NEMATODES IN ALFALFA PRODUCTION

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Abstract: Plant parasitic nematodes are nonsegmented-microscopic roundworms which are frequently present in alfalfa fields. Although more than 10 different genera have been found in alfalfa fields in California, two (stem and bulb, and root knot) are most commonly associated with damage. A management plan to fit a particular growing situation should be developed using a combination of techniques including: planting site selection, certified seed, clean equipment, weed and irrigation management, resistant varieties, crop rotation, fallow, organic amendments and chemical nematicides.

Keywords: nematode, *Ditylenchus*, stem and bulb, *Meloidogyne*, root knot,

INTRODUCTION

Plant parasitic nematodes are nonsegmented-microscopic roundworms which are frequently present in alfalfa fields. Whether or not alfalfa is to be planted in a nematode infested area, a grower should be knowledgeable about nematodes. If nematodes are present, both pre and postplant management strategies should be developed for pathogenic species. If an alfalfa field or a potential planting site is not infested, a grower should be aware of techniques available to prevent the introduction of harmful species. For growers to carry on a nematode pest management program they need to be familiar with the following: nematode biology; symptoms and signs of nematode damage; how nematodes injure plants; how to sample for nematodes; and the principles underlying various management techniques including: planting site selection, the use of certified seed, the importance of using clean equipment and irrigation water, weed management, the use of resistant varieties, crop rotation, fallow, organic amendments, and chemical nematicides.

BIOLOGY

Nematodes are aquatic animals that live in a variety of habitats including soil, fresh and saltwater, within plants, and within both vertebrate and invertebrate animals. Those living within plants and animals (e.g. hookworms, pin worms, and dog heartworm) may become damaging if their population level gets too high. Other nematode species such as those feeding on weeds, insects or decaying plants are considered to be beneficial to man and the environment. The nematodes which parasitize and damage alfalfa are less than one tenth of an inch long and are found in soil, within roots, or within the crowns, stems and leaves of plants. Within the soil, nematodes live and move within the film of water which lines soil pore spaces. They

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are small enough to move between individual soil particles. It is not uncommon for a single teaspoon of soil from an alfalfa field to contain 50 nematodes or for a single inch of alfalfa stem or feeder root to contain 200.

Nematodes have a relatively simple body structure. When viewed under a microscope, the external covering or cuticle is transparent permitting the viewing of major organs such as the digestive tract and reproductive system. They possess a spear or stylet which is used to pierce and feed on plant tissues. The nematode life cycle consists of an egg stage, four gradually enlarging juvenile stages, and an adult stage. The length of a single generation can vary from a few days to a full year depending on the species, the soil temperature, and other factors. Adult female nematodes can lay several hundred to a thousand eggs apiece.

At least 10 different types of plant parasitic nematodes have been found within alfalfa fields in California. Each of these has both a scientific (*Genus species*) and a common name which are used interchangeably by nematologists. The common name is derived from a morphological characteristic of the nematode, from plant damage symptoms, or from the typical location of the nematode within a host. Following are the scientific and common names of nematodes commonly associated with alfalfa in California: *Ditylenchus dipsaci* (stem and bulb), *Meloidogyne hapla* (northern root knot), *M. javanica* (Javanese root knot), *M. incognita* (southern root knot), *Meloidogyne arenaria* (peanut root knot), *M. chitwoodi* (Columbia root knot), *Pratylenchus penetrans* (lesion), *Xiphinema americanum* (dagger), *Longidorus africanus* (needle), *Criconemella curvatus* (ring), *Merlinius brevidens* and *Tylenchorhynchus* sp. (stunt), *Helicotylenchus* sp. (spiral), and *Trichodorus* sp. and *Paratrichodorus* sp. (stubby root). It is not uncommon for a single field to have several different genera of nematodes present. Root knot, lesion, stunt, and stubby root refer to plant damage characteristics. Stem and bulb refers to the locations in which these nematodes are found within plants. The cuticle of ring nematodes has prominent striations or rings. The stylets of dagger and needle nematodes are very long and prominent. When viewed through a microscope, spiral nematodes are often observed to be coiled.

The nematodes commonly found in alfalfa fields exhibit several different life history patterns. Stubby root, dagger, needle, ring, spiral, and stunt nematodes are ectoparasites. These nematodes use stylets to feed on roots, however, all stages of their life cycle are passed outside of roots in the soil. Stem and bulb and lesion are migratory endoparasites. Life cycle stages may be found within the plant (stems, leaves and crown for stem and bulb; roots for lesion) as well as soil. Root knot nematodes are sedentary endoparasites. The second stage juvenile enters a root, takes up a permanent feeding site and then develops to an immobile adult female within the root. The root cells around her head enlarge to form a gall or knot.

A knowledge of these life history patterns can be helpful when making management decisions. For example, some nematicides, parasites, or soil amendments can be expected to be active only in the soil and so would be more effective against ectoparasites than endoparasites. Other nematicidal agents might move systemically through a root or parasites might be able to penetrate roots and actively seek out nematodes and would then be effective against endoparasites.

Among all these nematodes, only a few are consistently associated with damage to alfalfa in California. The most important and those which will be discussed in the most detail are stem and bulb, and root knot. However, if any of the other nematodes mentioned above are present in a field in high numbers at the time of planting, they could cause stunting of seedling alfalfa.

More than 300 plant species are hosts for the stem and bulb nematode (*Ditylenchus dipsaci*). This nematode was first identified in Germany in 1881, and first found in the United States in 1923. Historically, it is thought to have been spread by debris in seed. This nematode is known to have at least 20 races which can only be distinguished by their different host range. When viewed under a microscope, these different races appear to be morphologically identical. Optimum temperatures for invasion and reproduction of this nematode in alfalfa are 59 to 68 F (15 to 20 C). Reproduction can occur from 41 to 86 F (5 to 30 C). A life cycle can be completed in 19 to 23 days in susceptible cultivars. In this species, the first larval stage occurs within the egg. All other larval and the adult stage are able to infect developing alfalfa bud tissue.

This nematode is known to be very sensitive to environmental conditions. Damage is most severe during moist cool weather, in cooler sprinkler-irrigated inland valleys, and foggy coastal areas. This nematode typically causes the most problems in the first and second cuttings. Hot dry weather occurring later in the growing season reduces its activity. When environmental conditions are unfavorable for development, the fourth stage larva can serve as a dessication resistant survival stage in which the nematodes metabolism slows to an almost undetectable level. During such conditions, large numbers may survive in the crowns of plants. When cool, moist conditions return, metabolic activity increases and development resumes. Typically only small numbers of this nematode are found in the soil. Under favorable conditions, they will move in a film of water from the soil to crowns where young leaves and shoots are developing, and they have been observed to enter plants through stomata.

As mentioned previously, five species of root knot nematode are associated with alfalfa in California. These species have wide and variable host ranges, different temperature optimums and different degrees of pathogenicity. In addition to causing problems on alfalfa, because of their wide host ranges, they create problems for many susceptible crops that might be grown in rotation. The range of temperatures for development of *M. incognita*, *M. arenaria*, and *M. javanica* is approximately 65 to 85 F (18 to 30 C) while that for *M. hapla* and *M. chitwoodi* is approximately 41 to 77 F

(5 to 25 C). Under these conditions, root knot nematodes can complete a generation in 4 to 6 weeks. The pathogenic situation for *M. chitwoodi* is further complicated by its having at least two morphologically indistinguishable races: only one of which is known to reproduce on alfalfa. The alfalfa race is not known to be widely distributed in California at the present time.

SYMPTOMS AND SIGNS OF NEMATODE DAMAGE

There are no distinctive signs of nematode damage to alfalfa that could not also be attributed to another problem. Stems of alfalfa plants infected with stem and bulb nematode will appear shortened, stunted and chlorotic. The nodes swell, and the internodes are short. Symptom development is associated with a release of digestive enzymes by nematodes, and a physiological imbalance of growth hormones produced by the plant.

Roots infested with root knot nematode may exhibit knots or galls and may branch excessively. Compared to galling on many other plants, those on alfalfa are typically much smaller. Root knot galls can be distinguished from nitrogen-fixing bacteria nodules by rubbing the roots between fingers. Galls, unlike nodules, are not easily dislodged. Above ground, plants may appear stunted, exhibit slower growth than expected, or have an unexplained dieback and chlorosis.

HOW NEMATODES INJURE PLANTS

Nematodes do not typically kill plants. They are plant stressors and act in conjunction with other stress factors in fields to reduce growth and yields. Penetration and movement by nematodes through plant tissues results in mechanical injury to cells and subsequent cell death and necrosis. Mechanical injury interrupts the uptake and flow of water and nutrients from roots and the flow of food from leaves to roots. In addition, nematodes create openings in roots through which other microorganisms can enter. For example, *M. incognita* can increase the severity of *Fusarium* wilt in alfalfa. *M. hapla* can increase the incidence of bacterial wilt in both resistant and susceptible alfalfa cultivars. All of the above increase the susceptibility of plants to environmental stress.

Because of the complex interactions that occur in an alfalfa field, it is difficult to say that problems observed are specifically caused by nematodes that may be present. A number of experiments have been conducted with various nematodes on alfalfa in either greenhouse or microplots where conditions can be more easily controlled. In these tests, the following nematodes have been shown to cause significant growth reductions: *D. dipsaci*, *M. hapla*, *M. incognita*, *Trichodorus* sp., *Tylenchorhynchus clarus*, and *Criconemella curvatus*.

SAMPLING FOR NEMATODES

Because there are no distinctive nematode diagnostic symptoms or signs, soil, root and plant tissue samples should be taken and sent to a diagnostic laboratory when vigor seems limited without an apparent cause. To begin the procedure, visually divide the field into sampling areas that represent differences in soil texture, drainage patterns, or cropping history. Take a separate sample from each area so that each can be managed separately. Because nematodes are usually not uniformly distributed within a field, it is necessary to take a series of subsamples from throughout the area to more accurately determine if nematodes are present. In a fallow field, samples should be collected randomly from the sample area. In an established field, collect separate subsamples from areas that show symptoms and from adjacent healthy areas. It is usually better to sample at the edge rather than the middle of an unhealthy area. This is because roots in the center of the area may be too decayed to support a good nematode population compared to more healthy areas around the edge. Samples should include feeder roots when possible, and be taken when soil is moist. Because nematodes feed on roots, they are more prevalent in the rooting zone of the current or previous crop and this is the area from which subsamples should be taken. Mix the subsamples together and place one quart of the mixed soil and roots into a plastic bag. Seal the bag, place a label on the outside of the bag, keep samples cool (do not freeze), and transport as soon as possible to a diagnostic laboratory. Be certain that the laboratory knows that alfalfa is the current or planned crop for the field so that they will use appropriate extraction techniques. Your local Farm Advisor can help you locate a diagnostic laboratory.

During recent years, increasing emphasis has been placed on the development and use of damage thresholds for making management decisions for pests. For many reasons, it is difficult to establish damage thresholds for nematodes. These include difficulties in obtaining representative samples and variability in extraction methods and efficiencies of different laboratories as well as the many biotic and abiotic factors that influence populations. However, sampling periodically to determine if plant parasitic nematodes are present, can be very helpful in establishing the need for, and the success of a nematode management program. If the nematodes discussed above as pathogens are present in a field with below normal growth and yield, and no other explanation of the problem can be found, it is likely they are contributing to the problem. In order to determine if the nematode population is increasing or is remaining stable, a grower should sample an infested field at least once a year and at the same time each year.

MANAGEMENT TECHNIQUES

Selection of Planting Site - Whenever possible, alfalfa should be planted in an area that is not infested with nematodes known to be pathogenic.

Certified Seed - should be used to minimize the chance of contaminating previously uninfested land. Until the importance of seed as a source of transmission of stem and bulb nematode was understood, as many as 17,000 nematodes were found per pound.

Clean Equipment - To minimize transfer of plant parasitic nematodes, water should be used to remove soil and plant debris from farm equipment prior to moving equipment between fields.

Irrigation Water - Although growers might not have a choice of sources for irrigation water, they should be aware that surface irrigation water has been shown to be a potential source of nematode contamination. Whether or not there is a serious potential for contaminating alfalfa fields from irrigation water has not been determined. However, it is clear that one should not use runoff water from a field known to be infested with nematodes to irrigate an uncontaminated field. If a serious contamination problem is suspected, settling ponds can be used to remove inoculum.

Weed Management - Burning weeds within infested fields in the fall has been shown to decrease problems from stem and bulb nematodes the following spring. Spring burning of weeds seems to make the problem worse.

Irrigation Management - Delaying cutting until the top two to three inches of soil is dry will minimize spread and reinfestation of stem and bulb nematode.

Choice of Variety - A number of factors in addition to nematodes should be considered when chosing a variety. From the standpoint of nematode management alone, varieties are available that are resistant to either stem and bulb nematode, to *M. hapla* or *M. incognita* but not to multiple pest species. This emphasizes the need to determine which nematodes are present in a field prior to choosing a variety. When discussing the merits of different plant varieties, it is important to understand the terminology used by nematologists. Immune varieties do not allow nematode feeding. Resistant or nonhost varieties may be invaded by nematodes and may show damage, but chemical or physical unsuitability of the plant will prevent or greatly limit nematode reproduction. Susceptible or host varieties allow normal nematode reproduction and may or may not tolerate nematode attack. Among susceptible varieties, tolerant hosts are able to withstand nematode feeding and penetration. Intolerant susceptible hosts will be damaged by nematodes. The degree of tolerance can be greatly influenced by environmental conditions. Your local Farm Advisor is a good source of information on which varieties are best adapted to your area.

The use of nematode resistant varieties is the most practical method of control. However, resistant varieties should not be planted in fields with high nematode populations. The field should be rotated to nonhost crops for at least two years.

Although nematodes are not able to reproduce in a resistant variety, they are able to infest resistant varieties, and can cause a hypersensitive response which can result in death of seedlings.

Resistance to the stem and bulb nematode was first found in a Turkistan alfalfa selection. From this selection, the cultivar Nemastan was selected which is also resistant to bacterial wilt. Lahontan (which also has resistance to spotted alpha aphid) was later selected from Nemastan. Resistance has also been found in the Turkish alfalfa introductionm Kayseri. Deseret, a selection from Kayseri, has resistance to downy mildew as well as to stem nematode. Additional cultivars reported to contain some degree of stem nematode resistance are: Washoe, Resistador, Caliverde 65, and Appalachee.

Resistance to *M. hapla* was first recorded from Vernal alfalfa and is present in Nevada Synthetic XX. Resistance has been found to *M. incognita* in nondormant African and Chilean, Sirsa, Mesa Sirsa, Moapa, Sonoran, and Synthetic YY cultivars.

Your local Farm Advisor is a good source of information on the most recent nematode resistant selections that may be adapted to your growing area.

Crop Rotation - California crops which have worked well in rotations for the race of stem and bulb nematode typically found on alfalfa include grain, beans, cotton, corn, sorghum, lettuce, carrots, and tomatoes. A three to four year rotation is usually recommended. Care should be taken to destroy all volunteer alfalfa from previous crops. Otherwise, they will serve as a source of reinfestation after the rotation is completed.

Because of the wide host ranges of the species of root knot nematode found in alfalfa in California, crop rotation is not usually a feasible method of managing this nematode.

Fallow - The length of time that many nematodes can survive in weed free fallow soil is not know. For the stem and bulb nematode, survival in the absence of hosts depends on environmental conditions. It can be as short as two years in rotation to grain in areas of high rainfall or irrigation or as long as 20 years in the dormant stage in fallow soil in areas with little rain.

Root knot nematode populations are likely to decrease by 80 to 90 percent within a years time. Until host roots or crowns and stems from a previous crop have rotted, they could continue to support nematodes.

Biological Control - Many soils contain predators and parasites of nematodes which may result in some level of natural biological control. There are no registered microbial nematicides.

Amendments - The addition of amendments to soil such as green manure, chitin, sesame chaff, animal manure, humic acid, organic fertilizer, compost and/or proprietary mixtures of beneficial microbials is generally proclaimed to be beneficial to plant growth. With respect to nematode management, such benefits may include: (1) stimulation of the growth of nematophagous fungi that may be present; (2) improvements in soil structure, in water retention, and in plant nutrition which would reduce stress on nematode infested plants; and (3) production of nematicidal breakdown products. Because of the complex nature of the interactions that may occur, interpretation of results following addition of soil amendments is difficult. Sufficient data is not available to predict with any certainity the nematode mortality that might be obtained with these materials. In some cases, the addition of amendments has resulted in phytotoxicity on some crops. Also, it is possible that nematode populations could increase following use of an amendment. If the amendment results in reduced stress on the crop, and the development of a healthier root system; this root system could support a larger nematode population. Leaving untreated areas for comparison to amended areas is a good method for judging the success or failure of soil amendments. Evaluation should include both nematode samples and plant yield.

Chemical Nematicides - Four chemicals are currently registered for preplant use on alfalfa in California: (1) 1,3-Dichloropropene (Telone II, 1,3-D), (2) chloropicrin, (3) metam-sodium (Vapam, Metam, Soil Prep, Sectagon etc.), and (4) methyl bromide. There are no nematicides registered for postplant use on alfalfa in California. The California Department of Food and Agriculture has suspended the issuance of restricted materials use permits for 1,3-D because they feel excessive amounts were being found in the air. Of the remaining materials, methyl bromide has shown the most consistent performance in nematode management. For each soil type, there is a range of soil temperature and moisture within which optimum performance will be achieved with this material. The Phytonematology Study Guide (UC ANR Publication 4045) contains an explanation of these phenomena, and a chart indicating the amounts of fumigant needed for various soil types and the ranges of temperatures and soil moistures over which soils can be successfully fumigated.

Nematicides will not erradicate nematodes from soil. Properly conducted applications will allow time for healthy root system development before nematode populations increase to damaging levels. Label usage recommendations should be followed precisely in all respects. Planting too soon after application can result in phytotoxicity. Mycorrhizal fungi, which are symbionts and essential to growth of many crops, can be killed by fumigation. The subsequent crop may not do well until these organisms are restored. Soil preparation is extremely important for successful use of any of the registered nematicides. Most nematicide failures result from the chemical not reaching the nematode in sufficient concentraton. For preplant applications, this is usually due to improper land preparation or applications outside of acceptable ranges of soil temperature and moisture.

CONCLUSION

Growers should consider using a combination of the management techniques that have been discussed. For example, a management program for stem and bulb nematode might involve the use of crop rotation, certified seed, the use of a nematode resistant variety, fall burning of weeds, and washing equipment before and after use in each field. Yearly soil sampling can help growers interpret stress symptoms in a crop. A proper nutrition and irrigation program can help to reduce stress caused by nematodes. The use of soil amendments in portions of fields which can be compared to unamended areas might provide growers with additional management tools for their particular growing conditions. The best results can be expected when alfalfa is not suffering from other biotic and abiotic problems in addition to nematodes. Local Farm Advisors can be a valuable resource on how to best integrate nematode management into a total crop management program.