Effects of Decenylsuccinic Acid on the Permeability and Growth of Bean Roots

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Summary. Decenylsuccinic acid (DSA) at 10^{-3} m has been reported to increase the permeability of bean root systems to water without seriously injuring the plants. We have confirmed the increase in permeability at 10^{-3} m, but have found that 10^{-4} m DSA reduces the permeability. Both concentrations cause leakage of salts from the roots and cessation of root pressure exudation. The roots of intact bean plants are killed by 1 hour’s immersion in 10^{-3} m DSA, but the plants may survive by producing new roots. Up to 4 hours in 10^{-4} m DSA causes only temporary cessation of growth. Comparisons are made between the effects of DSA and some metabolic inhibitors. It is suggested that DSA is acting as a metabolic inhibitor, and that increase in water permeability is the result of injury to the roots. Experiments with 3 other species indicated variations in response to 10^{-3} m DSA. These could be largely attributed to differences in susceptibility to injury.

Decenylsuccinic acid (DSA) \(^3\) was reported by Kuiper (3, 4) to increase the permeability of roots to water and to increase the resistance of plants to drought and frost. According to Zelitch (8) it causes stomatal closure when applied to leaves. Kuiper found that when bean roots were immersed in 10^{-3} m or 5 \times 10^{-4} m DSA for 2 hours their permeability to water increased greatly (ca. 8-fold at 30^\circ) and became only slightly dependent on temperature. He also tested the effect of 10^{-3}, 10^{-4} and 10^{-5} m DSA on growth of bean seedlings in vermiculite. When applied to the vermiculite with water it reduced growth, but when polyethylene glycol was added to provide osmotic stress DSA increased the growth and survival of the bean seedlings. This paper describes some further investigations of the effects of DSA on bean plants when applied to their roots.

Materials and Methods

Experiments were conducted on bean plants \(\textit{Phaseolus vulgaris} L.\) var. Bountiful) about 3 weeks old. The plants were grown in vermiculite with Hoagland’s solution, in a growth chamber with a 12-hour photoperiod, temperature 23^\circ in light, 18^\circ in darkness. About 18 hours before the start of an experiment the vermiculite was washed off, and the roots were suspended in aerated 0.1 strength Hoagland’s solution in a laboratory kept at about 23^\circ. This was to allow the roots to become equilibrated to the temperature and aeration conditions of the experiment. Root solutions were aerated during all experiments. For determining rates of water movement under suction, the stem was cut below the cotyledons, a graduated tube was attached, and a suction of 60 cm Hg applied. There were usually 4 plants per treatment. Most experiments were repeated 2 or more times, with similar results. Solutions of DSA were made up fresh on the day of the experiment. The DSA was first dissolved in warm 95% ethanol, 1 mole DSA per liter of ethanol. This solution was then added to water. For control solutions ethanol in water was often used, at the same concentration as in the DSA solution, e.g. 0.1 ml ethanol per liter for 10^{-4} m DSA. The DSA solutions were not buffered, because salts appear to alter the solubility of DSA. The pH of 10^{-3} m DSA is 3.65, of 10^{-4} m DSA 4.3.

Results

Permeability to Water. Figure 1 shows the effects of DSA on the rate of water movement through root systems under 60 cm Hg suction, at 23^\circ \pm 1^\circ. All root systems were in water before time zero. DSA (10^{-3} m) causes a large increase in permeability, as reported by Kuiper (3); but 10^{-4} m DSA

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\[^3\] The formula of decenylsuccinic acid is \(\text{CH}_2(\text{CH}_2)_{6}\text{CH} = \text{CH}-\text{CH}_2-\text{CH}(-\text{COOH})-\text{CH}_2-\text{COOH}\). The DSA used in these experiments was from lots 12E and 17, purchased from the Humphrey Chemical Company, North Haven, Connecticut.
Fig. 1. Rate of water movement through root systems at 23° under 60 cm Hg suction. Treatments started at time 0. ■, □ mean of 2 plants, others mean of 4 plants.

causes a large decrease. With either concentration the maximum effect is reached in about 30 minutes. The experiment with 10⁻⁴ M DSA was repeated 4 times, and in each case there was some recovery (i.e. increase) in permeability after 30 minutes. Two other concentrations were also tried: 3 × 10⁻⁴ M had a similar effect to 10⁻³ M; 3 × 10⁻⁵ M had little effect.

To test whether these effects of DSA could be due to pH alone, control solutions were adjusted to various pHs between 3.2 and 4.4 by adding HCl. The changes in permeability were always far smaller than those produced by 10⁻³ M or 10⁻⁴ M DSA. Figure 1A shows the response to pH 3.2 to 3.4, which is more acid than 10⁻³ M DSA.

Permeability to Electrolytes. During the suction experiments, and similar treatments without suction, measurements were made of the electrical conductivity of the solution surrounding the roots. Each root system was in an equal, separate root were measurement and experiments, more than those produced with 10⁻³ M DSA the maximum rate of electrolyte loss was reached in less than 10 minutes, and with 10⁻⁴ M it was reached in about 1 hour.

It might be expected from these results that DSA would reduce root pressure exudation. This was confirmed by experiments in which no suction was applied to the cut stem. Exudation ceased about 15 minutes after the roots were transferred to 10⁻³ M DSA, and about 3 hours after transfer to 10⁻⁴ M DSA.

Growth. The roots of intact bean plants were given one of the following treatments. There were 3 plants per treatment. A) Immersed in 10⁻³ M DSA for 1 hour, then in water for 3 hours. B) 10⁻³ M DSA in Hoagland’s solution, permanently. C) 10⁻⁴ M DSA 1 hour, water 3 hours. D) 10⁻⁴ M DSA 4 hours. E) Control: water 4 hours. After treatments A, C, D and E the plants were transferred to Hoagland’s solution, and all plants were placed in a greenhouse. Every 2 to 3 days the following measurements were made: 1) stem length, 2) length of median leaflet of third, fourth and fifth true leaves, 3) length of 2 marked main roots on each plant.

Stem and leaf growth showed similar responses to the treatments. The two 10⁻⁴ M DSA treatments (A and B) produced similar results to each other, and so did the two 10⁻³ M treatments (C and D). Figure 3 shows the leaf and root growth in treat-
ments A and C, expressed as a percentage of the growth of the control plants. In the 2 treatments with 10⁻³ M DSA (A and B), root growth stopped immediately, and it was never resumed. By day 6 the whole of the treated root system was clearly dead, but new roots were beginning to grow from the base of the stem. On day 9 the mean number of new roots per plant in each treatment was: A 18, B 19, C 2, D 5, E 1. On day 9 the new roots in treatment B were growing into the DSA, apparently without suffering injury; but by this time the DSA had flocculated, and presumably was no longer active. In treatments A and B leaf and stem growth declined to about zero on days 4 to 6, but recovered after the new roots began to form. Treatment with 10⁻⁴ M DSA for 1 or 4 hours (treatments C and D) nearly stopped stem, leaf and root growth during the first 2 days, but after that growth was gradually resumed, and the roots produced numerous new laterals. These experiments indicate that 1 hour in 10⁻³ M DSA kills the root system, but up to 4 hours in 10⁻⁴ M DSA produces only temporary cessation of growth.

Experiments with Other Species. The root systems of 10-week-old plants of tomato (*Lycopersicum esculentum* Mill. var. ‘Morglobe’) under 60 cm Hg suction were treated with 10⁻³ M DSA. At first the rate of water flow was reduced, but after 1.5 hours it began to increase, and it finally reached 60% above the rate before treatment. After 24 hours of treatment the roots were flaccid and dull in color, whereas the roots of control plants were turgid and looked healthy.

The roots of intact plants of red pepper (*Capsicum frutescens* L.) and water tupelo (*Nyssa aquatica* L.) were immersed in 10⁻³ M DSA. The plants were in a greenhouse in which the daytime temperature reached 35 to 40°, but control plants with their roots in dilute ethanol remained healthy. During the first day the leaves of the treated pepper plants remained turgid, but the roots became somewhat flaccid. After 24 hours the roots and lower leaves were extremely flaccid, and after 2 days the plants had collapsed completely. After 1 hour of treatment the tupelo leaves were noticeably wilted, and after a day many of the upper leaves were completely dried out and brittle. This suggests that the permeability of the roots to water was reduced. The plants were then moved to a dimly lit room at 23°, but the dried leaves did not recover, and more leaves later dried up. After 3 days the treated roots were darker in color than the controls, but were not noticeably more flaccid.

Evidently response to 10⁻³ M DSA varies from 1 species to another, but in none of the species tested could the effects be considered beneficial.

Comparison with Azide. Respiration inhibitors have been reported to decrease the permeability of the roots of several species to water. For instance, the permeability of tomato roots is decreased by sodium azide (5) and by potassium cyanide (6). Figure 1B shows the rate of water flow through bean roots treated with 3 × 10⁻³ M sodium azide. Figure 4 shows the conductivity of the solution surrounding roots treated in the same way. The sudden rise in specific conductivity by about 300 μhos after addition of azide is due to the conductivity of the azide itself, but the additional rise after that resulted from salts leaking out of the roots. In both these experiments the effects of azide are similar to those of 10⁻⁴ M DSA, though the azide acts more slowly. Azide also reduces and finally stops root pressure exudation.

Experiments to test whether DSA is a respiration inhibitor were performed with bean root tips in Warburg respirometers. During 3 hours after addition of 3 × 10⁻⁴ M DSA there was no reduction in O₂ uptake, indicating that DSA was not inhibiting respiration.

**Discussion**

The similarity between the effects of sodium azide and 10⁻⁴ M DSA on the permeability of bean roots suggests that DSA may function as a metabolic inhibitor. The fact that it does not inhibit O₂ uptake by bean roots does not preclude its inhibiting other processes. Ordin and Kramer (7) found that the phosphorylation uncoupler 2, 4-dinitrophenol (DNP) at 5 × 10⁻⁴ M decreased the permeability of *Vicia faba* root segments to water, but at 10⁻³ M increased the permeability almost 3-fold. After treatment with the higher concentration the root cells could not be
plasmolyzed and were regarded as dead. Hoagland and Broyer (1) found that bubbling CO₂ through the root solution caused an initial decrease in the rate of water flow through tomato root systems under suction, but after a few hours the rate began to increase and after 24 hours it was about twice the rate before treatment. As mentioned above, 10⁻³ M DSA has a similar effect on tomato, though it acts more rapidly.

It seems likely that the mode of action of CO₂, DNP, DSA and a number of other substances on root permeability is similar. At certain concentrations each inhibits some metabolic process which influences the permeability of the cytoplasm, and permeability is thus reduced. At higher concentrations or with longer treatment the cytoplasm is injured and permeability increases. The variation among the 4 species studied in their response to 10⁻³ M DSA could be largely accounted for by differences in susceptibility of the roots to injury by the treatment. Bean and pepper are apparently injured rapidly; in tomato injury begins after about 1.5 hours; and in water tupelo there was no definite evidence of injury reduced root permeability apparently continuing for at least a day. Kuiper's results (3, 4) suggested that 10⁻³ M DSA can increase the permeability of bean roots without harming the plants. Our results suggest, on the contrary, that a treatment with DSA which increases the permeability of bean roots also kills them, although the plant may survive by producing new adventitious roots. The decrease in temperature dependence of root permeability found by Kuiper (3) after treatment with 5 × 10⁻⁴ M DSA is also found in roots killed by dipping them in hot water (2). Kuiper stated (3) that DSA increases the permeability of cells to water by incorporation of the molecules into lipid layer of the cytoplasmic membrane. Our results and conclusions favor a different mode of action for DSA, but they leave one portion of Kuiper's evidence unaccounted for. He reported that the effect on root permeability of succinic acid derivatives related to DSA varies with different lengths of side-chain and also between substances with saturated and unsaturated side-chain. It would be desirable to test whether roots treated with these substances show a correlation between permeability and amount of injury.

The increased drought resistance of beans treated with DSA which Kuiper reported (4) cannot have resulted from increased root permeability, since 10⁻⁴ and 10⁻⁸ M DSA were effective and our experiments show that 10⁻⁴ M DSA greatly reduces root permeability. Perhaps some DSA reached the leaves and caused closure of stomates (8), thus decreasing transpiration. If this is so, it would be better to apply the DSA directly to the leaves, thus avoiding the danger of damaging the roots.

### Literature Cited