Emergence of Male-Sterile *Heliothis virescens* (Lepidoptera: Noctuidae) Backcross Moths at a Central Release Point and Their Resulting Spatial Distribution[1]

M. L. Laster, D. D. Hardee, and J. L. Roberson

USDA, Agricultural Research Service
Southern Insect Management Laboratory
Stoneville, Mississippi 38776

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ABSTRACT *Heliothis virescens* backcross moths are most effectively released in wide-area release programs from pupae placed in the field prior to moth emergence. A significantly greater moth emergence was obtained in the field from unharvested pupae in rearing trays than was obtained from harvested pupae. Mating, oviposition, and egg viability of emerged female moths were not adversely affected by handling procedures. Moths released from emergence containers were recovered in the most distant trap, 5.5 km, from the release point. However, the greatest number of released moths was captured in pheromone traps within 1.7 km of the release point.

KEY WORDS Insecta, tobacco budworm, dispersal, capture, backcross.

Chemical insecticides have been used as the control method of choice for the bollworm, *Helicoverpa zea* (Boddie), and the tobacco budworm, *Heliothis virescens* (F.), since the introduction of DDT in the 1940's. Generally, these materials have been effective, economical, and easy to apply. However, historically, a given insecticide has controlled these species for approximately ten years before losing its efficacy (Laster et al. 1988). The latest group of insecticides, the pyrethroids, was first widely used to control the bollworm and tobacco budworm in the late 1970's. Soon after their adoption for use on a broad scale, problems associated with their control of the tobacco budworm began to surface (Plapp 1981, Martinez-Carillo and Reynolds 1983). These problems have since spread and intensified (Plapp and Campanhola 1986, Plapp et al. 1987, 1990, Allen et al. 1987, Leonard et al. 1987, Graves et al. 1988, Luttrell et al. 1987, 1988) and strongly suggest the need for other management strategies for the tobacco budworm.

Backcross (BC) sterility resulting from the hybridization of *H. virescens* and *H. subflexa* (Guenée) (Laster 1972) was listed as one of the three autocidal systems that offer promise for controlling the tobacco budworm (Laster et al. 1988). This sytem entails mass rearing and release of the BC insects to compete with the target insects for mating and infusing the sterile trait into the native population. The program must be directed at the overwintered population because the native moth population is lowest at this time so the cost of rearing a sufficient number of BC moths to release in an effective ratio is most economical. Critical to the success of a release program is high survival of laboratory-reared BC moths and their ability to distribute themselves in a normal manner.

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Proshold et al. (1983), in release studies on St. Croix, U. S. Virgin Islands, reported that mating behavior was more nearly random between native and BC insects when BC insects were placed in the field as pupae and adults were allowed to emerge than when adults were placed directly in the field. From these studies, Proshold (1983) reported a moth emergence of about 70% from BC pupae harvested at Stoneville, Mississippi, shipped to St. Croix, and placed in the field. Laster et al. (1987) obtained a 57% moth emergence in the field from harvested BC pupae. Laster and Roberson (1990) reported that moths emerged from 59% of harvested pupae used in *Heliothis virescens* emergence studies. Because the effectiveness of a wide-area release program in suppressing *H. virescens* is dependent upon the released:wild ratio and the rearing cost is a large percentage of the total cost, low moth emergence and poor distribution significantly increase the cost of the program and decrease the overall likelihood of success. We conducted studies during 1989-90 to assess the emergence and distribution of BC moths from individual release points to determine the maximum spacing between release points to result in an approximately uniform distribution over the release area.

Materials and Methods

Heliothis virescens BC pupae, possessing the sterile male trait (Laster 1972), were used in these studies. The pupae were reared in the R. T. Gast Insect Rearing Laboratory (USDA, ARS), Mississippi State, Miss., using mechanized equipment as described by Sparks and Harrell (1976). The BC pupae were produced from larvae reared on a standard *Heliothis* diet (Brewer 1982) that incorporated a red dye (Calco Oil Red) to serve as an internal marker for identification (Hendricks et al. 1970). Treatments during both years consisted of: (1) harvested pupae placed in emergence containers (Laster et al. 1987), (2) unharvested pupae in rearing trays made of molded plastic cells and placed in a styrofoam box adapted for moth emergence, and (3) same as treatment 2 except that a standard corrugated cardboard box was used. Pupae were either harvested mechanically and placed in emergence containers or moths were allowed to emerge from the plastic-celled trays with the cover removed but without harvesting. Harvested pupae were removed from the trays with a mechanical harvester using procedures similar to those described by Harrell et al. (1974). Harvested pupae were mixed with vermiculite in trays 53 × 28 cm and placed in emergence cages as described by Laster et al. (1987). Plant material was placed on top of these trays to provide a resting area for emerging moths and to prevent the vermiculite from being blown from the trays. In treatments 2 and 3, emergence boxes holding the plastic trays were placed on stands about 1.5 m tall constructed with a plywood base secured to a 2.5-cm diameter galvanized pipe driven into the ground. Slits about 1.5 × 20 cm were cut, two near the top and two near the bottom on two sides of each box, to provide ventilation and allow the emerged moths to escape. The emergence boxes were 36 × 36 × 58 cm in size and capable of holding 16 plastic trays consisting of 64 cells each for a total of 1,024 cells per emergence box. The trays were placed in the boxes in a criss-cross fashion to avoid covering the open cells and preventing moth escape. Walls of the styrofoam insulated boxes were 0.5 cm thick and constructed with

a styrofoam core between two pieces of heavy paper (manufactured by Brick Associates, Inc., Bloomfield, N. J.). Treatments were arranged in a randomized complete block design with three replications in 1989 and eight replications in 1990.

Pupae were harvested August 7, 1989, and all treatments were placed in emergence containers in the field at Stoneville, Miss., August 8. Pupae were harvested May 1 and 8 in 1990 and all treatments were placed in emergence containers in the field on Australia Island, Madison Parish, La., May 2 and 9, respectively. Temperatures in the emergence containers were monitored with a hygrothermograph in 1989 and with a remote weather station (Micrologger Model CR21, Campbell Scientific, Inc., Logan, Utah) in 1990. A sample of female moths (10-12) taken randomly from inside the containers of each treatment and a sample of *H. virescens* females from the laboratory during 1989 were used to determine if high temperatures inside the containers affected reproduction. These females were paired with *H. virescens* males in the laboratory and observed for egg deposition. After oviposition, the females were sacrificed and mating was determined by the presence of spermatophores and their fertility was determined by egg hatch.

The release experiments were terminated August 24, 1989, and June 6, 1990. Moth emergence was determined by counting empty pupal cases and dead pupae in all trays. Data were analyzed by a one-way, randomized complete block analysis of variance (ANOVA) program from the CoStat (1986) statistical package; mean differences were separated by Duncan's (1955) New Multiple Range Test.

Dispersal of BC insects from the release on Australia Island in 1990 was studied using wire cone traps (Hollingsworth et al. 1978). Each trap had a 61-cm diameter opening at the bottom of the cone and a 35-cm diameter opening in the center of the inner apron. Australia Island is a peninsula almost entirely surrounded by Eagle Lake and has an area of about 1200 ha. BC moths emerged from 20,068 pupae (10,915 on $^{5}/_{2}$ and 9,153 on $^{5}/_{9}$) and dispersed from the emergence site at a central location on Australia Island.

A total of 24 cone traps was used to assess moth dispersal: 14 positioned at about 0.8 km distances on Australia Island and 10 positioned at about 3.2 km distances around and across Eagle Lake from the island. Trap distance from the release point ranged from 0.8 to 5.5 km, and traps were pooled into three distance intervals to reduce standard errors in numbers recaptured per trap-night as a function of distance from the release site. Traps were rebaited at 14-day intervals with a black polyvinyl chloride dispenser impregnated with about 6 mg of Z-11-hexadecenal: Z-9-tetradecenal (14.6:1) (V-2) (Hendricks et al. 1987). Moths were removed from the traps twice weekly, killed by fumigation with ethyl acetate, placed in labeled plastic bags and transported to the laboratory. The moths were then squashed to reveal the presence or absence of the red dye marker, and the number of moths captured was recorded for each trap number and location. Capture numbers were converted to the number captured per trap per night.

Results and Discussion

The numbers of pupae and percent moth emergence for the different treatments in 1989-90 are presented in Table 1. The 59.2 percent moth emergence obtained from harvested pupae in 1989 was similar to the 57.0 percent from harvested pupae reported by Laster et al. (1987) but was significantly lower ($F = 279.55$; df = 2, 8; $P < 0.05$) than that from unharvested treatments. Although moth emergence from harvested pupae in 1990 increased to 84.4%, emergence remained significantly lower ($F = 10.44$; df = 2, 23; $P < 0.05$) than that from unharvested pupae. Average emergence of 77.5% during the two-year study was significantly lower ($F = 17.34$; df = 2, 32; $P < 0.05$) than the two-year average emergence (> 94.8%) obtained from unharvested pupae.

Handling during the harvesting operation may have caused the generally low moth emergence from the harvested pupae relative to unharvested pupae. Because all pupae do not develop at the same rate as evidenced by the varied color and texture of the pupal case, and all pupae in a given tray must be harvested on the same day, pupae within a tray were harvested at various stages of development. The less mature pupae were apparently adversely affected by the harvest handling which probably caused most of the mortality and lower moth emergence from the harvested pupae indicated in Table 1. We believe that holding the pupae in the trays longer before harvest would have allowed more pupae to mature and increased the percentage of moth emergence. Moth emergence from pupae placed in release stations without harvesting was greater because all pupae were allowed to mature and were not subjected to handling prior to emergence.

Moth emergence from unharvested pupae placed in the styrofoam emergence box was not significantly greater than those placed in the cardboard box (Table 1). The lighter color and greater rigidity of the styrofoam box gave the pupae better protection from rain and extreme heat from sunlight than the cardboard box. Many of the cardboard boxes collapsed during heavy rain, and water had to be emptied from some of the trays.

Factors contributing to the increase in moth emergence from harvested pupae from 59.2% in 1989 to 84.4% in 1990 are not obvious. Placing the pupae in the field in August in 1989 and in May in 1990 may have contributed to this difference.

Maximum temperatures inside the cardboard box and for the harvested pupae in August reached 40° and 35°C, respectively. Temperature inside the styrofoam box was not obtained because of equipment malfunction. Maximum temperatures inside emergence containers during the May 1990 emergence period reached 33.3°, 32.7°, and 32.1°C for the harvested pupa container, cardboard box, and styrofoam box, respectively.

The average number of matings for female moths from the laboratory *H. virsecens,* harvested pupae, styrofoam box, and cardboard box was 3.0, 3.0, 2.3, and 2.7, respectively. The high temperature (40°C) in the cardboard emergence boxes apparently had no effect on female reproduction because larvae were produced by all mated females from all treatments. The effects of high temperatures on the BC males were not evaluated because these males are inherently sterile.

Table 1. Emergence of sterile *Heliothis* backcross moths from pupae treated as indicated in emergence cages in the field, Stoneville, MS 1989-90.

Treatment	No. Pupae		Percent emergence ± SE		
	1989	1990	1989	1990	1989-90
Harvested	3000	6037	59.2 ± 2.0 a	84.4 ± 9.1 a	77.5 ± 14.0 a
Unharvested (cardboard box)	3029	6833	94.8 ± 1.4 b	95.1 ± 2.2 b	94.8 ± 1.9 b
Unharvested (styrofoam box)	3022	7228	96.5 ± 2.6 b	95.9 ± 1.1 b	96.1 ± 1.5 b

Means in the same column followed by the same letter are not significantly different (P > 0.05; Duncan's [1955] multiple range test).

Capture of marked moths spanned a 30-day period from May 7 to June 7, 1990. A total of 18,256 moths emerged from 20,068 pupae placed in the field. During the 30-day capture period, 554 marked moths (3.0% of the total released) and 848 wild tobacco budworm moths were captured in the traps. Capture data are summarized in Table 2 as the number of male moths captured per trap per night (n = 30 and mean ± SE) for the three distance categories.

Proshold et al. (1983) reported that the greatest number of released moths captured per trap in studies on St. Croix, U. S. Virgin Islands was within the first 0.8 km of the release point. Our most distant trap from the release point was 5.5 km and released moths were captured in all traps. However, released moths were captured in greatest numbers to a distance of 1.7 km (Table 2). The trapping data suggest that by spacing release stations 3.4 km apart, movement of 1.7 km from each point should result in approximately uniform spatial distribution of moths in release programs.

Table 2. Average number of released, internally marked, BC and wild tobacco budworm males captured per pheromone trap per night at various distances (km) from a release point on Australia Island (Madison Parish, Louisiana) 1990.

Distance from release point (km)	No. traps	No. moths captured per trap per night ± SE	
		Marked	Wild
0 - 1.7	5	1.05 ± 0.93	1.01 ± 0.79
1.7 - 2.5	11	0.89 ± 0.54	1.27 ± 0.70
2.5 - 5.5	8	0.48 ± 0.38	1.16 ± 0.82

Smith and Snodgrass (1983) reported that numbers of tobacco budworm moths captured in pheromone traps were strongly influenced by trap location and habitat surrounding the trap. The numbers of both marked and wild moths captured in traps were variable as indicated by the standard errors (Table 2). The habitats surrounding these traps also varied, but no single physical or environmental factor was identified as a major influence on number of moths captured.

References Cited

Allen, C. T., W. L. Minter, R. R. Minzemayer and J. S. Armstrong. 1987. Development of Pyrethroid resistance in *Heliothis* populations in cotton in Texas. pp. 332-335. *In* Proceedings, Beltwide Cotton Production Research Conference, Dallas, January 4-8, 1987. National Cotton Council of America, Memphis, Tenn.

Brewer, F. D. 1982. Development and food utilization of tobacco budworm hybrids fed artificial diet containing oil soluble dyes. J. Georgia Entomol. Soc. 17: 248-254.

Co Stat. 1986. User's Manual for Co Stat (version 2.03). CoHort Software, Berkeley, Calif.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 11: 1-42.

Graves, J. B., B. R. Leonard and A. M. Pavloff. 1988. Status of pyrethroid resistance in tobacco budworm in Louisiana. pp. 335-356. *In* Proceedings, Beltwide Cotton Production Research Conference, New Orleans, January 9-14, 1988. National Cotton Council of America, Memphis, Tenn.

Harrell, E. A., A. N. Sparks and W. D. Perkins. 1974. Machine for collecting corn earworm pupae. USDA, ARS [Rep.] ARS-S-43, 4 pp.

Hendricks, D. E. and H. M. Graham. 1970. Oil-soluble dye in larval diet for tagging moths, eggs, and spermatophores of tobacco budworms. J. Econ. Entomol. 63: 1019-1020.

Hendricks, D. E., T. N. Shaver and J. L. Goodenough. 1987. Development and bioassay of molded polyvinyl chloride substrates for dispensing tobacco budworm (Lepidoptera: Noctuidae) sex pheromone bait formulations. Environ. Entomol. 16: 605-613.

Hollingsworth, J. P., A. W. Hartstack, D. R. Buck and D. E. Hendricks. 1978. Electric and nonelectric moth traps baited with the synthetic sex pheromone of the tobacco budworm. USDA, ARS-S-173. 13 pp.

Laster, M. L. 1972. Interspecific hybridization of *Heliothis virescens* and *H. subflexa.* Environ. Entomol. 1: 681-687.

Laster, M. L., W. F. Kitten, E. F. Knipling, D. F. Martin, J. C. Schneider and J. W. Smith 1987. Estimates of overwintered population density and adult survival rates for *Heliothis virescens* (Lepidoptera: Noctuidae) in the Mississippi Delta. Environ. Entomol. 16: 1076-1081.

Laster, M. L., J. E. Carpenter, M. D. Huettel, S. G. Miller, F. I. Proshold, R. T. Roush, J. C. Schneider and A. N. Sparks. 1988. Autocidal systems for *Heliothis* control. *In* Theory and tactics of *Heliothis* population management: III - emerging control tactics. So. Coop. Ser. Bul. No. 337, pp. 74-103.

Laster, M. L. and J. L. Roberson. 1990. *Heliothis* pupal handling and placement methods for improved moth emergence in wide-area release programs. pp. 311-312. *In* Proceedings, Beltwide Cotton Production Research Conference, Las Vegas, January 2-7, 1990. National Cotton Council of America, Memphis, Tenn.

Leonard, B. R., J. B. Graves, T. C. Sparks and A. M. Pavloff. 1987. Susceptibility of bollworm and tobacco budworm larvae to pyrethroid and organophosphate insecticides. pp. 320-324. *In* Proceedings Beltwide Cotton Production Research Conference, Dallas, January 4-8, 1987. National Cotton Council of America, Memphis, Tenn.

Luttrell, R. G., R. T. Roush, A. Ali, J. S. Mink, M. R. Reid and G. L. Snodgrass. 1987. Pyrethroid resistance in field populations of *Heliothis virescens* (Lepidoptera: Noctuidae) in Mississippi in 1986. J. Econ. Entomol. 80: 985-989.

Luttrell, R. G., J. B. Graves and J. R. Phillips. 1988. Performance of pyrethroid insecticides against *Heliothis* in the mid-south during 1987. pp. 232-236. *In* Proceedings, Beltwide Cotton Production Research Conference, Dallas, January 4-8, 1987. National Cotton Council of America, Memphis Tenn.

Martinez-Carillo, J. L. and H. T. Reynolds. 1983. Dosage mortality studies with pyrethroids and other insecticides on the tobacco budworm (Lepidoptera: Noctuidae) from the Imperial Valley, California. J. Econ. Entomol. 76: 983-986.

Plapp, F. W., Jr. 1981. Toxicity of synthetic pyrethroids to laboratory and field populations of the tobacco budworm in central Texas. J. Econ. Entomol. 74: 207-209.

Plapp, F. W., Jr. and C. Campanhola. 1986. Synergism of pyrethroids by chlordimeform against susceptible and resistant *Heliothis.* pp. 167-169. *In* Proceedings, Beltwide Cotton Production Research Conference, Las Vegas, January 4-9, 1988. National Cotton Council of America, Memphis, Tenn.

Plapp, F. W., Jr., G. M. McWhorter and W. H. Vance. 1987. Monitoring for pyrethroid resistance in the tobacco budworm. pp. 324-326. *In* Proceedings, Beltwide Cotton Production Research Conference, Dallas, January 4-9, 1987. National Cotton Council of America, Memphis, Tenn.

Plapp, F. W., Jr., J. A. Jackman, C. Campanhola, R. E. Frisbee, J. B. Graves, R. G. Luttrell, W. F. Kitten and M. Wall. 1990. Monitoring and management of pyrethroid resistance in the tobacco budworm (Lepidoptera: Noctuidae) in Texas, Mississippi, Louisiana, Arkansas, and Oklahoma. J. Econ. Entomol. 83: 335-341.

Proshold, F. I. 1983. Release of backcross insects on St. Croix, U. S. Virgin Islands to suppress the tobacco budworm (Lepidoptera: Noctuidae): Infusion of sterility into a native population. J. Econ. Entomol. 76: 1354-1359.

Proshold, F. I., J. R. Raulston, D. F. Martin and M. L. Laster. 1983. Release of backcross insects on St. Croix to suppress the tobacco budworm (Lepidoptera: Noctuidae): Behavior and interaction with native insects. J. Econ. Entomol. 76: 626-631.

Smith, J. W. and G. L. Snodgrass. 1983. Influence of surrounding habitat on *Heliothis* pheromone trap catch. Proc. 1982 Miss. Entomol. Assn., pp. 20-21.

Sparks, A. N. and E. A. Harrell. 1976. Corn earworm rearing mechanization. USDA Tech. Bull. 1554, 11 pp.