MASS REARING OF THE RED PALM WEEVIL, *RHYNCHOPHORUS FERRUGINEUS* OLIV., ON SUGARCANE AND ARTIFICIAL DIETS FOR LABORATORY STUDIES: ILLUSTRATION OF METHODOLOGY

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ABSTRACT

A method for laboratory mass rearing of the red palm weevil (*Rhynchophorus ferrugineus* Oliv.) (RPW) were developed. Weevils, initially obtained from the field, were maintained on the stems of sugarcane. Prior to mass rearing, several artificial diets were formulated and preliminary evaluated for development of the *R. ferrugineus*. Materials used for preparations of various diets were: oats, coconut cake, coconut fruit pieces, canned and/fresh pineapple, sucrose, molasses, egg yolk, salt, yeast, vegetable oil, potatoes, soybean flours, date palms leaves and palm fiber sheath, sugarcane fibers, bacto-agar, multi-vitamins, preservatives, and water. Oat and white bean diets were preferred by 1st to 3rd larval instars, while oats + fibers preferred by 4th to 5th larval instars. Larvae fully developed on artificial diets and molted four times during their development failed to construct cocoons because of the unavailability of fibers (palm or sugarcane. Facilities, materials required, diet preparation and procedures, and practical difficulties of rearing methods are discussed.

Additional Index Words: *Rhynchophorus ferrugineus*, pheromone, trapping, palm trees

INTRODUCTION

The red palm weevil (RPW), *Rhynchophorous ferrugineus* Oliver (Coleoptera: Curculionidae), is an economically important, tissue-boring pest of date palm in many parts of the world. The insect was first described in India as a serious pest of coconut palm (Lefroy, 1906) and later on date palm (Lal, 1917; Buxton, 1918). The insect is a major pest of date palm in some of the Arabian Gulf States including Saudi Arabia, United Arab Emirates, Sultanate of Oman, and Egypt (Cox, 1993; Abraham et al. 1998).

The agroclimatic conditions prevalent in this region and the unique morphology of the crop, coupled with intensive modern date palm farming, have offered the pest an ideal ecological habitat (Abraham et al., 1998).

Red palm weevil is a concealed tissue borer and spends all of its life stages inside the palm tree. Damage symptoms can be categorized by one or more of the following (Abraham 1998): presence of the tunnels on the trunk and base of leaf petiole made by the feeding grubs, oozing out of thick yellow to brown fluid from the tree, appearance of chewed up plant tissue in and around openings in the trunk, presence of a fermented odor from the fluid inside infested tunnels in the trunk, presence of adults and cocoons in the leaf axils, fallen empty pupal/chewed up frass on the ground around the palm, breaking of the trunk or toppling of the crown when the palm is severely infested.

Research on the biology and control of *R. ferrugineus*, for many projects conducted in the last four years at UAE University by various researchers, required large numbers of weevils of various stages. First attempt to develop a method for mass rearing of this pest was made by the authors during this period in the UAE. Rearing methods of this and several related species were reported in other countries and with similar species (Rahalkar et al., 1972, 1978, 1985; Rananavare et al., 1975; Giblin-Davis et al., 1989; Weissling and Giblin-Davis, 1995). The objectives of this study were to (1) illustrate the methodology of mass rearing *R. ferrugineus* on sugarcane, and (2) develop artificial diets for rearing *R. ferrugineus*.

MATERIALS AND METHODS

Insects

Various stages of *R. ferrugineus* were collected from infested date palm trees in Masafi area in Sharja Emirate in 1997. Each developmental stage was placed individually in covered plastic container. Portable wood saw was used to facilitate collecting weevils from heavily infested palm trees (Figure 1).

Figure 1. Collection of various stages of *R. ferrugineus* from infested date palm trees.

Rearing Room

Rearing of *R. ferrugineus* of various stages was carried out in a controlled rearing room at the Plant Protection Laboratory of the Plant Production Department, UAE University (Figure 2). The room was maintained at 25 ± 2ºC and 60-70% RH. The photoperiod was approximately 12:12 L:D. The room contained three large working benches, electrical outlets, stainless steel sink, side boards, autoclave, and a refrigerator. The room was also used as a media room for handling and preparing materials of artificial diets.

Figure 2. Room for rearing *R. ferrugineus* at the Faculty of Agricultural Sciences, United Arab Emirates University.

Equipments and Materials

Equipments and materials required for rearing *R. ferrugineus* on sugarcane and artificial diets are given in Table 1.

Rearing on Sugarcane

Adults. Adults collected from the field (Figure 1) were cleaned and kept (as group of at least 10 males and females but not sexed) in rectangular plastic boxes with press-on tight-fitting lids, or kept as individual pairs of males and females in 1 liter glass jars (Figure 3). All adults were provided with at least 5 absorbent cotton wicks saturated with a 10% honey solution for feeding and egg laying. Boxes and jars were staked side by side (or on the top of each other) on working benches. Few holes were made on all lids of boxes and jars for ventilation. Females lay their eggs on the cotton wicks (i.e., oviposition site). Association of both sexes for 24 h ensures fertilization of females and no further mating of females was necessary (Kaakeh, 1998). Adults after emergence from cocoons were sexed and kept separately in small jars for mating and egg laying. Sexing the adults was done according to the presence of a series of black hairs on the dorsa, frontal part of snouts of males and their absence in the females.

Table 1. Equipments and chemical materials used for rearing *R. ferrugineus* on sugarcane and artificial diets.

- Blanace (up to 3 kg; triple beam balance or others)
- Electric blender/mixer
- Autoclave
- Refrigerator
- Portable wood cutting saw
- Rectangular plastic boxes
- Polyethylene collecting buckets
- Aluminum and plastic trays or boxes (various sizes) with tight-fitting lids
- Glass and disposable plastic Petri dishes (50 and 100 mm In diameter)
- Camel-hair brushes (no. 2 preferred)
- Absorbent cotton or cotton wicks (sheets and bolls)
- Scissors
- Glass jars (1 liter)
- Measuring cylinders (50, 100, 200, and 500 ml)
- 40-mesh nylon netting
- Cork borer set
- Aluminum foil
- Transparent, polyethylene bottles, with tightly closing lid.
- Black fiber paper sheets
- Rubber bands
- Paper towels
- Absorbent cotton wicks
- Hand magnifiers
- Labpette digital micro-pipet, capacity 1-20 micro-liter
- Parafilm rolls (for sealing test tubes, flasks, Petri dishes, etc.)
- Eye wash bottles
- Disposable non-toxic particle masks
- Graduate and funnel brushes
- Glass shell and screw-cap vials

Figure 3. Rectangular plastic boxes and glass jars holding adults for mating and oviposition. Plastic containers contained unsexed groups of adults, while the glass jars hold pairs of sexed males and females.

Eggs. Cotton wicks holding eggs were removed from the oviposition sites (i.e., large plastic boxes and small glass jars in figure 3) and placed in separate boxes (Figure 4). Cotton wicks were wet with water to avoid drying. Other eggs were transferred with the camel hair brush and placed on wet filter papers inside the petri dishes for further studies. New cotton wicks, saturated with a honey solution, were placed in all containers holding the adult stages. After 2 to 3 days, larvae from hatched eggs were removed to separate containers and provided with pieces of sugarcane stems for feeding.

Larvae. Newly hatched larvae on cotton wicks were transferred with a camel's hair brush to pieces of sugarcane stems (at least 15 mm in diameter) (Figure 5). A small hole was made at the end of each piece of sugarcane stem using a cork borer. One week after feeding, larger larvae were transferred to larger fresh pieces of sugarcane stems (10 – 40 mm in diameter; this was based on the size of larvae at different developmental stages). Last larval instars made cocoons from the fibers inside the sugarcane stems. When sugarcane was infested with Drosophilla flies, yellow sticky traps were placed above the rearing containers as a control tool. Also, larvae were reared individually (Figure 5) in sugarcane stems to avoid cannibalism.

Figure 4. Egg collection from cotton wicks and filter papers.

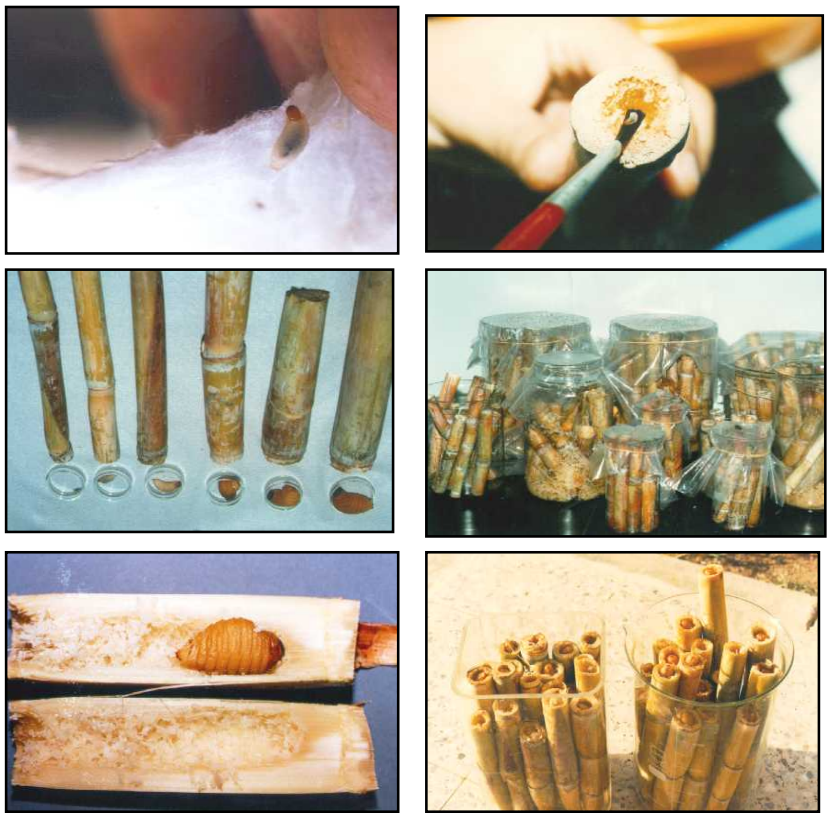

Figure 5. Handling and feeding of larvae in sugarcane stems.

Pupae. After 10-14 days of feeding of last larval instars, the stem pieces of sugarcane were split open and cocoons were collected. Cocoons were placed in a plastic containers or metal trays, wet with water as needed, and closed with lids. Two weeks after collecting the cocoons, they were checked daily for adult emergence. Adults were collected by hand and placed in plastic containers (as unsexed groups of adults) or placed individually in glass jars (as sexed, paired males and females).

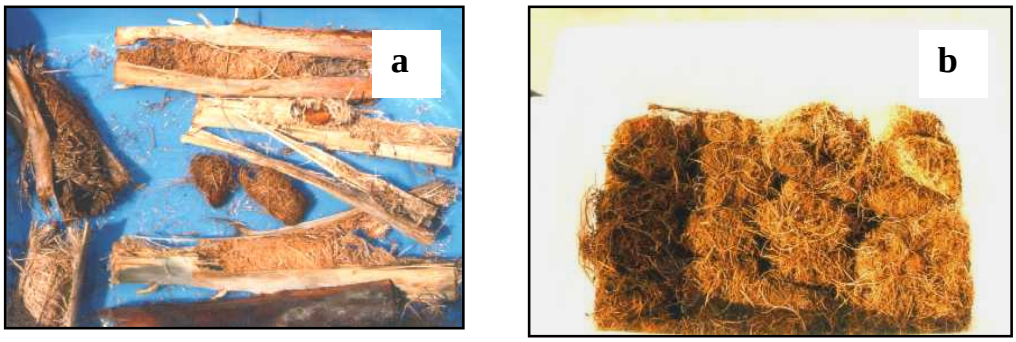

Figure 6. Collection of pupae of *R. ferrugineus* from pieces of sugarcane stems (a) and plastic containers holding cocoons (b).

Rearing on Artificial Diets

Preparation of Diets

Artificial diets were prepared for mass rearing of *R. ferrugineus* because of the unavailability of sugarcane in UAE and this was a limiting factor in culturing this insect. Diets were also developed to avoid the use of expensive palm tissues for culture of weevils.

Table 2 lists materials used for the preparation of several artificial diets. Different diets were evaluated in preliminary tests for larval and adult biomass gain, survival, and the rate of development Three diets were selected (Figure 7) and percentage of dry ingredients is listed in Table 3. Diet A is an mainly an oat diet, Diet B is an oat diet plus palm and coconut tissue, while diet C is mainly a white bean diet. Ingredients and water (1 - 2 liter of water for diets weighing 500 - 1000 g) were blended for approximately 5 minutes. All diets included bacto-agar, multi-vitamins, chemical preservatives. Bacto-agar was dissolved in water and added to other ingredients. The mixtures of all diets were then autoclaved for 20 min at 120ºC. Diets were poured in diet stainless-steel round trays or cups while still warm. All trays and cups were stored at room temperature until

required. Larvae were placed on diets after total coolness. As with rearing R. ferrugineus on sugarcane, when artificial diets were infested with drsophilla flies, yellow sticky traps were placed above the rearing containers as a control tool. Also, fungal and bacterial contamination may develop if chemical preservatives were not incorporated in the diets. This may also occur if high quantity of water was mixed with the dry ingredients of diets.

Table 2. Materials used for the preparation of artificial diet for rearing *R. ferrugineus* in the laboratory.

Food Materials	
Oats	Date palm leaves and fiber sheath
White beans	Canned or fresh pineapple
Sugarcane bagasse	Soybean flours
Fresh sugarcane stems	Coconut fruit pieces
Fresh coconut cake	Coconut fibers
Potatoes	Vegetable oil
Egg yolk	Wesson salt
Vitamin tablets*	Brewer's yeast or other brands
Honey	
Chemical preservatives	
Methyl para-hydroxybenzoate **	
Auromycin	
4M Potassium hydroxide***	
Sorbic acid****	
Bacto-agar	

* vitamin tablets contained vitamins A, B_1, B_2, B_6, B_{12}, D_2, E, K_1, Riboflavin, nictotinamide, and others.

** 14% solution In 95% ethyl alcohol. 5 ml water was added to 95 ml absolute ethyl alcohol (95% solution) and then 140 g methyl para-hydroxybenzoate was dissolved in 95% ethyl alcohol (amount of 15 ml solution per 1 kg diet was used)

*** 56 g potassium hydroxide in 250 ml distilled water (the amount of use 5 ml solution per 1 /kg of diet was used).

**** 12.5% stock solution In 95% ethyl alcohol. About 125 g sorbic acid was dissolved in 1 liter of 95% ethyl alcohol (the amount of 15 ml solution per 1 kg of diet was used) (Rahalkar et al. 1985).

Table 3. Percentage of dry ingredients of three artificial diets used for rearing *R. ferrugineus*.

Dry Ingredient	% Dry Ingredient in Diet A	Diet B	Diet C
Oats	57	48	15
White Beans	-	-	65
Palm and coconut tissues	-	15	-
Sugar	22	19	10
Molasses	11	10	-
Brewers yeast	9	7	9
Salt	1	1	1

Figure 7. Artificial diets in small plastic trays or cups or in stainless steel containers for feeding of larvae of various sizes.

Larval Feeding on Artificial Diets

Prior to mass rearing, several artificial diets were formulated and preliminary evaluated for development of *R. ferrugineus*. Second instar larvae were washed in tap water, weighed, and placed into holes made on artificial diets to facilitate feeding. Larvae were transferred one per diet cup with a fine camel hair brush (Figure 8). The weight for each larva (n = 8) was recorded weekly for 6 weeks, and was then compared with its starting

weight. When larvae reached the end of the last larval instar, they were placed in the stems of sugarcane for constructing cocoons. Diets were checked daily for any dead larvae in which they were replaced.

Figure 8. Feeding of larvae on artificial diets.

RESULTS AND DISCUSSION

Previous attempts that were made by several researchers followed several steps: Sugarcane was a good substitute for coconut for rearing *R. ferrugineus* (Rahalkar et al., 1972); sugarcane was later incorporated in nutrient agar for feeding young larvae and sugarcane stem pieces for feeding of older larvae (Rananavare et al. 1975). Rahalkar et al. (1978, 1985) improved the culture of *R. ferrugineus* by developing an artificial diet containing sugarcane bagasse (fiber), coconut cake, yeast, sucrose, minerals, vitamins, and preservatives. Giblin-Davis et al. (1989) cultured *R. cruntatus* and *R. palmarum* on decomposing pineapple. In our study, there were variations in the quality of developed diets made using different ingredients. Diet A was mainly made by oats, diet B was made using oats and fibers, while diet C was made using white beans. Diets A and B were preferred by young larvae (1st to 3rd larval instars) while diet B was preferred by older larvae (4th and 5th larval instars).

The average biomass of larvae at various stages, survival, percentage of larvae that went into cocoon, and biomass and percentage of emergence of adults varied greatly between the diets tested: a range of larval biomass after three weeks of feeding on artificial diets was 0.25 – 2.5 g and after 5 weeks was 0.5–4.5 g. The weight gained from feeding increased considerably in a three week period (i.e., larvae with weight ranged from 0.5 to 0.11g increased 40 to 80X, that is 4.18 to 4.54 g). The weight gained varied with the type of diet provided to the larvae. The percentage of larval survival ranged from 10 to 90%. The biomass of emerged adults ranged from 0.8 to 2 g. The percentage of adult emergence ranged from 10 to 80%.

Larvae were fully developed on artificial diets and molted four times during their development (Figure 9). These larvae failed to construct cocoons because of the unavailability of fibers (palm or sugarcane) or there was not enough quantities of high fiber substrates in diet B. All larvae fed by either of the three artificial diets were transferred to sugarcane stems for making cocoons. Additional studies are required to search for alternative food sources to develop diets that *R. ferrugineus* can fully develop on it without transferring last larval instar to sugarcane stems for cocoon construction.

Figure 9. Development of first instar larvae on artificial diet (a), and the molting of fourth instar larva to the fifth larval stage.

The success of our three artificial diets (Table 3) in providing the necessary nutritional requirement for molting and development was based on data recorded ing various life parameters of *R. ferrugineus* on sugarcane and artificial diets (a study conducted by the authors, see the next manuscript in this proceedings). The study on life parameters of *R. ferrugineus* included the following: fecundity or the number of eggs per female, egg viability or the percentage of hatch, larval developmental period, larval biomass, pupal

period, percentage of larval survival, percentage of adult emergence, fecundity, fertility, and female:male ratio.

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