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Growth Enhancement of Plants by Femtomole Doses of Colloidally Dispersed Triacontanol

Abstract. *Colloidal dispersions of crystalline 1-triacontanol in water, upon foliar application to corn* (Zea mays *L.*) *seedlings, resulted in growth increases at femtomole dosages (spray concentrations as low as 1 nanogram per cubic decimeter). The maximum growth increase occurred at 100 nanograms per cubic decimeter; at both higher and lower concentrations lessened growth increase was observed. The dispersions were prepared by sonication, with control of temperature and composition. Selected surfactants, which facilitate the dispersion process, are effective at 1 percent of the 1-triacontanol composition and are nontoxic.*

1-Triacontanol (TRIA) increases plant growth and sometimes increases crop yields (*1*). Attempts to exploit this discovery to increase the yield of food crops have met with variable success (*2*). The mode of application of the compound appears to have been a poorly controlled parameter and may have been partly responsible for this variability. We have addressed this problem by preparing a stable colloidal dispersion of crystalline particles of TRIA in water.

The administration of TRIA to the plant, as well as its transport within the plant, requires dispersal in water, either as a solution or as a colloid. The water solubility of TRIA can be estimated, by extrapolation of data on lower homologs (*3*), to be less than $2 \times 10^{-16} M$ (9×10^{-14} g/dm^3). Thus the solubility of TRIA is probably not a factor in its activity as a plant growth stimulant. Effective use requires that it be colloidally dispersed.

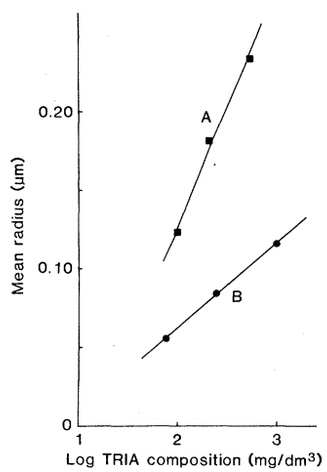

Fig. 1. Mean particle radius as a function of TRIA composition (A) without dispersant; (B) with dispersant (1 percent TAS relative to the TRIA composition). Each value of $\bar{r}$ is the mean of two data points. The average standard deviation for $\bar{r}$ from separate sets of similar determinations is 6.5 percent of $\bar{r}$ (ten sets of data; $N = 4$ in each set).

Methods for forming colloids fall into one of two classes. The colloidal particle is formed either by shearing apart a larger particle, or by assembling it from many smaller aggregates or from molecules. The second method has been the one most commonly followed. Specific procedures included precipitation by dilution of solutions in dimethyl sulfoxide (*4*) or acetone (*5*), dilution of a solution in a liquid nonionic surfactant (*6*), or dilution of TRIA solubilized in a micellar solution of mixed soap and nonionic surfactants (*2*).

In our study of the colloidal dimensions of dispersions, we have estimated particle size by a cumulant analysis of quasi-elastic light scattering (QELS) data (*7*). Dispersions prepared by dilution methods were highly and erratically polydisperse, having particle radii (r) ranging from 0.02 to 35 μm. At sufficiently high composition (10 to 100 mg/dm^3), dispersions formed by dilution are visibly heterogeneous. The micellar solution (*2*) appears to be metastable; over a period of months the solution deposits large crystals of TRIA. Thus, from all these test preparations, the fraction of TRIA that is effectively dispersed is uncertain. Further, the particle number concentration is likewise uncertain, uncontrolled, and relatively small (*8–11*).

We have now prepared uniform colloidal dispersions of TRIA in water having mean equivalent hydrodynamic particle radii ($\bar{r}$) of 0.05 to 0.3 μm and modest polydispersity ($\delta\bar{r} \cong 0.5\,\bar{r}$) by using ultrasonic dispersion of coarse suspensions (*12*). Such colloids are polydisperse, but the dispersity is reproducible and narrow compared to that of colloids formed by dilution. As would be expected, these colloids are stable over long periods of time. Although a small fraction of the material may generate a visible amount of flocculated material after some months, no substantial quantitative changes were evident in samples a year old.

Successful dispersion requires control of temperature, composition, sonic energy input, and it is facilitated by the presence of a dispersant. The temperature must be near or above the melting point (90°C), but below the boiling point of water. In the absence of a dispersant, the TRIA composition must be ~ 0.5 g/dm^3; the lower the composition, the smaller the particles formed (Fig. 1). Use of an effective dispersant decreases the resulting particle size at a given TRIA composition, or makes it possible to increase the composition to ~ 4 g/dm^3 and still form stable colloids (Fig. 1).

The dispersant presumably functions by retarding the coalescence of very small liquid TRIA droplets produced during the explosive energy flux released by cavitation (*12*). It must be surface

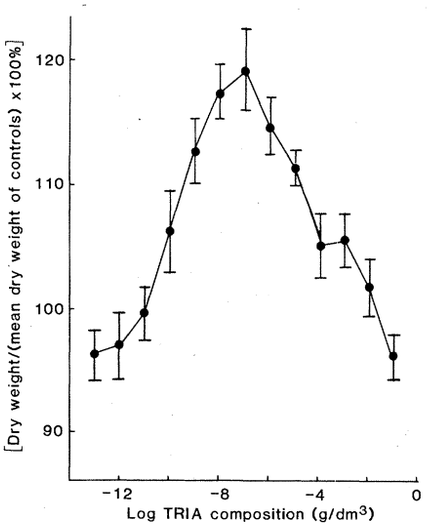

Fig. 2. Dry weight of corn shoots as a function of TRIA composition in the foliar spray, expressed as percent of controls. The controls were sprayed with TAS solution (10^{-3} g/dm^3). Seven-day-old shoots were sprayed and harvested 7 days later. The error bars calculated from shoot weight data on twelve pots, correspond to ± 1 standard error (standard deviation, $s/\sqrt{n}$). Data from two experiments of six pots each were combined. Each pot contained four shoots, weighed together. The mean shoot weight of the 12 control pots was 0.442 g (s = 0.032 g, or 7.3 percent of mean).

active, and all five surfactant hydrophilic group subclasses (*13*) were screened as possible dispersants. Compounds of the anionic, cationic, zwitterionic, and single bond (polyoxyethylene) subclasses were active, while those of the semipolar subclass were not. Screening was performed at surfactant concentrations such that no solubilization could have occurred ($\leqq 0.25$ of the critical micelle concentration).

For plant growth studies we used dispersions prepared by stepped microtip sonication in a conical flask at 90° to 92°C (40 W for 5 minutes) of 10-g samples. Each sample contained TRIA at 1.00 g/dm^3 and TAS (*14*) at 0.010 g/dm^3 (in distilled water). [The TRIA was prepared by the method of Gibson and Tulich (*15*), and was 97.7 percent triacontanol, 2.2 percent nonacosanol, and 0.25 percent octacosanol]. The QELS analysis gave $\bar{r} = 0.10$ μm, $\delta\bar{r}$ (for degree of fit = 3) = 0.05 μm.

Colloidal dispersions such as that described above are useful for biological or physical studies. First, the TRIA is presumably present as the pure crystal and thus it is at its maximum chemical potential.

Second, as a small particle it remains uniformly dispersed and is kinetically mobile. The mean diffusion coefficient of 0.1-μm radius particles (from QELS) is 2.6×10^{-12} m^2 sec^{-1} at 20.4°C, comparable to that of a soluble polymer, a vesicle, or a micelle. With a mean particle mass of about 3×10^{-15} g, the particle number concentration at, for example 1 μg/dm^3, is about 3×10^8/dm^3. Thus, if spray droplets with a volume of 10^{-9} dm^3 ($r = 62$ μm) are produced, only one spray droplet in three of this dispersion will contain a TRIA particle. Since dispersions formed by dilution are likely to have lower particle number concentrations (at a specified TRIA composition), the uniformity of application from these preparations will probably be poorer compared to that of our preparation.

Third, essentially all of the TRIA present is colloidally dispersed and the colloidal dimensions and composition are characterized prior to experimentation, thus providing control over and measurement of these variables.

Fourth, the level of dispersant utilized (0.01 g/g TRIA) is greatly reduced in comparison with other preparations which utilize surfactants [in (*2*) 3.85 and in (*6*) 1000 g dispersant per gram of TRIA]. Finally, an aqueous formula has practical safety or nonflammability advantages (or both) over solutions in organic solvents.

The growth response of field corn (cv. 'Pioneer' 3780) to colloidally dispersed TRIA was determined as follows (*1*). The concentrated preparation was diluted in distilled water, and this dispersion was sprayed to drip with a glass chromatography atomizer. The plants were sprayed in a hood to eliminate random drift of the aerosol about the laboratory, and the glassware was specially cleaned with 20 percent sodium hydroxide in ethanol and then Alconox solution; the cleaning agents were removed by thorough rinsing with water.

The gain in the dry weight of the plants after a week, relative to that of the controls, increased logarithmically with the composition in the spray to a maximum, and then decreased to that of the control (Fig. 2). At no composition did TRIA significantly retard the growth of these plants relative to controls, but an optimum spray composition for maximum weight gain does exist, at approximately 100 ng/dm^3. The reason for the decline in response at the higher compositions is not understood. As no TRIA sample is entirely free of homologs, the inhibitory effect of these impurities (*6*) may prevail at the higher levels.

Similar plant growth data resulted with rice seedlings grown in nutrient culture containing TRIA dispersions over the same composition range that was used in the corn studies.

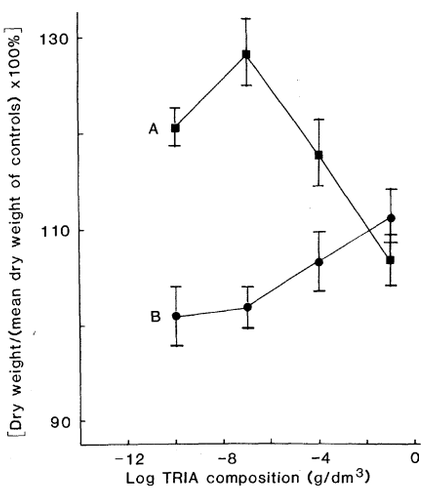

Fig. 3. Comparison of colloidal TRIA formulation (A) with suspensions formed by diluting a 0.1 percent chloroform solution of TRIA with 0.1 percent aqueous Tween 20 solution (B). Six-day-old shoots were sprayed and harvested 7 days later. Data are expressed as percent dry weight relative to controls, which were unsprayed. Error bars calculated from shoot weight data on eleven pots correspond to ±1 standard error. Data from two separate experiments were combined. Each pot contained four shoots, weighed together. The mean shoot weight of the 11 control pots was 0.297 g ($s = 0.032$ g, or 10.7 percent of mean).

The mode of preparation affects the plant response. The direct comparison in two experiments of the colloidally dispersed TRIA with a suspension of TRIA, formed by diluting a 0.1 percent solution of TRIA in chloroform with 0.1 percent Tween 20, demonstrates the different responses of corn to these two preparations (Fig. 3). The colloidally dispersed TRIA is effective at compositions several orders of magnitude lower than suspended TRIA, and the magnitude of the response is greater.

Colloidal TRIA produces a significant response in corn plants at spray compositions as low as 1 ng/dm^3. A 7-day-old corn shoot with a dry weight of 70 mg sprayed to drip retains about 50 μl of spray solution, which contains 5×10^{-14} g of TRIA (about 17 particles, 10^{-16} mole, or 7×10^7 molecules). Applied as a colloidal dispersion, TRIA appears to be one of the most active plant growth regulators reported.

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Exposure to Ethylene Oxide at Work Increases Sister Chromatid Exchanges in Human Peripheral Lymphocytes

Abstract. *Sister chromatid exchange rates increased significantly in the peripheral lymphocytes of a small group of hospital workers exposed to ethylene oxide for as little as 3.6 minutes per day regularly over a period of months. Results based on breathing zone exposure and task frequency estimates over a 6-month period for 14 workers suggest that sister chromatid exchanges are a sensitive indicator of exposure and that cumulative dose and dose rate are important predictors of sister chromatid exchange response.*

A sister chromatid exchange (SCE) is the visual manifestation of a four-stranded exchange in the DNA (*1*). Determining the average number of SCE's per cell is a sensitive method for measuring the chromosomal effects of some mutagenic and carcinogenic agents and correlates with other short-term tests for DNA damage (*2*). The exact mechanism of SCE formation is unknown, but is thought to reflect changes in DNA resulting from adduct formation or changes in conformational structure after exposure to exogenous agents (*3*). Mutations may be a subset of lesions that elicit SCE's (*4*).

Although SCE rates in human lymphocytes have been investigated as a biological monitor for exposure to harmful agents in the workplace (*5*), exposure data have been lacking. In the few studies that included estimates of airborne exposure, values were reported as air concentrations averaged over an 8-hour workday [time-weighted average (TWA)] without regard to specific patterns of exposure or epidemiological factors that may impinge on the specificity of the SCE response. Results presented here suggest that, when a precise description of short-term exposure is obtained, an exposure-response relation in humans is shown even though the 8-hour TWA exposure is low.

Ethylene oxide (ETO), a known mutagen and a suspected carcinogen, has been shown to increase SCE's in the rabbit in vivo (*6*). The current Occupational Safety and Health Administration (OSHA) standard for workplace exposure is 50 ppm as an 8-hour TWA. Previous studies showed an increase in SCE's in persons occupationally exposed to ETO; however, no breathing zone information on the levels of exposure that elicit this response was reported (*7*).

Our study was conducted in two hospitals that use ETO to sterilize supplies. There appeared to be no significant exposure to other chemicals in these work areas. A single-beam infrared spectrophotometer (Miran 1A, Foxboro) connected to a strip-chart recorder was used to measure and record short-term exposures of operators as they performed their work with sterilizers containing ETO. We had already determined that there was no extraneous exposure to ETO, such as might occur from leaking gaskets on the sterilizer doors.

To express an estimated dose of ETO received by a worker during a brief exposure it can be assumed that alveolar uptake and absorption of ETO approach 100 percent (*8*). The expression for estimated dose per task is then $m = \bar{V}_m \bar{c} T$, where m is estimated dose per task in milligrams, $\bar{V}_m$ is mean respiratory minute volume (estimated to be about 21 liters per minute for humans performing light work) (*9*), $\bar{c}$ is mean concentration of ETO in milligrams per cubic meter, and T is elapsed time (minutes) during task performance.

The tasks during which ETO exposure occurred were identified and defined. Each exposed the workers to a different dose (Table 1) (*10*). Estimates of the doses received by workers performing the same task at hospitals A and B differ considerably. This is attributable to differing ventilation systems, equipment,

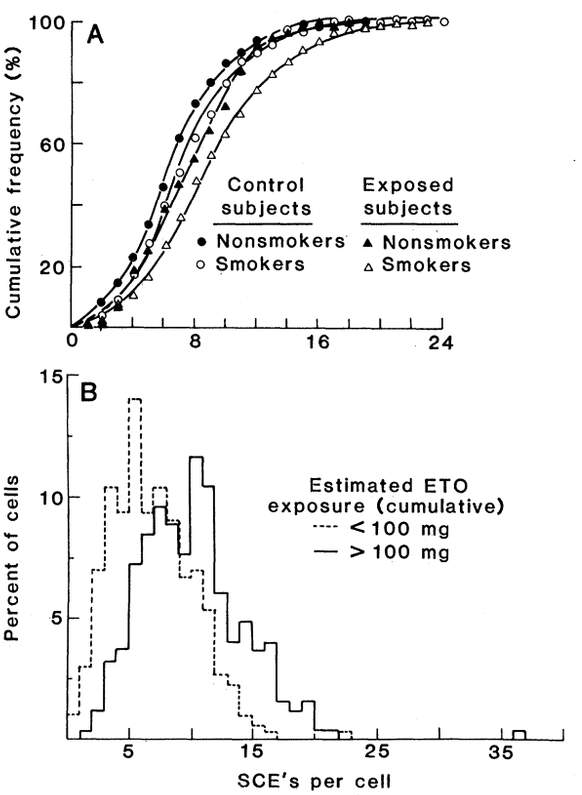

Fig. 1. (A) Cumulative frequency distribution of SCE's per cell. The mean number of SCE's per cell (± the standard deviation) was 7.04 ± 1.00 for control nonsmokers, 7.89 ± 0.92 for control smokers, 8.27 ± 2.06 for exposed nonsmokers, and 9.09 ± 2.00 for exposed smokers. (B) Frequency distribution of SCE's per cell in workers exposed to ETO at relatively low (D_w < 100 mg) and relatively high (D_w > 100 mg) cumulative doses.