ARTIFICIAL INFESTATION OF TRANSGENIC COTTONS WITH BEET ARMYWORM (LEPIDOPTERA: NOCTUIDAE) AND EVALUATION OF INSECT MORTALITY AND DAMAGE UNDER FIELD CONDITIONS

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Abstract

Transgenic cottons containing Bollgard®, Bollgard II®, and WideStrike™ traits along with non Bt cotton were grown during 2005-2009 to examine efficacy against beet armyworm, *Spodoptera exigua* (Hübner), (BAW) in field performance using natural and artificial infestations. Damage and mortality of BAW assessed by visual observation and ground reflectance spectra indicated that both dual-gene traits were significantly more efficacious than the single gene trait. The best method for field evaluations of effectiveness of different Bt and non-Bt cottons on BAW survival and leaf damage was artificial infestation with adults. Field efficacy bioassays of transgenic technologies need to be standardized. We demonstrated field bioassays with BAW on different transgenic cottons using natural and artificial infestations that can serve as a standard. The criteria needed for standardization of field methods for evaluations of efficacy of Bt cottons under artificial infestation are discussed.

Introduction

Assessing the efficacy of these Bt cotton varieties under new environmental and management regimes is of prime importance to companies that produce new or improved transgenic products; breeders, which create different varieties stacked with Bt endotoxins; and growers, which use them for cotton production. Field performance of cotton containing endotoxins under artificial infestation with different stages of lepidopteran should be standardized. Only this can provide accurate and stable data for insect control with different transgenic technologies.

The objectives of this study were to determine the mortality and damage of BAW in the field performance of transgenic cotton under natural and artificial infestations, and discuss new methodological approaches which can increase and stabilize the efficacy of transgenic technologies.

Materials and Methods

Field plots and plant material. The field trials were conducted in 2006-2009 at the North and South Farms of the Kika de la Garza Subtropical Agricultural Research Center ARS USDA, Lower Rio Grande Valley (LRGV) in Weslaco, Texas. Individual plots were 4-rows (on 76.2-101.6 cm row centers) by 75 m long and planted with transgenic cotton varieties containing the Bollgard, Bollgard II, WideStrike, and non-Bt traits that are recommended for the Valley. Each treatment was replicated three times in a randomized complete block design. Planting date, seeding rate, fertilizer, furrow irrigation, and other production factors were maintained according to local agronomic practices. The plots were used to evaluate BAW mortality and damage on Bt and non-Bt cottons under natural and artificial infestations them with insect eggs, larvae, pupae, and adult.

Insects. BAW were established from pigweed, *Amaranthus retroflexus* L., and maintained on a soybean-wheat germ diet (Shaver and Raulston 1971) at the Kika de la Garza Subtropical Agricultural Research Center in Weslaco, Texas.

Field assays. Artificial infestations of Bt and non-Bt cottons with egg-masses and larvae of BAW were in uncaged plot plants, while cages were used with pupae and adults. The cages were 1.8x1.8x1.8 m which covered two cotton plant rows and were produced commercially (BioQuip, Gardena CA). The cotton varieties used were: AMX 262R and PHY 425 RF - non Bt, DPL 444 BGIRR - Bollgard®, ST 4357 BGII/RR F -Bollgard II® and PHY 485 WRF – WideStrike ™.

Infestation with BAW egg masses. Egg masses were deposited to the wax paper placed in adult rearing cages (ice-cream cardboard liter containers). Egg masses of equal size (ca. 100-150 eggs/3 cm^2 wax paper) were attached with a pin to the underside of a mature leaf on every third plant. Infestations were conducted twice (60 and 90 days after planting (DAP). Beet armyworm populations were estimated per plot 8-10 days after infestations using a 1 .2 m drop cloth placed at 3 random locations within the center rows and plants were shaken to drop the larvae. We also determined condition of the eggs (desiccation, destruction by predators) and estimated the amount of leaf damage.

Visual observation. Infestations of Bt and non-Bt cottons with BAW under field conditions were recorded, as well as leaf damage by feeding of BAW larvae (percentage of leaf damage from total recorded) and rate of damage. Leaf rate of damage was estimated based on the following four categories: 0 - no apparent damage;

- 1 - light feeding damage or 10% of leaf area eaten;
- 2 - moderate damage or 10.1-30.0 % of leaf area eaten; and
- 3 - heavy damage or >30.0% of leaf area eaten.

Remote sensing observations. Ground reflectance spectra data were collected from the caged plots where BAW adults were released using a FieldSpec HandHeld spectroradiometer during 2007 and 2009. The spectroradiometer was sensitive in the visible to near-infrared (NIR) portion of the spectrum (350-1050 nm). The spectral readings were taken on 10 randomly selected canopies ‘from each plot. The average spectral readings for each of the four cotton varieties were calculated.

Infesting with BAW larvae. This study was initiated in 2006 at the North Farm. Each plot consisted of 2 cotton rows with a total of 90 plants (45 per row). Two infestations with neonate larvae (5-10 larvae per plant) were done 70 DAP using the Davis inoculator, and 80 DAP, as 1st and 2nd instars, using a salt shaker. Larvae were mixed with sterile corn cob grits (20/40 mesh) in the plastic inoculator’s bottle. After seven days, the number of live larvae and amount of leaf damaged were estimated as described above.

Infesting with BAW pupae. Pupae were released in commercial cages described earlier. A total of 180 pupae *(5 0%* female) were placed in each cage in a paper cup attached to the top of the cage at 80 DAP. The cotton varieties were evaluated for BAW emergence and leaf damage 10 days after the pupae were placed in the cage.

Infesting with BAW adults. Adults were released in commercial cages (125 adults, 50 % females/cage) at 50 and 90 DAP. After 10 days BAW larvae were sampled using drop cloths and leaves were inspected for damage.

Results and Discussion

Field assays

Infesting with BAW egg masses. The number of eggs that hatched 3-4 days after exposure on leaves of non Bt type ranged from 43.0 to 46.1%, while mortality ranged from 53.9 to 57.0%, due to heat and desiccation (23.8-31.8%) and from predators (25.2-30.1%). Survival of larvae on non-Bt cotton was the highest (43.6±2.0%), followed by single Bt endotoxin cotton (38.3±4.1%). Survival on non-Bt and single Bt endotoxin cottons were not significantly different from one another. Survival of larvae on dual Bt cottons was 17.3±4.5% which was significantly different from non-Bt and single Bt endotoxin cottons ($P = 0.024$). The percentage of leaf damage was 48.0+1.4, 33.7±4.0, and 18.3±1.7% on non-Bt, single, and dual Bt cottons ($P = 0.001$), respectively (Fugure1). The rate of damage was 1.7±0.2 (non-Bt), 1.5±0.2 (single Bt), and 0.8±0.1 (dual Bt cottons) ($P = 0.001$) (Figure 2). Average numbers of live larvae per one meter on non Bt type of cotton plot were 3.0±0.6, single Bt type plot - 2.2+0.7, and dual Bt types — 0.2±0.1 ($P = 0.006$) (Figure 3). Our previous data in LRGV of Texas showed that artificial infestation of cotton with eggs after 8:00 pm (after the heat and heat index had decreased significantly) can reduce mortality from abiotic factors by 2.0-fold, and by 1.6-fold from predators when substrate for attaching BAW egg-masses was green in color.

Infesting with BAW larvae. We observed 85% mortality of neonate larvae and 60% mortality of first instars on first-second days after cotton infestation (heat and physical damage). The surviving larvae damaged 25% non Bt-cotton, 16.3% single, and 2.5% dual Bt types.

Infesting with BAW pupae. These techniques were the least successful at establishing populations. All pupae were consumed by predators (i.e. fire ants).

Infesting with BAW adults. At 15-20 d after exposure, the average leaf damage on non-Bt cotton was 5.1-fold higher than on dual Bt cottons (P=0 .001), while the damage between non-Bt was only 1.4-fold higher than on single Bt cottons (*P=0.2*) (Figure 1). The average rate of leaf damage on non-Bt cotton was 8.9-fold higher than dual Bt cottons (*P=0.001*), while the rate on non-Bt was 1.5-fold higher compared to single Bt cottons *(P=* 0.1) (Figure 2). Average numbers of live larvae per one meter on non Bt type cotton were 7.0±1.6, single Bt type plot - 2.8±0.5, and dual Bt types — 0.09±0.06 (*P=0.006*) (Figure 3).

Figure 4 shows the reflectance spectral measurements of the three Bt cotton varieties and the non-Bt variety infested with BAW in the 2009 experiment. Both Bt II varieties had higher NIR reflectance than either the Bt cotton or the non-Bt cotton, and the Bt cotton had higher reflectance than the non-Bt cotton, indicating Bt cotton plants had less damage than non-Bt cotton after the artificial infestation. These results agree with those from the 2007 experiment.

Visual observations showed that season-long BAW leaf damage by natural infestation on non-Bt cotton was 3.6-fold higher than on genotypes containing dual Bt proteins (Bollgard II® and WideStrike) and 1.5-fold higher than on genotypes containing single Bt proteins (Bollgard varieties).

During the investigations, we observed variable data from the same treatments and the same initial insect infestations. These reduced accuracy of results and interpretation of data. Bioassay efficacy of transgenic technologies needs to be standardized. At first, a standard requirement should be developed to test insects and transgenic (no-transgenic) plants. For example, the initial insect survival must not be less than 95%, mass rearing insect programs should be operated with model and designed to develop different insect stages for artificial infestation in close proximity to their activity: egg-masses approximately 3-5 h to hatching (observe first neonate larva), larvae —50% 2nd instars in culture, pupae —first adults emerge, and adults — first adults start laying eggs. Larval mortality on transgenic plants can be assessed after plant tissue ingestion and the plants have received some level of damage (Hardee et al. 2001). We evaluated, when the larvae fed on less effective Bt type cotton leaves, they needed to consume more leaf material to reach the level of endotoxins that caused larval mortality. BAW need to feed on Bollgard Bt trait an average of 12.0 d, while bollworms (BW) only 7.0 d before larvae start to die. Besides, some larvae continue to survive after feeding stops. Mortality should be estimated not after *3-5* d, but after dead larvae are found. Initial population of test insects reared on artificial diet may cause reduction of feeding after transfer to natural host plants. For sampling plants need standardization relative to day when will be taking samples, how many times, side of plant, and amount of endotoxins in different plant structures. During sampling, the plants should not be sprayed with any synthetic chemicals.

Conclusions

1. The damage and mortality of the tested lepidopteran species assess by visual observation and ground reflectance spectra in field plots indicate that both dual-gene traits are significantly more efficacious than single Bt trait, while single Bt trait is more efficacious than non-Bt.
2. A standard requirement should be developed to test insects and transgenic plants.
3. The artificial infestation of cotton plants with BAW adults was the best method for field assays, while using pupae was the least successful techniques at establishing populations of BAW.
4. After infesting with BAW egg-masses, non-Bt type of cotton leaves, only 43 .0-46.1% eggs hatched and mortality of neonate larvae was 53.9-57.0% due to heat and desiccation.
5. When cotton plants were infested with neonate larvae, we observed 85% died (heat and physical damage) and 64.0% died of first instars. Artificial infestation with larvae need more studies to optimize the methodology.

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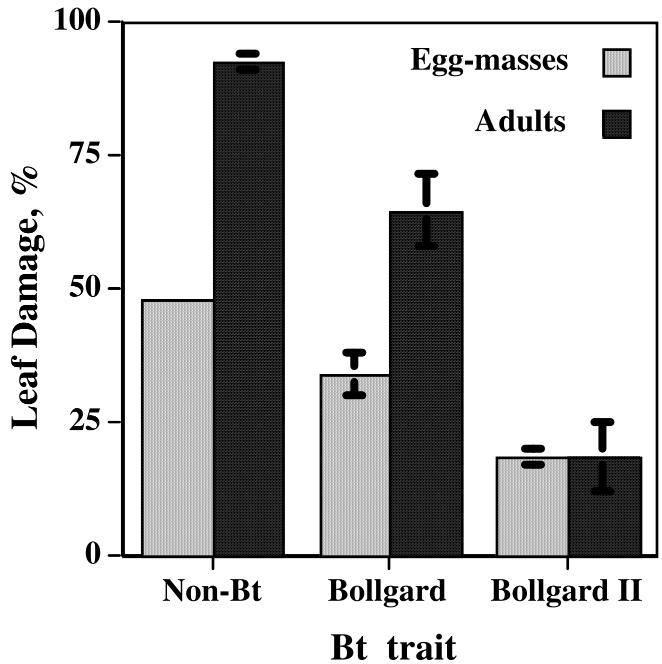

Figure 1. Leaf damage on different Bt trait cottons artificially infested with BAW egg masses and adults

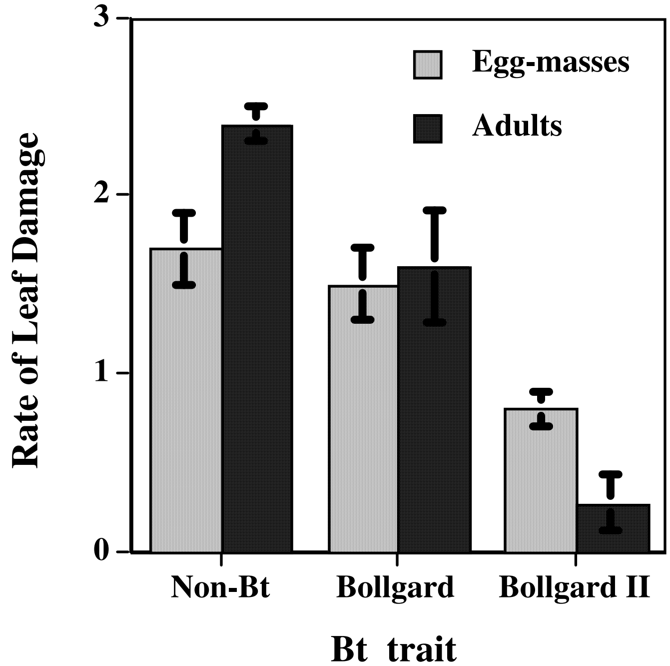

Figure 2. Rate of leaf damage on different Bt trait cottons artificial infested with BAW egg masses and adults

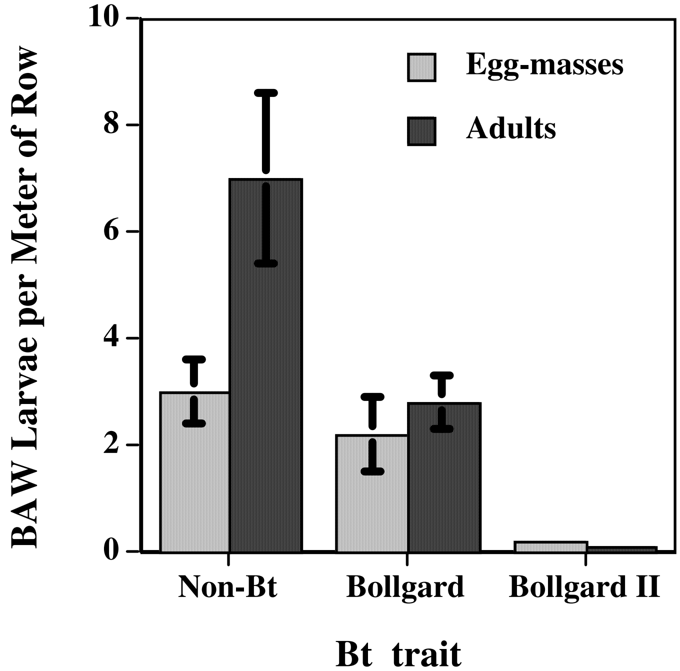

Figure 3. Alive larvae per meter per row on different Bt trait cottons artificial infested with BAW egg masses and adults

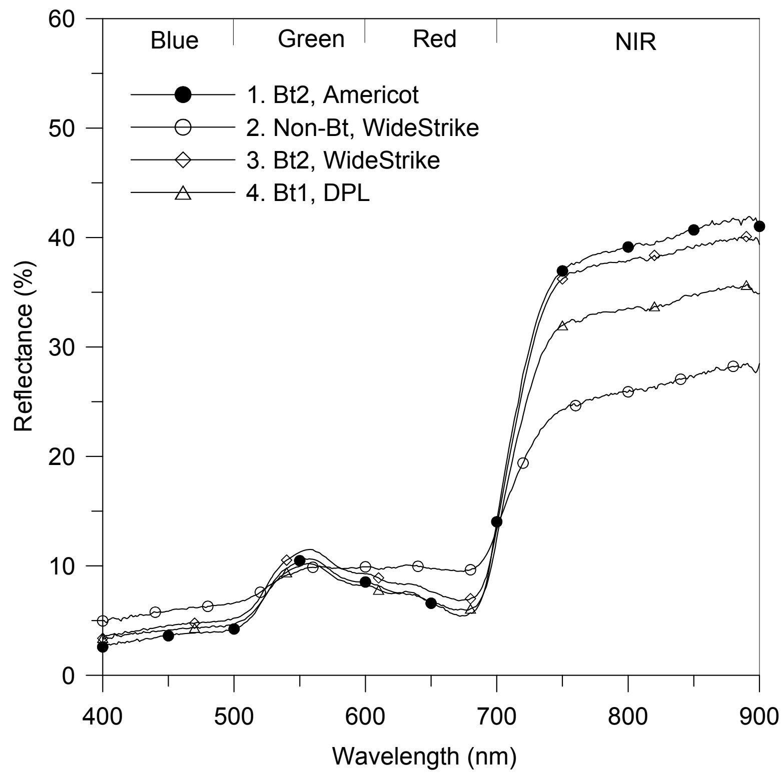

Figure 4. Ground reflectance spectra of Bt and non Bt cotton plants infested with BAW in the 2009 experiment.