Russian Journal of Nematology, 2015, 23 (2), 99 – 112

Effect of different water regimes on nematode reproduction, root galling, plant growth and yield of lowland and upland Asian rice varieties grown in two soil types infested by the rice root-knot nematode *Meloidogyne graminicola*

Pa Pa Win[1], Pyone Pyone Kyi[1], Zin Thu Zar Maung[2], Yi Yi Myint[3] and Dirk De Waele[4, 5, 6]

[1]**Plant Protection Division, Department of Agriculture, Ministry of Agriculture and Irrigation, Bayint Naung Road, West Gyogone, P.O. Box 1011, Insein, Yangon, Myanmar**
[2]**Agricultural Nematology Laboratory, 256 Giltner Hall, Michigan State University, 48823, East Lansing, MI, USA**
[3]**Department of Plant Pathology, Yezin Agricultural University, Yezin, Myanmar**
[4]**Laboratory of Tropical Crop Improvement, Department of Biosystems, Faculty of Bioscience Engineering, University of Leuven (KU Leuven), Willem de Croylaan 42, B-3001, Heverlee, Belgium**
[5]**Unit for Environmental Sciences and Management, North-West University, Private Bag X6001, 2520, Potchefstroom, South Africa**
[6]**Crop and Environmental Sciences Division, International Rice Research Institute (IRRI), DAPO Box 7777, Metro Manila, Philippines**
e-mail: dirkdewaele@pandora.be

Accepted for publication 21 October 2015

Summary. In a screenhouse experiment, plants of the lowland rice variety Thihtayin and the upland rice variety Kone Myint 2 were grown in two soil types (clay loam and sandy loam), inoculated with 3,000 *Meloidogyne graminicola* second-stage juveniles (J2) per plant and from 6 weeks onwards maintained until harvest under three water regimes: permanently flooded, intermittently flooded and upland (monsoon rainfed) conditions. Both varieties were susceptible to *M. graminicola* infection under all three water regimes and in both soil types but differences in susceptibility were observed between the two varieties and among the treatments. The effect of water regime on the number of eggs and J2 of *M. graminicola* inside the roots was lower than expected: with one exception no significant effects were observed of any of the water regimes on the root population density in both rice varieties in both soil types. This observation may be explained by the delayed flooding, which started 6 days after nematode inoculation for the permanent and intermittent flooding water regimes. In both varieties and in both soil types, the root galling index was significantly lower on permanently flooded plants (< 4.5) compared with plants that had been either intermittent flooded or grown under upland conditions (≥ 5.0). The highest root galling indices were always observed on plants grown under upland conditions (7.0-8.5). Permanent flooding prevented the suppression of most plant growth and yield-contributing traits measured. Moreover, permanent flooding also prevented significant yield loss in plants of both varieties grown in the clay loam soil and in plants of variety Thihtatyin grown in the sandy loam soil. The results of our study confirm again the enormous impact *M. graminicola* infection can have on the yield of both lowland and upland rice varieties. With the exception of one treatment, yield loss was always higher than 20% and even almost 100% (yield failure) in plants of both varieties grown in the sandy loam soil under upland conditions. Although yield losses caused by nematodes carried out under screenhouse experiments tend to result in an overestimation of these losses, the results of our screenhouse experiments show that yield losses caused on Asian rice by *M. graminicola* must be very high also under field conditions in the farmer's fields.

Key words: damage, intermittently flooded, multiplication factor, permanently flooded, plant growth traits, root galling, sensitivity, susceptibility, tolerance, upland conditions, yield-contributing trait, yield loss.

The rice root-knot nematode, *Meloidogyne graminicola* Golden & Birchfield, 1965, is considered to be by far the most damaging *Meloidogyne* species on Asian rice (*Oryza sativa* L.) (De Waele & Elsen, 2007). It occurs in all South Asian and Southeast Asian rice producing countries surveyed so far (Jain *et al.*, 2012), and can infect equally well lowland, upland and deepwater rice, and irrigated and rainfed rice (Bridge *et al.*, 2005). *Meloidogyne graminicola* is exceptionally well adapted to flooded conditions enabling it to continue multiplying in the host tissues even when the roots are deeply submerged in water (Fernandez *et al.*, 2014). Second-stage juveniles (J2) invade rice roots in upland conditions (*i.e.*, free draining, without surface water accumulation) just behind the root tip (Rao & Israel, 1973). They cannot invade rice in flooded conditions but quickly invade when infested soils are drained (Manser, 1968). Females develop within the roots and eggs are laid mainly in the cortex (Roy, 1976). The J2 of *M. graminicola* can remain in the maternal gall or migrate intercellularly through the aerenchymatous tissues of the cortex to new feeding sites within the same root (Bridge & Page, 1982). At 29/26°C, a *M. graminicola* population from the Philippines completed its life cycle on the susceptible Asian rice variety UPLRi-5 under simulated flooded conditions in 19 days (Fernandez *et al.*, 2014).

The population build up of nematode species on agricultural crops is not only influenced by the host response, but also by the environment in which the crop is growing, especially soil temperature, moisture and texture, and agronomic practices (Wallace, 1973; Trudgill & Phillips, 1997). In the case of *M. graminicola* it has been shown that soil moisture (and thus the water regime applied) is the most important environmental factor influencing especially the penetration of rice roots by this nematode species and thus the yield response (Plowright & Bridge, 1990; Prot & Matias, 1995; Tandingan *et al.*, 1996; Soriano *et al.*, 2000). Soil moisture even influenced the sensitivity (tolerance) of Asian rice varieties to damage and yield loss caused by *M. graminicola* (Tandingan *et al.*, 1996; Soriano *et al.*, 2000).

The influence of soil texture on the occurrence of *M. graminicola* is less documented. It has been reported that *M. graminicola* infects and damages a higher percentage of rice roots in light soils (sandy, sandy loam) compared with heavier soils (clay loam, clay) partly because of the greater ability of J2 of *Meloidogyne* spp. to migrate in a sandy soil than in a clay soil (Prot & Van Gundy, 1981; Prot & Matias, 1995). However, in Myanmar, even in clay loam and clay soils, the frequency of occurrence of *M. graminicola* in Asian rice roots and the root galling severity it caused were high (Win *et al.*, 2011).

In many areas in rice producing Asian countries, water is no longer easily available to keep the rice fields continuously flooded during the dry season since water for agricultural use is less and less available due to climate change and urbanisation (Bouman *et al.*, 2002; Tuong & Bouman, 2003). This water shortage process is further influenced by the increasing labour costs for irrigation and transplanting rice plants in the lowland rice ecosystems (De Waele & Elsen, 2007; Farooq *et al.*, 2011). Also, the development of early-maturing rice varieties and improved nutrient management techniques along with the increased availability of chemical weed control methods have encouraged many farmers in South Asia and Southeast Asia to switch from transplanting nursery-grown rice seedlings to direct wet seeding by broadcasting pre-germinated (in plastic bags) seeds (Farooq *et al.*, 2011) followed by delayed irrigation. These practices may favour the prevalence of *M. graminicola* and increase the economic significance of this highly pathogenic nematode species (Win *et al.*, 2011).

For about one decade, different agronomic practices have been developed to alleviate the water shortage problem in the Asian rice fields, such as intermittent irrigation, alternate wetting and drying, the cultivation of aerobic rice varieties (Bouman *et al.*, 2002). Therefore it is important to quantify the influence of water regime using less water on the damage potential of *M. graminicola* on rice. In this study, a screenhouse experiment was carried out to evaluate the effect of three water regimes on the damage and yield loss potential of *M. graminicola* on a commonly cultivated lowland (Thihtatyin) and upland (Kone Myint 2) Asian rice variety in two soil types.

MATERIALS AND METHODS

The experiment was carried out at the campus of the Plant Protection Division, Yangon, from December 2010 until April 2011 (*i.e.*, during the summer-irrigated rice growing season). The variety Thihtatyin was included in the experiment because it is the most commonly cultivated rice variety in clay soils in the summer-irrigated lowland rice ecosystem in Myanmar. Also, it had the highest prominence value of *M. graminicola*, and the highest root galling index during a nematological survey conducted in 2009 (Win *et al.*, 2011). Moreover, variety Thihtatyin is also cultivated in the lowland rice ecosystem in the Central part of Myanmar which has a lighter soil texture. The improved upland variety Kone Myint 2 was included in the experiment because it is the most commonly cultivated rice variety in sandy loam soils in the monsoon rainfed upland rice ecosystem in Myanmar (Win *et al.*, 2011). It is also able to grow under lowland conditions in a clay loam soil. The prominence value of *M. graminicola* on this variety observed during the above mentioned nematological survey (Win *et al.*, 2011) was < 1, while no root galling was observed.

Preparation of plants. Seeds of the varieties Thihtatyin and Kone Myint 2 were obtained from the Myanmar Rice Research Centre, Hmawbi, and the Aung Ban Research Farm, Shan State, respectively. The characteristics of these two Asian rice varieties are listed in Table 1. The seeds were first soaked in water overnight and pre-germinated on wet paper in Petri dishes at room temperature.

Nematode inoculum and inoculation. The nematode inoculum consisted of the offspring of a single *M. graminicola* female isolated from an irrigated Asian rice plant (variety unknown) in Pathein region, Ayeyarwady Delta (Lower Myanmar) and multiplied on the rice variety Thihtatyin under upland conditions (*i.e.*, field capacity) in a so-called sick plot at the campus of the Plant Protection Division, Yangon. Galled roots infected with *M. graminicola* were chopped into approximately 1-cm-pieces, macerated in a kitchen blender twice for 10 s and the J2 extracted from the resulting homogenate using the tray method (Whitehead & Hemming, 1965). Only freshly extracted J2 (*i.e.*, collected during a 24 h period) were used as inoculum.

Treatments and experimental set-up. To simulate the farmer's field conditions, the two soil types used were a clay loam and a sandy loam soil. The clay loam soil (42% clay, 25% loam, 32% sand) contained 0.18% nitrogen, 24.3 ppm P, 9.2 mg (100 g)$^{-1}$ K_2O at 5.8 pH. The sandy loam soil (10% clay, 13% loam, 75% sand), contained 0.16% nitrogen, 26.9 ppm P, 9.7 mg (100 g)$^{-1}$ K_2O at 5.6 pH. Three-day-old pre-germinated seeds were singly planted in 17-cm-diam. × 22-cm-high pots containing 1,500 ml of sterilised soil leaving 5 cm on the top for watering the plants. The soil in the pots was saturated (*i.e.*, 100% of the soil pore volume filled with water) at planting and at inoculation.

At the time of planting of the pre-germinated rice seeds in the saturated soil, six plants of each treatment of each rice variety were inoculated with 3,000 *M. graminicola* J2 per plant by pipetting three aliquots of the same volume in three 5-cm-deep holes around the base of the seedlings. Six plants of each treatment of each rice variety were not inoculated and acted as control plants. The pots were placed in a screenhouse at an air temperature ranging from 26 to 38°C. From 6 days after inoculation of the rice seedlings onwards, the pots were maintained under the following three water regimes until maturity (harvest) of the rice plants: *i*) permanently flooded: the soil in the pots was flooded to 5 cm above the soil surface and a layer of 3 to 5 cm standing water maintained by daily watering; *ii*) intermittently flooded: the soil in the pots was flooded to 3 cm above the soil surface and irrigated 3 times per week so that the soil was alternatively flooded, saturated and at field capacity; and *iii*) upland (monsoon rainfed): the soil in the pots was maintained at field capacity by watering as necessary.

All combinations of rice variety, water regime, soil type and nematode inoculum level were laid out in a split-split-split plot design with six replications. The two rice varieties (Thihtatyin and Kone Myint 2) were considered as main plots. The two soil types (clay loam and sandy loam) as subplots. The three water regimes (permanently flooded, intermittently flooded and upland) as sub-subplots. The two nematode inoculum levels (0 and 3,000 J2 pot^{-1}) as sub-sub-subplots.

Assessment of plant growth, yield-contributing traits and yield. The experiment was terminated when the panicles of each rice variety were mature and ready to harvest. Since the varieties matured at different times, they were harvested at different times. Non-inoculated plants of the same variety matured earlier than inoculated plants in all experiments. At harvest, the plants were carefully removed from the soil and the following three plant growth and six yield-contributing traits measured: plant height, fresh root weight, dry shoot weight, number of tillers per plant, number of panicles per plant, number of filled grains per panicle, percentage filled grains per plant, filled grain weight per plant and weight of 1,000 filled grains. The yield was estimated according to Yoshida (1981): grain yield (t ha^{-1}) = number of panicles m^{-2} × number of spikelets per panicle × percentage filled spikelets × 1,000 grain weight (g) × 10^{-7}. "Spikelets" included all filled, partially filled and unfertilised spikelets. Filled spikelets are called "grains". The number of panicles m^{-2} was calculated as the number of plants m^{-2} × number of panicles per plant. The grain yield was measured at 14% moisture content.

Assessment of *Meloidogyne graminicola* population densities and severity of root galling. At harvest, the rhizosphere soil of each plant was collected, mixed and the J2 were extracted from one 100 ml soil sub-sample using the tray method (Whitehead & Hemming, 1965). The roots from each up-rooted plant were chopped into approximately 1-cm-pieces and thoroughly mixed. One sub-sample of 3 g roots was macerated in a kitchen

Table 1. Characteristics of the lowland and upland rice varieties included in the experiment

Rice variety	Original name	Crop cycle (days)	Plant height (cm)	Grain yield (t ha^{-1})	Lowland or upland variety	Variety type
Thihtatyin	IR 13240-108-2-2-3	115	85-95	5-6	lowland	HYV[1]
Kone Myint 2	local	140-145	105-110	1.5-2.5	upland	traditional

[1] HYV: high-yielding variety.

blender twice for 10 s and the nematodes extracted from the resulting homogenate using the tray method (Whitehead & Hemming, 1965). After 24 h, the J2 that had moved through the sieve into the water were collected and concentrated in a 50-ml suspension. The same day, two 2-ml sub-samples were examined using a stereomicroscope and the J2 counted. The number of eggs in the sub-samples was also determined by re-extracting the eggs from the roots left on the tray sieve using a modified sodium hypochloride (NaOCl) extraction method (Stetina *et al.*, 1997). The roots were macerated in 0.5% NaOCl in a kitchen blender for 10 s. Then, the root suspension was passed through a series of 250-, 106- and 25-μm pore sieves. The eggs collected on the 25-μm pore sieves were counted using a stereomicroscope. The population densities of *M. graminicola* were calculated as the number of J2 (100 ml soil)$^{-1}$, J2 (g root)$^{-1}$, eggs (g root)$^{-1}$, J2 (root system)$^{-1}$ and eggs (root system)$^{-1}$. The final nematode population density was calculated as the number of J2 in the soil in the pots (1,500 ml) + J2 in the root system. The nematode muliplication factor (Mf^{-eggs}) was calculated as the final J2 population density/3,000 J2.

The severity of galling (root galling index) was visually assessed for each up-rooted plant by rating the percentage of roots with root tip galls on a 0-10 scale according to the rice root-knot nematode rating chart of Bridge & Page (1980).

Analysis of data. The data were analysed using STATISTICA 11.0 software (StatSoft Inc., Tulsa, USA). Prior to analysis of variance, plant growth and yield data, and nematode population densities were log(x+1) transformed while percentage filled grains per plant and root galling indices were arcsin(x/100) transformed to meet the assumptions of ANOVA (*i.e.*, normality and homogeneity of variances). The Shapiro-Wilk test was used to examine whether the dependent variable was normally distributed within groups while the homogeneity of the variances of the groups was tested with the Levene's test. The outliers were determined by calculating the standardised residuals falling outside the range from –2 to +2. When the assumptions for ANOVA were met, the data were analysed by using ANOVA. One-way ANOVA using Tukey's HSD test or t-test were performed for mean comparisons of root galling indices and nematode population densities, plant growth, yield-contributing traits and yield. Mean numbers are shown in the tables, after back-transforming the data, to facilitate interpretation.

RESULTS

***Meloidogyne graminicola* population densities.** *Meloidogyne graminicola* reproduced and multiplied on both rice varieties under all water regimes and in both soil types (Table 2).

Among the three water regimes, the soil population density of variety Thihtatyin was significantly ($P \leq 0.05$) higher under permanent flooding in the sandy loam soil (198 J2 (100 ml soil)$^{-1}$) compared with intermittent flooding and upland conditions (2 and 11 J2 (100 ml soil)$^{-1}$, respectively) in the same soil type but this was not the case in the clay loam soil. Among the three water regimes, the soil population density of variety Kone Myint 2 was significantly ($P \leq 0.05$) higher under permanent and intermittent flooding in both the clay loam and the sandy loam soil compared with upland conditions (a few hundred J2 *vs* a few tens J2 (100 ml soil)$^{-1}$). Under all three water regimes, except under upland conditions in the clay loam soil, the soil population densities of variety Kone Myint 2 were significantly ($P \leq 0.05$) higher compared with variety Thihtatyin.

Water regime did not influence the root population densities of either variety Thihtatyin or variety Kone Myint 2, except the number of J2 (g root)$^{-1}$ of variety Kone Myint 2 in the sandy loam soil. The number of J2 (g root)$^{-1}$ of variety Kone Myint 2 in the sandy loam soil under upland conditions was significantly ($P \leq 0.05$) higher compared with permanent flooding (11,013 *vs* 2,336 J2 (g root)$^{-1}$).

The soil and population densities of J2 and eggs on variety Thihtatyin were never significantly different between the two soil types under permanent and intermittent flooding. Sometimes the highest population density of J2 and eggs was observed

Table 2. Effect of water regime and soil type on the soil and root population densities of *Meloidogyne graminicola*, severity of root galling (RGI), and nematode multiplication factor (Mf^{-eggs}) of the lowland rice variety Thihtatyin and the upland rice variety Kone Myint 2

Water regime	Thihtatyin (V1)		Kone Myint 2 (V2)		Difference (V1-V2)	
	Clay loam (S1)	Sandy loam (S2)	Clay loam (S1)	Sandy loam (S2)	Clay loam	Sandy loam
	J2 (100 ml soil)$^{-1}$					
Permanent flooding	126±109[1] a[3] A[4]	198±225 a A	417±361 a A	398±226 a A	–291*	–200*
Intermittent flooding	47±17 a B	2±1 b A	1,016±369 a A	363±194 a A	–969*	–361*
Upland	40±39 a B	11±4 b A	33±7 b B	47±5 b A	+7	–36*
	J2 (g roots)$^{-1}$					
Permanent flooding	9,039±8,820 a A	11,761±10,257 a A	5,500±2,270 a A	2,336±2,492 a A	+3,529	+9,425
Intermittent flooding	12,060±5,649 a A	8,058±1,099 a A	5,718±3,664 a A	6,378±4,948 a b A	+6,342	+1,680
Upland	11,457±5,724 a B	28,560±24,988 a A	6,720±3,165 a B	11,013±1,324 b A	+4,737	+17,547*
	J2 (root system)$^{-1}$					
Permanent flooding	288,858±301,402 a A	408,563±363,634 a A	125,363±51,454 a A	30,031±35,508 a A	+163,495*	+378,532
Intermittent flooding	168,667±164,512 a A	60,043±10,190 a A	85,851±57,057 a A	45,728±19,815 a A	+82,816	+14,315
Upland	131,932±102,168 a B	212,719±173,603 a A	62,888±45,986 a B	97,710±5,328 a A	+69,044	+115,009*
	Eggs (g roots)$^{-1}$					
Permanent flooding	17,674±14,725 a A	10,915±9,343 a A	9,605±4,189 a A	13,436±15,173 a A	+8,069	–2,521
Intermittent flooding	15,929±15,880 a A	5,831±2,330 a A	12,825±5,390 a A	8,257±2,512 a A	+3,101	–2,426
Upland	5,430±3,003 a B	14,260±13,538 a A	12,513±4,195 a B	19,597±983 a A	–7,083	–5,334
	Eggs (root system)$^{-1}$					
Permanent flooding	339,661±294,835 a A	375,908±334,440 a A	230,408±122,527 a A	337,615±408,265 a A	+109,253*	+38,293
Intermittent flooding	274,274±334,911 a A	41,922±8,768 a A	164,816±109,858 a A	89,814±32,955 a A	+109,458	–47,892
Upland	75,196±50,562 a B	108,192±73,001 a A	110,863±83,207 a B	180,268±7,646 a A	–35,667	–72,076
	Root galling index[2] (RGI)					
Permanent flooding	3.5±1.9 b A	4.3±1.2 b A	3.0±1.2 b A	2.3±1 b A	+0.5	+2
Intermittent flooding	5.0±0.8 a A	7.3±1.0 a A	6.7±1.4 a A	7.3±0.8 a A	–1.7	0
Upland	7.5±0.4 a A	8.6±0.5 a A	6.9±1.3 a A	7.9±0.7 a A	+0.6	+0.7
	Mf^{-eggs}					
Permanent flooding	96.9	137.2	43.9	12.0	+53.0	+125.2
Intermittent flooding	56.5	20.0	33.7	17.1	+22.8	+3.0
Upland	44.2	71.0	31.1	32.8	+23.0	+38.2

[1] Data represent means ± SD (n = 6).
[2] Root galling index according to 0 = no swellings or galls, 1 = 10% galls, 2 = 20% galls, 3 = 30% galls, 4 = 40% galls, 5 = 50% galls, 6 = 60% galls, 7 = 70% galls, 8 = 80% galls, 9 = 90% galls and 10 = all roots of the root system galled.
[3] Means in the same column under each soil type, followed by the same lowercase letter, among the three water regimes, are not significantly different according to Tukey's HSD test ($P \leq 0.05$).
[4] Means in the same row, followed by the same uppercase letter, between the two soil types, are not significantly different according to Tukey's HSD test ($P \leq 0.05$).
(–): indicates a reduction in the inoculated plants compared to the non-inoculated plants. (+): indicates an increase in the inoculated plants compared to the non-inoculated plants.
* indicates that the change is significantly ($P \leq 0.05$) different according to the t-test.

Table 3. Effect of *Meloidogyne graminicola* infection on plant growth and yield-contributing traits of the lowland rice variety Thihtatyin (THY) and the upland rice variety Kone Myint 2 (KM 2) grown under three water regimes and two soil types

Water regime	**Clay loam (S1)**			**Sandy loam (S2)**			**Difference (S1-S2)**	
	UI	I	% change	UI	I	% change	UI	I
	Plant height (cm)							
	THY							
Permanent flooding	63.5±2.2[1] a[2]	61.5±5.9 a	–3.1	66.7±3.8 c	62.3±2.3 a	–6.6	–3.2	–0.8
Intermittent flooding	60.7±2.4 a	57.8±4.6 a	–4.8	59.0±6.0 b	60.5±5.8 a	+2.5	+1.7	–2.7
Upland	52.5±2.0 b	61.0±12.1 a	+16.2	52.3±3.9 a	40.4±6.7 b	–22.8*	+0.2	+20.6*
	KM 2							
Permanent flooding	90.5±6.3 a	80.2±10.4 a	–11.4	86.3±6.9 a	85.5±7.9 b	–0.9	+4.2	–5.3
Intermittent flooding	85.6±3.6 a	84.0±10.8 a	–1.9	88.5±7.6 a	69.2±9.7 a b	–21.8*	–2.9	+14.8
Upland	86.2±3.5 a	77.0±13.8 a	–10.7	82.5±7.4 a	48.8±17.0 a	–40.8*	+3.7	+28.2*
	Fresh root weight (g)							
	THY							
Permanent flooding	34.1±8.8[1] b[2]	27.5±6.2 b	–19.4	31.7±3.9 b	32.2±6.1 b	+1.6	+2.4	–4.7
Intermittent flooding	23.5±2.3 a	14.7±8.1 a	–37.4*	17.6±3.3 a	8.5±2.0 a	–51.7*	+5.9*	+6.2*
Upland	29.1±4.5 a b	14.3±5.7 a	–50.9*	20.6±3.6 a	6.6±3.6 a	–68.0*	+8.5*	+7.7*
	KM 2							
Permanent flooding	30.5±4.2 a	22.1±6.4 a	–26.3	27.2±3.8 b	21.0±5.4 b	–22.8	+2.8	+1.1
Intermittent flooding	28.5±5.8 a	17.9±4.0 a	–37.2	21.1±8.0 a b	10.9±3.4 a	–48.3*	+7.4	+7*
Upland	24.8±6.1 a	14.8±6.2 a	–40.3*	12.7±4.2 a	9.0±0.8 a	–29.1*	+12.1*	+5.8*
	No. of tillers per plant							
	THY							
Permanent flooding	20±2[1] a[2]	22±3 b	+11.1	23±4 a	20±4 b	–10.6	–3	+2
Intermittent flooding	26±3 b	14±5 a	–45.9*	17±4 a	8±3 a	–54.9*	+8*	+6*
Upland	21±3 a b	14±7 a	–33.7*	22±5 a	8±4 a	–61.9*	–1	+6
	KM 2							
Permanent flooding	12±1 a	14±2 a	+16.7	12±4 a	12±4 a	–5.7	0	+2
Intermittent flooding	13±4 a	13±4 a	+3.1	12±3 a	6±3 a	–46.6*	+1	+7*
Upland	11±3 a	9±4 a	–19.6	9±2 a	9±1 a	0.0	+2	0
	No. of panicles per plant							
	THY							
Permanent flooding	15.2±5.6 a	17.8±2.9 c	+17.1	21±2.3 b	15.8±4.8 b	–24.8	–5.8	+2
Intermittent flooding	17.3±5.2 a	10.7±2.9 b	–38.2*	11.8±2.6 a	6.2±3.1 a	–47.5*	5.5*	+4.5*
Upland	14.4±3.1 a	6.4±2.3 a	–55.6*	10.3±1.5 a	1.4±2.6 a	–86.4*	4.1*	+5*
	KM 2							
Permanent flooding	7.7±1.6 a	9.5±1.9 b	+23.4	8.2±1.3 a	8.8±5.3 b	+7.3	–0.5	0.7
Intermittent flooding	8.7±1.4 a	6.6±2.7 a b	–24.1	6.3±2.1 a	5.6±3.8 a b	–11.1	+2.4*	+1
Upland	8.0±1.1 a	4.8±2.6 a	–40.0*	7.2±2.3 a	2.5±0.6 a	–65.3*	+0.8	+2.3*
	No. of filled grains per panicle							
	THY							
Permanent flooding	49.4±24.7 a	37.5±6.7 a	–24.1*	35.8±12.9 a	37.7±15.8 a	+5.3	+13.6	–0.2
Intermittent flooding	36.7±11.8 a	36.4±12.1 a	–0.8	43.7±11.4 a	36.8±19.9 a	–32.7	–18	–0.4
Upland	37.9±14.7 a	36.8±3.1 a	–2.9	37.8±17.7 a	5.0±7.3 b	–86.8*	–0.1	+31.8*
	KM 2							
Permanent flooding	45.0±5.7 b	32.7±13.0 a	–27.3	43.0±6.4 b	33.9±15.4 b	–21.2	+2	–1.2
Intermittent flooding	39.4±7.6 a b	35.3±14.4 a	–10.4	38.2±9.7 a b	16.6±9.6 a b	–56.5*	+1.2	+18.7*
Upland	35.3±5.9 a	23.4±10.2 a	–33.7*	29.7±8.7 a	8.3±1.3 a	–72.1*	+5.6	+15.1*

Table 3. (continued) Effect of *Meloidogyne graminicola* infection on plant growth and yield-contributing traits of the lowland rice variety Thihtatyin (THY) and the upland rice variety Kone Myint 2 (KM 2) grown under three water regimes and two soil types

Water regime	Clay loam (S1)			Sandy loam (S2)			Difference (S1-S2)	
	UI	I	% change	UI	I	% change	UI	I
	Filled grains per plant (%)							
	THY							
Permanent flooding	76.4±18.6[1]a[2]	58.8±18.8 a	–23.0	60.5±19.1 a	60.8±15.7 a	+0.5	+15.9	–2
Intermittent flooding	62.1±11 a	66.6±7.8 a	+7.2	76.8±14.7 a	53.8±26.4 a	–29.9	–14.7	+12.8
Upland	65.9±9.7 a	76.6±8.4 a	+16.2	71.8±22.3 a	13.9±19.5 b	–80.6*	–5.9	+62.7*
	KM 2							
Permanent flooding	81.6±7.7 a	79.8±17.5 a	–2.2	87.9±6.6 a	72.5±25.0 b	–17.5	–6.3	+7.3
Intermittent flooding	86.5±5.0 a	79.3±17.9 a	–8.3	81.2±6.6 a	54.2±28.3 a	–33.3*	+5.3	+25.1
Upland	65.3±12.9 b	60.3±20.9 a	–8.1	75.3±19.9 a	26.7±1.7 a	–64.5*	–10	+33.3*
	Filled grain weight per plant (g)							
	THY							
Permanent flooding	13±2.1 b	11.0±3.1 b	–15.4	10.9±4.4 a	10.2±2.6 c	–6.4	+2.1	+0.8
Intermittent flooding	11.7±1.7 a b	6.8±2.4 a	–41.9*	9.9±3.1 a	4.4±2.9 b	–55.6*	+1.8	+2.4
Upland	7.9±3.7 a	6.2±1.9 a	–21.5*	7.7±2.5 a	0.2±0.3 a	–97.4*	+0.2	+6*
	KM 2							
Permanent flooding	8.0±1.8 a	6.7±2.1 b	–16.3	7.8±0.8 b	5.4±0.6 a	–30.8*	+0.2	+1.3
Intermittent flooding	7.8±0.8 a	4.7±0.8 a b	–39.7*	5.9±1.3 a b	3.1±0.6 b	–47.5*	+1.9 *	+1.6*
Upland	5.9±0.9 b	2.8±1.1 a	–52.5*	4.2±1.7 a	0.1±0.02 c	–97.6*	+1.7 *	+2.7*
	1,000 filled grains weight (g)							
	THY							
Permanent flooding	20.5±1.3[1] a[2]	18.9±0.9 a	–7.8*	17.9±6.5 a	18.1±0.3 b	1.1	+2.6	+0.8
Intermittent flooding	19.6±0.6 a	17.5±1.2 a	–10.7*	18.9±1.8 a	16.8±1.0 b	–11.1	+0.7	+0.7
Upland	18.4±2.1 a	19.0±3.4 a	3.3	17.5±1.9 a	3.6±7.3 a	–79.4*	+0.9	+15.4*
	KM 2							
Permanent flooding	23.2±1.4 a	22.5±1.2 a	–3.0	22.6±1.7 a b	22.8±1.4 b	0.9	+0.6	–0.3
Intermittent flooding	23.5±2.0 a	21.2±1.1 a	–9.8*	22.8±2.3 b	17.7±6.7 b	–22.4	+0.7	+3.5
Upland	21.3±2.3 a	23.5±2.8 a	10.3	20.1±1.4 a	6.3±1.1 a	–68.7*	+1.2	+17.2*
	Yield (t/ha)							
	THY							
Permanent flooding	4.7±0.8 b	3.7±1.0 b	–21.3	4.2±0.3 a	3.0±0.9 b	–28.6	+0.5	+0.7
Intermittent flooding	3.2±0.7 a	2.5±0.6 a	–21.9*	3.8±1.6 a	1.1±0.7 a	–71.1*	–0.6	+1.4*
Upland	2.9±0.8 a	2.2±0.7 a	–24.1*	2.7±1.3 a	0.04±0.06 a	–98.5*	+0.2	+2.2*
	KM 2							
Permanent flooding	3.0±0.8 a	2.6±1.1 b	–13.3	3.1±0.3 a	1.9±0.6 b	–38.7*	–0.1	+0.7
Intermittent flooding	3.1±0.4 a	1.7±0.4 a b	–45.2*	2.4±0.7 a b	0.6±0.5 a	–75.0*	0.7	+1.1*
Upland	1.9±0.5 b	0.8±0.4 a	–57.9*	1.5±0.7 b	0.07±0.02 a	–95.3*	0.4	+0.73*

[1] Data are means ± SD (n = 6).
[2] Means in the same column under each rice variety, followed by the same lowercase letter, among the three water regimes, are not significantly different according to Tukey's HSD test ($P \leq 0.05$).
(–): indicates a reduction in the inoculated plants compared to the non-inoculated plants. (+): indicates an increase in the inoculated plants compared to the non-inoculated plants.
* indicates that the change is significantly ($P \leq 0.05$) different according to the t-test.

in the clay loam soil and *vice versa*. The same observation was made for variety Kone Myint 2. However, under upland conditions, the root population densities of J2 and eggs on both the varieties were always significantly ($P \leq 0.05$) higher in the sandy loam soil compared with the clay loam soil.

With the exception of the soil population densities, the root population densities of J2 and eggs of the varieties Thihtatyin and Kone Myint 2 grown in either the clay loam or the sandy loam soil were, in general, not significantly different. A significant ($P \leq 0.05$) higher number of J2 (g root)$^{-1}$ on variety Thihtatyin under upland conditions in the sandy loam soil compared with variety Kone Myint 2 grown under similar conditions (28,560 *vs* 11,013 J2 (g root)$^{-1}$, respectively), and a significant ($P \leq 0.05$) higher number of J2 per root system on variety Thihtatyin under permanent flooding in the clay loam soil and under upland conditions in the sandy loam soil compared with variety Kone Myint 2 grown under similar conditions (288,858 and 212,719 J2 (g root)$^{-1}$ *vs* 125,363 and 97,710 J2 (g root)$^{-1}$, respectively) were observed. The soil population densities of variety Kone Myint 2 were significantly ($P \leq 0.05$) higher in both soil types under all water regimes with the exception of the clay loam soil under upland conditions.

The Mf^{-eggs} was the highest in variety Thihtatyin grown under permanent flooding in both the clay loam and the sandy loam soil (96.9 and 137.2, respectively). The Mf^{-eggs} was also the highest in variety Kone Myint 2 under permanent flooding in the clay loam soil (43.9) but the Mf^{-eggs} in variety Kone Myint 2 was only 12.0 when this variety was grown under permanent flooding in the sandy loam soil. Under upland conditions, the Mf^{-eggs} of variety Kone Myint 2 was similar in the clay loam and the sandy loam soil (31.1 and 32.8, respectively). Under all water regimes and in both soil types, the Mf^{-eggs} of variety Thihtatyin was higher compared with variety Kone Myint 2. The difference in Mf^{-eggs} between the two rice varieties ranged from very high (125.2 under permanent flooding in the sandy loam soil) to very low (3.0 under intermittent flooding in the sandy loam soil).

Severity of root galling. For both rice varieties and for plants grown in both soil types, the root galling index was significantly ($P \leq 0.05$) lower under permanent flooding compared with intermittent flooding and upland conditions (Table 2).

Effect on nematode infection on plant growth and yield-contributing traits. The effect of *M. graminicola* infection on the plant growth and yield-contributing traits of the rice varieties Thihtatyin and Kone Myint 2 is presented in Table 3.

In the clay loam soil, the three water regimes did not have a significant effect on the plant height of either non-inoculated or inoculated plants of both varieties with the exception of non-inoculated plants of variety Thihtatyin under upland conditions. In this soil type, no significant differences in plant height between non-inoculated and inoculated plants were observed. In the sandy loam soil, the three water regimes had a significant ($P \leq 0.05$) effect on non-inoculated and inoculated plants of both varieties with the exception of non-inoculated plants of variety Kone Myint 2 under upland conditions. In this soil type, the highest significant ($P \leq 0.05$) reduction in plant height (40.8%) was observed in variety Kone Myint 2 under upland conditions. In variety Thihtatyin the highest significant ($P \leq 0.05$) reduction in plant height was also observed under upland conditions (22.8%).

In the clay loam soil, the three water regimes had a significant ($P \leq 0.05$) effect on the fresh root weight of non-inoculated and inoculated plants of variety Thihtatyin but not of variety Kone Myint 2. In this soil type, the highest significant ($P \leq 0.05$) reduction in fresh root weight was observed in the varieties Thihtatyin and Kone Myint 2 under upland conditions (50.9 and 40.3%, respectively). In the sandy loam soil, the three water regimes had a significant ($P \leq 0.05$) effect on the fresh root weight of both non-inoculated and inoculated plants of both varieties. In this soil type, the highest significant ($P \leq 0.05$) reduction in fresh root weight was observed in variety Thihtatyin under upland conditions (68%) and in variety Kone Myint 2 under intermittent flooding (48.3%).

In the clay loam soil, the three water regimes had a significant ($P \leq 0.05$) effect on the dry shoot weight of non-inoculated and inoculated plants of both varieties with the exception of the dry shoot weight of non-inoculated plants of variety Kone Myint 2. In this soil type, the highest significant ($P \leq 0.05$) reduction in dry shoot weight was observed in variety Thihtatyin under intermittent flooding (47.7%) and in variety Kone Myint 2 under upland conditions (43.9%). In the sandy loam soil, the three water regimes had a significant ($P \leq 0.05$) effect on the dry shoot weight of non-inoculated and inoculated plants of both varieties. In this soil type, the highest significant ($P \leq 0.05$) reduction in dry shoot weight was observed in the varieties Thihtatyin and Kone Myint 2 under upland conditions (67.9 and 29.2%, respectively).

In both the clay loam and the sandy loam soils, the three water regimes only had a significant ($P \leq 0.05$) effect on the number of tillers of the non-inoculated and inoculated plants of variety Thihtatyin. Significant ($P \leq 0.05$) differences in number of tillers per plant between non-inoculated and inoculated plants were observed for variety Thihtatyin under intermittent flooding (45.9 and 54.9% in the clay loam and sandy loam soil, respectively) and under upland conditions (33.7 and 61.9% in the clay loam and sandy loam, respectively), and for variety Kone Myint 2 in the sandy loam soil under intermittent flooding (46.6%).

In the clay loam soil, the three water regimes had a significant ($P \leq 0.05$) effect on the number of panicles of the inoculated plants of both varieties. In this soil type, the highest significant ($P \leq 0.05$) reduction in number of panicles per plant was observed in the varieties Thihtatyin and Kone Myint 2 under upland conditions (55.6 and 40%, respectively). In the sandy loam soil, the three water regimes had a significant ($P \leq 0.05$) effect on the number of panicles per plant of non-inoculated or inoculated plants of both varieties with the exception of non-inoculated plants of variety Kone Myint 2. In this soil type, the highest significant ($P \leq 0.05$) reduction in the number of panicles per plant was observed in the varieties Thihtatyin and Kone Myint 2 under upland conditions (86.4 and 65.3%, respectively).

In the clay loam soil, the three water regimes had a significant ($P \leq 0.05$) effect on the number of filled grains of the non-inoculated plants of variety Kone Myint 2. In this soil type, the highest significant ($P \leq 0.05$) reduction in filled grains per panicle was observed in variety Thihtatyin under permanent flooding (24.1%) and in variety Kone Myint 2 under upland conditions (33.7%). In the sandy loam soil, the three water regimes had a significant ($P \leq 0.05$) effect on the number of filled grains of non-inoculated or inoculated plants of both varieties with the exception of non-inoculated plants of variety Thihtatyin. In this soil type, the highest significant ($P \leq 0.05$) reduction in number of filled grains per panicle was observed in the varieties Thihtatyin and Kone Myint 2 under upland conditions (33.7 and 72.1%, respectively).

In the clay loam soil, the three water regimes had a significant ($P \leq 0.05$) effect on the % filled grains in the non-inoculated plants of variety Kone Myint 2. In this soil type, no significant reductions in percentage filled grains per plant were observed for both varieties. In the sandy loam soil, the three water regimes had a significant ($P \leq 0.05$) effect on the percentage filled grains per plant of the inoculated plants of both varieties. In this soil type, the highest significant ($P \leq 0.05$) reduction in percentage filled grains per plant was observed in the varieties Thihtatyin and Kone Myint 2 under upland conditions (80.6 and 64.5%, respectively).

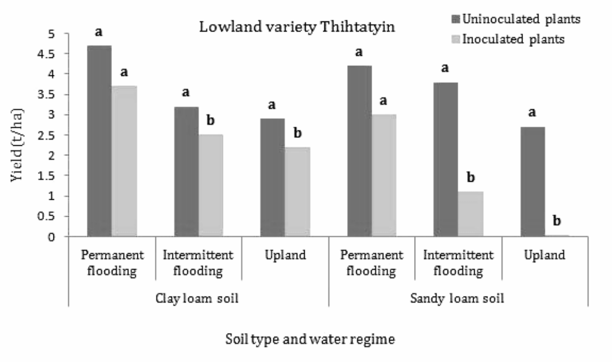
In the clay loam soil, the three water regimes did have a significant ($P \leq 0.05$) effect on filled grain weight of non-inoculated and inoculated plants of both varieties. In this soil type, the highest significant ($P \leq 0.05$) reduction in filled grain weight per plant was observed in variety Thihtatyin under intermittent flooding (41.9%) and in variety Kone Myint 2 under upland conditions (52.5%). In the sandy loam soil, the three water regimes had a significant ($P \leq 0.05$) effect on the filled grain weight per plant of non-inoculated or inoculated plants of both varieties with the exception of non-inoculated plants of variety Thihtatyin. In this soil type, the highest significant ($P \leq 0.05$) reduction in filled grain weight per plant was observed in the varieties Thihtatyin and Kone Myint 2 under upland conditions (97.4 and 97.6%, respectively).

In the clay loam soil, the three water regimes did not have a significant effect on the grain weight of non-inoculated or inoculated plants of both varieties. In this soil type, the highest significant ($P \leq 0.05$) reduction in the weight of 1,000 filled grains was observed in the varieties Thihtatyin and Kone Myint 2 under intermittent flooding (10.7 and 9.8%, respectively). In the sandy loam soil, the three water regimes had a significant ($P \leq 0.05$) effect on the grain weight of 1,000 filled grains of non-inoculated or inoculated plants of both varieties with the exception of non-inoculated plants of variety Thihtatyin. In this soil type, the highest significant ($P \leq 0.05$) reduction in the weight of 1,000 filled grains was observed in the varieties Thihtatyin and Kone Myint 2 under upland conditions (79.4 and 68.7%, respectively).

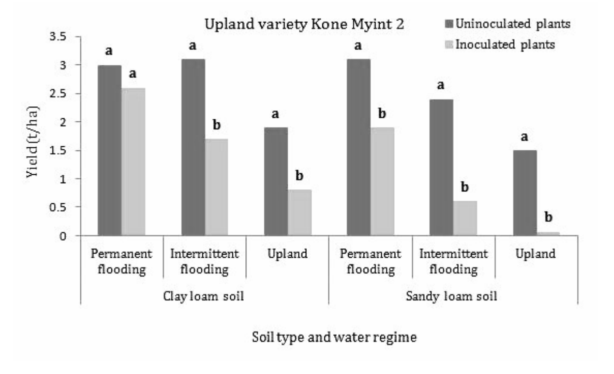
Effect of nematode infection on yield. The effect of *M. graminicola* infection on the yield of the rice varieties Thihtatyin and Kone Myint 2 is presented in Fig. 1A and Fig. 1B, respectively.

In the clay loam soil, the three water regimes had a significant ($P \leq 0.05$) effect on the yield of non-inoculated and inoculated plants of both varieties. In this soil type, yield of both varieties was not significantly reduced under permanent flooding. Under intermittent flooding and under upland conditions, yield of variety Thihtatyin was significantly ($P \leq 0.05$) reduced by 21.9 and 24.1%, respectively, while under these two water regimes yield of variety Kone Myint 2 was reduced by 45.2 and 57.9%, respectively. In the sandy loam soil, the three water regimes had a significant ($P \leq 0.05$) effect on the yield of non-inoculated or inoculated plants of both varieties with the exception of non-inoculated plants of variety Thihtatyin. In this soil type, yield of variety Thihtatyin was not significantly reduced under permanent flooding while under permanent flooding the yield of variety Kone Myint 2 was significantly ($P \leq 0.05$) reduced by 38.7%. Also in this soil type, under intermittent flooding and under upland conditions, the yield of variety Thihtatyin was reduced by 71.1 and 98.5%, respectively, while the yield of variety Kone Myint

2 was reduced by 75 and 95.3%, respectively. In both soil types, the yield of non-inoculated and inoculated plants of both varieties was significantly ($P \leq 0.05$) higher for plants grown under permanent flooding (3 t ha^{-1} or higher), with the exception of the yield of inoculated plants of variety Kone Myint 2, compared with plants grown under upland conditions (< 3 t ha^{-1}).



A



B

Fig. 1. Effect of *Meloidogyne graminicola* infection on the yield of A) the lowland Asian rice variety Thihtatyin and B) the upland Asian rice variety Kone Myint 2, grown under three water regimes in two soil types. Bars with the same letter, within a water regime, are not significantly different according to Tukey's HSD test ($P \leq 0.05$).

DISCUSSION

Both the lowland and the upland Asian rice varieties Thihtatyin and Kone Myint 2 were susceptible to *M. graminicola* infection under all three water regimes and in both soil types used in our study but differences in susceptibility were observed between the two varieties and among the treatments. In general, and based on the number of J2 in the roots, and on Mf^{-eggs}, variety Thihtatyin was more susceptible compared with variety Kone Myint 2 in all treatments. Also the severity of root galling was, in general, higher on variety Thihtatyin compared with variety Kone Myint 2 in all treatments. This observation is in agreement with previous reports (Win *et al.*, 2014, 2015).

In our study, in both soil types and under all three water regimes (with one exception), significantly more J2 were extracted from the rhizosphere soil of variety Kone Myint 2 compared with variety Thithayin. This observation may be, at least partly, have been caused by the different harvesting times of the two rice varieties. Variety Kone Myint 2 has a crop cycle duration that is about 20-25 days longer compared with variety Thihtatyin. Towards the end of a rice crop cycle, the J2 root population density of *M graminicola* declines and more J2 migrate in the soil (Win *et al.*, 2013). Thus, in the case of variety Kone Myint 2, the J2 had a few weeks more to migrate into the soil. Bridge & Page (1982) reported that under permanent and intermittent flooding J2 of *M. graminicola* migrate into the soil at maturity of the rice plants apparently because food in the roots becomes depleted but the J2 do not attack new roots and remain in the soil.

In the clay loam soil and for both varieties Thihtatyin and Kone Myint 2, the highest Mf^{-eggs} was observed under permanent flooding (96.9 and 43.9, respectively). In the sandy loam soil, the highest Mf^{-eggs} for variety Thihtatyin was also observed under permanent flooding (137.2) but for variety Kone Myint 2 under upland conditions (32.8). This observation suggests that the effect of water regime on the population density of *M. graminicola* is influenced by the soil type in which the rice plants are grown and that this effect can vary among rice varieties. This observation is in agreement with Soriano *et al.* (2000) who reported that the *M. graminicola* J2 root population densities on some Asian rice varieties (IR36, IR72, IR74 and IR 29) were higher in a clay soil compared with a sandy soil under permanent and intermittent flooding but that, in contrast, in another variety (IR 29) the J2 root population density was higher in a sandy loam soil compared with a clay soil under permanent flooding. A clay soil may have low oxygen concentrations, an unfavourable environment for organisms such as plant-parasitic nematodes (Van Gundy *et al.*, 1962). Also, a clay soil may limit the migration of nematodes in the soil because of the very small spaces between the soil particles (Young & Heatherly, 1990). Moreover, the soil type may have had an effect on root development and this, in turn, affects the nematode root population densities. The highest fresh root weight was observed in the clay loam soil under permanent flooding for both varieties (> 20 g) and in the sandy loam soil under permanent flooding for variety Thihtatyin (> 30 g). But, in contrast, for variety Kone Myint 2, the highest Mf^{-eggs} was observed in the sandy loam soil under upland conditions when the fresh root weight of the plants was lowest (9 g). This is probably caused by the high susceptibility to *M. graminicola* infection of the upland variety Kone Myint 2 under upland conditions in a sandy loam soil. In the sandy loam soil, the population density of *M. graminicola* J2 in variety Kone Myint 2 was about 4.7 times higher in plants grown under upland conditions compared with permanent flooded soils.

The effect of water regime on the number of eggs and J2 of *M. graminicola* in the roots was lower than expected: with one exception (the number of J2 of variety Kone Myint 2 grown in a sandy loam soil under permanent flooding *vs* upland conditions) no significant effects were observed of any of the water regimes on the root population density of both rice varieties in both soil types. This observation may be explained by the delayed flooding which started 6 days after nematode inoculation for the permanent and intermittent flooding water regimes. With this practice the farmers' water regime used in the direct seeded summer-irrigated lowland rice ecosystem in Myanmar was simulated. J2 of *M. graminicola* can penetrate rice roots within 5 h after inoculation (Rao & Israel, 1973) and once the J2 are inside the roots flooding does not affect their development and reproduction even when the plants are kept flooded until maturity (Netscher & Erlan, 1993; Prot & Matias, 1995; Soriano *et al.*, 2000). As mentioned above, flooding prevents penetration of rice roots by J2 of *M. graminicola* (Bridge & Page, 1982).

In both varieties Thihtatyin and Kone Myint 2, and in both soil types, the root galling index was significantly lower on permanently flooded plants (< 4.5) compared with plants that had been either intermittently flooded or grown under upland conditions (≥ 5.0). The highest root galling indices were always observed on plants grown under upland conditions (7.0 to 8.5). According to some authors, such as Amarasinghe *et al.* (2007), a higher number of galls per rice plant induced by *M. graminicola* J2 reduced plant growth and yield more owing to the higher disturbance to water and nutrient uptake by the roots. This is in agreement with the results of our study that the highest yield losses were usually observed under upland conditions for both rice varieties Thihtatyin and Kone Myint 2 in either a clay loam soil (24.1 and 57.9%, respectively) or a sandy loam soil (98.5 and 95.3%, respectively). The higher severity of root galling on plants grown under upland conditions compared with permanent or intermittent flooded plants cannot be explained by the presence of a higher number of J2 inside the roots of these plants because no effect of water regime on the number of J2 per root system was observed. This observation suggests that rice plants grown under upland conditions are more sensitive to the induction of root galling by *M. graminicola* J2 compared with rice plants grown under permanent or intermittent flooding. However, a higher sensitivity of rice plants to the induction of root galling by *M. graminicola* J2 under upland conditions compared with plants grown under either permanent or intermittent flooding is not the only possible explanation. Although permanent flooding does not limit the reproduction of *M. graminicola* inside rice roots (Bridge & Page, 1982), it may limit the migration and spread of J2 within the roots resulting in a smaller percentage of the roots being infected (Prot & Matias, 1995). Bridge & Page (1982) reported that larger galls were produced on flooded rice roots compared with roots grown in a well drained soil. When the soil is already flooded during the tillering growth stage of rice plants when numerous roots are produced, the J2 do not migrate to and penetrate the newly produced roots and the damage is thus low. This may be partly explained why root galling severity was lower under permanent flooding in our study in which the rice plants had been flooded during the tillering growth stage for the permanently flooded water regime. Also, Prot & Matias (1995) reported that flooding appears to favour the development of the rice root system. This may result in a 'dilution' of the severity of root galling. Bridge & Page (1982) and Fernandez *et al.* (2014) observed that under flooded conditions the root galls induced on rice plants by *M. graminicola* J2 tend to be larger compared with non-flooded plants. This observation seems to be in contradiction with our observation but the root galling index is based on the percentage of roots galled not on the size of the galls.

For both rice varieties, and in both soil types, permanent flooding prevented the suppression of most plant growth and yield-contributing traits measured. Moreover, permanent flooding also prevented significant yield loss in plants of both varieties grown in the clay loam soil and in plants of variety Thihtatyin grown in the sandy loam soil. Previous studies have reported that rice plants grown under flooded conditions had a heavier and more profuse root system compared with rice plants grown in a saturated soil (Pradham *et al.*, 1973) and that optimum rice yield was achieved under continuous shallow flooding (De Datta, 1981). The results of our study confirm the results of previous studies that (shallow) flooding reduces the percentage of roots damaged by *M. graminicola*, resulting in an absence of yield-reducing effects (Prot & Matias, 1995).

When all treatments are compared, of the plant growth traits measured, plant height was the least affected by *M. graminicola* infection while, in general, the highest percentage reductions in plant growth were observed when the plants were grown under upland conditions. These observations are in agreement with Win *et al.* (2014, 2015) who conducted experiments in which lowland and upland rice varieties were harvested at the stem elongation growth stage and at maturity.

It is difficult to conclude which of the yield-contributing traits measured had been the most affected by *M. graminicola* infection. Our results indicate that the damaging effects of *M. graminicola* on these traits were influenced by plant genotype, soil type and water regime but that a general conclusion cannot be made. For instance, no significant effect of nematode infection on the number of tillers per plant of variety Kone Myint 2 grown in the clay loam soil under all three water regimes was observed while a significant percentage reduction in number of tillers per plant of variety Thihtatyin grown in the same soil type under intermittent flooding and upland conditions was observed. By contrast, the number of tillers per plant of variety Kone Myint 2 grown in the sandy loam soil was significantly reduced in the plants grown under intermittent flooding. Another example is the percentage filled grains per plant which, in plants of both varieties grown in the clay loam soil, was not significantly reduced under any of the water regimes, while in plants grown in the sandy loam soil the percentage filled grains per plant was significantly reduced in plants grown under upland conditions.

The absence of a significant reduction in percentage filled grains per plant of variety Thihtatyin in plants grown in a clay loam soil under all water regimes in our study confirms the results of a previous study carried out also in a clay loam soil under intermittent flooding (Win *et al.*, 2015) that, on average (15 lowland rice varieties combined), a lower percentage reduction in the screenhouse or even no reduction in the field was observed. Interestingly, in our study, the number of tillers per plant of the upland rice variety Kone Myint 2 was not significantly reduced by *M. graminicola* infection under upland conditions in both soil types, but other yield-contributing traits such as number of panicles per plant and all traits associated with grain development were significantly reduced when the plants were grown under upland conditions in a sandy loam soil. This observation is, in general, in agreement with Win *et al.* (2015) that the highest percentage reductions in yield-contributing traits caused by *M. graminicola* infection to upland rice varieties in a sandy loam soil under upland conditions were observed on grain development, *i.e.*, filled grain weight per plant and number of filled grains per panicle compared with the percentage reduction in number of tillers per plant, although no significant reduction in percentage filled grains per plant was observed.

In the majority of the treatments in which significant percentage reductions in yield-contributing traits were observed, this observation was made on plants (of both rice varieties) grown under upland conditions but there were several exceptions. For instance, percentage reductions in number of panicles per plant of both varieties were the highest under upland conditions in both clay loam and sandy loam soil types. By contrast, no significant reduction in percentage filled grains per plant and the weight of 1,000 filled grains was observed on both varieties in the clay loam soil under upland conditions.

In general, the percentage reductions in yield-contributing traits were also higher in plants (of both varieties) grown under upland conditions compared with plants grown under permanent flooding. As a result, when the final yield, *i.e.*, the filled grain weight per plant (g) and yield (t ha^{-1}), were calculated, the percentage reduction of yield of both varieties was the highest in both soil types under upland conditions.

The results of our study confirm again the enormous impact *M. graminicola* infection can have on the yield of both lowland and upland rice varieties (Win *et al.*, 2015). With the exception of one treatment, yield loss was always higher than 20% and even almost 100% (yield failure) in plants of both varieties grown in the sandy loam soil under upland conditions. In the sandy loam soil, non-inoculated plants of the varieties Thihtatyin and Kone Myint 2 grown under upland conditions yielded 2.7 and 1.5 t ha^{-1}, respectively. Although yield losses caused by nematodes conducted under screenhouse experiments tend to result in an overestimation of these losses, the results of our screenhouse experiment show that yield losses caused on Asian rice by *M. graminicola* must be very high also under field conditions in the farmers' fields.

The results of our study further confirm the important effect of soil type and water regime on the yield losses caused by *M. graminicola* infection on Asian rice (Prot & Matias, 1995; Soriano *et al.*, 2000). A heavier soil and more water (permanent flooding) will alleviate this impact; a lighter soil and less water (upland conditions) will increase this impact.

The results of our study also show that the effect of soil type on the percentage reduction in plant growth and yield-contributing traits, and yield of both rice varieties caused by *M. graminicola* infection varies with water regime. For instance, plant height, number of filled grains per panicle, percentage filled grains per plant and weight of 1,000 filled grains of variety Thihtatyin were not significantly reduced in a clay loam soil under all water regimes. By contrast, significant percentage reductions of these traits were observed in a sandy loam soil under upland conditions.

The percentage reduction in yield of plants of variety Kone Myint 2 infected with *M. graminicola* grown in the clay loam soil under permanent flooding was 13.3% which was not significantly different from uninfected plants of the same variety grown under the same conditions. These plants yielded 2.6 and 3 t ha^{-1}, respectively. This observation indicates that in a lighter soil and under permanent flooding, variety Kone Myint 2 is tolerant to *M. graminicola* infection. When grown in the clay loam soil under permanent flooding even both varieties Thihtatyin and Kone Myint 2 were tolerant to *M. graminicola* infection. This observation supports the results obtained by Tandingan *et al.* (1996) that the sensitivity (including tolerance) of rice varieties to *M. graminicola* infection can vary under different water regimes. Tandingan *et al.* (1996) reported that the percentage yield reduction caused by *M. graminicola* infection under simulated rainfed upland conditions on the high-yielding lowland Asian rice varieties IR29 and IR74 exceeded 20% while the same varieties were tolerant (their yield was not affected) when they were grown in a permanently flooded clay loam soil. To our knowledge there are very few other examples of changes in tolerance of agricultural crops to plant-parasitic nematodes under changing water regimes.

ACKNOWLEDGEMENTS

This study was supported by a Flemish Interuniversity Council (VLIR-UOS) Ph.D. scholarship to P.P. Win. The authors express appreciation to the Plant Protection Division (Yangon) of the Ministry of Agriculture and Irrigation, Myanmar, for the facilities and assistance in conducting the experiments.

REFERENCES

AMARASINGHE, L.D., KARIYAPPERUMA, K.A.D.P.S. & PATHIRANA, H.N.I. 2007. Study on approaches to integrated control of *Meloidogyne graminicola* in rice. *Journal of Science of the University of Kelaniya* 3: 29-46.

BOUMAN, B.A.M., HENGSDIJK, H., HARDY, B., BINDRABAN, P.S., TUONG, T.P. & LADHA, J.K. 2002. *Proceedings of an International Workshop on Water-wise Rice Production.* The Philippines, International Rice Research Institute (IRRI). 356 pp.

BRIDGE, J. & PAGE, S.L.J. 1980. Estimation of root-knot nematode infestation levels on roots using a rating chart. *Tropical Pest Management* 26: 296-298.

BRIDGE, J. & PAGE, S.L.J. 1982. The rice root-knot nematode, *Meloidogyne graminicola*, on deep water rice (*Oryza sativa* subsp. *indica*). *Revue de Nématologie* 5: 225-232.

BRIDGE, J., PLOWRIGHT, R.A. & PENG, D. 2005. Nematode parasites of rice. In: *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture* (M. Luc, R.A. Sikora & J. Bridge Eds). pp. 87-130. Wallingford, UK, CABI Publishing.

DE DATTA, S.K. 1981. *Principles and Practice of Rice Production.* USA, John Wiley & Sons, Inc. 618 pp.

DE WAELE, D. & ELSEN, A. 2007. Challenges in tropical plant nematology. *Annual Review of Phytopathology* 45: 457-485.

FAROOQ, M., KADAMBOT, H.M.S., REHMAN, H., AZIZ, T., DONG-JIN, L. & WAHID, A. 2011. Rice direct seeding: experiences, challenges and opportunities. *Soil and Tillage Research* 111: 87-98.

FERNANDEZ, L., CABASAN, M.T.N. & DE WAELE, D. 2014. Life cycle of the rice root-knot nematode *Meloidogyne graminicola* at different temperatures under non-flooded and flooded conditions. *Archives of Phytopathology and Plant Protection* 47: 1042-1049.

JAIN, R.K., KHAN, M.R. & KUMAN, V. 2012. Rice root-knot nematode (*Meloidogyne graminicola*) infestation of rice. *Archives of Phytopathology and Plant Protection* 45: 635-645.

MANSER, P.D. 1968. *Meloidogyne graminicola*, a cause of root-knot of rice. *FAO Plant Protection Bulletin* 16: 11.

NETSCHER, C. & ERLAN, X. 1993. A root-knot nematode, *Meloidogyne graminicola*, parasitic on rice in Indonesia. *Afro-Asian Journal of Nematology* 3: 90-95.

PLOWRIGHT, R.A. & BRIDGE, J. 1990. Effect of *Meloidogyne graminicola* (Nematoda) on the establishment, growth and yield of rice cv. IR 36. *Nematologica* 36: 81-89.

PRADHAM, S.K., VARADA, S.B. & KAR, S. 1973. The influence of soil water conditions on growth and porosity of rice. *Plant and Soil* 38: 501-507.

PROT, J.C. & MATIAS, D.M. 1995. Effects 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 Ri5. *Nematologica* 41: 219-228.

PROT, J.C. & VAN GUNDY, S.D. 1981. Effect of soil texture and the clay component on migration of *Meloidogyne incognita* second-stage juveniles. *Journal of Nematology* 13: 213-217.

RAO, Y.S. & ISRAEL, P. 1973. Life history and bionomics of *Meloidogyne graminicola*, the rice root-knot nematode. *Indian Phytopathology* 26: 333-340.

ROY, A.K. 1976. Pathological effects of *Meloidogyne graminicola* on rice and histopathological studies on rice and maize. *Indian Phytopathology* 29: 359-362.

SORIANO, I.R., PROT, J.C. & MATIAS, D.M. 2000. Expression of tolerance for *Meloidogyne graminicola* in rice cultivars as affected by soil type and flooding. *Journal of Nematology* 32: 309-317.

STETINA, S.R., MCGAWLEY, E.C. & RUSSIN, J.S. 1997. Extraction of root-associated *Meloidogyne incognita* and *Rotylenchulus reniformis*. *Journal of Nematology* 29: 209-215.

TANDINGAN, I., PROT, J.C. & DAVIDE, R.G. 1966. Influence of water management on tolerance of rice cultivars for *Meloidogyne graminicola*. *Fundamental and Applied Nematology* 19: 189-192.

TRUDGILL, D.L. & PHILLIPS, M.S. 1997. Nematode population dynamics, threshold levels and estimation of crop losses. In: *Plant Nematode Problems and Their Control in the Near East Region* (M.A. Maqbool & B.R. Kerry Eds). pp. 45-58. Rome, Italy, Food and Agriculture Organization of the United Nations.

TUONG, T.P. & BOUMAN, B.A.M. 2003. Rice production in water-scarce environments. In: *Water Productivity in Agriculture: Limits and Opportunities for Improvements* (J.W. Kijne, R. Barker & D. Molden Eds). pp. 53-67. Wallingford, UK, CAB International.

VAN GUNDY, S.D., STOLZY, L.H., SZUSKIEWICZ, T.E. & RACKHAM, R.L. 1962. Influence of oxygen supply on survival of plant parasitic nematodes in soil. *Phytopathology* 52: 628-632.

WALLACE, H.R. 1973. *Nematode Ecology and Plant Disease*. UK, Edward Arnold Publishers Ltd. 228 pp.

WHITEHEAD, A.G. & HEMMING, J.R. 1965. A comparison of some quantitative methods of extracting small vermiform nematodes from soil. *Annals of Applied Biology* 55: 25-38.

WIN, P.P., KYI, P.P. & DE WAELE, D. 2011. Effect of agro-ecosystem on the occurrence of the rice root-knot nematode *Meloidogyne graminicola* on rice in Myanmar. *Australasian Plant Pathology* 40: 187-196.

WIN, P.P., KYI, P.P. & DE WAELE, D. 2013. Population dynamics of *Meloidogyne graminicola* and *Hirschmanniella oryzae* in a double rice cropping sequence in the lowlands of Myanmar. *Nematology* 15: 795-807.

WIN, P.P., KYI, P.P., MAUNG, Z.T.Z. & DE WAELE, D. 2014. Evaluation of the host response of lowland and upland rice varieties from Myanmar to the rice root-knot nematode *Meloidogyne graminicola*. *Archives of Phytopathology and Plant Protection* 47: 869-891.

WIN, P.P., KYI, P.P., MAUNG, Z.T.Z., MYINT, Y.Y. & DE WAELE, D. 2015. Comparison of the damage potential and yield loss of the rice root-knot nematode, *Meloidogyne graminicola*, on lowland and upland rice varieties from Myanmar. *Russian Journal of Nematology* 23: 53-72.

YOSHIDA, S. 1981. *Fundamentals of Rice Crop Science*. The Philippines, International Rice Research Institute (IRRI). 269 pp.

YOUNG, L.D. & HEATHERLY, L.G. 1990. *Heterodera glycines* investigation and reproduction of soybean grown in clay and silt loam soils. *Journal of Nematology* 22: 618-619.

Pa Pa Win, Pyone Pyone Kyi, Zin Thu Zar Maung, Yi Yi Myint and D. De Waele. Воздействие различных режимов полива на развитие нематод *Meloidogyne graminicola*, рост и урожай двух азиатских сортов риса при выращивании на двух типах почв.

Резюме. Проведены эксперименты в теплицах по заражению 3000 личинок *Meloidogyne graminicola* двух сортов риса: поливного Thihtayin и суходольного Kone Myint 2 при культивировании на двух видах почв и при трех режимах полива: постоянном затоплении, перемежающемся затоплении и поливе за счет муссонных дождей. При всех трех режимах нематоды поражали рис, хотя показатели существенно различались при различных параметрах эксперимента. Как правило, режимы полива не влияли на численность нематод в корнях. Напротив, индекс галлообразования был существенно ниже при постоянном затоплении (< 4.5), чем у периодически затопляемых (≥ 5.0). Наивысший индекс наблюдали на рисе, орошаемом только дождем (7.0-8.5). Постоянное затопление предотвращало подавление роста растений и снижение урожая у обоих сортов при выращивании на суглинках и у сорта Thihtatyin при выращивании на песчаных почвах. Наблюдали падение урожая более чем на 20% (и до 100%) на обоих сортах при выращивании на супесях при орошении дождем.
