Chemicals That Regulate Plants

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Five key plant hormones have commercially valuable uses in horticulture, and there is promise of more to come

Plants, like other living things, have complex internal mechanisms through which they coordinate their growth and respond effectively to changes in their surroundings. A fundamental concept of botany states that fluctuations in the amounts of a few key chemicals known as plant hormones, or "phytohormones," regulate practically every aspect of plants' functioning. Since the discovery of the first phytohormone nearly fifty years ago, botanists have learned much about these internal regulators, and the knowledge they have gained has been exploited successfully to develop practical uses for phytohormones in horticulture. Without question, research on phytohormones has already paid for itself. Moreover, scientists working on phytohormones believe that the prospects of finding new ways of manipulating plants with these chemicals are especially encouraging.

The Five Kinds of Phytohormones

Five distinct categories of phytohormones are recognized, each of which has characteristic molecular structures and physiological roles in plants: auxin, ethylene, cytokinin, gibberellin, and abscisic acid.

Auxin, the first phytohormone discovered, is probably the best understood of them all. The major form of auxin in plants is the chemical indole-3-acetic acid (IAA), which has been implicated in a variety of phenomena, including plant "architecture," the bending response to light, flower formation, leaf and fruit drop, and fruit maturation.

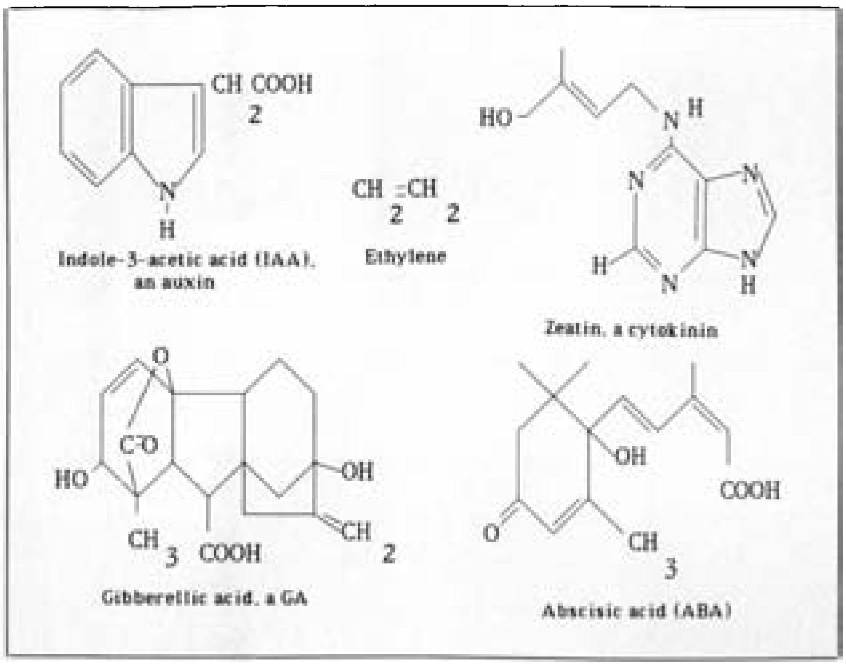
Ethylene, structurally the simplest hormone found in any living thing, regulates fruit drop, flowering, fruit ripening, and the death (senescence) of plant parts. After auxin, ethylene is the most important phytohormone in commercial practice.

The phytohormone cytokinin, on the other hand, currently is of limited practical use, even though tissue culture would be impossible without it. Because of its crucial role in tissue culture, cytokinin undoubtedly will assume increasing importance as advances are made in biotechnology. Cytokinin controls seed germination, plant architecture, the movement of gases between the interior of leaves and the atmosphere, fruit development, senescence, and fruit drop.

Gibberellin (GA), of which over sixty different chemical variants are known, regulates seed germination, stem growth, flowering, and fruit development. Many so-called "dwarf," or stunted, plants are actually defective in their abilities to produce GA.

Abscisic acid (ABA), the last phytohormone to be discovered, is responsible for seed dormancy. It also regulates the growth of roots and the exchange of gases between leaves and the atmosphere. To date, there are no important practical applications for abscisic acid, although the prospects are good that it will become a valuable chemical for increasing the capacity of plants to withstand drought, since it influences the amount of water that plants lose from their leaves.

In spite of all we know about phytohormones, an obvious question comes to mind: "Are there



Representative chemical structures of the five currently known classes of plant hormones Prospects for discovering additional kinds are considered to be especially good

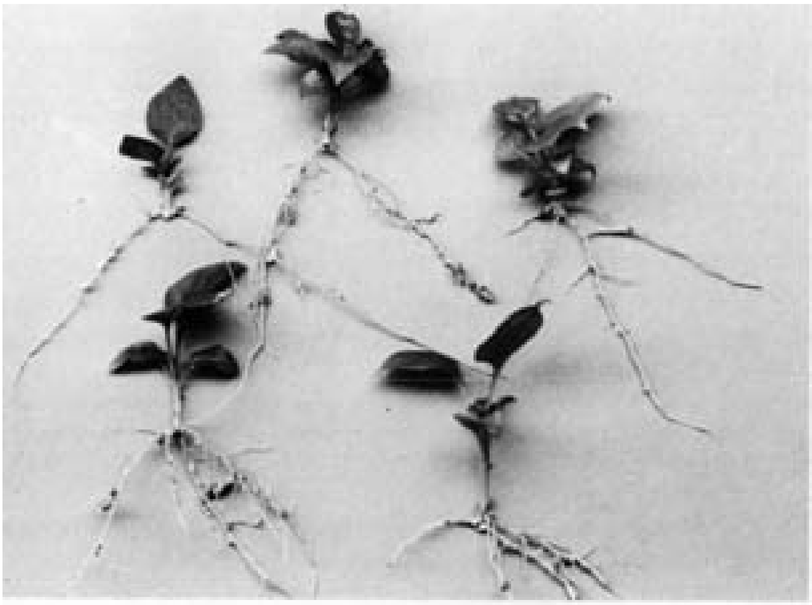
other kinds of phytohormones yet to be discovered?" The answer almost certainly is, "Yes." After all, over fifty different hormones are known among animals; it stands to reason, then, that plants have more than just five different hormone systems. In fact, there is evidence that the actual number of phytohormones is at least twice as large.

Horticultural Applications of Phytohormones

By far the greatest commercial use of phytohormones is as weed killers, that is, as herbicides. A practice begun in the 1930s, the use of excess doses of auxin as herbicidal treatments has become a multimillion-dollar industry. In the United States alone, over fifty million pounds of the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) are applied to millions of acres of agricultural land, as well as to golf courses, public parks, and lawns. An important aspect of this technology is the selectivity of the herbicidal effect: at the levels of auxin applied, dicotyledonous plants ("dicots"), such as dandelions, are killed, but monocotyledonous plants ("monocots"), such as grasses, are left unharmed.

While the use of phytohormones as herbicides might be considered a drastic measure because it involves excessive concentrations of auxin, most practical methods involve subtle alterations in the levels of hormones *inside* plants. An example is the regulation of seed germination with GA.

When a dry seed of a grain such as corn, barley, or oats is soaked in water, the seed produces enzymes that break down the protein and starch that are stored in the seed into their component "building blocks," which nourish the young seedling during the early stages of its growth. Obviously, the coordination of protein and starch breakdown with embryo growth and seedling development is of crucial importance. This is where



Cuttings of *Syringa* obtained through tissue culture and stimulated to produce roots by applying auxin. Photograph by the author.

GA plays a role. For example, one of the first events associated with water uptake by the seed ("imbibition") is the production of GA in the embryo. The GA produced diffuses from the embryo to a layer of cells immediately beneath the seed coat, where it activates the genes for enzymes that release stored reserve nutrients.

In the production of malt, which is popular as an additive for milk and is used to make beer, barley or oat grains are allowed to germinate only to the stage at which most of the starch in them has been converted to soluble sugar. At this point, development of the seedling is stopped by a heat treatment, and the resulting malt is ground to a powder. Sometimes, the grain is treated with GA during the imbibition period. This practice stimulates the breakdown of starch and ensures uniform malting. Several beers from Australia are produced from GA-treated barley.

One of the earliest applications of auxin in horticulture was to stimulate the formation of roots on cuttings. Nowadays, auxin in either liquid or powder formulations can be purchased at most garden-supply stores; it can be used at home to induce roots to grow on cuttings of most common horticultural plants. Essentially the same procedure is used by nurseries for propagating plants, especially when large numbers of identical individuals are needed.

In the last fifteen years, tissue culture has become increasingly important as a tool for propagating plants. (See "Biotechnology at the Arnold Arboretum" in the Summer 1984 issue of *Arnoldia*.) Known as "micropropagation," the usual method involves "shoot multiplication" in a nutrient medium, followed by the auxin-induced rooting of cuttings. After a shoot tip from a plant has been decontaminated, it is transferred to tissue-culture medium containing enough cytokinin to sustain growth and overcome apical dominance in elongating shoots. The result of this manipulation is the production of several, simultaneously growing shoot axes starting from only one tip, that is, shoot multiplication. Individual shoots are then used for further shoot multiplication in the next tissue-culture passage, or they are used as cuttings and rooted with an auxin treatment Theoretically, it is feasible to produce over a million plants from a single shoot tip through tissue-culture technology in just one year.

New Applications

According to the major scientific hypothesis relating phytohormones to the architecture of plants, shoot growth is a result of interactions between auxin, cytokinin, and GA. Auxin produced by the growing tip inhibits lateral buds in the axils of leaves, a phenomenon known as apical dominance that, in extreme cases, results in an unbranched (or "monopodial") axis Cytokinin, on the other hand, counteracts auxin, so that shoot systems in which cytokinin is produced at a high rate consist of several, simultaneously growing brances, a situation called "sympodial" growth. Branching, or its absence, therefore, is a consequence of the auxin–cytokinin balance. By contrast, the length of internodes (stem elongation) is regulated by GA.

Theoretically, the architecture of cultivated plants could be manipulated by altering the levels of any one of the three critical phytohormones. The common practice of nipping buds on houseplants, for example, effectively removes the source of auxin responsible for apical dominance. The result of this treatment is sympodial growth due to the liberation of lateral buds that the auxin produced in the tips of the stems had prevented from developing. Liberation of the lateral buds causes a bush-like, branched architecture. So-called growth retardants, many of which inhibit the production of GA, shorten the internodes of treated plants, resulting in dwarfed, compact architecture. Two examples of growth retardants are cyclocel and ancymidol.

The use of growth retardants has increased substantially in the last few years. Because they inhibit the elongation of stems, growth retardants reduce the need for expensive tree-trimming operations. As a matter of fact, several utility companies currently use growth retardants as a cost-saving measure along streets with aboveground power lines. Growth retardants are also applied to a major variety of wheat in West Germany that has a tendency to lodge (blow over) in high winds. They are also used on lawns to decrease the need for periodic mowing.

Sometimes, however, it becomes necessary to stimulate the growth of a lawn. Two days before the beginning of a national golf tournament, for example, the grounds crew mistakenly mowed an area designated for high grass (the "rough"). GA was put on the affected area, growth sped up, and the rough was restored just in time for the start of play.

Leaf drop, or abscission, is regulated by the relative concentrations of auxin and ethylene in the abscission zone. Ethylene tends to stimulate the process, while auxin inhibits it. In certain instances, cytokinin, GA, and ABA may also exert some control, although their effects vary widely according to the species of plant involved.

Probably the most important commercial use of phytohormones for defoliation involves cotton production. In normal practice, plants are sprayed a few days before harvest with an abscission stimulator (for example, an ethylene-generating chemical) that causes the leaves to drop but does not affect the cotton bolls, which can be harvested with a mechanical picker without harvesting leaves as well. Obviously, the savings that result from using phytohormones in cotton technology are substantial.

Other practical uses of phytohormone defoliants have been controversial. During the late 1960s in Vietnam, for example, phytohormones were sprayed from U.S. military airplanes to cause the leaves of rain-forest plants to fall off. According to official policy, this was done as a temporary, tactical measure, but the repeated treatments killed most major species of trees in the rain forests. The long-range consequences of this practice, both in terms of human health and the future of the Vietnamese ecosystem, are still being studied.

For years, chemicals have been applied to pineapple plants to stimulate flowering and, thereby, to synchronize fruit development and maturation. The value of this technology is realized in more efficient harvesting of ripe pineapples. When the practice was begun in the 1930s, smoke from fires was utilized. Today, an ethylene-generating compound known as ethephon is sprayed on plants.

Phytohormones also can be used to stimulate flowering in several other economically important plants. Fruit trees such as apple, pear, and peach are treated with chemicals to increase the number of flowers. During the commercial production of seeds for biennials such as carrot, beet, and cabbage, GA is used to cause flowering in the first year. Similarly, GA can shorten the time it takes for conifers to form cones, speeding up breeding programs with these plants.

In some instances, it is advantageous to inhibit flowering An especially dramatic example of this involves sugarcane. Chemicals are routinely utilized to prevent sugarcane from flowering during the time it is accumulating sugar. It is estimated that yield increases averaging 1.3 tons per acre are obtained in Hawaii as a result of this practice. Inhibitors of flower formation also are used to overcome "alternate bearing" in tree crops, the alternation of heavy ("on" years) and low ("off" years) flowering, with corresponding effects of fruit production. In the extreme case of mandarin oranges, alternate bearing causes fluctuations in fruit yield ranging from forty to zero boxes per tree in successive years. GA is used in Spain and Australia to reduce flower formation during "on" years. Similarly, in the United States, apple flowers are thinned during "on" years with an auxin treatment.

Phytohormones also affect cut flowers As soon as a flower is removed from a plant, the natural process of senescence speeds up, in large part through the agency of the phytohormone ethylene. Obviously, if flower senescence is to be delayed or prevented, the logical strategy is to counteract ethylene's effect. Various methods have already been devised for just this purpose, in fact, and some of them may have economic potential in the cut-flower trade. One way of extending the life of cut flowers is to refrigerate them, slowing down the metabolic reactions that result in senescence. A second method involves the treatment of flowers with anti-ethylene compounds Silver ion in the form of a silver nitrate solution, for example, inhibits the action of ethylene. Or, senescence can be retarded with inhibitors that block specific steps in the chemical pathway that leads to the production of ethylene by a plant.

Phytohormones are used extensively to regulate fruits, from their earliest stages of development through harvest, and even during postharvest storage. In fact, the major commercial use of GA in the United States involves seedless table grapes. By treating young grape clusters with GA, one can reduce the number of berries per bunch, but obtain larger and juicier individual fruits. There is no question that the GA-treated product is superior to the untreated one. In this case, phytohormone technology can boast a true success story. (See the inside back cover)

By contrast, phytohormone technology applied to tomatoes yields a definitely inferior product. Nonetheless, tomatoes are routinely harvested in the United States before they are mature, often with mechanical picking devices. The green fruits are then treated with ethylene to simulate ripening. The rationale for using this technology is that savings in the cost of harvesting outweigh the extra value of vine-ripened tomatoes. Moreover, it is argued, added ethylene only accelerates a process—ripening—that normally is under ethylene's control. Unfortunately, this latter assertion is a ridiculous oversimplification of what is involved. After all, who hasn't bought a "red" tomato that actually tasted "green"?

Because of its role in abscission, ethylene can be used effectively when fruits are harvested mechanically. In commercial practice, plants are sprayed with ethephon or some other ethylene-generating chemical a few days before harvest.

This treatment initiates formation of abscission zones that, in turn, loosen the fruits. Harvest then becomes a simple process of agitation—either shaking of the stem or a blast of air, followed by collection of the detached fruits. Ethylene-aided mechanical harvesting is a common procedure for cherries, blueberries, grapes, and oranges.

Sometimes, fruit abscission needs to be prevented. For example, when grapefruits and oranges reach maturity, they naturally drop from the tree as a result of abscission. To prevent this process and its associated economic losses, trees can be sprayed with auxin or with a mixture of auxin and GA when the fruits are quite young. The combination of the two hormones accomplishes two purposes: auxin keeps mature fruits on the tree, GA keeps them fresh.

Once a fruit has been harvested, senescence proceeds rapidly. (Senescence also occurs when flowers are removed.) To prevent this, fruits are usually stored at low temperatures to slow down their metabolism, and they are kept in a controlled atmosphere. Often, the amount of carbon dioxide in the air is artificially increased in storage because carbon dioxide tends to counteract ethylene's stimulatory effect on senescence, through a mechanism called "competitive inhibition."

Promising Areas for Applications Research

Other strategies currently are being used to control plants with phytohormones, but the examples given here illustrate the major strategies in use. Much already has been accomplished, with considerable economic impact, but much more could be done if the appropriate technology were developed.

For instance, it is conceivable that a plant's own defense systems for preventing diseases caused by viruses and microorganisms could be accentuated with chemicals. One class of compounds responsible for disease resistance (phytoalexins) has already been identified, and research currently is under way on phytohormones to stimulate the production of phytoalexins. If this research succeeds, we might be able to improve a plant's response to disease through the use of chemicals.

A second promising area involves so-called "bioregulators," which are chemicals that stimulate plants to make valuable products. For example, ethephon is used commercially to increase the production of rubber by *Hevea*. Another group of bioregulators is now being evaluated for their effects on the production of terpenoids by plants.

Of course, the greatest potential impact of phytohormones involves "biotechnology," the concerted application of different scientific disciplines to plant genetics. While most accounts of biotechnology emphasize the contribution of DNA biochemistry, biotechnology would not be feasible without the use of phytohormones, especially of cytokinin, to produce whole plants with new characteristics starting from single, genetically altered cells. Even today, phytohormones play a crucial role when tissue culture is used for the rapid, clonal propagation of plants that have superior characteristics, and for the production of plants, such as strawberries, that are free of virus and fungal infections.

Tissue culture, in spite of its performance, is still a relatively new technique. The common method for micropropagation takes advantage of the established role of cytokinin as a shoot-growth regulator and of the fact that shoot explants from many species can be grown on a medium consisting of basal nutrients plus cytokinin. During the last few years at the Arnold Arboretum, research has been conducted to determine whether this same method can be applied to woody plants in general. While this research is still under way, it has already made clear that micropropagation would be feasible with several groups of woody plants that are not now being exploited. For example, nearly half of the thirty-five families studied to date respond to cytokinin treatments in tissue cultures even though current micropropagation work with woody plants focuses on only two families—the

Rosaceae and the Ericaceae. On the other hand, it is also apparent that this technology will not work for all woody species. Obviously, we do not fully understand shoot growth in several species.

Before cytokinin was known, micropropagation of plants was not possible. Nonetheless, basic research on the internal control of shoot growth led not only to the discovery of cytokinin, but to a new and important practical use for phytohormones. Looking to the future, but reflecting also on fifty years of successful work with chemicals that regulate plants, we can feel almost certain that similar successes will occur. As we learn about phytohormones and discover new kinds, our ability to regulate plants will also increase As so often happens, botany and horticulture will complement each other.

John W Einset, a staff member of the Arnold Arboretum, is associate professor of biology in Harvard University His article on biotechnology at the Arnold Arboretum appeared in the Summer 1984 issue of Arnoldia *With the present article Professor Einset inaugurates a new column for* Arnoldia. *Called "Botany The State of the Art," the column will deal with practical application of botanical research to horticulture*