NEMATODE MANAGEMENT IN WALNUT ORCHARDS - 2000

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ABSTRACT

Several management strategies are being examined for lesion (Pratylenchus vulnus) and ring (Criconemella 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. 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 and DiTeraG. In 2000, this yield increase was only apparent for DiTera ES. All of the treatments reduced nematode populations in soil on at least one sampling date. When data for six sampling dates was combined, a reduction in soil nematode populations was evident for all treatments. 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 in both 1999 and 2000. The means of the three post-treatment samples demonstrated a reduction in soil nematode populations for both E3274 treatments. In the Stanislaus County trial initiated in 1998 with DiTera and E3274, a yield increase was observed only for E3274 at 2.5 pounds a.i./acre. In 1999, DiTera ES produced an increase in tree circumference. When data for 1999 and 2000 are combined, both DiTera ES and E3274 at 5.0 pounds a.i./acre increased tree circumference. All of the treatments except DiTera ES reduced nematode populations in soil on at least one sampling date. The mean of three post-treatment samples demonstrated a significant reduction in soil nematode populations for E3274 at 5.0 pounds a.i./acre.

The stress nematodes place on walnut trees could possibly be evaluated utilizing a pressure chamber (or pressure bomb) which has been developed to monitor water stress in perennial crops via measurement of midday stem water potential (in Bars) and trials are in progress to evaluate this method which could greatly facilitate evaluation of new methods for nematode management in walnuts at greatly reduced costs.

Three years of sampling to develop a picture of nematode population fluctuations has been completed. The 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.

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

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and trials to determine lethal times and temperatures for plant-parasitic nematodes that parasitize walnuts are in progress.

**OBJECTIVES**

1. Determine if pressure chamber measurements can provide indications of effective treatments for nematode management.

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. Determine if pressure bomb measurements can provide indications of effective treatments for nematode management.

The stress nematodes place on walnut trees could possibly be evaluated utilizing a pressure chamber (or pressure bomb) which has been developed to monitor water stress in perennial crops via measurement of midday stem water potential (in Bars). The technique consists of placing bags over individual leaves on trees for at least 2 hours to allow equilibration and then reading the pressure in the leaves with a portable unit between 1 and 3pm. Replicated measurements were taken weekly (grower practices permitting) from mid-June to mid-September in the nematicide trials being conducted in objectives 4 and 5 of this proposal to determine if treatment differences can be evaluated by this method.

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 was later expanded to sample three Solano County orchards of different ages. On each sampling date, four samples of roots and soil, each composed of four subsamples, were evaluated. Nematodes were extracted from soil via elutriation-sugar centrifugation and from roots in a mist chamber. Sampling has been completed and summary graphs are presented.
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 (110F, 115F, 120F, 125F, 130F) 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, 1999 and 2000 were conducted at the same temperatures but over a narrower range of times. Treatments in 2001, which will be the last year for treating non-infested rootstocks, will be determined following evaluation of previous treatments. Since all treatments are evaluated for 2 seasons following planting, evaluations will continue through 2003.

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

This objective was initiated in 1998. A trial is in progress in a young walnut orchard in Solano County that 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, 1999, and 2000. 1998, 1999 and 2000 harvest data have been 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.
5. Evaluate the effectiveness of a new Zeneca nematicide and DiTera for management of lesion nematode in two commercial walnut orchards.

This objective was initiated in 1999. Trials with identical treatments have been established in young walnut orchards in Stanislaus and Solano Counties that 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 a.i./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 and 2000. 1998, 1999 and 2000 harvest data have been obtained. At the present time, Zeneca has decided not to pursue development of E3274, so additional spring and fall treatments were not applied.

RESULTS AND DISCUSSION

Lesion (Pratylenchus vulnus) and ring (Criconemella xenoplax) nematodes reduce walnut yields through root damage from direct feeding and by placing trees under stress (Lownsbey, 1956; Lownsbey 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.

Evaluating the effectiveness of chemical and nonchemical products for nematode control on walnuts is time consuming and expensive. Chemical control trials for example require a minimum of two years (3 harvests including initial baseline data) before yield increases can be expected. Expenses include labor for applications, nematode sampling and processing, harvesting and grading samples and crop destruct costs for a large number of trees. Nematodes are plant stressors affecting the ability of water and nutrients to move through roots. During the past year we have been evaluating if pressure chamber measurements correlate with yields and/or nematode population changes. This method could greatly facilitate evaluation of new methods for nematode management in walnuts at greatly reduced costs. If the expected correlation is obtained, future nematode management trials would involve measurements taken on just a few days per season on a small number of treated and untreated trees. Such trials could rapidly provide useful evidence of the effectiveness of a large number of products at a number of rates. With the current trial logistics, we are typically limited to four or five treatments per trial. The pressure chamber technique would enable the screening of a large number of treatments, the best of which could then be more effectively tested in a more traditional trial setting. Statistical analysis of this data is in progress.

Research on grapes (Ferris and McKenry, 1974; Feil et al., 1997), prunes (Westerdahl et al., 1995), and peaches (Lownsbey, 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 three years of sampling are presented for soil (Figure 1) and root (Figure 2) populations of lesion nematode, ring nematode in soil (Figure 3), root biomass in soil samples (Figure 4), and percent soil moisture at the time of sampling (Figure 5). The 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, 1999, and 2000 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 6. In the spring of 2000, trees treated in 1998 and 1999 were evaluated for survival (Figures 7 - 10), trunk diameter based on surviving trees (Figures 11 - 14), trunk diameter based on all trees (Figures 15 - 18) and trunk diameter increase (Figures 19 - 22). 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.

The “biological” nematicide DiTera (a toxin produced by a fungus) is a promising postplant product. Abbott Laboratories has obtained a California EPA registration for DiTera 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 Solano County trial comparing DiTera and Enzone, initiated in 1997, a significant yield increase (pounds/acre) in 1999 was achieved with DiTera ES and DiTeraG. In 2000, this yield increase was only apparent for DiTera ES (Figure 23). All of the treatments reduced nematode populations in soil on at least one sampling date (Figure 24). When data for six sampling dates was combined, a reduction in soil nematode populations was evident for all treatments (Figure 25). Nematode populations within roots were not reduced by any of the treatments which is not surprising as none of the products tested are known to be taken up into roots (Figure 26).

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 in both 1999 (data presented in 1999 report) and 2000 (Figure 27). Analysis of nematode populations in soil demonstrated an increase in three treatments (DiTera ES and both E3274 treatments) compared to the check on one sampling date and a decrease in two treatments (E3274) on another (Figure 28). The means of the three post-treatment samples demonstrated a reduction in soil nematode populations for both E3274 treatments (Figure 29).

In the Stanislaus County trial initiated in 1998 with DiTera and E3274, a yield increase was observed only for E3274 at 2.5 pounds a.i./acre (Figure 30). In 1999, DiTera ES produced an increase in tree circumference (data presented in 1999 report). When data for 1999 and 2000 are combined, both DiTera ES and E3274 at 5.0 pounds a.i./acre increased tree circumference (Figure 31). All of the treatments except DiTera ES reduced nematode populations in soil on at least one sampling date (Figure 32). The mean of three post-treatment samples demonstrated a significant reduction in soil nematode populations for E3274 at 5.0 pounds a.i./acre (Figure 33). As in the previous trial, no reductions in nematode populations within roots were evident. (Figure 34). All of these results are encouraging particularly because of the large amount of variability typical of small plot trials.

REFERENCES


FIGURE 1.

Lesion Nematode average of three 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 $p = 0.05$. 

The number of lesion nematode per liter of soil is shown from January to December.
LESION NEMATODE
AVERAGE OF THREE 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.

NUMBER OF LESION NEMATODE PER GRAM OF ROOT

JAN  FEB  MAR  APR  MAY  JUN  JUL  AUG  SEP  OCT  NOV  DEC

0  200  400  600  800  1000  1200  1400  1600
**FIGURE 3.**

RING NEMATODE
AVERAGE OF THREE 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 THREE 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 $P = 0.05$. 
SOIL MOISTURE
AVERAGE OF THREE YEARS DATA
FROM THREE ORCHARDS

FIGURE 5.
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.

PERCENT SOIL MOISTURE AT TIME OF SAMPLING

JAN  FEB  MAR  APR  MAY  JUN  JUL  AUG  SEP  OCT  NOV  DEC

6  7  8  9  10  11  12  13  14  15  16  17  18  19  20
FIGURE 6.

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FIGURE 9.
1999 TREATMENT
2000 ENGLISH
PERCENT SURVIVAL
FIGURE 12.

1998 TREATMENT
2000 PARADOX
TRUNK DIAMETER (INCHES)
BASED ON SURVIVING
TREES

ERROR BARS
INDICATE 1
STANDARD
ERROR
FIGURE 13.

1999 TREATMENT
2000 ENGLISH
TRUNK DIAMETER
(INCHES) BASED ON
SURVIVING TREES

ERROR BARS
INDICATE 1
STANDARD
ERROR
FIGURE 14.

1999 TREATMENT
2000 PARADOX
TRUNK DIAMETER
(INCHES) BASED ON
SURVIVING TREES

ERROR BARS
INDICATE 1
STANDARD
ERROR
FIGURE 16.
1998 TREATMENT 2000 PARADOX TRUNK DIAMETER (INCHES) BASED ON ALL TREES

ERROR BARS INDICATE 1 STANDARD ERROR
FIGURE 17.

1999 TREATMENT
2000 ENGLISH
TRUNK DIAMETER (INCHES)
BASED ON ALL TREES

ERROR BARS
INDICATE 1
STANDARD
ERROR
FIGURE 19.

1998 TREATMENT 2000 ENGLISH TRUNK DIAMETER INCREASE (INCHES)

ERROR BARS INDICATE 1 STANDARD ERROR
FIGURE 20.
1998 TREATMENT
2000 PARADOX
TRUNK DIAMETER
INCREASE (INCHES)

ERROR BARS
INDICATE 1
STANDARD
ERROR
SOLANO COUNTY WALNUT TRIAL 1

FIGURE 24.

PROBABILITIES INDICATE DIFFERENCES FROM CHECK ACCORDING TO COVARIANCE ANALYSIS WITH TIME 0 AS THE COVARIATE.

- CHECK
- DITERA ES
- DITERA GR
- ENZONE

LESION NEMATODE / LITER OF SOIL

MONTHS AFTER FIRST TREATMENT

0 5 10 15 20 25 30 35

MAY MAY JUN OCT OCT MAR OCT

0 0.001 0.02 0.08 0.08 0.1
SOLANO COUNTY WALNUT TRIAL 1 - MEAN OF SIX SAMPLE DATES 1998 - 2000

FIGURE 25.

PROBABILITIES INDICATE DIFFERENCES FROM CHECK ACCORDING TO COVARIANCE ANALYSIS WITH TIME 0 AS THE COVARIATE.

ERROR BARS INDICATE 1 STANDARD ERROR

- CHECK
- DITERA ES
- DITERA G
- ENZONE
**Figure 27.**

**Solano County Trial 2 - Yield**

- **Probabilities above bars** indicate differences from untreated according to split plot analysis with time as the split plot.
- **Error bars** indicate 1 standard error.

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FIGURE 28.

PROBABILITIES INDICATE DIFFERENCES FROM CHECK ACCORDING TO COVARIANCE ANALYSIS WITH TIME 0 AS THE COVARIATE.
FIGURE 29.

SOLANO COUNTY WALNUT TRIAL 2
MEAN OF 3 SAMPLE DATES 1999 - 2000

PROBABILITIES INDICATE DIFFERENCES
FROM CHECK ACCORDING TO
COVARIANCE ANALYSIS WITH TIME 0 AS
THE COVARIATE.

ERROR BARS INDICATE
1 STANDARD ERROR

LOG (X+1) LESION NEMATODE / LITER OF SOIL

CHECK  DITERA ES  DITERA G  E3274-2.5  E3274-5.0

0.0  0.5  1.0  1.5  2.0  2.5  3.0  3.5  4.0
FIGURE 30.

STANISLAUS COUNTY TRIAL -
MEAN OF TWO SAMPLE DATES 1999 - 2000

ERROR BARS INDICATE 1 STANDARD ERROR

PROBABILITIES
INDICATE DIFFERENCES FROM CHECK ACCORDING TO COVARIANCE ANALYSIS WITH TIME 0 AS THE COVARIATE.
Figure 31.

Stanislaus County Trial - Mean of Two Sample Dates 1999 - 2000

Probabilities indicate differences from check according to covariance analysis with time 0 as the covariate.

Error bars indicate 1 standard error.
FIGURE 33.

STANISLAUS COUNTY TRIAL - MEAN OF THREE SAMPLE DATES 1999 - 2000

PROBABILITIES INDICATE DIFFERENCES FROM CHECK ACCORDING TO COVARIANCE ANALYSIS WITH TIME 0 AS THE COVARIATE.

ERROR BARS INDICATE 1 STANDARD ERROR
FIGURE 34.

STANISLAUS COUNTY TRIAL

PROBABILITIES INDICATE DIFFERENCES FROM CHECK ACCORDING TO COVARIANCE ANALYSIS WITH TIME 0 AS THE COVARIATE.