Inducible Flavone in Oats (*Avena sativa*) Is a Novel Defense Against Plant-Parasitic Nematodes

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ABSTRACT

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The induction of defense compounds in oats (*Avena sativa*) in response to invasion by parasitic nematodes and to application of the wound hormone methyl jasmonate was examined. Oats cv. Quoll seedlings were challenged with *Pratylenchus neglectus*, *Heterodera avenae*, and *Ditylenchus dipsaci* and treated with 1×10^{-4} M methyl jasmonate. Three compounds, isolated in methanolic root and shoot extracts of oats, exhibiting an absorbance spectrum typical of flavone glycosides, were induced by nematode invasion and methyl jasmonate. These were identified as flavone-C-glycosides by mass spectrometry. The effect of the flavone-C-glycosides on the invasion by and development of cereal cyst nematode

H. avenae was assessed using methanolic extracts of shoots and roots from methyl jasmonate-treated plants. Both extracts impaired nematode invasion and development. When the extracts were fractionated by high voltage paper electrophoresis, only one flavone-C-glycoside, *O*-methyl-apigenin-C-deoxyhexoside-*O*-hexoside, inhibited nematode invasion. The protective effect of the induction of flavone-C-glycosides in oats by methyl jasmonate was evaluated against *H. avenae* and *P. neglectus*. Treatment with methyl jasmonate reduced invasion of both nematodes and increased plant mass, compensating for damage caused by the nematodes, and is attributed to the active flavone-C-glycoside. The active compound, *O*-methyl-apigenin-C-deoxyhexoside-*O*-hexoside, has not been implicated previously in plant defense against any pest or pathogen, and appears to provide protection against the major cereal nematodes *Heterodera* and *Pratylenchus*.

Plant defense responses to parasitic nematodes have the potential to become part of management strategies that increase agricultural productivity (15,44,54,59). Both constitutive and induced defense mechanisms contributing to disease resistance are observed in plants (36,46,59). In plant-nematode interactions, a range of secondary metabolites is induced in plants after nematode invasion (64). Induction of phytoalexin synthesis in the roots in response to nematode parasitism has been documented (61). Proteins and enzymes, such as peroxidases of the phenylpropanoid pathway leading to the synthesis of lignin, suberin, flavonoids, phytoalexins, and coumarins, accumulate in plants, indicating an involvement in nematode resistance (7,19,21,45,65). Biosynthesis and accumulation of phenolic compounds in plant tissues following nematode infection is also considered a defense response (27). Although various compounds are associated with plant resistance to nematodes, the phytoalexin glyceollin in soybean provides the most convincing link for resistance mechanism against nematodes in plants (35,37). The limited information on direct involvement of phytochemicals in nematode resistance compared with that of insects and other pathogens is a major barrier for the development of novel nematode management strategies and highlights the need for further studies.

The induction of secondary metabolites by pests and pathogens, which limit further herbivory or infection (5,9,26,33), is indicative of their defense function. Therefore, induction of metabolites by mechanical damage or chemical treatment (e.g., jasmonic acid) is a widely used approach for the discovery of unknown defense compounds. Well-known potential defense compounds induced by jasmonic acid are proteinase inhibitors, benzophenanthridine alkaloids, nicotine, and phenolics such as flavonoids (6,24,30,41,43).

Oats (*Avena sativa*) are recognized among other cereals for their resistance to pests and diseases and are often grown in crop rotation to break the cycles of soilborne pests and diseases (52). Hence, it is possible that this species possesses secondary metabolites that impart resistance to nematodes. The presence of saponins, such as the avenacins, which are restricted to the genus *Avena*, has been implicated in disease resistance in oats (47). However, oats also produce and accumulate other secondary metabolites such as flavonoids, sterols, and other phenols (39,48,50) which are not characterized in the context of a possible involvement in resistance to nematodes. Accumulation of some compounds following nematode invasion has been reported and a possible involvement in plant defense has been suggested (4,9,14,22,27,45,49,61).

In this study, the inducibility of possible defense compounds in oats in response to invasion by parasitic nematodes with different feeding behavior and to application of methyl jasmonate was examined. Oat seedlings were challenged with *Pratylenchus neglectus* (root lesion nematode), a migratory endoparasite of roots, *Heterodera avenae* (cereal cyst nematode), a sedentary endoparasite of roots, and *Ditylenchus dipsaci* (stem nematode), a migratory endoparasite of shoots. The compounds induced in oats by nematodes and methyl jasmonate were extracted, characterized, and identified. To determine the biological activity of the inducible compounds, a bioassay was conducted by assessing the effects of shoot and root extracts from oats induced with methyl jasmonate on the invasion by and development of cereal cyst nematode, *H. avenae*, in wheat. The protective effects of the inducible compounds in oats against *H. avenae* and the root lesion nematode, *P. neglectus*, were also evaluated in vivo.

MATERIALS AND METHODS

Plant and nematode culture. Individual oat (*A. sativa* cv. Quoll) or wheat (*Triticum aestivum* cv. Egret) seedlings were grown in 300 ml of pasteurized Tailem Bend sand. Plants were

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maintained with addition of 50 ml of Hoagland solution per plant every other day in a growth room at near optimum conditions (18°C; 85% relative humidity; 12 h day and night) for the nematodes and plants.

The nematodes *D. dipsaci* (oat race), *H. avenae*, and *P. neglectus* were used in the study. Mixed stages of *D. dipsaci* and *P. neglectus* were cultured monoxenically in carrot callus using a modified protocol of Moody et al. (42). Invasive stage juveniles (J2) of *H. avenae* were hatched from eggs in soil organic matter containing cysts incubated in Whitehead trays (63) at 10°C.

Induction of compounds in oats. Oat seedlings with uniform growth and vigor were treated with a soil drenching of 10 ml of 10^{-4} M methyl jasmonate or inoculated with the nematodes *D. dipsaci*, *H. avenae*, and *P. neglectus* 15 days after sowing. Uninoculated and untreated plants were included as controls. Roots and shoots were sampled 3 days after treatment or inoculation by carefully washing off the soil from the roots of each plant and snap freezing the samples in liquid nitrogen. Methanol-extractable compounds from shoots and roots were detected and quantified as described below.

Extraction of inducible compounds. The frozen root and shoot samples were freeze-dried. These were then ground to fine powder (≈ 850 μ m of mesh) and 60 mg of each sample was extracted in 10 ml of methanol for 48 h. Methanol is commonly used as a general solvent for the extraction of secondary metabolites such as phenolics from plants (62). To remove the lipids in the extract collected, an aliquot of 7 ml of the methanol extract was diluted with water to make a 70% methanol extract solution and partitioned with 10 ml of hexane for 48 h. The aqueous methanol phase (8 ml) was removed and evaporated to dryness. The residue was dissolved in 5 ml of water and partitioned against 5 ml of butanol. A 4-ml aliquot of the butanol phase was evaporated and reconstituted in 400 μ l of 45% methanol prior to analysis.

Chemical analyses. The methanol-extractable compounds in shoot and root extracts were assayed by reversed-phase high-performance liquid chromatography (RP-HPLC) with a C-18 column (Waters Spherisorb octyldecyl silane [ODS-2], 4.6 mm $\times$ 150 mm, 5- μ m particle column). Isocratic elution was performed with water-methanol (55:45, vol/vol) as mobile phase at 1 ml/min at room temperature with detection at UV 254 nm. Rutin was used as a reference standard. Inducible compounds detected were collected and quantified using a UV-Vis spectrometer (Perkin-Elmer Lambda 5, Wilton, CT) with rutin as standard.

For structural analysis, HPLC-mass spectrometry (LC-MS) was performed. Separation of flavonoids was achieved on a reversed-phase column (Synergi Hydro-RP Phenomenex, Torrance, CA; 4 micron, 80A, 150 $\times$ 2 mm) with a flow rate of 180 μ l/min and injection volume of 20 μ l. Gradient elution was run with solvent A (1:19, formic acid/water) and solvent B (1:3:16, formic acid/water/acetonitrile) following a schedule of 10 to 35% solvent B (in water) for 35 min and then 35 to 60% solvent B for 25 min. The HPLC column was connected to a UV-Vis detector (HP1100, Hewlett Packard) monitoring at 280 and 340 nm followed by a mass spectrometer with an ion spray ion source (API-300, PE Sciex, Thornhill, Ontario, Canada). The mass spectrometer was operated in positive ion mode and was scanned from m/z 250 to m/z 1,000 in 1.88 s. Ion spray and orifice potentials were set at 5.5 kV and 30 V, respectively. Curtain and nebulizer gases were nitrogen and air, respectively. All mass spectral data were processed using Bio-Multiview software (version 1.2B3, PE Sciex).

Fractionation of shoot and root extracts of oats. Preparative high voltage paper electrophoresis (HVPE) was performed to separate the inducible compounds in both oat shoot and root methanolic extracts into fractions as well as to accumulate a sufficient amount of the inducible compounds to test the biological activity against nematodes. One milliliter of oat shoot and root extracts containing 268 and 124 mg of inducible compounds, respectively, in 45% methanol was blotted evenly along the cen-

tral axis on a sheet of Whatman 3 mm Chr chromatography paper (200 $\times$ 500 mm). Rutin was used as standard. Electrophoresis was carried out using borate buffer at pH 9.4 for 1.5 h at 400 V. Fraction sections were made based on electrophoretic mobility of the compounds in the extracts. Fluorescent illumination helped in the identification of bands. Each band was eluted in 45% methanol and passed through Sep-Pak C18 cartridges (Waters) to remove the borate. Several HVPE runs were performed and all the fractions collected were analyzed by RP-HPLC using the gradient run method in order to pool similar fractions from different runs. Prior to testing for biological activity, pooled fractions were evaporated and reconstituted in 1 ml of 10% methanol.

Test for biological activity of inducible compounds. Approximately 3,000 active invasive stage (J2) of *H. avenae* were treated for 24 h with 1-ml shoot and root extracts of oats. The oat shoot and root extracts were prepared by drying 50 μ l of the extracts used in the preparation of fractions described previously. These were resuspended in 1 ml of 10% methanol before treating the nematodes. The shoot and root extracts contained 3.39 and 0.16 mg of inducible compounds after diluting to 1 ml. The diluted amount was used for treating the nematodes because the concentrations of the inducible compounds were close to the adjusted concentrations of the pooled compounds A and B (1.04 and 0.82 mg, respectively) obtained from the fractionation by HVPE. The 16 fractions in 1 ml of 10% methanol obtained by HVPE were also used to treat nematodes. Preparation of the fractions was as described previously. Both water and 10% methanol controls were included. After treatment, the nematodes were rinsed with three changes of water. A known number (500 nematodes per plant) of treated and control nematodes were applied to 15-day-old wheat seedlings grown as described previously. Nematodes extracted from the sand by a flotation and sieving method (8) and stained in roots were counted 30 days after inoculation. Nematodes recovered from the soil were tested for vitality (53) by staining with 0.01% new blue R (GT Gurr Ltd., London, UK).

Protective effects of the methanol-extractable compounds. Oat seedlings were treated with a soil drench of 10 ml of 10^{-4} M methyl jasmonate 15 days after sowing (referred to as treated plants) to induce the compounds. Three days after induction, treated and untreated plants were inoculated with 500 each of *H. avenae* J2 and mixed stages of *P. neglectus* per plant. Uninoculated controls of both treated and untreated plants were included. Plants from each treatment were sampled and roots were washed thoroughly 5 and 10 days after inoculation. Nematodes were extracted and counted from both the soil and the roots as previously described. The vitality of nematodes recovered from the soil was tested. Additional plants were harvested to determine the fresh and dry weights of shoots and roots and for chemical analysis.

Experimental design and statistical analysis. Plants of uniform height and vigor were arranged in a randomized complete block design with six replicates before treatments in the experiments were applied. Data were transformed (logarithmic or arcsine transformation) when necessary for analysis. Analysis of variance was used to analyze the data and means were compared using Tukey's test.

RESULTS

Inducibility of compounds. Treatment of oats with methyl jasmonate (Fig. 1) increased concentrations of the methanol-extractable compounds from the roots (140%, $P < 0.01$) and shoots (131%, $P < 0.05$). The inducible compounds in the shoots and roots eluted as a single peak, $R_t = 4.34$ min, during isocratic RP-HPLC using 45% methanol (Fig. 2A). However, RP-HPLC gradient elution revealed the presence of three compounds (Fig. 2B and C). The three compounds, A, B, and C, were separated

using acetonitrile with retention times (R_t) of 20.89, 23.21, and 24.43 min for the shoot extract (Fig. 2B) and 20.95, 23.43, and 24.65 min for the root extract (Fig. 2C), respectively. Compounds A, B, and C comprised 77.3, 14.3, and 8.4% of the total amount of inducible compounds in oat shoot extract and 1.6, 37.2, and 61.2% of the total amount of inducible compounds in oat root methanolic extract, respectively.

Inoculation with the nematodes *H. avenae* and *P. neglectus* increased concentrations of these compounds in the roots by 288 and 305% ($P < 0.01$) and in the shoots by 127 and 141% ($P < 0.05$, Fig. 1), respectively. In contrast, *D. dipsaci* had no significant effect on the concentration of the compounds in the shoots or roots (Fig. 1). No other methanol-extractable compound was found to be inducible by methyl jasmonate or nematodes.

Bioassay of crude extract. The root and the shoot extracts were tested for biological activity against nematodes. The recovery from the roots of *H. avenae* treated with the methanolic shoot and root extracts was only 2 and 26%, respectively, compared with 65 and 59% for nematodes treated with water and MeOH, respectively (Fig. 3). Of the shoot and root extract-treated nematodes that invaded the roots (2 and 26%, respectively), only 2.8 and 5.4%, respectively, developed beyond the invasive stage (J2), in comparison to about 60% developing beyond J2 or early adult stage (J3 to J4) in the controls (data not shown).

Biological activity of the inducible compounds. Preparative HVPE of shoot extracts after induction by methyl jasmonate yielded 16 fractions. The compounds A and B were detected through RP-HPLC from the fractions obtained (Fig. 4). The average concentration of compounds A and B obtained from each electrophoresis run was 0.21 and 0.04 mg, respectively. Compound C, on the other hand, was not recovered. Two fractions, containing the inducible compound B, detrimentally affected the invasion of *H. avenae* in roots (Fig. 4). Only 28 and 34% of the nematodes treated with these fractions invaded the roots. Although the fractions containing compound A had a slight inhibitory effect, they were not significantly different from the controls, as with the remaining fractions (Fig. 4). The inducible compounds were not detected from fractions of the oat root extracts obtained by preparative HVPE, and these fractions did not significantly inhibit nematode invasion.

Protective effect of inducible compounds. The protective effects of induction of oats against *H. avenae* and *P. neglectus* were assessed. Methyl jasmonate treatment of oats affected the invasion of the nematodes into the roots (Fig. 5). A comparative proportion of nematodes, *H. avenae* (56%, $P < 0.01$) and *P. neglectus* (84%, $P < 0.01$), failed to invade and were recovered from the soil of treated versus untreated plants 10 days after inoculation. The

Fig. 2. A, B, and C within graphs indicate chromatograms of the inducible compounds in oats with (+MJ) and without (–MJ) methyl jasmonate treatment resolved by A, isocratic run using 45% methanol of shoots, and by acetonitrile/water gradient run of B, shoots and C, roots; DAD = photodiode array detection.

Fig. 1. Concentration of the inducible compounds in oat shoot (open) and roots (solid) after induction using nematodes *Ditylenchus dipsaci* (Dd), *Heterodera avenae* (Ha), and *Pratylenchus neglectus* (Pn), and methyl jasmonate (Mj) as detected by reversed-phase high-performance liquid chromatography; C = control.

Fig. 3. Proportion (%) of *Heterodera avenae* (inoculum = 500 nematodes per plant) recovered from soil (solid) and roots (open) of wheat cv. Egret 30 days after inoculation following treatment with water (control), 10% methanol (MeOH), and induced oat shoot (OS) and oat root (OR) extracts.

Fig. 4. Proportion (%) of *Heterodera avenae* (inoculum = 500 nematodes per plant) recovered from wheat cv. Egret roots 30 days after inoculation following treatment with fractions (1 to 16) of induced oat shoots and control (C); open bars are biologically active fractions; fractions containing compounds A and B are labeled accordingly.

Fig. 5. Proportion (%) of *Heterodera avenae* and *Pratylenchus neglectus* (inoculum = 500 nematodes per plant) recovered from the roots and soil 10 days after inoculation of methyl jasmonate-treated (open) and untreated (solid) oat seedlings (cv. Quoll).

Fig. 6. A, Concentration of inducible compounds in shoots and roots of methyl jasmonate-treated (open) and untreated (solid) oat seedlings (cv. Quoll) 10 days after inoculation of *Heterodera avenae* and *Pratylenchus neglectus*. B, Shoot and root dry mass of methyl jasmonate-treated (open) and untreated (solid) oat seedlings (cv. Quoll) 10 days after inoculation of *H. avenae* and *P. neglectus*.

proportion of nematodes that invaded the roots of treated plants decreased by 50% ($P < 0.01$) and 48% ($P < 0.01$) for *H. avenae* and *P. neglectus*, respectively, compared with that of untreated control plants. In contrast, the majority of the nematodes, *H. avenae* (68%, $P < 0.01$) and *P. neglectus* (79%, $P < 0.01$), were observed in the roots of untreated oats.

Ten days after inoculation of untreated plants, *P. neglectus* had induced 176 and 383% increase in methanol-extractable compounds in shoots and roots, respectively, compared with that of the untreated/uninoculated control (Fig. 6A). Likewise, *H. avenae* increased the concentration of the inducible compounds in the shoots and roots by 148 and 283%, respectively, compared with that of the untreated/uninoculated control 10 days after inoculation of untreated plants. The induction of the inducible compounds by inoculation with *P. neglectus* and *H. avenae* was equivalent to that induced by methyl jasmonate treatment, but combined treatment and inoculation did not increase concentrations over that of inoculation alone.

Inoculation of untreated oats with *P. neglectus* reduced root and shoot dry weight by 74 and 52% ($P < 0.01$), respectively, compared with that of untreated and uninoculated controls (Fig. 6B). Similarly, *H. avenae* in untreated oats reduced root and shoot dry weight by 42 and 54% ($P < 0.01$), respectively. In contrast, pre-inoculation induction of the compounds protected the plant from damage, resulting in growth equivalent to the uninoculated controls.

Identification of the inducible compounds. The absorbance spectra of the three compounds were typical of flavone glycosides (40). The λ_{max} (267, 336 nm) of A and B correspond to apigenin, while λ_{max} (267, 291, 349 nm) of C is similar to luteolin (Fig. 7).

Compounds A, B, and C have a mass ($M+H^+$) charge (m/z) of 565, 593, and 579, respectively (Fig. 8). Compounds A and C have similar ionization patterns (Fig. 8A and C), typical of a flavone-*C*-bisglycoside where the neutral loss of 132 m/z from the parent ions indicated the presence of an *O*-pentoside (2,3,11,56). The resulting ions of m/z 433 (A) and 449 (C) represent flavone-*C*-monoglycosides and were 162 m/z mass units greater than apigenin (m/z 271 $M+H^+$) and luteolin (m/z 286 $M+H^+$), which is consistent with the presence of a hexose. The successive loss of three molecules of water from m/z 433 (A) and 449 (C) as reflected by the major ions m/z 415 ($433 - H_2O$), 397 ($433 - 2H_2O$), and 379 ($433 - 3H_2O$) for compound A and m/z 431 ($449 - H_2O$), 413 ($449 - 2H_2O$), and 395 ($449 - 3H_2O$) for compound C, and the ions m/z 283 (433 less 150) and 299 (449 less 150) for compounds A and C, respectively, representing the methylene flavone ion were indicative of flavone-*C*-monoglycosides (11).

Compound B, on the other hand, has a different ionization pattern from compounds A and C. A neutral loss of m/z 146 from the parent ion (m/z 593) corresponds to the presence of a deoxyhexose. The remaining mass (m/z 447) is 14 m/z units greater than compound A (m/z 433), which suggests that compound B also has the *O*-methyl group like compound A. The typical flavone-*C*-glycoside ionization pattern exhibited by compounds A and C was not observed. However, other major ions at 327 m/z (447 – 120), 299 m/z (447 – 148), and 285 m/z (299 – 14) were consistent with apigenin-8-*C*-glycoside with an *O*-methyl group on the C7 position (11).

These data suggest that compounds A, B, and C were apigenin-*C*-hexoside-*O*-pentoside, *O*-methyl-apigenin-*C*-deoxyhexoside-*O*-hexoside, and luteolin-*C*-hexoside-*O*-pentoside, respectively. As the compounds were all flavone glycosides with related chromophores, the concentration of these compounds may be expressed as rutin equivalents since rutin is a commercially available flavone glycoside.

DISCUSSION

Our study revealed that three flavone-*C*-glycosides, *O*-methyl-apigenin-*C*-hexoside-*O*-deoxyhexoside, apigenin-*C*-hexoside-*O*-pentoside, and luteolin-*C*-hexoside-*O*-pentoside, were induced in oats by methyl jasmonate and nematode parasitism. Flavonoid glycosides are ubiquitous in the plant kingdom and are associated with a wide range of functions in plants (31). Aside from constitutive levels in plants, flavone-*C*-glycoside accumulation in response to attack of herbivores makes them candidates for defense components of plants (28,31). For example, levels of the flavonoid apigenin, as free aglycone, increased in herbivore-damaged plants (1), rendering them unpalatable to the herbivorous insect, *Spodoptera littoralis*. A constitutive flavone-*C*-glycoside in rice phloem sap has been implicated in resistance to the brown planthopper, *Nilaparvata lugens* (29). Hence, the accumulation of flavone-*C*-glycosides in oats can be interpreted as a possible defense response of oats and may indicate resistance in plant. The damage in tissues of oats caused by the nematodes in the study may be similar in some ways to herbivory of insects, which results in synthesis of flavonoid derivatives in plants including apigenin and luteolin glycosides (32,33).

Although flavonoids are generally accepted as defense metabolites of plants against both arthropod and mammalian herbivory (31), most of the studies only report an increase in the level of the flavonoids in challenged plants and do not provide direct evidence of the involvement of flavonoids in plant defense. Our study has demonstrated that an inducible flavone-*C*-glycoside is toxic to nematodes and supports the view that it is involved in plant defense and in resistance against nematodes. This was demonstrated by the inactivation of *H. avenae* upon treatment with the induced flavone-*C*-glycosides in oat shoot and root extracts. In this bioassay, the crude shoot extract immobilized most nematodes resulting in limited invasion of roots. The crude root extract, on the other hand, had less dramatic effects but significantly impaired development of nematodes in wheat roots.

Flavonoid phytoalexins other than flavone-*C*-glycosides have been reported to be involved in plant defense against nematodes. Coumestrol and psoralidin were compounds produced by lima beans in response to invasion by *Pratylenchus scribneri* and where coumestrol impaired motility of the nematode at 5 to 25 $\mu\text{g/ml}$ (51). The phytoalexin medicarpin occurs in higher concentrations in resistant alfalfa cultivars and immobilized *P. penetrans* in vitro (4). The same compound and 4-hydroxymedicarpin from *Taverniera abyssinica* were found to have nematicidal properties in bioassay with *C. elegans* (55). Another isoflavonoid, glyceollin, has been implicated in soybean resistance to *Meloidogyne incognita* and *H. glycines* (35,37). Glyceollin inhibited in vitro oxidative respiration and motility of *M. incognita* (38) and ad-

versely affected egg hatching and juvenile motility of *H. glycines* at 100 or 200 $\mu\text{g/ml}$ (58).

While the flavonoid content of oat leaves has been studied extensively (12,50), the flavonoids present in oat roots have not received much attention. There were four major flavone glycosides reported in oat leaves: 2''-*O*-rhamnosyl-8-*C*-glucosylapigenin, 7-*O*-methyl-2''-*O*-rhamnosyl-8-*C*-glucosylapigenin, 2''-*O*-arabinosyl-6-*C*-glucosylapigenin, and 2''-*O*-arabinosyl-6-*C*-glucosylluteolin (23). The mass spectra of the latter three flavone glycosides were consistent with the three observed in this study. Thus, it is suggested that *O*-methyl-apigenin-*C*-hexoside-*O*-deoxyhexoside is equivalent to 7-*O*-methyl-2''-*O*-rhamnosyl-8-*C*-glucosylapigenin, apigenin-*C*-hexoside-*O*-pentoside to 2''-*O*-arabinosyl-6-*C*-glucosylapigenin, and luteolin-*C*-hexoside-*O*-pentoside to 2''-*O*-arabinosyl-6-*C*-glucosylluteolin.

The analysis of the flavonoid profile obtained from RP-HPLC and mass spectrometry of the methanol extract of the shoot and roots of oats cv. Quoll showed that the concentration of apigenin-*C*-hexoside-*O*-pentoside was greatest in shoots and least in roots compared with the two other flavonoids. Luteolin-*C*-hexoside-*O*-pentoside was the reverse with lowest concentration in the shoots and highest in the roots and *O*-methyl-apigenin-*C*-deoxyhexoside-*O*-hexoside reached moderate concentrations in both shoots and roots.

Of the three inducible compounds, *O*-methyl-apigenin-*C*-deoxyhexoside-*O*-hexoside was found to be biologically active using the fractions from semipreparative HVPE. Apigenin-*C*-hexoside-*O*-pentoside was found to be inactive despite its high

Fig. 7. A, B, and C, Absorbance spectra of purified compounds A, B, and C from oat seedlings cv. Quoll.

concentration in the shoots. The activity of luteolin-*C*-hexoside-*O*-pentoside could not be established because it was not recovered from shoot extracts in sufficient quantity. Preparations from roots, where luteolin-*C*-hexoside-*O*-pentoside was predominant, did not yield sufficient material for the components to be tested. When the biological activity of the crude extracts from roots and shoots and the fractions from shoots containing *O*-methyl-apigenin-*C*-deoxyhexoside-*O*-hexoside is considered, it is likely that this is the sole active component in our study. Further studies are required to confirm the biological activity of luteolin-*C*-hexoside-*O*-pentoside. The biological activity of *O*-methyl-apigenin-*C*-deoxyhexoside-*O*-hexoside is further supported by the results obtained in the test using the semipurified compounds.

In methyl jasmonate-treated plants, the estimated concentration of *O*-methyl-apigenin-*C*-deoxyhexoside-*O*-hexoside reached 9.4 mg/g fresh weight (62.9 mg/g dry weight) in roots and 6.7 mg/g fresh weight (47.6 mg/g dry weight) in shoots. These were greater than the concentrations of the root (0.16 mg/ml) and shoot (0.47 mg/ml) extracts used in the bioassay as well as in the semipreparative HVPE (0.82 mg/ml) that were effective against nematodes. Thus indicating that the induced concentrations in the plant are sufficient to provide protection.

It is possible that induction and biosynthesis of the biologically active flavone-*C*-glycoside occurs in the roots since there was lack of induction by the stem invading *D. dipsaci* in this study.

However, Edwards et al. (22) reported that infection with stem nematode in alfalfa systemically elicited the accumulation of phytoalexin in the roots. In contrast, the accumulation of flavonoid glucosides in the shoots but not in the roots was observed upon infection with *D. dipsaci* in clover (14). Similarly, the flavonoid sakuranetin and a related phenylpropanoid in the leaves of rice were induced by *D. angustus* infestation and were considered to be responsible for the resistance of some cultivars (49).

There was a systemic response of the induction of flavonoids by root nematodes *H. avenae* and *P. neglectus* in this study similar to that reported for glyceollin by root knot nematodes in soybean (35,38). Cook et al. (14), on the other hand, showed a localized effect of the phytoalexin response in clover. This highlights the variation in defense response to nematodes among different plant species.

The protection provided by methyl jasmonate treatment of plants against nematodes *H. avenae* and *P. neglectus* adds support to the argument that the inducible flavonoids are involved in plant defense. In the absence of methyl jasmonate, nematodes were able to cause damage to the plant before sufficient flavone-*C*-glycoside accumulated. There was no obvious penalty associated with methyl jasmonate treatment, since shoot and root weights were similar to those of untreated controls. It is known in other plants that jasmonic acid induces genes involved in biosynthesis of phytoalexins (10,18) and phenolics (13,20,25,57) that are

Fig. 8. Fragmentation spectrum (mass spectrometry [MS]-MS) of the $[M+H]^+$ ion at **A**, m/z 565; **B**, m/z 593; and **C**, m/z 579 of compounds A, B, and C, respectively.

involved in plant defense (5,16,17,60). While methyl jasmonate may have induced other compounds in oats that were not detected by our system, the induction of the flavone-C-glycoside in high concentrations and the direct inhibitory effect of this flavonoid on the nematode are sufficient to account for the protection observed.

To our knowledge, this is the first report of a novel function and involvement of a flavone-C-glycoside, *O*-methyl-apigenin-C-deoxyhexoside-*O*-hexoside, in plant defense of oats against nematodes extending the known range of function of flavonoids in plants (31). Among cereals, oats has been identified to have useful levels of resistance to nematodes such as *Pratylenchus* and *Heterodera* spp. (34). The relatively high constitutive levels of flavone-C-glycosides and elicitor-induced accumulation may have contributed to the degree of resistance in oats to some nematodes. More studies to relate the association of the flavone-C-glycoside in resistance to nematodes in oats are underway comparing the flavonoid content and nematode resistance of different cultivars of oats. Further study on levels of enzymes and genes involved in this possible resistance mechanism to nematodes in oats is a logical step.

In summary, the inducible flavone-C-glycoside, *O*-methyl-apigenin-C-deoxyhexoside-*O*-hexoside, provided protection against the major cereal nematodes *Heterodera* and *Pratylenchus* in oats. This is the first report implicating the compound to plant defense against any pest or pathogen. The deliberate induction or breeding for enhanced constitutive production of defensive flavonoids in economically important plants may prove a useful approach for nematode population management.

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