SURVIVAL AND INFECTIVITY OF *MELOIDOGYNE GRAMINICOLA* IN FLOODED AND NON-FLOODED SOILS

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Summary. Two greenhouse experiments were conducted to assess the survival and infectivity of the rice root-knot nematode (*Meloidogyne graminicola*) after 0, 3, 6, 9, and 12 weeks incubation in flooded and non-flooded soils. Penetration and reproduction of *M. graminicola* in roots of rice bioassay seedlings changed little after nine weeks incubation in flooded soil. However, reproduction of *M. graminicola* decreased by 68 to 75% relative to its reproduction at the beginning of the experiment after twelve weeks incubation in flooded soil. By comparison, reproduction of *M. graminicola* in non-flooded soil declined by 60 to 93% after six weeks incubation and by 93 to 99% after 12 weeks, relative to its reproduction at the beginning of the experiment. This study demonstrated that survival and infectivity of *M. graminicola* is significantly greater in flooded soils than in non-flooded soils.

The evolution of anoxic conditions that occurs with flooding of soil can slow the movement and metabolic activity of second-stage juveniles of root-knot nematodes and may thus enhance their survival (Van Gundy, 1985). The capacity for prolonged nematode survival in flooded soils has important implications for *Meloidogyne graminicola*, which has been reported to cause substantial rice yield loss under simulated conditions of intermittent flooding (Rao and Israel, 1971; Soriano *et al.*, 2000). While *M. graminicola* is unable to penetrate rice roots in flooded soils, second-stage juveniles have been reported to remain viable under continuously flooded conditions for at least five months (Bridge and Page, 1982), and egg masses for as long as 14 months (Roy, 1982). The ability of *M. graminicola* to survive long periods under flooded rice production could negatively impact the growth of alternative host crops rotated with rice, such as occurs in South Asia's rice-wheat rotation system, as well as perpetuating problems for rice.

To assess the influence of flooding on the survival and viability of *M. graminicola* in soil, a study was undertaken to compare its reproduction on rice after prolonged incubation in flooded and non-flooded soils.

MATERIALS AND METHODS

Two experiments were conducted between May and December 2002 to compare the infectivity and reproduction of *M. graminicola* Golden *et* Birchfield, 1965, surviving in flooded and non-flooded soils for varying durations. The first experiment was a 2x2 factorial consisting of *M. graminicola*-infested soil maintained under flooded and non-flooded soil conditions, and non-infested soil maintained under flooded and non-flooded soil conditions for incubation periods of 0, 3, 6, 9, and 12 weeks. The second experiment had the same combination of treatments except that the incubation periods were 0, 3, 6, and 12 weeks.

The soil used in this experiment was a sandy loam with a pH of 7.1. The soil was obtained from the New York State Agricultural Experiment Station in Geneva, New York. It was steam pasteurized at 60° C for 40 minutes, after mixing four parts of the soil with one part of autoclaved sand. The *M. graminicola* population used in this experiment was originally recovered from rice roots grown in soil collected from a *M. graminicola*-infested field at the Bangladesh Rice Research Institute Regional Station in Rajshahi, Bangladesh. The population was maintained on rice (*Oryza sativa* L., var. BR11) in a greenhouse at Cornell University. Eggs of *M. graminicola* used as inoculum for this experiment were extracted from infected rice roots using a modification of the Hussey-Barker method (Hussey and Barker, 1973). Galled root pieces were placed in 1% NaOCl solution and pulse-blended in a Waring blender at 20 s intervals for three minutes. The nematode eggs were recovered on a #500 sieve and the eggs were washed for several minutes under running tap water to remove excess bleach.

Prior to inoculating the treatments, soil was placed in 400 cm^3 pots that were lined with a polyethylene bag to prevent the loss of nematodes. The treatments were infested with the nematode at a rate of three eggs per cm^3 soil by pipetting an *M. graminicola* egg suspension onto the soil, followed by shaking and mixing of the soil in the polyethylene bags. After thoroughly distributing the eggs in the soil, the bags were placed back in the pots. The pots were then placed on a greenhouse bench and lightly watered for three days to allow the nematode eggs to hatch, after which flooding to 3 cm depth was initiated by placing each pot inside a polyethylene-lined 1 litre pot filled with water. Soil of the two non-flooded treatments was kept moist throughout the experiment with daily watering. To reduce evaporation, no supplemental light source was used over the bench that contained the pots.

The treatments for all incubation periods were arranged in a randomised complete block design and replicated six times. At 0, 3, 6, 9, and 12 weeks after the initiation of flooding, the viability of *M. graminicola* was assessed with a rice seedling bioassay test. Pots of the two flooded treatments were removed from the flood water and drained to field capacity. The flooded and non-flooded treatments were then seeded with two 3-day-old pre-germinated rice seeds in each pot. Three days after sowing, the seedlings were thinned to one seedling per pot. The treatments were replicated six times, arranged in a randomised complete block design, and maintained in a greenhouse, at 24 °C for 30 days. The plants were watered once daily, and fertilized weekly with a liquid solution of 15-5-15 NPK with Ca and Mg.

Thirty days after sowing, the rice plants were removed from the pots, roots were washed, and root-galling severity was evaluated on a scale of 1 to 9, adapted from a 1 to 10 scale (Barker, 1985). A rating of 1 indicated no visible root galling; ratings of 2 to 9 indicated 1 - 3%, 4 - 10%, 11 - 25%, 26 - 35%, 36 - 50%, 51 - 65%, 66 - 80%, and > 80% of the root system galled, respectively. Nematode reproduction was assessed through counts of eggs extracted from the roots with 1% NaOCl. The eggs were stored in glass vials at 5 °C until counted under a stereoscope. Shoot height, shoot dry weight, fresh root weight, and tiller number were measured at the end of each bioassay test.

The experiment was repeated, with some modifications, in September through December 2002. The soil was infested with six *M. graminicola* eggs per cm^3 soil and placed in 950 cm^3 pots, after mixing thoroughly in a 2.5 litre plastic container. Pots of all treatments were sealed at the bottom with a double layer of adhesive tape prior to filling with soil. Flooding was initiated two days after nematode infestation. The nematodes were held in flooded or non-flooded conditions for 0, 3, 6, or 12 weeks. The bioassay procedure used in this experiment was similar to that described above except that five seedlings were planted per pot, and the test was carried out for 42 days. The higher density of *M. graminicola* inoculum and the longer duration for the soil bioassay test were employed for the second experiment in order to better detect differences in root-galling severity relative to that of the first experiment.

Data for the main effects and interactions of flooding and *M. graminicola* infestation were analysed using a general linear model for analysis of variance. Differences between individual treatment means were separated with a Tukey's significance test. Data for each bioassay period were analysed separately. All statistical tests were performed using Minitab, v. 13.1.

RESULTS

After 6, 9, and 12 weeks incubation in the first experiment, mean reproduction of *M. graminicola* on rice roots grown in the flooded soil was 86, 90, and 32% of mean *M. graminicola* reproduction measured at the week 0 (initial) bioassay (Fig. 1). Over the same intervals of incubation in the non-flooded soil, mean reproduction of *M. graminicola* on roots of bioassay plants was 40, 13, and 10% of mean *M. graminicola* reproduction measured in the week 0 bioassay. Differences in nematode reproduction between the flooded and non-flooded soil treatments were significant at weeks 6, 9, and 12 ($P < 0.01$). The bioassay test was not conducted on samples of the week 3 incubation period due to insect damage to several of the rice seedlings after emergence.

After weeks 6 and 9 of incubation, root-galling severity was significantly greater on roots of bioassay plants grown in flooded soil compared to those grown in non-flooded soil (Fig. 2), though actual root-galling differences were slight and overall root-galling severity was low throughout the experiment.

In the second experiment, mean *M. graminicola* reproduction on rice roots after 3, 6, and 12 weeks incubation in flooded soil was 92, 111, and 25%, respectively, of the mean reproduction measured in the week 0 bioassay (Fig. 3). Over the same incubation intervals, mean egg production of *M. graminicola* on roots of bioassay plants grown in the non-flooded soil was 112, 7, and 0.1% of the mean egg production measured in the week 0 bioassay. However, due to high variability in nematode reproduction among replications in each treatment, statistically significant differences were only detected in the week 12 bioassay ($P < 0.01$). Similarly, the only statistically significant treatment effect for root-galling severity occurred in the week 12 bioassay ($P < 0.05$), where mean root-galling severity in the flooded treatment was 3.3 compared with a mean value of 1.5 in the non-flooded treatment (Fig. 4).

No nematode eggs were recovered from, nor root-knot galls observed on, roots of bioassay plants grown in the non-infested soil in either experiment. Also, there were no significant plant growth differences between the *M. graminicola*-infested treatments incubated in flooded *vs* non-flooded soils in either experiment.

DISCUSSION

The infectivity and reproduction of *M. graminicola* was substantially greater after incubation in flooded than in non-flooded soils. For instance, after nine weeks of incubation there was only a 10% decrease in *M. graminicola* reproduction on bioassay roots grown in the flooded treatment as compared with a nearly 90% decline in nematode reproduction in roots grown in the non-flooded treatment in experiment 1. Similar results were obtained for the second experiment, though the decline in reproduction over time of *M. graminicola* in the non-flooded soils was greater than that measured in the first experiment. Differences in reproduction and root-galling severity were not as significant in the sec-

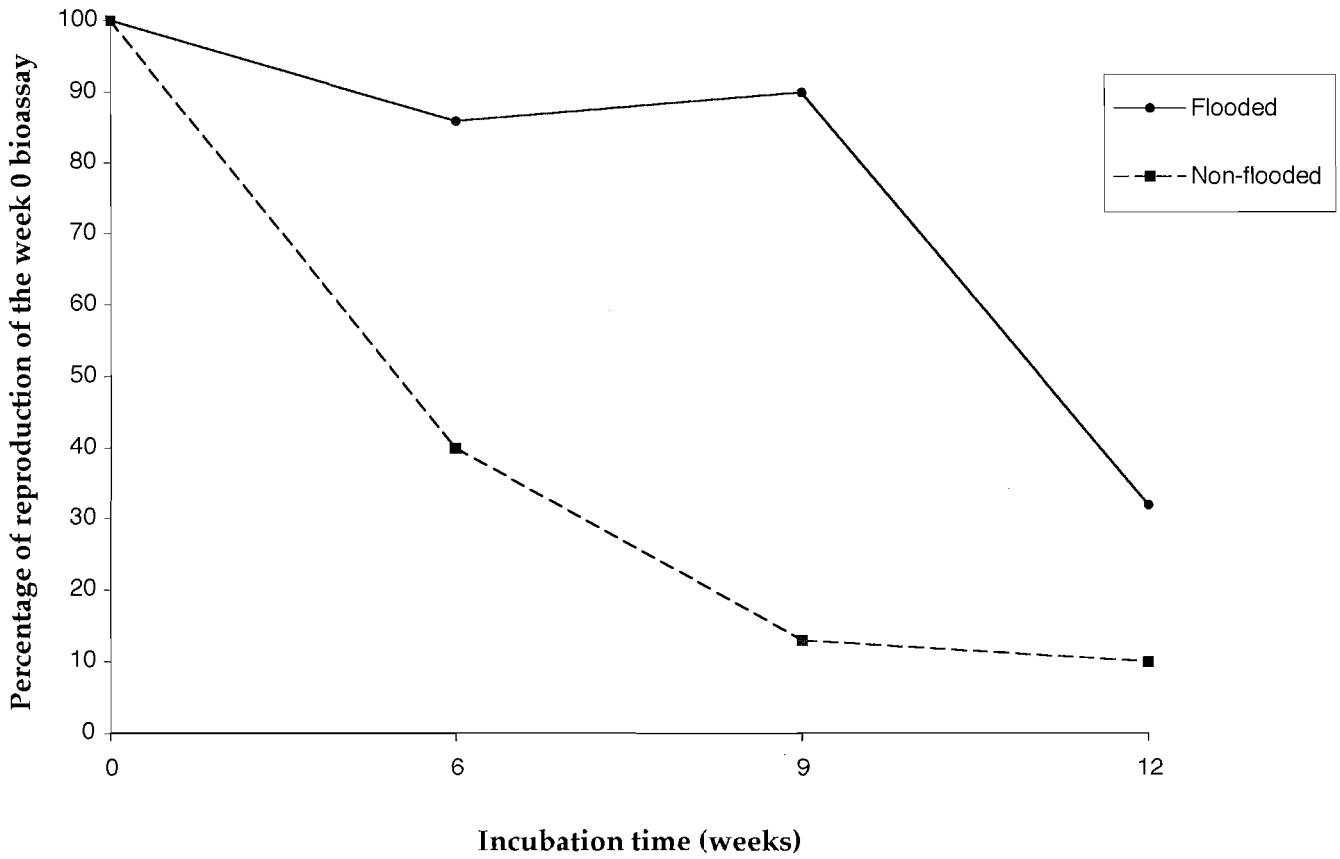

Fig. 1. Change in *Meloidogyne graminicola* reproduction in rice seedling bioassays following incubation under flooded and non-flooded soil conditions. Experiment 1: treatment differences for *M. graminicola* reproduction were significant in the week 6, 9, and 12 bioassay tests at $P < 0.01$ as determined by a Tukey's test.

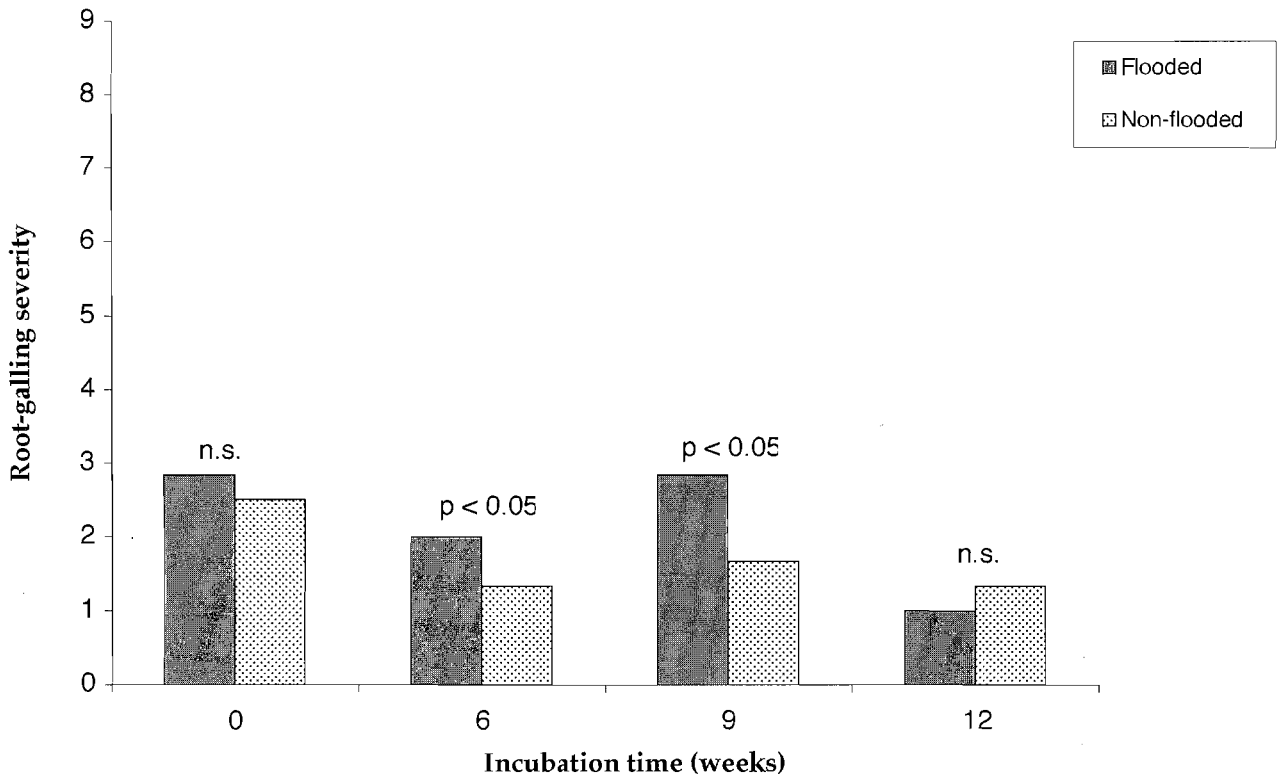

Fig. 2. Root-galling severity of rice seedling bioassays following incubation under flooded and non-flooded soil conditions. Experiment 1: root-galling severity was rated on a scale of 1 to 9. A gall rating of 1 indicates no visible galling. Ratings of 2 to 9 indicate 1-3, 4-10, 11-25, 26-35, 36-50, 51-65, 66-80 and >80% of the root system is galled, respectively.

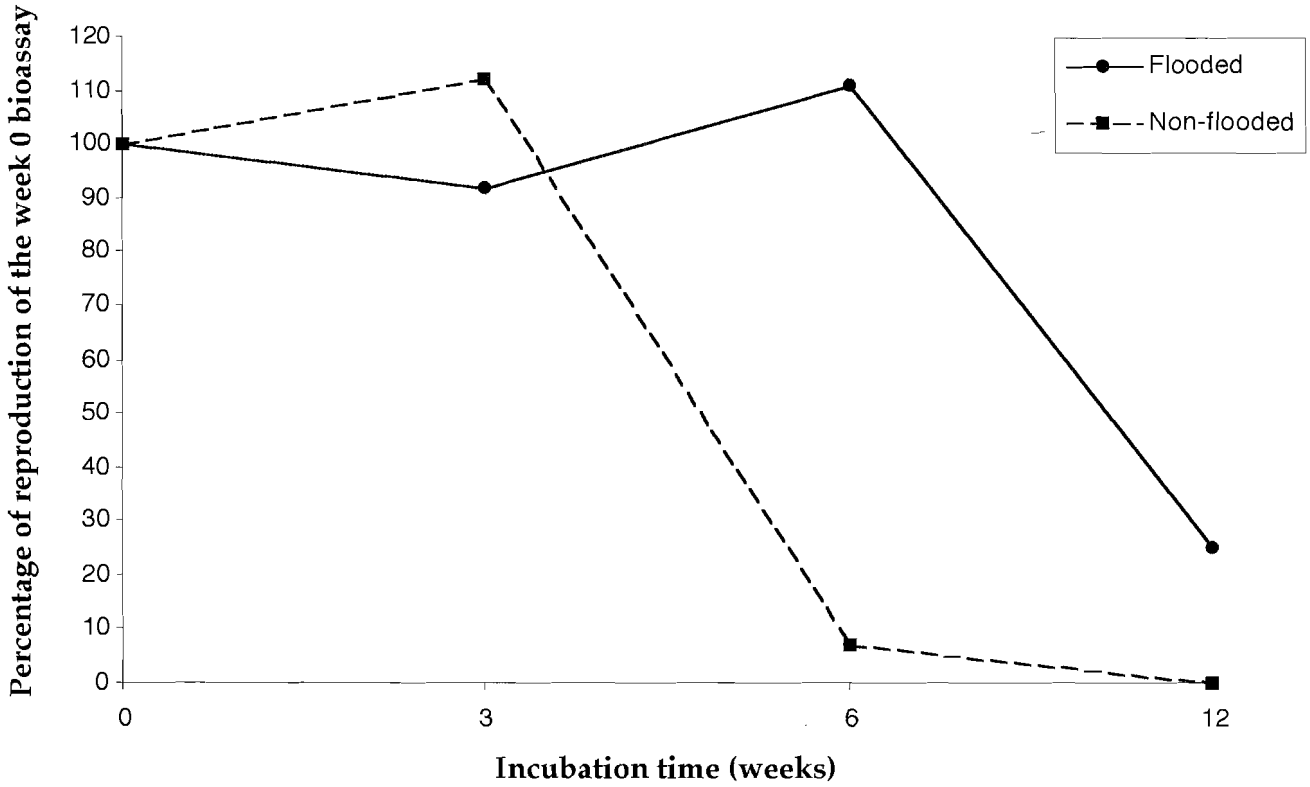

Fig. 3. Change in *Meloidogyne graminicola* reproduction in rice seedling bioassays following incubation under flooded and non-flooded soil conditions. Experiment 2: treatment differences in *M. graminicola* reproduction were significant in the week 12 bioassay at $P < 0.01$ as determined by a Tukey's test.

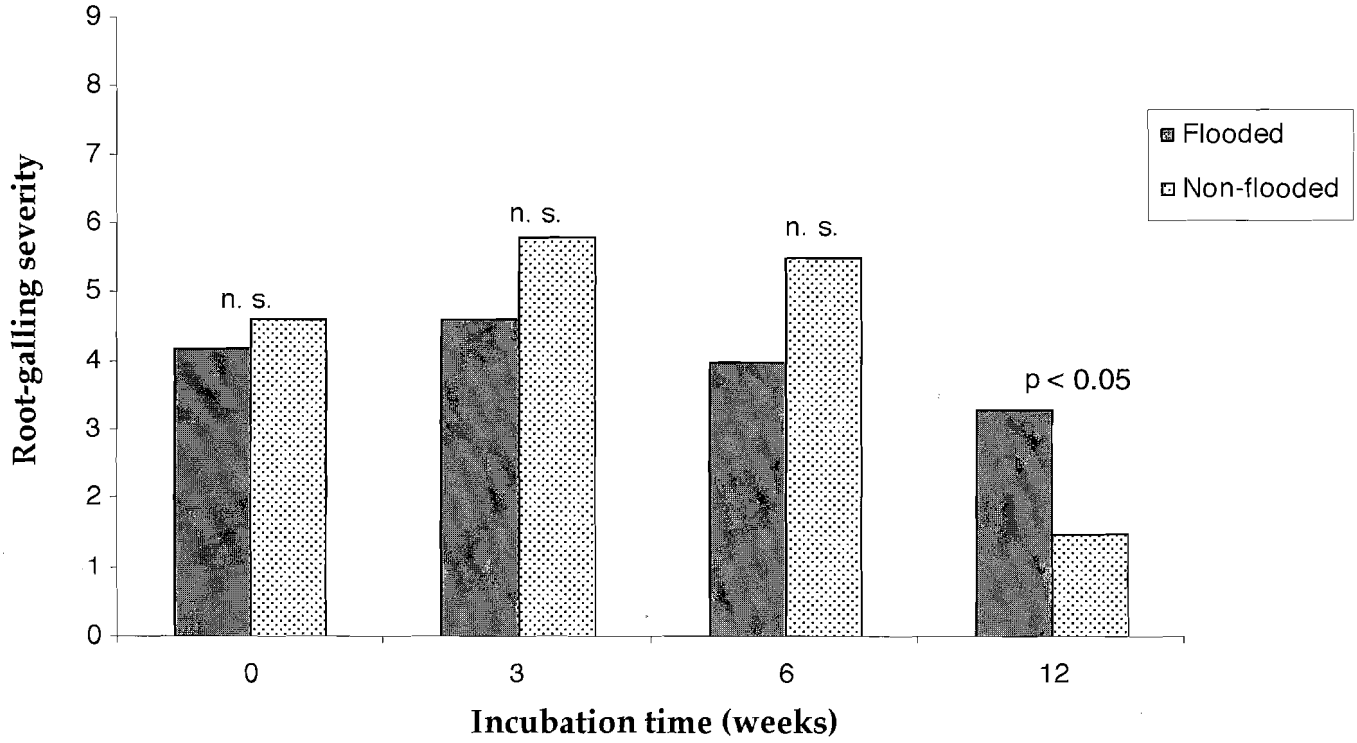

Fig. 4. Root-galling severity of rice seedling bioassays following incubation under flooded and non-flooded soil conditions. Experiment 2: root-galling severity was rated on a scale of 1 to 9. A gall rating of 1 indicates no visible galling. Ratings of 2 to 9 indicate 1-3, 4-10, 11-25, 26-35, 36-50, 51-65, 66-80, and >80% of the root system is galled, respectively.

ond experiment as in the first due to high variability in the egg production and root-galling severity data at all bioassay periods except week 12.

The decline in *M. graminicola* reproduction under conditions of aerobic incubation was greater than that reported in other soil-water studies involving *M. graminicola*. Roy (1982) observed no difference in reproduction of *M. graminicola* eight weeks after placing galled root pieces in moist and waterlogged soil. However, the physical protection provided by both the root tissue and egg sac matrix probably protected egg survival relative to experimental conditions where eggs are released from the egg sac (Orion *et al.*, 2001), as occurred in the present study. Soomro (1994) observed no change in the infectivity and reproduction of *M. graminicola* on *Echinochloa colona* after five months of storage in moist soil. The decline in survival and reproduction measured in the non-flooded soil treatment was similar to that observed for other root-knot nematode species, including *M. javanica* (Orion *et al.*, 2001; Townson and Apt, 1983), *M. incognita* (Rhoades, 1982), and *M. triticoryzae* (Chandel *et al.*, 2001).

The practice of soil flooding, where feasible, has been used to effectively diminish plant-parasitic nematode populations, such as in some organic soils. A flooded fallow of less than ten weeks was sufficient to significantly reduce damage from nematodes parasitic on banana in peat soils (Mateille *et al.*, 1988) and celery in muck soils (Thames and Stoner, 1953). Also, maintaining flooded soil conditions for rice production can effectively limit damage from plant-parasitic nematodes, including *Tylenchorhynchus* and *Paralongidorus* spp. in rice-producing areas of the southern USA and Australia, respectively (Hollis and Rodriguez-Kabana, 1966; Stirling *et al.*, 1989), and *M. graminicola* in lowland Asian rice systems (Prot and Matias, 1995).

While lessening any immediate damage to the rice crop, sustained flooding for rice production may not necessarily reduce the viability of nematodes surviving outside the host (Bridge and Page, 1982; Stirling *et al.*, 1989), as was measured for *M. graminicola* in this study. Even if flooded conditions are maintained during vegetative growth of rice, as is recommended for control of *M. graminicola* in Asia (Soriano *et al.*, 2000), nematode inoculum surviving in the soil can reinfect rice roots in the period between anthesis and maturity when rice paddies are typically not flooded. While late infection of rice would probably not impact yield, the rice roots could function as a bridge for the surviving nematodes between the cessation of flooding and the planting of an alternative host crop in a rotation with rice, such as wheat, as occurs throughout much of South Asia's rice-wheat system. Subsequently, the nematode population in the soil can be maintained throughout rice and wheat cropping cycles, increasing its potential to reduce crop yields.

ACKNOWLEDGEMENTS

Financial support for this research was provided by the United States Agency for International Development (USAID Grant No. LAG00-97-00002-00).

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Accepted for publication on 16 September 2003.