



Occurrence of the rice root nematode *Hirschmanniella oryzae* on monsoon rice in Myanmar

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ABSTRACT

During May-October 2007, soil and root samples from 539 fields were collected from 11 monsoon rice varieties in 12 regions in Myanmar. All regions surveyed and 90% of fields sampled were infested with the rice root nematode *Hirschmanniella oryzae*. The average *H. oryzae* population was 10/100 mL soil and 419/20 g roots respectively. In 6.9% of the fields sampled 50 *H. oryzae*/g root were found. The average root population densities were the highest (640/20 g roots) in Taungpyan variety and the lowest (155/20 g roots) in Immayebaw variety. The lowest soil and the second-lowest root populations of *H. oryzae* were observed in Shwethweyin which may indicate that this rice variety is less susceptible to *H. oryzae*. Among three cropping sequences, the highest frequency of occurrence (94%) was found in the rice-rice cropping sequence. Based on the prominence value (a combination of the frequency of occurrence and abundance) of *H. oryzae*, Hlaingtharyar was the most infested region where susceptible rice varieties are grown and rice-rice cropping sequence is practiced. The rice production in this region may be the most at risk of suffering important yield losses due to *H. oryzae*.

Keywords: *Oryza* spp., abundance, frequency of occurrence, prominence value, varietal susceptibility, varieties.

INTRODUCTION

In Myanmar, rice is the major agricultural crop and is grown throughout the country under different agro-ecosystems. It is grown both under rainfed lowland (monsoon) and upland conditions during the rainy season (from mid-May to mid-October) as well as under irrigated conditions during the dry summer (from mid-January to mid-May). The total annual rice growing area is 6.4 million ha or 60% of the total cultivated land area. Monsoon rice and irrigated summer rice (paddy rice) are grown on 4 and 2 million ha, respectively, mainly in the lowlands (Nwe, 2006). Rainfed upland rice is cultivated on about 400,000 ha, mainly in the mountainous northern regions of the country. During the past decades, cultivation of two and even three rice crops per year, and the development and adoption of improved rice varieties have significantly contributed to the increase of rice production (Nwe, 2006). As a result, rice yield in Myanmar increased almost three-fold, from 8 million tons in the 1960's to about 22.4 million tons annually in 2003-2005 or 14% of the total paddy rice production in Southeast Asia (FAO, 2006). Intensification of the rice production has also led to an increase in disease and pest problems. In Myanmar, rice is mainly grown by small-scale farmers. The crop is not only important for the food security of the majority of the population but also as a

source of cash income for smallholders who sell their harvest on the local markets and in the urban areas.

There are many constraints, both abiotic and biotic, that can reduce rice yield and seed quality. Among the biotic constraints are plant-parasitic nematodes (Bridge et al., 2005). In Myanmar, *Aphelenchoides besseyi*, *Ditylenchus angustus*, *Hirschmanniella oryzae*, *Meloidogyne graminicola* and several species of other genera such as *Pratylenchus*, *Hoplolaimus*, *Criconeoides* and *Tylenchorhynchus*, have been reported from rice (Swe, 1997; Than, 2003). Although no detailed information is at present available on the damage and yield loss plant-parasitic nematodes are causing to rice plants and rice production in Myanmar, preliminary observations have shown that several of these nematode species can cause reduction in rice yield and seed quality (Swe, 1997).

Worldwide, *H. oryzae* is the most common plant-parasitic nematode on irrigated rice especially in areas with a long history of irrigated rice cultivation and when the plants are constantly flooded (Bridge et al., 2005). This nematode is perfectly adapted to the constant flooded conditions in which irrigated rice is often being grown in the lowlands (Fortuner & Merny, 1979). It is one of the few plant-parasitic nematode species that can survive under anaerobic conditions (Babatola, 1981). In addition to Myanmar where there are indications that *H. oryzae* is widely spread in some rice producing areas, *H. oryzae* has

also been reported on rice in other Asian countries such as India, Pakistan, Bangladesh, Sri Lanka, Nepal, Thailand, Vietnam, Indonesia, the Philippines, China, Korea and Japan (Bridge et al., 2005). In microplots, *H. oryzae* caused a rice yield loss of 42 % when fertilizers were not applied (Fortuner, 1974) and 23% when fertilization was adequate (Fortuner, 1977). Yield reductions ranging from 27 to 39% were observed when rice seedlings were inoculated with inoculum levels of 1,200 nematodes/plant (Yamsonrat, 1967), 100 nematodes/plant (Mathur and Prasad, 1972) and 1-10 nematodes/g of soil (Jonathan & Velayuthan, 1987). Babatola and Bridge (1979) even observed a decrease in grain yield with 69% of rice plants inoculated with 1,000 *H. oryzae*. The number of panicles (Yamsonrat, 1967) and grain weight (Mathur and Prasad, 1972; Jonathan & Velayuthan, 1987) were affected. In Myanmar, preliminary observations showed that it can spread from infested rice nurseries to uninfested fields, and that it can cause root damage and decrease rice yield and seed quality (Than, 2003). There are also indications that *H. oryzae* is more pathogenic on the recently introduced high-yielding varieties compared with the traditionally grown cultivars (Thein, 2003).

The objective of the present study was to study the frequency of occurrence and abundance of *H. oryzae* in the most important monsoon rice producing areas of Myanmar in order to estimate the potential risk this nematode species may pose for monsoon rice production in the country.

MATERIALS AND METHODS

Site and plant selection

Because in Myanmar summer rice and other crops, especially pulses, are often grown in rotation with monsoon rice, the crop history of the fields sampled was also recorded. The survey was carried out during the 2007 monsoon rice growing season (May-October) in 12 regions of the Bago, Ayeyarwaddy and Yangon Divisions in Lower Myanmar and the Mandalay Division in Upper Myanmar. In each region, on average 45 rice fields were visited, and soil and roots were collected from one commonly grown local rice variety and the rice varieties Manawthukha and Sinthwelatt. All plants sampled had been transplanted 30-45 days before the sampling and were in the tillering growth stage.

Sampling

In each rice field, a survey area of 20 x 20 m was marked off by plastic ropes and within this area soil and root samples were collected from 16 plants evenly distributed over the survey area. Each selected plant was uprooted and rhizosphere soil collected up to a depth of 15 cm. The roots were roughly washed in the field to remove the soil from the root zone. Next, the plant shoots were cut off. The soil and root samples were placed in plastic bags and transported to the laboratory where they were kept at room temperature for one night, until extraction.

Estimation of *Hirschmanniella oryzae* population densities

In the laboratory, the soil samples from each rice field were pooled and the nematodes were extracted from a 100 mL sub-sample using Whitehead's tray method (Whitehead & Hemming, 1965). The root samples were washed again, pooled, chopped into approximately 1-cm-pieces and thoroughly mixed. A 20 g sub-sample was taken from each pooled sample, triturated in a kitchen blender for 15 s and the nematodes were extracted from the resulting homogenate using a modified Baermann funnel method (Prot et al., 1993). After 14-16 h, the nematodes which had moved through the sieve into the water were collected and concentrated in a 10 mL suspension. The same day, the suspensions were examined under a stereoscopic microscope and the juvenile and adult *H. oryzae* were counted. To estimate the susceptibility and resistance of the varieties surveyed, the prominence value (PV = nematode population density x $\sqrt{\text{frequency of occurrence}/10}$) of *H. oryzae* was calculated for each rice variety and for each region (De Waele et al., 1998).

RESULTS

In total, 539 soil and root samples were collected from 11 monsoon rice varieties (Table 1). The varieties Manawthukha and Sinthwelatt were cultivated in every region surveyed. The cultivation of the other varieties was restricted to one region (five varieties) or two to three regions (Immayebaw, Hmawbisan, Hmawbi 5 and Taungpyan). Rice (*Oryza sativa*)-blackgram (*Vigna mungo*) and rice-rice were the most common cropping sequences in six and five regions, respectively, and were practised in all divisions surveyed. The rice-sesame (*Sesamum indicum*) cropping sequence was common in only one region, Kyaukse, in Mandalay Division.

All 12 regions surveyed and 90% of the 539 monsoon rice fields sampled were infested with *H. oryzae* (Table 2). The lowest frequency of occurrence of *H. oryzae* (73.3%) was found in Nattalin. In Kyauktaga, Nyaungdone and Hlegu, all fields sampled were infested with *H. oryzae*. The number of *H. oryzae* found in the soil averaged 10/100 mL soil. The lowest average nematode soil population density (2/100 mL soil) was in Nattalin and the highest (30/100 mL soil) in Hlaingtharyar. The number of *H. oryzae* found in the roots averaged 419/20 g roots. The lowest average nematode root population densities (55/20 g roots) occurred in Pyay and the highest (1,318/20 g roots) in Hlaingtharyar region. In this region, the highest *H. oryzae* soil and root population densities were also observed: 76 and 4,551 nematodes, respectively. The highest prominence value in the soil and in the roots was also found in this region, Hlaingtharyar.

The highest frequency of occurrence of *H. oryzae* was observed in the rice-rice cropping sequence (94%) but the difference from the other two cropping sequences was

TABLE 1 - Cropping sequence and monsoon rice varieties sampled in Myanmar and their occurrence in four divisions and 12 regions

Division	Region	Cropping sequence	Monsoon rice varieties										
			HB5 ¹	MN H	MN P	ShT Y	H K	MT K	ST L	T P	IY B	HB S	AY M
Bago	Pyay	Rice-rice	-	-	-	-	-	+	+	-	+	-	-
	Nattalin	Rice-Blackgram	+	-	-	-	-	+	+	+	-	+	-
	Letpadan	Rice-Blackgram	-	-	-	-	-	+	+	-	-	-	-
	Kyauktaga	Rice-Blackgram	+	-	-	-	-	+	+	-	-	-	-
Ayeyarwaddy	Myaungmya	Rice-rice	-	-	-	-	+	+	+	-	-	-	-
	NYaungdone	Rice-Blackgram	+	-	-	-	-	+	+	-	-	+	-
Yangon	Hmawbi	Rice-Blackgram	-	+	-	-	-	+	+	-	-	-	-
	Hlegu	Rice-rice	-	-	+	-	-	+	+	-	-	-	-
	HLaingtharya	Rice-rice	-	-	-	-	-	+	+	+	-	-	-
Mandalay	Kyaukse	Rice-sesame	-	-	-	-	-	+	+	-	-	-	+
	Patheingyi	Rice-Blackgram	-	-	-	+	-	+	+	-	-	-	-
	Pyinmana	Rice-rice	-	-	-	-	-	+	+	-	+	-	-

¹HB5: Hmawbi 5, MNH: Manawhari, MNP: Manawpyae, ShTY: Shwethweyin, HK: Hnankar, MTK: Manawthukha, STL: Sinthwelatt, TP: Taungpyan, IYB: Immayebaw, HBS: Hmawbisan, AYM: Ayarmin.

small (Table 3). The average nematode population density of the soil in the rice-rice cropping sequence was higher than the other two cropping sequences. In the root, the average population density of *H. oryzae* was the highest in the rice-sesame cropping sequence and the lowest in the rice-blackgram cropping sequence. In the rice-rice cropping sequence, the highest *H. oryzae* population density (4,551/20 g roots) was observed in the roots.

The frequency of occurrence of *H. oryzae* on the different monsoon rice varieties ranged from 100% (Hmawbi 5, Manawhari and Manawpyae) to 80% (Hmawbisan and Ayarmin) (Table 4). The highest average nematode soil population density (19/100 mL soil) was in the variety Manawpyae and the lowest (4/100 mL) occurred in the varieties Shwethweyin and Ayarmin. The nematode average root population densities were highest (640/20 g roots) in the variety Taungpyan and lowest (155/20 g roots) in the variety Immayebaw. The highest *H. oryzae* population density in the soil was observed in the varieties Manawthukha and Taungpyan (76/100 mL soil) while the highest root population density was observed in the variety Manawthukha (4,551/20 g roots).

Among the different monsoon rice varieties, the highest prominence value of *H. oryzae* in the rhizosphere soil was found in the variety Manawpyae and the lowest in the varieties Shwethweyin, Immayebaw and Ayarmin. In the roots, the highest prominence value, observed in the variety Taungpyan, was four times higher than the lowest prominence value found in the variety Immayebaw.

On average, *H. oryzae* population densities in the root and soil were higher in the variety Manawthuka than in the variety Sinthwetatt (Table 5). On Manawthuka, both the soil and root population densities of *H. oryzae* were the highest in the Hlaingtharyar region (40/100 mL soil and 2,279/20 g roots, respectively) while the lowest *H. oryzae* soil and root population densities were observed in Nattalin (1/100 mL soil) and Kyauktaga (64/20 g roots), respectively. On Sinthwelatt, the highest *H. oryzae* soil and root population densities were both observed in the Hlaingtharyar region (26/100 mL soil and 878/20 g roots, respectively) while the lowest *H. oryzae* soil and root population densities were observed in Patheingyi (2/100 mL soil) and Pyay (43/20 g roots), respectively.

TABLE 2 - Frequency of occurrence, population density and prominence value of *Hirschmanniella oryzae* recovered from the soil and roots of monsoon rice in 12 regions in Myanmar

Division	Region	No. of fields (n)	Frequency of occurrence (%)	Population density		Prominence value (PV) ¹			
				No. of <i>H. Oryzae</i> /100 mL soil	No. of <i>H. Oryzae</i> /20 g roots	Soil	Roots		
				Min-max	Mean	Min-max	Mean		
Bago	Pyay	46	76.1	0-31	4	4-212	55 (0) ²	3	48
	Nattalin	45	73.3	0-9	2	40-1006	547 (1)	2	468
	Letpadan	50	74.0	0-23	4	15-849	362 (0)	3	311
Ayeerwaddy	Kyauktaga	36	100.0	2-44	17	2-266	59 (0)	17	59
	Myaungmya	50	98.0	0-28	8	36-792	254 (0)	8	251
	Nyaungdone	44	100.0	1-32	9	59-1577	649 (5)	9	649
Yangon	Hmawbi	45	97.7	0-45	10	44-1001	365 (1)	10	361
	Hlegu	38	100.0	4-53	25	60-991	337 (0)	25	337
	Hlaingtharyar	44	97.7	0-76	30	377-4551	1318 (21)	30	1303
Mandalay	Kyaukse	40	87.5	0-15	4	36-1739	537 (6)	4	502
	Patheingyi	45	84.4	0-14	3	1-1894	334 (3)	3	307
	Pymmana	56	98.2	0-52	14	7-879	208 (0)	14	206
Total or range		539		0-76		1-4551			
Mean			90.6		10		419 (37)	11	400

¹PV = nematode population density x $\sqrt{(\text{frequency of occurrence}/10)}$.²Number of fields in which *Hirschmanniella oryzae* population densities of more than 50 nematodes/g rice roots were found.

TABLE 3 - Frequency of occurrence and population density of *Hirschmanniella oryzae* recovered from the soil and roots of monsoon rice grown in different cropping sequences

Cropping sequence	No. of fields (n)	Frequency of occurrence (%)	Population density			
			No. of <i>H. oryzae</i> /100 mL soil		No. of <i>H. oryzae</i> /20 g roots	
			min-max	mean	min-max	mean
Rice-rice	234	94	1-76	16	4-4551	434
Rice-blackgram (<i>Vigna mungo</i>)	265	88	1-45	7	1-1894	386
Rice-Sesame (<i>Sesamum indicum</i>)	40	87.5	1-15	4	36-1739	537

TABLE 4 - Frequency of occurrence, population density and prominence value of *Hirschmanniella oryzae* recovered from the soil and roots of 12 monsoon rice varieties in Myanmar

Variety	No. of fields (n)	Frequency of occurrence (%)	Population density				PV	
			No. of <i>H. oryzae</i> /100 mL soil		No. of <i>H. oryzae</i> /20 g root		Soil	Roots
			Min-max	Mean	Min-max	Mean		
Hmawbi 5	20	100.0	2-32	13	17-1577	498	13	498
Manawhari	15	100.0	3-29	13	84-517	285	13	285
Manawpyae	15	100.0	5-41	19	106-430	256	19	256
Shwethweyin	15	93.3	0-14	4	1-1120	242	4	234
Hnarkar	15	93.3	0-28	9	71-585	273	8	264
Manawthukha	190	91.5	0-76	13	2-4551	527	12	504
Sinthwelatt	179	88.8	0-58	10	4-1540	328	10	309
Taungpyan	30	86.6	0-76	13	140-1505	640	12	596
Immayebaw	35	85.7	0-24	5	5-1283	155	4	144
Hmawbisan	10	80.0	0-13	5	188-1008	482	5	431
Ayarmin	15	80.0	0-9	4	184-1120	598	4	535
Total or range	539		0-76		1-4551			
Mean		90.8		10		389	9	369

¹PV = population density x $\sqrt{(\text{frequency of occurrence}/10)}$.

DISCUSSION

The frequency of occurrence of *H. oryzae* was very high: 90% of all monsoon rice fields surveyed were infested. Even in the region, Nattalin, with the lowest frequency of occurrence still about 70% of the monsoon rice fields were infested. In Asia, it is not uncommon to find such a high frequency of occurrence of *H. oryzae* in paddy rice (Ichinohe, 1988; Prot et al., 1994; Ravichandra et al., 2003). More remarkable is that during the examination of the root samples no other *Hirschmanniella* species and rarely other plant-parasitic nematode taxa were observed. Only in Pyay and Pyinmana regions were

Longidorus and *Meloidogyne* spp., respectively, sometimes also found. During similar surveys in other Asian countries usually more than one *Hirschmanniella* species and/or plant-parasitic nematode taxa, such as *Meloidogyne* spp., were found, often together with *H. oryzae*. In Japan, for instance, of about 200 paddy rice fields, *H. oryzae* occurred alone in only 10 % of the fields while in 72 % of the fields examined *H. oryzae* occurred together with *H. imamuri* (Ichinohe, 1988). In China, in addition to *H. oryzae* 15 other *Hirschmanniella* species were identified in association with rice in 17 provinces where rice is being cultivated (Liao et al., 2000).

TABLE 5 - Population densities of *Hirschmanniella oryzae* found in two commonly grown monsoon rice varieties in 12 regions in Myanmar

Division	Region	No. of <i>H.oryzae</i> /100 mL soil		No. of <i>H.oryzae</i> /20 g roots	
		Manawthukha	Sinthwelatt	Manawthukha	Sinthwelatt
Bago	Pyay	6	5	96	43
	Nattalin	1	4	383	520
	Letpadan	7	3	378	347
	Kyauktaga	16	4	64	282
Ayeyarwady	Myaungmya	11	7	346	171
	Nyaungdone	12	7	599	483
Yangon	Hmawbi	9	10	434	377
	Hlegu	31	24	514	252
	Hlaingtharyar	40	26	2279	878
Mandalay	Kyaukse	4	3	492	536
	Patheingyi	3	2	558	202
	Pyinmana	15	18	282	205
Mean		13	9	536	358

Based on the prominence value, a combination of the frequency of occurrence and abundance, Hlaingtharyar was the most infested region. In Hlaingtharyar, the rice-rice cropping sequence is the most common rotation and the farmers use the same rice variety for the monsoon and summer rice growing season. These two factors might have contributed to the high prominence value in addition to other conditions such as a long history of rice cultivation. Bridge et al. (2005) remarked that where there is a long history of rice cultivation *H. oryzae* is likely to be widespread and abundant. In the present study, the fields sampled were established with transplanted rice plants. Sato et al. (1970) described that the adult and juvenile populations of *H. oryzae* were larger in the roots of transplanted rice. High populations of *H. oryzae* were also reported in seedlings of flooded nurseries (Miura & Shoji, 1964; Nakazato et al., 1964). The rice varieties sampled in Hlaingtharyar were Manawthukha, Sinthwelatt and Taungpyan. All three of these varieties can be considered as good hosts of *H. oryzae*. In the present study, the latter variety had the highest average root population density. The soil texture of the rice fields in Hlaingtharyar is clay soil, which may be one of the factors for the detection of the highest population densities of *H. oryzae* in this region. Fortuner (1976) noted that the soil texture could explain the occurrence of *H. oryzae*. He found that the development of *H. oryzae* was better in clay soils than in sandy soils in Senegal. Mathur and Prasad (1971) also described that the highest *H. oryzae* populations were found in heavy clay soils in India.

The average soil and root population densities of *H. oryzae* in the monsoon rice fields surveyed were 10/100 mL soil and 400/20 g roots, respectively. Sampling was done at the right time: when the plants were in the active tillering growth stage. Fortuner & Merny (1979) stated that the maximum root population of *H. oryzae* can be found between tillering and heading stage of the rice crop. Data on the economic threshold level of *H. oryzae* on paddy rice are scarce. Grain yield was reduced about 1/3 in the presence of 100 (Mathur & Prasad, 1972) and 1000 (Babatola & Bridge, 1979) *H. oryzae* per plant. In China, 15 *H. oryzae*/g root at the end of the maximum tillering stage was considered as an acceptable economic threshold level for the control of this nematode (Ying et al., 1996). In the present study, in 6.9% of the fields sampled 50 *H. oryzae*/g root were found. The majority of these fields (almost 90%) were situated in Hlaingtharyar (56.8%) followed by Kyaukse (16.2%) and Nyaungdone (13.5%). But one should keep in mind that in the present study, the population densities of *H. oryzae* were most probably underestimated because of the extraction technique used (Prot et al., 1993).

It was found that Manawthukha and Sinthwelatt were the two most commonly grown monsoon rice varieties in every region surveyed. Due to the market demand and local preference, the other varieties grown locally differ from region to region. All monsoon rice varieties sampled were susceptible to *H. oryzae*. Among the varieties included in the survey, the lowest soil and the second-lowest root populations of *H. oryzae* were observed in Shwethweyin,

which may indicate that this rice variety is less susceptible to *H. oryzae*.

In the present survey, the variety Manawpyae had the highest population density per unit soil but a relatively low root population density compared with the other varieties, while the inverse observation was found in the variety Ayarmin. From this observation it can be assumed that a high soil nematode population density was not always correlated with a high root nematode population density.

The cropping sequence generally depends on climate, the adaptability of the crop and the economic return from the farm product. In the surveyed regions three different cropping sequences were recorded. The rice-blackgram cropping sequence was the most popular among the surveyed regions. This is because of the high economic return of blackgram due to export demand. There was not much variation in the frequency of occurrence of *H. oryzae* in the three cropping sequences. Rhizosphere soil of rice plants from the rice-rice cropping sequence contained on average more nematodes per 100 mL soil than that from the rice-blackgram and rice-sesame cropping sequences, but this was not the case for the root population densities of *H. oryzae*. The effect of cropping sequence, was therefore not clearly found in this study. This may be due to the survival of *H. oryzae*. Muthukrishnan et al. (1977) and Fortuner & Merny (1979) recounted that *H. oryzae* can survive in the soil for at least 7 months in the absence of a host plant and can stay longer than 12 months by means of quiescence in soil that is not continually wet. It can survive at high (35-45°C) and low (8-12°C) temperatures in fallow soil and can also survive in weeds and other hosts, in ratooning rice roots and in undecayed rice roots between crops (Mathur and Prasad, 1973; Ichinohe, 1988). In addition, Fortuner & Merny (1979) stated that the interval between two rice crops is important for the survival of *H. oryzae*. In Myanmar, the duration between two rice crops is about 4 to 5 months and this may not be enough time for effective lowering of the population of *H. oryzae* in the soil.

Based on the prominence value of *H. oryzae* (a combination of the frequency of occurrence and abundance), on the susceptibility to *H. oryzae* of the rice varieties grown and on the predominant rice-rice cropping sequence, Hlaingtharyar in the Yangon Division was the most infested region and rice production in this region may be the most at risk to suffer important yield losses due to *H. oryzae*. This region is the first where yield losses of monsoon rice due to *H. oryzae* should be investigated.

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