New Chemicals Promise Larger Crops

Synthetic analogs of brassinolide prove very effective, while the mystery of triacontanol's inconsistency may finally have been solved

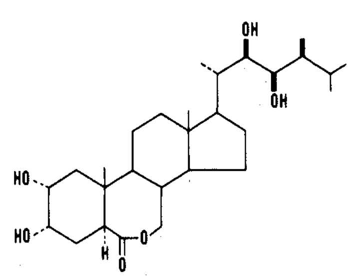
A new generation of plant growth regulators may soon exercise a strong influence on agricultural practices. Unlike earlier generations, which have already proved valuable for such diverse uses as inducing root growth, thinning fruit on trees, regulating crop maturation, aiding harvesting, and increasing the hardiness of plants, the new agents show promise of increasing crop yields and biomass for vegetables, cereal grains, and other produce. The prototypes of this new generation are brassinolide and triacontanol, both of which produce significant increases in crop yields. In recent months, scientists at the U.S. Department of Agriculture (USDA) have synthesized and tested two analogs of brassinolide that are inexpensive enough to use commercially, but that appear to be equally effective. Another scientist, meanwhile, seems to have discovered why results with triacontanol have often been inconsistent.

Brassinolide was discovered in a screening program for new plant hormones initiated at USDA's Beltsville Agricultural Center more than 10 years ago by John W. Mitchell. Mitchell, since retired, began screening pollens because he thought they would have a high concentration of hormones. The USDA group screened pollen from nearly 60 species and found that about half caused increased growth in bean seedlings. The greatest growth increases were obtained with pollen from alder trees and from rape (*Brassica napus* L.), a forage crop whose seeds yield an oil used as a lubricant and as a food. These two pollens shared an unusual characteristic. When an extract of the pollens was applied at high concentrations, the stem of the seedlings would split above the second pair of leaves because of the rapid growth, and then grow back together. No other compound has been found that produces this effect. The initial results also showed that the active substance was present in the pollen at a concentration of only parts per billion; most hormones are found at parts-per-million concentrations in plants.

Because this discovery was so promising, a Beltsville team headed by N. Bhushan Mandava undertook a crash program to isolate the active substance from rape pollen, which was more readily available than alder pollen. Michael Kozempel and his colleagues at USDA's Eastern Regional Research Center in Philadelphia then constructed a pilot plant for extracting large amounts of rape pollen with 2-propanol. From 225 kilograms of pollen, the USDA team obtained barely 10 milligrams of a pure, crystalline material that they named brassinolide.

On the basis of spectrometric studies by Mandava and his colleagues at Beltsville and by Michael D. Grove and his associates at USDA's Northern Regional Research Center in Peoria, Illinois, as well as x-ray crystallographic studies by Judith L. Flippen-Anderson of the Naval Research Center in Washington, D.C., the group reported in 1979 that brassinolide is a steroid. It is the first plant growth regulator that has been found to have a steroidal structure, and it is the first naturally occurring steroid that has been found to have a seven-membered lactone ring as part of the fused ring system. Application of pure brassinolide to bean seedlings produced significant increases in plant growth at concentrations of only 1 nanogram (10^{-9} gram) per plant and caused splitting of the stem at concentrations of 10 nanograms per plant.

Because isolation or synthesis of brassinolide itself was obviously too expensive for any significant use on crops, Mandava and Malcolm J. Thompson of Beltsville synthesized several analogs of the chemical to determine which parts of the molecule are most important for activity. They found that the hydroxyl groups on the A ring have to be in the alpha-conformation and that the two hydroxyls on the side chain are also necessary for activity. Last year, Mandava and Thompson synthesized two analogs—called brassinosteroids—and subjected them to field and hothouse trials.



Brassinolide

$CH_3(CH_2)_{28}CH_2OH$

Triacontanol

Mandava reported early this year at a regional meeting of the American Chemical Society that the synthetic brassinosteroids promote growth in the same manner as brassinolide, but that four to ten times as much of the synthetic compounds must be used. Even so, the total amount is still very small—only nanograms per plant. When applied to very young plants in field tests conducted by the Beltsville team, the brassinosteroids boosted the harvest of radishes and lettuce by 15 and 30 percent, respectively. Yields of bean and pepper plants in the field were increased by 6 to 7 percent, and that of potatoes in a small trial by about 25 percent. In greenhouse studies, treated bean and pepper plants weighed 35 percent more than untreated plants and produced a bean crop weighing 32 percent more than untreated plants. Field tests are now being conducted on potatoes in Colorado, and tests are planned for this year at several locations on vegetables, soybeans, and other major crops. Among other things, these trials are designed to determine the best time and method for applying the regulators.

Mandava estimates that 1 gram of either synthetic brassinosteroid would cost between \$5 and \$10 to produce on a commercial scale. That cost is not unreasonable since a gram would cover as much as 5 acres. The two Beltsville scientists are now undertaking the synthesis of brassinolide and one other analog using Chesapeake Bay oysters, which provide a relatively cheap and abundant supply of steroids as a starting material.

Meanwhile, John Siddal of the Zoecon Corporation in Palo Alto and Steven Fung, who is now with Ayerst Pharmaceuticals in Montreal, revealed that they have synthesized brassinolide. The same feat has also been accomplished by scientists at the Tokyo Institute of Technology. Synthetic brassinolide is too expensive for use on field crops, Siddal says, but Zoecon thinks it might prove useful for certain specialized crops and for ap-

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Split internodes

If too high a concentration of brassinolide is applied, the stem of a seedling splits above the second pair of leaves, then grow back together. [*Source: N. B. Mandava*]

plications in biochemical research. It might, for example, be used as an additive in media for culturing plants or in the regeneration of whole plants from cells or cuttings. The techniques currently used for laboratory manipulation of plant genetics are not good, says Siddal, and brassinolide could be quite useful.

Brassinolide and the brassinosteroids are not without some potential problems that require further research. Because they are steroids, it is possible that they could have some undesirable effects on insects, wildlife, and perhaps even man—although Mandava is quick to point out that no indication of such effects has been observed. Furthermore, because of the high value of vegetable and other crops on which the chemicals could be used, farmers may be reluctant to try them until their value has been thoroughly proved.

Prior to some current research by Andrew J. Welebir of Biochemical Research Corporation in Falls Church, Virginia, triacontanol's history has had several ups and downs. It was discovered in the mid-1970's by Stanley K. Ries of Michigan State University, who observed that placing chopped alfalfa in fields significantly increased the yield of tomatoes and other crops. When he isolated the active ingredient in 1977, he found that it was triacontanol, $CH_3(CH_2)_{28}CH_2OH$, a constituent of beeswax and a variety of plants observed as long ago as 1934. In his initial studies, Ries reported that triacontanol produced increases of 8 to 63 percent in yields of navy beans, asparagus, sweet corn, carrots, cucumbers, radishes, and tomatoes. The growth increases were rapid and, in some cases, even occurred in the dark. After 6 hours of darkness, for example, triacontanol-treated rice plants gained more than 10 percent in dry weight and 18 percent in protein, Ries reported.

Subsequent field tests of triacontanol by Ries and by USDA, universities, and chemical companies proved disappointing, however. Sometimes the chemical would produce a substantially increased yield, and sometimes nothing would happen. Many investigators gave up on the compound because it proved so unreliable. Ries attributes most of these problems to the method of formulation of triacontanol. The long-chain alcohol is very insoluble in water, and Ries and others have used detergents and surfactants to emulsify it and keep it in solution. Clearly this has not been adequate, since the inconsistent results have continued. Ries himself is discouraged and says he will work on the problem in the field for only one more season. If he has not achieved satisfactory results by then, he says, he will drop field research on triacontanol and go on to other projects.

Welebir, however, thinks that formulation is only part of the problem. Investigating the interactions of triacontanol with plant hormones, he observed that the activity of triacontanol is very sensitive to the concentration of metal ions, particularly calcium and lanthanum. Salts of calcium and lanthanum have previously been shown to inhibit action of hormones called auxins by increasing the binding of endogenous auxin to cell membranes. He has also found that plant response occurs only when the *p*H of the triacontanol solution is 8 or greater. He presented some of the results of this research at the American Chemical Society meeting in March.

Even before he developed a theoretical basis for their use, however, Welebir began adding calcium or lanthanum salts to preparations of triacontanol and found not only that the regulator worked consistently, but also that he obtained better results than other investigators. He suggests that the detergents and surfactants used to emulsify the triacontanol complex calcium ions so that they cannot participate in plant stimulation. He now uses a preparation of triacontanol dissolved in acetone and water; the concentration can be quite low, since it is effective when applied at a rate of 10 milligrams per acre. The cost is 2 to 4 cents per acre.

In preliminary field trials conducted last summer, Welebir found that the new formulation increased the weight of fresh sweet corn by 51 to 54 percent in three separate trials. Application to seedlings produced a 6 to 19 percent increase in ear length due to the formation of larger kernals and an increase of 20 percent in the number of ears. Field tests with tomatoes, Welebir says, showed early increases in yields of 65 to 72 percent, while the yield of beans increased by as much as 90 percent. Preliminary results with field corn, which had shown no effects in earlier studies with triacontanol, suggest that a 15 to 20 percent increase in yield can be obtained. Many new field trials on a variety of major crops will be conducted during the coming growing season to see if the results remain consistent.

Both the brassinosteroids and triacontanol will, of course, have to be approved by the Environmental Protection Agency (EPA) before they can be used on a commercial basis. Welebir, for one, sees little problem with this, since "triacontanol is ubiquitous in nature; corn tissue contains about 235 micrograms per gram, compared to the low nanogram levels each plant receives." Brassinolide also appears to be present in a large number of species. Biochemical Research Corporation is currently seeking an exemption from registration by EPA for triacontanol on this basis.

These new growth promoters, and others like them that seem certain to follow, should prove very valuable to American agriculture. As the amount of high-quality arable land continues to shrink at the same time that demand for food and biomass continues to grow, anything that can provide an increase in production per acre should find a good reception.

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