Selective Feeding of Soybean Looper (Lepidoptera: Noctuidae) on Meridic Diet with Different Concentrations of the *Bacillus thuringiensis* Cry1Ac Protein[1]

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J. Entomol. Sci. 39(2): 243-250 (April 2004)

Abstract The ability of soybean loopers, *Pseudoplusia includens,* (Walker), to selectively feed on meridic diet with different concentrations of Cry1Ac was investigated in a choice assay. Neonates selected non-treated meridic diet and diet with concentrations of Cry1Ac ranging from 0.1 to 1.0 µg/mL for feeding compared to diet with Cry1Ac concentrations of 5.0 and 10.0 µg/mL. Averaged across rating periods, means of 11.9 to 17.6% of larvae were observed feeding on diet with Cry1Ac concentrations ranging from 0.0 to 1.0 µg/ml, compared to 3.4% on diet with Cry1Ac concentrations of 5 µg/mL and 4.3% at 10 µg/mL. In a no-choice study, concentrations ranging from 0.1 to 1.0 µg/mL produced 4.3 to 40.8% mortality in soybean looper; while, concentrations of 5.0 µg/mL produced 58.5% mortality and 10.0 µg/mL produced 90.8% mortality. Also, diet with Cry1Ac concentrations of 5.0 and 10.0 µg/mL delayed the development of soybean looper larvae compared to diet with the other concentrations of Cry1Ac. Based on results of this study, soybean loopers can select diet with concentrations of Cry1Ac that result in low (<50%) levels of mortality.

Key Words *Pseudoplusia includens,* Bollgard, behavior

Bollgard® cotton has been grown commercially in the U. S. since 1996. During that time, Bollgard cotton cultivars have provided adequate control of the tobacco budworm, *Heliothis virescens* (F.), and bollworm, *Helicoverpa zea* (Boddie), except when bollworm populations are high and persist for long periods of time (Gore et al. 2003). However, other lepidopteran pests of cotton are not effectively controlled by the Cry1Ac protein in current commercial Bollgard cultivars (Stewart et al. 2001). For instance, the numbers of insecticide applications and costs of insecticide applications for soybean loopers, *Pseudoplusia includens* (Walker), were similar on non-Bollgard and Bollgard cottons during 2001 in Louisiana (Williams 2002). Currently, little information exists to fully explain the pest status of soybean loopers on Bollgard cotton.

Several factors can influence the pest status of lepidopteran larvae on Bollgard cotton. Soybean loopers are approximately 2 to 24 fold less susceptible to the Cry1Ac protein from *B. thuringiensis* than tobacco budworms (Luttrell et al. 1999). Therefore, cotton cultivars that express Cry1Ac at sufficient levels to control tobacco budworm may not effectively control soybean loopers. Also, expression of the Cry1Ac protein

[1]Received 30 May 2003; accepted for publication 15 September 2003.
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varies among cotton cultivars and some cultivars may provide better control of soybean loopers than other cultivars. Previous research has shown that Deltapine NuCOTN 33B and 458B/RR (Delta and Pine Land Co., Scott, MS) produce higher levels of the Cry1Ac protein than at least 11 other commercially available cultivars (Adamczyk and Sumerford 2001).

In addition to differential expression of the Cry1Ac protein among cotton cultivars, spatial variability in Cry1Ac expression exists among different plant parts within cultivars (Greenplate 1999, Adamczyk et al. 2001, Gore et al. 2001). Leaves, the preferred feeding site of soybean looper, produce more of the Cry1Ac protein than other plant parts (Greenplate 1999). Also, terminal leaves produce relatively high levels of Cry1Ac, and the amount of Cry1Ac decreases as individual leaves age (Adamczyk et al. 2001). If soybean loopers are able to selectively feed on leaves that produce low levels of Cry1Ac, supplemental control with insecticide applications may be needed to prevent economic losses in Bollgard cotton.

Previous studies have shown that bollworm (Jyoti et al. 1996, Gore et al. 2002) and tobacco budworm (Gould et al. 1991, Benedict et al. 1992, 1993) larvae avoid *B. thuringiensis* proteins. Bollworms migrate down Bollgard plants faster than they migrate down non-Bollgard plants (Gore et al. 2002). Consequently, bolls low in the plant canopy produce lower levels of Cry1Ac than terminal tissue and small squares, the preferred feeding sites of small bollworm larvae. Although bollworms appear to select feeding sites with low levels of Cry1Ac, no studies have determined if any species of lepidopteran larvae can distinguish between diets with different concentrations of *B. thuringiensis* proteins. This paper summarizes results of a laboratory experiment to determine if soybean looper larvae are able to selectively feed on meridic diet with different concentrations of Cry1Ac.

Materials and Methods

Soybean looper colony. A colony of soybean looper was established from collections made on non-Bollgard cotton (Stoneville 474 and Stoneville 4793R, Stoneville Pedigreed Seed, Stoneville, MS) in Washington Co., MS. Approximately 1500 larvae were collected from 29 August to 06 September 2002. Larvae were transported to the laboratory and maintained at 27 ± 3°C and 75 ± 5% relative humidity at a photoperiod of 14:10 light:dark. Soybean loopers were maintained in the laboratory for at least one generation to eliminate parasitoids and minimize pathogens. Larvae were fed a meridic diet (Thomas and Boethel 1993) in individual 30-mL plastic cups until pupation. Pupae were placed into 7.6-L plexiglass containers (approximately 100 per container). Moths were fed a 10% honey-water solution. Strips of paper toweling were suspended from the lid to provide a surface for oviposition. Egg sheets were harvested daily and placed into 3.79-L plastic bags. Upon eclosion, neonates were used in assays.

Choice study. Neonate soybean loopers were offered five concentrations (0.1, 0.5, 1.0, 5.0 and 10.0 μg/ml diet) of Cry1Ac incorporated into meridic diet along with non-treated diet in a choice feeding assay. Lyophilized powder of MVP II (Monsanto Co., St. Louis, MO) containing 19.7% Cry1Ac by weight was incorporated into diet to obtain the desired concentrations. The concentrations used were selected based on preliminary assays (data not shown) and historical data (Luttrell et al. 1999). Meridic diet with each Cry1Ac concentration or non-treated diet was poured into separate 100 mm diam Petri dishes (BD Falcon® No. 351029, VWR International) to a depth of 3 mm. Meridic diet disks (8 mm diam × 3 mm thick) were cut from each of the concentrations using a number three cork borer and placed into the test arenas so that each arena contained disks with all five Cry1Ac concentrations and a non-treated disk. Each test arena consisted of a 5.0-cm diam self sealing Petri dish (BD Falcon® No. 351006, VWR International). The experiment was arranged in randomized complete block design where date represented blocks (replicated over time). A total of 150 dishes (30 per block) was used in this experiment. The diet disks were randomly arranged around the edge of the dishes. Within a replication the randomization of Cry1Ac concentrations remained the same, while the randomization of concentrations was changed in subsequent replications. A single neonate was placed in the center of each dish. The location of larvae within each dish was recorded on each of five consecutive days. The percentage of larvae feeding on each concentration was calculated based on the total number of larvae per replication. Mean percentages of larvae feeding on each concentration were compared using repeated measures analysis of variance (PROC MIXED, Littell et al. 1996). Repeated measures analysis of variance was used because data were recorded from the same experimental units (dishes) over time. Therefore, the location of a larva within a dish on a given day will be more closely correlated to the location of the larva on previous days than to the location of a larva in another dish.

No choice study. To determine the effects of the Cry1Ac concentrations on soybean looper development, neonates were placed on approximately 12 mL of meridic diet with the same Cry1Ac concentrations described above in 30.0-mL plastic cups. Larvae were maintained at the environmental conditions described above. Thirty larvae were placed on each concentration for each of four consecutive days (a total of 120 larvae per concentration). Larval mortality was determined after ten days. Surviving larvae were allowed to complete development on the treated diet. Total mortality (based on the number of larvae at the beginning of each replication), time to pupation, and pupal weights were recorded. Mean larval mortality, time to pupation, and pupal weights for each concentration were compared with analysis of variance (PROC MIXED, Littell et al. 1996).

Results and Discussion

Based on results of the choice study, soybean loopers avoided diets that contained the Cry1Ac protein from *B. thuringiensis* when non-treated diet was available. A response of soybean looper was observed for Cry1Ac concentration ($F = 9.77$; df = 5, 24; $P < 0.01$) but not for time of evaluation ($F = 2.23$; df = 4, 96; $P = 0.07$). A significant interaction between Cry1Ac concentration and time of evaluation ($F = 0.62$; df = 20, 96; $P = 0.89$) was not observed. Neonate soybean loopers showed a preference for non-treated diet or diet with low concentrations of Cry1Ac (Fig. 1). More larvae were observed feeding on non-treated diet than on diet treated with 1.0, 5.0 or 10.0 μg/mL Cry1Ac. Soybean looper mortality averaged ±SEM 8.8 ± 1.4% mortality over the duration of the experiment.

In choice assays, tobacco budworm larvae avoid diets containing *B. thuringiensis* proteins, even at concentrations that exhibit no apparent biological activity in no-choice assays (Gould et al. 1991). In contrast, soybean loopers in the current study selected diets with concentrations of Cry1Ac that result in low (<50%) mortality compared to diets with concentrations that result in high (>50%) mortality when given a choice. More soybean loopers were observed feeding on diet with 0.1, 0.5, and 1.0

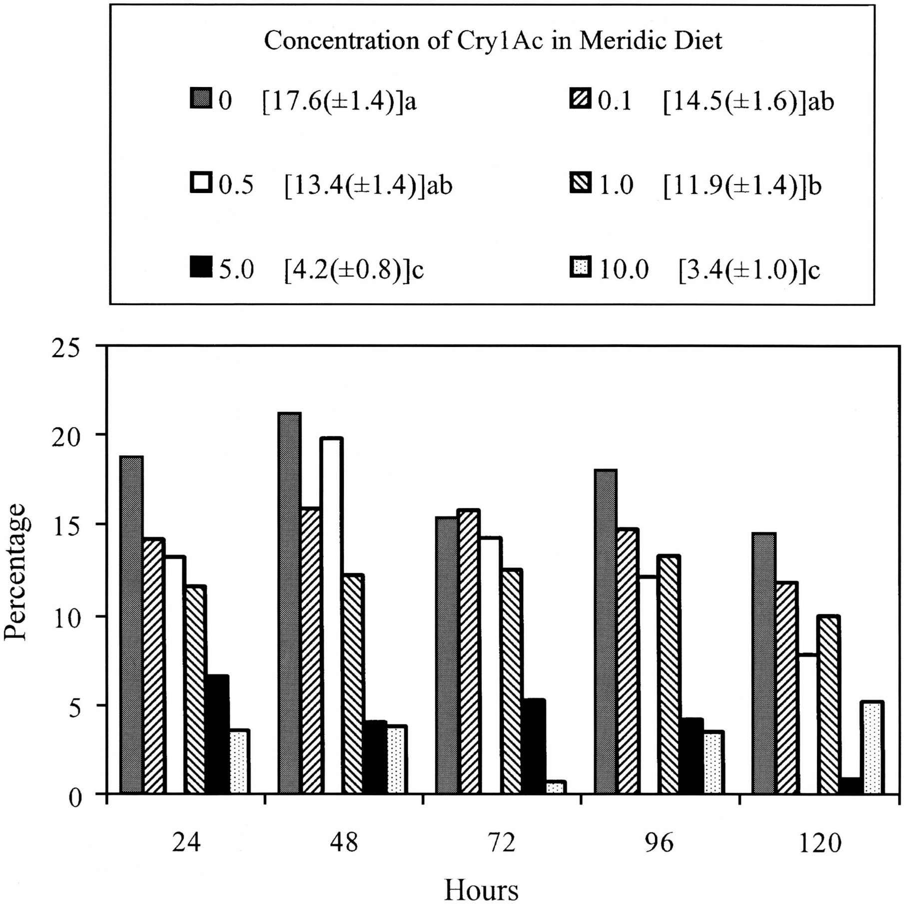


Fig. 1. Behavior of soybean looper, *P. includens,* on meridic diet with different concentrations of Cry1Ac in a choice feeding assay. Symbols on the graph represent the mean percentages of larvae feeding diet disks with the Cry1Ac concentrations at a given rating period. Numbers next to concentrations in the legend represent mean (±SE) percentages of larvae feeding on each concentration across rating intervals. Means followed by a common letter are not different (α = 0.05) according to Fisher's Protected Least Significant Difference.

µg/mL of Cry1Ac than on diet with 5.0, or 10.0 µg/mL Cry1Ac. This corresponds to data in the no-choice study where at 10 d ($F = 86.78$; df = 5, 18; $P < 0.01$) and at the time of pupation ($F = 30.44$; df = 5, 18; $P < 0.01$), soybean looper mortality was lower on diet with Cry1Ac concentrations ranging from 0.1 to 1.0 µg/mL than on diet with Cry1Ac concentrations of 5.0 and 10.0 µg/mL (Fig. 2). The same trend follows for developmental times of larvae.

In the no choice study, soybean loopers developed faster on the concentrations

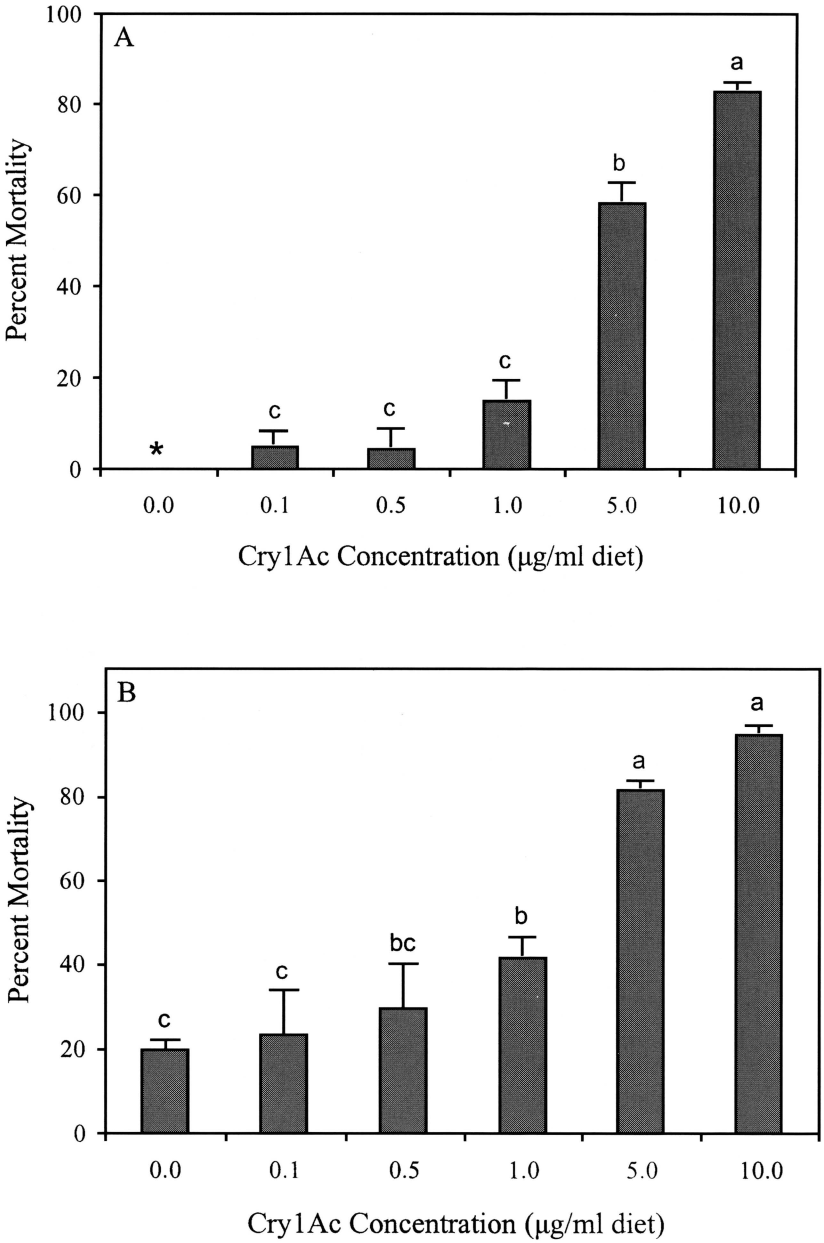
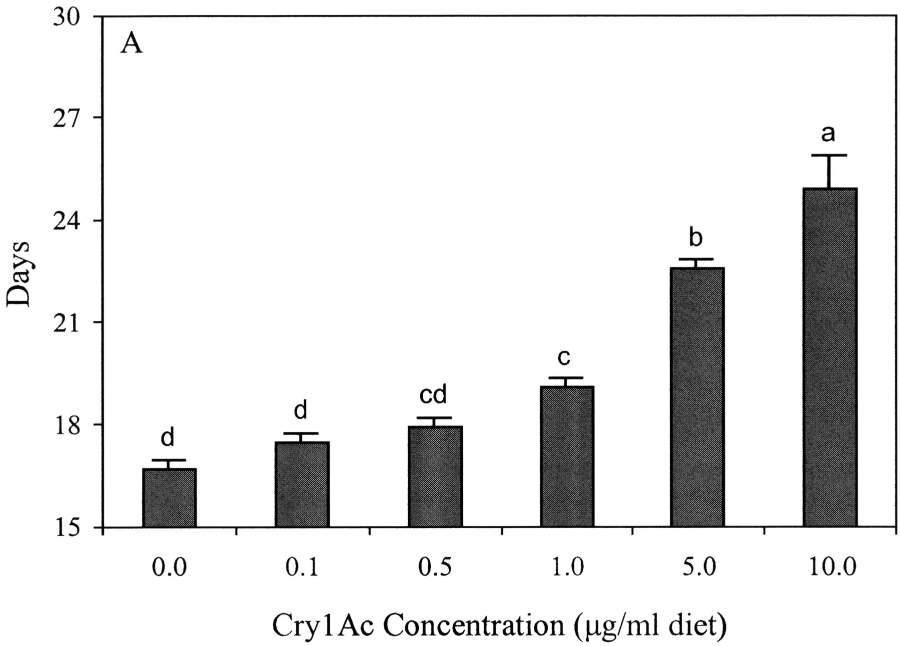


Fig. 2. Mortality of soybean looper, *P. includens,* at 10 days (A) and at the time of pupation (B) on meridic diet with different concentrations of Cry1Ac. Bars with a common letter are not different (α = 0.05) according to Fisher's Protected Least Significant Difference (* at 10 days, no mortality was observed on non-treated diet; therefore, it was excluded from the analysis).



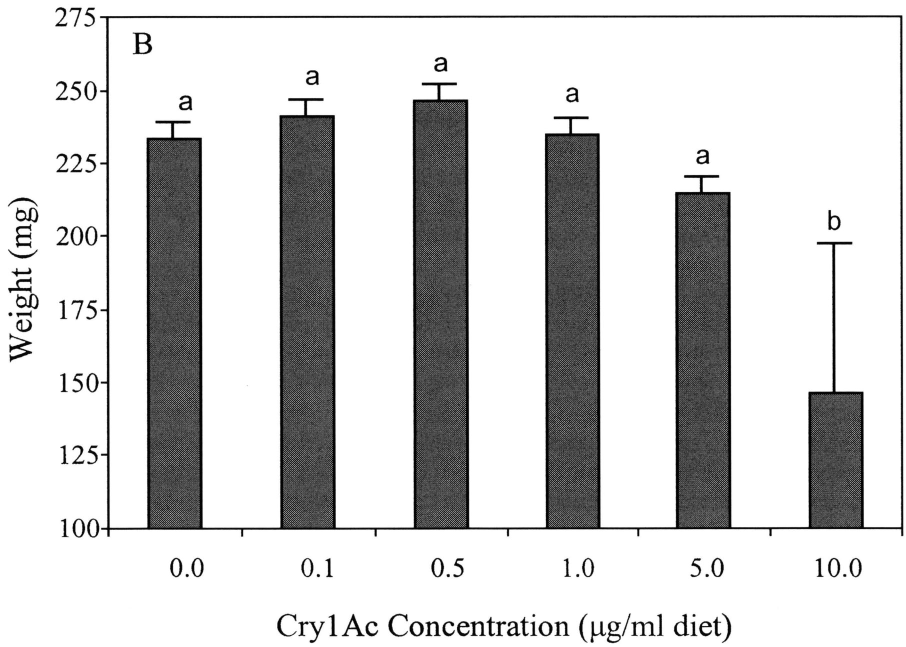


Fig. 3. Developmental times from neonate to pupa (A) and pupal weights (B) of soybean looper, *P. includens,* on meridic diet with different concentrations of Cry1Ac. Bars with a common letter are not different (α = 0.05) according to Fisher's Protected Least Significant Difference.

(0.1 to 1.0 μg/mL) that were selected in the choice study than on concentrations (5.0 or 10.0 μg/mL) that were avoided in the choice study ($F = 32.77$; df = 5, 17; $P < 0.01$) (Fig. 3A). In addition, pupae that completed development on diet with 10.0 μg/mL weighed less than larvae that completed development on the other Cry1Ac concentrations ($F = 2.97$; df = 5, 18; $P = 0.04$) (Fig. 3B).

Based on results of this study, soybean looper larvae appear to be able to selectively feed on diet with concentrations of Cry1Ac that result in low levels of mortality. This behavior, coupled with temporal and spatial variation in Cry1Ac expression in plants, may influence the pest status of soybean looper on Bollgard cotton if larvae are able to selectively feed on foliage that maximizes their growth and performance; thus, resulting in greater feeding injury to plants. Soybean looper adults typically oviposit on the abaxial surface of leaves in the mid- to lower-portions of the plant canopy (Felland 1992). Subsequently, the majority of larval feeding occurs in those areas. Future work should focus on determining if Cry1Ac levels vary among leaves where soybean loopers normally feed and if soybean looper larvae selectively feed on leaves with low expression in the field. Also, determination of how larvae selectively feeding on leaves with low concentrations of Cry1Ac might influence the development of resistance to Cry1Ac and other *B. thuringiensis* proteins should be researched.

Acknowledgments

The authors thank Leslie Price, Don Hubbard, Jennifer Holcomb and Leslie Hughes for technical assistance, Gordon Snodgrass and Roger Leonard for critical reviews of this manuscript, and Michelle Mullen and her staff for the preparation of treated diet. Mention of a commercial or proprietary product does not constitute an endorsement by the U. S. Department of Agriculture for its use.

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