HELPING PLANTS HOLD THE LINE

Within existing plants may lie the clues for practical alternatives to chemical pesticides

BY JULIE ANN MILLER

Fry, Cornell Univ.

Resistant strain of potato survives blight that downs less resistant type (foreground).

Nobody rolled up a sleeve in a recent experimental immunization program. The recipients were not children or old people or even guinea pigs. They were watermelon, green bean and cucumber plants.

Preventative medicine for humans has been around for a while, and now it may become essential to crop production. Researchers at the recent American Chemical Society meeting in Chicago reported studies indicating that plants can be stimulated to resist disease. In a special session on agricultural chemistry, other scientists described work they hope will lead to the breeding of crops more resistant to pests and disease.

There is certainly a pressing demand for economical, convenient and ecologically acceptable means of pest control. For safety reasons major pesticides, such as DDT, aldrin and dieldren, have been taken off the market, and toxaphene, the pesticide currently in heaviest usage in the United States, is being threatened by the Environmental Protection Agency. "EPA keeps banning these chemicals and telling us to use substitutes," says a Florida farm operator. "Well, we've just about run out of economical substitutes."

The search for new strategies in the battle against crop-damaging insects, worms and fungi has recently recruited chemists. Interest in the chemistry of resistance has increased not only with the advent of necessary microchemical techniques, but also with the expectation that the Food and Drug Administration will soon require chemical data to show that changes made in plants (even through conventional breeding methods) will not constitute a hazard to human or animal consumers, explains Paul A. Hedin of the Boll Weevil Research Laboratory in Mississippi State, Miss.

Some chemists are looking for substances that will damage pests, but not harm beneficial insects, fish, birds or mammals. Such substances might inhibit production of an insect component or, like pheromones, upset essential insect behavior. Still other scientists hope to discover the answer to the pest problem within the plants themselves.

"All plants, in their co-evolution with insects, must have chemical or physical means of resisting insect damage or they could not survive," says Tony Waiss of the U.S. Department of Agriculture Agricultural Research Service in Berkeley, Calif. "Corn, before people adapted it, had resistance. As people started breeding for higher yield and better color, they inadvertantly lost the resistance."

Waiss and others are determined to boost that innate resistance as a sensible approach to pest control. "Plants with built-in resistance have chemicals toxic to insects right where they are needed . . . on the plants," Waiss points out.

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Innoculation: Kuć sprays leaf with fungus.

Breeding plants to increase pest resistance has already been successful in several cases, such as rust-resistant wheat. Often the desired trait is found in an uncultivated variety. For example, in the battle against the lygus bug, which destroys cotton flower buds, 618 types of cotton were screened by agronomists at the Cotton Research Station in Shafter, Calif. The most promising candidate turned out to be the extra floral nectariless character in a wild Hawaiian species. Those plants reduce their pest problem by discontinuing the free lunch to the insects. The wild species lacks three of the four glands that secrete a sugary substance used by the bugs, explains Angus H. Hyer. He and Hubert B. Cooper Jr., Tom Leigh, Ward Tengey and John Benedict are now working to transfer that characteristic into a popular crop line of cotton.

Now the chemists are striving toward more efficient breeding strategies. "The use of unsubstantiated correlative factors, similar to the assumption that intelligent people wear glasses, has often impaired the success of a breeding program," Waiss says. On the other hand, if natural insecticides were identified within plants and measured directly in different strains, it should be much easier to select the most resistant strain.

Waiss and colleagues have identified several natural plant chemicals responsible for resistance and are trying to learn how these compounds act. Tannin is the common plant compound used to tan leather and responsible for the astringent taste in tea, wine and fruit. Waiss finds that tannin seems to provide a major defense of cotton against budworms. Other chemicals have also been implicated: two diterpene acids from the sunflower plant are toxic to sunflower moth larvae, and certain polyphenols from corn act against corn earworms. Because high concentrations of these chemicals are strategically located on the plant, they can be effective even though they are only one-thousandth as toxic as applied chemical insecticides. "Nature apparently knows what it's doing," Waiss says.

Cotton and tobacco plants contain other chemicals toxic to certain insects, Robert Stipanovic and Alois A. Bell have discovered. They examined more than 1,200 different cotton plants collected from the wild and from small plantings in Mexico, Central and South America and on Caribbean and Pacific islands. In 78 cases they found resistance to bollworms. They traced the resistance first to dark pigment glands on the leaves, stems and flowers and then to a series of compounds called heliocides (after *Heliothis*, the genus of insects that includes the cotton bollworm and tobacco budworm).

Rapid identification of these compounds makes extensive genetic studies feasible. Stipanovic has developed an analytical technique using thin-layer chromatography that requires only one leaf. "A three-man team can screen 240 progeny per day," Stipanovic says. This is much quicker than waiting to see whether or not the worms eat each plant.

Death of the pest is not absolutely essential for a crop to triumph; the true requirement is simply that the pest not eat the plants. A variety of plants contain chemicals that merely make them unappetizing to specific insects. This situation is reminiscent of the recent discovery at the University of Wyoming that coyotes will not kill sheep sprayed with synthetic tabasco sauce. The plant defense does not seem to be spicy. Hedin says, "Most of these compounds are bitter to humans, and some similar perception apparently occurs with insects." He also points out that although feeding deterrents do not directly kill the insect, most of those reported are, in fact, poisons.

An extensive survey has been conducted at Nagoya University in Japan for chemicals that are feeding deterrents to the tobacco cutworm. That insect is disasterous to Japanese agriculture; it attacks sweet potatoes, sugar cane, crucifers, taro and legumes.

Researchers measured antifeeding activity by determining the area that the insects consume of a disk cut from leaves and soaked in a plant extract. Of 53 different species, 19 showed antifeeding activity, Katsura Munakata reports. The scientists identified many new chemicals responsible for this activity including compounds in *Orixa Japonica* Thunberg, a plant that has long been used in Japan to insect-proof books.

In at least one group of antifeedants, Munakata found no linear relationship between bitter taste and ability to discourage insect feeding. In another group of chemicals, differences in activity were related to a group of atoms on one ring of the chemical structure.

Munakata thinks these antifeeding substances may be useful in developing resistant crop varieties and also in suggesting powerful synthetic insect antifeedants. "When used for insect pest management, antifeedants might be especially advantageous because they control the insects indirectly through starvation and they may not be harmful to parasites, predators, and pollinators. If crops were sprayed with efficient antifeedants, perhaps the pests would turn from the crops to weeds," Munakata says.

Breeding can fortify plants against the more sedentary disease agents, as well as against insects. William E. Fry of Cornell University has been examining varieties of potato plants for resistance to late blight. The late blight fungus was responsible for the Irish famine of the 1840s and is today probably the most important potato disease in the world. Although most potato varieties have little resistance, the study identified enough resistance in one of Cornell's breeding lines so that only half the usual amount of fungicide is needed to protect the plants in the field. This information about resistance is being provided to growers. No one yet knows what specific characteristic of the potato makes a variety fungus-resistant. "We don't even know how many genes are involved," Fry says.

If you cannot fortify the plant either with death-dealing or simply repulsive guards, the next best plan would be to mobilize troops effectively after the attack. That is the strategy the human body uses to defend against disease, and it also seems to work in plants. Thus, the booster shots for cucumbers.

"Plants can be protected from disease by mechanisms which resemble those used to immunize animals," Joseph Kuć told the ACS meeting. In the third year of field experiments at the University of Kentucky, he and Frank L. Caruso tested hundreds of watermelons, muskmelons and cucumbers. In each case about half the plants were deliberately infected with a small amount of a disease-causing fungus. The innoculations were successful. For example, among the watermelons only 1 out of 66 immunized plants died, while 47 out of the 69 unprotected plants succumbed. The immunized cucumbers had less than 2 percent of the leaf damage of the untreated plants. In further experiments, green bean plants were protected by immunization with a harmless form of fungus, rather than with the disease-causing agent. Researchers in other laboratories are developing immunization procedures for tomatoes, chestnut trees and plants susceptible to crown gall.

Kuć realizes that innoculating thousands of plants in a field would be tedious and expensive. He is, therefore, working to determine the chemical signals responsible for the protection. "The key to disease resistance in plants is the functioning of multiple mechanisms for resistance and the key concept in understanding their interaction is one of 'coordinated defense,'" Kuć says. When the mechanism of plant immunity is better understood, Kuć suggests, treatment of seeds could protect the young plants.

The detailed mechanism of plant immunity has attracted other scientists including Noel T. Keen and B. Breugger of the University of California at Riverside. They are studying a group of chemicals responsible for some, but not all, plant resistance responses. These small molecules, called phytoalexins, kill several groups of disease-causing microorganisms. Since the discovery of phytoalexins in 1940, 64 different types have been identified from 75 plant species.

Phytoalexins occur in very low or un-

Cotton harvests have already been enhanced by crop breeding.

Breeding the primitive spines (left) back into spineless varieties of safflower (right) may provide natural pest resistance.

Photos: Univ. of Calif., Division of Agricultural Sciences

detectable amounts in healthy plants. But they accumulate at the site where an invading organism has attacked. Unlike antibodies of the animal defense mechanism, phytoalexins do not seem to have specific targets. They may make cell membranes more leaky, which could harm a wide variety of microorganisms.

Several laboratories, including Keen's, are examining the biochemical process that dictates when and how a plant responds to a microorganism. The best hypothesis, Keen says, is that specific receptors on the plant bind specific products of the invader. Keen is examining how the genes responsible for the phytoalexin production are controlled. "We hope by knowing how plants are normally resistant, we can approach the problem of plants not being resistant," Keen says.

Although much research remains to be done on the extent of the mechanism and the details of its operation, Keen says it is time to consider how phytoalexins can be vehicles for practical plant disease control in the field. Applications may eventually include breeding plants that make the best phytoalexins against a specific agent. Researchers also have some evidence of the existence of a class of chemicals with promising characteristics. These "sensitizers" neither kill invaders nor stimulate phytoalexin production, but rather seem to strengthen the plants' immune reaction to later infection. This effect is indistinguishable from the plant being genetically more resistant, Keen says. "So far none of the approaches are close to being practical," Keen admits.

Waiss says, "Susceptibility to pests is the exception, resistance is the norm." But it will take much work to retrieve that norm and balance it with the desirable traits now bred into crops. The new genes for resistance will probably come from the wide range of uncultivated plants. J. B. Kendrick Jr. of the University of California Agricultural Experiment Station says in the September *California Agriculture,* "We must not yet abandon basic genetic resources, particularly the collection and conservation of wild species and primitive varieties of plants that carry the genes for traits we may desperately need in the future." □