

The rice root-knot nematode, *Meloidogyne graminicola*, on deep water rice (*Oryza sativa* subsp. *indica*)

John BRIDGE and Sam L. J. PAGE

ODA Tropical Plant Nematology Laboratory, Nematology Department,
Rothamsted Experimental Station, Harpenden, Heris, England.

SUMMARY

In a series of experiments designed to simulate deep water rice growing conditions, *Meloidogyne graminicola* Golden & Birchfield was found to survive in flooded soils and cause serious damage to cultivars of deep water rice from Bangladesh.

M. graminicola significantly reduced growth of deep water rice before flooding and to a greater extent after flooding. On submergence, the majority of plants (72%) infested with large numbers of the nematode were unable to grow above the water and were thus drowned out. By contrast, plants without nematodes had vigorous growth and all rapidly elongated above the flood water.

Juveniles did not invade rice roots growing in shallow or deep flooded soils, but they remained viable in the soils for at least 27 days and immediately infested roots once the water was removed. Nematodes that invaded roots before flooding developed and reproduced normally within the tissues for 36 or more days. Females laid their eggs mainly within roots and their progeny also remained within the roots producing new infection sites. A greater number of large galls were produced in flooded roots than in roots growing in well drained soils. *M. graminicola* was not found in flooded rice roots after five months at water depths of 29 cm and 1 m but survived in the flooded soils for this period and caused severe galling to rice planted immediately after the water was removed. Movement of nematodes was detected in the flood water. The life cycle of *M. graminicola* on rice in well drained soils was completed in nineteen days at an ambient temperature of 22-29°.

RÉSUMÉ

Le nématode galligène Meloidogyne graminicola, du riz flottant (Oryza sativa subsp. indica)

Dans une série d'expériences simulant les conditions de culture du riz en eau profonde, il a été observé que *Meloidogyne graminicola* Golden & Birchfield, survit dans les terrains inondés et cause de graves dégâts aux cultivars de riz flottants du Bangladesh.

M. graminicola réduit de façon significative la croissance du riz en eau profonde avant et, encore davantage, après la submersion. Une fois submergées, la majorité des plantes fortement infestées par le nématode sont incapables de dépasser la surface de l'eau et, en conséquence, sont noyées. Par contraste, les plantes non infestées croissent vigoureusement et dépassent rapidement la surface de l'eau.

Les juvéniles ne peuvent infester les racines du riz cultivé en eau profonde ou peu profonde, mais ils restent vivants dans le sol pendant au moins 27 jours et infestent immédiatement les racines une fois le terrain exondé. Les juvéniles qui infestent les racines avant la submersion se développent et se reproduisent normalement à l'intérieur des tissus pendant au moins 36 jours. Les femelles pondent leurs œufs principalement à l'intérieur des racines et leur descendance demeure dans celles-ci, causant de nouveaux centres d'infestation. Les grosses galles sont produites en plus grand nombre sur les racines submergées que sur les racines poussant dans des terrains bien drainés. Après cinq mois, on n'observe plus de *M. graminicola* sur les racines de riz croissant sous des profondeurs d'eau de 29 cm et 1 m, mais le nématode survit dans ces terrains inondés pendant cette période et cause de grosses galles sur le riz planté immédiatement après exondation. Le déplacement des nématodes a pu être détecté dans les eaux d'inondation. La durée du cycle de *M. graminicola* sur riz, dans des terrains bien drainés, est de dix-neuf jours à une température ambiante de 22 à 29°.



Fig. 1. Drowned out deep water rice in Bangladesh.

The rice root-knot nematode, *Meloidogyne graminicola* Golden & Birchfield, is recognised as one of the important pests of rice. It has been recorded from rice growing regions in Laos (Manser, 1968), India (Israel, Rao & Rao, 1963; Roy, 1973), Thailand (Buangsuwon *et al.*, 1971), U.S.A. (Golden & Birchfield, 1968; Yik & Birchfield, 1979), and Bangladesh (Hoque & Talukdar, 1971; Page *et al.*, 1979). It has been found mainly on rice growing in upland conditions and in nurseries (Buangsuwon *et al.*, 1971; Israel, Rao & Rao, 1963; Manser, 1968; Rao & Israel, 1971, 1972), and is reported to be absent when the rice crop is grown in flooded fields (Buangsuwon *et al.*, 1971; Manser, 1968) and to occur in small numbers in poorly drained soils (Rao & Israel, 1971). There have been few reports of *M. graminicola* or other *Meloidogyne* species infesting deep water rice apart from the observations that a species of *Meloidogyne*, referred to as *M. exigua*, occurs in the deep water rice region of Thailand (Hashioka, 1963; Kanjanasoon, 1962; Ou, 1972). However, in Bangladesh *M. graminicola* commonly caused galling of deep water rice roots in flooded conditions sometimes to a depth of 1.5 m during the early growth stages of the crop (Page *et al.*, 1979).

Deep water rice, also known as floating rice, is adapted to areas where deep flooding occurs and effectively there is no water control. It has an indeterminate growth habit and the capacity for rapid internode elongation when subjected to rising water levels or submergence, ensuring growth above the water level even when abrupt and deep flooding occurs. The major deep water rice growing areas are in the Ganges Delta of Bangladesh, the Mekong River Delta of Vietnam, the Irrawadi Delta in Burma, the Chao Phaya Delta in Thailand, Indonesia and other delta areas of S.E. Asia. Bangladesh is

the biggest producer of the crop with over 1.8 million ha under deep water cultivation (Kanter, 1978).

In Bangladesh we observed that *M. graminicola* was associated with yellowing and stunting of deep water rice (Page *et al.*, 1979). It was also associated with drowning out when many plants remain submerged and die after rapid and deep flooding (Fig. 1). This latter association, and aspects of the biology and survival of the nematode in flooded conditions, were investigated.

The isolate of *M. graminicola* used in these experiments was collected from deep water rice roots in Bangladesh and cultivated on rice in a heated glasshouse. All seed of the rice cultivars used was obtained from Bangladesh.

Effect of *M. graminicola* infestation on growth and elongation of deep water rice before and after flooding

MATERIALS AND METHODS

Seeds of deep water rice cv. Habiganj VIII were germinated in water and then grown singly in steam sterilised clay loam in 9 cm pots for three weeks. Active juveniles of *M. graminicola* were extracted from infested rice roots left on 90 μ m nylon sieves in Petri dishes of water for 48 hours. 4 000 second-stage juveniles of *M. graminicola* were introduced into the soil around each rice seedling in 22 pots. Ten ml of decanted water from nematode extractions, checked to be free of nematodes, was added to soil around seedlings in each of a further eighteen pots which were left as controls. All pots were arranged in a fully randomised design in a heated glasshouse at 21 to 36° and watered normally for five weeks. The pots were then submerged to a depth of 50 cm above soil level in large 100 l plastic bins. Eighteen plants with nematodes and eighteen without nematodes were fully randomised in three bins and NPK fertiliser was added to the water. The four extra plants with nematodes were used to estimate nematode populations in roots before flooding. Heights of plants to the tip of the tallest leaf, were measured fourteen days before and at flooding. The water in the bins was maintained at 50 cm above soil level for eighteen days before the pots were removed from the water. Plants growing erect above the water surface were noted, and all the plant heights, fresh root weights and leaf dry weights were measured. Nematodes were examined directly in roots after staining in boiling 0.05-0.1% acid fuchsin in a 1 : 1 : 1 glycerol/lactic acid/distilled water solution. Roots were cleared in a solution of equal parts of glycerol and distilled water and nematodes counted.

Table 1
Effect of *Meloidogyne graminicola* on growth and elongation
of deep water rice (cv. Habiganj VIII) before and after flooding

Treatment	Plant height (cm) ⁽¹⁾			Plants emerging above water (%) 18 days after flooding	Top dry weight (g) 18 days after flooding
	i) 14 days before flooding	ii) At flooding	iii) 18 days after flooding		
Untreated	47.3	60.5	103.2	100	0.43
Plants infested with <i>M. graminicola</i>	41.9 N.S.	48.1 **	54.1 ***	27.8	0.19 **

⁽¹⁾ Means of 18 plants. N.S.: Not significant, ** Significantly different ($P < 0.01$), *** Significantly different ($P < 0.001$).

RESULTS

M. graminicola significantly reduced ($P < 0.01$) growth of deep water rice plants in upland conditions before flooding and the reduction in growth was even more marked ($P < 0.001$) during flooding (Tab. 1). Rice plants with nematodes had severely galled roots but there was no significant difference in fresh root weight between infested and control roots. Mean root populations of *M. graminicola* were 339 females/g root before flooding and 490 females/g root eighteen days after flooding. All rice plants without nematodes grew very rapidly after submergence in water. They increased in height by as much as 55 cm in eighteen days and they all emerged above the water level and had healthy, erect growth. In contrast, plants infested with *M. graminicola* generally grew slowly and only five of the eighteen plants (27.8%) emerged above the water level during flooding (Fig. 2). Plants remaining submerged had flaccid and decaying leaves with no new growth and most were either dead or dying after the eighteen days under water. It was thus confirmed that the drowning out of the deep water rice crop in Bangladesh can be caused by *M. graminicola*.

Invasion in flooded rice roots

MATERIALS AND METHODS

Seeds of deep water rice cv. Habiganj II were germinated in water and planted singly into steam sterilised soil in 9 cm pots. When seedlings were well established they were immersed in water for a period

of 44 days to stimulate rapid growth to a height greater than 50 cm. Pots were removed from the water and allowed to drain. All pots were inoculated with 1 000 *M. graminicola* juveniles/pot into holes around the rice roots and covered with additional sterilised soil. The pots were then immediately separated into three treatments with 24 pots per treatment. Treatments were, 1 : shallow flooding-pots immersed in water to a depth of 10 cm above soil level, 2 : deep flooding-pots immersed in water to a depth of 50 cm above soil level, and 3 : well drained soils watered normally. All treatments were maintained for 27 days at a temperature of 22-29° in a heated glasshouse. Pots were removed from each treatment every two to four days, roots were washed free of soil, stained, and examined for nematodes.

RESULTS

There was no invasion of flooded rice roots when *M. graminicola* juveniles were introduced into soils immediately before flooding and no nematodes were found in either shallow or deep flooded roots over a nineteen day period. In well drained soils, *M. graminicola* invaded roots and completed its life cycle within nineteen days at an ambient temperature of 22-29°; third and fourth-stage juveniles were present in roots after eight days, immature females after twelve days, mature females with eggs after fifteen days, and mature females with embryonated eggs and second stage juveniles by nineteen days.

To test if viable second stage juveniles were still present in the soils after 27 days flooding, remaining rice plants and soils from shallow and deep flooded

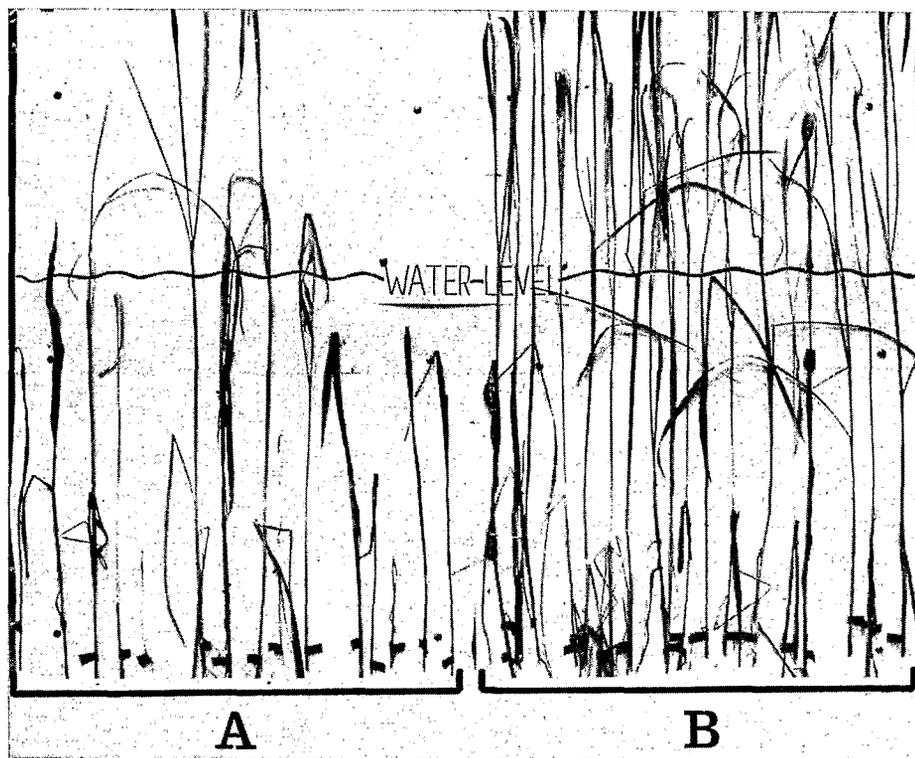


Fig. 2. Emergence of deep water rice plants above water level after 18 days of flooding. A : Plants infested with *M. graminicola*. B : Untreated plants.

treatments were removed from the water and drained. Juveniles invaded the roots and mature females had developed in roots of both treatments after fifteen days.

Development in shallow flooded roots

MATERIALS AND METHODS

Seeds of deep water rice cv. Habiganj VIII were germinated in water and planted singly into steam sterilised soil in 9 cm pots. Suspensions of 700 *M. graminicola* juveniles/pot were introduced around eleven day-old seedlings in all pots which were left for one week on a heated glasshouse bench. Half of the pots were immersed in bowls of water giving a depth of 10 cm water above soil level. The remaining pots were left on the bench and watered normally. Pots and bowls were arranged in randomised blocks and kept in an ambient temperature of 21 to 36°. Three pots were removed from each treat-

ment every seven or eight days ; roots were washed free of soil, weighed and stained. The different development stages of *M. graminicola* were identified and counted in the whole root system of each plant.

RESULTS

When nematodes invaded rice roots before flooding, development of *M. graminicola* continued for at least 36 days submergence in 10 cm of water (Tab. 2). Females laid their eggs mainly within root tissues, and lines of hatched juveniles were often observed in the root tissue leading away from the galls. There were almost four times the number of root galls greater than 0.5 cm in length on roots growing in flooded compared to well drained soils. There was some initial delay in population increase of nematodes in flooded roots compared to those in well drained soils up to 22 days, but numbers rapidly increased in submerged roots and high numbers of females with embryonated eggs were found in root tissues from both treatments after 36 days.

Table 2
Development of *M. graminicola* in flooded rice roots

Days after flooding	Treatment	Nos. of nematode stages/root system ⁽¹⁾			
		Juveniles	Immature ♀♀	Mature ♀♀	Mature ♀♀ + eggs
8	a. Well drained	0	0	<1	14
	b. Flooded	1	<1	1	11
22	a. Well drained	56	17	9	19
	b. Flooded	6	3	6	7
36	a. Well drained	14	18	17	394
	b. Flooded	80	87	86	500

(¹) Means of 3 replicates.

Survival in deep water rice soils

MATERIALS AND METHODS

A galvanised water tank 1.83 m deep was used to simulate deep water rice growing conditions. The tank was painted internally with non-toxic paint and fitted with a submerged heating coil and water circulator. It was set in a heated glasshouse and water in the tank was maintained at 28-31° throughout the experiment. Deep water rice seeds cv. Habiganj II were germinated in water before planting out singly into 15 cm pots in heat sterilised clay loam. Pots were placed in perforated trays and suspended over the tank with their bases just submerged in the water. Soils with eight day old rice seedlings were inoculated with 2 000 juveniles of *M. graminicola* per pot in each of four treatments with seven replicates per treatment. Seven pots without nematodes were included as controls in each treatment. All pots were left on the surface of the water for one week after inoculation of nematodes and then, over a three week period, pots of some treatments were gradually lowered into the water. Four treatments were established on the basis of soil depths in water, 1 : soil level above surface of water, 2 : soil level just below water surface, 3 : at a depth of 29 cm, and 4 : at a depth of 1 m. The pots were lowered at the rate of growth of the rice, which was stimulated by flooding, and upper leaves always remained above water level. NPK fertiliser was added to the water in the tank at commencement of the experiment. Rice plants were left at their different depths in the water for five months until maturity. The pots were then lifted from the

tank and roots were carefully removed leaving the soil in the pots. Roots were washed free of adhering soil, weighed and stained, and numbers of mature female nematodes in the roots counted.

All the pots containing soil from the deep water tank were allowed to drain and then planted to seedlings of rice cv. BR3, sown as a pre-flood crop in Bangladesh. They were arranged in a fully randomised design on a bench in a heated glasshouse at a temperature of 26-32° and watered normally. After six weeks, roots were removed from the pots, washed free of soil, weighed and stained, and numbers of female *M. graminicola* counted.

RESULTS

Rice plants at 1 m depth had become detached from their anchor roots in the pots and had assumed their natural floating habit. Roots remaining in these pots were old anchor roots and some nodal roots.

No *M. graminicola* were found in flooded rice roots after five months submergence in water depths of 29 cm and 1 m (Tab. 3). Few nematodes were found in roots from saturated soils or in roots growing in soils just below the water surface. Although no nematodes were present in deep flooded rice roots many had survived in the soil. When soils were removed from the water, planted to rice and kept well drained for six weeks, large numbers of *M. graminicola* developed in all the roots, including those growing in soils which had previously been submerged in 1 m of water (Tab. 4), causing severe root galling in all treatments (Fig. 3). *M.*

graminicola was also recovered from the rice roots growing in some of the soils which had not been inoculated with nematodes showing that there was movement of nematodes in the flood water, although not in all treatments (Tab. 4). This uneven movement could be accounted for by the pattern of water circulation in the tank.

Table 3
Numbers of female *M. graminicola* per g root of flooded deep water rice cv. Habiganj II after five months submergence ⁽¹⁾

Treatment	Depth of water above soil level			
	Above surface	1 cm	29 cm	1 m
Plants infested with <i>M. graminicola</i>	18.2	2.8	0	0
Control	3.2	0	0	0

⁽¹⁾ Means of 7 replicates.

Table 4
Numbers of female *M. graminicola* per g root of rice cv. BR 3 after 6 weeks in well drained soils previously flooded for five months ⁽¹⁾

Treatment	Previous depths of water above soil level			
	Above surface	1 cm	29 cm	1 m
Plants infested with <i>M. graminicola</i>	241	190.3	93.7	411
Control	54.9	0	42.4	1

⁽¹⁾ Means of 7 replicates.

Discussion

Our results have shown that *M. graminicola* is a serious pest of flooded rice and it is well adapted to the flood conditions prevailing in the deep water rice areas. The behaviour of *M. graminicola* does appear to be modified once flooding occurs. If

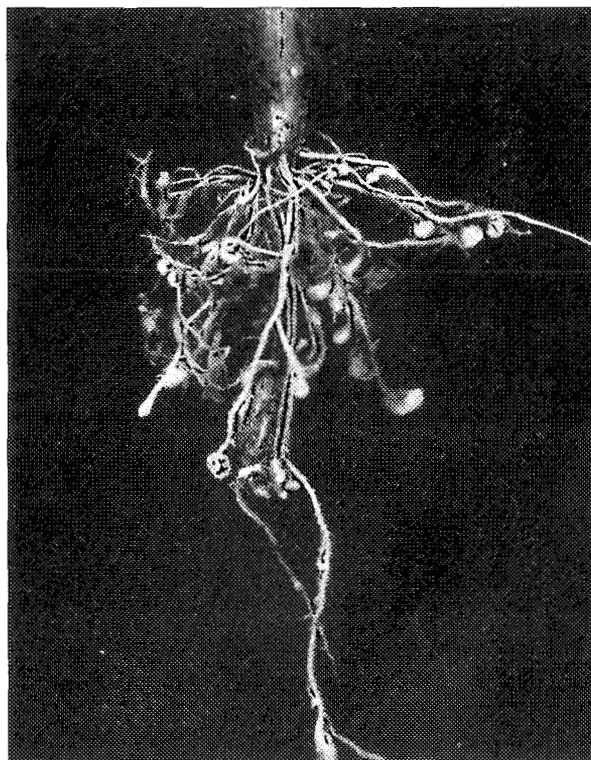


Fig. 3. *M. graminicola* galls on cv. BR 3 rice roots.

nematodes invade roots before flooding, they can develop and reproduce within the flooded roots. In Bangladesh, *M. graminicola* was present in deep water rice roots that had been under water for three to six weeks (Page & Bridge, 1978). Rice roots growing in flooded soil are not reinvaded by *M. graminicola*, which accounts for the apparent disappearance of *M. graminicola* in mature plants of deep water rice. The majority of eggs are laid within the root tissues and juveniles hatch and remain in the maternal gall or migrate intercellularly through the aerenchymatous tissues of the cortex to new feeding sites within the roots. Flooded rice roots have a greater number of large galls compared to roots growing in well drained soils. As feeding sites in the primary roots become exhausted, nematodes released into the flooded soil do not invade new roots. However, these second stage juveniles remain viable and survive flooding for a minimum of four weeks and can invade roots when the standing water recedes or is removed. Large populations of *M. graminicola* can survive in the soil, probably both as eggs and juveniles, for at least five months under deep water. Manser (1968) observed in Laos that *M. graminicola* is usually

absent from irrigated rice plants under constant irrigation but, should the irrigation stop, newly formed roots will become galled. Movement of viable eggs or juveniles does occur in flood water, as shown by the presence of nematodes in roots growing in untreated control soils in deep water. This indicates that the nematode will be rapidly and widely disseminated in the open flood waters of deep water rice areas, making any attempts to restrict their spread very difficult. In our experiments, the life cycle (J2 to J2) was nineteen days at 22-29°. A life cycle of 23-27 days at 26° has been recorded with a U.S.A. isolate of *M. graminicola* (Yik & Birchfield, 1979), and an isolate from India took 26-51 days according to the time of year (Patnaik, 1969).

There is clear evidence from our studies and earlier observations in Bangladesh that *M. graminicola* can cause substantial losses of deep water rice by preventing emergence above water once they have become submerged during abrupt flooding. It appears that root infection in the early stages of growth prevents the vigorous growth needed to avoid drowning out as was suggested in an earlier paper (Page *et al.*, 1979). Although plants infested with *M. graminicola* were shorter than the controls before flooding, the depth of submergence in water was comparatively moderate and growth or survival of the rice plants with nematodes in water was not related to the height of plants at flooding. Deep water rice can normally withstand submergence for a limited period and some varieties grow as fast as 25 cm per day (Kanter, 1978). The mean increase in height of plants without nematodes during flooding was 43 cm with a maximum of 55 cm. Increasing the water depth over the period would have stimulated greater growth.

Drowning out of deep water rice is likely to be most serious when rapid flooding occurs early in the monsoon period. In some areas in Bangladesh deep water rice is sown directly in well drained soils and often has to withstand drought for long periods until the onset of flooding which may be eight weeks or more after sowing. This allows time for populations of *M. graminicola* to build up in rice roots and drought conditions have been shown to increase numbers of the nematode (Rao & Israel, 1971). Our results indicate that rice raised in flooded soils would escape invasion by *M. graminicola*. Submergence can occur at any time during the growing season but plants subjected to a gradual rise in flood levels will produce adventitious nodal roots that absorb nutrients from the flood waters compensating for any nematode damage to the primary, anchor roots. It is unlikely that losses as high as the 72% that we experienced will always

be associated with *M. graminicola* in the field because root populations vary considerably (Page & Bridge, 1978), however, the nematode is potentially a very important pest of deep water rice which has hitherto gone unrecognised. In Bangladesh the nematode was found in 30% of deep water rice fields sampled (Page *et al.*, 1979). Other root nematodes found on deep water rice in Bangladesh, such as *Hirschmanniella oryzae* and *Tylenchorhynchus annulatus* (Page *et al.*, 1979) could have a similar effect on the growth or elongation capacity of the plants.

ACKNOWLEDGEMENTS

The authors thank the staff of the Deep Water Rice Pest Management Project BRRI, Bangladesh for their help in this work, and Sunniva Jordan and Mr. A.J. Callewaert for their valuable technical assistance. This work was carried out under MAAF Licence No. PHF 26/61 and 62 issued under the Import and Export (Plant Health) (Great Britain) Order 1980 and the Plant Pests (Great Britain) Order 1980, and was supported by the U.K. Overseas Development Administration.

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Accepté pour publication le 4 janvier 1982.