

HOST SUITABILITY OF AN ENDOPHYTE-FRIENDLY TALL FESCUE GRASS TO *MESOCRICONEMA XENOPLAX* AND *PRATYLENCHUS VULNUS*

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ABSTRACT

Nyczepir, A. P. 2011. Host suitability of an endophyte-friendly tall fescue grass to *Mesocriconema xenoplax* and *Pratylenchus vulnus*. *Nematropica* 41:45-51.

Tall fescue grass [*Schedonorus arundinaceus* (Schreb.) Dumont] cultivars with or without endophytes were evaluated for their susceptibility to *Mesocriconema xenoplax* and *Pratylenchus vulnus* in the greenhouse. Four tall fescue cultivars evaluated included, i) wild-type Jesup (E+, ergot-producing endophyte present), ii) endophyte-free Jesup (E-, no endophyte present), iii) Jesup (Max-Q, non-ergot producing endophyte) and iv) 'Georgia 5' (E+). Peach (susceptible Nemaguard rootstock) was included as the control. Nematode reproduction criteria were used in evaluating tall fescue susceptibility. Peach supported greater ($P \leq 0.05$) reproduction of *P. vulnus* and *M. xenoplax* than all tall fescue cultivars. Furthermore, all tall fescue cultivars were rated as poor hosts for *P. vulnus* and good hosts for *M. xenoplax* based on nematode reproduction factor. It was also observed that root endophyte status was not related to nematode reproduction and host susceptibility. These results provide useful insights into the potential use of tall fescue grass as a preplant groundcover alternative to chemical control of *P. vulnus*.

Key words: *Festuca arundinacea*, host parasitic relationship, management, *Mesocriconema xenoplax*, *Pratylenchus vulnus*, resistance, tall fescue grass, *Schedonorus arundinaceus*.

RESUMEN

Nyczepir, A. P. 2011. Evaluación de la reproducción de *Mesocriconema xenoplax* y *Pratylenchus vulnus* en un pasto con hongo endofítico. *Nematropica* 41:45-51.

Se evaluó la susceptibilidad a *Mesocriconema xenoplax* y *Pratylenchus vulnus* de cultivares del pasto *Schedonorus arundinaceus* (Schreb.) Dumont, con y sin hongo endofítico, en el invernadero. Los cuatro cultivares evaluados fueron i) Jesup tipo silvestre (E+, hongo endofítico productor de ergot presente), ii) Jesup libre de hongo endofítico (E-, hongo endofítico ausente), iii) Jesup (Max-Q, hongo endofítico no productor de ergot) y iv) 'Georgia 5' (E+). Se incluyeron plantas de duraznero (portainjerto susceptible Nemaguard) como controles. Se utilizó la reproducción del nematodo como criterio para evaluar la susceptibilidad del pasto. Se obtuvo mejor reproducción ($P \leq 0.05$) de *P. vulnus* y *M. xenoplax* en duraznero que en todos los cultivares de pasto evaluados. Se obtuvo baja reproducción de *P. vulnus* y alta reproducción de *M. xenoplax* en todos los cultivares. También se observó que la presencia del hongo endofítico no afectó la reproducción del nematodo o la susceptibilidad del pasto. Estos resultados son útiles al considerar la posibilidad de utilizar pastos en coberturas de presiembra como alternativa al control químico de *P. vulnus*.

Palabras clave: *Festuca arundinacea*, manejo, *Mesocriconema xenoplax*, *Pratylenchus vulnus*, relación hospedante parásito, resistencia, *Schedonorus arundinaceus*.

INTRODUCTION

In Georgia and South Carolina it is not uncommon to find more than one economically important plant-parasitic nematode genus within the same peach orchard, such as ring, *Mesocriconema xenoplax* (Raski, 1952) Loof & de Grisse, 1989 [= *Criconemoides xenoplax* (Raski, 1952) Loof and de Grisse, 1967]), root-lesion (*Pratylenchus* spp.), and root-knot (*Meloidogyne* spp.) nematodes (Nyczepir *et al.*, 1985). Ring, root-lesion, and root-knot nematodes are all important pathogens of peach in the United States and other parts of the world (Nyczepir and Esmenjaud, 2008).

In the southeastern United States the productive life span of peach [*Prunus persica* (L.) Batsch] trees does not exceed 6 to 10 years on some sites due to premature tree death (Brittain and Miller, 1978). Two causes of early tree mortality are a disease complex known as peach tree short life (PTSL) and Armillaria root rot (Savage and Cowart, 1942; Miller, 1994). Peach tree short life is caused by a predisposition of trees to cold injury, bacterial canker (*Pseudomonas syringae* pv. *syringae* van Hall) or a combination of both which results from parasitism by the ring nematode (Brittain and Miller, 1978; Nyczepir *et al.*, 1983). In closed-end field microplots peach trees died of cold injury after 4 years of parasitism by *M. xenoplax*, while trees in uninfested soil survived (Nyczepir *et al.*, 1983). Moreover, development of PTSL on land not planted with peaches for ≥ 75 years varies with exposure of trees to the cumulative population levels of *M. xenoplax* (Nyczepir *et al.*, 2004). Such evidence indicates that PTSL complex is a nematode-associated disease and the presence of *M. xenoplax* is required for the disease to occur.

Additionally, *M. xenoplax* was responsible for making peach trees (cv. 'Suwanee') more susceptible to bacterial spot (*Xanthomonas arboricola* pv. *pruni*) (Shepard *et al.*, 1999). Trees growing in *M. xenoplax*-infested soil show evidence of more severe bacterial spot damage than trees in soil where the ring nematode populations had been suppressed.

At least nine *Pratylenchus* spp. have been reported on peach throughout the world, but only *P. vulnus* Allen & Jensen, 1951 is of primary concern in California and the southeastern United States (Nyczepir and Esmenjaud, 2008). In Georgia, Fliegel (1969) was the first to report *P. vulnus* being associated with reduced peach tree vigor and rapid deterioration and reduction of feeder roots, which are distinct below-ground symptoms associated with root-lesion nematode feeding (Castillo and Vovlas, 2007). However, even with this evidence, many discounted the economic importance of *P. vulnus* to the peach industry in the southeastern United States until it was further demonstrated 32 years later in field microplots that *P. vulnus* (GA-peach isolate) was associated with reduced peach tree growth of 'Guardian[®]', 'Lovell',

and 'Nemaguard' rootstocks (Nyczepir and Pinochet, 2001). Reduction in marketable fruit size and yield has also been associated with peach trees growing in *P. vulnus* infested soil in California (McKenry, 1989). Managing *M. xenoplax* and *P. vulnus* is essential for establishment and optimizing yield of a peach orchard. The current preplant nematicide recommendation for managing these two plant-parasitic nematodes in the southeastern United States includes fumigation with Telone II (1,3-D) or Vapam (metam-sodium) (Horton *et al.*, 2010). These are the only two preplant soil fumigants available to peach growers, since the importation and manufacture of methyl bromide was banned in the United States and Western Europe after January 2005 (Clean Air Act, 1990), due to its role in ozone depletion. As a result of the reduced availability of pre- and post-plant nematicides in the agricultural market, alternatives to chemical control methods, such as rootstock resistance and nematode-suppressive groundcovers, are being investigated.

In the southeastern United States, Guardian[®] peach rootstock is recommended over other rootstocks previously used by this industry because trees on Guardian[®] rootstock have a lower mortality rate on PTSL sites even with *M. xenoplax* reproduction (Okie *et al.*, 1994a; Okie *et al.*, 1994b). Although, Guardian[®] has not exhibited resistance/tolerance to *P. vulnus* (Nyczepir and Pinochet, 2001), tall fescue grass has potential as an IPM preplant groundcover rotation crop for controlling *Pratylenchus* spp. (Bernard *et al.*, 1998). Additionally, tall fescue grass could be used as a post-plant groundcover between tree rows to address problem issues with soil erosion and poor orchard trafficability.

Tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumont. = *Lolium arundinaceum* (Schreb.) Darbysh., formerly *Festuca arundinacea* Schreb.] is the most widely grown perennial, cool-season turf and forage grass species that is well-adapted in the transition zone between the temperate northeast and subtropical southeastern United States. 'Kentucky 31' tall fescue is the dominant variety grown throughout the United States and its popularity among other forages is the result of it being vigorous, widely adaptable, able to withstand poor soil conditions, and resistant to pests and drought (Ball *et al.*, 1993). These favorable growth characteristics have been attributed to the symbiotic relationship with the endophytic fungus *Neotyphodium coenophialum* (Morgan-Jones & W. Gams) Glenn, C. W. Bacon, & Hanlin (West *et al.*, 1988; Bacon, 1995). The presence of the endophyte in tall fescue also has been reported to confer resistance to *Pratylenchus* spp. (Bernard *et al.*, 1998). For example, populations of *P. scribneri* were lower in soil planted to 'Kentucky 31' tall fescue in the presence of the endophyte than when the endophyte was not present (West *et al.*, 1988). Suppression of *P. scribneri* populations has also been associated with different strains of *N. coenophialum*

and tall fescue (Timper *et al.*, 2005). Populations of *P. scribneri* were suppressed in cv. 'Georgia 5' tall fescue containing the non-ergot endophyte strain AR584, whereas this endophyte did not suppress *P. scribneri* populations in cv. 'Jesup'.

The presence of the endophyte infection is not always beneficial; it has been associated with the "fescue toxicosis" syndrome in grazing cattle (Bacon *et al.*, 1977; Bouton, 2002). Tall fescue infected with *N. coenophialum* contains a group of ergot alkaloids produced by the endophyte. Fescue toxicosis is manifested when sensitive animals graze on *N. coenophialum*-infected grass resulting in elevated body temperature, poor weight gain, and (or) reduced prolactin concentrations (Bouton, 2002). One method to overcoming fescue toxicosis is to use nontoxic strains of *N. coenophialum* (i.e., endophyte-friendly) (Bacon and Siegel, 1988). An endophyte-friendly tall fescue provides beneficial effects for the plant without producing fescue toxicosis in animals. One such endophyte-friendly commercial tall fescue variety is 'Max-Q' [i.e., Jesup (Max-Q)]. Max-Q is the result of a novel, non-toxic endophyte strain (i.e., AR542) being inserted in 'Jesup' and 'Georgia 5' tall fescue in place of the toxic endophyte strain; thus providing beef producers the opportunity of avoiding fescue toxicosis (Phillips and Aiken, 2009). Jesup (Max-Q) tall fescue susceptibility to *P. vulnus* and *M. xenoplax* is unknown. The objectives of this research were to evaluate the host susceptibility and nematode reproduction of endophyte-infected (E+), endophyte-free (E-) and Jesup (Max-Q) tall fescue to *P. vulnus* and *M. xenoplax*.

MATERIALS AND METHODS

Nematode source and inoculum

Pratylenchus vulnus, which originated from a peach (*Prunus persica* L. Batsch) orchard in Byron, Georgia was reared monoxenically on carrot (*Daucus carota* L.) disk cultures (Moody *et al.*, 1973) and incubated at 22°C for multiplication. The *M. xenoplax*, which originated from a PTSL orchard in Byron, Georgia was cultured on Nemaguard peach seedlings in the greenhouse. Root-lesion nematode inoculum was prepared by macerating the nematode infested carrot disks in water in a commercial blender for four times at 5-second intervals. The nematode/carrot suspension was then concentrated using a 250- μ m sieve nested on a 38- μ m sieve (60 and 400-mesh, respectively). The carrot debris collected on the 250- μ m sieve was discarded, whereas the content on the 38- μ m sieve was placed on a Baermann funnel, from which the nematode inoculum was obtained. The *M. xenoplax* inoculum was extracted from the culture medium using centrifugation.

Assessment of tall fescue with (E+) or without (E-) ergot endophyte to P. vulnus and M. xenoplax

Four tall fescue cultivars with (E+) or without (E-) ergot endophyte were evaluated for host susceptibility to *P. vulnus* and *M. xenoplax* in the greenhouse. Fescue cultivars evaluated included, i) wild-type Jesup (E+, ergot-producing endophyte present), ii) endophyte-free Jesup (E-, no endophyte present), iii) Jesup (Max-Q, non-ergot producing endophyte) and iv) 'Georgia 5' (E+). Peach (*Prunus persica* L. Batsch) (susceptible Nemaguard rootstock) was included as the control. Individual 67-day-old Nemaguard peach seedlings or sets of five seed of each tall fescue cultivar were planted in separate 15 cm diameter plastic pots containing 1,500 cm³ steam pasteurized loamy sand (86% sand, 10% silt, 4% clay; pH 6.1; 0.54% organic matter). Pots without plants were designated as a fallow treatment to compare survival of nematodes in the absence of any plant with survival in soil planted with tall fescue or peach. Approximately 14 days later, the tall fescue seedlings were thinned to one plant per pot and the soil in all treatment pots (including fallow) was infested with 3,000 *P. vulnus* adults and juveniles or 1,000 *M. xenoplax* adults and juveniles in 40 ml water (Nyczepir *et al.*, 1987); which is equivalent to 200 *P. vulnus*/100 cm³ soil or 67 *M. xenoplax*/100 cm³ soil, respectively. Ten replications of each plant species or cultivar and of the fallow treatment were arranged in randomized complete blocks on benches in an air-conditioned greenhouse (27 + 5°C). Plants were watered and fertilized with Osmocote® (14-14-14) as needed. The *P. vulnus* and *M. xenoplax* experiments were terminated 130 days and 160 days, respectively, after soil infestation and nematode population densities in soil and roots were quantified. Nematodes in soil were extracted from a 100-cm³ subsample with a semi-automatic elutriator (Byrd *et al.*, 1976) and centrifugal-flotation and all life stages counted. *Pratylenchus vulnus* in roots were extracted by randomly cutting an approximately 8 gram fresh weight part of the root system and placing it on a fine screen in a Seinhorst mistifier chamber (Hopper, 1970) for 9 days at 23°C. After extracting the nematodes from the roots, the dry root weight (dried at 70°C in aluminum foil until no more loss in weight occurred) of each tissue extraction sample was determined. The remaining root systems were dried to a constant weight and then combined with the tissue extraction sample weights for total dry weight. The nematode reproduction factor [Rf = final population density (Pf) of all life stages divided by initial population density (Pi)] was calculated as a measure of host susceptibility among the different treatments. Test hosts were grouped into three classifications based on the nematode Rf rating, as follows: nonhost, Rf = 0 (highly resistant); poor host (resistant), Rf = 0.01-0.99; and good host (susceptible), Rf \geq 1. The experiment was repeated one time with minor modifications, which included inoculating approximately 25-day-old established peach and tall fescue grass seedlings and terminating the *P. vulnus*

Table 1. Reproduction of *Mesocriconema xenoplax* on tall fescue grass and peach cultivars in the greenhouse 160 (test 1) and 159 (test 2) days after soil infestation.

Plant species	Cultivar ^w	Rf ^v		<i>M. xenoplax</i> gravid females (Nematodes/100 cm ³ soil)	
		Test 1	Test 2	Test 1	Test 2
Peach	Nemaguard	96.95 ^{xy}	145.51 ^{xy}	707 ^{xy}	121 ^{xy}
Tall fescue	WT Jesup (E+)	3.05 a ^z	5.04 a	8 a	6 a
	EF Jesup (E-)	2.04 a	15.69 a	17 a	68 a
	Jesup (Max-Q)	1.32 a	9.07 a	6 a	11 a
	GA 5 (E+)	1.16 a	20.66 a	6 a	27 a
	Combined fescue	1.89	12.62	9	28
Fallow		0.00	0.09	0	0

Data are means of 10 replications.

^vRf = reproductive factor (Pf/Pi), where Pf = final population density and Pi = initial population density of 67 *M. xenoplax* per 100 cm³ soil.

^wWT = wild-type Jesup (E+, ergot-producing endophyte present); EF = endophyte-free Jesup (E-, no endophyte present), iii) Jesup (Max-Q, non-ergot producing endophyte) and iv) GA 5 = 'Georgia 5' (E+).

^xThe single-degree-of-freedom comparison between the means for peach vs. combined tall fescue cultivars and peach vs. fallow was highly significant ($P \leq 0.01$).

^yData were transformed [$\log_{10}(x + 1)$] before analysis and nontransformed data are shown in table.

^zMeans within tall fescue cultivars and column followed by the same letter are not different ($P \leq 0.05$) according to LSD.

Table 2. Reproduction of *Pratylenchus vulnus* on tall fescue grass and peach cultivars in the greenhouse 130 (test 1) and 153 (test 2) days after soil infestation.

Plant species	Cultivar ^v	Rf ^u		<i>P. vulnus</i> per			
		(soil + roots)		100 cm ³ soil		Gram dry root	
		Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
Peach	Nemaguard	11.18 ^w	26.00 ^w	745 ^{wx}	4,545 ^{wx}	391 ^w	2,681 ^w
Tall fescue	WT Jesup (E+)	0.01 b ^y	0.06 a	2 b	12 a	< 1 b	< 1 a
	EF Jesup (E-)	0.06 a	0.01 a	12 a	0 a	2 a	< 1 a
	Jesup (Max-Q)	0.00 b	0.03 a	0 b	6 a	0 b	< 1 a
	GA 5 (E+)	0.03 ab	0.06 a	6 ab	11 a	< 1 ab	< 1 a
	Combined fescue	0.03	0.03	5	7	< 1	< 1
Fallow		0.15	0.07	30	14	--- ^z	---

Data are means of 10 replications.

^uRf = reproductive factor (Pf/Pi), where Pf = final population density and Pi = initial population density of 200 *P. vulnus* per 100 cm³ soil.

^vWT = wild-type Jesup (E+, ergot-producing endophyte present); EF = endophyte-free Jesup (E-, no endophyte present), iii) Jesup (Max-Q, non-ergot producing endophyte) and iv) GA 5 = 'Georgia 5' (E+).

^wThe single-degree-of-freedom comparison between the means for peach vs. combined tall fescue cultivars and peach vs. fallow was highly significant ($P \leq 0.01$).

^xData were transformed [$\log_{10}(x + 1)$] before analysis and nontransformed data are shown in table.

^yMeans within tall fescue cultivars and column followed by the same letter are not different ($P \leq 0.05$) according to LSD.

^z --- = not included.

and *M. xenoplax* experiments 153 days and 159 days, respectively, after soil infestation.

Statistical analysis

All data were subjected to a general linear model procedure of SAS (SAS Institute, Cary, NC). Appropriate preplanned single degree of freedom comparisons were used to detect differences among treatment means as follows: Nemaguard peach vs. combined tall fescue cultivars and peach vs. fallow means following a significant F test. Means within a plant species were analyzed using LSD. Nematode data were transformed to $\log_{10}(x + 1)$ values and only nontransformed means are reported in tables. Only significant differences ($P \leq 0.05$) will be discussed unless stated otherwise.

RESULTS AND DISCUSSION

Nemaguard peach supported greater ($P \leq 0.01$) reproduction (Rf) and more gravid females of *M. xenoplax* than the tall fescue cultivars combined in both tests (Table 1). Even though peach was a better host for *M. xenoplax* than tall fescue, all individual tall fescue cultivars did support nematode reproduction and had gravid females present in soil. Furthermore, differences in *M. xenoplax* reproduction were not detected among the tall fescue cultivars as measured by nematode reproductive factor. Moreover, *M. xenoplax* reproduction does not appear to be influenced by endophyte status as has been reported for other ectoparasitic nematodes such as *Helicotylenchus dihystrera* and *Paratrichodorus minor* (Bernard *et al.*, 1998). Results indicate that all tall fescue cultivars tested would be rated as susceptible (good host) based on nematode reproduction factor $Rf \geq 1$. Similar results were observed by Zehr *et al.* (1986) in that the one tall fescue grass tested (cultivar unknown) maintained ring nematode reproduction (i.e., $Rf = 1.01$). All tall fescue cultivars in the current study exhibited Rf values and numbers of gravid females per 100 cm³ soil between 1.16 to 20.66 and 6 to 68, respectively. Utilizing tall fescue grass as an alternative to preplant fumigation control of *M. xenoplax* does not appear to be a practical recommendation for peach growers in the southeastern United States.

In both tests, 'Nemaguard' peach (known susceptible) supported greater ($P \leq 0.05$) reproduction of *P. vulnus* than all tall fescue cultivars combined and fallow as indicated by Rf value, number of *P. vulnus*/100 cm³ soil, and (or) number of *P. vulnus*/gram dry root (Table 2). Differences in *P. vulnus* reproduction as influenced by endophyte status among the individual tall fescue cultivars (i.e., E+ vs. E- vs. Max-Q) were inconclusive between test 1 and test 2. In test 1, EF Jesup (E-) supported greater nematode reproduction than WT Jesup (E+) and Jesup (Max-Q) based on Rf value, number of *P. vulnus*/100 cm³ soil, and number

of *P. vulnus*/gram dry root; indicating a possible association between endophyte status and nematode reproduction. However in test 2, differences among individual tall fescue treatments were not significant. Additionally, all individual tall fescue cultivars did not support *P. vulnus* reproduction in both tests ($Rf = 0.00 - 0.06$); indicating all tall fescue cultivars would be rated as poor hosts (resistant, $Rf = 0.01 - 0.99$) to *P. vulnus* infection.

It is evident from this study that tall fescue grass differed in its ability to suppress nematode reproduction of *M. xenoplax* and *P. vulnus*. In the current study, tall fescue grass is a poor host (resistant) for *P. vulnus*, but a good host (susceptible) for *M. xenoplax*. One explanation for the differences in nematode reproduction may be related to the different parasitic feeding habit between these two nematode genera. Nematode feeding sites on roots differ between a migratory endoparasite, such as the root-lesion nematode, and an ectoparasite, such as the ring nematode. The root-lesion nematode feeds in the root cortex, moving through and between the parenchyma cells which result in visible necrotic lesions (Castillo and Vovlas, 2007). The visible necrotic lesions are believed to result from a combination of both secretion of cell-wall degrading enzymes and mechanical force from the stylet (i.e., pressure from labial region and thrusting) of *Pratylenchus* spp. In contrast, ring nematodes feed from individual cortical cells for up to eight days and then move to a new feeding site along the outside of the root (Hussey *et al.*, 1992); which is modified into discrete food cells. Both nematode genera are cortical feeders, but one difference may be where the nematode is physically located in the root while feeding. The mode of action of nematode suppression in these tall fescue cultivars was not addressed in this study, but two possible mechanisms of root-lesion nematode suppression maybe i) the inability of *P. vulnus* to complete its life cycle and (or) ii) the occurrence of natural plant metabolites in tall fescue grass regardless of the presence/absence of the endophyte fungus. Several ergot alkaloids (e.g., ergovaline and α -ergocryptine) and polyphenolic compounds (e.g., chlorogenic acid) have been identified in tall fescue roots as being nematocidal or nematostatic to *P. scribneri*, respectively. However, only the polyphenolic compounds were not correlated with endophyte status (Bacetty *et al.*, 2009). Possibly the polyphenols are involved with suppression of *P. vulnus* in tall fescue grass and warrants further investigation.

Finding one alternative to chemical control for all three peach nematode pathogens in the southeastern United States (i.e., *M. xenoplax*, *Meloidogyne* spp., and *P. vulnus*) is difficult. Currently, Guardian[®] peach rootstock is recommended over other rootstocks previously used by this industry because trees on Guardian[®] survive longer on PTSL sites infested with *M. xenoplax* and are less prone to stunted growth in the presence of some *Meloidogyne* spp. Unfortunately,

Guardian® is not resistant to *P. vulnus* (Nyczepir and Pinochet, 2001). Therefore, tall fescue grass may have potential as a preplant ground cover IPM tool for suppressing the population density of *P. vulnus* in peach orchard establishment and warrants further investigation. Also, it would be important to select the appropriate tall fescue cultivar [e.g., Jesup (Max-Q)] if grazing cattle prior to orchard establishment is an option.

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