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PARASITISM OF SOYBEAN LOOPERS, PSEUDOPLUSIA INCLUDENS, IN BOLLGARD AND NON-BT COTTON

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Abstract

We investigated the effect of various cotton types on parasitism of soybean loopers, *Pseudoplusia includens*, by the polyembryonic, egg-larval parasitoid *Copidosoma floridanum* in the field. Since the parasitoid is present in the host throughout the host's entire leaf-feeding period, there is potential for adverse effects of Bt toxin on this parasitoid through prolonged exposure. Three types of cotton were used: Non-Bt (DPL 5690RR), Bollgard (DPL 458), and Bollgard II (DPL 468). Weekly shake samples were conducted, and loopers were counted, collected and monitored for parasitism. General population densities of soybean loopers were comparable among the three cotton types, and parasitism was likewise comparable. Mean parasitism rates for loopers during the sampling period in the 3 cotton types were: 21.2% for non-Bt cotton, 16.0% for Bollgard cotton, and 23.0% for Bollgard II cotton. Similarly, the number of parasitoids emerging from hosts did not differ in response to cotton type (1056 emerged per host from Non-Bt; 1114 per host from Bollgard, and 1061 per host from Bollgard II), or in relation to the instar at which the loopers were removed from the field. These results suggest that the Cry1Ac and Cry2Ab toxins do not adversely affect this parasitoid through the host. However, overall sample sizes were limited, and definitive conclusions will require additional work.

Introduction

The parasitoid *Copidosoma floridanum* (Dalman) attacks eggs of the soybean looper [*Pseudoplusia includens* (Walker); Lepidoptera: Noctuidae], laying a single egg in the host's egg. The parasitoid egg is polyembryonic, meaning that multiple parasitoid larvae arise from a single egg (Ode and Strand 1995). Adult *C. floridanum* emerge from the prepupal stage of the soybean looper. This parasitoid can become very abundant late in the season in Georgia cotton, and is generally the dominant parasitoid of soybean loopers during this period (JRR, pers. obs.). The effectiveness of *C. floridanum* as a natural enemy of the soybean looper is its impact on late-season population growth through reducing the size of the subsequent generation. Within the generation of parasitism, damage actually can be increased by parasitism, as parasitized soybean loopers consume more foliage and gain more weight than unparasitized loopers in order to sustain the parasitoids developing within (McPherson 1993).

The relationship of *C. floridanum* and the soybean looper provides an opportunity to evaluate nontarget effects of Bt-transgenic cotton on a host-specific parasitoid. The soybean looper is able to survive and develop on currently-deployed cotton plants genetically modified to express endotoxins of the bacterium *Bacillus thuringiensis* (Bt); specifically, cotton plants expressing Cry1Ac toxin (Bollgard) and plants expressing both Cry1Ac and Cry2Ab (Bollgard II), although survival is reduced and development delayed somewhat relative to plants not expressing Bt toxins. The parasitoid develops within the host from the host's egg stage until the host is preparing to pupate. Thus, the parasitoid is an intimate associate of the host throughout the entire duration of the host's feeding period, and throughout may be exposed to any toxins the host might ingest and sequester. Further, numerous parasitoids emerge from each host (nearly 3,000 wasps have been reported to emerge from a single host; Ode and Strand 1995), allowing quantification of toxic effects on brood size.

Nontarget effects of Bt-transgenic crops on natural enemies have been studied extensively in recent years, with most of the focus on predators (e.g., Obrycki et al. 2004, O'Callaghan et al. 2005). Parasitoids have been more difficult to study because in many cases elimination of the host will obviously eliminate the parasitoid. However, when the effects of the toxin are sublethal it is possible to examine the effects of the toxins on parasitoid-host interactions. There is precedent for concern regarding the effects on Bt toxins on parasitoids. Atwood et al. (1997) found that the parasitoid *Cotesia marginiventris* (Cresson) was adversely affected when developing in hosts [tobacco budworm, *Heliothis virescens* (F.)] that were provided a diet treated with Bt toxin. In studies of the parasitoid *Cotesia plutellae* Kurdjumov, Chilcutt and Tabashnik (1997, 1999) found that Bt toxin adversely affected parasitoids developing in susceptible and moderately-resistant hosts [diamondback moth, *Plutella xylostella* (L.)] reared on Bt-treated leaf material, but that intoxicated hosts were equally acceptable with healthy ones for parasitoid oviposition. Further, Bt toxin had no direct effect on *C. plutellae* through either ingestion or exposure to dried residues on leaf surfaces

(Chilcutt and Tabashnik 1999).

In studies of parasitoids attacking hosts developing on Bt-transgenic plants, similar adverse effects of host-mediated, negative effects have been observed. Bernal et al. (2002) noted that immature development was prolonged and survival reduced for the parasitoid *Parallorhogas pyralophagus* (Marsh) developing in larvae of the stemborer *Eoreuma loftini* Dyar on Bt-transgenic maize relative to non-transgenic maize. Most other life-history attributes of the parasitoid were, however, not affected. Baur and Boethel (2003) also found that the life history-traits of two parasitoids [the braconid *Cotesia marginiventris* (Cresson) and *Copidosoma floridanum*] were adversely affected when their hosts (soybean looper) developed on Bt-transgenic cotton. In the case of *C. floridanum*, the number of parasitoids emerging was reduced when parasitized hosts were reared on Bt cotton (DPL NuCotn 33B). Based on their results, Baur and Boethel (2002) also suggested that older plants may be less harmful to parasitoids. Given that the Cry1Ac titer tends to decrease as the plant ages (Greenplate 1999), and that the soybean looper is a late-season pest of cotton, it is possible that adverse effects observed in the laboratory may be ameliorated in the field.

This paper presents preliminary results from a field study of natural soybean looper parasitism by *C. floridanum* in conventional and Bt-transgenic cotton, extending the laboratory findings of Baur and Boethel (2002). The purpose of the study was to evaluate rates of parasitism of natural populations of soybean loopers in conventional and Bt-transgenic cotton, and to determine if there is an impact of cotton type on parasitoid brood size, as a measure of parasitoid fitness.

Methods

Plots of cotton were planted in Plains, Georgia, at the Southwest Branch Experiment Station of the University of Georgia on 2003. Each plot was 24 rows wide and 110 feet long. There were 3 cotton treatments in the trial: DPL 5690RR (non-Bt), DPL 458 (Bollgard I, with Cry1Ac), and DPL 468 (Bollgard II, with Cry1Ac and Cry2Ab). Each treatment was replicated 3 times in a randomized complete block.

Samples were taken weekly beginning in early August as soybean loopers appeared in the plots. Each sample consisted of 8 drop-cloth samples per plot using a standard drop cloth (1 meter square). Each drop-cloth sample involved the shaking of the cotton plants on each side of the cloth, after the cloth was placed on the ground between two rows of cotton. All soybean loopers falling on the cloth were counted and returned to the laboratory, where their instars were determined and they were placed on diet. Soybean loopers were held on diet until they had pupated or until parasitoids emerged from the host mummies. Parasitoids were counted for each host.

Numbers of soybean loopers in relation to cotton type were determined for each sample date. Percent parasitism for larvae collected on each sample date also was calculated, as was the number of parasitoids emerging from each host in relation to the instar of the host at time of collection, and the cotton type on which the host was collected. Assuming that the Bt toxins could exert some adverse effect on parasitoids, a working hypothesis is that fewer parasitoids would emerge from hosts collected in their later instars from plants because the parasitoids would have been exposed to ingested toxins for longer periods, and in increasingly greater amounts, as the larger host instars consume considerably more plant material than do younger instars.

Comparisons of looper larval populations and parasitism rates were made among treatments using repeated measures analysis of variance (PROC GLM, SAS Institute 1994). Values for parasitism rates were transformed (arcsin√%) prior to analysis due to heavy skewing of frequency data and heterogeneous variances.

Numbers of parasitoids emerging from hosts in relation to cotton type were analyzed using two-way analysis of variance (PROC GLM, SAS Institute 1994), with the two factors considered being instar of host at time of removal from the field, and plant type (all sample dates were pooled together for these analyses). It was hypothesized that if the Bt toxins exerted a cumulative effect on the parasitoids that parasitoids exposed to toxins for longer periods in the field (i.e., those collected and transferred to diet in the laboratory in later instars) would experience reduced survival/emergence relative to those removed from the field when their hosts were in younger instars.

Results and Discussion

Soybean looper abundance was not significantly different on the three cotton types, nor was the seasonal pattern of parasitism different (Table 1). Thus, parasitism in the field was unaffected by cotton type (overall mean of 21.2% for non-Bt cotton, 16.0% for Bollgard cotton, and 23.0% for Bollgard II cotton during the sampling period). The total numbers parasitized by *C. floridanum* were 31 out of 146 soybean looper larvae collected in non-Bt cotton, 23 out of 144 collected in Bollgard, and 26 out of 113 collected in Bollgard II. In addition to *C. floridanum*, we also reared the parasitoids *Meteorus autographae* (15 parasitized in non-Bt, 10 in Bollgard, and 6 in Bollgard II), *Cotesia autographae* (8 parasitized in non-Bt, 16 in Bollgard, and 6 in Bollgard II), *Cotesia marginiventris* (9 parasitized in non-Bt, 1 in Bollgard, and 2 in Bollgard II), a *Euplectrus* sp. (1 parasitized in Bollgard II), and at least one species of tachinid (1 parasitized by a tachinid in each crop treatment; one host in the non-Bt treatment was successfully parasitized by both a tachinid and *C. floridanum*) from collected soybean loopers. The other parasitoid species were not considered in further analyses.

Table 1. Relative abundance of soybean looper larvae (no. per row meter) and percent parasitism of the looper larvae by *C. floridanum* in non-Bt, Bollgard, and Bollgard II cotton (Plains, GA, 2003).

Sample date	Non-Bt (DPL 5690)		Bollgard (DPL 458)		Bollgard II (DPL 468)	
	Loopers/row meter	% para	Loopers/row meter	% para	Loopers/row meter	% para
8 Aug	2.3 ± 1.71	0	0	---	0.8 ± 0.50	33.3 ± 57.74
15 Aug	1.3 ± 0.96	0	03 ± 0.50	0	0	---
21 Aug	7.0 ± 3.46	22.9 ± 15.76	5.0 ± 4.24	5.0 ± 10.00	4.3 ± 3.3	6.3 ± 12.5
29 Aug	9.0 ± 5.60	12.5 ± 25.00	11.3 ± 4.57	13.7 ± 4.27	10.8 ± 6.85	21.0 ± 8.81
5 Sep	7.5 ± 1.29	8.3 ± 16.65	8.8 ± 3.77	23.8 ± 7.22	4.0 ± 2.16	18.2 ± 14.38
11 Sep	7.0 ± 3.56	6.3 ± 12.5	8.0 ± 2.71	0	4.3 ± 3.40	8.3 ± 16.65
18 Sep	2.5 ± 1.91	0	2.8 ± 2.22	0	4.3 ± 3.20	26.8 ± 20.52

Number of parasitoids emerging from parasitized loopers did not differ with respect to cotton type (F = 0.39, P = 0.6754) or in response to host instar at time of collection (F = 1.11, P = 0.3531; Fig. 2). The mean number of parasitoids emerging per host for the sampling period by cotton type (all instars pooled) was: 1056 ± 501.4 for non-Bt cotton, 1114 ± 431.5 for Bollgard, and 1061 ± 414.8 for Bollgard II. There was an obvious downward trend in the Bollgard II treatment as host instar increased, suggesting that there may be adverse effects due to Bollgard II, but the sample size was insufficient (26 parasitized larvae total: 3 2nd instar, 6 3rd instar, and 9 each in the 4th and 5th instar) to permit definitive conclusions to be drawn. No statistically significant interaction between host instar and cotton type was observed (F = 0.66, P = 0.7083), but sample sizes were necessarily limited.

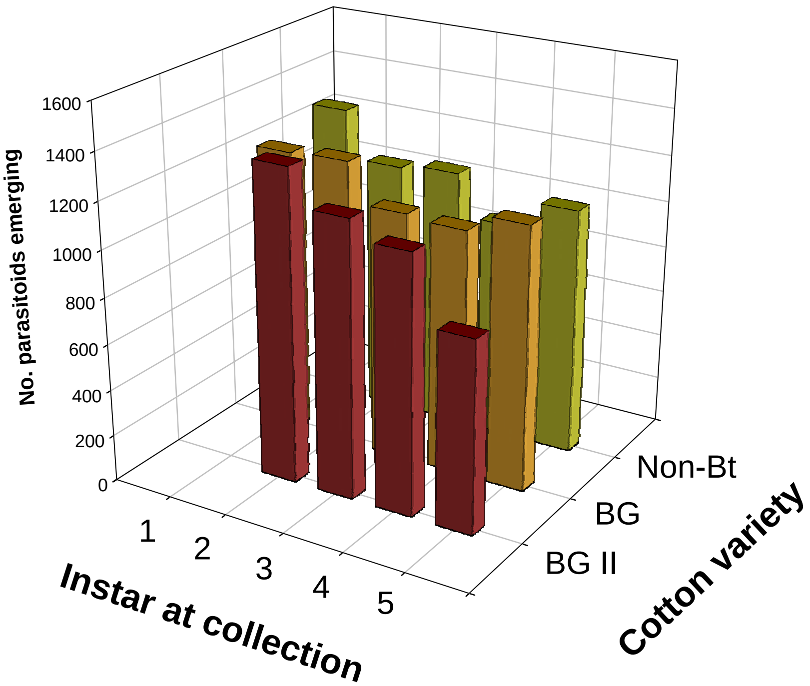

Fig. 2. Number of adult *C. floridanum* emerging per host from soybean looper larvae collected from three types of cotton plants, and during different host larval instars. Differences among and with cotton varieties are not significantly different (P > 0.3531).

In our study, parasitism of soybean loopers by *C. floridanum* (as expressed by successful parasitoid development in the hosts) was unaffected by the host's consumption of cotton plants expressing Cry1Ac and Cry2Ab toxins in the field. Rates of parasitism in the field did not differ with plant type, nor was the number of parasitoids emerging per host affected by plant type, or by instar at which the host was removed from the field. These results contrast with those of Baur and Boethel (2002). There may be several explanations for these differences. First, it is important to point out that the numbers of larvae we sampled for any given host instar were limited (as noted above for Bollgard II), and that the collected larvae completed their development on diet in the laboratory. Baur and Boethel (2002) exposed the parasitized hosts to the respective treatments throughout their development, which would have extended the period parasitoids were exposed to toxins relative to our study. Second, in our experiment, the hosts were naturally-occurring populations on mature cotton plants. Baur and Boethel (2002) used greenhouse-grown plants, and provided loopers with detached leaves. In our study, loopers occurred naturally and were able to select normal, preferred feeding sites. Some caterpillars are known to modify their feeding locations in response to Bt toxins (Gore et al. 2002) and it is possible that soybean loopers may selectively seek tissues with lower toxin levels. Third, expression of Bt toxin likely varied between the two studies. Baur and Boethel (2002) produced their cotton plants in the greenhouse, and did not necessarily use plants at the stage that corresponds with normal looper abundance in the field. Our plants were field-grown, and were colonized late in the season, when Bt toxin levels tend to decline in the plants (Greenplate 1999). Thus, the loopers in our study may have been exposed to lower toxin levels than were those in the Baur and Boethel (2002) study. Baur and Boethel (2002) did, however, point out that when they used leaves from older cotton plants in their study, the adverse effects of the Bt plants on *C. floridanum* were reduced.

Overall, our limited data suggest that Bt-transgenic cotton expressing Cry1Ac and Cry2Ab toxins in the field may have very little impact on *C. floridanum*, in contrast with the findings of Baur and Boethel (2002) in the laboratory. Additional work will be necessary to substantiate these results. However, it is important to note, as others have done (Candolfi et al. 2004, O'Callaghan et al. 2005), that understanding the effects of pesticide-incorporated plants on nontarget organisms will require examining the species in question under natural conditions. Laboratory studies provide insights and can help to verify mechanisms, but field studies provide the most realistic assessment of outcomes.

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