

# COMPARISON OF COTTON PLANT RESPONSE TO SQUARE LOSS FOLLOWING MANUAL REMOVAL OR TARNISHED PLANT BUG FEEDING – RESULTS FROM FIELD TRIALS IN 2001

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## Abstract

Effects of early square (floral bud) removal on cotton (*Gossypium hirsutum* L.) development were evaluated in normal and extremely late plantings in southern Arkansas in 2000 (Teague et al 2001). The experiment was repeated in a normal date of planting in Northeast Arkansas in 2001. Squares were removed either by feeding by tarnished plant bug (*Lygus lineolaris* Palisot de Beauvois) or manually by crushing. Injury treatments were initiated when first squares were visible, 41 days after planting (DAP), and continued at 48 and 55 DAP. All visible squares were crushed on each treatment date. For plant bug treatments, 3 nymphs, 2nd to 3rd instar, were released per plant. Plant response was monitored using COTMAN™ in-season with final plant mapping done using COTMAP. Square shed of 1st position squares at 59 DAP ranged from 8% in uninjured (protected) cotton to 39 and 45% following Bug or Crush treatments, respectively. No. of days to physiological cutout (nodes above white flower (NAWF) = 5) was delayed by 8 days for plants with insect induced injury compared to plants protected by insecticide. Differences in final plant structure and crop compensation following plant bug injury compared to manual square removal were observed. Mean maturity date for plants injured by plant bugs was 6 days later than either manually injured plants or protected plants. Plots were picked 5 times, and results indicated significant differences between treatments in the first 4 harvests. By the final harvest on 19 Oct, there were no statistical differences in final cumulative yield. Data from the 2001 study were similar to observations made in 2000 and indicated that crop response to injury from tarnished plant bug feeding was measurably different from response to manually induced injury. Results provide evidence that researchers should use insects in their research protocol rather than manually induced injury, if their goal is to accurately study plant response to feeding by tarnished plant bug.

## Introduction

Research efforts in Arkansas have been focused on development of decision guides for managing square retention prior to first flowers, concentrating on how square and boll retention affects crop carrying capacity and yield potential. These guides eventually will be incorporated into the COTMAN™ system (Danforth and O'Leary 1998). An accurate evaluation of crop response to insect induced injury is critical to this work.

Researchers have studied crop response to loss of fruiting structures by manually removing squares and bolls to simulate insect injury (Pitman et al. 2000, Herbert et al. 1999, Mann et al. 1997, Phelps et al. 1997, Ihrig et al. 1996, Montez and Goodell, 1994, Brook et al. 1992abc, Lentz 1990, Ungar et al. 1987, and others). Plant response to pest feeding injury may differ between insects species because of differences in time and duration of the injury and the feeding habit of the insect, including injurious effects of digestive enzymes (Sadras 1995). Squares injured by caterpillar feeding generally will shed. Feeding by tarnished plant bug, a key pest in Midsouth cotton, will result in small square shed. Larger squares typically are more tolerant. When anthers are hardly visible, the bug feeds on the totality of the floral bud, and they shed. As the square grows, the anthers reach a large enough size for the bug to feed on individual pollen sacks. When feeding is localized on the anthers, squares rarely shed. Squares with extensive anther damage may shed as bolls (Pack and Tugwell 1976).

It is unknown if results from crop compensation studies that have used manual square removal methods adequately simulate plant bug injury. Results from our study conducted in 2000 indicate that crop response to manual injury is different to that caused by bugs. This study was repeated in 2001 with the objectives: 1) to compare crop response to square loss caused by plant bugs and by manual removal and 2) to assess plant responses with standardized procedures that synthesize information involving boll loading, metabolic stress, and crop carrying capacity.

## **Materials and Methods**

The 2001 experiment was conducted at Wildy Farms, a commercial farm in Northeast Arkansas near Manila. The growing season is May through October, and the latest possible cutout date (that date with a 50% or 85% probability of attaining 850 DD60s from cutout) for this production area is 9 Aug or 31 July, respectively (Zhang et al. 1994 and Danforth and O'Leary 1998).

The cultivar, Stoneville 4892 was seeded on 8 May 2001. The soil was a Roton-Dundee-Crevasse Complex (sand). Furrow irrigation was initiated beginning 15 May, and continued at weekly intervals until 28 Aug. Rainfall in May, June, July, Aug, Sept and Oct was 5.27, 1.33, 2.04, 1.30, 2.67 and 5.82 inches, respectively. Defoliant was applied on 1 Oct (15 oz Folex and 8 oz Superboll).

There were 3 injury treatments: 1) release of tarnished plant bug nymphs (Bug), 2) manual crushing of squares (Crush) and 3) no injury and sprayed with insecticide (Protected). The experiment was arranged in a randomized complete block design, and each treatment was replicated 4 times. Plots were 12 rows wide, 25 ft long. Three sections of row, each 10 ft long, were selected in each plot for injury treatments. Tarnished plant bugs were obtained from a colony maintained on artificial diet at the USDA-ARS Biological Control and Mass Rearing Research Unit at Mississippi State, MS (Cohen et al 2000). Three tarnished plant bug nymphs (2nd and 3rd instar) were aspirated from rearing containers into a 1.5 inch long section of tubing. Tubes were placed at the base of each plant's main stem (Teague et al. 2001). Bugs were released on 18, 25 June and 2 July (41, 48 and 55 days after planting). For Crush treatments, all visible squares on plants were crushed using forceps. Care was taken to minimize touching the plants. Insecticide (Provado 0.047 lb(AI)/ac) was sprayed in the protected plots 11, 19, 26 June and 2 July. Insecticide applications were made across all plots on 18 July (Orthene (1/3 lb/ac)) and 1 and 11 Aug (Centric (3oz/acre)).

Plants were monitored in each plot from the early squaring period through cutout using the COTMAN system. Five consecutive plants in 2 treatment rows were monitored weekly. Prior to first flowers sampling included measurement of plant height, number of squaring nodes, and sheds of first position squares. Square shed data were divided into 3 categories of square size: total, large and small. Total squares were all first position squares. Small squares were 1<sup>st</sup> position squares located in the top 3 sympodial nodes, and large squares were 1<sup>st</sup> position squares located in sympodial node 4 and below. After first flowers, nodes above white flower were monitored. In all plant monitoring activities, samplers touched the plants as little as possible to minimize possible thigmonastic effects.

Final plant mapping was performed on 25 Oct using COTMAP (Bourland and Watson 1990). Ten plants in one row per plot were examined for node number of first (lowest) sympodial branch on the main axis, no. of monopodia, and no. of bolls on sympodia arising from monopodia. Bolls located on main stem sympodia (1<sup>st</sup> and 2<sup>nd</sup> position) were recorded as well as bolls located on the outer positions on sympodial nodes (>2<sup>nd</sup> position). The highest sympodium with 2 nodal positions and no. of bolls on sympodia located on secondary axillary positions were also noted. Plant height was measured as distance from soil to apex.

Plots were hand harvested from 2 rows per plot over 5 dates, 18, 27 Sept and 2, 10 and 19 Oct.

## **Results**

Bug and Crush treatments were initiated at 41 days after planting (DAP) with 1st plant monitoring data recorded 4 days later. Square shed differences were noted between Crush and Bug injured treatments and Protected treatments on every sample date (Table 1). Four days following the final injury treatment (59 DAP), total square shed was at 38.6 and 45.4 % for Bug and Crush treatments, respectively compared to 8.5 % for Protected. By 66 DAP, plants were flowering, and total square shed in Protected was 3.1% compared to 34.3 and 30.4% shed in Bug and Crush. Percent shed of 1<sup>st</sup> position squares was similar for Crush and Bug for most of the sample dates; however, small square shed on 48 DAP was significantly higher where plant bugs were allowed to feed compared to either Crush or Protected treatments. Differences in small square shed between Crush and Bug treatments were noted in 2000 as well. Small squares are difficult to crush without severely damaging other meristematic tissues in the terminal. Plant bugs will feed on tiny squares in the terminal (Tugwell et al 1976).

There were no differences in mean no. of squaring nodes per plant until after 1<sup>st</sup> flowers (Table 2). Similar observations were made in 2000. Squaring node data are plotted as nodes above 1<sup>st</sup> square and nodes above white flower in COTMAN growth curves (Figs. 1, 2). Boll loading results in metabolic stress reducing terminal growth and therefore a rapid decline in NAWF. Differences in rate of NAWF decline in the Bug compared to Protected and Crush injury treatments indicate reduced boll loading stress associated with lower fruit retention. Days to physiological cutout (no. of days from planting until mean NAWF = 5) were higher, 95 days compared to 89 days for Crush and 87 days for Protected (Table 3).

Significant differences in plant structure were observed between injury treatments in final plant mapping (Table 4). Percent early boll retention, defined as 1<sup>st</sup> plus 2<sup>nd</sup> position bolls on the 5 lowest sympodia, was significantly higher in the Protected treatment compared to Crush and Bug injury treatments. There were significantly higher numbers of sympodia with 1<sup>st</sup> position bolls in Protected and Bug compared to Crush treatments; however, % boll retention of 2<sup>nd</sup> position bolls and mean no. of sympodia with 2<sup>nd</sup> position bolls was significantly lower in Bug compared to Protected and Crush.

Significantly lower yields in the 1<sup>st</sup> through 4<sup>th</sup> harvests were observed between treatments (Table 4). By the 5th harvest, there were no statistically significant differences in final yield, although there were 300 lbs less lint produced in the injured compared to sprayed plots. Mean maturity date (Bourland et al 2000) indicated a 6 day delay for Bug compared to Crush or Protected ( $P > F$  0.01).

Heat unit accumulation from flowering date of the last effective boll population (NAWF=5) until application of defoliant on 1 Oct was 862, 820, and 692 DD60s for Protected, Crush and Bug treatments, respectively. Heat unit accumulation from physiological cutout to each harvest date for each treatment are shown in Fig 3. By final harvest, Protected, Crush and Bug treatments had accrued a mean of 923, 880 and 752 DD60s, respectively.

### Discussion

A major concern with pest effects of tarnished plant bug in cotton has been with crop delay in addition to yield loss. In TPB infestation studies in squaring cotton in Marianna, AR, Holman (1996) showed that TPB nymphs reduced cotton yield at increasing rates when 1<sup>st</sup> position square shed exceeded 26%. Lint yields of treatments that sustained 1 to 7% shed rates were not significantly different from those which sustained 19% square shed. Yields were numerically higher for treatments at the 19% square shed rate; however there was one day of delay associated with each 4 % of first position square shed. Interestingly, the relation of 1 day delay for each 4% shed was similar to that observed in our 2001 and 2000 trials. Total square shed rates in plant bug injury treatments in 2001 were maintained between 34 and 38% before 1<sup>st</sup> flowers, and physiological cutout was delayed by 8 days. In our 2000 study conducted at Mariana, 1<sup>st</sup> position square shed associated with plant bug feeding in the normal (16 May) date of planting was maintained between 41 and 40%; physiological cutout was delayed 12 days in the Bug treatment compared to the Protected treatment.

In their review of crop development for cotton pest management, Hearn and Room (1979) listed 2 types of time-dependent compensatory responses to loss of fruiting structures: 1) *time dependent tolerance* - when fruiting structures that would have shed physiologically replace those previously damaged or 2) *time-dependent compensation* – when loss of fruiting structures delays metabolic stress from boll loading stress therefore lengthening the time of squaring and allowing some of the additional squares to set bolls. We observed both types of compensatory response in 2000 and 2001; however, in 2001 with a cool fall and at a more northern location, compensation was not equivalent to that observed a year earlier. In the 2000 experiments, with a similar date of planting and cutout date, Protected treatments were able to accrue 1250 DD60s from physiological cutout until application of defoliant. In the 2001 study, DD60 accumulation from physiological cutout to date of defoliant application was 894 (Table 3). Total DD60s accrued from latest possible cutout date to 1 Nov was 819. Production areas in the northern extremes of the US Cotton Belt typically have less time for compensation; thus growers in these regions must be concerned with any management practice or pest that results in crop delay.

There were not great differences in square abscission rates between Crush and Bug treatments; however, NAWF measures, final plant mapping results, yield, and mean maturity dates indicate that the crop did respond differently to square loss induced manually compared to square loss associated with TPB feeding. Why these differences occurred is unknown. Plants may have responded to the handling associated with manual crushing; plant handling was minimal in the Bug treatment. Plant bugs can damage small squares; tiny squares were not accessible with manual methods. All injury came on the day of square crushing; injury from plant bug feeding would have been spread over the period from release to time of plant monitoring. Injury from insect feeding perhaps was more extensive than the manual removal; possible systemic effects of TPB digestive enzymes on plant response were not determined.

In this study, crop response to injury from tarnished plant bug feeding was measurably different from plants in treatments with manually induced injury. Results provide evidence that researchers should use insects in their research protocol rather than manually induced injury, if their goal is to accurately study plant response to feeding by tarnished plant bug.

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Table 1. Total, large and small square shed (% of first position floral buds) as influenced by injury treatments.<sup>1</sup>

Date of Planting	Square Size <sup>2</sup>	Time of injury (DAP) <sup>3</sup>	Sample time (DAP)	Mean no. shed squares (%)				LSD <sub>05</sub>
				Bug <sup>4</sup>	Crush	Protected <sup>5</sup>	P > F	
8 May	Total	41	45	21.2	14.9	2.4	0.24	
		48	52	37.3	30.7	4.8	0.03	9.9
		55	59	38.6	45.4	8.5	0.15	
		66	66	34.3	30.4	3.1	0.04	2.4
	Large	41	45	65.4	41.6	5.9	0.004	25.3
		48	52	58.2	62.0	7.3	0.008	19.3
		55	59	55.8	68.2	11.9	0.002	21.5
		66	66	48.3	45.2	5.0	0.002	25.2
	Small	41	45	5.1	6.9	0.8	<0.001	3.4
		48	52	15.8	3.3	2.5	0.005	10.1
		55	59	11.7	10.8	3.3	0.005	11.5
		66	66	7.5	0.0	0.0	0.008	17.2

<sup>1</sup>Square shed percentages were determined from 10 plants per plot using standard COTMAN procedures.

<sup>2</sup>Small squares were 1<sup>st</sup> position squares in the top 3 sympodia; large squares were all squares from the 4<sup>th</sup> sympodia down the plant; total were all 1<sup>st</sup> position squares.

<sup>3</sup>Days after planting (DAP).

<sup>4</sup>Three nymphs, 2<sup>nd</sup> to 3<sup>rd</sup> instar, were released per plant.

<sup>5</sup>Provado (0.047 lb ai/ac) applied on 11, 19, 26 June and 2 July.

Table 2. Squaring node number as influenced by square injury treatment.<sup>1</sup>

Sample Date (DAP) <sup>2</sup>	Mean no. squaring nodes			P > F	LSD <sub>05</sub>
	Bug	Crush	Protected		
18 June (41)	3.0	2.8	2.8	0.80	
22 June (45)	4.0	3.8	4.0	0.61	
29 June (52)	5.5	5.8	6.0	0.64	
6 July (59)	7.7	7.4	7.7	0.72	
27 July (80)	6.9	7.1	7.5	0.27	
6 August (90)	6.1	5.1	4.6	0.05	1.17
10 August (94)	5.7	4.4	4.0	0.09	

<sup>1</sup>Data are means of 3 replications. Squaring nodes were counted on 10 plants per plot using standard COTMAN procedures.

<sup>2</sup>Days after planting (DAP).

Table 3. Effect of injury treatments on no. of days to physiological cutout, and mean no. of heat units (DD60s) accumulated from date of physiological cutout until application of defoliant.

<b>Injury treatment</b>	<b>Mean date of physiological cutout<sup>1</sup></b>	<b>Mean no. days to cutout</b>	<b>DD60s from cutout to defoliation<sup>2</sup></b>
Bug	11 Aug	95.7	723
Crush	05 Aug	89.0	816
Protected	03 Aug	87.3	894
	<i>P &gt; F</i>	<i>0.16</i>	<i>0.17</i>

<sup>1</sup>Date at which mean NAWF = 5.

<sup>2</sup>Defoliation occurred 1 October.

Table 4. Plant response to injury treatments - results from final plant mapping following defoliation<sup>1</sup>.

<b>Category</b>	<b>Bug</b>	<b>Crush</b>	<b>Protected</b>	<b>LSD<sub>05</sub></b>
1 <sup>st</sup> Sympodial Node	7.1	6.7	<b>6.8</b>	
No. of Monopodia	2.1	1.5	1.9	
Highest Sympodia with 2 nodes	15.0	12.9	12.5	
Plant Height (inches)	52.9	44.6	44.3	
No. of Effective Sympodia	14.2	12.4	12.5	
No. of Sympodia	18.6	16.8	16.9	
No. of Sympodia with 1 <sup>st</sup> Position Bolls	5.2	3.9	5.6	<i>0.6</i>
No. of Sympodia with 2 <sup>nd</sup> Position Bolls	1.0	2.3	2.2	<i>1.1</i>
No. of Sympodia with 1 <sup>st</sup> & 2 <sup>nd</sup> Bolls	2.5	1.8	2.7	
Total Bolls/Plant	17.4	18.3	19.0	
% Total Bolls in 1st Position	45.2	32.2	43.6	<i>10.5</i>
% Total Bolls in 2nd Position	20.6	22.4	25.4	
% Total Bolls in Outer Position	13.2	20.4	14.3	
% Total Bolls on Monopodia	19.4	24.8	16.2	
% Boll Retention - 1st Position	41.5	34.3	49.0	
% Boll Retention - 2nd Position	23.1	32.6	38.9	<i>6.5</i>
% Total Bolls on Extra - Axillary	1.6	0.2	0.5	<i>1.0</i>
% Early Boll Retention	19.4	20.0	59.7	<i>10.6</i>
Total Nodes/Plant	24.7	22.5	22.7	
Internode Length (inches)	2.2	2.0	2.0	

<sup>1</sup> means of 10 plants per plot.

Table 5. Cumulative mean lint yield over 5 harvest dates for each injury treatment.

<b>Injury treatment</b>	<b>Cumulative lint yield (lb/ac) at each date of hand harvest<sup>1</sup></b>				
	<b>18 Sept</b>	<b>27 Sept</b>	<b>2 Oct</b>	<b>10 Oct</b>	<b>19 Oct</b>
Bug	164.5 c	347.3 c	541.3 c	761.5 c	1112.2
Crush	397.2 b	637.8 b	827.3 b	988.5 b	1183.6
Protected	623.0 a	768.3 a	970.3 a	1257.4 a	1426.5
	<i>P &gt; F</i>	<i>0.001</i>	<i>0.0002</i>	<i>&lt;0.0001</i>	<i>0.0002</i>
	<i>LSD<sub>05</sub></i>	<i>154.4</i>	<i>108.0</i>	<i>56.7</i>	<i>193.0</i>

<sup>1</sup>Calculations of lint yield were based on 33% turnout from seedcotton weights.

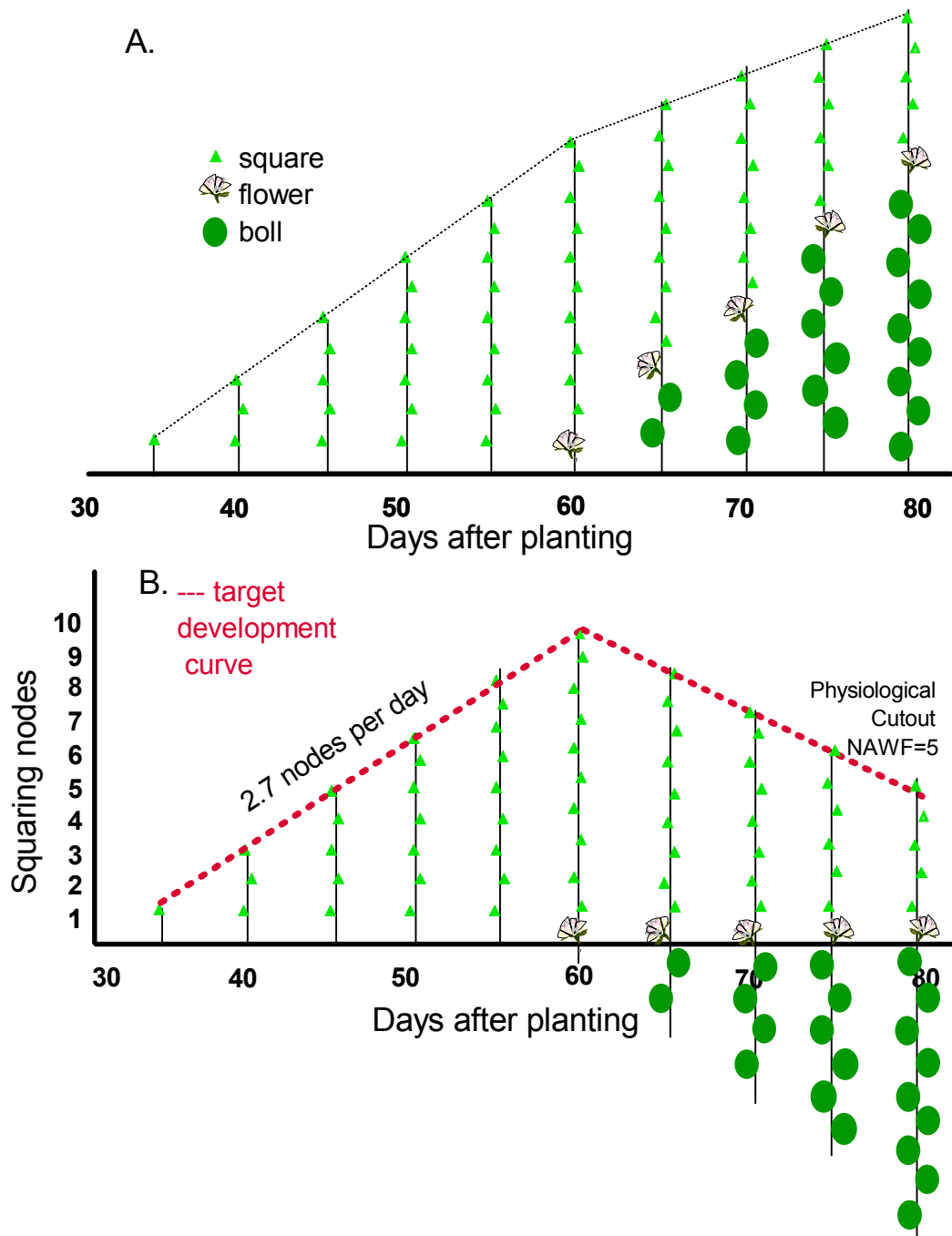


Figure 1. Squaring nodes are fruiting branches that have not yet flowered. Once cotton begins to square, plants will produce 1 squaring node every 2.7 days until 1st flowers when rate of squaring node production slows and finally stops as the crop reaches carrying capacity. After 1st flowers, boll loading results in metabolic stress which will affect rate of squaring node production. A rapid decline in squaring nodes is expected after 1st flowers in response to boll loading. The dominant physiological sink prior to first flowers is the terminal. With boll loading comes a shift of the dominant sink from the terminal to the developing fruit (bolls). In response the terminal growth rate declines; this is easily measured by counting squaring nodes (=NAWF). As the crop approaches carrying capacity, it will reach physiological cutout, the date of flowering of the last effective boll population. Eventually, production of squaring nodes will completely stop. When squaring nodes are plotted against days after planting, the resulting reference curve is the COTMAN Target Development Curve (B).

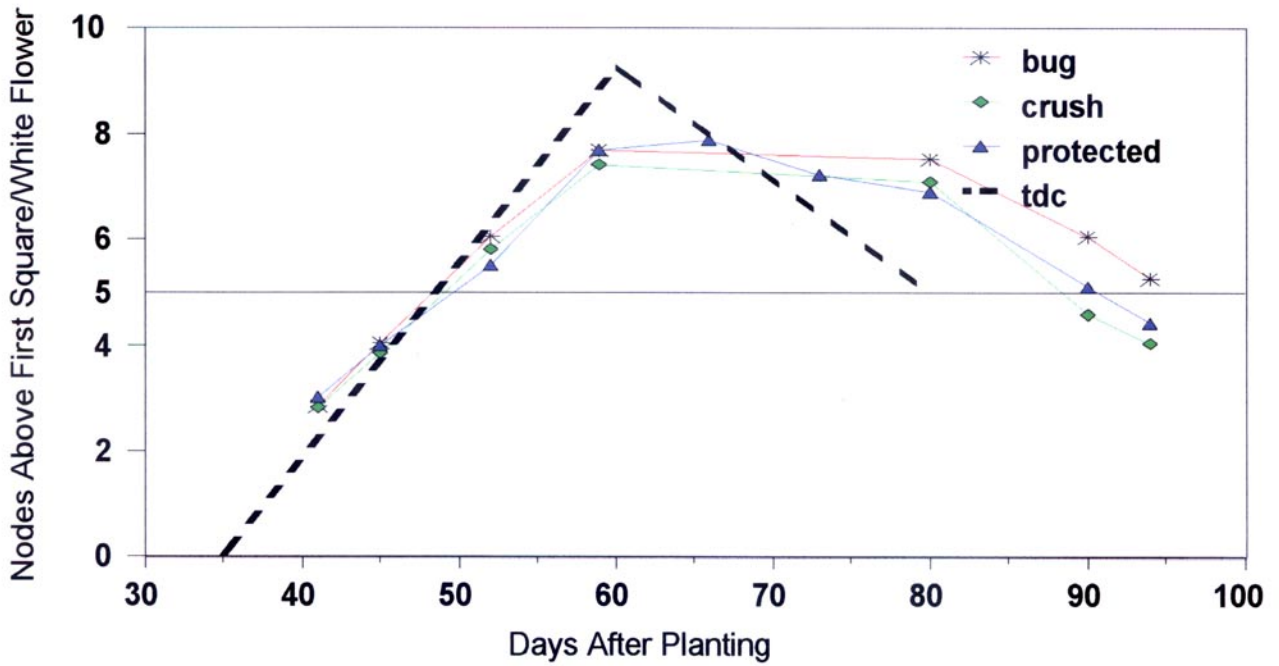


Figure 2. COTMAN growth curves plants exposed to tarnished plant bug nymphs, plants with manually removed squares or plants protected with insecticide.

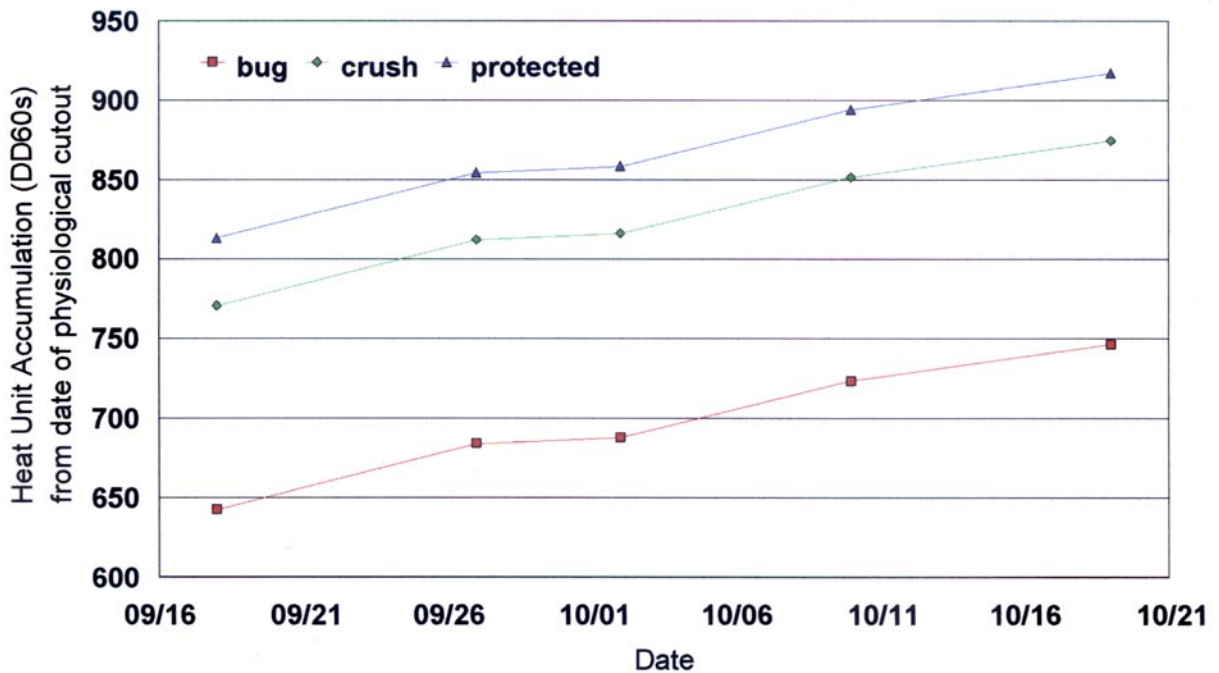


Figure 3. Heat units (DD60s) accrued by each treatment following physiological cutout for each date of harvest.