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# Physiologic Response of Cotton to the Insecticide Imidacloprid under High-Temperature Stress

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**Abstract** The insecticide imidacloprid (tradename Trimax™) has been shown to increase cotton (*Gossypium hirsutum* L.) yield in the absence of insects, but the explanation for this is not clear. Growth room studies were designed to investigate changes in the physiology and biochemistry of imidacloprid-treated cotton plants and provide information on the mode of action of yield enhancement. Imidacloprid was applied at the pinhead square growth stage at the rate of 52.3 g ai/ha and plants were exposed to day temperatures of 30, 33, 36, and 39°C. Increased levels of photosynthesis and higher values of chlorophyll fluorescence yield, measured two days after imidacloprid application, showed an advantage of imidacloprid-treated over untreated plants. The effect of imidacloprid was greater at the higher temperatures of the growth chamber studies. The results suggested that the imidacloprid-treated plants suffered less temperature stress. This suggestion was supported by findings of reduced glutathione reductase in the imidacloprid-treated plants in the growth chamber, indicating that the untreated plants were experiencing more stress, necessitating the activation of this defense mechanism.

**Keywords** *Gossypium hirsutum* · Imidacloprid · Temperature stress · Photosynthesis · Chlorophyll fluorescence · Antioxidant enzymes

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## Introduction

The cotton (*Gossypium hirsutum* L.) plant is a perennial with an indeterminate growth habit and is grown as an annual. Furthermore, cotton is reputed to have the most complex plant structure of all major field crops (Mauney 1984). This complex growth habit is also associated with an extreme sensitivity to adverse environmental conditions, reflected in excessive fruit abscission (Cothren 1999). The cotton crop is subjected to a variety of biotic and abiotic factors throughout its life cycle, and these play a key role in determining plant growth and yield. Insects have long been a major problem in cotton that decrease yields and raise production costs.

The development and use of synthetic organic insecticides has made possible the production of higher yields of cotton in areas where insects would have otherwise prevented the production of an economical cotton crop (Ridgway 1984). Insecticides are widely used in cotton production, although the amount used has decreased considerably with the advent of transgenic cultivars with the Bt (*Bacillus thuringiensis*) endotoxin. Growth and yield improvements have been reported for some insecticides. For example, White and Bourland (1986) suggested that enhanced cotton yields related to chlordimeform (tradenames Galecron, Fundal) were not all from insect control. Similarly, Bauer and Cothren (1990) reported that chlordimeform increased physiologic activity, as measured by radish (*Raphanus sativus*) cotyledon expansion. Aldicarb (tradename Temik®) also has been reported to enhance growth, promote earliness, and increase the number of bolls and squares on cotton plants grown at different temperatures (Reddy and others 1990).

Imidacloprid (tradename Trimax™) is an insecticide designed for control of the major sucking/piercing insects that affect cotton. However, there also have been anecdotal

**Table 1** Diurnal Changes in Temperature, Radiation, and Relative Humidity in the Growth Chamber Studies

| Diurnal time | Temperature treatments | | | | Radiation (μmol/m$^2$/s) | Humidity (%) |
|---|---|---|---|---|---|---|
| | 1 (°C) | 2 (°C) | 3 (°C) | 4 (°C) | | |
| 2:00 | 20 | 20 | 20 | 20 | 0 | 75 |
| 10:00[a] | 24 | 27 | 27 | 27 | 350 | 75 |
| 11:30 | 28 | 31 | 34 | 34 | 350 | 75 |
| 13:00 | 30 | 33 | 36 | 39 | 750 | 75 |
| 20:00 | 28 | 31 | 34 | 34 | 350 | 75 |
| 22:00[b] | 24 | 27 | 27 | 27 | 0 | 75 |
| 24:00 | 22 | 25 | 25 | 25 | 0 | 75 |

[a] Lights automatically turned on

[b] Lights automatically turned off

reports of imidacloprid causing yield increases in the absence of insect pests (for example, Trimax Technical Bulletin, BayerCrop Science, Raleigh, NC). Field studies in 2004 in Arkansas have also shown growth and yield enhancement by imidacloprid in the absence of insects (Gonias and others 2006). The authors reported an increase of 16.1% for crop dry weight, 12.7% for leaf area, and 10.6% for number of fruiting positions at three weeks after the first flower stage of growth, and at harvest an increase in lint yield by 7%. These increases have been attributed to improved plant metabolism, but the evidence is lacking. The imidacloprid molecule has a chloropyridine side chain that structurally resembles nicotinamide and nicotinic acid (niacin). These molecules have been shown to have antioxidant activity, for example, inhibition of protein oxidation and reactive oxygen species–induced apoptosis, and scavenging of reactive oxygen species has been reported for nicotinamide *in vitro* (www.pdrhealth.com). In addition, Ogata and others (2002) reported that many niacin-related compounds have scavenging activity against hydroxyl radicals.

We hypothesized that foliar application of imidacloprid to cotton lessens the effect of temperature stress on the plant due to the chloropyridine side chain and thereby enhances plant growth. The current study was designed to understand how cotton grown under high-temperature stress responds to foliar application of imidacloprid, with particular emphasis on the physiologic and biochemical changes that occur and how these may affect the development of yield.

## Materials and Methods

The response of imidacloprid-treated cotton plants to increasing day temperature was evaluated using large growth chambers (Model PGW36, Conviron, Winnipeg, Canada). The studies were conducted in the Altheimer Laboratory, Arkansas Agricultural Research and Extension Center in Fayetteville in February 2005, and repeated in November 2005.

The experiments were arranged in a completely randomized design with six replications. The cotton cultivar ST474 was planted in twelve 2-L pots containing Sunshine mix (Sun Gro Horticulture Distribution Inc., Bellevue, WA), four times in 3-day intervals for a total of 48 pots. The plants were grown in day/night temperature regimes of 20/30°C and were watered with half-strength Hoagland's nutrient solution (Hoagland and Arnon 1950) as necessary. The four temperature treatments were imposed as shown in Table 1. At the pinhead square stage of growth, imidacloprid (formulated as Trimax by Bayer CropScience) at 52.3 g ai/ha (the recommended rate for field applications) was applied to half of the pots from the first planting date. These 12 plants, together with the remaining 36 plants, were kept in the growth chamber at 20/30°C (day/night temperature) for two days, after which measurements were taken. Thereafter, the day temperature of the growth chamber was raised to 33°C and the procedure was repeated with the pots from the second planting date. The same format was used for the pots from the other two planting dates for 36°C and 39°C, respectively. The purpose of the design of this study was to acclimate the cotton plants to a higher day temperature before increasing the temperature by 3°C.

The growth chamber studies were designed to observe changes in cotton plant physiology, especially leaf photosynthesis, chlorophyll fluorescence, membrane integrity, leaf chlorophyll content, specific leaf weight, as well as changes in the biochemical parameters, antioxidant enzyme activity (catalase, glutathione reductase, and peroxidase), and total soluble proteins. All the measurements were recorded close to midday on the uppermost fully expanded main-stem leaf located four nodes below the terminal of the plant. The fourth main-stem leaf was then collected and the leaf area and dry weight were determined. Prior to the physiologic measurements, the fifth main-stem leaf of each

plant was collected and immediately stored in an ultrafreezer (-80°C) for antioxidant enzyme and protein extraction.

A Li-Cor 6200 portable photosynthesis system (Li-Cor Inc., Lincoln, NE) was used for measuring photosynthesis. Chlorophyll fluorescence yield was recorded by an OS1-FL modulated chlorophyll fluorometer (Opti-Science, Tyngsboro, MA) using the light-adapted test. Membrane leakage was measured with an automatic seed analyzer (Applied Intelligent Systems Inc., Ann Arbor, MI), using 2 ml of deionized water in each cell and a 1-cm-diameter leaf disk. The samples were incubated for 48 h before measuring the electrical conductivity. Leaf chlorophyll content was estimated by a SPAD-502 chlorophyll meter (Konica Minolta Sensing Americas, Ramsey, NJ).

Antioxidant enzymes were measured using the extraction procedure described by Anderson and others (1992) and a BioSpec-1601 enzyme analyzer (Shimadzu Inc., Columbia, MD). Initially, the frozen tissue was ground in liquid nitrogen with a mortar and pestle. The sample was then placed in a 35-ml centrifuge tube containing 0.5 g insoluble polyvinylpyrrolidone (PVP), one drop of antifoam A (Sigma-Aldrich, Milwaukee, WI), and 4 ml of ice-cold extraction buffer and homogenized with a Polytron homogenizer. The tubes were then centrifuged at 12,000 rpm and 4°C for 20 min, and the supernatant was passed through a PD-10 column (Amersham Biosciences, Uppsala, Sweden) for purification before measurement in the spectrophotometer.

For catalase the protocol of Beers and Sizer (1952) was followed. The disappearance of $H_2O_2$ was measured as the decrease in absorbance at 240 nm for 1 min at 25°C. The assay by Schaedle and Bassham (1977) was used for glutathione reductase. The glutathione-dependent oxidation of NADPH+H at 340 nm for 1 min at 25°C was recorded. Peroxidase activity was measured by monitoring the hydrogen peroxide-dependent oxidation of 2,3,6-trichloroindophenol at 675 nm for 1 min at 25°C, as described by Nickel and Cunningham (1969). For each of the antioxidant enzyme assays, three measurements per sample were recorded. Total soluble proteins were measured according to the technique described by Bradford (1976).

Treatment differences within temperature were detected using analysis of variance (ANOVA). Data means were separated at probability values $\alpha \leq 0.05$. Statistical analysis was performed using JMP 6 software (SAS Institute Inc., Cary, NC).

## Results

### Chlorophyll Fluorescence

The effect of imidacloprid application on the chlorophyll fluorescence yield of cotton at different day temperatures is summarized in Figure 1A. At a day temperature of 30°C, no significant effect of foliar application of imidacloprid was observed on chlorophyll fluorescence yield. Although not significantly different ($p = 0.06$), a higher value of chlorophyll fluorescence yield was recorded at 33°C for imidacloprid-treated plants compared with that of the untreated control. The effect of imidacloprid application on chlorophyll fluorescence yield became evident at day temperatures of 36°C and 39°C, where the imidacloprid-treated plants had significantly higher values of chlorophyll fluorescence yield compared with the untreated control. Higher values of chlorophyll fluorescence yield is an indication that the imidacloprid-treated plants were experiencing less stress. These results were validated by the second growth chamber study (Figure 1B).

Factorial analysis was also performed to detect main effects and interactions among temperature, treatment, and study (fixed factor). As no significant three-way interaction (temperature × treatment × study) was observed for chlorophyll fluorescence, the factor "study" was not considered. The two factors of factorial analysis (temperature and treatment) revealed no significant interaction, while the

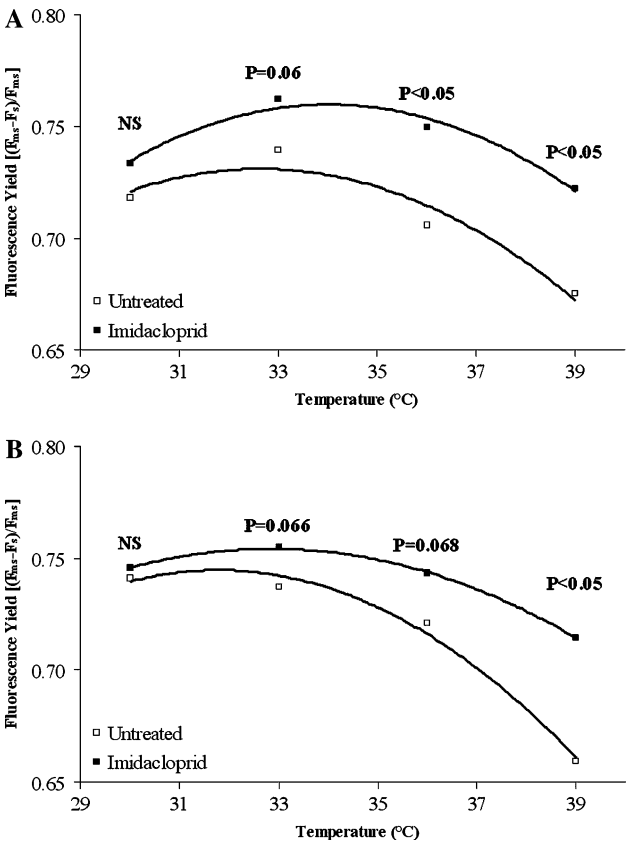


**Fig. 1** Effect of imidacloprid on chlorophyll fluorescence with increasing day temperatures measured two days after application in repeated growth chamber studies (**A**, **B**). *p* values for comparison of treatment means are shown, NS = nonsignificant ($p > 0.05$)

effect of temperature was close to significant ($p = 0.0587$), and the effect of treatment on chlorophyll fluorescence was significant ($p < 0.05$).

## Leaf Photosynthesis

In general, imidacloprid-treated plants exhibited higher photosynthetic rates than untreated control plants, indicating greater productivity (Figure 2A). Similar to chlorophyll fluorescence, no significant effect of imidacloprid application on leaf photosynthesis was recorded at 30°C and 33°C ($p = 0.153$). However, at 36°C and 39°C, significantly higher rates of leaf photosynthesis were recorded in the imidacloprid-treated plants compared with those of the untreated control plants. Results were similar in both growth chamber studies (Figure 2B).

Similar to chlorophyll fluorescence, no three-way interaction of the factors was detected for leaf photosynthesis. Therefore, the fixed factor "study" was not included in the analysis. The two-way interaction (temperature × treatment) was not significant, but the main effects of temperature and treatment were statistically significant ($p < 0.05$).

## Antioxidant Enzymes

Application of imidacloprid did not significantly affect catalase and peroxidase activities with increased temperature (Figures 3A and 4A). Similarly, no statistically significant differences were observed for glutathione reductase activity at 30, 33 and 36°C between imidacloprid-treated and untreated control cotton plants (Figure 5A). However, significantly lower levels of glutathione reductase activity were recorded at 39°C after application of imidacloprid (Figure 5A). Similar effects of imidacloprid application on the activity of the three antioxidant enzymes were documented in both growth chamber studies (Figures 3B, 4B, and 5B).

To determine if imidacloprid had an overall effect on the antioxidant enzyme system, as measured by the three enzymes, multivariate analysis of variance (MANOVA) was performed. For both studies, a statistically significant treatment effect was recorded for decreased antioxidant

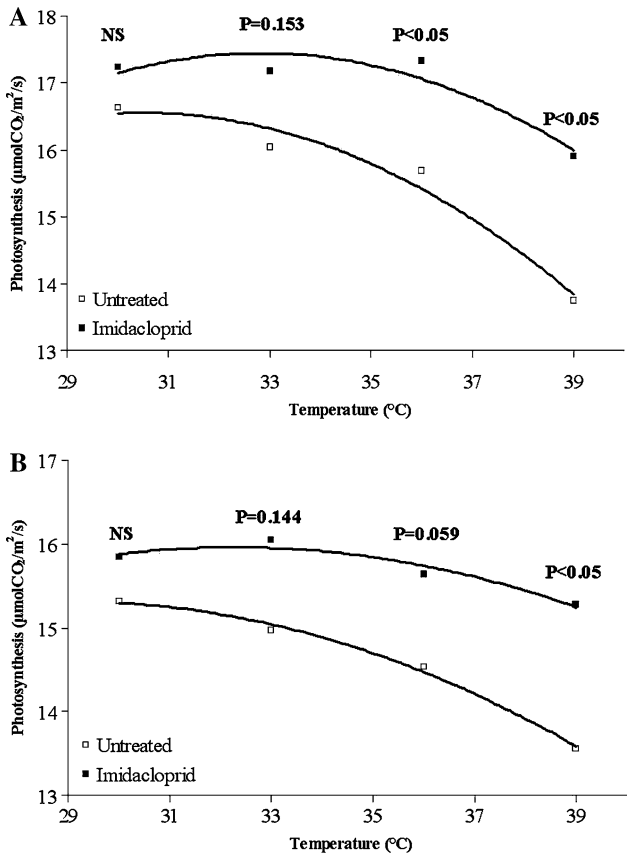


**Fig. 2** Effect of imidacloprid on photosynthesis with increasing day temperatures measured two days after application in repeated growth chamber studies (**A**, **B**). *p* values for comparison of treatment means are shown, NS = nonsignificant ($p > 0.05$)

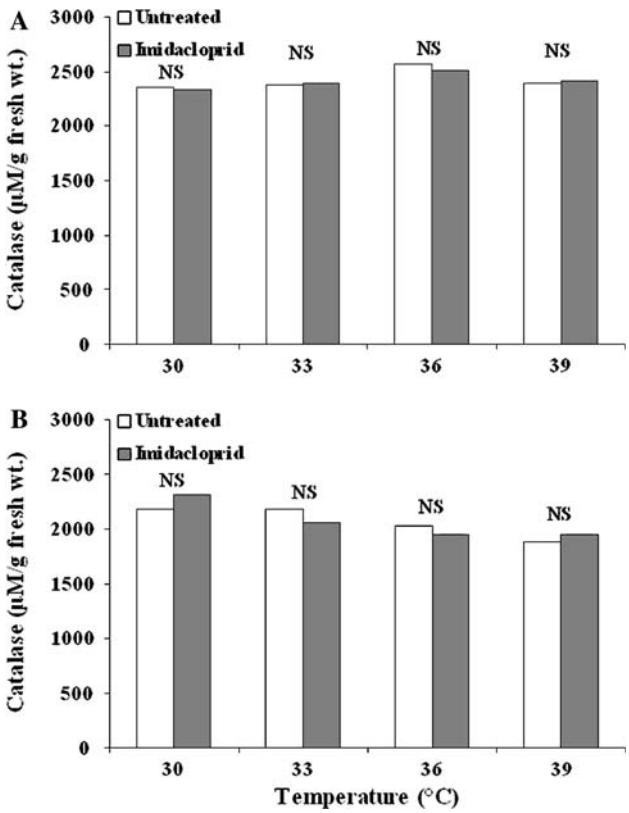


**Fig. 3** Catalase activity as affected by imidacloprid with increasing day temperatures measured two days after application in repeated growth chamber studies (**A**, **B**). *p* values for comparison of treatment means are shown, NS = nonsignificant ($p > 0.05$)

enzyme activity at 39°C after imidacloprid application. The lower antioxidant enzyme activity indicated that the imidacloprid-treated plants were experiencing less stress.

### Other Measured Parameters

No significant differences at all the temperature regimes were observed in membrane leakage, chlorophyll content, specific leaf weight, and total soluble proteins in both growth chamber studies after foliar application of imidacloprid (data not shown). Leaf area and dry weight of the fourth main-stem leaf showed a significant temperature and imidacloprid treatment effect, with increasing temperature decreasing both parameters (data not shown). Imidacloprid application increased leaf area by 5.9% and dry weight by 8.1%.

## Discussion

Changes in physiologic and biochemical parameters after foliar applications of imidacloprid help to explain the

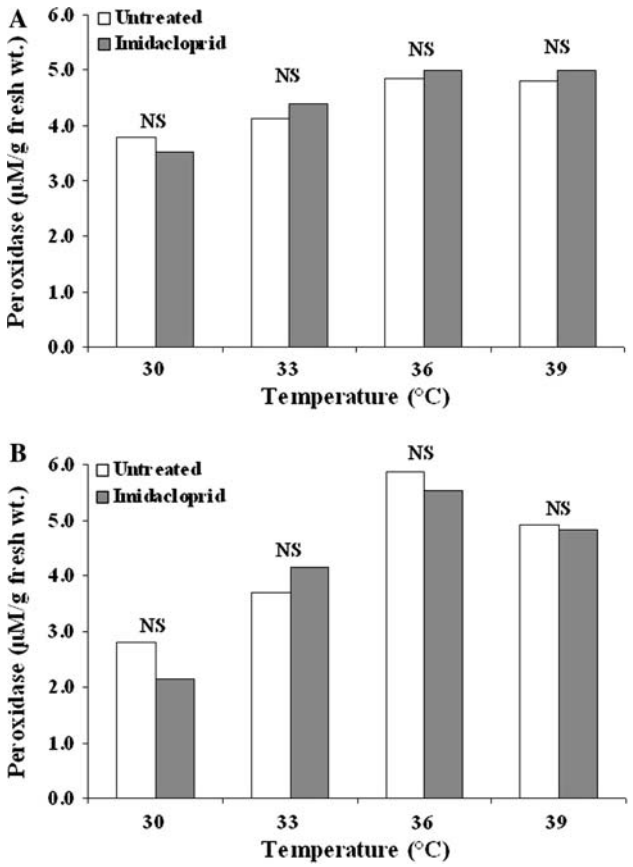


**Fig. 4** Peroxidase activity as affected by imidacloprid with increasing day temperatures measured two days after application in repeated growth chamber studies (**A**, **B**). *p* values for comparison of treatment means are shown, NS = nonsignificant ($p > 0.05$)

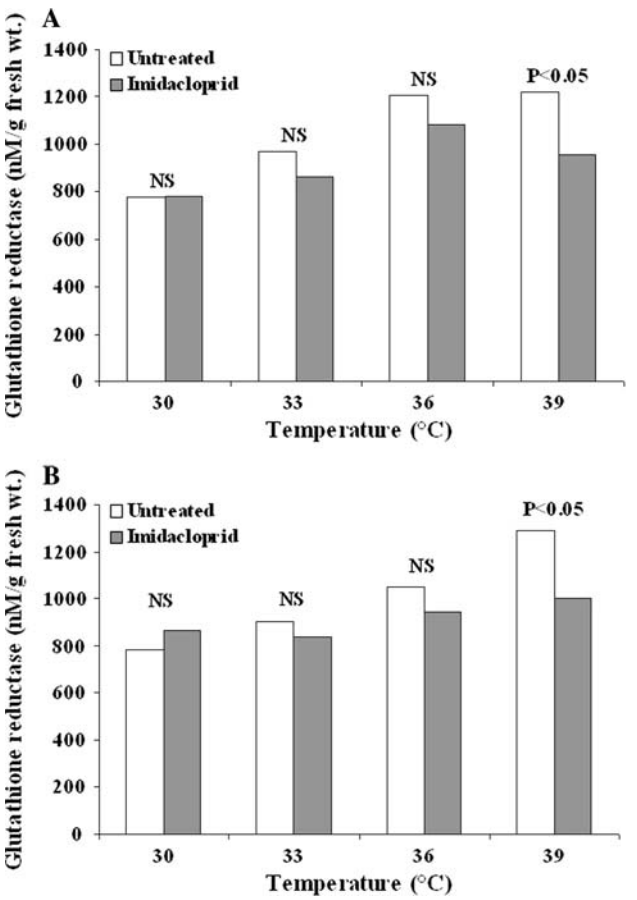


**Fig. 5** Glutathione reductase activity as affected by imidacloprid with increasing day temperatures measured two days after application in repeated growth chamber studies (**A**, **B**). *p* values for comparison of treatment means are shown, NS = nonsignificant ($p > 0.05$)

reported crop growth improvement and lint yield increase of imidacloprid-treated cotton. The results of this study suggest that the beneficial effect of imidacloprid was more prevalent under high-temperature stress.

The trend for imidacloprid-treated plants was to maintain increased physiologic activity even under very stressful conditions, that is, at temperatures over 36°C. Cothren (1999) stated that temperatures above 35°C have detrimental effects on cotton plant metabolism, causing poor flower survival and fruit production. Similarly, Bibi and others (2005b) showed that photosynthesis and leaf extension growth in cotton was significantly reduced at temperatures above 35°C. Imidacloprid-treated plants had higher photosynthesis and higher chlorophyll fluorescence yield values as temperature increased above 36°C. Gross photosynthesis of cotton can be reduced at temperatures above 32°C (Perry and Krieg 1981). However, imidacloprid-treated plants maintained higher photosynthesis at 36°C, and even at 39°C, the highest temperature tested in this study, whereas photosynthesis of the untreated control plants was dramatically reduced (Figure 2). The enhanced metabolism was reflected in the higher values of leaf area

and dry weight of the fourth main-stem leaf after application of imidacloprid. Similar results have been shown in field studies, where measurements of leaf photosynthesis indicated an 8.3% increase ($p = 0.103$) after application of imidacloprid (unpublished data).

Glutathione reductase has been shown to be the major antioxidant enzyme associated with both abiotic and biotic stress in cotton. Bibi and others (2005a) showed that the activity of glutathione reductase was more sensitive to high-temperature stress than that of catalase and peroxidase, with the activity of glutathione reductase increasing with increased temperature. In addition, glutathione reductase, but not catalase and peroxidase, activity was significantly increased in cotton after aphid herbivory (Gomez and others 2004). Reduced levels of glutathione reductase activity suggest that imidacloprid-treated plants were experiencing less injury from high-temperature stress compared with untreated plants. It is possible that the unique chloropyridine side chain of imidacloprid may have antioxidant properties similar to that of nicotinamide and nicotinic acid, which have a similar structure to the chloropyridine chain. The side chain can act as a scavenger of reactive oxygen species so that the glutathione reductase antioxidant defense mechanism for plant detoxification is not as active.

Improved metabolism of imidacloprid-treated plants is clearly shown by higher values of chlorophyll fluorescence yield and higher levels of photosynthesis. This enhanced metabolism advantage in imidacloprid-treated plants was reflected in improved crop growth and increased cotton yields reported in field studies (Gonias and others 2006).

This research suggests that imidacloprid can act as an oxidative stress-reducing factor by lowering the need for increased antioxidant enzyme activity. The concept of decreased antioxidant enzyme activity due to exogenous application of compounds that exert antioxidant behavior has been reported in plants (Penuelas and others 2005). These authors showed that in the presence of exogenous isoprene, expression of antioxidant compounds of *Quercus ilex* at high temperatures was suppressed. The improved protection of plant metabolism allows the plants to maintain growth and yield potential under elevated environmental stress.

## References


Anderson JA, Chevone BI, Hess JL (1992) Seasonal variation in the antioxidant system of Eastern white pine needles: evidence for thermal dependence. Plant Physiol 98:501–508

Bauer PJ, Cothren JT (1990) Growth-promoting activity of chlordimeform. Agron J 82:73–75

Beers R, Sizer I (1952) A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem 195:130–140

Bibi A, Oosterhuis DM, Gonias E, Bourland FM (2005a) Evaluation of techniques for quantifying the physiological response of cotton to high temperature. Summaries of Arkansas Cotton Research, 2004. University of Arkansas Agricultural Experiment Station Research Series 533:34–38

Bibi A, Oosterhuis DM, Gonias E, Bourland FM (2005b) Screening a diverse set of cotton cultivars for high temperature tolerance. Summaries of Arkansas Cotton Research, 2004. University of Arkansas Agricultural Experiment Station Research Series 533:39–43

Bradford MM. (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Cothren JT (1999) Physiology of the cotton plant. In: Smith CW, Cothren JT, editors. Cotton: Origin, History, Technology and Production. Danvers, MA: John Wiley & Sons, chap 2.2, pp 207–268

Gomez SK, Oosterhuis DM, Rajguru SN, Johnson DR (2004) Foliar antioxidant enzyme responses in cotton after aphid herbivory. J Cotton Sci 8:99–104

Gonias ED, Oosterhuis DM, Bibi AC (2006) How the insecticide Trimax™ improves the growth and yield of cotton. Proceedings of the Beltwide Cotton Conference, San Antonio, TX, January 4-6, 2006. Memphis, TN: National Cotton Council of America [CD-ROM]

Hoagland DR, Arnon DI (1950) The water-culture for growing plants without soil. Calif Agric Exp Stat Circ 347:32

Mauney JR (1984) Anatomy and morphology of cultivated cottons. In: Kohel RJ, Lewis CF, editors. Cotton. Agron Monogr 24. Madison, WI: American Society of Agronomy

Nickel RS, Cunningham BA (1969) Improved peroxidase assay method using Ieuco 2,3,6-trichloroindophenol and application to comparative measurements of peroxidase catalysis. Anal Biochem 27(2):292–299

Ogata S, Takeuchi M, Teradaira S, Yamamoto N, Iwata K, Okumura K, Taguchi H (2002) Radical scavenging activities of niacin-related compounds. Biosci Biotechnol Biochem 66:641–645

Penuelas J, Llusia J, Asensio D, Munne-Bosch S (2005) Linking isoprene with plant thermotolerance, antioxidants and monoterpene emissions. Plant Cell Environ 28:278–286

Perry SW, Krieg DR (1981) Gross net photosynthesis ratios of cotton as affected by environment and genotype. Proceedings of the Beltwide Cotton Producers Research Conference. Memphis, TN: National Cotton Council, p 51

Reddy KR, Reddy VR, Baker DN (1990) Influence of aldicarb on growth, development and photosynthesis of cotton. Proceedings of the Beltwide Cotton Producers Research Conference. Memphis, TN: National Cotton Council, p 44

Ridgway RL (1984) Cotton protection practices in the USA and world. In: Kohel RJ, Lewis CF, editors. Chapter 9, Section A: Insects, Cotton. Agron Monogr 24. Madison, WI: ASA, CSSA, and SSSA, pp 266–287

Schaedle M, Bassham JA (1977) Chloroplast glutathione reductase. Plant Physiol 59:1011–1012

White BW, Bourland FM (1986) Effects of chlordimeform on early boll retention and yield of cotton. Proceedings of the Beltwide Cotton Producers Research Conference. Memphis, TN: National Cotton Council, pp 521–524