

The Use of Organic Biostimulants to Reduce Fertilizer Use, Increase Stress Resistance, and Promote Growth

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Abstract-High quality organic biostimulants can stimulate plant growth, providing excellent growth and yield, with up to a 50% reduction in fertilizer use. These compounds are essentially, a stress vitamin mix for plants. They were developed for use in tissue culture of refractive or sensitive tissue explants. Research and practice has shown that these compounds can work under soil conditions, as well as in tissue culture. They work best on plants under water, cold, nutrient, and/or biotic stress. In well watered and heavily fertilized plants little effect of these compounds may be manifested.

The carrier for most biostimulants is a mix of humic substances and marine algal extracts; the carrier itself has slight activity as an organic biostimulant, however, the most active components are the stress vitamins. Ascorbate is the most active substance, followed by casein hydrolysate. In addition to acting as an antioxidant, ascorbate appears to promote xylem formation. Under proper conditions, organic biostimulants promote nutrient uptake, acting as both a phosphorus, nitrogen, potassium and micronutrient pump. Thus, seedlings treated with them develop better vascular systems (in the case of pines-more and larger diameter tracheids and thicker walls) to transport water and nutrients, and are more efficient in nutrient uptake due to a larger root system. Recent results in our laboratory suggest that the biostimulant treated plants are more resistant to insects, possibly because they are more vigorous, and can produce more of the energetically expensive defensive compounds like polyphenols.

Organic biostimulants, a by-product of biotechnology research, can improve field competitiveness, nutrient use efficiency, and stress resistance of plants (see Berlyn and Beck 1980; Berlyn et al. 1990; Russo and Berlyn 1990; 1992; Russo and Berlyn 1994). Because of these properties, they can decrease reliance on chemicals and tillage, and improve plant health. We do not claim that this approach can completely replace the use of agricultural chemicals. It is a truism that where agricultural chemicals are used, people have enough to eat, and where they are not used, people do not. The modern agricultural practices of developed countries have been highly successful in food production. However, these systems rely on high mechanization, high fertilizer, high herbicide, high fungicide, high pesticide, and high use of fossil fuel energy. Any alternative system that can decrease reliance on fossil fuels and minimize the disturbance of the ecosystem, promotes sustainability and helps preserve the food and fiber supply for future generations. This is increasingly important in view of the limited reserves of fossil fuels that are so necessary in the current high energy agricultural, forestry, and horticultural systems. Our approach is to provide one type of alternative that has less reliance on xenobiotic chemical inputs, and more reliance on the inherent physiological capacities of plants and the other organisms of the rhizosphere. This methodology can be used in urban and suburban systems, as well as in nurseries, agriculture and forested ecosystems.

Roots™ is an organic biostimulant developed by this laboratory. It stimulates plant growth,

providing optimum yield with up to a 50% reduction in fertilizer use. Essentially, Roots™ is a stress complex for plants. It utilizes many of the organic supplement components developed for use in biotechnology over the past 40 years. These components are used in standard plant tissue culture media where the object is to stimulate fragile tissues and cells to grow in an in vitro (foreign) environment that is stressful to them. These biostimulants alleviate some of the stress, and permit these explants to grow and differentiate depending on the other constituents of the tissue culture media. In the 1970's, the senior author experimented with using these same supplements for stress alleviation in the soil environment. Roots™ and its derivatives are the current result of this research. It is based on long experience in plant tissue culture (cf. Berlyn 1962). Despite their potential autotrophic capacity, plants are seldom situated in optimal conditions where such capacity can be fully realized. For example, when isolated plant roots are grown in tissue culture, they have to be supplied with B-vitamins because roots do not synthesize the B-vitamins they need for growth. In the intact plant, roots get most, if not all, their 13 vitamins from the leaves. In a well watered and fertilized plant growing in a growth room or greenhouse at optimal temperatures, the leaves are able to supply all the B-vitamins (and other substances) the roots need, but under the multiple stresses usually present in the field, this may not be the case. In such situations we have demonstrated that organic supplements improve plant health (Russo and Berlyn 1990). It is difficult to mimic these multiple stress environments in greenhouses or growth rooms and, in general, potted plant environments are quite different from those in the field. Nevertheless, we have found field data to show fairly good correspondence with our tissue culture, greenhouse, and growth room results over the past decade.

The main active ingredients in the current Roots™ formulae are ascorbic acid (vitamin C), casein hydrolysate, myo-inositol and alpha tocopherol (vitamin E), and thiamin. These substances are combined in an aqueous carrier of humic acid and marine algae extracts. The carrier itself has slight activity as an organic biostimulant. However, the stress vitamin supplements are far more active, even when used alone (Cho and Berlyn, unpublished). The ascorbate is the most active substance followed by casein hydrolysate. In addition to acting as an antioxidant, especially in the glutathione-ascorbate oxidation reduction cycle in the chloroplasts, ascorbate also promotes xylem formation. Roots™ promotes nutrient uptake, acting as both a phosphorus, nitrogen, potassium and micronutrient pump (Russo 1990). Seedlings treated with Roots generally develop better vascular systems (in the case of pines- larger diameter tracheids, larger lumen diameter and thicker walls [under some conditions]) to transport water and nutrients, and are more efficient in nutrient uptake due to a larger root system.

Although the increased efficiency of nutrient uptake might be expected to increase susceptibility to insects, this does not appear to be the case. Recent results in our laboratory suggest just the opposite. Other studies have corroborated this effect and demonstrated that vitamin C and E are active in insect deterrence (Neupane and Norris 1991). The fact that the biostimulant treated plants are more vigorous may enable them to produce more of the energetically expensive defensive compounds like polyphenols. Results in our laboratory have suggested that this is indeed the case for oaks and eastern hemlock (Sivaramakrishnan et al. 1996). This work involves a comparison of the feeding effects of two important insect pests, the spruce moth *Lymantria dispar* L. (Lepidoptera: Lymantriidae) and the hemlock

woolly adelgid (*Adelges tsugae* Armand, Homoptera: Adelgidae) on white oak *Quercus alba* L. and eastern hemlock *Tsuga canadensis* (L.) (Carr.) respectively. The hypothesis is: (1) insect feeding alters plant anatomy and physiology, leading to a loss of tree health and vigor, and directly or indirectly to death in extreme cases; and (2) organic biostimulants can increase host plant resistance to insect attack by improving health and vigor through improved cell structure and increased production of secondary compounds, such as polyphenols (Sivaramakrishnan, et al. 1996).

We have used a modified solution of Roots2™, and mineral nitrogen to create populations of trees of varying degrees of plant health and vigor by varying nutrient, herbivory and defoliation levels. Anatomical and physiological measurements were made to determine the effect of the different treatments. Physiological status is being determined by measurements of photosynthesis, leaf chlorophyll, chlorophyll fluorescence, total phenolics, starch, and carbohydrates, as well as by measurements of growth and plant leaf area. Anatomical changes (cell numbers, cell wall thickness, lumen and tracheid diameters) are being measured using image analysis in conjunction with histological techniques. Secondary chemistry has been measured using the Folin-Denis test. Response of gypsy moth larvae to the experimental treatments is being measured with feeding tests on the leaves. In the case of hemlock fecundity of the hemlock woolly adelgid on the treated and control plants, it is being used to measure the impacts of the treatments on host resistance.

In a preliminary study to measure the effect of organic biostimulants on gypsy moth feeding on red oak seedlings, we found that while no significant reduction in feeding was observed, total phenolics in red oak leaves were found to be elevated in foliage treated with organic biostimulants and fed on by gypsy moth larvae. The results were highly significant (Figure 1). Higher levels of total phenolics in oak leaves fed on by gypsy moth larvae have been reported by other workers, and these are negatively correlated with larval growth. In a study conducted on infestation levels of the hemlock woolly adelgid, we found that infestation levels were significantly lower on trees treated with organic biostimulant (Figure 2). This reduction of infestation occurred despite the fact that the trees treated with organic biostimulants grew significantly more in both height and diameter.

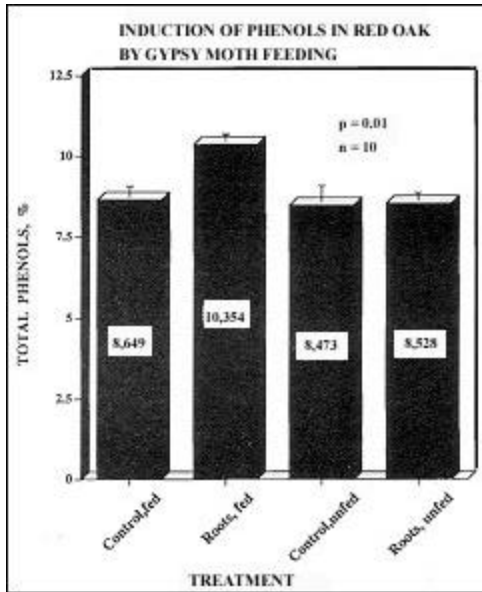


Figure 1. Induction of phenols in red oak by gypsy moth feeding.

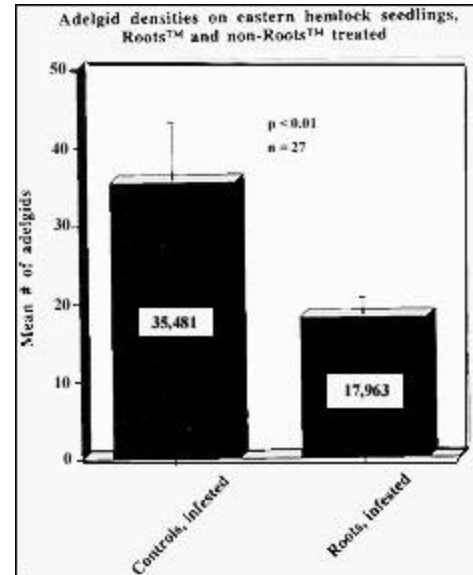


Figure 2. Hemlock woolly adelgid densities on seedlings treated with an organic biostimulant as compared to control.

Over the past decade, the various versions of the biostimulant have been tested on many species, and it has been beneficial in almost all these tests which included pines, beans, peas, lettuce, maples, radishes, oaks, tomatoes, alders, coffee, *Gliricidia*, *Leucaena*, orchids, *Hypoestes*, marigolds, *Araucaria*, and many others. In coffee we were able to increase photosynthesis and obtain better growth at half fertilization (as recommended by the Costa Rica Coffee Board). In addition, phosphorus, nitrogen and many other nutrients were enhanced in the biostimulant treated plants even with the 50% reduction in fertilization (Russo, 1990).

Nitrogen fixation was stimulated in *Alnus* (Berlyn and Russo 1990). *Roots2™* was also beneficial in micropropagation using tissue culture systems. In a recent test, *Roots™* with casein hydrolysate far surpassed superabsorbent gels alone in tomato yield. However, the highest yield occurred in plants treated with a combination of *Roots2™* and a polyacrylamide superabsorbent gel (Figure 3). We are experimenting with these superabsorbent gels, trying to find the most environmentally benign gel for use with *Roots™*. These gels have potential benefits in arid zone agroforestry, agriculture, and urban horticulture. They may also be useful in reducing irrigation frequency, and even conserving watering in home gardens and lawns, i.e., wherever water is in limited supply.

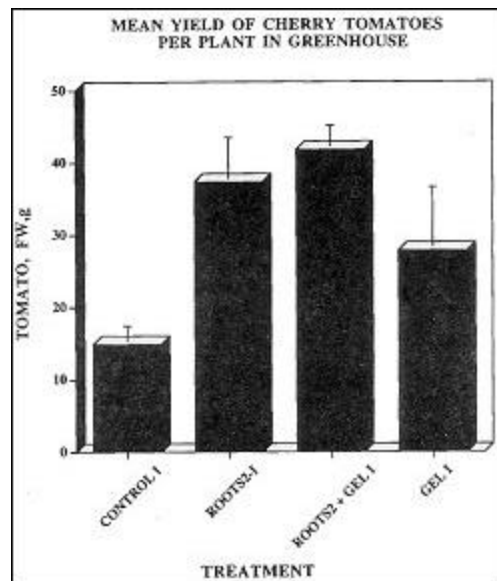


Figure 3. Mean fresh weight yields of tomatoes treated with: control, organic

biostimulant (Roots), organic biostimulant plus polyacrylamide superabsorbent gel, and polyacrylamide superabsorbent gel alone.

Roots™ promotes nutrient turnover in soils (reduces thatch in turf), and in another recent test, enhanced the rate of metabolism of ¹⁴C-labeled benzene by soil microorganisms (Liptak and Berlyn, unpublished data). We are always trying to improve the product and/or develop new ones. We are also interested in testing its effects in different species, systems, and conditions.

Organic biostimulants improve tree health and vigor in a number of ways. In pines we found Roots™ promoted a large increase in xylem cell number, cell wall thickness, and lumen diameter. Water flow is proportional to the fourth power of lumen diameter. Thus, a feedback loop is set up whereby the increased root biomass takes up more moisture, and increased hydraulic conductivity of the xylem means that the leaves are provided with reduced water stress. This coupled with enhanced antioxidant capabilities would lead to greater carbon assimilation. This additional photosynthate is allocated to enhancing the roots and the transport system itself, providing even more water and nutrients to the leaves, which in turn leads to greater carbon assimilation. Surplus carbon can be allocated to reserve compartments, and with greater carbon reserves, the trees are able to produce more secondary compounds, such as polyphenols, that may deter herbivory. As this loop cycles around, you get larger roots and stems, and more secondary compounds, as additional carbon becomes available for these functions. This permits a greater harvest of soil resources, and the loop recycles. This is a modified version of the super plant hypothesis (see Arnone 1988).

The biostimulant does not function as a growth regulator in the usual sense. No synthetic plant hormones are added. Roots™ does contain marine algal extract and marine algae are known to contain natural cytokinins. However, the cytokinin content of the working solution recommended for Roots™ (1 % of the master solution, 10 ml of Roots™ per liter of water) contains at most 0.1 mg/L of cytokinin. This is probably too low to exert a biological effect when added to soil, although it is at the low end of activity in tissue culture and hydroponics. In these systems the recommended concentration of Roots is 0.1% (one milliliter of the master solution per liter of water). We have also tested the ingredients separately and found the vitamin mix and casein hydrolysate to be the main active components.

In many studies, (Coffee, Alnus, Pinus, grasses, Populus) the rate of development was greatly increased as evidenced by the greater number of leaves formed in a given time period (Figure 4). The time between formation of successive leaves is termed the plastochron. This is a useful concept because plant development is more directly keyed to the plastochron than it is to chronological time. The primary vascular system consists of stem bundles (axial, cauline) and the leaf traces that diverge from them and enter the leaves. These complexes are termed sympodia, and in some species, they develop free in the ground tissue (each leaf is connected to only one axial bundle) and in others they are closed because each leaf is connected to two sympodia. The leaf traces begin development as procambial strands before the leaf primordia to which they will be connected are formed by the shoot apical meristem.

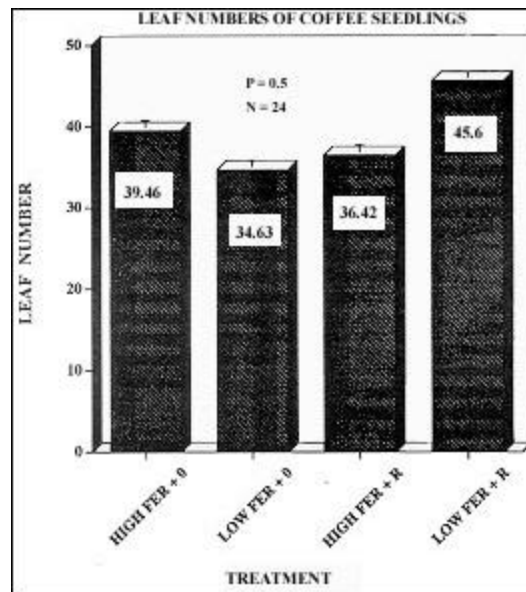


Figure 4. Leaf numbers produced by coffee seedlings over a given time period in response to treatment: HIGH FER=recommended fertilizer rate, LOW FER=half the fertilizer in the HIGH FER treatment, R=plants treated with Roots™, and 0=control plants (Russo 1990).

At a predictable point in space and developmental time, a vascular cambium is formed, and initiates the secondary plant body in woody plants. Many plants not ordinarily classified as woody plants, such as soybeans, also form a cambium and some secondary tissue. Thus, when the plastochron is shortened by treatment with Roots™, many aspects of development, in addition to the increased number or rate of formation of leaves, are accelerated. However, leaves are the collectors of solar energy and the transducers of solar energy into chemical bonds. Certain useful aggregations of these bonds we humans call food. Thus, making and maturing leaves faster and making more leaves increases the carbon fixing capacity of the plant and the planet.

It has been suggested that biostimulants induce an increase in membrane permeability. Such an increase could arise from several sources. There could be an increase in permeases, specific enzyme protein carrier molecules in the membrane, or an increase in activity of existing carriers. Increase in membrane permeability can also account for increases in cold resistance because increased permeability means less resistance to flow to extracellular ice. As temperatures approach freezing, the viscosity of water increases, and membrane permeability, decreases and flow to extracellular ice is decreased. If temperature change is sufficiently rapid, intracellular ice can form and result in protoplasmic damage in the symplast. If biostimulants can maintain higher membrane permeability at lower temperatures and increase wall storage space, they could decrease cold damage. If biostimulant treatment could effect a 2 degree protection over untreated plants, it would be of considerable importance for citrus and wheat growers.

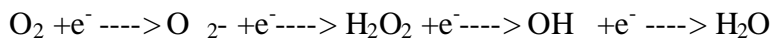
ANTIOXIDANTS IN GENERAL

Several of the ingredients of Roots™ are antioxidants, and additional antioxidants are being tested for possible use in the product. Plants have special requirements for antioxidant activity because of the nature of the photosynthetic process. Under high light fluxes more energy absorbed by chlorophyll than can be utilized in photosynthesis. Under these conditions the excess energy may lead to the formation of toxic free radicals. Plants use antioxidants to detoxify these by-products, and thus prevent severe consequences. Under sufficient stress, plants cannot produce the optimum amount of antioxidants and exogenously applied antioxidants like ascorbate can help in the detoxification process (Berlyn and Beck 1980).

Free radicals are energetic molecules that have one or several impaired electrons. They are formed when excess energy is transferred to the abundant oxygen molecules in the chloroplast that are present as a result of the splitting of water and evolution of oxygen during the process of photosynthesis. Free radicals are a serious problem for plant growth and development because they are extremely destructive to chlorophyll and other essential components of the chloroplast, such as: proteins, nucleic acids, lipids, and membranes. While the potential for free radical formation is greatest in the light absorbing and oxygen rich chloroplasts, free radicals can also be formed in many parts of the living cells, such as the mitochondria. These organelles function as an oxygen sink since oxygen is consumed in respiration at these sites.

However, photosynthesis is the most important source of electrons for free radical formation. In photosystem one (PS I) there are electron acceptors such as ferredoxin. When photoelectron flow is blocked due to lack of oxidized acceptors in the photoelectron transport chain, or due to lack of NADP⁺, to reduce to NADPH, the electrons can still be transferred to

oxygen in the illuminated chloroplast. The superoxide radical, O₂^{•-} is one of the destructive free radicals formed in this process. This oxygen derivative can be detoxified through the mediation of superoxide dismutase (SOD) leading (through additional electron donation) to formation of hydrogen peroxide. In turn, this can be reduced to water by the ascorbate/glutathione cycle. If not detoxified, hydrogen peroxide, upon additional electron donation, can lead to the formation of the hydroxyl free radical. The latter has no known biochemical detoxification system in the plant, and is extremely toxic, but further donation of an electron can reduce it to water.



Singlet oxygen is also very destructive and can be formed directly in photosynthesis. After chlorophyll is excited by absorption of a photon, it may decay to the triplet state before returning to the ground state. This triplet state of chlorophyll is relatively long lived, and this increases its probability of donating electrons to oxygen forming the toxic singlet oxygen, ¹O*₂. The toxic reactions of these activated oxygen species is termed photocatalytic destruction, photooxidation, oxidative photodestruction, or photodynamic effects. They can

lead to chain reactions that destroy biological membranes. In the face of all this potential destruction, plants in general thrive and function. This is due to antioxidant pathways. Most cells have an organelle, the peroxisome, that contains the enzyme catalase that can detoxify hydrogen peroxide to water and normal oxygen. However, the chloroplast does not contain catalase, but instead detoxifies hydrogen peroxide through the ascorbic acid glutathione chain. It does this at the expense of NADPH, and is an energy requiring reaction. However, this reaction sequence prevents the formation of the hydroxyl free radical (see Alscher and Hess 1992).

Other important antioxidants in the plant are atocopherol (vitamin E) and the carotenoids. Betahydroxy-beta methyl butyric acid (HMB) is another antioxidant that is produced in very small quantities by plants, but its physiological role in detoxification, if any, is not clear as yet. Carotenoids are extremely important in plant growth and development. They absorb harmful UV light, and also in the blue wavelengths. The action curve of photosynthesis of intact leaves shows much more activity in the wavelengths between the blue and red peaks, and a part of this is thought to be due to resonance transfer of light energy from carotenoids to chlorophyll. A key to its protective function may be found in its distribution in the chloroplast. It is high concentration in the chloroplast envelope where it is in a position to protect the chloroplast (Anderson and Beardall 1991; Mohr and Schopfer 1995). It also functions in the thylacoids by the radiationless deexcitation of excess energy absorbed by antennae chlorophyll. The xanthophyll chain of violaxanthin, antheroxanthin, zeaxanthin have been shown to be especially important in this regard (see Salisbury and Ross 1992; Mohr and Schopfer 1995). Any stress whether natural (water, nutrient, excessive light, temperature) or anthropogenic (acid rain, sulfur dioxide, ozone) increases the production of toxic oxygen derivatives (Foyer et al. 1994). The proper use of organic biostimulants provides a means of ameliorating the stress, or its consequences, and therefore improving plant health.

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