NEMATODE MANAGEMENT IN WALNUT ORCHARDS - 1999

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ABSTRACT

Several management strategies are being examined for lesion (Pratylenchus vulnus) and ring (Cironemella xenoplax) nematodes. The effectiveness of the “biological” nematicide DiTera (a toxin produced by a fungus) is being evaluated in three trials in two commercial walnut orchards. An experimental nematicide from Zeneca E3274 is also being evaluated in two of the trials and Enzone (sodium tetrathiocarbonate) is being evaluated in one trial. Monthly sampling over a period of three years is in progress to develop a picture of nematode population fluctuations. This picture should demonstrate the optimum times of the year to sample in order to detect the presence of nematodes, provide data for comparing samples taken at different times of the year, and provide the background information needed to initiate trials to determine if there is an optimum time of the year to apply post-plant treatments to walnut orchards. The predicted loss of methyl bromide will seriously affect the ability of nurseries to produce nematode free planting stock. Although research is in progress to develop alternative soil treatments, it is unlikely that any will be as reliable as methyl bromide in producing nematode free planting stock. If alternative soil treatments fail, it would be useful to have recommendations in place for cleaning up planting stock prior to planting. Hot water treatments of walnut rootstocks and trials to determine lethal times and temperatures for plant-parasitic nematodes which parasitize walnuts are in progress.

OBJECTIVES

1. Evaluate the effectiveness of Nematicur and DiTera for management of lesion and ring nematode in a commercial walnut orchard.

2. Optimize sampling strategies and treatment timing for lesion and ring nematode on walnuts.

3. Develop hot water treatments for preplant management of nematodes on paradox and English rootstocks.

4. Evaluate the effectiveness of DiTera and Enzone for management of lesion nematode in a commercial walnut orchard.

5. Evaluate the effectiveness of a new Zeneca nematicide and DiTera for management of lesion nematode in two commercial walnut orchards.
PROCEDURES

1. Evaluate the effectiveness of Nemacur and DiTera for management of lesion and ring nematode in a commercial walnut orchard.

A trial consisting of 4 replicates of 5 treatments in a randomized complete block design has been completed in a walnut orchard infested with lesion and ring nematode in San Joaquin County. Each replicate consisted of a single row of 11 trees. Baseline harvest data consisting of total yield per replicate and a 2,000 gram subsample graded by Diamond Walnut (large sound, baby walnut, edible yield, reflected light, % offgrade and predominant reason, % insect damage, % external damage and predominant type, % internal damage and predominant type) was obtained in October of 1996. Baseline nematode samples from each replicate were also taken in September 1996. Each sample consisted of 5 subsamples per replicate taken to a 2 foot depth. Additional nematode samples were taken in the Spring and Fall of 1997 and 1998. 1997 and 1998 harvest data were obtained. Nematodes were extracted from soil via elutriation-sugar centrifugation and from roots via mist chamber extraction. In elutriation-sugar centrifugation, a measured volume of soil is mixed with a larger volume of water, and poured through a fine mesh sieve which retains the nematodes. Nematodes washed from the sieve are then mixed with a sugar solution in a plastic tube and centrifuged. During this process, nematodes float in the sugar solution while any remaining soil particles are pulled to the bottom by the centrifugation. Nozzles at the top of a mist chamber periodically spray heated water on roots held in a mesh basket nested on top of a funnel. The stem of the funnel resides in a test-tube. Nematodes emerge from the roots and are captured at the bottom of the test-tube while excess water flows over the top of the tube. For both techniques, extracted nematodes are then counted under a microscope. Treatments (DiTera ES at 50 and 100 pounds/acre, DiTera granules at 100 pounds/acre, Nemacur at 1.5 gallons/acre and an untreated control) were applied with commercial equipment in November 1996, and June 1997. In the Fall of 1997, Bayer indicated it no longer planned to pursue a registration for Nemacur on walnuts. Following the 1997 harvest, the plots previously treated with Nemacur were treated with DiTera granules at 50 pounds/acre.

2. Optimize sampling strategies and treatment timing for lesion and ring nematode on walnuts.

In 1996, we proposed to sample a single orchard in Solano County infested with lesion and ring nematode at monthly intervals over a three year period. This objective has been expanded in that we are sampling three Solano County orchards of different ages. On each sampling date, four samples of roots and soil, each composed of four subsamples are being evaluated. Nematodes are extracted from soil via elutriation-sugar centrifugation and from roots in a mist chamber as described in the previous objective. Observed fluctuations will be related to physical (e.g. soil temperature and moisture) and(or) biological environmental factors.
3. Develop hot water treatments for preplant management of nematodes on Paradox and English rootstocks.

In 1997, we proposed to work only on Paradox rootstock. This objective has been expanded to include English rootstock as well. In 1996, both rootstocks were treated for 5 different lengths of time at each of 5 temperatures (110°F, 115°F, 120°F, 125°F, 130°F) with 5 replicates per treatment to establish baseline parameters for thermal tolerances. Treated rootstocks were planted in a 1-acre site at the USDA-ARS research station in Parlier and are being evaluated for survival, vigor (visual rating), and trunk circumference. Roots will be examined for evidence of nematode and fungal infestation. Planting material for use in subsequent years has been planted in a location known to be infested with lesion nematode (P. vulnus) and ring nematode. Laboratory experiments are in progress to determine the thermal tolerance of the ectoparasite C. xenoplax, the sedentary endoparasites Meloidogyne incognita and M. arenaria (root-knot nematode, a pest of English walnut), and to confirm those reported in the literature for P. vulnus. Treatments in 1998 and 1999 were conducted at the same temperatures but over a narrower range of times. Treatments in 2000 will be determined following evaluation of previous treatments.

4. Evaluate the effectiveness of DiTera and Enzone for management of lesion nematode in a commercial walnut orchard.

This was a new objective for 1998. A trial is in progress in a young walnut orchard in Solano County which has a high population of lesion nematode (P. vulnus). The trial consists of five replicates of four treatments in a randomized complete block design. Each replicate consists of 3 trees. Treatments (DiTera ES and granules at 50 pounds/acre, Enzone applied via drip irrigation at 1,000 ppm a.i. for 4 hours [ca 50 gallons product/acre] and an untreated control) were applied in October 1997 after baseline harvest data and pretreatment nematode samples were obtained. Additional treatments and nematode sampling were conducted in the spring and fall of 1998 and 1999. 1998 and 1999 harvest data have been obtained.

5. Evaluate the effectiveness of a new Zeneca nematicide and DiTera for management of lesion nematode in two commercial walnut orchards.

This is a new objective for 1999. Trials with identical treatments have been established in young walnut orchards in Stanislaus and Solano Counties which have high populations of lesion nematode (P. vulnus). These trials consist of five replicates of five treatments in a randomized complete block design. Each replicate consists of 3 trees. Treatments (E3274 liquid from Zeneca applied at 2.5 and 5 pounds ai/acre and DiTera ES and granules at 50 pounds/acre and an untreated control) were applied in November 1998 after baseline harvest data and pretreatment nematode samples were obtained. Additional DiTera treatments were applied and nematode samples were taken in the spring and fall of 1999. 1998 and 1999 harvest data was obtained. At the present time, Zeneca has decided not to pursue development of E3274, so additional spring and fall treatments were not applied. We will continue to evaluate the trial through an additional harvest.
RESULTS AND CONCLUSIONS

Lesion (*Pratylenchus vulnus*) and ring (*Criconemella xenoplax*) nematodes reduce walnut yields through root damage from direct feeding and by placing trees under stress (Lownsbury, 1956; Lownsbury et al., 1978). Lesion nematodes are likely to be found within roots as well as in soil, while ring nematodes are external parasites. At the present time, there are no chemical or cultural management techniques available for reducing nematode damage to walnuts to an economically acceptable level in established orchards. Improving yields and prolonging the life of existing orchards via postplant nematicides should be examined as an alternative to preplant fumigation with chemicals such as methyl bromide.

The “biological” nematicide DiTera (a toxin produced by a fungus) is a promising postplant product. Abbott Laboratories has recently obtained California EPA registration for DiTera for several crops and is interested in obtaining a registration on walnuts. This is a very water soluble product which has potential for penetrating to depths inhabited by walnut roots and which has performed well in a number of trials on annual crops. Research on other perennial crops indicates that two years of treatments are often required before significant yield differences become evident. In the trial initiated in 1996, small but statistically significant yield differences were evident in edible yield, offgrade, large sound, and baby walnut in the 1997 harvest (1997 yield and nematode data were presented in the 1998 report). 1998 yield data indicated significant differences in: 1) total yield for DiTera ES at 100 pounds/acre (Figure 1), 2) percent large sound for Nemacur and DiTera ES at 50 pounds/acre (Figure 2), 3) insect damage for DiTera ES at 100 pounds/acre (Figure 3), and 4) internal damage predominantly due to mold for DiTera granule and ES at 100 pounds/acre (Figure 4). Trends toward increases in edible yield and reflected light were evident but not statistically significant (Figures 5 and 7) as were trends toward decreases in baby walnut, and offgrade due to mold and shivel (Figures 6 and 8).

Research on grapes (Ferris and McKeny, 1974; Feil et al., 1997), prunes (Westerdahl et al., 1995), and peaches (Lownsbury, 1959) in California has demonstrated that nematode populations fluctuate throughout the year. Not knowing when these fluctuations occur in walnut orchards makes it difficult to provide recommendations on the best time of the year to sample to demonstrate the presence of nematodes, to interpret and compare samples taken at various times of the year, and to provide advice on optimum timing of treatments to mature orchards. Monthly sampling of lesion and ring nematodes in walnut orchards over a period of three years should provide a reproducible picture of nematode population fluctuations. This picture should demonstrate the optimum times of the year to sample in order to detect the presence of nematodes, provide data for comparing samples taken at different times of the year, and provide the background information needed to initiate trials to determine if there is an optimum time of the year to apply postplant treatments to walnut orchards. The results of the first two years of sampling are presented for soil (Figure 9) and root (Figure 10) populations of lesion nematode, ring nematode in soil (Figure 11), root biomass in soil samples (Figure 12), and percent soil moisture at the time of sampling (Figure 13). The first two years data indicate that lesion nematode populations within roots are lowest December through March while remaining abundant in soil. This information combined with the finding that feeder root biomass is lowest in March could lead to improved treatment timings for nematicides not expected to penetrate into roots.
Current nursery practices rely on producing clean stock by planting in soil treated with methyl bromide, by planting in soil not previously infested with plant parasitic nematodes, or by planting in soil in which nematode populations have been lowered sufficiently by other means so that populations are not detectable by sampling prior to harvest. The predicted loss of methyl bromide will seriously affect the ability of nurseries in California to produce nematode free planting stock. Although research is in progress to develop alternative soil treatments, it is unlikely that any will be as reliable as methyl bromide in producing nematode free planting stock. If alternative soil treatments fail, it would be useful to have recommendations in place for cleaning up planting stock prior to planting. Hot water treatments of planting stock have been developed for a number of nematode/crop combinations (reviewed by Bridge, 1975), have been used commercially for daffodils and garlic planting stock, and approved by CDFA for treatment of grape rootstocks following methyl bromide failures. Hot water treatments of Paradox and English rootstocks were initiated in 1997 with treatment times and temperatures based on information available in the literature for other crops. A wide range of times was selected with high mortality expected at the longest treatment times. Treatment lengths for 1998 and 1999 were shortened based on the results of the 1997 treatments. The treatment lengths tested for each temperature are indicated by the outlined boxes in Figure 14. In the spring of 1999, trees treated in 1997 and 1998 were evaluated for survival (Figures 15 - 18), trunk diameter based on surviving trees (Figures 19 - 22), trunk diameter based on all trees (Figures 23 - 26) and percent trunk diameter increase (Figures 27 and 28). The general trends illustrated so far are towards better survival and vigor for English versus Paradox and at lower versus higher temperatures. In these trials, we have had relatively poor survival of Paradox versus English rootstocks even among untreated trees. At the lower temperatures tested, there is actually a trend for better survival of hot water treated Paradox than for untreated trees.

In the Solano County trial comparing DiTera and Enzone, initiated in 1997, a significant yield increase (pounds/acre) in 1999 was achieved with DiTera ES (Figure 29). In the trial initiated in 1998 comparing DiTera and E3274, a significant yield increase was achieved with the Zeneca E3274 product at 5 pounds a.i. / acre (Figure 30). In the Stanislaus County trial initiated in 1998 with DiTera and E3274, a yield increase was not observed in 1999, but DiTera ES produced an increase in tree circumference (Figure 31). All of these results are encouraging particularly for the first year trials as research on other perennial crops indicates that two years of treatments are required before significant yield differences become evident.

REFERENCES


**FIGURE 2.**

1998 SAN JOAQUIN COUNTY - LARGE SOUND

Probabilities above bars indicate differences from untreated according to covariate analysis with 1996 as covariate:

- UNTREATED
- NEMACUR
- GRANULE
- DITERA100ES
- DITERA50ES

Error bars indicate 1 standard error.
1998 SAN JOAQUIN COUNTY - INSECT DAMAGE

PROBABILITIES ABOVE BARS INDICATE DIFFERENCES FROM UNTREATED ACCORDING TO COVARIATE ANALYSIS WITH 1996 AS COVARIATE:

ERROR BARS INDICATE 1 STANDARD ERROR

PERCENT

UNTREATED  NEMACUR  GRANULE  DITERA100ES  DITERA50ES

0.0  0.5  1.0  1.5  2.0  2.5  3.0
1998 SAN JOAQUIN COUNTY - INTERNAL DAMAGE (MOLD)  

ERROR BARS INDICATE 1 STANDARD ERROR  

PROBABILITIES ABOVE BARS INDICATE DIFFERENCES FROM UNTREATED ACCORDING TO COVARIATE ANALYSIS WITH 1996 AS COVARIATE:  

- UNTREATED  
- NEMACUR  
- GRANULE  
- DITERA100ES  
- DITERA50ES  

PERCENT
1998 SAN JOAQUIN COUNTY - EDIBLE YIELD

ERROR BARS INDICATE
1 STANDARD ERROR

PROBABILITIES ABOVE BARS
INDICATE DIFFERENCES FROM
UNTREATED ACCORDING TO
COVARIATE ANALYSIS WITH 1996
AS COVARIATE:

PERCENT

UNTREATED  NEMACUR  GRANULE  DITERA100ES  DITERA50ES
1998 SAN JOAQUIN COUNTY - REFLECTED LIGHT

FIGURE 7.

ERROR BARS INDICATE 1 STANDARD ERROR

PERCENT

51.6
51.4
51.2
51.0
50.8
50.6
50.4
50.2
50.0
49.8

UNTREATED  NEMACUR  GRANULE  DITERA100ES  DITERA50ES
1998 SAN JOAQUIN COUNTY - OFFGRADE (SHRIVEL / MOLD)

ERROR BARS INDICATE
1 STANDARD ERROR

PERCENT

UNTREATED  NEMACUR  GRANULE  DITERA100ES  DITERA50ES

FIGURE 8.
FIGURE 9.

LESION NEMATODE
AVERAGE OF TWO YEARS DATA FROM THREE ORCHARDS

TWO POINTS WHICH ARE NOT IDENTIFIED BY THE SAME LETTER ARE SIGNIFICANTLY DIFFERENT FROM EACH OTHER ACCORDING TO FISHER'S PROTECTED LSD TEST AT P = 0.10.
LESION NEMATODE
AVERAGE OF TWO YEARS DATA FROM THREE ORCHARDS

TWO POINTS WHICH ARE NOT IDENTIFIED
BY THE SAME LETTER ARE
SIGNIFICANTLY DIFFERENT FROM EACH
OTHER ACCORDING TO FISHER'S
PROTECTED LSD TEST AT P = 0.05.
RING NEMATODE AVERAGE OF TWO YEARS DATA FROM THREE ORCHARDS

TWO POINTS WHICH ARE NOT IDENTIFIED BY THE SAME LETTER ARE SIGNIFICANTLY DIFFERENT FROM EACH OTHER ACCORDING TO FISHER'S PROTECTED LSD TEST AT P = 0.05.
ROOT BIOMASS IN SAMPLES
AVERAGE OF TWO YEARS DATA
FROM THREE ORCHARDS

TWO POINTS WHICH ARE NOT IDENTIFIED BY THE SAME LETTER ARE SIGNIFICANTLY DIFFERENT FROM EACH OTHER ACCORDING TO FISHER'S PROTECTED LSD TEST AT P = 0.05.
Two points which are not identified by the same letter are significantly different from each other according to Fisher's protected LSD test at $P = 0.05$. 

Figure 13. Percent soil moisture at sampling average of two years data from three orchards.
FIGURE 18.

1998 TREATMENT
1999 PARADOX
PERCENT SURVIVAL
FIGURE 20.
1997 TREATMENT 1999 PARADOX TRUNK DIAMETER (INCHES) BASED ON SURVIVING TREES

ERROR BARS INDICATE 1 STANDARD ERROR
FIGURE 21.
1998 TREATMENT
1999 ENGLISH
TRUNK DIAMETER (INCHES)
BASED ON SURVIVING
TREES

ERROR BARS
INDICATE 1
STANDARD
ERROR

Check
FIGURE 24.

1997 TREATMENT
1999 PARADOX
TRUNK DIAMETER (INCHES)
BASED ON ALL TREES

ERROR BARS
INDICATE 1
STANDARD
ERROR
1998 - 1999
SOLANO COUNTY TRIAL 1 -
YIELD

ERROR BARS INDICATE
1 STANDARD ERROR

PROBABILITIES ABOVE BARS
INDICATE DIFFERENCES FROM
UNTREATED ACCORDING TO SPLIT
PLOT ANALYSIS WITH TIME AS THE
SPLIT PLOT

FIGURE 29.

POUNDS / ACRE

0.03
1998 - 1999
SOLANO COUNTY TRIAL 2 - YIELD

FIGURE 30.

0.05

PROBABILITIES ABOVE BARS INDICATE DIFFERENCES FROM UNTREATED ACCORDING TO SPLIT PLOT ANALYSIS WITH TIME AS THE SPLIT PLOT

ERROR BARS INDICATE 1 STANDARD ERROR

POUNDS/ACRE

<table>
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<th>98, DITERAG50</th>
<th>98, ZENECAS2.5</th>
<th>99, ZENECAS</th>
<th>99, CHECK</th>
<th>99, DITERAES50</th>
<th>99, DITERAG50</th>
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1998 - 1999
STANISLAUS COUNTY TRIAL 1 -
TRUNK CIRCUMFERENCE

ERROR BARS INDICATE
1 STANDARD ERROR

CIRCUMFERENCE (INCHES)

CHECK  ZENECA2.5  ZENECA5  DITERAES50  DITERAG50

PROBABILITIES ABOVE BARS
INDICATE DIFFERENCES FROM
UNTREATED ACCORDING TO SPLIT
PLOT ANALYSIS WITH TIME AS THE
SPLIT PLOT

FIGURE 31.