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BEHAVIOR OF BT RESISTANT AND NON-RESISTANT PINK BOLLWORM *(Lepidoptera: Gelechiidae)*

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Abstract

Pink Bollworm moths resistant to the Bt endotoxin Cry1Ac found in current varieties of transgenic cotton were not found to respond to pheromone traps as well as normal APHIS males. Bt resistant female moths did not call native male moths into traps as often or as well as normal APHIS female Pink Bollworm. This is important in considering future refugia strategy.

Introduction

In the Desert Southwest of the United States, Pink Bollworm (PBW) *Pectinophora gossypiella* (Sanders) is the key pest targeted for use of genetically modified cotton. That Bt cotton expresses Cry1Ac, the endotoxin produced by *Bacillus thuringinesis*. Use of genetically modified cotton requires the integration of strategies relying on untreated refuges to prevent the development of resistance in PBW. These strategies are based on the belief that resistant and non-resistant insects are equally competitive. The following studies provide data, which can be used to assess the competitiveness of Bt resistant insects (Btr) in cotton.

Materials and Methods

All PBW used in these studies were reared with the standard mass rearing containers and procedures normally used to produce sterile PBW in the USDA/CDFA mass rearing facility (Stewart 1984, Edwards et al 1996, and Miller et al 2001). However, our test insects were not reared in the normal facilities. They were reared in isolation in The Decision Support and Pest Management Systems Laboratory, a completely separate building. Normal non-resistant PBW insects were reared from eggs obtained from the USDA/CDFA facility and will be termed APHIS insects. Resistant, Btr, insects were reared from eggs obtained from a Btr resistant line maintained at the University of Arizona's Extension Arthropod Resistance Management Laboratory. Age after pupation was used to standardize maturity level of test moths. All tests used 2-3 day old moths for first release or field exposure.

We conducted three types of studies:

1. *Male responsiveness to standard survey traps*: In this study we used the ability of Btr and APHIS male moths to reach a standard PBW survey trap (Foster et al 1977) as an index of male responsiveness to the pheromone used by the female in mating. Tests were conducted in eight 12 ft. x 24 ft. x 6 ft. cages, each placed over three rows of cotton. The cotton was specifically grown for these studies and was located away from other cotton and in an area where no Bt cotton has ever been produced to further ensure that there was no hazard of developing a resistant population. This plot was destroyed before harvest to eliminate or drastically reduce the chance of any resulting over wintering population. As a final precaution all test insects released were irradiated at 10 KR, a dosage that eliminates fertile F2 progeny. All cages received both Btr and APHIS male moths. These insects were marked externally with different colors of day glow dye. All insects had the same internal fat-soluble dye from our diet. All test insects were released when a sufficient number reached the 2-day adult age stage. Slightly fewer APHIS than Btr males were available at the appointed time. Recapture rates of both resistant and non-resistant moths were then measured with traps until no males were captured in any trap.
2. *Female mating propensity*: The ability of Btr and APHIS female moths to attract and mate with either Btr or APHIS PBW male moths was tested three times in the second series of tests. In this test series, one female of each type was placed in a "mating station". Six mating stations were placed in each previously described field cage. Two additional cages were used. One wing was amputated from each female to prevent flight from the station. The station was a 2.5 qt. ice cream carton attached to a wooden stake at

plant canopy height and contained one of each type of female. A repellent ring of axel grease was placed around the rim of the carton to prevent moths from escaping, and a small cotton terminal and leaf section placed in the bottom of the carton provided a resting area for the females. Moths were placed in these stations in the first hour after sundown and recovered in the hour before sun up. Male moths were released 24 hours before female moths were released. Each cage received 100 male moths of either APHIS or Btr type. In the first test, a five-cage test, only APHIS male moths were available in sufficient numbers. In test 2 and 3 both types of male moths were used. Five cages were randomly chosen for release of male Btr moths and 5 for release of APHIS male moths. At sun up females were recovered from the mating stations and returned to the laboratory where they were categorized to origin by mark and dissected for spermataphore as an index of presence or absence of successful mating.

3. *Female attractiveness*: To more precisely test the ability of female PBW of each type to produce pheromones and attract male moths for mating a new assay procedure was established. This system will work only in areas where high levels of male moths are available for capture. A late season refugia field in the Phoenix area proved to be ideal with its extremely high level of moths available for trap captive. To conduct these tests a small cage for confining the female moths was constructed by cutting off the end of a 9 drum Thornton Plastic snap top vial and covering it with a screen. The tops of each vial were also modified with a screened opening. These cages were placed inside standard PBW traps and were attached to a small metal platform to keep them off the adhesive base of the trap. In two separate tests, thirty 2-day-old females of each category (Btr and APHIS) were placed in cages centrally in a delta trap. These traps were placed in the field in a paired design with each female baited trap placed at canopy height at the end of a non-Bt cotton field. The distance between each member of a pair was 1 meter apart. Pairs were separated by 3 meters. On the first night of the experiment the traps and their female "baits" were placed in the field in the hour after sundown. The labeled female cages were then recovered and moths counted and recorded that were captured in each trap in the hour after sun up. The female moths in the cages were returned to the laboratory and fed a 10% sucrose solution during the day/2 days before they were returned to the field for further nights of testing. These test procedures were used for two tests.

Results

In the first test Btr males did not respond as well as normal APHIS males. This difference was consistent in all 8 replications of this test. Results are shown in Table 1. It is important to note that normally a small insect does not respond as well in traps as a larger moth. Our Btr male moths were actually larger in weight than their non-resistant counterparts (19 mg vs. 16 mg). This is a result of greater larval density in the rearing container of the smaller moths.

In the second group of tests both an APHIS female and a Btr female were in the same mating arena. There were no consistent differences in rates of mating (Table 2). When only APHIS male moths were used (Test 1) there was less than 3% difference. In Test 2 we had some concerns that Btr males might be seeking Btr females. This data however was only from 30 females in five cages full of Bt males. In Test 3 this difference is much less apparent. Further testing will be conducted in 2002 to ensure that this was simple variations as expected.

In female trap capture studies (Table 3), the attraction rates of males to a single Btr female was down in two separate studies compared to APHIS females. The second of two studies where moths lived longer is shown. There were consistently fewer males captured (approaches) in traps with Btr females throughout. Only in the first night did Btr female moths bring males into more traps than APHIS females. APHIS females still attracted more total moths. Throughout the remainder of the test the ratio of numbers calling to the numbers of positive traps, was higher with APHIS females than with Btr females.

Conclusions

In our studies Btr moths appear to pay a fitness price for having acquired the ability to survive Cry1Ac exposure at high levels. Recapture rates of Btr males were appreciably less than APHIS moths even though we would have expected the larger Btr moths to perform better.

In examination of female mating success studies, the APHIS males appeared to mate equally with Btr and APHIS females. Btr males appeared in the 2nd test to mate most frequently with Btr females, however this trend was not as notable in the 3rd test. Females in non-competitive arenas as well as competitive arenas should be examined in future tests.

In our last series of tests APHIS females attracted more males per active trap. With the exception of only the first night of the study APHIS females were also attractive in more traps i.e. they had higher ratios of traps in which males were captured. We

concluded that obtaining Btr x Btr mating would be more difficult for this group of insects than expected if all their behavioral characteristics were equal.

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Table 1. Response of Btr and APHIS male PBW moths to standard survey traps when released in 8 field cages.

	Total Released	Total Recovered	%
Btr Males	540	201	37.22
APHIS Males	470	266	56.45

Conclusion: Btr males do not respond as well as APHIS males.

Table 2. Female mating propensity of Btr and APHIS insects with one of each female in the same arena.

	Test malesreleased	% APHIS females mated	% Btr females mated
Test 1	APHIS	50	47.6
Test 2	APHIS	34.6	24.1
	Btr	17.8	46.4
Test 3	APHIS	48	48.2
		53.8	67.86

Conclusion: We need better definition of Btr males x APHIS and Btr females interaction.

Table 3. Attraction of native PBW males by single Btr and APHIS females.

	APHIS					
Date	**9/23**	**9/24**	**9/26**	**9/28**	**10/3**	**10/5**
# calling	13/30	16/30	12/30	13/29	18/26	18/23
Males captured	24	52	40	33	168	225
			Btr			
# calling	16/30	9/30	11/30	11/30	10/30	7/30
Males aptured	22	16	15	11	56	23

Conclusion: Rate and intensity of female Btr calling is down.