# Plant Biochemistry: Two New Ways to Fight Pests

Plants exist in a delicate balance with insects and other pests, such as viruses, bacteria, and fungi. It has long been known that the pests are able to disrupt the metabolism of plants, but only recently has it been recognized that plants produce chemicals with the same effect on pests. It is now becoming apparent that at least some plants have rather remarkable defense mechanisms that help them ward off attacks by pests.

Experimental manipulation of these mechanisms for defense against plant pests has been hampered by the limited knowledge of how they operate. But key elements of two different mechanisms were reported at the recent Centennial Meeting of the American Chemical Society (ACS). One group of investigators reported the identification of a group of naturally occurring inhibitors of juvenile hormone, a chemical that is important in insect development. A second reported the identification of a substance that elicits in plants the accumulation of a chemical that is analogous to interferon in humans. Both discoveries represent potential first steps in the development of new types of pesticides that would be safer for the environment.

Juvenile hormones are relatively simple compounds that are present in all insects throughout most of their normal development. They are absent only during metamorphosis, the period when a larva changes into an adult insect. Juvenile hormones and synthetic analogs have been used as insecticides because their application to insects during metamorphosis results in the production of deformed adults that are incapable of further development and soon perish. These chemicals have limited utility, however, because they have very little effect on insects in the other stages of life.

Many investigators have observed that surgical removal of the corpora allata, the organ that produces juvenile hormone, produces effects that are detrimental to the insect. Immature insects undergo precocious metamorphosis, while adult female insects typically are rendered sterile; some insects are also rendered unable to produce their normal sex pheromones, and others are forced into diapause, a period of dormancy in which they do not feed, mate, or reproduce, regardless of the time of year. William S. Bowers of the New York State Agricultural Experiment Station in Geneva, speculated that inhibitors of juvenile hormone secretion, termed antiallatotropins, might produce the same effects.

It has been shown that some plants contain chemicals with juvenile hormone activity. Bowers thus reasoned that plants might also contain chemicals with antijuvenile hormone activity. Three years ago, therefore, he began screening plant extracts for such activity, and he announced at the ACS meeting that he has found two. They were isolated from the common bedding plant *Ageratum houstoniatum*, which has been observed to be relatively resistant to insect attack. The two compounds are 7-methoxy-2,2-dimethylchromene and 6,7-dimethoxy-2,2-dimethylchromene. Because the compounds cause precocious metamorphosis in insects, Bowers has named them precocene 1 and precocene 2, respectively. Both compounds had previously been synthesized by other investigators for different purposes.

### Premature Metamorphosis

Bowers has demonstrated that contact with the precocenes causes premature metamorphosis in milkweed bug nymphs (the principal insect species he has studied), cotton stainers, and other *Hemiptera* species. Precocenes cause the nymphs to skip one or more of their five instars (stages of life between molts) to become imperfect, miniature adults with shortened life spans. The ovaries of precocious female adults do not undergo development or yolking, and the females are sexually unreceptive. Exposure of normal adult females to precocenes shortly after metamorphosis also prevents ovarian development. Exposure of mated milkweed bugs causes the eggs to be resorbed and the ovaries to regress to the undeveloped condition. If mature eggs are present at the time of the exposure, the eggs are deposited and hatch normally, but undergo precocious metamorphosis.

The precocenes produce other effects that are detrimental to adult insects, such as blocking the production of sexual pheromones in cockroaches. One of the most striking activities is the induction of diapause in Colorado potato beetles: after exposure to precocenes, the beetles simply burrow into the ground and go to sleep for extended periods. But not all insects respond to precocenes in the same fashion, Bowers says. The development of some of those tested, for example, was not accelerated. But females of nearly all the species that he has so far tested became sterile when exposed to the chemicals before their sexual development was complete.

It seems likely that the precocenes are in some fashion interfering with the secretion of juvenile hormones. Bowers has demonstrated, for instance, that precocious maturation does not occur when milkweed bug nymphs are exposed to a combination of precocene and juvenile hormone. Similarly, adults exposed to the combination undergo normal mating, ovarian development, and oviposition. Bowers suggests that there are at least half a dozen different mechanisms by which this interference could occur.

Juvenile hormones and their analogs have frequently been termed the third generation of insecticides. Bowers suggests that the antiallatotropins could become the fourth generation. Even if the precocenes should be shown to affect only a limited range of pests, their discovery has demonstrated a new mode of insect control. Several pesticide manufacturers have already contacted Bowers about his findings. Other antiallatotropins will almost certainly be discovered now, and analogs with even greater activity may be synthesized. The chemicals should also be desirable from the standpoint of their environmental effects since they would be expected to be nonpersistent and to have a relatively specific activity that would not interfere with other biota.

The second development is somewhat more complicated and may require much more effort before it is put to practical use. It involves phytoalexins, a class of plant compounds first reported in 1940 by the late K. Müller of Germany. Phytoalexins are lipidlike chemicals synthesized by plants; they are toxic to fungi and bacteria, and there is some evidence that they might be toxic to other pests. The chemicals are produced in response to an attack by the pest, and might thus be considered analogous to interferon, the antiviral chemical that is produced in humans in response to a viral infection. At least 100 different phytoalexins have been isolated and characterized by several investigators. They are thought to act, in part, by preventing fungus spores from germinating.

In 1958, K. Uehara of the University of Osaka in Japan observed that phytoalexins accumulated in plant tissue exposed to filtrates from cultures of fungi or other pathogens. This suggested that there are specific compounds in the filtrate, termed elicitors, that elicit this response. At least three candidate elicitors have previously been reported. I. A. M. Cruikshank and his associates at the Commonwealth Scientific and Industrial Research Organization in Canberra, Australia, have isolated and partially purified a polypeptide with such activity. Noel T. Keen of the University of California at Riverside has isolated what he believes to be a low-molecular-weight chemical with elicitor activity. And Roland Rohringer and his associates at the Winnipeg Rust Research Laboratory in Manitoba have detected what they believe is a nucleic acid elicitor.

Cruikshank's polypeptide, however, acts as an elicitor in only one species. Some investigators thus think that it is an artifact rather than a true elicitor with a role in the disease process. Neither Keen's nor Rohringer's compound, furthermore, has been adequately characterized yet. Thus, Peter Albersheim and his associates at the University of Colorado in Boulder reported at the ACS meeting that they had, for the first time, isolated and characterized a phytoalexin elicitor.

The elicitor was initially obtained from filtrates of cultures of *Phytophthora megasperma* var. *sojae* (PMS), a fungus that attacks soybeans. It stimulates the accumulation of the phytoalexin glyceollin, which had previously been characterized by Robert Sidney Burden and John A. Bailey of Wye College at the University of London. The most crucial aspect of his group's work, Albersheim says, was the development of a sensitive and quantitative assay for elicitor activity that would make it possible to monitor the course of the purification.

The Albersheim group's assays are, in fact, refinements of semiquantitative assays developed by other investigators. In essence, the techniques require application of the test substances to wounds on the cotyledon (the first leaf to be developed by the embryo of a seed) or hypocotyl (the segment of stem directly below the cotyledon) of soybean seedlings, and incubation for 20 to 26 hours. Glyceollin is then extracted in a quantitative fashion and assayed either by spectroscopy or by a combination of thin-layer chromatography and spectroscopy. These tests are sensitive enough to identify as little as 0.2 microgram of the suspected elicitor.

Preliminary evidence indicated that the elicitor isolated from filtrates was a heterogeneous group of carbohydrates with masses as large as 100,000 daltons. Albersheim speculated that they might be parts of the cell wall from dying PMS cells. To test this theory, he broke down PMS cell walls with a heat treatment technique developed by other investigators for isolating surface antigens. Purification of this material yielded four separate fractions with elicitor activity—one of which was virtually identical to the

## The Moon: Not So Different from Earth After All

One of the principal scientific questions behind the Apollo flights was the origin of the moon. Lunar samples and surface experiments seemed initially to show that the moon is chemically very different from the earth, low in metallic iron and volatile elements, enriched in refractory materials such as aluminum and uranium. The differences were great enough to boost the view that the moon originated elsewhere in the solar system and was captured by the earth. Certainly, those who believed in a common origin for the two bodies faced difficulties in explaining their apparent chemical disparities.

This past year, however, three separate pieces of evidence indicating that the moon is not after all so different from the earth have come to light. While some substantial chemical differences remain, the two bodies are thought by a growing number of investigators to have been formed from basically similar materials. Capture models have been losing favor for several years, and the latest results seem likely to end speculation that the moon was formed in some far corner of the solar system.

Two of the new results bear on the supposed refractory character of the lunar materials. Laboratory experiments by A. E. Ringwood of the Australian National University at Canberra show that basaltic rocks from the lunar maria, formed deep in the moon's interior and presumably more characteristic of its bulk constituents than rocks formed on the surface, cannot be made from materials very rich in refractory elements such as aluminum and calcium. He concludes that the moon is not so refractory as was previously thought and that the parent materials for the earth and the moon were, in this respect, more similar.

Several years ago heat flow measurements at two locations on the lunar surface also seemed to indicate high concentrations of another key refractory element, uranium. Recent revisions of these experimental results by Marcus Langseth and his colleagues at the Lamont-Doherty Geological Observatory in New York, however, give a lower estimate of lunar uranium concentrations, about 40 parts per billion. Although there is some debate about the heat flow on the earth, Langseth's estimate (an average of 2 microcalories per centimeter$^2$ second) would give a comparable uranium value for the earth's mantle, 42 parts per billion. In any case, Langseth now believes, there is not a great difference in the uranium concentrations of the two bodies.

The third piece of evidence comes from analyses of oxygen isotopic ratios in terrestrial, lunar, and meteoritic samples by Robert Clayton of the University of Chicago. The data (see *Science*, 21 May 1976, p. 772) indicate that the earth, the moon, and certain highly evolved meteorites were all made from a similar batch of material, isotopically distinct from most meteorites and, presumably, from original material in the parts of the solar system where these meteorites were formed. As a result, Clayton proposes that the earth and the moon were both formed from the parent bodies of the highly evolved meteorites, which he views as sizable planetesimals 10 kilometers or more in diameter—large enough to have undergone internal melting. Accumulation of these planetesimals—rather than direct condensation of the earth and moon from the solar nebula or capture of a complete moon—is thus what he envisages for the origin of both the earth and the moon.

The paucity of volatile elements and metallic iron on the moon means that it is distinct from the earth, but, these new results seem to argue, not so chemically distant as had once been believed.—ALLEN L. HAMMOND material isolated from filtrates. Further studies showed that each fraction was composed of a carbohydrate core, which was apparently responsible for elicitor activity, combined with other material from the cell wall.

Digestion of the isolated materials with appropriate enzymes revealed that the smallest fragment with full elicitor activity was a $\beta$-glucan fragment with a mass of about 10,000 daltons. The $\beta$-glucan is a highly branched polymer of glucose connected through the 3- and 6-positions. The molecule also contains about 4 percent mannose, another sugar. Albersheim has subsequently isolated a nearly identical $\beta$-glucan from the cell walls of brewers' yeast, which is not a plant pathogen, but which could be a large source of the material.

Albersheim argues that this is the first time that a material isolated from the cell wall of a pathogen has been shown to be involved in the defensive response of a plant. And that response appears to be very specific. Less than 0.01 nanomole ($10^{-11}$ mole) of elicitor applied to a single soybean hypocotyl results in the accumulation of 150 nanomoles of glyceollin, a 15,000-fold amplification. This is apparently accomplished, he has further shown, by stimulation of the activity of phenylalanine ammonia-lyase, one of the first enzymes involved in the synthesis of phytoalexins. This effect is not obtained, Albersheim adds, with compounds that are highly similar in structure to the $\beta$-glucan, even when they are applied in quantities that are as much as 1000 times larger.

Albersheim isolated what is apparently the same elicitor from three different races of PMS, one of which would grow on the particular type of soybeans he was using and two of which would not. He found that each of the elicitors stimulated the accumulation of approximately the same quantities of glyceollin in the soybean. This suggests to him that production of the phytoalexin is not a specific determinant of a plant's resistance to infection. Such a possibility seems to receive confirmation from his observation that simultaneous exposure of the plants to the elicitor and the PMS race that normally grows on the plant does not impede that growth. Growth is halted, however, if the elicitor is applied 10 hours before the fungus. Albersheim thus suggests that resistance—at least in the PMS-soybean system—might be the result of the growth rate of the attacking pathogen. If the growth rate is slow, phytoalexins can accumulate in toxic concentrations before the pathogen can spread; if it is fast, the pathogen's spread can outdistance the phytoalexins.

These results are in direct contradiction to those that have been achieved by Keen, whose elicitor has also been derived from PMS. Keen finds that the elicitor from the PMS race to which the plant is resistant produces much less accumulation of glyceollin than does the elicitor from the race that grows on soybeans. Albersheim attributes Keen's results to impurities in the elicitor preparation. But Keen argues that Albersheim has isolated only a general elicitor whereas he, Keen, has isolated a specific elicitor that is actually involved in the disease process and that is responsible for species resistance to pathogens. These differences will probably not be resolved until Keen has more fully characterized his elicitor.

Albersheim's results, like those of Bowers, show potential for the development of new types of pesticides. Unfortunately, the $\beta$-glucan is such a large molecule that it may not enter plants readily. If that should be the case, Albersheim speculates, it might be possible to prepare derivatives of the elicitor that could readily be taken up by the plant, as has already been accomplished with drugs for animals. Application might also be easier if simpler chemicals such as that Keen thinks he has isolated, are also found to have elicitor activity. The elicitors would also be expected not to persist in the environment and to be so specific in their activity that, again, no harm to other biota would be expected.

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# Endangered Bird Species: Habitat Manipulation Methods

The endangerment and extinction of avian species, subspecies, and local populations due to environmental poisoning, hunting, and habitat destruction have prompted a new conservation endeavor: crisis management to bolster failing populations until they, and the ecosystems on which they depend, recover so that they can again be self-sustaining.

Unlike classic conservation techniques—education, legal protection, and habitat preservation and management—these new methods are highly manipulative. Biologists intercede directly in the afflicted birds' life cycles, either by moving or manipulating the birds themselves, or by manipulating their immediate breeding or feeding habitat.

Because threatened birds are perceived by both scientists and the public as an extremely valuable resource, there is strong pressure to interfere with them only on the basis of carefully defined, testable hypotheses, whose confirmation provides justification for disturbing the birds. Thus far in most cases it has not been possible to wait for this kind of proof. But as the discipline develops, its practitioners hope, it will become increasingly possible to intervene with one species using a technique already tested in another.

A case in point is the osprey (*Pandion haliaetus*). Demographic studies in the 1960's by Charles J. Henny, Jr., of the U.S. Fish and Wildlife Service (USFWS) indicated that local populations of ospreys in North America must produce between 0.95 and 1.30 young per breeding pair annually to maintain their numbers. In the mid-1960's, productivity fell below 0.50 among ospreys in coastal New Jersey and southern New England, due in large part to DDT, dieldrin, and possibly polychlorobiphenyls.

### Egg Transfer and Double-Clutching

A manipulative method to halt this decline was proposed by a biology student at Wesleyan University, Connecticut, Paul Spitzer. He conceived of the idea of tiding the Connecticut ospreys over their crisis by replacing their pesticide-laden, infertile eggs with "cleaner" eggs taken from reproductively healthy ospreys breeding in less contaminated areas. He called this technique "egg transfer."

Eggs and also osprey hatchlings were obtained from Chesapeake Bay. They tolerated the move by boat, car, and plane. The adult Connecticut ospreys tolerated the disturbance of being frightened from their nests so the eggs could be switched. They returned to brood the transferred eggs, most of which hatched. The young developed and fledged uneventfully.

The key question was whether, in 3 or 4 years, they would return to breed on the Connecticut coast or would stop at Chesapeake Bay, where they had been conceived and where, it might be supposed, they were genetically programmed to go. In fact, of 45 transferred osprey eggs and chicks that fledged, 7