Journal of Plant Diseases and Protection (2023) 130:105–113
https://doi.org/10.1007/s41348-022-00681-w

ORIGINAL ARTICLE

Host status of cover crops for root-lesion nematodes (*Pratylenchus* spp.) associated with apple orchards in South Africa

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Received: 7 June 2022 / Accepted: 1 November 2022 / Published online: 11 November 2022

Abstract

The host response of 17 cover crop cultivars to infection by single-species populations of *Pratylenchus hippeastri*, *Pratylenchus vulnus* and *Pratylenchus penetrans*, the three most common root-lesion nematode species in apple orchards in South Africa (SA), was evaluated under glasshouse conditions. Reproduction of the three *Pratylenchus* species was assessed 12 weeks after infection of the cover crop plants based on the reproductive potential (RF) and their (relative) susceptibility compared with the most susceptible control plant included in the experiment. Most of the cover crop cultivars (12 out of 17) were poor hosts of all three *Pratylenchus* species: brown mustard 'Caliente 199', white mustard 'Braco', canola/rapeseed 'Garnet', tillage radish 'Groundhog', barrel medics 'Jester', ribwort plantain 'Captain', creeping fescue (cultivar unknown), black oats 'SAIA', perennial ryegrass 'Champion blend', tall fescue 'Speedway', triticale 'Korog' and french marigold 'Naughty Marietta'. These 12 cover crop cultivars did not support reproduction of *P. hippeastri*, *P. vulnus* and *P. penetrans*, and have thus the potential to limit the build-up of large, damaging populations of these three root-lesion nematode species. Five out of the 17 cover crop cultivars were identified as good hosts of one or more of the three *Pratylenchus* species: pink serradella 'Margarita' and subterranean clover 'Aarbei Klawer' were good hosts of *P. penetrans* only; rye 'Duiker Max' was a good host of both *P. hippeastri* and *P. penetrans*; Indian buckwheat (cultivar unknown) and garden nasturtium 'Jewel Choice mix' were good hosts of all three *Pratylenchus* species. These five cover crop cultivars have thus the potential to enable the build-up of large damaging populations of either one, two or three of the root-lesion nematode species and may act as primary sources of infection of apple roots. Their use as cover crops in apple orchards infested with root-lesion nematodes should be avoided.

Keywords Apple · Cover crops · Host-parasite interaction · Pest management · *Pratylenchus* · Reproduction · Root-lesion nematodes · Susceptibility

Introduction

Apple (*Malus pumila* Mill.) is an important deciduous fruit tree cultivated worldwide. In South Africa (SA), apples are the largest component of the deciduous fruit crop with more than 25,000 ha harvested annually (Hortgro 2020). Root-lesion nematodes (*Pratylenchus* spp.) are the most common plant-parasitic nematodes present in apple orchards in SA (Hugo 1994; Hugo and Storey 2017; Knoetze et al. 2021). Root-lesion nematodes are migratory endoparasitic nematodes (Duncan and Moens 2013). Next to root-knot and cyst nematodes, root-lesion nematodes are considered to have the greatest impact on agricultural crop production worldwide (Castillo and Vovlas 2007; Jones et al 2013). They can cause severe damage by feeding and migrating through the cortical tissues of their hosts. Apple trees, especially

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younger trees, infected with root-lesion nematodes show poor growth resulting in gradual yield decline. Also, damage to the roots may predispose infected plants to infection by primary and secondary pathogens. According to the South African Plant-Parasitic Nematode Survey (SAPPNS), *P. delattrei*, *P. flakkensis*, *P. loosi*, *P. neglectus*, *P. penetrans*. *P. pratensis*, *P. thornei*, *P. scribneri*, *P. vulnus, P. zeae* and *P. hippeastri* have been reported from apple orchards in SA (Marais 2021). However, in a recent survey, conducted in all the major apple production areas in SA only *P. hippeastri*, *P. penetrans* and *P. vulnus* were detected. *P. hippeastri* was by far the most common species (84% of samples), whereas *P. vulnus* and *P. penetrans* were found in only about 10% of the samples (Knoetze et al. 2021).

So-called orchard floor management for maintaining soil health and controlling weeds is an important component of the management of perennial crop orchards (Hogue and Nielsen 1987; Merwin 2003). Cover crops, the practice of growing plants, usually in the off-season, leaving their biomass on the field to provide various benefits for the agroecosystem (Kaye and Quemada 2017) are also increasingly being used in perennial crop orchards, especially in woody perennial crop systems where crop rotation is not possible and ultimately replanting is necessary to restore production levels (Vukicevich et al. 2016; Fourie et al. 2021). Direct benefits of cover crops include, inter alia, the reduction in erosion, build-up of soil organic matter by fixing atmospheric nitrogen and reducing nitrogen leaching, phosphorus cycling improvement and the management of weeds (Thapa et al. 2018; Osipitan et al. 2018; Hallama et al. 2019; Beniaich et al. 2019; Wang et al. 2021; Webber et al. 2022). Through allelopathic effects, biofumigation and the interaction of cover crops with the soil microbial community, indirect benefits may include the suppression of soilborne pathogens including plant-parasitic nematodes (Hooks et al. 2010; Vukicevich et al. 2016; Brennan et al. 2020; Richards et al. 2020; Parajuli et al. 2022). Also, climate change mitigation and adaptation may be additional, important ecosystem services provided by cover crops (Kaye and Quemada 2017).

In principle, most plant species can be used as cover crops but agronomic requirements, such as rapid growth and high biomass production, restrict the number of plant species that are eligible as cover crops (Kaye and Quemada 2017). In selecting cover crops for use either in annual or perennial crop production systems, it is also important to ensure that the selected cultivars are poor hosts of soilborne pathogens that may be present in the orchards. Therefore, the aim of our study was to evaluate the host response of selected cover crops often used in orchard floor management in SA to infection by *P. hippeastri*, *P. vulnus* and *P. penetrans*, the three most common *Pratylenchus* species present in apple orchards in SA.

Materials and methods

Three glasshouse experiments were carried out simultaneously to assess the host response of 17 cover crop cultivars to infection by single-species populations of *P. hippeastri*, *P. vulnus* and *P. penetrans*. The experiments were first conducted during winter (May–July 2018) and repeated during late winter and spring (August–October 2019).

Preparation of nematode inoculum

Populations of *P. hippeastri*, *P. vulnus* and *P. penetrans*, maintained in vitro on carrot discs placed in Petri dishes (Coyne et al. 2014) at the Agricultural Research Council (ARC) Infruitec-Nietvoorbij research institute in Stellenbosch, SA, were multiplied to obtain the nematode inoculum. These populations were originally isolated from apple orchards in the Western Cape, SA (Knoetze et al. 2021). The nematodes were extracted from the carrot disc cultures using a modified Baerman tray technique (Marais et al. 2017) and collected in a sterile test tube. When the nematodes had settled on the bottom of the tube, the excess water was removed with a sterile pipette. To surface-sterilise the nematodes, an equal amount of 6000 ppm streptomycin sulphate was added to the nematode suspension to obtain a final concentration of 3000 streptomycin sulphate. The test tube was shaken and kept at room temperature for 1 h after which the streptomycin sulphate solution was removed by three rinses with sterile water. The carrots were first cleaned, peeled and surface-sterilised by flaming with 95% ethanol. Then, the sterilised carrots were cut into 0.5-cm-thick discs, 3 to 4 cm in diameter, using a sterile knife, and placed in Petri dishes. The carrot discs were inoculated with the surface-sterilised nematodes by transferring approximately 100 nematodes (a mixture of juveniles and adults) with a micropipette to the margin of each carrot disc. The Petri dishes were sealed with parafilm and kept at 25 °C in the dark in an incubator for 8 weeks.

Preparation and inoculation of the cover crops

Seventeen cover crop cultivars were included in the experiments together with four cultivars known to be susceptible to infection by root-lesion nematodes (http://nemaplex.ucdavis.edu) and who served as control plants (Table 1). The cultivars were selected on the basis of having potential to be used in apple orchards. Seeds of the various cultivars and control plants were sowed in 18-cm-diameter pots containing a sterilised sandy soil. After 4–8 weeks, when the plants had reached the two-leaf-stage, they were thinned to three plants per pot.

Table 1 Cover crops and control plants included in the study, and their use

Common name	Cultivar	Latin name	Family	Use
Celery (control plant)		*Apium graveolens*	Apiaceae	Control plant
Indian buckwheat		*Fagopyrum esculentum*	Asteraceae	Pollinator beneficial
Brown mustard	Caliente 199	*Brassica juncea*	Brassicaceae	Biofumigation
White mustard	Braco	*Sinapis alba*	Brassicaceae	Biofumigation
Canola/Rapeseed	Garnet	*Brassica napus* var. *napus*	Brassicaceae	Biofumigation
Tillage radish	Groundhog	*Raphanus sativus*	Brassicaceae	Reducing effects of soil compaction
Barrel medics	Jester	*Medicago truncula*t*a*	Fabaceae	Nitrogen fixing
Pink Serradella	Margarita	*Ornithopus sativus*	Fabaceae	Nitrogen fixing
Subterranean clover	Aarbei Klawer	*Trifolium subterraneum*	Fabaceae	Nitrogen fixing
Sweet pea (control plant)	Bijou mixed	*Lathyrus odoratus*	Fabaceae	Control plant
Ribwort plantain	Captain	*Plantago lanceolata*	Plantaginaceae	Limiting erosion; weed suppression
Creeping red fescue		*Festuca rubra*	Poaceae	Limiting erosion; weed suppression
Black oats	SAIA	*Avena trigosa*	Poaceae	Increase organic and carbon soil content; weed suppression
Perennial ryegrass	Champion blend	*Lolium perenne*	Poaceae	Increase organic and carbon soil content; limiting erosion
Rye	Duiker Max	*Secale sereale*	Poaceae	Reducing effects of soil compaction; limiting erosion
Sweet corn (control plant)	Star 7714	*Zea mays* convar. *saccharate* var. *rugosa*	Poaecae	Control plant
Tall fescue	Speedway	*Festuca arundinacea*	Poaceae	Limiting erosion; weed suppression
Triticale	Korog	x *Triticosecale*	Poaceae	Increasing organic and carbon soil content; weed suppression
French marigold	Naughty Marietta	*Tagetes patula*	Polygonaceae	Allelopathic effect
Apple rootstock (control plant)	G969	*Malus domestica*	Rosaceae	Control plant
Garden nasturtium	Jewel Choice mix	*Tropaeolum majus*	Tropaeolaceae	Weed suppression

To expose the plants to nematode infection, one nematode-infected carrot disc was buried about 3-cm-deep in the soil at a distance of 1.5–2.0 cm from the stems of the plants in each pot. To assess the initial nematode inoculum density (Pi), nematodes were extracted from five randomly-chosen carrot discs using a modified Baerman tray technique (Marais et al. 2017) before inoculation of each experiment and the average number of nematodes per carrot disc counted. On average, the Pi value was approximately 1,500 nematodes carrot disc^{-1}.

The nematode-infested pots were maintained in a glasshouse set at an ambient temperature of 25 °C. The glasshouse could be cooled but not heated. The plants were subjected to a standard watering and insect pest control regime during the duration of the experiments.

Assessment of nematode reproduction and host status of the cover crops

Twelve weeks after the nematode-infected carrot discs were added to the pots, the root systems of the three plants in each pot were carefully removed and rinsed with tap water to remove adhering soil particles. After the combined fresh root weight of the three plants was measured, the nematodes were extracted from one 5 g root sub-sample of each root system, using the sugar flotation method (Marais et al. 2017), whereby the 5 g root sub-sample is first macerated in a kitchen blender, then decanted through stacked 710 and 25 µm aperture sieves, and the nematode suspension retrieved from the 25 µm sieve subjected to centrifugation in a sugar solution.

Nematodes (juveniles and adults) in two 1-ml-aliquots from each 5 g root sub-sample were counted in a 1-ml Peters' slide using a light microscope. Based on the fresh root weight, the final number of nematodes root system^{-1} (Pf) and number of nematodes g^{-1} roots were calculated. Oostenbrink's reproduction factor ($Rf = Pf/Pi$) was used to calculate the reproductive potential of each of the three *Pratylenchus* species on each of the selected cover crop cultivars and control plants. An Rf value > 1 indicated susceptibility to nematode infection. In addition to the RF value, the relative susceptibility (S%) of each selected cultivar was calculated for each experiment as the number of nematodes g roots^{-1} of each cultivar as a percentage of the number of nematodes g^{-1} roots of sweet corn, in most experiments the most susceptible control plant. An *S*% value < 30% indicated poor host status for nematode infection.

Experimental design and data analysis

For all experiments, a randomised complete block design lay-out was used with seven replicates for each selected cover crop cultivar and control plant. Data were subjected to a one-way analysis of variance (ANOVA) using the General Linear Models Procedure (PROC GLM) of SAS software (Version 9.4; SAS Institute Inc, Cary, USA). Nematode counts were log(x + 1)-transformed before statistical analysis in order to conform to normal distribution. Fisher's least significant difference (LSD) test ($P \leq 0.05$) was used to compare means (Ott and Longnecker 2001).

Results

Pratylenchus hippeastri

In the winter experiment, the population density of *P. hippeastri* on the selected cover crop cultivars ranged from 0 (perennial ryegrass 'Champion Blend') to 17 (pink serradella 'Margarita') nematodes g^{-1} roots; in the late winter/spring experiment (excluding Indian buckwheat and French marigold 'Naughty Marietta' which were only included in the late winter/spring experiment) from 1 (ribwort plantain 'Captain') to 464 (garden nasturtium 'Jewel Choice mix') nematodes g^{-1} roots (Table 2). In both experiments, the population densities of *P. hippeastri* g^{-1} roots were significantly ($P \leq 0.05$) lower in all cultivars included in the experiments compared with the control plants, with the exception of Indian buckwheat, rye 'Duiker Max' and garden nasturtium 'Jewel Choice mix' in the late winter/spring experiment. In both experiments, the RF value of all cultivars was < 1 and significantly ($P \leq 0.05$) lower compared with the control plants, with the exception of pink serradella

Table 2 Reproduction of *Pratylenchus hippeastri* on selected cover crops and control plants (n = 7 plants). 1st experiment was carried out during winter (May–July 2018), 2nd experiment during late winter and spring (August–October 2019)

Cover crop	Fresh root weight (g)		No. of nematodes g^{-1} roots		RF[1]		S%[2]	
	1st exp	2nd exp	1st exp	2nd exp	1st exp	2nd exp	1st exp	2nd exp
Indian buckwheat	–	1.2	–	6.31 (738.1)[3] a[4]	–	0.22 (0.3) e	–	89
Brown mustard	10.7	7.4	1.15 (6.2) def	2.10 (10.6 ef)	0.03 (0.0) b	0.03 (0.0) e	1	1
White mustard	2.8	2.2	1.12 (5.1) def	1.64 (9.8) efg	0.01 (0.0) b	0.02 (0.0) e	0	1
Canola/Rapeseed	16.4	12.7	1.77 (11.3) cde	3.53 (37.8) d	0.14 (0.2) b	0.23 (0.3) e	1	5
Tillage radish	12.7	15.2	0.59 (1.7) ef	0.59 (1.4) gh	0.01 (0.0) b	0.02 (0.0) e	0	0
Barrel medics	7.8	1.5	0.92 (5.0) ef	4.16 (88.4) cd	0.04 (0.0) b	0.05 (0.1) e	0	11
Pink Serradella	7.0	14.1	2.30 (16.6) bcd	4.90 (175.0) bc	0.08 (0.1) b	0.68 (1.1) cd	2	21
Subterranean clover	12.3	13.6	2.51 (13.3) bc	3.66 (65.7) d	0.14 (0.2) b	0.33 (0.5) de	1	8
Ribwort plantain	45.6	41.1	0.32 (1.0) f	0.39 (0.9) h	0.05 (0.1) b	0.01 (0.0) e	0	0
Creeping red fescue	15.4	10.0	0.51 (1.3) ef	2.09 (18.8) ef	0.02 (0.0) b	0.05 (0.1) e	0	2
Black oats	14.2	7.8	0.27 (0.7) f	2.32 (17.4) e	0.01 (0.0) b	0.08 (0.1) e	0	2
Perennial ryegrass	27.3	20.6	0.00 (0.0) f	0.47(0.9) h	0.00 (0.0) b	0.01 (0.0) e	0	0
Rye	11.8	6.6	1.12 (6.0) def	5.50 (312.2) ab	0.07 (0.1) b	0.62 (0.9) cd	1	37
Tall fescue	53.0	26.4	0.61 (2.3) ef	1.12 (3.1) fgh	0.06 (0.1) b	0.04 (0.0) e	0	0
Triticale	8.5	5.8	0.55 (1.7) ef	4.08 (83.5) cd	0.02 (0.0) b	0.16 (0.2) e	0	10
French marigold	–	9.6	–	0.48 (1.5) h	–	0.00 (0.0) e	–	0
Garden nasturtium	6.1	5.1	3.60 (99.1) b	5.73 (464.3) ab	0.23 (0.3) b	0.71 (1.5) c	9	56
Sweet corn (control)	9.7	19.7	6.20 (1,052.0) a	6.34 (833.4) a	1.89 (8.5) a	1.89 (7.6) a	100	100
Apple rootstock (control)	–	12.7	–	6.13 (563.4) a	–	1.42 (3.9) b	–	68

[1]RF = reproduction factor = Pf (final nematode population/Pi = initial nematode population)

[2]S% = relative susceptibility = Pf of each cover crop/Pf of the most susceptible control plants X 100. In this experiment sweet corn was the most susceptible control plant

[3]Log(x + 1)-transformed values. Real means in brackets

[4]Means within the same column followed by the same letter(s) are not significantly different at ($P \leq 0.05$) according to Fisher's LSD test

'Margarita' (RF = 1.1) and garden nasturtium 'Jewel Choice mix' (RF = 1.5) in the late winter/spring experiment. The RF values did not differ significantly among the cultivars in the winter experiment; in the late winter/spring experiment, the RF values of pink serradella 'Margarita', rye 'Duiker Max' and garden nasturtium 'Jewel Choice mix' were significantly ($P \leq 0.05$) higher compared with the other cultivars. In the winter experiment, all cultivars had an S% value of either 0, 1 or 2, with the exception of garden nasturtium 'Jewel Choice mix' (S% = 9). In the late winter/spring experiment, 14 out of the 17 cultivars had an S% value of < 30 while rye 'Duiker Max', garden nasturtium 'Jewel Choice mix' and Indian buckwheat had S% values = 37, 56 and 89, respectively. In 12 out of 15 cultivars included in both experiments, fresh root weight was higher during the winter experiment compared with the late winter/spring experiment. Fresh root weight of sweet corn 'Star 7714', the control plant included in both experiments, was higher in the late winter/spring experiment compared with the winter experiment (19.7 vs 9.7 g, respectively).

Pratylenchus penetrans

In the winter experiment, the population density of *P. penetrans* on the selected cover crop cultivars ranged from 4 (black oats ''SAIA') to 539 (garden nasturtium 'Jewel Choice mix') nematodes g^{-1} roots; in the late winter/spring experiment (excluding Indian buckwheat and French marigold 'Naughty Marietta' which were only included in the late winter/spring experiment) from 1 (ribwort plantain 'Captain') to 589 (garden nasturtium 'Jewel Choice mix') nematodes g^{-1} roots (Table 3). In both experiments, the population densities of *P. penetrans* g^{-1} roots were significantly ($P \leq 0.05$) lower in all cultivars included in the experiments compared with the control plants, with the exception of Indian buckwheat, pink serradella 'Margarita', subterranean clover 'Aarbei Klawer', rye 'Duiker Max', triticale 'Korog' and garden nasturtium 'Jewel Choice mix' in both experiments and rye 'Duiker Max' in the winter experiment. In both experiments, the RF value of all cultivars < 1 and significantly ($P \leq 0.05$) lower compared with the control plants,

Table 3 Reproduction of *Pratylenchus penetrans* on selected cover crops and control plants (n = 7 plants). 1st experiment was carried out during winter (May–July 2018), 2nd experiment during late winter and spring (August–October 2019)

Cover crop	Fresh root weight (g)		No. of nematodes g^{-1} roots		RF[1]		S%[2]	
	1st exp	2nd exp	1st exp	2nd exp	1st exp	2nd exp	1st exp	2nd exp
Indian buckwheat	–	1.0	–	4.53 (130.0) bcd	–	0.11 (0.1) def	–	32
Brown mustard	17.8	7.4	2.82 (23.8) def	4.12 (88.5) cdef	0.37 (0.5) e	0.41 (0.5) def	4	22
White mustard	2.8	1.7	1.95 (15.1) efg	3.76 (78.3) def	0.05 (0.1) e	0.11 (0.1) def	3	19
Canola/Rapeseed	13.4	8.0	1.97 (11.0) efg	4.25 (102.4) cde	0.30 (0.5) e	0.51 (0.8) d	2	25
Tillage radish	9.9	11.3	2.64 (26.0) def	3.28 (32.4) efg	0.27 (0.4) e	0.31 (0.4) def	4	8
Barrel medics	8.7	3.8	3.02 (29.4) de	3.93 (58.2) def	0.39 (0.6) e	0.17 (0.2) def	5	14
Pink Serradella	8.5	11.4	5.25 (239.0) ab	5.81 (391.7) a	1.35 (3.8) bc	1.60 (4.7) b	41	97
Subterranean clover	14.9	10.6	4.77 (138.0) abc	5.10 (212.9) abc	1.54 (4.2) abc	1.05 (2.6) c	24	53
Ribwort plantain	22.8	35.7	1.60 (8.0) g	0.62 (1.4) i	0.06 (0.1) e	0.05 (0.1) f	1	0
Creeping red fescue	13.6	7.5	2.11 (11.0) efg	1.96 (11.1) h	0.27 (0.4) e	0.07 (0.1) ef	2	3
Black oats	13.9	8.2	1.17 (3.7) g	2.72 (23.4) gh	0.08 (0.1) e	0.16 (0.2) def	1	6
Perennial ryegrass	22.2	28.8	2.23 (8.0) efg	2.52 (19.1) gh	0.22 (0.3) e	0.40 (0.6) def	1	5
Rye	9.8	9.6	4.56 (115.9) bc	5.63 (418.6) a	1.04 (2.0) cd	1.35 (3.5) bc	20	104
Tall fescue	45.8	27.9	1.59 (6.0) gf	2.72 (18.6) gh	0.38 (0.6) e	0.37 (0.5) def	1	5
Triticale	4.9	10.1	3.80 (109.0) cd	4.03 (63.2) def	0.45 (0.7) de	0.47 (0.7) de	19	16
French marigold	–	7.5	–	0.16 (0.3) I	–	0.00 (0.0) f	–	0
Garden nasturtium	6.9	6.8	5.52 (539.1) ab	6.06 (588.7) a	1.67(7.1) ab	1.33 (3.0) bc	92	146
Sweet corn (control)	6.7	9.7	6.14 (587.0) a	5.76 (403.7) a	1.96 (8.4) a	1.36 (3.3) bc	100	100
Apple rootstock (control)	–	13.4	–	5.39 (262.0) ab	–	1.43(4.0) bc	–	65
Celery (control)	10.9	23.8	4.58 (247.1)[3] bc[4]	5.81 (381.4) a	1.24 (6.1) bc	2.15 (8.6) a	42	94

[1]RF = reproduction factor = Pf (final nematode population/Pi = initial nematode population)

[2]S% = relative susceptibility = Pf of each cultivar/Pf of the most susceptible control plants X 100. In this experiment sweet corn was the most susceptible control plant during the 1st experiment; celery during the 2nd experiment

[3]Log(x + 1)-transformed values. Real means in brackets

[4]Means within the same column followed by the same letter(s) are not significantly different at ($P \leq 0.05$) according to Fisher's LSD test

with the exception of pink serradella 'Margarita', subterranean clover 'Aarbei Klawer', rye 'Duiker Max' and garden nasturtium 'Jewel Choice mix' which had RF values which were not significantly ($P \leq 0.05$) different compared with the control plants but significantly ($P \leq 0.05$) higher compared with the other cultivars. In the winter experiment, all cultivars had an S% value < 30%, with the exception of pink serradella 'Margarita' (S% = 41) and garden nasturtium 'Jewel Choice mix' (S% = 92). In the late winter/spring experiment, 12 out of the 17 cover crops had an S% value < 30 while Indian buckwheat, subterranean clover 'Aarbei Klawer', pink serradella 'Margarita', rye 'Duiker Max' and garden nasturtium 'Jewel Choice mix' had S% values = 32, 53, 97, 104 and 146, respectively. In 10 out of 15 cultivars included in both experiments, fresh root weight was higher during the winter experiment compared with the late winter/spring experiment. Fresh root weights of sweet corn 'Starr 7714' and celery, the two control plants included in both experiments, were higher in the late winter/spring experiment compared with the winter experiment (6.7 vs 9.7 g and 10.9 vs 23.8 g, respectively).

Pratylenchus vulnus

In the winter experiment, the population density of *P. vulnus* on the selected cover crop cultivars ranged from 0 (rye 'Duiker Max' and tall fescue 'Speedway') to 288 (garden nasturtium 'Jewel Choice mix') nematodes g^{-1} roots; in the late winter/spring experiment (excluding Indian buckwheat and French marigold 'Naughty Marietta' which were only included in the late winter/spring experiment) from 0 (perennial rye 'Duiker Max') to 75 (garden nasturtium 'Jewel Choice mix') nematodes g^{-1} roots (Table 4). In both experiments, the population densities of *P. penetrans* g^{-1} roots were significantly ($P \leq 0.05$) lower in all cultivars included in the experiments compared with the control plants, with the exception of Indian buckwheat and garden nasturtium 'Jewel Choice mix' in the late winter/spring experiment. In both experiments, the RF value of all cultivars < 1 and significantly ($P \leq 0.05$) lower compared with the control plants, with the exception of garden nasturtium 'Jewel Choice mix' which had an RF value = 1.2 in the late winter/spring experiment. In the winter experiment, all cultivars had an S% value of either 0 or 1, with the exception

Table 4 Reproduction of *Pratylenchus vulnus* on selected cover crops and control plants (n = 7 plants). 1st experiment was carried out during winter (May–July 2018), 2nd experiment during late winter and spring (August–October 2019)

Cover crop	Fresh root weight (g)		No. of nematodes g^{-1} roots		RF[1]		S%[2]	
	1st exp	2nd exp	1st exp	2nd exp	1st exp	2nd exp	1st exp	2nd exp
Indian buckwheat	–	0.7	–	4.80 (159.2) a	–	0.15 (0.2) d	–	123
Brown mustard	7.7	9.7	0.80 (2.2)[3] cdef[4]	1.01 (5.1) def	0.00 (0.0) c	0.11 (0.1) d	0	4
White mustard	1.9	4.4	1.33 (5.6) cd	0.16 (0.3) fg	0.00 (0.0) c	0.00 (0.0) d	1	0
Canola/Rapeseed	14.4	12.0	0.18 (0.3) ef	1.02 (2.6) defg	0.00 (0.0) c	0.04 (0.1) d	0	2
Tillage radish	10.0	13.9	0.37 (0.7) cdef	0.70 (1.4) efg	0.00 (0.0) c	0.04 (0.0) d	0	1
Barrel medics	3.4	4.3	0.66 (3.0) cdef	0.81 (2.9) efg	0.00 (0.0) c	0.03 (0.0) d	0	2
Pink Serradella	5.3	15.7	1.26 (8.6) cde	1.48 (7.1) cde	0.01 (0.0) c	0.18 (0.2) d	1	6
Subterranean clover	16.7	9.9	1.45 (6.3) c	2.23 (13.4) bc	0.03 (0.0) c	0.18 (0.2) d	1	10
Ribwort plantain	24.5	37.9	0.40 (1.7) cdef	0.47 (0.9) efg	0.01 (0.0) c	0.05 (0.1) d	0	1
Creeping red fescue	12.9	13.0	0.73 (2.0) cdef	0.75 (1.7) efg	0.01 (0.0) c	0.04 (0.0) d	0	1
Black oats	13.6	8.3	0.32 (1.0) def	1.43 (8.0) cde	0.01 (0.0) c	0.12 (0.1) d	0	6
Perennial ryegrass	25.3	27.5	0.52 (3.7) cdef	0.00 (0.0) g	0.02 (0.0) c	0.00(0.0) d	0	0
Rye	12.1	11.0	0.00 (0.0) f	2.08 (19.8) bcd	0.00 (0.0) c	0.21 (0.3) d	0	15
Tall fescue	47.9	30.1	0.00 (0.0) f	0.60 (1.4) efg	0.00 (0.0) c	0.08 (0.1) d	0	1
Triticale	9.2	6.2	0.34 (1.1) def	2.61 (17.4) b	0.00 (0.0) c	0.19 (0.2) d	0	14
French marigold	–	8.4	–	0.16 (0.3) fg	–	0.01 (0.0) d	–	0
Garden nasturtium	9.1	6.6	5.37 (288.0) b	4.03 (75.1) a	0.52 (0.7) b	0.64 (1.2) c	26	58
Sweet pea (control)	3.8	26.4	6.54 (1,122) a	4.55 (129.1) a	0.64 (1.0) a	1.82 (7.5) a	100	100
Apple rootstock (control)	–-	16.1	–	3.97 (127.1) a	–	1.18 (3.7) b	–	99

[1]RF = reproduction factor = Pf (final nematode population/Pi = initial nematode population)

[2]S% = relative susceptibility = Pf of each cover crop/Pf of the most susceptible control plants X 100. In this experiment sweet pea was the most susceptible control plant

[3]Log(x + 1)-transformed values. Real means in brackets

[4]Means within the same column followed by the same letter(s) are not significantly different at ($P \leq 0.05$) according to Fisher's LSD test

of garden nasturtium 'Jewel Choice mix' (S% = 26). In the late winter/spring experiment, all cultivars had an S% value < 30%, with the exception of garden nasturtium 'Jewel Choice mix' (S% = 58) and Indian buckwheat (S% = 123). In 7 out of 15 cultivars included in both experiments, fresh root weight was higher during the winter experiment compared with the late winter/spring experiment. Fresh root weight of sweet pea 'Bijou mixed', the control plant included in both experiments, was higher in the late winter/spring experiment compared with the winter experiment (26.4 vs 3.8 g, respectively).

Discussion

Our results, based on the treshold of an S% value < 30, show that most of the cover crop cultivars (12 out of 17) included in our study were poor hosts of all three *Pratylenchus* species. In these cultivars, nematode population densities were in general (87.5%) < 25 nematodes roots g^{-1} in both winter and late winter/spring experiments. Interestingly, almost half of the nematode population densities higher than 25 nematodes g^{-1} roots were observed in the late winter/spring experiment with *P. penetrans*. These 12 cover crop cultivars do not support reproduction of *P. hippeastri*, *P. vulnus* and *P. penetrans*, and have thus the potential to limit the build-up of large, damaging populations of these three root-lesion nematodes in apple orchards. In previous studies, resistance to *P. penetrans* infection has also been found in cultivars of barrel medics, creeping red fescue, black oats, perennial ryegrass and French marigold but, in contrast with our study, not in white mustard and rapeseed (Marks et al. 1973; Thies et al. 1995; Vrain et al. 1996; Belair et al. 2002; Ball-Coelho et al. 2003; Lamondia 2006). In these studies, however, different cultivars of white mustard and rapeseed were evaluated compared with our study. Resistance of tall fescue cultivars to *P. vulnus* infection has also previously been reported (Nyczepir 2011). Differences in the host response to infection by plant-parasitic nematodes might exist among cultivars of the same plant species and care should be taken not to generalise the host response of one cultivar. Therefore, the host response to infection by *P. hippeastri*, *P. vulnus* and *P. penetrans* of cover crop cultivars that were not included in our study but could be used as cover crops in apple orchards in the Cape Peninsula should be evaluated.

Five out of the 17 cover crop cultivars included in our study were identified as good hosts of one or more of the three *Pratylenchus* species: pink serradella 'Margarita' and subterranean clover 'Aarbei Klawer' were good hosts of *P. penetrans* only; rye 'Duiker Max' was a good host of both *P. hippeastri* and *P. penetrans*; Indian buckwheat and garden nasturtium 'Jewel Choice mix' were good hosts of all three *Pratylenchus* species. Our observation that Indian buckwheat was a good host of *P. penetrans* confirms earlier observations (Belair et al. 2002). In these five cultivars, nematode population densities were, with very few exceptions, > 100 nematodes roots g^{-1} during both the winter and late winter/spring experiments. Population densities higher than 500 nematodes g^{-1} roots were observed on Indian buckwheat (*P. hippeastri*; in the late winter/spring experiment) and garden nasturtium 'Jewel Choice mix' (*P. penetrans*; in both winter and late winter/spring experiments). These five cover crop cultivars have thus the potential to enable the build-up of large damaging populations of either one, two or three of the root-lesion nematode species that are common in apple orchards in SA and may act as primary sources of infection of apple roots. Their use as cover crops in apple orchards infested with root-lesion nematodes should be avoided.

Pratylenchus species may occur in mixed-species populations in apple orchards. In about 10% of the apple orchards examined by Hugo (1994), mixed-species populations of two to three *Pratylenchus* species were found while in a more recent survey conducted in all the major pome fruit production areas in SA, mixed populations of *P. hippeastri, P. vulnus* and *P. penetrans* were also found (Knoetze et al, 2021). In view of the possibility that mixed-species populations of *Pratylenchus* may occur in an orchard, either the presence of *Pratylenchus* species should be examined in each apple orchard or only cover crop cultivars which are poor hosts of all *Pratylenchus* species which may be occur in apple orchards in SA should be included in the development of a root-lesion nematode management practice based on the cultivation of poor host cover crops in between apple trees.

Our results show that *P. penetrans* has the widest cover crop host range of the three *Pratylenchus* species included in our study: it could reproduce on five out of the 17 cover crop cultivars vs *P. hippeastri* and *P. vulnus* which could reproduce on three and two cover crop cultivars, respectively. *Pratylenchus penetrans* has a very wide host plant range ranging from maize, potato, vegetables to fruit trees (Castillo and Vovlas 2007; Duncan and Moens 2013). In contrast, *P. hippeastri* has a narrow host range being reported only from amaryllis, bromeliads, apples, grapevines and Cape Willow trees (Handoo et al. 2020) while *P. vulnus* infects mostly woody plants (Duncan and Moens 2013).

The experiments were carried out during two seasons and differences in weather conditions, especially temperature, may have had an effect on the reproduction of the *Pratylenchus* species. However, a comparison of the host response of the 12 cover crop cultivars that were poor hosts of all three *Pratylenchus* species shows that their poor host response was consistent between the two experiments indicting that differences in weather conditions had not affected their response. In contrast, differences in weather conditions may have affected the host response of the cover crop cultivars that were identified as susceptible to either one, two or all three *Pratylenchus* species. For instance, plants of subterranean clover 'Aarbei Klawer' infected with *P. penetrans* had an S% value of 24, in the winter experiment vs 53 in the late winter/spring experiment; plants of rye 'Duiker Max' infected with *P. hippeastri* and *P. penetrans* had S% values of 1 and 20, respectively, in the winter experiments vs 37 and 104, respectively, in the late winter/spring experiment. Both *P. penetrans* and *P. vulnus* have a worldwide distribution but *P. penetrans* mainly occurs in temperate climates vs *P. vulnus* in subtropical and Mediterranean climates (Duncan and Moens 2013). In our study, the population densities of *P. penetrans* on garden nasturtium 'Jewel Choice mix" (a good host of all three *Pratylenchus* species included in our study) were substantially higher in both experiments compared with *P. vulnus*: 539 and 589 nematodes g^{-1} roots in the winter and late winter/spring experiment, respectively, vs 288 and 75 nematodes g^{-1} roots, respectively. In contrast, the population densities of *P. hippeastri* were substantially higher in the late winter/spring experiment compared with the winter experiment (99 vs 464 nematodes g^{-1} roots) which may suggest that *P. hippeastri* prefers higher soil temperatures. Originally, *P. hippeastri* has been found in tropical regions (Florida, USA; Inserra et al. 2007; DeLuca et al. 2010).

Although the results of our study already provide information for apple producers to make informed decisions regarding the choice of cover crop cultivars to grow in apple orchards infested with root-lesion nematode species, the efficacy of these poor host cover crop cultivars to either suppress or limited the build-up of damaging population densities of these *P. hippeastri*, *P. vulnus* and *P. penetrans* should be further examined in situ. Also, in contrast with *P. hippeastri*, which has only been recorded in SA in two provinces on apple and *Salix mucronata* (Knoetze et al. 2021; Shokoohi 2019; Marais 2022), *P. penetrans* and *P. vulnus* are widespread in SA having been recorded in all provinces on a wide range of agricultural crops (maize, potato, vegetables, apple, grapevine, cotton, etc.; Marais 2022). The cover crops cultivars identified in our study as poor hosts of either *P. penetrans* and/or *P. vulnus* could also be used in these important agricultural crops to avoid damage that may be caused by these *Pratylenchus* species.

Acknowledgements The authors thank Bongani Sokomani, Murel Knipe, Dawn Hinds and Christopher Paulse for valuable technical assistance and Dr Mariette Marais (ARC-Plant Health and Protection) for supplying the information contained in the South African Plant-Parasitic Nematode Survey (SAPPNS) data bank.

Author's contribution RK and MA contributed to the conception, design and execution of the study. Preparation of the materials, data collection and analysis were performed by Rinus Knoetze. The first draft of the manuscript was written by Rinus Knoetze. DW commented on and edited the manuscript. All authors read and approved the final manuscript.

Funding The Agricultural Research Council and Hortgro (Project: P04000116) are acknowledged for funding this study.

Data availability All data generated and analysed in this study are included in this paper.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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