# SURVIVAL OF SECONDARY LEPIDOPTERAN PESTS ON BOLLGARD TECHNOLOGIES

**Kelly V. Tindall**
**Univ. of Missouri**
**Portageville, MO**
**B. Roger Leonard**
**LSU AgCenter**
**Winnsboro, LA**
**K.D. Emfinger**
**LSU AgCenter, Macon Ridge Station**
**Winnsboro, LA**

## Abstract

An experiment was conducted to examine the survival and development of three secondary lepidopteran pests (fall armyworm, saltmarsh caterpillar, and yellow-striped armyworm) on Bollgard, Bollgard2 and non-Bt cottons. At least 300 larvae per species were fed both Bollgard, Bollgard2 and non-Bt cotton cottons and larvae were rated every 2-3 days until pupation or death occurred. Fall armyworm survival was 26, 8, and less than 1% on non-Bt, Bollgard, Bollgard2, respectively; yellow striped armyworm survival was 34, 26, and 5% on non-Bt, Bollgard, and Bollgard2, respectively; and saltmarsh caterpillar survival was 26, 14 and 0% on non-Bt, Bollgard, and Bollgard2, respectively. Only one fall armyworm pupated on Bollgard2; it took 36 days to pupate compared to approximately 27.5 days for Bollgard and non-Bt. The weight of the pupa was 40% larger than those fed Bollgard or non-Bt cotton. Mean time for yellow-striped armyworm to pupate was 5-6.5 days longer on Bollgard2 than Bollgard and non-Bt and pupal weights were 17-26% smaller on Bollgard2 than non-Bt and Bollgard. No saltmarsh caterpillars survived to the pupal stage on Bollgard2.

## Introduction

There are numerous pests of cotton, but research typically addresses primary pests; however, it is also important to study the secondary pests, especially when there is high adoption of planting varieties that are engineered to be toxic to Lepidopteran pests. Insect protected transgenic cotton has gene(s) from the soil bacterium *Bacillus thuringiensis kurstaki* (Bt) and targets Lepidopteran pests. The first generation of insect protected transgenic plants consisted of one product, Bollgard® (Monsanto Company), which had only one Bt gene, *cry1Ac,* that offered control of primary pests like tobacco budworm (*Heliothis virescens*), and pink bollworm (*Pectinophora gossypiella*) and suppression of bollworm (*Helicoverpa zea*). The second generation of insect protected transgenic plants offers a broader spectrum of lepidopteran pest control because more than one gene has been inserted into the plant to produce multiple proteins that have activity on many secondary lepidopteran pests and enhanced efficacy on bollworm. Bollgard2® (Monsanto Company) is a second generation Bt cotton that has *cry1Ac* and *cry2Ab* genes and it has excellent activity against tobacco budworm, bollworm, pink bollworm, cabbage looper (*Trichoplusia ni*), soybean looper (*Pseudoplusia includens*), saltmarsh caterpillar (*Estigmene acrea*), European corn borer (*Ostrinia nubilalis*), beet armyworm (*Spodoptera exigua*), fall armyworm (*Spodoptera frugiperda*), and southern armyworm (*Spodoptera eridania*) (Anonymous 2007).

Figure 1 shows the ranking of several secondary lepidopteran pests over the past 20 years. There are no apparent trends in the severity of each pest. Fall armyworm and beet armyworm have historically been the most important of the secondary pests; however in the past few years beet armyworm has dropped in status. Whereas, salt marsh caterpillars increased in importance over the last two years. Because of the non-predictable nature of outbreak potential of these pests, it is important to gather information on these pests when possible to have reference data for comparison purposes in the future to assist in detecting the development of resistance. Although it is known that Bollgard2 provides effective control against many of the secondary lepidopteran pests, many of those data have come primarily from efficacy trials. Therefore, studies were conducted to better understand the survival and development of select secondary lepidopteran pests on Bollgard2 cotton.

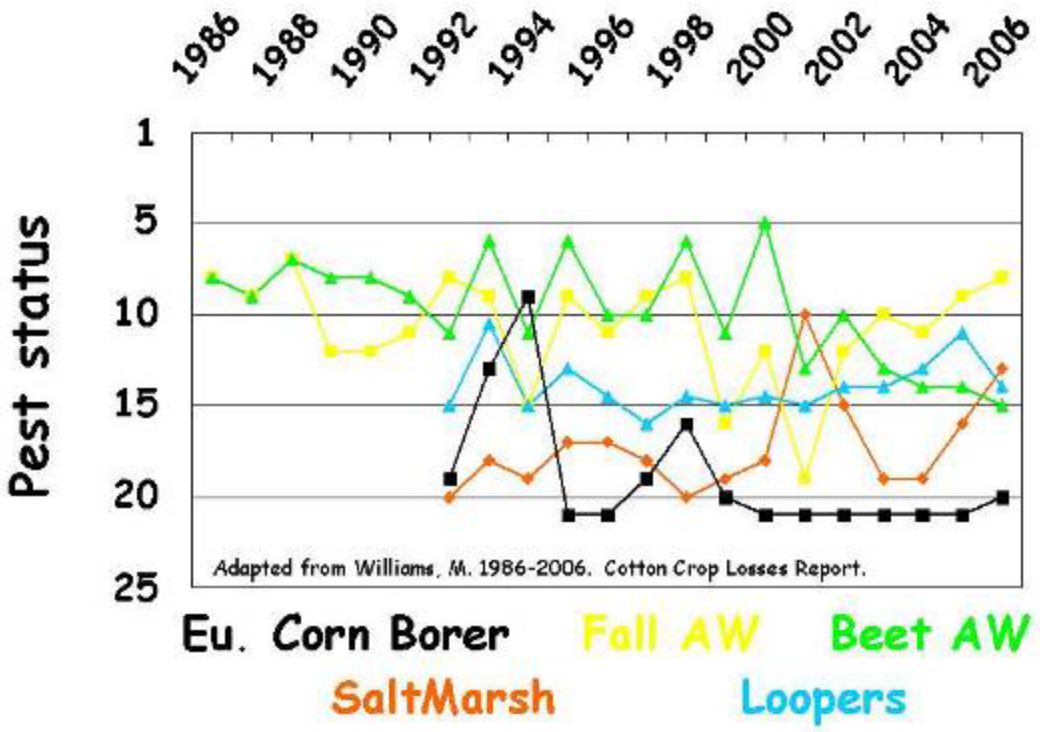


Figure 1. Ranking of importance of secondary Lepidopteran pests based on acres treated.

## Materials and Methods

Experiments were conducted in the summer of 2005 at the LSU AgCenter Macon Ridge Research Station near Winnsboro, LA in Franklin Parish and the MU Delta Research Center, near Portageville, MO in Pemiscot Co. in 2007. Feeding assays were conducted in the laboratory with fall armyworm, yellow-striped armyworm and salt marsh caterpillar. In 2005, fall armyworm were feed reproductive structures from Bollgard (STV5599BR), Bollgard2 (STV 4646B2R) and a non-Bt (PHY415RF). In 2007, yellow striped armyworm and saltmarsh caterpillar were feed foliage that from Bollgard (ST4575BR), Bollgard2 (ST4554B2RF) and non-Bt cotton plants (ST4664RF). All plant tissues were collected randomly from plants. Experiments were initiated with 10-20 neonates in a 30 ml plastic cup (FAW) or Petri dish (YSAW and SMC). Two days after infestation, larvae were rated for mortality and live larvae were transferred to individual feeding arenas with plant tissue. After being separated into individual feeding arenas, larvae were rated every 2-4 days until the larvae died or pupated. Once larvae reached late 3rd-4th instars, larvae were given new plant tissue daily to provide a continual food source. Three hundred saltmarsh caterpillar and yellow striped armyworm and 500 fall armyworm were fed tissue from each cotton variety. Mortality, date of pupation and pupal weights were recorded. Mortality data were analyzed using repeated measures with PROC MIXED in SAS (SAS 1998). Analysis is based on the percentages of larvae recovered, i.e., although experiments were initiated with 300 larvae, if only 290 were recovered, percent mortality would be based from the 290 recovered, not the 300 initial larvae. Date of pupation and pupal weights were analyzed in SAS using PROC MIXED.

## Results and Discussion

There was less than 1% survival on Bollgard2 cotton for fall armyworm; one larvae of 505 survived to the pupal stage (Figure 2). Fall armyworm survival on Bollgard was intermediate between Bollgard2 and non-Bt. The number of days necessary for fall armyworm to pupate on Bollgard2 was 36 compared to approximately 27.5 days for Bollgard and non-Bt (Table 1). There was only one larva that survived to the pupal stage, so caution should be used to draw conclusions about pupal data. However, if refuge requirements are developed so that resistant moths

are able to mate with susceptible moths, moths that are temporally separated by nearly 10 days are not likely to be able to locate one another. The single pupa reared on Bollgard2 was 40% larger than when fed Bollgard or non-Bt cotton.

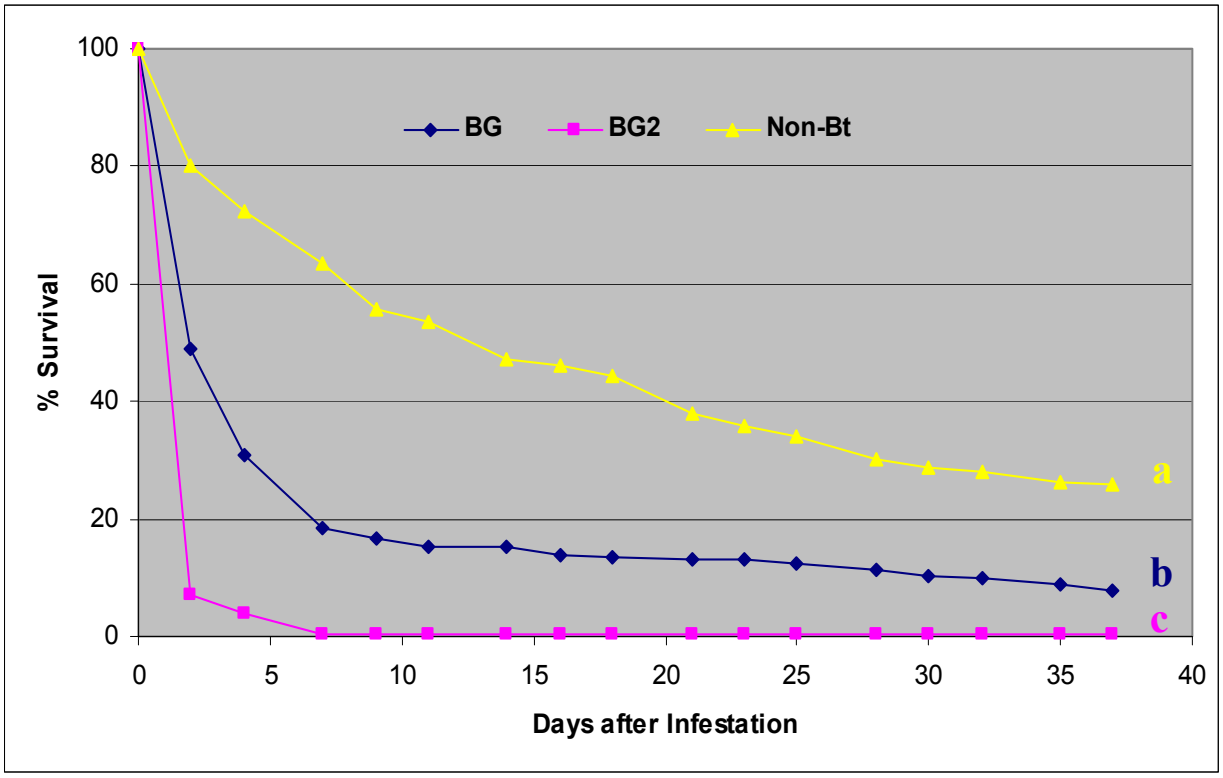


Figure 2. Survival of first instar fall armyworm fed Bollgard, Bollgard 2, or conventional cotton until pupation. Data analyzed repeated measures in PROC MIXED. trt: F-val=460.86, df=2,10, P-val<.0001; Day: F-val=37.24 , df=15,75, P-val<.0001; trt*Day: F-val=5.30, df=30,150, P-val<.0001. Lines with different letters are significantly different. Means were separated by Tukey's studentized range test a = 0.05.

Table 1. Mean number of days required for fall armyworm (FAW), yellow-striped armyworm (YSAW) and salt marsh caterpillars (SMC) larvae to either pupate or die on WideStrike cotton or non-Bt cotton and the respective pupal weights for those that survived to pupal stage.

| | FAW | | YSAW | | SMC | |
|---|---|---|---|---|---|---|
| | DTP[1] | Pupal wt. (g) | DTP | Pupal wt. (g) | DTP | Pupal wt. (g) |
| Non-Bt | 27.7 ± 0.5a[3] | 0.105 ± 0.35b | 20.4 ± 0.2b | 0.309 ± 0.030a | 27.8 ± 0.4b | 0.668 ± 0.018a |
| BG | 27.4± 0.4a | 0.112 ± 0.39 b | 18.8 ± 0.2c | 0.276 ± 0.004a | 28.7 ± 0.8a | 0.624 ± 0.018a |
| BG2 | 36 ± [3]a | 0.181 ± [3]a | 25.4 ± 0.5a | 0.228 ± 0.009b | N/A | N/A |
| | df=2,60 | df=2,58 | df=2,137 | df=2,144 | df=1,35 | df=1,36 |
| | F=1.69 | F=5.17 | F=79.97 | F=18.70 | F=5.00 | F=0.79 |
| | P =0.1925 | P=0.0086 | P<.0001 | P<.0001 | P =0.0319 | P=0.3804 |

[1] DTP= Means number of days required for larvae to pupate
[2] Means in the same column followed by different letters are significantly different. Means were separated by Tukey's studentized range test $\alpha = 0.05$.
[3] Mean is representative of only one larva surviving to pupal stage.

Approximately 5% of yellow striped armyworm fed Bollgard2 survived to pupae while survival on Bollgard and non-Bt was 26 and 34%, respectively (Figure 3). Mean time for yellow-striped armyworm to pupate was 5-6.5 days longer on Bollgard2 than Bollgard and non-Bt (Table 1). Yellow-striped armyworm reared on Bollgard2 were 17-26% smaller than non-Bt and Bollgard, suggesting that the may be less fecund than those fed Bollgard or non-Bt.

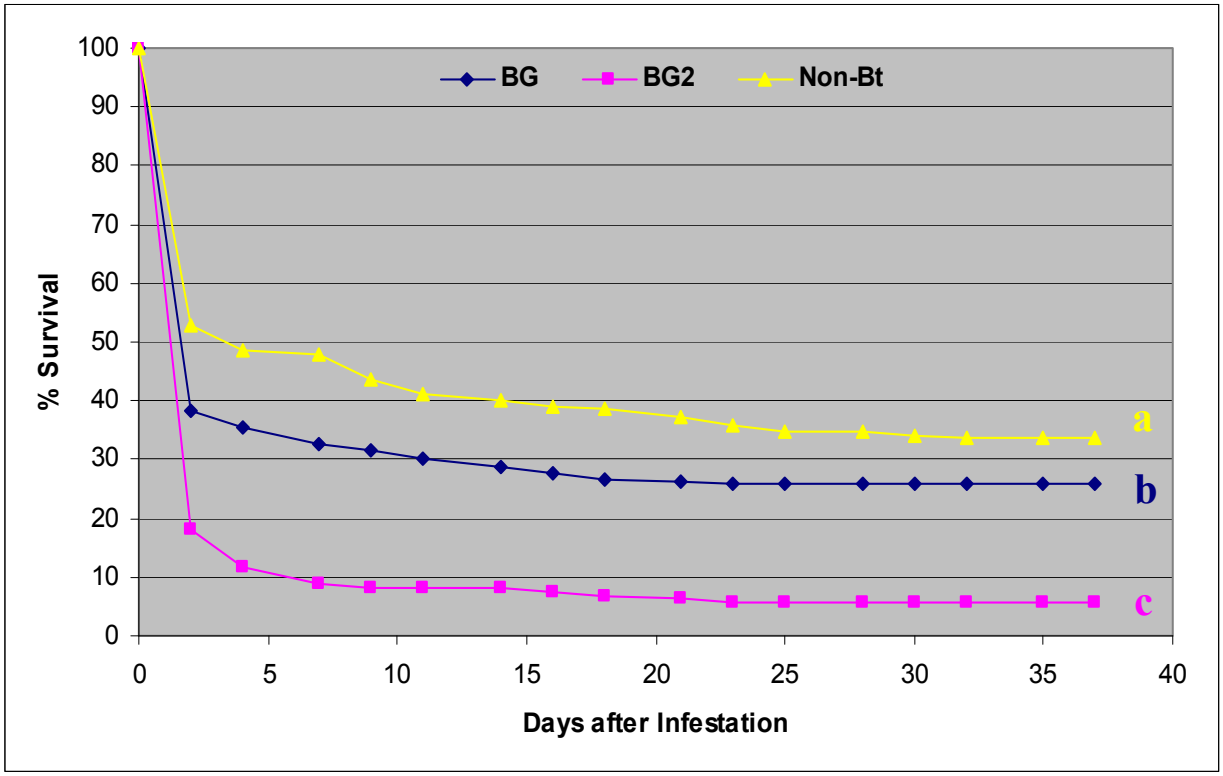


Figure 3. Survival of first instar yellow-striped armyworm fed Bollgard, Bollgard 2, or conventional cotton until pupation. Data analyzed repeated measures in PROC MIXED; trt: F-val=247.07, df=2, , P-val<.0001; Day: F-val=7.27, df=15,60, P-val<.0001; trt*Day: F-val=0.28, df=30,120, P-val=0.9999. Lines with different letters are significantly different. Means were separated by Tukey's studentized range test a = 0.05.

No saltmarsh caterpillars survived to the pupal stage on Bollgard2; furthermore, there was 100% mortality by the first rating date, two days after infestation (Figure 4, Table 1). There was one day difference in days to pupation on larvae fed Bollgard and non-Bt. The difference was significant; however, it is not likely that one day would have a biological significance. There were no significant differences in pupal weights.

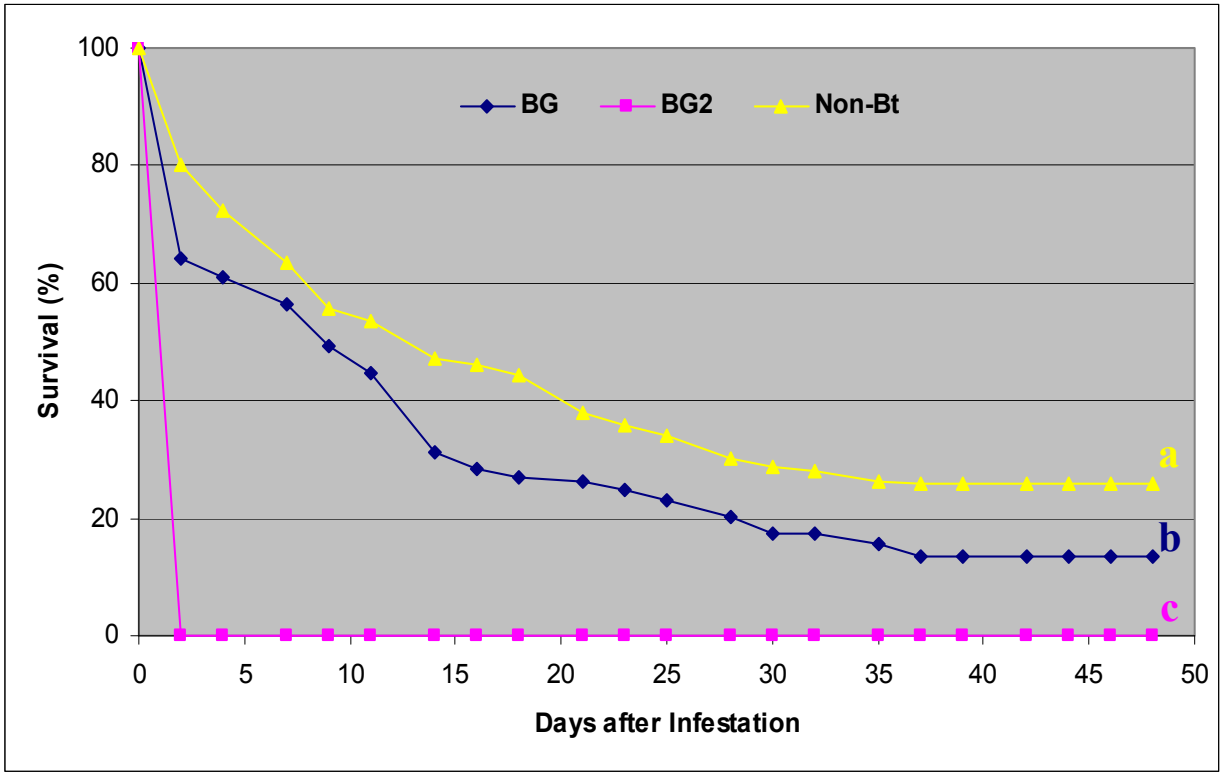


Figure 4. Survival of first instar saltmarsh caterpillar fed Bollgard, Bollgard 2, or conventional cotton until pupation. Data analyzed repeated measures in PROC MIXED. trt: F-val=218.63, df=2,6, P-val<.0001; Day: F-val=3.18, df=21,84 , P-val <.0001; trt*Day: F-val=1.10, df=42,126, P-val=.3377. Lines with different letters are significantly different. Means were separated by Tukey's studentized range test a = 0.05.

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