

OLIGIDIC DIETS FOR CULTURE OF *RHYNCHOPHORUS CRUENTATUS* (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

Several artificial diets were evaluated as alternatives to decomposing pineapple [*Ananas comosus* (L.) Merrill] for culture of *Rhynchophorus cruentatus* (F.) larvae. The most suitable diet tested for larval growth and survival was a combination of canned pineapple, oats, sucrose, molasses, brewers yeast, Wesson's salts, vitamins, and preservatives. Diets that were not supplemented with brewers yeast provided poor larval growth and survival. Larvae cultured from artificial diets were placed in sugarcane (*Saccharum officinarum* L.) for pupation.

Key Words: Palm weevil, palmetto weevil, laboratory rearing, artificial diets.

RESUMEN

Varias dietas artificiales fueron evaluadas como alternativas a la piña descomposta [*Ananas comosus* (L.) Merrill] para el cultivo de larvas de *Rhynchophorus cruentatus* (F.). La mejor dieta probada para el crecimiento y la supervivencia larval fué una combinación de piña enlatada, cebada, azúcar, melaza, levadura de cerveza, sales de Wesson, vitaminas y preservativos. Las dietas sin levadura de cerveza produjeron poco crecimiento larval y sobrevivencia. Las larvas criadas en dietas artificiales fueron colocadas en caña de azúcar (*Saccharum officinarum* L.) para su pupación.

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*Rhynchophorus cruentatus* (F.) is the only species of palm weevil in the continental United States (Wattanapongsiri 1966). Unlike several of its congeners, *R. cruentatus* is not considered a major pest of palms. However, this species will attack transplanted or otherwise stressed ornamental palms (Giblin-Davis & Howard 1988, 1989). In Florida, *R. cruentatus* is sympatric with the native cabbage palmetto, *Sabal palmetto* (Walter) Loddiges ex Schultes (Woodruff 1967), a palm often used as mature specimens in landscaping due to its low cost, natural abundance, and high transplanting survivorship.

Semiochemicals emanating from wounded or dying palms (Chittenden 1902, Wattanapongsiri 1966, Weissling et al. 1992, Giblin-Davis et al. 1994) and conspecific weevils (Weissling et al. 1993, 1994) are attractive to *R. cruentatus* adults. Females lay eggs in the leaf bases or directly into the wounds of dying host palms. The larvae molt several times and are voracious consumers of tissue within the bud and stem. Last-instar larvae migrate to the periphery of the host, prepare a cocoon from the fi-

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ber, and enter a pupal stage. Following eclosion, adults emerge from the tree. The life cycle of this weevil usually is completed in less than 84 d (see Giblin-Davis & Howard 1989 for detailed description).

Research on the biology of *R. cruentatus*, as well as its vector potential for the red ring nematode [*Bursaphelenchus cocophilus* (Cobb)], requires the collection of adults in the field, an expensive and time consuming endeavor. Thus, a laboratory rearing method for *R. cruentatus* was needed. Three other species of palm weevils have been reported to be cultured in the laboratory. *Rhynchophorus ferrugineus* (Olivier) (Rananavare et al. 1975) and *R. palmarum* (L.) (Wilson 1963) can be cultured using cut petiole or stem tissue of coconut palms, and *R. cruentatus* has been reared on buds of an alternate host, *Serrenoa repens* (Bartram) Small (Berger 1907). However, collection of palm tissue for culture of weevils is also expensive. Rahalkar et al. (1972) reported that sugarcane is a good substitute for coconut stem for rearing *R. ferrugineus*. This method was improved by incorporating sugarcane in nutrient agar for young larvae and whole sugarcane stem pieces for older larvae (Rananavare et al. 1975). Culture of *R. ferrugineus* was further improved by development of an artificial diet (Rahalkar et al. 1978, 1985) containing sugarcane bagasse (fiber), coconut cake, yeast, sucrose, minerals, vitamins, and preservatives. Using a combination of corn flour, sugarcane fiber, oats, sugar, coconut oil, and propionic acid for a larval medium and sugarcane for pupation, Sánchez et al. (1993) successfully cultured *R. palmarum*. Giblin-Davis et al. (1989) determined that *R. cruentatus* and *R. palmarum* could be cultured on decomposing pineapple [*Ananas comosus* Merrill] syncarp for young larvae and sugarcane stem for mature larvae. However, variation in the quality and availability of pineapple, and the inconvenience of working with decomposing material, prompted the development and evaluation of several agar-based diets for laboratory-culture of *R. cruentatus*.

#### MATERIALS AND METHODS

##### Insects

*R. cruentatus* adults were harvested as cocoons from infested *S. palmetto*, placed individually in covered 100-ml plastic cups with moistened tissue paper (Giblin-Davis et al. 1989) and stored at 29 °C until adult emergence. Females and males were placed as individual pairs in 500-ml covered containers with moistened tissue paper and a slice of apple (*Pyrus malus* L.). The apple slices were replaced at 1-3 day intervals, carefully dissected, and the eggs removed (Weissling & Giblin-Davis 1994). Eggs were transferred to petri dishes (15 × 100 mm) lined with moistened filter paper, sealed with parafilm, and stored at 29 °C until neonate larvae emerged.

##### Diet Preparation

All diets included 50 g bacto-agar (Difco Laboratories, Detroit, MI), 12.5 crushed vitamin tablets (Centrum; Lederle Laboratories Div., Pearl River, NY), and 1892 ml water. Each vitamin tablet weighed 1.4 g and contained: vitamins A (5000 I.U.), E (30 I.U.), C (60 mg), B<sub>1</sub> (1.5 mg), B<sub>2</sub> (1.7 mg), B<sub>6</sub> (2 mg), B<sub>12</sub> (6 µg), D (400 I.U.), and K<sub>1</sub> (25 µg), folic acid (400 µg), niacinamide (20 mg), biotin (30 µg), pantothenic acid (10 mg), calcium (162 mg), phosphorus (125 mg), iodine (150 µg), iron (18 mg), magnesium (100 mg), copper (2 mg), zinc (15 mg), manganese (2.5 mg), potassium (40 mg), chloride (36.3 mg), chromium (25 µg), molybdenum (25 µg), selenium (25 µg), nickel (5 µg), tin (10 µg), silicon (10 µg), and vanadium (10 µg). In addition, all diets contained the pre-

servatives: m-para-hydroxybenzoate [14% solution in 95% ethyl alcohol (25 ml)], sorbic acid [12.5% solution in 95% ethyl alcohol (37.5 ml) in preliminary tests or 6.28 g sorbic acid potassium salt in refined diets], and 4M potassium hydroxide solution (7.5 ml) (Rahalkar et al. 1985). All other ingredients and amounts are listed in Table 1. All materials except the crushed vitamin capsules and a 500-ml aliquot of water were blended for approximately 2 min and poured into a 5.7-liter stainless-steel bowl. The blender was rinsed with the remaining water and poured into the bowl. The mixture was then autoclaved for 20 min at 120 °C. As the diet cooled, the crushed vitamin tablets were added and the media was stirred. Diets were poured into diet cups while still warm. When diets cooled, a small hole was made in the diet surface to facilitate feeding and larvae were transferred one per diet cup with a fine camel hair brush. Diet cups were covered with a lid vented by several small holes. After 24 h, diets were checked and dead larvae were replaced. All tests were conducted in an environmental chamber at 29 °C with a photoperiod of 13:11 (L:D).

#### Preliminary Evaluation of Diets

Ten to twenty replicates of seventeen different diets (Table 1) were evaluated in preliminary tests for larval and adult biomass gain and survival. We also included the diet for culture of *R. ferrugineus* (Rahalkar et al. 1985). Experimental diets consisted of 100 g of diet in 100-ml cups. Preliminary observations indicated the need for fresh diet if larvae were left in culture for more than three weeks (unpublished data). Thus, larvae fed diets 1-17 and the published diet were removed from cups after three weeks and transferred to 100 g of fresh diet. During this transfer, larvae were rinsed with water, patted dry with paper toweling, and weighed. After an additional two weeks, larvae were removed, cleaned, and reweighed. To determine if larvae could be cultured for more than three weeks without a change in diet, we included diet 18 which was 200 g of diet 3. However, 100-ml cups were too small for this amount of diet so the media was placed in 500-ml cups. Larvae on diet 18 remained undisturbed for five weeks, at which time they were cleaned and weighed. After the final weighing, larvae from all diets were individually placed into holes (0.95 cm diam) drilled 10 cm into one end of 23-25 cm lengths of sugarcane (Giblin-Davis et al. 1989). Stems were wrapped in window screen secured at each end by elastic bands, and placed in an environmental chamber at 29 °C. After three weeks, stems were split open and cocoons, if present, were removed and placed in 100-ml vented cups with moistened tissue paper and stored at 29 °C until adult emergence. If larvae were still present, the cane was carefully re-wrapped, placed back in the environmental chamber, and checked one week later.

#### Culture Technique Modifications

The biomass and survival of larvae reared for three weeks on diets 2, 3, 12, 13, and 15 was good in preliminary tests (Table 2). Therefore, larvae were tested again on these diets when reared in 100-ml cups (100 g of diet) and then transferred to sugarcane after three weeks. This was done to determine if healthy weevils were produced under this time and resource saving regimen. Based on acceptable growth of larvae cultured on diet 18 in 500-ml cups (Table 2), we also compared larval growth on the diets described above when 200 g of media was placed in 500-ml cups and the larvae were allowed to feed undisturbed for five weeks before transfer to sugarcane. Based on poor biomass gain after three weeks in culture, but high final biomass after five weeks in preliminary tests (Table 2), larvae fed diets 14 and 17 were evaluated

TABLE 1. INGREDIENTS USED TO PREPARE DIETS FOR CULTURE OF *R. CRUENTATUS*<sup>1</sup>.

Ingredient	Diet No. and g of Each Ingredient																		
	Pub. <sup>2</sup>	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18 <sup>3</sup>
Canned palm hearts <sup>4</sup>	—	—	—	—	—	—	—	—	—	522.5	—	—	—	317.9	—	—	—	—	—
<i>S. palmetto</i> tissue	—	522.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Sugarcane bagasse	132.5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Coconut cake	150.0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Fresh potatoes	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	317.9
Canned pineapple <sup>5</sup>	—	—	162.5	161.5	143.7	142.7	280.4	—	142.7	—	485.0	522.5	161.5	—	317.9	—	472.5	—	161.5
Brewers yeast <sup>6</sup>	50.0	—	50.0	50.0	50.0	50.0	50.0	50.0	50.0	—	—	—	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Sucrose	190.0	—	125.0	125.0	125.0	125.0	125.0	125.0	125.0	—	—	—	125.0	125.0	125.0	125.0	—	125.0	125.0
Molasses <sup>7</sup>	—	—	65.0	65.0	65.0	65.0	65.0	65.0	65.0	—	—	—	65.0	65.0	65.0	65.0	—	65.0	65.0
Soybean oil	—	—	—	—	37.5	37.5	37.5	37.5	37.5	—	37.5	—	—	—	—	—	—	—	—
Cholesterol	—	—	—	2.0	—	2.0	2.0	2.0	—	—	—	—	—	2.0	2.0	2.0	—	—	2.0
Egg yolk	—	—	—	—	—	—	—	—	10.0	—	—	—	—	—	—	—	—	—	—
Oats <sup>8</sup>	—	—	157.5	156.5	138.7	137.7	—	280.4	137.7	—	—	—	156.5	—	—	317.9	—	—	156.5
Wesson's salts <sup>9</sup>	5.0	—	5.0	5.0	5.0	5.0	5.0	5.0	5.0	—	—	—	5.0	5.0	5.0	5.0	—	5.0	5.0

<sup>1</sup>All diets contained 50 g bacto agar, 12.5 crushed vitamin capsules, 25 ml of a 14% m-para-hydroxy benzoate stock solution, 37.5 ml of a 12.5% sorbic acid stock solution, 7.5 ml of a 4 M potassium hydroxide stock solution, and 1892 ml water (100 g diet in 100 ml cup).

<sup>2</sup>Artificial diet published by Rahekar et al. (1978, 1985) for culture of *R. ferrugineus*.

<sup>3</sup>Diet 3; 200 g in 500 ml cup, without three week subculture.

<sup>4</sup>Source: Costa Rica.

<sup>5</sup>Pineapple chunks in unsweetened pineapple juice; Dole Packaged Foods Corp.

<sup>6</sup>Debittered NF (Entomologically tested); Bio-Serv, Frenchtown, N.J.

<sup>7</sup>Grandma's Molasses "unsulphured", Mott's USA, Stamford, CT.

<sup>8</sup>Quick Oats; The Quaker Oats Co., Chicago, IL.

<sup>9</sup>Salt mixture, Wesson modification; United States Biochemical Corp., Cleveland, OH.

TABLE 2. BIOMASS AND SURVIVAL<sup>1</sup> OF *R. CRUENTATUS* LARVAE AND ADULTS FED ARTIFICIAL DIETS (PRELIMINARY TESTS<sup>2</sup>).

Diet	n	Larvae			Adults			Total Days in Sugarcane
		Biomass (g) 3 Weeks	Biomass (g) 5 Weeks	Survival %	Biomass (g)	Emergence (%)		
Pub <sup>3</sup>	20	1.14 ± 0.17 de	2.87 ± 0.23 ab	70.0 ± 10.5 abc	0.89 ± 0.09 bc	50.0 ± 11.5 ab	50.6 ± 1.9	
1	10	0.02 ± 0.00 e	0.02 ± 0.00 c	10.0 ± 0.0 cd	—	0.0 ± 0.0 b	—	
2	20	2.82 ± 0.18 abcd	3.52 ± 0.19 ab	50.0 ± 11.5 abcd	1.07 ± 0.15 bc	30.0 ± 10.5 ab	48.5 ± 1.1	
3	20	2.36 ± 0.18 abcd	3.25 ± 0.26 ab	65.0 ± 10.9 abc	1.06 ± 0.04 bc	45.0 ± 11.4 ab	50.0 ± 1.2	
4	10	1.67 ± 0.14 bcde	2.90 ± 0.21 ab	30.0 ± 15.3 bcd	0.90 ± 0.10 bc	30.0 ± 15.3 ab	48.7 ± 2.0	
5	10	1.89 ± 0.29 abcd	—	0.0 ± 0.0 d	—	0.0 ± 0.0 b	—	
6	10	1.15 ± 0.00 de	2.90 ± 0.00 ab	10.0 ± 10.0 cd	0.70 ± 0.00 bc	10.0 ± 10.0 ab	51.0 ± 0.0	
7	10	1.65 ± 0.34 bcde	—	0.0 ± 0.0 d	—	0.0 ± 0.0 b	—	
8	10	2.33 ± 0.00 abcd	2.67 ± 0.00 ab	10.0 ± 10.0 cd	0.80 ± 0.00 bc	10.0 ± 10.0 ab	52.0 ± 0.0	
9	20	1.38 ± 0.18 de	2.45 ± 0.18 b	70.0 ± 10.5 abc	0.83 ± 0.03 bc	55.0 ± 11.4 ab	50.2 ± 1.4	
10	10	1.47 ± 0.43 cde	2.68 ± 0.02 ab	30.0 ± 15.3 bcd	0.80 ± 0.15 bc	30.0 ± 15.3 ab	53.7 ± 1.2	
11	20	0.05 ± 0.01 e	0.10 ± 0.02 c	55.0 ± 11.4 abcd	0.50 ± 0.00 c	5.0 ± 5.0 b	65.0 ± 0.0	
12	11	3.15 ± 0.30 ab	3.28 ± 0.36 ab	54.5 ± 15.7 abcd	1.07 ± 0.07 bc	27.3 ± 14.1 ab	48.7 ± 3.3	
13	10	3.11 ± 0.33 abc	4.04 ± 0.16 ab	70.0 ± 15.3 abc	1.08 ± 0.03 bc	40.0 ± 16.3 ab	49.3 ± 2.7	
14	10	1.24 ± 0.21 de	3.13 ± 0.19 ab	100.0 ± 0.0 a	1.04 ± 0.07 bc	70.0 ± 15.3 a	52.0 ± 1.0	
15	10	3.40 ± 0.21 a	4.29 ± 0.34 a	40.0 ± 16.3 abcd	1.60 ± 0.20 a	20.0 ± 13.3 ab	52.5 ± 0.5	
16	10	1.64 ± 0.30 bcde	3.28 ± 0.17 ab	80.0 ± 13.3 ab	1.18 ± 0.06 b	50.0 ± 16.7 ab	54.0 ± 0.7	
17	10	1.15 ± 0.23 de	3.05 ± 0.31 ab	80.0 ± 13.3 ab	1.01 ± 0.05 bc	60.0 ± 16.3 ab	52.6 ± 0.7	
18	10	—	3.99 ± 0.20 ab	80.0 ± 13.3 ab	1.12 ± 0.05 b	60.0 ± 16.3 ab	49.2 ± 2.0	

<sup>1</sup>Means ± SEM.

<sup>2</sup>Means within a column followed by the same letter are not significantly different (P > 0.05; SNK).

<sup>3</sup>Artificial diet published by Rahaikar et al. (1978, 1985) for culture of *R. ferrugineus*.

further in 500-ml cups with 200 g diet. The biomass of these larvae was determined at five weeks before immediate transfer to sugarcane. Each diet and cup size combination was replicated 20 to 40 times.

### Statistics

Larval and adult survival were based on the initial number of test insects. All data except percentage values were square root ( $X + 0.001$ ) transformed. Percentage data were subjected to angular [ $\arcsin(\text{square root} + 0.001)$ ] transformation. Transformed data were subjected to analysis of variance using the Statistical Analysis System's general linear models procedure (SAS Institute 1985) for overall comparison of diets. Means were separated by Student Newman-Keuls' (SNK) test where significant ( $P < 0.05$ ) effects occurred. Diets evaluated in 100-ml cups were compared with diets in 500-ml cups using orthogonal contrasts (SAS Institute 1985).

## RESULTS

### Preliminary Evaluation of Diets

After five weeks in culture the average biomass of larvae and adults, as well as larval survival and percentage adult emergence, varied greatly (Table 2). The greatest mean larval biomass was observed for diet 15 both at three (3.40 g) and five (4.29 g) weeks but survival was relatively low (40%) (Table 2). The greatest larval survival (100%) occurred with larvae fed diet 14; mean biomass at five weeks (3.13 g) was not significantly different from that of diet 15 (Table 2). There was little gain in biomass of larvae fed diets 1 and 11, however, survival on diet 11 was relatively high (55%), whereas survival on diet 1 was low (10%) (Table 2). Adult emergence was greatest for diet 14 (70%) while no adults emerged from larvae fed diets 1, 5, and 7 (Table 2). Total time from placement of larvae in sugarcane to adult emergence varied from 48.5 days (diet 2) to 65 days (diet 11) (Table 2).

### Culture Technique Modifications

Overall, the greatest larval biomass was obtained from diet 2 in 500-ml (large) cups (3.5 g) while larvae fed diet 17 had the least biomass (1.39 g) (Table 3). The greatest larval survival was on diet 14 (92.5%) (Table 3). Adults reared as larvae on diets 2 and 3 (large cups) had the greatest biomass (0.94 and 0.92 g, respectively) while the greatest adult emergence was from larvae fed diet 12, small cups (55%) (Table 3). Total time from placement of larvae in sugarcane to adult emergence ranged from 33.8 days (diet 12, 500-ml cup) to 46.6 days (diet 17) (Table 3). Orthogonal contrasts indicated that overall larval and adult mass was greater for individuals cultured in 500-ml versus 100-ml cups ( $P < 0.01$ ). However, adult emergence was greater for larvae cultured in 100-ml cups ( $P < 0.03$ ).

## DISCUSSION

Using the diet described by Rahalkar et al. (1978, 1985), and several variations of this diet using readily available materials, we were able to achieve growth and development of *R. cruentatus* larvae. Sugarcane stem was, however, necessary for completion of the life cycle. Of diets tested in the preliminary experiments, seven were

TABLE 3. BIOMASS AND SURVIVAL<sup>1</sup> OF *R. CRUENTATUS* LARVAE AND ADULTS CULTURED ON ARTIFICIAL DIETS<sup>2</sup>.

Diet	Diet Cup Size <sup>3</sup>	n	Larval Biomass (g)	Larval Survival (%)	Adult Biomass (g)	Adult Emergence (%)	Total Days in Sugarcane	Males: Females
2	small	40	2.53 ± 0.10 c	87.5 ± 5.3 a	0.81 ± 0.04 abc	50.0 ± 8.0 a	43.7 ± 0.4	0.73
3	small	40	2.27 ± 0.12 cd	77.5 ± 6.7 ab	0.76 ± 0.04 abc	47.5 ± 8.0 a	44.8 ± 0.8	1.71
12	small	40	2.44 ± 0.11 c	70.0 ± 7.3 ab	0.79 ± 0.04 abc	55.0 ± 8.0 a	44.2 ± 0.9	1.44
13	small	40	2.10 ± 0.15 cd	60.0 ± 7.8 abc	0.70 ± 0.01 bc	45.0 ± 8.0 a	43.4 ± 0.6	0.80
15	small	40	1.88 ± 0.12 d	52.5 ± 8.0 bc	0.62 ± 0.04 bc	35.0 ± 7.6 a	44.2 ± 1.6	0.44
2	large	40	3.50 ± 0.14 a	82.5 ± 6.1 ab	0.94 ± 0.04 a	50.0 ± 8.0 a	44.6 ± 1.8	0.58
3	large	40	3.15 ± 0.10 b	65.0 ± 7.6 abc	0.92 ± 0.04 a	27.5 ± 7.1 a	43.5 ± 2.2	1.20
12	large	20	2.40 ± 0.22 c	36.8 ± 11.4 c	0.74 ± 0.07 abc	47.4 ± 22.1 a	33.8 ± 13.3	0.67
13	large	40	3.04 ± 0.10 b	75.0 ± 6.9 ab	0.82 ± 0.04 ab	37.5 ± 7.8 a	43.2 ± 1.5	0.88
14	large	40	2.22 ± 0.10 cd	92.5 ± 4.2 a	0.71 ± 0.03 bc	35.0 ± 7.6 a	45.0 ± 0.9	1.33
15	large	20	1.90 ± 0.11 d	50.0 ± 11.5 bc	0.66 ± 0.06 bc	35.0 ± 10.9 a	46.5 ± 1.9	1.33
17	large	20	1.39 ± 0.20 e	75.0 ± 9.9 ab	0.59 ± 0.08 c	30.0 ± 10.5 a	46.6 ± 2.9	0.50

<sup>1</sup>Means ± SEM.<sup>2</sup>Means within a column followed by the same letter are not significantly different ( $P > 0.05$ ; SNK).<sup>3</sup>Small, 100-ml cup with 100 g diet for 3 weeks prior to entry into sugarcane. Large, 500-ml cup with 200 g diet for 5 weeks prior to entry into sugarcane.

chosen for further evaluation in replicated tests based on larval and adult biomass and survival. Diets containing soybean oil (diets 4-8, and 10) resulted in high mortality of *R. cruentatus* larvae. The published diet (Rahalkar et al. 1978, 1985) was successful for culture of *R. cruentatus* larvae but was eliminated because of difficulty in obtaining coconut cake. Finely chopped field (*S. palmetto*, diet 1) and laboratory (pineapple, diet 11) hosts were unacceptable for growth, development, and survival of *R. cruentatus* larvae when brewers yeast, sucrose, molasses and salts were omitted (Table 2). However, when brewers yeast was added to chopped pineapple (diet 16), the diet was acceptable for larval growth, development, and survival (Table 2). This suggests that yeasts or some other undefined ingredients present in rotting host tissues are an important component in *R. cruentatus* nutrition. The importance of microbes is not surprising considering that many members of the Rhynchophorinae are associated with fermenting and rotting host tissues (Vaurie 1971). Further modification of the pineapple and brewers yeast diet (16) to include sucrose, molasses, oats, and salts with a reduction in the amount of pineapple (diet 2) resulted in improved larval performance. Supplemental cholesterol (diets 3, 4, 5, 6, 13, 14, 15, and 17) or the addition of egg yolk did not improve larval growth.

Weevils cultured by the methods of Giblin-Davis et al. (1989) had a mean generation time of 78-79 days, with 47-48 days from placement of larvae in sugarcane to adult emergence. Mean time to adult emergence in this study ranged from  $33.8 \pm 13.3$  days (diet 12, large cup) to  $46.6 \pm 2.9$  days (diet 17, large cup). Adults (sexes combined) emerging from field-collected cocoons average 1.14 g (Giblin-Davis et al. 1989), while adults cultured by the pineapple/sugarcane method of Giblin-Davis et al. (1989) had a mean biomass of 0.72 g (males) and 0.75 g (females). Weevils cultured by the pineapple/sugarcane method of Giblin-Davis et al. (1989) for over two years had a mean adult biomass of 0.89 g ( $n = 704$ ). Biomass of *R. cruentatus* adults cultured on artificial diets in this study (refined test) ranged from 0.59 g (diet 17, large cup) to 0.94 g (diet 2, large cup) (Table 3). These comparisons suggest that *R. cruentatus* cultured on artificial diets in this study are of comparable biomass to weevils reared using the pineapple/sugarcane method of Giblin-Davis et al. (1989), but adults are slightly smaller than those collected from the field.

Sugarcane appears to contribute very little to the continued growth of mature *R. cruentatus* larvae (Giblin-Davis et al. 1989). Using data from two years of culture on pineapple and sugarcane, we determined that the biomass of sugarcane pieces used for pupation is not correlated to biomass of adults (unpublished data). However, larval biomass is positively correlated with adult biomass (unpublished data). The biological significance of adult biomass and its importance in the fitness of *R. cruentatus* has yet to be explored. Weevils fed artificial diets in large cups had significantly greater larval and adult biomass than weevils reared in small cups. However, larvae were in culture for two weeks longer in the large cups. Thus, if larger larvae or adults are required, larvae should be cultured for longer periods of time with an increased amount of diet. The best diet tested for culture of *R. cruentatus* was diet 2 (supplemented pineapple and oats), in both sizes of diet cups (Table 3). The simplest diet tested that provided larval growth was diet 16, a mixture of canned pineapple and brewers yeast. Further testing indicated that by using a combination of 300 g of diet 3 in 500-ml cups and sugarcane, we were able to culture *R. palmarum* larvae to the adult stage ( $n = 5$ , 6.8 g larval mass, 80% larval survival, 2.5 g adult mass, 40% adult emergence) (unpublished data).

In conclusion, we were able to successfully culture *R. cruentatus* larvae by using readily available ingredients. We suspect that sugarcane is nothing more than a source of fiber from which the cocoon is constructed by *R. cruentatus* larvae. A limiting

factor in the culture of *R. cruentatus* to adults in many geographic locations may be the availability of sugarcane. We have tested alternative fiber sources added directly to 500-ml diet cups after larvae were in culture for 5 weeks but larvae failed to construct cocoons. Pupal induction in *R. cruentatus* needs further study but appears to require warm temperatures, low relative humidity, and a high fiber substrate.

The culture of *R. cruentatus* on artificial diets has several interesting implications. The use of agar-based diets, although somewhat odorous, has greatly improved working conditions in the rearing facility. In addition, larvae can be reared individually and their history can be documented. This will be important in future studies to determine the vector relationship of *R. cruentatus* with the red ring nematode, *B. cocophilus*. Larvae of *R. cruentatus* are large and would be well suited for use in physiological studies. For example, using the pupae of *R. palmarum* and *Sitophilus oryzae* (L.), Rahbé et al. (1990) identified a new class of hemolymph storage proteins. Finally, although we are aware of no human consumption of *R. cruentatus* larvae in the U.S., larvae of *R. palmarum* (Woodruff 1967), *R. phoenicis* (F.), and *R. ferrugineus* (DeFoliart 1990) are considered delicacies by some. The culture of *R. cruentatus* on artificial diets could be a potential advancement in developing a niche for consumption of our indigenous species by palm weevil gourmets or feeding burrowing owls in captivity (Yosef & Deyrup 1994).

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