IDENTIFICATION AND QUANTITATION OF VARIOUS INOSITOLS AND O-METHYLINOSITOLS PRESENT IN PLANT ROOTS RELATED TO SOYBEAN CYST NEMATODE HOST STATUS

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ABSTRACT

McDonald IV, L. W., S. C. Goheen, P. A. Donald, J. A. Campbell. 2012. Identification and quantitation of various inositols and o-methylinositols present in plant roots related to soybean cyst nematode host status. Nematropica 42:1-8.

Inositols and O-methylinositols in plant roots were extracted, isolated, and analyzed to test the hypothesis that these compounds may be involved in feeding site establishment for soybean cyst nematode. Root samples from soybean (*Glycine max*), tapia bean (*Phaseolus vulgaris*), crimson clover (*Trifolium incarnatum*), corn (*Zea mays*), sugar beet (*Beta vulgaris*), and peanut (*Arachis hypogaea*) were extracted with 80% ethanol, derivatized, and analyzed using gas chromatography/mass spectrometry (GC/MS). Fragment ions at m/z 260 and 265 were used to quantify the O-methylinositols and inositols, respectively. Pinitol, *D-chiro*-inositol, and *myo*-inositol were found in soybean samples and *myo*-inositol in tapia bean. Sugar beets contained *D-chiro*-inositol. Pinitol and *myo*-inositol were found in crimson clover. Corn contained *myo*-inositol. Pinitol, *D-chiro*-inositol, ononitol, and *myo*-inositol were found in peanuts. No correlation was found between the presence of any of the inositols or o-methyl inositols and the ability of these plants to host the soybean cyst nematode.

Key words: Inositol, O-methyl-inositol, Gas chromatography/mass spectrometry, Plant roots

RESUMEN

McDonald IV, L. W., S. C. Goheen, P. A. Donald, J. A. Campbell. 2012. Identificación y cuantificación de varios inositoles y o-metilinositoles presentes en raíces y su relación con la susceptibilidad al nematodo quiste de la soya. Nematropica 42:1-8.

Se extrajeron, aislaron y analizaron los inositoles y o-metilinositoles en raíces de plantas para probar la hipótesis de la participación de estos compuestos en el establecimiento de sitios de alimentación del nematodo quiste de la soya. Se incluyeron raíces de soya (*Glycine max*), fríjol (*Phaseolus vulgaris*), trébol rojo (*Trifolium incarnatum*), maíz (*Zea mays*), remolacha azucarera (*Beta vulgaris*) y maní (*Arachis hypogaea*) y se extrajeron las muestras con 80% etanol, se derivatizaron y se analizaron usando cromatografía de gases/espectrometría de masas (GC/MS). Se usaron fragmentaciones a m/z 260 y 265 para cuantificar los o-metilinositoles y los inositoles, respectivamente. En las muestras de soya se encontró pinitol, *D-chiro*-inositol y *myo*-inositol, y se encontró *myo*-inositol en el fríjol. La remolacha azucarera contuvo *D-chiro*-inositol. En el trébol rojo se halló Pinitol y *myo*-inositol. El maíz mostró *myo*-inositol y en el maní se encontró pinitol, *D-chiro*-inositol, ononitol y *myo*-inositol. No se halló ninguna correlación entre la presencia de ningúno de los inositoles o de los o-metilinositoles y la habilidad de estas plantas para ser hospedante del nematodo quiste de la soya.

Palabras clave: Cromatografía de gases/espectrometría de masa, Inositol, O-metil-inositol, Raíces

INTRODUCTION

Inositols are carbohydrates and cyclitols with the empirical formula $C_6H_{12}O_6$. They are polyols of cyclohexane and there are 9 possible stereoisomers. Inositols are ubiquitous in nature comprising an important component of living cells. Inositols are synthesized by many, but not all plants. Soybeans (Phillips *et al.*, 1982) and peanuts (Lee and Morris, 1963) are examples of good sources of inositols, but some fruits juices appear to have no inositol (Sanz *et al.*, 2004). Several fruit extracts contain *myo*-inositol but not *chiro*-inositol or *scyllo*-inositol (Sanz *et al.*, 2004). Also, inositols are soluble in water, so they can be widely distributed throughout the various tissues when they are present in any organism.

Several O-methylinositols serve an important role in plant biology by improving tolerance to heat stress, drought stress and salt stress (Ford, 1984; Guo and Oosterhuis, 1997; Manchanda and Garg, 2008; Sheveleva, *et al.*, 1997; Streeter, *et al.*, 2001). For example, pinitol is an important stress metabolite in soybean plants used to improve the plant's tolerance to water-deficit stress (Guo and Oosterhuis, 1997).

Soybean are the primary host for soybean cyst nematodes (*Heterodera glycines*, SCN) (Mueller, 2009). Soybeans are also a very rich source of inositol and related compounds (Phillips *et al.*, 1982). Corn, is not a host for SCN and has no detectible inositol (Garland *et al*., 2009). Klink *et al.* (2007) recently examined the response of soybean DNA to infestation by SCN. The authors reported several enzymes that were induced or suppressed by the SCN infection. One of these, SAM2, is involved in inositol synthesis. These relationships lead to our hypothesis that inositols in plants might be involved in SCN feeding site selection. Soybean plants are the most widely known host of SCN, causing significant annual yield losses. The relationship between abundant levels of several inositols in soybean and undetectable quantities of inositol in corn, a non host for SCN (Binder and Haddon, 1984), encouraged us to examine plant roots based on their SCN host status. Both soybean and tapia bean (Ichinohe, 1955) are good hosts for SCN while sugar beet is a marginal host (Riggs, 1992). In contrast, crimson clover (Riggs and Hamblen, 1962), and peanuts (Mueller, 2009) are non-hosts for SCN. These plants were selected to test our hypothesis that the presence of inositol was related to their susceptibility to SCN infection and/or reproduction.

Naturally occurring isomers of inositol are *myo*-inositol, *chiro*-inositol, *scyllo*-inositol, *muco*-inositol, and neo-inositol with *myo*-inositol being the most abundant (Wang *et al.*, 1990). Inositols and their various isomers are not all present in all plants or animals. As illustrated in Figure 1, *myo*-inositol is biologically synthesized in a two-step conversion of D-glucose 6-phosphate to L-*myo*-inositol-1-phosphate using catalyst L-*myo*-inositol 1-phosphate synthase (Hoffmann-Ostenhof and Pittner, 1982; Stieglitz, *et al.*, 2005). The L-*myo*-inositol-1-phosphate is then dephosphorylated through inositol monophosphates to produce free *myo*-inositol (Stieglitz *et al.*, 2005; Majumder, *et al.*, 2003). The L-*myo*-inositol 1-phosphate synthase is the key enzyme in the production of *myo*-inositol from D-glucose (Hoffmann-Ostenhof and Pittner, 1982). This enzyme has been isolated from various animals, plants, yeasts, and prokaryotes (Hoffmann-Ostenhof and Pittner, 1982). Furthermore, it has been suggested that all of the other inositol isomers are derived from *myo*-inositol via epimerization reactions (Hoffmann-Ostenhof and Pittner, 1982; Hipps, *et al.*, 1973). In the case of *D-chiro*-inositol, these epimerization reactions vary according to the species (Hoffmann-Ostenhof and Pittner, 1982). For example, in *Trifolium* species, *D-chiro*-inositol is synthesized from a methylation of *myo*-inositol to sequoyitol using NAD-specific sequoyitol dehydrgenase (Hoffmann-Ostenhof and Pittner, 1982). The sequoyitol is then epimerized to D-pinitol which is demethylated to *D-chiro*-inositol using NADP-specific D-pinitol dehydrogenase (Hoffmann-Ostenhof and Pittner, 1982). However, in algae, *D-chiro*-inositol is directly epimerized from *myo*-inositol without any intermediates (Hoffmann-Ostenhof and Pittner, 1982). The *O*-methylinositols are also derived from *myo*-inositol with pinitol being a major cyclitol in plants (Chiera, *et al.*, 2006). Therefore, the biosynthetic pathways of the inositol family are highly variable amongst plant species. The biosynthesis of pinitol follows a two step conversion of *myo*-inositol. *Myo*-inositol is first converted to ononitol through an inositol methyl transferase (IMT) (Chiera, *et al.*, 2006). This IMT takes the methyl group from S-Adenosyl-L-methionine and transfers it to *myo*-inositol forming ononitol. Ononitol is then converted to pinitol through an unknown epimerase (Chiera, *et al.*, 2006). These complex relationships between the various inositols and o-methyl inositols and their ability to be a good SCN host helped us determine whether there is a clear relationship between SCN damage and the presence of these metabolites.

MATERIALS AND METHODS

Inositols and o-methylinositols were extracted from plant roots using ethanol and water, derivatized,

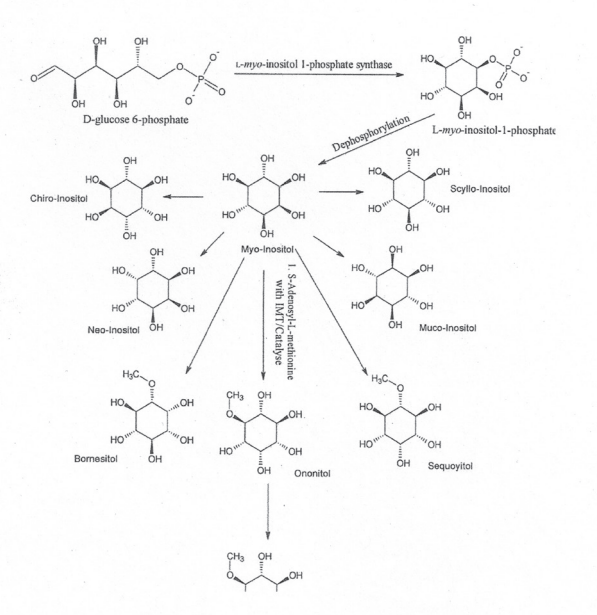

Figure 1. Biosynthesis of inositols and *O*-methyl-inositols from *myo*-inositols.

and analyzed by GC/MS. Samples were generated in triplicate and each extract was analyzed at least 3 times. Details of the procedures used are described below.

Myo-inositol, *scyllo*-inositol, *chiro*-inositol, *muco*-inositol, pinitol, β-D(+)-glucose, D-(-)-fructose, D-(-)-ribose, D-(+)-xylose, D-(+)-galactose, sucrose, D-(+)-glucose, α-lactose, D-(+)-maltose, D-(-)-mannose, D-mannitol, D-sorbitol, and D-lyxose standards were purchased from Sigma Aldrich. D-(-)-arabinose was purchased from Acros Organics (Fisher Scientific, Pittsburgh, PA). *Neo*-inositol and ononitol were provided as a gift from Dr. Laurens Anderson of the University of Wisconsin-Madison. Each standard was reported to be at least 95% pure. Purity as observed by GC/MS was confirmed to appear to be greater than 95%. Anhydrous sodium sulfate (ACS grade), trimethylsilylimidazole (derivatizing grade), toluene (99.8%) and ethyl alcohol (99.8%) (EtOH) were purchased from Sigma Aldrich. Ethyl alcohol (200 proof) was purchased from Sigma Aldrich then diluted with Milli-Q Plus deionized water.

Plant tissue sample preparation and derivatization

Seed of plants selected for analysis were germinated in modified sterile rag dolls, using laboratory paper towels at ambient temperature, until the radicals reached 3 cm. Roots were excised from each plant, placed at -80ºC for at least 24 h, placed on dry ice, and shipped overnight from the agricultural laboratory in Tennessee to the analytical lab in Washington State. Extraction of the frozen roots was carried out, using the methods as described by Skot *et al.* (1984). The thawed roots were thoroughly washed with approximately 10 ml of water then minced using a razor. The minced roots were then extracted using 10 ml of 80% EtOH at 70°C similar to the method of Phillips *et al.* (1982). The extract was centrifuged at 2,500 RPM (500 g) for 20 min. The centrifuge extraction was repeated three to five times for each plant, and each sample was transferred to a 5 mL glass vial, and dried under bottled nitrogen overnight using a heating block set at 23°C. Trimethylsilyl imidazole (TMIS) derivatives were prepared following the procedure established by Garland *et al.* (2009). Ten milliliters of 80% ethanol were added to the dried samples then stored at 4ºC for 18 hr to resolvate. After 18 hr, each mixture was vigorously vortexed. Five ml aliquots were volumetrically pipetted into new vials and dried under nitrogen overnight. The dried samples were then each derivatized using 100 µl of TMSI. This solution was left to react in a tightly capped 2 ml vial for 20 min at room temperature. After derivatization, a small amount of sodium sulfate was added to each vial and vortexed. The samples were then diluted to a final volume of 1 ml using toluene, and centrifuged at 2,500 RPM for 1 hr. The liquid aliquots were transferred to GC vials and stored in the refrigerator at 4°C until analysis using GC/MS.

GC/MS analysis

The concentration of *myo*-inositol, *scyllo*-inositol, *chiro*-inositol, *muco*-inositol, *neo*-inositol, pinitol, and ononitol were determined using GC/MS. Prior to analyzing the samples, derivatized standards of known concentration were injected to determine the retention time and mass spectrum of each cyclitol. One µl of the sample was injected on the RTX 1 capillary column (60 m x 0.25 mm ID, 0.25 µm thickness) in an Agilent 6890 gas chromatograph in the splitless mode. The temperature program was as follows: an initial temperature of 80ºC was held for 1 min and then increased at 20ºC/ min. to 180ºC followed by a 3ºC/min ramp to 240ºC with a final ramp at 20ºC-300ºC, and then held at 300°C for 1 min.

The column eluents were analyzed by an Agilent 5973 mass spectrometer in the electron ionization mode. The scan range ion was 50-550 amu with a 1 s scan time. Each root type analysis was averaged and the standard deviation about the mean was determined.

RESULTS

Standards of the inositols were analyzed per minute to determine their retention times and mass spectra and to create a standard calibration curve so the analytes in the root samples could be quantified. They eluted in the following order: pinitol (18.76 min.), *neo*-inositol (20.62 min), *muco*-inositol (21.15 min), ononitol (21.78 min), *D-chiro*-inositol (22.01 min), *scyllo*-inositol (23.74 min), and *myo*-inositol (25.47 min). Figure 2 illustrates the total ion chromatogram of the derivatized inositol and *O*-methylinositol standards. Table 1 shows the major ions present in the mass spectrum of each standard. All of the inositols had a common 265 m/z ion, easily distinguishing them from other monosaccharides. In addition, pinitol and ononitol were easily distinguished from the inositols due to their 260 m/z ion. This ion is consistent with the structure $C_{11}H_{24}O_3Si_2$, when analyzing each of the root samples, an extracted ion plot of 260 m/z for ononitol and pinitol, and 265 m/z for the inositols was selected to produce simpler spectra. Also, the area of either the 260 m/z or 265 m/z ion was used for quantitation depending on the compound.

The total ion chromatogram of a TMSI-derivatized soybean root extract is illustrated in Figure 3. The chromatogram was very clean containing only eight peaks in the region of interest. Six of these peaks were identified using the inositol, *O*-methylinositol, and sugar standards. Some of the peaks that appear in this and subsequent chromatograms were not identified because they were not target analytes.

The total ion chromatogram of a TMSI-derivatized tapia bean root chromatogram also consisted of peaks that were well resolved with *myo*-inositol being the only inositol or *O*-methylinositol found (Figure 4).

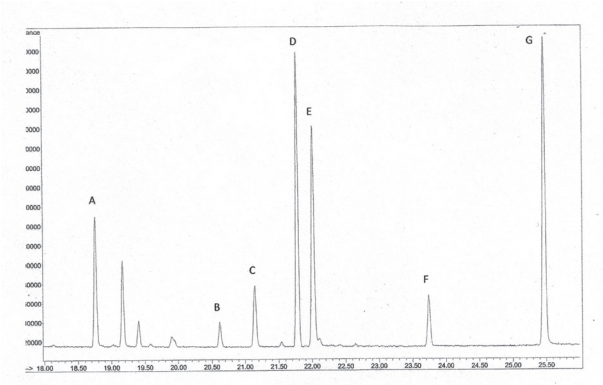

Figure 2. Total ion chromatogram of TMSI-derivatized 4 ppm inositol and O-methylinositol standards. A = pinitol, B = neo-inositol, C = muco-inositol, D = ononitol, E = D-chiro-inositol, F = scyllo-inositol, G = myo-inositol. All other peaks in the spectrum are impurities from the derivatizing reagent.

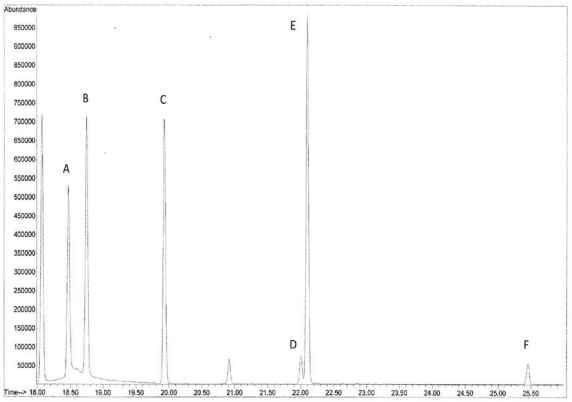

Figure 3. Total ion chromatogram of TMSI-derivatized soybean root extract. A = D-(-)-fructose, B = D-pinitol, C = D-(+)-glucose, D = *D-chiro*-inositol, E = β-D-(+)-glucose, F = *myo*-inositol.

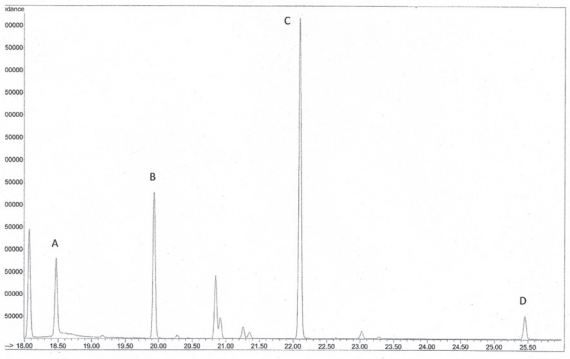

Figure 4. Total ion chromatogram of TMSI-derivatized tapia bean root extract. A = D-(-)-fructose, B = D-(+)-glucose, C = β-D-(+)-glucose, D = *myo*-inositol.

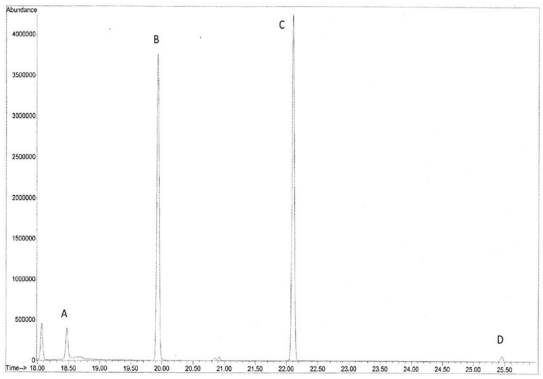

Figure 5: Total ion chromatogram of TMSI-derivatized corn root extract. A = D-(-)-fructose, B = D-(+)-glucose, C = β-D-(+)-glucose, D = *myo*-inositol.

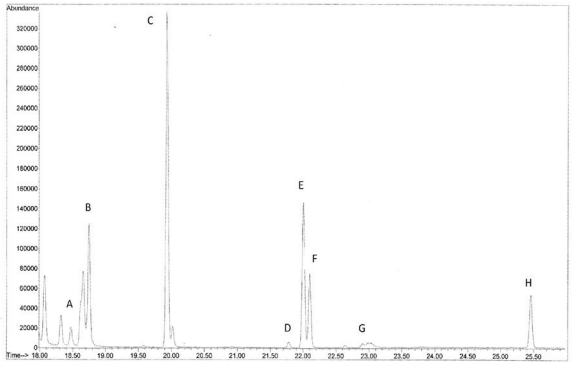

Figure 6: Total ion chromatogram of TMSI-derivatized peanut root extract. A = D-(-)-fructose, B = D-pinitol, C = D-(+)-glucose, D = ononitol, E = D-chiro-inositol, F = β-D-(+)-glucose, G = bornesitol, H = myo-inositol.

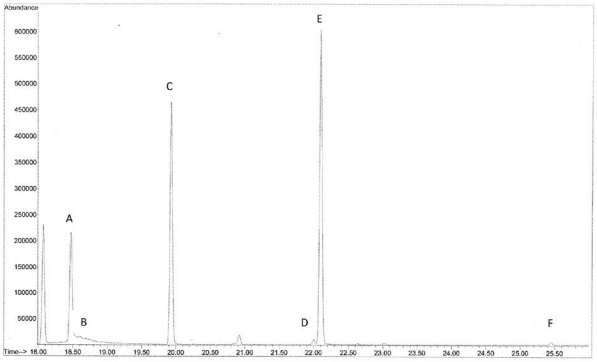

Figure 7. Total ion chromatogram of TMSI-derivatized sugar beet root extract. A = D-(-)-fructose, B = D-pinitol, C = D-(+)-glucose, D = *D-chiro*-inositol, E = β-D-(+)-glucose, F = *myo*-inositol.

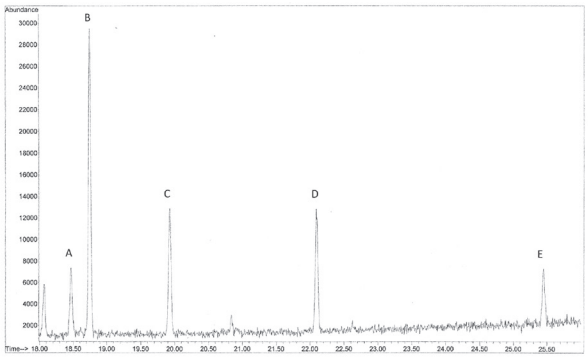

Figure 8. Total ion chromatogram of TMSI-derivatized crimson clover root extract. A = D-(-)-fructose, B = D-pinitol, C = D-(+)-glucose, D = β-D-(+)-glucose, E = *myo*-inositol.

β-D(+)-glucose was the major sugar observed in tapia bean roots. The concentration of *myo*-inositol in tapia bean roots was very similar to that found in corn roots (3.3 vs 4.3 mg/g respectively).

Comparatively, Figure 5 indicates the total ion chromatogram of a TMSI-derivatized corn root extract. The chromatogram contained only 5 major peaks. Both D-(+)-glucose, and β-D(+)-glucose were present in large amounts; *myo*-inositol was the only one of the inositol or *O*-methylinositol investigated.

A total ion chromatogram of a typical TMSI-derivatized peanut root extract is shown in Figure 6. Peanut roots had the greatest variety of inositols and *O*-methylinositols present of all the plants investigated in this study, as well as several identified carbohydrates. D-(+)-glucose was the most abundant sugar, but as shown in Table 2, the roots also contained pinitol (4.7 mg/g), ononitol (0.4 mg/g), *myo*-inositol (1.6 mg/g), and *D-chiro*-inositol (3.9 mg/g).

The TMSI-derivatized sugar beet root chromatogram was very simple containing large amounts of D-(+)-glucose, and β-D(+)-glucose. It also contained small amounts of pinitol (a peak was present but less than our quantitation limit), *D-chiro*-inositol (12 mg/g), and less than our quantitation limit of *myo*-inositol. There was more *D-chiro*-inositol in sugar beet roots than any of the other plants investigated. Those levels were more than twice that of either peanuts or soybeans.

The total ion chromatogram of a TMSI-derivatized crimson clover root extract is shown in Figure 8. From the simple spectrum it was very easy to identify pinitol as the major cyclitol (14 mg/g) while *myo*-inositol was also present (7.4 mg/g). These roots were the highest in *myo*-inositol compared to any of the other plants studied. Both D-(+)-glucose, and β-D(+)-glucose were also present in large amounts.

Table 2 summarizes the concentration of each of the cyclitols in the six plant roots. The cyclitols found in these six plant species were considerably different. Peanut was the only plant that contained quantifiable amounts of all four analytes. The limits of quantitation varied for each inositol. Pinitol and ononitol were quantifiable as low as 0.12 ppm, while *myo*-inositol, *D-chiro*-inositol, and *muco*-inositol were only quantifiable to 0.25 ppm. *Scyllo*-inositol was quantifiable to 0.5 ppm and neo-inositol was quantifiable above 2.5 ppm. A pinitol peak was observed in sugar beet root samples, but the concentration was below our quantitation limits.

No clear correlation was found between the presence of any of the inositols and the plants' ability to support SCN feeding site establishment. Table 2 shows soybean roots (a good host for SCN) had the highest concentration of pinitol, but no ononitol. Crimson clover roots had the second highest concentration of pinitol, no detectible *D-chiro*-inositol, but twice as much *myo*-inositol as soybean roots, yet, it is a non-host of SCN (Riggs and Hamblen, 1962). Similarly, peanut roots have all the inositols detected, but is also a non-host of SCN (Mueller, 2009). In contrast, tapia bean contained none of the inositols that were examined in this study with the exception of *myo*-inositol, yet it is a good host of SCN (Ichinohe, 1955). Finally, sugar beets are poor hosts (Riggs, 1992) with large amounts of *D-chiro*-inositol and only trace quantities of pinitol and *myo*-inositol. It remains possible that the inositols could be involved in SCN feeding site establishment, but no clear correlation is obvious from these results.

DISCUSSION

Pinitol was hypothesized to possess a critical role in the feeding site establishment of SCN. In this study, roots of soybean, crimson clover, tapia bean, corn, peanut, and sugar beet were examined. All plant species tested are hosts of a cyst nematode species but only soybean, tapia bean, and sugar beets are known to allow SCN feeding site establishment (Mueller, 2009; Riggs and Hamblen, 1962; Rigg *et al.*, 1980; Riggs 1992; Sharma, 1998).

There appeared to be no correlation between the ability of a plant to allow SCN feeding site establishment and the concentration or presence of either of the inositols or O-methylinositols. Soybean and tapia bean are good hosts of SCN. However, neither pinitol nor ononitol were detected in tapia bean. In contrast, sugar beets are poor hosts of SCN. Corn, peanuts and crimson clover are not hosts of SCN, yet of these, only corn appears void of pinitol and ononitol.

The observed cyclitols were similar to those already reported for soybeans (Phillips *et al.*, 1982; Streeter and Strimbu, 1998). Pinitol was easily identified at 18.76 min and observed to be one of the major cyclitols in soybean roots. The pinitol content of soybean roots observed here was similar to that observed previously in our laboratory (35 vs. 26 mg/g) and also similar to that observed by Streeter and Strimbu (1998) for the

Table 1. Relative intensities of common ions in the mass spectra of each standard. Percentages are based on the intensity of each ion relative to the most intense ion (base ion) peak of that standard. The dash (-) indicates the fragment was not observed in those components.

	Pinitol	Ononitol	*Myo*-inositol	*Scyllo*-inositol	*D-chiro*-inositol	*Muco*-inositol	*Neo*-inositol
73 m/z	100 %	69 %	52 %	44 %	53 %	46 %	41 %
147 m/z	53 %	43 %	40 %	26 %	37 %	29 %	27 %
191 m/z	44 %	42 %	41 %	34 %	34 %	30 %	21 %
217 m/z	85 %	100 %	86 %	58 %	65 %	66 %	49 %
260 m/z	93 %	44 %	-	-	-	-	-
265 m/z	16 %	13 %	22 %	6 %	26 %	18 %	17 %
305 m/z	48 %	42 %	100 %	73 %	82 %	96 %	23 %
318 m/z	74 %	43 %	55 %	100 %	100 %	100 %	100 %

Table 2: Dry weight concentrations of inositols and *O*-methylinositols in plant roots. Concentrations are given in µg/mg (dry weight). LOQ = Limits of quantitation. Components observed below the LOQ were identified but could not be quantified. *Scyllo, neo*, and *muco* inositol were not detected in any root samples. The last column depicts whether the plant is a good (yes), a non- (no), or a poor (poor) host of SCN.

	Pinitol	Ononitol	*Myo*-inositol	*D-Chiro*-inositol	SCN Host?
Corn *Zea mays*	ND	ND	4.3 ± 0.34	ND	No
Crimson clover *Trifolium incarnatum*	14 ± 1.2	ND	7.4 ± 0.72	ND	No
Peanut *Arachis hypogaea*	4.7 ± 0.85	0.41 ± 0.031	1.61± 0.93	3.9 ± 0.72	No
Soybean *Glycine max*	26 ± 3.3	ND	3.8 ± 0.28	4.9 ± 0.46	Yes
Sugar beet *Beta vulgaris*	Below LOQ	ND	Below LOQ	12 ± 1.8	Poor
Tapia bean *Phaseolus vulgaris*	ND	ND	3.3 ± 0.37	ND	Yes

ND = Not detected

petiole (27.1 mg/g), and pod (26.6 mg/g), but greater than the levels found in leaves (17.8 mg/g) and stems (15.6 mg/g) of soybean plants (Streeter and Strimbu, 1998). Similarly, ononitol was not observed in this or other studies of soybean extracts, but *myo*-inositol has been reported to be about the same concentration in leaves as our reported levels in roots (4.0 and 3.8 mg/g respectively) while the levels in pods was slightly higher (5.7 mg/g) and levels in petiole (1.2 mg/g) and stems (2.05 mg/g) was lower than what we observed in roots (Streeter and Strimbu, 1998). Levels of *D-chiro*-inositol was observed to be 4.9 mg/g in roots in our study, but less in other parts of the plant in a previous investigation (1.7 mg/g in leaves; 2.9 mg/g in pods; 1.4 mg/g in petiole, and 0.8 mg/g in stems; Streeter and Strimbu, 1998). While it is well known that pinitol is a major component in soybeans, the presence of pinitol in peanuts has also been recognized for almost 50 years (Lee and Morris, 1963).

The 80% ethanol extraction was similar to the

extraction method of Phillips *et al.* (1982). The extraction appeared to be effective as all of the inositols and *O*-methyl-inositols were soluble in this 70°C ethanol solution. After derivatization with TMSI, all of the extractable inositols were clearly identified using GC/MS. Furthermore, the method we used for extracting root samples provided data that was comparable to that of previous research (Phillips *et al.*, 1982; Sanz *et al.*, 2004; Lee and Morris, 1963).

Phillips reported pinitol, *D-chiro*-inositol, *myo*-inositol, and sequoyitol (5-O-methuyl-*myo*-inositol) in soybean plants. They also compared the relative pinitol content of foliage from 24 different cultivars against total carbohydrate in an 80% ethanol extract. Pinitol was typically 50-84% of the total carbohydrate. Lee and Morris (1963) isolated D-inositol from peanut flour. Binder and Haddon (1984) also reported pinitol, ononitol, bornesitol, *D-chiro*-inositol, and *myo*-inositol in peanuts. Using their mass spectrum and retention time, our study identified bornesitol in our peanut samples. Their extraction and separation technique described previously was more extensive utilizing fermentation of their aqueous extract for 16 hr separation using cation and anion exchange resins along with calcium oxide and phosphoric acid (Binder and Haddon, 1984). In contrast, Garland *et al.* (2009) used a methanol extraction to quantify pinitol levels in root samples and indicated a similar concentration of pinitol as a major cyclitol in soybeans.

The mass spectra of the derivatized inositols and *O*-methylinositols made them easily distinguishable from other monosaccharides. The 265 m/z ion was unique to the TMSI-derivatized inositols and the 260 m/z ion was unique to the TMSI-derivatized *O*-methylinositols. Upon comparison with other data, our mass spectra of pinitol and ononitol were consistent with those previously reported by Binder and Haddon (1984).

The results from this work confirm that extracting with 80% ethanol was effective in extracting inositols from frozen root samples as has been reported previously for extracting pinitol from soybeans (Phillips *et al.*, 1982). As the role of these species in biology and medicine continue to be better understood, their extraction and identification in natural products will become increasingly important. For the samples tested, peanut roots contained the highest concentrations of pinitol, *D-chiro*-inositol, ononitol, and *myo*-inositol. While these components were identified previously from peanuts (Lee and Morris, 1963), a direct comparison between the roots of these multiple species has not been examined in one study.

Since soybean, crimson clover, sugar beet, and peanut contained detectable amounts of *myo*-inositols and pinitol, they may all contain the inositol methyl transferase and S-Adenosyl-L-methionine to convert *myo*-inositol to ononitol, and the unknown epimerase to convert ononitol to pinitol. Both soybean root extracts and crimson clover root extracts have a large pinitol to *myo*-inositol ratio, but contain no ononitol. Future studies should investigate whether the conversion from *myo*-inositol to pinitol is just more efficient or if pinitol is produced by a different biosynthetic pathway. Furthermore, sugar beet had almost equal amounts of *myo*-inositol and pinitol but no ononitol. It is therefore possible that the conversions from *myo*-inositol to ononitol and from ononitol to pinitol follow different biosynthetic processes. The peanut root extracts had a small ratio of *myo*-inositol to pinitol when compared to soybean root extracts and crimson clover root extracts. Ononitol was also present in the peanut root extracts. Further studies should be conducted to determine if the biosynthesis of *myo*-inositol to pinitol is less efficient in the peanut roots compared to the soybean and crimson clover roots. It would be worthwhile to better understand the inositol biosynthetic pathways in these plants because SCN feeding site establishment requirements may still be linked to these or other nutrient pathways.

Although we were not able to find a clear correlation between the inositols and *O*-methylinositols and SCN feeding site establishments, there are likely to be compounds in soybean roots essential to the reproductive cycle of SCN. For example, SCN will infect corn roots, but cannot reproduce while residing in that plant. So, either corn produces an inhibitor or deterrent, or fails to produce one or more essential nutrients. It was unfortunate that we did not find such a nutrient, but that does not negate the hypothesis that one (or more) exists. The discovery of such a component or series of components has obvious value in the control of SCN infestations.

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