

Screening for Root Knot Nematode (*Meloidogyne hapla*) Using Lettuce

Laura Appleman

Faculty Sponsor: Deborah Hanmer, Department of Biology

ABSTRACT

Typically tomatoes have been used as an indicator plant to evaluate nematode galling, however it may take three months to receive results. In this study lettuce was assessed for its potential as an accurate and efficient indicator of *Meloidogyne* root galling. The number of days following germination required for lettuce to exhibit 70-80% galling acted as a measurement of its efficiency as an indicator. Galling increased dramatically after 42 days, with 70-80% galling being reached on the 45th day. These results confirm that lettuce can function as a rapid indicator of nematode galling and will shorten the screening time from 90 days to 45 days.

INTRODUCTION

Plant parasitic nematodes cause an estimated 14 % loss in economically important vegetable and fruit crops each year. However root knot nematodes in the genus *Meloidogyne* cause a striking 5% loss on their own (Agrios, 1997). In the northern United States *Meloidogyne hapla* is the most economically important species of root knot nematode because it is capable of surviving freezing temperatures (Jenkins, 1967).

Meloidogyne is most commonly controlled using resistant varieties and chemical application. Resistant varieties restrict entry or inhibit reproduction of nematodes (Jenkins, 1960). *Meloidogyne*, however, has a broad host range and fields often contain two or more species of parasitic nematodes. The two together make it difficult to prepare resistant varieties capable of withstanding multiple assailants.

Chemical control is more widely used than resistant varieties due to its non-selective nature in controlling nematodes. Unfortunately many nematicides are being phased out because of their toxic nature and ability to leach into groundwater. Also nonvolatile nematicides may have extensive residual qualities that restrict their application because they may be toxic to numerous mammals, as well as humans (Cremlyn, 1978).

As nematicides continue to be pulled from the market it is becoming increasingly important to develop an inexpensive, nonselective method of control. In recent years research has focused on biocontrol methods utilizing both bacteria and fungi that parasitize or trap nematodes. Manaku (1975) reports that a natural soil bacterium, *Bacillus penetrans* was able to effectively control root knot nematodes. Unfortunately *B. penetrans* cannot be cultured on any bacteriological media, and is therefore not a realistic candidate for a biocontrol agent.

Nematode trapping fungi have gained attention in recent years as possible biocontrol agents. These fungi normally trap nematodes as a nitrogen source using several different methods such as, adhesive traps, sticky conidia, or constricting rings (Jaffee, 2001). To this point, research has focused on deuteromycetes that naturally inhabit the soil such as *Arthrobotrys* and *Monacrosporium*. Although these fungi are naturally found in the soil it is difficult to establish them in large enough quantities to provide any control against root knot nematodes.

To this point tomatoes have been used to as indicator plants to assess the amount of galling produced by nematodes following treatment with various biocontrol agents. However, it can take up to three months in order to obtain any results. The objective of this study is to determine if lettuce can be used as a rapid indicator of the effects of *Meloidogyne* galling. The ability to obtain rapid results with lettuce would allow for the effectiveness of more fungal agents to be analyzed.

MATERIALS AND METHODS

Nematode cultures

The plant parasitic nematode used in this study was *Meloidogyne hapla*. *M. hapla* is an obligate parasite and cannot complete its life cycle without feeding on living plant roots. Tomato plants were used to maintain the *M. hapla* cultures. The tomato plants were grown in a study area consisting of 12 hydroponic ebb trays. A sodium

halide lamp provided light 12 hours a day and was regulated by a timer. The pots were watered as needed from below for two weeks following inoculation to provide adequate time for the nematodes to infest the root systems. Watering was achieved by filling the trays with approximately four inches of 80-degree tap water and draining them after three hours. After allowing two weeks for proper infestation the plants could be watered from above without fear of washing the nematodes out of the pots.

M. hapla eggs were harvested from the infested tomato roots approximately three months after inoculation. The roots were thoroughly cleaned and cut into 2-4 cm pieces. The roots were vigorously shaken for three minutes in a 2000mL flask containing a 0.5% NaOCl solution. The roots were poured into a 200-mesh sieve cradled on a 500-mesh sieve. The remaining root tissue and egg solution was extensively rinsed to remove all NaOCl before the eggs were collected from the 500-mesh sieve. A dissecting scope was then used to determine the number of eggs per ml.

Lettuce screening

The lettuce screening was performed to determine if lettuce could be used as a rapid indicator for nematode infestation, and how many days were required for 70-80% of the total root system to be galled. The lettuce was screened using 11; six-inch clay pots containing a 50:50 mixture of Schultz Professional Potting Soil Plus and silica sand. Each pot was inoculated with 500 nematode eggs. Ten lettuce plants were seeded into each pot, but following germination plants were thinned so that only six remained. Two weeks after germination pots were taken down every two to three days and the roots were scored according to Barker (1985) (Table 1).

Table 1. Barker's galling index.

| Percentage of total root system galled | Score |
|--|-------|
| 0 - 10 | 0 |
| 20 - 30 | 1 |
| 40 - 60 | 2 |
| 70 - 80 | 3 |
| 90 - 100 | 4 |

RESULTS

The lettuce plants showed 70-80% galling at 45 days following germination receiving a score of three. However, not all plants exhibited 70-80% galling. Four of the six plants assessed on this day exhibited only 40-60% galling (score of 2). Therefore the plants assessed on the 45th day had an average root score of 2.3 (Figure 1). Galling scores remained near zero after 41 days with a large increase on the 45th day. After peaking at 45 days, galling scores dropped as severe galling resulted in loss of root mass due to root rot. The scores dropped to an average of 1.8 after 48 days.

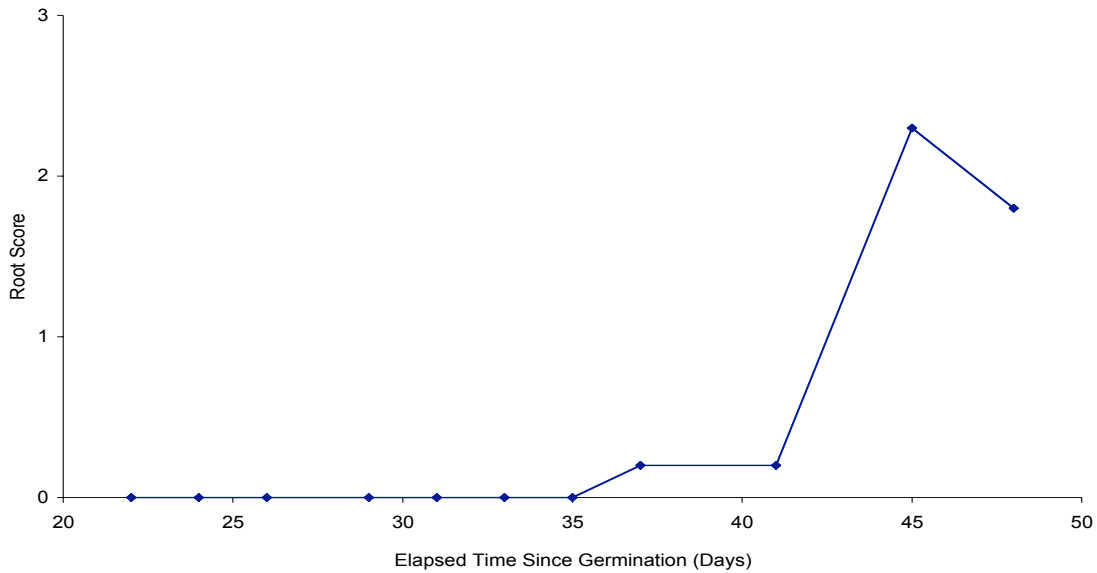


Figure 1. Average lettuce root scores indicating the percentage of galling following germination.

DISCUSSION

As hypothesized lettuce can be used as a rapid indicator of nematode galling, and can therefore be substituted for tomatoes as indicator plants. Using lettuce as an indicator plant will cut screening time from 90 days to 45 days. This will aid in the investigation for an effective biocontrol agent by shortening the screening time and allow for a greater number of potential agents to be evaluated.

ACKNOWLEDGEMENTS

I would like to thank Dr. Volk and Sean Westmoreland with their help in preparing fungal cultures.

LITERATURE CITED

- Agrios, G.N. 1997. Plant Pathology. 4th ed. Academic Press, San Diego. 635 pp.
- Baker, K.R., Carter, C.C. and Sasser, J.N. 1985. An advanced treatise on *Meloidogyne* vol II: methodology. North Carolina State University Graphics.
- Cremllyn, R. 1975. Pesticides. John Wiley & Sons, New York. 240 pp.
- Jaffee, B., D.Pearson, and L.Timm. 2001. Nematode- trapping fungi in conventionally and organic managed corn – tomato rotations. Mycologia. 93: 25-29.
- Jenkins, W.R. and D.P. Taylor. 1967. Plant Nematology. Reinhold Publishing Corporation, New York. 270pp.
- Jenkins, W.R., and J.N.Sasser, ed. 1960. Nematology. University Of North Carolina, Durham. 480 pp.
- Manakau, R. 1980. Biological control of nematode pests by natural enemies. Annual Review of Phytopathology. 81:415-440.