Libyan Agriculture Research Center Journal International 3 (1): 13-17, 2012
ISSN 2219-4304

Relationship Between Growth of Rice Seedlings and Time of Infection with *Meloidogyne graminicola*

[1]R.K. Jaiswal, [2]D. Kumar and [1]K.P. Singh

[1]Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005, India
[2]Division of Genetics and Plant Breeding, Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad - 224229, India

Abstract: Early infection of *Meloidogyne graminicola* significantly reduced the growth of rice plants. In general length and fresh weight of shoot and root decreased significantly with earliness in time of inoculation. Seedlings inoculated 24 h after germination recorded minimum growth of the plant, whereas growth parameters increased with increasing gap in time of inoculation. Onset of yellowing was observed on day 8 in the seedlings inoculated 24 h after germination while seedlings inoculated 2 days after 1st inoculation expressed it on day 10. Seedlings inoculated 14 days after 1st inoculation exhibited least effect of *M. graminicola* on the growth parameters followed by those inoculated 12 and 10 days after 1st inoculation. Nematode biomass in the roots of inoculated seedlings decreased with increasing gap in time of inoculation whereas root biomass consistently increased. The nematode-to-root biomass ratio was lower in the seedlings which were inoculated after 24 h of germination and 2 days after 1st inoculation which resulted into wilting. Nematode-to-root biomass ratio increased with increasing gap in time of inoculation that led to delay in onset of yellowing. Time of inoculation was positively correlated with shoot length ($R^2 = 0.90$), root length ($R^2 = 0.77$), fresh weight of shoot ($R^2 = 0.96$), fresh weight of root ($R^2 = 0.97$) and nematode-to-root biomass ratio ($R^2 = 0.20$) while it was negatively correlated with number of root galls ($R^2 = 0.75$), number of females ($R^2 = 0.82$), total population (eggs + juveniles) of nematodes ($R^2 = 0.77$) and nematode biomass ($R^2 = 0.82$).

Key words: Early infection % Meloidogyne graminicola % Nematode biomass % Symptoms

INTRODUCTION

Plant pathogens cause negligible to severe effect on the growth and yield of crops. However, severity of disease depends on the susceptibility of genotypes, virulence of pathogen, conducive environment and time of infection. Among these determinants, time of infection in combination with favorable environmental condition is a major factor that leads to seriousness of a disease. Like other phytopathogens, plant parasitic nematodes may cause complete destruction of crops when plants are infected at seedling stage [1]. Root-knot nematodes, *Meloidogyne* spp. mostly attack young roots of plants; hence, age of host becomes a predominant factor in infection and pathogenesis [2]. The amount of plant damage caused by a nematode is apparently related to the time of infection and age of the plant. Shane and Barker [3] reported that early infection of soybean seedlings by *M. incognita* severely reduced the growth of seedlings than late inoculated. Bergeson [4] also reported that inoculation of tomato seedlings in the cotyledon stage with *M. incognita* caused 81% reduction in growth, but had no adverse effect on seedlings in the 3-4 petiole stage. Similarly, Riedel [5] reported that 24-48 hours old onion seedlings were more severely damaged by *Ditylenchus dipsaci* than 72-96 hours old seedlings. Kumar and Singh [6] have also quoted that infection of young plants by nematodes at early stage is more devastating.

Root-knot nematode, *Meloidogyne graminicola* [7] is a serious pest of rice and has become a major constraint to rice production throughout the world [8-18]. The root-knot disease of rice caused by *M. graminicola* is characterized by abnormal swelling of roots (known as

Corresponding Author: R.K. Jaiswal, Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi - 221005, India.

root-knots or galls), yellowing, stunting and wilting of the plants. During our routine observations it was noted that seedlings infected immediately after germination showed severe stunting irrespective of number of root galls. Seedlings with a single root gall also caused severe stunting and wilting [19]. Such galls of course were invaded by large number of second stage of juveniles (J_2). From these observations it was hypothesized that severity of disease may be related to time of infection. To prove this hypothesis an experiment was conducted to explore the relationship between time of infection and rice seedling growth. Further nematode-to-root biomass ratios were also determined to find relationship with symptoms.

MATERIALS AND METHODS

The rice seeds (var. Ratna) were sown in several earthen pots each containing one kg steam sterilized soil. Ten seeds were sown in each pot after four days of soaking to ensure 100% germination. After 24 h of germination five pots were inoculated with active juveniles of *M. graminicola* @ 2000 J_2 per pot. This treatment represented 1^{st} inoculation. Subsequently other sets of five pots were inoculated at an interval of two days for each inoculation. For each treatment five pots were used as replication. Five uninoculated pots served as control. The pots were kept in a randomized block design and irrigated to maintain moisture to field capacity. 30 days after 1^{st} inoculation, observations on number of root galls, length of shoot and root, fresh weight of shoot and root per seedling were recorded. Further populations of eggs, J_2 and females were also recorded.

After recording data on growth parameters and number of galls, root systems of each inoculated seedling were placed in boiling 0.1% (w/v) acid fuchsine in lactic acid, glycerol and distilled water (1:1:1) for staining roots [20]. The stained root system of each seedling was placed in a separate culture tube and kept at room temperature. Thereafter, root galls of each root system were cut with a sharp blade and placed on a slide containing 2-3 drops of clear glycerol solution (equal parts glycerol and water). The galls were then teased with fine needles for release of the different stages of nematodes and their number was counted. After removing the gall debris from the slide, juveniles were covered with cover glass supported by bits of glass wool. Measurements of different stages of juveniles were taken from such slides. The biomass of nematodes was calculated by taking a minimum of 20 nematodes of each stage. For total biomass of the nematodes in a root system the number of nematodes showing different size was recorded. Further the number of eggs in a gall was also recorded. After counting the number of nematode in each developmental class, their measurements and the mean nematode biomass was calculated by the formula given by Andrassy [21] with slight modification.

$$G = \frac{a^2 b}{(1.6)(1,000,000)}$$

Where

G = Biomass (µg)
a = the greatest body width (µm)
b = body length (µm)
1.6 = constant for correcting volume of nematode; and 1,000,000 is a factor for converting μm^3 to µg

For calculating the biomass of females, biomass of neck and head and of the body was calculated separately. The width of the female body below the neck was measured at three points and the biomass was calculated with the average width to get the realistic value.

In order to determine the nematode-to-root biomass ratio in infected rice seedlings, estimation of the biomass of nematodes and associated roots were made 30 days after 1^{st} inoculation. The fresh weight of infected roots was considered the root biomass. Nematode biomass was then subtracted from infected root biomass to calculate net root biomass. The net root biomass of the inoculated seedlings was divided by the corresponding nematode biomass to obtain the nematode-to-root biomass ratio. This ratio was calculated from 10 rice seedlings and averaged. Onset of symptoms was also recorded.

Statistical Analysis: Plant response against the time of inoculation and biomass of nematodes were subjected to regression and correlation analysis.

RESULTS

From the observations it is evident that the growth parameters of rice seedlings were significantly affected by time of infection of *M. graminicola*. In general length & fresh weight of shoot and root decreased significantly with earliness in time of inoculation. Time of inoculation was positively correlated with shoot length (R^2 = 0.90), root length (R^2 = 0.77), fresh weight of shoot (R^2 = 0.96), fresh weight of root (R^2 = 0.97) and nematode-to-root biomass ratio (R^2 = 0.20) while it was negatively correlated with number of root galls (R^2 = 0.75), number of females

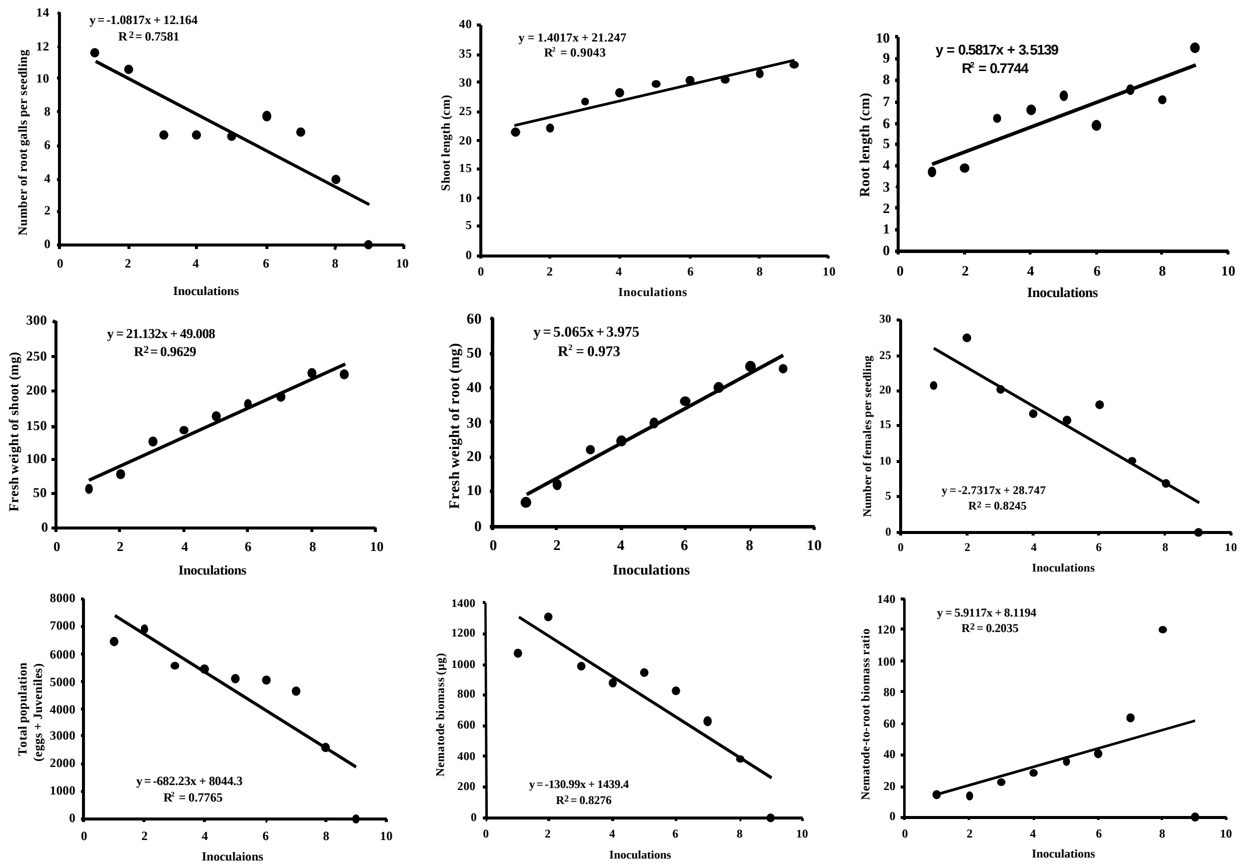

Fig. 1(a-i): Relationship between growth of rice seedlings and time of infection with *Meloidogyne graminicola*

(R^2 = 0.82), total population (eggs + juveniles) of nematodes (R^2 = 0.77) and nematode biomass (R^2 = 0.82) (Fig. 1). Seedlings inoculated 24 h after germination recorded minimum growth of the plant, whereas growth parameters increased with increasing gap in time of inoculation. Onset of yellowing was observed on day 8 in the seedlings inoculated 24 h after germination while seedlings inoculated 2 days after 1st inoculation expressed it on day 10. Seedlings inoculated 14 days after 1st inoculation exhibited least effect of *M. graminicola* on the growth parameters followed by those inoculated 12 and 10 days after 1st inoculation. The seedlings inoculated 4, 6 and 8 days after 1st inoculation were also adversely affected however, the magnitude of symptoms like drying of leaves decreased with increasing gap in time of inoculation. Nematode biomass in the roots of inoculated seedlings decreased with increasing gap in time of inoculation whereas root biomass consistently increased. The nematode-to-root biomass ratio was lower in the seedlings which were inoculated 24 h after germination and 2 days after 1st inoculation which resulted into wilting. Nematode-to-root biomass ratio increased with increasing gap in time of inoculation that led to delay in onset of yellowing. From the observations it is evident that the symptom manifestation is outcome of nematode-to-root biomass ratio which is determined by time of infection. This observation suggests that if time of infection is delayed by any management practice the severity of disease can be reduced.

DISCUSSION

The observations on time of inoculation of *M. graminicola* in rice seedlings clearly indicated that it plays an important role in determination of extent of loss. Severity of disease increased with earliness in infection which is evident from growth parameters. Shane and Barker [3] reported that early infection of soybean seedlings by *M. incognita* severely reduced the growth of seedlings than the late inoculated plant. Maximum reduction in growth parameters was recorded in those seedlings which were inoculated 24 h after germination while in subsequent inoculations the growth parameters were gradually reduced with same level of inocula.

This indicates that gap in time between seed germination and infection determines the root growth and their corresponding shoot growth. In the early inoculated seedlings the root biomass was minimum while the number of galls per seedling was higher with higher biomass of nematodes. Thus the nematode-to-root biomass ratios were lower in seedlings which were inoculated 24 h after germination or 2 days after 1st inoculation while in subsequent inoculations, ratios increased with increasing gap in time of inoculation. In early inoculated seedlings nematode established in the growing radicals, lateral roots or seminal roots inhibiting the growth of root system. Because of higher biomass of nematodes in poor root system, the absorption and uptake of water and nutrients must have been greatly reduced resulting in drastic reduction in growth parameters. Hussey [22] mentioned that nematodes remove plant nutrients, alter nutrient flow pattern and retard root growth, all of which may contribute to suppressed plant growth and yield. It is well established that photosynthates of infected plants are utilized by the growing nematodes established in the root system [23, 24]. In contrast, the seedlings getting late infection due to late inoculation recorded lower reduction in the growth of plants and reduced the number of galls and nematode population hence, lower nematode biomass. In such seedlings absorption of water, uptake of nutrients and utilization of photosynthates by nematodes are less affected. The best developed root system of the late inoculated seedlings is able to support both the plant growth as well as nematode without causing much adverse effect on seedlings. Thus the manifestation of symptoms is attributed to nematode-to-root biomass ratio within the root system [19]. Increase in nematode-to-root biomass ratio with increasing gap in time of inoculation supports the present findings. Bergeson [4] also reported that sensitivity of susceptible tomato plants to *Meloidogyne* species decreases rapidly with increase in age of the plant. In fields it is usually noticed that some times good growing plants register higher number of root galls than the severely diseased plants which is essentially due to difference in time of infection i.e. late infection. Early infection of *M. graminicola* may also predispose rice seedlings to premature death. Present work also exhibited that young plants are more vulnerable to nematode damage which supports the finding of other workers [5, 22, 25-27]. Thus this study gives a sound base for the management of rice seedlings from *M. graminicola* because if rice seedlings could be saved from early infection it may prevent a heavy economic damage due to this nematode pest.

ACKNOWLEDGEMENTS

Dr. R. K. Jaiswal is thankful to Banaras Hindu University, Varanasi, India for providing research fellowship.

REFERENCES

1. Agrios, G.N., 2005. Plant Pathology. 5th Edition, University of Florida, USA.
2. Dropkin, V.H., 1955. The relations between nematodes and plants. Experimental Parasitol., 4: 282-322.
3. Shane, W.W. and K.R. Barker, 1986. Effect of temperature, plant age, soil texture and *Meloidogyne incognita* on early growth of soybean. J. Nematol., 18: 320-327.
4. Bergeson, G.B., 1968. Evaluation of factors contributing to the pathogenicity of *Meloidogyne incognita*. Phytopathol., 58: 49-53.
5. Riedel, R.M., 1969. The influence of onion seedling age on the development of symptoms caused by *Ditylenchus dipsaci*. J. Nematol., 1: 24-25.
6. Kumar, D. and K.P. Singh, 2006. Assessment of Predacity and efficacy of *Arthrobotrys dactyloides* for biological control of root-knot disease of tomato. J. Phytopathol., 154: 1-5.
7. Golden, A.M. and W. Birchfield, 1968. Root-knot nematode (*Meloidogyne graminicola*) as a new pest of rice. Pl. Dis. Reportr. 52: 423.
8. Biswas, H. and Y.S. Rao, 1971. Toxicological assays of nematicides with rice nematodes. Indian Phytopathol., 24: 159-165.
9. Rao, Y.S. and H. Biswas, 1973. Evaluation of yield losses in rice due to the root-knot nematode, *Meloidogyne graminicola*. Indian J. Nematol., 3: 74.
10. Prasad, J.S., M.S. Panwar and Y.S. Rao, 1985. Occurrence of root-knot nematode *Meloidogyne graminicola* in semi-deepwater rice. Curr. Sci., 54: 387-388.
11. Plowright, R. and J. Bridge, 1990. Effect of *Meloidogyne graminicola* (Nematoda) on the establishment, growth and yield of rice cv. IR 36. Nematologica. 36: 81-89.
12. Netscher, C. and Erlan, 1993. A root-knot nematode, *Meloidogyne graminicola*, parasitic on rice in Indonesia. Afro-Asian J. Nematol., 3: 90-95.
13. Prot, J.C., L.M. Villanueva and E.B. Gergon, 1994. The potential of increased nitrogen supply to mitigate growth and yield reductions of upland rice cultivar UPL Ri-5 caused by *Meloidogyne graminicola*. Fund Appl. Nematol., 17: 445-454.

14. Prot, J.C. and D.M. Matias, 1995. Effect of water regime on the distribution of *Meloidogyne graminicola* and other root- parasitic nematodes in a rice field toposequence and Pathogenicity of *M. graminicola on* rice cultivar UPL Ri 5. Nematologica. 11: 219-228.
15. Tandingan, I.C., J.C. Prot and R.G. Davide, 1996. Influence of water management on tolerance of rice cultivars for *Meloidogyne graminicola*. Fund. Appl. Nematol., 19: 189-192.
16. Soriano, I.R., J.C. Prot and D.M. Matias, 2000. Expression of tolerance for *Meloidogyne graminicola* in rice cultivars as affected by soil type and flooding. J. Nematol., 32: 309-317.
17. Padgham, J.L., J.M. Duxbury, A.M. Mazid, G.S. Abawi and M. Hossain, 2004. Yield loss caused by *Meloidogyne graminicola* on lowland rainfed rice in Bangladesh. J. Nematol., 36: 42-48.
18. Poudyal, D.S., R.R. Pokharel. S.M. Shrestha and G.B. Khari-Chetri, 2005. Effect of inoculum density of rice root-knot nematode on growth of rice cv. Masuli and nematode development. Australasian Pl Pathol., 34: 181-185.
19. Singh, K.P., R.K. Jaiswal. N. Kumar and D. Kumar, 2006. Biomass of nematodes and associated roots: A determinant of symptom production in root-knot disease of rice (*Oryza sativa* L.). J. Phytopathol., 154: 676-682.
20. Bridge, J., S.J. Page and S.W. Jordan, 1981. An improved method for staining nematodes in roots. Report, Rothamsted Experimental Station, U.K. pp: 171.
21. Andrassy, I., 1956. Die Rauminhalts - und Gewichtstimmung der Fadenwürmer (Nematoden). Acta Zool Acad Sci Hung 11: 1-15 (English translation).
22. Hussey, R.S., 1985. Host-parasite relationships and associated physiological changes. In: An Advanced Treatise on *Meloidogyne* Vol. 1 Biology and Control, International *Meloidogyne* Project (Eds Sasser JN, Carter CC) pp: 143-153.
23. Bird, A.F. and B.R. Loveys, 1975. The incorporation of photosynthates by *Meloidogyne javanica*. J. Nematol., 7: 111-113.
24. McClure, M.A., 1977. *Meloidogyne incognita*: a metabolic sink. J. Nematol., 9: 88-90.
25. Seinhorst, J.W., 1965. The relation between nematode density and damage to plants. Nematologica. 11: 137-154.
26. Brodie, B.B. and P.D. Dukes, 1972. The relationship between tobacco yield and time of infection with *Meloidogyne javanica*. J. Nematol., 4: 80-83.
27. Khan, H., R. Ahmad. A.S. Akhtar. A. Mahmood. T. Basi and T. Niaz, 2000. Effect of inoculum density of *Meloidogyne incognita* and plant age on the severity of root-knot disease in tomato. International. J. Agric. Biol., 2: 360-363.