

IMPACT OF BOLLWORMS ON MATURITY AND YIELD BOLLGARD® AND BOLLGARD II® COTTONS

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Abstract

The bollworm, *Helicoverpa zea* (Boddie), is an important pest of cotton in the United States. Currently, a large percentage of Bollgard® cotton is treated with insecticides to control bollworms with little information about economic losses from these infestations. Also, Bollgard II® cotton was recently commercialized and no information is available about economic injury from bollworms on Bollgard II. The objective of this study was to determine the impact of bollworm infestations on maturity and yield of Bollgard and Bollgard II cottons. Bollworm infestations were established on non-Bollgard, Bollgard, and Bollgard II cottons in large field cages. Treatments included three and five levels of infestation for one to four weeks in 2002 and 2003, respectively. Bollworms significantly delayed maturity and reduced yields of Bollgard cotton when 50 or 100 percent of white flowers were infested for one to four weeks in 2002. In 2003, bollworms delayed maturity of Bollgard cotton when 100 percent of white flowers were infested for four weeks. Yields of Bollgard cotton were reduced when greater than 10 percent of white flowers were infested with bollworms for two to four weeks and when 10 to 100 percent of white flowers were infested for three to four weeks. Bollworm infestations did not delay maturity or reduce yields of Bollgard II cotton. Based on results of this study, insecticide applications targeting bollworms on Bollgard cotton should be initiated before infestation levels reach 10 percent of white flowers. In contrast, Bollgard II cotton was less susceptible to bollworms and is not likely to require insecticide applications as frequently as Bollgard cotton.

Introduction

The bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), are key pests of cotton throughout much of the southeastern and mid-southern United States. Historically, insecticides have been the primary tool used to manage these insects in cotton. However, the widespread occurrence of resistance to organophosphates and pyrethroids in the tobacco budworm made management difficult in the early- to mid- 1990's (Leonard et al. 1988, Plapp et al. 1990, Elzen et al. 1992). During the 1996 growing season, genetically engineered cottons that revolutionized integrated pest management in cotton were introduced for commercial production (Perlak et al 2001). These novel cottons produce the Cry1Ac protein from the soil bacterium, *Bacillus thuringiensis* Berliner var. *kurstaki*, and are sold under the trade name Bollgard® (Monsanto Co., St Louis, MO.) (Perlak et al. 1990). The Cry1Ac protein provides good control of the tobacco budworm and no insecticide applications have been needed to manage this insect on Bollgard cottons. In contrast, insecticide applications often are needed in Bollgard cotton to prevent economic losses from bollworms.

Several factors contribute to the pest status of bollworms on Bollgard cotton. First, bollworms are less susceptible to the *B. thuringiensis* Cry1Ac protein than tobacco budworms (Luttrell et al. 1999). Also, temporal and spatial variations in the expression of Cry1Ac among different plant parts results in some structures having lower expression than other structures (Greenplate 1999, Adamczyk et al. 2001b). Populations of bollworms surviving in Bollgard cotton tend to be associated with white flowers and small bolls within one to two days after anthesis (Gore et al. 2000). Previous research has shown that bollworm larvae move among different structures more frequently in Bollgard cotton than non-Bollgard cotton (Gore et al. 2002) and feed on structures (white flowers and small bolls) where their chance of survival is greatest (Gore et al. 2001). As a result, agricultural consultants and pest management specialists have adjusted their scouting protocols for Bollgard cotton. In non-Bollgard cotton, insecticide applications generally are based on damaged squares and numbers of live larvae found in terminals and squares; whereas, insecticide applications in Bollgard cotton are based on numbers of live larvae in small bolls.

The second generation of genetically engineered cotton is currently being developed. Of these, Bollgard II® cotton was released on a limited basis during 2003. Bollgard II cottons contain two genes that code for the production of two different insecticidal proteins from *B. thuringiensis* (Greenplate et al. 2000). These cottons produce Cry2Ab along with the Cry1Ac protein found in Bollgard cotton. The addition of a second protein has increased the efficacy against bollworms and broadened the spectrum of activity to include armyworms, *Spodoptera* spp.; soybean loopers, *Pseudoplusia includens* (Walker); and cabbage loopers, *Trichoplusia ni* (Hübner) (Stewart et al. 2001). Bollgard II cultivars are expected to require fewer insecticide applications for bollworms than Bollgard cultivars. However, little research has been conducted to determine when yield losses result from bollworm infestations in Bollgard or Bollgard II cottons. This paper reports a field-cage experiment designed to determine the impact of bollworm level of infestation and duration of infestation on maturity and yield of Bollgard and Bollgard II cottons.

Materials and Methods

Bollgard (2002, Stoneville 4892BR and 2003, Deltapine Suregrow 215BR) and Bollgard II (2003, Deltapine Suregrow 424BGII/RR) cottons were planted into large (0.05 hectare) field cages during 2002 and 2003 in a split-plot arrangement with three replications. Plots consisted of two rows by 1-m planted skip-row (2 planted rows and 1 skip row) with a 2-m alley between plots. Plots were planted skip-row to minimize intra-plot migration of larvae. Duration of infestation (weeks) was the main-plot factor and included four weeks during the flowering period. Cotton growth stages ranged from nine to five nodes above white flower during the four week period in 2002, and nine to four nodes above white flower during the four week period in 2003. Nodes above white flower counts were determined by counting the number of main stem nodes above the upper-most first position white flower as described by Bourland et al. (1992). Level of infestation was the sub-plot factor and included three (2002) or five (2003) levels of white flower infestation. The white flower infestation levels included 0, 50, and 100 percent during 2002. During 2003, the study was expanded to include more levels of infestation. The white flower infestation levels in 2003 included 0, 10, 25, 50, and 100 percent. Crop development was monitored throughout the season to determine the initiation of flowering and the proper time for artificial infestation of larvae. The entire test area was treated with insecticides weekly until two weeks prior to infestations to minimize injury from natural infestations of insect pests and eliminate natural enemies. Two weeks before artificial infestations, the cages were covered with translucent 32 mesh nylon (Synthetic Industries, Greenville, Georgia).

A colony of bollworms was established each year from field corn, *Zea mays* L. Approximately 200 to 300 large ($\geq 4^{\text{th}}$ instar) larvae were collected from corn ears each day for five days. Larvae were transported to the laboratory and maintained for one generation to obtain sufficient numbers of larvae at the proper stage for infestations. Larvae were fed a wheat-germ based meridic diet and maintained at $27 \pm 2^{\circ}$ C, $80 \pm 5\%$ relative humidity, and a photoperiod of 14:10 light to dark. After larvae completed development, pupae were put into 3.8-L cardboard containers (ca. 50 per container). The tops of the containers were covered with batiste cloth (egg sheet) to provide a surface for oviposition. The egg sheets were harvested daily and placed into 3.8 L plastic bags. Upon eclosion, neonates were offered meridic diet in 236-ml cardboard cups (ca. 100 per cup). Larvae were allowed to feed for 24 ± 4 -h before field use to minimize mortality from handling neonates in the field.

Beginning at nodes above white flower 9, white flowers were counted in each plot and larvae were placed into the designated number of white flowers (one larva per flower) corresponding to the level of infestation for each plot. Larvae were placed into white flowers daily for the designated number of weeks. Larvae were allowed to feed freely within the plots until they completed development. At the end of the season, the percentage of open bolls was determined as a measure of crop maturity. Additionally, the plots were harvested by hand and seedcotton weights were determined. Data for percent open bolls and seedcotton yield were analyzed with analysis of variance and means were separated according to Fisher's Protected LSD (PROC MIXED, Littell et al. 1996).

Results

Bollgard Cotton 2002

Bollworm infestations delayed maturity of Bollgard cotton compared to the non-infested plots during 2002. The percentage of open bolls was lower on Bollgard cotton when 100 percent of white flowers were infested for one week ($F=11.28$; $df=2, 6$; $P=0.01$), and when 50 or 100 percent of white flowers were infested for two ($F=29.17$; $df=2, 6$; $P<0.01$), three ($F=130.06$; $df=2, 6$; $P<0.01$), or four ($F=14.01$; $df=2, 6$; $P=0.01$) weeks (Figure 1). In addition to delayed maturity, bollworm infestations resulted in yield reductions of Bollgard cotton compared to the non-infested plots during 2002. Yields of Bollgard cotton were reduced when 50 or 100 percent of white flowers were infested with bollworms for one ($F=12.25$; $df=2, 6$; $P=0.01$), two ($F=11.35$; $df=2, 6$; $P=0.01$), three ($F=20.84$; $df=2, 6$; $P<0.01$), or four ($F=10.95$; $df=2, 6$; $P=0.01$) weeks (Figure 1).

Bollgard and Bollgard II Cottons 2003

The impacts of bollworms on maturity of Bollgard cotton in 2003 were not as great as those observed in 2002. There were no differences in the percentage of open bolls when white flowers of Bollgard cotton were infested with bollworms for one ($F=1.03$; $df=4, 9$; $P=0.44$), two ($F=1.82$; $df=4, 10$; $P=0.20$), or three ($F=0.27$; $df=4, 10$; $P=0.89$) weeks (Figure 2). The percentage of open bolls was reduced when 100 percent of white flowers were infested with bollworms for four weeks ($F=3.56$; $df=4, 10$; $P=0.05$). Bollworms did not reduce seedcotton yields of Bollgard cotton at any level of infestation when the duration of infestation was one week ($F=0.75$; $df=4, 9$; $P=0.58$) (Figure 2). Seedcotton yields were reduced when 25 or 100 percent of white flowers were infested with bollworms for two weeks ($F=3.46$; $df=4, 10$; $P=0.05$). Also, yields were reduced when 25 to 100 percent of white flowers were infested with bollworms for three weeks ($F=7.45$; $df=4, 10$; $P<0.01$) or when 10 to 100 percent of white flowers were infested for four weeks ($F=6.11$; $df=4, 10$; $P=0.01$).

Bollworms did not impact maturity of Bollgard II cotton for any duration of infestation. There were no differences in the percentage of open bolls for any level of infestation when white flowers were infested for one ($F=0.28$; $df=4, 10$; $P=0.89$), two ($F=2.60$; $df=4, 10$; $P=0.10$), three ($F=1.76$; $df=4, 10$; $P=0.21$), or four ($F=2.42$; $df=4, 10$; $P=0.12$) weeks (Figure 3). In addition, there were no differences in yield of Bollgard II cotton when white flowers were infested for one ($F=0.36$; $df=4, 10$;

$P=0.83$), three ($F=0.40$; $df=4, 10$; $P=0.80$), or four ($F=1.46$; $df=4, 10$; $P=0.29$) weeks (Figure 3). Yields were significantly lower from the plots when 10 or 25 percent of the white flowers were infested for two weeks ($F=9.27$; $df=4, 10$; $P<0.01$); however, this is probably an artifact resulting from the high level of variability in these data.

Discussion

White flowers provide little control of bollworms in Bollgard cotton (Gore et al. 2001). Consequently, injury to Bollgard cotton results from neonate bollworms and one to two day old larvae feeding in white flowers and migrating to other structures when they have attained a size where they are better able to tolerate the Cry1Ac protein in Bollgard cotton (Gore et al. 2003). Injury from those larvae is mostly to bolls, and to a lesser extent, squares (Gore et al. 2003). In the current study, bollworms delayed maturity or reduced yields of Bollgard cotton when 50 or 100 percent of white flowers were infested for at least one week in 2002 indicating the need for supplemental insecticide applications to prevent economic losses from bollworms.

During 2003, bollworms had less of an impact on Bollgard cotton. For instance, maturity was not negatively affected when white flowers were infested with bollworms for one to three weeks. However, yields were significantly reduced when white flowers were infested with bollworms for two to four weeks. Yields of Bollgard cotton were not significantly reduced when 10 percent of white flowers were infested for up to three weeks. This again supports the need for applications of insecticides to Bollgard cotton under certain situations to prevent yield losses from bollworms.

In contrast to the delays in maturity and yield losses observed on Bollgard cotton, bollworms did not adversely impact maturity or yields of Bollgard II cotton in 2003. The addition of the Cry2Ab protein apparently has increased the level of bollworm control and injury from bollworms in white flowers of Bollgard II is lower than that in Bollgard. Previous studies have shown high levels of bollworm mortality on various plant parts of Bollgard II cotton compared to Bollgard cotton (Adamczyk et al. 2001a, Stewart et al. 2001). Additionally, individual bollworms that were placed into white flowers of Bollgard II cotton damaged significantly fewer squares and bolls than larvae placed into white flowers of Bollgard cotton (Gore et al. 2003).

Applications of pyrethroid insecticides for bollworms do not significantly increase yields of Bollgard II cotton compared to that for non-treated Bollgard II cotton (Jackson et al. 2003). This further supports the findings in the current study that bollworms will not readily cause significant yield reductions in Bollgard II cotton. In contrast, when greater than 10 percent of Bollgard white flowers are infested with bollworms for one to four weeks or when 10 percent of white flowers are infested for four weeks, significant yield reductions may result. These data indicate that supplemental applications of foliar insecticides may be needed in Bollgard cotton to prevent economic losses from bollworms and applications of foliar insecticides to Bollgard II cotton may not provide economic returns in terms of a yield increase.

Control measures for any insect pest should be initiated to prevent an increasing population from reaching a level that will cause economic losses (Pedigo et al. 1986). Therefore, insecticide applications for bollworms on Bollgard cotton should be initiated before 10 percent of white flowers have been infested for more than one week. Currently, insecticide applications are recommended when five to eight live larvae are found per 100 small bolls in Mississippi (Mississippi State University Extension Service 2003), Georgia (Guillebeau 2001), South Carolina (Roof 2002), and Louisiana (Bagwell et al. 2002). Based on results from this study, these action levels appear to be appropriate for Bollgard cotton to prevent economic losses from bollworms. In contrast, insecticide applications for bollworms on Bollgard II cotton likely will not provide an economic return based on these results. However, the susceptibility of bollworm populations to *B. thuringiensis* proteins varies both temporally and spatially (Luttrell et al. 1999). Therefore, insecticide applications may be needed on Bollgard II cotton in some situations, especially when the cotton is under environmental stress that could compromise expression of the *B. thuringiensis* proteins. Thus, applications of insecticides to control bollworms on Bollgard II cotton will be needed less frequently than on Bollgard cotton.

References

- Adamczyk, J. J., Jr., L. C. Adams, and D. D. Hardee. 2001a. Field efficacy and seasonal expression profiles for terminal leaves of single and double *Bacillus thuringiensis* toxin cotton genotypes. *J. Econ. Entomol.* 94: 1589-1593.
- Adamczyk, J. J., Jr., D. D. Hardee, L. C. Adams, and D. V. Summerford. 2001b. Correlating differences in larval survival and development of bollworm (Lepidoptera: Noctuidae) and fall armyworm (Lepidoptera: Noctuidae) to differential expression of Cry1A(c) δ -endotoxin in various plant parts among commercial cultivars of transgenic *Bacillus thuringiensis* cotton. *J. Econ. Entomol.* 94: 284-290.
- Bagwell, R. D., J. W. Barnett, B. R. Leonard, E. Burris, S. Kelly, C. Pinnell-Alison, T. Erwin, M. Farris, and S. Micinski. 2002. Cotton insect control 2002. Louisiana Cooperative Extension Service, Baton Rouge, LA. Publ. 1083
- Bourland, F. M., D. M. Oosterhuis, and N. P. Tugwell. 1992. Concept for monitoring the growth and development of cotton plants using main-stem node counts. *J. Prod. Agric.* 5: 532-538.

- Elzen, G. W., B. R. Leonard, J. B. Graves, E. Burris, and S. Micinski. 1992. Resistance to pyrethroids, carbamate, and organophosphate insecticides in field populations of tobacco budworm (Lepidoptera: Noctuidae) in 1990. *J. Econ. Entomol.* 85: 2064-2072.
- Gore, J., B. R. Leonard, G. E. Church, J. S. Russell, and T. S. Hall. 2000. Cotton boll abscission and yield losses associated with first-instar bollworm (Lepidoptera: Noctuidae) injury to nontransgenic and transgenic Bt cotton. *J. Econ. Entomol.* 93: 690-696.
- Gore, J., B. R. Leonard, and J. J. Adamczyk. 2001. Bollworm (Lepidoptera: Noctuidae) Survival on Bollgard® and Bollgard II® Cotton Flower Bud and Flower Components. *J. Econ. Entomol.* 94: 1445-1451.
- Gore, J., B. R. Leonard, G. E. Church, and D. R. Cook. 2002. Behavior of bollworm (Lepidoptera: Noctuidae) larvae on genetically engineered cotton. *J. Econ. Entomol.* 95: 763-769.
- Gore, J., B. R. Leonard, and R. H. Gable. 2003. Distribution of Bollworm, *Helicoverpa zea* (Boddie), Injured Reproductive Structures on Genetically Engineered *Bacillus thuringiensis* var. *kurstaki* Berliner Cotton. *J. Econ. Entomol.* 96: 699-705.
- Greenplate, J. T. 1999. Quantification of *Bacillus thuringiensis* insect control protein Cry1Ac over time in Bollgard cotton fruit and terminals. *J. Econ. Entomol.* 92: 1377-1383.
- Greenplate, J. T., S. R. Penn, Z. Shappley, M. Oppenhuizen, J. Mann, B. Reich, and J. Osborn. 2000. Bollgard II efficacy: quantification of lepidopteran activity in a 2-gene product, pp. 1041-1043. *In* P. Dugger and D. Richter [eds.], *Proc. 2000 Beltwide Cotton Conf.*, San Antonio, TX. 4-8 Jan. 2000. *Natl. Cotton Counc. Am.*, Memphis, TN.
- Guillebeau, P. 2001. 2001 Georgia pest control handbook. Georgia Cooperative Extension Service, Special Bulletin 28, Athens, GA.
- Jackson, R. E., J. R. Bradley, Jr., and J. W. Van Duyn. 2003. Field performance of transgenic cottons expressing one or two *Bacillus thuringiensis* endotoxins against bollworm, *Helicoverpa zea* (Boddie). *J. Cotton Sci.* 7: 57-64. Online at <http://journal.cotton.org>.
- Leonard, R. R., J. B. Graves, T. C. Sparks, and A. M. Pavloff. 1988. Variation in resistance of field populations of tobacco budworm and bollworm (Lepidoptera: Noctuidae) to selected insecticides. *J. Econ. Entomol.* 81: 1521-1528.
- Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS® system for mixed models. SAS Institute, Cary, NC.
- Luttrell, R. G., L. Wan, and K. Knighten. 1999. Variation in susceptibility of Noctuid (Lepidoptera) larvae attacking cotton and soybean to purified endotoxin proteins and commercial formulations of *Bacillus thuringiensis*. *J. Econ. Entomol.* 92: 21-32.
- Mississippi State University Extension Service. 2003. Cotton insect control guide 2003, Publication 343. Mississippi State University Extension Service, Starkville, MS.
- Pedigo, L. P., S. H. Hutchins, and L. G. Higley. 1986. Economic injury levels in theory and practice. *Ann. Rev. Entomol.* 31: 341-368.
- Perlak, F. J., R. W. Deaton, T. A. Armstrong, R. L. Fuchs, S. R. Sims, J. T. Greenplate, and D. A. Fischhoff. 1990. Insect resistant cotton plants. *Biotechnology* 8: 939-943.
- Perlak, F. J., M. Oppenhuizen, K. Gustafson, R. Voth, S. Sivasupramaniam, D. Heering, B. Carey, R. A. Ihrig, and J. K. Roberts. 2001. Development and commercial use of Bollgard® cotton in the USA: Early promises versus today's reality. *Plant J.* 27: 489-501.
- Plapp, F. W., Jr., J. A. Jackman, C. Campanhola, R. E. Frisbie, J. B. Graves, R. G. Luttrell, W. F. Kitten, and M. Wall. 1990. Monitoring and management of pyrethroid resistance in the tobacco budworm (Lepidoptera: Noctuidae) in Texas, Mississippi, Louisiana, Arkansas, and Oklahoma. *J. Econ. Entomol.* 83: 335-341.
- Roof, M. E. 2002. Cotton insect management. Clemson University Cooperative Extension Service, IC 97, Clemson, SC.
- Stewart, S. D., J. J. Adamczyk, Jr., K. S. Knighten, and F. M. Davis. 2001. Impact of Bt cottons expressing one or two insecticidal proteins of *Bacillus thuringiensis* Berliner on growth and survival on noctuid (Lepidoptera) larvae. *J. Econ. Entomol.* 94: 752-760.

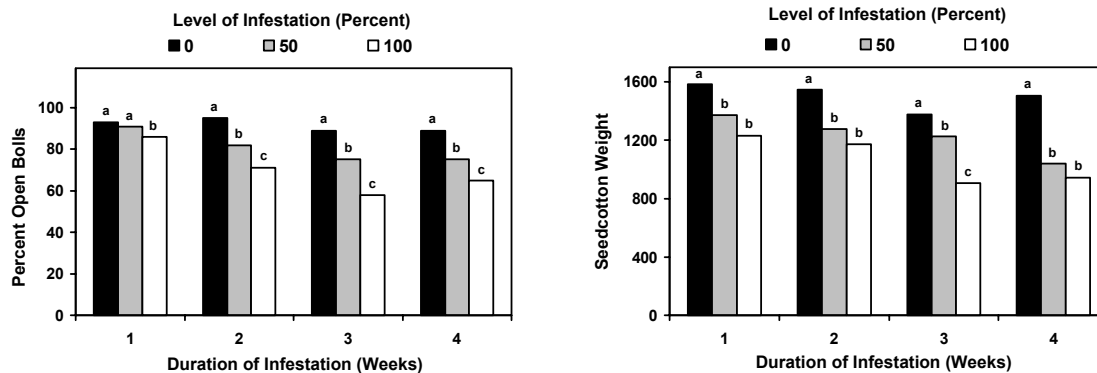


Figure 1. Impact of bollworms on maturity and yield of Bollgard cotton in 2002.

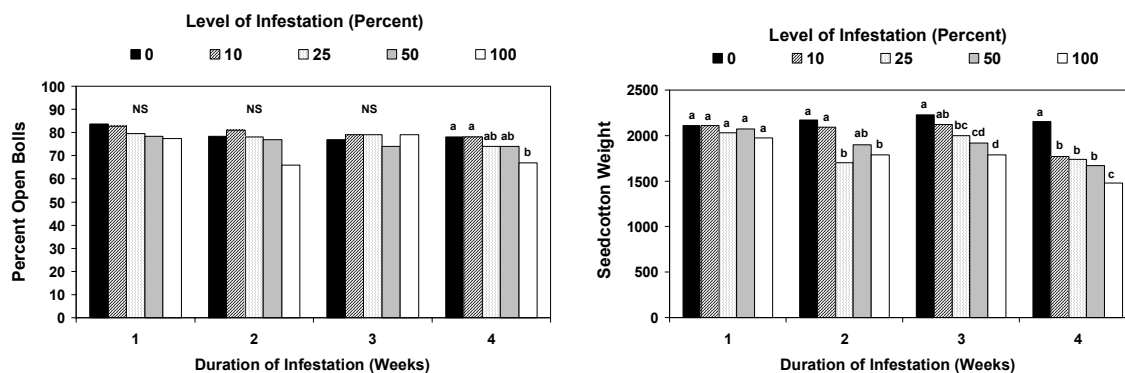


Figure 2. Impact of bollworms on maturity and yield of Bollgard cotton in 2003.

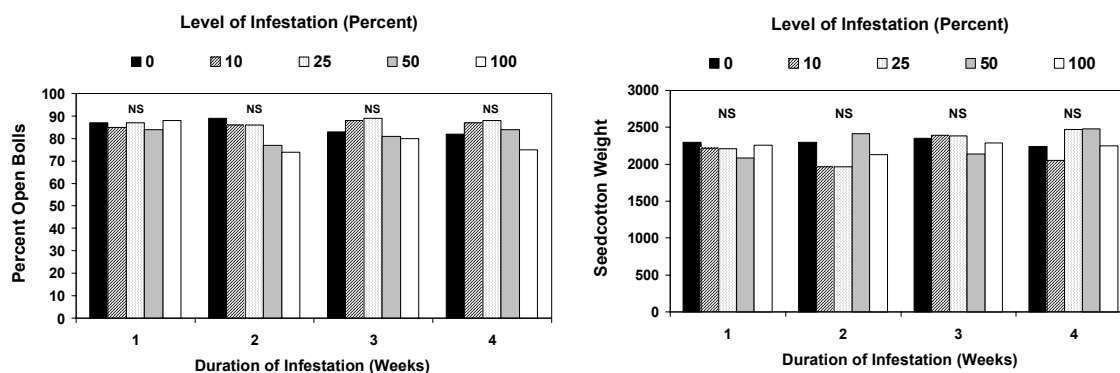


Figure 3. Impact of bollworms on maturity and yield of Bollgard II cotton.