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## Growth and Yield of Crops Treated with Triacontanol<sup>1</sup>

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**Abstract.** The growth of several vegetable and field crops in the greenhouse was increased by applications of 1-triacontanol to the foliage, soil, or seed. Neither the seed nor soil treatments increased the yield of crops in the field. However, foliar sprays ranging from 5 to 500 mg/ha significantly increased the marketable yield of 7 of 10 crops tested. The average yield increase was based on comparisons of all the different rates and time of 1-triacontanol applications with untreated controls. The response of tomato, carrot and wheat seed treatments with 1-triacontanol was shown to be positively correlated with temperature at time of germination and early growth.

Alfalfa (*Medicago sativa* L.) hay (4) and 1-triacontanol, [CH<sub>3</sub>(CH<sub>2</sub>)<sub>28</sub>CH<sub>2</sub>OH] isolated from alfalfa hay (5) has been shown to stimulate the growth of several crop species (5) as well as tobacco callus tissue (3). Triacontanol was previously shown to be the principal long-chain alcohol component of wax derived from alfalfa leaves (2).

Triacontanol applied in nutrient solutions to rice seedlings at  $2.3 \times 10^{-8}$  M (10 µg/liter) increased seedling dry wt and protein (nitrogen) content within 3 hr in the light or dark (6). Growth analysis indicated that the direct effect of triacontanol occurred within the first 24 hr after treatment (6). Treated plants continued growing larger than controls, but did not gain more dry wt per cm<sup>2</sup> of leaf area. This increase in dry wt had a V<sub>max</sub> of 4.9 mg/hr and a K<sub>dose</sub> of 25 min in the dark (1). This response was affected by both CO<sub>2</sub> and O<sub>2</sub> concn with the optimum response occurring at 200-300 µl/liter of CO<sub>2</sub> and 5% of O<sub>2</sub>. Dark grown seedlings treated with triacontanol did not fix more atmospheric CO<sub>2</sub> than did controls (1).

The consistent increase in growth due to triacontanol observed in these studies indicated that triacontanol may be useful in crop production. The objective of this research was to determine the best rates, methods, and times of triacontanol application for increasing the growth of several crops under greenhouse conditions and the yield under field conditions.

### Materials and Methods

**Greenhouse experiments.** Studies were carried out using a sterilized soil mix consisting of equal volumes of peat, sand and a sandy loam soil. Clay pots 10 and 18 cm in diam were used for small and large seeded crops, respectively. After seedlings emerged they were thinned to a uniform stand of 3 to 5 plants per pot. Plants were assigned to blocks based on uniformity of size. A random number table was used to assign treatments. They were grown in a greenhouse with a night temp maintained at about 25°C. In the temp studies, plants were grown

under an 8 hr night and a 16 hr day with an irradiance of 270 µEs/cm<sup>2</sup> (Lambda LI 185 with quantam sensor) at the soil level. After thinning, plants were watered twice a week with a soluble 20N - 8.6P - 16.6K fertilizer at the rate of 1 g/liter with 100 ml and 250 ml per pot for the 10 and 18 cm pots, respectively.

Triacontanol was applied to seeds by soaking the seed in dichloromethane containing triacontanol for 1 hr. Controls consisted of untreated seed and seed soaked in dichloromethane, except for the temp study where dichloromethane treated seed was the only control. A 2.5% emulsifiable concn of triacontanol (American Cyanamid, Princeton, New Jersey) was used for foliar applications. This formulation was still so concentrated that the rates used required the addition of Tween 20 at 0.1% by vol as a surfactant. Studies conducted in the greenhouse with the emulsion blank and Tween 20 used alone showed that neither influenced plant growth. The controls in these tests were all sprayed with either the commercial emulsion or chloroform and Tween 20. The sprays were applied with an atomizer until the liquid dripped from the leaves. The triacontanol solutions for the soil drenches were made up from dilutions of a 0.1 mg/liter stock solution. This was obtained by adding the pure chemical to water and stirring over heat for several days to get it into solution. The 1.0 mg/liter treatment was applied by dissolving the triacontanol in chloroform (1.0 mg/0.5 ml). The chloroform solution was added to water, rapidly shaken and added to the soil. The soil drenches were applied in 100 ml and 250 ml of water for the 10 and 18 cm pots, respectively. Plants were harvested at the soil surface and dried in an oven at 80°C for at least 2 days. Randomized complete block designs with 4 to 6 blocks were used for all tests. Means were compared by the LSD procedure.

**Field experiments.** Field tests were conducted at 3 locations in central Michigan on a Spinks sandy loam, Miami clay loam, or Park Hill clay loam. Normal cultural and pest control practices for crop production in Michigan were utilized in all field studies.

The foliar treatments were applied in the field with 1 or 2 flat fan nozzles with the same formulations used in the greenhouse. The Tween 20 was applied to controls when there was

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no emulsion blank available for comparison. The chemicals were applied in 194 to 484 liters of water per hectare.

Factorial experiments were utilized in most field tests with rate, time, and number of triacontanol applications as variables. Randomized complete block designs were used with the exception of the field studies on different nutrient levels. In these studies a split-plot design was utilized. Fertilizer levels were the main-plots and the triacontanol treatments were the sub-plots.

A minimum of 4 blocks was used in all tests. Studies initiated later in the summer had 6 blocks. Based on the coefficients of variation, it is believed that this is the minimum number of blocks required to show differences in yield for this type of research under the conditions of these field studies. A minimum of 1 row was used as a guard row on the outside and between each spray treatment on the cucumber, field corn, dry beans, and wheat.

The results of all tests were subjected to analysis of variance and relevant orthogonal and non-orthogonal comparisons were made.

All crops were harvested at what was considered to be the optimum harvest date. However, observations on the growth of plants in guard rows after harvest indicated that both the lettuce and carrots were probably harvested too early.

### Results and Discussion

*Greenhouse experiments.* Foliar applications in the greenhouse to vigorously growing seedlings of several crop species increased their dry wt (Table 1). The dry wt increase occurred at application rates as low as 0.01 mg/liter, and was maintained over a 100 to 1000-fold range in concn. The dose response curve for this large range in concn tended to be quadratic for the cucumber and carrots, but not for the field corn or soybeans (Table 1).

Application of the chemical in solution as a soil drench also improved the growth of several species (Table 2). There was very little difference in response to the different rates of triacontanol except for soybeans. The response was quadratic with soybeans in contrast to the results from foliar applications (Table 1).

Seeds soaked for 1 hr in dichloromethane containing triacontanol proved to be one of the most effective means of application in greenhouse studies (Table 3). The response was again relatively similar over a wide range of species and concn as indicated by the average % increase over the controls. Higher concn were not used because triacontanol was not soluble in dichloromethane at concn much higher than 10 mg/liter.

Several greenhouse tests were also conducted with multiple applications of triacontanol applied as a foliar spray 3 to 7

Table 1. Dry weight of several crops after a foliar application of triacontanol applied in the greenhouse when plants had 2 to 3 true leaves.

Triacontanol (mg/liter)	Biomass (mg dry wt/shoot)			
	Cucumber 'Green Star'	Field corn 'Michigan 396'	Soybean 'Swift'	Carrot 'Gold Pak'
	Growing time after treatment			
	15 days	15 days	22 days	33 days
Control	314	513	773	41
0.01	392	574	804	50
0.10	388	688	732	58
1.00	423	624	907	68
10.00	396	659	920	45
LSD 5%	47	67	98	16
1%	63	91	135	N.S.

Table 2. Dry weight of several crops after an application of triacontanol as a soil drench in the greenhouse when the plants had 2 to 3 true leaves.

Triacontanol (mg/liter)	Biomass (mg dry wt/shoot)			
	Rice 'IR-8'	Soybean 'Swift'	Barley 'Coho'	Cucumber 'Premier'
	Growing time after treatment			
	74 days	22 days	25 days	22 days
Control	1300	471	310	506
0.001	—	500	—	644
0.01	1727	592	341	607
0.10	2070	649	343	618
1.00	—	493	—	603
LSD 5%	587	96	23	136
1%	N.S.	129	N.S.	N.S.

days apart. In general, a single application was as effective as 2 or more applications.

Greenhouse research prior to planting crops in the fields clearly indicated that triacontanol would increase growth of several different crop species and it could be applied as a spray, soil drench, or seed soak. It did not indicate a definitive range of rates or an increased response from multiple applications. Therefore, both a wide range of concn and multiple applications were evaluated in the field.

*Field experiments.* Tests were conducted with 10 different crops in the field. Visual observations indicated that there were no differences in seedling growth from dichloromethane or triacontanol treatments to seed, except for tomatoes. The lack of visual differences was substantiated by the marketable yields obtained in the cucumber, carrot, lettuce, radish, field corn and sweet corn trials. In no case was the yield higher than in untreated controls. In the tomato experiment there was a dramatic effect, which apparently was due to the dichloromethane treatment and not triacontanol (Table 4). The differences in growth were obvious between thinning the seeded tomatoes and harvest. We have noted in past greenhouse studies and other unreported field research with seed treatments that occasionally controls with dichloromethane treated seeds produce more vigorous seedlings and higher yields. It is possible that the dichloromethane removes inhibitors from the seed.

In 2 soil drench tests with corn and tomatoes, there were also no increases in yield from the triacontanol treatments.

Applying triacontanol as a spray when the plants had 2 to 3 true leaves proved to be an effective method of treatment for several crops. In only a few cases could increased growth of the seedlings be observed or measured. The average height of all of the sweet corn treatments was 13% higher than the controls 15 days after treatment. This difference was significant at the 1% level. However, in 7 of the 10 crops receiving foliar applications, there were significant increases in yield. The dose response was similar to greenhouse studies, with no clear delineation in rate (Table 5). The best responses were with pickling cucumbers and sweet corn. In the test with carrots, which was harvested when the carrots were relatively small, there was an increase in yield at the low nitrogen level with a decrease in yield at the high nitrogen level (Table 5). This interaction has not been substantiated in a limited number of greenhouse studies. In tests with multiple applications at different rates, there were no appreciable differences between multiple applications or rates of application (Table 6). The test with cucumbers was conducted in a commercial field and the harvest was a simulated mechanical harvest. The increase in yield from the triacontanol treatments resulted in a 14% higher dollar yield per hectare. The higher yield in both

Table 3. Dry weight of several crops grown in the greenhouse after a seed treatment of one hour in dichloromethane containing triacontanol.

Triaccontanol (mg/liter)	Biomass (mg dry wt/shoot)					Avg increase as % of controls
	Carrot 'Gold Pak'	Cucumber 'Green Star'	Barley 'MSU Exp'	Sugar beet 'USH 20'	Tomato 'Heinz 1350'	
	Growing time after treatment					
	35 days	27 days	22 days	20 days	32 days	
Dry control	48	488	236	40	166	—
Solvent control	35	442	190	39	274	—
.01	64	534	350	73	365	48
1.00	92	652	286	58	382	50
10.00	85	725	372	56	420	50
LSD 5%	27	193	72	17	94	
1%	37	264	101	23	126	

cucumber studies was due to more cucumbers per plant, not to a difference in size of fruit. The sprays applied to transplanted tomatoes only increased the early yield. An asparagus test was conducted on an abandoned cultivar trial at the time the first spears emerged. Although the yield was increased significantly, the data are suspect because of the confounding with cultivars and the high coefficient of variation (28%).

*Temperature studies.* The lack of response in the field to seed treatments with triacontanol led to studies on the effect

Table 4. Yield of 'Heinz 1350' direct seeded tomatoes comparing seed soaking with triacontanol and alfalfa applied with the seed.

Treatment	Treatments		Yield (MT/ha) <sup>Z</sup>	
	Triaccontanol (mg/liter)	Alfalfa (kg/ha)	Early	Late
Control	—	—	6.2	25.9
Alfalfa	—	1	4.9	25.5
Alfalfa	—	10	3.9	24.6
DCM Control	—	—	21.9	36.4
DCM	0.01	—	21.4	35.9
DCM	0.10	—	20.4	37.0

<sup>Z</sup>F value for yield from all DCM treated seed compared to yield from seed not treated with DCM significant at 1% level.

Table 5. Yield of several vegetable crops sprayed with triacontanol in the field.

Triaccontanol (mg/liter)	Cucumber <sup>Z</sup> 'Premier' (\$/ha)	Radish <sup>Y</sup> 'Cherry Bell' (MT/ha)	Sweet Corn <sup>X</sup> 'Gold Cup' (MT/ha)	Carrot <sup>W</sup> 'Spartan Classic' (MT/ha)	
				supplemental N/ha	67 kg 123 kg
Control	810	1.81	10.76	27.4	29.0
Emulsion control	—	1.81	—	27.4	28.9
0.01	929	1.86	13.37	30.4	26.3
1.00	909	1.95	12.03	32.2	26.8
10.00	857	—	12.53	—	—

<sup>Z</sup>F value for quadratic trend for increased yield significant at 1% level.

<sup>Y</sup>F value for linear trend with rate of triacontanol significant at 5% level.

<sup>X</sup>F value for control vs. treatments significant at 1% level.

<sup>W</sup>F value for variance of linear trend in yield with different nitrogen levels significant at 5% level.

of temp on the growth response. Tomato, carrot, and wheat were studied sequentially in growth chambers. Temp for the different chambers were changed at the initiation of each test with a different crop. All 3 crops in this study grew best with the 20°C night temp and 25°C day temp (Table 7). The magnitude of the response to triacontanol was also greatest at this temp. When the average % increase of the treatments over the dichloromethane treated controls for all 3 crops was calculated,

Table 6. The yield of several crops receiving single or multiple applications of triacontanol. (2 or more rates were used with each crop with no significant difference between rates.)

No of applications	Cucumber <sup>Z</sup> 'Green Star' (\$/ha)	Dry bean <sup>Y</sup> 'Sanilac' (MT/ha)	Tomato <sup>X</sup> 'Heinz 1350' (MT/ha)	
			Early <sup>Y</sup>	Late
Control	1032	2.41	9.40	30.0
Single	1139	2.64	11.03	32.0
Multiple	1208	2.56	10.53	30.9

<sup>Z</sup>F value for control vs. treatments significant at 1% level.

<sup>Y</sup>F value for control vs. treatments significant at 5% level.

<sup>X</sup>Tomato grown at 2 fertility levels with no significant difference for fertility level or for the interaction of fertility level with treatment.

Table 7. Dry weight of wheat, carrot and tomato seedlings as influenced by triacontanol seed treatment at 4 different temp in growth chambers.

Temp (°C)	Treatment Triaccontanol (mg/liter)	Biomass (mg dry wt/shoot) <sup>Z</sup>		
		Wheat 'Ionia'	Carrot 'Spartan Classic'	Tomato 'Campbell 1327'
10-15	0	21.7	3.0	10
	1	23.8	4.0	12
	10	22.1	4.3	13
15-20	0	28.3	12.1	51
	1	38.7	17.9	60
	10	34.6	17.1	86
20-25	0	63.5	53.2	144
	1	88.7	82.0	292
	10	92.4	80.0	247
25-30	0	44.2	25.5	79
	1	75.3	48.9	159
	10	69.3	50.0	141

<sup>Z</sup>F value for variation in response of each crop to the triacontanol at the different growing temp significant at 1% level.

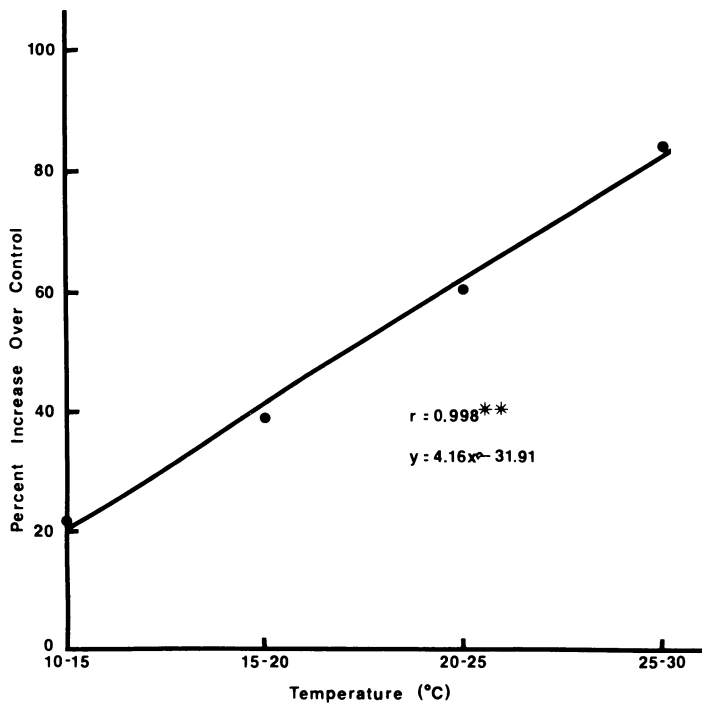


Fig. 1. The relationship between temp regimes and the response of carrot, tomato, and wheat to seed treatments with triacontanol dissolved in dichloromethane. The regression is expressed as the average % increase over the control of both concn of triacontanol with all 3 crops.

regression analysis indicated there was a positive correlation between temp and the growth response (Fig. 1). Even though the best crop growth occurred with the 20-25°C regime, the triacontanol response on a percentage basis was greatest with the 25-30°C regime. Average increase for all 3 crops over the

controls changed from about 20% at the lowest temp to 80% at the highest temp. This may explain the lack of response from the seed soak treatments with triacontanol which were planted during the relatively cool spring months.

Triacontanol stimulates the growth of plants quickly at rates as low as a few milligrams per hectare. All of the annual crop species tested in the greenhouse have responded indicating a lack of specificity. When plants respond to triacontanol in the greenhouse or field, it is usually over a very wide range of rates. Multiple applications do not seem to be beneficial, and our observations indicate that plants respond best when conditions for growth are optimal.

Foliar applications of triacontanol increased the yield of several crops in the field during this first year of testing, but much more research must be conducted before it can be used as another aid to increase agricultural crop production. Its efficacy must be proven under many different environmental conditions. The optimum method of triacontanol application in the field must be established, such as granular formulations, and also the rate and time of application to crops in the field.

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## Apple Fruit $\beta$ -galactosidase and Softening in Storage<sup>1</sup>

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**Abstract.** Fruit softening of apple (*Malus domestica* Borkh.) and the activity of  $\beta$ -galactosidase in 0.5 M citrate extracts of fruit cortex were compared for 'Lodi', 'McIntosh', 'Golden Delicious', and 'York Imperial' during storage at 0° or 3.3°, 10.0°, and 18.3°C. Enzyme activity per g fresh weight increased as fruits softened, but specific activity did not change. Cell wall galactose content also decreased during softening. The decrease in wall galactose was least in 'Lodi' which contained the lowest  $\beta$ -galactosidase activity. 'York Imperial' which softened most slowly showed the highest  $\beta$ -galactosidase activity at harvest and throughout storage. Concentrated enzyme preparations did not release measurable amounts of reducing sugar, uronic acid, or neutral hexose from isolated cell walls.

Enzymatic modifications of the cell wall are generally thought to be responsible for fruit softening. Knee (5) showed that the galactose content of apple cortex cell walls decreased as

the fruits ripened. Although the significance of this change in terms of wall structure and fruit firmness is unknown, a relationship to polyuronide solubilization was suggested (6); the increase in soluble polyuronide was closely correlated with apple fruit softening. Polygalacturonase has not been detected in apples, but Bartley (1) showed that  $\beta$ -galactosidase activity increased during ripening. He felt that this enzyme hydrolyzed a cross-linking galactan and thereby caused the release of polyuronide from the wall. The studies cited above were conducted

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