[RESEARCH NOTE]

Pathogenicity of *Pratylenchus pseudocoffeae* in chrysanthemum and its reproduction on 23 crops

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Pratylenchus pseudocoffeae Mizukubo is a root-lesion nematode recorded from Japan (Mizukubo, 1992), USA (Inserra *et al.*, 1998), Belgium (Waeyengerge *et al.*, 2000), and Iran (Mohammad Deimi *et al.*, 2009). In Japan, the species has been sporadically detected in chrysanthemum fields in Kyushu (Uesugi *et al.*, 2009). The known hosts of the species are chrysanthemum (Mizukubo, 1992; Mohammad Deimi *et al.*, 2009), artemisia (Mizukubo, 1992), aster (Inserra *et al.*, 1998), and grasses (Waeyengerge *et al.*, 2000).

The species causes chrysanthemum damage, such as root lesions and stunted and chlorotic above-ground parts (Mohammad Deimi *et al.*, 2009). Until now, there has been no experimental study or field observation that showed the pathogenicity of *P. pseudocoffeae* to chrysanthemum in Japan. The high soil nematode density in some fields (Uesugi *et al.*, 2009), however, suggested that the species could be involved in chrysanthemum damage. Clarification of the Japanese *P. pseudocoffeae* population's pathogenicity is important for the development of a chrysanthemum nematode control strategy.

Information about the host is essential for the control of *P. pseudocoffeae*, especially when we consider cultural control. Such host information, however, is limited to the above mentioned detection records, because no inoculation study has been performed.

Here, we report the high reproductive potential and pathogenicity of *P. pseudocoffeae* on one of the most common Japanese chrysanthemum cultivars 'Jimba.' We also describe the reproduction of this species on 23 crops including food, forage, ornamental, and nematode suppressive green manure crops.

The Yoron (27°02'N, 128°25'E, Kagoshima Prefecture, Japan) population of *P. pseudocoffeae* (the same population used in Uesugi *et al.* (2009)) was used for all tests. The population was propagated from a single female collected from commercially grown *Solidago* sp. and maintained in

chrysanthemum pot culture. Nematodes were extracted from chrysanthemum roots by using the maceration-filtration technique (Hooper, 1970), and aqueous suspensions of mixed-stage nematodes were used as inocula in tests 1 and 3-7. Chrysanthemum cultivar 'Jimba' obtained from the National Institute of Floricultural Science was used for all tests and pot culturing of the nematode. Steam sterilized andosol (15.8% sand, 24.9% silt, and 59.3% clay) was used for all tests, except that a mixture of the steamed andosol and nursery soil ('Kembyo', 'Yae Noge Co., Ltd., Japan) was used in test 2. Liquid fertilizer (HYPONeX 6-10-5; HYPONeX JAPAN CORP., LTD., NPK = 0.006%: 0.01%: 0.005% solution) was used for weekly fertilization in all tests.

Effect of *P. pseudocoffeae* on the formation of root lesions of chrysanthemum (test 1):

Seven-centimeter-long rooted cuttings of chrysanthemum were planted in a 3 × 3 × 4 cm plug seedling tray. One day after planting, chrysanthemums were inoculated with about 750 or 1,500 nematodes, suspended in tap water, and grown in an incubator (25 °C, 16L8D). As a control treatment, chrysanthemums were inoculated with the suspension after the nematodes were removed by sedimentation. Each treatment consisted of 16 replicates. Twenty-one days after inoculation, total fresh root weight was measured and basal part of the roots (approximately 1.5 cm), where lesions were observed, were rated as follows: 0, no lesions; 1, < 10% of roots have one lesion or more; 2, 10-50% of roots have lesions; 3, > 50% of roots have lesions. Nematodes were extracted from 70 to 116 mg of roots with lesions by incubation at 25 °C for three days (Hooper, 1970).

Effect of *P. pseudocoffeae* on chrysanthemum growth (test 2):

Soils having three different nematode density levels were prepared by mixing highly infested soil from chrysanthemum pot cultures with uninfested soil. Nematode densities of high, medium, and low density soils were 2,038, 446, and 56 nematodes/20 g soil, respectively, as counted after Baermann funnel extraction for three days. Nematode-free soil for the control treatment was prepared by heating high nematode density soil at 60 °C for four days. For each test sample, a 7 cm-long rooted cutting was planted in a polyethylene pot (10.5 cm diameter × 8.5 cm height) filled with each soil and grown in a greenhouse with ambient light and an average temperature of 27.0 °C. Each treatment consisted of nine or ten replicates. Ninety-two days after planting, shoot length, number of dead lower leaves, root weight, and root lesion ratings were examined. We modified the criteria for rating root lesions in test 2 as follows; 0, no lesion; 1, < 25% of roots have lesion; 2, 25-50% have lesions; 3, 50-75%

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have lesions; 4, > 75% have lesions. Nematodes were extracted from whole chopped roots and two 20 g soil samples by the Baermann funnel method for two and three days, respectively. The averages of two soil samples were used as data. Tests 1 and 2 were not repeated. Statistical analyses were performed using Ekuseru-Tokei 2008 (Social Survey Research Information Co., Ltd., Japan).

Host range of *P. pseudocoffeae* (tests 3-7):

The host range of *P. pseudocoffeae* was examined by examining 23 crops in five tests (Table 3). The 16 crops examined in tests 3 and 4 were selected from common food, forage, and ornamental crops. The seven crops examined in test 5 were selected from nematode-suppressive green manure crops targeting root-lesion and/or root-knot nematodes. Each plant was examined again in tests 6 and 7, except for garland chrysanthemum. All tests included chrysanthemum as the control. The seedlings or cuttings were prepared in plug seedling trays or polyethylene pots following the method described in Uesugi *et al.* (2011), with minor modification of the number of days from seeding to inoculation, at most four days longer or shorter than those reported in Uesugi *et al.* (2011). Exceptions were a six-day longer growth period for sweet potato in test 6 and a nine-day shorter one for oats in test 4. Lettuce, which was not used in Uesugi *et al.* (2011), was grown for 28 days before inoculation in a 3 × 3 × 4 cm plug seedling tray. The seedlings or cuttings were grown in five (tests 3-5) or seven replicates (tests 6 and 7), except that six replicates of cucumber were used in test 6. Plants were inoculated with about 300 nematodes and placed in a greenhouse (tests 3 and 5, average temperature, 26.4 and 27.3 °C, respectively) or in an incubator (tests 4, 6, and 7. Temperatures and photoperiods used were, respectively, 24 °C, 12L12D; 25 °C, 16L8D; and 25 °C, 12L12D). Forty days after inoculation, eggs and nematodes inside the roots were stained by the NaOCl-acid fuchsin method (Byrd *et al.*, 1983). Stained roots were macerated in 1.5% acetic acid in a blender, and a part of the suspension (1/4 to 1/50) was placed in a Petri dish to count eggs and nematodes. The total number of eggs and nematodes in the whole roots was estimated from the obtained data. For some samples, stained roots were not macerated but observed with a microscope after they were placed in acidified glycerin and pressed between glass plates.

Results of test 1 are shown in Table 1. The ratings of lesions on roots were significantly higher in nematode-inoculated treatments than in the control, although there was no significant difference in root weights. Nematodes were recovered from necrotic roots, indicating that *P. pseudocoffeae* caused root lesions of chrysanthemum as reported for

Table 1. Rating of lesions on roots of chrysanthemum 'Jimba' inoculated with *Pratylenchus pseudocoffeae*.¹

Number of inoculated nematodes	Root weight/plant (g)	Rating of lesions	Nematodes/0.1 g roots
1,500	1.50	2.9 a	919.4 a
750	1.58	2.7 a	412.5 b
0	1.61	0.0 b	0.0 c

¹ Means of 16 replicates. Pairs of means in a column followed by a different letter differ significantly at the 5% level according to the Steel-Dwass test.

an Iranian population (Mohammad Deimi *et al.*, 2009). The results of test 2 are shown in Table 2. One replicate of the medium density level treatment and two replicates of the low and high density level treatments were excluded from the data because their shoot growth was disturbed by self topping. The lesion ratings were significantly higher in nematode-infested treatments than in the control, as in test 1. Root weight was also significantly decreased in nematode-infested treatments. Shoot growth significantly decreased as nematodes increased from the initial density. Dead lower leaves were observed only in nematode-infested treatments. Nematode densities in soil and roots increased to high levels in all nematode-infested treatments. These results suggested that the Japanese population of *P. pseudocoffeae* has high reproductive potential on chrysanthemum and negatively affects its growth. We cannot attribute these negative effects totally to *P. pseudocoffeae* in test 2, because the edaphic or microbial condition was not strictly standardized among the treatments due to the mixing or heating of the soil used to control soil nematode density. The observed symptoms, such as root lesions, reduction of root weight and shoot growth, and increase of dead lower leaves, however, were consistent with those reported in the chrysanthemum root-lesion nematodes *P. penetrans* and *P. kumamotoensis* and in the Iran population of *P. pseudocoffeae* (Kobayashi, 1968; Sugimura and Kawasaki, 2008; Mohammad Deimi *et al.*, 2009). It is reasonable to list *P. pseudocoffeae* as a target species of chrysanthemum nematode control in southern Japan.

Results of tests 3-7 were shown in Table 3. One (test 7) or two (test 4) replicates of lettuce died before observation and were not included in the data. Chrysanthemum, edible burdock, lettuce, garland chrysanthemum, and common bean supported good reproduction of *P. pseudocoffeae*. Cultivation of these crops obviously has a risk of enhancing field populations of the species. Cucumber, egg plant, tomato, and soybean also showed reproductive rates higher than 1.0 in at least one of the two tests, although the reproductive rates were not so high as for the above mentioned five

Table 2. Effects of *Pratylenchus pseudocoffeae* on the growth of chrysanthemum 'Jimba'.¹

Initial nematode density	Shoot growth ² (cm)	Fresh root weight (g)	Number of dead leaves	Rating of lesion	Nematodes/ whole roots ³	Nematodes/ 20 g soil
High	49.7 a	1.5 a	2.6 a	3.9 a	23,328 ab	2,645
Medium	63.4 b	1.4 a	1.4 a	3.7 a	13,722 a	3,229
Low	72.9 c	1.7 a	0.9 ab	3.5 a	33,797 b	4,536
Control	79.7 d	4.5 b	0.0 b	1.0 b	0	- ⁴

¹ Means of 8-9 replicates. Pairs of means in a column followed by a different letter differ significantly at the 5% level according to the Steel-Dwass test.

² Shoot growth = final shoot length - initial shoot length.

³ Control treatment was not included in the statistical analysis.

⁴ Soil nematode density was not examined for the control treatment.

Table 3. Final nematode numbers of *Pratylenchus pseudocoffeae* in the whole roots of 23 crops at 40 days after inoculation¹.

Plant	Test no.	Eggs	A + J ²	P/P _i ³	Test no.	Eggs	A + J ²	P/P _i ³
Food, forage and ornamental crops								
Cabbage (<i>Brassica oleracea</i> L.) 'Shoshu'	4	0	132	0.4	7	24	71	0.3
Sweet potato (<i>Ipomoea batatas</i> (L.)) 'Koganengan'	3	0	13	0.0	6	1	27	0.1
Cucumber (<i>Cucumis sativus</i> L.) 'Tsubasa'	3	38	427	1.5	6	11	279	1.0
Squash (<i>Cucurbita maxima</i> L. × <i>C. moschata</i> Duch.) 'Tetsukabuto'	3	20	173	0.6	6	11	68	0.3
Peanut (<i>Arachis hypogaea</i> L.) 'Chibahandachi'	3	0	70	0.2	6	1	13	0.0
Soybean (<i>Glycine max</i> (L.)) 'Fukuyutaka'	3	78	590	2.2	6	3	129	0.4
Common bean (<i>Phaseolus vulgaris</i> L.) 'Hatsumidori-2go'	3	3,418	6,674	33.6	6	2,419	8,530	36.5
Oat (<i>Avena sativa</i> L.) 'Swan'	4	9	267	0.9	7	8	102	0.4
Upland rice (<i>Oryza sativa</i> L.) 'Hatafusamochi'	3	1	34	0.1	6	1	16	0.1
Pepper (<i>Capsicum annuum</i> L.) 'Kyohikari'	3	0	3	0.0	6	0	6	0.0
Tomato (<i>Solanum lycopersicum</i> L.) 'Pritz'	3	108	265	1.2	6	109	1,298	4.7
Eggplant (<i>Solanum melongena</i> L.) 'Kogorou'	3	48	118	0.6	6	88	1,197	4.3
Edible burdock (<i>Arctium lappa</i> L.) 'Tegaru'	4	322	484	2.7	7	3,229	6,438	32.2
Garland chrysanthemum (<i>Chrysanthemum coronarium</i> L.) 'Satoyutaka'				- ⁴	7	6,421	15,029	71.5
Lettuce (<i>Lactuca sativa</i> L.) 'Cisco'	4	700	1,033	5.8	7	2,108	6,738	25.3
Chrysanthemum (<i>Chrysanthemum morifolium</i> Ramat.) 'Jimba'	3	5,564	10,335	53.0	6	516	3,846	14.5
	4	988	2,959	13.2	7	1,500	8,764	34.2
	5	12,060	16,925	96.6				
Nematode-suppressive green manure crops								
Crotalaria (<i>Crotalaria spectabilis</i> Roth) 'Nemaking'	5	41	174	0.7	6	0	18	0.1
Chinese senna (<i>Senna obtusifolia</i> (L.)) 'Ebisugusa'	5	13	124	0.5	6	1	131	0.4
Black oat (<i>Avena strigosa</i> Schreb.) 'Hay oats'	5	4	89	0.3	6	2	18	0.1
Guinea grass (<i>Panicum maximum</i> Jacq.) 'Soilclean'	5	2	54	0.2	6	0	31	0.1
Hybrid sorghum (<i>Sorghum bicolor</i> (L.) × <i>S. sudanense</i> (Piper)) 'SS701 (Tsuchitaro)'	5	1	76	0.3	6	0	29	0.1
Sudan grass (<i>Sorghum sudanense</i> (Piper)) 'Nemaheraso'	5	2	52	0.2	6	0	32	0.1
French marigold (<i>Tagetes patula</i> L.) 'Centaur'	5	0	45	0.1	6	0	28	0.1

¹ Means of 3 to 7 replicates.

² A + J = adults + juveniles.

³ P/P_i (reproductive rate) = final nematode number/300, where final nematode number = number of eggs + number of adults and juveniles.

⁴ Not tested.

crops. The reproductive rates on the other 14 crops were less than 1.0. Of these crops, peanut, sweet potato, and French marigold were reported as suppressive against two chrysanthemum pest nematodes, *P. penetrans* and *P. kumamotoensis* (Sugimura and Kawasaki, 2008). Nematode-suppressive crops examined in test 5 were poor hosts for both *P. pseudocoffeae* and *P. kumamotoensis* (Uesugi *et al.*, 2011). These crops should be further examined as multi-target suppressive crops with respect to chrysanthemum nematode control.

We examined the pathogenicity and host range of one population of *P. pseudocoffeae* in the present study. Because intraspecific variation in reproductive potential on a certain plant is known in some species of *Pratylenchus* (Pinochet *et al.*, 1994; Mizukubo *et al.*, 2003), possible variations in *P. pseudocoffeae* populations should be examined in future studies.

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