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Stimulating Productivity of Hydroponic Lettuce in Controlled Environments with Triacontanol

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Additional index words. *Lactuca sativa*, 1-triacontanol (TRIA), controlled environments

Abstract. **Triacontanol (1-triacontanol) applied as a foliar spray at 10^{-7} M to 4-day-old, hydroponically grown leaf lettuce (*Lactuca sativa* L.) seedlings in a controlled environment increased leaf fresh and dry weight 13% to 20% and root fresh and dry weight 13% to 24% 6 days after application, relative to plants sprayed with water. When applied at 8 as well as 4 days after seeding, triacontanol increased plant fresh and dry weight, leaf area, and mean relative growth rate 12% to 37%. There was no benefit of repeating application of triacontanol in terms of leaf dry weight gain.**

Triacontanol (TRIA), a naturally occurring 30-carbon primary alcohol, has been found to increase yield of some crops (4, 8–11). However, effects of TRIA often have been inconsistent. For example, yield increases were not reported for maize (3), wheat (2), and some other crops (6). One reason for this inconsistency may involve formulation (9, 12). Since TRIA is almost insoluble in water, researchers have used detergents or surfactants to emulsify TRIA in effort to keep it in suspension (3, 10). Recently, an aqueous, colloidally dispersed formulation was developed that gives more consistent results than suspensions in chloroform–Tween 20 or acetone–NAA (9).

The biomass production group of the National Aeronautics and Space Administration Controlled Ecological Life Support System (CELSS) program seeks to define optimum growth requirements for crops to satisfy human nutritional requirements, waste recycling, and air revitalization for long-term space habitation (13). Research in our laboratory emphasizes optimizing productivity of high-yielding leaf lettuce cultivars as a model crop to supply minerals, vitamins, and fiber for a CELSS vegetarian diet in the shortest time and smallest growing space possible. The present study was conducted to investigate the potential of TRIA to enhance lettuce yield in an optimizing environment of CO_2 enrichment and radiation enhancement.

Seeds of 'Waldmann's Green', a high-yielding lettuce cultivar, were germinated and grown in nutriculture systems located within two enlarged and improved chambers of the Minitron system (1). Seeds were sown onto a cloth-lined trough wetted with half-strength modified Hoagland's No. 1 nutrient solution (5). Four days after sowing, seedlings were transplanted to individual holders within Minitron chambers. Plants were grown for the first 4 days in half-strength nutrient solution at pH 6.0, followed by a shift to single-strength solution containing double-strength N as 5 mM NH_4^+ + 25 mM NO_3^-. Photosynthetic photon flux (PPF) was 430 ± 5 $\mu mol \cdot s^{-1} \cdot m^{-2}$ (LI-COR, model LI-1800) at the top of the leaf canopy until 11 days from seeding, when it was increased to 900 ± 10 $\mu mol \cdot s^{-1} \cdot m^{-2}$. The lower PPF was achieved by 84% input wattage from cool-white fluorescent lamps and 16% from frosted incandescent lamps in the environmental room housing the transparent Minitron chambers. High PPF was provided by a bank of nine 150-W parabolic reflector flood lamps mounted above each chamber, in addition to the fluorescent + incandescent lamps on the ceiling of the growth room. A water/plexiglas barrier was placed between the lamp banks and each Minitron chamber. Photoperiod was 20 hr on a 24-hr cycle. Relative humidity was 75% ± 5% during the light cycle and 85% ± 5% during darkness. Shoot and rhizosphere temperatures were maintained at 25° ± 0.5°C day and night. Air flow rate through root ($4.9 liter \cdot min^{-1}$) and shoot compartments ($8.2 liters \cdot min^{-1}$), as well as CO_2 injection into the shoot atmosphere mixing chamber, were maintained with rotameters (Matheson, models 603, 604, and 610, respectively). Atmosphere flowing through the chambers contained ambient levels of CO_2 until 11 days from seeding. Atmospheric CO_2 was maintained at 69 ± 2 $mmol \cdot m^{-3}$ ($1500 \mu l \cdot liter^{-1}$) from 11 to 19 days of growth. Carbon dioxide in the flowing atmosphere was monitored with a non-dispersive infrared gas analyzer (Horiba, model PIR-2000).

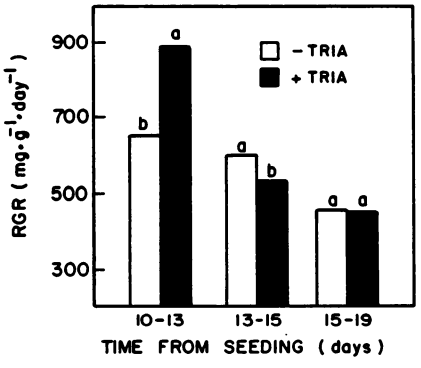

Fig. 1. Effect of 10^{-7} M triacontanol (TRIA) sprayed at days 4 and 8 after seeding on relative growth rate (RGR) of lettuce from 10 to 19 days of growth. Plants were grown at a PPF of 900 ± 10 $\mu mol \cdot s^{-1} \cdot m^{-2}$ and 69 ± 2 $mmol \cdot m^{-3}$ CO_2 from 10 to 19 days after seeding.

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Table 1. Effects of one appliccation of 10^{-7} M triacontanol (TRIA) on growth of 'Waldmann's Green' leaf lettuce from days 10 to 19 after seeding. Plants were grown at a PPF of 900 ± 10 $\mu mol \cdot s^{-1} \cdot m^{-2}$ and 69 ± 2 $mmol \cdot m^{-3}$ CO_2 from days 10 to 19.

Growth parameter	Time from seeding (days)	−TRIA	SE	+TRIA[z]	SE	Difference from −TRIA (%)
Leaf dry wt (mg/plant)	10	40	3[y]	64	2	+40
	12	106	11	134	7	+26
	15	635	21	767	23	+21
	19	4324	166	4832	126	+12
Root dry wt (mg/plant)	10	14	0	21	1	+50
	12	26	5	43	2	+65
	15	103	6	164	3	+59
	19	762	30	856	20	+12
Leaf number	10	4	0	4	0	0
	12	5	0	6	0	+20
	15	10	0	12	0	+20
	19	16	0	17	0	+6
Crop growth rate ($g \cdot m^{-2} \cdot day^{-1}$)	10–19	51.82	5.06	54.67	6.08	+5

[z]Sprayed at day 4 after seeding.
[y]One SE of the mean.

Table 2. Effects of two applications of 10^{-7} M triacontanol (TRIA) on growth of leaf lettuce. Plants were grown at a PPF of 900 ± 10 $\mu mol \cdot s^{-1} \cdot m^{-2}$ and 69 ± 2 $mmol \cdot m^{-3}$ CO_2 from days 10 to 19 after seeding.

Growth parameter	Time from seeding (days)	−TRIA	SE	+TRIA[z]	SE	Difference from −TRIA (%)
Leaf dry wt (mg/plant)	10	57	3[y]	64	2	+12
	13	216	8	249	11	+15
	15	1093	25	1275	70	+17
	19	4930	264	5623	212	+14
Root dry wt (mg/plant)	10	16	1	20	2	+25
	13	43	3	55	4	+28
	15	192	5	243	8	+27
	19	1037	57	1180	32	+14
Leaf area (cm^2/plant)	10	8.2	0.1	9.8	0.2	+20
	13	65.6	1.4	75.0	1.1	+14
	15	328.5	8.7	369.4	9.5	+21
	19	852	46.8	971	31.2	+13
Crop growth rate ($g \cdot m^{-2} \cdot day^{-1}$)	10–19	55.05	7.04	60.48	9.07	+10

[z]Sprayed at days 4 and 8 after seeding.
[y]One SE of the mean.

Each chamber initially accommodated 36 plants, which were harvested six times, beginning on day 10, with six plants/harvest. Plants were harvested systematically to preserve equal spacing of plants within each chamber. Previous studies using similar growth conditions indicated no significant differences in growth of plants sampled randomly or systematically within Minitrons (1). Final harvest was at 19 days after seeding to avoid subsequent complications arising from development of leaf tipburn, and to confine experimental treatments to the period of exponential growth.

At each harvest, leaf number (≥1 cm long), leaf, stem, and root fresh weight, stem length, and shoot diameter were determined. Leaf area also was determined for one experiment. Dry weight of plants was determined after 3 days at 75°C in a forced-air oven. Relative growth rate (RGR) was determined over paired harvest dates by regressing plant dry weight with time of treatment. Differences in RGR were expressed as differences in slope of regression lines according to *t* test at the 5% level. Crop growth rate was expressed as dry weight of leaves in a closed canopy per unit growing area per day between 10 and 19 days of growth.

Purified solutions of colloidally dispersed TRIA were provided by S. Ries (Michigan State Univ.) and H. Hathaway (Buckeye Cellulose, Memphis, Tenn). The colloidal dispersion of TRIA contained the dispersant sodium octadecyl sulfate (SOS) at 0.01 g SOS/g TRIA (9). The concentrated preparation was diluted to 10^{-7} μM TRIA with distilled water, and this dispersion was sprayed to run-off on 4-day-old lettuce seedlings. Since the level of SOS was only 0.1 $\mu g \cdot liter^{-1}$ (0.1 ppb) and no effect of this dispersant was found on dry weight gain by corn, rice (12), dry bean, and potato (J. Biernbaum, personal communication), distilled water was used as a control in our studies. Glass atomizers were used to dispense treatment solutions. Triacontanol was applied 7 hr into the photoperiod based on a report that this is an effective time of day for TRIA application (12).

The experiment was repeated after shifting treatment regimes between Minitron chambers, except that plants were sprayed at 8 days from seeding as well as on day 4. Before shifting treatment regimes, potential TRIA residues were removed by cleaning the growing system thoroughly with 20% (w/v) NaOH in ethanol followed by a detergent wash and exhaustive rinsing with deionized, distilled water. Care was taken to avoid contact of TRIA solutions with surfaces containing plasticizers. Environmental parameters were equalized as closely as possible between chambers.

Triacontanol applied once, on day 4, increased leaf and root dry weight, as well as leaf number, throughout the period of exponential growth from 10 to 19 days after seeding (Table 1). Leaf fresh weight was increased 17% by TRIA (data not shown). The gradual decline in growth stimulation during exponential growth by TRIA-treated plants may reflect actual inhibition of growth following initial stimulation. Triacontanol-treated plants were observed to develop leaf tipburn earlier than did −TRIA controls, which would contribute to inhibition of RGR. In spite of early stimulation followed by apparent inhibition of growth, the net effect of TRIA 15 days after application still was mild growth stimulation. Although crop (canopy) growth rate also was slightly greater for +TRIA plants during exponential growth (Table 1), several parameters were not affected by a single TRIA application, including harvest index (edible-to-total biomass ratio), stem length, stem fresh and dry weight, and leaf specific water content (data not shown). The fact that a single TRIA application on day 4 stimulated RGR 40% from days 10 to 11 after seeding indicates a prolonged residual effect of TRIA and also that TRIA-induced growth stimulation was not dependent on PPF elevation and/or CO_2 enrichment treatments that were initiated after RGR already had been stimulated.

If TRIA application was repeated on day 8 after seeding, growth enhancement by day 19 still was similar to that resulting from one application on day 4 (Table 2). However, the large stimulation of leaf and root dry weight gain at the onset of exponential growth when TRIA was applied only once (Table 1) was absent when application was repeated, suggesting that the additional TRIA hastened the onset of exponential growth, and RGR already was tapering off by day 10. Leaf dry weight of +TRIA plants increased in proportion to leaf area (Table 2), thereby negating any effect of TRIA on specific leaf weight. Double application increased leaf dry weight to the same extent as single application, further suggesting no additional benefit of multiple vs. single application, in agreement with findings by others (J. Biernbaum, personal communication).

Mean RGR 10 to 13 days after seeding was 37% greater for TRIA than for control seedlings (Fig. 1). This stimulation, 2 to 5

days after the second application, extends a previous observation of increased RGR beginning only 4 hr after rice was sprayed with TRIA (11). Relative growth rate generally increases as a plant develops, reaches a maximum value during exponential growth, and then declines (6). The 10% lower RGR of TRIA-treated plants relative to controls 13 to 15 days after seeding further suggests that maximum RGR was attained by TRIA-treated plants prior to that of control plants, or that TRIA had an outright inhibitory effect during later exponential growth. From days 15 to 19 of growth, RGR was not different between ±TRIA treatments. An analogous pattern of RGR changes occurred for plants treated only on day 4 (data not shown).

Mild stimulation of lettuce yield by TRIA followed by conditions of CO_2 enrichment and radiation enhancement indicates that TRIA has potential to enhance lettuce productivity in optimizing controlled environments. Many factors besides formulation are known to affect plant responsiveness to TRIA, including time of day applied, temperature, purity and stability of TRIA dispersions, and foliar vs. root-zone application (8, 12). In view of these potentially interactive effects on results obtained with TRIA, it is apparent that mode of action must be better understood before consistent, predictable increases in yield can be achieved. The present communication suggests that colloidally dispersed TRIA may be most consistent as a crop growth stimulant in controlled environments.

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Evaluation of Techniques to Measure Chilling Injury in Tomato

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Additional index words. chlorophyll fluorescence, electrolyte leakage, *Lycopersicon esculentum*, *L. hirsutum*, *L. pimpinellifolium*, *Solanum lycopersicoides*

Abstract. **Intact plants of four *Lycopersicon* species, *Solanum lycopersicoides* Dun. and a *L. esculentum* Mill. x *S. lycopersicoides* intergeneric hybrid at the four- to 11-leaf developmental stage were subjected to temperatures of 20° or 2.5°C for 72 hr. Plants were assayed for chilling injury by a visual rating of damage on specified leaflets (VRL), chlorophyll fluorescence (CF), electrolyte leakage (EL), and a visual rating of plants (VRP). Correlations of genotypic effects were significant between a) CF and VRL, b) CF and VRP, c) VRL and VRP, and d) VRP and EL. Correlations of the temperature × genotype interactions were highly significant between a) CF and VRL, b) CF and VRP, and c) VRL and VRP. CF was the most precise assay to quantify chilling injury. The means for CF for each of five leaflets from a single leaf produced similar separation of genotypes. Chilling resistance of an intergeneric hybrid between sensitive *L. esculentum* Mill. cv Sub-Arctic Maxi and resistant *S. lycopersicoides* suggested dominant, nuclear gene control.**

Chilling injury results if sensitive plant species are exposed to nonfreezing temperatures within the 0° to 12°C range (7). Tomato is sensitive to chilling temperatures, showing reduced vigor, yield, and length of growing season (2, 3). Since transplants are used predominantly for the establishment of tomato fields in midwestern United States, the young vegetative plant is the first developmental stage vulnerable to spring chilling temperatures.

The use of assays that quantify chilling injury should increase accuracy in identification of genotypic responses by eliminating the subjectivity associated with visual rating systems. Furthermore, quantitative biological assays may detect chilling injury prior to visible macroscopic symptoms, thereby saving time and preventing the destruction of plant material. The causes of chilling injury are not yet well-understood (8); therefore, progress in accurate quantification of injury has been slow.

Electrolyte leakage and chlorophyll fluorescence have been employed in genetic studies of chilling injury. The rate and magnitude of leakage (10, 15–17) have been used. The influence of temperature on chlorophyll has been expressed as the change in fluorescence measured over time (9, 11). Chilling stress responses of leaves may be distorted by detachment of leaves. Potvin (11) reported that the ranking of two species with regard to chilling tolerance was altered if leaves were detached. Kanuiga et al. (5) reported that photosystem II was damaged more extensively by darkness and by detachment of leaves than by the cold treatment. In contrast, Wolf et al. (16) reported that disks taken from the first fully expanded leaf of tomato seedlings could be used to differentiate sensitive from resistant genotypes.

This study was conducted to evaluate the correlations between screening procedures: a) chlorophyll fluorescence, b) a visual rating of leaflet samples, c) electrolyte leakage, and d) a visual rating of intact plants. The four assays were used to discriminate chilling sensitivity of nine tomato genotypes.

The five solanaceous species used in this study (Table 1) represent a range of presumed chilling sensitivities. The genotypes included three tomato cultivars with rela-

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