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Breeding Cotton for Resistance to the Tobacco Budworm: Techniques to Achieve Uniform Field Infestations[1]

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ABSTRACT

Techniques, equipment, and procedures for handling tobacco budworm, *Heliothis virescens* F., pupae, adults, eggs, and first instar larvae are described, as well as procedures for uniformly distributing first instar larvae on progeny rows of cotton (*Gossypium hirsutum* L.) in field plots. These techniques provide the uniformity and damage levels necessary to evaluate progeny rows for resistance to the tobacco budworm. In 1980, the techniques were used for 8 weeks. Egg production averaged 220,000 per day; and 90,000 to 100,000 plants were inoculated seven times each over the 8-week period. To illustrate the effectiveness of the techniques, data for the two check entries from 25 experiments are included herein. Mean yield losses due to *Heliothis* were 1,514 and 1,563 kg seed cotton/ha in 'Stoneville 213' and in a glandless, nectariless line of 'Stoneville 7A,' respectively. The system described is cost effective, rapid, uniform, and can be expanded easily to any desired size. The availability and use of these techniques should make it feasible for commercial cotton breeding companies to evaluate and select lines with increased levels of plant resistance to the tobacco budworm.

Additional index words: *Heliothis virescens* F., *Gossypium hirsutum* L., Host-plant resistance.

PRIMARY needs in breeding for insect resistance include techniques allowing scientists to obtain a uniform and repeatable level of pest infestation on progeny rows. Efforts to expand our breeding programs in cotton (*Gossypium hirsutum* L.) to include resistance to the cotton bollworm complex, *Heliothis* spp., were inhibited because of the inconstancy of natural insect populations and inadequate techniques.

Oliver et al. (1967) described a technique for growing *Heliothis* larvae on lyophilized square powder to determine the rate of larval growth. Parrott et al. (1978) described a technique useful for growing *Heliothis* larvae for weight-gain determinations on green terminal leaves or terminal tissue. However, both techniques were time consuming, expensive, and poorly suited for use on a large number of progeny rows. Therefore, we decided to develop techniques usable in the field and easily adapted by commercial cotton breeders. Hall et al. (1980) described techniques for placing eggs or larvae on cotton plants to obtain an infestation suitable for rating those plants on their levels of resistance. The distribution of first instar larvae was superior to eggs.

The production or rearing of lepidopterous insects is well documented (Berger, 1963; Burton et al., 1966; Burton, 1967; Raulston and Lingren, 1972; Sparks and Harrell, 1976; Davis, 1976; Davis et al., 1978). These rearing systems have been automated and are usually cost effective. Several commercial companies which produce hybrid corn (*Zea mays* L.) are presently rearing European corn borer (*Ostrinia nubilalis* Hubner), Southwestern corn borer (*Diatraea grandiosella* Dyar), or the fall armyworm (*Spodoptera frugiperda* J. E. Smith) for use in plant breeding programs.

The objective of this paper is to describe a system for producing larvae of the tobacco budworm (*H. virescens* F.) and for disseminating them uniformly to cotton plants so that different levels of resistance to this insect can be measured easily. Our method measures resistance as the ability of the plant to mature a large number of bolls when continuous populations of first instar budworm larvae are placed on the plant and allowed to grow and cause damage. It is realized that this method may not measure other potentially useful aspects of resistance. The following constraints were placed on the system: it must be 1) cost effective, 2) rapid, 3) uniform, 4) easily expanded to a size useful for commercial breeding, and 5) capable of measuring useful heritability.

METHODS AND MATERIALS

Moth House. An aluminum building (Fig. 1) was purchased for use as a moth house. We added 10 cm of insulation to the inside walls and 15 cm to the ceiling. Inside, we covered the walls and ceiling with 3.1 mm Masonite[3] paneling, applied two coats of polyurethane varnish, and installed vinyl floor covering (to make cleaning up easier and for protection in our high humidiy environment). A 5,500 BTU window air conditioner, an electric heater, and a humidifier were added to aid in humidity and temperature control. A 1.2 m section of flourescent lights set on a 12-hour light-dark cycle was added. Three Dome[3] micronaire electro-precipitator units were added to aid in moth scale control. A 5 cm fiberglass pre-filter was added to each unit. In addition, mask respirators (Comfo brand, assembly no. 7-201-1, with dust mist MSA filters TC-21C-133)[3] were worn while in the house.

A rack that held 20 oviposition cages was constructed along the 3.6 m side of the house opposite the door (Fig. 2). Twenty-watt incandescent lights, reduced in intensity to a slight glow with a rheostat and covered with two Styrofoam[3] picnic plates as dif-

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fusers, were placed between each two cages and were operated continuously. Temperature was maintained at 17 ± 1°C and relative humidity at 70 to 75%.

Cages. Cages for oviposition were built following the design by McWilliams (1977, Fig. 3). The cage frames were made of 2.5 × 23.5 cm wood. Grooves on both sides were fitted at top and bottom with aluminum-framed 20-mesh fiberglass screens, 63.5 × 63.5 cm. The moths oviposit on the screens. The wooden parts of the cage were painted black and then painted with a polyurethane coating. The slick surface tended to reduce oviposition on the cages.

Two 4-liter, round paper cartons were taped together for pupal emergence containers and fitted with a 6.4 cm hole. A 0.63 cm mesh rubber mat was shaped into a cone for the moths to rest on while they expanded and dried their wings. One day prior to emergence, vermiculite and 500 pupae were placed into the canister which was attached to the cage via a short piece of polyvinyl pipe, 6.4 cm in diameter. Moths emerged, dried their wings, and crawled or flew from the canister into the cage. Moths were fed a solution of 350 ml honey, 560 ml water, plus 5 cc of a complete vitamin mixture (Berger, 1963). The feeding chamber was a Telan[3] *Drosophila*-rearing cage. These chambers have an interlocking lid containing 13 holes < 1 mm in diameter. The base is 1.9 cm deep by 8.3 cm in diameter. A 2.5 × 10 cm piece of Mylar[3] was glued to the bottom of the feeding chamber to prevent it from falling through the hole in the cage. The chamber was then filled with the feeding solution, covered with a 7 cm circular piece of no. 2 qualitative filter paper which covered the 13 holes, and inverted into a hole in the top of the cage. Two feeding chambers were used per cage. The liquid nutrients soak into the filter paper from which the moths feed. Oviposition screens were changed easily by sliding a new one into the groove, thus forcing the egg-laden screen out the opposite end. Few moths escaped because the cage was not opened in this operation.

Egg Collection. Each day, the cages with egg-laden screens were removed from the house; the oviposition screens were changed, and the cages were then returned to the moth house. The screens with the eggs were placed into an egg collector (Fig. 4) 10 at a time. The collector was designed similarly to one used by McWilliams (1977). The screens were shaken horizontally at 250 strokes/min for 15 min in a 0.025% sodium hypochlorite solution made by mixing 1.96 liters of Clorox[3] in the 191 liters of water. The solution was then drained from the tank through a 0.84 mm and a 0.30 mm sieve. The eggs retained on the 0.30 mm sieve were washed onto filter paper and placed in an incubator to air dry.

[3]Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

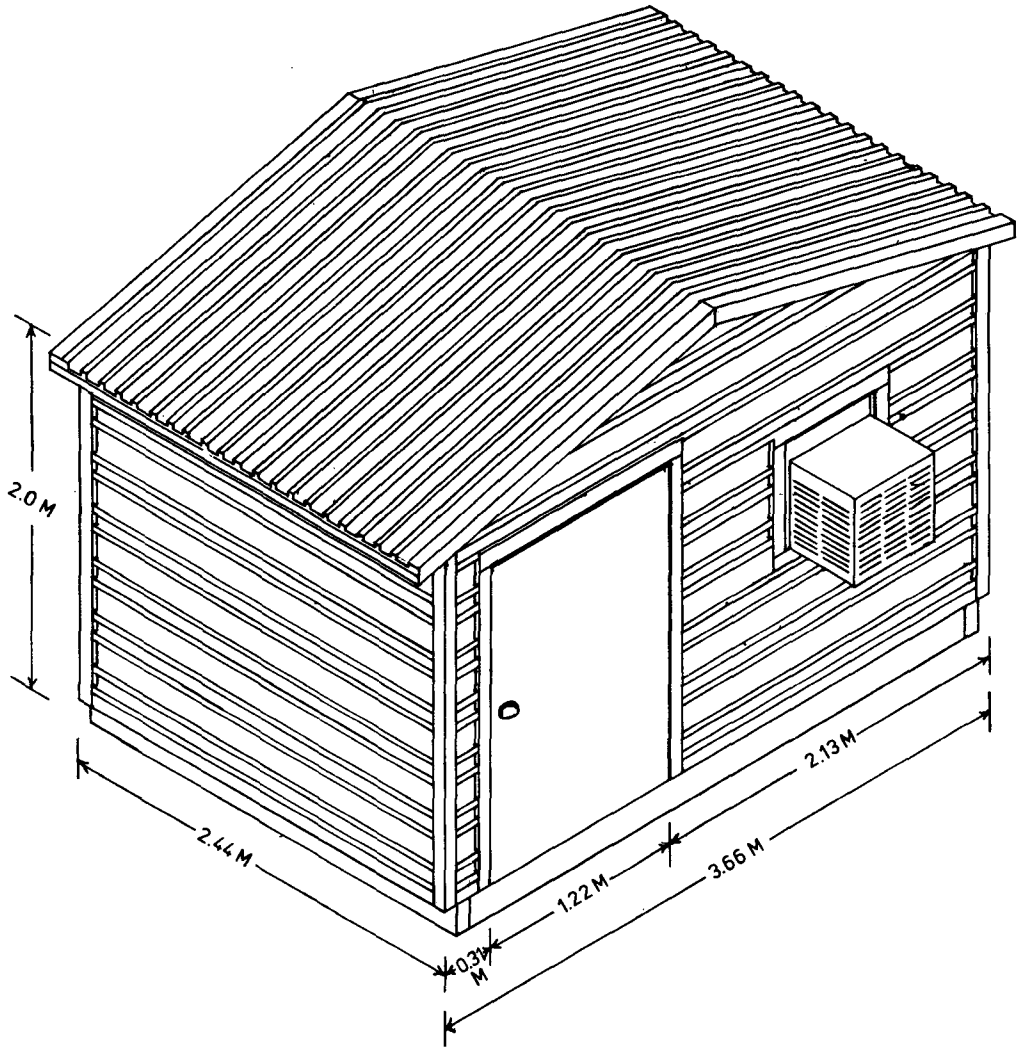

Fig. 1. Moth house, its external form and dimensions.

Sanitation. Each day, when the screens were changed, the cages were sponged off with a commercial germicidal (Blue Chip[3]) solution. The cages containing preovipositional females were handled the same way except the screens were not changed daily. The floor was also mopped daily with the germicidal solution. When a cage was rotated out of the moth house after 10 days (3 preoviposition and 7 for oviposition), it was washed with the germicidal solution. The rubber mats used to make the cones in the pupal canisters were soaked for 30 min in a 0.5% Clorox[3] solution. No disease problems occurred.

Handling of Eggs and Calibration of Larvae to Cob Grits Ratio. After the eggs had dried on the filter paper, they were gently brushed from the paper and weighed on an analytical balance. We calibrated applications by weighing eggs. Dry eggs weighed 76 mg/1,000. For our purposes, we placed 15,000 eggs and 25 g cob grits (No. 2040 Grit-o-Cobs[3] from Andersons, Maumee, Ohio) in a 4-liter glass jar. Each day, 50 eggs were also placed on moist filter paper in a petri dish to obtain percent hatching for quality control. Two Howard[3] incubators, set at 27 C with ambient relative humidity, were used for holding eggs until they hatched.

Twenty-four hours before the eggs hatched, a 10 × 40 cm piece of Hexcell[3] (HRH 10-1/8-3.0 cut to 0.27 thickness from Hexcell Corp., Arlington, Texas) was placed in the jar. As the larvae hatched, they moved into the Hexcell[3] which helped to prevent clumping. Clumping was a problem until 25 g cob grits plus the Hexcell[3] were added to the 15,000 eggs. The egg hatch in darkness also aids in controlling clumping.

Larvae required 974 degree hours above a developmental threshold of 12.6 C to hatch. Hatching on weekends was prevented by holding eggs collected on 2 days for a specified number of hours at 15.6 C (72 degree hours/24-hour period) before placing them into the 27 C (335.6 degree hours/24-hour period) incubators. This procedure allowed 7 days of eggs to hatch within a 5-day work week.

Larvae emerged between 0800 and 1200. At 1200, the mixing of the larvae and cob grits began. The Hexcell[3] was removed from a jar and tapped gently over a white porcelain tray to remove the larvae. Cob grits (200 g) were added to the tray and mixed with the larvae. The cob grits from the tray were added to the glass jar and mixed thoroughly, by gently shaking, to distribute the larvae uniformly throughout the grits. The mixture was poured into a plastic bottle and capped for transport to the field. Bottles of the larvae-grits mixture were attached to the Davis modified-CIMMYT inoculator (Mihm et al., 1978; Davis and Oswalt, 1979; Fig. 5); several applications were made onto a white surface which was checked for number of larvae per application. The inoculator dispenses 0.3 g of cob grits per application. By checking the percent hatch from quality control and knowing the number of eggs placed in each 4-liter jar, the proper amount of grits was added to the mixture for a specified number of larvae per application. For our routine work in 1980, 12 ± 2 larvae/plant (in one application) were applied.

The bottles of larvae-grits mixture were transported to the field in Styrofoam[3] ice chests with crushed ice. By keeping the larvae cool, they remained distributed throughout the grits. Larvae were dispensed in about 2 hours after mixing on a normal day. Larvae have been kept in the grits for 4 hours without adverse effects. After we had transported the larvae to the field and applied most of them to the cotton, we would frequently dispense one application of larvae-grits into a rearing cup containing artificial diet and then determine the number of larvae 4 days later as a quality control.

Field Plots and Larvae Application. Plants were grown in 9 m progeny rows 96 cm apart with 1.5 m alleys between rows. Cotton was planted on 6 to 9 May 1980 and emerged after about 1

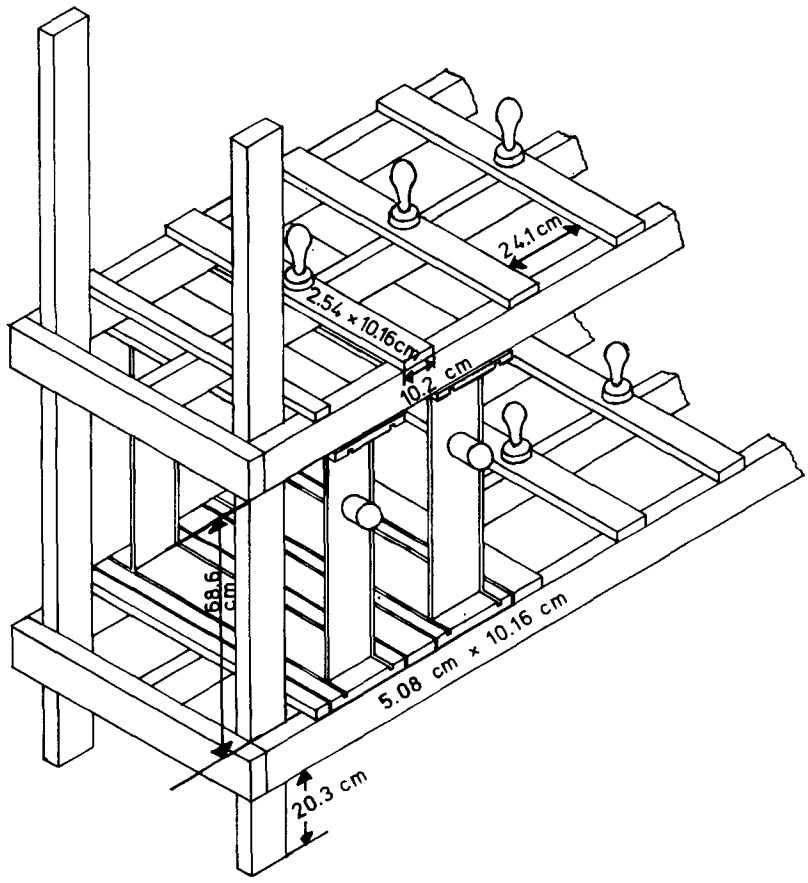

Fig. 2. Rack for holding 20 oviposition cages.

week. Plants were thinned to approximately 1 plant/22 cm when they were at the fourth to fifth node stage [i.e., the V_3 or V_4 stage (Elsner et al., 1979)]. Cotton began to square (produce flower buds) on 25 June when the first application of larvae was made by placing the grits-larvae mixture in the terminal area of each plant. Usually the application was placed in the terminal or on the first new leaf in the terminal. In 5 to 10 min on a hot day, the young larvae had crawled out of the grits mixture and were established in the terminal area or on the underside of the leaf. We estimated that at least one of the 12 larvae/plant would survive. Hall et al. (1980) recovered about 6% of the larvae they applied after 10 days.

In 1980, 5,000 pupae/week were received from the rearing group at the Bioenvironmental Insect Control Res. Lab., Stoneville, Miss. Twenty adult cages were kept in the moth house at all times when at full operation. Usually, cages of moths were kept for 10 days. Egg production averaged 220,000/day. We had 2,576 field plots involving about 90,000 to 100,000 plants. Thus, about 18,000 to 20,000 plants were treated/day. Because eggs were collected on a 7-day schedule and larvae were applied on a 5-day schedule, additional plots were treated 2 days/week. Therefore, all plots received larvae at approximately 7-day intervals. Treatment began 25 June and ended 20 August; each plot was treated seven times during the season. Relatively uniform infestation and damage levels were obtained.

In 1980, we conducted 25 separate experiments each involving about 25 lines being evaluated for resistance. 'Stoneville 213' (ST 213, a widely grown commercial cultivar) and Stoneville 7AGN (ST7AGN, a glandless, nectariless breeding line) were included in each experiment as checks. In each experiment, all entries were grown under two conditions: 1) fenvalerate was applied weekly for budworm control and azinphosmethyl {*O,O*-dimethyl *S*-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl) methyl] phosphorodithioate} as needed for boll weevil, *Anthonomus grandis* Boh., control; and 2) tobacco budworm larvae were applied as previously described with azinphosmethyl used as needed for boll weevil control. A randomized, complete block with four replications was grown for each experiment. The predominant soil type was Leeper silty clay loam (fine, montmorillonitic, nonacid, thermic, Vertic Haplaquepts) with inclusions of Catalpa silty clay loam (fine, montmorillonitic, thermic, Fluvaquentic Hapludolls).

RESULTS AND DISCUSSION

To illustrate the use and effectiveness of the techniques described in this paper, we present data in Table 1 from only the ST213 and ST7AGN entries in the 25 experiments conducted in 1980. Fruit damage rating, bloom counts, and visual observations showed that both ST213 and the glandless, nectariless line received extensive damage. Harvest data (yield of seed cotton in kilograms per hectare) were used to evaluate our materials for levels of resistance.

The mean seed cotton yields of ST213 and ST7AGN among the 25 experiments provide a measure of the re-

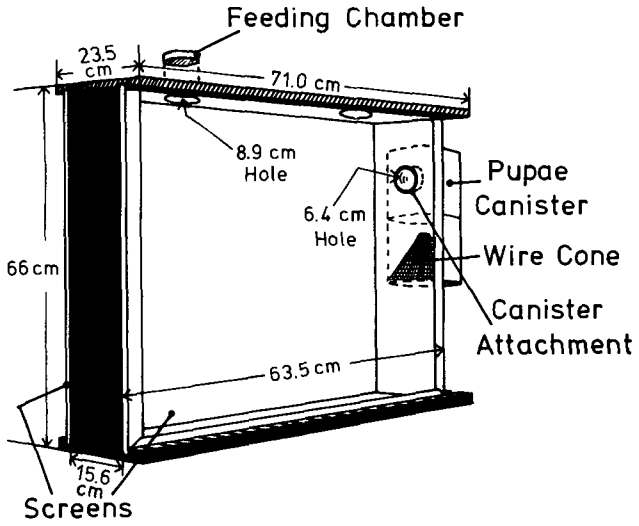

Fig. 3. Adult cage used for oviposition.

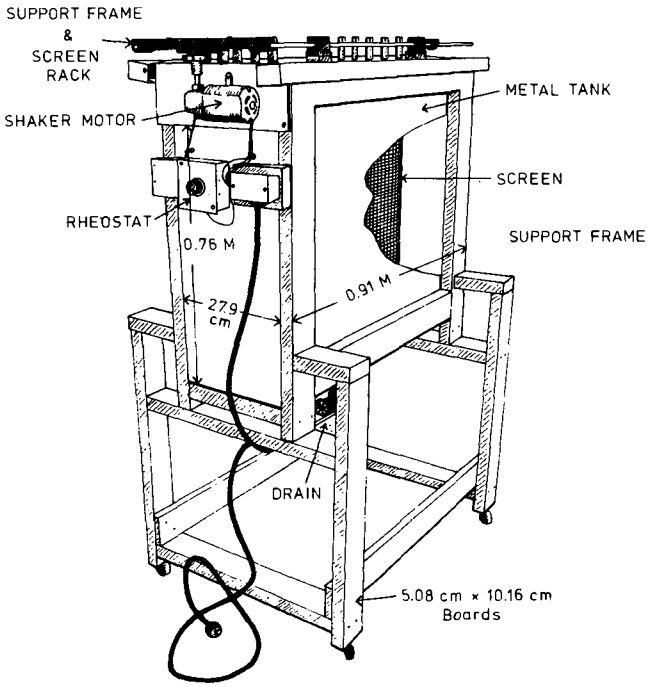

Fig. 4. Egg collector machine.

Fig. 5. Larvae inoculator.

Table 1. Seed cotton yield of the two checks common to 25 experiments in 1980.

Exp. no.	Stoneville 213			Stoneville 7AGN		
	Without *Heliothis*†	With *Heliothis*‡	Loss	Without *Heliothis*†	With *Heliothis*‡	Loss
	kg/ha					
33-1	1,708	666	1,042	1,532	336	1,196
33-2	2,255	616	1,640	1,495	463	1,033
33-3	2,391	1,264	1,127	1,892	240	1,652
33-4	2,139	799	1,341	2,248	172	2,075
33-5	2,490	721	1,768	1,748	290	1,458
33-6A	2,536	754	1,783	1,792	452	1,340
33-6B	2,251	896	1,355	1,564	384	1,180
33-6C	2,206	988	1,218	1,925	534	1,391
33-6D	2,539	945	1,594	1,742	374	1,367
33-7A	2,460	1,022	1,437	1,631	213	1,418
33-7B	2,467	903	1,564	1,609	221	1,388
33-7C	2,118	784	1,334	1,855	166	1,689
33-7D	2,715	348	2,367	1,766	277	1,489
33-8A	2,203	550	1,653	2,437	185	2,241
33-8B	2,100	693	1,407	1,558	227	1,331
33-8C	2,321	825	1,496	1,654	232	1,422
33-9	2,470	604	1,866	1,910	409	1,501
33-10	2,425	1,718	707	1,824	376	1,448
33-11	2,278	1,175	1,103	2,535	311	2,224
33-12	2,611	754	1,857	2,035	268	1,767
33-13	2,521	1,410	1,111	2,209	623	1,586
33-14	2,341	457	1,883	1,764	654	1,110
36-1	2,408	609	1,799	2,554	338	2,216
36-2	2,336	478	1,858	1,954	180	1,774
36-3	2,360	778	1,551	2,037	283	1,754
$\bar{x}$	2,345	839	1,514	1,891	328	1,562
SD	205	310	356	305	136	339
C.V.	8.8	37.4	23.5	16.1	41.3	21.7
L.S.D. 0.05	119	180	207	177	80	197

† Fenvalerate insecticide applied every 7 days.
‡ *Heliothis* larvae inoculated on plants every 7 days.

peatability of the infestation techniques as measured by yield reductions due to budworms (Table 1). Mean yield losses for the two entries are another (and probably superior) way of considering the data. The two entries had different yield potentials as is shown by their yields from insecticide-treated plots (ST 213, 2,345 kg/ha; ST7AGN, 1,891). The yield losses, however, were very similar; ST213 with 1,514 kg/ha ± 356 and ST7AGN 1,562 kg/ha ± 339. We were able to significantly reduce yields by applying tobacco budworm larvae. The amount of yield reduction desired in particular breeding programs or stages of breeding programs will differ; and this can be regulated by the numbers of larvae applied, frequency of application, or both.

As we considered screening for the tobacco budworm in our program, we chose to develop a system which could operate at the field or location level. We thus chose to develop a system which would begin with pupae and distribute first instar larvae. Pupae are easily shipped or stored and can thus be produced at a central location and shipped to field locations where the system is actually put into operation. The system was successfully used to inoculate 90,000 to 100,000 plants in 1980. Approximately 7 million larvae were grown and distributed in a uniform manner over an 8-week period. Each plant was infested seven times during this period.

The techniques described in this paper should make it feasible for commercial cotton breeding companies to evaluate lines for their relative levels of resistance to the tobacco budworm. These methods may also be used to produce uniform infestations for evaluations of chemical or biological control agents or other programs where uniform infestations are necessary. They should be easily adapted for use with the closely related cotton bollworm, *Heliothis zea* Boddie, and perhaps with other lepidopterous larvae on cotton and on other crops.

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