Senescence of Attached Bean Leaves Accelerated by Sprays of Silicone Oil Antitranspirants

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ABSTRACT

During an investigation into the use of oil emulsions in foliar sprays, it was found that silicone oil emulsions accelerated the senescence of the primary leaves of bean (Phaseolus vulgaris) plants. It was shown that accelerated senescence was not a result of the reduced transpiration rates found in silicone-sprayed leaves. Furthermore, the silicone oil emulsions did not induce leakiness in plant cell membranes. The senescence-enhancing effect seems to be connected with the ability of the silicone oil emulsions to penetrate into the leaf interior.

The efficiency of uptake foliar-applied solutes is reportedly increased by the addition of emulsified oils to leaf sprays (6, 11). Oily layers will often reduce the rate of evaporation from air-water interfaces and may thus prolong the period of relatively rapid solute absorption which is known to occur while the leaf surface remains moist (13). A disadvantage of oil emulsions and of most other film-forming materials, is that they reduce the rates of photosynthesis and respiration of treated areas by increasing the leaf diffusion resistance to CO₂ and O₂ (3, 8). However silicone oils, because of their relatively high permeability to oxygen and carbon dioxide (5, 8), are less inhibitory to gas exchanges, and several investigations into the use of silicone oils as antitranspirants have failed to reveal any toxic effects on cellular metabolism (1, 5, 8).

Initial investigations into the possible use of dilute silicone oil emulsions to promote uptake of foliar-applied solutes showed that these emulsions caused a rapid yellowing of the primary leaves of bean plants and this phenomenon was further investigated.

MATERIALS AND METHODS

Growth of Plants. Bean plants (Phaseolus vulgaris cv. Brittle-wax) were germinated for 3 days and then planted in vermiculite in 500-ml polyethylene pots, which were irrigated weekly with a half-strength Hoagland's solution. Plants were watered every 24 hr with distilled water, and were grown in a room at 25 to 26°C with fluorescent illumination (300 ft-c) and a 20-hr photoperiod. Plants were generally sprayed after 11 days growth in vermiculite, when the primary leaves were nearly fully expanded. As the plant grows, the primary leaves become increasingly shaded by new leaves and both transpiration and Chl levels in the primary leaves begin to decline after 10 days.

Spray Treatments. Dimethylpolysiloxane or silicone oils are chemically inert, nontoxic, clear, colorless liquids represented by the chemical formula shown below, where n determines the length of the polymer chain and the viscosity.

\[ \text{CH}_3 \left( \text{CH}_3 \right)_n \text{CH}_2 \]

Substitution of some of the methyl groups by phenyl or other groups alters the properties of these oils. The following oils were used in the experiments: (a) M.S.200: a dimethylpolysiloxane oil, viscosity 100 centistokes from Midland Silicones; (b) F 111/20: a dimethyldichlorosilane oil, viscosity 20 centistokes from I.C.I.; (c) M.S.702: a chemically modified silicone oil, viscosity 48 centistokes from Midland Silicones; (d) white light paraffin oil, purchased locally.

The oils were emulsified by blending with equal volumes of 10% (w/v) Tween 80 solutions. Such emulsions are stable but have a tendency to "cream" and must be shaken before use. Stock emulsions were diluted with distilled water before use. The diluted emulsions contained 5% (v/w) of the silicone or paraffin oil. Plants were sprayed to runoff and care was taken to prevent any spray solution from reaching the roots.

Measurement of Transpiration. Leaf blades were cut off the petiole, and the cut end was sealed with Vaseline. The blades were then weighed and transferred lower side up to a glass desiccator, which contained a hydrophilic silica gel. Water losses were estimated by removing the leaves from the desiccators and weighing them at 5-min intervals. Results are presented as leaf transpiration rates per cm² of leaf and are the averages of at least three determinations, using leaves from separate plants. Standard deviations from the means are given with the results.

Chl Determinations. Leaves were dried to constant weight in a forced draft oven at 70°C. Chl was extracted from the leaves with 80% aqueous acetone, and the yield in mg/g calculated from the A at 652 nm, according to Arnon (2). Results are the means of three to four extractions of leaves from separate plants.

Membrane Damage. Beet roots were purchased locally and cut into thin 1-cm diameter cylindrical discs. The discs were washed for 30 min in several changes of 0.5 mM CaSO₄, solution before being incubated in the solution to be tested. All solutions were made up in 0.5 mM CaSO₄, in order to provide the basic conditions for membrane integrity (4). After a 60-min incubation period, discs were rinsed free of incubation solution and batches of five discs transferred to 10 ml of 0.5 mM CaSO₄, solution. Membrane damage was quantitatively measured by the decrease in the partition of 14C in the discs as described by Neumann (9). Results are the means of three to four determinations using discs from separate plants.
assessed by spectrophotometric determinations of the amount of anthocyanin pigment that leaked into the incubation medium over a 24-hr period. Results are the means of three separate determinations.

**Surface Tension Determination.** Surface tensions of solutions were obtained with a Fisher surface tensiomat, using a 6-cm circumference platinum ring. Values are the mean of three determinations, corrected by using a standard graph supplied with the instrument.

**RESULTS AND DISCUSSION**

**Specificity of Senescence-enhancing Effect.** The primary leaves of bean plants sprayed with dimethylpolysiloxane oil emulsions took on a variegated appearance about 3 days after spraying. Yellow-green patches spread gradually over the whole leaf and eventually caused leaf fall. This effect was observed only when dimethylpolysiloxane oil emulsions were used. The same effect was observed with fresh or aged emulsions, so that the effect was not due to breakdown products from the oil or the Tween emulsifier. Samples of silicone oils from both Midland Silicones and from I.C.I. with viscosities of 20 and 100 centistokes were tested and all had the same effect. However, an emulsion of a chemically modified silicone oil used for high vacuum work (Midland Silicone 702) did not accelerate leaf senescence, and emulsions of light white paraffin oil were also without effect. The results in Table I illustrate the effect of the various treatments on leaf senescence as measured by Chl levels.

Two possible causes of the Silicone 200-induced reduction in Chl levels were at first considered. The first, that Silicone 200 oil interfered with respiration or photosynthesis and so accelerated senescence, seemed unlikely. Paraffin oil, which is known to interfere with these processes (12), had less effect on Chl levels than Silicone 200, which has a relatively low inhibitory effect on respiration and photosynthesis (8). The second possibility was that the well established antitranspirant effect of the Silicone 200 oils was the direct or indirect cause of accelerated senescence. The transpiration rates of leaves were therefore measured 2 and 10 days after spraying. The results in Table I show that the paraffin oil was nearly as effective as M.S. 200 Silicone oil in reducing transpiration but did not have the senescence-accelerating effect. Thus, it seems unlikely that reduced transpiration rates were the cause of accelerated senescence. This conclusion is in agreement with the findings of O'Leary and Knecht (7), that the growth of bean plants was not adversely affected by high humidity, which also greatly reduced transpiration.

**Surface Tension and Leaf Penetration.** Schönherr and Bukovac (10) showed that liquids with surface tensions below 30 dynes cm⁻¹ were likely to penetrate leaves spontaneously via the stomatal apertures. The surface tensions of various oil emulsions were measured in order to determine whether there was any correlation with the senescence-enhancing effect. It can be seen from Table I that only the Silicone 200 emulsion, which enhanced senescence, had a surface tension low enough for spontaneous stomatal penetration at time of spraying.

**Effect of Emulsions on Cellular Membranes.** In view of the likelihood of leaf penetration by unmodified dimethylpolysiloxane emulsions, it was decided to investigate the effect of these emulsions and of the Tween emulsifier on plant cell membranes. A solution of Aerosol OT detergent was also tested, to show the effect of a substance which effectively disrupts cell membranes.

Only treatment with Aerosol OT caused a leakage of anthocyanin from the cells (Table II). Beet disc membranes were apparently not affected by the 1-hr immersion in silicone oil emulsion. Similar experiments revealed no effect of silicone emulsions on membrane integrity after incubation periods of 24 hr. Thus, we concluded that the silicone oil emulsions did not enhance senescence by causing leakiness of cell membranes.

**Alternative Explanations.** The possibility that the silicone oil blocked transfer of water to the leaves was considered unlikely since the water contents of silicone oil-treated leaves were, if anything, slightly higher than those of the control leaves throughout the experimental period.

An effect of the dimethylpolysiloxanes on phloem transport or on the activity of membrane-bound enzymes may be postulated. It is noteworthy that specific histological changes were induced in the lung tissues of animals by brief exposures to dimethylpolysiloxanes (9).

The possibility that the straight chain dimethylpolysiloxane oils contained some toxic impurities, which were active even in diluted emulsions of the oil, cannot be ruled out. However, samples of oils manufactured by both I.C.I. and Midland Silicones gave the senescence-accelerating effect. A final possibility is that the dimethylpolysiloxane oils penetrated the leaves and spread to form a film which reduced the rate of escape of ethylene gas. This might lead to a build-up of ethylene within the leaf and senescence would result.

In conclusion, it would seem that silicone fluids of the dimethylpolysiloxane variety can have toxic effects on plants. Their use as plant antitranspirants or as substitutes for paraffin oils in aiding the uptake of foliar-applied solutes does not therefore seem advisable, at least where easily penetrable leaves, such as bean leaves are involved.

**LITERATURE CITED**