Biochemical Relationships

nematodes, plants, and linking soil components of complex problem of widespread, important pest of state's agriculture

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The problems associated with nematode diseases of plants can be visualized as one part of a system consisting usually of three components: nematodes, plants and the linking medium, most frequently soil.

This natural division can serve as the basis for separate approaches for fundamental study. For example, in an approach through the plant, the phenomenon of host preference or specificity or from another point of view the natural resistance of some plants to attack could be considered.

Scientific research normally results in the addition of small bits of information to accumulated knowledge. Occasionally one such finding observed may possess far-reaching implications. Probably, few people in 1928—at the time of the discovery with penicillium mold—would have predicted the tremendous antibiotic industry that provides a weapon against disease and gives agriculture a tool for greater production. The role of vitamins in nutrition is another example of an unsuspected consequence of what was initially just an interesting observation.

Geneticists can breed for plants of desirable character without knowing the details or the mechanism of action of the particular character, though that information would be helpful. On the other hand, chemical control of nematodes through the plant, without a knowledge of the mechanism of resistance to nematode attack, would compel reliance upon a fortuitous selection of an effective chemical agent. If, for example, the resistance of a plant were due to the presence or absence of some chemical agent or agents, it might be possible to design a substance for field application. To design such a substance, it would be necessary to know such things as the precise chemical character, sites of formation and action, ability to translocate within the plant and the concentration for activity.

The idea of chemotherapy—the application of poison which, when sprayed on a plant, is absorbed and translocated throughout the plant—has been used successfully by entomologists and plant pathologists in the control of some insect pests and fungus diseases.

A step toward chemotherapy as a control of nematodes has been taken with some grafting experiments now in progress. For example, by cross-grafts of resistant and susceptible plants it should be possible to determine whether a resistant top would confer resistance on a susceptible rootstock and whether a susceptible scion would cause a breakdown of the resistance of a rootstock on which it was grafted. The plants used in these experiments are of the genus *Beta,* domestic sugar beets which are susceptible to the sugar-beet nematode—*Heterodera schachtii*—and wild beets which are resistant to the sugar-beet nematode. The experiments are not yet completed, but the results to date with the wild beet—*Beta patellaris*—and the domestic sugar beet indicate that the rootstocks behave as the parent plant irrespective of top. The resistance and susceptibility appear to be properties of the root system of the plants. This observation agrees with the results of an English worker with the tomato-black nightshade cross-grafts and the golden nematode — *H. rostochiensis* — a close relative of the sugar-beet nematode. In

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ORNAMENTALS

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An ectoparasitic pin nematode—*Paratylenchus* sp.—also feeds on carnation roots, but has not been found so destructive as the ring nematode in recent tests. On other crops and under other conditions it could be equally damaging.

A nematode constantly found associated with diseased camellias in California is the spiral nematode—*Helicotylenchus erythrinae.* It has been observed with its spear inserted into camellia roots, feeding. The plants not only make little or no growth but they often show dieback, and develop hairy balls on the root system—actually a proliferation of rootlets. The condition and its association with the nematode are under further investigation.

The cyst nematode—*Heterodera fici*—is interesting because it appears to have a limited host range, as do other members of its genus. It has been found only on plants of the genus *Ficus.* Ornamental rubber plants—*Ficus elastica*—throughout California often have large populations of this nematode feeding on the roots. The effect on the plant is unknown.

Two species of foliar nematodes—*Aphelenchoides fragariae* and *A. ritzemabosi*—cause damage to ornamental plants in California. *A. fragariae* is an important pest of the Croft lily causing yellowing and distortion of the foliage as well as stunting of plants. Control is by hot water treatments. This nematode also attacks a variety of other plants including birdsnest fern. It can be controlled by the use of nematode-free propagating material and nematode-free soil. *A. ritzemabosi* injures chrysanthemum, African violet, Peperomia, fibrous begonia, gloxinia and many other kinds of ornamentals. It can be controlled by the use of clean propagating material and nematode-free soil. On chrysanthemum, control has been obtained with parathion at the rate of one-fourth pound actual per 100 gallons of water applied at weekly intervals.

The stem or bulb nematode—*Ditylenchus dipsaci*—attacks and injures a wide variety of ornamental plants. In California it frequently does severe damage to narcissus causing necrosis in the bulbs as well as leaf galls that are commonly called spikkles. Control has been obtained by hot water-formalin treatment of the bulbs. The treatment consists of a presoak period of two hours in water at 75°F followed by a two-hour treatment in water at 110°F. One point of commercial formaldehyde is added per each 25 gallons of water.

Phlox, hydrangea and primula have been found infested with *D. dipsaci* in California. Control is obtained by the use of nematode-free cuttings or seed. The damage to these plants consists of swollen shortened stems and distorted leaves. Severe infestations sometimes kill young plants.

The reniform nematode—*Rotylenchulus reniformis*—and the burrowing nematode—*Radopholus similis*—are exotic parasites, found in California only in nurseries, and eradicated when found.

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Cross-grafts. Sugar beet / *B. patellaris* left and *B. patellaris* / sugar beet right. Note differences in root character above and below graft union.

further tests the same worker found that, in cross-grafts of susceptible tomato and resistant Peruvian wild tomato, the scions of the resistant plant did not reduce the susceptibility of the tomato rootstock exposed to the root-knot nematode—*Meloidogyne incognita.* The hypothesis—that susceptibility and resistance could be conferred, initially based upon a guess—may provide useful information. If the final results bear out the indications of the earlier experiments, investigation into resistance to the sugar-beet nematode can be pursued by using chemical supplements in aseptic tissue cultures of root portions.

Another phase of the approach through the plant is exemplified by recent studies in Canada on pathogenicity of the root-lesion nematode — *Pratylenchus penetrans*—on peaches. Small peach seedlings were grown in cultures free of all organisms except the added root-lesion nematode. The characteristic injury of a darkened necrotic area around the point of entry into the root was demonstrated to be a response to the nematode attack. Further work provided evidence that the nematode secreted enzymes which could hydrolyze amygdalin, the bitter constituent of peach pits and almonds. The products of the hydrolysis are toxic to the plant cells, which die and give rise to the dark lesions. The hydrolysis and its toxic products could be the mechanism by which this nematode injures peach, cherry, apple, almond and other plants that possess amygdalin, but the root-lesion nematode also injures host plants that do not contain amygdalin. Therefore, the story is not complete, and awaits further studies.

The root-knot nematode also induces a characteristic response in host plants by causing knots, or galls, on the roots. Gall formation is a complex phenomenon involving the formation of giant cells around the head region of the nematode as well as some cell proliferation and enlargement. Experiments by a Russian worker provided evidence that some of the cell enlargement can be accounted for by osmotic changes as a result of hydrolysis of starch and proteins in the cells. The hydrolysis results in more molecules in the cell, which then absorbs water and swells. In other experiments, an alcoholic extract of galls dried, made up in gelatin and applied as a small block to a healthy root, produced swelling at the point of application. Though galls contain more amino acids than normal root tissue, and some amino acids promote cell enlargement, other agents may be involved in this swelling.

Based on oxygen consumption, there is a threefold increase in respiration of gall tissue over normal root tissue and probably an increase in the general metabolic rate. A number of things occur in the process of gall formation, one of which may become of practical interest and, perhaps, provide a foothold toward chemotherapy.

The second approach to fundamental study of the nematode problem involves the nematode itself. Some phases of this study are closely related to studies of plant response. For example, the hydrolysis of the starch and proteins of plant cells is brought about by secretions of the nematodes. If certain nematodes are stored in water for a period, then separated, the water is believed to contain enzymes that hydrolyze cellulose and chitin. Cellulose is a common constituent of plants, so the cellulose hydrolyzing enzyme can serve the nematode in its attack and movement through the plant. Chitin is not a normal constitutent of plants and the function of the chitin hydrolyzing enzyme is unknown.

Comparatively little is known about the nematodes themselves. For example, if 30–40 million worms like the stem or bulb nematode—*Ditylenchus dipsaci*—that attacks garlic, onion and alfalfa are dried, they weight about one gram, 10% of which is carbohydrate and 40% protein. Of this one gram, about one third is soluble in fat solvents, two fifths is soluble in water and about one fourth is soluble in neither. Nematodes have a cuticle that is layered, and it is known that for certain nematodes some amino acids usually present in proteins are absent from the cuticle. The functions of some of the internal organs and glands and what chemical reactions take place are not fully known but must be known before differences between the pest and the plant can be found and those differences used in control measures.

The third approach to the problem is through the soil, the linking medium between pest and plant.

How nematodes in soil get to the roots of plants is under investigation. There have been indications that roots of plants give off chemical substances which diffuse through the soil and nematodes are attracted by these chemicals and follow them to the roots—a process known as chemotaxis. If nematodes do find the roots in this way, it may be possible to do something to interrupt the process of attraction. If the bottom of a small dish is covered with a layer of wet sand and nematodes are added at the center and a germinating seed to one side, more nematodes are soon found on the seedling side. However, many nematodes remain in the clean sand opposite the seedling. The experiments have not been completed, but it begins to look as if this effect is not the whole explanation and there are more things involved.

Another event which takes place at long range, as does chemotaxis, is referred to as the hatching response. This effect appears most prominent with the cyst-forming nematodes, but has been observed with other types, for example, the root-knot nematode. Work in Europe and elsewhere, with the golden nematode—the cyst-forming relative of the sugar-beet nematode found in California—considered such things as soil type, temperature, moisture, aeration, hosts, and so forth for effects on hatch, but the isolation of the active material has not been accomplished.

A similar program with the sugar-beet nematode was initiated recently at Davis. It has been necessary to develop methods for obtaining and purifying large numbers of cysts and to learn how best to store them. It has been necessary also to find the best source of hatching factor that can be obtained on a large scale.

After this preliminary work a reliable routine system of assay needs to be established to measure the hatching activity of many samples.

The hatching factor is of considerable interest. What can stimulate a resting larva to continue its development is unknown. This phenomenon is not unique; it has been observed with other organisms. A knowledge of the mechanism involved in this response will be of help in leading to a better understanding of these pests.

Among the several aspects of nematode physiology and biochemistry, there may be found information of far-reaching importance in the problems of nematode control.

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Alexander Fleming, British bacteriologist, discovered penicillin in 1928.