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ENVIRONMENTAL FACTORS ALTERING THE
RESPONSE OF PLANTS TO
TRIACONTANOL

By

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ABSTRACT

ENVIRONMENTAL FACTORS ALTERING THE
RESPONSE OF PLANTS TO
TRIACONTANOL

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The growth regulating activity of triacontanol (TRIA) in seedling bioassays was dependent on various cultural and environmental conditions. In growth chamber studies, maize seedlings exposed to a 35°C air temperature for 1 hr prior to treatment with TRIA gained more dry weight than those exposed to 25°C prior to treatment. Under greenhouse conditions, TRIA applied at 1300 and 1700 HR but not 0900 HR increased maize seedling dry-weight accumulation. Night temperatures from 10°C to 25°C did not alter the magnitude of the response of maize seedlings to TRIA. The temperature and time of day conditions favoring a stimulation of seedling dry weight accumulation by TRIA also resulted in a temporary increase in seedling water potential. Dry-weight accumulation was not increased by TRIA when maize seedlings were grown in nutrient culture with aerated solution or calcined clay as the root media. In some tests where TRIA applications decreased growth, the response may have been due to higher levels of P in the soil and peat media. Even when the factors which were shown to affect the response were optimized, no protocol could be developed which provided consistent dry-weight increases of "soil"-grown maize seedlings to TRIA.

The yield response of crops to TRIA, formulated as a colloidal dispersion, was tested with 13 crop species in 45 field experiments over a 3-year period. Foliar application of TRIA resulted in treatment effects with 11 of the 13 crops and in 30 of the 45 experiments. The average yield increase was 13% at the optimum TRIA concentration in tests with positive yield responses and was 5% over all 45 experiments. In 7 experiments, significant yield decreases averaging 12% were measured with the same concentration that increased crop yield in other tests. The most active concentrations were generally 0.1 to 1.0 µg/liter but significant effects were measured at 0.01 and 1000 µg/liter. No morphological stage of crop development proved optimal for treatment of all crops. Observations and experiments testing environmental conditions indicated yield increases were most likely when TRIA was applied during mid to late afternoon, on warm sunny days, when environmental stress was not limiting growth.

This thesis is dedicated to the memory of Ayn Rand, author and philosopher. Her novels and philosophy of objectivism have provided order and direction for my development as a teacher, scientist and a human being. Her philosophy, in essence, is the concept of man as a heroic being, with his own happiness as the moral purpose of his life, with productive achievement as his noblest activity and reason as his only absolute.

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It goes without saying that my wife Patti and son Jake intimately share in this accomplishment and my success.

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INTRODUCTION

One potential method of increasing crop yield is the application of growth- and yield-enhancing chemicals to crops. Although many chemicals with yield-enhancing activity have been identified, few are used commercially because of a lack of consistent effects. One such chemical is triacontanol (TRIA), a 30-carbon primary alcohol found primarily in the cuticular wax of plants. TRIA was originally isolated from alfalfa hay that was found to stimulate growth of seedlings and yield of crops more than could be accounted for by its nutrient content (11). While much TRIA research is currently in progress, the widespread interest following the discovery of growth-regulating activity of TRIA has faded.

TRIA has been a difficult plant growth regulator (PGR) to study, due in part to the inability of the research community to define simple biological systems with reproducible responses. The initial isolation and identification of TRIA was based on its stimulation of seedling growth. Much of the research directed at investigating the metabolic response of plants to TRIA has also been done with seedlings of various species. Other early studies tested the effect of TRIA on crop yield, seed germination, senescence of leaf disks, hypocotyl growth and photosynthesis. The status of TRIA as a PGR and its effects on plant growth and crop yield were recently reviewed (14). Laboratory and controlled-environment research indicate that TRIA has biological activity but results of field tests have not been consistent enough to warrant commercial use of TRIA as a yield-enhancing PGR.

Lack of consistency with TRIA and other PGR's has often been attributed to application problems and to variations in cultural and environmental conditions. The development of an aqueous colloidal dispersion formulation of TRIA (8) has provided a better method of applying TRIA and has led to more consistent results with seedling bioassays (13,14) and other biological systems (4,7). This formulation is generally more active than previous formulations with an optimum activity at 0.1 to 1.0 µg/liter.

In these studies, seedling growth assays were used to investigate cultural and environmental factors altering the response of plants to TRIA in greenhouse and growth chamber studies. The colloidal dispersion formulation of TRIA and the effects of environmental factors, cultural practices and application methods on the response of crops to TRIA were also tested in yield trials under field conditions. The general objectives were to draw conclusions about the whole-plant response to TRIA and the inconsistancies associated with TRIA results.

PART I

ENVIRONMENTAL FACTORS ALTERING THE RESPONSE OF SEEDLINGS TO TRIACONTANOL: RELATIONSHIPS TO GROWTH, WATER STATUS, AND NUTRIENT AVAILABILITY

Abstract

Environmental factors altered the response of seedlings to foliar applications of triacontanol (TRIA). The factors studied were based on preliminary tests in which the growth regulating activity of TRIA in seedling bioassays was dependent on various cultural and environmental conditions. In growth chamber studies, maize seedlings exposed to a 35°C air temperature for 1 hr prior to treatment with TRIA gained more dry weight than those exposed to 25°C prior to treatment. Under greenhouse conditions, TRIA applied at 1300 and 1700 HR but not 0900 HR increased maize seedling dry-weight accumulation. The time of TRIA application was also important in growth chamber studies. Night temperatures from 10°C to 25°C did not alter the magnitude of the response of maize seedlings to TRIA. TRIA did not increase growth of maize seedlings grown in growth chambers at high temperatures (35°C day/30°C night). The temperature and time of day conditions favoring a stimulation of seedling dry weight accumulation by TRIA also resulted in a temporary increase in seedling water potential. The solute potential of the second leaf of 6-day-old maize seedlings, 1 hr after

treatment with TRIA, decreased 0.8 bar. The response to TRIA was also affected by root media and fertilizer treatments. Dry-weight accumulation was not increased by TRIA when maize seedlings were grown in nutrient culture with aerated solution or calcined clay as the root media. In some tests where TRIA applications decreased growth, the response may have been due to higher levels of P in the soil and peat media. Even when the factors which were shown to affect the response were optimized, no protocol could be developed which provided consistent dry-weight increases of "soil"-grown maize seedlings or solution-cultured rice seedlings to TRIA.

Introduction

The development of an aqueous colloidal dispersion formulation of TRIA has provided an application method which has led to more consistent results with seedling bioassays (22,36) and other biological systems (20,24). With the removal of many formulation inconsistancies, the frequency of differences in seedling growth was greater, but the magnitude of the differences varied from test to test (36). This variability was attributed to environmental factors affecting bioassays using greenhouse grown seedlings (36). Temperature, irradiance and relative humidity can fluctuate in the greenhouse diurnally, daily and seasonally and affect the growth of plants.

The first visible effect of TRIA on seedlings is a rapid growth response which is verified by increases in dry weight and leaf area (32,33). In plant tissue undergoing rapid growth, cell expansion is a function of wall loosening and synthesis, influx of water, and accumulation of osmotically active solutes to maintain turgor. Therefore, these processes should be accelerated if growth is stimulated after TRIA is applied. Factors which alter the availability of osmotically active solutes and of water may alter the response of seedlings to TRIA.

Since the accumulation of dry matter that occurs following TRIA treatment is not totally light dependent (33), it is likely a function of solute accumulation due to ion uptake and the hydrolysis of starch and other forms of readily mobile carbon to more osmotically active compounds. One of the first attempts to study the mechanisms of growth stimulation by TRIA showed a rapid increase in succinate and alpha-amino acids in TRIA-treated rice (Oryza sativa) seedlings. There was also increased incorporation of deuterium from D_2O into many metabolites, particulary glutamate and aspartate (11). Further research confirmed the rapid increase in concentration of free amino acids and reducing sugars following treatment of maize and rice seedlings with TRIA (35). These compounds have been shown to be major components of solute accumulation during osmotic adjustment in expanding tissues and drought-stressed plants (26,44). The change in solute potential of a solution can be calculated from changes in the molar concentrations of solutes (26). The change reported for free amino acids and reducing sugars following TRIA treatment (35) on a ug/g dry weight basis could account for a 0.5 to 1.0 bar decrease in solute potential on a whole-shoot basis (assuming 90% water content).

TRIA increased the activity of starch phosphorylase in cell-free supernatents from maize (Zea mays) seedlings and decreased levels of starch in rice and soybean leaves (Glycine max) (19). Decreased starch and increased soluble-sugar levels were also observed in winter wheat (Triticum aestivum) seedlings from TRIA-treated seed (7). Phosphenolpyruvate (PEP) carboxylase (18,19), glucose-6-phosphate dehydrogenase (G6PDH), and isocitrate dehydrogenase (IDH) (23) increase in activity following TRIA treatment of maize seedlings. Although the specific site or type of PEP carboxylase activity was not identified, the primary products of this reaction are organic acids. G6PDH and IDH are regulatory enzymes in the pentose-phosphate shunt and the citricacid cycle, two primary metabolic pathways. Increased activity of these enzymes could result in increases in soluble metabolites.

In previous studies, when rice plants were placed in the dark for 6 hr following treatment with TRIA, the increase in dry weight was the same whether plants were treated at the beginning or end of the 16-hr photoperiod (5). This was interpreted as indicating that immediate products of photosynthesis were not involved in the dark response. However, the increased rate of dry-weight accumulation in the light following TRIA treatment compared to the dark (33) suggests some involvment of photosynthesis. No effect on $^{14}CO_2$ assimilation was measured in barley seedlings, even though growth was stimulated by TRIA (30). Labeling studies with rice and maize seedlings showed no effect of TRIA on the fixation or labeling pattern of $^{14}CO_2$ (19). However, TRIA increased $^{14}CO_2$ assimilation and the movement of photosynthate to the grain of rice plants treated with TRIA 10 days after anthesis (10). No increase in photosynthetic carbon assimilation was measured after the addition of TRIA to cell cultures of _Chlamydomonas reinhardtii_ (14) or root applications to tomato (_Lycopersicon esculentum_) in solution culture (12). Increased growth in these studies appeared to result from a reduction of O_2 inhibition of photosynthesis (12,14). However, in a recent study with TRIA treated _Chlamydomonas_ cultures, no effect on O_2 inhibiton of photosynthesis was found but CO_2 assimilation was increased (20). Increased CO_2 assimilation was mainly accounted for by an increased levels of ribulose-P_2 and in the specific activity of the enzyme ribulose-P_2 carboxylase/-oxygenase (20).

Both N and P nutrition of seedlings are altered by TRIA. It has been speculated that the increase in the uptake of P_i (18,28,31) following treatment could be related to starch hydrolysis and the formation of sugar phosphates (20). Nitrogen and ash content increased when rice seedlings increased in dry weight in the dark following TRIA treatment (5). Nitrogen content also increased with TRIA-stimulated increases in dry weight of rice and maize seedlings (21,43). The uptake of Rb^+ by sorghum (Sorghum vulgare) seedlings increased following TRIA treatment (31). Rb^+ uptake is considered to be an indicator of K^+ uptake, a major ionic contributor to solute accumulation and osmotic adjustment. The activity of Ca^{++}- and Mg^{++}-dependent ATPase in isolated barley (Hordeum vulgare) root membrane vesicles is increased by TRIA (24). Although the specific type of ATPase activity was not identified, it could be an indication of an effect on ion uptake.

Accelerated cell-wall loosening and synthesis and water uptake must also take place if growth is stimulated. Increased growth stimulating activity of TRIA when formulated with IAA and NAA has been reported (45,47), but was not substantiated by others (36). Increased ATPase activity in TRIA-treated barley root membrane vesicles (24) could be associated with proton pumping and the acidification of the cell wall associated with wall loosening and growth.

Increased water uptake by rice seedlings was one of the first effects of TRIA reported (32). Water availability and the water status of treated plants may regulate the degree of response to TRIA. The sensitivity of seedling growth to small changes in water potential and factors influencing water potential has been documented (2,6,9). Leaf expansion of maize, sunflower (Helianthus annuus), and soybean seedlings decreased rapidly over changes of 0.5 to 1.0 bar water potential (6). While growth can be maintained under conditions of continued water stress by osmotic adjustment (26,27), short term water stress may result in a decreased growth rate temporarily, until the stress is relieved (2). The diurnal fluctuation in water potential of exposed leaf tissue even under conditions of adequate soil moisture (1,9) is an example of a small, temporary stress which could limit growth under greenhouse conditions commonly used for seedling bioassays with TRIA.

The objectives of this research were to identify cultural and environmental factors which alter the response of seedlings to TRIA. Temperature, time of day of application, plant water status and nutrient availability altered the response of maize seedlings to TRIA. It is proposed that TRIA promotion of growth and dry-weight accumulatiom will be greater under conditions favoring expansive growth.

Materials and Methods

The methods used in this study were similiar to those used for the initial identification of the growth-regulating activity of TRIA (32) and in subsequent studies with TRIA (5,33-36,38). Rice seedlings in solution culture and maize, tomato, soybean, and barley seedlings in pot culture were treated with foliar applications of TRIA. In some experiments, seeds of these crops were soaked for 1 hr in dichloromethane (DCM) containing TRIA. Maize and rice seedlings were used as the primary bioassays.

Maize seedlings. Maize seedlings (Cv. Pioneer 3780) were grown in a lapped glass greenhouse in pot culture. The root media varied over the course of this study but were primarily a mix of sandy loam field soil and course sand, peat, perlite, or vemiculite to provide good aeration, waterholding capacity and minimal fertility. The media was steam pasteurized following mixing. Eight seeds were sown per 18-cm clay pot and after the development of 2-3 leaves (5 to 7 days), the seedlings were thinned to the 4 most uniform per pot. Pots were then grouped according to plant size into blocks. Pots were fertilized with 250 ml of a solution containing 1.0 g/liter of a soluble 20N-8.6P-16.6K fertilizer 6 to 24 hr before treatment and then every 3 days. Foliar applications of TRIA were made under the planned conditions and the seedlings were grown for 4 to 6 days prior to harvest and oven drying of shoots (70^{o}C). Shoot dry weight was used as the primary measure of growth.

Photosynthetic photon flux (PPF) of mid-day ambient sunlight in the greenhouse ranged from 1000 to 1200 $\mu mol s^{-1} m^{-2}$ during summer months to 100 to 200 $\mu mols^{-1}m^{-2}$ during cloudy winter days. Plants were generally grown with 200 to 300 $\mu mols^{-1}m^{-2}$ supplemental irradiance from 1000-W multi vapor metal halide lamps for 16-hr per day. Plants were also grown under 150 to 200 $\mu mols^{-1}m^{-2}$ from 400-W high pressure sodium (HPS) lamps or 100 to 150 $\mu mols^{-1}m^{-2}$ from 1500-ma cool white fluorescent lamps in some tests. Day and night air temperatures fluctuated from season to season but generally were maintained at 28-30^{o}C day temperature (0600 to 2200 HR) and 20-22^{o}C night temperature. Plants in 1 m^2 reach-in growth chambers were grown with 300 to 400 $\mu mols^{-1}m^{-2}$ from 1500-ma or 800-ma cool white fluorescent and 60-W incandescent lamps with a 30$\pm$2oC, 16-hr day and 25$\pm$2^{o}C 8 hr night. Humidity and CO_2 were not controlled.

Rice seedlings. Rice seeds (10 g) were surface sterilized with mercuric chloride (100 mg/100 ml water) and planted in sterile calcined clay in 500 ml polyethylene cups. The clay was kept saturated with distilled water until transplanting. After 9 to 10 days the largest seedlings were transplanted to solution cultures in 220 ml cups wrapped in aluminum foil. Sponge foam discs were used to suspend 4 plants above the solution in each cup. Hoagland's solution number 2 (pH 4.5-5.0) at 1/4 strength was used initially but changed to 1/2 strength after 2 to 3 days. Solutions were renewed every 2 to 3 days thereafter. The plants were grown in growth chambers with 350 to 400 $\mu mols^{-1}m^{-2}$ from fluorescent and incandescent lamps for a 16-hr photoperiod. Air temperature during the light period was maintained at 30+2^{o}C and at 20+2^{o}C during the dark period.

Approximately 1 week after transplanting, or just before the emergence of the fourth leaf, the plants were sorted visually for size. The plants were sorted by size a second time the next day, grouped into blocks, and randomly assigned treatments. Short term, 24-hr growth tests were conducted with individual plants loosely placed in test tubes (15x2.2cm) with 15-30 ml of fresh nutrient solution. The roots were exposed to light, and a large portion of the stem was below the rim of the test tube for the 24 hr period during the test. After 24 hr the plants were harvested and oven dried at 70^{o}C. Plants grown for 3 days after treatment with TRIA were maintained in culture cups, with one cup containing 4 plants as an experimental unit. Oven-dried samples were weighed after equilibration with the laboratory environment and dry weight was used as a measure of growth.

Environmental conditions and plant water status. Temperature was measured with copper constantine thermocouples or a shielded, aspirated thermograph (Weather Measure model 611T). PPF was measured with a Licor model Li-185 and a quantum sensor. Water and solute potential were measured on 7-mm leaf disks using a Wescor model HR-33(T) dewpoint hygrometer and C-52 sample chambers (Wescor, Inc., Logan, UT). Samples were placed in the chambers in the test environment and allowed to equilibate (2 to 4 hr) under laboratory conditions. Leaf samples for solute potential were tightly wrapped in foil and frozen in liquid nitrogen. Equilibation time for solute potential samples was 1 to 2 hr. Pressure potential (turgor) was calculated as the difference between water and solute potential. Water potential of whole shoots was measured with a pressure chamber (model 3000, Soilmoisture Equipment Corp., Santa Barbara, CA). Maize plants were cut with a razor blade just above the first root and placed immediately in the chamber. Pressure was raised at the rate of 0.1 to

0.2 bar per second and most readings were complete within 60 seconds after cutting.

TRIA formulation and application. All TRIA used in these studies was provided by Proctor and Gamble Co., Cincinnatti, Ohio and was formulated as a colloidal dispersion. The purity of the TRIA was generally greater than 97.7% tallow alkyl sulphate (TAS) or sodium octedecyl sulphate (SOS) were used as dispersants in the stock formulations at 1% of the TRIA concentration. Stocks of TRIA dispersions were kept sterile to prevent breakdown of the dispersion and were autoclaved when necessary at 130°C and 150 KPa for 20 min. Limited autoclaving does not alter the activity of the colloidal dispersion (36). The occurrence of contaminants in the diluent or in sprayer components can affect the biological activity of TRIA (38). Stock TRIA dispersions were diluted with either tap water, distilled water or hexane-washed distilled water. Most TRIA applications were made with a hand-held polyethylene aerosol sprayer.

Experimental design and statistical procedures. A randomized complete block design was used in all experiments. The blocks were used to account for plant size at the start of the experiment and temperature and light gradients of the growth environment during the experiment. Most tests had 6 to 8 replicates and were repeated over time. All results were subjected to analysis of variance including trend analysis when applicable. Mean seperation was by Fisher's least significant difference (LSD) when the F value was significant at the 5% level or by relevent single degree of freedom F-tests.

Results and Discussion

Environmental variables. In preliminary tests, the effect of TRIA on maize seedling growth was tested in the greenhouse or outside in an area between 2 greenhouses. Temperature and irradiance level were not strictly defined or controlled. The response to TRIA was dependent on the location or environment of the test and the combination of natural and supplemental irradiance. In tests treated in the greenhouse during the summer months, TRIA increased dry weight accumulation of plants grown with no supplemental irradiance or under fluorescent lamps but did not stimulate growth of plants grown under HID lamps (Table 1, Test 1). In another experiment, TRIA increased growth of plants in the greenhouse, decreased dry weight accumulation of plants outside under full sun and had no effect on plants grown outside but shaded with saran (Table 1, Test 2). The greenhouse was shaded during summer months and cooled with evaporative coolers. The midday temperature, however, often exceeded 35^{o}C on bright sunny days. The 1000 W metal halide HID lamps emmitted a significant amount of long wave radiation which increased both leaf and media temperatures (by as much as 5^{o}C greater than fluorescent lamps) and the rate of water loss. When these tests were repeated during the winter months, however, the best response to TRIA was with plants grown under HID lamps (Table 1, Test 3).

In another test in July, only plants grown under fluorescent lamps after treatment increased in dry weight with TRIA treatment (Table 2, Test 1). When the test was repeated in October, TRIA

Table 1. The effect of location and irradiance level of the growth environment on the response of maize seedlings to 1.0 µg/liter TRIA. (14-day-old plants, 7 days after treatment)

Treatment		Dry wt (mg/shoot)[z]		
Location, Irradiance	TRIA	Test 1[y]	Test 2	Test 3
Greenhouse, Ambient	0	460	434	-
	+	496	487	-
Greenhouse, +Fluorescent	0	588	-	485
	+	639	-	583
Greenhouse, +HID	0	784	-	610
	+	744	-	707
Outside, Ambient	0	-	500	-
	+	-	462	-
Outside, Shaded	0	-	317	-
	+	-	315	-
LSD 5%		41	33	76
1%		55	45	NS

[z]F value for the interaction of growth environment with TRIA treatment was significant at the 1% level for Test 1 and 2.

[y]Average of 2 tests.

Table 2. Response of maize seedlings to 1.0 μg/liter TRIA when grown in the greenhouse with supplemental irradiance from different lamps. (13-day-old plants, 7 days after treatment)

Treatment			Planted 7/22		Planted 10/2	
Lamps before Treatment	Lamps after Treatment	TRIA	Dry wt (mg per shoot)	Increase over Control (%)	Dry wt (mg per shoot)	Increase over Control (%)
Fluorescent	Fluorescent	0	480		338	
Fluorescent	Fluorescent	+	550	15	370	9
Fluorecsent	HID	0	855		418	
Fluorecsent	HID	+	837	-	482	15
HID	Fluorescent	0	725		342	
HID	Fluorescent	+	775	7	374	9
HID	HID	0	1103		438	
HID	HID	+	1105	-	500	15
LSD 5%			NS[z]		40	
1%					53	

[z]F value for the interaction single degree of freedom comparing the response of plants held under fluorescent lamps after treatment with plants under HID lamps after treatment was significant at the 5% level for the first test and the 1% level for the second test.

increased the dry weight accumulation of plants grown under HID lamps after treatment (Table 2, Test 2). Plants were larger under HID lampsfor both experiments. Both light quality, quantity, temperature, and perhaps some unknown factors varied between these treatments. However, it appeared that temperature and the level of ambient solar radiation in combination with the supplemental irradiance altered the response to TRIA. It should also be noted that control of the greenhouse environment during these preliminary experiments was not adequate. Only 1 temperature set point (30°C) could be designated so night temperatures varied with the outside air temperature. Proper environmental controls were installed and greenhouse temperatures were monitored constantly during the remainder of the experiments.

Some tests of the effect of temperature on the response to TRIA have already been reported (36). Those experiments indicated that higher temperatures before treatment favored an increase in dry weight accumulation after treatment with TRIA but temperature after treatment did not affect the plant response to TRIA. This pattern was evident whether plants were held at the different temperatures in the light or dark. Rice seedlings held at different temperatures responded similarly whether TRIA was applied to the leaves or roots (36).

In further tests with maize seedlings in growth chambers, the effect of temperature before treatment was evident if plants were moved from a 25°C day temperature to 35°C for 64 min but not 4 or 16 min before treatment (Table 3, Test 1). The effect of reduced pretreatment temperature was also evident if plants were moved from a 35°C day temperature to 25°C before treatment (Table 3, Test 2). Moving plants

Table 3. Effect of growth chamber temperature before and after treatment with 1.0 μg/liter TRIA on the dry weight accumulation of maize seedlings. (1. 14-day-old plants, 7 days after treatment; 2. 13-day-old plants, 6 days after treatment)

1. Grown at 25°C day/15°C night					2. Grown at 35°C day/25°C night				
TRIA	min at 35°C Before	min at 35°C After	mg[z] per shoot	% over Control	TRIA	min at 25°C Before	min at 25°C After	mg[y] per shoot	% over Control
0	64	0	356	-	0	0	0	363	-
+	0	0	386	8	0	60	60	363	-
+	4	0	390	10	+	0	0	408	12
+	16	0	384	8	+	15	0	388	7
+	64	0	439	23	+	60	0	383	5
+	64	4	432	21	+	60	60	357	2
+	64	16	414	16	+	0	15	402	11
+	64	64	418	17	+	0	60	425	17
LSD 5%			27					34	
1%			36					46	

[z]F value for the single degree of freedom comparison of the 4 TRIA treatments held at 35°C for at least 64 min with the 3 TRIA treatments held less than 64 min at 35°C was significant at the 1% level.

[y]F value for the single degree of freedom comparison of the 3 TRIA treatments held at 25°C before treatment with the 3 TRIA treatments not at the lower temperature before treatment, was significant at the 1% level.

from higher temperatures before treatment to lower temperatures after treatment favored an increase in dry weight. Moving plants from a lower temperature before treatment to a higher temperature after treatment reduced or cancelled the effect of TRIA.

Based on temperature and irradiance studies, it was postulated that the time of day of treatment might alter the response to TRIA. Maize seedlings were treated with TRIA at 3 times of the day in the greenhouse, growth chambers and outside. Under greenhouse conditions with supplemental irradiance from HID lamps, TRIA applied at 1300 and 1700 HR increased dry weight accumulation (Table 4). TRIA applied at 0900 HR did not alter the dry weight of maize seedlings. Under growth chamber conditions, the only difference in dry weight obtained was when TRIA was applied at 1300 HR, even though light and temperature were constant during the photoperiod (Table 4). Plants grown in pot culture outside and treated with TRIA accumulated less dry weight than the control at all 3 times (Table 4). Air temperature, soil temperature, and irradiance were measured for each location at each treatment time. Irradiance levels were highest at 1300 HR and air temperature was similar at 1300 and 1700 HR in the greenhouse and outside. Daily fluctuations in minimum and maximum temperature were greater and light levels were higher outside. All plants were started in the greenhouse and moved to the other 2 locations the day before treatment until harvest. Two other experiments in the same location outside, but with plants grown outside from planting, resulted in no treatment differences (data not shown).

Table 4. Effect of time of day of treatment with 0.1 µg/liter TRIA on the dry weight accumulation of maize seedlings grown in 3 different environments. (12-day-old plants, 6 days after treatment)

Time (HR)	TRIA	Dry wt (mg/shoot)		
		Greenhouse[z]	Growth Chamber[y]	Outside[x]
Control	0	762	558	543
0900	+	797	597	509
1300	+	820	617	520
1700	+	843	585	519
LSD	5%	44	44	14
	1%	NS	NS	19

[z]F-value for the linear trend was significant at the 1% level. Each value is the mean of 3 experiments of 4 replicates each.

[y]F-value for the quadratic trend was significant at the 5% level. Each value is the mean of 3 experiments of 4 replicates each.

[x]Each value is the mean of 2 experiments totaling 10 replicates.

A consideration in interpreting the time of day effect was whether or not the effect was a result of a circadian rhythm. The activity of certain herbicides is dependent on time of treatment during the photoperiod in growth chambers, and in some cases this effect was entrained or occurred if the plants were held continuously in the dark after one photoperiod (39). Plants for these tests were all initially grown under greenhouse conditions. The plants were then moved to either growth chambers or outside, the day prior to treatment with TRIA. The start of the photoperiod was similar under greenhouse and growth chamber conditions but the optimum time of day for treatment was different for the 2 locations.

To test if differences in night temperature could alter the response to TRIA or the optimum time for TRIA applications, maize seedlings were placed in growth chambers with a 25°C or 15°C night temperature either the night before treatment, the night after treatment or both. There was no difference in the response to TRIA due to night temperature, and in each case, treatment at 1200 HR was better than at 1600 HR (Table 5). In a second set of tests, maize seedlings were grown at 30°C day temperature and 25°C or 10°C night temperature the night before and continuously after treatment. TRIA applied at all 3 times increased dry weight but with a 25°C night the response was greatest at 0900 and 1300 HR and with a 10°C night the response was greater at 1300 and 1700 HR (Table 6). Colder night temperature did not alter the magnitude of the response to TRIA.

Table 5. Response of maize seedlings to 1.0 μg/liter TRIA at 2 times of day averaged over 4 night temperature treatments for the night before and/or after TRIA treatment[z]. Each value is the mean of 2 tests with 4 temperature regimes and 8 replicates each. (13-day-old plants, 6 days after treatment)

Treatment		Dry weight	
Time (HR)	TRIA	mg/shoot	% over Control
-	0	521	-
1200	+	585	12
1600	+	545	5
LSD 5%		20	
1%		27	

[z]Plants were placed in growth chambers at the begining of the dark period (2200 HR)) when 6 days old and given either 25°C or 15°C nights. TRIA was applied during the next photoperiod and plants remained in growth chambers the following dark period. Half of the plants at each night temperature were moved to the other night temperature. Plants were moved to the greenhouse at 1000 HR the following day and grown for 6 days.

Table 6. Effect of 1.0 μg/liter TRIA applied at different times of day on the growth of maize seedlings grown in growth chambers at 30°C day temperature and 25°C or 10°C night temperatures. (13-day-old plants, 6 days after treatment)

Treatment		Night temperature[z]			
		25°C		10°C	
Time	TRIA	Dry wt (mg/shoot)	% over control	Dry wt (mg/shoot)	% over control
Control	0	570	-	437	-
0900	+	667	17	487	11
1300	+	693	22	520	19
1700	+	627	10	510	17
LSD 5%		33		33	

[z]F value for the interaction of night temperature with time of day was significant at the 5% level. The analysis was for 12 replicates over 2 tests.

Maize seedlings were also grown under 4 different day, night temperature regimes to test if temperatures altering the growth rate, altered the response to TRIA. TRIA increased the growth of maize seedlings in 3 of 4 temperature regimes (Table 7). The highest temperature regime was above the optimum for growth of maize seedlings and there was no response to TRIA. There was a stimulation of growth by TRIA when growth was limited by low temperature but not when growth was limited by high temperature. Results with seedlings grown from TRIA treated seed showed the opposite temperature response (34).

A generalization that faster growing plants or larger plants show greater stimulation of dry weight accumulation by TRIA does not adequately explain the results of these tests. For example, plants grown under HID lamps were always larger then plants grown under other lamps in the greenhouse, however, the response to TRIA was not always greatest under HID lamps. Treatment at different times of day and different pretreatment temperatures altered the response to TRIA. Apparently factor(s) other than long term growth rate before and after treatment alter the response to TRIA.

Water status. One hypothesis to explain these results is that plant water status at the time of and after treatment is affecting the plant response to TRIA. The effects of water potential on growth of seedlings under conditions comparable to those used in these tests have been studied (6,9,27). Expansive growth is perhaps the metabolic function most sensitive to changes in water potential.

The water potential of maize seedlings was measured as plants were placed at different temperatures in growth chambers prior to treatment

Table 7. Response of maize seedlings grown in growth chambers at 4 different temperature regimes to 1.0 μg/liter TRIA. (17, 15, 13, and 12-day-old plants, 7 days after treatment)

Temperature (^{o}C)		TRIA	Dry weight	
Day	Night		mg/shoot	% over control
20	15	0	260	
		+	287	11
25	20	0	367	
		+	410	12
30	25	0	527	
		+	583	11
35	30	0	365	
		+	373	2

[z]F value for the single degree of freedom comparison of the control versus TRIA at the highest temperature with the average of the control vesus TRIA at all other temperature regimes was significant at the 5% level.

and then returned to greenhouse conditions. Plants were moved from a 25°C greenhouse to a 35°C or 15°C growth chamber for 1 hr and then back to the 25°C greenhouse. When plants were moved to a 15°C chamber, the water potential increased. The water potential of plants moved to a 35°C chamber decreased (Fig. 1). After 1 hr at a given temperature, the temperature of the root medium approached the air temperature. Returning pots to the greenhouse after this time resulted in a temperature differential between the shoot and root zone which affected water status. When the plants were moved back to the 25°C greenhouse (1600 HR), the water potential of plants held at 15°C for 1 hr decreased, presumably due to cold root zone temperatures (16-17°C) which could have limited water uptake. The water potential of plants held at 35°C for 1 hr increased, presumably due to higher root zone temperatures (32-33°C) and reduced transpirational demands in the greenhouse compared to the high temperature chamber. Plants held at 35°C before treatment had water potentials favoring growth after treatment with TRIA and had the greatest response to TRIA.

Under greenhouse conditions, maize seedling water potential decreased from early morning until 1500 to 1600 HR, when as solar radiation and air temperature decreased, water potential increased. Leaf solute potential was not different from 1300 HR to 1700 HR and was approximately 0.5 bar higher at 2100 HR. The value of seedling water potential between midday and late afternoon ranged within the values where leaf expansion of corn seedlings is most sensitive (-4 bars midday to -2 bars early evening) (6). As in the temperature test, conditions of increasing water potential after treatment corresponded

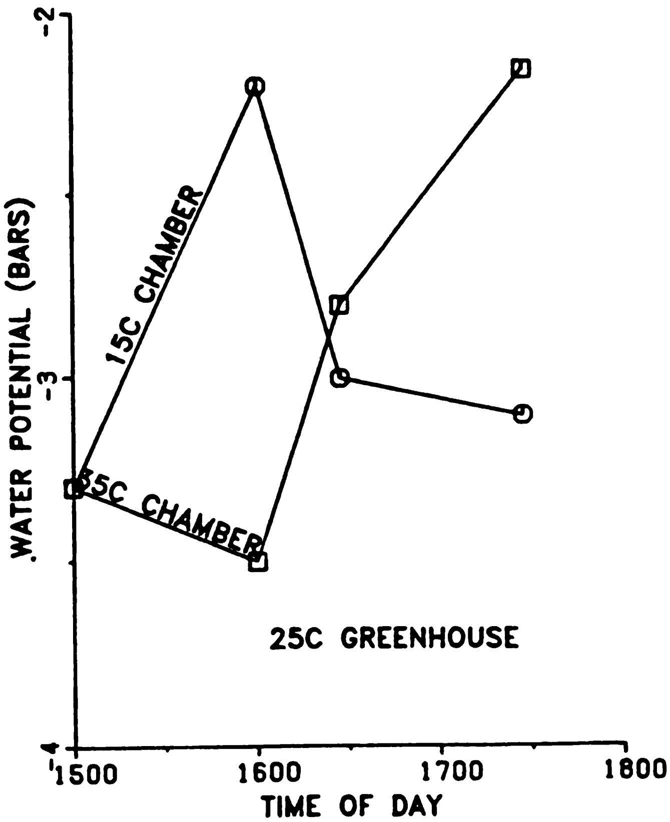

Figure 1. Change in water potential of maize seedlings moved from the greenhouse to growth chambers at 15°C or 35°C for 1 hr and then returned to the greenhouse.

to conditions where TRIA increased the dry weight accumulation of maize seedlings.

Water and solute potential, and stomatal conductance under growth chamber conditions showed only small differences during the 16-hr photoperiod. Dry weight of seedlings treated the same day as water status measurements showed no stimulation by TRIA at any time. In a later test, water and solute potential of untreated plants were measured during the 16-hr photoperiod and again showed only a small linear decrease over time (Table 8). A fourth TRIA treatment was applied at 2130 HR, 30 min before the light and temperature changed. This treatment resulted in the only difference in dry weight among treatments (Table 8). Water potential increased rapidly and water of guttation was visible on the plants within 10 to 15 min after the light and temperature were changed. This experiment was conducted during a period when the increased growth response to TRIA under greenhouse conditions could not be obtained for unknown reasons. This may account for the lack of response at 1300 HR as shown in previous experiments.

Midday water potential of seedlings in the greenhouse under different levels of supplemental irradiance from different lamps, was lower for seedlings grown under HID lamps than those grown under fluorescent lamps. The difference was greater when ambient solar radiation was high, or on a sunny day as opposed to a cloudy day. Air, soil, and leaf temperatures were also higher under the HID lamps.

During the course of this study, TRIA caused small but significant reductions in dry weight accumulation of corn seedlings in some tests.

Table 8. Response of maize seedlings to TRIA applications and water, solute and pressure potential of untreated plants at different times during the photoperiod under growth chamber conditions. (9-day-old plants, 3 days after treatment)

TRIA Treatments			Water Status at treatment[y] (bars)			
Time (HR)	TRIA	Dry wt[z] (mg per shoot)	Time (HR)	Water Potential	Solute Potential	Pressure Potential
Control	0	548				
0900	+	561	0900	-3.0	-8.1	5.1
1300	+	551	1300	-3.2	-7.9	4.7
1700	+	560	1700	-3.4	-8.3	4.8
2130	+	579	2100	-3.7	-8.3	4.6
			2215	-1.0	-	-

[z]F value for the single degree of freedom comparison of treatment at 2130 with all other treatments was significant at the 5% level.

[y]Measurements made on untreated plants in the same environment at the time of treatment. Each value is the mean of six determinations.

Several field tests also resulted in significant reductions in yield with TRIA treatment. Evaluation of these tests suggested that water stress may have been a common denominator. Corn and soybean seedling growth was compared when TRIA was applied to seedlings under standard greenhouse conditions and when seedlings were water stressed by withholding water prior to treatment. TRIA increased growth under standard conditions but not under conditions of water stress (Table 9). Seedlings were stressed by withholding water for a single episode just prior to application of TRIA. Seedling water potential reached -5 to -6 bars which was not to the point of wilting. Water and fertilizer applications were identical for the 2 treatments from the day after treatment with TRIA until harvest. Growth was not significantly decreased by the water stress or by TRIA applied to water stressed plants under these conditions.

When placed under a drought stress, plants will allocate more growth potential into the development of roots then shoots (41). One hypothesis which could explain the lack of increased shoot growth when drought stessed seedlings are treated with TRIA, is that TRIA stimulates root growth more than shoot growth. The corn seedling bioassay would not account for this possible effect since only shoot growth was measured. This hypothesis was tested with the rice seedling bioassay. Rice seedlings were treated under "standard" or drought stressed conditions imposed by a KCl or PEG (carbowax 6000) osmoticum. There were no significant effects under the "standard" conditions in these or several other tests with the rice seedling bioassay. In 1 of 3 tests with KCl at a -4 bar osmoticum added to 1/2 Hoagland's, there was a

Table 9. Dry weight of soybean and maize seedlings treated with 0.5 µg/liter TRIA at normal (-3 to -4 bar) or drought stressed (-5 to -6 bars) water potentials.

Soil Moisture	TRIA	Dry wt (mg/shoot)	
		Soybean[z]	Maize[z]
Control	0	443	337
	+	483	357
Drought Stressed	0	423	340
	+	403	337
LSD 5% level		32	13
1% level		NS	17

[z]The F value for the interaction of TRIA with soil moisture was significant at the 5% level.

small decrease in seedling dry weight (control seedlings 63.9 mg, TRIA seedlings 61.6 mg, significant at the 5% level). In 3 tests with a PEG osmoticum, there were no significant differences due to applications of TRIA. Growth was significantly decreased over the 24-hr test period with both the KCl and the PEG osmoticum. In each of these tests and in several independent tests, TRIA was also applied to nonstressed rice seedlings under condition previously reported for TRIA research. Growth stimulation with TRIA was measured in only 2 of 20 tests. The inability to get consistent positive growth responses with non stressed treatments with the rice seedling bioassay indicates that this protocol was not adequate for testing this hypothesis.

The hypothesis that the water status of seedlings altered the response to TRIA is also based on the changes in metabolic activity and the accumulation of osmotically active substrates following treatment of seedlings with TRIA (7,11,19,24,35). A decrease of 0.8 bar (more negative) in solute potential was measured for leaf disks taken from the emerging 2nd leaf of corn seedlings 1 hr after treatment with TRIA (Table 10). Two seedlings matched for size in the same pot were sprayed either with distilled water or distilled water with 0.5 ug/l TRIA. Tests were done in both greenhouse and growth chamber conditions. The measured effects are near the limit of detectable differences with the methods used but were significant in 3 of 4 tests (Table 10). One hr after treatment was selected as a sampling time based on previous rapid effects of TRIA (35).

These data indicate that the response of maize seedlings to TRIA is at least partially dependent on plant water status at the time of

Table 10. Change in solute potential of the expanding region of the first true leaf of 6-day-old maize seedlings 1 hr after treatment with 0.5 μg/liter TRIA.

Treatment	Solute Potential (bars)				
	Test 1 (4 reps)	Test 2 (6 reps)	Test 3 (6 reps)	Test 4 (4 reps)	Average
Control	-9.8	-9.4	-10.3	-9.5	-9.7
TRIA	-10.5*	-10.2*	-10.7	-10.5*	-10.5**

*,** F value was significant at the 5% (*) and 1% (**) level respectivlely.

and following treatment. There was not a clear separation of the degree of importance of temperature and other factors from plant water status. While seedling water potential affects expansive growth, plants can accumulate solutes to maintain pressure potential necessary for expansive growth. Further experiments in which leaf extension rate is compared to the response to TRIA are necessary.

Nutrient availability. As the supply of root media substrates used to grow maize seedlings changed over time, the growth-stimulating activity of TRIA sometimes varied with the corn-seedling bioassay. Preplant addition of fertilizer or premixed soilless media with fertilizer ammendments usually resulted in maize seedling leaf tip necrosis and lack of growth stimulation with TRIA. Problems with leaf tip necrosis were generally overcome by changing and mixing substrates. Field soil, sand, peat, perlite , and vermiculite in various proportions were mixed for good aeration and water holding capacity, minimal fertility and high pH.

Experiments with maize seedlings in soilless root media mixes, aerated solution culture or pot nutrient culture with a calcined clay substrate did not show significant differences in dry weight upon treatment with TRIA (data not shown). With solution culture, both standard Hoagland's solution #1 and #2 were used, starting at one quarter strength and gradually progressing to full strength. Variations in the concentration of complete Hoagland's, concentrations of N and P varied independently, and various ratios of NO_3:NH_4 N were investigated. While growth was altered due to fertilizer treatments, there was no dry weight response to foliar or root applications of

TRIA. Maize growth in nutrient culture and calcined clay was visually comparable to growth in the soil, sand, peat substrate.

Tip burning of maize leaves in some media was associated with conditions favoring high rates of transpiration and in some cases, but not all, higher rates of fertilization. The degree of necrosis was greater on plants grown under HID lamps in the greenhouse. Symptoms first appeared as a dark, water soaked area at the tip of older leaves. The symptoms generally appeared on the first true leaf, when seedlings were 8 to 9 days old or when the 4th leaf was emerging. Tissue necrosis followed in 1 to 2 days, progressing from the tip, down the margins of the leaf. The symptoms were similar to those reported for excessive soluble salts, however, saturated paste soil test values indicated a low to medium level of soluble salts in the root medium (0.5 to 1.4 x 10^{-3} dSm^{-1} or 0.5 to 1.4 mmho).

In several tests designed to test a new lot of root media, TRIA decreased growth of maize seedlings (Table 11). There was a quadratic trend of maize seedling dry weight with TRIA concentration as in experiments with growth stimulation. Both control and treated plants developed tip burning and marginal necrosis as previously described. This media was later found to be contaminated with NaCl, to have low initial P levels (1ppm) and was fertilized with a high P (15N-20P-13K) water soluble fertilizer before treatment rather than the standard fertilizer.

The levels of Na (81 ppm compared to normal values of \15 ppm)and Cl present in the media used for the test in Table 11 did not raise the soil test level of soluble salts (1.4 x 10^{-3} dSm^{-1}) above the

Table 11. Response of maize seedlings in a soil-based root media with high levels of Na and Cl, to different rates of TRIA. (1. 11-day-old plants, 3 days after treatment)

TRIA (μg/liter)		Dry wt (mg/shoot)
control		219
0.01		213
0.10		205
1.00		209
10.0		206
100		216
LSD	5% level	10
	1% level	13

F value for the quadratic trend of shoot dry weight with TRIA concentration was significant at the 1% level.

acceptable range. However, maize is less tolerant to Na than many species (25) and metabolic energy is expended to limit Na uptake. Based on elemental analysis maize seedlings, tissue levels of K were lower and of Na were higher under the conditions resulting in decreased growth. In the case of plants grown in a calcined clay absorbant, which did not show any affect of TRIA in repeated testing under various conditions, high levels of Na were found in the shoots and particularly the roots (˜5000 ppm). TRIA had no effect in experiments testing the effect of pre plant incorporation of N and P fertilizer salts in the K, Ca, or Na form on the response of maize to TRIA (data not shown).

Leaf tip necrosis was also evident in plants grown in a commercial peat-lite root media which had P fertilizers incorporated. A sample of the media had an initial soil test value of 40 ppm P where a value of 20 ppm is considered very high. Floride is a common contaminant of P fertilizer and the leaf necrosis symptoms were similar to those reported for F toxicity (42). Raising the pH of the root media and addition of charcoal have been shown to decrease the uptake of F (42). Raising the pH of this media and increasing the concentration of regular fertilizer applications resulted in increases in maize seedling growth with TRIA treatment (Table 12). TRIA decreased the growth of plants grown in this media without any fertilizer. Analysis of leaf tissue with and without marginal necrosis showed levels of F in necrotic tissue (127 ppm) were 4 times higher then in control tissue (30 ppm). Floride was determined colorimetrically with SPANDS reagent after distillation of water extracts of plant tissue. The analysis may

Table 12. Stimulation and inhibition of dry weight accumulation of maize seedlings with 1.0 μg/liter TRIA depending upon fertilization level and pH of a peat based media (3 peat-lite:1 sandy loam). (11-days-old plants, 4 days after treatment)

Fertilizer	TRIA	Dry wt (mg/shoot) Root media pH	
		6.2-6.3	6.8-6.9
None	0	191	180
	+	173	170
1 g/liter water soluble 20N-8.6P-16.6K	0	183	180
	+	184	185
2 g/liter water soluble 20N-8.6P-16.6K	0	188	184
	+	189	202
LSD 5% level		NS	9

be suspect, however, due to the difficulty of removing interfering ions from the sample.

Various salts were added with fertilizer in an attempt to create the leaf tip necrosis. Addition of 250 ml of of 15 mM Cl^- (530 ppm), F^- (285 ppm), NH_4^+ (210 ppm), and Na+ (345 ppm) did not not result in marginal necrosis of corn seedlings. The addition of 15 mM P (465 ppm) from reagent grade KH_2PO_4 and $NH_4H_2PO_4$ or 20N-8.6P-16.6K (4 g/liter) resulted in severe tip burning and marginal necrosis, confirming the earlier observation that tip burning was associated with P fertilizer.

The tip burning and marginal necrosis observed in these maize seedlings were similar to symptoms of P toxicity, particularly in grasses (4,29,40). Levels of 2 to 5% P on a dry weight basis were reported for leaf tissue exhibiting toxicity symptoms. P toxicity was associated with plants grown in soils with a low P fixation capacity and low pH. Studies with clover (Trifolium repens) and oats (Avena sativa) showed that higher media pH and higher levels of NO_3-N fertilizer alleviated the problem (40). In certain cases, toxicity will occur without excessive soil levels of P. If plants are grown under low P levels, and then P applied, there can be a rapid uptake of P to toxic levels (13). Salt stress (NaCl) and high levels of P may also interact resulting in increased uptake of P (3,29).

When conditions of high levels of media P, low pH (pH 5.5-5.7) and moderate levels of NaCl (100 mg/liter media) were created in the commercial peat-lite media used for the tests in Table 12, there was no effect of TRIA on maize seedling dry weight (Table 13). TRIA

Table 13. Dry weight of maize seedlings grown in peatlite media in 10-cm plastic pots and treated with 1.0 μg/liter TRIA or 100 mg/liter gibberellic acid (GA). (12-day-old plants, 6 days after treatment)

	Dry wt (mg/shoot)[z]	
Treatment	pH 7.0 to 7.2 N and K Fertilizer	pH 5.3 to 5.5 P fertilizer + NaCl
0	230	244
TRIA	243	234
GA	247	241
LSD 5% level	12	

[z]F value for the TRIA by amendment interaction was significant at the 5% level. Average of 2 tests with 6 replicates each.

stimulated growth when the same media was limed to a high pH (pH 7.0-7.2) and fertilized with only K and N (no NaCl). This was the first increase in growth due to TRIA in this study for maize grown in a peat based soilless media. Growth of the controls was similar under the 2 pH and fertility conditions.

A foliar application of gibberellic acid (GA_3) was included in this test to measure possible growth stimulation by GA under the different fertility conditions. In 2 experiments, seedlings treated with GA increased in dry weight or showed no effect of GA dependent on the fertility or media ammendments (Table 13). The effect of GA on dry weight was similar to TRIA. Unlike TRIA, however, in both media the GA treated plants appeared taller than the controls, but in only 1 treatment was the dry weight increased.

An increase in P uptake following TRIA treatment has been reported (18,28,31,43). The decreased dry weight accumulation of maize seedlings associated with P fertilization may be related to a) a stimulation of P uptake after TRIA treatment which may reach "toxic" levels, or b) altered carbohydrate metabolism at high levels of tissue P (15), or c) high levels of tissue P creating an osmoticum and water stress on the plant. The level of cytoplasmic P_i plays and important role in regulating carbohydrate movement from the chloroplast (15). High levels of P_i may upset this process. Corn and sorghum in solution culture are sensitive to levels of P generally used in solution culture (15-30 mg/liter) and best growth was obtained at low P levels (1-3 mg/liter) (8).

In several experiments the response to TRIA was altered but overall growth of the controls for each fertilizer treatment was not different. This could account for bioassay conditions where growth is apparently "normal" but stimulation of growth with TRIA is negligible. Problems with various media and fertilizer treatments could be related to water stress. The water potential of corn seedlings was altered by media and fertilizer treatments in some tests. Excess moisture and water soluble fertilizer in the root media under high light and temperature decreased water potential similar to lack of water.

Conclusions

Environmental variables altered the response of seedlings to TRIA. Evidence presented supports the hypothesis that environmental conditions favoring temporary increases in water or pressure potential enhance the TRIA stimulation of seedling growth. Conditions imposing a water stress limited the effects of TRIA on dry weight. The environmental conditions identified which altered the response to TRIA only favored or enhanced the frequency of obtaining a stimulation of growth. They did not increase the magnitude of the response significantly more than initially reported (32). The response of maize seedlings to TRIA was dependent on the root media used to grow seedlings for the bioassay. Growth of maize seedlings grown in nutrient culture with solution or calcined clay as the root media was not altered by TRIA. In some experiments where TRIA application decreased growth, the response may have been due to higher levels of P in the root media. Even with the incorporation of temperature, time, water and nutrient conditions identified as favoring a growth response to TRIA, consistent results could not be obtained.

Based on experiments in which the response to TRIA was altered by environmental conditions, I hypothesised that factors favoring expansive growth would favor the response to TRIA. Plant water potential affects expansive growth and was altered by the temperature and time of day conditions which altered the response to TRIA. However, expansive growth is influenced by more factors than seedling water potential. The pressure potential neccessary for expansive growth can be maintained at low water potentials by solute accumulation. Low temperatures can also limit expansive growth even under conditions of high pressure potential. Light transitions, night temperatures (9), and N and P nutrition can also affect leaf expansion. Experiments must also be conducted to separate the effect of environmental conditions on the potential for expansive growth from the effects on drying time and penetration of TRIA.

To accurately test this hypothesis, it is necessary to have a protocol in which applications of TRIA consistently result in greater growth of treated plants than the control. Despite extensive effort, there is still not a simple reproducible method for testing the effect of TRIA on whole plants, that works in the hands of all researchers. During certain periods of this research differences in growth with TRIA treatments were measured in a majority (60 to 70%) of tests. There were also periods when only 10 to 20% of tests resulted in differences in growth with TRIA treatments. While several potential problems have been identified, there are obviously still undefined variables. My interpretation of this research is that the problem(s) or lack of consistent effect is related to nutritional and water status factors altering the growth of seedlings. An understanding of how these conditions alter the response to TRIA may help us elucidate the way that TRIA regulates plant growth.

The inhibition of growth in some tests, with the same concentrations that stimulated growth in other tests or conditions, is likely an important clue to discovering the factor(s) responsible for inconsistencies with TRIA research. Inhibition of growth has been reported in some other studies (16,17), sometimes associated with higher rates of TRIA. The observations relating to water stress and inhibition of growth by TRIA in this study may have some bearing on the report that TRIA increased growth of a drought tolerant line of sorghum but decreased growth of a drought intolerant line of sorghum (31).

The factors identified in this research may also alter the affect of TRIA on the yield of crops. Cultural and environmental variables including the time of day and conditions at treatment were investigated in yield trials under field conditions and the results are presented in Part II.

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PART II

FIELD STUDIES WITH CROPS TREATED WITH COLLOIDALLY DISPERSED TRIACONTANOL

Abstract

The yield response of crops to triacontanol (TRIA), formulated as a colloidal dispersion, was tested with 13 crops in 45 field experiments over a 3-year period. Foliar application of TRIA resulted in treatment effects with 11 of the 13 crops and in 31 of the 45 experiments. The average yield increase was 13% at the optimum TRIA concentration in tests with positive yield responses and was 5 % over all 45 experiments. In 7 experiments, significant yield decreases averaging 12% were measured with the same concentration that increased crop yield in other tests. The most active concentrations were generally 0.1 to 1.0 µg/liter but significant effects were measured at 0.01 and 1000 µg/liter. No general stage of crop development for treatment was optimal for all crops. Yield increases were most likely when TRIA was applied mid to late afternoon, on warm sunny days, when environmental stress was not limiting growth. Based on the results of these studies, TRIA cannot be recommended for commercial application to crops.

Introduction

A potential for increasing yield with TRIA has been demonstrated, however, the lack of consistent effects has not allowed its commercial use as a yield-enhancing plant growth regulator (PGR) (32). New field tests with TRIA should consider the availability of new methods of formulating TRIA and the identification of factors affecting the activity of TRIA on seedling growth. Formulation of TRIA, which has a very low solubility in water, is believed to be one of the poorest controlled variables in TRIA research (14). In most previous field research, TRIA precipitated from chloroform or acetone when the solvent was mixed with water, forming a suspension of particles. The particles in this suspension are unstable and variable in size. Formulation of TRIA as a colloidal dispersion gives smaller and more uniform particles. This was believed to be an important discovery which might eliminate inconsistencies of yield enhancement with TRIA (14). In laboratory and greenhouse studies using this formulation, TRIA had more consistent activity and was active at lower concentrations (14,31).

Application time and the temperature preceeding application alter the growth-stimulating activity of TRIA as measured by seedling dry weight accumulation under controlled environment conditions (31). These factors may affect the response of crops to TRIA under field conditions. It is also necessary to avoid interference by other chemicals in application water or systems due to the molecular nature of TRIA and the low application rate (12,33). When dilutions of the colloidal dispersion formulation of TRIA were collected after passing through various agricultural sprayers used for TRIA research, there was a reduction of seedling-growth-stimulating activity (33).

The objectives of this research were to test the efficacy of colloidally dispersed triacontanol in yield trials under field conditions and to identify cultural and environmental factors which could alter the response of crops to TRIA. TRIA concentration, application time during the day, weather variables and stage of crop development at treatment were investigated. The experimental methods and parameters studied were also a continuation of experiments conducted during the first 4 years of field research with other formulations of TRIA at Michigan State University (18,27). TRIA did not consistently increase crop yield. Based on the analysis of 45 experiments, treatment conditions favoring yield enhancement by TRIA are outlined.

Literature Review

Field research at Michigan State University (MSU) during the 4 years (1977 to 1980) following the discovery of the growth-regulating properties of TRIA indicated many inconsistencies in response. The first year of trials in 1977 tested the effect of TRIA on 10 crops. Yield increases ranged from 7 to 22% and averaged 12% for the 7 crops responding to foliar applications (27,29). There was no effect from seed treatment or root treatment. There was a response to foliar sprays ranging in TRIA concentration from 0.1 to 100 mg/liter, with no clear optimum rate, and there were generally no visual differences in growth in cases where yield was increased significantly (29). Of the 5 crops treated by foliar applications in 1978, only cucumber (Cucumis sativa) clearly responded to TRIA. There was a 13% increase in crop dollar value. Small effects of TRIA on the yield of wheat (Triticum aestivum) were dependent on cultivar and location of the test. Sweet corn (Zea mays) showed similiar small effects dependent on TRIA concentration and the rate of NH_4NO_3 fertilizer applied. Second year conclusions were that single applications were as good as multiple applications and there was no apparent optimum growth stage for TRIA application. Experiments with carrot (Daucus carota) and sweet corn were interpreted as showing better responses to TRIA under low N fertilizer levels (27). Concern was expressed (27) that lower volumes of spray were applied in the field in 1978 then 1977 and that greenhouse tests indicated a high volume and complete plant coverage were necessary (12).

Field tests were continued in 1979 with the chloroform/Tween 20 formulation and in 1980 with an acetone formulation (18). TRIA increased wheat yield 7% with a high spray volume (1470 liters/ha) but not with a lower spray volume (470 liters/ha). Sweet corn yield was increased 22%, and cucumber value 18%, but there was no effect on yield of tomato (Lycopersicon esculentum) transplants treated before or after transplanting in 1979. Tests in 1980 showed no effect on 5 cool season crops, no effect on sweet corn or cucumbers grown at 2 locations and with different N and P levels, no effect on tomato and muskmelon (Cucumis melo) transplants which suffered early chilling damage, and both increases and decreases in the yield of wheat dependent on location of the test and formulation of TRIA. No specific optimum stage of crop development or optimum concentration for TRIA application was identified. It was also concluded, with little supporting data, that there was a differential trend in response of corn and tomato to TRIA when grown at different levels of P fertility in the field. Yield was increased by TRIA applications in approximately 40% of experiments with the chloroform/Tween 20 formulation and 10% with the acetone formulation (18).

In cooperation with Michigan State University, American Cyanamid Company conducted extensive field studies during 1977-79 to determine the potential use of TRIA as a plant biostimulant (2). An aqueous formulation containing TRIA, the triethanolamine salt of oleic acid (1.05% w/w) as an emulsifier and Triton X-100 (2.8%) as a surfactant was used. This formulation was active in greenhouse and field experiments at Michigan State University. However, with the exception of 1 test with rice (Oryza sativa) in Japan, in 46 tests with 10 crops in 20 locations around the world there was no significant yield enhancement.

The Biochemical Research Corporation has done extensive field testing of various formulations of TRIA disolved in organic solvents, particularly acetone, and then diluted with water (35,36). They reported increases of 15 to 75% in yield of several crops, dependent on the addition of metallic salts of the Hoffmeister series and other hormones to the TRIA formulation. Results of their studies are available only in abstract form or in patent applications. The methods used in these field studies have not been subject to critical review. Their formulation was found to be no more effective than other formulations in promoting seedling growth (31).

Several investigators have tested TRIA on field corn. Based on 3 years of research, a patent was filed for a method of increasing field corn yield with TRIA, claiming that the stage of crop development at the time of application was critical (21,22). Application rates of 2 to 56 mg per acre were equally effective in increasing yield 4 to 6% (0.4 to 1 MT/ha) when applied when the unemerged tassle was approximately 1 to 2 cm as opposed to when the tassle was 8 to 10 cm in length. Both chloroform/Tween 20 and acetone TRIA formulations were used in this study. Others reported TRIA increased corn yield 3 to 7% but found that time of TRIA application was not critical as long as it was applied during vegetative stages (23). In another study, TRIA did not affect field corn yield in 3 of 4 experiments with 2 corn cultivars for 2 years (4 experiments) under irrigated and nonirrigated conditions (26). Where yield was affected, there was an increase in irrigated plots and a decrease of the unirrigated plots. There was no effect of TRIA on the yield of hydroponically grown field corn (8).

Other reports of TRIA's effect on wheat, tomato, sugar beet (Beta vulgaris), tabasco pepper (Capsicum frutescens), muskmelon, sweet potato (Ipomoea batatas) and cotton (Gossypium hirsutum) have been published. TRIA applied as a soil drench increased the yield of tabasco pepper (17). Foliar sprays of the chloroform/tween 20 formulation increased the yield of tomato (7,34) and sugar beet (24) grown in pot culture but had no efffect on the yield of sweet potato (5), muskmelon (4) or winter wheat (3) in the field. Flower production and lint yield of cotton were increased by applications of TRIA formulated as a colloidal dispersion (6).

There are a number of reports from Asia of significant yield enhancement with TRIA. A recent review (32) of some of these studies, noted that yield was increased 10 to 20% with many crops treated with several different formulations of TRIA isolated from beeswax and synthetic TRIA. Several possible explanations for the apparent discrepancy in success ratio between regions of the world were considered (33) resulting in the conclusion that formulations were not significantly different, but that application methods could have had some effect. Chemical and organic contaminants from agricultural chemicals and application equipment in the United States may inactiviate the small amount of TRIA applied to stimulate growth (33).

Materials and Methods

Cultural Procedures. Cultural practices were similar to standard commercial production practices in Michigan and to those reported previously (18,26). Experiments with field crops and asparagus (*Asparagus officinalis*) were set out in uniform sections of large production areas either at MSU farms or on private farms. Cucumber, snap bean (*Phaeseolus vulgaris*), lettuce (*Lactuca sativa*), sweet corn, and onion (*Allium cepa*) were grown at the MSU East Lansing Horticulture Farm. Plots were seeded with either a cone or v-belt planter and irrigated after planting to stimulate uniform germination. Tomato, muskmelon and onion transplants were started in greenhouses. Muskmelons were direct seeded into peat pots, thinned to 2 plants per pot and transplanted with 4 to 6 leaves. Tomatoes were pricked out of seed flats, transplanted to 72 plants per flat, and grown as bare root transplants. Both muskmelon and tomato transplants were sorted and assigned to blocks according to size (small,medium,large) prior to planting. Starter solution (250 ml of 9N-20P-13K at 5.7 g/liter) was applied to each transplant at planting. Plots at the MSU East Lansing Horticulture Farm were hand cultivated to prevent weed competition and possible herbicide interactions. Fertilizer was broadcast prior to preparation of the seed bed and sidedressed later in the season according to standard cultural practices. Regular fungicide and insecticide applications were necessary at this location and were made with a large air blast sprayer at recommended rates and scheduling. When natural rainfall was not adequate overhead irrigation was used to provide 4 to 7 cm of moisture over a 2 to 3 hr period.

Uniform plant density was established when possible. This was necessary for estimation of treatment effects on fruit number and size. Guard rows were included between adjacent plots in most experiments and on the perimeters of all experiments.

TRIA formulation and application. All TRIA used in these studies was provided by the Proctor and Gamble Co., Cincinnatti, Ohio and was formulated as an aqueous colloidal dispersion. The purity of the TRIA was generally greater than 97.7% and as high as 99%. Tallow alkyl sulphate (TAS) or sodium octadecyl sulphate (SOS) were used as dispersants in the stock formulations at 1% of the TRIA concentration. Stocks of TRIA dispersions should be kept sterile to prevent breakdown of the dispersion and stocks were autoclaved at 130°C and 150 KPa for 20 minutes when necessary. Limited autoclaving did not alter the activity of TRIA stocks (31).

The occurrence of contaminants in the diluent or in sprayer components can affect the biological activity of TRIA (33). Stock TRIA dispersions were diluted with either tap water, distilled water or hexane washed distilled water in these studies. Most TRIA applications were made with a back-pack spayer with compressed CO_2 as the propellent. Dispersions were diluted in 2-liter polyethlyene "soft drink" bottles and applied through neoprene pressure tubing and a metal wand fitted with a 6503 flat fan nozzle. Some applications were made with a 4-liter galvanized tank fitted with rubber pressure tubing, a brass wand, and hand pump. These sprayers correspond to numbers 5 and 6 from previous studies (33). Applications were made at 214 KPa and 374 liters/ha. In 1981, controls were treated with the dispersant

TAS. In 1982 and 1983 controls were not treated since low levels of TAS do not affect growth (14).

Treatment Conditions. Many variables could influence the effect of foliar applications of TRIA. Collection of data defining cultural and environmental conditions improved as the study progressed. The location of each experiment and relevent experimental information are presented in Table 1. The cultivars used for each test are listed with the results.

Temperature was measured with a shielded mercury thermometer or a shielded thermograph (Weather Measure model 611T). Photosynthetic photon flux (PPF) was measured with a Licor model LI-185 and a quantum sensor. Water and solute potential were measured on leaf disks using a Wescor model HR-33(T) dew point hygrometer and C-52 sample chambers (Wescor, Inc., Logan Utah). Samples were taken from exposed, recently matured leaves. Equilibration times were determined for each crop and ranged from 2 to 4 hr for water potential and 1 to 2 hr for solute potential. Water potential samples were placed in the chambers in the field and allowed to equilibrate under laboratory conditions. Solute potential samples were tightly wrapped in foil and frozen in liquid nitrogen. Pressure potential was calculated as the difference between water and solute potential. Stomatal conductance was measured with a Licor model 1600 steady state diffusive resistance porometer.

Harvest and Yield. Marketable yield at the proper stage of maturity and fruit or grain number and size were determined. All plots

Table 1. Experimental, cultural and treatment conditions for all experiments.

Expt	Crop	Year	Loc[z]	Plot Size (M)	Reps[y]	Dates: Plant	Dates: Harvest	Dates: Trmt	Stage of Development at Treatment	Treatment Conditions: Time(HR)	Treatment Conditions: Temp(C)	Treatment Conditions: Radiation
1	Alfalfa	82	1	0.9x6.1	6	4/81	6/6	5/13	3 days post cut	1500	28	clear
							7/21	6/17	11 days post cut	1500	20	partial
2		82	1	0.9x6.1	6	4/81	8/12	6/9	4 days post cut	1500		
3	Dry Beans	81	5	0.9x7.6	6	6/25	10/9	7/23	preanthesis	1245	22	
4		81	5	0.9x7.6	6	6/25	10/9	7/23	preanthesis	1200	22	
5		81	5	0.9x7.6	6	6/25	10/9	7/31	mid anthesis	1130	24	clear
6		83	5	0.6x7.6	8	6/12	9/21	7/13	5-6 trifoliate	1800	29	clear
								8/16	mid pod fill	1600	30	clear
7		83	5	0.6x7.6	8	6/12	9/1	7/13	5-6 trifoliate	1800	29	clear
8	Field Corn	81	3	1.5x4.6	8	5/22	9/15	6/11	3-4 leaves	-		
								6/26	tassle 1-2cm	-		
								7/3	tassle 5-6cm	-		
9		82	3	1.5x4.6	8	5/26	10/26	6/8	3-4 leaves	1430	30	clear
10		82	1	0.8x6.1	6	6/11	9/23	7/8	7-8 leaves	1400	28	clear
11		82	3	1.5x7.6	8	-	10/26	6/8	6-7 leaves	1500	29	clear
12		83	2	1.5x7.6	8	6/2	9/12	7/8	tassle 1-2cm	1600	28	clear
13	Potatoes	81	5	0.9x7.6	6	-	10/12	7/14	early anthesis	1230	24	
14		83	5	0.9x7.6	8	5/27	9/28	6/13	tuber initiation	1500	33	clear
								8/16	tuber bulking	1400	31	clear
15		83	5	0.9x7.6	8	5/17	9/13	6/13	tuber initiation	1530	33	clear
16	Wheat	81	2	1.2x4.6	6	10/80	-	4/28	tiller	1500	21	rain
								6/1	boot	0830	24	clear
17		81	4	1.2x4.6	6	10/80	-	4/30	tiller	0900	7	clear
								6/2	boot	1000	24	cloudy

Table 1. continued.

Expt	Crop	Year	Loc[z]	Plot Size (M)	Reps[y]	Dates: Plant	Dates: Harvest	Dates: Trmt	Stage of Development at Treatment	Treatment Conditions: Time(HR)	Temp(C)	Radiation
18	Wheat	82	2	1.2x4.6	6	10/81	7/28	6/8	heading	3 times	warm	clear
19		82	4	1.2x4.6	6	10/81	7/24	5/29	boot	3 times		partial
20		83	2	1.2x4.6	6	10/82	7/24	6/10	heading	3 times	warm	clear
								6/15	anthesis	3 times	cool	cloudy
								6/22	post anthesis	3 times	warm	clear
21		83	2	1.2x4.6	6	10/82	7/24	6/13	heading	1600	30	clear
22		83	4	1.2x4.6	6	10/82	8/1	6/17	anthesis	3 times		partial
23		83	4	1.2x4.6	6	10/82	8/1	6/14	heading	1600	30	clear
24		84	2	1.2x4.6	6	10/83	7/19	6/14	heading	3 times	warm	clear
								6/24	anthesis	3 times	warm	clear
25	Asparagus	81	1	1.2x13	5	-	5/1-6/9	4/28	spears emerging	-		
26		81	1	1.2x7.0	6	-	5/7-6/9	5/5	spears emerging	-	10	cloudy
27		82	6	1.2x15	10	-	5/9-6/21	5/7	spears emerging	1500	16	partial
28		83	6	1.2x15	9	-	5/27-6/20	5/27	spears emerging	1700	20	clear
								6/14	mid season	1700		clear
29	Cucumbers	81	1	1.2x6.1	6	6/11	7/27	6/29	2-3 leaves	-		
								7/8	7-8 leaves	-		
								7/13	20-25 leaves	-		
30		82	1	0.6x6.1	8	6/25	8/17	7/22	15-20 leaves	1800	28	cloudy
31		83	1	1.2x9.1	6	6/10	7/22	7/15	8-9 leaves	4 times	warm	hazy
32	Snap Beans	82	1	0.6x7.6	8	6/25	8/16,25	7/21	2-3 trifoliates	4 times	warm	clear
33		83	1	0.6x7.6	9	6/10	8/3	7/12	3-4 trifoliates	4 times	warm	clear

Table 1. continued.

Expt	Crop	Year	Loc[z]	Plot Size (M)	Reps[y]	Dates: Plant[x]	Dates: Harvest	Dates: Trmt	Stage of Development at Treatment	Treatment Conditions: Time(HR)	Temp(C)	Radiation
34	Lettuce	82	1	0.6x7.6	8	4/29	6/28	6/2	6-7 leaves, heading	4 times		clear
35	Muskmelons	81	1	1.5x7.6	6	5/28T	8/4-8/27	6/12	-	-	21	clear
36		82	1	1.2x6.1	7	5/25T	8/4-8/27	7/1	branched, anthesis	1600		partial
37	Onions	81	1	0.6x7.6	9	4/28T	8/17	5/29	3-4 leaves	-		
38		83	1	1.2x4.9	6	4/12	9/14	7/6	first bulbing	1700	29	clear
39	Sweet Corn	81	1	0.9x6.1	4	5/26	8/14,17	6/17	5-6 leaves	-	24	clear
								7/7	tassle visible	-	32	clear
40		81	3	0.9x6.1	4	6/3	8/20	6/26	5-6 leaves	-		
								7/14	tassle visible	1500	27	clear
41		83	1	0.9x9.1	3	6/10,23	8/5,29	7/15-17	5-6 leaves/tassle	1800	33	clear
42	Tomatoes	81	1	1.2x6.1	5	5/28T	8/12-9/2	7/7	anthesis	-	24	
43		81	3	1.2x6.1	3	6/2 T	8/20-9/3	-	anthesis	-		
44		82	1	1.2x7.6	6	5/25T	8/19-9/9	7/1	anthesis	1630	26	partial
45		83	1	1.2x7.6	7	5/31T	8/13,30	6/14	preanthesis	1600	27	clear
								7/16	postanthesis/fruit	1600	29	clear

[z]Location codes and soil types: 1) MSU East Lansing Horticulture Farm, Spinks Sandy Loam; 2) MSU East Lansing Crops Farm, Conover Loam; 3) MSU Clarksville Horticulture Farm, Drysden Sandy Loam; 4) Saranac Wheat Farm, Matherton Loam; 5) Stanton, Montcalm County, sandy loam; 6) Hart, Oceana County, sandy loam.

[y]Number of replicates or blocks in the experiment.

[x](T)-set out as transplants.

except alfalfa (Medicago sativa), dry beans, and wheat were hand harvested and weighed with a spring scale accurate to 0.05 kg. Alfalfa plots were cut with a Gerry mower and raked. Dry bean plots were hand harvested and the beans recovered with a mechanical thresher. Wheat plots were harvested with a small-plot mechanical harvester and thresher. Grain weight after air drying (11-12% moisture) was determined with a top-loading Mettler balance accurate to 0.01 g. A random sampling of 200 wheat grains was weighed to determine treatment effects on seed size. Economic yield of pickling cucumbers was calculated by grading harvested fruit into standard size classes and then calculating dollar values dependent on the current season's market value. Asparagus and muskmelon were harvested every 2 to 3 days throughout the season. Tomatoes were harvested 3 times in 1981 and 1982 and twice in 1983. Sweet corn was also harvested twice, 3 to 5 days apart.

Experimental design and statistical analysis. Randomized complete block designs were used to account for variability within the field or plot. In experiments with 2 or more factors, split plot designs were used with TRIA treatments as subplots. Most tests had 6 to 8 replicates (Table 2). The results of all tests were subjected to analysis of variance including trend analysis when applicable. Mean separation was by Fisher's least significant difference (LSD) when the F value was significant at the 5% level or greater or relevant single degree of freedom F-tests.

Results

Results are presented by crops, which have been categorized as field crops or vegetable crops and alphabetized within each group.

Alfalfa. Foliar applications of 0.5 ug/liter TRIA to alfalfa with 3 to 11 days of regrowth after cutting increased the dry matter yield of alfalfa hay harvested 4 to 5 weeks later for 2 of 3 cuttings (Table 2). The average increase in yield for all 3 cuttings was 12%. Alfalfa was one of the few crops in this study where yield is a direct measure of vegetative growth.

Dry Beans. Two cultivars of dry beans in a commercial production field were treated in 1981 with 4 concentrations of colloidally dispersed TRIA to determine the optimum concentration for increasing yield. 'Black Turtle' and 'Seafarer' navy bean were sprayyed prior to the onset of anthesis. A second test of 'Seafarer', adjacent to the first, was treated during anthesis. TRIA increased yield in both tests sprayed prior to anthesis (Table 3). The average increase in yield for the 10 ug/liter rate was 11%. There was no effect of TRIA on the yield of the 'Seafarer' plot treated during anthesis.

Dry beans were treated in 1983 on a different farm in the same county in a field where plantings of 2 different cultivars merged. Three rates of TRIA were applied at the 5 to 6 trifoliate stage, prior to anthesis. 'Dark Red Kidney' was treated a second time during mid pod fill. There were no differences in yield for either cultivar (Table 3). The plots were located in an irrigated field.

Table 2. Dry matter yield of 'Saranac' alfalfa treated with 0.5 μg/liter TRIA. Two tests within the same field were treated 3, 11, and 4 days after cutting. Each observation is the mean of 6 replicates.

	Hay Yield (MT/ha)			
	Test 1		Test 2	
TRIA	Harvest 1	Harvest 2	Harvest 1	Average
0	2.62	3.21	2.71	2.85
+	2.80	3.77**	3.00*	3.19**

*, ** F value was significant at the 5% and 1% level respectivly.

Table 3. Yield of navy, kidney, and cranberry dry beans treated with TRIA.

1981[z]				1983[y]		
	MT/ha				MT/ha	
TRIA (μg/l)	'Black Turtle'	'Seafarer' Test 1	'Seafarer' Test 2	TRIA (μg/l)	'Dark Red Kidney'	'Taylor Cranberry'
0	1.60	1.71	1.69	0	3.21	3.45
0.1	1.65	1.86	1.59	0.2	3.30	3.50
1.0	1.62	1.89	1.43	1.0	3.38	3.47
10.0	1.84	1.84	1.61	5.0	3.31	3.51
100	1.66	1.72	1.80			

[z]F value for difference in average response of beans to TRIA compared to the controls of 'Black Turtle' and 'Seafarer' Test 1 compared to 'Seafarer' Test 2 was significant at the 5% level.

[y]No significant differences.

Field Corn. Five experiments with field corn were designed to determine the optimum concentration of TRIA, the effect of soil P availability on the response of corn to TRIA, and the best stage of crop development for treatment. In 1981, TRIA was applied to field corn at the MSU Clarksville Horticulture Farm at 3 stages of crop development, 3 rates of P applied as a supplemental band, and 6 rates of TRIA in a split-split-plot design. TRIA was applied at the 3-to-4 leaf stage (June 11), the 1-to-2 cm tassle stage (June 26) and the 5-to-6 cm tassle stage (July 3). Supplemental P was banded on June 11 at 0, 20 and 40 kg/ha P as triple super phosphate. With no supplemental P, the response to TRIA was similiar for all 3 application dates. When averaged over all P levels and application dates, the optimum TRIA concentration of 0.1 µg/liter increased yield 5% (Table 4). The increase in yield was due to increased weight per ear.

Two experiments were conducted with field corn in 1982 testing the effect of P availability on the response to TRIA. Three rates of TRIA were applied at the 3 to 4 leaf stage at the MSU Clarksville Horticulture Farm with 0, 40 or 90 kg/ha supplemental P. There was no effect of P or TRIA on total yield. The second experiment was planted at the MSU East Lansing Horticulture Farm in an area used for vegetable production. The plant density and yield were lower than at the Clarksville Farm. Three rates of TRIA were applied at the 7 to 8 leaf stage when the unemerged tassle was 2 to 3 cm long. The number of ears (multiple ears per stalk) and yield per plot increased linearly with P rate (data not shown). Total yield was not increased with TRIA

Table 4. Yield of field corn (15% moisture) treated with TRIA.

TRIA	MT/ha (unshelled)			
(µg/l)	1981[z] Clarksville (Dekalb 25A)	1982[y] Clarksville (Dekalb XL32A)	1982[y] East Lansing (Pioneer 3780)	1983[x] East Lansing (Great Lakes)
0	10.09	10.95	7.45	8.45
0.01	10.23			
0.1	10.64			9.14
1.0	10.31	10.70	7.86	8.66
10.0	10.17	10.95	8.13	8.55
100	10.01	11.28	8.00	

[z]F value for the quadratic trend was significant at the 1% level. Each value is the mean of 3 P levels and 3 treatment dates.

[y]No signifficant differences. Each value is the mean of 3 P levels.

[x]F value for comparison of lowest rate of TRIA versus the control or all other treatments was significant at the 5% level.

(Table 4) but the weight per ear was 6% larger than the control with 1.0 ug/liter TRIA (data not shown, significant at the 5% level).

A third experiment was conducted in 1982 at the MSU Clarksville Horticulture Farm to test the effect of TRIA application with a standard agricultural sprayer. A 12-row multi-nozzle sprayer was rinsed thoroughly with water and liquid ammonia prior to use. TRIA at 0.5 µg/liter was applied to 500 meter long areas, 12 rows wide at 308 liters/ha and 214 kPa. There were 8 dry control and 8 treated sections. Sections 7.6 meters long in the 2 middle rows of each plot were harvested. Control plots yielded 13.6 MT/ha and treated plots 14.5 MT/ha (unshelled weight). The treatments were significantly different at the 5% level. The difference was primarily due to a difference in the number of ears harvested per plot.

In a test with field corn at the MSU Crops Farm in 1983, there was an 8% increase in yield with 0.1 µg/liter TRIA applied at the 1 to 2 cm stage (Table 4). The increase was due to an increased weight per ear. The grain was shelled from the ears and the grain weight per treatment was similiar to the pattern of unshelled weight (data not shown).

With field corn studies the response to TRIA varied with TRIA concentration and the level of supplemental P. The levels of P added were above those usually recommended for corn. Field corn yield increased when TRIA was applied at the 3 to 4 leaf stage and when the unemerged tassel was 1 to 2 cm. In an analysis of 43 replicates from the 5 experiments over the 3 year period (base P_2O_5 level) comparing the control versus TRIA (between 0.1 and 1.0 µg/liter) the average yield increase was 8.3% or 839 kg/ha (significant at the 1% level).

Potato. Potato (Solanum tuberosum) yield increased 18% with 1.0 ug/liter TRIA and showed a quadratic trend with concentration in 1981 (Table 5). Treatments were applied during anthesis, when greater than 50% of the foliage was fully developed. The increase in yield was not specifically due to either an increase in tuber weight or number. Two potato plots were treated with 3 rates of TRIA in 1983. Foliage was 30 to 35% developed at location 1 and 75 to 80% developed at location 2 but tubers were initiated at both locations. There was 2 weeks difference between the planting dates of the 2 locations. Plots at location 1 were resprayed during tuber bulking. At location 1, all 3 rates of TRIA yielded more than the control (Table 5). At location 2, there was a decrease in yield with TRIA for all rates (Table 5). The difference in yield at both locations was neither an effect of tuber number or weight. The reason for this difference in effect on yield between the 2 locations is not known. The design of the experiments did not allow determination of whether this was an effect of location or stage of development at the time of TRIA treatment. The 2 experiments were located approximately 1 mile apart and possibly subject to different irrigation, cultural, or soil conditions.

Soft winter wheat. Wheat plots treated in 1981 at 2 locations in Michigan at the early tiller and boot stages with 5 concentrations of TRIA showed no differences in yield between treatments (data not shown). Wheat tests in 1982 were designed to study the effect of P availability on the response to TRIA (similiar to the 1981 field corn

Table 5. Yield and tuber size of 'Russet Burbank' (1931) and 'Monona' (1983) potatoes treated with TRIA.

1981			1983				
				Location 1		Location 2	
TRIA (µg/l)	MT/ha[z]	g/tuber	TRIA (µg/l)	MT/ha[y]	g/tuber	MT/ha[y]	g/tuber
0	31.5	142	0	33.1	132	40.1	147
0.1	34.3	141	0.2	36.7	139	37.0	142
1.0	37.3	144	1.0	36.3	136	38.1	139
10.0	35.0	162	5.0	35.4	136	37.6	135
100	34.0	143					

[z]F value for comparison of control versus 1.0 ug/l was significant at the 5% level.

[y]F value for comparison of control versus all 3 rates was significant at the 5% level.

studies) and the effect of TRIA applied at different times of day. Controlled environment research during the summer of 1981 indicated that high temperature prior to treatment favored TRIA stimulated increases in seedling dry weight (31). In greenhouse tests, the growth-stimulating activity of TRIA was greater when applied at 1700 HR compared to 0900 or 1300 HR (31). Split plot designs with supplemental P fertilizer as main plots and TRIA at different times of day as subplots were replicated at 2 locations. At the MSU East Lansing Crops Farm, supplemental P was applied as a 2% foliar spray of KH_2PO_4 two days after TRIA treatment since soil conditions prohibited banded P applications. TRIA was applied at 0900, 1300 and 1700 HR on a clear sunny day with the wheat at the boot stage. There was no effect of P sprays on yield and no interaction with TRIA treatment. The only significant yield effect with TRIA was at 1700 HR (Table 6). At the MSU Saranac Wheat Breeding Farm, triple super phosphate (0N-20P-0K) was banded between the 12 inch rows approximately 2 to 5 cm deep at the rate of 52 kg/ha P. Cool, cloudy or rainy weather postponed TRIA treatment for 9 days after P application. Treatments were applied at early heading stage. The 0900 HR application was made as a heavy fog was breaking up and the plants were wet with dew. The 1300 and 1700 HR applications were under warm, clear skies. There was an interaction of supplemental P with TRIA at different times of day. Under base P fertility (P_1) conditions the only response to TRIA was at 1700 HR (Table 6). With supplemental P (P_2) only treatment at 0900 HR was different from the control. When time of day treatments are averaged

Table 6. Yield of 'Augusta' soft winter wheat treated with 0.5 µg/l TRIA at different times of day. Each value is the mean of 6 replicates.

Treatment		Yield (MT/ha)										
		1982				1983			1984			
		East Lansing		Saranac[y]								
Time	TRIA	P_1	P_2[z]	P_1	P_2[z]	Day 1	Day 2	Day 3	Day 1	Day 2	Average[x]	
Control	0	5.07	5.18	3.47	3.40	4.83	4.80	4.63	3.76	3.82	4.33	
0900	+	5.25	5.26	3.47	3.70	4.73	4.90	4.98	3.70	3.57	4.39	
1300	+	5.10	5.01	3.45	3.65	5.31	4.91	4.70	3.70	3.78	4.40	
1700	+	5.48	5.38	3.70	3.50	5.08	4.88	5.00	3.78	3.73	4.50	
LSD 5%		0.27		0.29		0.44	NS	NS	NS	0.22		

[z]East Lansing P_2 was 2% KH_2PO_4 foliar spray after treatment; Saranac P_2 was 52 kg/ha P from triple superphosphate applied 9 days before TRIA treatment.
[y]F value for the interaction single degree of freedom comparison of P with the quadratic trend of time was significant at the 5% level.
[x]When all 9 sets of means are treated as random effects and analyzed over years and conditions the F value for the linear trend with time was significant at the 5% level.

over both locations and P levels, the only significant difference was with treatment at 1700 HR.

Based on tests from controlled environment studies (accompanying paper), it was postulated that the response to TRIA was greater during the period of recovery from mid day water deficits. Diurnal fluctuation in water, solute and pressure potential and the effect on leaf extension rate in field grown corn and sorghum have been reported (1). Experiments in 1983 were similiar to tests in 1982 and incorporated measurement of certain environmetal and water status parameters. At the Saranac wheat farm there was no effect of 3 concentrations of TRIA applied at 3 different times of the day (data not shown). The experiment at the MSU Crops Farm was designed to test TRIA application at 3 times on different days. PAR, temperature, and water and solute potential were measured at 0900, 1300, 1700, and 2100 HR on each day. The first treatments were applied on June 10 (day 1), on a hot, clear day. Applications of TRIA at 1300 and 1700 HR resulted in higher yields than those of the control or TRIA application at 0900 HR (Table 6). The largest yield was with treatment at 1300 HR. Water, solute, and pressure potential all decreased from 0900 to 1300 HR and water and pressure potential increased from 1300 to 2100 HR (Fig. 1). There were no differences due to treatments applied on June 15 (day 2), a cool, overcast day with rainshowers (0.5 cm) from 1430 to 1530 HR and sudden clearing after 1630 HR. Rapid clearing late in the day, just prior to the 1700 HR sampling, probably resulted in the sudden decrease in pressure potential at 1700 HR (Fig. 1). TRIA applied on June 22 (day 3), a warm clear day, did not increase yield. Soil moisture

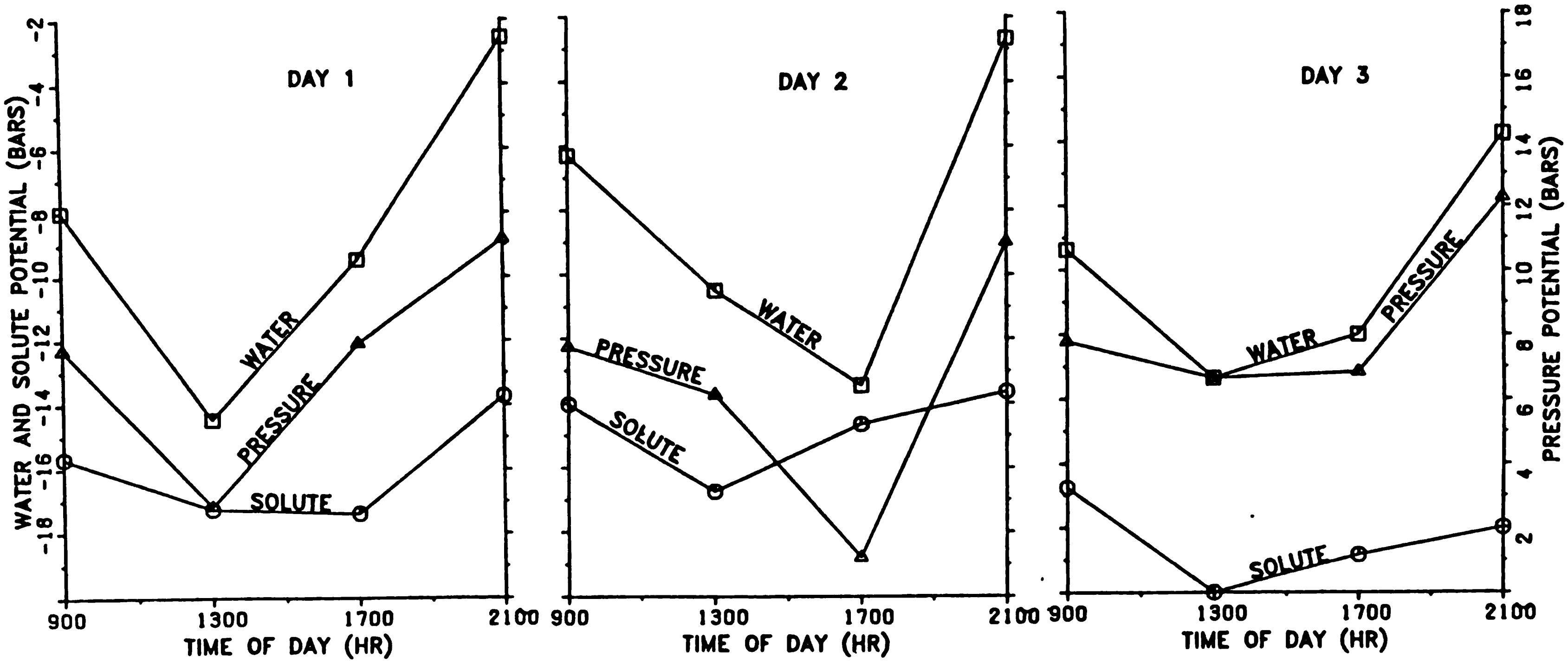

Figure 1. Water status of the flag leaf of 'Augusta' soft winter wheat on 3 days when plots were treated with TRIA.

conditions were dryer on day 3 than on day 1 and was reflected by the comparison of water potential at 0900 and 2100 HR for the 2 days (Fig. 1). The only rainfall for the 2 weeks prior to June 22 was 0.5 cm on June 15. The effect of environmental conditions for the 3 days is confounded with stage of development since there was rapid progression towards anthesis during this time period.

Wheat was the only crop treated in the field with TRIA in 1984 as part of this research. Treatments were applied on 2 days at different times as in 1983. The spring of 1984 was very dry at the MSU Crops Farm with no significant rainfall from the time the head was observed in the boot stage until 1 week prior to harvest. First treatments were applied on June 14, a warm sunny day, when the wheat was heading. There were no differences in yield due to TRIA treatment (Table 6). At the time of the second applications on June 24, a hot, dry and sunny day, water stress had progressed to the point that the margins of many flag leaves were rolling by mid day and many lower leaves had started to senesce. The yield of plots treated with TRIA at 0900 HR was lower than control plots or treatment at other times (Table 6).

The activity of 3 different colloidal dispersion formulations at 3 different concentrations was evaluated in 1983 at both East Lansing and the Saranac Wheat Farm. The colloidal disperion formulation made from 97.3% TRIA and applied at 0.1, 1.0 and 10 µg/liter was compared with a colloidal dispersion from 66% TRIA applied at 10, 100 and a 1000 µg/liter and a substituted 30 carbon compound applied at 1.0, 10, and 100 µg/liter. Each formulation was tested with a control in a split plot design. The concentrations selected were based on greenhouse tests with corn seedlings. There was a quadratic trend with concentration averaged over formulation at both locations. However, the trend was for increasing yield at East Lansing and decreasing yield at Saranac (Table 7). Treatments were applied on 2 consecutive days under nearly identical developmental and environmental stages. Rainfall data indicated less rain during head development at the Saranac location, but this was not reflected in the yield.

Asparagus. Effects of TRIA applications on asparagus yields were limited to the first 5 to 6 harvests after treatments were applied in 1981. In Test 1 TRIA increased spear production and in test 2 TRIA decreased spear production and lowered yield (Table 8). The number of spears harvested was increased over a 12 harvest period in plots treated with 5.0 μg/liter TRIA in 1982 (Table 8). There was no effect of TRIA treatment on yield in 1983 (Table 8). Increased yield of TRIA treated asparagus was reported previously (29) but the results were considered suspect due to the high experimental variability which is typical of the heterogeneous asparagus cultivars available.

It is difficult to explain a stimulation of asparagus yield without assuming some distally translocated effect which affects the asparagus buds since treated portions were harvested 2 to 3 days after treatment and effects were measured for several harvests. The buds for the current years harvest are present at the first harvest. Increased yield due to an increased number of spears per plot was also measured by researchers at Davis, California in 1982 (personal communication).

Processing Cucumbers. There were no differences in yield, number of fruit per plot or dollar value per acre for 'Greenstar' processing

Table 7. Yield of 'Augusta' soft winter wheat at 2 locations in Michigan treated with 3 rates and averaged over 3 formulations of TRIA.

TRIA Concentration (avg of 3 formulations)	Yield[z] (MT/ha)	
	East Lansing	Saranac
Control	4.67	5.01
Low	4.77	4.81
Medium	4.92	4.90
High	4.70	4.94

[z]F value for the quadratic trend of concentration averaged over formulation was significant at the 5% level for both locations.

Table 8. Yield of snapped 'Martha Washigton' asparagus treated with TRIA at MSU in 1981 and in a commercial production area in Oceana County in 1982 and 1983. ("No." designates the "Number of spears per plot".)

	1981								1982			1983[w]			
	Plot 1				Plot 2							Spray 1		Spray 2	
TRIA	Harvest				Harvest				TRIA	Harvest		Harvest			
(µg/l)	1 to 6[z]		7 to 15		1 to 5[y]		6 to 15		(µg/l)	1 to 12[x]		3 to 11		12 to 22	
	MT/ha	No.	MT/ha	No.	MT/ha	No.	MT/ha	No.		MT/ha	No.	MT/ha	No.	MT/ha	No.
0	0.48	39	1.47	110	0.37	59	0.91	117	0	0.30	232	0.24	206	0.20	190
10	0.51	42	1.38	99					0.5	0.27	225	0.23	204	0.18	179
1000	0.60	52	1.53	115	0.28	44	0.91	112	5.0	0.31	251	0.24	202	0.18	180

[z]Number of spears per plot with 10 and 1000 ug/liter TRIA was significantly greater than the control at the 1% level.

[y]Total weight and number of spears were significantly different from the control at the 1% level.

[x]Number of spears per plot with 5.0 ug/liter TRIA was significantly greater than the control at the 1% level.

[w]No significant differences.

cucumbers treated in 1981 and 1982 with TRIA (data not shown). Treatments of 1.0 and 10 ug/liter were applied at the 2, 7, and 20-25 leaf stages in 1981 and plots were harvested 3 times. Six different rates and volumes of TRIA were applied with a flat fan nozzle and a controlled droplet applicator in 1982 and plots were harvested once to simulate mechanical harvesting. Increased yield of 'Greenstar' cucumber with TRIA treatment was previously reported (18,29).

TRIA (1.0 µg/liter) was applied to 'Castlehy 2012' cucumbers at 4 different times of day in 1983. Vines were flowering and small fruit were just visible. In a simulated machine harvest 1 week after treatment, there were more fruit from plots treated with TRIA at 1300 HR compared to all other treatments (Table 9). The increase was due to more smaller fruit and fewer oversize fruit. This also resulted in a higher market value. Greater fruit number and smaller fruit are probably an indication of greater plant vigor and development of more than the crown fruit. Water, solute and pressure potential data are presented in Fig. 2. Water and pressure potential decreased from 0900 to 1700 HR and then increased from 1700 to 2100 HR. Environmental conditions were very warm and bright, but hazy as opposed to clear or partly cloudy.

<u>Snapbeans.</u> There was no effect of 1.0 µg/liter TRIA on snapbean yield in either 1982 or 1983, regardless of the time of day of application (Table 9). Plant populations were low in 1982 due to poor germination and pod development of the cultivar 'Spartan Arrow' dictated 2 harvest dates. Plant populations were high and uniform in 1983 and plants matured uniformly for a once over harvest.

Table 9. Yield of 'Castlehy 2012' processing cucumbers, 'Spartan Arrow' (1982) and 'Bush Blue Lake' (1983) snap beans and 'Bibb' lettuce treated with TRIA (1.0 µg/l) at different times of day.

		Cucumbers[z]			Snap Beans[y]		Lettuce[y]	
TRIA	Time (HR)	MT/ha	Fruit per Plot	$/ha	1982 MT/ha	1983 MT/ha	Time	MT/ha
0	Control	4.98	65	427	10.60	8.61	Control	9.84
+	0900	4.46	65	404	11.21	8.47	0900	9.74
+	1300	5.10	70	475	10.83	8.54	1200	8.88
+	1700	4.92	65	442	11.32	8.81	1500	9.94
+	2100	4.92	63	427	11.03	8.67	1800	10.13

[z]F value for comparison of TRIA at 1300 HR versus all other treatments was significant at the 5% level for fruit per plot and $/ha.

[y]No significant differences.

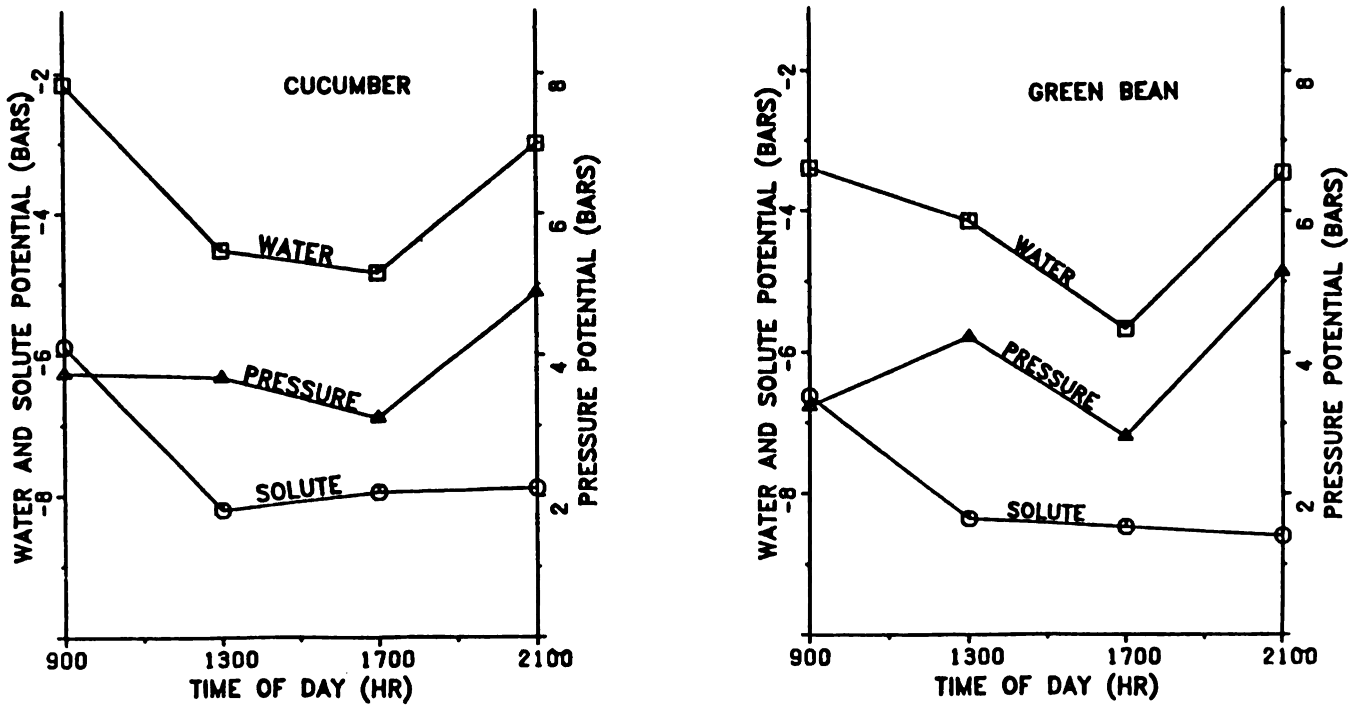

Figure 2. Water status of the most recently expanded leaf of cucumber and snap beans on the day plots were treated with TRIA.

Environmental and water relation patterns were measured in 1983 and followed diurnal trends similiar to wheat and cucumbers (Fig. 2). Plots were irrigated throughly the day before treatment and the measurement of plant water status.

Lettuce. Lettuce also did not respond to 1.0 ug/liter TRIA applied at 4 different times of day in 1982 (Table 9). Treatments were applied during the early vegatative growth stage and plots had been sidedressed with NH_4NO_3 and irrigated 2 days prior to treatment.

Muskmelons. The yield of muskmelons was increased approximately 40% in both 1981 and 1982 with TRIA application at 1.0 ug/liter (Table 10). Both 1.0 and 100 ug/liter increased yield similiarly in 1981. In 1982 an experiment was designed to test the observation made in controlled environment studies that a higher temperature of the water used for application increased TRIA effectiveness. Occasionally in early greenhouse studies, TRIA formulated as an aqueous suspension with Tween 20 was applied in warm water (˜25^{o}C) due to the warming of the mixture of water and TRIA dissolved in chloroform to drive off the chloroform. Preliminary experiments designed to test this variable with controlled environment seedling bioassays, indicated warm water increased TRIA's growth-stimulating activity (not published). Although the results of this test support that observation, further controlled environment tests were not consistent. This observation was incorporated into this research to the extent that TRIA dilutions were not made in cold water and generally were made in water above 25^{o}C. A repeat of this test was planned in 1983 but the melon plots were severely damaged by chilling temperatures early in the season.

Table 10. Yield of 'Roadside' muskmelons treated with different rates of TRIA in 1981 and with TRIA (1.0 µg/l) in heated water in 1982.

1981[z]			1982[y]		
TRIA (µg/l)	MT/ha	kg/melon	TRIA (Temp)	MT/ha	kg/melon
0	13.56	1.52	Control	12.17	1.54
0.01	15.38	1.65	20°C	11.32	1.43
1.0	18.80	1.54	40°C	15.40	1.41
100	18.86	1.53	60°C	17.55	1.66

[z]F value for linear increase in yield and number of fruit with TRIA rate was significant at the 1% level.

[y]F value for the control and 20°C versus 40°C and 60°C was significant at the 5% level for yield and number of fruit.

The increased yield was due to a greater number of fruit harvested per plot. The difference in yield could be accounted for by 1 additional melon per 3.0 m^2. TRIA applications were made when the vines were branched and flowering but prior to visible fruit set. There is no obvious reason for the large increases in yield of muskmelons relative to other crops tested in this study. Others (4) found no effect of TRIA on the growth, yield, or soluble solids of muskmelons.

Onions. Greenhouse grown onion transplants were planted on 10 cm centers in rows 0.61 m apart and treated with TRIA early in the season (3 to 4 leaves). TRIA application at 0.01 ug/liter increased onion yield 18% (Table 11). Although not a commercial practice in Michigan, onions were direct seeded on mineral soil at the MSU East Lansing Horticulture Farm in 1983. Three rates of TRIA were applied to plots in a split-plot design. Main plots were treated with either no additional fertilizer or sidedressed with 448 kg/ha 15N-13P-13K the day of TRIA treatment. Treatments were applied prior to the initiation of bulbing, when 5 to 6 leaves were present. There was no effect of banded fertilizer or TRIA on yield (Table 11). Plants and bulbs were much smaller than those direct seeded on organic soils. Water availability may have limited growth and yield.

Sweet Corn. Sweet corn plots at East Lansing and Clarksville in 1981 were treated with 4 rates of TRIA during vegetative growth (5 to 6 leaves) and when the tassle was visible. There were no increases in yield for either stage or location. There was a quadratic trend of decreasing yield with TRIA concentration at Clarksville when plots were treated at the early stage of growth (Table 12). Differences in yield

Table 11. Yield response of 'Sweet Sandwich' onions from transplants in 1981 and direct seeded in 1983 to foliar applications of TRIA. Fertilizer treatment (Fert 2) in 1983 was 448 kg/ha 15N-13P-13K banded the day of TRIA treatment.

1981[z]			1983[y]				
				Fert 1		Fert 2	
TRIA (µg/l)	MT/ha	g/bulb	TRIA (µg/l)	MT/ha	g/bulb	MT/ha	g/bulb
0	14.5	141	0	20.8	68	22.0	66
0.01	17.1	160	0.2	19.4	62	21.7	67
1.00	15.3	137	0.5	21.0	67	20.2	63
			2.5	21.7	65	22.5	68

[z]F value for yield at 0.01 ug/l versus the control was significant at the 1% level.

[y]No significant differences.

Table 12. Yield of 'Gold Cup' (1981) and 'Harmony' (1983) sweet corn treated with TRIA at 2 growth stages. Plots were planted at 1 time and treated on different days in 1981 and planted at different times and treated on the same day in 1983.

STAGE	TRIA (µg/l)	1981 Clarksville[z] MT/ha	1981 Clarksville[z] ears /plot	1981 East Lansing[y] MT/ha	1981 East Lansing[y] ears /plot	1983 TRIA (µg/l)	1983 East Lansing[x] MT/ha	1983 East Lansing[x] ears /plot
5th	0	15.7	24	15.0	22	0	11.3	42
to	0.01	13.1	21	12.5	19	0.2	12.0	45
6th	0.1	13.8	21	14.7	23	1.0	11.7	43
leaf	1.0	14.5	24	12.4	20	5.0	11.2	42
	10.0	16.3	24	12.3	20	25.0	12.3	44
Tassle	0	14.7	24	16.9	25	0	17.8	57
Visible	0.01	15.6	25	14.4	21	0.2	20.6	64
	0.1	14.0	23	16.3	25	1.0	19.6	62
	1.0	13.9	23	16.1	24	5.0	20.1	63
	10.0	14.6	24	14.7	23	25.0	19.9	63

[z]F value for quadratic trend of yield with TRIA concentration at the 5th to 6th leaf stage was significant at the 5% level.

[y]No significant differences.

[x]F value for comparison of control versus all rates averaged over stage was significant at the 1% level for yield and 5% level for ears/plot.

were due to the number of harvested ears (second ears) as opposed to ear weight. Two experiments treated at different growth stages in 1982 were not harvested due to severe smut infestations which prevented accurate determination of marketable yield.

Tests investigating the effect of stage of crop development at treatment might be confounded by different environmental conditions at treatment. To avoid this problem, sweet corn plots in 1983 were planted at different times within a split-plot design so that they could be treated on the same day but at different stages of development. Sub-plots were designed so treatments could be applied on 3 consecutive days under different conditions of soil moisture. Day 1 applications were made the day of regularly scheduled irrigation and irrigation was postponed 1 day. Treatments were applied on day 2 and irrigation started 30 min later. Treatments on day 3 were applied 24 hr after irrigation. All treatments were applied at 1800 HR under hot (30-33^{o}C), clear sky conditions. Four concentrations of TRIA were applied as sub-sub-plots. The main effect of all 4 concentrations of TRIA versus the control was a 9.5% increase in yield. When averaged over TRIA rate, the increase in yield over the control was 6.4%, 7.2% and 14% for day 1, 2 and 3 respectively. Differences in yield were due to differences in the number of ears, ie. an increase in the number of second ears. The importance of effect of stage of development for the TRIA response is still not confirmed due to differences, mainly moisture availability and temperature, during grain fill for the 2 planting dates which affected the final yield (first planting 62 ears/45 plants and second planting 43 ears/45 plants per plot).

Tomato. The yield of tomatoes was not altered by any of the 16 treatment combinations of seed and foliar TRIA treatments at the East Lansing Horticulture Farm (data not shown). A smaller experiment with 8 treatments at the Clarksville Farm resulted in decreased yield from transplants grown from TRIA treated seed (Table 13). Foliar applications of 10 ug/liter at anthesis had no effect on transplants from TRIA treated or dichloromethane (DCM) soaked control seed but increased the yield of transplants from unsoaked control seed. Since transplants at both locations were from the same lot, if the seed treatment effect was present prior to transplanting, it should have affected yield at both locations unless some other factor influenced yield. If the treatment effect was after transplanting, then some difference between locations must have influenced the response. Increased yield from transplants grown from seed treated with TRIA and from DCM soaked seeds relative to unsoaked seed has been previously reported (18,27).

Tomato tests in 1982 were designed to test the hypothesis that P availability and fertility level influence the response of plants to TRIA. Phosphorus treatments were designed so that additional P was applied and available prior to treatment or additional P was applied at the time of TRIA treatment. Under the conditions of this experiment, supplemental banded P had no effect on yield or response to TRIA. TRIA increased the yield of tomatoes 4.5% with and without supplemental P (Table 13). The addition of a supplemental fertilizer

Table 13. Yield of 'Pic Red' tomatoes in 1981 treated with TRIA as a seed treatment, foliar spray, or a combination of seed and foliar treatment, and yield of 'Castlehy 2043' tomatoes in 1982 grown at 5 fertility levels and sprayed with 1.0 μg/liter TRIA.

1981			1982[z]		
TRIA Treatment		Yield	Fertilizer	TRIA	Yield
Seed[z]	Foliar	(MT/ha)			(MT/ha)
Dry Control	0	68.0	Control	0	97.3
Dry Control	+	80.6		+	102.0
DCM Control	0	88.8	+ P at Transplant	0	95.5
DCM 1.0 μg/l	0	76.9		+	100.2
DCM 10 μg/l	0	79.6	+ P at Treatment	0	95.5
DCM Control	+	80.6		+	99.1
DCM 1.0 μg/l	+	69.0	+ 224 kg/ha 16N-8P-14K	0	108.7
DCM 10 μg/l	+	68.7		+	108.7
			+ 448 kg/ha 16N-8P-14K	0	115.7
LSD 5% level		11.0		+	106.3

[z]Seeds were soaked for 1 hr in dichloromethane (DCM) or DCM containing TRIA.

[z]F value for the single degree of freedom interaction comparison of control and TRIA at control and +P fertilizer treatments with control and TRIA at the highest fertilizer level was significant at the 5% level.

band along the row at the time of treatment either cancelled this effect at 224 kg/ha or caused a reduction in yield at 448 kg/ha. Plants in the high fertilizer treatments were visually greener late in the season compared to other treatments and showed no damaging or toxic effects. Control plants showed a linear increase in yield with fertilizer treatment.

The stage of crop development at the time of treatment was also investigated with tomatoes in 1983. Two rates of TRIA were applied 2 weeks after transplanting (preanthesis), 4 weeks after transplanting (postanthesis), or at both times. There were no differences between treatments for marketable yield over 2 harvests. There was a significant interaction of fruit size and number for the early harvest, which accounted for 14% of the total harvest. Preanthesis TRIA treatment decreased the number of fruit and increased fruit size while postanthesis spray increased the number of fruit and decreased the fruit size. The combination of pre- and post-anthesis sprays had no effect (data not shown). It is possible, that TRIA treatment had an effect on early fruit number or development but that some other factor limited yield.

Summary of all tests. Differences in yields were measured in 30 of 45 experiments with TRIA treated crops (Table 14). Yield increases in 25 tests ranged from 5 to 40% and averaged 13%. Decreases in yield in 7 tests ranged from 4 to 24% and averaged 12%.

Table 14. Summary of results for all tests.

Expt[z]	Crop	Year	Avg Yield (MT/ha)	Optimum Concn (μg/l)	CV[y] (%)	Stat[x]	% change in yield with best concn and avg of all concn
1	Alfalfa	82	2.71	0.5	10	NS	+ 7
			3.49	0.5	11	*	+17
2		82	2.85	0.5	4	*	+11
3	Dry Beans	81	1.67	10	12	*	+15 (+6)
4		81	1.80	1.0	22	*	+11 (+7)
5		81	1.62	1.0	13	*	-15 (-5)
6		83	3.30	1.0	9	NS	+ 5 (+4)
7		83	3.48	-	9	NS	- (+1)
8	Field Corn	81	10.2	0.1	11	*	+ 5 (+2)
9		82	11.0	-	12	NS	-
10		82	7.86	-	7	*	(+7) (* for wt/ear)
11		82	10.9	0.5	5	*	+ 7
12		83	8.70	0.1	3	*	+ 8 (+4)
13	Potatoes	81	34.4	1.0	13	*	+18 (+12)
14		83	35.4	0.2	6	**	+11 (+9)
15		83	38.2	0.2	7	*	- 8 (-6)
16	Wheat	81	5.40	-	4	NS	No differences
17		81	-	-	-	NS	No differences
18		82	5.21	0.5	7	*	+ 6 at 1700 HR
19		82	3.54	0.5	9	*	+ 5 avg (P*time)
20	Day 1	83	4.99	0.5	7	*	+10 at 1300
	Day 2		4.87	0.5	4	NS	- (+2 all times)
	Day 3		4.83	0.5	9	NS	- (+6 all times)
21		83	4.77	-	6	*	+ 5 (+3)
22		83	4.98	-	6	NS	No differences
23		83	4.91	-	4	*	- 4 (-3)
24	Day 1	84	3.73	0.5	6	NS	No differneces
	Day 2		3.73	0.5	5	*	- 7 at 0900 HR

Table 14. Continued.

Expt[z]	Crop	Year	Avg Yield (MT/ha)	Optimum Concn (µg/l)	CV[y] (%)	Stat[x]	% Change in yield for best concn and avg of all concn
25	Asparagus	81	1.99	1000	-	**	+25(** for spear No.)
26		81	1.23	1000	-	**	-24(** for spear No.)
27		82	0.29	5.0	-	*	+ 3 (* for spear No.)
28		83	0.42	-	13	NS	No differences
29	Cucumbers	81	-	-	15	NS	No differences
30		82	25.0	-	17	NS	No differences
31		83	4.88	1.0	16	*	+ 2 (* for fruit No.)
32	Green Beans	82	11.0	1.0	9	NS	+ 7 at 1700HR
33		83	8.62	1.0	11	NS	+ 2 at 1700HR
34	Lettuce	82	9.71	1.0	15	NS	-10 at 1200 HR
35	Muskmelons	81	16.7	100	20	*	+40(+31)
36		82	14.1	1.0	31	*	+40(+21) (water temp)
37	Onions	81	15.6	0.01	-	*	+18(+12)
38		83	21.2	-	9	NS	No differences
39	Sweet Corn	81	14.5	-	17	NS	No differences
40		81	14.6	0.01	14	*	-17(-8)
41		83	15.7	-	8	*	- (+9)
42	Tomatoes	81	76.5	-	16	NS	+10 foliar
43		82	76.5	-	8	*	+18 foliar,-13 seed
44		82	103	1.0	26	*	+5 low,-8 high fert
45		83	59.3	-	10	*	early fruit size,num

[z]Correspond to numbers in Table 1.

[y]Coefficient of variation.

[x]NS, *, **, F value was not significant (NS), or significant at the 5% (*) and 1% (**) level respectively.

Discussion

TRIA formulated as a colloidal dispersion. Previous studies with other formulations indicated that the active concentration range was broad and that there was no single optimum concentration (18,27,29). Experience with colloidally dispersed TRIA under controlled environment conditions indicated that there was an optimum concentration and that the concentration applied was more important than the rate of application (14,31). The optimum concentration of the colloidal dispersion for stimulating seedling growth is 0.1 to 1.0 µg/liter which is 100 to 1000 times lower than concentrations used with other formulations (14,31). One primary objective of this research was to determine if there was a consistant optimum concentration of TRIA formulated as a colloidal dispersion for use under field conditions. In several field tests with 3 or more concentrations there was 1 concentration which gave a maximum yield. The majority of tests in this study indicate a narrow active concentration between 0.1 and 1.0 ug/liter (Table 14). In some tests, however, a wide range of TRIA concentrations gave similiar yields and effects on yield were obtained with 0.01 and 1000 µg/liter.

Different batches of colloidal dispersion were used throughout this study. An effort was made to keep the purity of TRIA and the particle size of the dispersions consistant since these factors have been shown to affect the optimum concentration (31). At 0.5 ug/liter the activity of TRIA formulated as a colloidal dispersion increased with increasing purity between 96% and 99.4% (31). In this study, 2 tests with wheat indicated a 100-fold difference in the active concentration dependent on TRIA purity. These tests with wheat and preliminary greenhouse tests indicated that less pure sources of TRIA formulated as a colloidal dispersion are active but at higher concentrations. The nature of the impurities may be as important as the quantity of the impurities in determining growth regulating activity since certain long-chain alcohol analogues of TRIA have been shown to inhibit TRIA activity (12).

Stage of crop development for treatment. Treatments were applied during early vegetative growth, anthesis, early fruit set, and during dry matter accumulation of reproductive or storage organs (Table 1). Treatment effects were measured for crops treated at each stage. The last developmental stage was not tested properly, because the crops treated during final stages of dry matter accumulation of reproductive or storage organs had also been treated at earlier stages. Asparagus, alfalfa, snapbeans, dry beans, lettuce, and onions were treated during vegatative stages of growth. Potatoes, tomatoes, cucumbers, and muskmelons were treated at early anthesis when vegatative growth was still progressing. Wheat was treated after the boot stage and up to late anthesis. In general, results support the conclusions of Ries and Houtz (32), that "applications to leafy vegetables should be when vegetative development is progressing rapidly and for fruiting vegetables and grains at anthesis" or when fruit/grain set is being determined. Field and sweet corn may be exceptions to this generalization since they were only treated during vegetative growth stages in this study.

Based on the results of this study and previous field studies by other researchers, the effect of TRIA is not crop specific or dependent on the plant part harvested. Vegetative, grain, fruit, bulb and tuber yield were affected by TRIA in this study. Vegetative growth of alfalfa and asparagus, the number of grains per head in wheat, ear size and number in field corn and sweet corn, fruit number in cucumber and muskmelon, bulb size in onion, and both fruit number and size in potato and tomato contributed to differences in yield. In some cases yield differences could not be attributed to any specific component of yield.

TRIA was initially isolated from alfalfa after side dressings of alfalfa hay increased yield of crops and growth of seedlings more than could be accounted for by several factors including the nutrient content of the hay (28). Without proposing how the increased growth came about, early field studies assumed that applications of TRIA during early growth stages that increased vegetative growth or biomass would result in greater yield (29). In the first year's field studies (29), increased yield from 7 of 10 crops treated with foliar applications at early growth stages supported this assumption. However, tests in later years (18,26) indicated the stage of growth at treatment time was not critical and that results were not predictable. Although increased biomass often results in increased yield, it is not assured (9). Controlled environment studies to date indicate a rapid periodic response to TRIA (13,30,32), as opposed to some permanently altered state of metabolism. The results of this study suggest that the effect of TRIA on plants may result in increased vegetative growth at early growth stages, but may result in altered fruit or grain number or carbohydrate partitioning applied at later growth stages. The application of TRIA to certain crops at later stages of fruit and grain development will need to be investigated further.

Stategies for enhancing yield with growth regulators have recently been directed at a few key mechanisms. These mechanisms often revolve around the question of whether yields are source or sink limited, and include increasing photosynthetic activity or efficiency, optimizing photosynthetic carbon partioning, increasing fruit/grain set or number, and enhancing nutrient and water uptake and/or utilization. The possible relationship of TRIA's metabolic effect to these mechanisms has not been studied. Since the initiation of this field study, TRIA has been shown to alter plasmalemma ATPase activity in barley root membrane vesicles (16), to alter the activity of several enzymes in carbon metabolism pathways (11,15), and to increase photosynthetic rate in studies with the algae Chlamydomonas reinhartti (10). The tissue concentration of reducing sugars, free amino acids, and soluble protein (30), as well as the uptake of Rb and P (24) were shown to increase after treatment of seedlings with TRIA. One study indicated increased CO_2 assimilation and movement of photosynthate to the grain of rice plants treated 10 days after anthesis (7). These physiological effects could alter yield via several mechanisms. It was not the objective of this study to identify specific physiological effects of TRIA under field conditons, however, knowledge of these effects may define conditions and stages of development which increase the probability of enhancing yield.

Environmental Conditions. Conditions at the time of application and/or after application have an effect on the response of plants to TRIA. Applications resulting in increased yield were generally made on warm sunny days with minimal air movement, with treatments applied mid to late afternoon (1300 to 1700 HR) under conditions favoring growth (Table 1). However, not all tests treated with TRIA under these conditions resulted in increased yield and some tests treated under other conditions did increase yield.

Time of day of application is important but it cannot be said that treatments applied in the morning will not affect yield. In 2 tests with wheat, applications of TRIA at 0900 HR either increased or decreased yield. The likelihood of enhancing yield, however, may be improved by applying TRIA mid to late day. When nine comparisons of wheat treated at different times of day (Table 6) were combined in one analysis of variance with each test or main plot of 6 replicates treated as a random effect in analysis of variance, the comparison of treatment at 1700 HR versus all other treatments was significant at the 5% level. The increase in yield at 1700 HR was 4% over the control. Wheat tests conducted in 1981 were treated in the morning or on a rainy afternoon and showed no TRIA effect on yield.

Treatments were generally applied under the highest temperature conditions possible but the effect of temperature could not be separated from other environmental conditions such as radiant energy, humidity, and moisture availability. Applications during mid to late afternoon were during the warmest part of the day. A combination of factors favoring growth or limiting stress are probably more important than any one specific condition alone. If TRIA only increases vegetative growth, it is likely that when environmental conditions limit growth, TRIA will not increase yield. Actual growth rate at the time of treatment was not measured in any of these tests.

Observations from controlled environment research and earlier field tests indicated that plant water status might alter the response to TRIA. Water potential data for some tests are presented as an indication of plant water status and potential for growth. Since the samples were taken from exposed, recently matured tissue, the data may not be representative of the water status of the elongating or meristamatic tissue (19). At this point, only a speculative relationship can be suggested between the effect of time of day on TRIA's growth regulating activity and diurnal fluctuations in water potential and expansive growth. Further evidence will be necessary to test the hypothesis that the response to TRIA is greater under environmental conditions favoring expansive growth. However, available evidence indicates that TRIA should not be applied to crops under a water stress.

In at least one experiment in this study, and one reported previously (29), TRIA increased yield at lower fertility levels but decreased yield when additional fertilizer was applied. In both cases, controls at the higher fertilizer level yielded more than unfertilized controls. Although Regher concluded that it was not likely that environment influenced the effect of dinoseb and TRIA on corn yield, in at least one experiment he reported that TRIA increased yields under irrigated conditions and decreased yields under nonirrigated conditions (26). Other experiments in this study with dry beans, potatoes, wheat, and asparagus resulted in increased yield of plots treated at one location or time and decreased yield in plots treated at the same time at different locations or the same location at different times. When the results of all experiments were summarized, the number of tests resulting in decreased yield led to an attempt to evaluate experiments with yield decreases to identify potentially important variables. Controlled environment studies with seedlings also identified conditions when TRIA decreased seedling dry weight accumulation. The potential for undesirable depression of yield with TRIA will need to be investigated further. If the enhancement or depression of yield are due to conditions at the time of or following TRIA application, it is likely that some conditions between the two extremes will result in no effect on yield. This may explain the lack of effectivness in some studies. Nutrient availability and "water" stress were 2 factors that were identified in both field and greenhouse studies as important variables associated with the depression of growth and yield with TRIA.

Conclusion. This research was undertaken with the concept that a general set of conditions could be defined where consistent yield enhancement with TRIA could be achieved for most crops. I suggest, in contrast, that such a goal is not practical. A more acceptable approach to field use of TRIA for commercially increasing yield may be to define a specific set of conditions for a limited set of crops where TRIA works most consistently. Conditions have been outlined which favored yield enhancement from TRIA application in these tests. These conditions are presented as starting points and not as absolutes. Treatment effects at different stages of development could not be totally separated from environmental effects, even with carefully planned experimental designs. It is likely that a combination of both developmental and environmental factors affect the response of crops to TRIA. Empirical field testing is less likely to identify a proper stage of treatment than are studies directed at identifying the fundamental effect of TRIA on plant metabolism.

The increase in yield with TRIA in these studies are not sufficiently consistent to warrant large-scale commercial application. The positive yield effects are large enough, however, to warrant future investigations to define conditions favorable for increasing crop yields with TRIA and how TRIA can influence yield relative to emerging stategies for enhancing yield with plant growth regulators. An understanding of these factors may also enhance the effective use of other plant growth regulators.

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SUMMARY

In both field and controlled environment tests, the plant response to TRIA varied with environmental constraints on growth and yield. Conditions favoring growth and yield enhancement as well as depression of growth with TRIA have been outlined. These conditions are based on available but limited data. It is clear from these studies that an extensive effort would be necessary to clearly define all environmental factors influencing the response of crops to TRIA and that attempts to do so may be impractical.

As with many proposed PGR's, the transition from consistency in the laboratory to consistency in the field has been difficult. This is not suprising, however, considering the complexity of biological systems and our incomplete knowledge of the mechanisms regulating crop growth and yield. There is a concensus that the chemical manipulation of yield is closely linked to cultural protocols and environmental factors. Two abstracts of papers presented at the 1984 Plant Growth Regulator Society of America meeting concluded with the following statements: a) "but there is an indication that fertility level is an important factor in eliciting beneficial responses with bioregulants" (3); and

> b) "Overall, weather may be the single most important factor in crop productivity. It may also play an important role in determining the response of a crop to a yield enhancement treatment or a PGR. Variability of weather conditions across locations may explain the lack of performance of a yield enhancer to date. This obstacle must be overcome before the goal of yield enhancement by a chemical can be realized" (1).

Many similiar statements were reported in the recent CRC review of PGR's (10). One view in particular which seems applicable to TRIA was in the review of PGR use in soybean production. It was concluded that since research has shown it is impractical to expect a compound to produce consistent results, we should develop recommendations for specific crops and conditions where success is evident (16).

The physiological basis of crop yield is complex and much effort is being directed toward assembling a model of the interacting components. While TRIA and other PGRs may not be used exstensively to increase yield due to environmental constraints, an understanding of how yield is increased by TRIA under certain conditions may provide clues to processes which can be altered in other ways, more consistently than with current chemicals such as TRIA. The future of hormonal and chemical manipulation of growth and yield may be dependent on the involvement of molecular biology with applied agricultural sciences. Effort concentrated on understanding the mechanism of a few yield altering compounds, so that the generated information can be used as predictors for new compounds, may be as important as the generation of new PGR's from screening programs.

This research has been part of a concerted effort to develop an understanding of the growth regulating activity of TRIA. TRIA has been approached from a chemical or formulation perspective; from a fundamental biochemical and physiology perspective with isolated membrane systems, unicellular algae and seedlings; and in this research from a whole plant, agricultural application perspective. Formulation methods have been improved, but not without complications. Floculation of TRIA particles by cations (4), interference of the small amount of TRIA by contaminants in application systems (15) concentration dependence of activity (5,13), loss of activity over time under nonsterile conditions (13) and the necessity for attention to detail during formulation are some of the potential problems which must be taken into account when working with the colloidal dispersion. However, the formulation has proved reliable enough in certain bioassays that it was probably not the primary cause of inconsistent effects in this research.

The effects of TRIA on the metabolism and enzyme activity of maize seedlings (6,12) and on CO_2 assimilation of Chlamydomaonas (4) suggest a range of effects but not a primary activity. Based on research with isolated membrane systems, a relationship between TRIA's activity and membrane structure and function is suggested (7,8). This is only a beginning of the type of work that will be necessary to identify how TRIA affects growth. The results of the field and greenhouse studies reported here, do not provide a strong basis for conclusion about the mode of action of TRIA, but they provide some circumstantial evidence. Possibly the most important observation from this study is that treatment of crops and seedlings with TRIA under certain conditions can decrease yield and shoot dry weight accumulation. Other chemicals found to stimulate growth have also on occassion resulted in yield depression (10). In the yield study, decreases were as large as increases although much less frequent. In the seedling study, decreases in growth were small and as difficult to reproduce as increases in growth. This apparent potential for both increases and decreases in growth and yield could account for the lack of effect in some tests in this and other studies.

Evidence is accumulating to support a regulatory role of TRIA in plant growth. The cuticle is often viewed as a static barrier between the plant and the environment. Recent evidence has shown that the cuticle composition and thickness changes with variations of light, temperature and humidity (2). TRIA's function as a component of leaf cuticular wax, may therefore be related to its growth regulating activity. The synthesis of cuticular waxes may be involved in the plant response to environmental stress and resulting osmoregulation.

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