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Improving the use of rye (*Secale cereale*) for nematode management: potential to select cultivars based on *Meloidogyne incognita* host status and benzoxazinoid content

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Summary – Six geographically diverse cultivars of rye (*Secale cereale*), a wheat (*Triticum aestivum*) cultivar and hairy vetch (*Vicia villosa*) cultivar unstated were screened for *Meloidogyne incognita* host suitability. Chemical constituents of rye can suppress *M. incognita*, so the rye cultivars and wheat were also tested for benzoxazinoid content to determine if rye cultivar selection could be based upon plant chemistry. There was variation in *M. incognita* host status among the rye cultivars. Cultivars Aroostook, Elbon, Oklon and Wrens Abruzzi were the most resistant rye cultivars, with low numbers of *M. incognita* eggs/g dry root. Cultivar Wheeler had somewhat more eggs/g root than these cultivars, while cv. Merced supported nearly three times more eggs/g root than cv. Wheeler. Most of the rye cultivars were similar to each other in total benzoxazinoid content, although cv. Aroostook had the lowest amount of total benzoxazinoids. When data from roots and shoots were combined, more than 79% of the total benzoxazinoids in all six of the rye cultivars were comprised of the non-methoxy-substituted forms: *i*) (2*R*)-2-β-D-glucopyranosyloxy-4-hydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA-glucoside); *ii*) 2,4-dihydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA); and *iii*) benzoxazolin-2(3*H*)-one (BOA). In the rye cultivar roots there was little difference among cultivars in amounts of the methoxy-substituted benzoxazinoids: *i*) (2*R*)-2-β-D-glucopyranosyloxy-4-hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIMBOA-glucoside); *ii*) 2,4-hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIMBOA); *iii*) 6-methoxy-benzoxazolin-2(3*H*)-one (MBOA); and *iv*) 2-hydroxy-7-methoxy-(2*H*)-1,4,-benzoxazzin-3(4*H*)-one (HMBOA). However, cv. Aroostook roots had the lowest concentration of non-methoxy-substituted benzoxazinoids. Rye cultivars were generally similar to each other in amounts of benzoxazinoids in shoots. The shoots had much lower concentrations of methoxy-substituted benzoxazinoinds than the roots but much higher concentrations of non-methoxy-substituted forms. Cultivars with the lowest numbers of eggs/g root and the highest amounts of benzoxazinoids (with potential for action against nematodes in soil after incorporation as a green manure) are possible candidates for optimal nematode management.

Keywords – BOA, cultivars, DIBOA, DIBOA-glucoside, DIMBOA, DIMBOA-glucoside, hairy vetch, hydroxamic acid, MBOA, wheat.

Rye (*Secale cereale*) cover crops are an important component of many crop rotation systems in the eastern and southeastern United States. This cover crop provides multiple agronomic benefits including reduced erosion, increased soil tilth, sequestration of nutrients, and weed and plant-parasitic nematode suppression. The incorporation of a rye cover crop temporarily suppressed numbers of *Meloidogyne incognita* juveniles in soil (Johnson & Motsinger, 1990; McSorley & Dickson, 1995). Although rye reduced *M. incognita* activity, there was no correlation between the production of low molecular weight aliphatic organic acids and nematode suppression, suggesting that other mechanisms were responsible for the nematode suppressiveness of rye (McBride *et al*., 2000).

Another potential nematode suppressive mechanism by rye is the production of the secondary metabolites benzoxazinoids, which are found in the Poaceae, Acanthaceae, Lamiaceae, Ranunculaceae and Scrophulariaceae. The compound 2,4-dihydroxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIBOA) and its breakdown product benzoxazolin-2(3*H*)-one (BOA) have each been implicated in the allelopathy of rye (Barnes & Putnam, 1987; Sicker *et al*., 2000; Sicker & Schulz, 2002). Additional allelopathic compounds have been identified in rye, includ-

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ing 2,4-hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one (DIMBOA) and its degradation product 6-methoxybenzoxazolin-2(3*H*)-one (MBOA) (Hofman & Hofmanova, 1969; Tang *et al*., 1975; Rice *et al*., 2005). The benzoxazinoids DIBOA and DIMBOA occur as glucosides in intact rye. Upon tissue disruption β-glucosidase is released and the glucosides are rapidly hydrolysed to release DIBOA and DIMBOA, which subsequently decompose in water to form BOA and MBOA, respectively.

In a previous study we determined the toxicity of selected benzoxazinoids found in rye, and of their degradation products, against *M. incognita* and *Xiphinema americanum* (Zasada *et al*., 2005). DIBOA was generally more toxic than DIMBOA to *M. incognita* second-stage juveniles (J2). However, although DIBOA was toxic to *M. incognita* when the nematode was immersed in the compound, a proportion of the nematodes recovered when the compound was removed, indicating that the effects were not permanent. MBOA was also toxic to J2 of *M. incognita* and mortality increased even after the compound was removed. BOA was the least nematotoxic compound tested.

Understanding the toxicity of several of the chemical constituents contained in rye is part of improving nematode management using a rye cover crop; companion studies are needed to determine the locations and quantities of these compounds in the plant. Most studies have focused on the shoots of plants and on the non-methoxy-substituted (DIBOA-glucoside, DIBOA, BOA) benzoxazinoids, which are the compounds most commonly implicated in the allelopathic potential of rye. Little is known about the chemical composition of rye roots or about the methoxy-substituted (DIMBOA-glucoside, DIMBOA, MBOA) benzoxazinoids found in rye roots and shoots (Rice *et al*., 2005).

Rye is a very widely planted cover crop and it is important to maximise any plant-parasitic nematode suppression that it may provide. Factors to consider in selecting a cultivar include nematode host status of rye cultivars, variability in benzoxazinoid content of rye cultivars and plant phenology. The specific objectives of this research were: *i*) to screen a geographically-diverse set of rye cultivars against *M. incognita*; and *ii*) to determine the benzoxazinoid content and composition of the shoots and roots of the cultivars, in order to use these two factors as aids in determining specific cultivars to use for nematode management.

Materials and methods

Meloidogyne incognita host status studies

To determine the host status of rye cultivars, 655 cm^3 of steamed soil (1:5 compost:sand mixture) was placed in a 6.4 × 25.4 cm Deepot (Hummert International, Earth City, MO, USA). The soil was moistened to 70% of water holding capacity. One seed per pot of each rye cultivar, and of wheat and vetch, was planted 2.5 cm deep. The rye cultivars tested were: Aroostook, Elbon, Merced, Oklon, Wheeler and Wrens Abruzzi. Wheat (*Triticum aestivum*) cv. Anza and hairy vetch (*Vicia villosa*), cultivar unstated, were included as comparisons. The seeds were allowed to germinate and 5 days after planting the seedlings were each inoculated with 5000 *M. incognita* eggs. Three holes, approximately 10 cm deep, were made around each seedling and nematode eggs in water suspension were pipetted into the holes and covered with soil. *Meloidogyne incognita* race 1 (originally isolated from a field near Salisbury, MD) cultured on glasshouse-grown pepper (*Capsicum annuum*) cv. PA-136 was used. Eggs were extracted from the roots of 3-month-old pepper plants with 0.5% sodium hypochlorite and used immediately.

Plants were maintained in a glasshouse with temperatures between 24 and 29°C and natural and supplemental lighting were combined to achieve a 16-h day length. There was a total of eight pots per cultivar per trial and the experiment was repeated once, for a total of two trials. The pots were arranged in a completely randomised design on a glasshouse bench. Experiments were terminated 49 days after planting. Shoot dry weights (all plant parts above the soil line) were determined after removal of the plants from the pots and oven-drying for 7 days at 60°C. Roots were processed to collect nematode eggs. Roots were placed in a container with 0.5 % sodium hypochlorite and shaken for 3 min on a reciprocal shaker. The roots and solution were poured over 20- and 500-mesh sieves, and the eggs were rinsed with water and collected from the 500-mesh sieve. All aqueous egg suspensions were refrigerated until counted. Aliquots were counted to estimate numbers of eggs/g of dry root. Root dry weights were determined after oven-drying for 7 days at 60°C.

Benzoxazinoid extraction

Approximately 200 seeds of each rye cultivar and wheat were sown 2.5 cm deep in 1500 cm^3 of 1:5 steamed compost:sand mixture in 20.3 cm diam. pots. There was a total of five pots per cultivar. After 10 days the plants were

harvested. Soil was washed from the roots, and roots and shoots were separated for analysis. Ten g (wet weight) per pot of root and of shoot material were stored at −80°C until benzoxazinoid extraction. Dry weight of excess root and shoot material was determined after oven-drying for 7 days at 60°C. The experiment was conducted twice.

Benzoxazinoids were extracted by grinding fresh plant material in liquid nitrogen and immediately transferring the material to a 125 ml Erlenmeyer flask and adding 50 ml of 100% methanol at 65°C (5:1 volume methanol/wet weight material). The plant/methanol slurry was covered and placed in a 65°C water bath for 15 min. The extracted benzoxazinoids in methanol were decanted into 50 ml polyethylene tubes and centrifuged at 2000 *g* for 5 min. The supernatant was transferred to another 50 ml polyethylene tube and stored at 4°C until analysis. Prior to analysis, portions of the extracts were filtered using a 0.7 μm Whatman glass microfibre filter and these methanol extracts were diluted with an equal volume of water and injected directly onto an LC column connected to a high performance liquid chromatograph coupled to a triple quadrupole mass spectrometer (LC/ESI/MS-MS; Micromass LC Quattro, Micromass, Manchester, UK).

Analytical methods

Authentic standards were used to quantitate the benzoxazinoids found in the rye samples. The following compounds were included: DIBOA–glucoside ((2*R*)-2-β-D-glucopyranosyloxy-4-hydroxy-2*H*-1,4-benzoxazin-3(4*H*)-one); DIBOA; BOA; DIMBOA-glucoside ((2*R*)-2-β-D-glucopyranosyloxy-4-hydroxy-7-methoxy-(2*H*)-1,4-benzoxazin-3(4*H*)-one); DIMBOA; MBOA; and HMBOA (2-hydroxy-(2*H*)-7-methoxy,1,4-benzoxaxin-3(4*H*)-one).

The analytical method followed procedures described by Rice *et al*. (2005), with minor modifications to include HMBOA (standard provided by Dr Dieter Sicker, Leipzig University, Leipzig, Germany). Briefly, the primary method involved separation using a Waters X-Terra MS C-18 column (Waters, Milford, MA, USA) 5 μm 2.1 × 150 mm and a solvent mixture of 65:35 (v/v): 65 parts of 'A', a 30:70 (v/v) mix of methanol and 1% formic acid in deionised water, and 35 parts of pure deionised water supplied at a flow rate of 0.3 ml min^{-1} and a column temperature of 35°C. The LC/ESI/MS-MS was operated in electrospray ionisation mode acquiring ions in both positive and negative operation. Non-specific mass spectrometer settings were as follows: capillary voltage 3.0 kV; source and desolvation temperatures were 140°C and 400°C; and nitrogen was generated to supply the nebuliser and desolvation systems: gas flow rates were approximately 70 and 600 l h^{-1}, respectively. Argon was used as the collison-induced decomposition gas to fragment the parent ions; typical pressure 3×10^{-5} mBar.

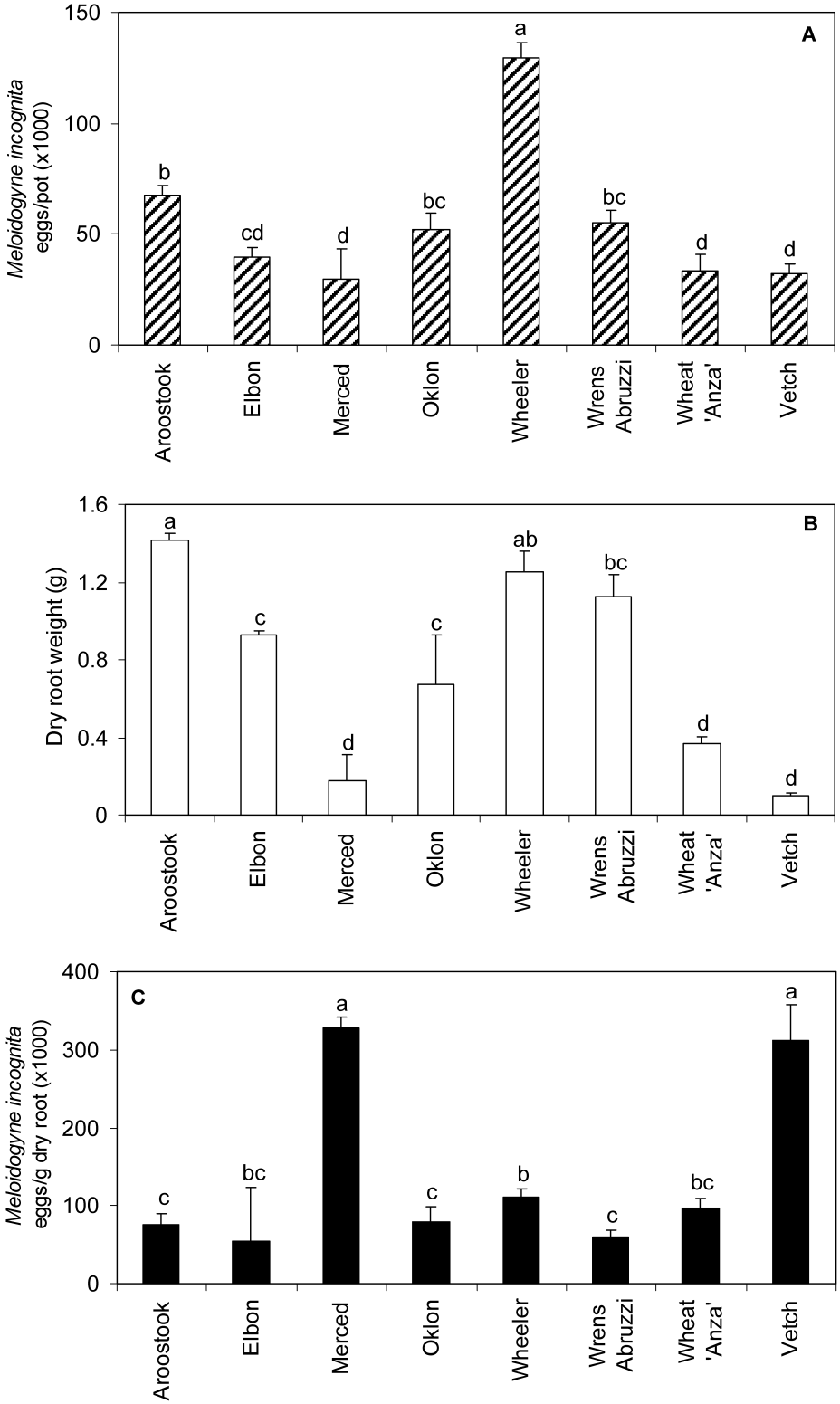
Statistical analysis

Results from *M. incognita* host status studies were similar between trials ($P > 0.3$); therefore, they were combined for analysis. A variance-weighted ANOVA was used and significant differences between cultivars were determined using PROC MIXED (SAS Institute, Cary, NC, USA). Means were compared with Tukey's adjustment for multiple comparisons ($P < 0.05$). Benzoxazinoid concentrations of rye cultivar shoots and roots were log-transformed prior to analysis, when necessary, to fit the assumptions of the model; non-transformed data are presented. Significant differences among benzoxazinoids among cultivars were determined using PROC MIXED. Means were compared with Tukey's adjustment for multiple comparisons ($P < 0.05$).

Results

Meloidogyne incognita host status of rye cultivars

Meloidogyne incognita reproduced on all of the plant species (Fig. 1A). All of the rye cultivars except Merced and Elbon had significantly greater numbers of eggs/pot than wheat and vetch. Cultivar Merced had the fewest number of eggs/pot and was different ($P < 0.001$) from all other rye cultivars except cv. Elbon. The greatest numbers of eggs/pot were present on cv. Wheeler. Cultivars Aroostook, Oklon and Wrens Abruzzi were intermediate in *M. incognita* egg production. The greatest ($P < 0.003$) rye root biomasses were recorded from cvs Aroostook and Wheeler followed by Wrens Abruzzi, while cv. Merced had the lowest of the rye cultivars (Fig. 1B). Calculation of eggs/g dry root demonstrated that number of eggs/pot was not a good indicator of rye cultivar host status. The cultivar with the lowest root biomass, Merced, had the highest ($P < 0.001$) number of eggs/g dry root of any rye cultivar, and was similar to vetch (Fig. 1C). While cv. Anza also had low root biomass, eggs/g dry root was lower than cv. Merced or vetch. Cultivars Wrens Abruzzi, Aroostook and Elbon appeared to be the most resistant ($P < 0.001$) rye cultivars with low numbers of eggs/pot and high root biomass resulting in low eggs/g dry root.


A
Meloidogyne incognita
eggs/pot (x1000)
150
100
50
0
b
cd
d
bc
a
bc
d
d
B
Dry root weight (g)
1.6
1.2
0.8
0.4
0
a
c
d
c
ab
bc
d
d
C
Meloidogyne incognita
eggs/g dry root (x1000)
400
300
200
100
0
c
bc
a
c
b
c
bc
a
Aroostook
Elbon
Merced
Oklon
Wheeler
Wrens Abruzzi
Wheat 'Anza'
Vetch

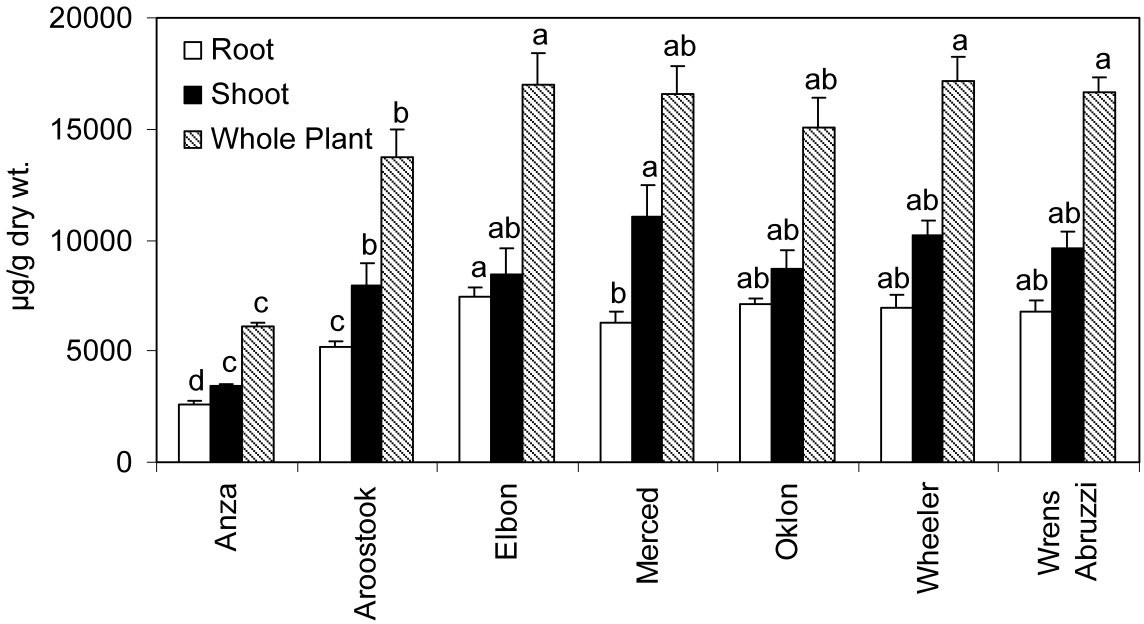


Fig. 2. *Total benzoxazinoids (µg/g dry wt) (DIMBOA-glucoside, DIMBOA, DIBOA-glucoside, DIBOA, HMBOA) and their degradation products (MBOA and BOA) in root, shoot and whole plant tissue 10 days after planting of rye cultivars and wheat cv. Anza. Data are from two experiments, and each bar is the average of ten replicates (± one standard deviation). Bars with different letters are different (*P *< 0.001) according to Tukey's adjustment for multiple comparisons.*

BENXOZAZINOID CONTENT OF RYE CULTIVARS

The levels of the benzoxazinoids were relatively high in these samples; therefore all of them were initially prepared in 50 ml methanol. The lowest working standard of 0.5 μg ml^{-1} was found to be sufficient for determining the concentrations of benzoxazinoids in the samples. This amount of standard equates to a functional detection limit of 50 ng g^{-1} dry weight in the tissue. For the comparisons called for in this study, this level of detection was sufficient. For precision, the relative percent difference for three duplicate pairs was 17%. Three sequential extractions of the same sample indicated that an average of 70% of all of the analytes was recovered in the first extraction; therefore, all comparisons were made using single-methanol extraction results. The average recovery of the DIBOA-glucoside spikes (n = 5) was 100%.

Total benzoxazinoid concentrations were the lowest ($P < 0.001$) in wheat cv. Anza (Fig. 2). Cultivar Aroostook had the lowest ($P < 0.001$) total concentration of benzoxazinoids of any of the rye cultivars screened, and was significantly different from cvs Elbon, Wheeler and Wrens Abruzzi. Total benzoxazinoids in shoots of cv. Aroostook were lower than the concentration in shoots of only one cultivar, Merced, while root concentrations in cv. Aroostook were lower ($P < 0.001$) than in all of the other rye cultivars (Fig. 2). The composition of the whole rye plants showed that >79% of the benzoxazinoid chemistry comprised the non-methoxy-substituted form. This was in contrast to wheat, which contained >99% of the methoxy-substituted form.

Wheat differed greatly ($P < 0.001$) in its benzoxazinoid shoot and root composition and in respective total concentrations from the rye cultivars (Table 1). Wheat shoots were comprised of >99% methoxy-substituted benzoxazinoids (DIMBOA-glucoside, DIMBOA, MBOA, HMBOA) compared to on average <1% in the rye culti-

Fig. 1. *A: Number of* Meloidogyne incognita *eggs/pot; B: Dry root weights; C: Number of eggs/g dry root obtained from rye cultivars, wheat and vetch planted in glasshouse pots. Data are from two experiments, and each bar is the average of 16 replicates (± one standard deviation). Pots were inoculated with 5000* M. incognita *eggs and experiments were terminated 49 days after planting. Bars with different letters are different (*P *< 0.001) according to Tukey's adjustment for multiple comparisons.*

var shoots. The same proportion of methoxy-substituted to non-methoxy-substituted (DIBOA-glucoside, DIBOA, BOA) constituents occurred in the roots of wheat (Table 1) as was observed in the wheat shoots.

The majority (99%) of the benzoxazinoids in the rye shoots, regardless of cultivar, were the non-methoxy-substituted form (Table 1). Cultivars Aroostook and Elbon had the lowest concentrations of total non-methoxy-substituted benzoxazinoids in the shoots, but these concentrations were only significantly different from the cultivar, Merced, which had the highest. Cultivars Oklon, Wheeler and Wrens Abruzzi were intermediate in total non-methoxy-substituted benzoxazinoids. There was no difference in the concentration of total methoxy-substituted forms in the shoots among the rye cultivars tested.

The roots of the rye cultivars also comprised a mixture of methoxy-substituted and non-methoxy-substituted benzoxazinoids (Table 1). The benzoxazinoids of the rye cultivar roots, except Aroostook, comprised >67% of the non-methoxy-substituted form. The benzoxazinoid composition of cv. Aroostook roots was 58% non-methoxy-substituted and 42% methoxy-substituted benzoxazinoids. Aroostook was the only rye cultivar that differed significantly from the other cultivars in concentration of total non-methoxy-substituted form in the roots. Cultivars Aroostook, Oklon and Wheeler roots had similar concentrations of the methoxy-substituted benzoxazinoids as wheat cv. Anza roots. The lowest concentrations of total methoxy-substituted forms were found in the roots of cvs Elbon and Merced.

Discussion

The goal of this study was to improve the use of a rye cover crop as a nematode management tool through the selection of an appropriate cultivar. We wanted to identify a cultivar that did not support high *M. incognita* reproduction, and which also had the potential to release large concentrations of benzoxazinoids when incorporated into soil. The rye cultivars evaluated in this study did differ in their *M. incognita* host status, but did not vary greatly in their benzoxazinoid composition or concentrations. Therefore, host status is a primary consideration when selecting a rye cultivar as a cover crop for *M. incognita* management.

This was the first side-by-side comparison of such a diverse set of rye cultivars. The majority of previous rye-*M. incognita* host tests were conducted on cv. Wrens Abruzzi (Opperman *et al*., 1988; Johnson & Motsinger, 1989; Ibrahim *et al*., 1993). Results with this cultivar have been variable. Although cv. Wrens Abruzzi was a good host for *M. incognita* in one of the previous studies (Ibrahim *et al*., 1993), it was a poor host for three *Meloidogyne* spp., as was the wheat cv. Florida 301, when both were grown in field microplots (Opperman *et al*., 1988). However, when cv. Florida 301 was screened in a constant temperature growth room, this cultivar supported nematode reproduction. Others have suggested that reproduction of *Meloidogyne* spp. on rye in the field may be limited by low winter temperatures (Johnson & Motsinger, 1989). In our glasshouse study, cv. Wrens

Table 1. *Methoxy-substituted (DIMBOA-glucoside, DIMBOA, MBOA and HMBOA) and non-methoxy-substituted (DIBOA-glucoside, DIBOA, BOA) benzoxazinoids in 10-day-old rye and wheat shoot and root tissue*[1].

Cultivar	Shoots		Roots	
	Total methoxy-substituted	Total non-methoxy-substituted	Total methoxy-substituted	Total non-methoxy-substituted
Aroostook	63 ± 9 b[2]	7868 ± 997 b	2172 ± 168 abc	3024 ± 224 b
Elbon	74 ± 10 b	7562 ± 1495 b	1816 ± 297 c	5389 ± 621 a
Merced	50 ± 9 b	10956 ± 1681 a	1750 ± 99 c	4649 ± 416 a
Oklon	60 ± 15 b	8610 ± 864 ab	2309 ± 103 ab	4789 ± 251 a
Wheeler	39 ± 13 b	10183 ± 721 ab	2170 ± 185 abc	4816 ± 459 a
Wrens Abruzzi	59 ± 9 b	9593 ± 710 ab	1981 ± 126 bc	4767 ± 566 a
Wheat 'Anza'	2968 ± 127 a	9 ± 4 c	2567 ± 159 a	33 ± 6 c

[1] Extracted with methanol and analysed with LC/ESI/MS-MS.

[2] Values are in μg/g dry wt and are the means (± one standard error) of ten replications (data from two experiments combined). Values followed by different letters are different ($P < 0.001$) according to Tukey's adjustment for multiple comparisons.

Abruzzi had one of the lowest numbers of *M. incognita* eggs/g dry root.

Although cv. Wrens Abruzzi is a popular rye cultivar, other cultivars are incorporated into rotations depending upon geography. Therefore, it is important to have information regarding the host status of other cultivars. In our study, cv. Merced was found to support much higher numbers of eggs/g dry root than any other tested rye cultivar. Glasshouse screening of rye cultivars provides a valuable starting point for the selection of varieties for nematode management. Promising cultivars and their associated nematodes must next be challenged in the field.

Plant material was harvested 10 days after planting to evaluate benzoxazinoids. While this is not the time during the plants' phenology when it would be incorporated into soil, it was anticipated that this would be a time when the cultivars would demonstrate a difference in chemistry because numerous reports indicated that the concentration of these compounds peak early in the phenology of rye and wheat (Copaja *et al*., 1999; Wu *et al*., 2001). No large differences were detected in benzoxazinoid concentrations between the cultivars tested in our study, although cv. Aroostook was significantly lower in total amounts of these compounds than cvs Elbon, Wheeler or Wrens Abruzzi. The quantities of benzoxazinoids found in the rye cultivars were similar to those mentioned by Mayoral *et al*. (1994).

Others have demonstrated differences among cultivars, in non-methoxy substituted benzoxazinoids (DIBOA and BOA) in field-grown plant material (Burgos *et al*., 1999; Reberg-Horton *et al*., 2005). For example, cv. Bonel was the highest producer of DIBOA and BOA compared to cvs Aroostook and Oklon (Burgos *et al*., 1999). At a similar sampling date in another study (Reberg-Horton *et al*., 2005), cvs Bonel and Wrens Abruzzi were similar in DIBOA concentrations, but both cultivars contained lower DIBOA concentrations than cv. Wheeler. Environmental conditions have a profound impact on plant chemistry (Mawaja *et al*., 1995), and may account for the difference between studies. In addition, those studies (Burgos *et al*., 1999; Reberg-Horton *et al*., 2005) focused only on the shoots of plants and the non-methoxy substituted benzoxazinoids. In our study, the majority (99%) of the benzoxazinoids in the rye shoots, regardless of cultivar, was the non-methoxy-substituted forms; this is similar to previous findings (Zùñiga *et al*., 1983). We measured almost twice as much DIBOA-glucoside, the precursor to DIBOA and BOA, in the shoots as there was in the roots. This result demonstrates that to achieve lethal concentrations of DIBOA in soil, above-ground plant material will have to be incorporated.

Few researchers have discussed the methoxy-substituted benzoxazinoid constituents in rye, or studied the chemistry of rye roots; the latter are not always included in extraction routines. We found that the methoxy-substituted benzoxazinoids were more concentrated in roots than in shoots, and the non-methoxy-substituted forms less concentrated in root. As with shoots, there was a mix of the methoxy-substituted and non-methoxy-substituted benzoxazinoid forms in roots. However, the ratio of non-methoxy-substituted to methoxy-substituted forms was far higher in shoots than in roots. As the amount of benzoxazinoids present upon incorporation will be partially dependent upon plant biomass, it is unlikely that the methoxy-substituted benzoxazionoids (DIMBOA and MBOA) will play a large role in nematode suppression because of the small amount of root biomass present at cover crop termination.

Our study indicated that the tested rye cultivars varied in host status to *M. incognita*, and also demonstrated that the benzoxazinoid compositions of the rye cultivars evaluated in this study did not differ greatly early in plant development. Biomass produced in the field is an important factor in the effects of benzoxazinoids on nematodes, and the total benzoxazinoid concentration present at cover crop incorporation will be a function of biomass and plant chemistry (Burgos *et al*., 1999; Reberg-Horton *et al*., 2005). Based upon lethal concentration values for DIBOA, DIMBOA, BOA and MBOA against *M. incognita* reported previously (Zasada *et al.*, 2005), the benzoxazinoid concentrations measured in this study were sufficient to result in nematode mortality if plant material was incorporated into soil. An essential question remains: after planting and incorporation of a rye cover crop, will nematodes be exposed to lethal concentrations of these compounds in soil? Factors which will influence the fate of these compounds in soil include soil texture, organic matter, moisture and microbial degradation, and these parameters need to be studied to determine the importance of rye benzoxazinoids in affecting nematode populations.

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