Why Nutrition Is Important in Rooting Native Azaleas—Part 2

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As we continue our discussion of factors affecting rooting success with native azaleas, I think it is important to consider several aspects. In native azaleas, adventitious roots can be defined as roots that develop from shoots and stems. Rooting is a key step in the vegetative propagation of unique varieties of native azaleas, and difficulty with rooting often results in commercial nurseries having to use more costly propagation methods such as tissue culture. It is clear from our current research that adventitious root formation is a complex process known to be affected by factors such as plant hormones, enzyme co-factors, phenolic compounds, light, carbohydrate accumulation, nutrients, and other environmental conditions. I would ask that we also consider another factor—genetics.

While there has been considerable work completed on the physiology of adventitious root formation, the genetic and molecular aspects involved have not been adequately addressed and are poorly understood. In the first installment of this paper we addressed the possible role of phenolic compounds and their role in adventitious rooting. Now we will examine some of the other factors affecting rooting. We will leave light, genetics, and environment for a later essay and focus on hormones, co-factors, carbohydrate accumulation, and nutrients.

We will begin with a quick review of plant hormones. Plant hormones are chemicals that regulate and affect all aspects of plant growth and development. Plant hormones can be produced and act locally or be transported throughout the plant using the vascular tissue and diffusion.

Just like with human cells, not all plant cells respond to hormones; the cell must have a receptor for the hormone in order for it to respond. Plant cells typically respond at specifically defined points in their growth cycle. Plants require the hormones at certain locations within the plant during these times to control growth and development. Because of this, plants tend to produce and sequester hormones at sites of active cell division. The two most active sites of cell division in plants are within shoot and root apical meristems (SAMs and RAMs).

The amount of hormone required for plant responses tends to be extremely low, sometimes just a few parts per million per liter can have the desired effect. Due to the low concentrations needed, studying plant hormones, their effects, and interactions has been extremely difficult. Only since the late 1970's and early 1980's have plant physiologists begun to piece together their overall effects and relationships. What is clear is that plant hormones have a significant effect on gene expression and transcription on many levels, controlling cell division and growth.

While there are some lesser known plant hormones, it is generally accepted that there are five major classes of plant hormones. Each class can be grouped together based on their chemical structure and similar effect. There are a few other plant growth regulators which can't be placed into one of the five hormone classes. These exist naturally and include chemicals that can inhibit plant growth or interrupt physiological processes within plants. Within each hormone class there are chemicals that stimulate and inhibit functions. Many of these hormones have complex interactions with each other. A few of the interactions fall into permissiveness of synergism, while others are clearly antagonistic. The five major classes are:

Abscisic Acid or ABA

This class is composed of one chemical compound typically produced in plant leaves when the plants are under stress. In general, it acts as an inhibitory compound that affects bud growth as well as seed and bud dormancy. It causes bud dormancy and influences the last set of leaves to form into protective bud covers. It also accumulates within seeds preventing them from germinating before winter.

ABA is broken down by cold temperatures, and is one of the chemicals responsible for dormancy in seeds. Scientists are still working out the complex interactions between ABA and other plant hormones. It is not known if ABA has any effect on the production of adventitious root formation.

Auxins

Auxins are a class composed of compounds that stimulate cell enlargement, bud formation, and root initiation. Auxins also interact with other hormones and together with cytokinins, control the growth of stems, roots, flowers, and fruits. They were the first class of growth regulators discovered.

Auxin levels decrease in light and increase in dark. They stimulate cambium cells to divide and inhibit bud growth affecting apical dominance. In this paper we are primarily concerned with their ability to promote lateral and adventitious root formation. Auxins, especially 1-Naphthaleneacetic acid (NAA) and Indole-3-butyric acid (IBA), are commonly used in the horticulture industry to stimulate root growth in cuttings. The most common auxin found occurring naturally in plants is indoleacetic acid or IAA. It is not clear if IBA is converted to IAA in the plant. Some natural IBA has been detected in a few plant species. If IBA is not converted, then it may stimulate the conversion of some pre-cursor molecule into IAA.

Cytokinins or CKs

CKs ae a group of chemicals that influence cell division and shoot formation. They were called kinins when first isolated from yeast cells. They help delay senescence or the aging of tissues, are responsible for mediating auxin transport throughout the plant, and affect internodal length and leaf growth. They have a highly-synergistic effect with auxins, and the ratios of these two plant hormones affect most major growth periods during a plant's lifetime. Cytokinins counter the apical dominance induced by auxins; and in, conjunction with ethylene, promote abscission of leaves, flowers, and fruits.

Ethylene

Ethylene is a gas that forms from the breakdown of methionine, which is in all cells. Ethylene is virtually insoluble in water and therefore usually does not accumulate within the cell. As a plant hormone its effectiveness depends on its rate of production versus its rate of escaping into the atmosphere. Ethylene is produced at a faster rate in rapidly-growing and dividing cells, especially in darkness. New growth and newly germinated seedlings produce more ethylene than can escape the plant, which leads to elevated amounts of ethylene, inhibiting leaf expansion. As the new shoot is exposed to light, reactions by photochrome in the plant's cells produce signals causing ethylene production to decrease, allowing leaf expansion.

While the effects on fruit ripening in response to ethylene are well known, its other effects are less well known. Studies seem to indicate that ethylene affects stem diameter and plant height. When plants are subjected to lateral stress, greater ethylene production occurs, resulting in thicker stems. Ethylene also has been shown to regulate other hormones, especially abscisic acid.

Gibberellins or GAs

Gibberellins include a large range of chemicals that are produced naturally within plants and by fungi. They were first discovered when Japanese researchers noticed a chemical produced by a fungus called *Gibberella fujikuroi* that produced abnormal growth in rice plants. They play a major role in seed germination, producing an enzyme that affects food production for new cell growth. They promote flowering, cellular division, and growth after germination. Gibberellins also reverse the inhibition of shoot growth and dormancy induced by ABA.

Other identified plant growth regulators include:

- Brassinolides—plant steroids chemically similar to animal steroid hormones. First isolated from pollen of the mustard family and extensively studied in *Arabidopsis*. They promote cell elongation and cell division, differentiation of xylem tissues, and inhibit leaf abscission. Plants found deficient in brassinolides suffer from dwarfism.

Element	Form Available to Plants
Macronutrients	
Carbon	CO_2
Oxygen	CO_2
Hydrogen	H_2O
Nitrogen	NO_3^-, NH_4^+
Sulfur	SO_4^{2-}
Phosphorus	$H_2PO_4^-$, HPO_4^{2-}
Potassium	K^+
Calcium	Ca^{2+}
Magnesium	Mg^{2+}
Micronutrients	
Chlorine	Cl^-
Iron	Fe^{3+}, Fe^{2+}
Boron	$H_2BO_3^-$
Manganese	Mn^{2+}
Zinc	Zn^{2+}
Copper	Cu^+, Cu^{2+}
Molybdenum	MoO_4^{2-}
Nickel	Ni^{2+}

▲ Figure 1

- Salicylic acid—activates genes that assist in the defense against pathogenic invaders.

- Jasmonates—produced from fatty acids and seem to promote the production of defense proteins that are used to fend off invading organisms. They are believed to also have a role in seed germination, the storage of protein in seeds, and seem to affect root growth.

- Signalling peptides

- Systemin

When it comes to carbohydrate accumulation in plant stems and its role in rooting, there seems to be some disagreement. Nevertheless, it has been generally observed that stock plants that have a high-carbohydrate-to-low-nitrogen ratio root with greater success.

There are several methods used to produce the high carbohydrate/low nitrogen balance in stock plants. One method is to withhold nitrogen fertilizer while growing stock plants in full sunlight. Root pruning and girdling shoots with rubber bands to block downward translocation are also effective.

One of the newest methods involves the use of chemical growth retardants such as Cycocel, CEPA, or a combination of both. Such pre-treatments cause plant growth to cease and carbohydrate accumulation to increase.

Our research indicates that shoot selection is also important. Lateral shoots in which growth has stopped and carbohydrates have been accumulating are better than rapidly growing terminal shoots. We collect our cuttings from shoots toward the base and middle of the plant, using only the basal portion of the shoot, as opposed to the terminal. The basal and middle portions of the shoot contain more carbohydrates and less nitrogen than the stem tip.

Lastly, we come to nutrients and co-factors. In Figure 1 I have listed the essential mineral nutrients and the forms in which they are available to the plant. A nutrient is considered to be essential if its absence prevents a plant from completing its life cycle or it has a clear physiological role. They are divided into macro- and micro-nutrients depending on whether they are required in relatively large or small amounts. Pay particular attention to the last three elements listed under macronutrients: potassium, calcium, and magnesium. They are all positively charged ions and are known to serve as co-factors in energy-producing cellular pathways.

Potassium is a co-factor for more than 40 enzymes. It is the major cation for establishing plant cell turgor pressure and instrumental in controlling cell membrane potential. Calcium contributes to cell wall and cell membrane structure and acts in signal transduction. Magnesium is a co-factor in enzymes required for phosphate transfers (energy production) and a component of chlorophyll.

Beginning in the spring of 2007, rooting experiments were performed using pre-soak solutions of four different salts and water. Thirty-six cuttings were used in each treatment. All cuttings were taken from May 10 to May 15 from clones of a *Rhododendron austrinum* selection. The terminal sections and all but two or three leaves were removed from each 4- to 6-inch cutting. The basal stems were wounded using a sharp potato peeler. Each group was placed in a one gallon zip-lock bag and subjected to a refrigerated 12-hour presoak in one of the solutions. After pretreatment each cutting was quick dipped in a solution of 3000 PPM KIBA, 1000 PPM NA and 1% DMSO. All cuttings were stuck in 606 trays filled with a mix of 50 percent aged pine bark and 50 percent coarse perlite. All five trays were placed on a 70 degree heat mat under mist that came on for five seconds every 20 minutes between 9 a.m. and 4 p.m.

The results from this experiment are given in the table below:

Pre-Treatment	No. Stuck	No. Rooted	Percentage
0.05M MgCl	36	36	100
0.05M CaCl	36	30	83.3
0.05M KCl	36	28	77.7
0.05M NaCl	36	25	69.4
Distilled H20	36	27	75.0

In 2008 a similar experiment was conducted using pre-treatments of MgCl and distilled water on four different species. *R. calendulaceum*, *R. arborescens*, *R. periclymenoides*, and *R. atlanticum*. In every case, the plants pre-treated with MgCl rooted in higher percentages, with the *R. arborescens* and *R. atlanticum* rooting better than 90 percent. Unfortunately the aftercare of these plants was overlooked and all 288 plants perished when the cooling system for the greenhouse failed and they went un-watered for six days.

Discussion

If you have a system that works for you, I would encourage you to try pre-treating your cuttings with a soak in a cold solution of MgCl. Then use your normal protocol and see if you have better success.

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