

# EFFECT OF TRIMAX™ INSECTICIDE ON THE PHYSIOLOGY, GROWTH AND YIELD OF COTTON

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## Abstract

Trimax™ is a new insecticide from Bayer CropScience specifically for use on cotton that provides excellent control of major sucking/piercing insects in cotton. In addition, significant yield enhancement benefits have been observed even in situations without economic target insect infestations. However, information is lacking on how Trimax affects plant growth and thereby enhances yield. The current studies were designed to understand plant response to foliar application of Trimax with particular emphasis on the physiological and biochemical changes that occur and how these may effect the development of yield. Field and growth chamber studies were designed to supply information which will permit us to quantify plant growth response to Trimax. There was a consistent trend for Trimax to increase yield in both years and locations, although only one experiment was significant. There were no significant effects of Trimax on plant growth parameters; however, there were significant effects on the physiology and biochemistry of the plants, in particular membrane integrity, carbohydrate content, polyols, and antioxidant enzymes activity. The interpretation of these responses is not clear and additional research is planned. These studies should also allow us to determine, or at least formulate, an explanation of how Trimax is affecting the growth and development of the cotton plant, and therefore enhancing yield.

## Introduction

Trimax™ is a new insecticide from Bayer CropScience registered specifically for use on cotton. It was discovered in 1985 and was the first commercially introduced insecticide in the class of chloronicotinyl insecticides. Trimax provides excellent control of the major sucking/piercing insects in cotton (aphids, cotton fleahopper, banded winged whitefly, plant bugs (excluding *Lygus hesperus*), green stinkbug and southern stinkbug). It also has ovicidal effects on bollworm and budworm. In addition, Trimax has strong antifeeding effect providing excellent protection from damaging pests feeding on cotton. Trimax can be applied up to five times per growing season, allowing for multiple applications in sustained pressure and multiple pest situations. The active ingredient in Trimax is imidacloprid, the only insecticide in the nitroguanidine subclass of chloronicotinyl insecticides with a chloropyridine side chain. This distinguishing side chain is structurally related to compounds like nicotinamide and chloronicotinic acid known as systemic plant resistance inducers. These substances help plants to better tolerate environmental stress during drought, disease and insect attacks.

## Enhanced Growth and Yields from TRIMAX

Pest management and plant metabolism/health benefits of Trimax, especially when used in multiple application spray programs beginning early to mid-season, have resulted in enhanced yields (Anon., 2002). Significant yield enhancement benefits have been observed even in situations where insect infestation is below economic threshold levels. Information is lacking on how Trimax affects plant growth and thereby enhances yield. Therefore, the current study was designed to understand plant response to foliar application of Trimax with particular emphasis on the physiological and biochemical changes that occur and how these may effect the development of yield. In order to understand the biochemical changes induced by Trimax on cotton, the activity of antioxidant enzymes after foliar application on cotton leaves was also determined.

Trimax gives the plant an apparent growth advantage and, due to the specific nature of the side chain on the imidacloprid molecule, it is hypothesized that this is due to physiological and biochemical changes in the plant that lessens the effect of environmental stresses. One of these changes is the activation of antioxidant enzymes that detoxify the plant of free radicals which are always present due to the numerous environmental stresses that crops face daily. The objectives of these studies were to (1) investigate the effect of Trimax on the growth and yield of cotton, (2) quantify the physiological and biochemical responses of Trimax-treated cotton to explain the mode of action of yield enhancement, and (3) determine the response of Trimax-treated plants to high temperature stress.

## Materials and Methods

Field studies were conducted at the University of Arkansas Delta Branch Experiment Station, Clarkedale in northeast Arkansas, and also at the Main Experiment Station in Fayetteville in northwest Arkansas, for two growing seasons, 2002 and 2003. The cotton (*Gossypium hirsutum* L.) cultivar Stoneville 474 was used for the studies. Trimax was applied with a CO<sub>2</sub> backpack sprayer starting at pinhead square.

## **2002 Field Studies**

The studies were planted on May 10, 2002 in Clarkedale, in a Dundee silt loam, and on May 12, 2002 in Fayetteville, in a Captina silt loam. A Randomized Complete Block (RCB) design with six replications was used for both locations. Treatments consisted of an untreated control, and Trimax applied at the recommended field rate and timing of 1.5 oz/acre (1 oz/acre = 70.1 g/ha) three times at weekly intervals, starting at pinhead square.

## **2003 Field Studies**

The 2003 study at Fayetteville, AR was planted on May 21, 2003, in a Randomized Complete Block (RCB) design with six replications. The treatments consisted of (1) untreated control, (2) Trimax at 1.5 oz/acre applied once, and (3) Trimax at 1.5 oz/acre applied three times at weekly intervals, starting at pinhead square.

The 2003 study at Clarkedale, AR was planted on May 13, 2003, in a Randomized Complete Block (RCB) design with eight replications. The treatments consisted of an untreated control, Trimax at 0.5, 1.0, 1.5, 2.0 oz/acre applied once, Trimax at 0.5, 1.0, 1.5, 2.0 oz/acre applied three times at weekly intervals, and Trimax at 8 oz/100 lb seed (seed treatment).

## **Growth Chamber Study**

The effect of increasing day temperature on the growth response of cotton to Trimax was evaluated using two growth chambers. The experimental design utilized for the Trimax study was a RCB with four replications. This study was conducted in the Alzheimer laboratory growth rooms, Fayetteville, AR, in October 2003. The first chamber was programmed for a 12-h photoperiod, with day/night temperature of 20/30°C and relative humidity of 75%. The cotton cultivar Stoneville 474 was planted in 2-L pots filled with Sunshine potting media. The second growth chamber was used to expose the plants to increased day temperatures of 33, 36 and 39°C. Treatments consisted of an untreated control and Trimax at 1.5 oz/acre, split for day temperature. Trimax was applied at pinhead square using of programmed spray chamber.

## **Measurements**

The measurements taken included classical growth analysis (biomass and leaf area), leaf photosynthesis, specific leaf weight (SLW), canopy temperature, chlorophyll content, chlorophyll fluorescence, membrane integrity, nonstructural carbohydrate concentrations of leaves, lint yield, and antioxidant enzymes (catalase, ascorbate peroxidase, peroxidase, and glutathione reductase). For the classical growth analysis, 1-m lengths of row were sampled from each plot three weeks after first flower. Physiological measurements were made one week after each Trimax application. Photosynthesis was measured with a portable LI-6200 photosynthesis system (Li-Cor Inc, Lincoln, NE). Membrane leakage was measured with an automatic seed analyzer (Applied Intelligent Systems Inc. Ann Arbor, MI). Canopy temperature was recorded with a handheld infrared thermometer (Everest, CA). Chlorophyll was measured with a Minolta SPAD meter, and chlorophyll fluorescence was measured with an OS1-FL modulated chlorophyll fluorometer (Opti-Science, Tyngsboro, MA). Carbohydrates and sugar alcohols were measured using HPLC. The extraction procedure from leaf tissue, to determine antioxidant enzymes was described by Anderson et al. (1992) and a BioSpec-1601 enzyme analyzer (Shimadzu Inc., Columbia, MD) was used for the analysis. At final harvest, lint yield was determined by mechanical harvest at Clarkedale and 2-m hand-sampling at Fayetteville.

## **Results**

### **Effects of TRIMAX on Lint Yield**

Increased lint yield was observed in 2002, at the two locations of the experiment, but only the increase in Fayetteville was significant (Figure 1). Trimax also increased lint yield in both locations in 2003, but the differences were not significant (Figure 2). The overall mean lint yield from two locations and two years of Trimax at 1.5 oz/acre applied three times compared to the untreated control was 157.7 kg/ha (+14.04 %). In 2003 the 2.0 oz/acre rate of Trimax applied three times gave the highest yield, i.e. 134.1 kg/ha above the control (data not presented). The seed treatment of TRIMAX was not significantly different ( $P > 0.05$ ) from the untreated control.

### **Effects on Plant Growth (Field Study)**

Applications of Trimax during square development appeared to have a stimulatory effect on plant growth, although most of the parameters measured were not significantly different ( $P > 0.05$ ) from the untreated control (Table 1). In 2003, Trimax had no significant effect on plant growth measurements (data not presented), due presumably to the mild environmental conditions experienced during the reproductive period.

To evaluate our hypothesis that the growth advantage imposed by Trimax was, in part, due to the activation of antioxidant enzymes to detoxify the plant of free radicals (Gould, 2003), we evaluated the effect of Trimax on antioxidant enzyme activity with time after application (Figure 3). Trimax caused an immediate increase in glutathione reductase and decrease in ascorbate peroxidase. However, all enzyme activity tended towards the control value two days after Trimax application. Trimax obviously stimulated some antioxidant enzyme activity but the interpretation of this is not apparent and future research will attempt to explain these responses.

Trimax significantly decreased specific leaf weight and increased chlorophyll content in 2002, but had no effect on photosynthesis and canopy temperature (Table 2). It was suggested that this was associated with improved carbohydrate production and movement out of the leaf.

The application of Trimax caused a significant increase in the content of carbohydrates in leaves, and an increase in the myo-inositol (sugar alcohol) content (Figure 4). This result was related to improved efficiency of carbohydrate production and increased stress resistance from the increase in polyols.

### **Effect of Increased Temperature (Growth Chamber Study)**

To evaluate the effect of Trimax under temperature stress, measurements were taken to record changes in fluorescence (FL), membrane integrity (MI) and antioxidant enzymes (AO) under conditions of increasing temperature. At 33°C there was no significant effect on MI (Figure 5) or FL (Figure 6), but AO activity was significantly affected (data not presented). Whereas at 36°C both MI and FL were significantly decreased by Trimax. Furthermore, at temperatures above 36°C the effect of TRIMAX declined.

### **References**

Anderson, J.V., B.I. Chevone, and J.L. Hess. 1992. Seasonal variation in the antioxidant system of Eastern white pine needles. *Plant Physiology* 98: 501-508.

Anonymous. 2002. Bayer CropScience Technical Bulletin TRO211, Raleigh, NC.

Gould KS. 2003. Free radicals, oxidative stress, and antioxidants. pp. 9-16. In: Thomas B, Murphy D, Murray B (eds) *Encyclopedia of Applied Plant Science*. London, Academic Press.

Table 1. Effect of Trimax on plant growth and development measured three weeks after first flower, Clarkedale, Arkansas, 2002.

Clarkdale, Arkansas, 2002.												
Treatments	LAI (m <sup>2</sup> /m <sup>2</sup> )	Dry weight						Plant area <sup>†</sup>	# Squares (/m <sup>2</sup> )			
		Leaf (g/m <sup>2</sup> )		Fruit (g/m <sup>2</sup> )		Total (g/m <sup>2</sup> )						
Control	2.9	a <sup>‡</sup>	115	a	27.6	a	303	a	590	a	193	a
TRIMAX	3.3	a	132	a	36.6	a	356	a	755	b	187	a

<sup>†</sup> Plant area = plant height × canopy width (Measured July 17, 2002)

<sup>‡</sup> Numbers followed by the same letter are not significantly different (P≤0.05)

Table 2. Effect of Trimax on physiological parameters, measured three days after first flower, Clarkedale, Arkansas, 2002.

Treatments	Specific		Canopy	
	Leaf Weight (g/m <sup>2</sup> )	Chlorophyll (SPAD units)	Photosynthesis (μmol[CO <sub>2</sub> ]/m <sup>2</sup> /s)	Temperature (°C)
Control	64.1 b <sup>†</sup>	45.7 b	23.3 a	26.7 a
TRIMAX	56.8 a	51.7 a	24.5 a	26.6 a

<sup>†</sup> Numbers followed by the same letter are not significantly different (P≤0.05)

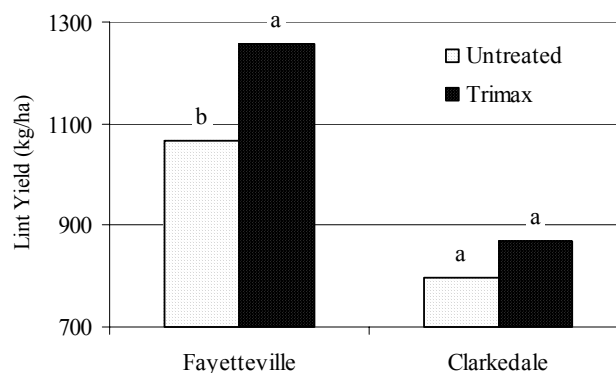


Figure 1. Effect of Trimax on lint yield in 2002. Columns superseded by a different letter are significantly different (P≤0.05).

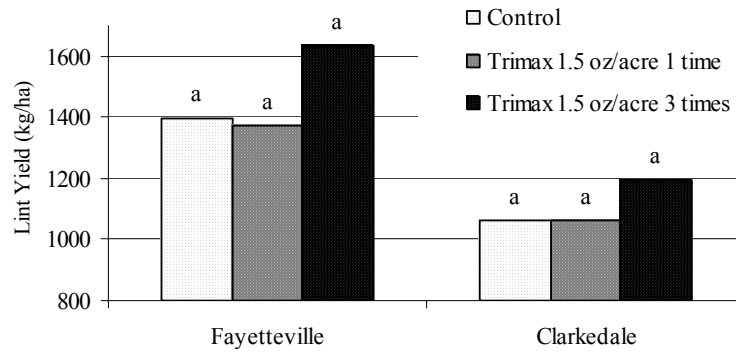


Figure 2. Effect of Trimax on lint yield in 2003. Columns superseded by a different letter are significantly different ( $P \leq 0.05$ ).

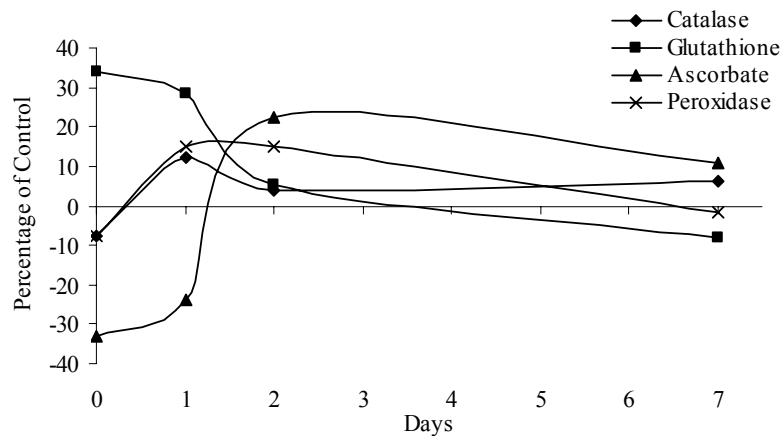


Figure 3. Effect of Trimax on antioxidant enzyme activity for seven days after foliar application. Measurements started 3 hours after application.

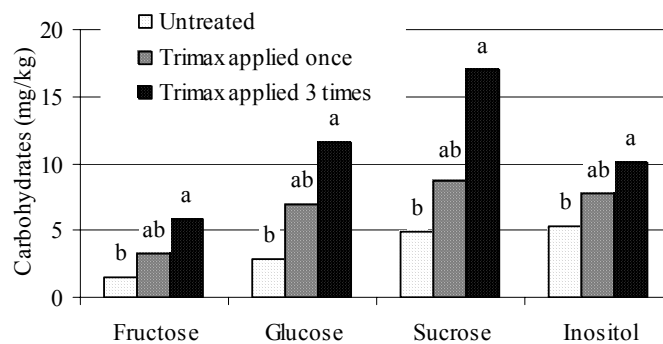


Figure 4. Carbohydrate contents in leaves three weeks after first flower, Fayetteville, 2003. Columns superseded by a different letter are significantly different ( $P \leq 0.05$ ).

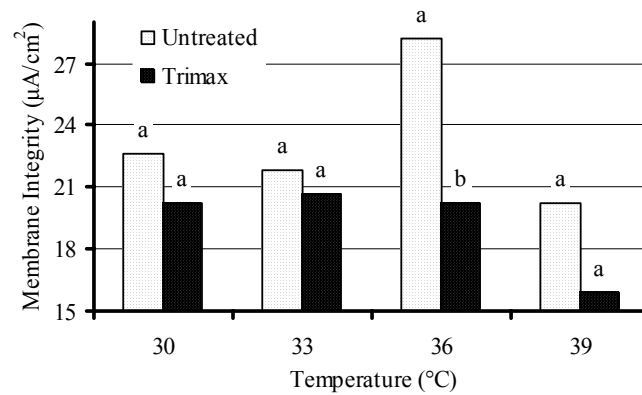


Figure 5. Effect of Trimax on membrane integrity at high temperatures. Pairs of columns superseded by a different letter are significantly different ( $P \leq 0.05$ ).

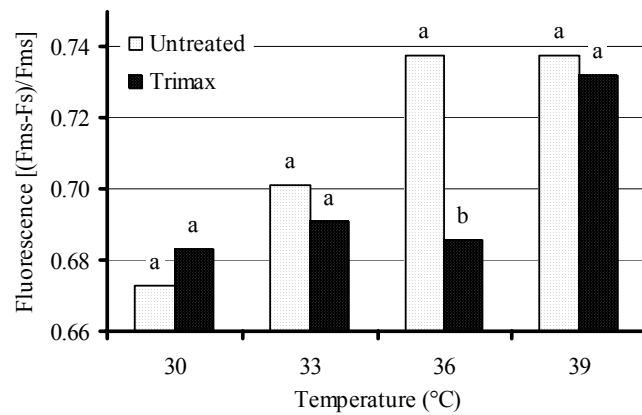


Figure 6. Effect of Trimax on chlorophyll fluorescence at high temperatures. Pairs of columns superseded by a different letter are significantly different ( $P \leq 0.05$ ).