

INCREASED TOLERANCE OF FALL ARMYWORMS
(LEPIDOPTERA: NOCTUIDAE) TO CRY1AC δ -ENDOTOXIN
WHEN FED TRANSGENIC *BACILLUS THURINGIENSIS* COTTON:
IMPACT ON THE DEVELOPMENT OF SUBSEQUENT GENERATIONS

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ABSTRACT

Increased tolerance to Cry1Ac protein was found in a population of fall armyworms, *Spodoptera frugiperda* (J. E. Smith), after selection for a single generation with transgenic *Bacillus thuringiensis* Berliner (Bt) cotton foliage. When fed Cry1Ac treated artificial diet, larvae whose parents had fed on transgenic Bt cotton leaves had significantly higher larval weights and a shorter time to pupation than those larvae whose parents had fed on conventional cotton leaves. In addition, there was no evidence to suggest any fitness or vigor differences existed from progeny of fall armyworms that fed previously on conventional or transgenic Bt cotton. Furthermore, tolerance of fall armyworms to Cry1Ac had a heritable component in the subsequent generation based on larval weights and time to pupation. These data show that using a common approach designed to control all intrinsically tolerant lepidopteran species of transgenic Bt cotton identically may not be desirable.

Key Words: *Spodoptera frugiperda*, plant-resistance

RESUMEN

Aumento en tolerancia a la proteína Cry1Ac fue encontrado en poblaciones del gusano de otoño *Spodoptera frugiperda* (J. E. Smith), después de escogimiento para una generación singular de follaje de algodón con *Bacillus thuringiensis* Berliner (Bt) transgénico. Al ser alimentadas una dieta artificial tratada con Cry1Ac, las larvas con padres que se alimentaron de hojas de algodón Bt transgénica mostraron pesos larvales significativamente mayores y menos tiempo a pupación que las larvas quienes padres se alimentaron con hojas de algodón convencionales. También, no hubo evidencia para sugerir que existen diferencias de salud o vigor en progenie de *S. frugiperda* que se alimentaron previamente con algodón convencional o Bt transgénico. Además, la tolerancia de *S. frugiperda* a Cry1Ac tuvo un componente heredable en la generación subsecuente basado en pesos larvales y tiempo a pupación. Estos datos demuestran que pudiera no ser deseable usar una practica común diseñada a controlar idénticamente a todas las especies de lepidóptera intrínsecamente tolerantes al algodón Bt transgénico.

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is a destructive migratory pest of many crops in the Western Hemisphere (Sparks 1979; Young 1979). Historically, this pest has been a sporadic, but serious pest of conventional cotton in the southern United States (Bass 1978; Smith 1985). This pest has the potential to damage both conventional cotton bolls and transgenic cotton bolls that contain an insecticidal Cry1Ac δ -endotoxin from the soil bacterium, *Bacillus thuringiensis* Berliner (Bt). The damage to bolls of transgenic Bt cotton caused by the fall armyworm can be more extensive than other lepidopterous pest of cotton including bollworms, *Helicoverpa zea* (Boddy), tobacco budworms, *Heliothis virescens* (F.), and beet armyworms, *Spodoptera exigua* (Hübner) (Bagwell 1994; Adamczyk et al. 1998a). Although application rates of foliar insecticides are often reduced for bollworms found on transgenic Bt cotton, possibly due to reduced fitness or vigor of individuals (Brickle et al. 1999), local outbreaks

of fall armyworms on transgenic Bt cotton often need full application rates of foliar insecticide treatments to keep these populations below economic injury levels (Hood 1997; Smith 1997).

Although certain lepidopterous pests of cotton are very susceptible to current transgenic Bt technology [e.g. tobacco budworms and pink bollworms, *Pectinophora gossypiella* (Saunders)], fall armyworms, bollworms, and soybean loopers, *Pseudoplusia includens* (Walker) are only sub-lethally effected by the Cry1Ac δ -endotoxin (MacIntosh et al. 1990, Wilson et al. 1992, Halcomb et al. 1996, Adamczyk et al. 1998b, and Sumerford & Solomon 2000a). It seems that the Cry1Ac δ -endotoxin found in current transgenic Bt cotton varieties does not provide sufficient mortality to fall armyworm larvae, but only slows larval development (Adamczyk et al. 1998b). Thus, application of foliar insecticides must be used to control this pest on current transgenic Bt cotton varieties (Smith 1997).

Few studies have examined the impact Cry1Ac δ -endotoxin has on subsequent generations of Lepidoptera. Lambert et al. (1998) showed that the increased tolerance of bollworm larvae to transgenic Bt cotton can occur in subsequent generations, although complex interactions (i.e., genetic vs. environmental) were not sufficiently resolved. Furthermore, in a similar study using fall armyworms, the effect of selection for a single generation with transgenic Bt cotton foliage on survival and development of fall armyworms could not be fully characterized (Adamczyk et al. 1998b).

It seems that tolerance to Bt is heritable among certain species of Lepidoptera. Sumerford & Solomon (2000b) showed a genetic component for variation in larval development among *H. zea* feeding on Cry1Ac diet. The authors also found that selecting for more optimally growing larvae was correlated with improved survivorship when larvae were exposed to Cry1Ac. This study was conducted in two parts: 1) to determine if increased or decreased tolerance of Cry1Ac δ -endotoxin was found in a population of fall armyworms after selection for a single generation with transgenic Bt cotton foliage, and 2) to determine if offspring of more tolerant individuals also exhibited greater tolerance of Cry1Ac during the subsequent generation.

MATERIALS AND METHODS

P_1 Generation

A fall armyworm colony (obtained from Dr. Frank Davis (retired), USDA, ARS, CHPRRU at Mississippi State University) was utilized in all tests. Females from this colony are annually outcrossed with wild, pheromone trapped males to maintain genetic heterogeneity and traits present in field individuals. Larval and adult rearing as well as egg harvesting were conducted as described in Adamczyk et al. (1998b).

Three colonies of fall armyworms were established from the original colony mentioned above. Larvae were reared until pupation on artificial diet, conventional cotton leaves, and transgenic Bt cotton leaves as described in Adamczyk et al. (1998b) and modified in Adamczyk et al. (2000). Individual pupae were separated based on larval host (colony designation: NBT, BT, and DIET; reared on conventional leaves, transgenic Bt leaves, and artificial diet, respectively) and equal numbers of pupae (100) were then placed in 3.79 liter cylindrical containers for moth emergence. Adult rearing and egg harvesting were conducted as described in Adamczyk et al. (1998b).

G_1 Experiment

To examine the effects Cry1Ac δ -endotoxin had on a subsequent generation of fall armyworms, G_1 neonates from all colonies were placed on arti-

cial diet incorporated with a lyophilized powder of MVP II containing 19.7% Cry1Ac by weight (purified Cry1Ac; Monsanto Co., St., Louis, MO) using the method described in Sumerford & Solomon (1999). Thirty neonates were placed in 28.6 ml cups (1 per cup) containing approximately 5.0 ml of Cry1Ac diet and replicated twice. In addition, the same cohort of individuals from the same three colonies was reared on non-Cry1Ac diet as a control to determine if vigor differences existed among colonies. In a preliminary experiment, it was determined that a dose of 10.0 $\mu\text{g/ml}$ of Cry1Ac slowed larval development of fall armyworms very similar to transgenic Bt cotton. Therefore, this diagnostic concentration was used in all tests. Survival of larvae at 7 days after exposure (DAE), survival to pupae, larval weights at 7 DAE, and time to pupation were recorded. Survival analysis between colonies for each dose was conducted with G-tests using PROC FREQ (SAS Institute 1998). All mean weights and times were log transformed before analyzed using REML-ANOVA (PROC MIXED; Littell et al. 1996).

G_2 Experiment

To determine if tolerance of fall armyworms to Cry1Ac had a heritable component, moths from the above colonies were pooled and the subsequent generation tested. Regardless of what the P_1 larvae fed upon, equal numbers of G_1 larvae from all three colonies that fed on non-Cry1Ac diet were allowed to pupate, pooled, and adults mated as described above. This G_2 colony (REG) served as a control again to account for any fitness or vigor differences among colonies.

Pupae from larvae reared the previous generation on Cry1Ac diet were separated based on time to pupation of G_1 individuals. Those that had pupated at 15 DAE were termed the FAST colony and those individuals that pupated at 19 and 20 DAE were termed the SLOW colony. These pupation times were selected to insure that similar numbers of pupae were available to develop adequate colonies. All G_2 colonies (REG, SLOW, and FAST) were maintained as described above. Survival of larvae at 8 DAE, survival to pupae, larval weights at 8 DAE, and time to pupation were recorded. Survival analysis between colonies for each dose was conducted with G-tests using PROC FREQ (SAS Institute 1998). All means weights and times were log transformed before being analyzed using REML-ANOVA (PROC MIXED; Littell et al. 1996).

RESULTS AND DISCUSSION

G_1 Experiment

Based on very high (>85%) survival data, rearing fall armyworms on transgenic Bt cotton had

no effect on mortality in the subsequent generation. In addition, there were no significant differences ($P > 0.05$) in larval survival at 7 DAE ($0 \mu\text{g/ml}$: $\chi^2 = 2.21$, $df = 2$, $P = 0.33$; $10 \mu\text{g/ml}$: $\chi^2 = 3.33$, $df = 2$, $P = 0.19$) and survival to pupae ($0 \mu\text{g/ml}$: $\chi^2 = 1.46$, $df = 2$, $P = 0.48$; $10 \mu\text{g/ml}$: $\chi^2 = 0.29$, $df = 2$, $P = 0.87$) among all three colonies.

Larvae that were reared on Cry1Ac diet weighed significantly less ($P < 0.05$) and took significantly more time to pupate ($P < 0.05$) than those larvae reared on non-Cry1Ac diet which is a reported sub-lethal effect observed for fall armyworms feeding on transgenic Bt cotton (Adamczyk et al. 1998b) (Figs. 1 and 2). Significant differences ($P < 0.05$) among colonies (larval weights: $F = 12.27$; $df = 2$, 345 ; $P < 0.001$, time to pupation: $F = 13.19$; $df = 2$, 319 ; $P < 0.001$) and diet (larval weights: $F = 541.24$; $df = 1$, 345 ; $P < 0.001$, time to pupation: $F = 329.58$; $df = 1$, 319 ; $P < 0.001$) were observed as well as colony by diet interactions (larval weights: $F = 5.76$; $df = 2$, 345 ; $P = 0.004$, time to pupation: $F = 17.47$; $df = 2$, 319 ; $P < 0.001$). In addition, based on larval weight and time to pupation for larvae feeding on non-Cry1Ac diet, again there was no evidence to suggest any fitness or vigor differences existed among the three colonies ($P > 0.05$).

It appears that rearing fall armyworms on transgenic Bt cotton caused increased tolerance in the subsequent generation to Cry1Ac. When fed Cry1Ac diet, larvae whose parents had fed on transgenic Bt cotton leaves (BT) had significantly ($P < 0.05$) higher larval weights at 7 DAE and a shorter time to pupation than those larvae whose parents had fed on conventional cotton leaves (NBT) (larval weights: $t = 5.24$, $df = 345$, $P < 0.001$

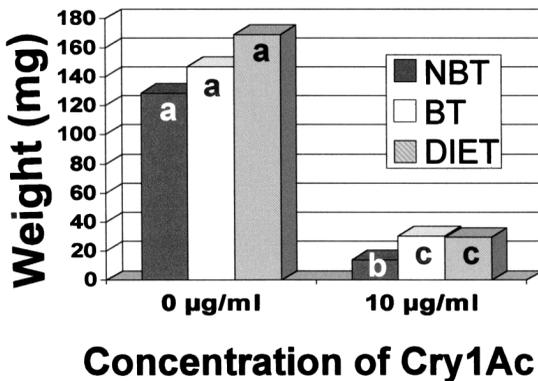


Fig. 1. Mean larval weights at 7 days after exposure (DAE) for fall armyworms fed non-Cry1Ac diet ($0 \mu\text{g/ml}$) or Cry1Ac diet ($10 \mu\text{g/ml}$). NBT, BT, and DIET colonies = previous generation reared on conventional cotton leaves, transgenic Bt cotton leaves, and non-Cry1Ac diet, respectively. Columns with the same letter are not significantly different ($\alpha = 0.05$) from one another (REML-ANOVA; PROC MIXED; Littell et al. 1996).

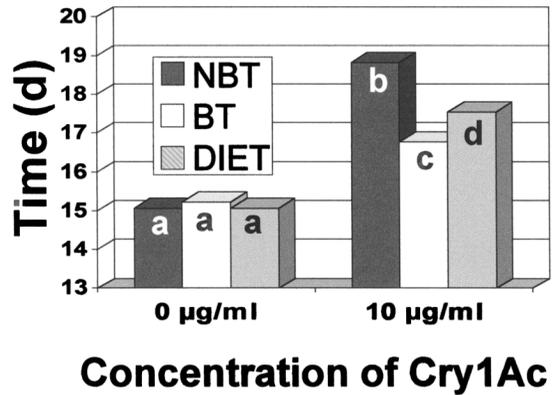


Fig. 2. Mean time to pupation for fall armyworms fed non-Cry1Ac diet ($0 \mu\text{g/ml}$) or Cry1Ac diet ($10 \mu\text{g/ml}$). NBT, BT, and DIET colonies = previous generation reared on conventional cotton leaves, transgenic Bt cotton leaves, and non-Cry1Ac diet, respectively. Columns with the same letter are not significantly different ($\alpha = 0.05$) from one another (REML-ANOVA; PROC MIXED; Littell et al. 1996).

(LSMEANS); time to pupation: $t = -7.59$, $df = 319$, $P < 0.001$ (LSMEANS)] (Figs. 1 and 2).

G_2 Experiment

As in the G_1 experiment, there was no indication that G_2 larvae were less fit or vigorous from feeding on Cry1Ac diet than from feeding on non-Cry1Ac diet. In fact, survival of pupae for the FAST colonies was significantly higher than the SLOW or REG colonies (Fig. 3).

Based on larval weights and time to pupation, increased tolerance to Cry1Ac was inherited among individuals in the subsequent generation (Figs. 4 and 5). Significant differences ($P < 0.05$) among colonies (larval weights: $F = 20.78$; $df = 2$, 348 ; $P < 0.001$, time to pupation: $F = 38.06$; $df = 2$, 309 ; $P < 0.001$) and diet (larval weights: $F = 695.25$; $df = 1$, 348 ; $P < 0.001$, time to pupation: $F = 1360.96$; $df = 1$, 309 ; $P < 0.001$) were observed as well as colony by diet interactions (larval weights: $F = 15.00$; $df = 2$, 348 ; $P < 0.001$, time to pupation: $F = 24.27$; $df = 2$, 309 ; $P < 0.001$). When fed Cry1Ac diet, larvae from the FAST colony had significantly ($P < 0.05$) higher larval weights at 8 DAE and a shorter time to pupation than those larvae from the SLOW (larval weights: $t = 8.06$, $df = 348$, $P < 0.001$ (LSMEANS); time to pupation: $t = -9.66$, $df = 309$, $P < 0.001$ (LSMEANS)] or REG colonies (larval weights: $t = -5.46$, $df = 348$, $P < 0.001$ (LSMEANS); time to pupation: $t = 4.55$, $df = 309$, $P < 0.001$ (LSMEANS)]. Furthermore, based on time to pupation, a heritability estimate was calculated that further suggests tolerance to Cry1Ac was inherited in the subsequent generation ($h^2_{\text{FAST}} = 0.49$). In addition, based on larval

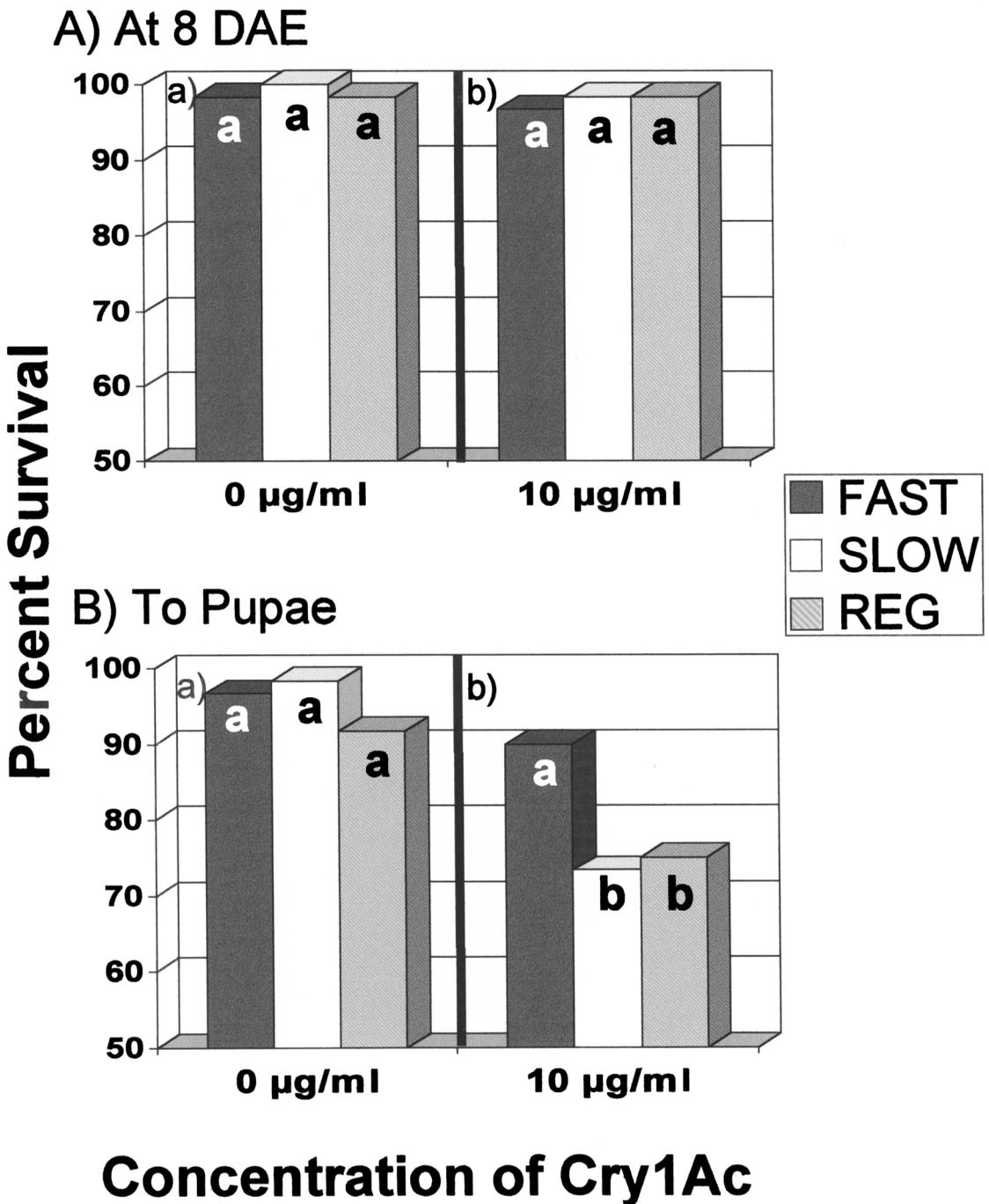


Fig. 3. (A) Mean larval survival of fall armyworms at 8 days after exposure (DAE) and (B) to pupae when fed: (a) non-Cry1Ac diet (0 µg/ml) or (b) Cry1Ac diet (10 µg/ml). FAST and SLOW colonies = previous generation pupated at 15-16 DAE and 19-20 DAE, respectively; REG colony = previous generation reared on non-Cry1Ac diet. Columns separated by dose with the same letter are not significantly different ($\alpha = 0.05$) from one another (likelihood ratio chi-square analysis using PROC FREQ; SAS Institute 1998).

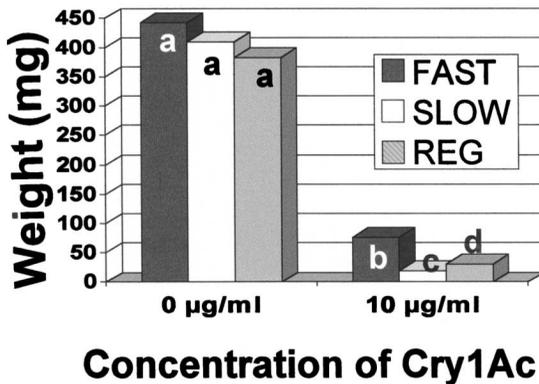


Fig. 4. Mean larval weights at 8 days after exposure (DAE) for fall armyworms fed non-Cry1Ac diet (0 µg/ml) or Cry1Ac diet (10 µg/ml). FAST and SLOW colonies = previous generation pupated at 15 DAE and 19-20 DAE, respectively; REG colony = previous generation reared on non-Cry1Ac diet. Columns with the same letter are not significantly different ($\alpha = 0.05$) from one another (REML-ANOVA; PROC MIXED; Littell et al. 1996).

weight and time to pupation for larvae feeding on non-Cry1Ac diet, there was no evidence to suggest any fitness or vigor differences existed among the FAST and SLOW colonies ($P > 0.05$), although the REG colony took significantly longer to pupate than either the FAST or SLOW colonies ($P > 0.05$).

The assumption that sub-lethal effects from a single generation of exposure of fall armyworms to transgenic Bt cotton has a negative impact on fitness or vigor in the subsequent generation seems to be inaccurate. Although some studies

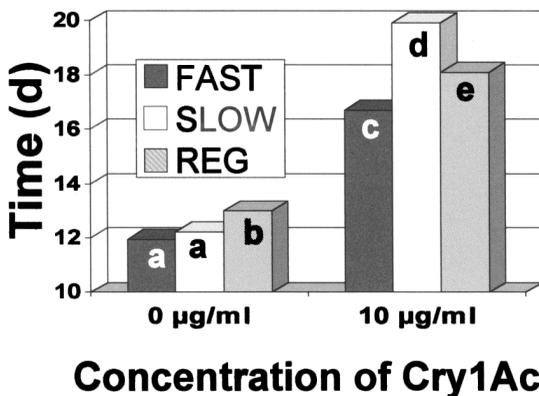


Fig. 5. Mean time to pupation for fall armyworms fed non-Cry1Ac diet (0 µg/ml) or Cry1Ac diet (10 µg/ml). FAST and SLOW colonies = previous generation pupated at 15 DAE and 19-20 DAE, respectively; REG colony = previous generation reared on non-Cry1Ac diet. Columns with the same letter are not significantly different ($\alpha = 0.05$) from one another (REML-ANOVA; PROC MIXED; Littell et al. 1996).

have suggested that negative maternal effects can be transmitted by *H. zea* parents feeding on transgenic Bt cotton to their offspring (Lambert et al. 1998), no indications of this occurred with fall armyworms. Studies have further shown that reduced application rates of foliar insecticides can be used for *H. zea* on transgenic Bt cotton compared to conventional cotton, possible due to reduced vigor of larvae feeding on Cry1Ac (Brickle et al. 1999). Our data suggests that not all lepidopterous pests of cotton that are intrinsically tolerant to Cry1Ac may be controlled identically on transgenic Bt cotton. Because more than one generation of fall armyworms can attack transgenic Bt cotton in one season, future work will be needed to determine if larvae are more tolerant to Cry1Ac in later generations compared to previous generations in naturally occurring populations.

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