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Efficacy of Methionine Against Ectoparasitic Nematodes on Golf Course Turf

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Abstract: Plant-parasitic nematodes are important pathogens of intensely-managed turf used on golf courses. Two of these nematodes that are common in the southeastern US are *Belonolaimus longicaudatus* and *Mesocriconema ornata*. Currently, there is a lack of effective treatments that can be used to manage these important pests. Turfgrass field trials evaluated DL-methionine as a turfgrass nematicide against *B. longicaudatus* and *M. ornata*. One trial was on a bermudagrass putting green, the other was on zoysiagrass maintained under putting-green conditions. Two rates of methionine, 1120 kg/ha in a single application, and 112 kg/ha applied twice four weeks apart, were compared with untreated control and fenamiphos treatments. Measurements collected included soil nematode counts, turf density, and root lengths. In both trials, 1120 kg/ha of methionine reduced numbers of both nematode species ($P \leq 0.1$), and 112 kg/ha of methionine reduced numbers of both nematode species after two applications. Bermudagrass turf density responded favorably to both methionine rates and root lengths were improved by the 1120 kg/ha rate. Zoysiagrass showed short-term phytotoxicity to methionine, but quickly recovered and treated plots were improved compared to the untreated controls by the end of the trial. These trials indicated that methionine has potential for development as a turfgrass nematicide, but further research is needed to determine how it can best be used.

Key words: Belonolaimus longicaudatus, bermudagrass, *Cynodon*, *Mesocriconema ornata*, nematode management, ring nematode, sting nematode, turfgrass, *Zoysia*, zoysiagrass.

Plant-parasitic nematodes are very important pests of turfgrasses, particularly in the southeastern United States (Crow, 2007). Sting nematode (*Belonolaimus longicaudatus*) is considered the most damaging nematode to most grass species in this region. Other ectoparasitic nematodes that commonly affect turfgrasses in the southeast include various Criconematoidea including *Mesocriconema ornata*.

The organophosphate nematicide fenamiphos has been the most commonly used nematicide on golf courses for many years. However, fenamiphos is no longer being manufactured or sold in the United States. 1,3-Dichloropropene (Curfew Soil Fumigant, Dow Agrosciences, Indianapolis, IN) is currently registered for nematode management on turf in the coastal states from Texas to North Carolina. Although this nematicide is very effective against *B. longicaudatus* and certain other plant-parasitic nematodes (Crow et al., 2003; 2005), it has several drawbacks including: application cost, invasive injection methods, buffer zone, reentry time, and geographic restrictions. There are other nematode management products marketed for turfgrass use, but most of those evaluated have not shown consistent efficacy (Crow 2005). Currently, there is a great need for turfgrass nematicides that are: i) effective, ii) not harmful to humans and animals, and iii) economical.

Methionine is an essential amino acid that is produced in plants solely as L-methionine. However, when produced synthetically it occurs in equal parts of the L and D chiral forms. DL-Methionine is produced commercially and currently its primary industrial use is as an animal feed supplement. Methionine is classified by the United States Environmental Protection Agency (EPA) as a List 4B "Other ingredients for which EPA has sufficient information to reasonably conclude that the current use pattern in pesticide products will not adversely affect public health or the environment" (Anonymous, 2004).

It had been suggested that amino acid "antimetabolites" and chemical analogues of methionine, notably the D-methionine stereoisomer or DL-methionine racemate, would interfere with essential intracellular metabolic pathways and enzymes in nematodes. Overman and Woltz (1962) postulated that nematodes exposed to antimetabolites such as methionine would be unable to feed, and in pot experiments found that DL-methionine reduced populations of *Paratrichodorus minor* in soil and galling of tomato roots by *Meloidogyne incognita*. Similarly, DL-methionine reduced the number of *Heterodera avenae* females in wheat (Prasad and Webster, 1967) and *Globodera rostochiensis* on potato (Evans and Trudgill, 1971). More recently, DL-methionine was shown to suppress hatching of eggs and mobility of *M. incognita* J2 in laboratory experiments (Talavera and Mizukubo, 2005).

The greatest limitation to the commercial use of methionine as a nematicide in the past has been that the high rates required (hundreds of kg/ha) have been considered too costly to be practical. However, large amounts of DL-methionine are currently produced for use in animal feed. With increased production, economies of scale have led to comparatively lower prices today than in the past. Also, the high value of certain recreation turfgrasses allows for pesticide costs that are higher than practical for many row crops. In 2009, an application of Curfew Soil Fumigant for nematode management on putting greens was almost $9700 US/ha. The objective of this research was to evaluate the effectiveness of DL-methionine as a nematicide for *B. longicaudatus*

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and *M. ornata* on golf course turf. This study focused on the nematicidal potential and not the economics of DL-methionine based nematicide.

Materials and Methods

In 2008, two field trials were conducted to evaluate DL-methionine as a nematicide for management of *B. longicaudatus* on turfgrasses. One site was 'PristineFlora' zoysia (*Zoysia japonica* × *Z. tenuifolia*) growing at the Plant Science Research Unit in Citra, Florida, USA that was managed under putting green conditions. The other site was a 'Tifdwarf' bermudagrass (*Cynodon dactylon* × *C. transvaalensis*) nursery green at the Eagle Ridge Golf Club in Summerfield, Florida, USA. Both of these sites were naturally infested with damaging numbers of *B. longicaudatus*, and also with *M. ornata*. Soil at both sites was > 95% sand and < 2% OM.

The experimental treatments evaluated were crystallized DL-methionine produced as an animal feed supplement (Evonik Degussa, Theodore, Alabama, USA) applied at two rates. The methionine rates evaluated were 224 kg/ha applied in two applications of 112 kg/ha each at four week intervals, and a single application of 1120 kg/ha. These applications were compared with untreated controls and the industry standard nematicide fenamiphos (Nemacur 10G, Bayer CropScience, Research Triangle Park, North Carolina, USA) at 1.12 kg a.i./ha. The experimental design was randomized block with 5 replications. Plots were 1.5 m^2 with 0.6 m untreated borders between plots. Plots were assigned to blocks based on initial *B. longicaudatus* population densities in order to minimize initial treatment variance. Data collected included plant-parasitic nematode population density, turf density, and turf root length.

Fenamiphos and methionine treatments were applied on 2 July. The second application of the lower methionine rate was applied on 30 July. Methionine and fenamiphos treatments were applied topically using a shaker apparatus. Following applications, the entire experimental area was irrigated with 0.65 cm of water using the pre-existing commercial irrigation system. Subsequently, the turf was maintained by the facility staff using their standard maintenance practices. Turf at both locations was maintained at 0.6 mm height.

Nematode samples were collected before treatment on 28 June, and after treatment on 17 July and 15 August. Nematode samples consisted of 9 cores 10-cm-deep × 1.9-cm-diam. from each plot. The top layer of the plugs consisting of leaves, stolons, and rhizomes, along with associated organic thatch layer whch was discarded and the remaining soil fraction was thoroughly mixed. Nematodes were extracted from a 100 cm^3 subsample by centrifugal-flotation (Jenkins, 1964). The plant-parasitic nematodes extracted were then identified and counted.

Turf density was evaluated before treatment on 2 July, and after treatment on 17 July, 30 July, and 15 August. Turf density was measured by taking a digital photo of each plot using a tripod-mounted camera. Digital image software (SigmaScan Pro 5, SPSS Inc., Chicago, Illinois, USA) was used to determine the percentages of pixels in each image that were designated green (Richardson et al., 2001); these percentages are termed as "turf density".

Root samples were collected on 15 August. Root lengths were measured from two cores 3.8-cm-diam. × 15.25-cm-deep cores taken from each plot. These two cores had a combined turf surface area of 22.8 cm^2 and soil volume of 350 cm^3. The thatch layer was removed from the cores and the remaining portions of the two cores from each plot were combined. The soil was washed across a 1 mm-mesh sieve to catch the roots and remove most of the soil. The sieve was then placed in a tray of water and roots were picked out manually for analysis. Roots collected were placed into a clear plastic tray and scanned with an desktop scanner (Epson perfection 4990, Epson America, Long Beach, California, USA) to obtain bitmap images of the root system. The bitmap images were then analyzed using WinRHIZO (Regent Instruments, Quebec, Canada) software. This program measures root lengths in millimeters.

For plant-parasitic nematode data, analysis of covariance (ANCOVA) with the initial measurement as the covariate was used to evaluate effectiveness of treatments; this takes into account how the measurement changes over time compared with the untreated control. ANCOVA does not allow for direct comparisons among experimental treatments, rather, it allows for determination of which treatments were different from the untreated control. ANCOVA also was used to evaluate turf density data. Root length data were compared using the contrast procedure with each treatment contrasted with the untreated control. Differences indicated by ANCOVA or the contrast procedure are shown according to the *P*-value generated ($P = 0.1, 0.05, 0.01$), where no differences are indicated $P > 0.1$.

Results

In both trials, fenamiphos and 1120 kg/ha of DL-methionine reduced numbers of *B. longicaudatus* in soil ($P \leq 0.05$) relative to the untreated controls 2 weeks after the initial applications (Table 1). However, in the zoysia trial numbers of *B. longicaudatus* in the fenamiphos treatment were not different ($P \geq 0.1$) from the untreated control 6 weeks after treatment whereas with 1120 kg/ha of DL-methionine they were different ($P \leq 0.05$). Two applications of DL-methionine at 112 kg/ha were required to reduce numbers of *B. longicaudatus* in soil relative to the untreated control ($P \leq 0.1$) in both trials.

Only DL-methionine at 1120 kg/ha reduced ($P \leq 0.1$) numbers of *M. ornata* on bermudagrass relative to the untreated control, and only 2 weeks after

TABLE 1. Effects of DL-methionine treatments and fenamiphos on population densities of *Belonolaimus longicaudatus*/100 cm^3 over time on bermudagrass and zoysia in separate field experiments. Nematode samples were collected before treatment (Pi), 2 weeks after the first treatment applications (Pm), and 6 weeks after the first treatment applications (Pf).

	Bermudagrass			Zoysia		
Treatment	Pi	Pm	Pf	Pi	Pm	Pf
Untreated	110	18	15	99	35	60
Fenamiphos	110	7**	3***	99	17*	40
Methionine 1120 kg/ha	112	< 1***	2***	98	6***	27**
Methionine 224 kg/ha	110	16	9*	100	31	36*

Data are means of 5 replications.
*,**,*** Different from untreated according to analysis of covariance, $P = 0.1$, 0.05, 0.01, respectively.

TABLE 2. Effects of DL-methionine treatments and fenamiphos on population densities of *Mesocriconema ornata*/100 cm^3 on bermudagrass and zoysia in separate field experiments. Nematode samples were collected before treatment (Pi), 2 weeks after the first treatment applications (Pm), and 6 weeks after the first treatment applications (Pf).

	Date					
	Bermudagrass			Zoysia		
Treatment	Pi	Pm	Pf	Pi	Pm	Pf
Untreated	76	50	60	545	768	356
Fenamiphos	89	71	99	100	446*	215***
Methionine 1120 kg/ha	117	30*	33	596	296***	104***
Methionine 224 kg/ha	62	41	54	406	573	159***

Data are means of 5 replications.
*,**,*** Different from untreated according to analysis of covariance, $P = 0.1$, 0.05, 0.01, respectively.

treatment (Table 2). On zoysia, both fenamiphos and DL-methionine at 1120 kg/ha reduced numbers of *M. ornata* 2 and 6 weeks after treatment ($P \leq 0.1$). Two applications of DL-methionine were required to reduce numbers of *M. ornata* relative to the untreated control ($P \leq 0.1$) on zoysia.

Turf density of bermudagrass was improved ($P \leq 0.05$) by all the treatments relative to the untreated control (Table 3). On zoysiagrass, the 1120 kg/ha rate of DL-methionine caused an initial reduction in turf density, but this was followed by an increase in turf density by the final observation date. Fenamiphos improved turf density 2 weeks after treatment, but not at subsequent evaluation dates. The lower rate of DL-methionine 2 and 4 weeks after the first application improved turf density, but had no affect on turf density following the second application. Root lengths of bermudagrass were improved by fenamiphos and DL-methionine at 1120 kg/ha, but did not improve root lengths of zoysiagrass. Two applications of DL-methionine decreased root lengths of zoysiagrass relative to the untreated control.

DISCUSSION

In these trials DL-methionine showed great potential as nematode management tool on turf. However, further research is required to determine how practical DL-methionine would be as a turfgrass nematicide. These studies should be viewed as an initial step to determine nematicide potential and identify other questions that need to be answered.

Our observed enhancement of bermudagrass growth by methionine can be attributed to pesticidal activity and plant regulatory effect. D- and L-methionine were shown to enhance root tip spiraling and root hair proliferation in *Brassica rapa* (Hasegawa et al., 2003). Methionine contains both nitrogen and sulfur. The nitrogen content of methionine is in excess of 9%. Therefore, at the high rate of methionine used in these experiments about 100 kg/ha of N was applied. This is about 2/3 of the yearly N fertility recommended for growing most turf in Florida. At the 1120 kg/ha rate of DL-methionine the bermudagrass experienced rapid greening (data not shown) indicative of a nitrogen fertility response rather than a nematode response. Over a period of several weeks the turf density increased, which is more characteristic of a nematode response (Luc et al., 2007). Similarly, the initial phytotoxicity to zoysiagrass from the high-rate DL-methionine treatment can be explained by "nitrogen burn." After this initial phytotoxicity, the turf density of zoysia increased similarly to that of bermudagrass. If high rates of DL-methionine are to be applied for nematode management the fertilizer affects should be considered. Perhaps N fertilizer application should be suspended following a methionine treatment or substituted with DL-methionine. Also, excessive nitrogen and resulting problems may limit the amount of DL-methionine that can be applied to established turf in a single application. Further studies are needed to better evaluate these fertilizer effects.

A single application of 1120 kg/ha was effective against the nematodes evaluated, but a single application of 112 kg/ha was not. However, after two applications of 112 kg/ha, populations of *B. longicaudatus* were reduced compared to the untreated controls in both trials. Further studies that include multiple rates and application frequencies are required to determine practical application use patterns. Further research also should explore the efficacy of additional rates to determine if there are rates lower than 1120 g/ha that would give acceptable results in a single application. Also, because golf course turf is intensively managed sequential applications of lower rates would be practical for this commodity. The long-term effects on turf and nematodes should be explored. Because the DL-methionine used in these studies was largely non-formulated methionine crystals applied topically, more research is needed

TABLE 3. Effects of DL-methionine treatments and fenamiphos on turf density (0-100%) of bermudagrass and zoysia in separate field experiments.

	Date							
	Bermudagrass				Zoysia			
Treatment	July 2	July 17	July 30	Aug 15	July 2	July 17	July 30	Aug 15
Untreated	65	56	78	61	94	78	88	92
Fenamiphos	78	72***	92***	69**	91	86***	87	90
Methionine 1120 kg/ha	78	60**	93***	81***	94	38***	91	98***
Methionine 224 kg/ha	66	61**	87***	73***	94	85**	95***	92

Data are means of 5 replications.
*,**,*** Different from untreated according to analysis of covariance, $P = 0.1, 0.05, 0.01$, respectively.

to evaluate other formulations and/or application technologies. This might allow adequate nematode control using lower amounts of active ingredient.

Future research also should evaluate performance on additional nematode species and grasses. While DL-methionine was effective against the ectoparasites evaluated in these studies, there also are endoparasitic nematodes that are important on turf, i.e., root-knot (*Meloidogyne* spp.) and lance (*Hoplolaimus* spp.) nematodes. Additional studies should be conducted to evaluate the efficacy of DL-methionine against endoparasites on established turf.

Many of the reduced-risk alternatives to organophosphate nematicides on turfgrasses that have been evaluated by nematologists have turned out to have little or no efficacy in the field (Crow, 2005). DL-Methionine is one of the few that appears to be safe yet effective. Clearly, a great deal more research is needed for development of methionine-based nematicides. Only after the effective use rates and formulation are determined can the economic viability of a methionine-based nematicide be addressed. However, much of this research will be initiated within the next year and should yield the information required to develop this exciting nematode management technology.

TABLE 4. Effects of DL-methionine and fenamiphos on root lengths (cm of root/350 cm^3 of soil) of bermudagrass and zoysia in separate field experiments.

Treatment	Bermudagrass	Zoysia
Untreated	359	591
Fenamiphos	485**	459
Methionine 1120 kg/ha	503**	510
Methionine 224 kg/ha	358	446**

*,**,*** Different from untreated according to analysis of covariance, $P = 0.1, 0.05, 0.01$, respectively.

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