USE OF BRASSICACEOUS SEED MEAL EXTRACTS FOR MANAGING ROOT-KNOT NEMATODE IN BERMUDAGRASS

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ABSTRACT

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Plant-parasitic nematodes are difficult to control in intensively managed subtropical turfgrasses. Chemical options for nematode control are limited because of high management costs and increasing health concerns, so there is increased interest in finding alternatives to conventional synthetic nematicides. The objective of this study was to determine the effectiveness of brassicaceous seed meal extracts for control of root-knot nematode (*Meloidogyne graminis*) in 'TifEagle' bermudagrass [Cynodon dactylon (L) Pers × C. transvaalensis Burtt-Davy] maintained as a putting green. Laboratory and field experiments were carried out to evaluate the efficacy of seed meal extracts from *Sinapis alba*, *Brassica juncea*, and *B. napus* for managing root-knot nematodes. In the laboratory assay, infested soil treated with each of the seed meal extracts had significantly reduced *M. graminis* J2 numbers compared to a nontreated control. *Brassica juncea* treatments resulted in the fewest J2 in the samples, followed by *S. alba* and *B. napus*. In a field evaluation, regardless of seed meal type used, high concentrations (90 or 120 g seed meal/L) reduced J2 populations in a bermudagrass putting green compared to the nontreated control. Seed meal type significantly affected turfgrass quality. *Brassica juncea* at the 90 or 120 g seed meal/L suppressed nematodes with no phytotoxicity, and showed potential to be used for managing root-knot nematode in bermudagrass. Studies to enhance the nematicidal efficacy and lower phytotoxicity are needed to further examine the full potential of using brassicaceous seed meals for management of nematodes in subtropical turfgrasses.

Key words: bermudagrass, *Brassica juncea*, *Brassica napus*, management, *Meloidogyne graminis*, seed meal, *Sinapis alba*, turfgrass.

RESUMEN

Handiseni, M., W. Cromwell, M. Zidek, X. G. Zhou, y Y.-K. Jo. 2017. Uso de extractos de harina de semilla de brassicas para el manejo del nematodo agallador en el césped Bermuda. Nematropica 47:55-62.

Los nematodos fitoparásitos son difíciles de controlar en el césped subtropical bajo manejo intensivo. Las opciones para el control químico del nematodo son limitadas debido al alto costo de manejo y al aumento de problemas en la salud. Por lo tanto, hay un interés creciente en el descubrimiento de alternativas para los nematicidas sintéticos convencionales. El objetivo de este estudio fue determinar la eficacia de extractos de harina de semilla de brásicas para el control del nematodo agallador (*Meloidogyne graminis*) en un césped subtropical, grama 'TifEagle' [*Cynodon dactylon* (L) Pers × *C. transvaalensis* Burtt-Davy] mantenido como césped para golf. Experimentos en laboratorio y campo se llevaron a cabo para evaluar la eficacia de tres harinas de semilla de brásicas, *Sinapis alba*, *Brassica juncea* y *B. napus* para el manejo del nematodo agallador en la grama TifEagle. En el ensayo en laboratorio, el tipo de harina de semilla, la tasa de concentración y el tiempo de exposición significativamente interactuaron en la afectación del número de J2 de *M. graminis*. Cualquier extracto de harina de semilla a cualquier concentración y a cualquier tiempo de exposición mayormente resultaron en un reducido número de J2 de *M. graminis* en comparación con el control. Los tratamientos a base de *Brassica juncea* resultaron en los menores J2 en las muestras, seguido por *S. alba* y *B. napus*. En la evaluación en campo, independientemente del tipo de harina de semilla, altas concentraciones (90 o 120 g de harina de semilla/L) de extractos de harina de semilla redujeron las poblaciones de J2 en una grama para golf en comparación con el control no tratado. El tipo de harina de semilla significativamente afectó la calidad del césped. Concentraciones altas de *Brassica juncea* (90 o 120 g de harina de semilla/L) proveyeron un efecto nematicida alto y no causaron fitotoxicidad. La harina de semilla de *Brassica juncea* demostró el potencial a ser usado como un nematicida

biológico para el manejo del nemátodo agallador en grama. Enfoques para reducir la fitotoxicidad necesitan ser examinados más a fondo para alcanzar el potencial total del uso de harina de semilla de brásicas para el manejo de nematodos en el césped subtropical.

Palavras chave: grama, *Brassica juncea*, *Brassica napus*, manejo, *Meloidogyne graminis*, harina de semilla, *Sinapis alba*, césped.

Plant-parasitic nematodes are a common soilborne pathogen group found in turfgrass. They can cause severe damage to subtropical turfgrasses used for golf courses, particularly where the plant is grown in sand-based soil and intensively managed (Van Gundy, 1985). Damage often leads to a loss of turfgrass in fairways and putting greens where most recreational activity occurs, thus incurring significant economic costs to the golf industry (Crow, 2011; McClure *et al.*, 2012).

The lack of effective chemical treatment methods for plant-parasitic nematodes on established golf course turf has become a major issue in the United States. The last effective organophosphate nematicide, fenamiphos (Nemacur, Bayer Environmental Science, Research Triangle Park, NC), was banned from turfgrass use due to environmental concerns (Environmental Protection Agency, 2008). Alternatives have been sought, but with limited success. For example, a botanical derivative, furfural (Multiguard Protect, Agriguard, Cranford, NJ) (Crow *et al.*, 2003) and neem oil (Akhtar, 1999) have been labeled as nematicides in turfgrass but are reported to be inconsistent in field efficacy under varying environmental conditions.

This lack of effective, dependable options for controlling nematodes poses a serious problem in subtropical turfgrass management. There is a need for new nematicidal active ingredients for turfgrass management. The use of seed meal extracts from certain *Brassicaceae* species for plant pest management has garnered attention in recent years. Brassicaceous seed meal releases nematicidal substances into the soil through hydrolysis of glucosinolates (Zasada and Ferris, 2003). These substances also stimulate proliferation of antagonistic soil microbial communities that provide unfavorable conditions for nematodes (Cohen *et al.*, 2005). Efficacy of brassicaceous seed meals on plant-parasitic nematodes (Mojtahedi *et al.*, 1991; Johnson *et al.*, 1992), soil-borne pathogens (Ramirez-Villapudua and Munnecke, 1988; Subbarao *et al.*, 1999), and weeds (Brown and Morra, 1995) has been well documented. Cox *et al.* (2006) showed *Brassica juncea* seed meal provided 92 and 99% mortality rates of sting nematode (*Belonolaimus longicaudatu*) in irrigated and non-irrigated soils, respectively. Similarly, Zasada *et al.* (2009) tested seed meals of four *B. juncea* varieties as soil amendments against the lesion nematode (*Pratylenchus penetrans*) and the root-knot nematode (*Meloidogyne incognita*), and showed that *B. juncea* seed meal caused >90% nematode mortality.

Given the strong evidence of the effectiveness of brassicaceous seed meal as a nematicide, there is great potential for its use in managing nematodes in turfgrass. However, brassicaceous seed meals have had only limited use in turfgrass. It is because the primary application method for seed meal in the field is to directly incorporate dry seed meal into soil, which is not practical in established turfgrass and may result in severe phytotoxicity. Therefore, research on modified material preparation and different application protocols for brassicaceous seed meals was necessary. The objective of this study is to evaluate water-soluble extracts of seed meal from three *Brassica* species for nematode management in subtropical bermudagrass.

Seed meal extract preparation

Seed meal extracts from *Sinapis alba* 'IdaGold' (Brown *et al.*, 1997), *B. juncea* 'Pacific Gold' (Brown *et al.*, 2004), and *B. napus* 'Dwarf Essex' were used for both a laboratory assay and a field trial to evaluate their nematicidal effects. Seed meal was first ground into powder using an Oster hand-blender (Sunbeam Products, Boca Raton, FL). Seed meal powder (30, 60, or 90 g) was added to an Erlenmeyer flask filled with distilled water (dH_2O) up to 1 L. The flask was placed on a shaker for 30 min to mix thoroughly, then the suspension was filtered using a piece of fusible cloth (Pellon Consumer Products, St. Petersburg, FL). The resulting solution was stored in 4°C until use.

Laboratory assay

Soil and turfgrass composite samples were collected from a 'TifEagle' bermudagrass [*Cynodon dactylon* (L) Pers. × *C. transvaalensis* Burtt-Davy] putting green at the Texas A&M Turf Research and Education Center, College Station, TX, that was infested with the root-knot nematode, *Meloidogyne*

graminis (Cromwell *et al.*, 2014). An aliquot of 100 cm^3 sandy loam sample in a cone-tainer was treated with 25 ml of seed meal extract of *S. alba*, *B. juncea* or *B. napus* (90, 60, 30, or 0 g seed meal/L) for varying exposure times (0, 3, 7, or 10 days) at room temperature (~25°C). The treatments were arranged in a completely randomized design with three replications, and the experiment was conducted twice. After the allotted exposure time passed, the 100 cm^3 soil samples were placed into a Baerman funnel. After 48 h incubation at room temperature, nematodes were filtered out and collected using a No. 500 U.S.A. Standard Test Sieve (0.025 mm-diameter pore size, Newark Wire Cloth Company, Clifton, NJ). Second-stage juveniles (J2) of *M. graminis* were counted using an inverted compound microscope at 40× magnification (Leeds Instruments, Inc., Minneapolis, MN).

The collected data were subjected to Analysis of Variance (ANOVA) using SAS statistical software (9.3 version, SAS Institute, Cary, NC). A square root transformation was used on the data of J2 counts to better fit a generalized linear model for ANOVA. Differences between treatment means were examined using Fisher's Least Significant Difference test (LSD $P < 0.05$). Variances between repeated experiments were unequal by the Bartlett test of homogeneity, and thus each experiment was analyzed and presented separately.

Seed meal type, concentration, and exposure time affected the survival of *M. graminis* J2 in soil samples in Experiment 1, but there were no interactions among main effects (Table 1). Seed meal extracts from *B. napus* and *S. alba* suppressed the number of nematodes from soil in comparison to the nontreated control (42 and 21 J2/100 cm^3 soil for *B. napus* and *S. alba*, respectively, vs. 55 J2/100 cm^3 for the control). Treatment with *B. juncea* most effectively lowered nematode numbers (3 J2/100 cm^3). Both exposure time and seed meal concentration resulted in decreases in the number of nematodes recovered from the soil as the lowest numbers recovered at the highest concentration and longest exposure times (data not shown).

In Experiment 2, seed meal type and concentration, but not exposure time, affected nematode recovery. Also there were significant interactions in seed meal type × exposure time, concentration × exposure time, and the three-way interaction (Table 1). Most seed meal extract treatments resulted in reduced *M. graminis* J2 compared to the nontreated control, except at a few instances such as 25 g/ml seed meal concentration at 7 and 10 days exposure (Table 2). Soils treated with *B. juncea* seed meal extract consistently resulted in greater reduction of *M. graminis* J2 compared to soils treated with the other *Brassica* seed meal extracts and the nontreated control (Table 2). As the exposure days and seed meal extract concentration increased, the *M. graminis* J2 numbers decreased.

Field evaluation

A field trial was carried out at the Texas A&M University Turfgrass Center on a 'TifEagle' bermudagrass putting green from November 2012 to November 2013. Each plot (1.2 × 1.2 m) was arranged in a completely randomized block design with four replicates. Respective seed meal types were weighed, and 120, 90, 60, or 30 g was placed in a flask filled with dH_2O up to 1 L. The flask was placed on a shaker for 30 min to mix thoroughly, then the suspension was filtered using a piece of fusible cloth. Filtered seed meal extracts of *S. alba*, *B. juncea*, or *B. napus* at varying concentrations (120, 90, 60, 30, or 0 g seed meal/L) were applied once per month at a rate of 40 ml/m^2 except during winter dormancy between December 2012 and

Table 1. Analysis of Variance (ANOVA) of the effect of brassicaceous seed meal concentration and exposure time on *Meloidogyne graminis* in soil in laboratory experiments.

	Experiment 1			Experiment 2	
Source of variance	DF	MS	*P* value	MS	*P* value
Seed meal type (S)	2	193.4	<0.0001	162	<0.0001
Concentration (C)	3	32.7	0.0342	130.8	<0.0001
Exposure time (T)	3	48.1	0.0145	0.97	0.8753
S x C	6	1.2	0.9953	9.39	0.2681
S x T	4	6.28	0.6866	41.5	0.0003
C x T	6	5.9	0.7823	26.4	0.0022
S x C x T	12	18.36	0.0810	25.6	0.0001

Table 2. Number of *Meloidogyne graminis* J2 in 100 cm^3 affected by *Brassica* seed meal type, application concentration and exposure time on the soil associated with bermudagrass in Experiment 2 of the laboratory assay.

	3 days				7 days				10 days			
	Concentration (seed meal g/L)				Concentration (seed meal g/L)				Concentration (seed meal g/L)			
	25	50	75	100	25	50	75	100	25	50	75	100
Seed meal type	*M. graminis* J2 in 100 cm^3											
Control	11 a[z]	11 a	11 a	11 a	7.8 a	7.8 a	7.8 a	7.8 a	8.4 b	8.4 a	8.4 a	8.4 a
Brassica napus	5.5 b	4.1 b	1.9c	0.7 c	6.1 a	4.9 b	5.2 b	3.9 b	11.2 a	5.4 b	1.7 b	0 b
Sinapis alba	2.9 c	5.1 b	6.9 b	4.9 b	4.2 b	3.1 b	2.2 c	1.2 c	10.2 a	6.6 b	2.9 b	0 b
Brassica juncea	0 c	1.4 c	0 d	0 c	9.7 a	0 c	0.5 c	0 c	1 c	0 c	0 c	0 b

[z]Means having the same letter within a column do not differ significantly from each other.

Table 3. Analysis of Variance (ANOVA) for the effects of brassicaceous seed meal type, concentration, and sampling time on *Meloidogyne graminis* J2 and turfgrass quality in the bermudagrass field plots.

	Number of J2			Turfgrass quality		
Source of variance	DF	MS	*P* value	DF	MS	*P* value
Seed meal type (S)	2	7.4	0.3009	2	2.6	0.0017
Concentration (C)	4	44.3	0.0001	4	1.6	0.0074
Time (T)	7	968.6	<0.0001	7	5.6	<0.0001
S x C	6	10.2	0.1294	6	1.42	0.0019
S x T	14	3.74	0.8590	14	0.37	0.5344
C x T	21	5.84	0.5260	21	0.27	0.8530
S x C x T	42	10.1	0.0084	42	0.26	0.9453

March 2013. The treatment solutions were delivered at 275.8 kPa using a carbon dioxide boom sprayer equipped with two TeeJet 8002 nozzles (TeeJet Technologies, Springfield, IL) at 300 ml/m^2 water volume. Plots were irrigated up to field capacity immediately after seed meal application. Soil and turfgrass composite samples were collected from each plot with a soil probe (2.5 cm in diameter and 6-cm deep) every two weeks after seed meal extract application. Each composite sample consisted of 6 soil cores. *Meloidogyne graminis* J2 were extracted and counted from a 100 cm^3 sample as described above. Turfgrass quality was evaluated monthly using a 1 to 9 scale with a range of 6 = acceptable and 9 = the best possible (Krans and Morris, 2007).

The collected data were subjected to ANOVA using SAS. Second-stage juvenile (J2) counts were square-root transformed before analysis. Differences between treatment means were examined using Fisher's LSD test ($P < 0.05$). Three-way interaction (seed meal type × concentration × sampling time) was significant for the *M. graminis* J2 found in the field plots (Table 3). Mean number of *M. graminis* J2 in the plots gradually decreased from December 2012, reached to the lowest number in August and September 2013, increased thereafter, and reached to the highest number in November 2013 (Fig. 1). Whilst there was no clear trend on the impact of the seed meal type × concentration × sampling time interaction on *M. graminis* J2, *S. alba* and *B. juncea* consistently outperformed the control.

The main effects of seed meal type, application concentration, and rating time all affected turfgrass quality (Table 3). The interaction between seed meal type and concentration was also significant for turfgrass quality. Average turfgrass quality in all plots was above the acceptable level (> 6) during the period of the study regardless of seed meal extract type and concentration (Table 4). Increased concentration of seed meal extracts affected turfgrass quality differently depending on seed meal type. High concentrations (60 to 120 g seed meal/L) of *S. alba* and *B. juncea* were not phytotoxic on the turfgrass while the low concentration (30 g seed meal/L) of *B. napus* and *B. juncea* lowered turfgrass quality.

Our laboratory assay and field trial demonstrate that certain brassicaceous seed meal extracts may be useful in suppressing *M. graminis* in bermudagrass with limited phytotoxicity, and may be useful for managing root-knot nematodes (*Meloidogyne* spp.) in established turfgrass systems as previously shown in other field crops (Mojtahedi *et al.*, 1991, Lazzeri *et al.*, 2004; Rahman and Somers, 2005; Henderson *et al.*, 2009; Zasada *et al.*, 2009). Higher concentration of allyl glucosinolates in *B. juncea*, compared to those in *S. alba* and *B. napus*, could explain why nematicidal effectiveness of *B. juncea* was higher than the other seed meals tested in this study. A primary glucosinolate in *B. juncea* is allyl isothiocyanate, accounting for over 99% of total glucosinolates in defatted seed meal (Handiseni *et al.*, 2011). These compounds, particularly 2-propenyl isothiocyanate, which is generated from enzymatic degradation of 2-propenyl glucosinolate by myrosinases, are known to be highly toxic to nematodes (Lazzeri *et al.*, 1993; Donkin *et al.*, 1995; Buskov *et al.*, 2002; Zasada and Ferris, 2003). The more positive performance of *B. juncea* seed meal as a nematicide in our laboratory and field experiments agrees with previous findings with plant-pathogenic nematodes in various crops (Mazzola *et al.*, 2009; Zasada *et al.*, 2009; Rahman and Somers, 2005).

Turfgrass quality showed no immediate correlation with the fluctuation of *M. graminis* J2 in bermudagrass as has been previously described (Cromwell *et al.*, 2014). However, changes in turfgrass quality throughout the field evaluation were associated with seed meal types and application rate, likely due to influences on nematode populations and phytotoxicity to the turfgrass. The lowest turfgrass quality was observed in the plots treated with low concentrations (30 or 60 g seed meal/L) of *B. napus*, which contains lower levels of allyl glucosinolate than *B. juncea* (Mazzola *et al.*, 2007; Mazzola *et al.*, 2009; Zasada *et al.*, 2009). Low turfgrass quality ratings on the plots treated with *B. napus* are probably due to low nematicidal activities and consequent stress from high number of nematodes. Low turfgrass quality in the plots treated with *S. alba* seed meal extracts may be associated with phytotoxicity by certain hydrolytic products of glucosinolates that these brassicaceous seed meals contain (Earlywine *et al.*, 2010). *Sinapis alba* seed meal has been reported

Table 4. Effects of seed meal extract type and application concentration on turfgrass quality in the bermudagrass field plots. The highest turfgrass quality was observed in September, but the lowest quality in December and August (data not shown).

	Concentration (seed meal g/L)			
Seed meal type	30	60	90	120
Control	6.7 a[z]	6.7 a	6.7 ab	6.7 a
Brassica napus	6 c	6.2 b	6.5 b	6.8 a
Sinapis alba	6.7 a	6.5 a	6.5 b	6.6 a
Brassica juncea	6.6 b	6.6 a	6.8 a	6.6 a

[z]Means having the same letter within a column do not differ significantly from each other ($P < 0.05$). The scale of the turfgrass quality is 1 to 9 with a range of 6 = acceptable and 9 = the best possible.

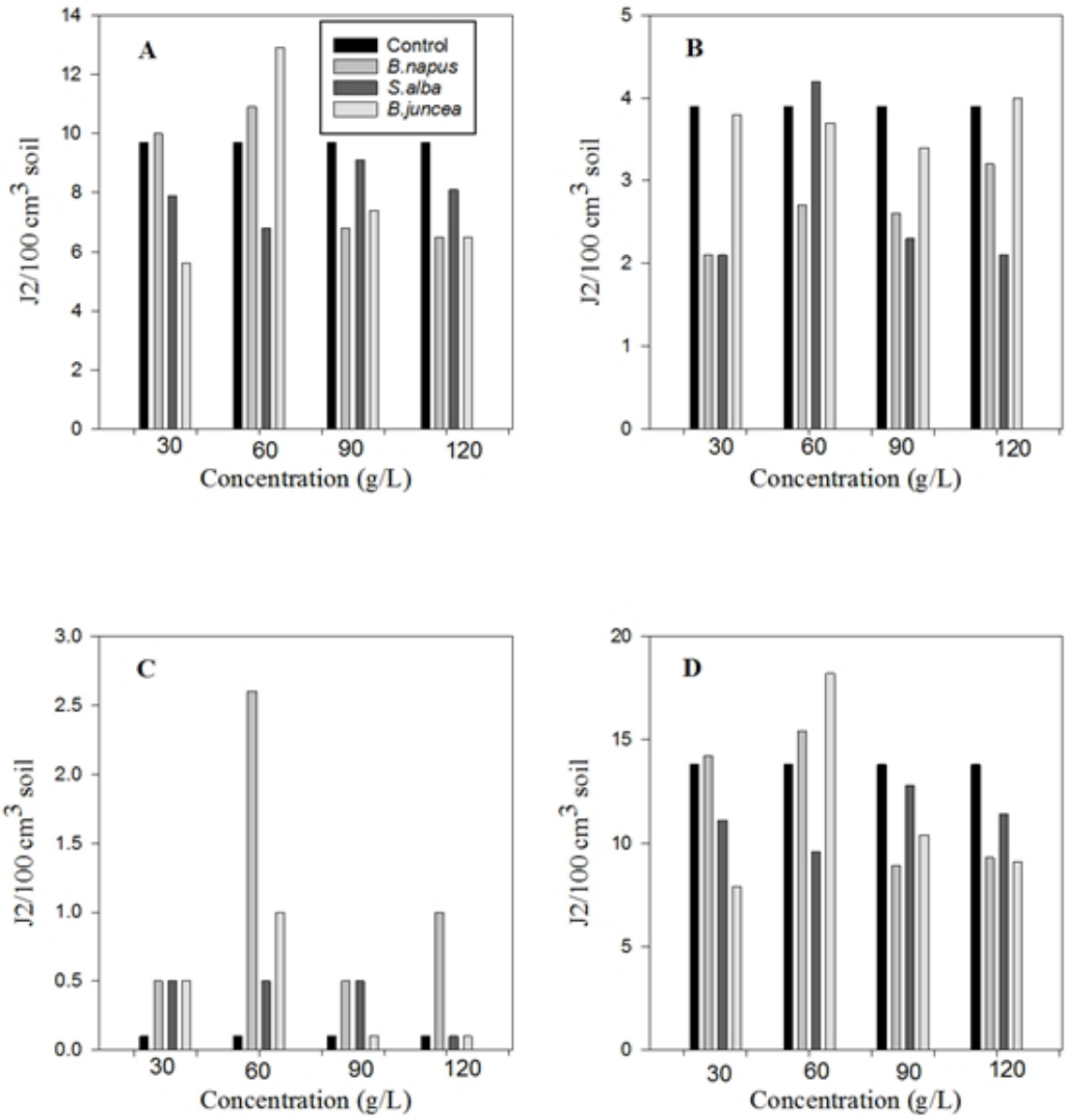

Fig. 1. *Meloidogyne graminis* J2/100 cm^3 soil in bermudagrass plots treated with seed meal extracts in December 2012 (A), and June (B), September (C), and November (D) 2013.

to have higher herbicidal effect on grassy weeds than *B. juncea* (Handiseni *et al.*, 2011). In the case of *B. juncea*, high concentrations of seed meal extracts (90 or 120 g seed meal/L) did not compromise turfgrass quality.

This study showed that use of *B. juncea* seed meal as a soil treatment has potential as a biological alternative to conventional synthetic nematicides in bermudagrass. Season-long application regiments including various application dosages, times of application, and conditions of reducing phytotoxicity need to be further examined so as to achieve full potential of using brassicaceous seed meals for management of pathogenic nematodes in subtropical turfgrasses.

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