Multicellular Rearing Methods for the Beet Armyworm, Soybean Looper, and Velvetbean Caterpillar (Lepidoptera: Noctuidae)[1]

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ABSTRACT Methods used for rearing *Heliothis* spp. larvae in a multicellular container were modified for rearing beet armyworm (BAW), *Spodoptera exigua* (Hübner), soybean looper (SBL), *Pseudoplusia includens* (Walker), and velvetbean caterpillar (VBC), *Anticarsia gemmatalis* (Hübner). Polyester-cotton cloth used for *Heliothis* oviposition was unsatisfactory for BAW, SBL, and VBC. A polyester-cotton cloth with a dimpled surface was discovered which was suitable for all three species. Beet armyworm eggs were removed from the oviposition cloth and disinfected with sodium hypochlorite. This procedure could not be used for SBL or VBC as it resulted in significant reduction in egg hatch. New methods were developed for placing BAW, SBL, and VBC eggs into a multicellular container. A separator and high volume blower used to harvest *Heliothis* pupae was also used to harvest BAW and VBC pupae. SBL pupae, because of webbing spun by the larvae prior to pupation, could not be harvested in this manner. A pupal harvest method was developed for SBL. A phosphoric-propionic acid mix incorporated into the larval diet controlled contaminants. These procedures have been used since 1986 at Stoneville and more than ¾ million pupae and 100 million eggs were reared in 1987 and 1988.

KEY WORDS Beet armyworm, soybean looper, velvetbean caterpillar, multicellular container, rearing, *Spodotera exigua, Pseudoplusia includens, Anticarsia gemmatalis.*

The multiple insect species rearing section within the Southern Field Crop Insect Management Laboratory at Stoneville, Mississippi, maintains up to 15 insect colonies in support of entomological research. Over 100 scientists in USDA, universities, and private industry are routinely supplied with insects for use in research studies. In order to supply high quality insects at a low cost, we routinely search for ways to simplify and economize rearing operations. Two *Heliothis* species and a sterile backcross from one of these species have been reared for several years in our laboratory using a multicellular container (Hartley et al. 1982). The simplicity, reliability, and economy experienced by using the multicellular container prompted efforts to adapt this technique for rearing the beet armyworm (BAW), *Spodoptera exigua* (Hübner), soybean looper (SBL), *Pseudoplusia includens* (Walker), and velvetbean caterpillar (VBC), *Anticarsia gemmatalis* (Hübner). Adaptation of the multicellular container to rearing these insect pests required considerable modification in techniques.

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Materials and Methods

Adult Holding. To assure optimum mating and oviposition, BAW and VBC pupae were sexed with the aid of a stereoscope (King et al. 1985). Although possible, SBL pupae were not sexed because their pale green coloration makes distinguishing of sculptural differences between male and female pupae difficult. VBC male and female pupae were placed together in oviposition cages (30 × 30 × 45 cm) constructed of 3.1 mm thick clear acrylic sheet at the rate of 150 pair. Moths emerged, mated, and oviposited on a dimpled-surface, white polyester-cotton cloth (Pikka-Pukka Pattern Springmaid Industries, New York, NY) vertically suspended through 3.2 mm × 25 cm slits in the top of the oviposition cage. Paper towels originally used as oviposition sheets disintegrated in the chemical solutions required to remove and disinfect the eggs. Polyester-cotton cloth used in *Heliothis* production (King et al. 1985) did not provide the texture to stimulate maximum oviposition. A cotton pad (7.5 cm diam.) was placed in a petri dish inside the cage and saturated daily with a 5% sucrose-Schlitz® beer solution which served as a food and water source. Eggs were collected every other day and the moths were discarded 9 days after emergence.

BAW male and female pupae were placed in separate cardboard buckets (3.8 liter) covered at the top with white polyester cloth. As the adults emerged 100 males and 100 females were transferred to 20 × 30-cm cages constructed of clear acrylic plastic tubing 3.1 mm thick. The cage top and bottom was also made of 3.1-mm-thick, clear acrylic plastic. The bottom was permanently attached and the removable top had a circular groove cut to fit the circumference of the cage. Four slots, 3.2 mm × 12.5 cm each, were cut in the cage top to allow strips of white polyester-cotton cloth identical to that used for VBC oviposition (ca. 10 cm wide) to be inserted and removed.

A food and water source was provided by placing a cotton pad (7.5 cm diam.) in a petri dish inside the cage and saturating the pad daily with 5% sucrose solution. Eggs were collected every other day and the moths were discarded after 4 days.

SBL pupae were randomly mixed with 200 placed in each oviposition cage and allowed to emerge, mate, and oviposit. The cages were identical to those described for the BAW. Eggs were collected every other day, and the moths were discarded 9 days after emergence.

All oviposition cages for BAW, SBL, and VBC had a hole (1.9 cm diam.) cut about 2.5 cm from the bottom to allow hookup to a scale collector identical to that reported by King et al. (1985). Containment of wing and body scales in a rearing laboratory is essential, as they are a serious health hazard (Ridgway and Whittam 1970). The adults of all species were held at 24 ± 1° C and 80 ± 10% RH with a 14:10 (L:D) photoperiod.

Larvae Rearing. The larval rearing container was identical to the multicellular unit reported by Hartley et al. (1982). The BAW was reared in cell units which consisted of 903 cells, each 1.25 × 1.25 × 2.8 cm, and the SBL and VBC were reared in cell units consisting of 648 cells. The dimensions of each cell in this unit was 1.4 × 1.4 × 2.8 cm. Artificial diet similar to that described by King and Hartley (1985) was used to rear all three species. SBL rearing required addition of 25 ml of raw linseed oil per 3.8 liters of diet. Because methyl paraben and sorbic acid did not control the mold, *Aspergillus niger* (Van Tieghem), 10 ml of a stock propionic-

phosphoric acid solution was incorporated into each 3.8 liters of diet (Powell and Hartley 1987). The stock solution of propionic-phosphoric acid was prepared by mixing 418 ml of propionic acid and 42 ml of phosphoric acid with 540 ml of distilled water. No harmful effects were detected in the egg, larval, pupal, or adult stages in any of the three insect species. *Aspergillus niger* was eliminated by the addition of this acid mix.

BAW egg masses were removed from the polyester-cotton cloth and separated into individual eggs by immersion in 0.2% sodium hypochlorite (NaOCl) for 2 min. This procedure was used to remove and disinfect *Heliothis* eggs by King et al. (1985). SBL and VBC eggs removed and disinfected in this manner failed to hatch. SBL and VBC eggs were removed from the polyester-cotton cloth by washing for 5 min in a 50-50 solution of water and industrial ammonia containing 5% ammonium hydroxide (NH4OH). Eggs of all three species were rinsed in six 1-min changes of distilled water to remove ammonium hydroxide and sodium hypochlorite. *Heliothis* eggs are routinely placed into multicellular containers using an aluminum sheet "planter" originally described by Raulston and Lingren (1972) and modified by Hartley et al. (1982). BAW, SBL, and VBC eggs placed into multicellular containers using this planter were severely damaged. To place the eggs into the multicellular container, they were measured volumetrically (1 ml = 7,000 BAW, 7,000 SBL, 7,000 VBC) and ¼ ml, mixed with 50 ml of distilled water in a washing bottle, was sprayed onto white paper towels (Scott 1 ply roll towels, Scott Paper Co., Philadelphia, PA) cut to the size of the diet surface in the multicellular container. After paper towels with eggs dried, they were laid on the diet surface with the side containing the eggs facing the diet. Care was used in drying the paper towels and eggs because too much exposure resulted in desiccation and reduced egg hatch. Multicellular containers were then sealed, wrapped in a single layer of brown wrapping paper (18.2 kg wt.) to prevent entrance of microbial contaminants, and held at 29.5 ± 1° C and 55 ± 5% RH for 16 days.

Pupal Harvest. BAW and VBC pupae were harvested dry by removing the cell unit and bumping it against the tray, which caused the pupae to fall into the tray. Diet and frass were then removed from the pupae by use of a two-part separator and high volume blower. These techniques and equipment were described by Hartley et al. (1982) for harvesting *Heliothis* spp. pupae.

SBL pupae could not be harvested by the above method because the pupae are attached to the cell unit by webbing spun by the larvae prior to pupation. Cell units containing SBL pupae, diet, and frass were removed from the container tray and immersed in a 114-liter plastic vat containing 76 liter of 1% sodium hypochlorite maintained at 24°C. The webbing was dissolved by the sodium hypochlorite in about 5 min. The freed pupae float to the top and can be screened from the surface. The pupae are rinsed for 1 min in a no. 4 sieve with 24°C tap water to remove the sodium hypochlorite and small particles of diet or frass. SBL pupae are spread on paper towels and allowed to air dry. We found that one vat of 1% sodium hypochlorite solution is sufficient to dissolve the webbing of ca. 3,000 pupae. A fresh solution of sodium hypochlorite was prepared when pupae were not freed from the cell unit after soaking for ca. 5 min.

Results and Discussion

In 1987 and 1988, 173,200 BAW pupae, 19,481,000 BAW eggs, 384,200 SBL pupae, 42,795,000 SBL eggs, 308,200 VBC pupae, 48,306,000 VBC eggs were produced using the procedures described in this paper. There were no outbreaks of disease, and problems with the mold *Aspergillus niger* were eliminated by the phosphoric-propionic acid mix. The ability to produce these three insect species (BAW, SBL, VBC) and the corn earworm (CEW) in large numbers made possible the American Soybean Association's Insect Distribution Program to support entomological research on soybeans. Through this program, for a small fee the USDA Agricultural Research Service of Stoneville, MS, in cooperation with the American Soybean Association of St. Louis, MO, supplies eggs and pupae of BAW, SBL, VBC, and CEW to soybean researchers in government, industry and universities. Based on documented research by USDA scientists (Lambert and Hamer 1988) and conversations with industry researchers, e.g. (John Hicks, Pioneer Seed Co. and Doris Paroonagian, Dow Chemcial Co.), all BAW, SBL, and VBC reared by these techniques performed well in field, greenhouse, and laboratory tests.

Average pupal production per container, based on pupae collected from 100 containers, was 556, 604, and 813 for the BAW, SBL, and VBC, respectively. Average pupal wts. based on 50 males and 50 females collected from 100 containers were: BAW male 136 mg, female 154 mg; SBL male 202 mg, female 187 mg; and VBC male 249 mg, female 211 mg. This compares favorably with pupae reared in 22.5 ml plastic cups, which produced average pupal wts. of: BAW male 136 mg, female 160 mg; SBL male 178 mg, female 167 mg; and VBC male 254 mg, female 216 mg. The cup rearing system is not economical for large scale production, but is widely used to produce high quality insects which can be used for comparison.

The rearing of all three species using the same basic soybean flour-wheat germ diet used for rearing other species [tobacco budworm, *Heliothis virescens* (F.), bollworm, *H. zea* (Boddie), *H. subflexa* (Guenée), sterile *Heliothis* backcross, sugarcane borer, *Diatraea saccharalis* (F.), and *Bactra verutana* (Zeller)] at our laboratory not only simplified diet preparation, but also enable increased efficiency through purchase of diet ingredients in bulk. The ingredients to prepare 3.8 liter of soybean flour-wheat germ diet currently cost about $3.00. This is about $1.00 per 3.8 liter less expensive than the pinto bean diet that is widely used for rearing many Lepidoptera species (Leppla 1985).

The procedures described here simplified and economized rearing of the BAW, SBL, and VBC at the Stoneville laboratory. Previously, all three species were reared in 22.5-ml plastic cups which were expensive and required excessive labor to infest with larvae and harvest pupae. Disposable plastic cups (22.5 ml) with a waxed-paper lid cost about $0.02 each verus about $20.00 for construction of a reusable multicellular larval rearing container (648-903 cells). Cups cannot be easily reused, whereas the multicellular unit can be cleaned and reused for 10 years or longer with little additional expense. Infestation of eggs and pupal harvesting were accomplished much more rapidly using the multicellular container, thus saving approximately one half the time required for comparable rearing in 22.5-ml plastic cups.

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