

ASSESSING BEET ARMYWORM DAMAGE ON BT AND NON-BT COTTONS BY VISUAL OBSERVATIONS AND REMOTE SENSING

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Abstract

We evaluated damage, survival, and yield of the beet armyworm, *Spodoptera exigua* (Hübner) populations on Bollgard II® (ST 4357 BGII/RRF and AMX 1532RGII/RR), WideStrike™ (Phy 485 WRF), Bollgard® (DPL 444 BRR), and non-Bt cottons (AMX 262R, Phy 425 RF) in the Lower Rio Grande Valley (LRGV) of Texas. Experiments were conducted under natural infestations in field plots and by artificially infesting cotton in field cages where damage assessment was evaluated by visual ratings, reflectance spectra, and airborne color-infrared (CIR) digital imagery. Visual observations showed that season-long beet armyworm leaf damage 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); larval survival rates on non-Bt cotton were 12-fold and 2.4-fold higher, respectively. During the cotton growing seasons leaf damage and larval survival rates between varieties belongs to the dual Bt type, or non-Bt cotton type were not significantly different. Only at the end of the season (110 d after planting), the damage of WideStrike™ cotton was 1.4-fold higher compared to Bollgard II® damage (dual Bt protein types). Ground reflectance spectra and airborne color-infrared (CIR) digital imagery were obtained from the test plots shortly before harvest. The obtained images were able to differentiate beet armyworm damage levels between genotypes containing dual Bt proteins and non-Bt cotton group of varieties. The yield of Bollgard II® was 1.2-fold (2005) and 1.4-fold (2006) higher than Bollgard and non-Bt cotton, but the yields from the latter two were not significantly different. We found that artificial infestation with adults is the best method for field evaluation on effectiveness of different Bt and non-Bt cottons on beet armyworm survival and leaf damage.

Introduction

The beet armyworm, *Spodoptera exigua* (Hübner), is a cosmopolitan species that attacks > 90 plant species in at least 18 families throughout North America, many of which are crop plants (Pearson 1982). Over the last 2 decades, it has become an increasingly destructive secondary pest of cotton in the United States. Although the beet armyworm historically has been perceived as an occasional late-season pest of cotton, population outbreaks experienced in the 1980s and early 1990s in Alabama, Georgia, Louisiana, and Mississippi (Douce and McPherson 1991, Burris et al. 1994, Layton 1994, Smith 1994) and late 1990s in Texas (Huffman 1996, Summy et al. 1996) have demonstrated the potential damage it may cause. Chemical control programs against this pest have been complicated by its propensity to develop insecticide resistance (Cobb and Bass 1975, Bremer et al. 1990). Outbreaks of beet armyworm in cotton are often associated with multiple treatments with broad-spectrum insecticides, especially in the boll weevil, *Anthonomus grandis grandis* Boheman, eradication zones, which severely reduce natural enemy numbers as well (Mascarenhas et al. 1996, Ruberson 1999). Both Bt cotton and boll weevil eradication have great value for cotton production in the USA. Bt cotton has proven itself to be a useful tool in the process of eradicating the boll weevil, and in non-eradicated boll weevil zones by minimizing risk of outbreaks of lepidopteran secondary pest problems, which could augment activity of beneficial insects. Current and experimental varieties can contain the Cry1Ac-endotoxin alone (Bollgard®), or they can be stacked with Cry2Ab (Bollgard II®, Monsanto Ag. Co.), or Cry1Fa (WideStrike™, Dow Agrosciences, Indianapolis, IN) (Adamczyk and Gore 2004). Available Bt cotton varieties are highly effective against tobacco budworm, *Heliothis virescens* (F.), and provide suppression of bollworms, *Helicoverpa zea* (Boddie). Information about effectiveness of Bt cotton on beet armyworm is fragmentary and insufficient. Adoption of this technology has been slow in the LRGV of Texas, with only 4,300 hectares (5.8 %) planted in 2005 (Williams 2006). Therefore, assessing the efficacy of Bt cotton of different varieties under environment and management regimes is of prime importance to the growers.

The objectives of this study were (1) to determine the seasonal species composition of Noctuidae that effect cotton in the LRGV of Texas; (2) to evaluate the efficacy of some commercially of naturally infested Bt and non-Bt cottons against beet armyworm; and (3) to assess different methods of artificially infesting cotton with beet armyworm under field conditions and their efficacy on commercially Bt and non-Bt cottons by visual observation and remote sensing.

Materials and Methods

Field trials were conducted in 2004-2007 at the North and South Farms of the KSARC-ARS-USDA, Weslaco Texas. Bt types, traits and varieties used in the experiments described in Table 1. All plots were maintained according to local agronomic practices. Insecticides were applied only for boll weevil control. Beet armyworms survival and damage were monitored on cotton plants weekly at 40 days after cotton was planted (DAP) until defoliation.

Pheromone traps (beet armyworm, bollworm, and tobacco budworm, 10 traps per each species) were installed around the perimeter of plots at 1.2 m above the ground on sticks, with 50 m distances between traps. Each trap contained a dispenser (Pherocon cap) which was replaced weekly. Both traps and dispensers are available commercially (Trece, Inc. Salinas, CA USA). The traps were checked weekly.

Visual observation of naturally infested Bt and non-Bt cottons with beet armyworm was conducted by walking diagonally from one corner to another and examining at least 25 individual plants at random. The numbers of different stages of beet armyworm (egg masses, larvae) were recorded, as well as leaf damage by feeding beet armyworm 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 - not apparent damage; 1 - light feeding damage or $\leq 10\%$ leaf area eaten; 2 - moderate damage or 10.1-30.0 % leaf area eaten; and 3 - heavy damage or $>30.0\%$ leaf area eaten.

Artificial infesting Bt and non-Bt cottons with different stages of beet armyworms (egg masses, larvae, pupae, adults) under two types of cages (one - wood frame covered with aluminum screen, 4.5x1.8x1.0 m, and made by coworkers of KSARC; the second - produced commercially, BioQuip, Gardena CA, metal tubes covered with net, 1.8x1.8x1.8 m, two cotton plants rows).

Beet armyworm laboratory culture. Beet armyworm egg masses, pupae, and adults were obtained from a laboratory colony maintained at the KSARC in Weslaco, TX, while larvae were obtained from Benzon Research Inc., Carlisle, PA. Beet armyworm were reared exclusively on soybean-wheat germ diet (Shaver and Raulston 1971).

Infesting with beet armyworm 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/3.0 cm² wax paper sample) were attached with a pin to the underside of a mature leaf on every third plant. Under each screened wooden cage were two cotton rows with a total of 90 plants (45 per row). Infestations were conducted twice (70 and 90 DAP). Beet armyworm populations were estimated per plot at 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. Besides that, we appraised condition of eggs (desiccation, destroy by predators). In addition, we also estimated leaf damage using same as earlier a visual rating system.

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

Infesting with beet armyworm pupae. Pupae were released in commercial cages. A total of 180 pupae (50% female) were released in each cage by placing them in a paper cup attached to the top of the cage at 95 DAP. The cotton varieties (PHY 425 RF - non Bt, DPL 444 BG/RR – Bollgard®, ST 4357 BGII/RR F – Bollgard II® and PHY 485 WRF – WideStrike™) were evaluated for emergence and leaf damage 10 days after the pupae were placed in the cage.

Infesting with beet armyworm adults. Adults were released in commercial cages (same cottons as for pupae) (125 adults, 50 % females/cage) at 110 DAP. After 10 days beet armyworm larvae were sampled using drop cloths and leaves were inspected for damage.

Cotton yield was estimated by hand harvested samples (two rows per treatment - 13.75 ft row long) and processed on an Eagle laboratory gin.

Statistical Analyses. One-way analysis of variance (ANOVA) was conducted, and means were separated by Tukey Studentized range honestly significant difference (HSD) test ($\alpha=0.05$; Wilkinson et al. 1992).

Remote sensing observations. Ground reflectance spectra were collected from the caged plots using a FieldSpec HandHeld spectroradiometer on 8 August 2007. The spectroradiometer was sensitive in the visible to near-infrared (NIR) portion of the spectrum (350-1050 nm). Airborne color-infrared (CIR) digital imagery was acquired using an imaging system from the cotton field on 9 August 2007. The imaging system consisted of three digital charge coupled device (CCD) cameras, which were filtered for spectral observations in the green (555-565 nm), red (625-635 nm), and NIR (845-857 nm) wavelength intervals, respectively. Mean digital count values were derived from each of the three bands in the CIR image for two types of cages. The normalized difference vegetation index (NDVI) was calculated.

Results

Seasonal Noctuidae composition. The most prevalent Noctuid captured over the three year trapping periods (2005-2007) was the beet armyworm (74.4-84.5%), followed by the bollworm (11.7-20.1%), and tobacco budworm (3.9-5.5%) (Table 2).

Natural infestation of beet armyworms. Because the cotton varieties in the studied years were different, we combined mean data for all years from all varieties belonging to non-Bt, single endotoxin, or dual endotoxin Bt types across years for comparative evaluation of their influences on beet armyworm damage and survival larvae. In 2004, the natural infestation of beet armyworm larvae per 100 plants was low and not significantly different ($t = 0.19$; $P = 0.85$) on non-Bt (2.2 ± 0.9) and dual endotoxin Bt type (2.3 ± 0.7). The mean percentage of leaf damage on non-Bt was 2.8 ± 0.3 and on dual endotoxin Bt type was 2.4 ± 0.4 . Damage ratings were 0.608 ± 0.09 on non-Bt and 0.525 ± 0.07 on dual endotoxin Bt type. Leaf damage and damage ratings were not significantly different from one another ($P=0.517$ and $P=0.469$, respectively). During the next 2 years (2005 and 2006) the natural densities of beet armyworm larvae were relatively high on non-Bt cotton (17.8 ± 0.9 in 2005, and 21.0 ± 0.4 in 2006) compared with significantly low densities on dual endotoxin Bt type (3.3 ± 0.7 in 2005, and 9.0 ± 0.2 in 2006) which is likely associated with larvae mortality ($P = 0.009$ and $P = 0.015$, respectively) (Fig. 1A). Seasonal leaf damage by beet armyworm was significantly different on Bt and non-Bt cottons when the natural insect densities were high. Leaf damage on non-Bt cotton on average were 1.5-fold higher than on single endotoxin Bt type and 3.6-fold higher than on dual endotoxin Bt type; the damage leaves on single endotoxin Bt type were 2.3-fold higher than on dual endotoxin Bt type ($F = 18.8$, $df = 3, 36$, $P = 0.001$, 2005; $F = 15.6$, $df = 3, 34$, $P = 0.001$, 2006; and $F = 10.2$, $df = 3, 34$, $P = 0.009$, 2007) (Fig. 1B). The same trend was observed at the rate of damage: on non-Bt cotton this index was 1.5-fold higher than on single endotoxin Bt type and 2.7-fold higher than on dual endotoxin Bt type; on single endotoxin Bt type > dual endotoxin Bt type at 1.8-fold ($F = 23.3$, $df = 3, 36$, $P = 0.001$, 2005; $F = 25.8$, $df = 3, 36$, $P = 0.001$, 2006; and $F = 23.1$, $df = 3, 34$, $P = 0.001$) (Fig. 1C). The differences between varieties of genotypes containing dual Bt endotoxin cottons (Bollgard II and WideStrike) during the cotton growing seasons were not significantly different. Only at the end of the season (110 d of cotton age), the damage to WideStrike cotton variety (Phy 485 WRF) was 1.4-fold higher than Bollgard II variety ST 4357 BG2RF.

Yield. In 2005, dual Bt endotoxin cottons produced (1,170.3 lb/ac) significantly more lint ($P = 0.007$) than varieties containing Bollgard (973.2 lb/ac) and non-Bt (961.8 lb/ac) cottons. In 2006, dual Bt endotoxin cotton produced lint 624.1 lb/ac, while varieties containing Bollgard produced 451.2 lb/ac, with non-Bt producing the least at 430.3 lb/ac lint ($P = 0.002$), but the yield from the latter two was not significantly different.

Artificial infestation of beet armyworms

Infesting with beet armyworm egg masses. The number of eggs that hatched 3-4 days after exposure to leaves of none 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 ($43.6 \pm 2.0\%$), followed single Bt endotoxin cotton ($42.3 \pm 8.1\%$). Survival on non-Bt and single Bt endotoxin cottons were not significantly different from one another. Survival of larvae on dual Bt cottons was $17.3 \pm 4.5\%$ which was significant different from non-Bt and single Bt endotoxin cottons ($P = 0.024$). The percentage of leaf damage was 53.4 ± 7.9 , 53.7 ± 2.1 , and $33.4 \pm 1.7\%$ on non-Bt, single, and dual Bt cottons ($P = 0.04$); and the rate of damage was 1.4 ± 0.2 (non-Bt), 1.3 ± 0.2 (single Bt), and 0.9 ± 0.2 (dual Bt cottons) ($P = 0.169$) (Fig. 2).

Infesting with beet armyworm larvae and pupae. These techniques were the least successful at establishing populations. We believe this could be caused by inaccurate infestation of single larvae rather than egg masses and pupae were consumed by predators (i.e. fire ants).

Infesting with beet armyworm adults. At 5, 10, and 20 d after exposure, the average leaf damage on non-Bt cotton was in 5.1-fold higher than on dual Bt cottons ($P=0.001$), while the differences between non-Bt and single Bt cottons were not significant ($P=0.7$). 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 ($P=0.1$) (Fig. 2).

Ground reflectance spectra and airborne multispectral image. Figure 3 presents the reflectance spectra of Bt and non-Bt cotton plants infested with beet armyworm within six short cages in the 2007 experiment. Infested Bt cotton, especially dual Bt cottons, had higher NIR reflectance and NDVI values than infested non-Bt cotton, indicating Bt cotton plants were healthier and had less damage than non-Bt cotton after the artificial infestation. Figure 4 shows a CIR digital image of Bt and non-Bt cotton in the six short cages and four tall cages. Because of the small cage areas and coarse image resolution, it was difficult to distinguish the differences among the varieties from the image. However, the image contained digital spectral data for each plot. Table 3 shows the mean spectral values for the three bands and NDVI for the caged plots based on the CIR image. Similar to reflectance spectra, Bt cotton had higher NIR and NDVI values than non-Bt cotton. Although these preliminary results are promising, more experiments with replications are necessary to statistically test the differences in tolerance to beet armyworm damage among the Bt and non-Bt varieties.

Conclusions

- Beet armyworm is the most prevalent Noctuid on cotton in the LRGV of Texas.
- Seasonal (2005-2007) cotton leaf damage and their rates were significantly different between Bt and non-Bt cottons when natural infestation with beet armyworm larvae was relatively high.
- Artificial infestation with beet armyworm adults is the best method for field evaluation of the effectiveness of different Bt and non-Bt cottons on beet armyworm survival and damage.
- Artificial infestation of different Bt and non-Bt cotton in field plots with beet armyworm egg masses and larvae need more studies to optimizes.
- Remote sensing techniques, including ground reflectance spectra and airborne CIR imagery, can be a useful tool for assessing beet armyworm damage between Bt and non-Bt cotton, though more research is needed.

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Table 1. Bt cottons used in experiments

| Bt Type* | Bt Trait | Variety | Bt Endotoxins | Owner of Bt Trait | Owner of Variety | Year |
|----------|-------------|----------------------|---------------|-------------------|--|------------------|
| Single | Bollgard | DPL 444BG/RR | Cry1Ac | Monsanto | Delta & Pineland (Monsanto) | 2006, 2007 |
| Dual | Bollgard II | Americot1532 BGII/RR | Cry1Ac+Cry2Ab | Monsanto | Americot | 2007 |
| Dual | Bollgard II | St 4357 BGII/RRF | Cry1Ac+Cry2Ab | Monsanto | Stoneville seed Co. (Bayer Crop-science) | 2007 |
| Dual | Bollgard II | DPL424 BGII/RR | Cry1Ac+Cry2Ab | Monsanto | Delta & Pineland (Monsanto) | 2004, 2005, 2006 |
| Dual | WideStrike | Phy 485 WRF | Cry1Ac+Cry2Fa | DowAgro-science | DowAgro-science | 2006, 2007 |
| None | Non-Bt | Americot 262R | None | None | Americot | 2006, 2007 |
| None | Non-Bt | Phy 425 RF | None | None | DowAgro-science | 2006, 2007 |
| None | Non-Bt | DPL 5415 RR | None | None | Delta & Pineland (Monsanto) | 2004, 2005 |

*Endotoxin

Table 2. Percentage composition of Noctuid species caught by pheromone traps

| Noctuidae* | Number captured per pheromone trap** | | | Total captured during the season (all traps) | | | Percentage of total number of Noctuid captured | | |
|------------|--------------------------------------|-----------|----------|--|-------|-------|--|------|------|
| | 2005 | 2006 | 2007 | 2005 | 2006 | 2007 | 2005 | 2006 | 2007 |
| BAW | 8.5±0.7a | 10.2±0.6a | 9.6±0.9a | 548.0 | 797.0 | 583.0 | 74.4 | 84.5 | 84.4 |
| TBW | 0.6±0.1c | 0.5±0.1c | 0.6±0.3c | 40.0 | 47.0 | 27.0 | 5.5 | 5.0 | 3.9 |
| BW | 2.3±0.2b | 1.1±0.1b | 0.9±0.6b | 148.0 | 99.0 | 81.0 | 20.1 | 10.5 | 11.7 |

*BAW-beet armyworm, BW-bollworm, TBW-tobacco budworm

**Means ±Se within a column followed by the same letter are not significantly different (Tukey honestly significant difference, $P < 0.05$)

Table 3. Mean spectral values for the caged plots based on a color-infrared image from a cotton field in south Texas in 2007

| | Cage | Bt Trait | Variety | NiR | RED | GREEN | NDVI ^a |
|----|--------------------|-------------|-----------------------|-----|-----|-------|-------------------|
| 1 | Short ^a | Non-Bt | Americot 262R | 204 | 90 | 158 | 0.386 |
| 2 | Short | Non-Bt | Phy 425RF | 195 | 97 | 160 | 0.336 |
| 3 | Short | WideStrike | Phy 485WRF | 223 | 82 | 156 | 0.461 |
| 4 | Short | Bollgard II | Americot 1532 BGII/RR | 229 | 77 | 153 | 0.497 |
| 5 | Short | Bollgard II | St 4357 BGII/RRF | 237 | 80 | 155 | 0.497 |
| 6 | Short | Bollgard | DPL 444BG/RR | 211 | 86 | 155 | 0.422 |
| 7 | Tall ^b | Non-Bt | Phy 425RF | 177 | 91 | 151 | 0.320 |
| 8 | Tall | WideStrike | Phy 485WRF | 189 | 79 | 145 | 0.412 |
| 9 | Tall | Bollgard II | St 4357 BGII/RRF | 187 | 80 | 144 | 0.403 |
| 10 | Tall | Bollgard | DPL 444BG/RR | 191 | 78 | 144 | 0.420 |

^aShort cages- 4.5x1.8x1.0 m (Long X Wide X Height);^bTall cages- 1.8x1.8x1.8 m

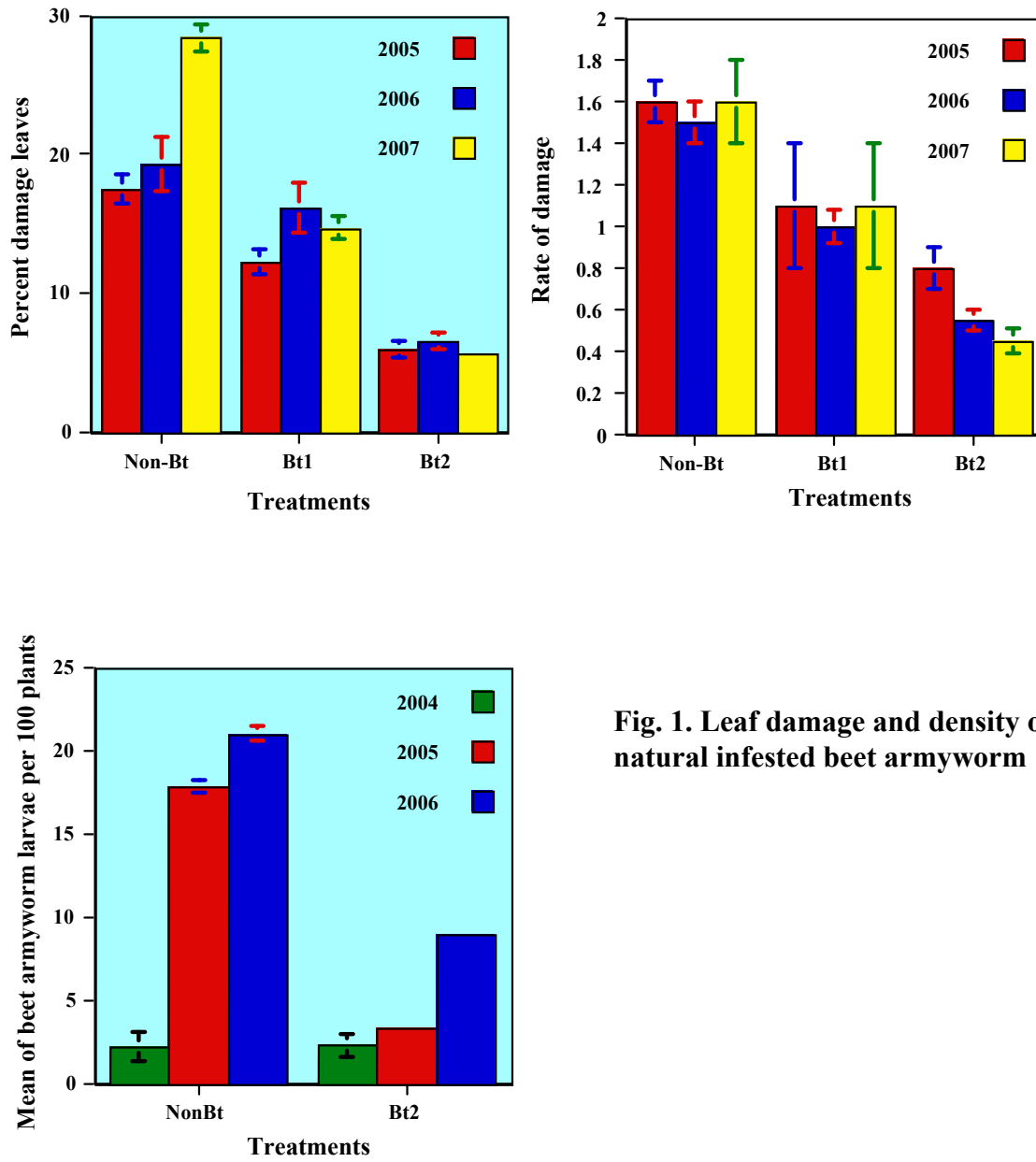


Fig. 1. Leaf damage and density of natural infested beet armyworm

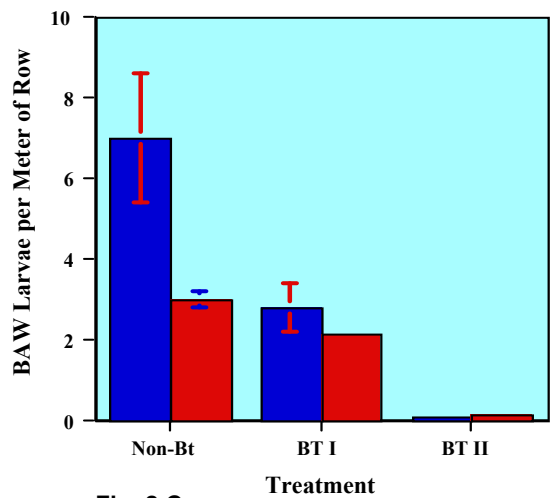
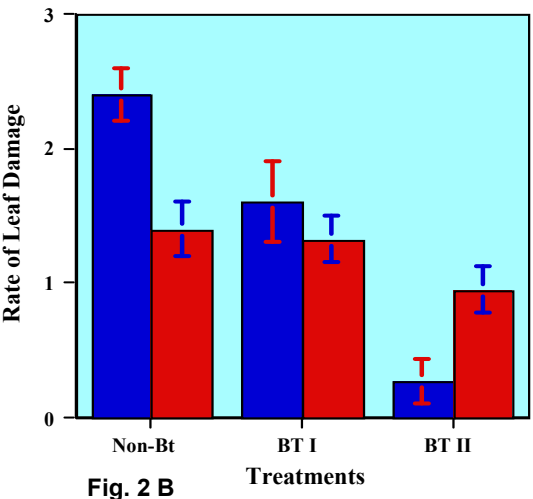
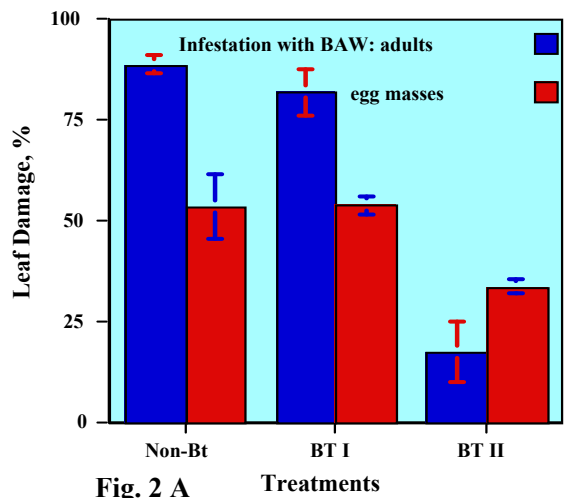


Fig. 2. Leaf damage and density of artificial infested cotton plots with egg and adult beet armyworms

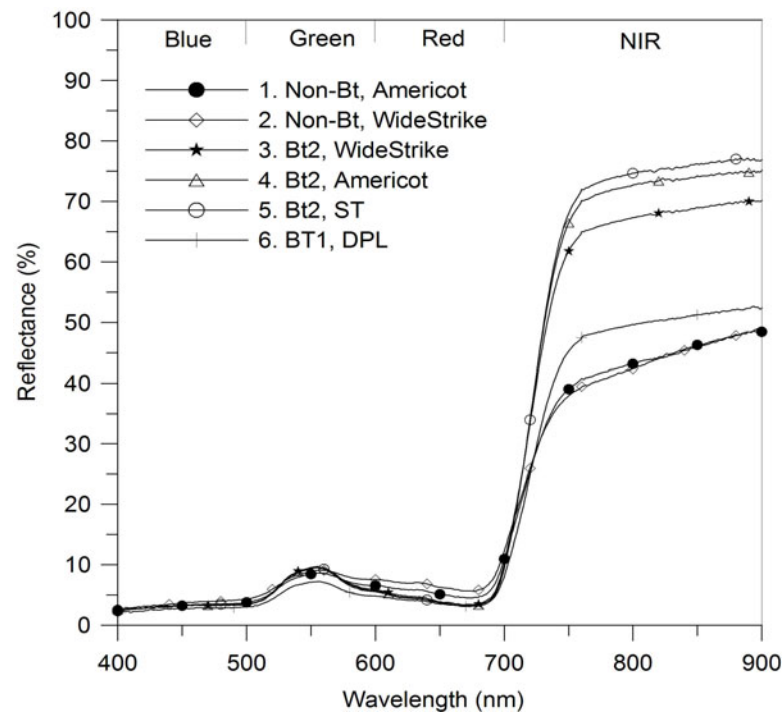


Figure 3. Ground reflectance spectra of Bt and non-Bt cotton plants infested with beet armyworm in six short cages in the 2007 experiment.

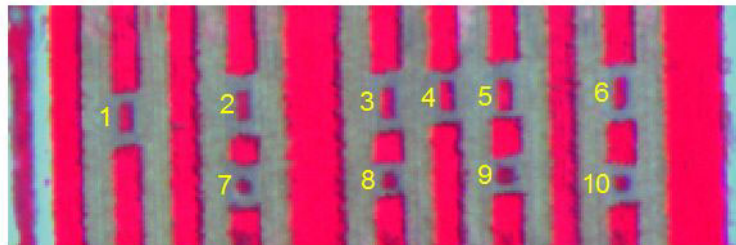


Figure 4. Color-infrared digital image of BT and non-Bt cotton in six short cages (1-6) and four tall cages (7-10) in the 2007 experiment. The numbers next to the plots are defined in Table 2.