INCREASING THE EFFECTIVENESS OF
1-TRIACONTANOL APPLIED
TO PLANTS

By

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ABSTRACT

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Triacontanol (TRIA) is a 30 carbon primary alcohol which stimulates the growth of plants and may increase crop yield. The increase in growth and yield has been variable in greenhouse and field studies. Possible sources of this variability were investigated, in an attempt to develop practical recommendations for the use of TRIA on food crops. The factors studied included formulation, dose, pH of spray solutions, night and day temperatures, and nitrogen and phosphorus nutrition. In growth chamber and greenhouse studies, a colloidal dispersion of TRIA applied at concentrations of 0.1 to 10 μg/L stimulated growth of corn (*Zea mays* L.) seedlings. These doses are 100 to 1,000 times less than the relatively low doses used with the original chloroform Tween 20 formulation in prior research. The pH of the spray affected the response of plants to TRIA. Water suspensions with a pH of 8.0 or more were found most effective with the original formulation when applied to corn, rice (*Oryza sativa* L.) and soybeans (*Glycine max* L.) Night temperatures influenced the response of corn seedlings to TRIA. Night temperatures of 10°C and 15°C prior to TRIA application increased the response of corn to TRIA. The temperatures during the day after treatment did not alter the magnitude of the response of corn to TRIA.

Field research in Michigan in 1979 and 1980 and in Ontario during 1981 showed inconsistent yield responses in several crops. TRIA increased the yield of winter wheat (Triticum aestivum L.), sweet corn, tomatoes (Lycopersicon esculentum Mill.) and cucumbers (Cucumis sativus L.) in some tests during this period. Approximately 40% of the tests in the field with the chloroform or colloidal formulations resulted in a positive response, with 10% for the acetone emulsion. Tomato yields varied with dose of TRIA applied to the foliage. The highest yields in the field were obtained with applications of about 10 μg/L of the colloidal dispersion. Treatment of tomato seeds with TRIA or application to roots of tomato transplants increased the yield. The same colloidal dispersion of TRIA increased the dry weight of corn at 0.1 μg/L in controlled environment studies. There was a differential trend of response of corn and tomatoes to TRIA when grown at different levels of phosphorus fertility in the field. It is postulated that TRIA may be more effective on plants growing under mild stress conditions, such as low night temperatures.

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INTRODUCTION

The world is facing an ever-increasing population, consequently an ever-increasing demand for food. A major problem is to supply sufficient food for the increasing population. It is important to investigate the potential usefulness of any compound or technique which may improve the yield and/or quality of crops. Triacontanol (TRIA) is one such compound. Applications of a few mg/ha have increased crop yield.

TRIA is a 30 carbon primary alcohol first isolated as a component of alfalfa leaf wax by Chibnall et al (7). Its ability to stimulate plant growth was first reported by Ries (22).

Since the original discovery of plant growth regulating activity TRIA has been shown to stimulate the yield of several annual crop species (21, 24). Single foliar applications of TRIA at concentrations of 0.01 to 10 mg/L to seedlings increased the yields of sweet corn (*Zea mays* L.) cucumbers (*Cucumis sativa* L.), soybeans (*Glycine max* L.) and wheat (*Triticum aestivum* L.) an average of 12%. Little information is available on the effect of TRIA on yield components or quality, but TRIA may stimulate plant growth. The implications of consistent increased crop yields resulting from very small amounts of TRIA are enormous. However, plants have not consistently responded with increased yield or bio-mass production to applications of TRIA under field or greenhouse conditions. The reasons for this failure to consistently stimulate yield or bio-mass production are not clear. Furthermore, the exact environmental conditions, TRIA concentrations, timing and method of TRIA application and other factors required to obtain increased yields have not been delineated. However, if techniques are developed so that consistent yield increases could be obtained, then TRIA would be an excellent management tool for increasing crop productivity.

The objectives of this research were to:

(1) Investigate the effect of the pH of different formulations on the response of plants to TRIA.

(2) Investigate types of formulation and doses of TRIA.

(3) Evaluate the effect of pre and post treatment temperatures on the response of plants to TRIA.

The major problem delaying the commercial use of TRIA in crop production both in the field or greenhouse is this lack of a consistent response. This was also the central problem in this research. The major difficulty in this study was to find a protocol that would consistently reproduce the response of plants to TRIA, so that desired environmental and physical variables could be investigated in detail. Much emphasis was placed on environmental factors in order to develop such a protocol.

LITERATURE REVIEW

Triacontanol (TRIA, CH_3 $(CH_2)_{28}$ CH_2OH) is a 30 carbon primary alcohol which was identified as a component of alfalfa (Medicago sativa L). leaf wax by Chibnall et al in 1933 (7). Coarsely chopped alfalfa hay banded at 117 kg/ha was reported by Ries et al (22) to increase the yield of tomatoes (Lycopersicon esculentum Mill.) by 10 tonne/ha. The yields of cucumbers (Cucumis sativus L.) and lettuce (Lactuca sativa L.) were also increased by this treatment. The increase in yield was greater than that expected from the added nitrogen from the alfalfa. It was subsequently demonstrated that a chloroform extract of alfalfa hay as well as ground alfalfa applied pre-plant to the soil increased the dry weight of corn (Zea mays L.) seedlings grown in a greenhouse (22). The chloroform extract added at the equivalent of 0.1 to 10 mg/L of emulsion also increased the growth of rice (Oryza sativa L.) grown in solution culture. TRIA was isolated and identified as the active component of the extract (22). Synthetic TRIA, both when applied in the nutrient solution to rice plants and to the foliage of corn seedlings grown in soil increased the dry weight compared to untreated plants (22).

The response of plants to TRIA under laboratory conditions. The overall response of plants to TRIA is characterized by a very rapid increase in growth rate as measured by dry weight accumulation and increases in leaf area (23). Ries and Wert (23) demonstrated increased dry weight and leaf area of rice plants in solution culture within 3 hr of treatment with 10 µg/L of TRIA in the nutrient solution. Kjeldahl-detectable nitrogen also increased due to the treatment. They showed that the net assimilation rate (NAR) of rice was highest the first 8 hr after treatment; however, there was no effect on the NAR after 24 hr (23). This suggests a very short-term direct effect of TRIA on plant metabolism.

Ries and Wert (23) showed that the increase in plant dry weight would occur when rice was treated for 6 hr in the dark. This evidence suggests that plant response to TRIA is not wholly dependent directly on the photosynthetic pathway, and that metabolism of a stored product may be involved. If the growth response to TRIA depends on the metabolism of a stored product, then the manipulation of night temperatures should induce differences in the response. Lower night temperatures would lead to greater food reserves, hence a larger pool available to respond to TRIA application. Effects of night temperatures on this possible TRIA effect have not been investigated. Bittenbender et al (2) further investigated the dark response of rice to TRIA. Carbon dioxide was shown to have an apparent regulatory role on dry weight gain in the dark in response to TRIA (2). Rice plants did not respond to TRIA in the dark when the CO_2 level around the plant was less than 50 or greater than 360 µL/L. The maximum dark response occurred when CO_2 levels were 100 to 200 µL/L, i.e. less than ambient atmospheric levels. The effect of CO_2 on the daytime response of plants to TRIA is not known. The role of CO_2 in the response of plants to TRIA remains unclear.

Eriksen et al (8) demonstrated a 30% increase in dry weight of tomatoes grown in solution culture after 24 days when 100 µg/L TRIA was added to the solution before flower bud formation. No effect of TRIA was observed with a single application; TRIA needed to be supplied with every change of the nutrient solution. No effect on corn was observed in solution culture with similar treatments (8). In these experiments, Eriksen et al also observed an apparent effect on the photosynthetic system of tomatoes (C3 plant) treated with TRIA under several concentrations of oxygen. Photosynthetic efficiency of treated and control plants was compared at 21% O_2 and 2% O_2. Net photosynthesis was reduced by 39% in control plants and 27% in TRIA-treated plants when compared at 21% vs 2% (8). This would indicate more available photosynthate in TRIA-treated plants. There was no effect of TRIA on photosynthesis of corn (8). The results agree with those of Ries and Wert (23) who showed no apparent effect of TRIA on rice (C4 plant) photosynthesis. It may be possible that TRIA affects C_3 and C_4 plants differentially.

Short-Term biochemical responses of plants to TRIA. In laboratory experiments rapid changes in metabolism induced by TRIA have been demonstrated. For example, changes in Kjeldahl-detactable nitrogen have been reported within 80 min (11, 13, 14, 25). Ries et all (25) showed increased in Kjeldahl-detectable soluble protein, free amino acids and reducing sugars within 4 min of treatment. Dry weight was shown to increase in 10 min and leaf area within 20 min (25). As many of these experiments were 80 min in duration, the long-term biochemical effects of treatment with, if any, TRIA are not known. The relationship of these short-term biochemical events with long-term effects of TRIA on plants remains to be demonstrated.

Houtz (11) has shown that phosphate uptake from the nutrient solution increased as a consequence of treating rice with TRIA. Ramani and Kannan (20) demonstrated a TRIA-induced increase in uptake and transport of phosphate and rubidium ions by sorghum (*Sorghum vulgare* L.) grown in solution culture. Drought resistant cultivars of sorghum absorbed more phosphate and rubidium ions than did drought susceptible cultivars (20).

It is evident that TRIA influences the uptake of some mineral ions, and that cultivar differences exist. Bittenbender et al (2) showed that the TRIA response of rice was not influenced by the source of nitrogen (NH_4^+ vs NO_3^-). Singletary (18) demonstrated with soybeans (Glycine max L.) that there was no effect on nutrient uptake grown in solution culture. He found however, that TRIA at 10 µg/L increased the growth of corn seedlings grown in solution culture with half of the normal N and P levels compared to those grown in full strength solutions. Clearly, there is an effect of TRIA on the short-term nutrient status of the plant. However, the effect of the plants' initial nutritional status on its ability to respond to TRIA needs clarification.

Formulations of TRIA. Originally, crystalline TRIA was dissolved in chloroform to make a stock solution. Aliquots of this stock were added to water containing 0.1% v/v Tween 20 to form a stable suspension (23). An acetone-$CaCl_2$-water emulsion developed by Weliber (26) was used for a period of time in 1980. In 1981, a stable colloidal dispersion of TRIA was developed by Laughlin et al (14). This formulation of TRIA has several advantages: it is an aqueous dispersion, virtually eliminating problems of safety as compared with using organic solvents. It is prepared under controlled conditions, as opposed to previous formulations of chloroform or acetone. Furthermore, the colloidal dispersion of TRIA has been shown to be as much as 1,000 times as effective as the original TRIA-A formulation in short-term studies with rice and corn (14). This formulation of TRIA has been shown to stimulate the growth of rice in solution culture when applied at concentrations as low as 1 ng/L (2.3×10^{-12}M) (14). These minute quantities are about the physiologically active endogenous levels of known naturally-occurring plant hormones. This suggests a profound physiological role for TRIA in the plant.

In fact, the concentrations of applied TRIA that have elicited responses in plants are much lower than those used for other exogenously applied growth regulators, often applied at concentrations up to several hundred ppm. This suggests that TRIA has a very potent effect on plant metabolism.

Jones et al (12) showed that other long-chain hydrocarbons such as octacosanol ($CH_3 (CH_2)_{26} CH_2OH$) were powerful inhibitors of the activity of TRIA. Octacosanol at 2.4×10^{-12}M was shown to inhibit TRIA applied to rice at 2.3×10^{-8}M (100 µg/L) (12), thus small amounts of impurities in the formulation may drastically effect the activity of the TRIA. However, the nature of the type of inhibition is unknown.

Response of plants to TRIA under field and greenhouse conditions. Single foliar applications of TRIA ranging from 20 mg/ha to 2.2 g/ha increased marketable yields an average of 13% for sweet corn, 14% ($/ha) for cucumbers (more fruit/plant), 10% soybeans, 17% for tomatoes (more early fruit) and 8% for wheat (21, 24). Howeveer, in these studies an effect of TRIA could not be consistently obtained in every experiment. Also the optimum concentration of TRIA was not determined. Bouwkamp and McArdle (4) showed that TRIA applied at a concentration of 100 µg/L to sweet potatoes (*Ipomoea batatas* L.) in the field had no effect on yield of roots at harvest. Leaves were sampled at 14 hr, 5 and 57 days after treatment; weighed and analyzed for nitrogen. The dry matter of leaves was increased by TRIA at 14 hr and 5 days, while % nitrogen increased after 5 days. There were no differences at harvest. It is evident that a short-term effect of TRIA was obtained but this effect was not reflected in an increased yield of sweet potato roots.

Bosland et al (3) did not obtain an increase in yield or change in soluble solids of 'PMR-45' muskmelons (Cucumis melo L.) when plants were treated in the 8 to 10-leaf stage with 0.01 to 10.0 ppm TRIA as a foliar spray. This stage of growth is later than that used by Ries et al (24) when TRIA increased the yield of cucumbers, indicating that age may be a factor in the lack of response.

In greenhouse experiments TRIA increased the dry weight of cucumber, field corn, soybean and carrot (Daucus carota L.) seedlings 34%, 34%, 19% and 65% respectively of TRIA at concentrations of 0.01 to 10 mg/L (24). However, consistent responses to TRIA were not obtained and optimum doses and conditions were not defined. Pocock (17) reported variable responses in potted sugar beets (Beta vulgaris L.) with plant age and concentration of TRIA. TRIA applied at concentrations of 0.001 to 1 mg/ha when the first tree leaves are expanding decreased the growth of beets by 10%. However, the dry weights increased when TRIA was applied after the first two leaves were fully expanded. Three weeks after treatment these plants had increased dry weight by 30% as compared to the controls (17). There was no effect of TRIA on sugar concentration at harvest (17). Application of TRIA to the seeds of several species by soaking in TRIA dissolved dichloromethane mixtures has stimulated dry weight production in greenhouse studies an average of 50% (24). No effect of TRIA could be found with direct-seeded crops in the field (24). However, marketable yield of tomatoes was increased 27% when grown from transplants produced from treated seeds (24). Reasons for this lack of consistency are not known. Ries et al (24) investigated the response of wheat, corn and tomato seedlings from treated seeds grown at night and day temperatures of 10/15^{o}C, 15/20^{o}C, 20/25^{o}C and 25/30^{o}C. Percent dry weight increases over control and temperature were linearly related, indicating that temperature influenced the response. Increases due to the temperatures studied varied from 20 to 80%, indicating that temperature influenced the magnitude of the response to TRIA.

Henry and Primo (9) reported increased bio-mass of 11-day-old 'Great Lakes' lettuce, but not 'Grand Rapids' grown in solution culture in a growth chamber indicating a cultivar difference in response to TRIA under these conditions. Charlton et al (6) reported no effect of TRIA at 0.1 to 100 μg/L on several analogues applied to the seeds of durum wheat in greenhouse experiments. Hoagland (10) demonstrated no effect of TRIA at 10^{-5}M on seed germination of 15 crop and weed species. As with the effect of TRIA on crops grown in the field, there are inconsistent reports of its effect on growth in shorter germ greenhouse studies. The reasons for this lack of consistency are not clear.

General comments on the action of TRIA on plants. Since the original discovery of the growth stimulating activity of TRIA, various reports (17, 21, 24) show increased long-term growth, and 4 show no effect (3, 4, 6, 8). Increased yield was not obtained in every field experiment (21). No successful responses on crops grown as perennials have been reported to date. Little is known about the effect of TRIA on various yield and quality parameters. Reasons for the inconsistent responses of plants to TRIA are not clear. Climatic, soil conditions, species, cultivar, concentration and formulation and formulation quality may all influence the ability of plants to respond positibely to TRIA. The optimum dose of TRIA, time of application, stage of growth and the mode of application required to obtain the maximum response of plants all remain undefined for any species. In addition, another unknown factor is the effect of spray pH on the plants' response to TRIA. A low pH has been shown to increase the uptake of NAA (16). However, the effect of pH on the physiological activity of long-chain alcohols is unknown.

The action of TRIA on plants has been most consistent in short-term lab oratory studies (2, 11, 12, 13, 14, 22, 23, 25). Consistent increases in dry weight, Kjeldahl-nitrogen, free amino acids and reducing sugars have been obtained in rice and corn seedlings in short-term biochemical studies (11, 13, 25). Short-term biochemical responses to TRIA in these studies have been shown to be rapid, in some cases a matter of minutes. Reasons for the inconsistency between field, greenhouse and laboratory studies are not known, and the relationship between observed biochemical responses and total plant behaviour remain unclear. Since yield is a function of the total integrated plant-environmental interaction during its life, it is difficult to evaluate the importance of short-term, transitory biochemical effects on long-term growth and yield.

TRIA can be a plant growth stimulant. However, little is known about the conditions required to induce positive effects on the yield of crops. If a means is found to consistently increase yields of crops when treated with TRIA, then it may become an important management tool in crop production.

MATERIALS AND METHODS

Formulations. A solution of TRIA was prepared with the TRIA dissolved in chloroform (10 mg/10 ml). Aliquots were added to distilled water containing Tween 20 (polyoxyethlene sorbitan monolaurate) 0.1% v/v as a surfactant (23). This emulsion was heated to drive off the chloroform, and is referred to as TRIA-A. TRIA was also prepared by dissolving in acetone, aliquots of one which were added to a 2% acetone/water emulsion (26). This will be referred to as TRIA-B. The colloidally-dispersed formulation (14) was added directly to distilled water and will be referred to as TRIA-C. All control treatments were made as their individual formulations, but without TRIA.

A 1 mM or 10 mM phosphate buffer was used to obtain various spray pH's with the TRIA-A formulation. Sodium hydroxide and sulphuric were used to adjust the pH of the TRIA-B and TRIA-C formulations.

Typically, TRIA was applied to the run-off point, using a glass 250 ml chromatography sprayer (A.H. Thomas Co., Philadelphia, PA) with air as the propellant. Concentrations of TRIA tested ranged from 0.1 to 10,000 ug/L.

Procedures in controlled environments. Greenhouse experiments were conducted under the following environmental conditions, supplemental lighting, both fluorescent and High Intensity Discharge lamps (67 W/m^2 at 1.2 m), the photoperiod was 16 hr, the night temperature was approximately 21°C. 'Heinz 1350' tomatoes and 'Greenstar' cucumbers were grown in 10 cm clay pots and 'Pioneer 3780' field corn in 18 cm clay pots. A 1:1:1 soil mix of sand, peat and loam was used for the period from March 1979 to September 1980 and a 1:1 soil:peat mix was used after this time. Nutrients were applied twice per week at 250 ml per pot (100 ml for 10 cm pots) with 1.6 g/L solution of 12:21.1:6:6 (N:P:K) or 1 g/L of 20:8.8:16.6 soluble fertilizer.

All water was applied in measured amounts once the experiment was started. Plants were thinned to 4 uniform seedlings per pot and carefully blocked for size in all experiments. Typically corn was treated at the early three-leaf stage, 6 to 8 days after planting, depending on time of year and harvested 7 days later. 'Corsoy' soybeans were sprayed when the first true leaves appeared. The procedures for the rice experiments were previously described (23). Plants were dried to a constant weight at 70°C prior to weighing.

In the growth chamber studies night temperatures of 10°C, 15°C, 20°C and 25°C nights were used and the day temperatures were maintained at 30°C. For several of the night temperature experiments, corn heat units (CHU) were calculated using the formula of Brown (5). Plants were treated at the number of CHU accumulated to the third-leaf stage of corn grown with 22°C nights. Plants grown at 22°C nights were harvested after 5 days (430 CHU); plants from the other treatments were harvested after the same heat units had accumulated.

Plants were watered by hand with 250 mL 2 times per week. Fertilizer was applied as in the greenhouse experiments. Light levels were measured with a Lambda Li 185 with Quantum Sensor (Lincoln, Ne.) and a Weston F56 sunlight illumination meter (Newark, N.J.) and were 10,000 lux at 0.75 m at East Lansing and Simcoe respectively. Sixteen-hour day and 8-hour nights were used for most experiments.

After the end of March 1981, both controlled environment and field research was conducted at the Horticultural Experiment Station, Simcoe, Ontario. In 1981 all of the TRIA used was the colloidally-dispersed TRIA-C. Corn experiments were similar to those at M.S.U. with the exception that 15 cm plastic pots and peat soil mix (Metro 200) were used and the sprays were applied at 350 L/ha with a trigger-type atomizer.

Seed treatments. Seeds of 'Earlibright' tomatoes were treated for 30 minutes in surfactant and concentrations of TRIA-C, ranging from 0.001 to 1.0 mg TRIA/L. The surfactant control was 0.1 mg/L. Seeds were removed at the end of treatment and dried overnight at room temperature. For pregermination, seeds were placed in 1.5 kg plastic freezer bags with 250 mL of high resistance deionized water. At the end of 2 hr the water was drained off and approximately 100 mL of fresh, deionized water was added. The bags were filled with air and sealed with a twist tie and the seeds germinated at room temperature (21^{o}C) for 3 days.

In order to investigate the possibility that with the colloidal dispersion, the wax on the seed coat was preventing TRIA from reaching the embryo, the seeds were soaked 30 min in DCM with constant stirring. After soaking, the seeds were dried overnight at room temperature.

Field experiments. Normal cultural practices for Michigan were used in Michigan in 1979, 1980 and those recommended for Ontario were used in 1981. Cultural practices in both areas are similar. Wheat experiments in 1979 were conducted at both East Lansing and Saranac, Michigan. The East Lansing experiment used several TRIA-A treatments applied at 2 different stages of growth on 4 cultivar combinations and doses of TRIA-A. The following cultivars of soft winter wheat were used in 1979: 'Augusta', 'Tecumseh', 'Ionia' and 'Genesee'. 'Ionia' and 'Genesee' were lost due to severe lodging. Treatments were applied to drip in the test at Saranac in order to wet the plants thoroughly. It was found that CO_2 as a propellant lowered the spray solution pH, therefore all further experiments were sprayed with a pump-up compressed air sprayed to avoid this complication. Further experiments in 1979 and 1980 were sprayed to the run-off point to completely wet the plants, as with the greenhouse experiments.

Experiments were conducted to elucidate the optimum dose of TRIA-A for 'Gold Cup' sweet corn and 'Greenstar' cucumbers. Ears of sweet corn of marketable size were picked by hand, weights were recorded before and after husking.

Pickling cucumbers were harvested once-over and graded based on Pickle Improvement Committee of the International Pickle Packers Association standard prices. Yield was converted to $/ha as follows: fruit up to 2.7 cm in diameter was given a value of $13.25/100 kg; 2.7 to 3.8 cm $6.23; 3.8 to 5.1 cm $4.42; 5.1 to 5.7 cm $2.2; 5.7 to 6.4 $1.11; greater than 6.4 cm $0.55.

'Heinz 1350' tomatoes were harvested by hand and the weight of ripe marketable fruit was recorded.

'Gold Cup' sweet corn and 'Greenstar' cucumber experiments were conducted at the East Lansing and at the Carksville Horticultural Research Stations in 1980 to investigate the effect of low nitrogen and high phosphorus nutrition on the response of crops to TRIA. The Dryden sandy loam soil at Clarksville has a higher natural fertility than the Spinks sandy loam soil at East Lansing. Similar rates of nitrogen (80 kg/ha) and phosphorus (160 kg/ha) were applied to the corn and cucumbers at both locations as a sidedressing.

'Pikred' tomatoes were hand-weeded as it was felt that the stress induced by cool, drying winds may have weekened the plants, and possibly predisposed them to injury from the herbicide in combination with the TRIA treatments. Harvest methods in 1980 were similar to those used in 1979.

In 1980, experiments were conducted at East Lansing, Saranac and Tuscola County with 'Augusta' and 'Frankenmuth' soft-white winter wheats. Different rates of TRIA-A and TRIA-B were compared. Plots were sprayed at the early jointing stage and prior to anthesis. The East Lansing plot was lost due to winter injury. An experiment was conducted to evaluate the effect of phosphorus (0.5% P) applied to the leaves and TRIA at the early filling state of wheat.

Several cool season crops were screened for response to TRIA. It was hypothesized that the cooler night temperatures in the spring may favor the response. 'Improved Progress' peas (*Pisum sativum* L.) were direct-seeded, cole crops, 'Market Prize' cabbage (*Brassica oleracea* L. var *capitata*), 'Waltham' broccoli (*B. oleracea* L. botrytis group), 'Imperial 10-6' cauliflower (*B. oleracea* L. botrytis group), 'Early hybrid G' chinese cabbage (*B. rapa* L. chinesis group) were started in the greenhouse and transplanted to the field.

In 1981 field research was conducted at the Horticultural Experiment Station, Simcoe, Ontario. The 1981 experiments were designed to find the optimum dose of TRIA-C to use on 'Snow Crown' cauliflower, 'Earlivee' sweet corn and direct-seeded 'Veepro' tomatoes. Experiments were conducted to evaluate effects of nitrogen and phosphorus fertilizer and the dose of TRIA on sweet corn and tomatoes. Nitrogen at 45 kg/ha was applied pre-plant and 20 kg/ha of phosphorus was banded after planting. Plots were split for an additional 45 kg/ha N at the recommended time of application for both crops to evaluate the effect of 'low' and 'high' nitrogen. In addition, an experiment with or without starter fertilizer (10:23:8) at 1 kg/100 L to the roots of bare root 'Earlibright' tomato transplants. Tomato seeds were treated with TRIA-C as in greenhouse experiments and grown for transplants or direct-seeded.

The two direct-seeded tomato experiments were harvested using a Hart-Carter harvester. For multipick hand-harvest experiments, tomatoes were picked at or beyond the breaker stage and graded according to size. The large were over 5.0 cm and the small 2.5 to 5.0 cm in diameter. At the final harvest, vines were stripped of all fruit over 2.5 cm diameter to obtain an estimate of total fruit yield.

Cauliflower was harvested 3 times per week. At harvest, the trimmed head was weighed and the florets removed and weighed.

Sweet corn of marketable size was harvested by hand. The number of harvested plants were recorded and number of ears/plant were calculated. After weighing, the husks were removed, the cobs graded as marketable or unmarketable and reweighed. A cob was considered unmarketable if it was short (under 15 cm), unfilled, diseased or damaged.

Experimental designs and statistical analysis. Experiments were usually arranged in randomized complete block designs. Split plot designs were used in some field experiments. Analysis of variance was conducted on all data using appropriate statistical methods (19). Trend analyses or orthogonal/non-orthogonal comparisons were made where relevant.

RESULTS

CONTROLLED ENVIRONMENTS

Spray pH. After observing a decrease in spray pH after spraying with the CO_2 propelled sprayer, an experiment was conducted to evaluate the effect of pH on the response of corn to TRIA-A. There was a differential growth response of corn treated with TRIA-A applied in various pH buffered sprays (Table 1). TRIA-A did not stimulate growth of corn seedlings when applied at a pH of 6.8, reduced growth when applied at pH 7.7 and stimulated growth at pH 8.0. In a further experiment TRIA-A differentially affected the growth of corn seedlings when applied in buffered or unbuffered sprays usingcompressed air or CO_2 as the propellant (Table 2). The source of propellant had no effect other than that induced by changing the pH of the spray. The increased growth of corn seedlings in response to concentration of TRIA-A in pH 8.0 sprays was best described as a quadratic trend. The decreased growth of corn to TRIA-A treated with low pH sprays was best described as a linear trend. Furthermore, the growth of corn was reduced by the high pH sprays. No effect of spray pH on the response of corn to TRIA was found when the plants were grown outdoors (Table 3). In another experiment there was no effect of pH on the growth of corn treated with TRIA-A (Table 4). In one experiment TRIA-C, applied in a spray at pH 9.0 (356 mg/plant) stimulated the growth of corn seedlings, as compared to controls (326 mg/plant), but did not stimulate growth when applied at pH 5.0 (346 mg/plant) or 7.0 (336 mg/plant). Controls sprays at a pH of 8.0

Table 1. Growth of field corn seedlings as affected by TRIA-A applied to run-off in sprays of various pH's obtained with phosphate buffer.

TRIA-A (100 ug/L)	Dry Weight (mg/shoot)[z] pH 6.8	7.7	8.0
-	428	452	379
+	442	359**[x]	468**

[z] The F value of the interaction for pH x TRIA was significant at the 1% level.

[x]**The F values for the comparison of the control with TRIA pH 7.7 and 8.0 were significant at the 1% level.

Table 2. Differential growth responses of corn seedlings to TRIA-A applied to run-off in sprays of various doses and pH's with 10 mM phosphate buffer[z].

Treatments		Dry Weight (mg/shoot)[y]		
		TRIA-A (ug/L)		
Buffer	Average Post spray pH	0	100	1000
-	6.1	458	437	424**
+	8.0	400	443	432**[w]

[z] Carbon dioxide or air was used as the spray propellant.

[y] The F value for the interaction of pH x TRIA-A was significant at the 1% level.

[x]* The F value for the linear trend of TRIA-A with dose was significant at the 5% level.

[w]**The F value for the quadratic trend of TRIA-A with dose was significant at the 1% level.

Table 3. Growth of corn in pots outdoors as affected by the pH of the spray and TRIA-A.

pH	Phosphate buffer (1 mM)	TRIA-A (100 ug/L)	Dry weight*[z] (mg/shoot)
6.3	0	0	381
6.3	0	100	409
8.0	+	0	392
8.0	+	100	441

[z]*The F value for TRIA was significant at the 5% level. There was no significant effect of pH on the growth of plants.

Table 4. The response of corn seedlings to TRIA-A applied at various spray pH's in 10 mM phosphate buffer[z].

Treatments	Dry weight*[y] (mg/shoot)
pH	
6.0	304
7.0	301
8.0	331
TRIA (ug/L)	
0	297
100	328

[z] There was no effect of pH on the response of plants to TRIA.

[y]*The F value for TRIA was significant at the 1% level.

Table 5. The response of soybeans to sprays of TRIA-A at different pH's.

pH	Phosphate buffer (1 mM)	TRIA (ug/L)	Dry weight (g/shoot)^{z}
6.3	0	0	1.16**^{y}
6.3	0	100	1.04
8.0	+	0	1.06
8.0	+	100	1.19**

^{z} The F value for pH x TRIA was significant at the 0.05% level.

^{y}*,**The F values for the comparison of pH 6.3 control with pH 8.0 control was significant at the 1% level. The F value for the comparison of control with TRIA at pH 8.0 was significant at the 5% level.

Table 6. The response of rice seedlings to TRIA-A in a 10 mM phosphate buffered emulsion.

pH	TRIA (100 ug/L)	Dry weight*^{z} (mg/plant)
5.6	0	76
5.6	+	79
8.0	0	72
8.0	+	87

^{z}*The F value for TRIA is significant at the 5% level.

decreased the growth of soybeans (Table 5). TRIA-A applied in sprays of pH 8.0 overcame this inhibitory effect. Growth of rice seedlings was stimulated by TRIA-A, the greatest increase occurred when treated in pH 8.0 emulsions (Table 6).

Phosphate buffers were not used with TRIA-B as precipitates formed when the emulsion was prepared.

Night temperatures. Growth of field corn seedlings increased in response to treatment with TRIA-A at $10^{o}C$ and $15^{o}C$ nights (Table 7). Additionally, plant dry weight was increased by growing in the lower night temperatures. Plants grown with $10^{o}C$ nights often had injury on the leaves. This may have been due to guttation fluids or chilling. Several other tests were conducted with similar temperature regimes; however, little effect of TRIA was obtained in these experiments.

When night temperatures were changed after spraying with TRIA-B, the greatest response occurred with plants grown at $15^{o}C$ night temperatures before treatment (Table 8). Plants grown at $15^{o}C$ nights continuously were the largest but had a lower percent increase than those receiving $25^{o}C$ nights post-treatment. In a similar experiment conducted with TRIA-C, night temperatures of $15^{o}C$ ($30^{o}C$ days) favoured the response, while pre-treatment night temperatures of $25^{o}C$ inhibited the response of TRIA (Table 9).

In further experiments day temperatures were investigated as well as spray dispersion temperature. Plants grown at $30^{o}C$ or $35^{o}C$ day ($15^{o}C$ nights) were larger than those grown in the greenhouse. There was no effect of day temperature on the response of corn seedlings to TRIA-C. TRIA-C was applied in sprays at $20^{o}C$, $50^{o}C$ to test the hypothesis that solution temperature would influence the response of TRIA. At a $30^{o}C$ solution temperature, 0.1 µg TRIA-C/ℓ inhibited the growth of corn. This effect, however, could have been due to the warm dispersion rather than TRIA.

Table 7. Dry weight accumulation of corn seedlings grown at different night temperatures in response to TRIA-A at 100 μg/L in pH 8.0 1 mM phosphate buffer[z].

Day/Night Temperatures (°C)[z]	Dry weight[y] (mg/shoot) Control	TRIA
30/10	510	648**[x]
30/15	383	440*
30/22	410	410

[z]Plants were harvested after approximately 430 corn heat units had accumulated.

[y]The F value for temperature linear and quadratic were significant at the 5% level.

[x]*,**The F values for the comparison of control with treatment at the respective temperatures were significant at the 5% levels respectively.

Table 8. Response of corn to pre and post treatment night temperatures with 30°C day temperatures to TRIA-B.

TRIA-B (100 μg/L)	Dry weight[z] (mg/shoot) Night temperature °C (Pre/post treatment) 15/15	15/25	25/15	25/25
-	488	353	473	358
+	508	393	485	388

[z]The F value for the interaction of pre x post night temperatures and TRIA-B was significant at the 5% level.

Table 9. Growth of corn seedlings at different night temperatures and $30^{\circ}C$ days before and after treatment with TRIA-C.

TRIA-C (1.0 µg/L, 350 L/ha)	Dry weight[z] (mg/shoot) Night temperature °C (Pre/post treatment) 15/15	15/25	25/15	25/25
-	323	360	370	378
+	354*[Y]	395*	352	384

[z]The F value for pre treatment x TRIA-C was significant at the 5% level.

[Y]*The comparison of control vs TRIA-C with $15^{\circ}C$ nights prior to treatment was significant at the 5% level.

Table 10. The response of 8-day-old corn seedlings to dose of TRIA-A sprayed to run-off[z].

TRIA-A (µg/L)	Dry weight *[Y] (mg/shoot)
0	682
100	848
1000	746

[z]Unbuffered spray.

[Y]*The F value for quadratic trend of dose of TRIA significant at the 5% level.

Dose of TRIA. Over the course of this work many experiments were conducted to find the optimum concentration of TRIA to use with all 3 formulations both when sprayed to drip or at set volume per unit area.

The response of corn seedlings to applications of increasing concentrations of TRIA was best described as a quadratic curve with 100 μg/L of unbuffered TRIA-A being maximal (Table 10). The response of cucumbers exhibited a cubic trend, with the largest increase from 100 μg/L (Table 11). Decreased growth resulted with 10 μg/L TRIA-A. The response was also found in the stem, cotyledon and the first true leaf. No effect was found in the second or third (both still small and expanded) leaves.

In one of the pH experiments 100 μg/L of TRIA-A applied at pH 8.0 proved superior to 1,000 μg/L (Table 2). The growth response of corn to unbuffered spray of TRIA-A at 100 μg/L applied in various volumes induced a growth response best described as a quadratic trend (Table 12). The largest increase in dry weight of corn occurred between 410 and 820 L/ha (40 to 80 mg/ha) of spray applied.

In a test with TRIA-C applied in concentrations of 0 to 100 μg/L at 350 L/ha, 0.1 μg/L TRIA-C stimulated growth (Table 13). It appears that the concentration of TRIA-C required to elicit the response is less than the optimum for TRIA-A (100 μg/L). Other evidence for this increased effectiveness of TRIA-C was apparent in the studies with night temperatures when TRIA-C stimulated growth at 1 μg/L (Table 9).

Seed soaks. TRIA increased the rate of germination of tomato seed as measured by emergence of the radicle after 72 hr (Table 14). However, after planting in the greenhouse, this effect was lost. In another similar experiment there was no effect on germination. However, TRIA-C at 0.1 mg/L increased growth measured by height and stem diameter after 17 days and dry weight and height at harvest (25 days) (Table 15). The effect on stem

Table 11. Growth of cucumbers in response to doses of TRIA-A with 1 mM pH 8.0 phosphate buffer sprayed to run-off.

TRIA-A (ug/L)	Dry weight*^{z} (mg/shoot)
0	527
10	444
100	560
1,000	539

^{z}*The F value for cubic trend to dose of TRIA is significant at the 1% level.

Table 12. Increased dry weight of corn seedlings treated with various unbuffered volumes of spray of 100 ug TRIA-A/L.

Spray volume (L/ha)	TRIA-A (100 ug/L)	Dry weight**^{z} (mg/shoot)
1,000	0	356
200	+	387
400	+	410
600	+	401
800	+	409
1,000	+	353

^{z}**The F value for quadratic trend to dose of TRIA is significant at the 1% level.

Table 13. The effect of different spray concentrations of TRIA-C applied at 350 L/ha on the growth of corn seedlings.

TRIA-C (ug/L)	Dry weight (mg/shoot)
0	380
0.1	412*[z]
1.0	397
10.0	382
100.0	388

[z]*The F value for the comparison of control with 0.1 ug/L TRIA-C was significant at the 5% level.

Table 14. Germination of tomatoes after a 30-min seed treatment with TRIA-C. Lots of 50 seeds were counted after germination for 72 hr.

TRIA-C (mg/L)	Germination[z] %
Dry (untreated)	12
Surfactant	35
0.01	52
0.1	71
1.0	46

[z]The F value for the following comparisons were significant (% level):

untreated with surfactant (1%);
surfactant with 0.01 mg/L TRIA-C (5%);
surfactant with 0.1 mg/L TRIA-C (1%);
TRIA 0.01 mg with 0.1 mg/L (5%).

diameter was lost by harvest, Larger plants were obtained from pre-germinated seeds. Furthermore, there was a differential effect of pre-germination and TRIA-C on the height at harvest (Table 16). Plants from pre-germinated seeds treated with 0.1 mg/L were shorter than those at 0.01 mg/L. However, this effect did not exist after 17 days, indicating that the effect was lost in the 6 days prior to harvest.

In the first 2 experiments, excess seed was treated and the remainder saved and planted at a later date. There was no effect of TRIA-C on seeds held for 6 weeks at room temperature. Seeds from experiment 2 were planted in 2 locations, to investigate the ability to 'hold' the response to TRIA and any differential response in the greenhouse and growth room. There was no effect of TRIA at either location.

The soaking time for seed treatment of tomatoes with TRIA-C dispersions was investigated; however, there were no effects of TRIA-C due to time of seed treatment.

The lack of consistent effects of TRIA in tomato seed treatments indicated that seed size and indirectly, vigour might influence the seeds' ability to respond to TRIA. Visually, in most cases, plants would show responses; however, there was a lot of interplant variability which may have masked the response. In 2 experiments, seed was sized and showed that there was no interaction of seed size with TRIA on the growth of the seedlings in one test. In this test, tomato seed about 3.1 mm in diameter treated with TRIA-C resulted in larger plants after 11 days. However, the effect was not detectable 17 days after treatment.

In another experiment, there was an increase in seedling dry weight for the larger seed sizes. In some of the treatment combinations the TRIA-C reduced the height of the tomato seedlings, producing stockier plants which would be better for transplants than the control plants.

Table 15. Growth of tomato seedlings after treatment of the seeds with TRIA-C at different concentrations for 30 min. Seeds were sown with or without pre-germination for 72 hr.

Seed Treatment	TRIA (mg/L)	Dry weight (mg/shoot)	Stem diameter (mm)
0	0	379	4.5
Water	0	323	4.6
Surfactant	0	350	4.8
Surfactant	0.01	387	4.9*
Surfactant	0.1	448*[z]	5.1*
Surfactant	1.0	391	5.0*

[z]*The F value for the comparison of TRIA-C with controls was significant at the 5% level.

Table 16. Differential effect of pre-germination and TRIA-C on the height of tomato seedlings 25 days after planting.

	Height (cm)					
	TRIA (mg/L)					
Pre-germination	0 (Dry)	0 (H_2O)	0 (Surfactant)	0.01	0.1	1.0
0	10.1	9.8	10.5	11.2*[z]	12.4**	10.8
+	10.9	10.6	10.4	12.0*	11.0	11.6

[z]*,**The F value of the comparison of treatment with mean of control is significant at the 5% and 1% levels respectively.

Table 17. Differential effect of treatment water temperature on the response of tomatoes to TRIA-C.

Temperature °C	Dry weight (mg/shoot)[z]			
	TRIA (mg/L)			
	Surfactant	0.01	0.1	1.0
20	375.4	377.9	316.7*[y]	318.3*
50	360.4	363.3	370.0	362.9

[z] The F value for treatment temperature x TRIA-C was significant at the 5% level.

[y]*Comparisons of TRIA-C vs control was significant at the 5% level.

Table 18. Dry weight of tomato seedlings from seeds treated with different pH's of TRIA-C.

pH	Dry weight (mg/shoot)[z]			
	TRIA (mg/L)			
	0 (Surfactant)	0.01	0.1	1.0
5	439	415	471	528**
7	528	516	475	452*
9	435	474	496	457

[z] The F value of the interaction for pH x TRIA-C was significant at the 5% level.

[y]*,**The F values for the linear trend of TRIA-C were significant at the 5 and 1% levels respectively for that pH of treatment dispersion.

Soaking seed in hot water is recommended for disease control for many crops. It was hypothesized that increasing the treatment solution temperature might affect the seedlings' response to TRIA, resulting in both disease control and plant stimulation. TRIA-C decreased tomato plant dry weight with 20°C treatment water (Table 17).

Work with foliarly-applied sprays indicated that a higher pH of TRIA-A favoured the plants' response to TRIA, hence the pH of the water for seed treatment was investigated. There was a differential effect of pH and TRIA-C on the growth of tomato seedlings (Table 18). At pH 5.0 increasing TRIA increased plant dry weight (20%) at pH 7, TRIA decreased plant dry weight, while at pH 9.0 TRIA-C had no effect on plant dry weight. The largest tomato plants occurred in treatments at a pH 5.0, containing 1.0 mg/L and in the surfactant control at a pH of 7.0, with 528 mg/shoot each (Table 18). There is no reasonable explanation for the increased growth of the surfactant control at pH 7.0.

FIELD EXPERIMENTS.

1979. The yield of 'Augusta' winter wheat was increased in 1979 by foliar applications of 1,000 μg/L TRIA-A applied in 1,400 L/ha of spray (Table 19). The amount of spray and dose applied to the plants appeared to be a factor, since 1,000 μg/L applied to wheat at 1,400 L/ha provided higher yields than the same concentration applied at 470 L/ha. The upright leaves of the wheat were difficult to wet with spray. TRIA-A applied to wheat at anthesis at 100 μg/L induced a slight reduction in yield.

The yield of "Goldcup' sweet corn increased linearly with dose of 100 or 1,000 μg/L TRIA-A at a pH of 8.0 (Table 20). The weight of husked ears was 18 and 25% larger than the control after treatment of plants with 100 or 1,000 μg/L TRIA respectively. In a second experiment with sweet corn, there was no response to TRIA-A that could be measured.

In 1979, TRIA-A had no effect on tomatoes sprayed in the greenhouse several days prior to transplanting, or after establishment.

TRIA-A at 100 µg/L increased the yield by 18% as measured in dollars/ha (based on Pickling Cucumber Improvement Committee Standard prices) of 'Greenstar' cucumbers (Table 21).

1980. TRIA-B applied to tomatoes grown on unmulched soil and on black or silver mulches did not increase the marketable yield of tomatoes. There was an increased yield of the tomatoes planted on both kinds of mulch.

Likewise in a study with different formulations of TRIA-B there was no difference in tomato yield. The addition of $CaCl_2$ and $CaCl_2$ + Tween 20 to the spray solution had no effect on the response of plants to TRIA.

It has been reported that TRIA increased the rate of phosphate uptake in rice (11). It was postulated that TRIA applied with a starter solution might also enhance plant growth. However, research to test this hypothesis showed no significant response from plants treated with TRIA in the transplant water. Yields in this trial were low, due to inclement weather in the spring. The transplants were also damaged by cold, dry winds several days after transplanting. Plants without starter fertilizer suffered severely, while those with the fertilizer were damaged less. This may have been a factor in all three tomato experiments (as well as muskmelon), as all suffered chilling damage.

An experiment to determine the effect of TRIA on soluble solids of muskmelon fruit showed no significant difference in yield or soluble solids. These results agreed with the research of Bosland (3).

Table 19. Increased yield of soft winter wheat treated with various volumes and concentrations of unbuffered TRIA-A.

Spray applied L/ha	TRIA ug/L	Tonne/ha^{z}
1,400	0	5.42
470	100	5.00
470	1,000	5.38
1,400	100	5.23
1,400	1,000	5.80

^{z}*The F value for 1,000 ug/L TRIA applied in 1,400 L spray/ha was significantly greater than all treatments at the 5% level.

Table 20. Yield of sweet corn when sprayed to drip with TRIA-A at a pH 8.0, obtained with a 1 mM phosphate buffer.

TRIA-A (ug/L)	Weight of husked cobs*^{z} (tonnes/ha)
0	18.5
100	21.9
1,000	23.2

^{z}*The F value for the linear trend to dose of TRIA is significant at the 1% level.

Table 21. Dollar return of cucumbers treated with TRIA-A applied in pH 8.0 1 mM phosphate buffer to run-off[z].

TRIA-A (ug/L	Yield*[y] ($/ha)
0	444
50	459
100	525
200	401

[z] Dollar returns based on pickling cucumber improvement committee prices.

[y]*The F value for cubic trend to dose of TRIA is significant at the 1% level.

Table 22. Yield of soft winter wheat treated with TRIA-A and TRIA-B applied to run-off in two locations.

TRIA (ug/L)	Yield (tonne/ha)[z]			
	Location			
	Saranac ('Frankenmuth')[y]		Tuscola ('Augusta')	
	TRIA-A	TRIA-B	TRIA-A	TRIA-B
0	4.43	4.35	5.34	5.57
100	4.29	4.53	5.45*[x]	5.32*

[z] The F value for formulation x TRIA was significant at the 5% level in both locations.

[y] The F value for the comparison of TRIA-A with TRIA-B was significant at the 5% level.

[x]*The F values for the comparison of TRIA with control were significant at the 5% level.

Experiments were conducted to assess the effect of supplemental nitrogen and phosphorus on the response of sweet corn and pickling cucumbers treated with TRIA-B. There was no effect of nutrients on the response of plants to TRIA. Cucumbers and corn responded to supplemental applications of fertilizer at East Lansing but not at Clarksville, perhaps because the soil fertility as measured by soil tests was higher at Clarksville than at East Lansing.

Small trials with several cool season crops failed to indicate any yield increase in response to TRIA-B applied in 1980.

Winter wheat trials conducted in 1980 with both TRIA-A and TRIA-B formulations, two cultivars, 2 times of application and 2 locations showed different results (Table 22). At Saranac there was no response with the cultivar 'Augusta'. TRIA-B increased the yield of 'Frankenmuth', whereas TRIA-A was ineffective. The opposite held for 'Augusta' at Saranac. TRIA-A and TRIA-B decreased the yield of 'Frankenmuth' winter wheat at Tuscola (5.49 vs 5.18 t/ha for TRIA). In East Lansing there was no significant difference among any of the treatments where TRIA was applied during the grain filling period. The addition of phosphate had no effect on the TRIA response nor did addition of $CaCl_2$ to the spray emulsion.

1981. TRIA-C did not increase the yield of 'Snow Crown' cauliflower in 1981. The surfactant and TRIA-C at 1,000 µg/L reduced the weight per plant of 'Earlivee' sweet corn (Table 23). TRIA at 0.1 µg/L increased the weight of unmarketable cobs. Most of the unmarketable ears were short and not completely filled to the tip. Furthermore the percentage of harvested plants varied with different combinations of TRIA and amount of spray applied (Table 23). There was no effect of TRIA-C on ears per plant. An increase in number harvested increased with TRIA-C at 670 and 1,010 L/ha.

The harvest of more plants in TRIA treatments may explain the increase in number of unmarketable ears due to the smaller cobs from smaller or later plants. During a longer season, these cobs would have likely been acceptable resulting in higher total yields from TRIA-treated plants.

Yield of ripe tomato fruit was increased 34% and 50% by 0.1 and 10 μg/L of TRIA-C respectively, while there was no response at 1.0 μg/L (Table 24). There was no effect of volume of spray applied. The highest yields of ripe fruit occurred when the seedlings were treated with 10 μg/L of TRIA-C. Total yield followed that of ripe fruit. The percentage of green fruit was least from plants treated with 0.1 and 10.0 μg TRIA-C/L.

Soil tests at the Simcoe Experiment Station indicated the presence of high levels of phosphorus and potassium, hence little response in yield from supplemental phosphorus was expected. There was also no response of sweet corn to supplemental applications of nitrogen. Phosphorus increased the marketable ears per plant and total number of ears/plant regardless of TRIA treatment. There was a differential response in number and weight of unmarketable ears to phosphorus and applications of TRIA. As with the previous corn experiment, most unmarketable ears were short and not filled to the tip. With no additional phosphorus, TRIA-C at 0, 0.1, 100 μg/L decreased the weight of unmarketable ears (717, 678, 453 kg/ha); when 20 kg/ha phosphorus was added TRIA-C increased the weight of unmarketable ears (445, 453, 750 kg/ha).

Application of nitrogen to direct-seeded tomatoes increased the yield of ripe fruit. There was no effect of TRIA-C on either total tomato yield or number of ripe fruit. However, random samples of 25 red fruit showed differential responses of weight/fruit to TRIA and phosphorus (Table 25). One μg/L TRIA-C resulted in lower fruit weight with no phosphorus and slightly higher with added phosphorus. The effect of TRIA on size and numbers

Table 23a. Increase in unmarketable sweet corn in response to TRIA-C applied at the 3-leaf stage in different volumes of spray. There was no effect of spray volume nor an effect of TRIA-C on marketable yield.

Treatment TRIA (ug/L)	Marketable ears (husked) (g/plant)	Unmarketable ears (kg/ha)
Water	319	828
Surfactant	288*[z]	865
0.1	321	1305*
1.0	323	733
1000	288*	923*

[z]*The F value for the comparison of treatment with water control was significant at the 5% level.

Table 23b. Percentage of sweet corn plants harvested[z].

	TRIA (ug/L)*[y]			
L Spray	Controls	0.1	1.0	1000
340	93.4	74.8**	78.4	85.9
670	81.2	92.5	92.2	97.1*
1010	86.2	82.1	91.2	93.7

[z] The F value of Liters spray applied x TRIA-C was significant at the 5% level.

[y]*,**The F value for the comparison of TRIA-C with controls were significant at the 5 and 1% levels respectively.

Table 24. Yield of tomatoes treated with various concentrations of TRIA-C. There was no difference between 340, 670 and 1010 L/ha of spray at the different concentrations.

TRIA-C µg/L	tonnes/ha Ripe	tonnes/ha Total	Percent Green
0	38.14**[z]	60.27*	34.6*
0.1	50.93	67.48	19.4
1.0	36.80	51.74	30.1
10.0	57.34	79.48	21.5

[z]*,**The F value for cubic trend to dose of TRIA was significant at the 5% and 1% levels respectively.

Table 25. The weight of 25 randomly selected ripe tomato fruit as affected by dose of TRIA-C and Phosphorus[z].

	Weight/fruit (g)		
	TRIA-C (µg/L)		
Phosphorus (kg/ha)	0	1.0	10.0
0	80.0	72.8	79.2
20	80.0	85.6*[y]	76.8

[z]The F value for Phosphorus x TRIA-C was significant at the 5% level.

[y]*The comparison of TRIA 1.0 µg/L at 0 with 20 kg/ha P was significant at the 5% level.

as the yield of ripe fruit/clump was the same.

Foliar applications with a pH of 9.0 did not affect the weight of marketable corn. One µg/L of TRIA-C at 340 L/ha reduced the marketable ears/plant and number of marketable cobs (Table 26). Similar to the other corn experiment (Table 23), TRIA-C increased the number of unmarketable ears.

TRIA-C stimulated early yield of tomatoes when applied to the roots before transplanting (Table 26). Addition of the fertilizer increased the amount of small fruits in the 'early'harvests. TRIA at 10 µg/L increased the yield of early red fruit; however, the weight/fruit was less, indicating that the increase was due to number of fruit rather than size of fruit. There was a differential response to starter fertilizer and TRIA for small fruit in the early harvest. More small fruits were harvested from plants treated with TRIA-C and no starter (5.1 t/ha) and fewer small fruits when the starter was added with TRIA-C (4.5 t/ha). No differences in total yield were obtained.

Direct-seeded tomatoes yielded less than transplants. Transplants for the trial were from a test in which TRIA-C increased the rate of germination (Table 14). The surfactant treatment and 1.0 mg/L increased the amount of early small fruit as compared to the 'dry' control (Table 28). In the later yields, 0.01 mg/L decreased the number of breakers, while in early harvests TRIA-C at 0.1 mg/L decreased the weight of fruit. There was no difference in total yield, indicating that TRIA may affect only early yields. It would appear that the surfactant (tallow alkyl sulphate) or soaking the seeds in water may affect growth under certain conditions.

Table 26. Marketable yield of sweet corn as affected by TRIA-C applied at 340 L/ha in phosphate buffers at pH 5.0 and 9.0.

TREATMENT TRIA (μg/L)	Marketable ears/plant
0.0	.968
1.0	.840*[z]
100.0	.913

[z]*F value for the comparison of control with L μg TRIA-C was significant at the 5% level.

Table 27. Yield of tomatoes in response to TRIA-C applied to the roots for 5 minutes.

Treatment TRIA (μg/L)	Ripe fruit Early harvest tonnes/ha	wt./fruit early (g)
0	9.1	101
Surfactant	8.3	96
1.0	9.7	94**
10.0	10.7*[z]	95**

[z]*,**The F value for the comparison of control with 10 μg TRIA/L was significant at the 5% and 10% levels.

Table 28. Tomato yield from plants grown from TRIA-C treated seeds that had been direct-sown or grown for transplants[z].

TRIA (mg/L)	Time of Harvest: Early (small) (tonne/ha)	Time of Harvest: Late (breakers) (tonne/ha)	Total (tonne/ha)	Wt/fruit early (g)
0 - untreated	1.33	14.79	38.4	105
0 - surfactant control	2.73*[y]	15.13	40.3	107
0.001	2.00	19.60**	46.2	107
0.01	1.39	15.89	36.8	096*
1.0	2.61	14.36	38.1	109

[z]Surfactant (tallow alkyl sulphate) was used at 1% of the highest dose of TRIA.

[y]*,**The F value for the comparison of treatments with untreated control was significant at the 5% and 1% levels respectively. There was no effect of TRIA-C on the weight of green fruit.

DISCUSSION

Spraying with CO_2 as the propellant reduced the pH of foliar sprays to approximately 5.0. This was due to CO_2 dissolved into the water forming carbonic acid. In subsequent experiments, the low pH was found to reduce the response of plants to TRIA-A. High spray pH was found to favour the response of plants to TRIA-A. However, spray pH is not likely the sole factor involved in obtaining a response to TRIA-A because as in several previous experiments, resulting in more growth and/or higher yield, high pH sprays were not used. The penetration of leaves by certain herbicides, e.g. entry of NAA can be enhanced by a lower pH of the solution (16). However, the effect of pH on the activity of primary alcohols is not known. The pH is more likely to affect the plant than the TRIA. In several experiments, the high pH controls reduced plant growth as compared to controls at lower pH. In fact, the greatest growth occurred with the low pH controls. TRIA-A apparently overcame the 'stress' induced by low pH.

Acid-induced growth has been suggested as a hypothesis to explain cell wall expansion; it may be that the depressed growth found in the high pH controls is due to a high pH induced stress on the plant. TRIA somehow overcomes this stress, resulting in more growth.

In one seed soak experiment, the treatment pH affected growth of the seedlings. One possible explanation for this effect is that pH may affect water soluble inhibitors in the seed coat.

The effect of the spray solution pH on plant response to TRIA-A is not likely due to the phosphate buffer. Increased growth was obtained in experiments where the low pH was attained with CO_2 or buffer.

Clearly, there is an effect of pH on plant response to the TRIA-A formulation. This pH effect may explain some of the inconsistency in prior research, particularly with field experiments with TRIA-A, where CO_2 was used as a propellant for the plot sprayer. However, most of the data is with TRIA-A formulation and the pH of the colloidal dispersion (TRIA-C) does not appear to be an important factor.

Lower night temperatures enhanced the response of plants to all formulations of TRIA. Bittenbender (2) suggested that a stored product was involved in the TRIA response. Cooler nights should result in more food reserves due to less respiration. These reserves may be available for utilization during the TRIA-induced growth response, resulting in more growth. If a stored product is mobilized due to treatment with TRIA, increasing the level of stored product should increase the response to TRIA, assuming that the stored product is limiting. Low night temperatures at the seedling stage reduced growth, application of TRIA increased the growth to that occurring with warm temperatures. Larger plants were obtained with 10^{o}C nights in the first night temperature experiment as the plants were harvested after the same accumulation of heat. These plants were some 10 days older than those grown at 25^{o}C. Due to lower night temperatures those plants would have had more food reserves to utilize for growth.

Some of the research suggests a possible mode of action for TRIA. Since TRIA is a long-chain hydrocarbon, and lipoidal, it may have its effect on the phospho-lipids of the membrane, thus modifying membrane function.

In some tests, the controls, as expected, increased in growth with increased temperature. With the low temperatures before treatment, TRIA applications gave the same response as the temperatures. TRIA appeared to ameliorate the low temperature stress, which had induced slower growth. TRIA may, under low temperature stress conditions, allow the plant to attain its yield potential. It may well be that there are similarities between this and chilling injury. Temperatures of 15^{o}C are approaching temperatures where chilling injury occurs in many species of tropical origin. Chilling injury occurs around 12^{o}C and induces phase changes in membranes, which lead to metabolic imbalances (15). Among the manifestations of chilling injury are increased enzyme activation energy (due to conformation changes) and loss of membrane integrity, with attendant solute leakage.

It is also possible that TRIA may temporarily reverse the changes in cellular function induced by low temperatures, bu altering membranes to a state normally found at warmer temperatures. The fact that TRIA had no effect when applied before the low temperatures indicates that it cannot prevent low temperature stresses. TRIA may temporarily alter the structure of the membrane or perhaps membrane proteins (or both), hence affecting activity and biochemical reactions.

Prior research by Ries et al (22) and Richman (21) with seed treatments indicated that TRIA would not stimulate the growth of plants when these seeds were direct-seeded in the field. Seed treatments increased the growth of plants grown in the greenhouse. The increased yield of direct-seeded tomatoes treated with TRIA-C in this study is the first report of successful stimulation of yield with treated seeds directly seeded. It is unknown if this yield increase was due to the use of TRIA-C or environmental conditions.

Soaking tomato seeds with TRIA-C indicated that TRIA-C increased the rate of germination. This is the first evidence indicating that TRIA had a favourable effect on germination. Hoagland, using TRIA dissolved in DMSO (10) reported no significant effect of TRIA on germination of several species of weed and crop seeds. Seeds from the first treatment experiment with TRIA-C showed increased germination. Some of these pre-germinated seeds were planted in the greenhouse, whereupon the effect was lost prior to harvesting of the seedlings. However, plants obtained from the same lot were grown for field transplants. Seedlings grown from TRIA-C treated seeds yielded more fruit in the field. This indicates that a response to TRIA can occur and subsequently be lost.

Previous work in treating seeds with TRIA have been based on dissolving TRIA in an organic solvent, e.g. DCM (24), DMSO (10). These solvents may also dissolve seed coat waxes and allow their components to permeate the seed along with the synthetic TRIA. It is possible that natural TRIA in the seed coat (if present) is dissolved and moved into the seed, hence making it available to the young seedling. Also TRIA inhibitors such as C-28 alcohols, e.g. octacosanol, may move into the embryo and inhibit the TRIA response. Jones et al (12) have shown that octacosanol is a potent inhibitor of TRIA. Soaking in DCM resulted in increased dry weight of seedlings from TRIA-treated seeds, but in one test in the field only DCM-treated seeds had an effect on the yield of tomatoes (21).

The new TRIA-C formulation developed by Laughlin (14) has proved to be more effective. This formulation has the advantage of having no organic solvents. Hence, there should be less of a problem with inhibitors solubilized by the organic solvents. The seed coat waxes should not be affected unless they absorb the triacontanol or act as a barrier. However, the effect of the surfactant on yield of seed-soaked tomatoes suggests that the surfactant used in the TRIA-C may stimulate growth or solubilizes some stimulant under certain conditions. The increase in yield due to TRIA-C applied to the seed would be an important tool if it consistently resulted in larger seedlings. Application of TRIA to seeds would be a simple and effective technique for treatment.

The dose of TRIA applied to the plants is an important factor affecting their response to TRIA. The response of plants to TRIA dose may best be described by a quadratic or cubic trend. This curvilinear response to concentration of TRIA was also reported in other research (21, 24). Depending on the concentration used, TRIA may stimulate, decrease or have little effect on growth and yield in both controlled environment and field experiments. It is not known if the optimum dose to use will vary with environmental conditions. Laughlin et al (14) have shown a smooth curve of dry weight increases in response to TRIA-C over a wide range of concentrations in short-term growth chamber studies. The plants in these studies were sprayed to run-off. Cubic responses to concentration of TRIA in longer term greenhouse and field studies may indicate that there may be several 'optimum' doses of TRIA. Cubic responses to concentration of TRIA were found in prior research (21, 24). Another possibility is that there may be a critical ratio of TRIA to some internal factor, or a ratio of TRIA to long-chain alcohol contaminants. The appropriate dose for maximum response of crops to TRIA-C appears to be in the range of 0.1 to 10 μg/L. This is about 1,000-fold less than with TRIA-A or TRIA-B.

Coverage of the plant as well as concentration of TRIA appears to be essential. TRIA at 1,000 μg/L and 1,400 L/ha (1.4 g/ha) increased yield of winter wheat (Table 21). The morphology of the plant may be an important factor influencing coverage. The upright slender leaves of wheat are more difficult to wet. The actual dose of TRIA absorbed by these plants is unknown.

There is no consistent method of obtaining the drip point. Hence, the dose of TRIA applied could vary considerably. If 10 pots of corn are sprayed to drip, it can be observed that the plants are differentially wet. This is particularly true with TRIA-C where there is no spreader-sticker used. The variation in applied dose may be responsible in part for inconsistency of significant increases in dry weight or yield. TRIA is virtually insoluble in water, less than 2×10^{-16}M (14), hence the quality of the formulation is important, especially for the very low concentrations used. If the dispersion of TRIA is not uniform, some plants may receive excess, while others do not receive enough. TRIA-C is probably more effective because the colloidal dispersion is uniform and results in a more uniform dose of TRIA to the plant.

Due to the problems with foliar applications of TRIA to the plant seed treatments and dips to the roots at transplanting may be a more reliable method of applying TRIA. In the 1981 research TRIA-C applied to seeds or roots of transplants stimulated the yield of tomatoes. Seed treatments with TRIA-A have stimulated growth of corn, tomatoes and wheat seedlings grown in growth chambers (24) and applied to the roots of rice grown in solution culture (23). For example, by adding TRIA to transplant water, or to the seed before planting, the cost of an extra application with a sprayer would be saved.

Much of the effort in this research was devoted to studying the lack of consistency of the response of plants to TRIA, both under controlled environments and the field. TRIA has consistently increased dry weight, water soluble proteins, free amino acids and reducing sugars in short-term studies in growth chambers (11, 13, 25). It is likely that some environmental factor occurring before or after treatment or both influences the plant's ability to respond to TRIA. However, difficulties with formulation, method and time of application cannot be ruled out at this point. Many times in this research a visual response was observed, but at harvest it could not be measured. Bouwkamp and McArdle (4) have also reported a loss of effect of TRIA on sweet potatoes. They measured an increase in dry weight and % nitrogen 5 days after treatment, but there was no effect on yield at harvest. A prime example of this problem can be found with the 1981 seed treatment data, where a stimulation in germination carried through to increased yield in the field but not increased growth in the greenhouse. The environmental factors which induced this effect are not known.

Greenhouse studies with corn treated with TRIA-B at 7 days (early 3-leaf stage) had 27% greater dry weight than controls. Plants treated at the 2-leaf (5 days old) or early 4-leaf (9 days) responded with an increase of 5%, and a decrease of 2% in dry weight respectively. It is not known if the decrease at 9 days was due to physiological age or difficulty in sorting the plants into uniform blocks. TRIA applied to very young sugar beet seedlings reduced growth, when applied when the first true leaves were expanded, it stimulated growth (17). This implies that there is an effect of age on a plant's ability to respond to TRIA.

Nitrogen nutrition has been reported to influence the response of plants to TRIA (21, 18). TRIA-C increased the weight per fruit of tomatoes treated with supplemental phosphate in 1981 but had little effect when no phosphate was added. Other interactions of nutrition with TRIA applications were not observed in this research. Natural soil fertility, climatic and environmental conditions vary appreciably, and other environmental conditions may have a greater effect than nutrition on the plant's ability to respond to TRIA.

In this research, the response of plants treated with TRIA was too inconsistent for commercial use. In field studies in 1979 and 1981 using TRIA-A and TRIA-C respectively, a positive response was obtained in approximately 40% of the trials. In 1980 positive responses were encountered in one trial out of 11, using TRIA-B. In greenhouse and growth chamber studies, the positive response rate was approximately 10%, 1% and 30%, using TRIA-A, TRIA-B and TRIA-C respectively. This lack of consistent response was the central difficulty in this research. The reasons for this lack of consistency in response to TRIA are not clear, and have to be solved before TRIA can be recommended as a commercial growth regulator. Since the short-term biochemical studies show greater consistency in the response of plants to TRIA, it may be that some environmental factor(s) may affect longer-term field responses of plants. Furthermore, the differences in growth obtained in short-term biochemical studies may or may not be sufficiently large to serve as a basis for differential growth over longer periods of time.

TRIA can stimulate plant growth. However, whole plant response to TRIA has not been consistently reproducable in this or prior research in both field and controlled environment studies. The main thrust of this research became a study of this variability of plant response to TRIA. However, this was not possible to accomplish because of the difficulty in developing a standard protocol for conducting tests both under field and controlled environment conditions. Due to these problems, many tests conducted were not included because there was no measureable effect of any of the treatments. A protocol for the application of TRIA to plants must be developed before TRIA may be recommended for use as a commercial plant growth stimulator. Unfortunately, no such reproducable protocol was developed in this research.

SUMMARY

The overall objective of this work was to investigate parameters influencing a plant's ability to respond to TRIA, and attempt to develop a set of recommendations for commercial use of TRIA. Lack of consistency of the response of plants to TRIA remains the major problem preventing commercial use of TRIA. Much effort was devoted to investigate this lack of consistency, and to develop a standard protocol for testing factors influencing plant response to TRIA. Unfortunately, no such protocol has been developed that results in consistent increases in dry matter or yield under either field or greenhouse conditions.

The following factors influence the response of plants to TRIA: (1) the spray pH of TRIA-A should be at 8 or above to favour a growth stimulus, however, the effect of pH on other formulations was not consistent; (2) night temperatures should be less than 20°C, for example 15°C nights have been shown to be effective. TRIA may be effective under 'mild' stress conditions such as low night temperatures. Most crops do not have optimal conditions during their entire life cycle. If we can learn when and how to apply TRIA, food production may be increased.

TRIA-C appears to be a superior formulation of TRIA, and is effective at 10 ug/L or less.

The problems associated with lack of consistent results remain. The above recommendations do not guarantee success. They only influence the likelihood of a positive response. Other factors as yet undefined, appear to govern the plant's ability to carry the TRIA response through to crop maturity. The reasons for these inconsistent responses by plants remain unclear and offer a challenge to researchers with plant growth regulators.

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