Cry 1Ab and Cry 9C Transgenic Corn Hybrid Effects on Laboratory Populations of Indianmeal Moth (Lepidoptera: Pyralidae) and Angoumois Grain Moth (Lepidoptera: Gelechiidae)[1]

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Abstract Several *Bacillus thuringiensis* Berliner (Bt) Cry proteins including Cry 1Ab, Cry 1Ac, and Cry 9C, have been observed at relatively high levels in Bt corn grain using the CaMV 35S promoter. Thus, a laboratory experiment was conducted to quantify the impact of DeKalb 679 BTY Cry 1Ab (MON 810) and Garst 8600 BLT Cry 9C (CBH 351) transgenic grain on Indianmeal moth, *Plodia interpunctella* (Hübner), and Angoumois grain moth, *Sitotroga cerealella* (Olivier), survival, development and fecundity. Eggs of Indianmeal moth or Angoumois grain moth were added to cracked or whole kernel corn. Emergence and fecundity were recorded for 5 wks. Emergence and fecundity of both moth species was reduced on both Cry 1Ab and Cry 9C-transformed corn, but only Cry 1Ab-transformed corn delayed development of Indianmeal moth. Results indicate that populations of these moths may be negatively impacted in grain bins by Bt corn hybrids and that lepidopteran populations should be monitored in field-scale assays to examine the effects of the presence of Bt corn hybrids on insects in storage environments.

Key Words Indianmeal moth, Angoumois grain moth, transgenic grain, Cry 9C, Cry 1Ab, *Plodia interpunctella, Sitotroga cereallella,* stored grain, *Bacillus thuringiensis*

Transgenic insecticidal cultivars expressing *Bacillus thuringiensis* Berliner (Bt) Cry proteins were developed to provide a novel means of insect control for field pests. Plants expressing delta-endotoxin in various tissues convey protection against several lepidopterous pests such as European corn borer, *Ostrinia nubilalis* (Hübner) (Leemans et al. 1990, Koziel et al. 1993, Armstrong et al. 1995, Labatte et al. 1996, Davis and Coleman 1997), fall armyworm, *Spodoptera frugiperda* (J. E. Smith), southwestern corn borer, *Diatraea grandiosella* Dyar (Williams et al. 1997), and corn earworm, *Helicoverpa zea* (Boddie) (Pilcher et al. 1997). Two non-target stored grain moth pests, Indianmeal moth [*Plodia interpunctella* (Hübner)] and Angoumois grain moth [*Sitotroga cerealella* (Olivier)], also are susceptible. In addition, Bt transgenic corn hybrids have been developed with activity targeting important coleopteran pests in addition to Lepidoptera (Ostlie 2001).

It was recently reported that some Bt-11 and MON 810 Cry 1Ab delta-endotoxin transformed corn hybrids negatively impact Indianmeal moth and Angoumois grain moth emergence, fecundity and development (Sedlacek et al. 2001). Giles et al.

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(2000) found that several Cry 1Ab hybrids and a Cry 9C hybrid lowered Indianmeal moth emergence as well. Several Cry proteins including Cry 1Ab, Cry 1Ac, and Cry 9C have been observed at moderately high levels in Bt corn grain using the CaMV 35S promoter. The release of elite Bt corn hybrids, including the controversial and ill-fated StarLink™ (Aventis CropScience, Research Triangle Park, NC) containing Cry proteins in the grain, suggest that screening for impact on stored product lepidopterans be a continuing line of investigations. Thus, the objective of this study was to quantify the impact of DeKalb 679 BTY Cry 1Ab (MON 810) and Garst 8600 BLT Cry 9C (CBH 351) transgenic grain on Indianmeal moth and Angoumois grain moth survival, development and fecundity in the laboratory.

Materials and Methods

Colonies of Indianmeal moth and Angoumois grain moth were reared at the Stored Grain Insect Management and Ecology Laboratory at Kentucky State University, Frankfort, KY. Colonies were originally established from wild unselected Bt-susceptible moths obtained at the Kentucky State University Agricultural Research Farm. Indianmeal moth was reared on a wheat-based diet as described by McGaughey and Beeman (1988). Several hundred Indianmeal moth eggs were obtained by placing 50 adult Indianmeal moths in an empty 0.47-L jar overnight. Eggs collected were then placed into 60 to 80 g of this diet in 0.47-L Mason jars and incubated in darkness for 24 h at 27 ± 1°C and >60% RH.

The Angoumois grain moth was reared in 0.95-L Mason jars containing 300 g of shelled DeKalb 679 hybrid corn kernels and an ear of corn standing vertically in the jar. Fifty adults were introduced into these jars and allowed to oviposit for 48 h. To maintain the colony in the laboratory, emerging adults were allowed to oviposit on the grain and the ear of corn. After oviposition, the corn ears were removed and replaced. The removed ears (with moth eggs) were then placed in Mason jars containing fresh corn kernels. Angoumois grain moth colonies were incubated in darkness for 24 h at 27 ± 1°C and >60% RH.

DeKalb 679 BTY and its isoline DeKalb 679 corn were grown in Sacramento, KY, and Monticello, KY, respectively, and harvested and shelled in the fall of 1999. They were then transported to the stored grain bin research complex at the Kentucky State University Research Farm in March 2000. One thousand bu of each was placed in each of 2000 bu grain bins. An adequate amount of corn to conduct the experiment was removed and transported to the Stored Grain Insect Management and Ecology Laboratory on the campus of Kentucky State University. Garst 8600 BLT corn and its isoline Garst 8600 were provided by Aventis CropScience and grown in Janesville, WI, and Minooka, IL, in 1999. All corn was kept frozen at 0°C until used. Separate experiments for each moth species and each transgenic hybrid were conducted using a completely randomized design with five replicates (Hanley 2001).

One hundred seventy grams of each corn type was cracked and added to 0.45-L Mason jars. Corn to be used in Indianmeal moth assays was cracked in a standard kitchen blender for 10 s because Indianmeal moth is a secondary colonizer requiring some type of physical damage to the kernels. Fifty, 24-h-old, Indianmeal moth or Angoumois grain moth eggs from Kentucky State University colonies were added to each jar and placed into an environmental chamber at 27 ± 1°C and >60% RH in continuous darkness. Insects were allowed to develop from egg to the adult stage. Adult emergence was used to quantify survivorship of both species. Daily checks for emerging adults began after 27 d of incubation because this is the minimum duration required for the species to complete development under these conditions in the laboratory (Howe 1965, Harein and Subramanyam 1990). Duration of development was defined as the number of days required to develop from egg to adult. To determine Indianmeal moth fecundity, adults were collected daily for 5 wks and allowed to oviposit in empty 0.45-L jars for as long as they lived. Angoumois grain moths were collected for the same duration and placed in jars containing fluted filter paper to determine fecundity. Experiments were repeated three times using identical procedures and conditions.

Survival, duration of development and fecundity data were analyzed using analysis of variance (ANOVA) for DeKalb and Garst hybrids (SAS Institute 1999).

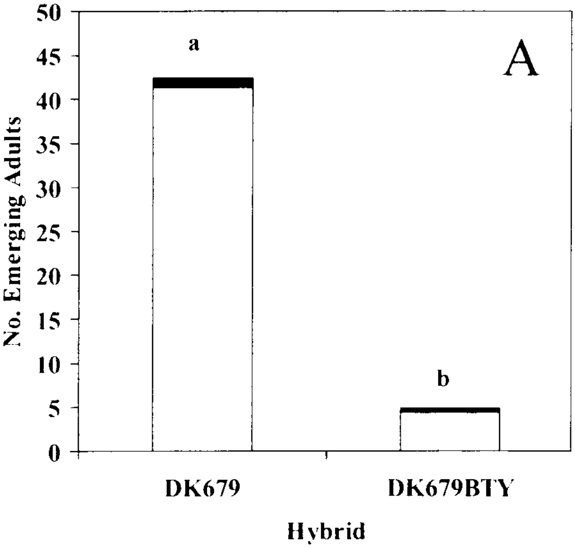
Results

Adult Indianmeal moth and Angoumois grain moth emergence was lower when these species were reared on the Bt transgenic corn hybrids compared to the non-Bt isolines. Emergence of Indianmeal moth was 89% lower using DeKalb 679 BTY, a MON 810 transformed hybrid ($F = 841.58$; df = 1, 26; $P < 0.0001$), 72% lower using WI-Grown Cry 9C Garst 8600 BLT, CBH 351 transformed, and 83% lower using IL-Grown Cry 9C Garst 8600 BLT, CBH 351 transformed grain [WI ($F = 386.15$; df = 1, 26; $P < 0.0001$); IL ($F = 785.40$; df = 1, 26; $P < 0.0001$)] (Figs. 1A, 2A). Similarly, emergence of Angoumois grain moth was 85% lower using DeKalb 679 BTY ($F = 75.06$; df = 1, 26; $P < 0.0001$) and 78% lower using WI-Grown or IL-Grown Cry 9C Garst 8600 BLT compared to non-Bt isolines [WI ($F = 112.44$; df = 1, 26; $P < 0.0001$); IL ($F = 182.15$; df = 1, 26; $P < 0.0001$)] (Figs. 1B, 2B).

Mean duration of development of Indianmeal moth differed significantly between the Bt and non-Bt hybrids, ranging from 34.4 to 45.9 d for DeKalb hybrids ($F = 231.70$; df = 1, 26; $P < 0.0001$) (Fig. 3A). Angoumois grain moth duration of development also differed significantly among individuals reared on DeKalb hybrids, ranging from 46.6 to 41.2 d ($F = 17.01$; df = 1, 24; $P = 0.0004$) (Fig. 3B). In addition, this same trend was observed in Indianmeal moths reared on Garst hybrids with duration of development ranging from 34.0 to 47.7 d [WI ($F = 116.95$; df = 1, 26; $P < 0.0001$); IL ($F = 139.23$; df = 1, 26; $P < 0.0001$)] (Fig. 4A). In both cases, Indianmeal moths reared on the DeKalb 679 BTY and Garst 8600 BLT hybrids had longer durations of development. Duration of development for Angoumois grain moths reared on Garst 8600 BLT and Garst 8600 were not statistically different [WI ($F = 0.28$; df = 1, 26; $P = 0.6016$); IL ($F = 0.05$; df = 1, 26; $P = 0.8222$)] (Fig. 4B).

Fecundity was higher during the first 2 wks of sampling on non-Bt corn compared to later sampling dates in both Indianmeal moth and Angoumois grain moth. This is consistent with the normal biology of these insect pest species (Sedlacek et al. 1995). Adults of larvae reared on DeKalb 679 BTY and both Garst 8600 BLT hybrids oviposited fewer eggs during the first 2 wks and during the entire period examined compared to their non-Bt isolines. Indianmeal moth fecundity was significantly lower on DeKalb 679 BTY, than on its non-Bt isoline DeKalb 679, representing 56.3% fewer eggs oviposited per female ($F = 161.62$; df = 1, 26; $P < 0.0001$) (Fig. 5A).

Angoumois grain moth fecundity was lower per female on the DeKalb 679 BTY than on its isoline DeKalb 679 ($F = 58.27$; df = 1, 25; $P < 0.0001$) (Fig. 5B). Angoumois grain moths reared on DeKalb 679 BTY laid 58.5% fewer eggs per female. In addition, Indianmeal moth fecundity was lower on both Wisconsin and Illinois grown Garst



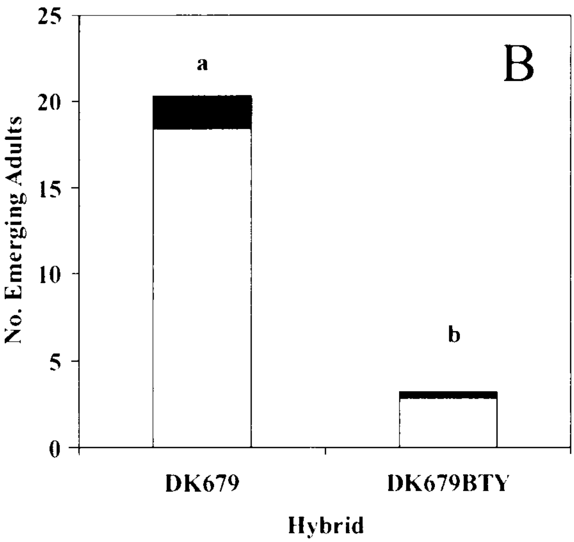


Fig. 1. Emergence of Indianmeal moth (A) and Angoumois grain moth (B) as influenced by DeKalb 679 BTY (Cry 1Ab) and DeKalb 679 (isoline) corn hybrids. Area at the top of each bar represents standard error; bars with different letters are significantly different ($P < 0.05$) as determined by ANOVA.

8600 BLT than on its non-Bt isoline Garst 8600 [WI ($F = 30.41$; df = 1, 26; $P < 0.0001$); IL ($F = 73.70$; df = 1, 26; $P < 0.0001$)] (Fig. 6A). This is 54.7% fewer for Wisconsin Garst 8600 BLT and 74.1% fewer eggs oviposited per female Indianmeal moth in Illinois Garst 8600 BLT, compared to their isolines.

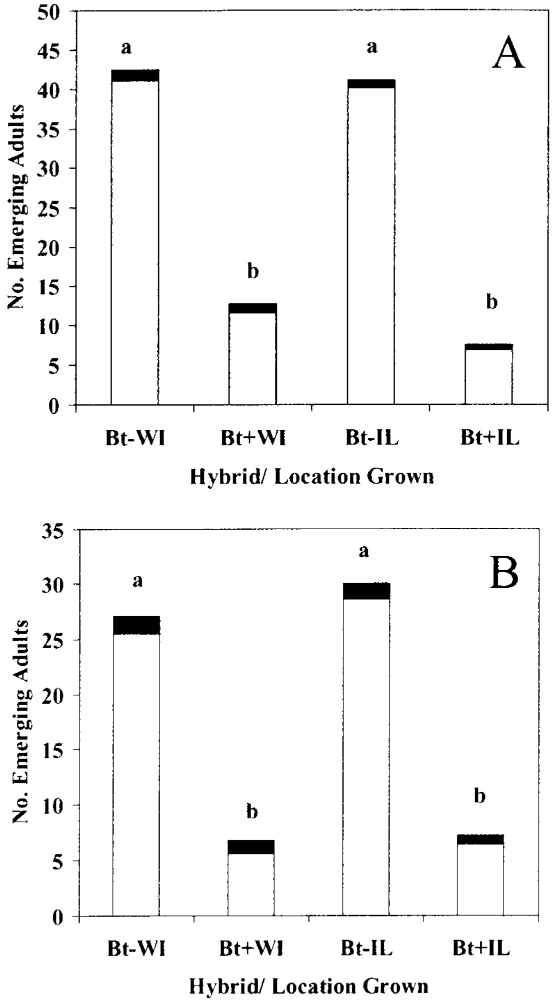


Fig. 2. Emergence of Indianmeal moth (A) and Angoumois grain moth (B) as influenced by Garst 8600 BLT (Cry 9C) and Garst 8600 (isoline) corn hybrids. Area at the top of each bar represents standard error; bars with different letters within a growing location are significantly different ($P < 0.05$) as determined by ANOVA. WI and IL refer to the location at which each of the corn hybrids was grown.

Mean number of eggs oviposited per female Angoumois grain moth only differed significantly among individuals reared on the Wisconsin-grown Garst 8600 BLT [WI ($F = 26.82$; df = 1, 26; $P < 0.0001$); IL ($F = 0.12$; df = 1, 26; $P = 0.7371$)] (Fig. 6B). In this case, 56.9% fewer eggs were oviposited per female Angoumois grain moth compared to the non-Bt isoline.

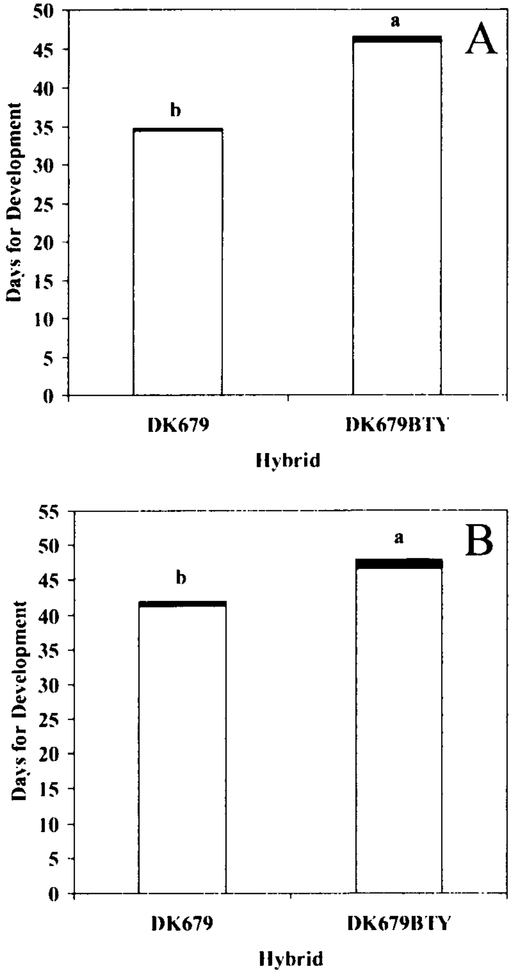


Fig. 3. Mean duration of development of Indianmeal moth (A) and Angoumois grain moth (B) as influenced by DeKalb 679 BTY (Cry 1Ab) and DeKalb 679 (isoline) corn hybrids. Area at the top of each bar represents standard error; bars with different letters are significantly different ($P < 0.05$) as determined by ANOVA.

Discussion

Results of this research indicate that Cry 1Ab MON 810 and Cry 9C CBH 351 Bt transformed corn kernels reduced Indianmeal moth and Angoumois grain moth populations in laboratory assays. Cry 1Ab MON 810 Bt transformed corn reduced emergence, delayed development of larvae, and reduced fecundity in both species. In addition, Cry 9C CBH 351 Bt transgenic corn emergence and fecundity were lower and duration of development was longer in Indianmeal moth. Angoumois grain moth

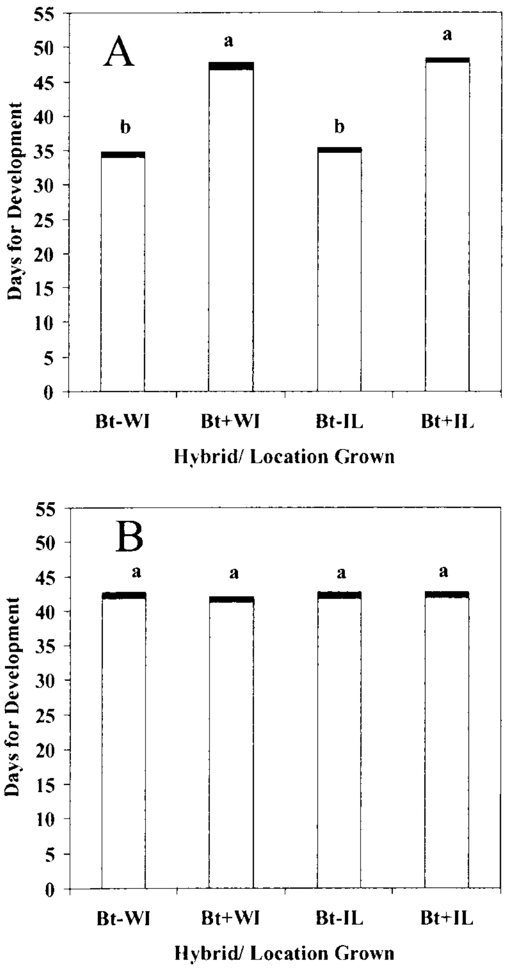


Fig. 4. Mean duration of development of Indianmeal moth (A) and Angoumois grain moth (B) as influenced by Garst 8600 BLT (Cry 9C) and Garst 8600 (isoline) corn hybrids. Area at the top of each bar represents standard error; bars with different letters within a growing location are significantly different ($P < 0.05$) as determined by ANOVA. WI and IL refer to the location at which each corn hybrid was grown.

emergence and fecundity also was lower. These data are in accord with those reported by Giles et al. (2000) and Sedlacek et al. (2001) showing similar impact of transgenic corn modified with Cry 1Ab and Cry 9C Bt proteins using the MON 810 and CBH 351 transgenic events, respectively.

Toxin content of kernels used in the experiment was not quantified, but experimental data from Environmental Protection Agency (EPA) biopesticide fact sheets showed that Cry 1Ab protein expressed in MON 810 corn grain is between 0.19 and

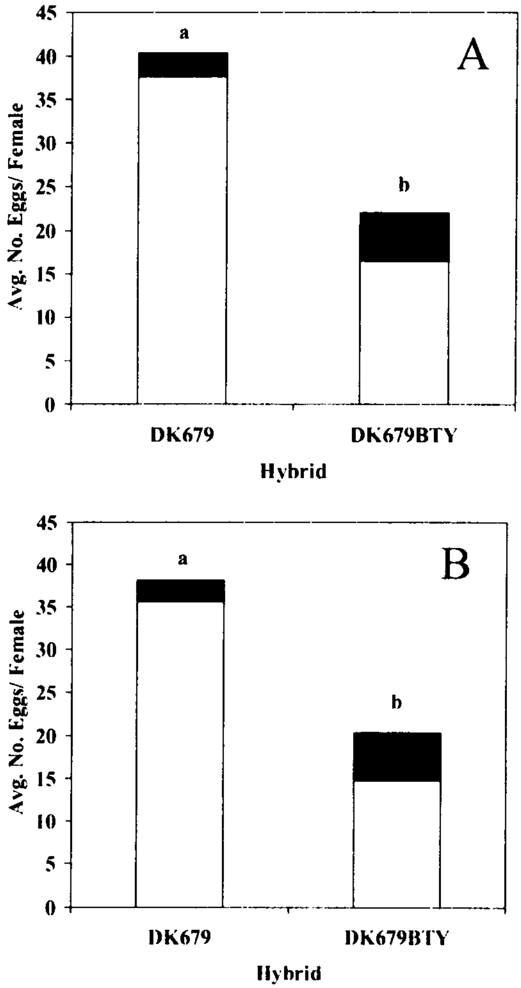


Fig. 5. Fecundity of Indianmeal moth (A) and Angoumois grain moth (B) as influenced by DeKalb 679 BTY (Cry 1Ab) and DeKalb 679 (isoline) corn hybrids. Fecundity is expressed as the mean total number of eggs laid by individual females during the study. Area at the top of each bar represents standard error; bars with different letters are significantly different ($P < 0.05$) as determined by ANOVA.

0.39 µg toxin/g of corn grain (Environmental Protection Agency 2000a). Cry 9C protein expressed in CBH 351 corn grain was shown to be at most 18.6 µg toxin/g of corn grain (Environmental Protection Agency 2000b).

The Cry 9C protein present in CBH 351 corn did not affect development time of Angoumois grain moth larvae. With Indianmeal moth on Cry 1Ab and Cry 9C kernels, the data suggest that surviving larvae were exposed to sub-lethal doses of Bt toxin,

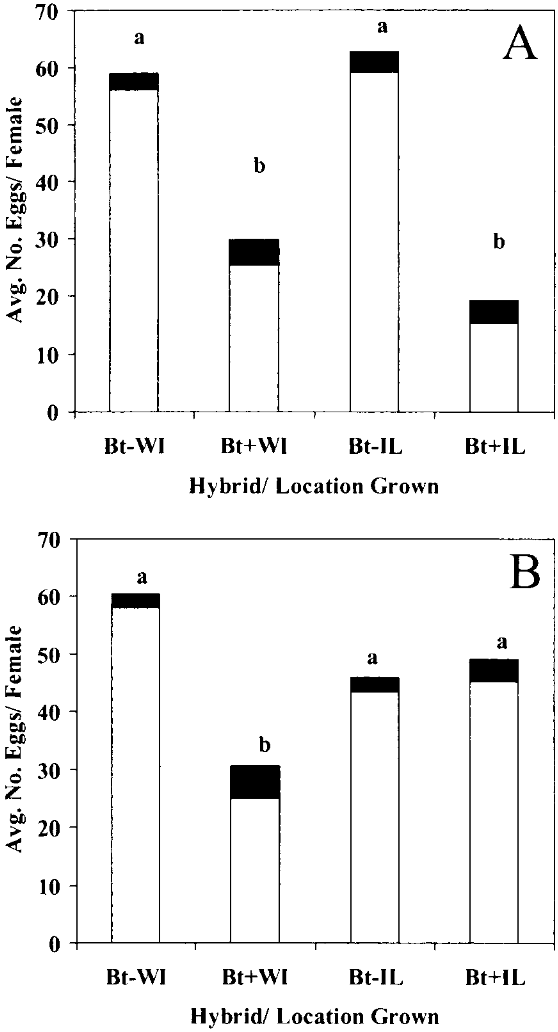


Fig. 6. Fecundity of Indianmeal moth (A) and Angoumois grain moth (B) as influenced by Garst 8600 BLT (Cry 9C) and Garst 8600 (isoline) corn hybrids. Fecundity is expressed as the mean total number of eggs laid by individual females during the study. Area at the top of each bar represents standard error; bars with different letters within a growing location are significantly different ($P <$ 0.05) as determined by ANOVA. WI and IL refer to the location at which each corn hybrid was grown.

because larvae exposed to the toxin had development times much longer than those observed in the isolines. Variable toxin expression among individual kernels could also lead to sub-lethal dosing as well. In addition, Indianmeal moth susceptibility to Bt toxins could be much higher than in Angoumois grain moth.

Some insects develop resistance to Bt and to Bt corn. Indianmeal moth can develop resistance to Dipel™ (Abbott Laboratories, North Chicago, IL) (McGaughey and Johnson 1992, Hanley 2001), and European corn borer can develop resistance to Bt corn (Bolin et al. 1999). Because >25% of the corn grown in the United States expresses Bt Cry proteins in kernels, research examining impact of Cry 1Ab grain on moth populations in grain bins and aspects of resistance of Indianmeal moth to Bt corn grain should continue.

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