Insectary Rearing of the Pink Hibiscus Mealy Bug and Its Parasitoids in a Desert Environment

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INTRODUCTION AND FACILITIES

During the winter of 1999-2000, in cooperation with USDA-APHIS and Imperial County, personnel form the Calif. Dept. of Food & Agriculture (CDFA) and Office of the Agric. Commissioner setup an insectary for the production of the pink hibiscus mealybug (PHM) and its parasitoids. Procedures were adapted from those describe by Meyerdirk et al. 2002. These procedures combined with those used by commercial insectaries for producing parasitoids of other mealybug species were integrated to meet our specific needs within the desert southwest climate, where summer temperatures often exceed 105^{o}F. Two trailers with three rooms each were rented, measuring 60 x 11 ft. One trailer is used to house the mealybug culture and the other to house each parasitoid culture in separate rooms. Outdoor entrances into each trailer consist of a closed porch with fine mesh screening [hole size= 0.095 mm ^{2} (52x52 strands/inch)] and two doors. The parasitoid cultures are maintained on PHM reared primarily on Japanese pumpkins. More recently, several alternative squash varieties have been used as well.

PUMPKIN PRODUCTION AND STORAGE

Based on the past experience of growing Japanese pumpkins at several locations in California, the growing seasons for Japanese pumpkins occurs from February to June [harvest mid-May through mid-June] in the low desert region of the state, July to November [harvest October-November] in southern intermediate valley areas near the city of Riverside and from May through October [harvest August through October] in the Sacramento Valley. Presumably, it would be feasible to grow a crop from March through June as well near Riverside. Frequent high temperatures exceeding 100^{o} F for more than several days, causes a rapid decline in mature plants. Growing procedures are typical for cucurbit varieties, including plant spacing of approximately 24-30 inches within-row and 6-7 ft. between-rows.

Pumpkins are harvested when they have a dull green appearance and 50% or more of the pumpkin is covered with a waxy bloom (see pumpkins in photo with "gray frost"). This is the time they are most suitable for rearing PHM. They will not store well if picked when the fruit are shiny green or yellowing from age.

Prior to use or placing in storage, each pumpkin is washed using a soft bottlebrush using water only, followed by soaking in a 5% household bleach solution for 10 minutes and subsequently thoroughly rinsed with tap water and open-air dried.

Storage is conducted in a cold room maintained at 50^{o} F and a humidity level of approx. 50% RH (maintained using a portable dehumidifier). Mold occurs rapidly at levels above 65% RH.

ALTERNATE PHM FOOD

Several alternative squash/pumpkin varieties can be used to rear the PHM. It is essential that they are purchased from an organic producer and that they are not surface treated with wax or oil products. Varieties include sweet dumpling, carnival, delicata, and acorn squash. Sweet dumpling squash is highly desirable for this use because of its size (4-5 in. dia.) and suitability as a PHM host, as well as remaining in storage for long periods (2-3 mos.). Acorn squash stores for a shorter period.

Potatoes can be used to rear the PHM, however, the El Centro insectary has done so simply to support a maintenance culture. It can take a considerable length of time to develop a culture capable of being maintained on potato sprouts. Sprouted red and white varieties can be used. We have been most successful utilizing sprouted russet potatoes, however, other insectaries have had greater success with red potato

varieties. After washing potatoes in a mild bleach solution as stated above, potatoes are held in a dark room at 50^{o} F and 50% RH to encourage sprouting. Potatoes with 2-4 in. sprouts are used for rearing PHM. In general, only small numbers of mealybugs are produced using potato sprouts. Because pumpkins are more attractive to PHM than to potatoes, it is important to avoid inoculating and holding the two in the same cabinets.

PHM REARING

Containment - Mealybug cultures are typically maintained in closed, dark room facilities. This reduces crawler movement and escape. By utilizing double door screened entrance ways, along with placing fine mesh screening over all room vents and openings, PHM cultures can be maintained free of contaminants. In addition, all food items used in culture are cleaned, and cultures are housed in ventilated cabinets [4'x 2'x 4' high]. This provides a second level of isolation, and in the event that the colony in one cabinet becomes contaminated, allows for the destruction or otherwise isolation of that portion of the colony from the remainder. Furthermore, these cabinets with sealed doors greatly reduce the number of escaped crawlers moving throughout the room. Cloth mesh size needed to prevent crawler escape must be ultra fine [anti-virus screen, approx. hole size = 0.02mm^{2}].

Temperature/humidity/lighting - Room temperature ranging from 74 to 84^{o} F (day/night) provides for a 30-37 day PHM generation time. Room humidity and most importantly cage/cabinet humidity should be maintained near 40-50% RH. Humidity levels maintained for lengthy periods above 60% will induce mold. Dehumidifiers and household room vaporizers work very well for maintaining desired levels of RH. In addition, 8-10 inch dia. fans work well to circulate the air within a room, preventing stratification and aid in moving air through cages/cabinets. One or two fans pointing down from the upper wall area in each room are recommended. They can be pointed directly at the screened areas of cages/cabinets to facilitate air movement within. Although each trailer has a central air temperature control unit, we placed auxiliary window air conditioners in each room along with a space heater. This allows us to modify room temperature by room and provides emergency room cooling in the event of a breakdown of the primary central air heating and cooling unit. The doors and windows of the trailers were poorly insulated; therefore, aluminum foil covered foam board insulation (approx. 1 in. thick) was placed over the inside of each exterior door, as well as over each window. The PHM rearing rooms are kept dark except when cages are being serviced.

Inoculation of pumpkins – A holding cabinet was modified to collect PHM crawlers. The shelf is covered by a heavy piece of white cardboard that has a square cutout (approx. 8 x 8 in.) on the front, center edge. A piece of heavy weight white paper is placed within the cutout area with its edges overlapped by the cardboard sheet. The ventilated cabinet includes a low watt bulb (4 watt) suspended 12-18 in. from the shelf surface. Foil was molded into a tube and place around the bulb so that a beam of light projects downward onto a sheet of heavy weight paper on the shelf surface. Old pumpkins containing ovisacs that are about to hatch are removed from the holding cabinets and placed around the periphery of the shelf. Attracted by light, crawlers move from the old infested pumpkins unto the cardboard surface and eventually to the paper surface under the beam of light. On a daily basis, crawlers may be collected by simply removing the paper and pouring them on to new pumpkins. Alternatively, new pumpkins may be placed around the edge of the lit area for 12–24 hours, then removed and located in holding cabinets for rearing another generation of PHM. The level of infestation is based on a qualitative assessment. For Japanese pumpkins, it is desirable to have noticeably high crawler densities in the deeply grooved lines of the fruit. Infestation levels should not be so great that the overall surface of the pumpkin is pink with crawlers. A similar approach can be used for other pumpkin varieties, such that localized surface areas contain high crawler densities of a similar form. Survivorship of crawlers on potatoes is very low, typically requiring repeated inoculation over several days.

PARASITOID REARING

Containment – Cages are required that are approximately two cubic feet in size, and have a large viewing area and substantial ventilation to prevent excessive humidity. Preferably, they should have two, arm entrance holes with cloth socks.

Temperature/humidity/lighting – Temperature and humidity conditions are maintained similar to those used in the mealybug insectary. Fluorescent room lights are set to a 16:8 day/night photoperiod. It has been suggested that they can be reared in darkness as well.

Adult parasitoid food & water – Clean honey without water added should be finely streaked onto the walls of the cage several times a week. Alternatively, honey can be streaked onto one or two inverted paper water cups set in the middle of the cage. Water is provided by saturating paper towels cut to fit within an inverted petri dish lid.

REARING DETAILS – PHM infested pumpkins should be selected that predominantly (approx. 60%) contain the most appropriate mealybug life stage for parasitization by the given parasitoid

species. ***Anagyrus kamali*** readily attacks mid-third instar PHM nymphs. Although older adult mealybugs are attacked as well, it has been shown that the adult PHM is more likely to encapsulate the parasitoid eggs, preventing them from hatching. The most appropriate PHM life stages for ***Gyranusoidea indica*** are the late second or early third instars. ***Allotropa mecrida*** successfully attacks second instar PHM. These respective mealybug stages should be the most common life stage present for the particular parasitoid species at the time of cage setup. However, keep in mind that the inclusion of earlier PHM life stages will provide host materials on future days.

For all three parasitoid species, three to four well-infested pumpkins are placed in a cage with 800 to 1000 parasitoids, having an approximate sex ratio of 1:2 M/F. From the time a PHM is stung to parasitoid emergence, development of *A. kamali* is approximately 18 days, 22 days for *G. indica*, and approx. 26 days for *Allotropa*. Following setup, cages are maintained for at least 35 days, keeping in mind that the predominant oviposition period is approximately 7-10 days.

When setup as previously described, a cage with *A. kamali* will commonly yield 4,000 to 5,000 parasitoids. At times we have had cages that yield upwards of 8,000. *A. kamali* production is highly variable, and we have frequently had cages that only produce 2,000 individuals. A cage similarly setup for *G. indica* production commonly yields 8,000 to 10,000 individuals. We have often had cages that produce in excess of 12,000. The production of *G. indica* is characteristically far less variable than *A. kamali*. As a result, to achieve equal production of the two species, two cages of *A. kamali* are set up for every one-cage setup for *G. indica* production. *Allotropa* sp. nr. *mecrida* is a gregarious species, producing several parasitoids per host. Production generally ranges from 5,000 to 12,000 individuals per cage.

Harvesting parasitoids – Parasitoids are collected using a standard aspirator attached to a vacuum pump station. To operate effectively, it is recommended that a filter be applied to the vacuum line along with a restrictor clamp (or perforated vacuum line) to control the strength of the vacuum and keep wax and other debris from damaging the vacuum unit. To further minimize injury to parasitoids, it is useful to place a piece of foam on the bottom of the collecting vial. To feed the parasitoids, honey is applied to a piece of nylon screening that is secured to the underside of the vial lid. It is essential that the honey is contained within the squares (i.e., matrix) of the screening and does not extend above the surface of the screening. Otherwise, the chilled parasitoids are likely to become stuck to the honey during shipment. Lastly, parasitoid vials are wrapped in a portion of white paper towel and tape is applied. This provides a buffer against temperature extremes in a cold box with ice, and more importantly prevents exposure to sunlight, risking over heating, during release. The parasitoids store well for at least several days at 55^{o} F. The presence of honey is essential at higher temperatures.

REFERENCES

Meyerdirk, D.E., R. Warkentin, B. Attavian, E. Gersabeck, A. Francis, J. Adams, and G. Francis. 2002. Biological control of pink hibiscus mealybug project manual. United States Dept. of Agric., Animal and Plant health Inspection Service, Plant Protection and Quarantine. 194pp. <http://www.aphis.usda.gov/ppq/manuals/pdf_files/phm.pdf>

Sagarra, L.A., C. Vincent, N.F. Peters and R.K. Stewart. 2000a. Effect of host density, temperature, and photoperiod on the fitness of *Anagyrus kamali*, a parasitoid of the hibiscus mealybug *Maconellicoccus hirsutus*. Entomologia Experimentalis et Applicata 96: 141-147.

Kairo, M.T.K., A.E. Cross, V.F. Lopez, D.D. Peterkin and P. Ram. 1997. Biological Control of the hibiscus mealybug: Rearing the hibiscus mealybug, *Maconellicoccus hirsutus*, and the parasitoid *Anagyrus kamali* Moursi; 33 pp. Trinidad: International Institute of Biological Control.

---------- Supplemental Information ----------

PUMPKIN/SQUASH SOURCES

Potential Northern California producers:

Full Belly Farm, P.O. Box 251, Guinda, Ca 95637. Phone 530 796-2214 [www.fullbellyfarm.com]. Organic producers of sweet dumpling, delicata, carnival and acorn squash. These varieties are typically available from August through November. Shipping is available.

Terra Firma Farms, LLC, P.O. Box 836, Winters, CA 95694
(530) 756-2800 (530) 795-2473. [www. Terrafirmafarm.com]

Riverdog Farm – contact: Trini Campbell or Tim Mueller, P.O. Box 42 17635 County Road 61, Guinda,CA 95637 Ph. 530-796-3802 (530) 796-4100

Live Oak Farm – (530) 796-4084

SEED SOURCES

japanese pumpkin (*Cucurbita moschata* cv. Chirimen)

Stokes Seeds
P.O. Box 548

Buffalo, New York 14240-0548
Phone: 1-800-263-7233
716 695-6980
FAX: 716 695-9649 Est. $95/lb.

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