エンバク「たちいぶき」における3種ネコブセンチュウの侵入・発育性および増殖性

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Tsukuba Office, Agriculture, Forestry and Fisheries Research Council Secretariat
Invasion, development, and reproduction of 3 Meloidogyne species on oat cultivar Tachiibuki, a nematode-suppressive fall crop

Yasushi Tateishi1,2, Hideaki Iwasori1,2, Kenta Uesugi1 and Masaaki Katsura1,3

The degree of susceptibility of oat cultivars Tachiibuki and Haeibuki to infection by 3 Meloidogyne species (M. incognita, M. arenaria, and M. hapla) were examined. Reproduction rates of 3 M. incognita populations on Tachiibuki in greenhouse experiments were low, and this confirmed our previous field experiments. The reproduction rates of 3 M. arenaria populations were low on Tachiibuki and on Haeibuki, whereas the latter is a suitable host for M. incognita. With regard to invasion of second-stage juveniles (J2), similar rates were observed for M. incognita and M. arenaria on Tachiibuki and Haeibuki roots 5 days after inoculation at 25°C. After 30 days, however, Tachiibuki roots inoculated with M. incognita or M. arenaria contained only a small number of mature females compared to the susceptible nematode host. Most J2 nematodes on Tachiibuki roots were undeveloped and a relatively high percentage of males was observed. We conclude that the low rates of reproduction of these 2 root-knot nematode species on Tachiibuki can be attributed to the inhibition of development from the J2 stage to mature females. For the 3 M. hapla populations, reproduction was close to zero and root invasion was minimal on both oat cultivars. Nematol. Res. 41 (1), 1-7 (2011).

Key words: Avena sativa, host suitability, Meloidogyne arenaria, Meloidogyne hapla, Meloidogyne incognita

INTRODUCTION

In recent years, oat cultivation during late summer and autumn (fall cropping) has become widespread in Kyushu, a region with a warm climate in Japan. We previously reported that the reproduction rate of nematodes belonging to the species Meloidogyne incognita (Kofoid & White) on an oat (Avena sativa L.) cultivar, Tachiibuki, was low compared to the reproduction rate on other oat cultivars (Tateishi et al., 2008). Cultivar Tachiibuki has several characteristics that make it suitable for fall cropping (Katsura et al., 2001). Moreover, damage caused by nematodes to sweet potatoes (Ipomoea batatas (L)) grown in a field in which Tachiibuki had been cultivated during fall was reduced compared to the damage on sweet potatoes in fields pre-cultivated with other oat cultivars (Tateishi et al., 2008). Therefore, Tachiibuki is promising as a novel, nematode-suppressive forage or green manure crop, which can be grown during the non-cropping period in the fall, for instance, after forced cropping of sweet potato.

Apart from M. incognita, which causes serious damage to sweet potato and many other crops, 2 other nematode species—M. arenaria (Neal) and M. hapla Chitwood—are also prevalent in the upland fields of southern Kyushu (Gotoh, 1976; Iwahori et al., 2000; Iwahori, 2010). These species too cause damage to many crops. However, there is little information on the reproduction rates of these species on Tachiibuki. To be able to use cultivar Tachiibuki as a nematode-suppressive crop in practice, it is essential that the rates of development and reproduction of these major Meloidogyne species on this cultivar are investigated.

In the present study, we compared the reproduction rates of M. incognita, M. arenaria, and M. hapla on Tachiibuki in greenhouse experiments. Subsequently, invasion and post infection development of these nematodes, which are closely related to nematode reproduction, were examined under controlled conditions.

MATERIALS AND METHODS

Nematode species:

Single egg mass populations were isolated from 3 root-knot nematode species collected from several regions of Japan, as shown in Table 1. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis (Iwahori et al., 2000) of mitochondrial DNA was used for species identification of the nematodes. Each population was propagated on tomato cultivar Pritz, under greenhouse conditions, for 2 months prior to the start of the experiments. Aqueous suspensions of J2 nematodes, freshly

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were used in the pots and carefully washed. The root systems were
Nematode reproduction assay:

volume of 20 ml of a liquid fertilizer

Seeds of the 2 oat cultivars were sown in plastic
Žd
et al.

met 0.015% phloxine B for

masses were immersed in an aqueous solution of 10% gelatinous matrix. The eggs and

root systems were recovered from the

Nematode root invasion assay:

were classified into the following 5 categories:
(1) J2 nematodes without spike-like tails; (2) sausage-shaped J2 nematodes with spike-like tails; (3) sausage-shaped nematodes without spike-like tails; (4) pear-shaped nematodes;

hatched from egg masses obtained from tomato roots, were

used as inocula.

Oat cultivars:

Two oat cultivars, Tachiibuki and Haeibuki (Ueyama et al., 2001) obtained from Snow Brand Seed Co. Ltd., Japan, were used in the experiments.

Nematode reproduction assay:

The reproduction test was performed as described previously (Tateishi et al., 2008), with some modifications. Seeds of the 2 oat cultivars were sown in plastic, wedge-shaped pots (27 × 27 × 42 mm), filled with 12 ml of steam-sterilized Melanandus, consisting of 15.8% sand, 24.9% silt, and 59.3% clay. The pots were placed in a greenhouse at an average temperature of 21°C. After 3 weeks, the oat seedlings were transplanted to polyethylene pots (5 cm in diameter) filled with 100 ml of the same soil. Five days after transplanting, a nematode inoculum of 500 J2 nematodes in several milliliters of water was injected into each pot. A volume of 20 ml of a liquid fertilizer, containing 1.45 mg nitrogen, 2.41 mg phosphorus, and 1.21 mg potassium, was applied to each pot once every 2 weeks. After 63 days of cultivation, the oat root systems were recovered from the pots and carefully washed. The root systems were immersed in an aqueous solution of 0.015% phloxine B for 15 min to stain the egg masses, and the numbers of egg masses were counted. Up to 12 intact egg masses were randomly chosen from each root system. The selected egg masses were immersed in an aqueous solution of 10% sodium hypochlorite (NaOCl; active chlorine, 8.5–13.5%) for 10 min to dissolve the gelatinous matrix. The eggs and eggshells liberated from the egg masses were counted, and the average number of eggs deposited in a single egg mass was calculated. The tomato cultivar Pritz was used as a control host for each of the 3 nematode species.

Nematode root invasion assay:

Seeds of the 2 oat cultivars were germinated on moist filter paper in Petri dishes. The germinated seeds were planted in plastic wedge-shaped pots (35 × 35 × 50 mm) filled with 20 ml of the same soil as that used in the reproduction assay. Ten ml liquid fertilizer, containing 0.72 mg nitrogen, 1.21 mg phosphorus, and 0.60 mg potassium, was applied to each pot. The pot was placed in a growth chamber (LH-30CCFL-8CT; Nippon Medical & Chemical Instruments Co. Ltd., Japan) at a constant temperature of 25°C, and with a photoperiod of 16L:8D (6,050 lux). After 2 weeks, a nematode inoculum of 200 J2 nematodes in 1 ml of water was injected into each pot. Garden balsam (Impatiens balsamina L.) was used as a control host for each of the 3 nematode species tested. Five days after inoculation with the J2 nematodes, the root systems were recovered from the pots, and washed with care. The NaOCl-acid fuchsin-glyc-erin technique (Byrd et al., 1983) was applied to stain the nematodes. Each of the processed root systems was spread on a watch glass and the stained nematodes were observed and counted under a stereomicroscope. Each combination of plant and nematode population was replicated 9 or 10 times.

Characterization of the rates of development of the M. incognita Nishigoshi and M. arenaria Kikuyo nematode populations:

The preparation of oat seedlings and the inoculation with J2 nematodes were performed as described for the invasion assay. Ten ml liquid fertilizer, containing 0.72 mg nitrogen, 1.21 mg phosphorus, and 0.60 mg potassium, was applied to each pot at the moment of planting, together with the inoculation of the J2 nematodes, and 15 days after inoculation. The root systems of 3 to 10 plants were recovered at 6 time points, with 5-day intervals, and up to 30 days after inoculation. They were subjected to the NaOCl-acid fuchsin-glyc-erin staining procedure described above, and the numbers of nematodes inside the roots were counted under a biological microscope at 60–300 fold magnification. The developmental stages of the nematodes were determined based on morphological characteristics, and they were classified into the following 5 categories: (1) J2 nematodes with no or minor swelling; (2) sausage-shaped J2 nematodes with spike-like tails; (3) sausage-shaped nematodes without spike-like tails; (4) pear-shaped nematodes;

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality of population</th>
<th>Original host</th>
<th>Name of population</th>
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<tbody>
<tr>
<td>M. incognita</td>
<td>Koshi City in Kumamoto Pref.</td>
<td>Sweet potato</td>
<td>Nishigoshi</td>
</tr>
<tr>
<td>M. incognita</td>
<td>Miyakonnoyo City in Miyazaki Pref.</td>
<td>Sweet potato</td>
<td>Miyakonnoyo</td>
</tr>
<tr>
<td>M. incognita</td>
<td>Tsukuba City in Ibaraki Pref.</td>
<td>Sweet potato</td>
<td>Tsukuba</td>
</tr>
<tr>
<td>M. arenaria</td>
<td>Kikuyo Town in Kumamoto Pref.</td>
<td>Soybean</td>
<td>Kikuyo</td>
</tr>
<tr>
<td>M. arenaria</td>
<td>Miyazaki City in Miyazaki Pref.</td>
<td>Taro</td>
<td>Miyazaki</td>
</tr>
<tr>
<td>M. arenaria</td>
<td>Kochi City in Kochi Pref.</td>
<td>Eggplant</td>
<td>Kochi-1</td>
</tr>
<tr>
<td>M. hapla</td>
<td>Takamori Town in Kumamoto Pref.</td>
<td>Strawberry</td>
<td>Takamori</td>
</tr>
<tr>
<td>M. hapla</td>
<td>Koshi City in Kumamoto Pref.</td>
<td>Strawberry</td>
<td>Koshi</td>
</tr>
<tr>
<td>M. hapla</td>
<td>Kochi City in Kochi Pref.</td>
<td>Hypericum sp.</td>
<td>Kochi-2</td>
</tr>
</tbody>
</table>
(5) male nematodes. Percentages of nematodes in each category were calculated for each incubation period. The categorization of nematode development was performed according to Eisenback and Triantaphyllou (1991).

RESULTS

Reproduction rates of the 3 Meloidogyne species:

Numbers of egg masses produced by the 3 populations of M. incognita on Tachiibuki were significantly smaller than those on Haeibuki (Mann-Whitney's U test, \( P < 0.001 \)) (Table 2). Each of the 3 populations of M. arenaria produced only a few egg masses on both oat cultivars, although the number of egg masses produced by the Kikuyo population of M. arenaria on Tachiibuki was significantly larger (\( P < 0.05 \)) than those on Haeibuki (Table 3). None of the 3 M. hapla populations produced any egg masses on either oat cultivar (Table 4).

For the M. incognita populations, the average numbers of eggs per egg mass were 100 or fewer on Tachiibuki, and several hundreds on Haeibuki. For the M. arenaria populations, numbers of eggs per egg mass varied from fewer than 100 to over 400 on oat cultivars. Both Meloidogyne species produced larger numbers of eggs on tomato than on the 2 oat cultivars.

Root invasion of the 3 Meloidogyne species:

No significant differences were observed in root invasion between the 2 oat cultivars, Tachiibuki and Haeibuki, for any of the 3 Meloidogyne species, at 5 days post inoculation (Table 5). The only exception was the M. arenaria Kikuyo population. From this population, significantly larg-
er numbers of individuals ($P < 0.01$) were detected inside the roots of Tachiibuki than of Haeibuki. Very small numbers of nematodes were observed for the *M. hapla* populations on either of the 2 oat cultivars. For each of the nematode populations examined, consistently larger numbers of nematodes were detected on the control crop garden balsam than on the oat cultivars.

Rate of development of the *M. incognita* Nishigoshi population on Tachiibuki and Haeibuki:

Up until 5 days after the inoculation with J2 nematodes, all nematodes observed remained in the second-stage with no or minor swelling on all of the examined plants (oat cultivars and control crop) (Table 6). Even after 10 days, most nematodes were at a stage of no distinct swelling on either oat cultivar. In contrast, on garden balsam, 63.4% of the nematodes had adopted a sausage-like shape. The fractions of sausage-shaped nematodes on the 2 oat cultivars started to increase after 15 days. By 20 days, clear differences in nematode development became apparent between Tachiibuki, Haeibuki, and garden balsam. On Tachiibuki, the percentage of sausage-shaped nematodes without spike-like tail (stage 3) was still fairly low at this point, and even after 25 days, approximately half the nematodes were still at stage 2 (sausage-shaped with a spike-like tail). On Haeibuki, on the other hand, 14.2% of the nematodes had already developed into the pear-shaped stage (stage 4). Moreover, adult, egg-laying females were already observed on Haeibuki and garden balsam at this time point. After 30 days, 35.6% and 99.8% of the nematodes were in the pear-shaped stage on Haeibuki and garden balsam, respectively, whereas on Tachiibuki only 22.2% had reached this stage. On Tachiibuki, 60% of the nematodes was still at stage 1 (J2 with no or minor swelling) or stage 2 (sausage-shaped with a spike-like tail). Compared to garden balsam, the 2 oat cultivars contained relatively high numbers of male nematodes.

Rate of development of the *M. arenaria* Kikuyou population on Tachiibuki and Haeibuki:

Up until 10 days after inoculation, *M. arenaria* nematodes developed in a similar fashion to *M. incognita* nematodes on the 2 oat cultivars. On Tachiibuki, *M. arenaria* even showed similar development to *M. incognita* until 30 days after inoculation, with a high proportion of nematodes still at the second-stage juvenile stage, and a low percentage of pear-shaped nematodes (Table 7). On Haeibuki, relatively high percentages of nematodes without swelling were found, and the first sausage-shaped nematodes without spike-like tail (stage 3) were only detected after 20 days. No pear-shaped nematodes were detected after 30 days.

### Table 6. Comparison of development of *Meloidogyne incognita* on 2 oat cultivars in an incubator at a constant temperature of 25°C and a photoperiod of 16L:8D.

<table>
<thead>
<tr>
<th>Days after inoculation</th>
<th>Haeibuki</th>
<th>Tachiibuki</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Developmental category (%)</td>
<td>Developmental category (%)</td>
</tr>
<tr>
<td></td>
<td>J2 with no or minor swelling</td>
<td>J2 with a spike-like tail</td>
</tr>
<tr>
<td></td>
<td>tail</td>
<td>tail</td>
</tr>
<tr>
<td>5 days</td>
<td>97  100  0  0</td>
<td>26  100  0  0</td>
</tr>
<tr>
<td>10 days</td>
<td>88  92.1  7.9  0</td>
<td>70  98.6  1.4  0</td>
</tr>
<tr>
<td>15 days</td>
<td>94  97.7  2.3  0</td>
<td>88  96.7  3.3  0</td>
</tr>
<tr>
<td>20 days</td>
<td>96  97.4  2.6  0</td>
<td>96  96.7  3.3  0</td>
</tr>
<tr>
<td>25 days</td>
<td>98  97.4  2.6  0</td>
<td>96  96.7  3.3  0</td>
</tr>
<tr>
<td>30 days</td>
<td>99  97.4  2.6  0</td>
<td>96  96.7  3.3  0</td>
</tr>
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</table>

1. Percentage of total root system nematodes in each developmental category after inoculation with 200 J2s per plant.
2. Total nematode number tested for developmental stage in 10 plants.
DISCUSSION

The previous findings, which showed that *M. incognita* reproduced poorly on the oat cultivar Tachiibuki (Tateishi et al., 2008), were confirmed by the modified small-pot assay used in the present study (Table 2). Our results also confirmed that there are differences in susceptibility to *M. incognita* in oat cultivars (Pedersen and Rodriguez-Kabana, 1987; Opperman, 1988; Ibrahim et al., 1993). Invasion rates of *M. incognita* J2 nematodes were lower on Tachiibuki than on garden balsam in all the 3 populations examined. In contrast, invasion rates on Tachiibuki and Haeibuki were similar, despite the fact that reproduction was significantly higher on the latter cultivar (Tables 2 and 5). These results indicate that the low reproduction rate of *M. incognita* on Tachiibuki is not due to a low invasion rate. On observing the development of the *M. incognita* Nishigoshi population in Tachiibuki roots, we discovered that, even at 30 days after inoculation, nearly 60% of the individuals were still at the J2 stage. In addition, the percentage of males was relatively high, and only a small number of pear-shaped individuals was detected (Table 6). Michell et al. (1973) reported that an oat cultivar named Wintok, which was a poor host for *M. naasi* Franklin race 5, induced a high percentage of males in this nematode population, and slowed the development of this race compared to other compatible races. Thirty days after inoculation, males constituted 59% of the remaining individuals, and egg-bearing females constituted only 30% of the population. In the present study, few pear-shaped nematodes were observed on Tachiibuki, even at 45 days after inoculation (data not shown). Since no necrotic cells were observed around invading nematodes, the low reproduction rate of *M. incognita* on Tachiibuki must be attributed to the inhibition of development of the nematodes into females.

The relatively low reproduction rates of *M. arenaria* populations on Tachiibuki were similar to those of the *M. incognita* populations (Table 3). Previous studies have found that reproduction rates of *M. arenaria* are different on different oat cultivars (Opperman, 1988; Ibrahim et al., 1993). For the *M. arenaria* Kikuyu population, significant differences in reproduction and root invasion rates were observed between the 2 oat cultivars tested (Tables 3 and 5). Interestingly, in contrast to the *M. incognita* populations, this *M. arenaria* population developed very poorly on Haebiki, illustrated by the fact that not a single pear-shaped nematode was observed in this cultivar (Table 7). Similar results were obtained in another assay, in which the rate of development of the *M. arenaria* Miyazaki population was analyzed (data not shown). Even if the parasitic reac-
tion of *M. arenaria* on particular oat cultivars, such as Haeibuki, is essentially different from that of *M. incognita*, it is clear that the poor development of *M. arenaria* resulted in a low reproduction rate.

In general, plant species belonging to the Poaceae are regarded as nonhosts for *M. hapla*. Ichinohe and Yuhara (1956) reported that no gall formation was observed on any of the 11 different gramineous plant species examined, including oat, in a field infested with *M. hapla* in northern Hokkaido. Moreover, Bélair (1992) reported low population densities of *M. hapla* after cultivation of the oat cultivar, Dorval. However, Ogbuji and Jensen (1974) observed that 2 populations of *M. hapla* reproduced on an oat cultivar named Lee. In the present study, minor invasion events, but zero reproduction, were consistently observed for the 3 *M. hapla* populations on the 2 oat cultivars used in this study (Tables 4 and 5). In addition, the nematodes that managed to invade the roots looked as if they had not established normal feeding sites. The populations were only followed until 5 days after the inoculation. Thus, this result showed that Tachiibuki and Haeibuki are nonhosts or very poor hosts, for the tested *M. hapla* populations under the experimental conditions used.

It was shown previously, that fall cropping of the oat cultivar Tachiibuki resulted in a reduction in nematode population densities in field plots infested with *M. incognita* and *M. arenaria* (Tateishi et al., 2008). This study shows that the 3 major *Meloidogyne* species reproduce and develop poorly on Tachiibuki, which confirms that this oat cultivar can be used as a root-knot nematode-suppressive agent in fall cropping practices in southern Kyushu. *Pratylenchus coffeae* Zimmermann, another major nematode pest in sweet potato production in southern Kyushu (Gotoh, 1974), is able to reproduce on Tachiibuki. Fortunately, population densities of this nematode were not disturbingly high after fall cropping of this cultivar (Usugi et al., 2010).

Further study is required to clarify the inhibition of normal development of nematodes on Tachiibuki. This includes a detailed examination of the giant nurse cells induced by nematodes invading the roots. Furthermore, as it appears improbable that the reduction in nematode damage in the field after fall cropping of Tachiibuki (Tateishi et al., 2008) is explained exclusively by the reduced reproduction rate of the nematodes in the oats, some reports suggest that *Meloidogyne* species are suppressed in an indirect way by the production of plant derivatives during cropping of gramineous nematode hosts (Sipes and Arakaki, 1997; Zasada et al., 2007). Therefore, it is important to characterize in detail the nematode-suppressive factors involved in the cultivation of sweet potato in combination with fall cropping of Tachiibuki under field conditions.

**LITERATURE CITED**


英文論文（本報・短報）の和文摘要

エンパク「たちいぶき」における3種ネコブセンチュウの
侵入・発育性および増殖性

立石 喜・岩屋英春・上杉健太・植 眞昭 ……………1

夏播き栽培（秋作）によって後作サツマイモのネコブセン
チュウ害抑制が認められているエンパク品種「たちいぶ
き」における、南九州地域の主要ネコブセンチュウ3種の
侵入、発育、および増殖を、対照品種「はえいぶき」と比
較した。小型ポットを用いた調査により、「たちいぶき」
におけるサツマイモネコブセンチュウ（以下センチュウ省
略）およびアレナリアネコブ各3個体群の低い増殖性が示
された。アレナリアネコブでは「はえいぶき」における増
殖性も低かった。サツマイモネコブとアレナリアネコブ2
期幼虫（J2）は、いずれの品種の根にも同様に侵入した。
接種30日後の「たちいぶき」根では、好適寄主と比較して、
未発育J2と成虫の割合が高く、逆に成虫の割合が低く、
こうした発育特異が両種虫種の低い増殖性をもたらしてい
ると考えられた。キタネコブ3個体群は、「たちいぶき」根
へのJ2の侵入もわずかで、増殖も認められなかった。

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