DEVELOPING A FRUIT INJURY BASED ACTION THRESHOLD FOR BOLLWORMS AND FIELD VALIDATION

D. Kerns
Texas A&M University
College Station, TX
S. Brown
LSU AgCenter
St. Joseph, LA
F. Yang
Louisiana State University
Baton Rouge, LA
S. Stewart
The University of Tennessee
Jackson, TN
G. Lorenz
University of Arkansas
Lonoke, AR
N. Seiter
University of Arkansas
Monticello, AR
G. Studebaker
University of Arkansas
Keiser, AR,
J. Gore
D. Cook
Mississippi State University, Stoneville, MS
A. Catchot
Mississippi State University
Starkville, MS
M. Jones
University of Missouri
Portageville, MO

Abstract

Since the introduction of Bt cotton in the United States in 1996, management of the Heliothines including the bollworm, *Helicoverpa zea*, has become much less problematic. However, there are still incidents where unacceptable fruit injury is experienced and insecticidal over sprays are utilized to prevent yield loss. There has been much speculation surrounding the reasons for control failures among Bt cotton technologies including Bt resistance or tolerance, inadequate expression of Bt toxins, and expression due to plant phenology or environmental stressors. The objective of this project was to determine economic injury levels and an economic threshold across Bt technologies based on fruit injury, and to validate that threshold the following year. Economic injury levels varied from 3.54-14.76% injured fruit depending on crop value, yield potential and control cost. On average, across varied crop values and yield potential, the economic threshold was approximately 6% injured fruit based on cotton with a yield potential of 1,200 lbs-lint/ac, a crop value of $0.70/lbs-lint with a control cost set at $24/acre. This estimate falls within the range of most current state extension recommendations. In 2016, a 6% injury economic threshold was validated in 8 field trials across 5 states. The 6% fruit injury threshold was compared to a preventative threshold and a non-treated across non-Bt, WideStrike and Bollgard 2 cotton varieties. A profitability analysis was conducted where cotton value, insecticide + application cost, and Bt technology fees were set at $0.70, $22.50 and $27.00 per acre. Using these criteria, there was no significant differences in profitability across technology (varieties), and although both the 6% threshold and the preventative threshold were statistically similar, both were more profitable than the non-treated.

Introduction

The introduction, in 1996, of transgenic cotton containing genes expressing *Bacillus thuringi*ensis (*Bt*) proteins ushered in a new era in cotton insect pest management. The first Bt cotton introduced in the U.S. was Bollgard I which expressed the Cry1Ac endo-toxin. This toxin was highly effective towards tobacco budworm, *Heliothis virescens*, but moderately toxic towards bollworm, *Helicoverpa zea*. Insecticide applications targeting tobacco budworm were completely eliminated, while those targeting bollworm were greatly reduced. To increase efficacy and for resistance management, dual and multi-Bt gene cotton varieties have since been introduced including Bollgard II, WideStrike and WideStrike 3. Although these introductions have increased the efficacy of transgenic cotton targeting lepidoteran pests, including bollworm, there are still incidents where unacceptable fruit injury is experienced and insecticidal over sprays are required to preserve yield.

The objective of this project was to evaluate the efficacy of second and third generation Bt cotton for efficacy towards bollworms and to determine if over spraying Bt cotton results in a reduction in damage and increased yields relative to non-Bt cotton. Additionally, we report an incidence where bollworms were able to survive and damage WideStrike 3 cotton, and our attempt to demonstrate susceptibility of these larvae on WideStrike 3 leaf tissue.

Materials and Methods

Tests were conducted across the Mid-South at five and seven locations in 2014 and 2015 respectively, to determine the impact of foliar insecticide applications targeting lepidopteran pests on injury and yields in second and third generation Bt cottons. The Bt cotton technologies evaluated included: TwinLink™ (TL; Cry1Ab, Cry2Ae), Bollgard II® (BG2; Cry1Ac, Cry2Ab), WideStrike® (WS; Cry1Ac, Cry1F), and WideStrike 3® (WS3; Cry1Ac, Cry1F, Vip3A). A non-Bt variety (NBT) was included as a check. All tests were 5 x 2 factorials with factor A being the cotton technology and factor B being entries sprayed for bollworms with Prevathon at 17-20 fl-oz/ac, or non-sprayed. Plots were 4 rows wide x 40-60 ft in length. Each factorial combination was replicated 4 times. Foliar applications were made in accordance with the occurrence of larvae in the non-Bt cotton plots at each individual location.

Insect densities, and square and boll injury were determined prior to foliar treatment and weekly thereafter using either a modified whole plant sampling procedure or by sampling 25-50 squares and bolls per plot. The modified whole plant sampling procedure was performed by sampling 20-25 plants per plot. For each plant, the top 4-5 nodes plus one white or pink bloom and one small to medium-sized square and boll were sampled. If larvae or fresh injury was observed, the entire plant was sampled. Total counts of larvae, total numbers of damages squares, and total numbers of damaged bolls were recorded in each plot. Fruit injury data was normalized across sampling procedures by converting each test site replicate into a percentage change relative to the non-Bt, non-sprayed plot. For purposes of this report, all fruit injury was pooled and averaged across the season and larval counts were excluded. All plots were harvested and yields were determined. All fruit injury data were analyzed using PROC GLIMMIX ($P < 0.05$) with random effects of site-yr, rep(site-yr) and variety*rep(site-yr). Where significant interactions where detected the SLICEDIFF option of the LSMEANS statement was used to separate sprayed vs non-sprayed for each variety.

To determine the relationship between fruit injury and yield, a linear regression model was used where yield was the dependent variable and percentage fruit injury was the independent variable. The ROUT method (Q = 1) was used to detect outliers. The economic injury level (EIL) equation:

$$\text{EIL (\%fruit injury)} = (\beta_0 - (\text{\% yield potential}))^{-\beta_1}$$

Where β_0 is the y-intercept regression, β_1 is the slope of the regression and % yield potential is the maximum yield potential – the gain threshold (GT). GT was calculated based on the equation:

$$\text{GT} = (C^{-(V \times Y)}) \times 100$$

Where C is the estimated control cost of an insecticide application, set at $22.50 per acre, V is the crop value, $0.60-$1.00 per lbs of lint in $0.05 increments, and Y is the crop yield potential varied at 800, 1,200 or 1,400 lbs-lint per acre.

The economic threshold (ET) was set at 70% of the EIL.

The ET determined from the 2014 and 2015 data, where cotton was valued at $0.70 per lbs-lint and using a crop yield potential of 1,200 lbs-lint per acre was determined to be approximately 6% injured fruit. In 2016, the 6% injured fruit threshold was validated. Threshold validation tests were conducted at 8 locations across 5 states in the Mid-South. Plots were 4 rows wide x 40-50 ft in length in a 3 × 3 factorial with 4 replications. Factor A consisted of cotton technologies: non-Bt, WideStrike or Bollgard 2. Factor B consisted of either a non-treated, sprayed at a threshold of 6% injured fruit (squares and bolls), or a preventative threshold sprayed at first occurrence of bollworm egg lay and/or small larvae. The insecticide application at all locations was Prevathon at 19 fl-oz per acre.
Square and boll injury were determined prior to foliar treatment and weekly thereafter by arbitrarily sampling 25 squares and bolls per plot. All plots were harvested and yields were determined. Profitability (P) was determine for each location base on the equation:

$$P\ (\$^{-acre}) = V - ((C_I \times A) + C_T))$$

Where V is the crop value (lbs-lint/acre × $0.70), C_I is the cost of the insecticide and insecticide application (set at $22.50 per acre), A is the number of applications, and C_T is the cost of the Bt technology set at $27.00 per acre for both WideStrike and Bollgard 2. Profitability was pooled across each location by variety for analysis. Data were analyzed using PROC GLIMMIX ($P < 0.05$) with random effects of site, rep(site) and variety*rep(site). Where significant ($P < 0.05$) LSMEANS were separated using Tukey's HSD ($P < 0.05$).

Results and Discussion

Across all locations, non-Bt cotton treated with Prevathon averaged a 60.79% decrease in fruit injury in yield over non-sprayed non-Bt cotton (Fig. 1). Among the Bt entries, WideStrike benefitted the most from foliar over sprays, with an average reduction in fruit injury of 22.07%, while TwinLink and WideStrike 3 saw a 7.07% and 6.98% reduction in injury, respectively. Bollgard 2 was the only technology that did not demonstrate a significant ($P > 0.05$) reduction in fruit injury when treated for bollworms.

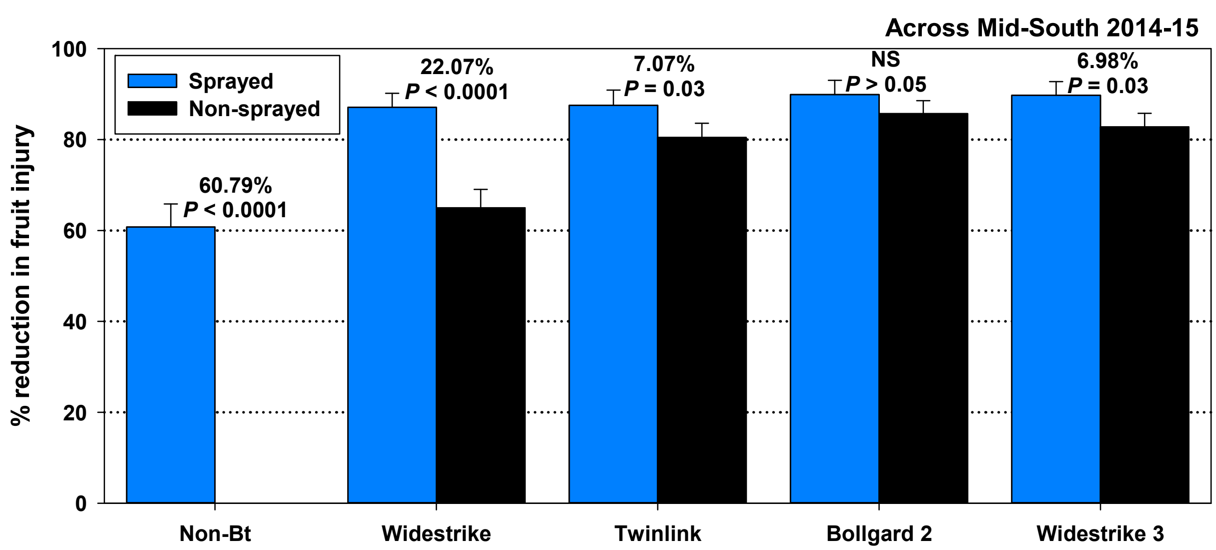

Figure 1. Percent reduction in injured fruit (square and bolls pooled) among five cotton technologies when treated with Prevathon at 19 fl-oz/acre.

Differences between sprayed and non-sprayed treatments was also observed for yield for the non-Bt and WideStrike entries (Fig. 2). Spraying non-Bt for bollworms resulted in a 21.3% increase in yield while WideStrike resulted in an 8.1% increase in yield. Although significant differences were not observed for TwinLink, Bollgard 2 or WideStrike 3, there were individual site locations where a benefit was observed within each technology. Using 5% injury as a benchmark, 66.7% of non-Bt plots saw an increase in yield, whereas WideStrike and TwinLink benefitted 33.3%

and 16.7% of the time, respectively (Fig. 3). Bollgard 2 and WideStrike 3 were least affected, both benefitting at 8.3% of the site locations.

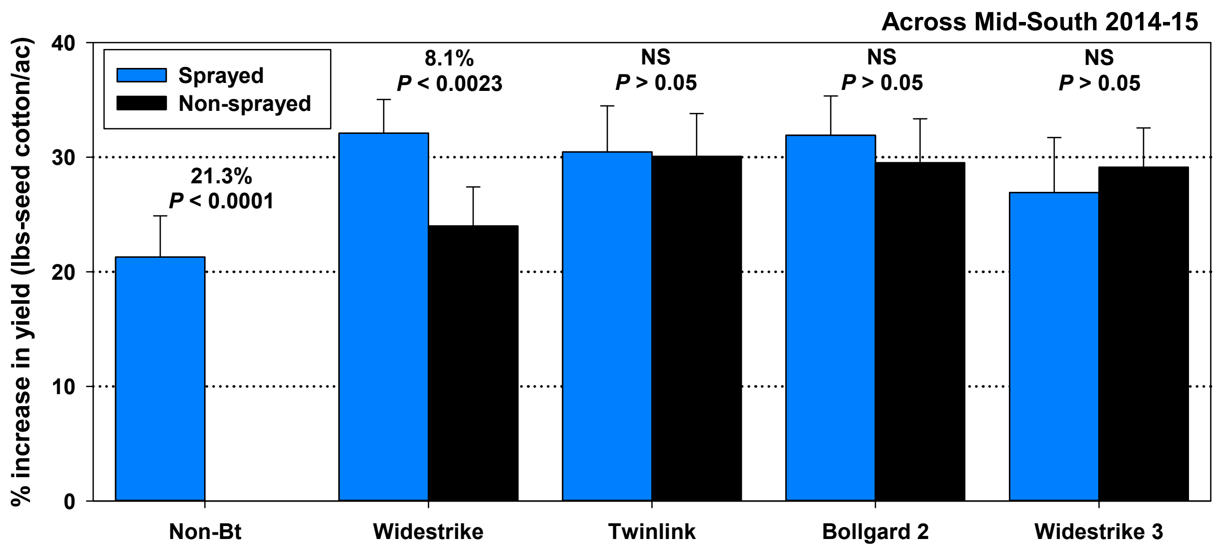

Figure 2. Percent increase in yield among five cotton technologies when treated with Prevathon at 19 fl-oz/acre.

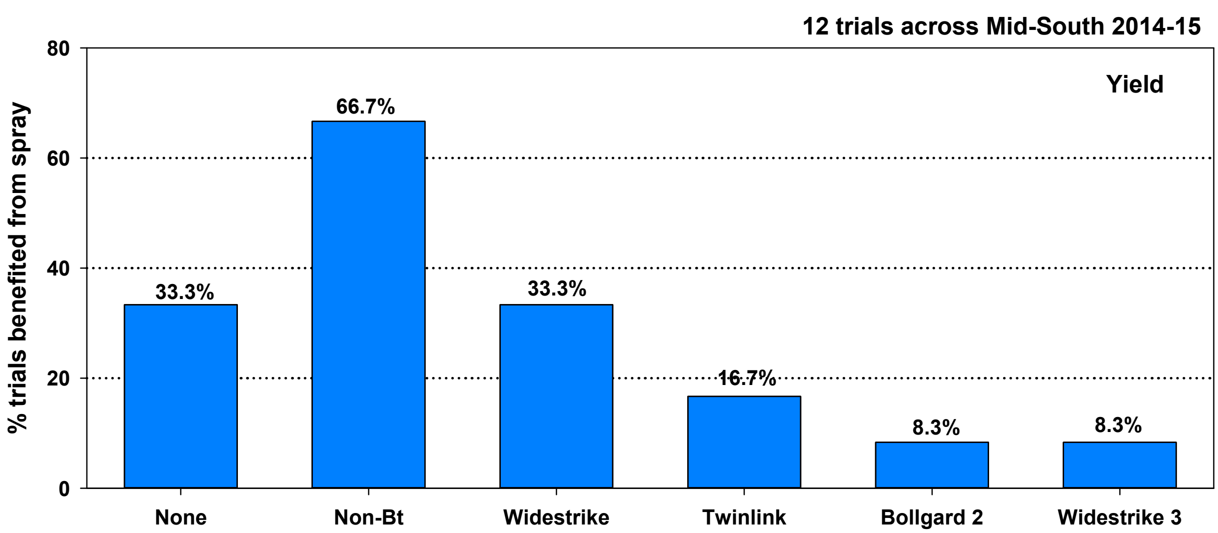

Figure 3. Percentage of trials that exhibited a ≥5% increase in yield among five cotton technologies.

Based on normalized pooled data across all site locations, cotton varieties and sprayed vs non-sprayed treatments, the linear regression of percentage injured fruit to relative yield showed a significant relationship ($P < 0.0001$) (Fig. 4). We observed that percent fruit injury was negatively correlated with yield (F = 91.69; df = 435; $P < 0.0001$; $r^2 = 0.17$). The y-intercept, 39.78 ± 1.34, represents the % change in injured fruit between the non-Bt sprayed and non-sprayed plots. The slope of the line, -0.34 ± 0.04, indicates that for every 1.0% increase in fruit injury, there was a 0.34% reduction in yield.

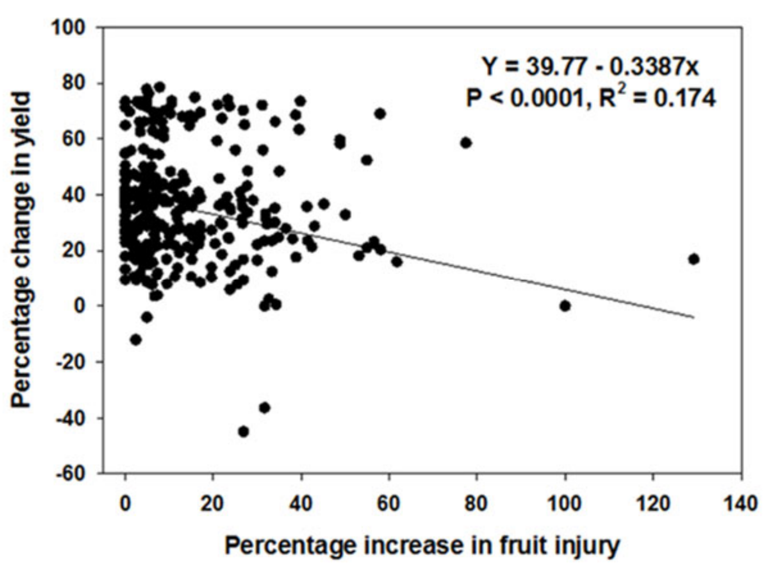

Figure 4. Regression analysis of the correlation of percentage change in yield as influenced by percentage in

The GT represents the amount of crop value, based on reasonable yield potential and market price, which can be lost to a pest before management is justified. Thus GT = Management Cost ($/acre) / Market value ($/acre). Based on the values we have estimated for cost of control and crop market value, GTs range from 1.71% to 5.00% yield loss in lbs-lint/acre, across 3 yield potentials (800, 1,200 and 1,400 lbs-lint/acre) where cost of control was set at $22.50/acre and market values ranged from $0.60 to $1.00/lbs-lint (Table 1).

Table 1. Gain threshold based on varied crop value and yield potential.

Crop value ($/lbs)	Low yield 800 lbs-lint/acre	Med yield 1200 lbs-lint/acre	High yield 1400 lbs-lint/acre
0.60	5.00	3.33	2.86
0.65	4.62	3.08	2.64
0.70	4.29	2.86	2.45
0.75	4.00	2.67	2.29
0.80	3.75	2.50	2.14
0.85	3.53	2.35	2.02
0.90	3.33	2.22	1.90
0.95	3.16	2.11	1.80
1.00	3.00	2.00	1.71

The calculated EILs ranged from 5.06% to 14.76% injured fruit, averaging 8.49% (Table 2). Although there is almost a 10% difference between the low and high EILs, this value is representative of a wide range of yield potential. ET is based on a stagnate buffer of 70% of the EIL to lessen the likelihood that the EIL is reached or exceeded. The calculated ET ranged from 3.54% to 10.33% injured fruit depending on crop value and yield potential. For cotton valued at $0.70/lbs-lint, the ET is 5.06%, 5.90% and 8.86% for cotton with yield potentials of 1,400, 1,200 and 800 lbs-lint/acre, respectively.

Table 2. Economic injury levels (EIL) and economic thresholds (ET) set at 70% EIL, based on percent fruit injured by bollworms across varied crop values and yield potentials.

Crop value	Low yield 800 lbs-lint/acre		Med yield 1200 lbs-lint/acre		High yield 1400 lbs-lint/acre	
($/lbs)	EIL	ET	EIL	ET	EIL	ET
0.60	14.76	10.33	9.84	6.89	8.44	5.90
0.65	13.63	9.54	9.08	6.36	7.79	5.45
0.70	12.65	8.86	8.44	5.90	7.23	5.06
0.75	11.81	8.27	7.87	5.51	6.75	4.72
0.80	11.07	7.75	7.38	5.17	6.33	4.43
0.85	10.42	7.29	6.95	4.86	5.95	4.17
0.90	9.84	6.89	6.56	4.59	5.62	3.94
0.95	9.32	6.53	6.22	4.35	5.33	3.73
1.00	8.86	6.20	5.90	4.13	5.06	3.54

In 2016, we chose 6% fruit injury (cotton with a 1,200 lbs-lint/acre yield potential valued at $0.70/lbs-lint) as a ET for field validation of our threshold. Regardless of the threshold utilized, 6% fruit injury or preventative, the non-Bt required the same number of insecticide applications for bollworm control (Table 3). WideStrike cotton exceeded the 6% threshold at 6 out of 7 locations and resulted in 25% fewer insecticide applications relative to the preventative threshold. Fruit injury in the Bollgard 2 plots exceeded the 6% threshold only twice, and resulted in 75% fewer insecticide applications than the preventative threshold.

Table 3. Number of applications of Prevathon at 19 fl-oz/acre applied at either a 6% injured fruit or a preventative threshold among seven locations.

	Non-Bt		WideStrike		Bollgard 2	
Site location	6%	Prevent	6%	Prevent	6%	Prevent
Starkville, MS	1	1	0	1	0	1
Pine Bluff, AR	1	1	1	1	0	1
Rowher, AR	2	2	1	2	1	2
Winnsboro, LA	1	1	1	1	0	1
Stoneville, MS	1	1	1	1	1	1
St. Joseph, LA	1	1	1	1	0	1
Jackson, TN	2	2	1	1	0	1
Mean[1]	1.29	1.29	0.86	1.14	0.29	1.14
% reduction in applications[2]	0		25		75	

[1]Mean number of applications within a threshold across site locations.
[2]Percent reduction in number of insecticide applications where the 6% fruit injury threshold was used relative to a preventative threshold.

Based on a profitability analysis using actual yields, insecticide cost + application fees of $22.50/acre, and Bt technology fees set at $27.00/acre, there were no statistical differences in profitability ($/acre) among the three cotton technologies, non-Bt (DP 1441 RF), WideStrike (PHY 499 WRF) and Bollgard 2 (ST 4946 BG2RF), (P = 0.0926). Note: herbicide technology cost was not included in the calculation. Likewise, there was no interaction between cotton technology and spray threshold (P = 0.8705). However, the threshold utilized was significant (P = 0.0017). The profitability of the 6% fruit injury threshold and the preventative threshold does not differ and were greater than then non-treated (Fig. 5).

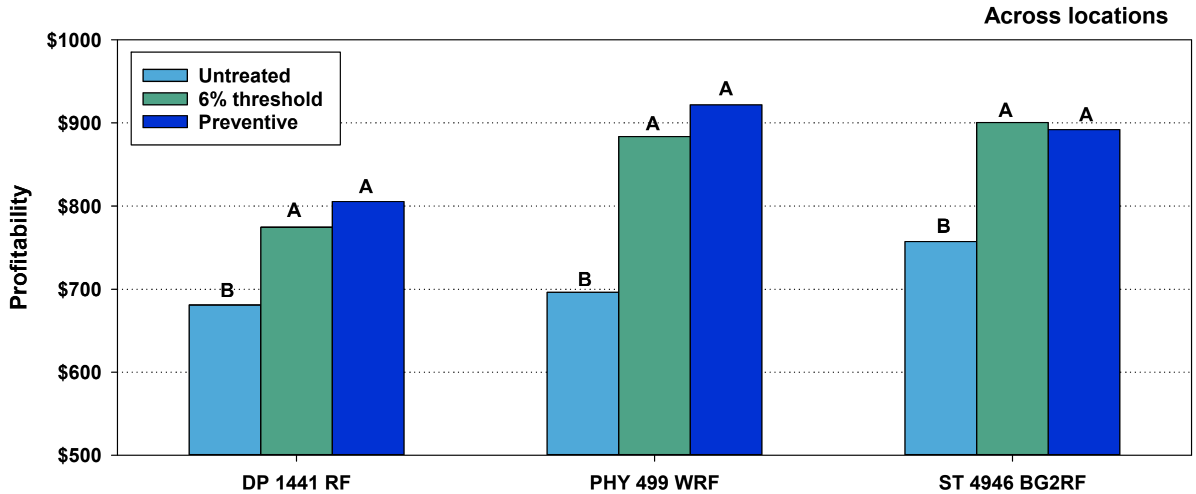

Figure 5. Profitability ($/acre) of non-Bt (DP 1441 RF), WideStrike (PHY 499 WRF) and Bollgard 2 (ST 4946 BG2RF) technologies either non-treated, sprayed at a threshold of 6% injured fruit or sprayed preventatively, across 7 locations in the Mid-South.

These results suggest that using percent fruit injury due to bollworm feeding is a viable threshold. This threshold can vary depending on crop value, crop yield potential and control cost. Field validation demonstrated that using the threshold resulted in fewer insecticide applications and profitability equivalent to preventative sprays.