ALGINATE FILMS FOR ASSESSMENT OF PARASITISM OF *MELOIDOGYNE INCOGNITA* EGGS IN SOILS TREATED WITH ORGANIC AMENDMENTS[†]

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ABSTRACT

Chavarría-Carvajal, J. A., and R. Rodríguez-Kábana. 1998. Alginate films for assessment of parasitism of *Meloidogyne incognita* eggs in soils treated with organic amendments. Nematropica 28:41-48.

Alginate films containing *Meloidogyne incognita* eggs were used to evaluate the effect of organic amendments on parasitism of nematode eggs in treated soils. A total of five greenhouse experiments were conducted. In experiments one through four, alginate films impregnated with *M. incognita* eggs were placed into soils amended with 0, 10, 20, 30, 40, and 50 g/kg dried foliage of velvetbean (*Mucuna deeringiana*) and kudzu (*Pueraria lobata*), pine bark or paper waste. In the fifth experiment, the soil was amended with urea-N at rates of 0, 0.15, 0.30, 0.45, 0.60, 0.75, and 0.90 g/kg. To determine the percentage of parasitism of root-knot nematode eggs in each individual substrate, microscopic observations were made at 0, 5, and 10 weeks after treatment. Most rates of velvetbean, kudzu, pine bark, and paper waste increased the percentage of parasitism on *M. incognita* eggs between 5 and 10 weeks after treatment. The percentage of parasitism in soils treated with 0.30 to 0.75 g urea-N/kg soil was significantly improved, but only at 10 weeks after treatment.

Key words: alginate, *Meloidogyne incognita*, *Mucuna deeringiana*, organic amendments, parasitism, *Pueraria lobata*.

RESUMEN

Chavarría-Carvajal, J. A. y R. Rodríguez-Kábana. 1998. Películas de alginato pare la evaluación del parasitismo de huevos de *Meloidogyne incognita* en suelos tratados con enmiendas orgánicas. Nematropica 28:41-48.

Películas de alginato impregnadas con huevos de *Meloidogyne incognita* fueron utilizadas pare evaluar el efecto de enmiendas orgánicas sobre huevos del nematodo nodulador en suelos tratados. Un total de cinco experimentos de invernadero fueron realizados. En los primeros cuatro experimentos, las películas de alginato fueron puestas en suelos tratados con 0, 10, 20, 30, 40 y 50 g/kg de follaje seco de mucuna (*Mucuna deeringiana*) y kudzu (*Pueraria lobata*), corteza de pino, y papel de desecho. En el quinto experimento, el suelo fue enmendado con urea-N a dosis de 0, 0.15, 0.30, 0.45, 0.60, 0.75 y 0.90 g/kg. Se realizaron observaciones microscópical a 0, 5 y 10 semanas después del tratamiento, pare determinar el porcentaje de huevos parasitados en cada sustrato individual. La mayoría de las dosis de mucuna, kudzu, corteza de pino, y papel incrementaron el parasitismo de huevos de *M. incognita* entre las 5 y 10 semanas después del tratamiento. El porcentaje de parasitismo fue solamente mejorado de una forma significativa por urea-N al final del experimento, cuando el suelo fue tratado con las dosis de 0.30 a 0.75 g/kg.

[†]Portion of a Ph.D. thesis submitted by the first author to Auburn University.

Palabras claves: alginato, enmiendas organicas, *Meloidogyne incognita*, *Mucuna deeringiana*, parasitismo, *Pueraria lobata*.

INTRODUCTION

The relative abundance of antagonists in soil may determine whether a phytonematode species can develop in sufficient numbers to cause significant damage to crops (Rodríguez-Kábana *et al.*, 1994). Inhibitory activities by microorganisms decomposing organic soil amendments may be one of the more practical biocontrol practices against phytonematodes (Mankau, 1981). Organic amendments have been associated with the suppression of nematode populations through stimulation of an antagonistic soil microflora during their decomposition (Baby and Manibhushanrao, 1993). Nematode eggs when exposed to soil are subject to colonization by different species of soil microorganisms, particularly fungi (Chen *et al.*, 1994; Chen *et al.*, 1996; Godoy *et al.*, 1982; Stirling, 1991).

Traditional strategies for determination of the colonization of nematode-eggs have been based on dilution techniques and selective media (Godoy *et al.*, 1983), buried glass slides and agar discs coated with selective agar media (Culbreath, 1985; French and Hebert, 1982) and the use of capillary tubes and nylon gauze impregnated with substrates (Johnson and Curl, 1972). However, all of these techniques have serious constraints and limitations, such as the difficulty of preparation, and overstimulation of fungal egg-colonizers by the selective culture media. Also, some of these specialized techniques require the extraction, isolation, and counting of antagonistic organisms, and are not suitable for obligate parasites or microorganisms that do not sporulate readily in culture media (Mankau, 1981). However, a method developed by Rodríguez-Kábana *et al.* (1994), allowed the delivery of nematode inoculum embedded in alginate films and the evaluation *in vivo* of microbial antagonistic activity against nematode eggs in soil.

The objective of this research was to evaluate the effects of organic amendments (velvetbean, kudzu, pine bark, paper waste, and urea-N), using the alginate film method proposed by Rodríguez-Kábana *et al.* (1994), on the parasitism of *M. incognita* eggs.

MATERIALS AND METHODS

Preparation of alginate solution. The alginate solution was prepared by adding medium viscosity sodium alginate (Sigma Chemical Co., St. Louis, MO, USA) to warm (60°C) demineralized water in a blender at a 3% (w/v) concentration. The suspension was blended for approximately 20 seconds to obtain a homogeneous solution. The solution was heated for 30 seconds (80°C) in a microwave oven and put at room temperature for 6 hours to obtain a clear solution free of air bubbles.

Extraction of nematode eggs. Meloidogyne incognita eggs were extracted from 12-week-old infected tomato roots cv. Rutgers (*Lycopersicum esculentum*), using the method described by Barker (1985). Roots were cut into 0.5-1 cm sections and shaken in 200 ml of 1% NaOCl for 3 minutes. The suspension was then passed through a 200-mesh sieve, nested over a 500-mesh sieve to collect nematode eggs. The eggs retained on the 500-mesh sieve were rinsed 10 times with demineralized water and collected in a beaker.

Inoculum Preparation. Alginate films with *M. incognita* eggs were prepared essentially as described by Rodríguez-Kábana *et al.* (1994). Polyvinyl chloride-coated fiberglass screens (commercial porch screens) of 2.5 × 5.0 cm in size, were placed for 1 hour in petroleum ether, followed by 95% methanol overnight to eliminate the oil from the screen surface. Alginate solution was mixed carefully to avoid bubble formation, with an aqueous suspension of freshly-extracted *M. incognita* eggs. The final alginate concentration was 2%. The polyvinyl screens (2.5 × 5.0 cm) were dipped into the alginate-egg suspension for 3-4 seconds and removed with forceps. Each screen was placed between two glass rods and pulled up to make a uniform film. Alginate screens were dipped in 0.25 M $CaCl_2$ for 3-4 seconds and washed in three consecutive demineralized water dips. The final *M. incognita* egg concentration was 6.3 eggs per grid square, approximately 3,213 eggs per alginate film (2.5 × 5.0 cm).

Greenhouse experiments. Five greenhouse experiments were conducted. In the first two experiments, green foliage and stems of velvetbean (*Mucuna deeringiana*) and kudzu (*Pueraria lobata*) were dried (25°C) and ground into a powder (particle size = 250 µm). In the third experiment, commercially available dry pine bark nuggets from slash pine (*Pinus elliottii*) and loblolly pine (*Pinus taeda*) were ground (250 µm) and used as a soil amendment. In the fourth experiment, paper waste from cardboard (Tascon Inc., Houston, TX, USA) was evaluated. The amendments were added to soil at rates of 0, 10, 20, 30, 40, and 50 g/kg. In the fifth experiment, urea (34% N) was applied to soil at concentrations of 0, 0.15, 0.30, 0.45, 0.60, 0.75, and 0.90 g N/kg soil. The experimental design for each experiment was a randomized complete block with eight replications per treatment. Two non-amended controls with twice-autoclaved soil and builders' sand were included in every experiment. In each experiment the soil was apportioned in 1-kg quantities in 4-L capacity polyethylene bags. The amendment was added to the bag and after thorough mixing was transferred into 1-L, 10-cm-diameter PVC pots. Pots with the amended soils were placed in a greenhouse (25-30°C) and kept moist (60% field capacity) throughout the duration of the experiments. Alginate films with *M. incognita* eggs were placed for 4 days into amended soils at 0, 5, and 10 weeks after treatment. The films were removed from the substrate with a metal spatula and washed with tap water. Soil particles were removed by brushing carefully with a camel hair brush, as described by Rodríguez-Kábana *et al.* (1994). Each individual film was then transferred to a 75 × 25 mm microscope slide and covered with a 24 × 50 mm cover glass. Microscopic observations were made from an area of 2.5 × 1.5 cm of the alginate film, about 130 grid squares (ca. 800 nematode eggs). The percentage of parasitism of *M. incognita* eggs was then determined from approximately 6 400 eggs per treatment.

Data were analyzed using standard procedure for two-way analysis of variance (ANOVA) (Steel and Torrie, 1980). Means were compared for significance using Least Significant Differences (LSD) when F values were significant ($P = 0.05$).

RESULTS AND DISCUSSION

Velvetbean powder applied to soil at rates between 30 and 50 g/kg significantly increased the percentage of parasitism of *M. incognita* eggs between 5 and 10 weeks after treatment (Fig. 1). Kudzu powder applied at 30 and 40 g/kg increased the percentage of parasitism of rootknot nematode eggs 5 weeks after treatment (Fig. 2).

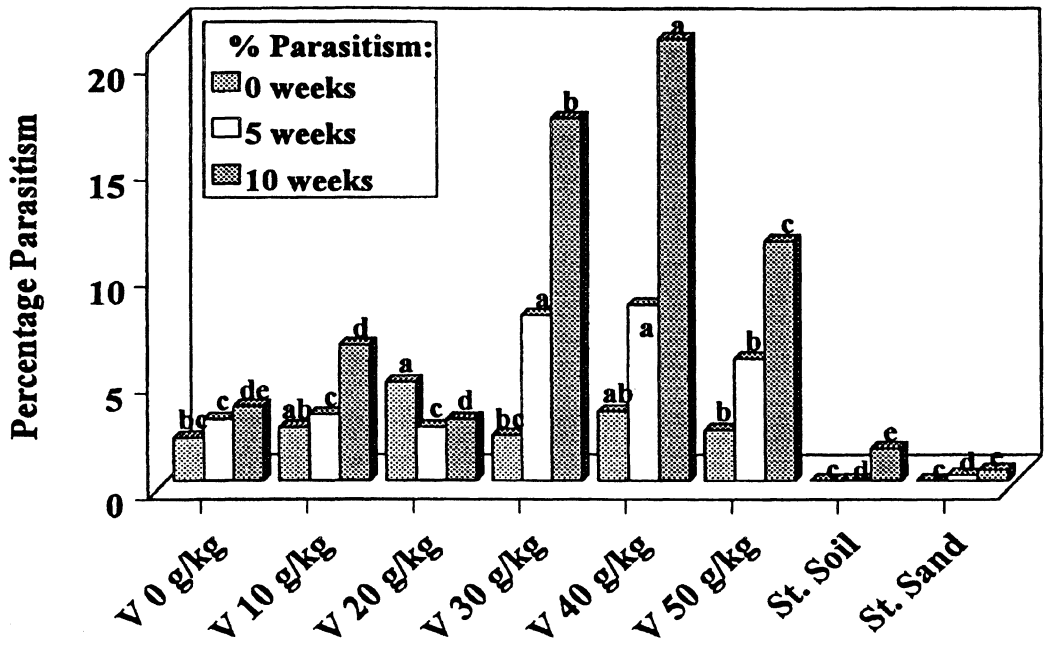

Fig. 1. Percentage of parasitism by fungi of *Meloidogyne incognita* eggs in a soil amended with velvetbean (V). Columns with common letters are not significantly different at $P < 0.05$. St. = Sterilized.

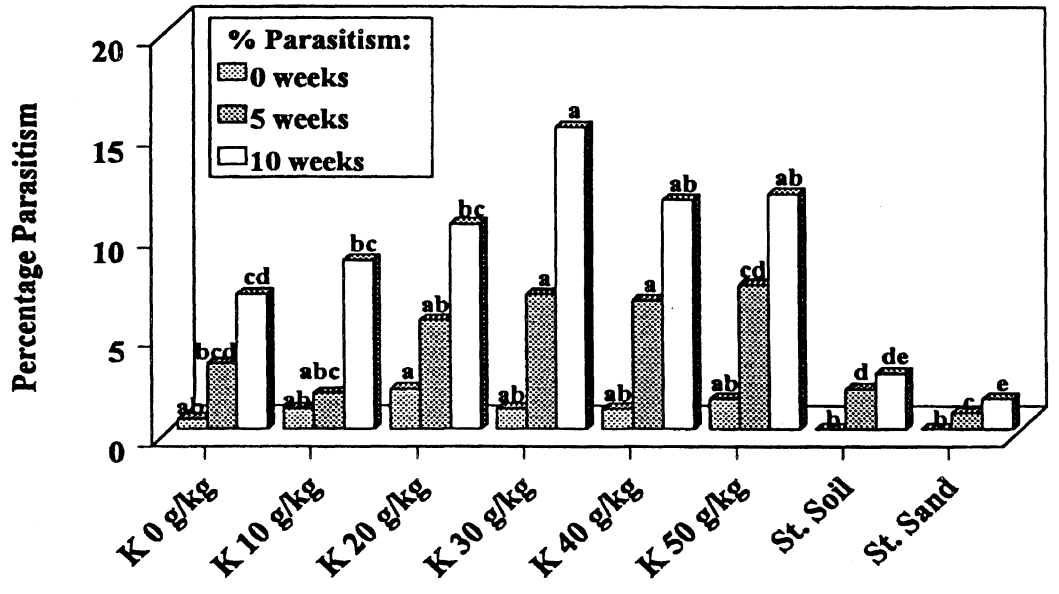

Fig. 2. Percentage of parasitism by fungi of *Meloidogyne incognita* eggs in a soil amended with kudzu (K). Columns with common letters are not significantly different at $P < 0.05$. St. = Sterilized.

At 10 weeks, kudzu rates of 30 to 50 g/kg significantly increased the percentage of parasitism when compared with the non-treated control, sterile soil and sterile sand. Soils amended with pine bark (30 to 50 g/kg) also showed an increase in parasitism 5 weeks after treatment (Fig. 3). Ten weeks after application, pine bark at rates of 20 to 50 g/kg resulted in increased percentage of parasitism. Paper waste applied between 10-50 g/kg increased egg parasitism at 10 weeks after treatment (Fig. 4). Soils treated with urea-N (0.30 to 0.75 g/kg) showed an increase in the percentage of parasitism of nematode eggs at the end of the experiment (Fig. 5). No increase in parasitism was observed with urea at 0 and 5 weeks after application.

The results indicate that amendments with velvet bean, pine bark and paper waste are effective for the stimulation of an antagonistic soil microflora responsible for increased parasitism of *M. incognita* eggs. Most parasitized eggs showed fungal mycelium growing around or above the egg surface, suggesting that fungal species were responsible for most of the parasitism. Additionally, parasitism invariably increased with time, linking increases in antagonistic activity to decomposition of organic matter. Previous microscopic observations of egg penetration by soil microorganisms (Bursnall and Tribe, 1974; Godoy *et al.*, 1983; Morgan-Jones *et al.*, 1981; Morgan-Jones and Rodríguez-Kábana, 1981) suggested that some degree of natural biological control is present in agricultural soils (Ownley-Gintis *et al.*, 1983; Kerry *et al.*, 1982; Rodríguez-Kábana and Canullo, 1992). Our results demonstrate that it is possible to enhance parasitism of nematode eggs and increase suppression of *M. incognita* populations

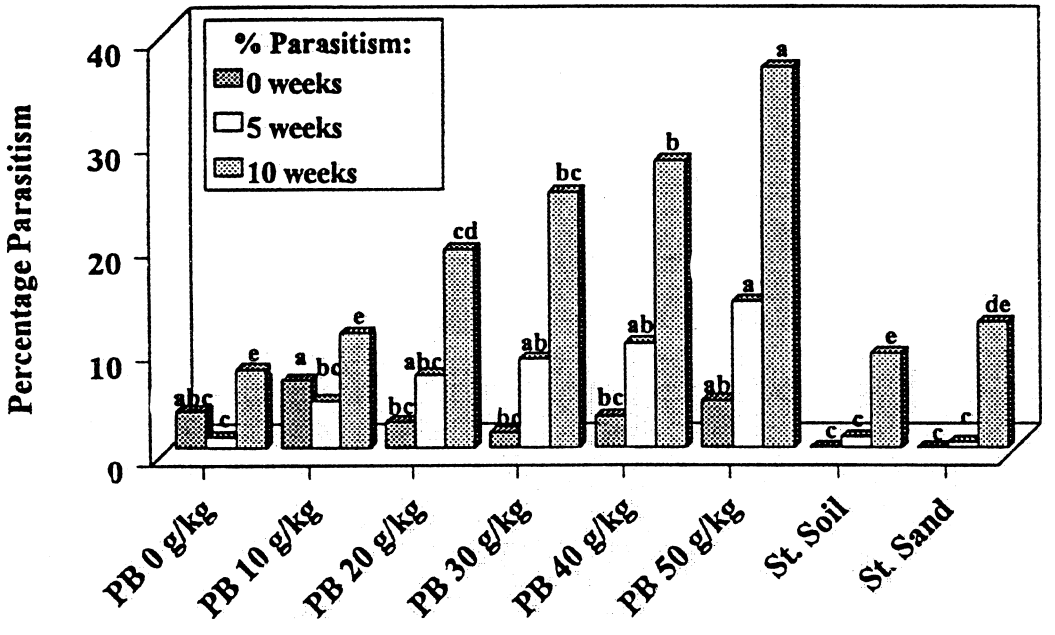

Fig. 3. Percentage of parasitism by fungi of *Meloidogyne incognita* eggs in a soil amended with pine bark (PB). Columns with common letters are not significantly different at $P < 0.05$. St. = Sterilized.

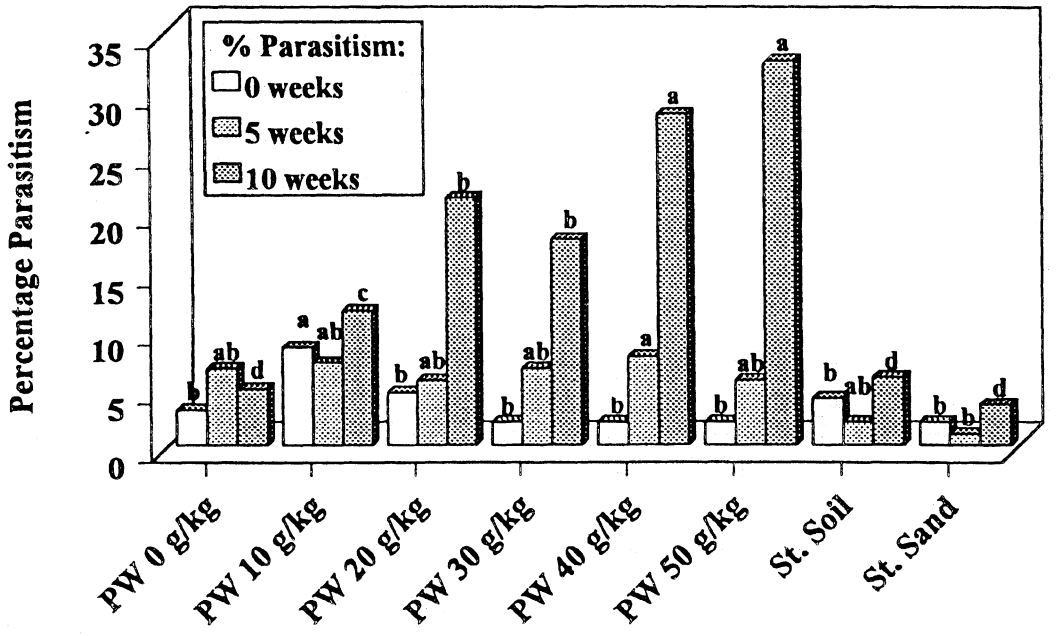

Fig. 4. Percentage of parasitism by fungi of *Meloidogyne incognita* eggs in a soil amended with paper waste (PW). Columns with common letters are not significantly different at $P < 0.05$. St. = Sterilized.

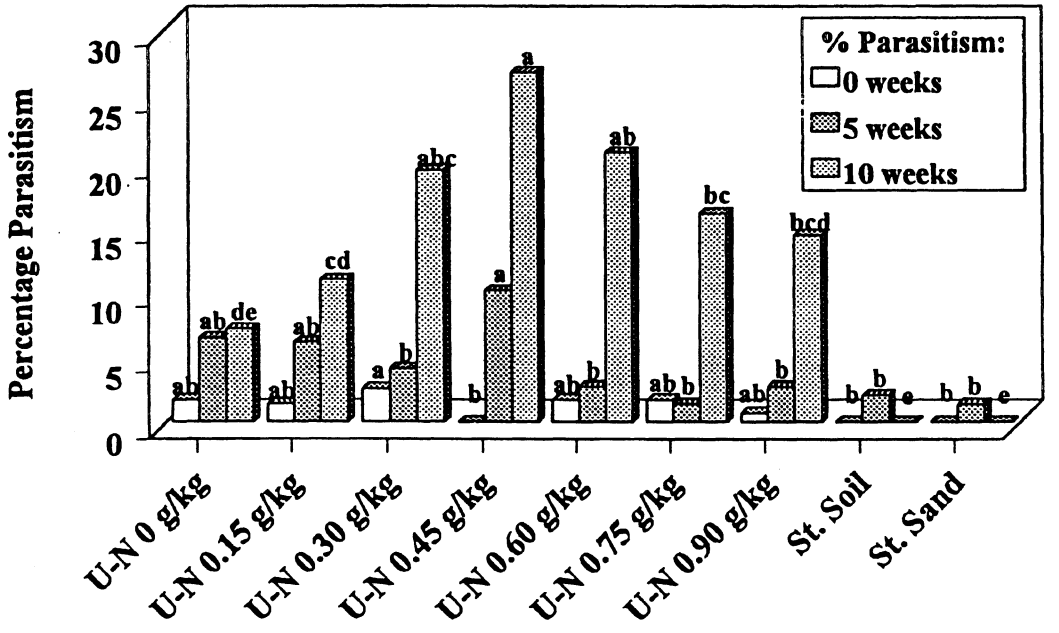

Fig. 5. Percentage of parasitism by fungi of *Meloidogyne incognita* eggs in a soil amended with urea-N (U-N). Columns with common letters are not significantly different at $P < 0.05$. St. = Sterilized.

with some organic materials. Egg parasitism was also increased in soils amended with chitin or flax seed meal (Rodríguez-Kábana *et al.* (1994), compared with unamended soil. When chitin was added to soil, there was an increase in parasitism of nematode eggs by fungi (Rodríguez-Kábana and Morgan-Jones, 1987; Rodríguez-Kábana, 1986).

In summary, the alginate film is an inexpensive and simple technique, useful to measure parasitism of nematode eggs under experimental conditions. The technique makes possible *in vivo* evaluation of parasitism of nematode eggs, and overcomes some drawbacks of specialized techniques used in the past, which required the extraction and isolation of antagonistic microorganisms in selective media, or the use of complicated agar disks, capillary tubes, and other materials or methods that are difficult to use.

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