FULVIC ACID AND ADVENTITIOUS ROOT FORMATION

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Summary—Root initiation was observed in 5-cm hypocotyl segments of beans after treatment with aqueous solutions (pH 6–7) of a soil fulvic acid and plant growth regulators. In most instances the effects were additive; although some synergism was detected with 3-indoleacetic acid and with 1-naphthaleneacetic acid, some results with 2,4-dichlorophenoxyacetic acid indicated an antagonistic trend.

The possibility that the polyphenolic fulvic acid may act in a manner relatively independent of endogenous 3-indoleacetic acid is considered.

INTRODUCTION

Although phenolic compounds of known chemical structures have been reported to modify the growth of plants, little is known about modifications caused by water-soluble soil humic substances. Previously, the authors (Schnitzer and Poapst, 1967) reported that the absorption of 750–1500 μg of fulvic acid by 5 cm bean hypocotyl segments increased root initiation by more than 300 per cent. This prolific initiation of roots suggested that other plant parts might be altered also; a point that was confirmed by the observation that fulvic acid concentrations from 500 to 4000 ppm inhibited stem elongation in peas in the presence and absence of added 3-indoleacetic acid (Poapst et al., 1970b). Thus it was indicated that the addition of fulvic acid may redirect resources within the plant and substantially influence morphological development.

Fulvic acid is a water-soluble low molecular weight humic substance widely distributed in soils and waters. It can complex di- and tri-valent metals (Schnitzer, 1969) and so influence nutrient availability to roots. Because of commercial practices and the decay of plant material, plant growth regulators such as 2,4-dichlorophenoxyacetic, 1-naphthaleneacetic, and 3-indoleacetic acids may be detected in the rhizosphere. All of these compounds are known to promote root initiation (Avery et al., 1947), which is also one of the best known physiological properties of fulvic acid. The possibility that a growth regulator and fulvic acid could interact to produce additive or nonadditive responses in rooting seemed likely; this could have practical implications in field management and in certain horticultural practices. For this reason, and also to increase our comprehension of the influence of soil organic matter fractions on morphogenesis, the combined effects of fulvic acid and growth regulator were investigated in root initiation tests using bean stem sections.

METHODS AND MATERIALS

The fulvic acid (FA) originated from the Bh horizon of the Armadale, a poorly drained podzol soil in Prince Edward Island, Canada. Methods of extraction, purification and drying, as well as a number of physical and chemical characteristics of this material have been described (Barton and Schnitzer, 1963). Chemical analyses showed 50.9% C, 3.4% H and 44.7% O; and also 9.1, 3.3, 3.6 and 3.1 m-equiv. of COOH, phenolic OH, alcoholic OH
and C=O respectively, per gram of dry ash-free material. Its number-average molecular weight was 951. The molecular formula calculated from these data was C_{24}H_{16} (COOH)_{9} \quad (OH)_{2}(CO)_{3}. The purified FA contained 0·26% ash and was completely soluble in water.

1-Naphthaleneacetic acid (NAA), 2,4-dichlorophenoxyacetic acid (2,4-D), 3-indoleacetic acid (IAA) and indole were highest reagent grade.

Root initiation was observed in 5 cm hypocotyl sections of Phaseolus vulgaris L, cv ‘Contender’ grown from seed obtained from commercial sources.

The procedure (Poapst et al., 1967), originally designed for testing phenols, was as follows: cotyledons were removed from 11-day seedlings which were grown on vermiculite in a controlled environment with continuous illumination (25°C, 90% relative humidity, 1500 m-photons, Sylvania Model No. F96T12-CW-235). Roots and etiolated stem tissue were trimmed off 5 cm below the cotyledonary node. The resulting leafy stem cuttings were allowed to absorb 0·25 ml of 0·025M NaHCO_{3} solution, either with or without FA and growth regulators, from individual vials, a process requiring less than 3 hr. The pH of the control solution was 8·4, and those of the FA solutions were 7·1, 6·6 and 5·8. Cuttings were then trimmed through the cotyledonary node and the retained 5-cm hypocotyl sections were planted vertically, node end exposed, 4·8 cm deep, in pots of Perlite previously drenched with distilled water. Pots were then overwrapped with polyethylene and held in the controlled environment described above. After 6 days combined counts of roots and root initials were made, and averages and standard errors calculated. Each sample consisted of 22 segments. Synergism (Sy) or antagonism (An) was computed using the formula described by Gorter (1969)

\[ Sy \text{ or } An = z - [(x - c) + y] \]

where \( x - c \) = no. of roots promoted by compound A
\( y - c \) = no. of roots promoted by compound B
\( z \) = no. of roots promoted by compound A + B
\( c \) = no. of roots promoted by control solution (NaHCO_{3})

When \( z = (x - c) + y \), the results were said to be additive.

Differences from this equilibration (null point) were tested for significance.

The possible effect of pH on root initiation was tested with the following solutions: distilled water, 0·025M NaHCO_{3}, and 0·01M phosphate solutions (H_{3}PO_{4}, KH_{2}PO_{4}, Na_{2} HPO_{4}), adjusted to pH 5·0, 6·0, 7·0, 8·0 and 9·0. There were no apparent differences between any of the treatments.

Capability of detecting synergistic response was tested with aqueous solutions of 5–10 ppm IAA and 50 ppm of the known synergist indole (Gorter, 1958). Tests gave \( t \) values equal to 4, or greater, with 21 degrees of freedom.

**RESULTS AND DISCUSSION**

Data from a typical experiment with each growth regulator are shown in Table I. Data show results exhibited by a large number of experiments, which were repeated several times over an 8-month period and analyzed separately. All treatments increased the numbers of adventitious roots over the controls. Synergism and antagonism, however, did not always recur at exactly the same concentrations. This was attributed to the fact that the phenomena occurred within small ranges in concentration and to intrinsic differences in seed lots.
The use of hypocotyl sections to detect synergistic response proved interesting. Conceivably the upper plant parts, especially the leaves could contain functional requisites for this response. The removal of leaves and upper stem tissues removed resources and manufacturing capacity. This obviously militated for a sharply increased specificity of action on the part of the stimulants. All previous root initiation tests for synergism reported in the literature were performed on leafy cuttings. However, it appears that the synergistic response with some stimulants at least, can be localized in leafless (hypocotyl) tissues.

<table>
<thead>
<tr>
<th>Test substances* (ppm)</th>
<th>Root count + SE</th>
<th>Test substances* (ppm)</th>
<th>Root count + SE</th>
<th>Test substances* (ppm)</th>
<th>Root count + SE</th>
<th>Interaction†</th>
</tr>
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<tbody>
<tr>
<td>FA 1500</td>
<td>6·09 ± 0·78</td>
<td>IAA 5·00</td>
<td>5·64 ± 0·67</td>
<td>FA 1500</td>
<td>6·00 ± 0·77</td>
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<td></td>
<td>10·00</td>
<td>6·36 ± 0·77</td>
<td>1500 + 5·00</td>
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<td>13·45 ± 1·31</td>
<td>Sy</td>
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<tr>
<td></td>
<td>20·00</td>
<td>6·82 ± 0·82</td>
<td>1500 + 10·00</td>
<td></td>
<td>13·50 ± 1·84</td>
<td>Sy</td>
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<tr>
<td>FA 3000</td>
<td>8·05 ± 1·03</td>
<td>NAA 0·25</td>
<td>5·55 ± 0·56</td>
<td>FA 1500</td>
<td>6·77 ± 0·64</td>
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<tr>
<td></td>
<td>1·00</td>
<td>8·27 ± 0·78</td>
<td>1500 + 1·00</td>
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<td>10·27 ± 0·87</td>
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<td></td>
<td>2·00</td>
<td>9·86 ± 1·19</td>
<td>1500 + 2·00</td>
<td></td>
<td>15·00 ± 1·71</td>
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<tr>
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<td>5·77 ± 0·60</td>
<td>1500 + 0·25</td>
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<td>8·41 ± 0·61</td>
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<td>6000</td>
<td>7·24 ± 0·55</td>
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<td>12·44 ± 1·31</td>
<td>Sy</td>
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<td>2,4-D 0·025</td>
<td>7·45 ± 0·65</td>
<td>FA 1500</td>
<td>8·86 ± 0·98</td>
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<td></td>
<td></td>
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<td>12·55 ± 1·76</td>
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<td>3·39 ± 0·30</td>
<td>2,4-D 0·0625</td>
<td>1500 + 0·0625</td>
<td>6000</td>
<td>12·41 ± 1·73</td>
<td>Add</td>
</tr>
</tbody>
</table>

* Test substances: IAA (3-indoleacetic acid); NAA (1-naphthaleneacetic acid); 2,4-D (2,4-dichlorophenoxyacetic acid); FA (fulvic acid).
† Interaction considered to be Sy (Synergistic) or An (Antagonistic) when the effect of the combined components differed from the sum of the effects of the individual components Add (additive) with a probability > 95%.
‡ The control solution contained 2100 ppm NaHCO₃.

The tendency to increase the rooting effect of FA, either additively or synergistically, by the addition of growth regulators has obvious connotations that are both favorable and unfavorable for agriculture. In addition, the data also give some insight into the nature of the rooting stimulus of FA. The compatibility of FA with applied IAA, exemplified by additive effects, suggests that such a state extends to the relatively small amounts of natural IAA in situ. It is likely that a portion of the relatively large amount of applied IAA reaches the action sites and is 'indistinguishable' from the endogenous IAA. Since the effect of applied IAA on rooting is linear, within certain limits (Luckwill, 1956; Poapst et al., 1967),
one may deduce from those tests that showed additive results that the net balance after subtracting the IAA effect is clearly identified with FA promotion. It may then follow from the above assumptions, that the polyphenolic FA has a characteristic and separate action on rooting, not mediated completely by the plant hormone: this point merits further investigation. Gorter (1969) observed a synergistic effect of phenols and attributed it to reactions which inhibit IAA-oxidase, and to reactions which deaminate tryptophan. The synergistic response in Table 1 might seem to fit such an explanation. Curiously however, the previously mentioned stem elongation tests with peas clearly showed inhibition of growth by FA in the presence and absence of added IAA (Poapst et al., 1970b). There would thus appear to be other causes for synergism, and for the root initiation stimulus in general. Possibly this low molecular weight polyphenolic compound selectively inhibit other enzymes, and thus cause a redirection of metabolism. In the case of indole, a substance which has slight rooting properties per se (Gorter, 1962), there may be difficulty in penetrating action sites, thus reducing the potential rooting properties of this compound, while the simultaneous addition of auxin with its consequent effect on permeability (Veldstra, 1951) may help to overcome this deficiency. Such an effect might apply equally well to FA. It is further considered that the permeability effect would be selective and thus NAA may also cause synergism, while 2,4-D produces antagonism.

FA on the other hand, is perhaps not very different from certain products of phenol oxidation which have been shown to be potent rooting factors when administered in alkaline solution (Poapst and Durkee, 1967; Poapst et al., 1970a), a medium which favors the persistence of free radical activity (Blois, 1955). It is noteworthy that stable free radicals have been detected in FA (Schnitzer and Skinner, 1969) and the possible role of these free radicals in root initiation is presently under investigation. It is probable that hydrophilic polymers such as FA, pass through the intra-cellular spaces, after they are taken into the hollow stelae of the bean stem. Conceivably, they may effectively charge the wall stelae and this may explain root initiation and synergistic influences.

Audus (1965) has deplored the fact that little is known about the real action of synergists. It is hoped that this study will stimulate some thought on this subject.

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REFERENCES

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