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PRINCIPLES OF PLANT AND ANIMAL PEST CONTROL

VOLUME 4

Control of Plant-Parasitic Nematodes

SUBCOMMITTEE ON NEMATODES
COMMITTEE ON PLANT AND ANIMAL PESTS
AGRICULTURAL BOARD
NATIONAL RESEARCH COUNCIL

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Volume 1 Plant-Disease Development and Control
Volume 2 Weed Control
Volume 3 Insect-Pest Management and Control
Volume 4 Control of Plant-Parasitic Nematodes
Volume 5 The Vertebrates That Are Pests: Problems and Control
Volume 6 Effects of Pesticides on Fruit and Vegetable Physiology

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Foreword

The objective of the project on Plant and Animal Pest Control was to outline, for each of the several classes of pests, the principles of control where these are established; to call attention to effective procedures where true principles are not yet established; and to indicate areas of research that appear to warrant early attention. The reports are not intended to be textbooks in the usual sense, nor encyclopedias, but are intended to deal with basic problems, the principles involved in controlling pests, and the criteria that should be considered in conducting research and in evaluating published information. Specific instances of control practices are cited only to illustrate principles and procedures. It is hoped that these reports will be useful to researchers at all levels, to pest-control agencies, to administrators seeking guidance on priorities for application of resources, and to general field workers in the United States and elsewhere.

The National Academy of Sciences selected a committee of outstanding scientists to represent the diverse aspects of the problem and assigned to them responsibility for carrying out the project. To assist that committee, six subcommittees of specialists were appointed. Appropriate members of the parent committee were assigned as liaison members of the subcommittees, and in due time all reports were reviewed by the parent committee.

Some seventy scientists have collaborated over a four-year period to produce this series. Many others have contributed, to a lesser degree, in preparing statements and in reviewing and commenting on drafts of individual sections. Final responsibility for the content of these volumes rests with the parent committee. The Agricultural Board, under whose direction the Committee on Plant and Animal Pests operated, has reviewed and approved each manuscript.

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Preface

This volume considers the principles involved in control of plant-parasitic nematodes and points to research avenues that might lead to improved methods in the future. The biology of the organisms themselves and the environmental stresses to which they are subjected are included because this information is crucial to progress toward their control. We hope the material will prove useful to scientists trained in areas other than nematology who find themselves confronted with nematode-control problems as well as to students newly moving into this area of study and research.

Specific nematocides are cited only in support of general statements relative to control principles, and trade names are used only when there is no widely recognized common name or when specific reference is made to a given commercial product. The first time such a common or trade name appears in a chapter, the principal active ingredient is indicated. The abbreviations DBCP, EDB, and 1,3-D are used to designate the compounds 1,2-dibromo-3-chloropropane, ethylene dibromide, and 1,3-dichloropropene, respectively.

In preparing this material, sections were developed by individual Subcommittee members. The whole was then reviewed and integrated by the group collectively. Selected references are provided in literature citations following each chapter. The views of fellow workers were solicited and incorporated in the discussions, and we are greatly indebted to S. D. Van Gundy,

A. M. French, R. Mankau, and J. F. Spears, who served as consultants in this endeavor.

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Contents

PART I

INTRODUCTION

CHAPTER 1

The Science of Nematology

Although worldwide recognition of nematodes as important causal agents of plant diseases did not occur until the middle of this century, nematodes were studied in both the British Isles and Europe more than 100 years earlier. Four milestones mark these studies: in 1743, the first observation of a plant-parasitic nematode—the wheat gall nematode (*Anguina tritici*); in the 1850's, the discovery that a root-knot nematode (*Meloidogyne* sp.) caused galls on cucumber roots; recognition that the sugar-beet nematode (*Heterodera schachtii*) damaged sugar beets; and, shortly thereafter, the publication of the first comprehensive paper on free-living nematodes. A great deal of credit for the early progress of general nematology belongs to workers in Europe and the British Isles.

In the United States, limited attention was given to the study of nematodes during the early 1900's. Several significant discoveries during 1945–1955 accelerated the development of plant nematology as a separate discipline. These were the introduction of practical nematocides; the discovery of the golden nematode (*Heterodera rostochiensis*) in a major potato-producing region of the United States; the demonstration that the burrowing nematode (*Radopholus similis*) was the cause of spreading-decline disease of citrus in Florida; recognition of the serious damage caused by nematodes feeding at root surfaces; recognition of the many interactions between nematodes and other soil-inhabiting organisms in plant-disease complexes, including breakdown of disease resistance; and the discovery of the transmission of viruses by certain nematodes. Because of these and other developments, research in nematology received increased attention and financial support.

In the United States, early research in nematology was conducted by a few scientists, some trained in Europe, working in the U.S. Department of Agriculture and various experiment stations of the agricultural colleges. Many of the important contributions in nematology prior to 1950 were made by U.S.D.A. nematologists. Some experiment-station plant pathologists and entomologists trained themselves, almost unaided, to identify plant-pathogenic nematodes and to conduct research on the biology and control of important species. When the importance of nematodes to crop production was recognized and funds for both research and personnel were available, the research was handicapped by the scarcity of trained nematologists. To meet this need, teaching programs in nematology were developed or expanded, primarily within departments of plant pathology or entomology. Universities then employed nematologists to teach and to work cooperatively with plant pathologists and other biologists in solving many nematological problems. In 1940, the number of scientists engaged in plant nematology in the United States was less than 25; today it is more than 300.

Because of the introduction of undergraduate and graduate courses for the training of nematologists at several universities, the availability of textbooks emphasizing plant and soil nematodes, new research equipment and techniques, and regional cooperative research throughout the United States, progress in nematology should continue. The establishment of regional centers where nematologists could study all aspects of a particular phase of nematology would further accelerate progress.

The training of nematologists varies widely. In England and Europe, most undergraduate and graduate students receive instruction in broad fields such as mathematics, physics, chemistry, and biology rather than in nematology. At the postgraduate level, only a few formal courses in nematology are offered; most postgraduate students receive training on an informal basis, working with a nematologist at a laboratory.

Professional societies serve to bring scientists together for discussion and exchange of ideas. The founding of the Society of European Nematologists in 1953 and its subsequent growth resulted from the nematologist's need for contact and collaboration with others in the same field. To satisfy this need, the Society conducts a symposium every two years and issues a newsletter twice a year.

Nematologica, an international journal of nematological research, includes research papers on nematodes of agricultural importance, articles on free-living nematodes, and general papers on morphology, taxonomy, ecology, and physiology.

In all countries, and particularly in the United States, the role of the societies is important to nematologists, because of the fragmentation of the subject matter among other disciplines. In 1910, five scientists founded the

Helminthological Society of Washington. The Society publishes its Proceedings, in which many important research papers in all areas of nematology appear. By tradition, the study of nematodes parasitic to man and animals is called "helminthology," and the study of plant-parasitic and free-living nematodes is called "nematology."

Papers, symposia, and informal discussions on nematode diseases of plants have been presented for many years at annual meetings of the American Phytopathological Society. Numerous papers on this subject are published in *Phytopathology*, the official journal of this society, first issued in 1910.

In 1962, the Society of Nematologists was formed as an outgrowth of the American Phytopathological Society. Membership is open to anyone interested in any phase of nematology. The Society publishes a newsletter, will publish the first volume of the *Journal of Nematology* in 1969, and holds annual meetings at which papers representing many aspects of nematology are presented.

Although important contributions in nematology are coming from laboratories in numerous countries, greatly expanded research programs on all kinds of nematodes are urgently needed. Rapid development of this science, based on worldwide cooperation, would bring immeasurable benefits to all peoples of the world.

CHAPTER 2

Nematodes and Their Importance to Man

Nematodes are probably the most numerous multicellular animals in the world. They escape notice because most kinds are so minute that they cannot be seen without the aid of a microscope. However, not all nematode species are small. One, parasitic in whales, is comparable in length to a 25-foot section of garden hose. Nematodes abound everywhere and are found in nearly every biological niche that will support life—including deserts, the ocean bottom, Antarctic ice, and hot springs. Some of the soil-inhabiting nematode species feed on microorganisms such as bacteria, fungi, algae, and other nematodes. Many soil-dwelling species feed only on higher plants. Both insects and higher animals have nematode parasites. Many of the animal parasites are several centimeters long and are easily detected. Some of them cause man great physical discomfort and debilitation. For these reasons, the animal parasites were the first nematodes to be recorded and studied. Domesticated animals suffer similar parasitism and debilitation, with resultant indirect losses to man.

PLANT-PARASITIC NEMATODES IMPORTANT TO MAN

In addition to the nematode parasites of man and higher animals, there are many species that parasitize our food and fiber crops, thus reducing supplies of both throughout the world. The earliest records of plant-parasitic nematodes were published in the mid-1700's. At that time, nematodes were scientific curiosities that were used to explore the capabilities of the microscope, which Leeuwenhoek had recently developed. During the next century, a great deal of effort was directed toward the application of science to agriculture,

especially in Europe. One of the results of this effort was the discovery that certain plant-parasitic nematodes, such as the wheat nematode (*Anguina tritici*) and the sugar-beet nematode (*Heterodera schachtii*), often were the principal limiting factors of the growth of crop plants.

Until the 1940's, knowledge of other nematode species important to agriculture accumulated slowly. The chemical industry then introduced the soil fumigants 1,3-D (principal active ingredient 1,3-dichloropropene) and EDB (ethylene dibromide), which reduced nematode populations in soils at much less expense than was previously required. Improvement of plant growth and yield following the use of nematocidal soil fumigants led to rapid grower acceptance of the importance of nematodes to agriculture. Only a decade after the introduction of these soil fumigants, 5 percent of all pesticide expenditures were for such materials. Improved plant growth following soil fumigation does not prove nematode pathogenicity, but these chemicals are far more effective as nematocides than as bactericides or fungicides. Plant-growth responses following the use of nematocides indict nematodes by associating them with poor growth. Further means for testing nematode pathogenicity are discussed in Chapter 5.

Virtually every crop has its complement of nematode parasites. More than 150 nematode species are being studied to determine their role in plant disease, and many new nematode parasites are discovered every year. In experimental studies, it has been found that plant weight is usually inversely proportional to the number of pathogenic nematodes added to the soil around the roots of plants. This relationship varies with the particular crop and nematode and is influenced by environmental factors such as fertility, moisture, temperature and soil type. Given an adequate food supply and proper environment, nematodes, like other organisms, increase logarithmically. Perennial crops provide a constant food supply and hence are especially vulnerable to nematode damage. Similarly, annual crops grown as a monoculture intensify nematode problems. As pathogens, nematodes affect crop yield and quality or both. They limit the utilization of nutrients by plants, thus causing waste of fertilizers. They predispose perennial plants to winter injury. Nematode-infected plants wilt more readily than noninfected ones, which necessitates more frequent irrigation. Certain nematode species act as vectors for pathogenic viruses. Others alter the physiology of their host so that it becomes more susceptible to fungal diseases, or they provide avenues of entry for pathogenic bacteria. Unrecognized nematode infestations confound and often totally negate experiments designed to study other factors that limit plant growth.

Quarantine actions by federal, state, and local agencies against such well-known nematode pathogens as the golden nematode of potato (*Heterodera rostochiensis*) in New York, the burrowing nematode of citrus (*Radopholus*

similis) in Florida, and root-knot nematodes (*Meloidogyne* spp.) and lesion nematodes (*Pratylenchus* spp.) in California have acquainted many growers, and segments of the general public, with plant-parasitic nematodes and the damage that they cause.

PRINCIPAL CHARACTERISTICS OF NEMATODES

Nematodes are roundworms that are bilaterally symmetrical, mostly microscopic in size, but complex in organization, possessing all the major physiological systems of higher animals except respiratory and circulatory systems. Species parasitizing man and higher animals vary in length from 2 to 300 cm. Plant-parasitic species are comparatively small, ranging from 0.5 to 3 mm and 0.01 to 0.5 mm in width. Most nematodes are cylindrical and slender, tapering toward the head and the tail (Figure 1), but females of some of the plant-parasitic species assume varying forms, such as pear, lemon, or kidney shapes. Most soil-inhabiting nematodes are semitransparent. With a compound light microscope and an oil-immersion objective, sufficient anatomical detail can be seen to identify nematode specimens to species. Increasing use of electron microscopy promises to add greatly to information about nematodes.

The nematode body lacks internal segmentation and is covered with a multilayered cuticle which has various surface markings. Underlying and inside the cuticle is the hypodermis, which is a thin unicellular layer. The hypodermis protrudes into the body cavity ventrally, dorsally, and laterally, producing longitudinal ridges called chords.

Nematodes possess two types of muscles—the somatic muscles (Figure 1) and specialized muscles. The somatic muscles occur as a layer of longitudinal cells underlying the hypodermis between the chords. Specialized muscles are connected with the stylet, esophagus, intestine, and reproductive organs.

The major center of the nervous system, called the nerve ring, surrounds the esophagus in the region of the isthmus, where associated ganglia also are concentrated. Somatic nerves are contained in the hypodermis, and they connect with sensory organs in the head region and with the esophagus, intestine, and reproductive systems.

The excretory system usually opens to the exterior by a pore that is located ventrally at about the level of the nerve ring. Leading to the pore is a duct that is often lined with cuticle. This duct connects with tubules that extend most of the length of the body and may be free in the body cavity or contained in the lateral chords.

The digestive system of nematodes is tubular and is divided into three main regions: esophagus, intestine, and rectum. The anterior oral opening is terminal and is usually surrounded by various types of lip structures and sensory organs (papillae).

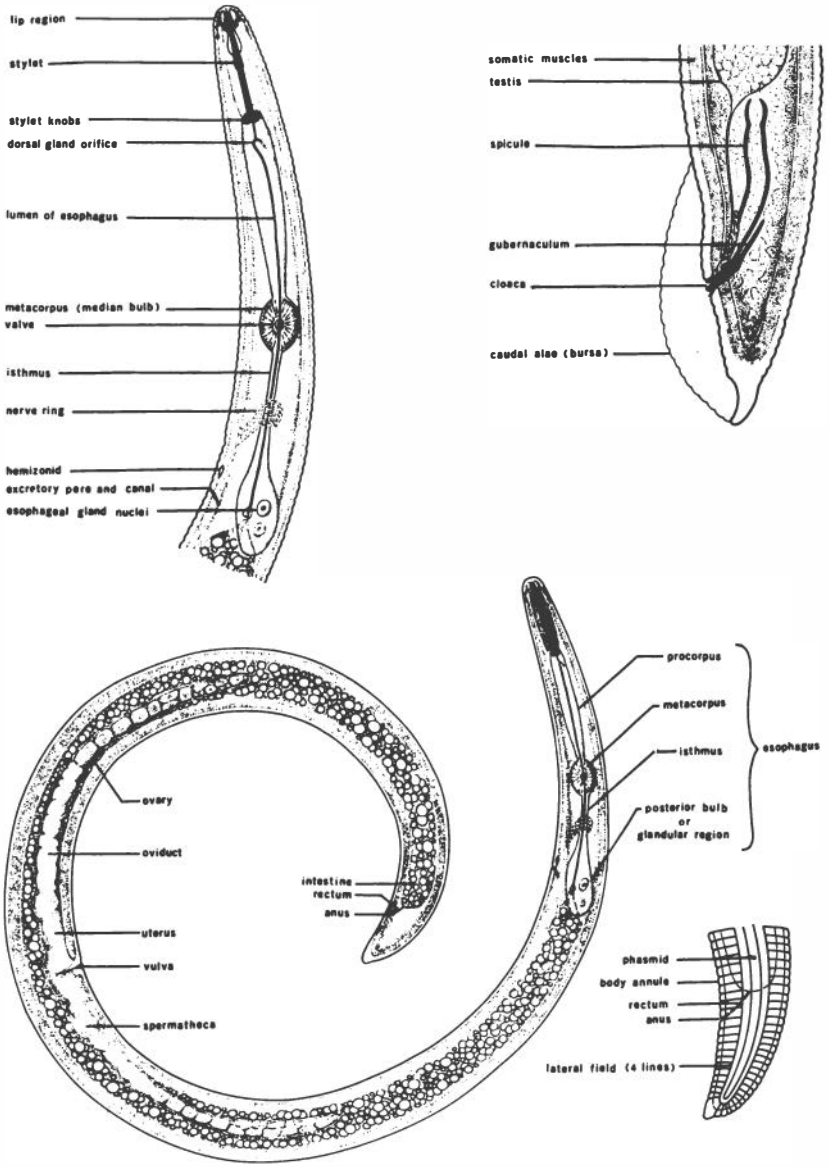

FIGURE 1 A typical plant-parasitic nematode, *Rotylenchus breviglans* Sher, 1965. (Courtesy of the Department of Nematology, University of California, Riverside.)

Plant-parasitic forms possess a stylet, which is usually hollow and is used for piercing and feeding on plant cells. Most plant-parasitic nematodes are members of the taxonomic order Tylenchida, a group characterized by a three-part esophagus. In most species of the Tylenchida, the esophageal region behind the stylet (the procorpus) is slender, and the midregion (metacorpus or median bulbar region) is swollen and equipped with a cuticularized valvular apparatus, which appears to function as a pump. Behind the metacorpus, the esophagus narrows to a slender isthmus and ends in a glandular region. There are usually three esophageal glands, one dorsal and two subventral, although there may be as many as six in some nematodes, such as the genus *Hoplolaimus*. In two groups of the order Tylenchida (superfamilies Tylenchoidea and Criconematoidea), the two subventral glands open into the lumen of the esophagus near the valve in the median bulb, and the dorsal gland opens into the lumen farther forward, just posterior to the stylet. In the third superfamily (Aphelenchoidea), all three esophageal glands open into the lumen near the esophageal valve.

Some plant-parasitic nematodes, and all that are known to transmit viruses, belong in another order, the Dorylaimida. Nematodes in this order have a two-part esophagus consisting of a slender anterior region that leads to a shorter, swollen glandular region. Some of the nematodes in this group, such as dagger nematodes (*Xiphinema* spp.) and needle nematodes (*Longidorus* spp.), have hollow stylets, but others (stubby-root nematodes, *Trichodorus* spp.) have a toothlike stylet (onchiostyle).

The intestinal wall is composed of a single layer of cells, which are determinate in number. The intestine ends in a constricted region that leads to the prerectum, then the rectum, and finally opens to the outside by a ventral, subterminal, or terminal anus in the female or by a cloacal opening in the male.

Male and female nematodes are usually similar in appearance except for the reproductive systems. However, pronounced dimorphism occurs in some species: females become swollen, and males remain slender and cylindrical. Reproduction without males is common, and in some species females produce both spermatozoa and eggs. Usually, the females have one or two tubular ovaries, a spermatheca, oviduct, and uterus. Eggs are deposited through a slitlike opening called the vulva. The vulva is usually ventral and subterminal to midway in the body, but it may be terminal. Males usually have one testis, rarely two; two cuticularized spicules; and a gubernaculum or guiding apparatus. They may have caudal alae, which are lateral extensions of the cuticle in the region of the spicules and are thought to be clasping organs used in copulation. The male intestine joins the reproductive system posteriad, forming a true cloaca.

LIFE CYCLE

There is no true metamorphosis in nematodes. The young are smaller, but in other respects they generally resemble adults, and they could correctly be called juveniles. However, the term "larva" is firmly entrenched by usage in nematological literature. There are five stages in the life cycle. Inside the egg the developing embryo grows, elongates, and differentiates to become the first-stage larva. In most nematode species, the first-stage larva continues to develop and molts to the second stage while still within the eggshell. The second-stage larva usually emerges from the egg. The nematode then feeds, develops, and molts twice more while passing through the third and fourth larval stages to become a fully developed adult. There are exceptions to this cycle. The larvae of certain animal parasites develop to the third stage before emerging from the egg. Larvae of *Xiphinema index* and certain *Rhabditis* spp. are reported to emerge from the egg before the first molt. At the end of each larval stage, the cuticle is shed, including its extensions into the oral opening, excretory pore, vulva, and the rectum or the cloaca.

Based on their life habits, the plant-parasitic species can be classified in two groups. The soil inhabitants normally complete the entire life cycle in the soil, in or about the roots of plants. The aboveground parasites may begin their cycle on the soil or in the shallow surface layers, often in host-plant residues; but when suitable host plants develop and favorable conditions prevail, the aboveground parasites ascend the plant or attack the growing seedlings and mature aboveground. Trunks, stems, petioles, leaves, flowers, and seeds are known to be attacked.

SOIL INHABITANTS

Ectoparasitic Species

Some nematode species spend their entire life cycle free in the soil, feeding externally on the roots of host plants. They usually stop feeding and detach themselves when the roots are disturbed. Eggs are deposited in the soil. Examples of this kind of parasite are species of ring nematodes (*Criconemoides* spp.), pin nematodes (*Paratylenchus* spp.), and stubby-root nematodes.

Other species, such as a sheath nematode (*Hemicycliophora arenaria*) and the walnut nematode (*Cacopaurus pestis*), are similar to the above nematodes but differ in that attachment of the adult female to the host root is more permanent. These have long stylets that penetrate deep into the root. In most cases, these nematodes are essentially sedentary after attachment to the root.

Endoparasitic Species

Some endoparasites, such as the lesion nematodes, are migratory in the cortical parenchyma of host roots. They move through the root tissues, feeding on cells, multiplying, and often causing necrosis of root tissues. Overwintering occurs in the roots, or in soil about the roots, without any known special mechanism for protection from adverse conditions.

Other endoparasitic types penetrate rootlets as second-stage larvae, become sedentary before molting, and remain through the remainder of the life cycle. Eggs are deposited in a matrix, as in the case of root-knot nematodes, or may be retained inside the body of the female, as in the cyst nematodes (*Heterodera* spp.). The cuticle of the cyst nematodes undergoes chemical changes and becomes a durable, brown, so-called cyst that protects the eggs from adverse conditions. Most larvae remain inside eggshells within the cyst until favorable conditions occur, finally emerging to wander free in the soil and to find new infection sites.

Intermediate forms are found in *Tylenchulus* and *Rotylenchulus*, in which younger stages feed ectoparasitically and later stages penetrate host tissues. The posterior end of the body remains outside the root and becomes swollen and reniform in shape. Eggs are deposited in a gelatinous matrix. Second-stage larvae hatch from the eggs, and the cycles are continuous.

ABOVEGROUND PARASITES

Bud and Leaf Nematodes (Aphelenchoides *spp.*)

The bud and leaf nematode (*Aphelenchoides ritzemabosi*), parasitizing chrysanthemum, is an example of a nematode that is endoparasitic in leaves. Individuals enter leaves through stomata and live in intercellular spaces. Development is continuous, and all stages occur together. Nematodes also live in dormant buds or in growing points in crowns of plants, and they may be found in all stages of development in dead leaves on or in the ground. They may survive, quiescent, in dried leaves for at least two years and then revive when the leaves are moistened.

The spring crimp nematode of strawberry (*A. fragariae*) typifies ectoparasitism in buds. The nematodes survive adverse conditions in the soil or deep within developing buds. When a film of moisture is present on the plant, development is continuous, and the nematodes remain outside the plant tissues feeding ectoparasitically on epidermal tissues near the growing points of buds.

Seedgall Nematodes (Anguina *spp.*)

The wheat nematode (*A. tritici*) will serve as an example. Quiescent second-stage larvae survive within seedgalls that drop to the ground at harvest or that are harvested with the healthy grain. Up to 70,000 larvae per gall have been reported. When galls are moistened, the larvae emerge and migrate to the growing points of developing grain seedlings. Here they feed ectoparasitically, protected within the leaf sheaths. When seed primordia begin to form, the larvae enter the embryo and become endoparasitic. Within the primordial tissues, the larvae grow rapidly and molt several times to become adults. After mating, the females deposit eggs inside the transformed seed tissues. These eggs develop and hatch so that second-stage larvae remain free inside the gall. There is only one cycle per year.

Stem Nematode (Ditylenchus dipsaci)

This species has a life cycle similar to that of the chrysanthemum foliar nematode, except that it invades stems as well as leaves. Development is continuous, and several life cycles are completed in one season, but the principal stage that overwinters or survives unfavorable environmental conditions is the fourth-stage or preadult larva. This stage is exceptionally resistant to drying and to chemical toxicants.

SIGNIFICANCE OF LIFE HABITS

Life histories of nematodes should be understood when control measures are considered. The wheat nematode may be effectively controlled by crop rotation with nonhost plants, because the emergence of larvae from the galls is virtually complete when soil moisture and temperature become favorable. Larvae die when they are outside the gall in the absence of the host. Control of certain cyst nematodes by crop rotation, however, is much more difficult. These nematodes remain encysted in the absence of a host. They can survive for long periods in this state. The protection afforded endoparasitic nematodes by roots often makes them more difficult to control than ectoparasites.

GEOGRAPHIC DISTRIBUTION

Plant diseases caused by nematodes have been found in every country and region where nematological investigations have been conducted. It is difficult

to find data from which the origin of the various economic nematode pests can be deduced. Many species occur wherever their host crops are grown and appear to have been spread as the culture of the crop spread. The sugar-beet nematode is one of many species that appears to have been spread in this manner. A few nematodes appear to be native parasites of wild plants but have become adapted to cultivated crops when these were grown in the area; the false root-knot nematode (*Nacobbus batatiformis*) in Nebraska and adjoining states is probably such a nematode. Water and, in some cases, wind are responsible for local spread of nematodes, but long-range distribution is largely by man's movement of plants and soil.

Are nematode diseases increasing, decreasing, or being contained? Current discoveries often reflect the initiation or extension of surveys and investigations rather than new introductions. There is usually a lag of several years between the time of infestation and the time when the nematode population reaches a detectable level. For these reasons, the question is difficult to answer with precise data. We believe nematode diseases are increasing because of spread by man as well as man's tendency to monoculture plants. In recent years, the spread of nematode diseases has been accompanied by development of a variety of methods of prevention of nematode disease. In general, these methods are being used only in countries with an advanced agricultural technology. Countries with greatest food needs are doing the least and are the most poorly equipped to control nematodes. In the future, the extension of nematode-control methods to new areas and the development of improved methods will be important means for increasing the world food supply.

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PART II

FACTORS INFLUENCING NEMATODE CONTROL

CHAPTER 3

Ecological Relationships

Knowledge of the ecological relationships between plant-parasitic nematodes and their environment is important for understanding some of the principles of nematode control. Agricultural land represents a specialized environment, ranging from a dry, barren waste to a moist, lush jungle of plant growth. Plant-parasitic nematodes are mostly those soil-inhabiting species that are capable of withstanding the frequent changes caused by man's agricultural practices. Some of these nematodes, such as species of spiral (*Helicotylenchus* spp.), stunt (*Tylenchorhynchus* spp.), and sheath (*Hemicycliophora* spp.) nematodes, can live in a wide variety of habitats. Some, such as the rice nematode (*Hirschmanniella oryzae*) in aquatic habitats, are widely distributed but are limited to particular combinations of environmental conditions. Still others, such as sting nematodes (*Belonolaimus* spp.) in the sandy soils of the southeastern United States, are found only in very special situations. Therefore, it is difficult to recommend control practices for such diverse kinds of nematodes without first knowing how they live and survive in the soil and in host plants.

VERTICAL DISTRIBUTION OF NEMATODES

The vertical distribution of nematodes in cultivated soil is usually irregular but is generally closely related to the distribution of plant roots and the area adjacent to roots, which is called the rhizosphere. Since the movement of nematodes in the soil by their own activities is limited at most to a few feet per year, it is obvious that the number of plant-parasitic nematodes is greater

in soils containing plant roots than in soils without plant roots and is correlated with the distribution of roots of present and previous hosts. Nematodes are mainly concentrated in the top foot of soil, and as many as six billion have been estimated in the top inch of an acre of soil. Little information exists on the distribution of nematodes deeper than 1 foot in soil; however, a root-knot nematode (*Meloidogyne incognita*) has been found at a maximum depth of 17 feet in grape vineyards. Consequently, in a chemical treatment the soil must be treated to a greater depth for deep-rooted than for shallow-rooted crops, thus requiring more chemical and more expensive equipment. The fallowing of soil for an adequate period will generally reduce the number of nematodes present. Plant-parasitic nematodes survive longer in the absence of food sources than most nonparasitic nematodes. The cyst nematodes (*Heterodera* spp.), the most persistent of the plant-parasitic nematodes, decline in soil at a steady rate of 35 to 60 percent per year, depending on soil type, moisture, and temperature, regardless of the density of the initial population per unit of soil. Many questions remain unanswered concerning the influence of soil type, moisture, aeration, and other factors on distribution and the response of nematodes to them.

NEMATODE SURVIVAL

Plant-parasitic nematodes are able to survive despite unfavorable conditions such as cold and dry periods between host crops. Except in the tropics and heated areas, such as greenhouses, they do not grow and reproduce throughout the year. Nematodes survive unfavorable environments in a dormant condition, which is a quiescent or inactive state that is often associated with a lowered metabolic rate. The length of the quiescent period is usually limited by the amount of food reserves in the nematode and the environmental conditions. Quiescence may serve to extend a comparatively short life cycle of 20 to 40 days to periods varying from a year for many plant parasites to 20 to 30 years for such nematodes as the stem nematode (*Ditylenchus dipsaci*) and the wheat nematode (*Anguina tritici*).

NEMATODE POPULATIONS

In agricultural soil, the upper population limit for any plant-parasitic nematode species depends on the nematode's reproductive potential, the host-plant species, and the length of time the nematode remains in an environment favorable for reproduction. Generally, the reproductive potential of the specialized endoparasites and aboveground parasites is greater than that of

many of the ectoparasites. Some nematodes have only one or two generations a year, while others have several generations during the growing season. The latter include such important nematode pests as root-knot and cyst nematodes, lesion nematodes (*Pratylenchus* spp.), and the citrus nematode (*Tylenchulus semipenetrans*). The population level of each of these nematodes is dependent on the nematode's ability to live successfully in soil.

The importance of a nematode as a plant parasite depends largely on whether or not the population limit exceeds the level at which economic damage occurs to a crop plant. This concept of population threshold (Figure 2) at which yield loss begins is often used in connection with crop pests to determine the tolerance level of a host crop. For any set of environmental conditions, each host crop has its own tolerance level for a nematode species. Thus, a nematode causes economic damage only if its population density exceeds the tolerance level of the plant grown in the field. For example, the population density of root-knot nematodes is generally much higher than the tolerance level of many host plants, thus accounting for their importance as a nematode pest. In crop rotation, the cultivation of good host crops is alternated with poor or nonhost crops. Estimates of thresholds vary with seasons and from field to field. A clear understanding of nematode populations is important to nematode control.

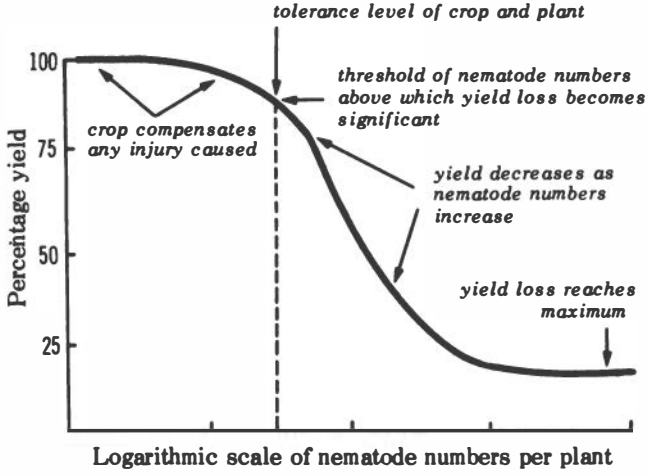

FIGURE 2 A diagrammatic relationship between plant-parasitic nematode populations and crop loss. (After Jones, 1965.)

THE SOIL ENVIRONMENT

All plant-parasitic nematodes inhabit soil for varying lengths of time during their life cycles (Figure 3). For example, the ectoparasitic nematodes spend their entire lives in the soil, usually in the rhizosphere of the plant. The more specialized endoparasites enter plant tissue and thereby spend less of their lives in the soil and rhizosphere. The aboveground parasites are mostly inside plant tissues and spend very little of their lives in the soil. Due to the nematode's life habitat in the soil, it is easier to control ectoparasitic than endoparasitic nematodes.

The principal factors in the nematode's soil environment are temperature, moisture, texture, aeration, and the chemistry of soil solution. Only in the

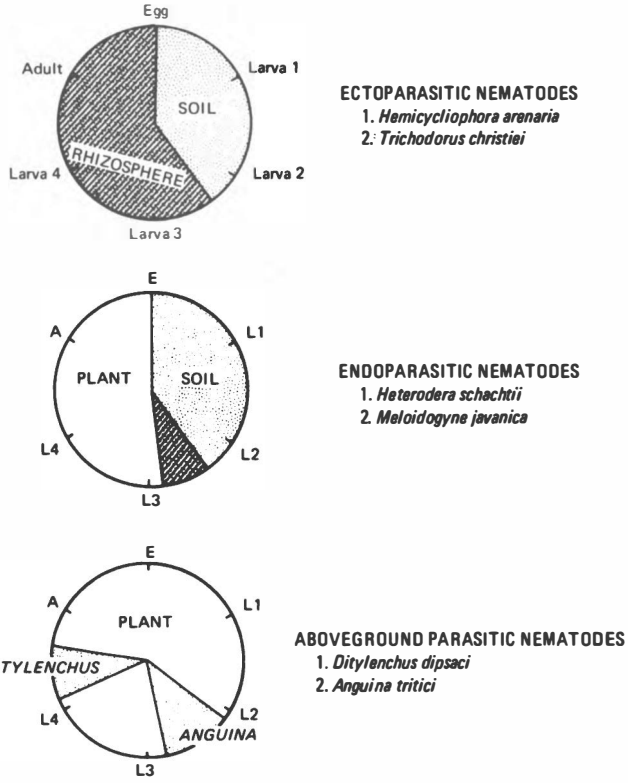

FIGURE 3 The relationship between parasitic habits of some nematodes and the portion of their life cycle spent in the soil, plant, and rhizosphere.

laboratory is it possible to investigate this complex, constantly changing environment; yet, from laboratory data it is difficult to relate varied factors such as nematode distribution, population levels, and pathogenicity to any one factor. Results of field-population studies are necessary to determine the influence of the interdependent and interacting environmental factors.

TEMPERATURE

Temperature affects nematode activities such as hatching, reproduction, movement, development, and survival and also affects the host plant. Most plant-parasitic nematodes become inactive at a low temperature range of 5 to 15°C, have an optimum range of 15 to 30°C, and become inactive at a high temperature range of 30 to 40°C. Temperatures outside these extremes may be lethal. Little information exists on the effect of constant or alternating temperatures on specific activities of individual nematode species. The Javanese root-knot nematode (*M. javanica*) is of little concern in the northern states, where it does not overwinter out-of-doors in deeply frozen soils, but the northern root-knot nematode (*M. hapla*) overwinters and may be a serious pest in these areas. Temperatures, however, do not limit the establishment of some nematodes: the sugar-beet nematode (*Heterodera schachtii*) is a serious pest in the north as well as in the south, where the soil temperature may exceed 35°C. The less adaptable stem nematode is restricted to cool climates or to warm climates where the host is winter-grown.

Determining the influence of temperature on nematode reproduction in plants is complicated, because temperature influences the growth of the plant itself. Changes in plant growth produce corresponding changes in root morphology and physiology. Temperature partially determines the choice of crop plantings and rotations. In areas of the United Kingdom, Europe, and the United States, some varieties of potatoes and sugar beets are grown in early spring, when the soils are too cold for reproduction of the potato-cyst and sugar-beet nematodes but are not too cold for the growth of the plants. Protection from nematodes during the early part of the growing season reduces nematode damage at harvest.

MOISTURE

Fluctuating soil moisture due to rainfall or irrigation is one of the chief factors influencing nematode-population increases. Dry soil conditions may depress populations of a ring nematode (*Criconomoides xenoplax*), a dagger nematode (*Xiphinema americanum*), and root-knot and cyst nematodes. Although dry

conditions may depress nematode activity and resulting populations, all nematodes may not be killed. Eggs of most nematodes as well as certain other nematode stages, such as preadult stages of pin nematodes (*Paratylenchus* spp.), survive drying. Dry fallowing of field soils may not be a practical control measure except in some hot, dry regions, where it reduces the numbers of nematodes so that a profitable crop can be obtained.

Saturated soils are not generally favorable for nematode pests of agricultural crops. In tropical rain belts and in flooded fields, populations of some species of root-knot, cyst, stunt, and pin nematodes have been reduced by excess water, lack of oxygen, and toxins of anaerobic organisms. However, high populations of some nematodes, such as species of *Dolichodorus*, *Radopholus* and *Hirschmanniella*, are found chiefly in wet locations.

It is thought that nematodes are constantly active in soils that have a moisture content of between 40 and 60 percent of field capacity. In dry and wet soils they are quiescent for varying periods. Nematodes need free water films in the soil for hatching and movement, but the influence of moisture on the nematode is little known. Since the interrelationship of soil moisture and soil structure is responsible for the aeration properties of the soil, the oxygen level may be the fundamental factor influencing some activities of nematodes. As soil moisture increases, soil aeration decreases, so that soils become low in oxygen immediately after heavy rains, flooding, or irrigation. From studies of a few plant-parasitic nematodes, it appears that individual nematodes are capable of fermentative as well as oxidative metabolism, which enables them to survive for varying periods of time without oxygen. Low levels of oxygen may induce quiescence and enable nematodes to survive. Growth and development of nematodes, which are important in determining population levels, are oxygen-dependent; therefore, high populations are usually found in moist, well-aerated soils.

SOIL TEXTURE AND STRUCTURE

Soil texture describes the sizes of soil particles. A coarse-textured soil usually contains a high percent of sand and has large pores that drain more quickly than the small pores of fine-textured soil, which has a high proportion of clay and silt. Because of the wide variation of the biotic, physical, and chemical environments within textural categories, it is difficult to generalize among soil type, nematode activity, and distribution. Many of the cyst, root-knot, lesion, and stubby-root (*Trichodorus* spp.) nematodes are found in large numbers in coarse-textured sandy soils. However, the stem, sugar-beet cyst, and some species of lesion and stunt nematodes are numerous in

clay soils. Still others, such as the citrus nematode, occur frequently in both sandy and clay soils.

The speed of nematode movement through soil is related to soil pore diameter, soil particle size, diameter of the nematode and its relative activity, and the thickness of the soil-water films. A nematode cannot move between soil particles when the pore diameters are less than the nematode width. As mentioned previously, soil structure, moisture, and aeration are interrelated. When the soil pores are full of water, a nematode moves inefficiently, and, when aeration becomes limiting, the nematode becomes inactive. In very dry soils, there is good aeration but not enough water to form films, so that the nematodes do not move. Only a soil of intermediate moisture has sufficient aeration and water films for efficient nematode movement.

SOIL SOLUTION

The chemical constitution of the soil solution, a major constituent of the soil environment, includes soil salinity, pH, organic matter, fertilizers, insecticides, and nematocides. Plant-parasitic nematodes probably derive few nutrients from the soil solution. The hatching of eggs and the survival of larvae may be influenced by various salts and ions. During dry and wet periods, soil nematodes are subjected to variable salt concentrations in the soil solution. However, nematodes can tolerate osmotic pressures up to about 10 atm, at least for short periods. This is considerably higher than the maximum 2 atm occurring in most agricultural soils. A soil pH ranging between 5.0 and 7.0 has little effect on nematodes. Lime, often used to neutralize soil acidity, causes no decrease in population. Fertilizers and organic matter may influence nematode populations indirectly by increasing host-plant growth. Occasionally, the use of nematocides and insecticides in soil may eliminate some nematode enemies, thus leading to an increase in population of a plant parasite.

CLIMATE

Rainfall and temperature above soil level are extremely important to the growth and development of both nematodes and plants. These factors are usually responsible for seasonal fluctuations in nematode populations and may even determine the success of a species in becoming established in a new habitat or region. Climatic factors affecting humidity are particularly important to aboveground parasitic nematodes, which are able to invade seedlings and move upward on plant surfaces covered by water films or droplets.

These nematodes may be subjected to severe desiccation and great extremes of temperature owing to the more violent changes in aerial climate compared with soil climate. Perhaps, as an adaptation to this, certain stages of these nematodes, such as second-stage larvae in wheat nematodes and fourth-stage larvae in stem nematodes, are capable of withstanding long periods of desiccation. Specific information on the influence of the microclimate of the plant surface on nematode activities is lacking.

THE PLANT ENVIRONMENT

The plant-host environment, consisting of either root or stem and leaf tissue, greatly influences the endoparasitic nematodes. The plant tissues that are usually attacked are apical meristems that contain cells with thin walls and offer a chemically rich environment. The epidermis and cell wall offer mechanical barriers to nematode entrance and movement. The plant tissue protects endoparasitic nematodes from the soil environment and is their sole source of food, and the quality and quantity influence nematode growth and reproduction. Thus, host susceptibility, tolerance, and resistance to nematodes are dependent on properties of individual plant cells and tissues. Much remains unknown about the nature of these factors and their effect on the nematodes. The periderm and necrotic areas, which are formed in some plants in response to nematode feeding, may affect nematode growth and reproduction, because the quality and quantity of nutrients are deficient in these areas, or the nematodes may be excluded from suitable plant cells by these areas. In plant parasites such as root-knot, cyst, and citrus nematodes the host cells are modified to provide specialized feeding sites, and their physiological and nutritional dependence on the host become delicately balanced. Recently, this intricately balanced system has been studied to try to find ways of controlling nematodes by the use of chemotherapeutic agents or antimetabolites to modify the host-plant environment to one unsuitable to nematodes. This area of research needs emphasis to gain an understanding of nematode nutrition and host-parasite relations and to aid in developing methods for systemic control of nematodes.

THE RHIZOSPHERE

In addition to serving as a source of food for nematodes, plant roots may also modify the soil environment by lowering the concentration of mineral nutrients, depleting moisture, increasing carbon dioxide, reducing oxygen, and contributing a variety of organic substances by exudation and sloughing off

of cells. The rhizosphere, the zone immediately around the plant roots, is a dynamic environment, where the relationships among nematode, host, and environment are often of a chemical nature. A root exudate stimulates the hatching of eggs of the cyst nematodes. Hatching is usually stimulated by chemicals from a wide range of plants, some of which are nonhosts. The composition of hatching chemicals is unknown, despite more than 20 years of research by numerous workers. The eggs of root-knot nematodes, as well as most other plant-parasitic nematodes, hatch freely in water; but in soil, plant-root exudates significantly increase the hatching of root-knot nematode eggs as compared with a water hatch. They stimulate the metabolism of larvae after hatching and may account for their directional movement toward plant roots. The exudates also influence the molting of preadult larvae of the pin nematode. Such examples of stimulation by plant roots appear to be a refinement of parasitism.

Root exudates and other chemicals may also inhibit egg-hatching or may repel nematodes. From a few observations, it appears that some plants, such as marigold, asparagus, and tobacco, produce chemicals that repel or even kill some species of nematodes. Little is known about the identity of these exudates and other chemicals, the nature of the reactions on nematodes, or the receptors in the nematodes. There is also evidence that nematodes may be repelled by small quantities of nematocides.

Microorganisms in the rhizosphere may significantly influence nematodes in several crops by antagonism, by competition for food and oxygen, or by excretions that may stimulate or inhibit nematodes. Research in this area should not be overlooked.

The ecological system that illustrates the complex interrelationships among plant-parasitic nematodes, plant, climate, and soil environment is summarized in Figure 4. Although the information now available is substantial, the interrelationships for any one nematode–plant combination are not completely understood. Comprehensive coordinated information on nematode activities, such as length and stage of life cycle, and mechanisms for nematode survival in unfavorable environments and in the absence of a host is limited.

A critical evaluation of vertical distribution of nematodes in soil, particularly at depths below 2 feet, is needed to determine the possible crop loss caused by these nematodes and the need for their control. Other areas needing ecological studies include host–parasite relations, mixed populations of plant parasites, the influence of other microorganisms in the rhizosphere, the influence of plant microclimate on aerial plant parasites, the influence of soil factors on population levels of plant parasites, and the application of nematode-hatching chemicals to infested soil. New methods, fresh approaches, and long-range programs aimed at developing integrated ecological concepts of plant-parasitic nematodes are necessary for progress toward more effective and economical control.

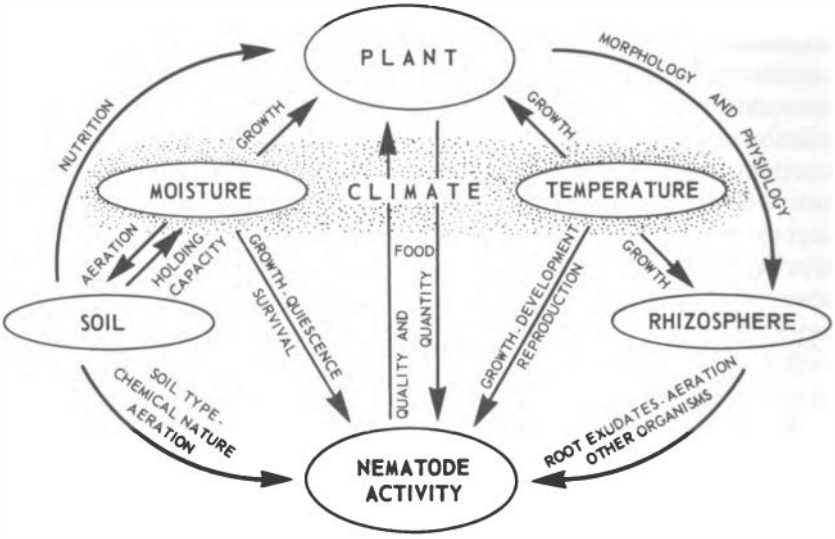

FIGURE 4 The ecological system, showing the complex interrelationships among plant-parasitic nematodes, plant, climate, and soil environment.

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CHAPTER 4

The Physiology of Nematodes in Relation to Control

Investigations of nematode metabolism and biochemistry, mechanisms of action of nematocidal agents, biochemical bases for plant resistance to nematodes, and nematode nutrition are increasing. Few basic studies on nematode physiology were conducted before 1950, and, even now, relatively few investigators are conducting research on such subjects. One reason for the limited research on many aspects of nematode physiology has been the difficulty of obtaining adequate quantities of specific plant-parasitic nematodes. While the information in this section may not bear directly on specific control measures, it may give insight as to why some of the commonly applied methods are successful.

CHEMICAL COMPOSITION

Nematodes, like other animals, contain carbohydrates, proteins, lipids, nucleic acids, vitamins, hormones, minerals, and numerous other chemicals, but not much is known of the precise kinds or amounts of these substances present in nematodes. Although the composition of animal-parasitic nematodes has received considerable study, it is doubtful if all these data are also applicable to the plant parasites. Chemical composition of nematodes, which affects longevity, degree of resistance to temperature extremes, desiccation, atmosphere, osmotic conditions, and chemicals, undoubtedly relates closely to the success of the various control measures utilized.

Glucose, fructose, and 15 free and protein amino acids were identified from two species of plant-parasitic nematodes. Plant-parasitic nematodes

generally contain far more lipid than do animal parasites. The high lipid content of nematodes may have a bearing on the success of the lipophilic halogenated hydrocarbons as nematocides and also on longevity of nematodes in the absence of host tissue. The cuticle, which protects nematodes from their environment, is composed primarily of protein but also contains lipids, polyphenols, enzymes, and nucleic acids.

METABOLISM

Intermediary metabolism in plant-parasitic nematodes is presently not understood well enough that we can make comparisons with the metabolism of animal parasites. Portions of the metabolic sequences of sugar breakdown and terminal oxidation in plant nematodes have been elucidated, but in no instance have all the steps in any one sequence been demonstrated. Research on these fundamental metabolic cycles is needed, since variations in the sequences of chemical reactions might be important factors in governing the parasitic nature of nematodes. Such variations may also represent weak points in metabolism, toward which attempts to develop specific control measures could be directed. Synthesis of amino acids by two species of plant-parasitic nematodes incubated in ^{14}C-labeled glucose and acetate solutions in the absence of plant tissues has been shown, illustrating that it is not imperative for nematodes to feed on cells in order to take in chemicals.

Nematodes seem able to digest a variety of polymeric plant components outside the body. It has been reported that enzymes that break down starch, sucrose, pectin, cellulose, protein, and glycosides have been discharged by plant-parasitic nematodes. Presumably, these enzymes break down plant components outside the nematode body before they are ingested by the parasite. Homogenates or extracts of nematodes contained all the above plus other digestive enzymes. In addition to being active outside the nematode, such enzymes are very likely active in the further degradation of plant substances in the digestive tract of the nematode. Possession of specific digestive enzymes may determine if a nematode can parasitize higher plants or fungi or both.

RESPIRATION

Both plant- and animal-parasitic nematodes require oxygen. Animal parasites consume oxygen at a constant low rate, despite the ambient oxygen concentration, and build up an oxygen debt under low oxygen tensions. Nematodes may move actively in the presence of low oxygen, but such conditions may inhibit development and egg hatch.

Recent studies with plant-parasitic nematodes demonstrated that species differ in sensitivity to lack of oxygen. Egg production and survival of molting nematodes and males were most sensitive to low oxygen tension. Hatch of eggs was less sensitive and movement and survival of preadult and mature females were least sensitive to low oxygen. Control of nematodes through flooding of soil may be partially due to lowering of oxygen levels below those required for nematode survival.

Oxygen consumption by intact specimens of several species of plant-parasitic nematodes has been measured by microrespirometry. Rates of oxygen uptake varied according to nematode species, condition of the nematodes, and carbon dioxide and osmotic concentrations of the incubation solution. Fresh homogenates of animal-parasitic nematodes initially consumed oxygen far more rapidly than did intact specimens, but the rate of uptake soon dropped off. This difference was probably caused by a low rate of diffusion of oxygen into intact nematodes.

TEMPERATURE

Since nematodes lack a means of controlling body temperature, metabolic and physical activity are dependent on the ambient temperature. In general, the optimum temperature for growth and development of plant-parasitic nematodes is in the range of 5 to 30°C. Desiccated nematodes can withstand both higher and lower temperatures than fully hydrated ones, probably because enzymes are more resistant to temperature inactivation in the dehydrated than in the hydrated condition. Moisture plays an important role in determining the thermal stresses that nematodes can withstand.

In addition to its direct effects on survival, temperature influences other aspects of nematode biology. Infectivity, sex determination, and rate of development of plant-parasitic nematodes are affected by temperature. Cysts of the oat cyst nematode (*Heterodera avenae*) are reported to require a period of exposure to low temperatures before eggs will hatch. In soybeans grown at 24°C, the developing soybean cyst nematodes (*H. glycines*) are mostly females, while at 31°C they are mostly males. A few days of exposure of young infected plants to 35°C followed by growth at 24°C was sufficient to cause most of the nematodes to develop as males. Root-knot nematodes (*Meloidogyne* spp.) produce few viable eggs above 35°C, whereas 25 to 32°C is optimum for egg production; however, the golden nematode (*Heterodera rostochiensis*) develops and produces eggs most rapidly at 18°C. Optimum temperatures for development of the host plant, as well as fluctuating temperatures, may also affect the rate of development of nematodes. Although temperature may influence nematode biology directly, other environmental factors may

influence the effect of temperature. Advantage is taken of the temperature tolerance of nematodes when control measures such as hot-water treatments of nematode-infected plant material, heat sterilization of soil, and early planting of crops to escape nematode damage are utilized.

MOISTURE

The natural environment of nematodes is aquatic. The soil solution that covers soil particles and is in soil pores is the medium in which nematodes live and through which they move to contact plant tissues. It is also the medium in which the physiological functions of nematodes occur, such as gas exchange and discharge of excretory products. For nematodes inside plant tissues, ample solution is available to satisfy their requirements. Water is also the medium in which nematocidal chemicals come in contact with the nematode body.

Water moves freely into and out of the nematode body through the cuticle, although recent findings show that nematodes have at least some control over loss of body water under conditions of water stress. However, when desiccated, eggs and tanned cysts are resistant to water loss. Under conditions of ample moisture, solutes in the nematode body cavity maintain a constant turgor pressure within the body. All nematodes can probably withstand some water stress, but the degree of water stress which is lethal varies among species. Nematodes inhabiting the aboveground parts of plants, as well as certain soil-inhabiting nematodes such as the cyst stage of some *Heterodera* spp., are especially resistant to desiccation. Some nematodes will survive freeze-drying, while other species are killed when soil containing them is air-dried. When the moisture content of soil approaches the wilting point for plants, activity of nematodes in soil is curtailed. Desiccation, heat, and starvation are involved when fallow is used to control nematodes.

Most free-living and plant-parasitic nematodes withstand severe and repeated osmotic changes with little or no adverse effects. In hypertonic solutions, nematodes shrink and become inactive because of loss of water, but, when transferred to isotonic or hypotonic solutions, they quickly become turgid and active again. Eggs are not plasmolyzed in hypertonic solutions. Although solutions of high or fluctuating osmotic concentration may not kill nematodes, movement and egg hatch are inhibited at relatively low solute concentrations. Solute concentrations of 0.1 to 0.2 M inhibit the hatch of eggs of several plant-parasitic nematodes. Ions seem to move into and out of the nematode body with changes of osmotic concentration of the ambient solution, but movement of particular ions is somewhat selective. Specimens of the animal parasite, *Ascaris* sp., regulate the concentrations of certain ions

in the body fluid, particularly potassium, calcium, and magnesium. Although water and certain ions pass directly through the cuticle of all nematodes, the excretory system appears to regulate the osmotic concentration of the body fluid as well as functioning in the elimination of certain body wastes. Radioactive tracers may aid in elucidating mechanisms of controlling osmotic concentration in nematodes. Finally, it must be remembered that water interacts with other physical and chemical factors affecting nematodes, as was described earlier. Soil is a complex medium in which the dynamic interaction of various physical and chemical factors combine to influence all aspects of nematode activity.

DORMANCY AND LONGEVITY

Several species of plant-parasitic nematodes become dormant during periods of unfavorable environmental conditions and then revive when conditions become favorable again. Nematodes that are parasitic on the aboveground parts of plants survive repeated periods of unfavorable conditions enroute to tissues upon which they will feed. Plant tissues suitable for growth and reproduction of the wheat nematode (*Anguina tritici*) are available only for the short time of flower embryo development, after which weeks or months may elapse before plant tissues are again available. Larvae of the wheat nematode have been revived from galls after 28 years of storage, stem nematodes (*Ditylenchus dipsaci*) after 23 years of storage in infected plant material, and bentgrass nematodes (*Anguina agrostis*) after 4 years of storage. Quarantines, the use of nematode-free propagative materials, and rotations including nonhost or resistant plants are some measures that are often used to control these types of nematodes.

The developmental stage in which nematodes survive periods of dormancy varies with the species. Only second-stage larvae of the wheat nematode survive dormancy, while in the stem, bud, and leaf nematodes (*Aphelenchoides* spp.) the resistant stages are the fourth-stage preadult larvae and the fifth-stage adults, respectively. Larvae inside eggs in cysts of the golden nematode and the sugar-beet nematode (*Heterodera schachtii*) remain alive for years when stored dry on the laboratory shelf. Larvae in cysts of certain other cystforming species are less resistant to drying. Dormant stages of nematodes are particularly resistant to the action of nematocidal chemicals because of the protective cyst wall in cyst nematodes and the lowered metabolic activity or other physiological factors in both cyst and other nematode species.

In the presence of growing host plants in a suitable environment, most nematodes can be maintained indefinitely. Longevity in the absence of host plants, an important factor in nematode control, is greatly influenced by

environment. Nematodes of many species can survive in soil for at least a year in the absence of a suitable host, probably on body food reserves. In soil, nematodes survive over a wide temperature range, but they survive longer at low than at high temperatures. This is caused primarily by slow depletion of body food reserves and low metabolic rate.

Certain plant-parasitic nematodes, such as the golden nematode, persist for years in soil in the absence of host plants. However, in some of the reports, total exclusion of possible hosts, such as particular weeds, that might maintain populations was not certain. Populations of some cyst nematodes in soil without host plants declined at a rate of 35 to 60 percent per year, whereas populations of other types of nematodes were reduced by 75 to 95 percent in the first year. In most experiments, a few nematodes were still being recovered from soil when the tests were terminated. The reduction of populations of plant-parasitic nematodes in fallow soil thus follows a hyperbolic curve. Yet, even after extended periods of time, a few specimens, which are important for perpetuation of the species, may survive to serve as inoculum to rebuild populations once host plants are again available.

HATCHING, MOLTING, GROWTH, AND SEX DETERMINATION

Eggs of most nematode species hatch freely in water, soil solution, or other aqueous solutions of low osmotic concentration. However, hatch of eggs of certain cyst nematodes is greatly increased by incubation in solutions leached from the host-plant root system. This hatching factor has been studied most intensively with the golden nematode, in which the hatching reaction is highly specific. The cabbage cyst (*Heterodera cruciferae*), hop cyst (*H. humuli*), and carrot cyst (*H. carotae*) nematodes also respond to specific hatching factors. Egg hatch in several other cyst nematodes and some root-knot nematodes also may be stimulated by root exudate, but appreciable hatch occurs in water alone. In some cases, root exudate from nonhost plants stimulates egg hatch, and investigations indicate that more than one hatching factor occurs in some root exudates.

In addition to substances in root exudates, many laboratory chemicals will stimulate the hatch of nematode eggs, but never to the degree obtained with root exudates. Chemicals stimulating hatch include various dyes, amino acids, sugars, and inorganic salts. No means of nematode control utilizing artificial stimulation of egg hatch has yet been developed.

When an egg begins to hatch, the larva moves about within the eggshell, at which time enzymes secreted by the larva are thought to hydrolyze the inner layer of the eggshell. Destruction of this layer allows entrance of water into

the egg. This is then taken up by the larva and causes it to swell. Finally, it seems that a combination of enzymatic action weakening the eggshell and mechanical activity of the enlarged larva, both through bodily movement and stylet thrusts against the shell, are sufficient to rupture the eggshell and free the nematode larva. Hatching factors are thought to act by increasing the permeability of the eggshell, thereby hastening activation of the larva.

The stimuli that induce molting in nematodes have been little investigated. Growth and molting of many plant-parasitic nematodes require feeding on plant tissues, but in species such as the reniform nematode (*Rotylenchulus* spp.), eggs will hatch and the nematodes will grow and molt to the preadult stage in a dish of water in the absence of host tissues. Complete development of adults and production of viable eggs, however, require feeding on host tissues. Molting, or exsheathing, in animal parasites seems to require enzymes produced by the nematode. The process of molting in animal- or plant-parasitic nematodes is not well understood, although certain root diffusates stimulated molting in some of the pin nematodes (*Paratylenchus* spp.).

When food is in ample supply, growth in nematodes is a continuous process except during molts. The cuticle can make limited growth between molts. Since nematodes are determinate in cell number, growth is primarily expressed as an increase in cell size. Growth may be accompanied by changes in body shape of the females, such as those that occur in root-knot, cyst, reniform, and several other nematodes; but, in most kinds of nematodes, males and females remain vermiform throughout the life cycle.

Sex determination in nematodes seems to be governed by both genetic and nutritional mechanisms. Results of several studies indicate that when the food supply becomes limited the sex ratio is shifted toward males. Males often predominate in nematode populations developed in the presence of resistant plants, which may be a reflection of nutritional deficiency. Possible control of sex by hormones in plant-parasitic nematodes has not been investigated.

NUTRITION AND LABORATORY CULTURING

Several free-living and animal-parasitic nematodes, and one stylet-bearing form, have been propagated *in vitro*. No nematode has been propagated on a completely defined medium, despite intensive efforts toward this goal. All media that support *in vitro* propagation of free-living nematodes have failed to support reproduction of plant-parasitic species. The free-living nematode *Caenorhabditis briggsae* is known to require six B-vitamins and ten amino acids.

Although all plant-parasitic nematodes require living plant tissues for reproduction, there is great variation in the host ranges of different nematodes.

For instance, the golden nematode will reproduce only on a very few plants, while the host plants of certain root-knot nematodes number in the hundreds. Attempts at *in vitro* culturing of plant-parasitic nematodes would be most likely to succeed with species that do not induce specialized plant reactions and that have wide host ranges.

Laboratory culturing of plant-parasitic nematodes on a scale adequate for biochemical studies has been successful with about 12 nematode species. Sterile root cultures, grown on nutrient agar media, were first utilized for culturing nematodes. Plant callus cultures, particularly of alfalfa, proved highly useful. This culturing technique is presently the most promising method of propagating large quantities of plant-parasitic nematodes in the laboratory.

CONCLUSIONS

The physiology and biochemistry of plant-parasitic nematodes is only now beginning to receive proper attention. To aid biochemical studies, supporting techniques such as those involving culturing and handling of many kinds of plant-parasitic nematodes still need to be developed. Indications are that as this area of research expands, novel and perhaps surprising variations from conventional metabolic patterns, which will contribute basic information not only to nematology but also to fundamental biochemistry, will be elucidated. Results of biological studies with plant-parasitic nematodes have indicated some very interesting phenomena, which, however, can only be explained by future biochemical and physiological investigations.

Progress in biochemical nematology is presently hampered not only by a shortage of investigators but also by a lack of suitable positions and facilities for some of the few competent, enthusiastic, highly trained young graduates. Until more support is provided for such research, progress in this area will continue to be slow.

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CHAPTER 5

Pathogenic Relationships

NEMATODE DISEASE SYMPTOMS AND DISEASE DIAGNOSIS

The symptoms of nematode disease are commonly those of root impairment, such as growth reduction (Figure 5), increased wilting, mineral-deficiency symptoms, decreased winter-hardiness, and dieback in perennials. It is often difficult to prove conclusively whether nematodes, other microorganisms, other limiting factors, or combinations of these are the cause of root impairment. Before discussing the solution of this difficulty, however, those nematode disease symptoms that are easily recognized in plants should be considered.

Top Symptoms and Signs

Certain species of seedgall nematodes (*Anguina* spp.) transform floral parts, producing characteristic galls in place of normal seeds (Figure 6). Other species of *Anguina* produce galls and distortion in leaves and stem. The stem nematode (*Ditylenchus dipsaci*) causes swelling and distortion of stems and leaves (Figure 7). Bud and leaf nematodes (*Aphelenchoides* spp.) frequently cause foliar discoloration in a characteristic pattern (Figure 8). Where symptoms suggest nematode infection, the actual presence of nematodes should be determined by dissecting or extracting nematodes from the tissues and identifying them.

FIGURE 5 Reduced growth of corn associated with the stubby-root nematode, *Trichodorus christiei.* (After J. R. Christie. Courtesy of *Phytopathology.)*

Root Symptoms and Signs

The galling (Figure 9) caused by root-knot nematodes (*Meloidogyne* spp.) is easily recognized but can be confused with the more apical root-galling (Figure 10) caused by certain sheath nematodes (*Hemicycliophora* spp.) or with the bending and apical galling caused by dagger nematodes (*Xiphinema* spp.). Lesion nematodes (*Pratylenchus* spp.) produce characteristic lesions in the root cortex of some host plants (Figure 11). Female cyst nematodes (*Heterodera* spp.) can be seen on the roots of host plants (Figure 12) if the soil is carefully removed from the roots. Soil clings to a gelatinous material secreted by the citrus nematode (*Tylenchulus semipenetrans*), causing infected citrus roots to appear "dirtier" than uninfected ones. The stubby roots, excessive root proliferation, and root necroses that accompany root infection by other kinds of root-feeding nematodes are often not sufficiently distinctive to permit sure diagnosis.

FIGURE 6 A normal seed head of wheat (left), and a head with seeds galled (right) by the wheat-gall nematode, *Anguina tritici*. (Courtesy of Shell Development Company.)

EXPERIMENTAL DETERMINATION OF PATHOGENICITY

In general, because nematodes are unobtrusive plant parasites, their pathogenicity must be established experimentally. The procedures used to establish pathogenicity attempt to parallel those outlined in Koch's postulates. This attempt is not entirely successful, for two reasons. First, nematodes parasitic on higher plants are obligate parasites. Second, and more important, the activities of most plant-parasitic nematodes occur in soil, a microbiologically complex medium. Nematologists are circumventing the first difficulty. Some of the more important plant-parasitic species are now maintained in plant-tissue cultures free of all other microorganisms. Large numbers of microbiologically sterile nematodes for experimental use can be obtained from such cultures. In time, this approach will probably succeed with many additional species. The second difficulty, extrapolation from results of simple experiments involving only nematode and plant to the complex field situation has not been

overcome. This difficulty is shared by plant pathologists working with other soil microorganisms.

In the absence of a satisfactory stepwise series of tests, such as Koch's rules of proof, nematologists make observations and conduct experiments to judge association with disease, include experimental treatments that will test involvement in disease, and conduct still other experiments to decide the role of a nematode in a disease.

To judge association of a nematode with a plant disease, the nematode must first be identified to species. Nematodes show greater morphological specialization then do bacteria and fungi, and identification can usually be made with greater precision, although physiological races and morphologically similar species do occur. Specimens can be killed with hot water and preserved in formalin or passed into glycerine and mounted for future reference. Methods for recovering and preserving nematodes are discussed in Chapter 7. Observation of the nematode feeding on or in the host is useful at the association stage. Differences in nematode population levels can be created by soil fumigation, by previous cropping, or by adding nematodes to uninfested soil. Subsequent plant growth in soil thus treated can be correlated with nematode population level. When adding nematodes to soil, it is useful to apply them in numbers on a logarithmic scale (e.g., 100; 1,000; 10,000; and 100,000). Such a

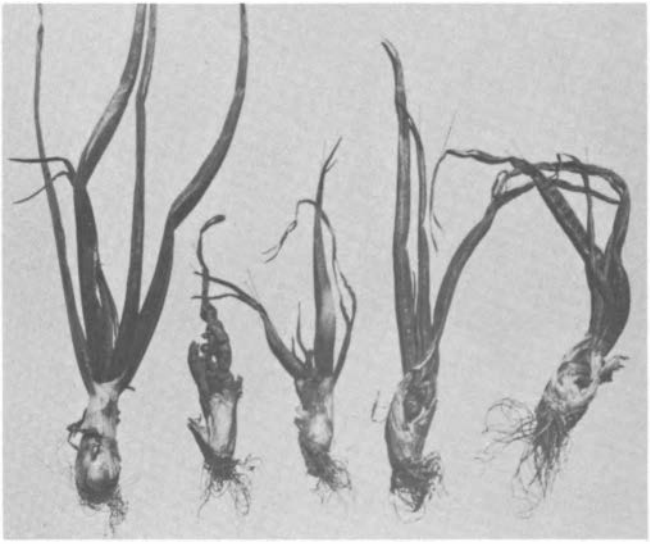

FIGURE 7 Distortion of onion by the stem nematode, *Ditylenchus dipsaci*; one healthy onion on the left. (Courtesy of the Department of Nematology, University of California, Davis.)

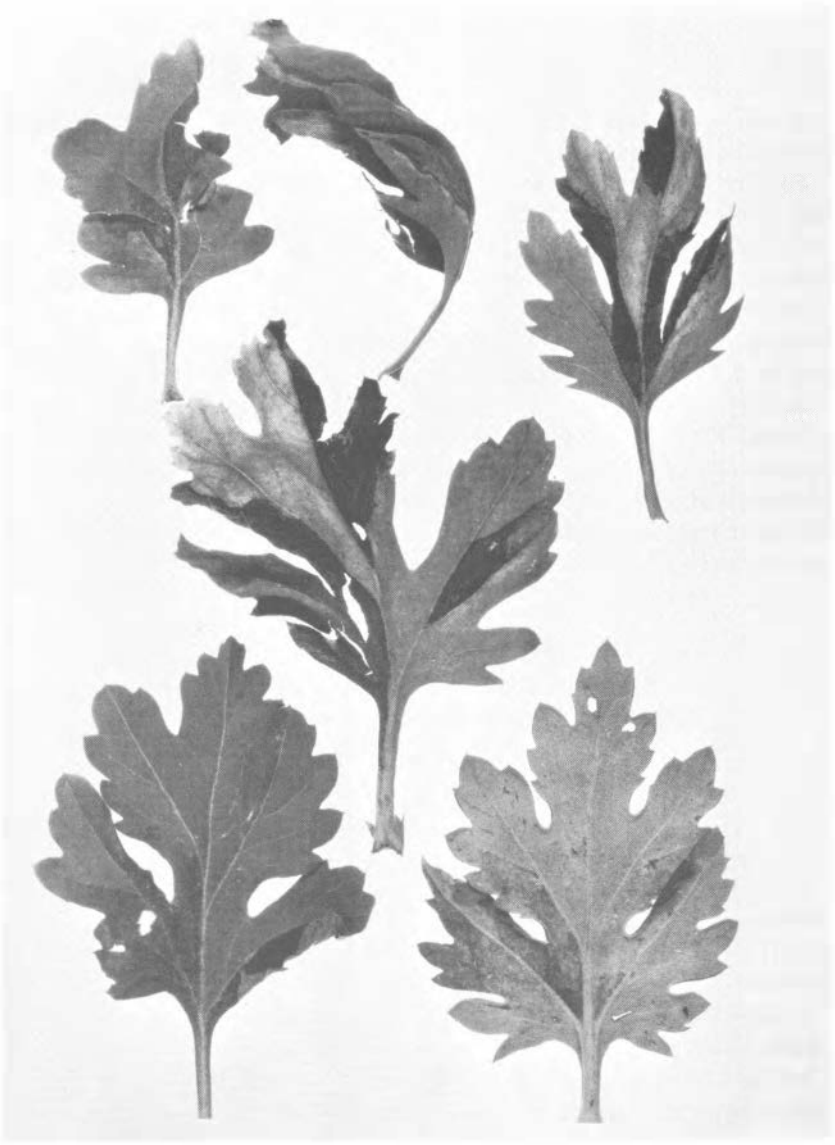

FIGURE 8 Angular discoloration produced by the chrysanthemum foliar nematode, *Aphelenchoides ritzemabosi*, in chrysanthemum leaves. (Courtesy of Nematology Investigations, USDA.)

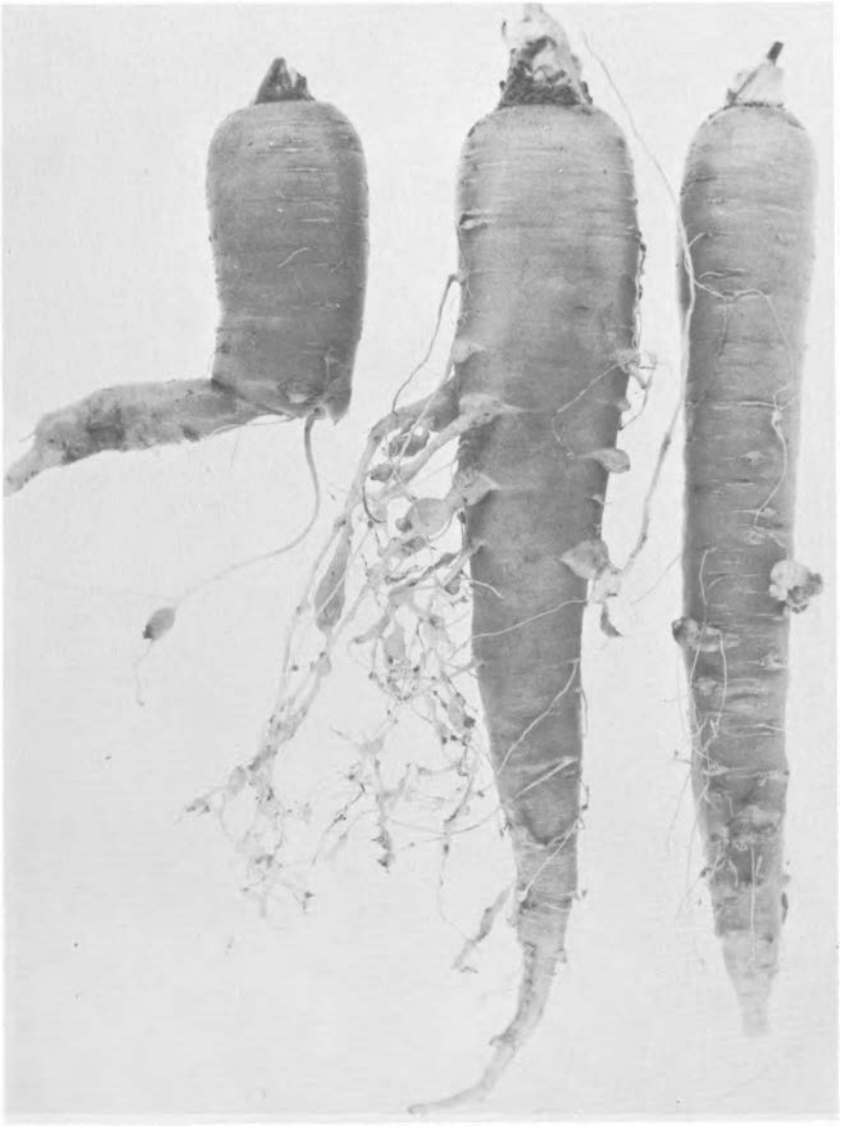

FIGURE 9 Galling of carrots by a root-knot nematode, *Meloidogyne incognita*. (Courtesy of the Department of Nematology, University of California, Riverside.)

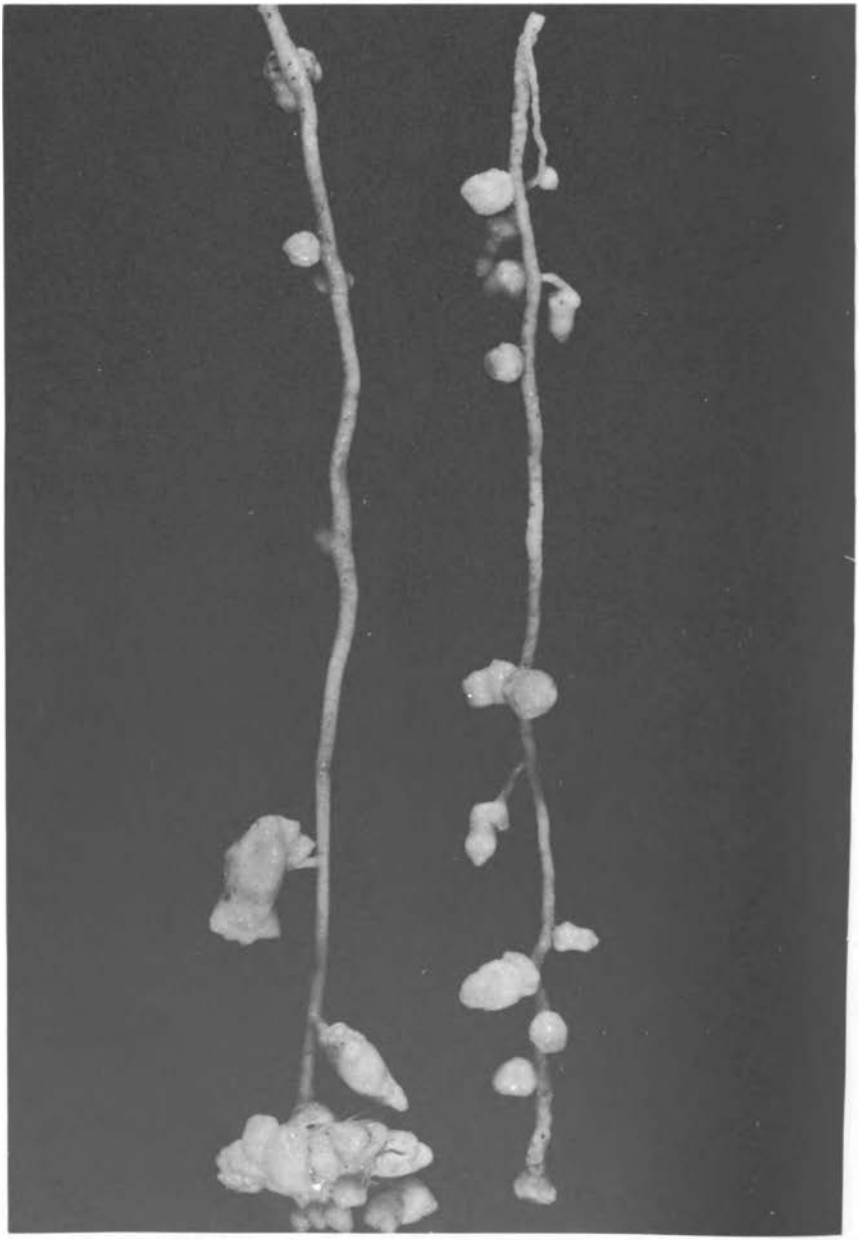

FIGURE 10 Citrus root galled by the sheath nematode, *Hemicycliophora arenaria*. (After S. D. Van Gundy. Courtesy of *Plant Disease Reporter*.)

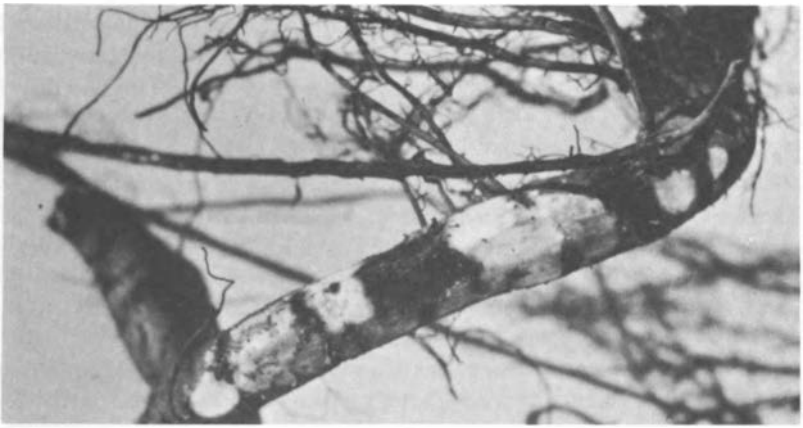

FIGURE 11 Lesions on walnut root associated with a lesion nematode, *Pratylenchus vulnus*. (Courtesy of the Department of Nematology, University of California, Davis.)

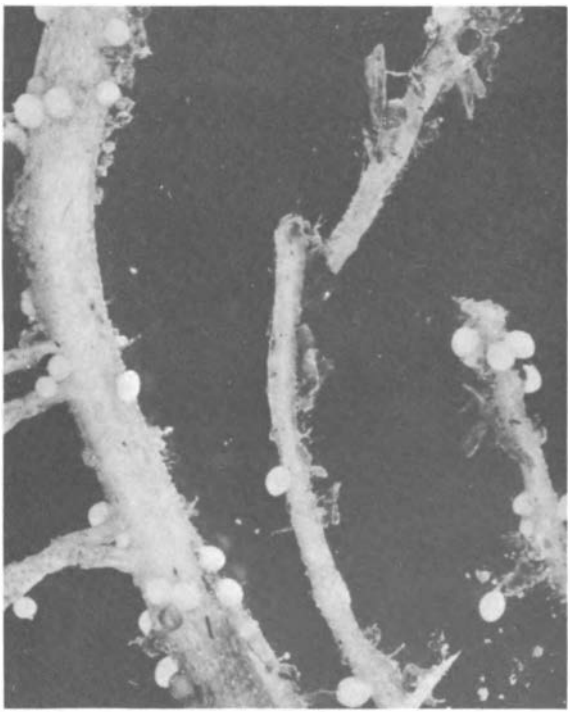

FIGURE 12 Mature females of the golden nematode, *Heterodera rostochiensis*, on potato roots. (Courtesy of the Department of Plant Pathology, Cornell University.)

series approximates various stages during the natural increase in a nematode population with time and enables determination of both the tolerance limit of the plant in terms of nematode numbers and the nematode population density that can maintain itself on the plant being tested. Depending on the similarity between field and experimental conditions, this information may be applied to field situations. Observations and experiments used to judge association with disease usually include either microorganisms other than nematodes or soil treatments that have microbiological or chemical effects in addition to the nematocidal effect. The data from these experiments can be used to judge association with disease of unknown cause but cannot be used to prove involvement in disease or its pathogenicity.

Involvement of a nematode species in a plant disease may be judged by comparing plants inoculated with a water suspension of nematodes isolated from roots or soil with plants inoculated with an otherwise similar suspension but freed of nematodes by a technique such as sieving. Freeing the suspension of nematodes by sieving provides a valid check on associated microorganisms if these microorganisms pass through the sieves that are employed. The experimental goal would not be attained, for example, if fungus inoculum was present in the suspension in the form of mycelium, since mycelium, as well as the nematodes, would be removed by sieving. Bacteria and many fungus spores will pass through the fine sieves used in nematode extraction. If removal of the nematode (and nothing else) from the inoculum eliminates disease, that nematode must be involved in the disease. Other organisms may also be involved. Whether the nematode is a pathogen, an incitant, an aggravator, or a vector must be determined by further and different experimentation.

The role of a nematode in plant disease can only be proved with nematodes that are freed of other microorganisms. This can often be accomplished by surface sterilization of the nematodes with chemicals, if the nematode suspension is free of organic fragments. Because surface sterilization frees plant-parasitic nematodes from other microorganisms, and because attempts to isolate organisms from inside these nematodes have usually failed, most plant-parasitic nematodes are believed to be internally free of live microorganisms. An exception may be the American dagger nematode (*Xiphinema americanum*), in whose reproductive tract a bacterium was reported.

Considered singly, studies of association, involvement, or role usually will not prove satisfactorily that a nematode is or is not the cause of a disease as it occurs in the field. Consideration of observations and results of experiments at all three of these levels are usually required to understand the relation of a nematode to a plant disease, but disease-control efforts need not await complete understanding of the disease. The initial judgment of association, and attempts at control by soil fumigation, often proceed together profitably.

HISTOPATHOLOGY OF NEMATODE-PARASITIZED PLANTS

Microscopic study of the tissue and cellular alterations associated with parasitism is termed histopathology. Details of histopathological changes induced in tissues by nematode parasitism, starting with initial stages of parasitism and continuing until the interrelationship is well established, must be correlated with physiological and biochemical studies. By correlating findings from these studies, it may be possible to determine the fundamental basis for plant-tissue alterations that occur during nematode parasitism.

For convenience, the gross symptoms of nematode parasitism of plant tissues may be separated into galling; necrosis, or death, of cells or tissues; distortion; and inhibition of growth. In many nematode–plant-tissue associations a single symptom may be observed, but, in others, two, three, or all four symptoms may be seen. For instance, galling is the dominant symptom of infection in alfalfa seedlings by the stem nematode, but inhibition of apical growth and distortion of shoots may occur simultaneously with galling, and in older infections necrosis may develop. However, the only gross visible symptom of tobacco stunt nematode (*Tylenchorhynchus claytoni*) damage to plant roots may be retarded growth of the root system because of the inhibition of growth of many root apices.

Gall Formation

Species of nematodes in several genera induce swellings, or plant galls, both in roots and aboveground parts of plants. The site of galling on the plant, i.e., seed, leaf, stem, or root, is determined by the feeding site of the particular nematode species involved. A plant gall possesses certain anatomical features that are more or less peculiar to the nematode species inducing the gall, although these features may vary somewhat, depending on the particular plant involved and environmental conditions such as soil type, fertilizer levels, moisture, and temperature.

Perhaps the simplest types of plant galls induced by nematodes are those caused by dagger and sheath nematodes on the root apices of certain plants. These nematodes feed as ectoparasites, with only the stylet, and not the nematode body, penetrating plant tissues. Feeding occurs on a root apex, stimulating hyperplasia (cell division) and hypertrophy (cell enlargement) of root-tip cells, which results in a gall composed of a compact mass of cells (Figure 13).

Galls induced by the stem nematode on various plants and different aboveground parts are essentially similar histopathologically. Gall formation in alfalfa seedlings is well documented and serves as the example here (Figures 14 and 15). Within a few hours after inoculation, nematodes penetrate the epidermis in the axils of cotyledons, forming cavities in the cortical tissue just

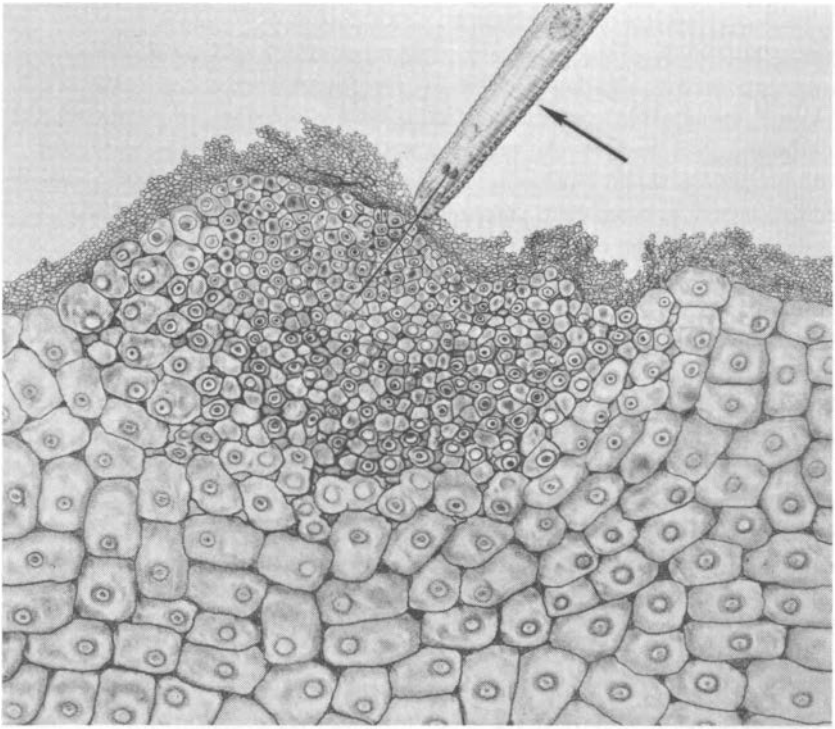

FIGURE 13 Response of citrus-root cortical parenchyma tissue to feeding by a specimen of *Hemicycliophora arenaria* shown with stylet penetrating plant root (arrow). (After S. D. Van Gundy. Courtesy of *Phytopathology*.)

below the plant apex. One day after inoculation, swelling of the stem below the cotyledons is visible. Further gall development is principally an amplification of earlier anatomical changes. Galls enlarge by hyperplasia and hypertrophy of cortical parenchyma cells, while nematodes are simultaneously destroying cortical cells and thus forming cavities. Galls may continue to enlarge while the plant is actively growing. Many of the undestroyed parenchyma cells become enlarged, misshapen, and separated from one another. Xylem tissue is little affected directly. Apical growth is usually inhibited, and, although nematodes do not penetrate the apex itself, these cells may become distorted when adjacent to galls. Although these nematodes penetrate roots in small numbers and form cavities in the cortex, no galling is induced.

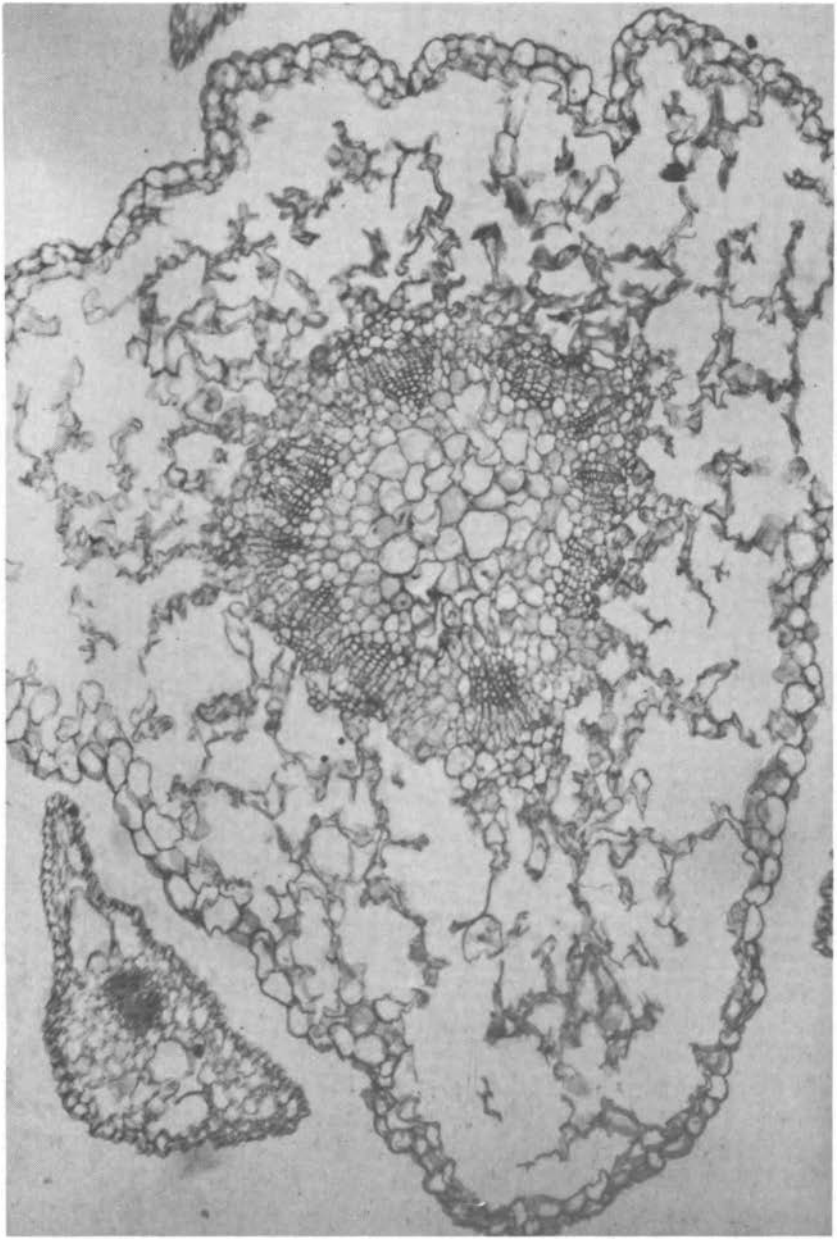

FIGURE 14 Cross section of an alfalfa stem galled by *Ditylenchus dipsaci*, 20 days after nematode inoculation (After L. R. Krusberg.)

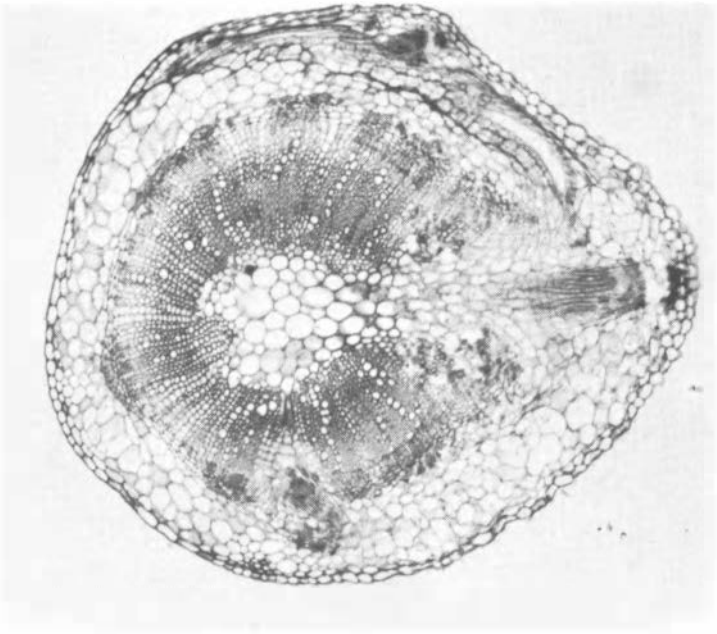

FIGURE 15 Cross section of a normal alfalfa stem. (After L. R. Krusberg. Reproduced by permission of E. J. Brill.)

The beach-grass root nematode, *Ditylenchus radicicola*, causes galls on roots of several grasses (Gramineae). These galls are determinate and develop only to specific size. The galls are histopathologically similar to galls caused by the stem nematode in aboveground parts of plants, except that hypertrophy and hyperplasia are more pronounced (Figure 16).

The most common and well-known gall-inducing nematodes are the root-knot nematodes. Second-stage larvae penetrate the epidermis near the root tip, and within 24 hours they move between or through cells to the feeding site in the vascular cylinder. A root tip may be penetrated by several larvae, in which case apical growth is usually inhibited. When larvae begin feeding, two to five cells in the vascular tissue around the head of the nematode enlarge, and nuclei within these cells may divide several times without division of the cytoplasm, thus resulting in multinucleate cells (Figure 17). Under the continued influence of nematode feeding, walls between enlarging cells and adjacent cells dissolve, the protoplasts combine, and, as additional surrounding cells are incorporated, so-called giant cells are formed (Figure 18). Giant cells continue to enlarge for several days or weeks, and they provide food for the

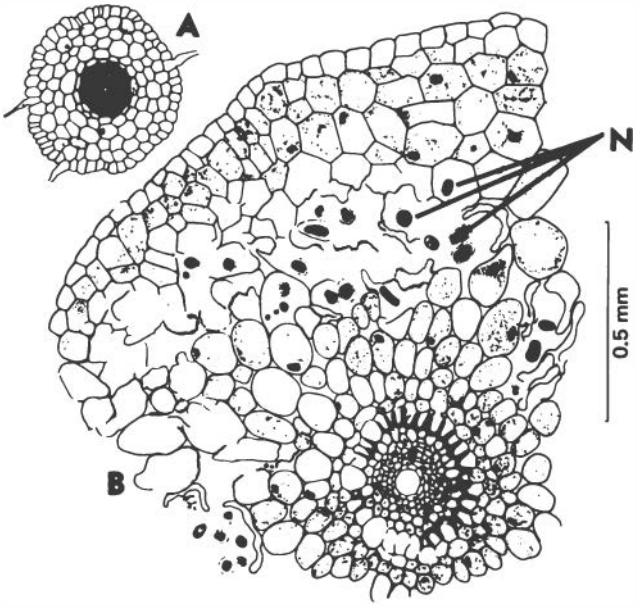

FIGURE 16 A. Cross section of a normal root of *Elymus arenarius*. B. Cross section through a gall induced by *Ditylenchus radicicola* (N) on a root of *E. arenarius*. (After T. Goodey. Reproduced by permission of *J. Helminthol.*)

developing nematode. Typically, developed giant cells are multinucleate and contain viscous cytoplasm with many mitochondria, proplastids, and golgi bodies. They possess a well-developed endoplasmic reticulum and are very active metabolically. Walls of giant cells are usually thick except in areas where adjacent cells are being incorporated. Living nematodes are required for initiation and continued development of giant cells. Giant cells may also form from cortex or storage parenchyma cells as well as from vascular tissues.

Simultaneous with giant-cell formation, other changes occur in plant tissues surrounding the feeding root-knot nematode. Hypertrophy and hyperplasia occur in the pericycle, cortex, and epidermis adjacent to the enlarging nematode, so that it is continually enveloped by plant tissues (Figure 18). As the nematode matures in small roots, the posterior portion extends close to the epidermis, so that extruded eggs lie outside the plant root. When egg-laying is nearly completed, degenerative changes such as granulation of giant-cell cytoplasm, dissolution and coalescence of giant-cell nuclei, cork formation around the nematode and giant-cell complex, and pronounced thickening of giant-cell walls may occur in gall tissues. When females die or mature males

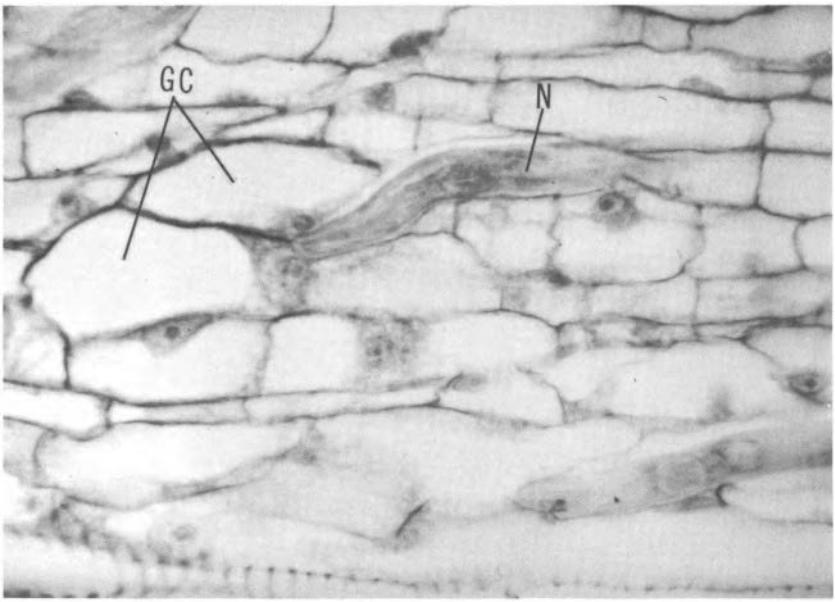

FIGURE 17 Initiation of giant cells around the head of a larva of *Meloidogyne incognita acrita* in a sweet-potato root 3 days after nematode inoculation. Figure shows nematode larva (N) and initial formation of giant cells (GC). (After L. R. Krusberg. Courtesy of *Phytopathology*.)

leave roots, there is evidence that the plant resorbs the galled tissues to some degree.

Cyst nematodes also induce giant cells in plant tissues, but galls are not formed, as there is little or no hypertrophy or hyperplasia in cells surrounding the nematode. Instead, the nematode breaks out of the root as it grows, so that only the head and neck are embedded in the root when the nematode reaches maturity (Figure 19). Giant cells induced by root-knot and cyst nematodes are similar in most respects.

Leaf and seed galls induced by *Anguina* spp. vary in complexity. In general, a central cavity containing nematodes is surrounded by a few layers of parenchymatous cells, which appear to serve as the source of food. Layers or groups of other types of cells, such as sclerenchyma, may surround the parenchyma tissue (Figure 20). Galls are determinate, and they form by hypertrophy and hyperplasia of parenchyma cells at the infection site.

Nematodes of a few other genera, such as *Nacobbus, Meloidodera*, and *Hypsoperine*, also induce root-galling, which generally follows one or another of the patterns described above.

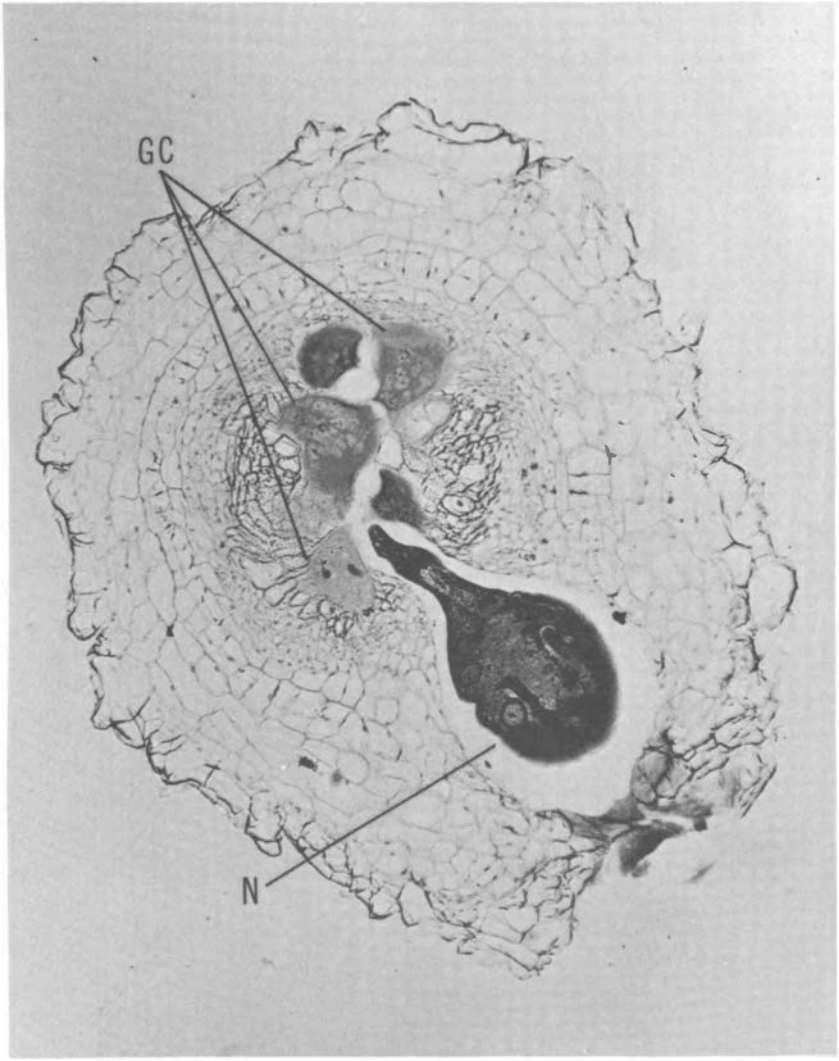

FIGURE 18 Cross section of a tomato root gall showing a female, *Meloidogyne* sp. (N), giant cells (GC), and other abnormal tissues in the root. (Reproduced by permission of Rothamsted Experiment Station.)

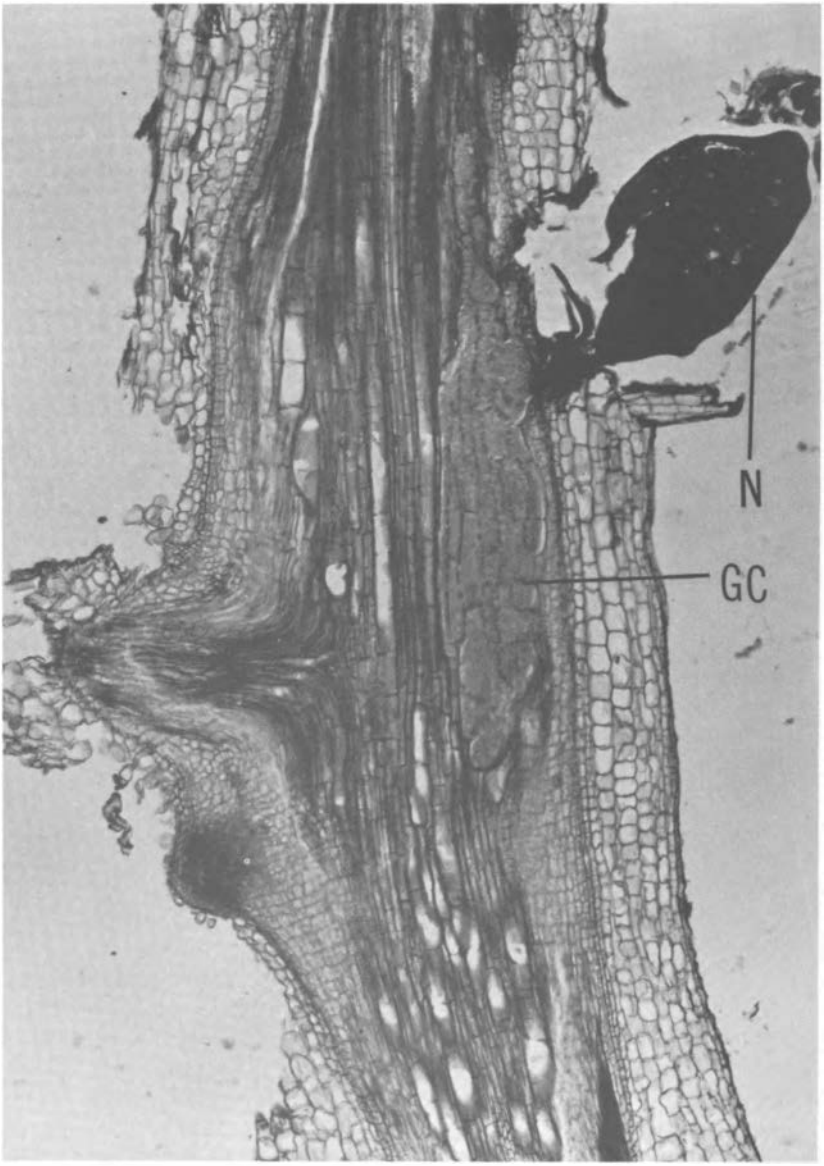

FIGURE 19 Longitudinal section of a soybean root infected by a female soybean cyst nematode, *Heterodera glycines*, showing position of the nematode (N) and giant cells (GC) in root tissues. (After B. Y. Endo. Courtesy of *Phytopathology*.)

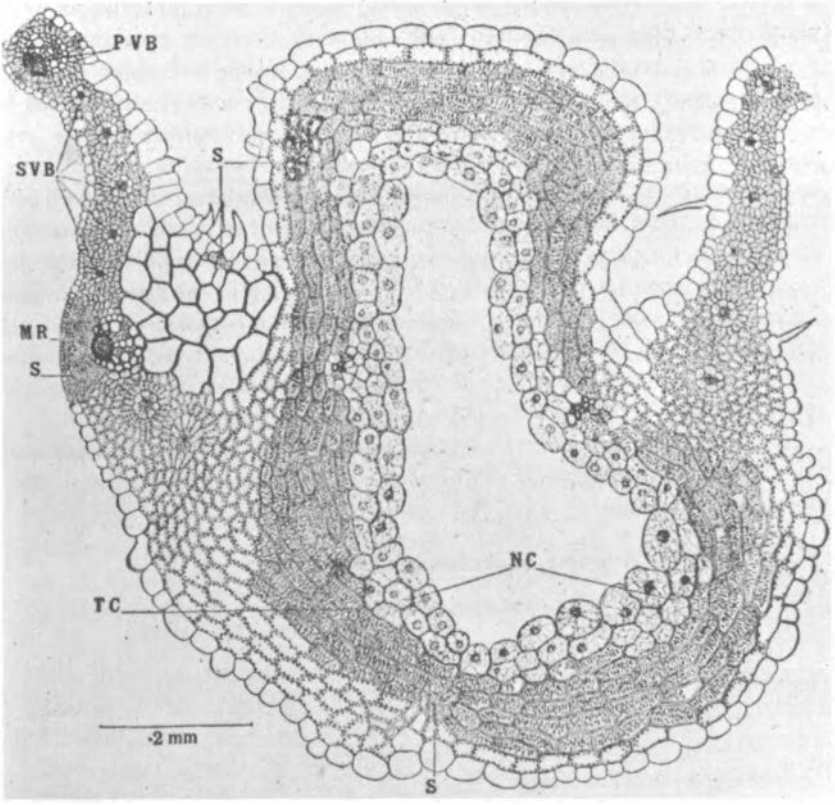

FIGURE 20 Cross section through a gall induced by *Anguina cecidoplastes* in the lamina of a leaf of *Andropogon pertusus*, showing: MR, midribs; NC, nutritive cells; PVB, primary vascular bundles; S, sclerenchyma cells; SVB, secondary vascular bundles; and TC, thickened cells. (After T. Goodey. Reproduced by permission of *J. Helminthol.*)

Lesion Formation

Nematodes of several genera, including ectoparasitic and endoparasitic species, cause lesions in roots or aboveground parts of plants. Except in a few instances, little is known about progressive histopathological changes occurring in host tissues during penetration of nematodes into plants and establishment of the parasitic relationship.

Lesion nematodes apparently penetrate almost anywhere along the roots. In the cortical parenchyma, the nematodes are present in cavities formed by

their feeding. Root tissues of many but not all plants discolor quickly following nematode infection. In heavily infected roots, the cortex surrounding the vascular tissue may become permeated by cavities, resulting in sloughing off of the cortex and, finally, death of the root. Secondary microorganisms, such as bacteria and fungi, may aggravate root necrosis. Surface-sterilized lesion nematodes produce necrosis in sterile root tissues, but necrosis caused under field conditions is usually more extensive. The burrowing nematode *(Radopholus similis)* causes lesions in the cortical parenchyma of citrus roots similar to those caused by lesion nematodes, feeds on phloem and pericycle tissues, and may cause slight swelling of roots due to stimulation of cell division in the pericycle (Figure 21). A lance nematode (*Hoplolaimus coronatus*) also burrows into the cortex of roots, usually feeding in phloem tissue and occasionally penetrating xylem tissue.

The potato rot nematode (*Ditylenchus destructor*) causes rot in underground parts of several plants. In potato tubers, nematodes feed on parenchyma cells, creating a network of tunnels through the tissues and leaving vascular strands intact. Infected tissues shrink and crack, allowing secondary microorganisms to enter and add to the destruction. Potato tubers are successfully colonized by the potato rot nematode only in the presence of fungi, although this nematode can be cultivated on callus cultures of several plants in the absence of fungi.

The citrus nematode feeds primarily on cortical parenchyma cells of citrus-feeder roots. Occasionally, several second-stage larvae may enter a citrus root and form a cavity in the cortex. Usually, a second-, third-, or fourth-stage larva becomes partially embedded in a root, with the head several cells deep in the cortex, where it feeds on cortical parenchyma while maturation occurs. Only cells directly penetrated by the nematode body are destroyed; the cells on which the nematode feeds are altered but not destroyed (Figure 22).

Localized cortical necrosis of plant roots is caused by spiral (*Helicotylenchus* spp. and *Rotylenchus* spp.), pin (*Paratylenchus* spp.), ring (*Criconemoides* spp.), and other nematodes. These nematodes generally penetrate only a few cell layers into cortical tissues.

The red ring disease of coconut palm, caused by *Rhadinaphelenchus cocophilus*, is characterized by discoloration in stems of this plant (Figure 23).

Browning of chrysanthemum leaves is caused by infection with chrysanthemum foliar nematodes (*Aphelenchoides ritzemabosi*). Only older leaves become diseased, probably because intercellular spaces in the mesophyll are large enough to permit free movement of the nematode. Nematodes enter young leaves through the stomata but are restricted to the substomatal chamber and do not cause damage. Within a few days after infection, large numbers of mesophyll cells in the older leaves are destroyed by nematode feeding, causing large sections of leaf tissues to die, turn brown, and collapse.

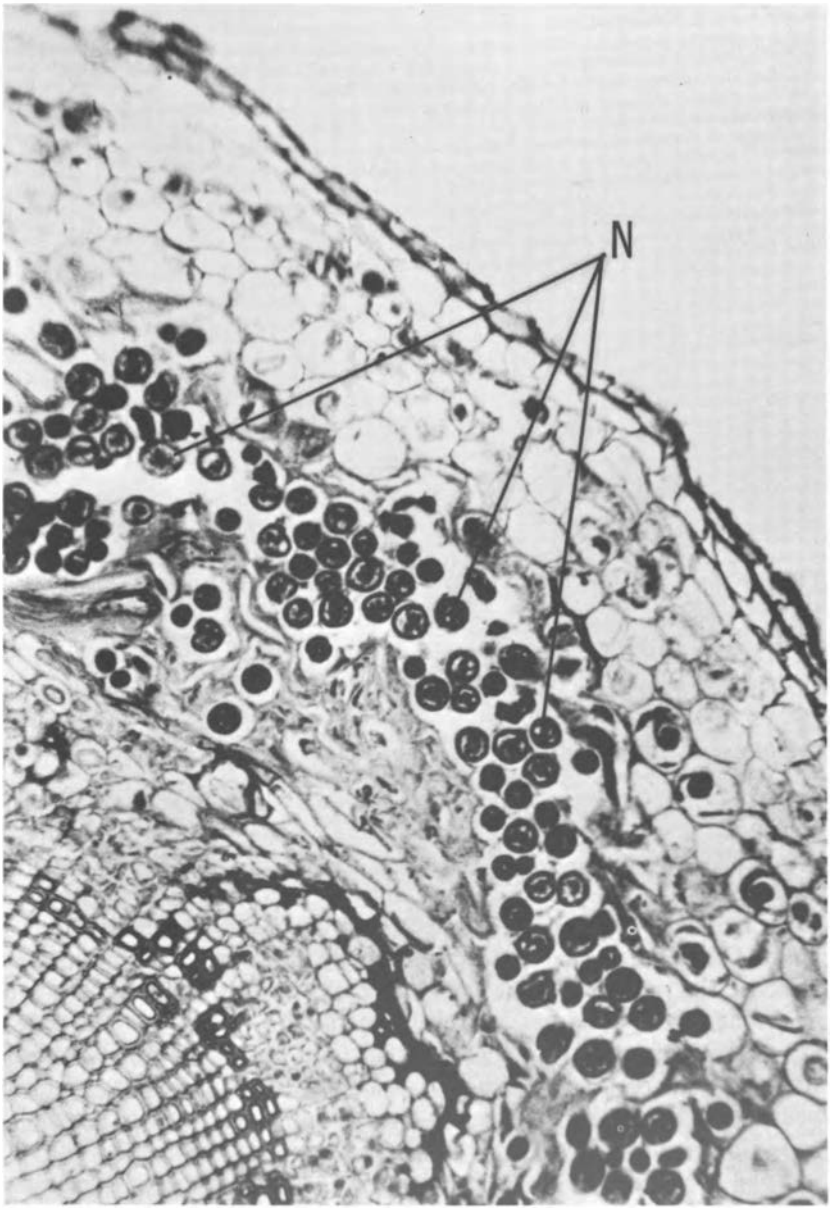

FIGURE 21 A cross section of a grapefruit root infected by the burrowing nematode, *Radopholus similis*, showing cavities in cortical parenchyma containing nematodes (N). (After DuCharme. Courtesy of *Phytopathology*.)

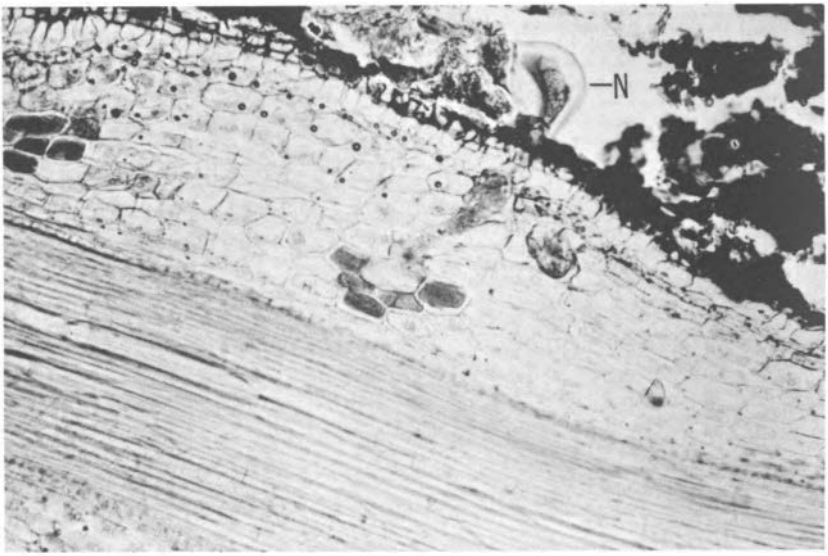

FIGURE 22 Cross section of a sour orange root, showing alteration of cells in the cortical parenchyma tissue by the citrus nematode, *Tylenchulus semipenetrans* (N). (After S. D. Van Gundy.)

Inhibition of Apical Growth

Inhibition of apical growth of plant tissues may accompany galling or necrotic responses to nematode parasitism. However, growth inhibition may be the only visible response to nematode feeding. For example, the awl (*Dolichodorus* spp.), sting (*Belonolaimus* spp.), and stubby-root nematodes (*Trichodorus* spp.) inhibit apical growth caused by ectoparasitic feeding on root apices. Cells in affected apices, including meristematic cells, become mature, enlarged, and vacuolate. In the presence of dense nematode populations, root apices may be distorted and destroyed. Feeding by a stubby-root nematode (*T. christiei*) stimulates formation of lateral roots behind affected root tips. As these lateral roots emerge through the cortex, they also are attacked by nematodes, thus finally resulting in a severely restricted plant root system, consisting of numerous short, stubby roots (Figure 24).

Stunt nematodes (*Tylenchorhynchus* spp.) and sometimes pin nematodes elicit a milder response in roots of many plants than do sting or stubby-root nematodes. Such plants, when grown in the presence of dense nematode populations, merely have smaller root systems than do plants grown in nematode-free soil. These nematodes apparently feed on plant cells and reduce root

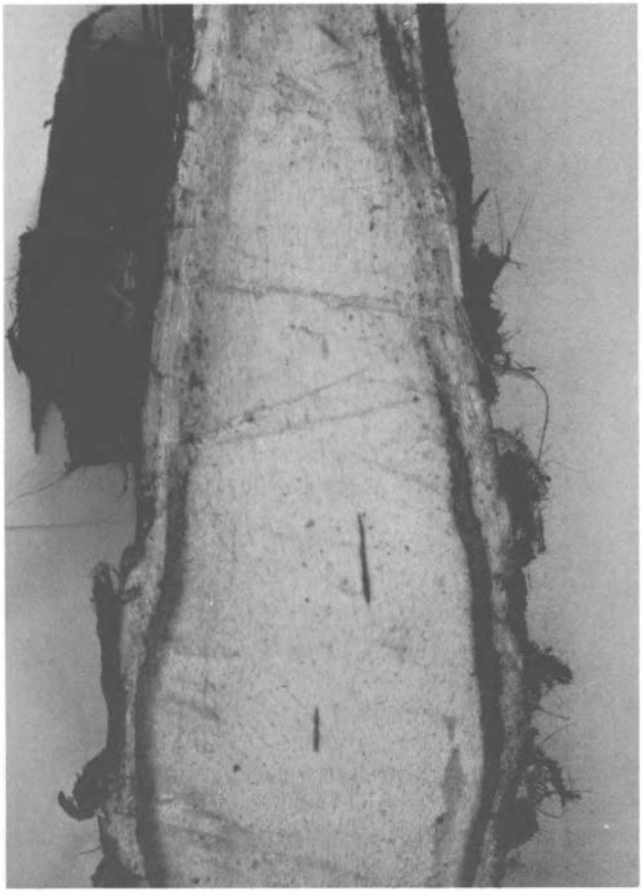

FIGURE 23 Discoloration in the lower stem of a coconut palm, caused by *Rhadinaphelenchus cocophilus*.

growth without destroying cells. No histopathological evidence of feeding was found in sections of alfalfa roots on which specimens of tobacco stunt nematode were microscopically observed feeding on epidermal cells.

Certain nematodes feeding ectoparasitically near meristematic tissues of aboveground plant parts may also inhibit apical growth. Apical growth was stopped, and apical cells matured and became highly vacuolated, when alfalfa seedlings were inoculated with chrysanthemum foliar nematodes (Figure 25). This and other species of *Aphelenchoides* not only inhibit apical growth but also kill epidermal cell layers and cause malformation of young tissues.

FIGURE 24 Root system of a corn plant injured by the stubby-root nematode, *Trichodorus christiei*. (After J. R. Christie. Courtesy of Soil Science Society of Florida.)

PHYSIOLOGY OF DISEASED TISSUES

An increase in the respiratory rate of tissues is characteristic of plant diseases caused by fungi and bacteria. Because nematode-diseased tissues are reported to absorb oxygen more rapidly, at the same rate, or less rapidly than comparable healthy plant tissues, no generalizations about the effect of nematodes on the respiratory rate of plant tissues are possible. Increased activity of oxidase enzymes, including the oxidases that destroy the plant growth hormone, indole-3-acetic acid (IAA), was found in extracts of plant galls caused by stem and root-knot nematodes. Solutions in which live nematodes were incubated also contained IAA-destroying enzymes. Such enzymes may be involved in inhibition of apical growth in plants affected by nematodes.

Recent studies have elucidated some of the biochemical changes resulting from nematode infection of plant tissues. Results of histochemical tests on root-knot nematode galls demonstrate that giant-cell walls contain cellulose and pectin but no lignin, suberin, starch, or ninhydrin-positive substances. However, giant-cell protoplasm contains carbohydrate, fat, ribonucleic acid, and a large amount of protein. Starch disappears in galled tissues, while cellulose, sugars, phosphorylated intermediates, keto acids, free amino acids, protein, nucleic acids, phosphorus, and nitrogen increase when compared with

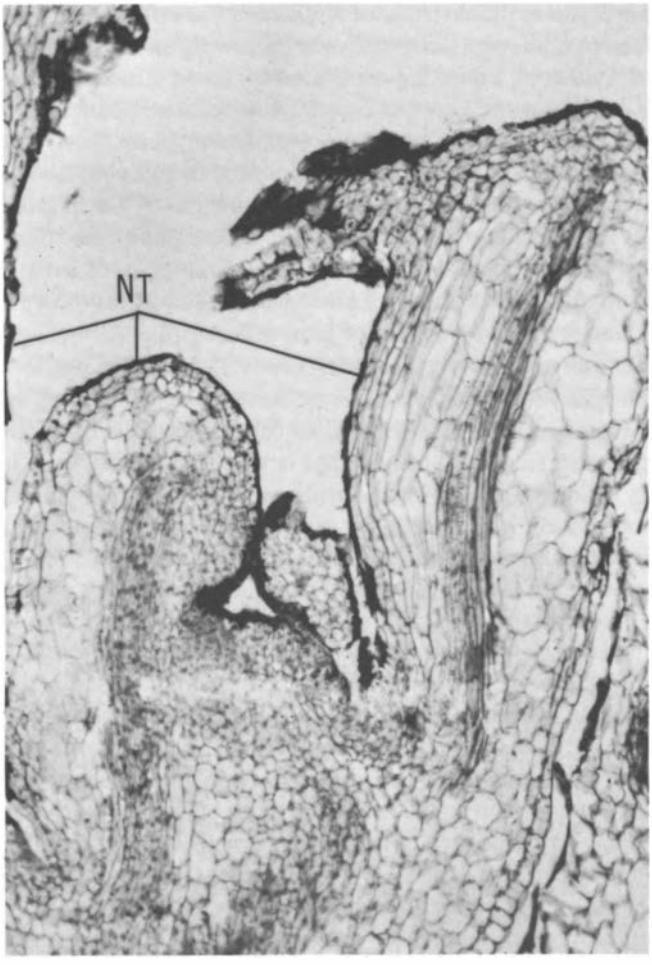

FIGURE 25 Longitudinal section through the apical region of an alfalfa seedling inoculated with *Aphelenchoides ritzemabosi*, showing necrotic-epidermal and outer-cortical parenchyma tissue (NT), caused by nematode feeding. (After L. R. Krusberg. Reproduced by permission of E. J. Brill.)

healthy tissues. Levels of several metals and sulfur remain the same as in comparable healthy tissues. These findings, as well as results from histopathological studies, indicate that root galls induced by root-knot nematodes are very active metabolically.

Levels of certain biochemicals in plant tissues affected by other nematodes were investigated. Free and protein amino acids accumulated more rapidly

and to higher levels in shoot tissues of alfalfa and pea infected with stem nematode and in citrus roots infected with burrowing nematode than in comparable healthy tissues, indicating stimulated synthesis of these substances. Plant roots infected with *Nacobbus batatiformis* accumulated starch, whereas in other nematode-diseased tissues starch usually disappears.

In a few instances, radioactive tracers were used to study metabolism of root-knot nematode-galled plant tissues. Accumulation of ^{32}P in galls occurred when infected roots were incubated in labeled phosphate solution. Results from recent studies demonstrated the incorporation of tritiated thymidine and uridine into giant-cell protoplasm. Uridine was incorporated into nuclei, nucleoli, and cytoplasm of giant cells regardless of the age of the nematode associated with the giant cells. However, thymidine was incorporated into nuclei of giant cells upon which larvae were apparently feeding at the time of treatment and only into those giant cells associated with growing nematodes, that is, second- to fourth-stage larvae. These results were interpreted as an indication that DNA was synthesized in giant cells only when nematodes were feeding, but RNA was synthesized independent of nematode feeding. Little tritium was accumulated in nematode bodies.

The mechanism of nematode-induced galling of plant tissues obviously involves plant-growth regulating mechanisms, but no incitant has been identified. Most investigations concern host–parasite interactions of root-knot and stem nematodes. Based on paper chromatographic and biological activity assays, galled plant tissues were reported to contain more auxin or indole compounds than healthy tissues. In a recent study, eggs and larvae of three species of root-knot nematodes and tomato roots galled by each species contained indole plant-growth regulators. Occurrence of each of the four identified growth regulators varied with the particular nematode species involved; those found in a specific nematode were also present in extracts of roots galled by that nematode species. Data from studies with plant-growth inhibitors also suggest that the mechanism of nematode-induced galling of plant tissues is growth-regulatory in nature. When applied to the tops of plants, the plant-growth inhibitor, maleic hydrazide, inhibited development of root-knot nematode galls on tobacco roots. The significance of these findings as related to nematode-induced galling has yet to be determined.

A few studies have concerned mechanisms by which nematodes induce necrosis in plant tissues. In peach roots infected by a lesion nematode, *Paratylenchus penetrans*, browning was due to breakdown of the plant glycoside, amygdalin, by β-glycosidases of plant and nematode origin to release HCN, which was toxic to the root tissues. Phenolic compounds accumulate in lesion nematode-infected tobacco roots and stored-carrot root tissues, but the identities of these compounds and their mechanisms of formation and accumulation are unknown. In chrysanthemum leaves infected by chrysanthemum foliar nema-

todes, rapid browning was thought to be caused by very active nematodes that quickly punctured and destroyed many mesophyll cells. Such tissues were thought to lack some nutrient that was required by the nematode and that stimulated hyperactive feeding. The rapid browning was interpreted as a sign of plant resistance. In chrysanthemum leaves which browned slowly, it was thought that nematodes were able to obtain required nutrients easily, so cells were punctured and destroyed at a slow rate.

The interrelationships of nematode parasitism and mineral nutrition of plants has been the subject of many studies. In general, plants grown under conditions of marginal or deficient mineral supply are more susceptible to nematode injury than plants grown in the presence of optimal or excess mineral-nutrient supply. Nematode parasitism also may alter the balance of mineral elements in a parasitized plant, but the effects of nematode parasitism on specific nutrients in a plant are not well understood.

CONCLUSIONS

Judgments of nematode pathogenicity should be based on three complementary kinds of evidence: nematode association with disease, nematode involvement in disease, and nematode role in disease.

The majority of histopathological studies of nematode–plant interrelationships have concerned tissues in which parasitic relationships were already established at the time of sampling. Although some fine detailed studies have been conducted, more are needed to develop a comprehensive picture of the plant-tissue alterations associated with plant diseases caused by different nematodes. Especially needed are studies of tissue changes during the critical period of the establishment of the host–parasite relationship; also needed are comparisons of reactions in resistant and susceptible plant tissues. Ideally, histopathological, cytological, and histochemical studies of plant tissues should be conducted simultaneously, and, where feasible, electron microscopy should be used. Such comparative studies, combined with physiological and biochemical studies of nematode-diseased plant tissues, should provide the information needed to understand the fundamental basis of nematode parasitism of plants. These kinds of studies should be encouraged.

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CHAPTER 6

Nematode Interrelationships with Other Plant-Disease Organisms

FUNGI AND BACTERIA

One of the most significant developments in nematology and plant pathology during the last decade has been the demonstration of the many interactions between nematodes and other soil-inhabiting pathogens, including fungi, bacteria, and viruses, in causing plant diseases. The performance of several crop varieties that were selected for resistance to specific fungal or bacterial pathogens in nematode-infested fields figured heavily in this discovery. Increases in growth of these disease-resistant varieties in fumigated soils over those in nonfumigated soils were surprisingly large. Controlled greenhouse tests proved conclusively that certain plant-pathogenic nematodes greatly enhance development of diseases caused by fungi and bacteria in plants that are usually resistant to these diseases. Specific examples are the tobacco varieties developed for resistance to the black shank fungus, *Phytophthora parasitica* var. *nicotianae* (Figure 26), and the Granville wilt bacterium, *Pseudomonas solanacearum* (Figure 27), and cotton and tomato varieties resistant to the *Fusarium* wilts interacting with root-knot nematodes (*Meloidogyne* spp.).

Although various theories of the role of the nematode have been advanced, the mechanisms that render nematode-infected plants susceptible to fungi and bacteria have not been elucidated. In some bacterial diseases, it is thought that endoparasitic nematodes, as they invade roots, merely provide avenues for entrance by bacteria. In some nematode–fungus–plant interactions, the role of the nematode is believed to be more complex than simple wounding of the plant, since wounds are not required for infection of plants by the fungi involved. Recent investigations indicate that host physiology is altered by

FIGURE 26 The interaction between the root-knot nematode, *Meloidogyne incognita*, and the black shank fungus, *Phytophthora parasitica* var. *nicotianae*, on the highly resistant black shank tobacco variety Coker 139. The plants on the right were grown in soil inoculated with the black shank fungus only; the plants on the left were grown in soil inoculated with the black shank fungus and root-knot nematodes. Root-knot nematodes alone, not shown, caused only slight stunting. (After Powell and Nusbaum. Courtesy of *Phytopathology*.)

FIGURE 27 Dixie Bright 101 tobacco plants 21 days after inoculation with *Pseudomonas solanacearum* and *Meloidogyne incognita*. Left: root-knot nematodes added at transplanting and *P. solanacearum* (concentrated suspension) added 24 hours after transplanting. Right: *P. solanacearum* alone (concentrated suspension) added to soil 24 hours after transplanting. Root-knot nematodes alone, not shown, caused only slight damage.

nematode infection of plant tissue. For example, the black shank fungus develops more vigorously and extensively in tobacco root tissues galled by root-knot nematodes than in nongalled roots of resistant varieties. Investigations on how nematode infections affect host physiology are needed, as well as basic studies on the interactions between nematodes and fungi.

Synergistic relationships between phytopathogenic fungi and nematodes in increasing the severity of plant diseases have also been demonstrated. For example, the incidence and severity of wilt of eggplant, caused by the soilborne fungus *Verticillium dahliae*, were increased in the presence of a lesion nematode, *Pratylenchus penetrans*, and the number of nematodes within eggplant roots was increased in the presence of the fungus. In addition to increasing the rate of nematode reproduction, fungus-infected roots were more attractive to and were more readily invaded by these nematodes than were noninfected roots. These relationships, however, are not well understood. Research is needed to determine the influence of individual environmental factors that affect development of pathogens and disease in plants simultaneously exposed to various combinations of organisms.

NEMATODES

Information of the relationship between two or more nematodes in causing a plant disease is fragmentary. One relationship that must exist is that of competition. It is not unusual to find that certain species are primary in a given disease situation; however, whether or not a given nematode is the predominant species in causing disease in a plant will depend on factors such as the host, the initial population level of that nematode species as well as other nematodes, relative reproductive rates of the species involved, soil type, and other environmental factors. Furthermore, any advantage one nematode may have over another in a given situation is likely to be temporary and may change with the planting of a different crop and as the environment is otherwise modified.

VIRUSES

Soil treatments that kill nematodes reduce the incidence of certain diseases caused by soilborne viruses. When certain nematodes were allowed to feed on virus-infected plants and were then transferred to healthy plants, the healthy plants became diseased (Figure 28). Most of the soilborne viruses were really nematode-borne (nematode-transmitted) viruses. These fall into two distinct groups: the round- or polyhedral-shaped (NEPO-Viruses), transmitted only by

FIGURE 28 Chile peppers showing healthy and diseased plants. Left: healthy. Right: diseased, showing stunting and leaf mottle caused by tobacco rattle virus transmitted by the stubby-root nematode, *Trichodorus allius*. (Courtesy of the Department of Nematology, University of California, Riverside.)

species of dagger (*Xiphinema* spp.) and needle nematodes (*Longidorus* spp.); and the rod- or tubular-shaped (NETU-Viruses), transmitted by species of stubby-root nematode (*Trichodorus* spp.). Examples of the NEPO-Viruses include tomato ring spot, tomato black ring, tobacco ring spot and the grapevine fanleaf. The NETU-Viruses include pea early browning and tobacco rattle. Nematodes of some species retain the ability to transmit viruses for several weeks after feeding on an infected plant, indicating that the viruses are probably carried internally in the nematodes. The fanleaf disease of grapes was the first virus disease shown to be transmitted by nematodes. Nematode species in three genera—*Xiphinema, Trichodorus,* and *Longidorus*—are now known to transmit some 20 viruses, many of economic importance, which together attack a wide range of host plants.

PRACTICAL APPLICATIONS

An understanding of interrelationships among various kinds of soil-inhabiting plant pathogens is extremely important. Most agricultural soils are infested with plant-parasitic nematodes of one to six or eight genera, as well as various

phytopathogenic fungi, bacteria, and viruses. Root systems of plants are often attacked simultaneously by several different soil-inhabiting organisms. Diseases resulting from such multiple infection are frequently classified as root-rot complexes. Their diagnosis is difficult. Since multiple infections of plant roots by several pathogens are common, it is necessary to gain a thorough understanding of the contributions of each component pathogen and the factors that favor their individual activity before it is possible to understand complex interrelationships. The breeder developing plant varieties resistant to specific diseases needs to be aware of plant-disease complexes caused by interacting pathogens and, if possible, to incorporate resistance to each of the pathogens involved.

Recognition that nematodes interact with other pathogens in causing disease has to some extent promoted nematode control by the use of nematocides. Growers may elect to accept plant-yield losses caused by some nematode species, especially where the direct damage is slight, but they cannot afford the losses resulting from nematodes interacting with other organisms that cause such destructive diseases as black shank and Granville wilt of tobacco. In many instances, control of nematodes by soil fumigation results also in effective control of other diseases that would become economically damaging in the presence of the nematode.

At present, soil nematocides are cheaper and more effective than soil fungicides or soil bactericides, and, for this reason, soil fumigation is currently the cheapest means of control for many disease complexes. If less-expensive means for control of soil fungi or bacteria or if multiple resistance factors in plants are developed, the situation could change.

FUTURE RESEARCH NEEDS

Disease complexes involving plant-pathogenic nematodes and one or more other soil-inhabiting plant pathogens are now well recognized. Perhaps least understood is why the over-all effects of two pathogens interacting on a host are greater than the additive effects of two pathogens occurring singly. Also, the range and combinations of organisms involved, whether soilborne or aerial pathogens, are not known. For example, the effects of nematode parasitism of plant roots or other parts on leaf diseases caused by fungi, bacteria, and viruses as well as the physiological and biochemical bases for changes in susceptibility or disease expression need to be investigated.

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CHAPTER 7

Considerations Basic to Nematode Control

The purpose of nematode control is to improve crop yields and quality. In a broader sense, nematode control aims to maximize the efficient and effective use of arable lands to meet the increasing need for food and fiber throughout the world. The biology of the nematode, its ecological relationships, methods of spread, value per acre of the host crop, and the cultural practices used in the particular area are important factors which must be considered in developing control measures.

NEMATODES–PRIMARILY A SOIL PROBLEM

All plant parts, including roots, leaves, stems, buds, flowers, seeds, and even the trunks of trees, are subject to attack by nematodes. Most plant-parasitic species live either in the roots or in the soil around the roots of host plants, or in both. Even the foliar parasites spend at least part of their life cycle in the soil or in plant parts associated with the soil.

Nematode eggs, larvae, or adults may be present in the soil and are usually more vulnerable to toxic chemicals or cultural practices than are nematodes within plant tissues. Certain developmental stages of some nematodes, such as the cysts of the sugar-beet nematode (*Heterodera schachtii*), are more resistant to desiccation or starvation than are active stages. However, it is not certain whether nematocidal chemicals are less effective against resistant than against active stages. More reliable information is needed in this important area of research.

NEMATODE INFESTATIONS IN THE FIELD

In some glasshouses or plant beds, where all the growing media can be treated with heat and chemicals or replaced with pasteurized soil, it is possible to eradicate nematode infestations. Under field conditions, however, eradication of nematodes is not possible. Suitable crop rotations, chemical treatments, or use of resistant varieties may reduce infestations to levels below those that cause economic losses in crop yield. But a few nematodes survive even under the most rigorous control programs, and, under favorable conditions and with repeated plantings of suitable host plants, these survivors inevitably build up again to damaging levels. For these reasons, it is justifiable to try to prevent the introduction of nematode parasites into noninfested fields or areas.

The most common and efficient means of nematode dispersal are the activities of man. Farm equipment moved from field to field, or even hundreds of miles across state boundaries, often carries viable nematodes in the soil or plant parts that cling to the equipment. Infected rootings, cuttings, bulbs, runners, and even seeds shipped for planting in other areas provide an ideal and direct means for nematodes to be carried from one site to another.

HAZARDS OF MONOCULTURE

Because it is difficult to detect low nematode populations in the soil and impossible to eradicate an infestation once it is established, crop rotation is used to prevent the buildup of damaging infestations. It is a sound precaution to use crop rotations, even when repeated samplings fail to show the presence of plant-pathogenic nematodes. It may take years, even under monoculture, for a trace infestation of a particular species to build up to detectable and eventually economically damaging levels. Once present, however, a high population is difficult to reduce and makes economic production of a susceptible crop extremely difficult. It is much harder to produce profitable crops once a severe nematode problem is present than it is to prevent the introduction and buildup of the pathogenic nematodes. Furthermore, the choice of a crop to use in a rotation is restricted when high levels of a destructive nematode are present.

CONTROL METHODS AND LOCAL SITUATIONS

Numerous measures may be successfully employed against a given nematode problem. As in any pest-control operation, local conditions may exclude some or all possibilities. Control measures must take into account the particular

area or situation involved. For example, it is important whether the host crop is a perennial or an annual and whether established plantings or future plantings are contemplated or planned. Variations in soil type are also important: dispersion of fumigants is greatly restricted in fine-textured clay, organic peat, or muck soils. This restricted dispersion limits the direct control of nematodes by fumigation, although practical economic responses by host plants are known from fumigation of both clay and peat types of soil. Further research is needed for a better understanding of factors affecting chemical control in these types of soil.

Another example of the importance of local situations concerns resistant plant varieties. The first problem is availability of resistant varieties; certain resistant varieties are not always available in supply adequate to meet demand, and many plants have no known sources of resistance to important nematode species. Furthermore, resistance bred for one nematode species usually does not hold true for all populations of that species or for other species. Local differences in nematode populations or species may be an important factor. The nature and extent of these differences are fruitful areas for research.

The grape rootstocks Dogridge and Saltcreek, bred for resistance to root-knot nematode (*Meloidogyne* spp.), illustrate the importance of local conditions in the successful use of plant varieties for nematode control. These rootstocks, especially Saltcreek, are not suitable for use in fertile sandy loam or finer-textured soils, because they produce excessive vegetative growth, which makes control of fruit production difficult or impossible and affects the quality of fruit.

Rotation schemes are highly effective control programs, but differences in local conditions must again be considered, because not all crops that are suitable for rotation can be used profitably.

Differences in nematode life cycles and feeding habits must be considered in the development and application of control measures. Ectoparasitic nematodes, which feed at root surfaces, are easily controlled in living plants by a side-dressing treatment with DBCP (1,2-dibromo-3-chloropropane). Control of the citrus nematode (*Tylenchulus semipenetrans*) on citrus and a dagger nematode (*Xiphinema index*) on grape are good examples of the successful use of DBCP. However, endoparasites, which feed and reproduce inside plant root tissues, such as root-knot nematodes, are not as readily killed in living plant roots; consequently, control has not been satisfactory when measured in growth response and yields.

Another example of the importance of differences in life habits is encountered when field soil is turned repeatedly during the summer to control nematodes. Theoretically, this treatment exposes nematodes to higher than normal temperatures and desiccation, either of which may be lethal. However,

the larvae of some species of cyst nematodes, such as the sugar-beet nematode, are protected inside eggs held in the cysts; thus, they are better able than unprotected nematodes to resist desiccation and exposure to higher temperatures. The mortality of other species, such as the root-knot nematodes and ectoparasitic species (*Trichodorus, Xiphinema,* and *Criconemoides*), is very high when the nematodes are exposed by soil cultivation.

In a few situations, those species that attack the aerial parts of plants can be controlled by chemical sprays, or, if visual symptoms are well-defined and easily detected, the removal of infected plants may be practical. Parasites of roots present a different problem. Many root infections produce symptoms that are not diagnostic or that may be overlooked because they are underground. Nematodes in the soil are more difficult to recover for identification than those in plant tissues.

Control of nematodes in perennial tree and vine crops is more difficult than in annual or herbaceous crops. The long-term nature of perennial crops makes rotation schemes, which are successfully used with annual crops, impractical. Furthermore, with annual crops, preplant soil fumigants can be applied every year or every other year, as needed, and the nematode kill does not have to be as complete as it must be for tree and vine crops. Often, successful annual crops can be grown following fumigation or fallow periods, even though nematode populations build up during that growing season to the same or higher levels than in untreated areas. With perennial crops, nematodes that survive fumigation or fallow have time to recover and build up to destructive levels. The cost of removing old plantings of tree or vine crops and bringing new plantings into bearing is very high. Surviving roots of excised plants provide a source of nutrient for nematodes and in part negate the effects of control practices. This has been studied in detail for grapevines, where it is known that some nematodes survive for four to five years after the vines are removed. There is also some evidence that citrus-nematode larvae survive for as long as nine years after removal of their tree hosts.

IDENTIFICATION OF NEMATODES AND DEGREES OF INFESTATIONS

The first step in diagnosing a crop-production problem in which nematodes may be a factor is to collect and identify the nematodes that are present in the involved soil or plant material. Accurate identification to species is essential because of differences in nematode life habits, host ranges, and pathogenic effects on various host plants. Distribution data are also important for determining whether the nematodes are associated or coincident with the disease problem.

COLLECTION OF SOIL AND PLANT SAMPLES

The collection of soil may be made with a variety of augers, with tubes, or simply with a shovel (Figure 29). Moist soil, preferably in the vicinity of plant roots, rather than dry surface soil should be sampled. Each sample should contain feeder roots whenever possible. To avoid drying, plastic bags are preferable to other containers for soil and plant samples. Storage of samples at cool temperatures is essential.

EXTRACTION OF NEMATODES FROM SOIL AND PLANTS

MECHANICAL ISOLATION

Small pieces of plant parts, such as roots, stems, leaves, buds, and seeds, may be examined in clear water by tearing the tissue apart with dissecting needles. If nematodes are present, they will usually float free in the water and are readily seen.

Food blenders (Figure 30) are sometimes used to macerate plant tissues to free the nematodes. The resulting suspension of plant parts and nematodes can be examined directly or can be fractionated with sieves before examination.

SEPARATION BY SPECIFIC GRAVITY, SIZE, AND SHAPE

Wet Sieving of Soil

A volume of soil is mixed with approximately twice its volume of water in a pan. About 10 seconds are allowed for settling of coarse particles. Supernatant is then decanted through a series of sieves with openings ranging from 0.8 to 0.04 mm (Figure 31). The nematodes caught on the screens are washed into a beaker. The resultant suspension contains some small soil and plant particles along with the nematodes in water. This is examined under a binocular microscope equipped with a substage and a flat mirror to give diffuse, indirect light through a clear-glass stage (Figure 32). Final specific identification is usually possible only by using a compound microscope with 900 to 1,000 magnification (oil immersion).

Elutriation

To increase precision, elutriation is sometimes substituted for the decanting of supernatant (Figure 33). *Heterodera* cysts are lighter and easier to separate

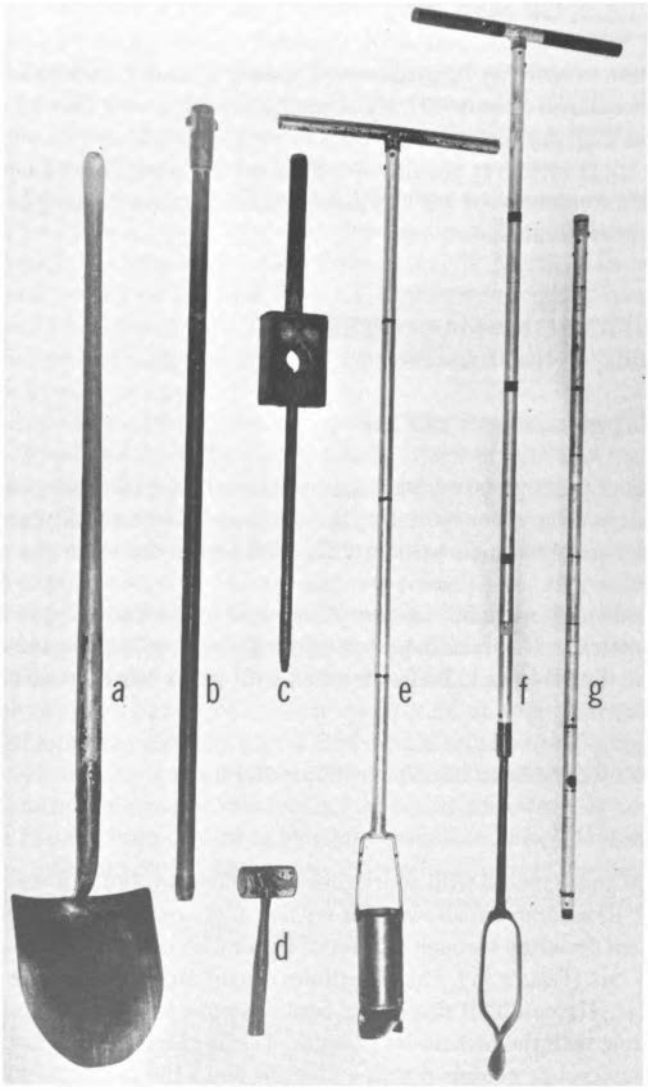

FIGURE 29 Equipment for collecting soil and root samples: a, shovel; b, Veihmeyer soil tube; c, hammer to drive Veihmeyer tube into ground; d, wrapped leather mallet to tap soil from bucket auger; e, 3-inch bucket-type auger; f, Dutch soil auger; g, extension used with e and f for deep samples.

FIGURE 30 Food blender for macerating plant parts.

FIGURE 31 Soil sieves, pans, and beakers for wet-sieving soil.

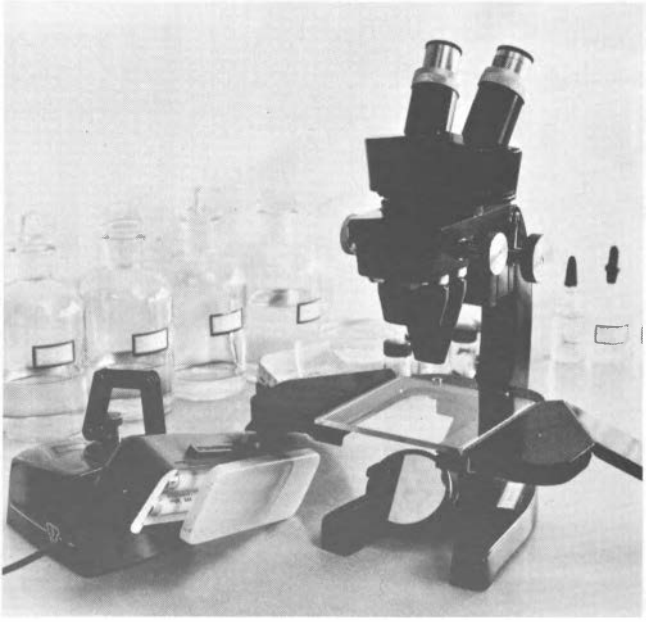

FIGURE 32 Dissecting microscope with clear-glass stage and substage mirror. Lamp with cover partly removed to show fluorescent bulbs.

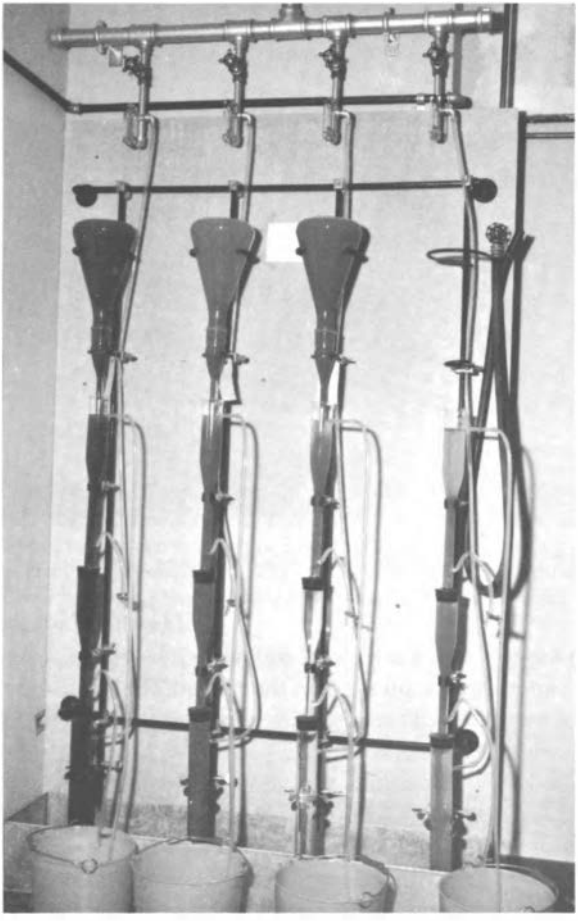

FIGURE 33 Seinhorst soil elutriators.

from soil than other nematode forms, and special apparatus have been devised for their recovery (e.g., Figure 34). Increasing the specific gravity of the soil solution with sugar and subsequent centrifugation is another commonly used method.

SEPARATION BY NEMATODE MOVEMENT

The equipment most widely used for separation by nematode movement is the Baermann funnel. A wire screen is fitted across a glass funnel about half

FIGURE 34 Fenwick can to separate *Heterodera* cysts from soil.

an inch below the top rim. A sample of soil, wet-sieve residue, or plant parts is placed on a paper tissue supported on the screen. The tissue used is preferably of the silicone-treated type, such as Kimwipes (Kimberly-Clark Corp., Neenah, Wisconsin), which do not disintegrate when wet. The bottom of the funnel may be extended by rubber or plastic tubing and closed with a pinchcock or a small vial inserted in the end of the tubing (Figure 35). The funnel is then filled with water to a level to make contact with the sample on the wire. The nematodes move through the paper and screen into the water in the funnel. They settle by gravity to the bottom of the funnel, where they are collected for identification.

A modification of this method is one set up inside a mist chamber (Figure 36). Substitution of a fine mist for the water bath allows better aeration and results in less microbial and chemical interference. In using a mist, the rubber tubing attached to a funnel can be inserted into a large test tube. The funnel is then placed in a mist chamber, where it is exposed to intermittent, regular fine sprays. The water filters through the sample, down the funnel, and into the test tube, where it accumulates and ultimately overflows. The nematodes carried in the water settle by gravity into the bottom of the tube. Both the Baermann-funnel and mist-chamber techniques depend on nematode activity and ability to move through soil or out of plant material and through the tissue paper into the funnel.

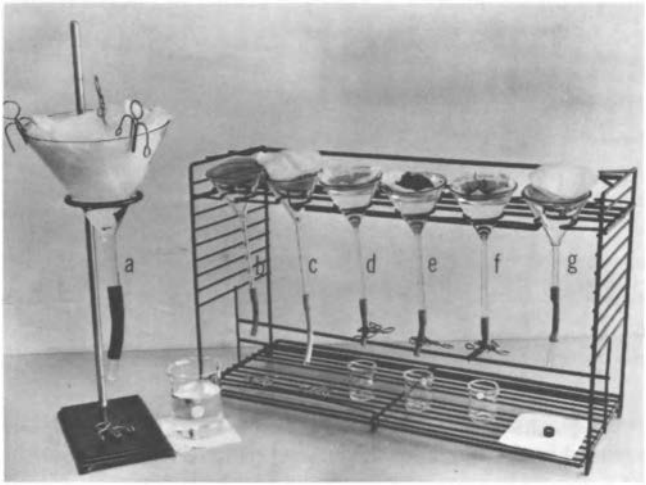

FIGURE 35 a, Large Baermann funnel fitted with several layers of cheesecloth to separate *Xiphinema* spp. from soil sievings; b, funnel showing wire screen in place; c, funnel with screen and tissues; d, funnel with pinchcock and water added; e and f, funnels with soil and root pieces on tissues; g and a are funnels fitted with small vials instead of pinchcocks.

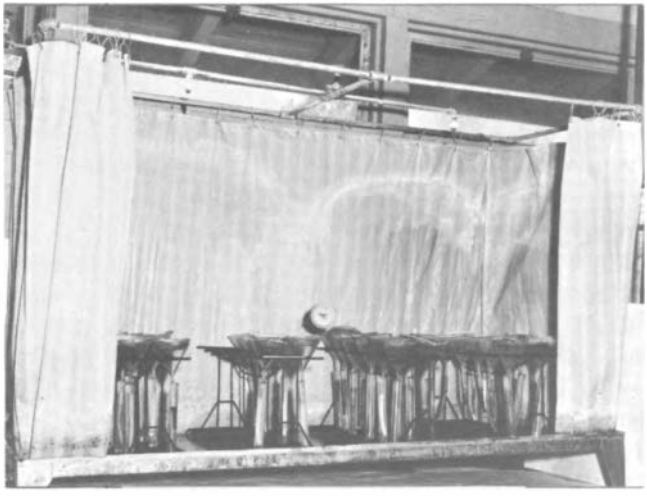

FIGURE 36 Baermann funnels on racks in mist chamber. Funnel tips are inserted directly into large test tubes instead of being closed by pinchcocks or vials.

Endoparasitic species can be recovered from inside roots or other plant material by washing the plant parts free of soil and storing in a Mason jar or closed plastic bag (Figure 37). The lid of the Mason jar should be reversed and screwed down lightly enough to avoid desiccation but not so tightly as to lead to suffocation. The nematodes emerge from the roots, and after several days storage they are recovered by rinsing the jar with fresh water. The rinse water can be examined directly or cleared through the Baermann funnel.

After the nematodes have been recovered by any of these or similar methods, identifications can be made from temporary mounts or specimens prepared in glycerine. The nematodes are picked up by a pipette, by a slender pick made of a bamboo splinter (Figure 38), or by nylon bristle from a tooth brush and transferred to a separate drop of water on a glass slide. Gentle heat from a small flame, sufficient only to stop movement, will kill the nematodes in the water. Before placing a cover glass on the drop of water, small glass rods are placed in the water to prevent the nematodes from flattening. A fingernail-polish or paraffin seal prevents drying and permits examination for several hours.

Permanent mounts are made by killing the nematodes in water by gentle heat and fixing in 2½ percent formalin for 24 hours. The nematodes are then transferred to 2½ percent glycerine in 30 percent alcohol for 24 hours in a

FIGURE 37 Moist roots in plastic bag or in Mason jar.

FIGURE 38 Tools and equipment for killing, fixing, preserving, and mounting nematodes for identification.

sealed cavity slide and finally to 5 percent glycerine in 30 percent alcohol in a B.P.I. dish stored in a petri dish. After the mixture dries to a viscous condition (about 7 to 10 days), it is placed in a desiccator for 24 hours, and the specimens are mounted in dehydrated glycerine. Glass rod supports are again required to prevent flattening. Zut slide ringing compound (Bennett's, Salt Lake City, Utah) is used to seal the cover slip.

It is not always possible to prepare permanent slides for reference and restudy, in which case it is essential that mass collections be made whenever possible. Nematodes recovered in water are concentrated by allowing to settle for an hour or more, decanting the excess liquid, and adding hot 5 percent formalin in equal volume. Again allow to settle, decant, and store in small vials sealed by paraffin. Preserved material of this kind can be made into permanent mounts any time in the future when and if specific identification must be checked. The fact that there are usually mixtures of various plant-parasitic species in the same soil, often two or more species of the same genus that could be confused or overlooked, is further reason for keeping mass collections.

Taxonomy of nematodes becomes increasingly complex and technical every year, as many new and closely related species are described. For this reason, increased training of scientists in this field is needed. Whenever practical, there should be at least one trained taxonomist in every laboratory where nematological research is conducted.

POPULATION LEVELS AND PREDICTION OF PLANT DAMAGE

For most plant-parasitic nematode species, there is insufficient information on the levels of population or density of infestations that are likely to cause damage to particular crop plants. One notable exception is the golden nematode (*Heterodera rostochiensis*). In Great Britain and the Netherlands, advisory services are established for the control of this nematode by crop rotation. Soil samples are examined to calculate nematode populations surviving in the cyst stage. Based on the number of cysts with viable contents per gram of soil, advice is given to growers as to whether to plant potatoes or to continue to plant nonhost crops. Similar advisory services are now available in the Netherlands for other nematodes pathogenic to various agricultural crops. The growers pay a fee for this service, which includes identification of nematode infestations in soil, calculations of nematode population densities, and advice as to types of crops to plant, based on the nematode analyses.

Before such services can be developed further, much information is needed on the host-range preferences of many plant-parasitic nematode species, the minimum sampling procedures on which to judge infestations, and how to predict damage from population densities. Unfortunately, examination of soil samples is laborious, time-consuming, and expensive. Because of this high cost it is probable that similar advisory services will be developed only for agricultural crops of high acre value.

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PART III

BASIC PRINCIPLES OF CONTROL

CHAPTER 8

Prevention of Spread

Plant-pathogenic nematodes move only relatively short distances under their own power; therefore, the most usual means of nematode spread is the transportation of infested soil and infected vegetative plant parts by man. Nematodes may also be carried by wind, water, and domestic or wild animals and birds. Active stages of most nematodes are susceptible to desiccation; thus, the resistant or resting stages are the ones most important in long-distance spread. Establishment in a new area occurs only when sufficient numbers of viable nematodes are transported to a location where susceptible hosts are subsequently planted and where the environment is suitable for reproduction of the nematode species.

MEANS OF DISSEMINATION

SOIL AND PLANT TISSUE

Soil and vegetative plant parts, because they often protect nematodes from desiccation and are frequently transported by man, are important carriers of nematodes over both short and long distances. Soil is also important because most plant-pathogenic nematodes spend at least part of their lives in soil, and soil thus infested is commonly transported along with plant materials.

Nematodes are frequently present on the surface of true seed and in associated debris or soil. For example, cysts of the sugar-beet nematode (*Heterodera schachtii*) have been found on sugar-beet seeds. Only a few nematode species, such as seedgall nematodes (*Anguina* spp.) and the stem nematode

(*Ditylenchus dipsaci*), infect true seed, but many kinds of plant-pathogenic nematodes infect vegetative plant materials used for propagation, such as transplants, ornamentals, nursery stock, bulbs, and corms. Since these high-acre-value plants are cultivated on the best soil available, one crop is grown frequently or continuously on the same land, often resulting in damaging nematode populations in plant materials and in the soil. Infected propagation materials are particularly important in nematode spread, because materials from a relatively small area are used to plant much larger and often widely separated areas. A pathogenic nematode introduced into a field in this manner is likely to spread throughout the field (Figure 39), to adjacent fields on the same farm, and to adjacent farms. Although experimental data indicate that plant-parasitic nematodes passing through the digestive tracts of animals are killed, infected plant parts and soil associated with manure are important in spreading nematodes.

MACHINERY, REUSABLE CONTAINERS, AND FERTILIZER

Nematode-infested soil and infected plant materials may be transported along with machinery and reusable containers, such as burlap bags and crates. Nematodes moved by these means are often deposited in soil in which susceptible crops are grown later. Nematodes may also be carried from infested to clean

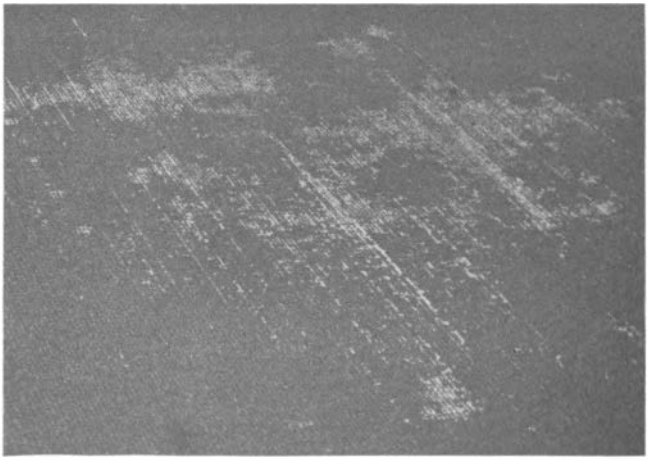

FIGURE 39 Areas of poor growth shown in aerial view of sugar-beet field indicate pattern of spread of sugar-beet nematode (*Heterodera schachtii*).

fields on automobiles, especially in soil clinging to tires and fenders. The peanut lesion nematode (*Pratylenchus brachyurus*) is spread by the use of infected peanut hulls as a conditioner in fertilizers.

The golden nematode (*Heterodera rostochiensis*), an introduced pest, was found in more than 30 fields of one grower on Long Island, New York. These fields were on various farms located among uninfested fields. It was concluded that nematode spread was associated with the movement of machinery and used burlap bags.

ANIMALS

Undoubtedly, nematodes are disseminated in mud or plant debris clinging to birds and other animals, but there is little published information on the extent of this means of spread. Potentially, stages of plant-pathogenic nematodes resistant to drying could be carried for long distances by migrating birds. Some animals, such as rodents and insects, live in the soil and thus are often contaminated with infested soil. Horses and other animals used to pull farm implements may transport infested soil within and between fields. Many kinds of nematodes may be spread on clothing, shoes, hand tools, or hands. *Rhadinaphelenchus cocophilus*, the causal agent of red ring of coconut, is disseminated by the palm weevil and other insects.

WATER

Although nematodes may be carried for relatively long distances in irrigation water, only local spread generally occurs in surface water. Nematodes may be transported short distances by spattering raindrops. Living nematodes may be carried by the movement of underground water, but dead or inactive nematodes are not carried through soil, even by water at high flow rates.

In coarse-textured soils of Florida citrus orchards, spread of the burrowing nematodes (*Radopholus similis*) downhill in surface-drainage water was eight times the rate of uphill movement. Also, in Florida citrus orchards this nematode moved under a hard-surface road in underground water. In an experiment in which a column of coarse-textured Florida soil 42 inches high and 3 inches in diameter was watered intermittently from the top, the burrowing nematode was moved from top to bottom in 30 hours.

Nematode spread by water, particularly long-distance spread, depends on resistance of the nematode to submersion in water, and resistance varies among species and among stages of a species.

Irrigation, which is used more in crop production every year, effectively transports nematodes. More information of the importance of this means of spread is urgently needed.

WIND

Although wind is often mentioned as an important factor in nematode dissemination, there is little evidence of long-distance spread by wind or of extensive local spread in the direction of prevailing winds. Large quantities of soil and small pieces of plant debris are blown from field to field, farm to farm, and for even greater distances, but only nematodes resistant to drying survive such dissemination, and most stages of the majority of nematodes are killed easily by desiccation. Large numbers of cysts of the golden nematode, which are highly resistant to drying, were found in snow along roads adjacent to Long Island, New York, potato fields infested with this nematode. It appears doubtful that these relatively heavy cysts would be carried by wind for long distances.

NATURAL BARRIERS

Natural barriers such as mountains and oceans reduce nematode spread by water, wind, animals, and by soil adhering to farm machinery. However, the use of refrigerated ships and trucks and of airplanes makes possible the transportation of infected plant materials over or around practically all natural barriers. Infested soil may be carried along with both host and nonhost plants.

If the climate is unsuitable, an introduced nematode species either will not survive or its population increase will be so slow that it will not become an economically important pest. Climatic factors not only influence nematode survival directly but also influence it indirectly by their effect on kinds of crop plants that can be grown.

Although a nematode is transported to a new area with favorable environment, it will not become established unless one or more cultivated or wild hosts is present. If a cultivated host is grown infrequently in a crop rotation and wild hosts are absent, the introduced nematode may die from starvation. However, any species of plant-parasitic nematode should be considered a potential threat to agriculture in areas free of that species, and all means should be taken to prevent its entry.

PRACTICES TO RESTRICT SPREAD

SANITATION

It is particularly important to control nematodes in nurseries, because plant materials from them are widely distributed. All soils in nurseries should be treated with steam or nematocides. Floors, benches, containers, tools, and storage areas of buildings in which plant materials are handled or stored should be thoroughly cleaned or fumigated. Splashing of water should be avoided, and hose nozzles should be kept off the floor. Clothing and shoes worn by workers in nematode-infested areas should not be worn in nematode-free areas.

The identity of new plant material should be maintained and the material isolated until it is known to be free of plant-parasitic nematodes. Nematode-contaminated plant material should be isolated and either discarded or treated, if satisfactory treatments are available.

NEMATOCIDAL TREATMENTS

The control of plant-pathogenic nematodes in nurseries by the use of heat and nematocidal chemicals results in the production of high-quality nursery stock and reduction of nematode spread. Moist heat, when properly used, will eliminate nematodes from soil, but it is expensive and thus practical only for relatively small areas or quantities of soil. Nematocidal chemicals, when used commercially as preplant treatments, will not eradicate nematodes from soil but will kill a high percentage of them. Because of application problems and phytotoxicity of most nematocidal chemicals, the treatment of soil around living plants is generally less effective than preplant treatments. Control, which approaches eradication of nematodes inside roots of some plant species, has been achieved by dipping bare roots in aqueous solutions of nematocidal chemicals. Although extensive plant damage often results, some hot-water treatments kill nematodes in plant tissues. For example, hot water is used to treat garlic bulbs infected with stem nematodes, grape rootstocks infected with root-knot and lesion nematodes, and citrus rootstocks infected with burrowing nematode and citrus nematode (*Tylenchulus semipenetrans*) (Figure 40). To increase the effectiveness of nematocidal treatments, research should be directed toward developing inexpensive, effective, nonphytotoxic chemical-dip treatments for bare-rooted stock; safe and effective drenches or fumigants for established plants and container-grown or balled stock; and effective systemic nematocides.

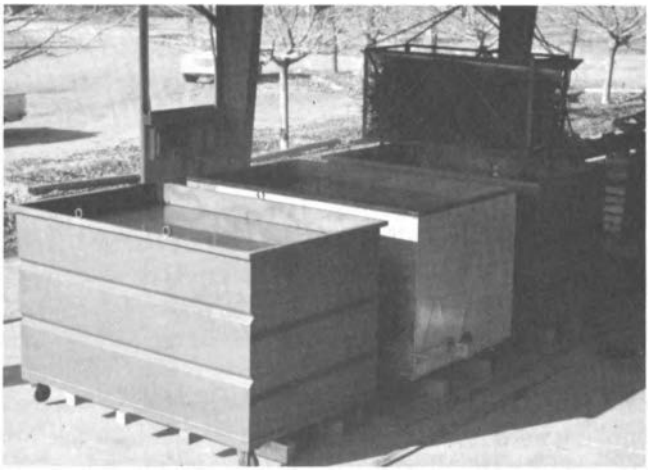

FIGURE 40 Hot-water treatment of grapevine rootings for eradication of root-knot (*Meloidogyne* spp.) and lesion (*Pratylenchus* spp.) nematodes: right tank for presoaking; center tank for treatment, 51.5°C for 5 minutes; left tank for cooling. (Courtesy of the Department of Nematology, University of California.)

CERTIFIED PLANT MATERIALS

The production of vegetative plant-propagation material that is certified to be nematode free or to have a specified level of infection is accomplished by growing clean plants in clean soil or other media. Strict sanitation must be practiced, and both the material and the rooting medium must be periodically checked for the presence of pathogenic nematodes. Vegetative seed of banana free of the burrowing nematode, potato seed pieces free of the golden nematode, garlic cloves free of the stem nematode, and strawberry plants free of root-knot and lesion nematodes are produced commercially.

The use of resistant or immune plant varieties also reduces nematode spread. Of course, resistant varieties, which are symptomless hosts of a pathogenic nematode, reduce chances of nematode detection and thus increase spread.

QUARANTINES AND REGULATIONS

Practically all countries and subdivisions of countries have some type of plant disease and pest act under which exclusionary measures are promulgated.

Quarantine measures are fundamentally regulatory and prohibitory. They prohibit the introduction into a specified area of a particular plant or possible carrier of a pest, whether or not it is known to be carrying the pest, but nearly every quarantine includes provisions that allow for the introduction of plants or possible carriers that in some manner have been protected against or freed from contamination by the pest against which the quarantine is established. These exceptions to total exclusion regulate rather than prohibit the movement of the pest and of plant parts with which it is associated.

For example, the United States federal soybean cyst-nematode (*Heterodera glycines*) quarantine prohibits the movement of root crops from the regulated areas where they are grown, but it also provides that root crops (except sugar beets) may be exempted "if cleaned free of soil." The prohibition of movement is a quarantine measure, while the provision for cleaning the root crops is a regulation allowing free movement of plant materials freed of the pest. The New York State golden-nematode quarantine prohibits the movement of plants of tomato or eggplant grown on infested or dangerously exposed fields.

Quarantine acts may be either local or general. A local or district quarantine, of which the soybean cyst-nematode quarantine is an example, prohibits introduction of plants or other potential carriers from specified districts in which the nematode pest is known to occur. A general quarantine forbids the importation of plants or carriers from any area, regardless of whether or not the pest is known to occur there.

The California quarantine against the burrowing nematode is not a general quarantine, as it prohibits introduction of soil and rooted plants from Florida, Hawaii, and Puerto Rico, with various specified exceptions and regulatory provisions. However, administrative instructions added as an appendix to the quarantine require that host plants in nine specified genera must be intercepted and inspected for burrowing nematodes by laboratory methods, regardless of their origin. These latter provisions, in effect, extend the quarantine into a general one in regard to certain plants.

Provisions included in quarantines, and other regulatory measures, may involve a wide variety of measures aimed at giving assurance that the pest is not being introduced with the exempted plants or articles. Certification of origin is one of the most common provisions. The California burrowing-nematode quarantine provides, for example, that plants may be exempted if they bear an official certificate stating that they were grown where surveys failed to detect the pest. The same quarantine includes another type of provision that deals with the conditions under which the plants were produced, viz., "above ground in sterilized soil or other suitable material prepared or treated to assure freedom from burrowing nematode."

Another common regulatory measure requires that plants or carriers be subjected to a specified treatment for destruction of the pest. Several counties

in California's central valley restrict the entrance of sugar-beet harvesting machinery unless it has been thoroughly steam-cleaned, and similar requirements are in effect in soybean cyst-nematode and golden-nematode quarantines. United States federal plant-quarantine officials at ports of entry require methyl bromide fumigation of used bags that may be contaminated with golden-nematode cysts.

Regulations may place stringent controls over every aspect of the growing of crops to prevent the possibility of spread of nematode pests. The New York State Golden Nematode Act of 1947 placed the planting, growing, and harvesting of potatoes under the direction of the project administrator and included regulations relating to crop rotation, topsoil movement, and soil treatment.

In addition to compulsory regulations issued in direct connection with quarantines, other types of regulatory measures may be used on a voluntary basis to exclude nematode pests or restrict their spread. These include various programs mentioned earlier for certification, registration, or inspection of nursery stock, bulb crops, seed potatoes, seed garlic, or other types of agricultural seed. While these programs may contribute to the purposes of quarantine and are often enforced by quarantine officials, their principal object is improvement of quality of the planting stock, and they are usually instigated by the growers themselves.

Accurate knowledge of the distribution of a nematode is essential before the promulgation of a quarantine or regulation involving that nematode. Before the distribution can be determined, the nematode species must be correctly identified by a taxonomic specialist.

In general, a nematode is quarantined or regulated only when it is of known economic importance in one area and unknown, or occurs as a localized or incipient infestation, in the area to be protected. Occasionally, as a safeguard, a quarantine or regulation is established against a nematode believed to be new to an area and suspected of being economically important.

When the barley root-knot nematode (*Meloidogyne naasi*) was identified in a small area in the Tulelake Basin of northern California, regulations on the movement of root crops and machinery were established. The economic importance of the nematode was not well known, but it was the only known infestation of this nematode in North America, and safeguards against spread appeared to be warranted.

Inspections for the presence of nematode pests, for enforcement of quarantines and regulations, may be made at either the point of origin or destination. Inspection at origin offers several advantages over destination inspection: nematodes are more readily detected in fresh soil or plant samples collected from the growing crop; infested areas may be effectively delimited; duplication of sampling of material shipped to many destinations is avoided; and shipping costs of contaminated materials are avoided. Bulb crops and other

ornamentals grown in the Netherlands are inspected annually for presence of the golden nematode before they are shipped to the United States.

In the enforcement of the U.S. Plant Quarantine Act and subsequent regulations and quarantines, federal port and border inspections annually intercept numerous important plant-pathogenic nematodes. During the fiscal year 1964–1965, the golden nematode was intercepted 101 times; the oat cyst nematode (*Heterodera avenae*), 42 times; and root-knot nematodes, 66 times.

State regulatory agencies also make quarantine or regulatory inspections of plant materials in interstate shipments. In 1965, the nematology laboratory of the California Department of Agriculture examined about 1,250 soil and plant samples taken from shipments originating outside the state: 31 of the samples contained nematodes not known to be established in California, and nematode pests of significant economic importance to agriculture were found in 275 of the samples.

Although careful inspections and surveys are of great value, all nematodes are not detected by present methods. Thus, populations below the detectable level must be considered in an over-all control program.

The detection of nematodes by quarantine and regulatory workers is difficult, because plant symptoms are not diagnostic for most of the important root-feeding nematodes. Although visual inspection for nematodes is widely used, it is recognized that usually only heavy infections of root-knot and lesion nematodes can be detected in this manner.

Despite the limitations of quarantines and regulatory measures and the difficulties in measuring their effectiveness, they have played an important role in limiting the spread of plant-pathogenic nematodes. Furthermore, they encourage growers to produce clean plant materials and discourage the shipment of nematode-infected materials.

Basic information from future research in such areas as nematode taxonomy, host-range studies, pathogenicity, and soil- and plant-sampling is urgently needed for improving methods used to prevent nematode spread.

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CHAPTER 9

Reduction of Nematode Populations through Land-Management and Cultural Practices

Most plant-parasitic nematodes can be controlled to varying degrees by land-management and cultural practices. These include fallow, the practice of keeping the land free of all plant growth; flooding; growing cover crops; crop rotation; time of planting; organic manuring; removal or destruction of infected plants; trap and antagonistic crops; nutrition and general care of host; and sanitation and the use of nematode-free planting stock.

The specific principles involved in control of nematodes by land-management and cultural practices differ; however, all are based on the inability of nematodes to survive, multiply, and cause disease under the conditions imposed on them by the use of these practices. Most of these practices reduce nematode populations gradually over a period of weeks, months, or even years, as opposed to rapid kill such as that obtained with heat or toxic chemicals. Furthermore, as control is relative, satisfactory economic control may not be achieved by any single practice but by a combination of several practices. The fact that a practice that reduces the nematode population considerably may not be economically effective at present does not preclude the possibility that it may be economically feasible in the future. With the advance of knowledge of the effect of specific practices on nematode populations and with more efficient implementation of practices, for example, through improved machinery, a high degree of control may be possible. Because of this possibility, those practices that are known to reduce nematode populations to a measurable extent are discussed even though they may not be economically feasible or widely used at this time.

FALLOW

Fallow is the practice of keeping land free of all vegetation for varying periods by frequent tilling of the soil by disking, plowing, harrowing, or by applying herbicides to prevent plant growth. At least two principles of nematode control are represented by this practice.

The first principle, and perhaps the most important, is starvation of the nematode. Plant-parasitic nematodes are obligate parasites, depending on living hosts for the food necessary to develop to maturity and to reproduce. Therefore, in the absence of a host plant, the nematode will die after the stored food in the body has been depleted. Some of the cyst nematodes (*Heterodera* spp.) can survive as unhatched eggs or dormant larvae in cysts in the soil in the absence of a host for at least 14 years, but these are exceptions. In upper soil layers, most plant-parasitic nematodes probably do not survive for more than 12 to 18 months, and many do not survive the first 6 months. Compared with upper soil layers, soil at lower depths is more constantly cool and moist, increasing the length of nematode survival. Survival is also influenced by the amount of infected root debris remaining in the soil from the previous crop.

The second principle involved in fallow is death through desiccation and heat. With some exceptions, nematodes of most species, depending on stage of development, will die if exposed to the drying action of the sun and wind. When fallow land is tilled frequently to destroy vegetation, the surface strata of soil are exposed to the drying and heating effects of wind and sun. Fallow is especially effective in areas of low rainfall and high soil temperatures or in areas where rainfall is seasonal, thus resulting in long periods, perhaps 6 months or more, of dry conditions.

There are several objections to the practice of fallow: the operations necessary to maintain lands completely free of vegetation are difficult and expensive; fallow in areas of high rainfall is a poor soil conservation practice and is likely to impair the physical structure of the soil; and fallow land does not contribute to farm income.

FLOODING

Flooding of fields to control nematodes is not widely accepted. Results of early investigations indicated that flooding for 12 to 22 months is required to rid soil of root-knot nematodes (*Meloidogyne* spp.). Where water is plentiful and level land can be taken out of production for long periods, flooding may be a useful control practice. Certain crops, such as rice, can grow under flooded conditions. Experiments showed that rice seeded in water and kept flooded for 4 to 6 weeks had only a trace of the white tip disease caused by

the white-tip nematode (*Aphelenchoides oryzae*), whereas rice drilled and flooded after the seedlings were 3 to 4 inches tall was 60 percent diseased. In this case, the nematodes were seed-borne rather than soilborne, but this is a special situation, since flooded fields are not generally planted.

The principles of control involved in flooding are not completely understood. Presumably, flooding eliminates all host plants, and the nematodes die from starvation. In addition, flooding decreases the oxygen content of the soil and may kill nematodes by asphyxiation. It has been shown, however, that stored foods of some nematodes are not used as rapidly under conditions of low oxygen, and this may actually extend the length of survival. Chemicals lethal to nematodes, such as butyric and propionic acids, hydrogen sulfide, and perhaps others, often develop in flooded soils of low pH containing large amounts of rapidly decomposing organic matter. Flooding of rice fields in Louisiana gives good control of certain nematodes that are parasitic on rice. It should be remembered, however, that nematodes are essentially aquatic, and some species may persist but will not reproduce in saturated soils. Some disadvantages of flooding include the possibility of introducing new pests, as well as changes in structure, fertility, and pH of the soil.

COVER CROPS

Cover crops are grown in the winter as a soil-conservation measure and to provide forage for livestock or in the summer between rows of widely-spaced crops such as fruit trees. Populations of some nematode species may decline on cover crops, but others undoubtedly increase. A reduction in population is probably caused by resistance of the cover crop to the particular nematode, and, conversely, any increase is due to susceptibility of the crop to the species that increased. Low winter temperatures also may limit populations of parasitic species so that they do not increase substantially even though the cover crop is susceptible. With some sedentary endoparasites, the "trap-crop principle" may be operative. In trap-cropping, the larvae enter the roots, and many develop to an immobile stage but fail to develop into adults; thus, they are trapped within the root tissues. The addition of organic matter resulting from the plowing under of green-manure crops increases the population and predacious activity of nematode-trapping fungi, predacious nematodes, and of the internal parasites of nematodes. Also, nematocidal substances, such as butyric acid, form during the decomposition of cover crops such as rye and timothy. The importance of these substances in biological control of plant-parasitic nematodes, however, is little understood. Although some investigators are optimistic about the potential of biological control, no practical control of nematodes by predacious fungi, toxic substances resulting from decomposition of crop residues, or other biological agents is known. This subject is discussed more fully in Chapter 10.

CROP ROTATION

The use of crop rotation to reduce nematode populations is without question the most effective and most widely used land-management practice. This practice was used by growers long before its significance as a means of nematode control was recognized. To be an effective control practice, crops that are unfavorable hosts for the nematode must be included in the rotation sequence. Some of the more serious nematode pathogens, such as the golden nematode of potatoes (*Heterodera rostochiensis*), the soybean cyst nematode (*H. glycines*), the stem nematode of alfalfa (*Ditylenchus dipsaci*), and some species of the root-knot nematode, are comparatively host specific, which makes selection of unfavorable hosts relatively easy. Furthermore, in areas where one of these nematodes occurs, it is usually the predominant species, and growth of a resistant crop for two to four years will greatly decrease the population of that species by starvation. Growth of a resistant crop for one year is generally inadequate. Two resistant crops between susceptible crops may give fair control, but three or four years and, with some nematodes, seven or eight years are necessary for effective control (Figures 41–44).

Although crop rotation is widely used and is effective in nematode control, it has important limitations. First, the degree of control is based on the level of resistance of the rotation crops and on the number of years between susceptible crops. Also, populations of other species of nematodes may occur on the alternate crop. Furthermore, the nonhosts or resistant crops grown in the rotation may be of low acre value and consequently contribute little to the farm income.

TIME OF PLANTING

Certain pathogenic nematodes are inactive during the winter months because low temperatures inhibit their activities. For example, California sugar-beet yields in fields infested with the sugar-beet nematode (*Heterodera schachtii*) are much higher if the beets are planted in January or February than if planted in March or April. Similarly, the root-knot nematode seldom damages the spring potato crop in North Carolina, since the crop grows at temperatures lower than those at which the nematodes are very active. Potatoes planted in the late spring, however, grow most during the hot summer months and are harvested in the fall. Under these conditions, root knot can be a serious problem unless otherwise controlled. In Britain, the golden nematode disease of potatoes may be controlled effectively by early planting of early potato varieties; potato roots develop and grow at a lower temperature than is favorable for hatching, movement, and development of the nematodes.

FIGURE 41 The effect of crop rotation on the control of a root-knot nematode (*Meloidogyne incognita*) on tobacco. Above, 3 years of tobacco; below, tobacco after 2 years of fescue. (Courtesy of C. J. Nusbaum.)

FIGURE 42 The effect of crop rotation on the control of a root-knot nematode (*Meloidogyne hapla*) on peanut. Above, 3 years of peanuts; below, peanuts after corn and tobacco. (Courtesy of C. J. Nusbaum.)

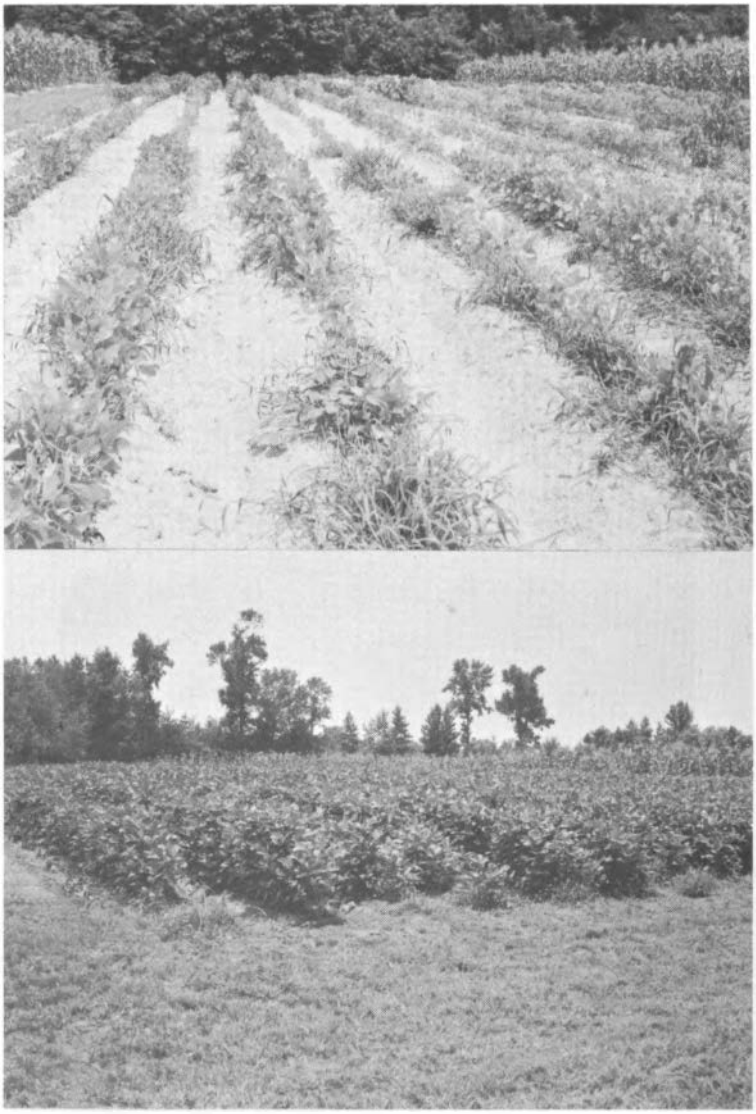

FIGURE 43 The effect of crop rotation on the control of the soybean cyst nematode (*Heterodera glycines*) on soybeans. Above, 3 years of soybeans; below, soybeans after 2 years of corn.

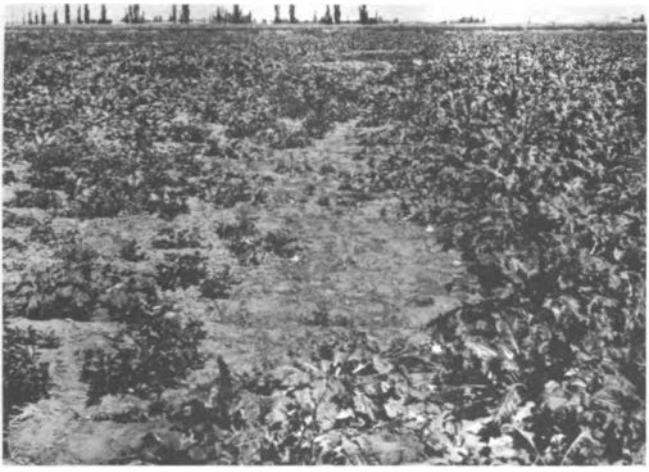

FIGURE 44 The effect of crop rotation on the control of the sugar-beet nematode (*Heterodera schachtii*) on sugar beets. Left, sugar beets following sugar beets; right, sugar beets following 1 year of grain.

ORGANIC MANURING

Several investigators have found a reduction in the population levels of plant-pathogenic nematodes following the addition of organic manures to soil. In most cases, increased activity of microorganisms in the soil followed these treatments, and reductions in nematode populations were assumed to be caused by the buildup of nematode-destroying organisms in the soil. However, in few cases were the actual factors responsible for killing the nematodes determined.

REMOVAL OR DESTRUCTION OF INFECTED PLANTS

Because of sucker growth, some annual crops, such as tobacco, continue to live for several weeks after harvest is completed. This sucker growth is sufficient to keep the root systems alive, and, consequently, parasitic nematodes present in the roots continue to reproduce. One or two additional generations may develop between the end of harvest and the time the plant is killed by frost. Experiments show, however, that if the stalks are cut soon after harvest and the root system of the plant is then turned out and exposed (Figure 45), the population of nematodes, especially of the root-knot nematode, is reduced

FIGURE 45 Field showing tobacco roots exposed to the drying effects of the sun and wind. (Courtesy of F. A. Todd.)

through the drying action of the sun and wind. Two control principles are utilized in this practice: the destruction of the host plant by cutting the stalk and uprooting the plant, thus preventing further reproduction of the nematode; and the killing by desiccation of large numbers of nematodes concentrated in the soil around the root system and inside the roots.

TRAP AND ANTAGONISTIC CROPS

Early investigators, employing the trap-crop principle in attempts to control certain species of the cyst and root-knot nematodes, planted highly suscep-

tible crops in infested fields and allowed the crop to grow only long enough for the second-stage larvae to enter the roots and begin their development into adults. Since only the second-stage larvae of nematode species in both these genera are infective, any development beyond the second stage renders the nematode immobile, and death occurs if the crop is destroyed prior to maturity of the nematodes. This practice is undoubtedly effective in reducing the populations of certain species, but it can result in even higher populations if destruction of the crop is not accomplished before nematodes complete their development and reproduce. In fact, the population can increase several-fold above the original infestation if reproduction occurs. In addition, the grower has the expense of planting and destroying a crop that brings in no revenue. The trap-crop method of nematode control, while theoretically feasible, is rarely used commercially.

A more effective approach to the use of trap crops is to plant crops that are highly susceptible to invasion by the nematode but are resistant to the development of larvae into adults. In this case, the crop does not have to be destroyed but can be harvested or used as a cover crop and turned under as green manure. *Crotalaria* has been successfully used to reduce populations of certain species of root-knot nematodes.

Roots of certain plant species have recently been found to exude toxic chemicals, thus reducing the soil-population levels of some nematode species. Marigolds and asparagus are examples of such plants. While the use of antagonistic crops reduces populations of certain nematode species under some conditions, little is understood concerning principles involved, and such practices are not yet developed to the point of practical control of nematodes.

NUTRITION AND GENERAL CARE OF HOST

The deleterious effects of nematode damage to certain crops can be offset to some degree by proper nutrition, moisture, and protection from adverse conditions, such as cold, which place the plant under stress. For this reason, greenhouse plants can usually tolerate much higher populations of nematodes than can field-grown plants. Practices tending to offset the damage caused by nematode attack are irrigation, conservation of moisture by mulching, fertilization, protection of plants on cold nights, and control of root and foliar diseases caused by other pathogens. It should be pointed out, however, that these are only delaying tactics, and, if susceptible crops are grown continuously, the nematode population will reach proportions that will cause serious damage. The rapidity of disease development and the magnitude of the damage will depend on the host and nematode species involved, the re-

sistance or tolerance of the host, and on various factors of the environment that favor or deter development of the disease.

Results of some recent investigations showed that soil-population levels of several nematode species may be differentially altered by host nutrition and, similarly, that disease development and severity are more pronounced in infected plants that are deficient in one or more essential nutrients. Also, nematode infection caused an increase or decrease in concentration of one or more minerals in leaf or root tissue. The interactions among host, parasite, and nutrition are complex, and the application of such information to fertilization programs designed to minimize damage caused by nematodes to crop plants is just beginning.

SANITATION AND USE OF NEMATODE-FREE PLANTING STOCK

The land-management and cultural practices discussed above reduce nematode populations in fields to varying degrees. Most of these measures have limitations: the degree of control is erratic, and sometimes those factors actually responsible for the reduction in nematode populations are not fully understood. However, sanitation and the use of nematode-free planting stock are sure and effective means of nematode control. Cost of these practices is small, yet many growers continue to use nematode-infected transplants or seed pieces of crops such as tomato, pepper, strawberry, peach, sweet potato, tobacco, and potato, as well as infected bulbs, corms, rhizomes, and tubers of many other plants. Examples of nematode-infected seeds and plants are alfalfa seed infected with stem nematode, wheat seed with wheat nematode (*Anguina tritici*), and rice seed and strawberry and chrysanthemum plants with species of bud and leaf nematodes (*Aphelenchoides* spp.). Nursery planting stock harboring nematode parasites is shipped all over the world. Although pathogenic nematodes are already widespread, indiscriminate use of nematode-infected plants and plant parts introduces new species into many fields and consequently complicates control measures. Furthermore, nematodes introduced in this manner are in a favorable position for survival, since they are already in or close to host-plant tissues. The greatest yield loss, however, is probably not in the plants on which the nematodes were introduced but in plants grown subsequently in the newly infested field.

FUTURE RESEARCH NEEDS

Reducing nematode populations through land-management and cultural practices is dependent on two approaches: the prevention or depression of

reproduction of the nematode on host crops and the acceleration of population decline of resting stages of nematodes through practices tending to shorten the survival of forms that persist for several years in the absence of a host.

The first of these objectives can be accomplished by fallow, which removes any source of food for the nematode, and by planting resistant host plants which may range from completely resistant (immune) to slightly resistant. Since living host plants are necessary for nematode development and reproduction, elimination of a suitable food source will effectively stop population increase. The net result is a lower nematode population than would occur through the use of susceptible plants, the degree of reduction being dependent on the level of resistance of the crop planted. Therefore, tests of relative resistance or susceptibility of as many crops as possible to the pathogenic species of nematodes in a given area are urgently needed. Without this, selection of crops to use in a rotation sequence designed to reduce the nematode population will mostly be guesswork.

The second objective, the acceleration of the death rate in the soil of nematodes neither feeding nor reproducing, but merely persisting, will depend on knowledge of the effect on nematode survival of soil environment or cultural practices applied to the soil. Therefore, studies should be conducted to determine the influence of soil environment and cultural practices on the survival rate of nematodes. For example, factors responsible for nematode mortality when fields are flooded, or when the over-all microflora is changed due to one or more of the above practices, should be determined. Such investigations are necessary before practical application of these practices can be used to accelerate the death of nematodes in soil.

CHAPTER 10

Biological Control of Nematodes

USE OF BIOTIC AGENTS TO CONTROL NEMATODES

There is abundant empirical evidence that plant-parasitic nematodes are attacked by numerous and varied soil organisms, but the activities of such organisms and their effects on nematode populations in agricultural or nonagricultural soils are little understood. Most soils are inhabited by some microorganisms that are parasites and predators on many different kinds of soil nematodes, including plant-parasitic species. In addition, there are soil microfauna that may be predacious on nematodes. The sedentary plant-parasitic nematodes protruding from roots may be especially vulnerable to all these parasites and predators.

FUNGI

Over 50 species of fungi capture and consume nematodes. Of these fungi, only those included in several genera of the Hyphomycetes and the Zoopagales have received attention as biological control agents. All the known fungal parasites of plant-parasitic nematodes also attack other widely diversified types of soil nematodes. Spores of some parasitic fungi are ingested by the nematode, while other fungi trap nematodes by various devices, such as a sticky material adhering to fungal mycelium. The trapping of nematodes is apparently only through chance contact with the fungal traps. Large, robust nematodes may escape, but most soil nematodes are small enough to be held fast by trapping fungi (Figure 46). Large quantities of fungal mycelium and a

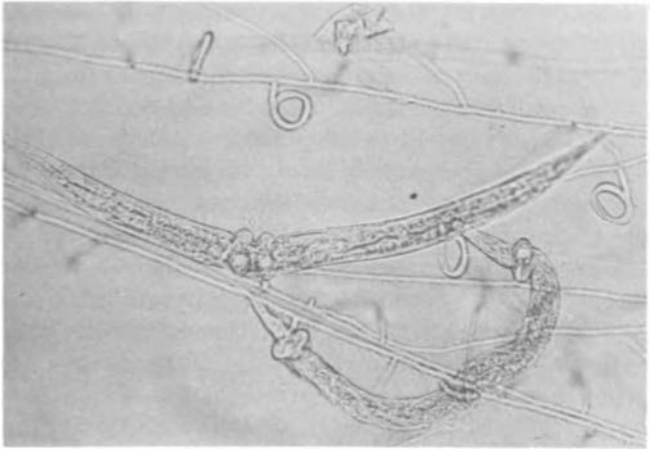

FIGURE 46 Citrus nematode larvae (*Tylenchulus semipenetrans*) trapped and penetrated by *Arthrobotrys dactyloides*. This predacious fungus occurs commonly in the rhizosphere of nematode-infested citrus plants.

high specificity between host and parasite, or predator, are necessary if fungal traps are to be important in controlling nematodes.

The physiology of predacious fungi, especially those factors inducing trap formation, has been studied by mycologists and microbiologists. A morphogenic substance believed to induce trap formation in *Arthrobotrys conoides* has been purified. However, some isolates of this fungus produce traps spontaneously in pure culture, as do many other predacious fungi; therefore, other mechanisms of trap induction must also be involved. Different predacious fungi have widely varied reactive thresholds and responses to trap-inducing substances. The various species of nematode-trapping fungi are divided into two ecological groups, based on the pattern of germination of conidia exposed to soil: a sensitive group, where conidia or tubes quickly give rise to trapping organs; and an insensitive group, in which little trap formation occurs. Fungi belonging to the sensitive group are efficient predators and poor saprophytes compared with the inefficient predators and able saprophytes of the insensitive group. The nutritional requirements of *A. conoides*, a member of the insensitive group, are similar to the requirements of other soil fungi; biotin, thiamine, and zinc are required for growth in a glucose–inorganic salts medium. However, very little is known about the nutrition and physiology of those species belonging to the sensitive group. Further information on the predacious fungi is needed.

Despite some research in this area, possible ways to utilize these biotic agents in effectively controlling nematodes have not yet been discovered. Greatest emphasis has been on the use of nematode-trapping fungi. While their effectiveness *in vitro* and in pot tests has been encouraging, attempts to use them for nematode control on a field scale have been unsuccessful. Annual cultivation reduces the density of these fungi in agricultural soil, but they are apparently able to persist under these conditions. In citrus orchards, three or four species of nematode-trapping fungi are frequently intimately associated with infestations of the citrus nematode (*Tylenchulus semipenetrans*).

Artificial application of nematode-trapping fungi to soil would seem appropriate only if they were absent from the soil in question, if it were known that a given fungus species was more effective than fungi already present against a particular nematode pest, or if greater population densities of the fungi would result. The latter may be difficult to attain in view of demonstrated antagonisms to such introduced predacious fungi. Very little is known about the trapping activity in the natural soil environment. Some evidence indicates that trapping increases for a short time after the addition of organic matter to the soil and then declines. Evidence from laboratory tests shows that various species of nematode-trapping fungi differ considerably in their ability to trap nematodes in soil. This may account for the inconsistent results obtained with these organisms.

Certain little-known phycomycetous fungi, which develop internally in their hosts, parasitize nematodes. Those phycomycetes studied are only weakly parasitic on nematodes and are probably saprophytic on dead or injured fauna of the soil.

The efficiency of fungal predators and parasites is probably limited by lack of mobility, inability to seek out prey or hosts, and by their low population densities. In the confined biotic community of the soil, the production of large quantities of motile spores capable of spreading in soil-water films would be a desirable characteristic of an effective biological control agent. More information is needed about the occurrence and biology of these microorganisms and the susceptibility of nematodes attacked by them.

BACTERIA AND VIRUSES

Bacteria are often found attacking nematodes maintained in the laboratory, but the deteriorated condition and unfavorable environment of the nematodes generally preclude judging the significance of such associations. A widespread bacterial infection of soil populations of a dagger nematode (*Xiphinema americanum*) was reported, but, as with other reports of bacterial diseases of nematodes, the observations are not unequivocal. A disease of a root-knot

nematode (*Meloidogyne* sp.), transmissible and apparently caused by a virus, was noted, but the observation was never confirmed.

Certain myxobacters, or protozoa-like bacteria, isolated from soil, produced lytic enzymes that dissolved or lysed some bacterium-feeding nematodes in the genera *Caenorhabditis, Rhabditis,* and *Panagrellus*. The lytic substances from these myxobacters did not lyse the fungus-feeding nematode (*Aphelenchus avenae*) or the plant-parasitic clover cyst nematode (*Heterodera trifolii*); thus, differences in the chemical composition of the nematodes are implied. Although bacterial enzymes attack certain nematodes in a simple laboratory system, it is unlikely that their effectiveness in soil, with its physical, chemical, and biological complexity, would be specific for plant-parasitic nematodes. Obtaining and developing bacterial or viral pathogens of nematodes should receive increased attention from nematologists studying plant-parasitic nematodes.

PROTOZOANS

A sporozoan parasite, generally referred to as *Duboscquia penetrans*, is often found parasitizing plant-parasitic and other soil nematodes, but its small size and complex life cycle make it difficult to study. The effectiveness of sporozoans as biological control agents is unknown. Their widespread occurrence and ability to destroy the reproductive organs of nematodes or to kill their nematode hosts indicate the potential control value of these organisms; considering this, the biology and ecology of such sporozoans merit further study.

A large amoeboid proteomyxan organism, *Theratromyxa weberi*, although frequently observed ingesting nematodes, is not considered of practical importance in the control of plant-parasitic nematodes. Other soil protozoa probably have only an incidental predatory relationship to nematodes.

OTHER NEMATODES

Certain carnivorous nematode species, including many of the *Mononchidae*, comprise one of the most important and least studied groups of organisms predacious on soil and plant-parasitic nematodes. The small predatory species of *Seinura* feed voraciously on nematodes and may, in some cases, be of considerable value in nematode control. Various widespread predators in the nematode superfamily Dorylaimoidea may play a significant role in the biological balance of soil organisms (Figure 47). Because information on the effectiveness of these predacious nematodes is derived almost entirely from observation rather than experimentation, further investigation is needed.

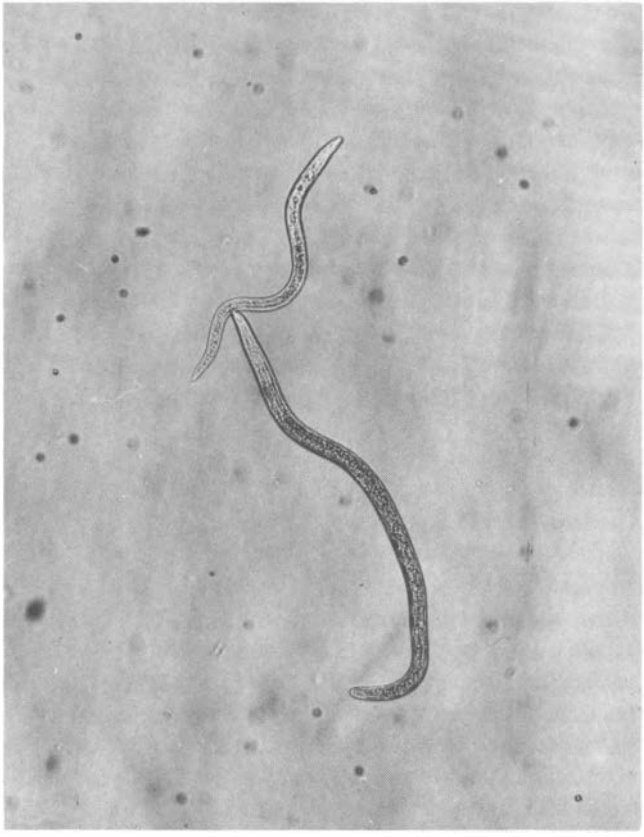

FIGURE 47 Predacious *Eudorylaimus* sp. feeding on larva of *Aphelenchus avenae* in agar culture.

OTHER INVERTEBRATES

Tardigrades, small animals found chiefly in water films surrounding leaves of terrestrial mosses and lichens, but sometimes numerous in the soil, will kill nematodes. Despite recent study of the role of tardigrades in soil-nematode population dynamics, relatively little is known of the biology of these curious organisms. Under laboratory conditions, a soil-inhabiting turbellarian flatworm has been observed ingesting large numbers of root-knot nematodes.

Since parasites and predators of nematodes are widespread, biological-control studies should be primarily ecological and should aim at modifying both the physical and biological characteristics of their environment in an

effort to reduce nematode populations. Despite increasing studies in the special area of soil biology dealing with nematodes, knowledge of fundamental factors affecting the biological equilibrium and biotic potential of nematode pests is inadequate.

INFLUENCE OF ORGANIC MANURING ON NEMATODE CONTROL

In some instances, the addition of large amounts of organic materials to soil results in reduced populations of plant-parasitic nematodes and in higher crop yields. The reduction in numbers of plant-parasitic nematodes is thought to be caused, at least in part, by an increase in natural enemies of nematodes. In addition, the presence of decomposing organic materials in the soil apparently provides host plants with some tolerance to nematode attack. Decomposition products of organic matter and plant residues may also be detrimental, directly or indirectly, to plant-parasitic nematodes. Certain highly concentrated volatile fatty acids produced by decomposing rye residues in soil are toxic to a root-knot (*Meloidogyne incognita*) and a lesion nematode (*Pratylenchus penetrans*) but not to the saprophagous species tested. It may be significant that certain saprophagous nematodes appear to be more tolerant than plant-parasitic nematodes to the halogenated hydrocarbon nematocides.

PLANT-ROOT EXUDATES TOXIC TO NEMATODES

Roots of several plants contain chemicals that, on leaching into the soil, are toxic to plant-parasitic nematodes. A compound found in asparagus roots and tentatively identified as a glycoside is toxic to several species of plant-parasitic nematodes.

The French marigold, when grown on soil infested with lesion nematodes (*Pratylenchus* spp.), suppresses the population of these nematodes and reduces the numbers found in the roots of susceptible host plants. The African marigold behaves similarly. A population of stunt nematode (*Tylenchorhynchus dubius*) also was suppressed, but populations of spiral nematode (*Rotylenchus robustus*) and certain other Tylenchida were unaffected. Three compounds of an α-terthienyl type, toxic to nematodes, were identified in root exudates from these plants.

There is little doubt that the roots of many plants release chemicals toxic to nematodes into the soil. Possible uses and the economic value of plants such as the marigold should be studied.

The majority of plant-parasitic nematodes inhabit soil during all or part of their life cycles. The impossibility of directly observing a phenomenon

occurring in soil, coupled with the physical and biological complexity of the soil, make research in biological control of plant-parasitic nematodes extremely difficult. However, results of research conducted to date demonstrate the great diversity of natural control agents. The combining of chemical and biological control measures offers advantages such as reduced cost for nematocidal chemicals and reduced problems of pesticide residues.

The search should continue for more specific parasites of injurious nematodes: parasites able to persist in soil, parasites able to attack endoparasitic nematodes and continue their lethal activities after the nematodes escape the soil environment and enter the host roots, and, finally, effective parasites obtainable by man in large numbers for wide dissemination in agricultural soils.

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CHAPTER 11

Plant Resistance

Potentially, the most economical and effective method of controlling nematodes is the use of nematode-resistant plant varieties. At present, largely because of limited research in this area, few nematode-resistant varieties of plants are available to the commercial grower. Plant breeders and nematologists have developed cotton, cowpeas, lespedeza, tobacco, lima beans, soybeans, peppers, tomatoes, and grape and peach rootstocks resistant to root-knot nematodes (*Meloidogyne* spp.) (Figure 48); clover and alfalfa resistant to the stem nematode (*Ditylenchus dipsaci*); potatoes resistant to the golden nematode (*Heterodera rostochiensis*); barley resistant to the cereal root nematode (*H. major*); soybeans resistant to the soybean cyst nematode (*H. glycines*) (Figure 49); citrus rootstocks resistant to the citrus nematode (*Tylenchulus semipenetrans*); and corn resistant to a stunt nematode (*Tylenchorhynchus claytoni*). Commercial varieties of cotton, lima beans, and soybeans resistant to the root-knot nematode, and varieties of alfalfa, oats, and barley resistant to the stem nematode are grown extensively. Undoubtedly, some commercial varieties of a number of crops developed primarily for high yield and quality of product are tolerant to one or more nematodes.

Nematode resistance has been discovered in both cultivated and wild plant species. For example, root-knot resistance was found in wild species of *Lycopersicon* from South America, in small-fruited hot peppers, in Chilean and African alfalfa varieties, in selections from alfalfa varieties cultivated in the United States, and in phylloxera-resistant grape hybrids and pure species of American origin. Resistance to the golden nematode is present in wild species of *Solanum* and in selections from commercial potato varieties from South America. A lima bean variety, selected over a number of years by the Hopi

FIGURE 48 Young peach trees of the same age growing in soil infested with a root-knot nematode (*Meloidogyne incognita*). Left, on susceptible Lovell rootstock; right, on Rancho-resistant rootstock. Another rootstock now available, Nemaguard, is also resistant to another root-knot nematode (*M. javanica*). (Courtesy of the Department of Nematology, University of California.)

FIGURE 49 Comparison of cyst nematode-resistant and susceptible soybean varieties in soil infested with soybean cyst nematodes (*Heterodera glycines*). Left, Picket; right, Lee. (Courtesy of *Phytopathology*. After Brim and Ross.)

Indians of Arizona for adaptability to their soils, was found to be resistant to the root-knot nematode.

Few plants are immune to nematode attack. Resistance is usually relative or incomplete; therefore, selection of resistant and susceptible plants, either by nematode increase or by degree of plant damage, is often difficult in breeding programs.

DEVELOPMENT OF NEW VARIETIES

Although a few nematode-resistant plant varieties have been developed by selection from commercial varieties, such as a Swedish red clover resistant to the stem nematode, the most common method of obtaining resistant varieties is to cross plants having desirable commercial characters with those possessing nematode resistance. An original cross between a commercial variety and a nematode-resistant source, followed by repeated back crosses to commercial varieties, is generally used in breeding programs to incorporate resistance into a commercially acceptable plant variety.

Unrelated plant species, especially those with different chromosome numbers, are difficult to hybridize. Some crosses can be made only by using specialized culture techniques; a root-knot resistant tomato hybrid obtained by this method has been used as a source of resistance by plant breeders.

Undesirable horticultural characters are often linked to nematode resistance, making transfer of resistance without these characters difficult. A wild cotton (*Gossypium barbadense* var. *darwinii*) is highly resistant to a root-knot nematode (*M. incognita*), but this resistance is inherited recessively and is probably polygenic. More time and work are required to transfer such resistance to commercial varieties than to transfer resistance owing to a single gene.

In developing nematode-resistant varieties, a rapid and accurate method of determining the degree of resistance is necessary. With the exception of nematodes from a few genera, such as *Meloidogyne, Heterodera,* and *Ditylenchus,* difficulty in obtaining large quantities of uniformly viable nematode inoculum is an important limiting factor in breeding for resistance to many plant diseases caused by nematodes.

Roots infected with root-knot nematodes are commonly used as inoculum in breeding experiments. Infected roots containing many egg masses are chopped and equal quantities are added to the soil in which the test plants are to be grown. The success of this method depends on a high percent of nematodes remaining viable in the plant roots during the inoculation procedure.

Inoculum of most of the cyst nematodes (*Heterodera* spp.) is relatively easy to obtain. Cysts (dead bodies of swollen females containing eggs and larvae) are washed from soil by flotation and sieving. These cysts are freed of

accompanying debris, proportioned by counting or weighing, and added to soil. The number of larvae present is estimated by counting the number of eggs or larvae contained in samples of cysts. Adding approximately equal numbers of larvae hatched from cysts is more precise but is time consuming and difficult to adapt to routine tests. Furthermore, free larvae are more susceptible to adverse environmental conditions than larvae in cysts and thus are more likely to die before reaching plant roots.

Large quantities of stem nematodes for use in breeding programs are grown routinely on host tissue. As these nematodes are generally found inside stems or bulbs, their recovery is relatively easy. Stem nematodes for testing resistance of rye seedlings are obtained by inoculating potato tubers with this nematode and storing them at room temperature for several months. Aseptic inoculum of this nematode also may be produced on callus-tissue cultures.

Infected roots are often used as sources of inoculum of migratory endoparasitic nematodes. Large quantities of some species, primarily lesion nematodes (*Pratylenchus* spp.), are grown on callus-tissue or excised-root cultures. Although this method is not now used extensively to produce inoculum for breeding programs, it probably will be widely used in the future.

Inoculum of ectoparasitic root feeders, nematodes that spend most of their lives in the soil feeding on outer root cells, is particularly difficult to obtain because of the time required to extract these nematodes from soil and to separate the test species from other species. Although large numbers of a stunt nematode (*T. claytoni*) can be produced on callus-tissue cultures under aseptic conditions, for most ectoparasitic root feeders the only practical method is to test resistance in the field or greenhouse in soil infested by more than one nematode species. Unfortunately, natural soil populations of most of these nematodes fluctuate greatly over relatively short time periods and thus are unreliable sources of inoculum. When it is possible to produce large numbers of most ectoparasitic forms on callus tissue or excised roots or *in vitro*, breeding for resistance to those species will be facilitated.

In testing for resistance, nematode-infected plant material is uniformly mixed with soil or placed in the vicinity of the test-plant roots, or extracted nematodes are added by pipettes, hypodermic needles, or other devices to the root zone or to soil at the time of transplanting or sowing. Whichever method is used, the unprotected nematodes must be placed in the vicinity of plant tissue. Suitable moisture and temperature and other requirements of the particular nematode species should be considered. The active stages of most nematodes are particularly sensitive to drying.

Nematodes from more than one source should be included in the inoculum, because different collections of nematodes of a single species may vary in pathogenicity. Although such variability has not been demonstrated for all plant-parasitic nematodes, it occurs in a high percent of the species tested.

Generally, nematode resistance or tolerance is tested by measuring plant performance and rating disease symptoms, such as root galls. Because growers are primarily interested in yield and quality of product, these are very important criteria. However, the rate of nematode reproduction also should be determined.

Good plant performance in the presence of pathogenic nematodes may result from either plant resistance or tolerance. A resistant variety is a poor host and does not support high nematode populations. Many levels of plant resistance occur, varying from immune plants on which no nematodes develop to those supporting populations almost as high as susceptible varieties. A tolerant variety, however, is a good host, possessing low susceptibility to the disease caused by the nematode. If a tolerant crop is grown frequently in a rotation, the nematode population may increase above the tolerance level of that or other crops in the rotation. When available, resistance is preferable to tolerance. Occasionally, crop damage results from the feeding of large soil populations of nematodes on immune or highly resistant plants.

NATURE OF RESISTANCE

Substances given off by plant roots, which stimulate hatching of nematode eggs or attract nematodes to roots, are not related to the resistance of plants to nematodes. Stimulants or attractants from roots of immune or resistant plants are sometimes more potent than such substances from susceptible ones.

Nematodes enter roots of most resistant plants, but often in smaller numbers than they enter roots of susceptible plants. An exception is that larvae of a root-knot nematode (*M. hapla*) do not penetrate roots of rye and oat plants.

The number of endoparasitic nematodes penetrating plant tissues may be influenced by age of the tissues. Although young chrysanthemum leaves are entered by chrysanthemum foliar nematodes (*Aphelenchoides ritzemabosi*), the nematodes apparently cannot move among the mesophyll cells because of the compactness of the tissue at that stage of growth; therefore, the nematodes cannot feed and reproduce. In older leaves, the mesophyll cells have intercellular spaces sufficiently large for easy movement and colonization by the nematodes; therefore, the nematodes can live and reproduce successfully. It is unknown whether morphological features are solely responsible for lack of or limited penetration of plant parts. Perhaps biochemical or physiological factors also are involved in these phenomena.

The most common reaction of nematodes to resistant plants may be failure of all or a high percent of females to develop to maturity even though infective stages of nematodes enter plant tissue. In most cases, development of females does not proceed further than the third stage. Although females fail to

develop, or develop at a slower rate, males may mature normally. Plant resistance may cause not only slower nematode development but also production of fewer eggs by females.

It has been postulated that resistance may, in many cases, be related to the failure of the host to supply some nutrients necessary for rapid reproduction or even for survival of the nematode. The formation of few or no giant cells at feeding sites of maturing female nematodes is associated with plant resistance to cyst and root-knot nematodes. The cytoplasm in these giant cells is less dense than that in giant cells induced in susceptible varieties. Perhaps these nematodes can obtain food suitable for maturation from only these enlarged cells. Infection by stem nematodes causes conspicuous enlargement and separation of parenchymatous cells in susceptible plants but only slight cell enlargement and separation in resistant plants.

Nematode populations around the roots of resistant plants sometimes decline at a more rapid rate than can be explained by starvation, and it is presumed that toxins of plant origin are responsible. In two instances, specific biochemical substances, which were present as inherent constituents of the plants and were thought to be responsible for plant resistance to nematodes, were identified or partially identified. α-Terthienyl compounds isolated from some species of marigold and a glycoside isolated from asparagus were identified as the toxic factors. These compounds have a wide spectrum of activity against nematodes. Substances specific in their toxicity towards nematode species, or even races or biotypes, are responsible for varietal resistance, rather than compounds such as those mentioned above. Perhaps phytoalexins, substances formed only after infection of plant tissue by the nematode, are also involved in plant resistance to nematodes. Results of reciprocal grafting studies indicate that resistance factors are associated with individual plant cells attacked by the nematode and are not generally translocated in the plant. In one case, however, a resistance factor from a resistant *Lycopersicon* scion was translocated to a susceptible stock, as evidenced by lowered penetration and egg production by a root-knot nematode (*M. incognita*) infecting the stock roots.

Death of cells hypersensitive to nematode feeding and browning of tissues around infecting nematodes occurs in many resistant plants in response to infection by several different nematode species. Death of cells usually occurs within a day or two after infection. Larvae of cyst and root-knot nematodes die in reacting tissues of a number of plant species, but stem nematodes do not die in tissues of resistant pea despite death of plant cells. Death of hypersensitive cells may cause extensive damage to a particular plant species even though the nematode species causing the damage cannot reproduce on the plant. Hypersensitive reactions have been postulated for resistance of chrysanthemum to the chrysanthemum foliar nematode; red clover, alfalfa, and pea

to the stem nematode; citrus to the citrus nematode; lima bean to the lesion nematode; and soybean to root-knot nematodes. Browning of plant tissues as a result of feeding of a lesion nematode (*P. penetrans*) appears to be caused by accumulation and oxidation of phenolic compounds. In apple and peach rootstocks, lesion formation, as a result of feeding of lesion nematodes, is roughly correlated with the amygdalin content of rootstocks. Whether such compounds are basically responsible for resistance is unknown.

Formation of wound periderm or corky layers, which wall off and retard the development of nematodes in plant tissues, has been reported. Cork layers in old yam tubers retard entrance of root-knot nematode larvae, and cork layers around developing females retard their development. Wound periderm is also present around feeding sites of developing citrus nematode larvae in roots of resistant citrus plants.

Altered mineral nutrition may change factors such as organic constituents in cells and physiology of the plant and may influence nematode reproduction and pathogenicity. For example, soybean and lima bean are more resistant to root-knot nematodes when grown under low-potassium fertilization than when grown under medium or high levels of potassium. When potassium is deficient, nematodes on lima bean develop slowly and egg production is delayed, but the pathogenic effects of the nematode are severe; when potassium is high, the nematodes develop rapidly and lay many eggs, but the pathogenic effects are less severe than those when potassium is deficient. However, a negative correlation exists between potassium levels and development of lesion nematodes in cherry. The apparent discrepancy may be explained by the levels of potassium used, duration of the experiment, plant species used, or nematode species used. Levels of several elements, rather than a single one, and relative levels of several elements interacting, may influence nematode reproduction and pathogenicity in plant tissues.

The vigor of a plant influences resistance to nematodes. For example, populations of a lesion nematode (*P. penetrans*) on Wando pea plants increase as root development and plant growth are restricted by low but not deficient nutrition, low light intensity, fruiting of the plants, and late defoliation. Treatments that greatly reduce root development and plant growth, such as girdling the stem by scalding and defoliating plants after two days, reduce population levels of *P. penetrans*.

INFLUENCE OF ENVIRONMENT

Although resistance is a characteristic of the host plant, environmental factors may alter symptom expression of plants and development of the nematode. Temperature may affect the rate of nematode entry into the plant host,

nematode reproduction, and pathogenicity. Factors such as moisture, pH, and other microorganisms also may be important. Some kinds of plant-pathogenic nematodes apparently cause greater crop losses in coarse-textured soils than in fine-textured soils. This effect may be caused partly by greater nematode movement in the coarse-textured soils.

GENETIC BASIS FOR RESISTANCE

Resistance to nematodes of several genera has been found in a wide variety of plant species, and the mode of inheritance of this resistance has been determined. Inheritance in both diploid and tetraploid plants has been described.

In general, resistance to nematodes is caused by one or a few completely or incompletely dominant genes. The resistance of grape to a root-knot nematode (*M. incognita acrita*) is caused by a single dominant gene, whereas resistance of cowpea to one root-knot nematode (*M. incognita*) involves two dominant genes. In addition to major genes, modifying genes or those with minor effects may be involved in resistance to some nematodes, such as in resistance of oats to the stem nematode. However, resistance of soybean to the soybean cyst nematodes is governed by three independently inherited recessive genes, and resistance of corn to the ectoparasitic stunt nematode (*T. claytoni*) also appears to be recessive. The resistance of wild cotton (*G. barbadense* var. *darwinii*) to a root-knot nematode (*M. incognita*) is inherited recessively and appears to be polygenic. Resistance caused by a specific plant gene has been found responsible for resistance to one to four nematode species.

RESISTANCE TO COMPLEX DISEASES

In plant-breeding programs to control disease caused by nematodes and microorganisms such as fungi or bacteria, it is important to breed for resistance to all organisms but particularly for nematode resistance. For example, although root-knot nematodes damage cotton in the absence of other microorganisms, these nematodes further depress cotton yields by increasing the severity of fusarium wilt, a disease caused primarily by a fungus, *Fusarium oxysporum* f. *vasinfectum*.

RESISTANCE-BREAKING BIOTYPES

Potato varieties resistant to the golden nematode, if repeatedly grown in some infested fields, bring about a change in the pathogenicity of the nematode

population from one that is almost entirely composed of nonresistance-breaking individuals to one composed almost entirely of those capable of breaking resistance. Furthermore, in a rotation including potatoes every 4 to 5 years, and no other hosts of the golden nematode, it is estimated that within 20 years there will be a complete change from nonresistance-breaking to resistance-breaking populations. Because movement of nematodes from field to field and area to area is usually more restricted than that of most fungi, spread of resistance-breaking biotypes of nematodes is probably slower than that of most fungi.

Resistance-breaking biotypes have been reported for many but not all nematodes. Oat varieties resistant to the stem nematode have been widely grown in Wales, but no resistance-breaking population of the nematode has appeared.

FUTURE RESEARCH

More effective methods for testing resistance must be devised, and the importance of correctly identifying nematodes must be emphasized. Progress in breeding for resistance to root knot increased rapidly after the root-knot nematodes were separated into a number of species differing in host range.

The high cost of direct control measures, and persistence of nematodes in the soil, point to a need for emphasis on breeding resistant varieties of plants. The development of many plant varieties with nematode resistance will result in untold benefits, especially to growers of low-acre-value and of perennial crops, such as tree fruits and forage crops. Before major progress is possible in this field, there must be a marked increase in research workers and research teams, such as nematologists and plant breeders, actively engaged in cooperative programs.

The biochemical basis of resistance in plants to nematodes has been little studied. Research in this area should aid in developing resistant varieties of plants and, perhaps, in the discovery of new chemical means of nematode control.

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CHAPTER 12

Control by Physical Factors

The application of heat, a widely used pest and pathogen control practice, is the most important of the various physical factors used in nematode control. Other physical factors, such as irradiation, plasmolysis, or electricity, are harmful or lethal to nematodes, but their potential for effective nematode control is not yet realized; further research and advanced technology may make these physical factors important for control in the future.

CONTROL BY HEAT—BASIC CONSIDERATIONS

With control by heat, time of exposure to a specific temperature is a most important relationship; for every temperature there is a minimum exposure time required for the heat to produce a particular effect on an organism. In addition to this time–temperature combination, specific heat and heat exchange are used in calculating the heat energy involved and the subsequent cost, an important practical factor. Engineering computations for cost and efficiency are readily adaptable to pathogen control methods utilizing heat and are very useful if the biological aspects are given due consideration.

Heat affects nematodes in many ways, ranging from immediate destruction by burning or searing at extremely high temperatures to the coagulation of cytoplasm at approximately 65°C, a relatively low temperature. Temperatures below this coagulation point, but above normal, cause lethal or sublethal injury to nematodes; however, the exact nature of the injury is not known. Such temperatures are effective for nematode control despite the fact that the nematodes may not be killed immediately. As with other control

methods, the immediate death of the nematode is not necessary for disease control, a point sometimes overlooked in evaluating new control methods and materials.

Each developmental stage of a nematode has its own level of heat sensitivity, its own time–temperature curve. These curves differ for each species, for certain temperature-tolerant life-cycle stages or states of being, and under conditioning influences of moisture content and rates of temperature change. These biological details are especially significant in disinfecting a living host of nematodes when the heat susceptibilities of parasite and host differ only slightly. They are also important in efficiently disinfesting nonliving objects, so that areas of inadequate heating are eliminated and reinfestation is prevented. The term "disinfestation" refers to the control of nematodes associated with nonliving materials; "disinfection" refers to the control of nematodes associated with living materials.

Heat treatments that kill other pests and pathogens of plants are also effective and practical for nematode control, enabling the use of a single treatment for control of several pests. The following figures, illustrating the lethal temperatures for various organisms, can serve as a guide for disinfestation treatments with heat. A few highly resistant seeds and some plant viruses require 30 minutes of exposure at 100°C under moist conditions to be killed. Most seeds and viruses, all plant-pathogenic bacteria and fungi, various worms and mollusks, and all arthropods are killed when held at 82°C for 30 minutes. Most nematodes are destroyed when held at 49°C for 30 minutes. In practice, when infested soils are heated thoroughly and uniformly by tumbling in a continuous-flow treatment system, using dry heat or steam, a temperature of 82°C for 30 minutes is satisfactory for nematode control. If treated soils are in containers or in a stationary mass, a final temperature of 100°C for 30 minutes is recommended.

DISINFESTATION BY HEAT

Heat disinfestation usually involves soil and other substrates, containers, and inert surfaces that become infested with undesirable nematodes. Heat must be present long enough to control the nematodes, usually by causing their death.

Nematodes on exposed surfaces are easily reached by heat, so that maintaining the lethal temperature on the surface for a sufficient time is all that is required. Nematodes inside porous substrates can be reached by transferring heat throughout the substrate by agents such as hot water, steam, or perhaps hot air, or by crumbling and spreading the substrate into a thin layer to obtain exposed surfaces and then applying heat.

In heat disinfestation, it is advantageous to conserve the heat after application to the substrate, thus allowing maximum time for heat penetration and thereby increasing the effectiveness of the treatment. Heat retention also provides the possibility of nematode control at lower temperatures because of longer exposure time. It is necessary to apply heat carefully to avoid leaving relatively cool areas where nematodes can survive.

DRY HEAT

Dry heat may be applied in a quick searing manner, as when surface-borne organisms are contacted briefly by flame, such as the use of a weed burner to disinfest the surfaces of a potting bench or soil-mixing base. Efficiency depends largely on accessibility of the organisms to the direct heat. In applying flash-heat to soil or sand, the use of an open flame moving over a thin, loose layer of the substrate is necessary.

Dry heat may also be applied so that the entire mass being heated is brought to an over-all desired temperature. Any ovenlike enclosure, heated by a suitable source, serves for dry-heat disinfestation. Heat may be applied externally, internally, or in combination. Convenience in loading, with minimum effort and damage to enclosed heating elements, is important in the oven design.

A continuous-flow soil system is particularly useful in applying dry heat. In some systems, soil is fed into one end of a heater unit and collected at the other end. The rate of flow through the heater and the distance traveled in contact with the heat source are regulated by the machine design to give the desired amount of heat. The equipment provides for exposure to open flame, passage over heated metal plates, or both.

In another continuous-flow system, a machine picks up soil to a desired depth in a path, passes the soil in a thin layer through the heat chamber, and then deposits the treated soil behind as the machine slowly advances.

The burning of wood or brush on the surface of an intended planting site, although wasteful of fuel, is effective if sufficient heat is generated to obtain penetration.

The convenience of the continuous-flow system in treating soil as it moves past a heat source is obvious, but the intense heat required in this type of system destroys organic materials in the soil. An oven permits the use of a lower temperature, thereby avoiding the destruction of organic materials. Treatment of a moving and tumbling soil mass by dry heat assures uniform heat distribution. However, none of these methods is widely used.

MOIST HEAT

Disinfestation by moist heat utilizes hot water, steam, or steam plus chemicals. Direct exposure to boiling water or steam is used to disinfest open surfaces. Scouring by flowing water, the force of steam, and brushing can be used with this method.

Soil can be disinfested by moist heat, either as hot water or steam. Precautions to ensure uniform heat distribution and penetration throughout the substrate are necessary. Soils should be pulverized to eliminate clods and should not be waterlogged. Soil moist enough for planting is ideal for steam treatment, since the organisms are hydrated and pore spaces are open. Because of its greater caloric content, steam is a more efficient heat carrier than hot water; a pound of water at the boiling point of 100°C requires 970 BTU to be converted to a pound of steam at 100°C, and this heat is released when steam is converted back to water. At atmospheric pressure, the maximum temperature reached is 100°C, so that the organic material in the soil is not destroyed. Autoclaving at elevated temperatures and pressures is sometimes used in laboratory experiments.

HOT WATER

Drenching or soaking objects in hot water effectively controls nematodes. The duration of heat exposure is easily regulated. Vigorously boiling water in adequate volume is necessary to attain and maintain the desired temperature. Since hot water has low heat energy, an excessive amount is necessary to produce sufficient heat to destroy other types of plant pathogens. Hot water is impractical for soil treatment, since the soil becomes waterlogged and undesirable leaching may occur.

STEAM

Steam disinfestation is adaptable to the needs of home growers, to the extensive operations of commercial nurserymen or mushroom growers, and to many other types of plant production where large acreages are not involved. Materials to be treated are brought to the equipment, or suitable equipment is moved to the growing site. Due to its lethal effect, free-flowing steam is widely used for disinfesting growing beds, containers, and media. Precautions such as breaking of soil clods, drainage of waterlogged soil, adequate dispersion of steam outlets, and elimination of cool spots in treatment beds are necessary to ensure thorough uniform penetration of the steam and effective heating.

Steam treatment of soils has many advantages. The treatment is short, easy to apply, and nontoxic to man. In many cases, planting can be done after the soil cools. A broad spectrum of kill, including weeds, is obtained. Treatments can take place near living plants. Lethal heat can penetrate unrotted crop refuse that may harbor pathogens. Steamed, fine-textured soils become more granular, with resulting improvement in aeration and drainage. Increased plant growth sometimes results from increased availability of nutrients, improved physical structure of the soil, and, perhaps, from favorable biological changes, in addition to the benefits of pest and pathogen destruction.

Steam treatment of soils also has disadvantages. Treated soils, especially those high in organic matter, may be toxic to certain kinds of plants, and seed germination may be reduced. In nurseries, the choice of proper ingredients in preparing soil mixtures can eliminate the problem. As the toxic effects may be temporary or may last for several months, postponing planting for several weeks after treatment is advisable when toxicity is suspected. Leaching the soil reduces toxicity, but it waterlogs the soil, is inconvenient, and costs time and money. The steam-generating equipment is cumbersome and expensive, and steaming large amounts of soil is laborious.

STEAM PLUS CHEMICAL

Combining volatile toxicants, such as formaldehyde at the rate of 0.2 to 0.4 fluid ounce per gallon of water, with steam may lower the cost of effective treatments by reducing the amount of heat required. A low-temperature steam–air mixture has been used without loss of effectiveness, and with reduced fuel costs. The application of a combination of a low-temperature steam–air mixture with volatile nematocides and other pesticides may offer a solution for problem situations.

ENDOGENIC HEAT

Heat generated by decomposition in compost piles or bins is sufficient to destroy plant-parasitic nematodes. Checking compost temperatures and turning any relatively cool compost materials into the pile will assure uniform nematode kill. The variability in the heat produced is dependent not only on location in the compost pile but also on the composition of the compost. Some control of the plant parasites present in composting materials can also result from the biological agents present. Saprophagous nematodes invariably are abundant; their high populations appear to induce increased populations of other organisms that prey upon the nematodes.

DISINFECTION BY HEAT

Control of nematodes associated with living plant materials requires removal or destruction of the nematodes without harming the living material.

When certain ectoparasitic nematodes are present on the root system or other underground plant parts, mechanical removal may be effective and economical. Washing the plant parts in a tank or with a stream of water, augmented by agitating, light brushing, or rubbing, may be sufficient. Such methods are applied to many ornamental plants and turf grasses that are bare-rooted at some time in their processing for distribution or replanting. A hot-water treatment or nematocidal dip is needed for plants contaminated with ectoparasitic nematodes, such as *Hemicycliophora* spp. or *Criconemoides* spp., that are difficult to dislodge by mechanical means because their long stylets are inserted in plant cells.

When endoparasitic nematodes are present within plant tissues or enclosed within protective layers of plant parts, they require a penetrating chemical or physical agent to kill them. Heat is most commonly used. Heat applied externally is absorbed by the plant propagule and spread within to reach pathogens. When a differential in heat susceptibility exists between plant tissue and nematode, with the latter being more sensitive, effective heat treatment is possible.

To develop satisfactory treatments, the thermal–time–death curves must be determined for all nematode stages present in the infected plant parts. The plant parts, also, are tested for tolerance to temperature–time combinations that kill the nematodes. Because different stages in the development and dormancy of the plant materials may have different heat tolerances, the most tolerant stage is selected for treatment. The procedure is thoroughly tested on a small scale before large lots of plants are treated.

Because water transfers heat rapidly and excludes any possibility of harming plant tissue by desiccation, it is preferable to air as the medium for effecting moderate temperature changes in treated materials. A pretreatment soak of plant parts is desirable to rehydrate nematodes and make them more susceptible to heat injury, to bring the plant tissues to a uniform starting temperature, and to dispel insulating air bubbles trapped by the plant parts. The presoaked materials are drained, then transferred to the hot-water bath, and the treatment timing is begun. The temperature of this bath is held constant within about 0.3°C. This is achieved by having present a large volume of water compared with the volume of plant materials being treated, having adequate heater capacity, or having additional hot water available to add to the bath for quick correction of temperature drops (see Figure 40, Chapter 8). Thorough mixing and agitation of the water in the treatment tank also are necessary for accurate temperature control. If necessary for plant survival, the

treatment temperature can be brought down to normal rapidly by a post-treatment immersion in another tank of water held at a suitable temperature. Otherwise, the plant parts are cooled to the ambient temperature on removal from the treatment tank. Some plant propagules may require drying. Even forced drying may be necessary to avoid other disease hazards likely to be involved in subsequent storage. Some plant parts require protection from excess drying. Plants in a dormant state are most likely to withstand heat treatment.

The effectiveness of hot-water treatments for control of nematodes and other pathogens is increased by adding suitable chemicals to the treatment baths. In some instances, this may be necessary for effective treatment. A few examples of recommended hot-water treatments indicate the general range of temperature–time combinations found useful: narcissus bulbs infected with stem nematode (*Ditylenchus dipsaci*), treat 4 hours at 43.5°C in 0.5 percent formalin in water; iris bulbs with the potato rot nematode (*D. destructor*), 3 hours at 43.5°C in water and formalin; Easter lily bulbs with spring crimp nematode (*Aphelenchoides fragariae*), 1 hour at 44°C in a water and formalin bath; citrus rootstock with burrowing nematode (*Radopholus similis*), 10 minutes at 50°C; seed with bentgrass seedgall nematode (*Anguina agrostis*), 15 minutes at 52.2°C in water containing a wetting agent; wheat seed with wheat nematode (*A. tritici*), 30 minutes at 49°C or 10 minutes at 50°C; begonia plants with spring crimp nematode, treat by submerging pot and contents for 1 minute at 49°C, 2 minutes at 47.8°C, or 3 minutes at 46.8°C; sweet potatoes with root-knot nematodes (*Meloidogyne* spp.), 65 minutes at 46.8°C; and grape rootings with root-knot nematodes, 30 minutes at 47.8°C, 10 minutes at 49°C, 5 minutes at 51.6°C, or 3 minutes at 53°C.

Hot-water disinfection may be the only practical way to control nematodes infecting some plants. Heat treatments can also be valuable in controlling viruses and other plant pathogens, thus giving multiple benefits for one treatment. The treatment can often be worked into the normal processing of the plants. The disadvantages are the lack of protection against reinfection, the possibility of spreading other pathogens by water, the rather precise conditions required, and the expensive and cumbersome equipment employed.

CONTROL BY LOW TEMPERATURE

Little information is available on either the effect of cold on nematodes or the possible use of cold in controlling nematodes. Cold storage of tubers or bare-root nursery stock does not normally result in disinfection. Study of cold in relationship to nematode survival offers possibilities for research that may lead to new control methods for nematode diseases.

CONTROL BY ELECTRICITY

Electrocution, electrotaxis (galvanotaxis), heating, and diathermy are possible ways electricity might be used in controlling nematodes.

In practice, it is difficult to electrocute small organisms such as nematodes, even at electrical potentials that are hazardous to man. The lethal effect of electricity on a small organism within an electrical field depends on the difference in potential (voltage) existing from one side of the organism to the other and on the amount of current flow (amperage) passing through the organism. In addition to the small size of the nematodes in an electrical field making the resultant voltage differential and current flow in the organism small, nematodes usually are in an environment containing electrical conducting pathways of lesser resistance than the nematodes. For example, the electrolytes in moisture films of soil or in tissues of a plant may conduct electricity more readily than the nematodes.

Electricity is known to influence the direction of nematode movement, and it may be possible to devise some practical means of applying electricity to the plant or surrounding soil to ward off nematodes.

Resistance to the flow of electricity through a substrate, such as soil, produces heat. Containers with metallic plates to serve as terminals distributing the flow throughout the soil within the container are easily constructed. With suitable moisture content and source of electrical power, usually changed to high voltage with a transformer, the soil can be brought to a temperature lethal to nematodes. Wire-mesh grids have been placed at two levels in greenhouse benches to accomplish the same result. These techniques do not electrocute nematodes, but they destroy them with heat.

Electrical energy applied at radio-wave frequencies, similar to diathermy, killed encysted golden nematodes (*Heterodera rostochiensis*) located within bales of burlap bags. Such use of electrical energy for nematode disinfestation deserves further investigation.

CONTROL BY IRRADIATION

The few studies concerning lethal effects of irradiation on nematodes have been discouraging. Published data indicate a remarkably high tolerance of nematodes to radiation.

Cathode rays (electron-beam radiation) such as those produced by electron accelerators have limited power of penetration but can sterilize on very brief exposure. No information is available on their possible use for nematode control.

Radiation sterilization techniques used in food-processing, or of sterilization of surgical supplies and drugs, may be adaptable to disinfestation treatments of objects involved in plant production.

Ultraviolet rays, particularly in the shorter wavelengths, are harmful to some types of organisms. Since these rays are unable to penetrate matter, only surface-borne organisms exposed directly to the rays are affected. It is commonly assumed that the ultraviolet light of longer wavelengths, filtered through the earth's atmosphere, is harmful to exposed nematodes.

Although prospects of using irradiation to control nematodes do not appear promising, the different kinds of radiation, such as x rays, gamma rays, ultraviolet rays, and cathode rays, should be tested.

CONTROL BY MISCELLANEOUS PHYSICAL FACTORS

Physical methods, such as ultrasonics, osmotic concentration, mechanical destruction, pressure, desiccation, and mechanical seed-cleaning, have been insufficiently tested or have been found unfeasible.

Sugar, used to increase the osmotic concentration of the soil solution, was found to kill all nematodes within 24 hours when applied at 1 to 5 percent of soil by weight, but 10 to 50 tons of sugar per acre would be required.

Ultrasonics are ineffective for killing nematodes in soil but may be effective in water.

Modern mechanical seed-cleaning methods, although not widely used at present, are used to remove infected wheat kernels (cockles) or galls, which contain large numbers of the wheat nematode, from wheat seed. Seed-cleaning methods might be further developed to remove small particles of debris infected with the stem nematode (*D. dipsaci*) from seed of crops such as clover and alfalfa.

CHAPTER 13

Control by Chemicals

Practical control of plant-parasitic nematodes with nematocidal chemicals is a relatively recent development. The discovery of the nematocidal properties of a 1,3-dichloropropene–1,2-dichloropropane mixture (1,3-D or D-D Soil Fumigant) and EDB (1,2-dibromoethane or ethylene dibromide) in 1943 and 1945, respectively, had a profound influence on progress in the field of nematology. Initial trials with these nematocides resulted in economically effective control under field conditions and strikingly demonstrated the destructive potential of certain plant-parasitic nematodes, particularly the root-knot nematode. Since then, the use of nematocides has developed rapidly from a few hundred pounds in 1943 to an annual total exceeding 60 million pounds in 1963.

Chemical control has limitations and cannot completely replace crop rotation, fallow, and the use of resistant varieties. Interactions among soil, chemicals, and nematode pests are not yet well understood. Despite these problems, a rapid increase in the use of chemicals has occurred, and an even more rapid increase in their use is expected.

CHARACTERISTICS OF NEMATOCIDES

TYPES

The most successful and effective nematocides in use today are volatile halogenated hydrocarbons. It is generally considered that a material must have high vapor pressure to spread through the soil and contact nematodes in the water films surrounding soil particles. The first material used on a field scale

was carbon bisulfide, but, because of the high dosage required for adequate nematode control (800 to 1,000 pounds per acre) and the explosive hazard involved, its use was never widespread. The use of chloropicrin, another of the early soil fumigants, is limited by high cost and the need for a surface seal because of its relatively high vapor pressure. It is especially valuable where serious fungus and nematode problems occur in the same field. Methyl bromide, although it is expensive and requires the use of soil covers, is used extensively in high-acre-value plant beds and nurseries. Weed control is an important added benefit from its use.

A trend toward less volatile materials began with the development of DBCP (1,2-dibromo-3-chloropropane). The vapor pressure of this material at 21°C is 0.8 mm Hg, in contrast to 29 mm Hg for 1,3-D and 17 mm Hg for EDB.

Recently, promising new compounds that are almost nonvolatile have been developed. These must be mixed with the soil or distributed through water in the soil or through the plant itself. None is widely used at present, but with further testing some may find commercial use. Since many nonvolatile compounds are relatively stable in soil, phytotoxicity and residues in plant and soil may be disadvantages.

Compounds such as Vapam (sodium-*N*-methyldithiocarbamate), Trapex (20 percent methyl isothiocyanate), and propargyl bromide are nematocidal but are used principally to control disease complexes in which fungi, bacteria, insects, or weeds, in addition to nematodes, are involved.

Combinations of nematocides and fungicides, such as Vorlex (1,3-D and methyl isothiocyanate) and Trizone (chloropicrin, methyl bromide, and propargyl bromide), are used to increase the activity of the pesticide.

Several organic phosphates show some activity as nematocides but are primarily insecticides. Examples of these are: diazinon [*0,0*-diethyl *0*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate]; Zinophos (*0,0*-diethyl *0*-2-pyrazinyl phosphorothioate); Thimet or phorate {*0,0*-diethyl *S*-[(ethylthio) methyl] phosphorodithioate}; and several experimental compounds not registered for commercial use.

PHYTOTOXICITY

Phytotoxicity must always be considered when chemicals are added to soil to control nematodes, insects, or fungi. All the early commercial nematocides, such as carbon bisulfide, chloropicrin, EDB, and 1,3-D, can be phytotoxic and, depending on the dosage used, must be applied several weeks or months before the crop is planted. Phytotoxicity is influenced by soil type, temperature, moisture, soil tilth, and kind of plant grown. Some plants are severely injured

by traces of certain nematocides in soil. The use of less-volatile, slower-acting chemicals increases the need for compounds with low phytotoxicity. Although the general phytotoxicity of these chemicals seems to be low, toxicity to specific plants has tended to increase. For example, many crops can be planted immediately following DBCP soil fumigation, and a number of crops will tolerate a nematocidal dosage of DBCP to the root zone of the established plant. Certain crops, however, show sensitivity to DBCP; namely, red and sugar beets, Fordhook lima beans, garlic, onions, peppers, sweet and white potatoes, and tobacco.

RESIDUES IN PLANTS AND SOIL

With the trend to more stable, less volatile, and more persistent nematocides, the danger of excessive residues in the soil and plant is increased. Highly volatile nematocides usually present few residue problems, since a portion of the material passes out of the soil as vapor into the air and the remainder is quickly broken down or leached out, leaving only traces of degradation products in the soil. Only occasional problems with residues have been found with EDB and DBCP. With these compounds, the uptake of bromine is a problem in certain crops, such as peanuts and citrus pulp, used for livestock feed. The burning quality of tobacco may be affected by the uptake of halogens when certain fumigants are used. Excessive chlorine can be controlled by reducing the amount of chlorine applied in the fertilizer. In those cases where bromine residues are a problem, they can be controlled by reduced use of bromine-containing chemicals.

With increasing commercial use of stable compounds, the residue problem will become more serious. Long residual action may limit the use of some compounds that are highly toxic to nematodes. Chemical residues in plants grown in treated soil may be harmful or unpalatable to man or animals consuming the plants. Isolated instances of "off taste" or "taint" in crops following the application of chemicals to soil are usually due to misuse, such as an overdose of chemical, or application when weather or soil conditions are unfavorable for escape or breakdown of the chemical.

FACTORS INFLUENCING NEMATODE KILL IN SOIL

Nematodes live in thin water films intimately associated with and surrounding soil particles. To be effective, nematocidal chemicals must penetrate and diffuse into pores or crevices in the soil to contact the nematodes and, in addition, must penetrate the moisture films surrounding the nematodes. Highly

water-soluble compounds are effective in penetrating these films but do not diffuse readily in soil. Rapid diffusion of the chemical through the soil occurs in the vapor phase rather than through the soil water. Many chemicals toxic to nematodes fail as nematocides because of limited penetration and diffusion or because of inactivation when applied to soil. As most nematocides in commercial use are volatile, their nematocidal activities in soil are influenced by factors such as soil type and condition, soil moisture and temperature, and chemical-application rate and depth.

SOIL TYPE

The diffusion of volatile compounds is definitely influenced by soil type. Clay particles or organic matter may adsorb the compound and reduce the dispersion of the material.

In fine-textured clay soils, pore spaces are much smaller than those in sandy or sandy-loam soils. Such small pores are likely to be blocked by excess moisture or compaction, resulting in noncontinuous passages into which vapors are unable to diffuse. As a consequence, fumigation may be incomplete, especially if the nematocide is highly volatile or short-lived in the soil. Sandy soils, however, contain large pores that are less likely to be blocked by excess moisture or compaction; but a surface seal, necessary to prevent rapid loss of vapor, is more difficult with such coarse-textured soils. For effective nematode control, peat and other organic soils require two to three times the amount of nematocide needed for mineral soils. The high rates are necessary to compensate for the adsorption of the nematocides on the organic matter of the soil.

SOIL CONDITION

Soil preparation is very important for best results with volatile soil fumigants. Deep plowing or chiseling aids downward penetration of fumigant vapors. Where the soil has been tilled to the same depth by disking and plowing for many years, a compacted layer called a plow-sole layer results. This layer may be almost impervious to penetration by water, roots, and fumigants. If it is broken up by chisel or plow, the fumigant, depending on the dosage applied, is able to move down 2 to 6 feet.

In addition to eliminating the plow-sole layer, the surface foot of soil should be well pulverized and smoothed before the chemical is applied. A drag or roller following the injection equipment will then effectively seal the surface and prevent the vapor from escaping too rapidly. The surface seal is

important in increasing the concentration of the vapor in the surface inch or two of soil and thereby increasing nematode control in that zone as well as in the subsoil. The soil should not be disturbed for at least one week after treatment and for longer if deep penetration into the subsoil is desired.

Since living nematodes are protected inside undecayed roots, crop residues should be incorporated into the soil and allowed to decompose before fumigant is applied. EDB, DBCP, and chloropicrin usually give poor control of nematodes inside plant residues, but fumigants containing 1,3-D are more effective in penetrating root tissue. Coarse residue or trash also can prevent an effective surface seal and create channels through which the chemical vapors escape. Alfalfa and corn stubble decrease effectiveness of fumigants unless several months are allowed between turning in the plant residue and application of the fumigant. Adsorption of the fumigant by undecayed organic matter in the soil also may decrease nematode kill by inhibiting complete diffusion of the chemical.

SOIL MOISTURE AND TEMPERATURE

Both soil moisture content and temperature are important to successful fumigation, especially of fine-textured and very-coarse-textured or sandy soils. In sandy-loam soils, these factors are not as critical. In general, most effective gas diffusion of nematocides occurs at a temperature of 18 to 24°C at an 8-inch depth in coarse-textured soil that is moist but is below field capacity in moisture content. If the moisture content is too high—approaching field capacity—a high proportion of the pore spaces are filled with water, and gas diffusion is retarded. Poor nematode control frequently results from fumigation of fine-textured, cold, wet soil. With increased clay content, soil moisture and temperature conditions should be more favorable before fumigation is attempted.

APPLICATION DEPTH

The depth at which volatile fumigants are applied varies with vapor pressure, dosage, temperature, moisture, soil type, nematode species to be controlled, and depth of control desired.

For minimum dosage rates under optimum soil conditions, application is generally made at a depth of 6 to 8 inches. If the application is too deep, the vapors will not reach the surface inch or two of soil; if it is too shallow, the vapors are lost through the soil surface.

As dosage is increased, depth of application also should be increased. For example, 1,3-D at 20 gallons per acre should be injected at 8 inches, while 60 to 80 gallons per acre should be injected at 12 to 14 inches. Increased depth and rate of application are associated with high nematode kill to deep soil levels and little loss of vapors from the soil surface. If soil temperature is above 27°C and soil moisture is considerably below field capacity, depth of application should be increased and, to retain the vapors in the soil, the surface of the soil should be well compressed following application.

In-the-row applications in beds should be injected at greater depth than flat or over-all applications. The usual practice is to inject to a depth of 12 to 13 inches in the bed. When cotton is planted in the bed after fumigation, the surface 2 to 3 inches of soil is pushed off by the planter. This removes the surface layer, which, due to the deep injection, may not have received an effective dosage of fumigant. Recent studies indicate that, if injected at 18 to 22 inches in conjunction with deep tillage, the fumigant may be more effective than it would be with more-shallow injections. Where plow-sole layer or hardpan occurs, deep tillage alone increases the growth of cotton.

APPLICATION RATE

The rate of chemical application is dependent on the properties of the particular chemical and the crop to be grown. For annual crops, it is generally uneconomical to apply a higher dosage of nonpersistent fumigant than that necessary to grow one profitable crop. For crops spaced wider than 2 feet, row application has been effective in many cases and may cost up to 50 percent less than over-all treatment.

For perennial crops such as orchards and vineyards, recommended dosages are usually at least double those used for annual crops. A higher degree of control to a greater depth is needed for perennials than for annuals, because perennials are deep-rooted and grow for many years. Until compounds that could be used safely for treatment of living plants were developed, the need for highly effective preplant treatments was critical. With the advent of DBCP, which can be applied safely to the root zone of many living plants, nematodes on many perennial plants can be kept at low levels. The use of DBCP as a postplant treatment does not eliminate the need for preplant treatment, but, to keep the nematode population low, it should be utilized in conjunction with the preplant treatment. With present nematocides, eradication of nematode populations under field conditions is not practical.

SEALING SOIL SURFACE AFTER APPLICATION

Vapors of volatile soil fumigants may be lost rapidly through the soil surface if the material is not injected to a sufficient depth or if the surface of the soil is not well sealed. Vapor loss through the soil surface may be reduced by using various methods of surface-sealing, depending on the volatility of the compound, the type of application, and the result required.

For highly volatile compounds such as chloropicrin and methyl bromide, a vapor-proof cover is necessary, especially if the material is used without dilution. The conventional method of applying methyl bromide is to release the vapor under a plastic film. The vapor is distributed over the soil surface under the cover and penetrates downward into the soil from several inches to several feet, depending on the dosage used. Chloropicrin may be applied by injection, but a seal is necessary.

For best results with chloropicrin, a vapor-proof cover should be used, but a satisfactory seal can be obtained by sprinkling the soil surface immediately after injection with enough water to wet the soil to a depth of 0.5 inch.

With less-volatile nematocides, such as 1,3-D, EDB, or DBCP, a roller or drag used immediately after application provides a satisfactory seal.

A soil-surface seal is desirable following application of any volatile nematocide, regardless of soil type, temperature, moisture, or preparation. The seal improves nematode control in the surface inch of soil and, in addition, increases retention of vapors in the soil and penetration of vapors, both laterally and vertically.

APPLICATION METHODS

FORMULATIONS

The method of applying a nematocide varies with the type of chemical. Some commercial nematocides can be formulated to suit the type of applicator available to the grower, but highly volatile compounds must be used as fumigants. The most common method of applying methyl bromide and chloropicrin, which are gases under field conditions, is by injection, after which water is sprinkled on as a surface seal or the soil is covered with a vapor-proof film or cover. Equipment is available which efficiently injects these volatile compounds and covers the soil surface with polyethylene tarps in one operation. The gases may also be released directly under the cover. Ease in handling and effectiveness increase when these volatile materials are combined with other less volatile compounds, but a surface seal is still necessary.

To reduce volatility and make handling easier, some volatile compounds are formulated as granules adsorbed on an inert carrier. Granules are not always satisfactory, because the nematocide may be so tightly adsorbed to the carrier that the vapors cannot escape. In one case, 1,3-D was held so tightly that phytotoxicity resulted in two consecutive crops of sugar beets after a single application. A granular formulation of a chemical does not basically alter the method of application, except that the material can be metered into the soil with a fertilizer spreader rather than with a liquid injector. To obtain maximum nematode kill, the granules must be applied under the soil surface and allowed to diffuse from a point or band. Up to 50 percent of the activity is lost if the granules are spread on the soil surface and mixed into the soil. The lower the vapor pressure of the fumigant, the more successful it is in a granular formulation.

Some of the carriers used are Attaclay (an attapulgite-type carrier), Hi-Sil (synthetic silicon dioxide), vermiculite, and carbon. Carbon is not satisfactory with some volatile compounds, because they are bound so tightly that the vapors are released too slowly to attain concentrations that provide satisfactory nematode control. Hi-Sil is reported to hold compounds tightly until the granules are moistened, at which time the fumigant is released. Additional work on granular formulations is needed, especially with the more volatile materials.

Nonvolatile compounds are best used as granules or liquids that can be spread on the soil surface and then mixed into the soil by rototiller or disk harrow. Soil injection is not satisfactory for nonvolatile nematocides unless the toxicant is taken up by the roots, thus controlling nematodes by systemic activity.

Granular formulations of nematocides can be mixed with fertilizer, or the nematocide can be adsorbed on the fertilizer granules. Although applying nematocides directly with fertilizer is a logical and economical method of application, it is not altogether satisfactory, because, to be most effective, the nematocide should usually be applied deeper and at an earlier date than the fertilizer. Combinations of nonvolatile, residual-type nematocides and fertilizers may prove satisfactory. However, such nematocides may require over-all application, with incorporation into the soil, to obtain effective nematode control, while the fertilizer may be used best when carefully applied in a specific location with respect to the roots of the plant. The same problems occur with combinations of nematocides and liquid fertilizers.

Some materials, such as DBCP, are effective at such a low dosage rate that the equipment commonly available cannot be used. In such cases, the chemical is diluted with solvent to increase the volume, or, when a suitable solvent is not available, an emulsifiable concentrate is used. Water can be used as a

diluent with an emulsifiable concentrate, and the formulated material can be applied in irrigation water, by either flood or furrow.

Nonvolatile compounds, which must be mixed with the soil to be effective, may be formulated in a solvent or as an emulsion and sprayed on the soil surface before being mixed into the soil by disk or rototiller.

The application of soluble or emulsifiable concentrates of nematocides in irrigation water has been attempted with a variety of compounds but is feasible with only a few. At this time, DBCP and Vapam are applied commercially in this way. The chemical may be metered into the irrigation water by gravity flow or through a centrifugal pump.

The gravity-flow technique is successful if the nematocide is introduced into water with sufficient agitation for thorough mixing, such as a drop over a weir or head gate, or when the water comes from a pipeline with sufficient force for mixing.

Pump application is needed when the material must be metered into very-slow-moving irrigation water. To obtain mixing, both the inlet and outlet hoses of the pump are placed in the stream of water. A portion of the water is pumped through the centrifugal pump and back into the stream. The chemical is metered into the inlet side of the pump, where it is mixed thoroughly with the water (Figure 50).

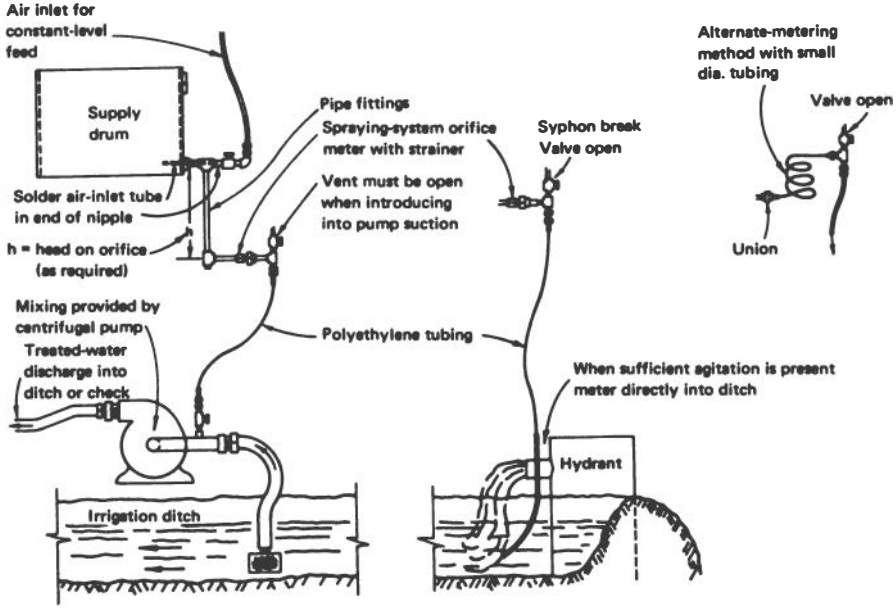

FIGURE 50 Diagram of gravity-flow and centrifugal-pump application of DBCP in irrigation water. (Courtesy of Shell Development Company.)

For sprinkler irrigation, the material may be metered into the pump or introduced into the sprinkler lines. The latter method requires some type of pressurized vessel with a pressure higher than that in the irrigation line in order to force the chemical into the system.

Application of DBCP by sprinkler irrigation has not been as effective as application by flood irrigation. In areas where flood irrigation is not possible, sprinkler application is utilized to a limited extent, even though up to 80 percent of the material may be lost into the air as vapor. Less volatile materials could be more effectively used by sprinkler application, if sufficient penetration into the soil could be achieved.

Additional research on the effects of temperature, humidity, wind velocity, sunlight, and soil penetration, and on the application of chemicals in irrigation water is needed.

APPLICATORS

Injectors

The first nematocidal chemicals used on a field scale were volatile liquids; consequently, the first applicators were adapted to apply these materials beneath the soil surface to prevent vapor loss to the atmosphere. Commonly used applicators are hand injectors and shank or chisel injectors.

Hand injectors are metering devices designed to apply a measured amount of material into the soil through a hollow tube or spike at a given depth. Several methods of metering have been used, but the displacement pump with a spring-loaded valve has been most successful. For small areas such as home gardens, planting sites for a limited number of trees, and experimental plots, the hand injector is very useful. The area to be treated is first marked off into squares varying from 10 to 18 inches in size, depending on chemical, soil type, temperature, and moisture. A measured amount of chemical is then injected at the intersection of each of the lines, but, for best coverage, injections should be made both at the intersections and at a point halfway between intersections on alternate rows. For most of the volatile compounds, 12-inch spacing is satisfactory. As the dosage rate is increased, the spacing also may be increased. The injection hole should be sealed with the foot immediately after the spike is withdrawn (Figure 51).

For large-scale applications, the tractor-drawn or tractor-mounted shank or chisel applicator is used (Figure 52). With this type of applicator, the chemical is injected into the soil through tubes fastened to the back edge of the chisel or shank. The material is usually metered through orifices from a pressurized manifold. The manifold may be pressurized from a gear pump mounted on

FIGURE 51 Application of volatile soil fumigants by hand injector. (Courtesy of Shell Development Company.)

FIGURE 52 Tractor-mounted chisel-injection applicator. Cultipacker behind for sealing. (Courtesy of Shell Development Company.)

the power takeoff of the tractor or by compressed gas, such as nitrogen, carbon dioxide, or air. If compressed gas is used, a tank, properly constructed to withstand the pressures required for accurate metering of the nematocide, must also be used. Shipping drums or similar containers are not suitable. A simple, inexpensive method of metering liquid nematocides is by gravity flow through metering orifices or coils from a constant-head container. The constant-head container is a sealed vessel with the liquid outlet and air inlet located at the same level, near the bottom of the drum (Figure 50). By this

arrangement, air is taken into the drum to displace the liquid removed. By introducing the air at the bottom of the container, a varying vacuum is produced over the liquid to maintain atmospheric pressure at the liquid outlet. Therefore, regardless of depth of liquid in the drum, the flow is constant. The pressure head on the orifice is dependent only on the vertical distance between the outlet and the metering orifice ("h," Figure 50). For this type of metering, the shipping drum is a satisfactory container.

The gravity flowmeter may be used with the chisel method of application (Figure 53). To maintain a uniform rate of application with these devices, the tractor must be driven at a constant speed. More recently, land-driven displacement pumps, which eliminate the need for constant speed and are used

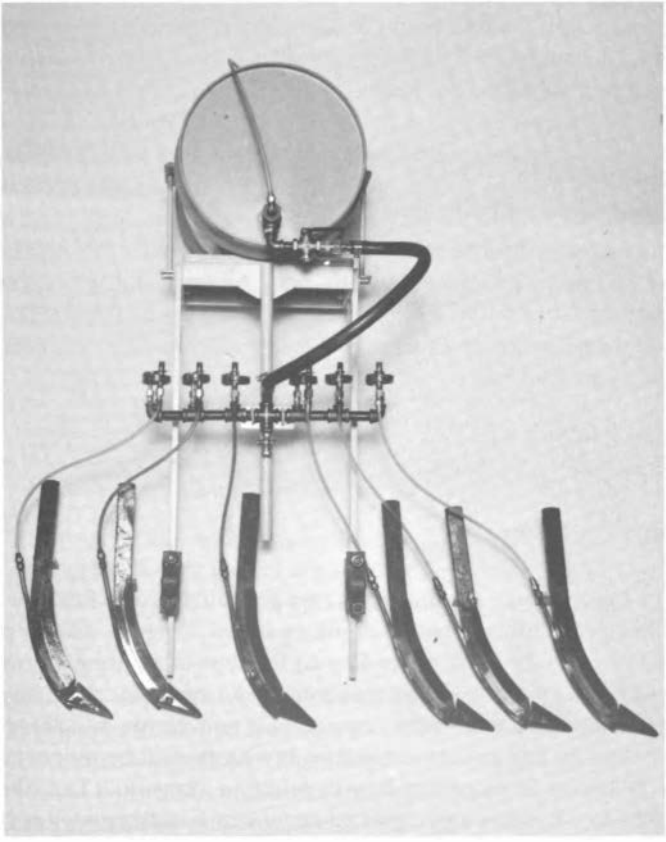

FIGURE 53 Gravity-flow equipment for chisel injection. Copper-tubing coils meter the chemical into the soil. (Courtesy of Shell Development Company.)

with metering orifices for multiple-chisel application, have been devised. The pump is geared to the ground so that a given amount of material is passed through the pump per linear foot of travel, regardless of the speed of the tractor.

Plow Application

Some growers apply nematocides to small fields with a gravity-flow applicator mounted on a moldboard plow. The chemical is metered in the same manner as for chisel injection, but it is introduced directly in front of the plow in the bottom of the furrow and is covered immediately as the soil is plowed. This method may be used for a single plow or a gang of plows, providing the chemical is applied in the furrow in front of each plow (Figure 54). To seal the chemical in the soil, the soil should be harrowed and smoothed immediately following application.

Nonvolatile materials must usually be incorporated into the soil by the use of either a disk harrow or a rototiller. Neither type of equipment will mix deeper than 4 to 6 inches, but water-soluble compounds may be carried deeper by rain or irrigation. With either method of mixing, the material must be distributed evenly over the soil surface. This can be accomplished with either a sprayer or a fertilizer distributor, depending on the formulation of the chemical. For liquid application, any pressure applicator with spray nozzles mounted on a boom serving as the metering device is suitable. For granular application, any spreader that distributes granules evenly over the soil surface is satisfactory.

TYPES OF TREATMENT

PREPLANT TREATMENT

As most of the commercial nematocides are phytotoxic, they must be applied to the soil before seeds are planted or plants are set. Over-all, row, or planting-site applications can be used, depending on the type of planting. In over-all (broadcast) application, the entire area is treated, usually by chisel application at 12- to 14-inch spacing or, if the compound is nonvolatile, by spreading it uniformly over the soil surface and mixing it with the soil by disk or rototiller. With high dosage rates, to obtain deep penetration, down to 8 feet, the chisels may be as far as 18 inches apart. Heavy equipment is necessary when the application depth is more than 14 inches. The 12-inch spacing and 8-inch depth of application are used for low dosage rates when controlling nematodes on fast-growing annual crops such as carrot, lettuce, and squash.

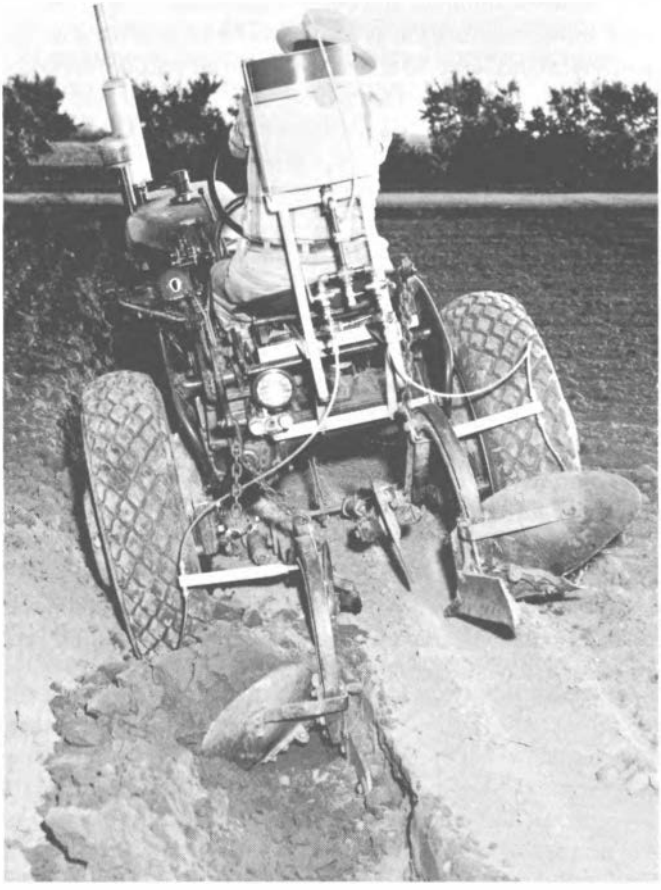

FIGURE 54 Gravity-flow equipment for application by plow. Chemical applied directly in front of plow. (Courtesy of Shell Development Company.)

An important way to reduce the cost of soil fumigation is by row rather than over-all application. For crops grown in rows more than 2 feet apart, row application is widely practiced. Depending on spacing, the cost of chemical is from one half to one tenth of that for over-all application. Not only is there a saving in the amount of chemical needed, but also a reduction in application cost, because the chemical can usually be applied during land preparation. Crops grown on beds can be treated when the beds are formed. For bed or row application, either one or two chisels are used per bed or row. If one

chisel is used, which is common practice, the chemical dosage must be high enough to kill most nematodes in at least a 16- to 18-inch band of soil. The tomato root system in Figure 55 shows root-knot nematode (*Meloidogyne* spp.) galling of roots that have extended beyond the treated strip. An illustration of reduction in dosage by row placement is the fumigation for control of root knot in cotton fields: 20 gallons per acre of 1,3-D-type nematocide is required for an over-all treatment, but with 38-inch row-spacing, 9 gallons per acre, a reduction of over 50 percent, is adequate. As the row-spacing is increased, the saving in chemical is correspondingly increased. For tomatoes, with rows 5 feet apart, a dosage of 5 to 6 gallons per acre of 1,3-D provides effective nematode control.

A strip or row preplant treatment can also be used for some perennial crops, such as peach, grape, citrus, and walnut, saving as much as 50 percent of the chemical as compared with over-all application. A disadvantage of this procedure is that recontamination from cultivation or irrigation is almost certain. Dosages of 40 to 100 gallons per acre of 1,3-D are required for deep-rooted perennials, but, where this is not practical, a high dosage applied in the planting rows is preferable to low dosage over the entire area. For example, it is better to treat the planting area, or one half of the total area, at the rate of 40 gallons per acre of 1,3-D rather than the total area at 20 gallons per acre. Despite the higher cost, the trend is toward over-all treatment on perennials rather than treatment of the planting strip only.

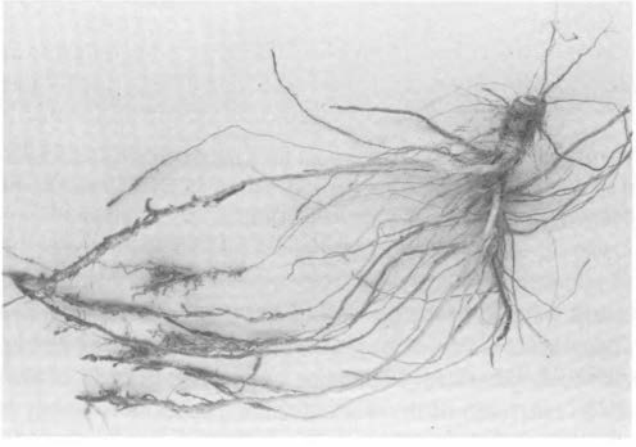

FIGURE 55 Tomato root from plant grown in row-fumigated soil. Galled roots have grown out of treated zone. (Courtesy of the Department of Nematology, University of California, Riverside.)

The same principles apply to planting-site application as to row or strip applications. The treatment of sites is complicated mechanically by the need to interrupt application between planting sites. However, this treatment, more adaptable to hand injectors than to power equipment, is commonly used for replant problems in established orchards or vineyards. Experimentally, it has been demonstrated to be feasible with wide-spaced annual crops such as melons. For melons, a treating site of 2 square feet is sufficient, while tree crops require a minimum area of 6 to 8 feet. Although the planting-site method reduces the amount of chemical used, the saving in chemical may be more than offset by the increased cost of application.

TREATMENT AT PLANTING TIME

Most commercial nematocides are too phytotoxic for use immediately before a crop is planted. Some materials are tolerated at nematocidal dosages by certain plants, but none is completely nonphytotoxic to all plants. Peach is quite tolerant of DBCP and can be planted immediately following application, without phytotoxicity. Although cotton can be planted immediately following application of DBCP without serious injury, it is advisable to wait at least one week. Pineapple can be planted immediately following application of 1,3-D; however, it is not because the pineapple roots are exceptionally tolerant but because the fleshy slips do not start to put out new roots for a week or more following planting.

In general, it is necessary or at least desirable to delay planting until the chemical has dissipated from the soil. A waiting period is beneficial even with relatively nonphytotoxic compounds, because nematode control is more effective when planting is delayed than when planting immediately follows treatments. Evidently, in the latter situation, some of the larvae are able to invade roots before the chemical has had time to act.

When the soil is wet or cold, phytotoxicity may result after an otherwise adequate waiting period, because volatilization, diffusion, degradation, and escape of the chemical are retarded. Nitrification may be depressed under these conditions, resulting in the accumulation of toxic levels of ammonia.

POSTPLANT TREATMENT

The application of chemicals to established plants for control of plant-parasitic nematodes, especially root parasites, is a recent development, used mostly with perennial crops such as trees, vines, and certain ornamentals. Increased yields from postplant treatments usually occur only after the plant

has had time to recover from nematode damage; thus, this treatment is more applicable to long-lived crops, such as those in orchards and vineyards, than to the short-lived annuals.

As mentioned previously, DBCP is the compound used most widely in postplant treatments. Several other chemicals, which have been tested on turf and other living plants with varying success, are still in the experimental stage. Results of experiments with new chemicals on annual crops such as melons, cucumbers, and cotton showed yield increases, but preplant treatments with the same compound or standard commercial nematocides gave superior results.

Two effective methods for applying nematocides to established plants include side-dressing with a chisel applicator or hand injector and application in irrigation water. If the land is level and the soil type is satisfactory, application by irrigation is preferred, because it is easy, requires little equipment, and coverage is uniform, especially close to vines or trees. In nonirrigated areas or areas that are not level enough to flood-irrigate, the chisel method of application is used. Disadvantages of this method are the danger of root injury by the chisels and the difficulty of injecting close to the base of the tree or vine (Figure 56). More care is also necessary in preparing the land for injection than for irrigation application. Sprinkler irrigation has been used in areas where flood irrigation is not possible, but results are erratic because of excessive loss of the chemical to the atmosphere during application.

FIGURE 56 Side-dress of citrus by chisel injection of DBCP for control of citrus nematode (*Tylenchulus semipenetrans*). (Courtesy of the Department of Nematology, University of California, Riverside.)

BARE-ROOT DIP

Considerable effort has been directed toward finding a chemical that would control nematodes on bare-rooted nursery stock as a dip without the need for high temperatures. Although eradication has not been achieved, three materials are reported to be highly effective: Zinophos, Dasanit {*0,0*-diethyl *0*[-*p*-(methylsulfinyl) phenyl] phosphorodithioate}, and SD 4965 (1,6-hexanedithioldiacetate).

The two phosphate materials used at 600 ppm as a 30-minute dip are reported to achieve near-eradication on some ornamentals. Extreme care must be exercised in handling these compounds, because they are highly toxic to mammals. SD 4965 is relatively nontoxic to mammals, but a 24-hour dip is recommended, and, even after such a long treatment, eradication is not always achieved.

It is doubtful whether a material can be found that will be effective on all plants and toxic to all stages of all species of plant-parasitic nematodes and that will also readily penetrate plant tissue, roots, bulbs, rhizomes, and corms without injury to the plant. The most difficult nematode species to control are the migratory endoparasites (*Pratylenchus* spp. and *Radopholus similis*), the sedentary endoparasites (*Meloidogyne* spp.), bulb and stem nematodes (*Ditylenchus dipsaci*), and bud and leaf nematodes (*Aphelenchoides* spp.). These forms, present as larvae, eggs, or adults, may be deeply embedded in plant tissue. The surface feeders, such as *Xiphinema* spp., *Trichodorus* spp., and *Belonolaimus* spp., are easier to control, since they are present on the surface of the plant tissue or in the soil surrounding the roots.

The most promising types of compounds are organophosphates or carbamates, which appear to be readily taken up by the plant tissue in nematocidal dosages. This systemic-type material may be more specific in activity than a nonsystemic type such as SD 4965, but, for specific plants and nematode species, it may be more effective and less phytotoxic than a nonsystemic type. It appears that no one compound will be effective on all plants and nematode species, but several specific types or combinations of chemicals will be necessary in some situations.

SEED TREATMENT

There is no commercially available nematocidal chemical that is effective for treating seeds; such a chemical must be systemic, that is, taken into the root and distributed throughout the root system. For bud and leaf nematodes, the chemical must be translocated to aboveground plant parts. The mode of action may be as either a toxicant or a repellent to the attacking nematodes.

Theoretically, repellency could result either from the chemical itself or from an alteration in plant metabolism, making the plant less attractive to the nematodes.

It does not appear possible to apply enough nonsystemic material to the seed to protect the root zone of the plant for an extended time, because the root system soon grows beyond the treated zone. A volatile compound capable of giving protection in the root zone could not be effective at the shallow depth of application necessary for a seed treatment. Volatile compounds must be applied 6 to 8 inches deep, while seed is usually planted less than 2 inches deep.

Compounds effective as seed treatments would probably also be effective in hopper-box or in-the-row applications with the seed, since the mode of action would probably be identical.

BASIC STUDIES ON NEMATOCIDES

The limited information available on the mode of action of nematocides is supplemented by knowledge of insecticidal and fungicidal action that may apply to nematocides.

In discussing the mode of action of nematocides, two different but related phenomena must be considered. One is movement of the nematocide to the site of action. Movement to the site of action can be further separated into two distinct areas: movement in or through the medium harboring the nematode and penetration into or uptake by the nematode.

MOVEMENT TO SITE OF ACTION IN SOIL

Most nematodes of agricultural importance spend at least part of their life cycles in the moisture films surrounding soil particles. Thus, if a soil-contact nematocide is to function, it must be capable of passing through or persisting in the soil without being either adsorbed or degraded biologically or physically until it has had time to act on the nematodes. The nematocide must be sufficiently water-soluble to diffuse into the moisture film surrounding the nematode. The widely used nematocides 1,3-D, EDB, and DBCP possess these characteristics. The water solubilities of 1,3-D, EDB, and DBCP are approximately 1,000; 2,700; and 930 ppm, respectively. For comparison, concentrations of 10, 5, and 2.5 ppm kill 50 percent of root-knot larvae in *in vitro* tests (LD_{50}). These chemicals are sufficiently water-soluble to accumulate to lethal dosages in soil solution. Systemic nematocides, such as certain organophosphorus compounds, are taken up by plant roots and move to the site of

action in the roots or in the aboveground parts of plants. In other cases, the nematocides are sprayed on the foliage to control foliar parasites. Sometimes nematode-infected plant parts are immersed in an aqueous solution of the nematocide, which then diffuses into the tissue.

PENETRATION INTO THE NEMATODE

Three principal layers can usually be distinguished in the cuticle of a nematode: the cortex, the matrix, and fiber. Some workers believe the cuticle is secreted collagen and the outer cortical layer is tanned protein. Others believe the cuticle is not secreted but is condensed outer layers of the hypodermis. The outermost layer of the cuticle is a thin, thermolabile, lipoid membrane, which is believed to be the main barrier to penetration of drugs, stains, and other chemicals. The nematode cuticle occupies the same position toward nematocides as the insect cuticle does toward insecticides; thus, closer study of its chemical and physical properties might be rewarding with regard to mode of entry of nematocide into the nematode.

Both water and lipoid solubility appear to be necessary characteristics of nematocides. It has been indicated that nematocidal properties of chlorinated hydrocarbons could be correlated with solvency of beeswax and cholesterol. The implication was that these properties were necessary for the nematocide to penetrate the nematode cuticle and cellular membranes.

Penetration of alkyl halide nematocides, such as EDB and DBCP, is relatively fast when nematodes are exposed to aqueous solutions of these chemicals. A dynamic equilibrium can be established between the internal and external concentration of the nematocide in as short a period as 15 to 30 minutes, depending on the specific nematocide and the nematode species. The internal concentration of the nematocide may be from 2.5 to 20 times the external concentration. The rate of accumulation of the nematocide is a function of the rate of penetration and the rate of release of the nematocide. At equilibrium, the rate of release equals the rate of penetration. Penetration appears to occur, for the most part, directly through the cuticle rather than through natural openings such as the amphids, mouth, anus, vulva, and excretory pore.

MODE OF ACTION OF THE NEMATOCIDE ON THE NEMATODE

Halogenated Hydrocarbons

Some possible mechanisms of the action of organic halide nematocides have been proposed: compounds that act by narcosis, that is, reversible inhibition,

a physical mechanism; those that act by some chemical mechanism, that is, irreversible inhibition; and other compounds whose toxicities are a result of both mechanisms.

One theory has been advanced to explain some of the relationships between chemical structure and toxicity of organic halides. Those compounds that are highly reactive in bimolecular nucleophilic displacement reactions are the most toxic, and those having low reactivity in this reaction are the least toxic. Thus, toxicity is believed caused primarily by the inhibition of some essential enzyme system in the nematode by chemical reaction of a reactive halide with some required nucleophilic (basic) center, such as sulfhydryl, amino, or hydroxyl groups present in enzymes. The reactive halide may also react chemically with biologically important proteins or peptides or some regulator of cellular metabolism, inhibiting its function. A schematic representation of such a reaction follows:

$$\begin{bmatrix}\text{Protein}\\ \text{or}\\ \text{Enzyme}\end{bmatrix} : + \; R{:}X \longrightarrow \begin{bmatrix}\text{Protein}\\ \text{or}\\ \text{Enzyme}\end{bmatrix} {:}R + {:}X$$

($R{:}X$ = organic halide)

Toxicity of organic halides parallels the ease of displacement of the halogens in the following order: I > Br > Cl. Toxicity to nematodes generally decreases with alkyl substitution, which is consistent with their rate of reaction in bimolecular nucleophilic substitution reactions. The 2,3-unsaturated allylic halides are usually more toxic than saturated forms, which correlates with their reactivity in both unimolecular and bimolecular nucleophilic substitution reactions.

Because of the rapid rate of solvolysis in various solvent systems, including water, some organic halide nematocides show a lower order of toxicity than is consistent with their rate of reaction in bimolecular nucleophilic substitution reactions. Fortunately, allyl alcohol and some of the halogen-substituted allylic alcohols are very good nematocides; therefore, solvolysis is not always detrimental.

Another possible mode of intoxication of nematodes by alkyl halides may involve the interaction of halo-organic nematocides with an iron center in the respiratory chain of the nematode. This hypothesis is supported by the rapid oxidation of dilute solutions of Fe^{II} porphyrins to the corresponding Fe^{III} halide complexes (hemino) by alkyl halides such as DBCP and 1,3-D.

Organophosphates

A number of organic phosphate insecticides and nematocides are highly toxic to nematodes when applied to soil, roots, bulbs, or foliage. However, because

of one or more factors, such as restricted movement in soil, selectivity, persistent residues, phytotoxicity, or high cost, they have not gained widespread use.

An acetylcholine-splitting enzyme, sensitive to cholinesterase inhibitors, has been demonstrated in plant-parasitic nematodes. Histochemical techniques were utilized to demonstrate that Thimet and a glycoside from asparagus inactivated the enzymatic hydrolysis of acetylthiocholine. Positive reactions, indicating the presence of cholinesterase, were obtained in nematodes of several plant-parasitic genera. This is not complete evidence for a cholinergic system in nematodes or for its inactivation by organic phosphate nematocides, but it is a strong indication that this is one mode of action of these compounds in nematodes.

Certain other nematocides, such as the aliphatic carbamoyloxime, 2-methyl-2-(methylthio)propionaldehyde-*O*-(methylcarbamoyl)oxime, may also kill nematodes by attacking the nervous system, since they are known to be potent inhibitors of acetylcholinesterase in man, animals, and insects.

Carbamates

Carbamate nematocides, such as Vapam, produce toxic volatile decomposition products such as methylisothiocyanate. The toxicity is believed to be caused by the chemical inactivation of biochemically important thiol groups within cells. The thiocyanate apparently reacts with enzymes containing free sulfhydryl groups.

Many organic compounds, including certain fatty acids, thiophenes, and a terthienyl compound from marigold, have been reported as toxic to plant-parasitic nematodes. In addition, several chemicals, including some of the organic phosphates, selenium compounds, maleic hydrazide, sodium fluoroacetate and fluoroacetamide, act as systemic nematocides in plants. As with the commercial nematocides and other biocides, there is little experimental evidence concerning their mode of action as nematocides. Additional research on nematode physiology and biochemistry as related to toxicology and mode of action of nematocides is needed.

SELECTIVITY OF NEMATOCIDES

Field and laboratory data suggest that nematocidal chemicals display considerable selective toxicity against nematodes. Unfortunately, most of the data on this selectivity comes from field plots and consequently is confounded with many important physical and biological variables affecting the efficacy of nematocides. Differences occur in toxicity to various stages in the life cycle of a given nematode species and to species of various nematode genera.

The common nematocides EDB, DBCP, and 1,3-D differ in their ovicidal properties. EDB and DBCP apparently are not as effective as 1,3-D in killing eggs of nematodes, and higher concentrations of all three nematocides are required to kill eggs than to kill larvae. In the case of DBCP, 200 times as much chemical is required to kill eggs in egg masses of *Meloidogyne javanica* as compared with free second-stage larvae. Eggs within cysts of the sugar-beet nematode (*Heterodera schachtii*) are highly resistant to nematocides.

EDB and DBCP are more toxic to the root-knot nematode and the citrus nematode than to the lesion nematode (*Pratylenchus scribneri*) and the stubby root nematode (*Trichodorus christiei*). As many as three- to fivefold increases in concentration are required to give similar levels of kill of the latter two nematodes as the former two. Propargyl bromide is about equally toxic to all four nematodes and considerably more toxic than 1,3-D, EDB, and DBCP. Cysts and larvae of the sugar-beet nematode are killed by lower dosages of 1,3-D than of EDB or DBCP.

Two new chemicals, the organic phosphate nematocide, Nellite (phenyl *N,N*^{1}-dimethyl phosphorodiamidate), and SD 7727 (2,4-dichlorophenyl methanesulfonate), are reported to have a high degree of selectivity for root-knot nematodes. Both are effective in controlling root-knot nematodes in soils at concentrations below 5 ppm, but neither is effective against numerous other plant-parasitic nematode species tested. SD 7727 is particularly interesting in that its mode of action appears to be inhibition of infection rather than direct toxicity.

The general trend is toward development of selective nematocides. The reasons for such selectivity by certain compounds are little known and offer an interesting area for research.

DEVELOPMENT OF RESISTANCE TO NEMATOCIDES

The potential development of resistance of nematodes to the present nematocides is of continual concern to nematologists. One hears reports that nematodes in a given field are more difficult to control with a certain nematocide than are nematodes in comparable fields, but no experimental evidence exists demonstrating that nematodes have actually developed resistance to the commonly used nematocides. That nematodes have not developed resistance is not too surprising considering the relatively low selection pressure to which most nematode populations are exposed. Most fields are treated only once a year, and nematocides have been in general use in most areas only since 1950. Just a few fields have been treated annually since 1943. In addition, many fields are row-treated, or treated with minimal dosages, so that the population that persists is not likely to consist merely of resistant individuals, if such

exist. Well-planned laboratory and field tests are needed to determine whether some of the important plant-parasitic nematodes can develop resistance to nematocides.

FUTURE RESEARCH NEEDS

Despite the outstanding progress in control of plant-parasitic nematodes by chemicals, additional research is needed. Very little is known concerning the mode of action of the commercial nematocides. Although such information would probably not increase the use of these compounds, it might help in the development of improved materials and in understanding the susceptibility of nematode species and their stages of development to nematocides.

The fate of volatile chemicals applied to the soil is not well known. Such materials spread in all directions—up, down, and laterally. Do they break down physically, microbiologically, or chemically? Do the intact materials leave the rhizosphere by leaching or by gaseous diffusion?

Some of the new chemicals are quite specific. Are they directly specific for certain nematode species or indirectly through the host plant, such as by repellent action?

Can nematodes become resistant to nematocides?

How do nematocides move in water? Many field observations have been made, but carefully planned, well-controlled tests are needed. Such information would be especially helpful in the application of chemicals in irrigation water, either by flood or sprinkler.

How do nematocides move in plants? It is agreed that a systemic material applied to the foliage of plants and translocated downward to control nematodes in the roots is desirable. Do nematocides follow patterns that have been worked out for insecticides and herbicides? Is it possible for a nematocide to act in this manner? Does the chemical need to be toxic to the nematode when it reaches the root, or can it be merely repellent, or perhaps change the metabolism of the plant, making it immune? Techniques for evaluating these actions are desirable.

Cheap, accurate, dependable application equipment, both for liquids and granules, is needed. In many areas, the development of nematocide usage has been delayed by lack of suitable equipment.

Improved equipment for injecting volatile chemicals into the root zone of established plants, without injuring the root system, is desirable. Probe or spike injection, high-velocity liquid injection (Spitnik), and water injection under pressure are examples of methods that are under trial or that have been suggested.

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CHAPTER 14

Evaluation and Selection of Control Measures

A nematode disease problem can usually be solved in a number of different ways. The research nematologist, agricultural specialist, and farmer are faced with the evaluation of the disease problem. From the various control methods available, they must select the method that is the most effective, most economical, or both. The method selected will depend on the nematode species, the host plant, the environmental situation, the cash value of the crop, and the relative cost of available control methods.

INTEGRATION OF CONTROL MEASURES

In most cases, practical control of a nematode disease involves integration of several diverse control measures. Some nematode diseases can be prevented merely by using nematode-free seed or vegetative propagating materials. For example, the disease caused by the wheat nematode (*Anguina tritici*) is prevented by planting clean seed in land that has not been planted to wheat for at least one year. The garlic disease caused by the stem nematode (*Ditylenchus dipsaci*) can be prevented by planting nematode-free garlic cloves in clean soil. The disease of banana caused by the burrowing nematode (*Radopholus similis*) can be controlled in banana plantations by eradicating the nematode from the rhizomes used to propagate bananas and planting this seed in soil that is free of the nematode. New citrus orchards can be kept free of either the citrus nematode (*Tylenchulus semipenetrans*) or the burrowing nematode by using trees produced in clean nursery soils and planting in orchard sites free of these nematodes.

The application of a preplant nematocide is often all that is required to control a nematode disease effectively. For example, diseases of a number of annual crops caused by the root-knot nematodes (*Meloidogyne* spp.) can be controlled by preplant soil fumigation. Several procedures, however, are necessary to control nematode diseases on a number of perennial and annual crops propagated from vegetative materials such as roots, bulbs, corms, rhizomes, slips, and transplants. The propagating stock and soil must be relatively free of nematodes. For perennials, such as tree and vine crops, the combination of nursery propagation of nematode-free plants and preplant soil fumigation is required when the soil is infested. In The Netherlands, the production of bulbs requires the use of treated bulbs in fields that have had carefully controlled rotations. In addition, the field must be surveyed prior to planting and must be free of the golden nematode (*Heterodera rostochiensis*) to prevent contamination of the bulbs by infested soil at harvest. Control of root-knot damage to sweet potatoes involves at least three phases of the production: first, the selection of seed roots that are either free of nematodes or freed of nematodes by hot-water or dry-heat treatments; next, the placing of the seed roots into beds of sand or coarse-textured soil that either is nematode-free or, if infested, is preplant fumigated, preferably under tarps, or steamed to eliminate the nematodes; and, finally, the transplanting of the clean "slips" into nematode-free soil or preplant-fumigated soil.

With many nematode problems, there is a tendency to rely too heavily on only one method of control. More research is needed on utilization and integration of nematode control measures.

EFFECTIVENESS AND ECONOMICS

The ideal way to control a nematode disease is to eradicate the nematode, or nematodes, involved. This has been accomplished only in very limited areas such as greenhouses or propagating beds. With the soil fumigants in use today, it is not feasible to eradicate nematodes that are distributed throughout the soil mass to a depth of 12 to 15 feet, such as root-knot nematodes on grape or burrowing nematode on citrus. In such cases, preplanting fumigation is directed at reducing the nematode population density to well below the tolerance level of young transplanted trees and vines. However, eradication of nematodes with present-day preplant soil fumigants is more likely for those nematodes distributed in the surface soil, such as the potato rot nematode (*Ditylenchus destructor*).

Large sums of money are spent for nematocides to limit spread and possibly achieve eradication of incipient infestations of introduced nematode pests that cause severe crop damage and are difficult to control by other means.

Such control involves high nematocide dosage rates, gas-proof tarps, and repeated treatment. Funds expended for this purpose are justified because of the potential crop loss if noninfested acreage becomes infested. The actual cost of restoring the infested acreage to a productive level is not involved.

On a more restricted basis, the same cost–effectiveness criteria can be applied to nurseries. With the recent advent of quarantine laws requiring close inspection of nursery plants moving in inter- and intrastate commerce, nematode problems have become much more acute for nurserymen. In some cases, nematocide at a cost in excess of $200 per acre is applied to nursery soils; the expenditure is justified, since noninfected nursery plants can generally move freely in the trade.

The use of nematocides for possible eradication of nematodes in nurseries has received considerable attention. New chemicals or combinations of chemicals for testing are continually available. New application equipment, such as mechanized tarp layers, has been developed. Research to determine which preplant nematocide to use and how to use it most effectively should be integrated with studies on the use of postplant nematocides designed to keep nematode populations below economic levels on living plants. Further studies on the detection of nematodes at very low population densities also are needed. This would assist in quarantine work and in the evaluation of the effectiveness of control practices.

In some cases, treating soil with high dosages of nematocides before planting perennial crops is justified purely on the basis of increased plant growth and production. An initial cost of $250 per acre for preplant treatment of citrus soil with 1,3-D (1,3-dichloropropene) can be less than the increased value of fruit in the first three years of production. The investment of $250 per acre for soil fumigation is not unreasonable for a crop such as citrus, for which the investment in land and trees may exceed $4,000 per acre.

The effectiveness of preplant soil fumigation of soils planted to perennial crops has been judged on the basis of how closely nematode control approximates eradication. This reflected the long-term nature of the crop, the inability to rotate, and the lack of an effective nematocide that could be used for postplant treatment. The development of DBCP (1,2-dibromo-3-chloropropane), which is effective in controlling a limited number of nematode species around the roots of living plants, changes this situation somewhat and points the way for additional research. A critical need remains for nematocides to use in postplant treatments to kill endoparasitic nematodes in the roots of perennial crops. Such chemicals would further reduce the necessity of striving for eradication, with its high cost in preplant treatments for perennial crops. After soil application, such a chemical would enter roots from the soil, or, after foliar application, it would move from the foliage to the roots. Various combinations of nematocide treatments could then be utilized to control all kinds of nematodes that parasitize plants.

The economic return from soil fumigation is an important consideration. A compilation of reports from the United States compares crop yields on plots treated with nematocides with yields on untreated plots. In 853 comparisons involving seven crops, an average increase in yield of 87 percent resulted from nematocide application. Yield increases for crops for which more than 25 comparisons were available are shown in Table 1. Although the evidence does not support the conclusion that differences were solely the result of nematode control, it is evident that nematocides have a favorable influence on the yield of several crops in many parts of the United States.

While these data provide strong circumstantial evidence that nematodes severely reduce crop yields, they indicate little about the economic returns from fumigation. An increase of 100 percent in the yield of sugar beets may not result in a net profit to the grower, whereas an increase of 13 percent in tobacco may be profitable. In California, yields of sugar beets are increased consistently when soil infested with the sugar-beet nematode (*Heterodera schachtii*) is fumigated with nematocides containing 1,3-D; but if yields are increased from 9 to 18 tons per acre, no profit is returned to the grower, because 18 tons per acre are required to pay production costs. Thus, although fumigation is successful from the standpoint of nematode control and increased sugar-beet yields, it is generally not considered economically successful and is not recommended. In other states, such as Colorado and Utah, soil fumigation consistently results in profits to sugar-beet growers and is recommended. In California, soil fumigation for root-knot nematode control on sugar beets in the San Joaquin Valley is recommended, because the nematode drastically reduces the tonnage of roots produced and also affects the quality of the root and, consequently, the ease with which sugar is extracted.

As another example, the average yield of cotton in the United States is about one bale per acre. Yet, in Arizona and California, soils producing two to three bales per acre respond to soil fumigation. When the soil is sandy loam and root-knot nematodes are prevalent, soil fumigation consistently improves yields. Nematodes are the primary limiting factor to plant growth in these

TABLE 1 Yield Increase Following Soil Application of a Nematocide

Crop Plant	Increase (%)
Lima bean	35
Cotton	91
Soybean	126
Sugar beet	175
Tobacco	13
Tomato	73

fields, since other crop inputs, including soil moisture, are rigidly controlled. On farms with large acreages, an increased return of $50 per acre is important.

Shortly after the introduction of such successful nematocides as 1,3-D and EDB (ethylene dibromide), it was suggested that the increase in crop value compared with the cost of fumigation should be a ratio of 4 to 1. Actually, no generalized practical formula is applicable: every nematode–crop interaction must be analyzed with respect to its individual geographic and economic circumstances. Data accumulated over a 20-year period on nematode problems and soil-fumigation results on a certain crop in a specific geographic location provide much better criteria for making fumigation recommendations than do arbitrary figures on returns per dollar invested in nematocides.

Root crops such as carrots, sweet potatoes, table beets, and white potatoes are markedly reduced in quality and in market acceptance by diseases caused by root-knot nematodes, even though the crop yield may not be materially affected. Cash returns on a crop of sweet potatoes are increased as much as $300 per acre from an expenditure of only $30 per acre for soil fumigation. This results from an increase in the pack-out of higher grades without any significant increase in total production.

In Georgia, control of the lesion nematode (*Pratylenchus brachyurus*) on peanuts by soil fumigation increases the yield of high-grade peanuts. Postplant treatment of Valencia and navel oranges with DBCP significantly increases the percent of larger fruit, thereby increasing profits.

At present, quality of crop produce as related to nematode control is usually considered only in terms of economics. However, with increased knowledge concerning the influence of nematodes on the nutrient status and physiology of host plants, it will be important to explore the effects of nematodes on factors such as fiber length and strength in cotton, flavor and vitamin content in edible annual and perennial crops, and nutrient content of forage crops for livestock.

Soil fumigation may adversely affect the growth and quality of some plants. Bromine-sensitive crops, such as onion, carnation, and citrus, are sometimes stunted by preplant soil fumigation with nematocides containing bromine. In some cases, this may be sensitivity to the nematocide itself rather than to bromine. These points need additional research for clarification.

LEVEL OF AGRICULTURAL DEVELOPMENT

RECOGNITION OF A PROBLEM

In some areas of the United States, and in many of the underdeveloped countries of the world, there are two primary obstacles to effective nematode

control. The first, and perhaps the most important, is lack of recognition that plant-parasitic nematodes are seriously limiting the potential yield of crops. The main reason for this is the shortage of agriculturists at all levels who recognize and understand nematode diseases. This situation is changing as nematologists increase in numbers and more agriculturists receive training in nematode control. The second obstacle is primarily economic and represents a lack of capital to invest in equipment and nematocides. Cultural control methods and resistant varieties should be used where feasible, since, for many nematode diseases, nematocides are too expensive. In some countries, import duties and marketing costs raise the price of fumigants to levels that are prohibitive for most uses. Under such circumstances, it is not surprising that soil fumigation is not a widespread practice in all agricultural areas of the world.

GROWTH OF SOIL FUMIGATION IN THE UNITED STATES

The increase of soil fumigation in the United States is impressive. Since its infancy in 1943, it has developed into an established agricultural practice. According to the U.S. Bureau of Census, 1963 Census of Manufacturers, production of soil fumigants in 1958 amounted to 25,446,000 pounds, as compared with 61,356,000 pounds in 1963. Under intensive cultural conditions, large quantities of methyl bromide are used for the control of weeds, nematodes, and soil insects. Large quantities are applied to tobacco seedbeds in southeastern states. In California, one of the oldest soil treatments is a mixture of methyl bromide and chloropicrin, which is applied, before planting, on a large proportion of the strawberry acreage at the rate of 300 pounds per acre, costing approximately $270.

An estimated 10 million pounds of EDB are used annually in the United States for soil fumigation. EDB and 1,3-D are used extensively in tobacco fields of the southeast. In the pineapple fields of Hawaii, 1,3-D is the principal preplant fumigant that is used. In the cotton fields of Arizona and California, 1,3-D is also widely used as a preplant fumigant. Use of DBCP as a soil fumigant has increased rapidly; in 1962, 1,545,000 pounds; in 1964, 5,314,000 pounds; and in 1966, 8,722,000 pounds were produced.

Although the use of nematocides is expanding, only a fraction of the world's soils that could benefit from nematocide applications are presently treated.

SPECIFIC METHODS OF EVALUATING CONTROL MEASURES

NEMATODE CONTROL

In previous sections, control measures were considered primarily from the standpoints of effectiveness in killing nematodes and of cost. The technical

problems involved in actually evaluating the relative effectiveness of the control measures were not emphasized.

With regard to nematode control, questions arise as to when, how, and where to sample fields for detection of crop-damaging nematodes. Detailed studies on sampling methods were made in England and The Netherlands on the cyst nematodes, *Heterodera schachtii* and *Heterodera rostochiensis*. Techniques that give consistent and statistically sound results were developed. With these nematodes, the level of infestation in the top 1 or 2 feet of soil is the primary concern.

Reliable estimates of soil populations to depths of 8 to 10 feet or more are needed for many nematode species, particularly for those that are parasitic on deep-rooted perennials. Several reliable techniques are available for extracting nematodes from soil and plant samples. New procedures for collecting samples and obtaining information on the number of samples required for reliable data are needed.

The most urgently needed information includes dependable procedures for making predictions on potential crop losses, based on the kinds and numbers of plant-pathogenic nematodes recovered from soil and root samples. On the basis of this information, recommendations can be made concerning whether or not soil fumigation of a particular area of land would be profitable. Such predictions cannot be made now even for fields infested with the most economically important species in the United States, namely the root-knot nematodes. The cropping history of the land is presently the important source of information concerning possible infestation with the root-knot nematodes and with certain other species.

ECONOMIC EVALUATION

Efficient techniques are available for determining the value of nematode control. These evaluations require detailed data on yield, quality, date of maturity, and other factors that might influence the value of the crop. The cost and effectiveness of alternate control methods also must be considered. The impact of nematode control measures on other cultural operations, such as rotation, must be taken into account. A nematode control measure, at first judged too expensive, may in practice prove economical if multiple benefits result, such as control of weeds and fungi, thereby eliminating weeding costs and insuring a uniform stand and maturity. Nematode control may also aid in more efficient use of fertilizer and water. Although an important factor in the overall economics of crop production, the economics of nematode control has yet to receive attention from the agricultural economists.

PART IV

RESEARCH NEEDS

CHAPTER 15

Future Research Needs

Plant diseases caused by nematodes have been found in all areas of the world, and many new nematode diseases are being discovered annually. Although the role of more than 150 nematode species in plant diseases is being studied, knowledge about nematodes and their control is far less than that available for other organisms, such as insects and fungi, that attack plants.

Nematodes are now recognized as pests of global concern, threatening world food supplies. This has prompted authorities in many countries and subdivisions of countries to enact regulatory and quarantine measures. Such action is particularly necessary when plant materials and soil are transported and infestations are heavy.

Although careful inspections and surveys by regulatory and quarantine workers are of great value, all nematodes are not detected by present sampling methods; thus, populations below the discovery level must be considered in any program to control nematodes by regulation or quarantine. Basic information in such areas as taxonomy, host ranges, plant pathogenicity, and soil and plant sampling is needed to improve methods of preventing nematode spread.

Correct species identification is basic to nematode control; thus, nematode taxonomy at all levels must be emphasized. In particular, taxonomists must be urged to study thoroughly the taxonomic relations among nematodes of relatively large groupings and the influence of environmental factors on morphological variation, and to search for means of differentiating nematodes, other than by morphology. Additional collections of permanently mounted nematodes must be established throughout the world. Investigators must be encouraged to make at least a correctly preserved mass collection of a nematode used in an experiment or series of experiments. Unless these steps are

taken, research data may become useless when taxonomic changes necessitate reidentification.

Most aspects of the life and survival of nematodes in soil and plants and many of the basic premises of cultural control of nematodes involve ecology encompassing the complex interrelationships among the organism, the soil, and the plant. There are, however, very few detailed research publications or long-range research programs devoted entirely to nematode ecology, and the published data, much of which are contradictory, are based mainly on observations and preliminary experiments. Although nematologists are aware that plant nematodes greatly influence and modify the productivity of soil, a lack of awareness of nematode populations on the part of the horticulturist, soil scientist, and plant pathologist has led in the past to misinterpretations not only of factors limiting crop response but also of experimental results. Future research on nematode ecology should be conducted by research teams, including investigators in related areas as well as nematologists.

In addition to those conducted under natural field conditions, pathogenicity experiments should be conducted in the glasshouse and the laboratory or growth chamber under aseptic conditions involving only nematode and plant. Conclusions should be reached only after evaluating comparative results from at least two types of these experiments. Progress in this and most other nematological research could be facilitated by improved methods of inoculating plants and extracting nematodes from soil and plant parts.

The development of versatile, economical, and easy-to-handle nematocides is greatly needed for more effective control of nematode diseases. For example, nematocides that are effective in cold, wet soils are needed. Also needed are nematocides that are nontoxic to a wide variety of plants, allowing application at planting time and in the vicinity of roots, thus resulting in better timing of applications and reduction in the amount of chemical needed. More rapid and effective methods of applying nematocides around roots, especially roots of perennials, need to be developed. An "ideal" nematocide for root pathogens would be a systemic chemical that, when sprayed on tops of plants, is translocated to the roots and kills or repels nematodes feeding on these roots, without imparting harmful residues to parts of the plant harvested for food or fiber. Using such a systemic chemical in place of a soil fumigant would require only a fraction of the quantity of nematocide, because the plant rather than the soil is treated.

Interactions among soil, nematocides, and nematodes are not well understood. Little is known about the intriguing problem of how and in what quantities nematocides enter nematodes and, after entering, how the nematodes are actually killed.

In many agricultural areas of the world, it is practical to apply nematocides in irrigation water. To refine this method of application, data are needed on

the effects of such factors as temperature, humidity, wind velocity, sunlight, and soil penetration of chemicals.

Despite these limitations resulting from the lack of basic information, the use of chemicals has increased rapidly and should increase even more rapidly in the future. Control need not await complete understanding. Basic research to gain an understanding of the nematode and the disease and attempts to control by methods such as soil fumigation often proceed together profitably.

The high cost of nematode control by methods such as soil nematocide treatments points to a need for development of a wide range of nematode-resistant plant varieties. Additional plant varieties with improved nematode resistance will result in untold benefits, especially to growers of perennial and low-acre-value crops and to crop growers in developing countries, where the cost of nematocide treatments is often prohibitive. However, before major progress in this field is possible, there must be a marked increase in numbers of research teams, consisting of both plant breeders and nematologists, actively engaged in such programs.

Future research must be directed toward improving methods for testing the resistance of plants to nematodes. The importance of nematode culture and correct identification must not be overlooked if progress in developing resistant plant varieties is not to be hindered.

The biochemical basis of resistance of plants to nematodes has hardly been studied. Research in this area might aid not only in developing nematode-resistant plant varieties, but also in the discovery of new nematocides.

Crop rotation is the oldest and still the most widely used field-control measure for nematodes. Rotations, selected on the basis of crop-yield results alone, without considering nematodes, often owe the resulting increased yields to the unwitting control of nematodes. Although, in recent years, data from nematode host-range and population studies have been used in planning rotations, there is a critical need for additional information in these important areas. The need for research in areas such as population dynamics and host ranges should be emphasized. Differences in host specificity of geographical isolates of a nematode species must be considered in any breeding program. Research to determine exactly how resistance-breaking nematodes arise on previously resistant varieties must also receive a high priority.

Many plant diseases, especially root diseases, are caused by nematodes in combination with other soil organisms. The fungus–nematode and the bacteria–nematode relationships are so numerous and varied that they present a wide-open field for profitable research. For example, relatively weak fungal and bacterial pathogens, once they gain entry into plant roots in the presence of feeding nematodes, can cause considerable damage. The possible biochemical or physiological role of the nematode in these disease complexes is not known. In fact, in most instances it is not known whether the presence of two

or more pathogens on a single host has only an additive or a synergistic effect on disease severity. Nematode attacks sometimes lower the resistance of plants to diseases caused by other organisms, such as resistance to vascular-wilt diseases caused by fungi and bacteria; however, the basis for this breakdown of resistance is not understood. Future challenging and interesting research areas include determining why plants with nematode-damaged roots are more susceptible to cold injury and why fungal infection of roots sometimes increases nematode buildup in these roots.

Although nematodes are known to transmit many soilborne viruses, the nature of virus transmission by nematodes is essentially unknown, and the importance of nematodes in the damage caused by these virus diseases is difficult to ascertain. To better understand the whole gamut of host–nematode–bacteria–fungus–virus relationships, nematologists need to know more about generally overlooked peripheral areas. These areas include changes induced in plant constituents by parasitism, immunology, and enzyme action, as well as the physiology, biochemistry, and genetics of nematodes.

New approaches to nematode control will undoubtedly be found. Perhaps through research, viruses and enemies such as fungi, bacteria, and invertebrate animals (including predacious nematodes), which greatly reduce nematode populations under certain naturally occurring conditions, can be used effectively to control specific diseases. Perhaps results of future research will show how attractants can be used to influence nematodes to move toward a nematocide, how male sterility can be used to reduce nematode populations, and how repellents rather than nematocidal chemicals can be used in control programs. Additional basic research in nematode biology is needed to further the development of such control measures. Progress in nematode control would be accelerated by the cooperative efforts of teams composed of such specialists as a nematologist, an organic chemist, a biochemist, soils chemist, plant pathologist, an agronomist, and a horticulturist.

Basic research on nematode physiology, biochemistry, ecological relationships, and host–parasite interactions, so important in developing control methods for nematode diseases, has gained impetus only recently. Even today only a few investigators are working in this area. Despite some research on plant-parasitic nematodes, the physiology and biochemistry of nematode parasites of man and animals have received considerably more attention. Because of the great differences in these types of nematodes and in their environments, it is dangerous to assume that data obtained with parasites of man and animals apply equally to plant parasites. However, it is probable that many of the concepts and techniques developed by helminthologists deserve more consideration by nematologists, and vice versa.

Nematodes, like other animals, contain carbohydrates, proteins, lipids, nucleic acids, vitamins, hormones, minerals, and numerous other substances,

but most of the existing information on types or amounts present concerns animal parasites. Too little is known of intermediary metabolisms in plant-parasitic nematodes to make comparisons with those of animal parasites. Research on fundamental metabolic cycles, such as sequences of sugar breakdown and terminal oxidation, is needed to understand the nematode's use of plant tissues for food.

Although water and certain ions pass directly through the nematode cuticle, the excretory system appears to be responsible for osmotic regulation of the body fluid, as well as for excretion. Information on osmotic regulation in nematodes is important to nematode control, because chemicals used to kill nematodes are transported in the water phase. The use of radioactive tracers may help to elucidate how nematodes control their osmotic concentration and to understand nematode metabolism.

A primary reason for the limited research on many important aspects of nematode physiology, biochemistry, and host–parasite relationships is the difficulty of obtaining specific plant-parasitic nematodes in large enough quantities for study. Although several plant-parasitic species can be grown on excised roots, on callus tissue, or in association with a fungus or a bacterium, the majority of species cannot be grown even using these methods. Ability to grow plant-parasitic nematodes on chemically defined media under sterile conditions would aid many types of nematological research.

Despite some progress in recent years, relatively little is known about mechanisms involved in changes induced in plant cells and tissues by plant-parasitic nematodes. For example, although it is known that the mechanism of nematode-induced galling of plant tissues involves growth regulators, little research effort has been directed toward specifically identifying the compounds involved. Furthermore, the majority of the microscopic studies of tissue and cellular alterations (histopathological studies) associated with nematode parasitism have dealt with plant tissues in which parasitic relationships were already established at the time of sampling. Especially needed are studies of tissue changes during the critical period of the establishment of the host–parasite relationship and comparisons of reactions in nematode-resistant and nematode-susceptible plant tissues. Ideally, histopathological, cytological, and histochemical studies of plant tissue should be conducted simultaneously, and, where feasible, electron microscopy should be used. Such comparative studies, combined with physiological and biochemical studies of infected and noninfected tissues, will provide the information needed to understand the fundamental basis of plant parasitism by nematodes.

Plant-pathogenic nematodes will become more important to agriculture within the next few decades because of their spread by man and because of more intensive plant culture, particularly monoculture, on the better agricultural soils of the world. Despite the widespread parasitism of every crop plant,

less than 100 nematode diseases are considered of serious proportions. Many other nematodes causing less obvious or less severe damage are generally unrecognized.

Well-trained nematologists are absolutely essential to fruitful research programs. Fortunately, many colleges and universities recognize the wisdom of training nematologists who will eventually teach and conduct research in this area. Some colleges in the United States and India have established departments of nematology. In most universities, instruction and research in nematology is centered in departments of plant pathology, entomology, and economic zoology; however, as the science of nematology gains stature and status, the establishment of separate departments of nematology will increase.

Nematologists should be trained with full recognition of the urgent needs of nematology as a rapidly growing area of biology as well as of the needs directly related to agriculture.

With recognition of the importance of nematology, the better training of nematologists, and more and higher caliber research in nematology, man's control of nematode infestations on important food crops may become one of the most dramatic developments in world food production in the latter half of the twentieth century.