EVALUATION OF TWO LABORATORY STRAINS OF TOBACCO BUDWORM USED FOR ARTIFICIAL INFESTATION OF COTTON

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Abstract

Two laboratory strains of tobacco budworm (*Heliothis virescens*, F.) were evaluated for the ability to cause fruiting structure damage on cotton (*Gossypium hirsutum*, L.) in a small plot field trial during the 1999 growing season. The two colonies are reared under different rearing systems as well as for different intended research purposes. The Dow AgroSciences (DAS) colony (Indianapolis, IN) is reared for the purpose of maintaining consistent year to year laboratory bio-assay evaluations of foliar applied instecticides. Alternately, the colony obtained from the insectary of the Corn/Cotton Host Plant Resistance Research Units, at the U.S.D.A, Agricultural Research Service (A.R.S.) facility at Starkville, MS is reared for the express purpose of artificially infesting field plots in order to evaluate cotton lines for host plant resistance. While there are various housing and minor nutritional differences between the two colonies, a major difference in the two rearing systems is that A.R.S. scientists out-cross their laboratory females with natural population "wild" males on an annual basis in the Fall of each year. The progeny from the cross are then used to begin a new colony and the old colony is discarded. The DAS budworm colony has not been out-crossed to a natural population in over 15 years. Sampling data averaged over 6 weeks showed that the A.R.S. tobacco budworm colony closely exhibited a 2:1 difference over the DAS colony in terms of greater damage and a 3:1 difference in greater larval presence in squares and bolls under field conditions. Seed cotton yield data showed that there was a reduction in yield in the A.R.S. budworm infested plots of 12% from the non-infested control plots and a 15% reduction in yield from the DAS budworm infested plots. There was no difference in yield in the DAS budworm infested plots when compared with the non-infested control plots. The research appears to show that laboratory reared colonies of tobacco budworm used for artificial infestation on cotton can differ in the ability to cause fruit damage under field conditions. Out-crossing a laboratory colony with natural population insects seems to play a major role in enhancing the ability of the progeny to aggressively damage fruit under field conditions when using artificial infestation methods. This in turn may increase the reliability of germplasm screening techniques for host plant resistance, or other pesticide screening research that involve plant/insect interactions.

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Introduction

Artificially infesting cotton with laboratory strains of insects as a way to supplement natural infestations has been successfully demonstrated to be a valuable tool for evaluating cotton lines for host plant resistance (Jenkins et. al., 1982). This same artificial infestation technique can also be used to evaluate transgenic cotton lines and foliar insecticides as well. These methods are often implemented because of year to year inconstancy in natural insect populations. A minimal natural insect population tends to cause inadequate damage to the susceptible check cotton plant lines and therefore increases the difficulties in detecting those plant lines that are more resistant to feeding damage.

A possible weak link in this evaluation method would be the utilization of a laboratory strain that is not well adapted to feeding on cotton under field conditions. While there are some consistent factors, rearing methodology quite often differs from laboratory to laboratory in terms of colony genetics, nutrition, and physical housing. Most rearing systems do involve feeding the larval stage of the TBW an artificial diet made of various soy protein/wheat germ based diet combined with vitamins while it is being confined within a small singular cell. However, containers used in rearing the adult stage usually differ in size and therefore in volume of adult habitation. Larger volume containers with a greater number of adults would probably allow an increased rate of random mating of adult pairs as well as increased opportunity for mobility through flight. Smaller containers may inhibit mobility and therefore may reduce adult fitness and perhaps even mating. Moths lose wing scales in moving around and these often collect in small cramped containers, which may have poor ventilation, and this could further increase stress on the adults. There may also be differences in the genetic backgrounds of particular colonies of insects. Some researchers are practitioners of "out-crossing to the wild". A term used to describe the out-crossing of laboratory female adults to natural population adult males. This involves capturing males in pheromone traps in the field and bringing them into the laboratory to mate with lab reared females. The immediate progeny from this cross are assumed to be genetically 50% natural population genes and 50% lab population genes. These progeny are then used to start a new colony. The old colony is discarded after several generations if the new colony is performing equivalent to it. It is generally believed that bringing in "wild genes" to the colony confers some natural population characteristics. Guthrie and Carter (1972) found that European corn borer *Ostrinia nubialis* H. reared continuously on artificial diet lost the ability to grow on a susceptible line of corn. In addition, they also found that only one back-cross to the wild population allowed the colony to regain survivability fitness on corn equal to the wild borers. Other laboratory feeding assay research has been conducted that indicated recently out-crossed insects should

perform better in the screening of cotton germplasms due to better survival on cotton leaf tissue (Mulrooney et al., 1991). Indeed, very recent research done by Carpenter and Wiseman (1999) showed that recently out-crossed fall armyworm *Spodoptera frugiperda* performed significantly better in establishing and causing damage to corn than a fall armyworm colony that had never been out-crossed. However, in light of this information, a point that should be made is that genetic uniformity of inbred strains adapted to the laboratory environment, are ideal subjects for bioassays and other research requiring little variation between individuals (Mulrooney et al. 1992). The objective of this particular study was to evaluate the ability of two laboratory populations of tobacco budworm reared under different systems to cause damage to cotton under field conditions.

Materials and Methods

Field Trial

A conventional cotton variety (JaJo9550) was planted on May 15th, 1999 in a 2 row planted, 1 skip row pattern with a row length of 40 ft. Plots were arranged in a randomized complete block design with five reps. Treatments consisted of:

1.) A.R.S. tobacco budworm neonate larvae infested plots and 2.) DAS tobacco budworm neonate larvae infested plots. Non-infested control plots, sprayed weekly with insecticides (Karate Z, Bidrin, Vydate, Provado, Orthene) at recommended rates for idealized control of all pests were planted adjacent to, but not randomized within, the budworm infested plots. This planting design helps to minimize pyrethroid insecticide drift between infested and non-infested plots due to the insecticides known efficacy against tobacco budworm in the Delta area of Mississippi. Since randomization of the control plots did not occur within the RCB design only numerical (non-statistical) comparisons can be made in reference to the worm-infested plots. All plots received recommended rates of insecticides throughout the growing season for non-lepidopteran species pests (boll weevil, plant bug, aphids, etc.) when economic thresholds were reached. These insecticidal sprays, consisting of the products Bidrin, Provado, and Vydate, are known to have very little efficacy against tobacco budworm and were done on Fridays to minimize deleterious effects on the budworm plots.

Budworm plots were artificially infested on Tuesday of each week with inoculators developed by Davis and Oswalt (1979) and in accordance with the technique used by Jenkins et al. (1982). Tobacco budworm neonate larvae from either the DAS colony or the A.R.S. colony were infested (rate = 10 larvae/row foot) once per week on the plots for six weeks. Beginning at pinhead square stage of plant growth the plots were then randomly sampled (20 plants/plot) on Thursday of each week for fruit damage and the presence of larvae. After six weeks of infesting plots and collecting sample data, all plots were sprayed with a pyrethroid (Karate Z) at labeled rates once per week for two weeks. After the growing season, plots were defoliated and harvested for yield on. Differences in pounds of seed cotton yield per plot were determined. Data for larvae in terminals, number of squares damaged, number of larvae in squares, number of damaged bolls, and number of larvae in bolls were analyzed using paired t-test comparisons (P= 0.05). Yield data were collected on September 12, 1999 using a two row mechanical picker modified for weighing individual plots and then analyzed by PROC ANOVA (Agronomix Software, Inc., 1999). Means were separated with Fisher's protected LSD (P= 0.05).

Feeding Assays

The feeding bioassays were conducted to determine the ability of each colony to survive and grow on cotton leaf tissue. Leaf samples were collected in the field, placed in insulated plastic beverage coolers and transported to the laboratory. A cork bore tool measuring ¾ of an inch in diameter was used to cut leaf sections for feeding to neonate larvae. Leaf discs were placed into plastic trays containing cells measuring ¾ inch (height) x ¾ inch (diameter) partially filled with 1-2 ml of 5% cooled agar to maintain freshness of leaf material. One budworm from each colony was transferred to each of 48 cells in the tray and sealed with a perforated self-adhesive lidding material. Trays were placed in a growth chamber at 80 degrees F with lighting adjusted for 10 hours of darkness and 14 hours of light. After 4 days the plant material was replaced with fresh leaf disks and larvae were allowed to feed for 3 additional days. The feeding assay was repeated three times (dates) over the course of the growing season using 6 replications of 8 larvae each (n= 48 larvae per test date, total N= 144). All live larvae in a replicate were counted, pooled, and weighed at day 7. Data were analyzed by PROC ANOVA or PROC GLM (Agronomix Software, Inc., 1999) for a randomized complete block design and the means separated by Fisher's protected LSD (P= 0.05).

Rearing Systems and Insect Colonies

The A.R.S. tobacco budworm colony was established in 1983 using females obtained from the A.R.S. facility at Stoneville, MS. Each year since establishment females from the colony are out-crossed with "wild" males collected from various sited in Mississippi to maintain genetic diversity in the population. This is done in the Fall of each year when the progeny of this cross are then used to start a new colony. The old colony is discarded after the new colony has proven to be adapted to laboratory rearing conditions. Larvae are fed an artificial diet of soy protein/wheat germ based growth medium (diet) mixed with essential vitamins in individual plastic growth cells until pupation according to developed methodology (Davis et al., 1990). Adults are housed in 36"x36"x36" steel cages with cloth screens which function as

a resting substrate as well as for egg lay. Adult moths are fed a diet of sugar water solution (10% sugar by concentration) via sponges mounted on top of the cages. The DAS tobacco budworm colony was obtained from Shell Chemical Company in Richmond, CA. The colony was established in an unknown year but has been reared on diet in the laboratory for over 15 years. No out-crossing to a natural tobacco budworm population has ever been done. Larvae are fed and housed in a like manner as described above. However, adults are housed in small 1 gallon cylindrical cardboard fonda containers with cloth screen tops for egg lay. Adults are fed a sugar water solution (10% sugar by concentration).

Results and Discussion

Sampling data averaged over 6 weeks showed that the A.R.S. tobacco budworm infested plots closely exhibited a significant 2:1 difference over the DAS budworm infested plots in terms of greater damage in squares and bolls (Table 1). Square damage results showed that there was an average 41% damage rate for the A.R.S. budworm infested plots compared with an average 21% damage rate for the DAS budworm infested plots. The average boll damage rate for the A.R.S. budworm infested plots was 18% compared with an average of 9% for the DAS budworm infested plots (Table 1). Data for larval counts on squares showed a significant 3:1 difference in larval presence in plots of the A.R.S. colony versus the DAS colony (Table 2). There was a 3:1 greater numerical difference in larvae in bolls for the A.R.S colony infested plots as well, but this was non-significant (P= 0.05). The presence of larvae in terminals for the A.R.S. budworm infested plots (16%) was only numerically greater (non-significant) than the DAS budworm infested plots (10%) (Table 2).

In the feeding assays, the larvae from the DAS colony fed for 7 days on terminal cotton leaf tissue did not differ from the A.R.S. colony in survivorship (Table 3). However, there was a significant difference in the growth weights with the DAS colony larvae growing less over the seven day period (Table 3). Perhaps this could indicate a decrease in the ability to digest and utilize the cotton tissue as a food source due to allelochemicals present in the plant tissue (Table 3). It is well known that gossypol is an important allelochemical in the resistance of cotton plants to the tobacco budworm (Stipanovic et al. 1977; Parrott et al. 1983; Hedin et al. 1981, 1983).

Seed cotton yield results showed a significant 15% decrease in seed cotton for the A.R.S. colony infested cotton (17.88 lb/plot) compared with the DAS colony infested cotton (20.96 lb/plot) (Table 4). There was a 12% reduction in yield of the A.R.S. plots from the non-infested control cotton plots (20.4 lb/plot) but this was only numerically different (Table 4). The DAS budworm infested plot yields and the non-infested control plot yields showed even less numerical difference (Table 4). In comparing the yield for the two worm infested plots it does appear that the A.R.S. colony of worms did the most damage to the fruiting structures.

Before harvesting the plots for yield, each of the treatments were mapped for differences in boll retention for fruit positions 1-3 on nodes 5 through 21. Site retention differences (numerical differences) were observed between treatments. It appeared that the A.R.S. colony of tobacco budworm caused more fruit loss at positions 1-3 on nodes 5 through 15. This may reflect a lack of feeding or searching aggressiveness in the DAS larvae. According to the mapping data, the DAS larvae appear to have caused loss to first position fruit throughout the plant but very little damage to positional sites 2 and 3. In addition, it should also be noted that in both worm treatments, there was actually an increase in retention at fruiting sites 2 and 3 on nodes 16 through 21 in comparison with the non-infested control plots. These differences could help to explain the minimal yield loss between the worm infested treatments and the non-infested control.

Summary

Although based on only one year of data, it would appear that these two laboratory colonies of tobacco budworm larvae reared under different systems differ in the ability to establish and cause fruit damage on cotton under field conditions. The rearing system used at the A.R.S. facility in Starkville, MS appears to produce tobacco budworm larvae that have an enhanced ability to establish and cause more vigorous damage to cotton fruit under field conditions. This in turn caused numerically greater yield loss in comparison with the cotton grown under the idealized conditions. Yield for the A.R.S. infested cotton plots was significantly less when compared with the DAS colony infested cotton plots. The possible reasons for this marked difference in adaptation to field conditions may include system variation in adult housing and nutrition. However, it appears that the most important difference is in how each facility handles the genetics of the colony. The DAS tobacco budworm colony is reared for uniformity of research conducted under laboratory conditions and therefore is not outcrossed to the natural population. The A.R.S. colony is outcrossed annually to maintain genetic diversity. This allows the colony to have behavioral and physiological characteristics similar to their wild cousins. Researchers interested in supplementing natural insect populations for field evaluations involving host plant resistance, insecticide screening, and other evaluations involving plant/insect interactions may want to consider obtaining insects from a laboratory colony that annually out-crosses to natural populations.

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Table 1. Percentage of larval feeding damage to cotton squares and bolls on JaJo9950 conventional cotton line after 6 weeks.*

	Average/plot	
Colony type	**Sq.Dam.**	**B. Dam.**
ARS	41 a	18 a
DAS	21 b	9 b
CONTROL	4	2

*Means in columns followed by sameletter do not differ using paired t-test (P=0.05)

Table 2. Percentage of larvae in terminals, squares, and bolls on JaJo9550 conventional cotton line after 6 weeks.*

	Average/plot		
Colony type	**Terminal**	**Square**	**Boll**
ARS	16 a	18 a	6 a
DAS	10 a	6 b	2 a
CONTROL	1	1	1

*Means in columns followed by same letter do not differ using paired t-test (P=0.05)

Table 3. Leaf tissue feeding assay results on JaJo9550 conventional cotton line after 7 days.*

Colony type	**Survivors****	**Total Weight (mg)#**
ARS	7.3 a	80.0 a
DAS	7.0 a	64.2 b
LSD(P=0.05)	0.3	6.5

*Means in columns followed by same letter do not differ
** Average of surviving larvae over reps and dates
#Average total of larval weight over reps and dates

Table 4. Average seed cotton yield.*

Colony type	**Yield (lb/plot)**
ARS	17.9 a
DAS	21.0 b
CONTROL	20.4
LSD(P=0.05)	2.8

*Means with same letter do not differ