[Research Note]**Detection of *Meloidogyne* species from wild herbaceous plants in non-crop fields in northwest Kyushu, Japan**Yoko Ishibashi¹ and Toyoshi Yoshiga^{1,*}

Root-knot nematodes (*Meloidogyne* spp.) were surveyed from suitable wild herbaceous plant hosts in non-crop fields at an elevation of less than 200 m of northwest Kyushu by pulling out and observing the root systems for galls. Nematodes were detected in 10 out of 49 surveyed sites (20%): *M. hapla* in nine sites and *M. incognita* in one site. Nematodes were identified by perineal patterns of adult females or size of amplicon of partial mitochondrial DNA. Our survey suggests that *M. hapla* is the predominant species in non-crop fields in northwest Kyushu. Nematol. Res. 46(1), 17–19 (2016).

Key words: *Bidens*, *Cirsium*, distribution, *Meloidogyne hapla*, *Meloidogyne incognita*

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) parasitize plant roots and cause the development of root galls in a wide variety of plants. A total of 13 *Meloidogyne* spp., which include described and putative species, have been detected in Japan (Narabu, 2002). Among these species, *M. incognita*, *M. arenaria*, and *M. hapla* are the important root-knot nematodes in Japan.

Information on the distribution of *Meloidogyne* spp. is important for crop production to avoid the damage caused by *Meloidogyne* spp. The nematode distribution in southern Kyushu, a major crop area located in a temperate region, has been studied since the 1960s and resulted in the detection of *M. incognita*, *M. arenaria*, *M. hapla*, and *M. javanica* from mostly crop fields (Maehara, 1960; Goto *et al.*, 1964; Iwahori *et al.*, 2000b). In northwest Kyushu, although there is no survey data on distribution of *Meloidogyne* species, *Meloidogyne* nematodes, considered to be mainly *M. incognita*, often cause damages on production of vegetables such as

cucumbers and carrots (Yoshiga, unpublished data). Those nematodes present in fields were probably introduced by human activities but there is no information on how these nematodes are introduced.

Soil in crop fields is sometimes introduced from non-crop fields in hilly or mountainous areas for soil amendment and soil dressing to avoid diseases caused by continuous cropping. On the other hand, soil amending and dressing may pose a risk of introducing new plant-parasitic nematodes but only limited information on the natural distribution of *Meloidogyne* spp. in non-crop fields is available (Gotoh, 1971). To prevent introduction of *Meloidogyne* spp. by soil amendment and soil dressing and to understand the distribution of *Meloidogyne* spp. in non-crop fields, we surveyed and identified *Meloidogyne* spp. from wild herbaceous plants in non-crop fields in northwest Kyushu.

MATERIALS AND METHODS

We surveyed root-knot nematodes mainly in non-crop and less disrupted fields near agricultural areas at an elevation of less than 200 m in Fukuoka, Saga, Nagasaki, and Kumamoto Prefectures in northwest Kyushu during 2003–2009 (Table 1). Sampling was mainly performed from September to October when the nematode density was expected to be high and egg masses could be collected. Wild herbaceous plants, such as *Bidens* sp., *Cirsium* sp., *Crassocephalum* sp., and *Farfugium japonicum*, which are suitable hosts for *M. incognita*, *M. arenaria*, *M. hapla*, and *M. javanica* (Yoshida, 1992; Takakura and Yuhara, 1992; Sano, personal communication), were pulled out from the ground with the help of a shovel. Identification of plants was mainly based on a compendium of Japanese flowering plants (Hayashi *et al.*, 1983) with the help of pictures on different internet sites. Plant roots with galls presumably caused by root-knot nematodes were collected and brought back to the laboratory. The galls were dissected under a stereo microscope and females were removed using a pair of forceps. Because of the low nematode density and difficulty obtaining several nematode samples in good condition, a single female from a plant or a single second-stage juvenile (J2) from an egg mass was used for the analysis.

Species identification was performed by comparing the perineal patterns of adult females according to the method described by Narabu (2002) or DNA fragment sizes of the region between the cytochrome oxidase subunit II (COII) gene and the large subunit of the 16S ribosomal RNA gene in mitochondrial DNA (mtDNA)

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after amplification using polymerase chain reaction (PCR) if the perineal patterns were not clear. DNA samples were prepared as follows: a single adult female or a single J2 was crushed on a small piece of filter paper (Iwahori *et al.*, 2000a), and the filter paper was incubated in 25 µl of worm lysis buffer [10 mM Tris-HCl (pH 8.2), 2.5 mM MgCl₂, 50 mM KCl, 0.45% Tween 20, 0.05% gelatin, and 60 µg/ml proteinase K] at 60°C for 20 min, followed by heat denaturation of proteinase K at 95°C for 15 min. PCR was performed in a 10 µl reaction mixture using primers mt-COII (C2F3) and mt-LrRNA (1108) (Orui, 1998) with slight modification. The PCR products were analyzed using 1% agarose gel electrophoresis.

RESULTS AND DISCUSSION

Root-knot nematodes were detected in 10 of the 49 surveyed sites (20%) located in Prefectures in northwest Kyushu (Table 1). *Meloidogyne* spp. were detected from the following host plants: *Bidens* sp., *B. tripartita*, *Cirsium* sp., *Crassocephalum* sp., *F. japonicum*, *Sanicula chinensis*, and *Siegesbeckia glabrescens*. Several lateral roots were observed to arise from the root galls of plants in nine out of the 10 sites, which is typical for *M. hapla*. The perineal pattern of these adult nematodes in the nine sites was classified as *M. hapla*, and the size of the PCR

products obtained from these nematodes was approximately 540 bp, which is the same size as *M. hapla* (Orui, 1998). Judging from these results, the nematode species in the nine sites was *M. hapla*. On the other hand, one *Meloidogyne* nematode on a host *Cirsium* sp. from the roadside of a crop field in Fuji-cho, Saga City, Saga Prefecture, was identified to be *M. incognita* based on the perineal pattern and PCR product size (1.7 kbp) which agrees with the size of *M. incognita* (Orui, 1998).

Meloidogyne hapla, the northern root-knot nematode, is distributed throughout Hokkaido and Honshu and is the predominant species present in Fukushima, Nagano, and Gifu Prefectures and northward, but is detected in only limited areas of Shikoku and Kyushu (Ito, 1966; Gotoh, 1972; Takakura and Yuhara, 1992). On the other hand, *M. incognita* is distributed mainly in the southern part of Japan (Ito, 1966; Gotoh, 1972) and *M. incognita* and *M. arenaria* are often detected from sweet potato and taro fields (Iwahori *et al.*, 2000b). In the present study, however, *M. hapla* was predominantly detected in non-crop fields at an elevation of less than 200 m in northwest Kyushu. Very limited information on distribution of root-knot nematodes in non-crop fields is available but Gotoh (1971) surveyed the hilly grasslands of Aso-Kuju Plateau

Table 1. *Meloidogyne* spp. detected from non-cropping fields in northwest Kyushu.

Location	Sampling dates	Number of sites	Number of detections	Host plants	Species detected
Fukuoka Prefecture					
Nakagawa Town	Dec. 2003	1	0		
Fukuoka City Hakata-ku	Dec. 2003	2	1	<i>Cirsium</i> sp.	<i>M. hapla</i>
Umi Town	Dec. 2003	1	0		
Yame City	Sep. 2009	2	0		
Saga Prefecture					
Imari City	Sep. 2003	1	0		
Kanzaki City Sefuri-machi	Sep. 2003	3	2	<i>Cirsium</i> sp.	<i>M. hapla</i>
Karatsu City Hamatama-cho	Sep. 2008	1	0		
Ishihara	Sep. 2008	1	0		
Kyuragi-machi	Sep. 2008	1	1	<i>Cirsium</i> spp.	<i>M. hapla</i>
Nanayama-mura	Sep. 2008	1	0		
Ushirogawachi	Sep. 2008	1	0		
Yamada	Sep. 2008	1	0		
Yobuko-cho	Sep. 2008	1	0		
Kouhoku Town	Sep. 2008	1	0		
Ogi City	Sep. 2003	1	1	<i>Cirsium</i> sp.	<i>M. hapla</i>
Omachi Town	Sep. 2008	1	1	<i>Sanicula chinensis</i> , <i>Farfugium japonicum</i>	<i>M. hapla</i>
Saga City Fuji-cho	Sep. 2003; Sep. 2008	4	2	<i>Cirsium</i> sp., <i>Bidens</i> sp.	<i>M. incognita</i> , <i>M. hapla</i> ¹
Mitsuse-mura	Sep. 2003	3	0		
Yamato-cho	Sep. 2003	4	0		
Shiroishi Town	Sep. 2008	2	0		
Takeo City	Oct. 2003; Sep. 2008	2	1	<i>Bidens tripartita</i> , <i>Siegesbeckia glabrescens</i>	<i>M. hapla</i>
Taku City	Sep. 2003	3	1	<i>Crassocephalum</i> sp., <i>Bidens</i> sp.	<i>M. hapla</i>
Tosu City	Dec. 2003	2	0		
Ureshino City Shiota-cho	Sep. 2008	1	0		
Nagasaki Prefecture					
Higashisonogi Town	Sep. 2009	2	0		
Kawatana Town	Sep. 2009	1	0		
Matsuura City Fukushima-cho	Sep. 2009	3	0		
Kumamoto Prefecture					
Nankan Town	Oct. 2009	2	0		
Total		49	10		

¹*Meloidogyne incognita* and *M. hapla* were detected from *Cirsium* sp. and *Bidens* sp., respectively at different sites.

in central Kyushu and found that *M. hapla* was the only species detected in the grasslands at an elevation of more than 650 m, although *M. incognita* was also detected in a corn field at a similar elevation. This information implies that *M. incognita* was introduced to the field during agricultural activities and *M. hapla* is the predominant species at least in hilly areas and could be also in non-crop fields in central Kyushu. In our study in northwest Kyushu, although *M. incognita* was detected from one site, it was on the roadside just next to agricultural fields and *M. hapla* was predominantly detected; thus, it is possible that *M. hapla* is predominantly distributed in the area and the *M. incognita* isolate was introduced from the agricultural fields. Our results together with the information from Gotoh (1971) suggest that *M. hapla* is the predominant species in non-crop fields in northwest Kyushu and at least hilly areas in central Kyushu, and it may be the native species in these areas.

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