Improvement of Carrot Stands with Plant Biostimulants and Fluid Drilling

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Abstract. We determined if application of certain naturally occurring compounds would stimulate emergence, growth, and development of carrot (*Daucus carota* L.). The commercially available biostimulants Agro-Lig, Enersol (humic acids), and Ergostim (folic acid) were added at a concentration of 1.5% (w/v) to Laponite 508 (magnesium sulfate) gel used in fluid drilling. Agro-Lig, Enersol and Ergostim increased carrot emergence > 2-fold as measured by number of roots as compared to untreated seed. Number of carrots increased 50% to 75% when biostimulants were incorporated into the gel, compared to fluid-drilled seed without the biostimulants. When biostimulants were applied as a drench over untreated seeds sown conventionally, the average root weight obtained was more than twice that from untreated seeds.

Some naturally occurring non-phytotoxic compounds composed of humic, folic, and fulvic acids act as plant biostimulants and are reported to stimulate plant growth and development in a variety of crops (Armstrong, 1979; Austin et al., 1969; Bryan, 1976; Himelrick, 1983; Lee, 1976; Russell, 1961; Senn and Kingman, 1975). Fluid drilling, a technique involving sowing germinated seed in a carrier gel, may promote earlier emergence of small-seeded crops and produce a more vigorous stand of seedlings resulting in increased yields (Salter, 1978).

The biostimulant Ergostim (Montedison, New York), consisting of L-cysteine (N-acetyl thiazolidan-4-carboxylic acid) and folic (pteroylglutamic) acid, has been found to increase yield in rice, corn, and apples when used as a soil amendment and has improved plant vigor, fruit uniformity, and total yield of strawberries when used as a foliar spray (Himelrick, 1983). Dry humate powder (Agro-Lig) and liquid humate (Enersol) (American Colloid Co., Skokie, Ill.) are humic acid compounds made from Leonardite shale, an organic substance mined in North Dakota. These compounds may enhance plant growth through increased root absorption of iron complexes (Bryan, 1976; Rauthan and Schnitzer, 1981). Humates also stimulated seed germination (Linham, 1978; Russell, 1961). In Florida field trials, Agro-Lig incorporated in the soil increased yield of potatoes and soybeans and, applied as a

sidedress, increased yield of tomatoes (Bryan, 1976; Freeman, 1968).

Carrot stand establishment is difficult, partly due to limited storage reserve in the small seed. The difficulty is exacerbated when soils crust and inhibit emergence. This study was initiated in an attempt to increase carrot stand establishment through the use of three selected biostimulants in conjunction with fluid drilling.

'Camden' carrot seeds were germinated at 20C in aerated columns of water for 4 days. One treatment consisted of distilled water; a second was distilled water with the liquid biostimulant Enersol at 1.5% (v/v); a third was Ergostim at 1.5% (v/v) in distilled water.

In a preliminary study, seeds treated as above and with radicles exposed were mixed in 1.5% (w/v) Laponite 508 gel, then planted 2 cm apart and 1 cm deep, by use of a cake icing syringe, in flats of sandy loam soil following the method of Salter (1978). Untreated seeds were planted by hand. Average day/night temperature was 30/22C in the greenhouse. Seeds germinated in distilled water were mixed with the gel, and the mixture was divided into three portions: one received a 1.5% (v/v) concentration of Enersol, one received a 1.5% (v/v) concentration of Ergostim, and the remainder was left untreated. A total of 48 seeds per treatment

were planted for each of four replicates. Flats were arranged in the greenhouse in a completely random experimental design and watered daily. Emergence counts were taken 3, 6, and 9 days after planting.

Seeds treated as described for the preliminary greenhouse study were planted at the Central Crops Research Station, Clayton, N.C. in Fall 1983. Before planting, trifluralin was soil-incorporated at 0.75 kg a.i./ha for weed control. Preplant fertilization was (kg-ha⁻¹) 54N-48P-90K. The soil type was a Norfolk sandy loam. The fluid drill was a modified frame of a belt-type precision seeder (Stan-Hay, Suffolk, England), having a gel carrier and peristaltic pump added to discharge the gel. The nozzle diameter was 75 mm and ≈ 156 ml·m⁻¹ row of gel was discharged. Drained, treated seed was uniformly incorporated into the gel. The untreated seed was sown using a fixed aperture seeder (Planet Jr., Bennettsville, S.C.). Seeding rates were ≈ 60 seed/m. Ten mm of irrigation was applied three times during the first week after seeding. A randomized complete-block design was used with four replications. Root number and root weight were recorded at harvest.

In June 1984, 'Camden' carrot seeds were germinated for 3 days in columns of aerated distilled water. No biostimulants were added to the germination water. Planting and irrigation were as described for the previous fall with field seeding on 10 June. A drench of 1.5% (v/v) Enersol and water was applied to the soil immediately after planting the germinated seed. Biostimulants had been added to the gel at 1.5% (v/w) as liquids. The experimental design was a 4 × 4 Latin square. Emergence counts in three random, 30-cm segments per plot and overall plot ratings were made at 5-day intervals. Carrots were harvested on 30 Nov. and roots separated into large (marketable for processing; > 3.5 cm in diameter at the shoulder) and small (< 3.5 cm in diameter at the shoulder). Root number and weight were recorded for each plot. Statistical analysis included single degree-of-freedom comparisons.

In a Sept. 1984 planting, treatments and procedures were the same as in the Fall 1983 experiment without the Ergostim and drench treatments. A hardening treatment, which consisted of alternately soaking and drying the seed at 20C for 3 consecutive days at 12-

Fig. 1. Average number of biostimulant-treated and nontreated carrot seedlings emerging during 7 weeks. Data represent three random 30-cm sections per 6-m plot.

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Table 1. Average carrot plant number and weight per 6 m at harvest as influenced by use of the biostimulants Enersol and Ergostim in germination water or in the gel at seeding (spring planting).

Treatment	Root no. ^a			Root wt (kg)	
	Large	Small	Total	Large	Small
1. None	32	20	52	5.0	0.8
2. Gel-seeded	42	72	113	6.5	2.7
3. Enersol in gel	49	219	268	5.5	5.2
4. Agro-Lig in gel	50	83	135	6.9	2.7
5. Enersol drench	24	11	34	5.5	0.3
6. Ergotism in gel	58	89	167	6.7	2.9
Comparison	Probability				
Trt. 1 vs. others	NS	**	**	NS	**
Trt. 3. vs. Trt. 4	NS	**	**	NS	**
Trt. 2 vs. Trt. 3, 4, 6	NS	**	**	NS	NS
Trt. 3 vs. Trt. 4, 6	NS	**	**	NS	**
Trt. 4 vs. Trt. 6	NS	NS	NS	NS	NS

^aLarge = greater than 3.5 cm in diameter at shoulder; small = less than 3.5 cm in diameter at shoulder, **^{NS} Significant at $P = 0.01$ or no significant difference, respectively.

Table 2. Influence of germinating carrot seeds in solutions of the biostimulants Enersol and Agro-Lig or hardening seeds on seedling vigor and subsequent total plant numbers and root weights 62 days after planting.

Seed treatment	Seedling vigor ^a Days after seeding			Average total roots/m	
	7	14	21	No.	Wt (g)
None	1.0	3.5	4.8	18	18
Pregerminated check	7.2	8.2	8.0	33	42
Enersol	8.8	9.0	8.8	40	100
Agro-Lig	8.8	8.8	8.5	39	61
Hardened	4.5	5.0	4.8	16	21
L.S.D. _{0.05}	0.7	0.5	1.8	9	56

^aVigor rating based on a 0 to 10 scale, where 10 = most vigorous seedling development.

^bAll treatments, except the untreated seed, were gel-seeded following 3 days of germination in the treatment solutions.

hr intervals, was added. Seeds were planted as a late fall crop and visually rated for seedling vigor 7, 14, and 21 days after planting using a 0 to 10 scale, with 10 being most vigorous. A randomized complete-block design with four replications was used. Roots were harvested in late November when growth had ceased for the season. At this time, the roots were not yet of marketable size, but treatment effects on early development were evident.

Results from the preliminary greenhouse study confirmed that germination was enhanced by the biostimulants tested and by fluid drilling (data not shown). Untreated seeds had 85% germination using a standard laboratory germination test, but only a 54% final emergence count in flats of soil in the greenhouse. The soil in the flats crusted similar to soil in the field, which probably contributed to reduced seedling emergence relative to the potential germination. Germinated seed planted in gel, with or without biostimulant added to the germination water, had a minimum emergence of 82% 9 days after planting in the flats. When biostimulant was added to the germination water, the rate of seedling emergence increased for seeds planted without gel. Three days after planting, no untreated seeds had emerged, 44% of the seeds germinated in water alone had emerged, but 66% and 54%, respectively, of the seeds germinated in water plus Enersol or Ergostim had emerged. Fewer than 25% of the final number of untreated seeds had

emerged 5 days after planting; emergence was complete after 7 days. Rapid emergence can be critical in establishing carrots in the field. Seeds emerging sooner often produce more vigorous plants (Salter, 1978). Synchrony of carrot emergence also contributes to more uniform root size. Ergostim and Enersol stimulated early emergence more when included in the germination water than when incorporated in the gel (data not shown).

In the first fall field seeding, no significant stand or yield differences were found between the germinated and untreated seed, with or without biostimulants (data not shown). While the recommended radicle length for gel-seeding is 2 mm, the radicles in this test were 3 to 5 mm at the time of seeding, which may have resulted in seedling injury during planting. Radicles of seeds germinated in water with biostimulants were ≈ 2 mm longer than the seeds germinated in pure water, indicating a stimulator effect of biostimulants on radicle elongation.

The following spring, when seeds were germinated in water without biostimulants for only 3 days before planting, twice as many carrots were produced by seed germinated in water and then seeded with gel than by untreated seed (Table 1)—results similar to those obtained in the preliminary greenhouse study. Total weight of large roots was about the same, whether seeding in gel or direct-seeding without gel, possibly due to compensation by the roots for lower plant density where gel was not used. When Enersol was added

to the gel, the total number of roots was more than twice that than when germinated seed was planted without a biostimulant and more than five times greater than when untreated seed was planted. The dense plant stand resulted in more small roots in the biostimulant treatments at harvest, which was particularly marked when Enersol was incorporated in the gel. Slight overseeding of carrots is customary to compensate for poor germination and emergence. These results indicate that overseeding may be unnecessary and possibly detrimental when biostimulants are used.

Results using Agro-Lig and Ergostim in the gel were not as pronounced as with Enersol, although the total number of roots harvested was still much greater than from untreated seed or gel alone (Table 1). Agro-Lig in the gel increased total root numbers by only 19%; Ergostim showed a 78% increase; and Enersol showed a 137% increase over the gel-seeded check. The Agro-Lig powder may not have mixed as uniformly with the gel as the liquids Enersol and Ergostim, although an effort was made to mix the gels thoroughly. The better performance of the humic acid biostimulant Enersol, compared to the folic acid biostimulant Ergostim, possibly may be due to the humates forming soil complexes. Humic acids can, through the ability to form complexes with metal ions and hydrous oxides, affect the availability of nutrients to plant roots and possibly facilitate the movement of metal ions, such as iron, within the plant (Schnitzer and Poapst, 1967). Humates may also act directly by inducing exudation of auxins, amino acids, and organic phosphates from the growing seedling (Armstrong, 1979; Senn and Kingman, 1975).

Enersol applied in the gel increased the total number of carrot roots harvested, compared to untreated seed or gel-seeding of seed germinated in pure water. The single-degree-of-freedom comparisons illustrate the effectiveness of biostimulants and gel-seeding (Table 1). The total number of roots in all gel-seeded treatments was greater than in the treatment not involving gel-seeding. Treatments involving gel-seeding with a biostimulant gave results superior to gel-seeding without the addition of biostimulants. While top weights obtained the previous fall did not indicate significant differences among treatments, carrot tops from seeds treated with biostimulants were observed to be much larger and more vigorous in field plantings than those without biostimulant or seeded without the use of gel. Of the biostimulants tested, Enersol in the gel gave the greatest increase in seedling emergence and survival, as indicated by root numbers at harvest. Weights or numbers of large roots were not significantly different with use of biostimulants, possibly due to the inability of roots to develop fully under the high plant populations obtained as a result of overseeding compounded by the high percent emergence. Plots were not thinned to duplicate as nearly as possible commercial production practice.

The average number of seeds emerged in

three random, 30-cm segments of each treatment during 7 weeks are shown in Fig. 1. The greatest number of emerged seedlings was obtained when Enersol was incorporated in the gel at seeding, followed by treatments using Ergostim or Agro-Lig in the gel. Although the gel-seeded treatments lost 25% or more of the emerged seedlings, more total harvested roots were produced than in the untreated control. Overcrowding of seedlings may have contributed to the high mortality. A combination of factors, including seedling survival, stand establishment, health of the crop, and plant spacing, contribute to high carrot yields.

Similar results in carrot weights and root numbers were obtained in Fall 1984 (Table 2). Seed germinated in water and seeded in gel again produced about twice as many carrots as using raw, untreated seed. Seeds germinated with biostimulants and then gel-seeded produced $\approx 16\%$ more carrots than gel-seeded without a biostimulant. The use of biostimulants also resulted in greater average root weights compared to when no biostimulant was used.

In contrast to other studies (Austin et al., 1969), seeds subjected to alternate imbibition and then drying (hardening) produced even fewer roots than seeds left untreated. Untreated and hardened seed did not differ significantly in average weight, total number of roots produced, or in plant vigor 21 days after seeding (Table 2). Seedling vigor was increased significantly by hardening the seed relative to untreated seed, but only up to 14 days after seeding. In addition, either gel-seeded or biostimulant-treated seedlings were much more vigorous than untreated seed. There were highly significant differences in seedling emergence when untreated seed was compared to gel treatments and when use of biostimulants was compared to untreated seed (Table 2). Enersol and Agro-Lig treatments did not differ in the total number of roots harvested, although roots from treatments receiving Enersol were heavier.

Results of this study indicate gel-seeding combined with biostimulants can have a beneficial effect on carrot stand establishment and root growth, especially under stressful environmental conditions. Overseeding may be unnecessary when biostimulants are used due to the increased seedling emergence.

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The Use of Vesicular-Arbuscular Mycorrhizae in Boston Fern Production: I. Effects of Peat-based Mixes

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Abstract. The horticultural Boston fern [*Nephrolepis exaltata* (L.) Schott cv. Verona] was micropropagated in vitro using commercial techniques. Rooted plantlets were transferred into pots containing one of three test substrates made of peat and vermiculite and subsequently inoculated with one of two species of *Glomus*. Survival of uninoculated control plants growing on a black peat-based mix was less than that on a brown peat-based mix. Vesicular-arbuscular mycorrhizal (VAM) inoculation significantly increased survival on the former, but not the latter, substrate. The growth of roots was enhanced in brown peatmoss, but VAM colonization was faster with black peatmoss. Compared to uninoculated controls growing under the same fertilization regime, inoculated plants had significantly higher frond P and N concentration and also showed better frond and root growth. On a growth-increment basis, our results suggested that the brown peat-based mixed was more suitable for fungal activity and fern growth.

Beneficial interactions between vesicular-arbuscular mycorrhizal (VAM) fungi and various woody ornamental plants (Nemec, 1987) or horticultural crops (Menge, 1983) are, in general, well-documented. Most of the experiments supporting these conclusions have been conducted in greenhouses

using pot cultures and sterilized soils. The use of mineral-type substrates was often preferred over organic substrates (Dehne and Backhauss, 1986; Plenchette et al., 1982). We found only a few investigations dealing with the potential use of peat-based substrates for VAM formation (Biermann and Linderman, 1983a; Biermann and Linderman, 1983b; Caron and Parent, 1988; Graham and Timmer, 1984; Graham and Timmer, 1985; Johnson and Hummel, 1986; Parent and Caron, 1987).

Little is known about the response of ferns to VAM inoculation (Cooper, 1975; Cooper, 1977; Stamps and Johnson, 1984), even though they are often mycorrhizal in the field (Berth and Kendrick, 1982; Boullard, 1957; Cooper, 1975). The VAM inoculation of ferns has been successful in various steam-sterilized mineral soils that contain organic inclusions (Cooper, 1975) and in a steam-sterilized potting medium containing equal parts of sand, peat, and perlite (Stamps and

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