stages needed to conduct studies of their biology, and behavior; and to obtain other information basic to achieving effective management of this pest.

In a tuberose plantation located in the Emiliano Zapata municipality, Morelos, Mexico, we collected damaged tuberose bulbs, from which we obtained *S. acupunctatus* larvae of different stages, which we separated in the laboratory and placed individually in 100-mL plastic containers each with a tuberose-bulb and moistened paper filter. Next the containers were placed in a Precision® model 818 incubator at  $27 \pm 1$  °C, 60-70% RH, and 12:12 h L:D until the emergence of the adults. We placed sets of 40 pairs of the emerged adults in  $5.8 \times 2.7$  cm plastic containers each with perforations in the container top to promote air circulation and to help avoid the growth of fungi; 20 g of the tuberose bulb was placed within each container for feeding and oviposition. This tuberose was replaced every 3 d, and with the aid of a Nikon stereoscopic microscope, part of the epidermis was removed to expose the eggs. The eggs were removed with a no. 1 marten hair brush and deposited in  $15 \times 60$  mm petri dishes with wet filter paper and stored in the incubator until eclosion.

We evaluated 4 diets; all diets included the following: 6.25 g agar (Bioxon); vitamins (Centrum, 1.45 g) (Wyeth, S. A. de C. V.); 15.62 g sucrose, 6.25 g brewer's yeast, 19.86 g wheat bran, 0.50 g cholesterol, 1.0 g Wesson salt mixture, all diets were prepared with 56 mL of distilled water to blend the ingredients plus 180 mL to dissolve the agar.

In 3 of the diets, we also used, as phagostimulant and as a growth promoter, part of the tuberose host-plant bulb of *Polygonum tuberosum* and jicama (*Pachyrhizus erosus* R. T. Clausen Urb.; Fabales: Fabaceae), also known as yam bean or Mexican turnip.

The jicama-based diets (J) 27.97 g and tuberose-based diets 27.97 g (diet A) included the same amounts of the following ingredients: 0.30 g sorbic acid, 0.97g ascorbic acid, 0.55 g methyl P-hydroxybenzoate. In diet B (30 g tuberose bulb) and diet C, (35 g tuberose bulb), the concentra-

tions of the antimicrobials was increased, i.e., 0.35 g sorbic acid, 1.02 g ascorbic acid, 0.60 g methyl P-hydroxybenzoate, in order to compensate for the greater proportion of tuberose, which could support greater microbial growth (yeasts, bacteria, and fungi) in the diets. The ascorbic acid content was increased because the deficiency of the latter in insects is associated with abnormalities in ecdysis (sclerotization). Ascorbic acid is a growth factor necessary for adequate development of the phytophagous insects (Chippendale & Beck 1964), sorbic acid serves as a phagostimulant, and sucrose is very important feeding stimulant for some species of curculionids (Kays et al. 1993). The need for minerals was met with a Wesson salt mixture. Cholesterol in the diet is important as the precursor of the insect steroidal molting prohormone, ecdysone. These ingredients were mixed in a blender and finally, agar was mixed in hot water. The diets were poured into the 4 × 1.5-cm plastic containers, and once these were solidified and chilled, the neonatal larvae, 90 per treatment, were individually placed on the diet in a container, and the containers, in turn, were placed in a Precision model 818 incubator at 27 ± 1 °C, 60-70% RH and 12:12 h L:D. These preparations were inspected daily and the diet was replaced at d 12. We used the natural diet as a control; the latter consisted of 20-g pieces of tuberose bulb. Response variables included larval weight at 12 d and 24 d, larval duration of development and survival, prepupal and pupal weights, % emergence of adults, fecundity, and fertility. The experimental design was completely random with 3 repetitions ( $n = 90$  neonatal larvae); we applied analysis of variance (ANOVA) and the Tukey test of separation of means. Percentage of mortality was calculated according to the formula of Abbot (1925).

When the adults on each of the diets emerged, we segregated them into groups of 8 pairs and provided each group with the appropriate diet. Each 8-pair group was placed separately in a 5.5 cm diam × 3-cm-high plastic container with 15 g wet weight of tuberose bulb for oviposition. These preparations were held at 27 ± 2 °C and 11:13 h L:D. After having copulated, the females laid

their eggs in the tuberose bulb; dissections was performed every 3 d to harvest the eggs and to place them in 60 mm diam Petri dishes in a moist chamber until the eggs had hatched. We recorded the number of eggs laid and the number of fertile eggs for each treatment during a 21 wk period.

All of the diets assayed were accepted by *S. acupunctatus* neonatal larvae, which indicated to us that the consistency, texture, and wetness of each of the diets was acceptable. The weights of larvae, prepupae, and pupae reared on each of the diets were analyzed statistically. ANOVA of larval weights at d 12 among the treatments were significant different (Tukey,  $P \leq 0.05$ ). Larvae fed with the tuberose C diet had the lowest weight (0.12 g), while those fed with the tuberose A and jicama had the highest weights (0.25 g, 0.22 g). At d 24, we observed a significant difference between the 4 meridic diets and the control diet (tuberose bulb), because larvae, prepupae and pupae in the control weighed less than in the meridic diet treatments.

The average % survival (Table 1) of *S. acupunctatus* larvae cultured on all 4 of the tuberose-based meridic diets at d 12 and d 24 was high (>84%). Also the % survival during the pupa stage was very high (>4%). Total survival from larva to adult was significantly high on tuberose B (97%) and tuberose C (94%) diets, while total survival for the jicama diet was considerably lower (60%) and even less than in the control (78%).

Average durations of development (d) (Table 2) of larvae reared on jicama, tuberose A, tuberose B and tuberose C diets were significantly longer than the control. Average prepupal development times was significantly the shortest on the jicama diet, and that the control (tuberose bulb) was significantly longer than on tuberose B, but not significantly longer than on tuberose A and tuberose C. Pupal development time of the control was significantly the longest. Total larval + pupal development times in the 4 meridic diet treatments (55.33-72.72 d) were significantly longer than the control (49 d); those on the tuberose A diet were significantly the longest (72.72 d) (Table 2).

Best performances were obtained when larvae were reared with the tuberose B and C diets, with

TABLE 1. PERCENT SURVIVAL (MEAN ± SEM) OF IMMATURE STAGES *SCYPHOPHORUS ACUPUNCTATUS* REARED ON ARTIFICIAL, MERIDIC DIETS.

	Diet Life stages				
	Larvae, day 12	Larvae, day 24	Prepupae	Pupae	Total
Jicama	84 ± 0.58	95 ± 2.0	80 ± 3.21	94 ± 3.51	60 ± 4.04
Tuberose A	96 ± 1.00	100 ± 0.0	96 ± 2.08	96 ± 1.52	90 ± 3.51
Tuberose B	100 ± 0.00	100 ± 0.0	96 ± 1.00	100 ± 0.00	97 ± 2.00
Tuberose C	100 ± 0.00	100 ± 0.0	94 ± 3.50	100 ± 0.00	94 ± 4.00
Control	87 ± 0.57	90 ± 3.0	100 ± 0.00	100 ± 0.00	78 ± 3.05

Values are mean survival (% ± SEM) at each stage. Total corresponds to mean survival (% ± SEM) of the initial number of neonatal larvae (initial  $n = 90$  neonates).

TABLE 2. DURATION OF DEVELOPMENT OF *SCYPHOPHORUS ACUPUNCTATUS* LARVAE, PREPUPAE, AND PUPAE REARED ON ARTIFICIAL, MERIDIC DIETS.

Diet	Days			
	Larvae	Prepupae	Pupae	Total
Jicama	54.60 ± 14.6 ab	2.46 ± 0.67 c	8.07 ± 1.94 b	65.13 ± 2.50 b
tuberosa A	60.95 ± 14.4 a	3.57 ± 1.57 ab	8.20 ± 2.65 b	72.72 ± 0.81 a
tuberosa B	54.92 ± 11.37 ab	2.84 ± 1.22 bc	7.46 ± 1.65 b	65.22 ± 2.44 b
Tuberosa C	44.83 ± 0.40 b	3.50 ± 1.29 abc	7.00 ± 2.64 b	55.33 ± 1.70 c
Control	33.05 ± 2.53 c	4.44 ± 1.57 a	11.52 ± 6.8 a	49.01 ± 2.90 d

<sup>1</sup>Means with the same letter(s) are not statistically significant according to the Tukey test ( $\alpha = 0.05$ ).

excellent emergence of adults (97% and 94%, respectively) (Table 1). Weissling and Giblin-Davis (1995) asserted that diets without Brewer's yeast result in low larval growth and survival; this element was added in all 4 meridic diets in this study. Mortality was minimal in the 3 tuberosa-based diets, which suggests that they possessed all of the elements essential for weevil development. However, % survival was only 60% on the jicama-based meridic diet and 78% on the control, which indicates either a nutrient deficiency or toxic element in jicama.

The results that we obtained with regard to the percentage of survival are very favorable in comparison with those obtained by other authors for other Curculionidae species, who reported survival rates ranging from 44-69% for the pepper weevil *Anthonomus eugenii* Cano (Toba et al. 1969), from 48-63% for *Hylobius radialis* Buchanan (Hunt et al. 1992), and 77.8-76% for *Aubeonyx mariaefranciscae* Roudier (Farinós et al. 1999).

According our results on laboratory rearing of *S. acupunctatus*, we think that the tuberosa B diet proved to be the most adequate, because we observed a constant pattern with respect to weight at d-12 and d-24, a high percentage of survival and about 65 d for larval plus pupal development. The formaldehyde levels employed induced no negative effect on the rates of development and survival of the larvae. The results that we obtained in the present work suggest that the sorbic acid and methyl-P-hydroxybenzoate doses were adequate, because the percentage mortality of larvae and pupae was low and microbial contamination was absent.

We found that the number of eggs laid daily per female reared on the jicama and tuberosa A diets ranged from 1-7; on tuberosa B, 1-11; on tuberosa C, 1-10; and on the control (natural tuberosa), 1-6. The average numbers of eggs laid per female per wk were as follows: jicama, 5; tuberosa B, 7.8; tuberosa A, 4.7; tuberosa C, 4.1; and the natural diet, 4.2 eggs.

That females that had been reared on the jicama diet had a high rate of oviposition during wk 1, which then decreased precipitously to a low level during the remainder of the period. Females

that had been reared on tuberosa B were the most fecund with a substantial but variable oviposition rate during the 21 wk period, but that tapered off during the last 4 wk of this period. Females that had been reared on the remaining 3 diets, i.e., tuberosa A, tuberosa C, and the natural diet, displayed no noteworthy differences in the number of eggs laid per female during the 21 wk oviposition period. But there was a difference with respect to the tuberosa B diet. With respect to the total number of eggs produced from females that had been reared on jicama, tuberosa A, B, C, and the natural diets, we observed egg to larvae eclosions of 72, 81, 82, 73, and 65%, respectively. The percentage of egg eclosion per wk was variable in all treatments but that it presents a high eclosion index.

With respect to females that had been reared on 1 of the 4 diets assayed, the percentages of fecundity and fertility remained fairly constant, with the highest values won by females reared on the tuberosa B diet in which the constituents of the preservatives were higher than in tuberosa A and the jicama-based diet. In other studies, it has been difficult to establish a point of equilibrium between efficacy of the antimicrobial agent and innocuity for the insect (Singh & House 1970; Burkholder et al. 1973; Kariluoto 1978), especially if the species' margin of tolerance of the compound is narrow.

These results show that the tuberosa B diet possesses the essential nutrients and the balanced proportions for growth, development, and reproduction of *S. acupunctatus* under laboratory conditions. Thus the use of the tuberosa B diet can serve as the basis for producing large numbers of weevils needed for different studies required to develop efficient technologies for managing populations of this pest in the field.

#### SUMMARY

Four meridic diets were evaluated as alternatives to the natural diet (*Polianthes tuberosa*) for laboratory rearing of *Scyphophorus acupunctatus*. The best diet tested for growth, survival, fecundity, and fertility was tuberosa B. The latter resulted mean larval weight that was higher than

the control, egg to adult survival of 97%, average number of eggs laid per female per wk of 7.8, and average eclosion of eggs of 82%.

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