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A LABORATORY METHOD FOR REARING *CATOLACCUS HUNTERI* (HYMENOPTERA: PTEROMALIDAE), A PARASITOID OF THE PEPPER WEEVIL (COLEOPTERA: CURCULIONIDAE)

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The pepper weevil, *Anthonomus eugenii* Cano, is a serious pest of cultivated *Capsicum* spp. peppers in the southern United States, Hawaii, Mexico, Guatemala, Honduras, Costa Rica, and Puerto Rico (Schuster et al. 1996). Eggs are deposited in flower buds and fruit, where larvae and pupae complete their development. Infested buds and fruit often abscise, but larvae and pupae can complete development if fallen buds and fruit do not desiccate. Yield losses can reach 90% in Florida, if the weevil is not controlled (Schuster & Everett 1982). Broad spectrum insecticides have been used most often to manage the pest but may lead to unintended consequences, including insecticide resistance and outbreaks of non-target pests. Biological control could be an alternative or adjunct to insecticides in managing the pepper weevil.

At least three species of predators and seven species of parasitoids have been reported to attack the pepper weevil (Riley & King 1994). The most abundant parasitoid recovered from the pepper weevil in Florida was *Catolaccus hunteri* Crawford (Hymenoptera: Pteromalidae) (Riley & Schuster 1992). While natural enemies generally are regarded as contributing little to control of the pest (Elmore & Campbell 1954), 50% parasitism of pepper weevil larvae by *C. hunteri* was observed in fallen jalapeno buds and over 20% parasitism in fallen bell pepper buds (Schuster et al. 1988). Augmentative releases of *C. hunteri* on alternative host plants during the summer off-season and on pepper at the initiation of flowering have resulted in reduced or delayed damage by weevil larvae (Schuster unpublished data). Because *C. hunteri* has shown potential for bio-control of the pepper weevil, a method of rearing the parasitoid in the laboratory is needed.

A commercial diet for rearing pepper weevil larvae is available (Bio-Serv, Entomology Division, Frenchtown, NJ); however, the diet was not used due to low egg hatch (Toapanta 2001). Because rearing the pepper weevil in pepper fruit is time and space consuming, an alternative host was sought. The cowpea weevil, *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae), was shown to be a suitable factitious host for rearing *Catolaccus grandis* (Burks) (Rojas et al. 1998), a closely related parasitoid of the boll weevil, *A. grandis grandis* Boheman. The *C. maculatus* larvae were encapsulated in Parafilm® (Pechiney Plastic Packaging, Inc., Menasha, WI) for presentation to parasitoid adults. This method was developed for exposing *A. grandis grandis* larvae to ovipositing *C. grandis* (Cate 1987) and was mechanized for mass production (Roberson & Harsh 1993). Methods also were developed for producing *C. maculatus* larvae in pieces of garbanzo beans (chick peas), *Cicer arietinum* L. (Leyva et al. 2002). The pieces were not large enough for larvae to complete their development within, thus forcing the larvae to exit the bean pieces. The larvae then were easier to collect prior to encapsulation. This method had been used successfully to rear *C. hunteri* in the laboratory. Life history parameters including pre-oviposition period, oviposition period, adult longevity, fecundity, and egg to adult development period of *C. hunteri* on *A. eugenii* were found to be the same whether the parasitoid had originally been reared on either *C. maculatus* or *A. eugenii* (Seal et al. 2002). Collecting larvae and encapsulating them in Parafilm represents extra investments in time and equipment. Therefore, a method was developed for rearing *C. hunteri* on *C. maculatus* larvae directly in garbanzo beans.

Two colonies of *C. maculatus* were maintained in a room at a temperature of about 27°C, relative humidity of about 60% and a photoperiod of 14L:10D. The colonies were maintained on black-eyed peas, *Vigna unguiculata* (L.) Walp., and on garbanzo beans. The black-eyed peas were used to maintain the colony of *C. maculatus* and the garbanzo beans were used for exposing *C. maculatus* larvae to the *C. hunteri* parasitoid.

Three times a week, six narrow-mouth 800-ml "Mason" glass jars (Ball Corporation, Muncie, IN) were filled with 300 g of black-eyed peas each. About 100 *C. maculatus* adults were collected with an aspirator connected to a vacuum pump and were deposited in each jar, which then was sealed with a screen, filter disc, and metal ring. These jars were stored upright. A new generation of bruchid adults emerged about every 30 d.

Three times a week, ca. 400 *C. maculatus* adults were collected with an aspirator and put into each of ten 800-ml glass jars that contained 300 g each of garbanzo beans. The *C. maculatus* adults were removed 48 h later by placing the beans and bruchids on a metal sieve placed in the large opening of a 25-cm diam galvanized funnel,

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the narrow end of which was attached to a wet/dry vacuum cleaner. The vacuum was operated until all *C. maculatus* adults were drawn through the sieve. The beans then were returned to the jars, which were laid on their sides. In about 21 d, the hatching larvae were 4th instars, the lifestage used previously for parasitism (Rodriguez-Leyva et al. 2000). The larvae form pupation cells and chew an emergence hole, leaving only the integument of the bean. These opaque "windows" can be seen readily and aid in the selection of beans with 3rd instars present. These jars were moved to the *C. hunteri* rearing room, which was maintained under the same conditions as the *C. maculatus* rearing room.

The beans were placed in trays (9 × 8 × 2 cm) with 115-125 beans in each tray. The trays were plastic strawberry baskets with the sides trimmed to 2 cm high (Fig. 1a). Corks were glued to the bottoms of the trays to elevate them, thus allowing more accessibility of the female parasitoids to the beans on the bottoms of the trays. Every Monday, Tuesday, and Wednesday, two trays were placed in each of two oviposition containers consisting of No. 6 (2.8 liter) plastic jars (Newell Rubbermaid Co., Wooster, OH) laid on their sides (Fig. 1b). Water was provided by inserting two water-filled, cotton-plugged 1-dram vials through two 1.3-cm diameter holes in the upper surface of each container. A cloth sleeve was attached to the mouth of each container and was sealed with a rubber band when not in use. Drops of honey were placed on the inside top of the containers to provide food and were replenished when consumed by the parasitoids. About 50 female and 50 male parasitoids were introduced into each oviposition container. The trays in the containers were changed three times a week for 26 days, at which time the oviposition containers were disassembled and cleaned for re-use.

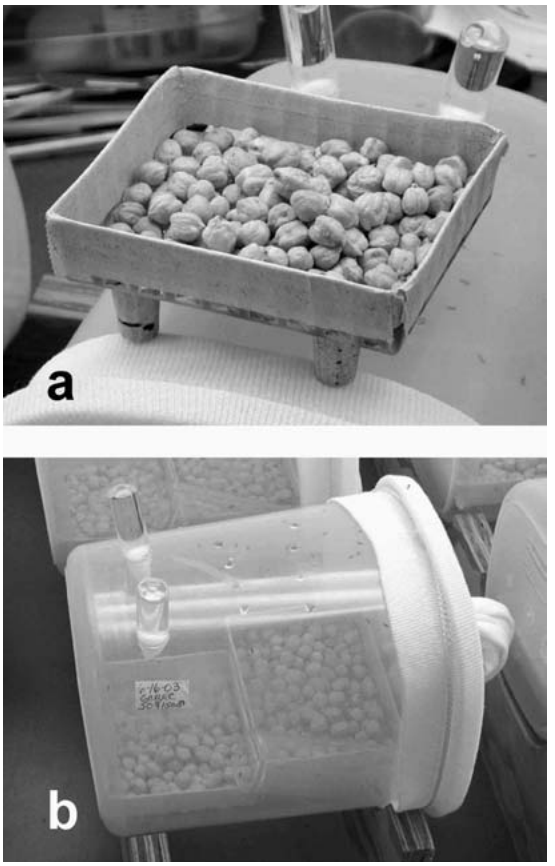


Fig. 1. Strawberry basket (a) for exposing garbanzo beans with 4th instar *Callosobruchus maculatus* in oviposition containers to adult *Catolaccus hunteri* (b) in the laboratory.

The beans that had been exposed 2-3 days to parasitoids were placed in No. 3, 2.4-liter rectangular, plastic containers (Newell Rubbermaid Co., Wooster, OH) with screen covered square holes cut in the lid to allow ventilation but prevent escape of emerging *C. maculatus* adults. The beans were divided into three containers and each container was placed individually in Plexiglas® (Atofina Chemicals, Inc., Philadelphia, PA) incubation cages (30.5 × 30.5 × 30.5 cm) with a cloth sleeve on one end. Two sides of the cage were covered with organdy fabric to allow ventilation.

After about 7 d, adult parasitoids began to emerge and were collected with a vacuum pump aspirator. The garbanzo beans were sifted to remove *C. maculatus* adults. The beans were then placed on a wax paper-lined fiberglass lunchroom tray (45 × 35 cm), one layer deep and the trays were placed on the shelves of an emergence box (Fig. 2a). The emergence boxes were constructed of wood and had 4-8 shelves with individual, sealable doors for each shelf. The shelves did not extend to the back of the emergence box and the bean-filled trays were not placed on the shelf all the way to the back. Thus, an open space was created at the back of the box from the bottom to the top. At the top of this open space, a hole (5 × 20 cm) was cut and covered with metal window screen that allowed passage of *C. hunteri* but prevented that of the *C. maculatus* adults. A Plexiglas collection chamber (32 × 32 × 21 cm) (Fig. 2b) was attached to the top of the emergence box over the screen-covered slot. The sides of the Plexiglas box had cloth sleeves installed, allowing access for collecting parasitoid adults with a vacuum pump aspirator. Two water-filled, cotton-plugged vials were placed in the bottom of the Plexiglas box and honey was streaked on the inside of the top and front. Both were replenished as needed. Trays were replaced within the emergence box every 23 days as new parasitoid-exposed, *C. maculatus*-infested beans were added. Once a week, the Plexiglas box was thoroughly cleaned with

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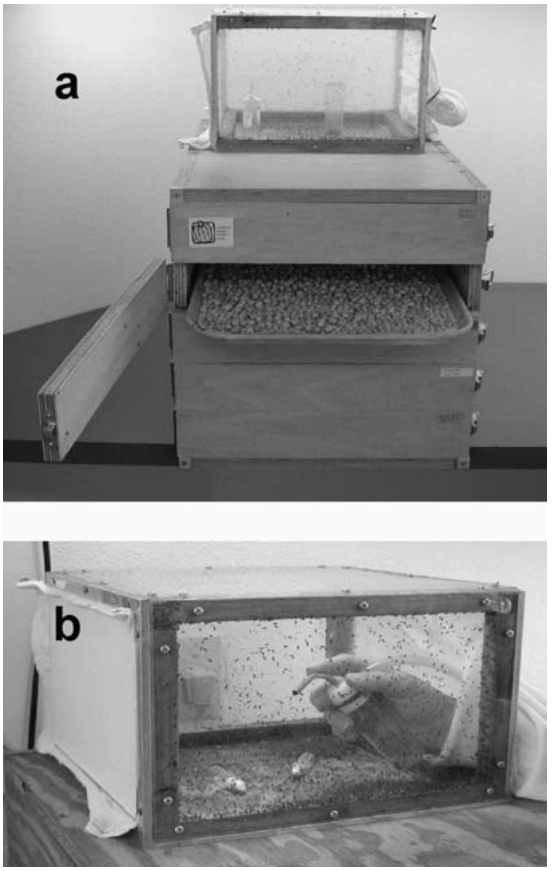


Fig. 2. Emergence box (a) with tray of garbanzo beans infested with 3rd instar *Callosobruchus maculatus* that have been exposed to adult *Catolaccus hunteri*. Aspirating *C. hunteri* adults from the Plexiglas® collection chamber (b).

Kimwipes® tissue (Kimberly-Clark Corp., Roswell, GA) moistened with water.

Approximately 18,000 parasitoids were produced weekly by these rearing methods with 26 oviposition cages. Start-up costs include about $133 for 180 "Mason" jars for rearing the *C. maculatus*; about $150 for a humidifier to maintain RH at 60% in the *C. maculatus* rearing room; about $315 for 26 *C. hunteri* oviposition cages including plastic jars, vials, cotton balls, honey, plastic berry baskets, corks, fabric (also used for incubation cages), twine rope, and rubber bands; about $80 for each of 9 *C. hunteri* larval incubation cages; and about $155 for labor and supplies to build each of three adult emergence cages. About 22 h/wk were required in the maintenance of both the *C. maculatus* and *C. hunteri* colonies. About 4 kg of black-eyed peas and 6 kg of garbanzo beans were used each week. At $8/h for labor and $1.22/kg for the peas and $0.90/kg for beans, the estimated recurring cost of production was about $186/wk.

Anecdotal observations have indicated that biweekly releases of 1,500 *C. hunteri* along one edge of pepper fields of different sizes during the summer and fall fallow season resulted in reduced infestations of the pepper weevil on pepper during the following spring season. In addition, experimental evidence on an organic farm indicated that weekly releases of the parasitoid at about 7,400/ha delayed the pepper weevil infestation (Schuster, unpublished data). In experimental plots, weekly releases of 1,500 *C. hunteri* in nightshade during the fallow, off-season followed by weekly releases at 7,400/ha in adjacent pepper in the spring resulted in 65-75% fewer pepper fruit infested by the pepper weevil.

It is estimated that for an organic grower with a 1-ha block to make releases of 1,500 *C. hunteri* adults every 2 wk for 32 wks (16 releases during the fallow off-season) would cost about $250 in recurring expenses. To add additional releases of 7,400/ha would cost about another $76/wk during the early pepper season. Neither of these cost estimates includes the cost of labor to release the parasitoid adults. The estimated cost for fallow season releases is probably cost effective but the in-season costs may be prohibitive; however, in discussions with organic producers, this latter cost may not be prohibitive in light of few effective alternatives for managing the pepper weevil. The current rate of parasitism in the *C. maculatus* host is about 40%. If the rate of parasitism could be increased without increasing production costs, the cost for releases of *C. hunteri* for managing the pepper weevil during the spring season could become more cost effective.

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Summary

Methodology was developed to rear *Catolaccus hunteri* Crawford, a parasitoid of the pepper weevil (*Anthonomus eugenii* Cano), on an alternative host, the cowpea weevil (*Callosobruchus maculatus* F.) in temperature controlled rooms at 27°C, 60% relative humidity and 14L:10D photoperiod. Black-eyed peas, *Vigna unguiculata* (L.) Walp., were used to maintain a colony of *C. maculatus*, and garbanzo beans, *Cicer arietinum* L., were used to expose the *C. maculatus* larvae to *C. hunteri* females. About 250 garbanzo beans containing 4th instar *C. maculatus* were exposed 48 to 72 h to 50 female and 50 male *C. hunteri*. Parasitoid-exposed beans were held for about 7 days and were placed into emergence boxes with screened-covered slots, which retained *C. maculatus* adults in the box but allowed *C. hunteri* adults to pass into a Plexiglas collection chamber. With an invest-

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ment of about 22 h/wk, about 18,000 parasitoids can be reared weekly at an estimated recurring cost of $186/wk for labor and supplies. Start-up costs for rearing containers and a rearing room humidifier totaled about $1,800.

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