12.3–13.4% soluble solids for 'Starking' and 'Golden Delicious' respectively). In 1975 detailed measurements of size and color were done on samples from each of the 3 in-row spacings (Table 2). Soluble solids were slightly lower and grade was slightly better for 'Golden Delicious' at the 1.22 m spacing, but no differences in size or quality were found for 'Starking' at the different spacings. 'Golden Delicious' quality was somewhat poorer in 1975 than in former years because of a very late bloom period and a cool summer. Long-term mean soluble solids for 'Golden Delicious' in this plot are 13.3%. The moderate amount of poorly colored fruit of both cultivars in 1975 was due in part to a luxury level of nitrogen (2.44% in leaves). We cannot explain why 'Golden Delicious' fruit had better color at the 1.22 m spacing than at 2.44 m, but it permits us to state with confidence that the higher density did not reduce color.

In the past, growers have resisted going to high density plantings because they believed the initial cost of trees to be too high. However, calculation of the costs vs. returns in the present study indicates the highest density would be the best economically (Table 3). If the cost of a tree (plus planting and establishment) is $2, and the value of the crop is $120 per metric ton, then the highest density 'Golden Delicious' produced a gross return (over initial establishment cost) of more than $128,000, which is over $14,000 more than that of the lowest density of 897 trees per ha. Moreover, the lowest density tested was more than 3 times the usual density of 247 trees per ha (100 trees per acre) currently being used in many areas of the U.S. It is important to note that even though the closest spacing resulted in the highest yield per ha, the yield per tree during the first 12 years was 42% less than that of the widest spacing. This indicates clearly that linear extrapolations of low density tree yield to higher densities as was done by Carlson and Oh (1) are not valid, and that actual spacing trials provide the most reliable estimate of yield per hectare.

Average yield in commercial apple orchards in the U.S. was about 330 boxes per acre (16 t/ha) in the heavy crop year of 1975 (from USDA data on acreage and production). This is less than 1/6 the yield of 'Golden Delicious' during the third 6-year period of this study. From these data it is apparent that actual yield in most orchards is far below the potential. The ultimate objective is to convert a maximum amount of sunlight into quality fruit with minimum cost. The use of special rootstocks at the proper spacing and in a favorable climate could approach this objective. A recent survey, by the California-based McCarthy Southdown Corporation, indicated that the Willamette Valley of Oregon is one of the favored climates in the U.S. for sustained high yield of apples. It has an average frost-free season of 213 days and according to grower and experiment station records (unpublished), there has not been a crop failure of 'Golden Delicious' or 'Rome Beauty' in the last 50 years due to frost or poor fruit set.

**Influence of Daminozide on the Levels of Root-promoting Substances in *Pelargonium hortorum* Bailey**

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Abstract. Levels of root-promoting substances from *Pelargonium hortorum* Bailey were detected by the mung bean rooting bioassay. Fractionated ethnicolic extracts from *Pelargonium* showed 1 major and 2 minor zones which promoted rooting of mung beans. The active fractions promoted rooting in the absence of indoleacetic acid (IAA), in contrast to results previously published. Levels of promoters fluctuated during the propagation period, decreasing to a low point just prior to root initiation. Active zones from extracts of *Pelargonium* treated with 2500 ppm succinic acid-2, 2-dimethylhydrazide (daminozide, SADH) did not differ from those of controls indicating that daminozide does not promote rooting by modification of the level of ethnicolic extractable root promoters.

Despite numerous investigations into the mechanism of daminozide action, the process is still vague. The inhibition of shoot elongation in many plant species by daminozide and other growth retardants has been attributed to interference with gibberellin (GA) biosynthesis and GA action (22, 23, 28, 29). However, daminozide did not affect GA production in *Fusarium* (16) or in *Echinocystis* seeds (4); and Pales et al. (17) found that it did not alter GA action in the barley endosperm test.

Others explain the action of daminozide in terms of the reduction of indoleacetic acid (IAA) levels either by inhibition of tryptamine oxidation (21) or by stimulation of peroxidase and IAA oxidase activity (7). Ryugo and Sachs (22) findings do not support Reed's proposals (21) that 1) the active portion of daminozide is the unsymmetrical dimethylhydrazine moiety and 2) the primary effect of daminozide is to inhibit IAA synthesis. Some evidence exists that IAA is unable to reverse...
Table 1. Effect of basal dip of daminozide on rooting of 'Irene' geranium cuttings.

<table>
<thead>
<tr>
<th>Daminozide (ppm)</th>
<th>Mean no. of roots</th>
<th>Mean dry wt of roots (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10.3 a²</td>
<td>41.6 a</td>
</tr>
<tr>
<td>1000</td>
<td>15.4 b</td>
<td>92.5 b</td>
</tr>
<tr>
<td>2500</td>
<td>18.2 b</td>
<td>111.5 b</td>
</tr>
<tr>
<td>5000</td>
<td>15.4 b</td>
<td>93.5 b</td>
</tr>
</tbody>
</table>

²Mean separation in columns by Student Newman-Keul’s test, 1% level. Means of 30 cuttings.

daminozide growth inhibition (15, 30).

The action of daminozide may not be directly related to either GA or IAA metabolism. In excised tissues, growth retardation caused by daminozide cannot be reversed by GA3 or IAA (24). The inhibition of both ethylene production and the development of the respiratory climacteric by daminozide has been reported for several fruit crops (1, 12, 14, 25). Undurraga and Ryugo (27) investigated daminozide transport and found that daminozide lowered membrane integrity, allowing vascular contents to diffuse rapidly into the external medium. Cleland (3) concluded from the diversity of tissue responses to growth retardants, that growth retardants possessed more than one mode of action.

Daminozide has been shown to be effective in the stimulation of rooting (2, 18, 19, 20), but its influence on root initiation is not readily explained. There is evidence that endogenous rooting factors, other than auxin, control rooting. Such factors may be present in easy-to-root cuttings, but in lesser amounts, if at all, in the difficult-to-root ones (5, 9, 10). Adventitious root production by mung bean, Phaseolus aureus Roxb., cuttings has been used to detect root promoting substances (11).

This investigation was undertaken to determine the effect of daminozide on the levels of root-promoting substances in 'Irene' geranium, using the mung bean rooting bioassay developed by Hess (8). Such findings might shed light on the mechanism of daminozide action in the stimulation of adventitious root formation.

Materials and Methods

Sampling and extraction technique. Uniform 7.6 cm 'Irene' cuttings³ were treated with 0, 1000, 2500 and 5000 ppm SADH applied to the basal portion of the cuttings as a 15-sec aqueous dip. The cuttings were rooted in sterilized sand under intermittent mist and with a rooting medium temperature of 24°C at a diurnal air temperature regime of 15.5-21°C. Samples of thirty 2.5-cm basal segments were taken after 1, 3, 7, 11, 15, or 19 days. The samples were frozen, lyophilized, and ground in a Wiley mill to pass a 40-mesh screen. The tissues were stored at -20°C until needed for assay.

Tissue samples, 0.5 g each, were extracted with absolute ethanol followed by 95, 80, and 60% ethanol as per Girouard's suggestion (6). For each ethanol concn used, the tissue samples were extracted for 30 min with 25 ml at 5°C. The extracts were combined and concentrated to 6 ml at 40°C.

Paper chromatography and bioassay. One ml of the concd extract was streaked on 15 cm-wide strips of Whatman 3M chromatography paper. The chromatograms were equilibrated for 6 hr and developed 30 cm at 22°CC by ascending chromatography in isopropyl alcohol and water (4:1 v/v). Chromatograms were cut into sections of 3 cm in length and each section inserted into 22 x 52 mm glass vial which contained 16 ml of deionized water. A chromatogram section from below the origin, and 1 section from above the solvent front served as controls. The root-promoting activity of the chromatogram sections was determined by the mung bean bioassay (8).

The general bioassay technique is described below. Mung bean seeds weighing between 34 and 44 mg each were soaked for 1 min in 0.3% sodium hypochlorite solution, then placed between 6 layers of cheesecloth for 24 hr. Seeds were planted in sterilized sand at a spacing of 2.5 x 2.5 cm in 47 x 54 x 9 cm flats. Plants were grown for 7 days under cool-white fluorescent lights at air temp of 24°C (day) and 21°C (night). Light intensity was 81 klx at plant level and a 16 hr photoperiod was used. Clear polyethylene was placed over the flats for the first 48 hr to maintain a high humidity. Seedlings were cut 3.5 cm below the cotyledonary node, the cotyledons removed and cutting bases placed in deionized water for 1 hr to regain turgidity. Five cuttings and a chromatogram section were placed in each 25 x 52 mm vial with 16 ml of deionized water. Vials were spaced 5 x 5 cm in trays and placed in a lighted chamber. The chamber was maintained at 25°C, 60% relative humidity, with 5.4 klx light intensity from cool-white fluorescent lights at plant level, with a 16 hr photoperiod. Deionized water was added daily to maintain the original water level. Roots were counted at 7 days with the aid of a binocular microscope. Each step in the bioassay was conducted at the same time of day.

Results and Discussion

A preliminary step in determining daminozide's effect on the level of root-promoting and inhibiting substances in 'Irene' geranium was to substantiate its promoting role in adventitious root formation. Daminozide enhanced rooting and 2500 ppm produced the greatest number and weight of roots (Table 1). Since 2500 ppm was the best concn of those tried, the levels of root-promoting and inhibiting substances were determined in geraniums treated with 0 and 2500 ppm.

Results of our preliminary experiments with the mung bean rooting bioassay were inconsistent if cuttings were transferred to deionized water after 24 hr. Repeatable results were obtained only when chromatogram sections were left in the vials as suggested by M. Kawase (personal communication). We also noted stimulation of root development on mung bean cuttings in vials containing blank chromatograms when compared to cuttings in vials containing only water.

One major (sections 5–7) and 2 minor (sections 2 and 10) root-promoting and no inhibiting zones were detected on chromatograms of ethanolic extracts of control and treated geranium cuttings for all sampling dates (Fig. 1). No significant differences were detected in rooting response of mung beans treated with the fractionated extract from control and daminozide-treated geraniums.

Hess (10, 11) reported 4 root promoting zones on chromatograms of ethanolic extracts from easily rooted juvenile Hedera helix L. Jackson and Harney (13) detected 1 active zone on the

Table 2. Rooting "units of promotion" values of geranium extracts as influenced by daminozide treatments and as determined by the mung bean rooting bioassay. "Units of promotion" were determined by totaling the no. of roots per cutting for each chromatogram section minus the number of roots per cutting on the control (average of chromatogram section 1 and 12).

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
<th>Daminozide (2500 ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>155.3 ab²</td>
<td>126.1 ab</td>
</tr>
<tr>
<td>3</td>
<td>98.7 ab</td>
<td>106.1 ab</td>
</tr>
<tr>
<td>7</td>
<td>89.2 ab</td>
<td>75.7 a</td>
</tr>
<tr>
<td>11</td>
<td>74.5 a</td>
<td>103.4 ab</td>
</tr>
<tr>
<td>15</td>
<td>119.1 ab</td>
<td>133.4 ab</td>
</tr>
<tr>
<td>19</td>
<td>163.3 ab</td>
<td>183.9 b</td>
</tr>
</tbody>
</table>

²Mean separation by Student Newman-Keul's test, 5% level. Means of 4 replications.
Fig. 1. Histograms showing activity of endogenous root promoters extracted from basal segments of control and daminozide (SADH) treated 'Irene' geraniums for 6 sampling dates, as determined by the mung bean rooting bioassay. Chromatogram sections 1 (below the origin) and 12 (above the solvent front) served as the controls. Means for a given sampling date with the same letter are not significantly different at the 1% level, by Student Newman-Keul's test. Each value represents the mean of 4 replications.

Chromatograms of lyophilized geranium tissue extracted with 70% methanol that promoted rooting of mung beans in the presence of 10 mg/liter IAA. They detected no significant rooting response when the extracts were assayed alone. In contrast, our fractionated geranium extracts promoted rooting when assayed without IAA.

Taylor and Odom (26) reported that "units of promotion," values based on the relative amounts of endogenous root promoting and inhibiting substances, were reliable indicators of rooting potential of hardwood and softwood pecan cuttings.
exposed to various preconditioning treatments prior to propagation. We found that “units of promotion” decreased through the root initiation period and then increased with root development (Table 2). Roots were visible on the geranium cuttings by the eleventh day. Even though there was no major change in the chromatogram active zones over the sampling dates (Fig. 1), at least a portion of the promoting substances were apparently metabolized during root initiation. In contrast, Zondag and Tukey (31) found that cofactor levels of 2 chrysanthemum cultivars fluctuated during the propagation period and reached a peak just prior to root initiation.

We conclude that modification of the levels of ethanolic extractable root-promoting substances as detected by the mung bean rooting bioassay is not the mechanism of action of daminozide in the promotion of adventitious root formation of geranium cuttings. The identification of the active fractions and the level of their activity in promoting Pelargonium rooting will require additional study.

**Literature Cited**


