Pearl Millet for the Management of *Pratylenchus penetrans* in Flue-Cured Tobacco in Quebec

Guy Bélair, Nathalie Dauphinais, and Yvon Fournier, Horticulture Research and Development Centre, Agriculture and Agri-Food Canada, St-Jean-sur-Richelieu, Quebec, Canada J3B 3E6; and Om P. Dangi, Agriculture Environmental Renewal Canada Inc. (AERC), Ottawa, Ontario, Canada K2E 7J6

ABSTRACT

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Two 1-year rotation experiments were conducted from 1998 to 2000 to assess the impact of forage and grain pearl millet (*Pennisetum glaucum*) on *Pratylenchus penetrans* populations in a tobacco field (*Nicotiana tabacum* cv. Delgold) in Quebec. Performance of these crops was compared with rye, the standard rotation crop. Rye plots were doubled to include a fumigated standard (application of metham sodium at 67.4 liters/ha on a band). Forage pearl millet increased tobacco yields by an average of 103% compared with nonfumigated rye. In 2000, leaf dry weights of tobacco following grain pearl millet CGPM H5 and CGPM H6 was increased by 70 and 73%, respectively, when compared with nonfumigated rye and were not significantly different from the fumigated plots following rye. Forage and grain pearl millet should be considered as a viable alternative to fumigation for controlling *P. penetrans* infestation in flue-cured tobacco production in Quebec.

The root-lesion nematode, *Pratylenchus penetrans* (Cobb) Filipjev & Schuur. Stekh., is a serious pest of many crops in North America. In eastern Canada, this endoparasitic nematode causes substantial economic losses in high-value crops (1,4,6,8,10,12,15,16). In Quebec, economically damaging population densities of *P. penetrans* have been recorded on tobacco (*Nicotiana tabacum* L.) (11).

In eastern Canada and Quebec, rye (*Secale cereale* L.) is commonly grown in rotation with flue-cured tobacco. It has been demonstrated that rye is a good host of the root-lesion nematode (3,5,14), so its use as rotation crop increases nematode populations on subsequent crops, thereby potentially decreasing yields. The reliance on soil fumigation with chemicals such as metham sodium is a common practice in Quebec to control preplant populations of *P. penetrans* and increase tobacco yields. The use of crops that are unfavorable for the root-lesion nematode may help to reduce or eliminate the use of nematicide in these soils. Forage and grain hybrids of

pearl millet (*Pennisetum glaucum* L.) have been developed by Agriculture Environmental Renewal Canada (AERC Inc., Ottawa, Ontario). The principal distinguishing characteristics of grain pearl millet are its dwarf stature, early maturity, and grain yield. Forage pearl millet is highly photo-period sensitive and produces high yields of green fodder with good forage quality. Both grain and forage type hybrids developed by AERC are well adapted to climatic and soil conditions of eastern Canada. These hybrids were shown to be poor hosts of *P. penetrans* in both greenhouse and field conditions in Canada (1,3,6,7).

The objective of this research was to assess the impact of forage pearl millet and grain pearl millet on populations of *P. penetrans* and their impact on subsequent flue-cured tobacco yields in Quebec.

MATERIALS AND METHODS

From 1998 to 2000, two 1-year crop rotation experiments were conducted on one experimental site at St-Étienne-des-Grès, Quebec (46°26'N; 72°46'W), on a sandy soil (85% sand, 8% silt, 7% clay, and 5.2% organic matter). Trial 1 was carried out from 1998 to 1999 and trial 2 from 1999 to 2000. In both trials, rotation crops were planted the first year and tobacco was grown the following year as the high-value crop. Trials were carried out in adjacent fields on the same farm, and tobacco was the previous crop. In both trials, five treatments were arranged in a randomized complete block design with four replications. The crops in trial 1 were forage pearl millet (hybrid CFPM 101), grain pearl millet (hybrid CGPM H6), grain sorghum

(cv. GS7), and rye (cv. Musketeer). Because grain sorghum was shown to be a good host of the root-lesion nematode, it was replaced by an early maturing grain pearl millet hybrid (CGPM H5) in trial 2. Rye, which promotes an increase in populations of the root-lesion nematode, was used as our positive control crop.

Eight plots of rye were included in the first year of each trial. Four of these plots were fumigated prior to planting tobacco and four were not fumigated. Tobacco and rye seeds were obtained from local seed suppliers, and pearl millet hybrid and grain sorghum seeds were provided by AERC. Forage and grain pearl millet hybrids were selected based on their performance, as genotypes that suppressed *P. penetrans* populations in a greenhouse screening study (7). In both trials, the four rye plots were fumigated with metham sodium (Vapam) at 67 liters/ha on 28 April 1999 (trial 1) and on 24 April 2000 (trial 2). The fumigant was injected at the 15-cm depth using a 4-row John Blue applicator equipped with one injector per row. Soil temperature was approximately 9°C at injection depth.

Plots were 6.1 m wide × 14 m long. In both trials, plots were sown with fall rye the year before the trial; and the following spring, plots to be sown to millet or sorghum were plowed 1 week before sowing. For trial 1, millet and sorghum were sown on 2 June 1998 and received 300 kg/ha N-P-K (23-11-20) fertilizer. A second planting was done on 13 July 1998 due to the poor establishment of the first seedlings. Millet and sorghum were sown with a 15-cm row spacing. Hybrids CFPM 101 and CGPM H6 were planted using a seed rate of 6 and 5 kg/ha, and grain sorghum CGS 7 was planted at 14 kg/ha to obtain a plant population of 150,000 plants per ha. The fall rye was densely planted using a seed rate of 120 kg/ha, which is similar to the traditional practice. In trial 2, millets were sown on 7 June 1999 and received 300 kg/ha N-P-K (34-0-0 and 10-10-20) fertilizer 1 week before sowing. In 1999, the seeding rate of all pearl millet hybrids was increased by 30% due to low germination recorded the previous year. Populations of grain pearl millet hybrids (seed rate of approximately 7 kg/ha) were about 265,000 plants per ha, and forage pearl millet (10 kg/ha) was about 388,000 plants per ha. Seeding rate used for rye was simi-

Corresponding author: G. Bélair
E-mail: belairg@agr.gc.ca

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lar to that in trial 1. In both trials, weeds were controlled manually several times during the season. Two cuts of the forage CFPM 101 were performed annually; one cut on the last week of July and a second on the second week of September. Each time, the forage was chopped and left on the soil. Rotation crops were incorporated as green manure by plowing in early October.

In trial 1, tobacco seedlings (*Nicotiana tabacum* L. cv. Delgold) were transplanted on 15 May 1999, 56 cm apart within a row and 107 cm between rows. In trial 2, seedlings were transplanted on 7 June 2000, 61 cm apart within a row and 107 cm between rows. All plots were fertilized with a broadcast application of 3-10-20 (4% Mg, 5% Ca) at 1,188 kg/ha before transplanting. Plots also received 224 kg/ha of 3-10-20 at transplanting and 224 kg/ha of 3-0-20 (50% calcium nitrate) on the last week of June.

Tobacco yields were collected based on biomass, but quality evaluations were not conducted. Leaf yield was determined by harvesting two tobacco plants per plot for trial 1 and six plants per plot for trial 2. Leaves were cut from the stem, and both were dried for 3 days at 65°C. Yield was expressed as dry weight in kg/ha. Plant height and number of leaves per plant were recorded by examining 10 arbitrarily chosen tobacco plants per plot for trial 1 and

36 plants for trial 2. Leaf width and length were recorded in trial 2. Tobacco yields were recorded on 29 July 1999 (trial 1) and on 8 August 2000 (trial 2).

Soil and root samples were collected at each site to assess population densities of *P. penetrans*. Sampling dates were 2 June 1998, 29 July 1998, 16 October 1998, 19 May 1999, 29 July 1999, and 8 September 1999 for trial 1, and 19 May 1999, 29 July 1999, 8 September 1999, 15 June 2000, 8 August 2000, and 29 September 2000 for trial 2. For the first sampling date, 15 soil cores (5 cm diameter × 20 cm deep) per plot were randomly collected within plots. For the midseason and harvest sampling, both soil and roots were collected. Roots from 15 plants per plot were removed arbitrarily, and the soil surrounding roots was kept for soil extraction. Both soil and roots were placed in plastic bags and stored at 4°C until nematode extraction. Nematode population density was estimated by processing two 50-g soil samples from each plot by the modified Baermann pan method (18). Roots were washed, and two 15-g root samples per plot were placed in a misting chamber for 2 weeks at 22°C (17). After the nematode extraction, roots were oven-dried (65°C) for 2 days and weighed. Nematodes were counted using a stereomicroscope and expressed as numbers per kilogram of soil and numbers per gram of dry root weight.

Statistical analysis. Nematode counts were transformed using $[\log_{10}(x+1)]$ before statistical analysis. Data were analyzed by the analysis of variance using the general linear model (PROC GLM) procedure (SAS Institute, Cary, NC). Waller's test was used to compare treatments when the analysis of variance showed significant differences among means ($P \leq 0.05$). Pearson correlation (PROC CORR) was run between means for the logarithm of the number of *P. penetrans* in tobacco roots at harvest and tobacco yields (SAS Institute).

RESULTS

In both trials, rotation crops influenced densities of *P. penetrans* in the soil and the roots during the rotation year (Table 1). In 1998 and 1999, average initial nematode densities were 4,258 and 407 per kilogram of soil, respectively. In trial 1, nematode densities in initial soil samples on 2 June 1998 and midseason root samples on 29 July 1998 were higher under rye than the other crops. Nematode densities in the final soil samples (16 September 1998) were the highest under rye and CGS 7, intermediate under CGPM H6, and the lowest under CFPM 101. In trial 2, based on initial soil densities on 19 May 1999, the plots were uniformly infested with *P. penetrans*. At midseason, soil and root densities (29 July 1999) were lower under

Table 1. *Pratylenchus penetrans* densities under rotation crops in 1998–1999

Rotation crop	Trial 1 (1998)					Trial 2 (1999)				
	<i>P. penetrans</i> per kg soil			<i>P. penetrans</i> per g dry root		<i>P. penetrans</i> per kg soil			<i>P. penetrans</i> per g dry root	
	Sampling dates									
	2 June	29 July	16 Oct.	29 July	16 Oct.	19 May	29 July	8 Sept.	29 July	8 Sept.
Forage millet (CFPM 101)	2,443 bA ^z	1,350 bB	875 cB	168 bB	293 cA	605 aA	775 bA	883 bcA	177 bA	74 aA
Grain millet (CGPM H5)	...	...	...	...	...	198 aB	680 bA	758 bcA	137 bA	250 aA
Grain millet (CGPM H6)	2,828 bA	1,825 bA	1,893 bcA	126 bB	367 cA	363 aA	743 bA	1,450 cA	68 bA	147 aA
Grain sorghum (CGS 7)	2,500 bA	710 cB	3,365 aA	114 bB	924 bA	...	...	...	...	...
Rye	6,635 aA	4,950 aAB	3,773 abB	411 aB	2,157 aA	300 aB	4,617 aA	3,073 abA	1,356 aA	910 aA
Rye (to be fumigated)	6,883 aA	11,438 aA	3,893 aB	644 aB	3,352 aA	570 aB	3,937 aA	5,160 aA	1,580 aA	601 aB
Probability > <i>F</i>	0.0005	0.0001	0.0016	0.0001	0.0001	0.0653	0.0001	0.0144	0.0001	0.4327

^z For each extraction method used to estimate nematode population, the logarithm of values within a column followed by the same lowercase letter and within a row followed by the same uppercase letter are not significantly different, as determined by Waller's test ($P = 0.05$).

Table 2. *Pratylenchus penetrans* densities under flue-cured tobacco crop in 1999–2000

Rotation crop	Trial 1 (1999)					Trial 2 (2000)					
	<i>P. penetrans</i> per kg soil			<i>P. penetrans</i> per g dry root		<i>P. penetrans</i> per kg soil			<i>P. penetrans</i> per g dry root		
	Sampling dates										
	19 May	29 July	8 Sept.	29 July	8 Sept.	15 June	8 Aug.	29 Sept.	8 Aug.	29 Sept.	
Forage millet (CFPM 101)	735 bB ^z	300 dC	1,753 bcA	544 bcB	1,853 aA	95 aB	25 cC	1,363 aA	158 cB	858 aA	
Grain millet (CGPM H5)	...	...	...	...	...	130 aB	323 bB	1,825 aA	517 bA	1,613 aA	
Grain millet (CGPM H6)	2,035 abA	598 cB	2,980 abA	595 bcB	3,764 aA	173 aB	250 bB	1,788 aA	232 bcB	2,035 aA	
Grain sorghum (CGS 7)	3,240 aA	1,133 bB	2,645 bA	850 abA	3,176 aA	...	...	...	...	...	
Rye	4,563 aA	2,287 aB	6,385 aA	1,672 ab	4,107 aA	380 aB	1,235 aA	2,538 aA	4,568 aA	2,687 aA	
Rye fumigated	238 cB	333 dA	928 cA	482 cB	1,447 aA	35 aB	118 bB	2,975 aA	1,387 bA	1,801 aA	
Probability > <i>F</i>	0.0001	0.0001	0.0003	0.0142	0.3315	0.4037	0.0003	0.1898	0.0001	0.1906	

^z For each extraction method used to estimate nematode population, the logarithm of values within a column followed by the same lowercase letter and within a row followed by the same uppercase letter are not significantly different, as determined by Waller's test ($P = 0.05$).

CFPM 101, CGPM H5, and CGPM H6 than with rye.

For trial 1 in 1998, soil densities of *P. penetrans* decreased by 64.2% during the season in the plots cropped with CFPM 101 ($P = 0.0028$) (Table 1). Density of *P. penetrans* in roots increased for all treatment crops. For trial 2 in 1999, soil nematode densities under rye and CGPM H5 increased from May to July, but remained unchanged throughout the entire season under CFPM 101 and CGPM H6. In 1999, average density of *P. penetrans* in millet roots ranged from 68 to 250 per gram of dry root and remained constant from July to September. Average nematode densities in rye roots ranged from 601 to 1,580 per gram of dry root and were reduced from July to September in plots of rye to be fumigated.

In the tobacco crop, densities of *P. penetrans* either in soil or in tobacco roots were affected by the previous rotation crop (Table 2). In trial 1, preplanting 19 May 1999 soil nematode densities were highest in plots previously in nonfumigated rye and CGS7, intermediate in CGPM H6, and lowest in CFPM 101 and fumigated rye plots. At midseason (29 July 1999), soil nematode densities were highest under rye and lowest following CFPM 101 and fumigated rye. Thus, the suppressive effect of the forage pearl millet (CFPM 101) and, to a lesser extent, CGPM H6 on populations of *P. penetrans* was detectable 1 year after the rotation crop (trial 1). In 1999, densities of *P. penetrans* under CFPM 101 were similar to those observed in fumigated rye plots. Final soil nematode densities (8 September 1999) were the highest in plots previously grown in nonfumigated

rye and the lowest in fumigated rye. In tobacco, final root nematode densities in September were not affected by previous rotation (Table 2).

In trial 2, midseason (8 August 2000) soil nematode densities were highest in plots previously grown in nonfumigated rye, intermediate in CGPM H5, CGPM H6, and fumigated rye, and lowest in CFPM 101. Midseason root nematode densities followed the same trend, except CGPM H6 was not significantly different from CFPM 101.

In 1999 (trial 1), soil densities of *P. penetrans* were at their lowest in July for all crops, except the fumigated rye plots, which had the lowest populations in May (Table 2). Root densities of *P. penetrans* in tobacco increased from July to September, except for plots previously grown in sorghum. In 2000 (trial 2), densities of *P. penetrans* in soil were at their lowest level in June and at the highest level in September. In 2000, nematode densities in tobacco roots increased during the growing season in plots previously in CGPM H6 ($P = 0.0021$) and CFPM 101 ($P = 0.0037$), but did not change significantly in the other previous rotation crops.

The growth of tobacco plants in both field trials was significantly affected by the rotation crops (Tables 3 and 4). In trial 1, plants were tallest following CFPM 101, intermediate following CGPM H6 and fumigated rye, and shortest after nonfumigated rye and CGS 7 (Table 3). Number of leaves was significantly reduced following CGS 7 compared with the other treatments. Tobacco leaf dry weights were increased by 43 and 22% after CFPM 101 and CGPM H6, respectively, and signifi-

cantly reduced by 41% following CGS 7 compared with nonfumigated rye. Tobacco plants grown in CFPM 101 plots were significantly taller than the ones in the fumigated rye plots, but were not different based on all other growth parameters.

In trial 2, tobacco plant height was significantly increased following millet rotation and soil fumigation (Table 4). Plants after CFPM 101 were significantly taller than the other treatments, including fumigated rye plots (+14%). Following both CGPM H5 and CGPM H6 rotations, plant height, number of leaves, leaf size, and leaf dry weight increased significantly compared with unfumigated rye and were not significantly different from the plants measured in the fumigated rye plots. Leaf dry weights of tobacco following CGPM H5 and CGPM H6 were increased by 70 and 73%, respectively, compared with unfumigated rye, but were not significantly different from the fumigated plots. Based on leaf dry weight, forage pearl millet CFPM 101 increased tobacco yields by an average of 103% compared with rye (Tables 3 and 4).

Tobacco growth in both trials was negatively correlated with densities *P. penetrans* in tobacco roots at harvest (Table 5). All plant growth parameters were better correlated to nematode densities in trial 2 than in trial 1.

DISCUSSION

In this study, rotation crops significantly influenced densities of *P. penetrans* in tobacco, and nematode densities had a negative effect on the growth of tobacco plants. These results support earlier reports of the susceptibility of tobacco to *P. penetrans* (6,8,10,13,15,16) and demonstrate that forage pearl millet hybrid CFPM 101 in rotation with tobacco significantly decreases densities of *P. penetrans* compared with rye (the traditional rotation crop). Based on both the field trials, forage pearl millet increased tobacco yields by an average of 103% compared with rye. In the second trial, both grain pearl millet hybrids (CGPM H5 and CGPM H6) were similar to forage pearl millet in their ability to suppress nematode populations and provided a 70 to 73% increase in tobacco yield, which was equal to soil fumigation. These results also support the hypothesis

Table 3. Effect of rotation crops on growth of flue-cured tobacco in 1999

Rotation crops	Tobacco growth			
	Plant height (cm)	Leaves per plant	Leaf dry weight (kg/ha)	Plant dry weight (kg/ha)
Forage millet (CFPM 101)	126.4 a ^z	16.3 a	2,847.0 a	3,999.5 a
Grain millet (CGPM H6)	119.3 b	15.7 ab	2,429.7 ab	3,300.6 ab
Grain sorghum (CGS 7)	96.5 d	13.6 c	1,181.4 c	1,503.6 c
Rye	111.0 c	15.5 b	1,992.2 b	2,600.6 b
Rye fumigated	121.0 b	16.0 ab	2,842.8 a	3,994.5 a
Probability > F	0.0001	0.0001	0.0002	0.0001

^z Values within a column followed by the same letter are not significantly different, as determined by Waller's test ($P = 0.05$).

Table 4. Effect of rotation crops on growth of flue-cured tobacco in 2000

Rotation crops	Tobacco growth					
	Plant height (cm)	Leaves per plant	Leaf width (cm)	Leaf length (cm)	Leaf dry weight (kg/ha)	Plant dry weight (kg/ha)
Forage millet (CFPM 101)	94.3 a ^z	17 a	24.1 a	54.2 a	1,662 a	2,598 a
Grain millet (CGPM H5)	82.7 b	16 b	22.6 b	50.7 b	1,246 b	1,861 ab
Grain millet (CGPM H6)	85.2 b	16 b	22.4 b	51.6 b	1,267 b	1,906 a
Rye	61.0 c	14 c	20.3 c	45.0 c	731 c	1,031 b
Rye fumigated	82.5 b	16 b	22.4 b	50.8 b	1,267 b	1,914 a
Probability > F	0.0001	0.0001	0.0001	0.0001	0.0001	0.0162

^z Values within a column followed by the same letter are not significantly different, as determined by Waller's test ($P = 0.05$).

Table 5. Pearson's correlation coefficient^z (*r*) between root *Pratylenchus penetrans* densities at harvest and tobacco growth data recorded in trial 1 (1999) and trial 2 (2000)

Tobacco growth	Number of <i>P. penetrans</i> in roots (log[x+1]/g dry root)	
	Trial 1	Trial 2
Plant height	-0.4643**	-0.7509*****
Number of leaves	-0.4079*	-0.7180*****
Leaf dry weight	-0.5369***	-0.7029*****
Plant dry weight	-0.5404***	-0.6665*****
Leaf width	...	-0.6838*****
Leaf length	...	-0.7156*****

^z *, $P \leq 0.10$; **, $P \leq 0.05$; ***, $P \leq 0.01$; ****, $P \leq 0.001$; *****, $P \leq 0.0001$.

that improved growth of tobacco was principally related to the reduced nematode pressure. Previous reports have shown forage pearl millet to be a poor host to *P. penetrans* (3,6), and that rye will increase their densities to damaging levels (3,5,9,14). Forage pearl millet CFPM 101 increases tobacco yields under Ontario's growing conditions (6).

In addition to forage production, forage pearl millet would be beneficial as a green manure crop on the sandy soils with poor inherent soil fertility and low levels of organic matter on which tobacco is traditionally grown. In these field trials, forage millet was cut down twice mechanically with residues returned to the soil each time as green manure. It is recommended the crop be mowed when it reaches a height of 75 to 100 cm, because mature pearl millet residues can be difficult to incorporate in the soil and slow to decompose, and may cause problems during planting and variability in growth of subsequent crops.

While the mechanism of biological control of *P. penetrans* by pearl millet is unknown, Ball-Coelho et al. (1) suggested that it could be related to S-containing compounds. CFPM 101 shoots and roots contained greater concentrations and amounts of S than plants exhibiting little or no suppression (rye). Marigold (*Tagetes erecta*) is a good alternative to chemical fumigation for Ontario flue-cured tobacco growers to manage *P. penetrans* (16). Pearl millet competes better with weeds,

produces greater quantities of biomass, and is more economical than marigold (2).

Future investigations on the poor host suitability of pearl millet should be considered to establish how this crop affects the reproduction of *P. penetrans*. New hybrids of grain pearl millet being developed should be evaluated for their potential to suppress root-lesion nematodes. Results of this study establish that nematode-suppressing grain and forage pearl millet are better rotation crops than the traditional rye or soil fumigation, which is hazardous to human health and the environment.

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