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Field Efficacy of Furfural as a Nematicide on Turf

W. T. Crow, J. E. Luc

Abstract: A commercial formulation of furfural was recently launched in the United States as a turfgrass nematicide. Three field trials evaluated efficacy of this commercial formulation on dwarf bermudagrass putting greens infested primarily with *Belonolaimus longicaudatus*, *Meloidogyne graminis*, or both these nematodes, and in some cases with *Mesocriconema ornatum* or *Helicotylenchus pseudorobustus*. In all these trials, furfural improved turf health but did not reduce population densities of *B. longicaudatus*, *M. graminis*, or the other plant-parasitic nematodes present. In two additional field trials, efficacy of furfural at increasing depths in the soil profile (0 to 5 cm, 5 to 10 cm, and 10 to 15 cm) against *B. longicaudatus* on bermudagrass was evaluated. Reduction in population density of *B. longicaudatus* was observed in furfural-treated plots for depths below 5 cm on several dates during both trials. However, no differences in population densities of *B. longicaudatus* were observed between the furfural-treated plots and the untreated control for soil depth of 0 to 5 cm during either trial. These results indicate that furfural applications can improve health of nematode-infested turf and can reduce population density of plant-parasitic nematodes in turf systems. Although the degree to which turf improvement is directly caused by nematicidal effects is still unclear, furfural does appear to be a useful nematode management tool for turf.

Key words: Belonolaimus longicaudatus, bermudagrass, *Cynodon dactylon*, furfural, *Helicotylenchus pseudorobustus*, management, *Meloidogyne graminis*, *Mesocriconema ornatum*, ring nematode, root-knot nematode, spiral nematode, sting nematode, turfgrass.

Bermudagrass (*Cynodon* ssp.) is a commonly grown, warm-season turfgrass that is susceptible to damage from many species of plant-parasitic nematodes, particularly in subtropical areas of the southeastern United States (Crow, 2007). Among the most important nematode pathogens of bermudagrass turf in Florida are sting (*Belonolaimus longicaudatus*) and root-knot (*Meloidogyne graminis*) nematodes, and to a lesser degree ring (*Mesocriconema ornatum*) and spiral (*Helicotylenchus pseudorobustus*) nematodes (Crow, 2005). Nematode damage leads to poor turf quality, reduced drought tolerance, reduced fertilizer uptake, increased weed problems, and reduced playability of golf and sports turf. The loss of fenamiphos has left turf managers with limited options for nematode control in turf systems (Crow, 2007). Currently 1,3-dichloropropene (1,3-D) is the industry standard turf nematicide used in the southeastern United States (Crow et al., 2003; 2005). Although effective, 1,3-D is restricted to a single application per year, and it has reentry, buffer, and geological restrictions that limit its use. There is great need for additional nematode management tools for turfgrass managers to use in integrated pest management programs. One such promising tool is furfural, a formulation of which, Multiguard Protect EC, was launched as a turfgrass nematicide in the United States in 2011.

Furfural (2-furancarboxaldehyde) is a naturally occurring aromatic aldehyde present in some essential oils, foods, and cosmetic products. It is industrially prepared from organic agricultural residues, such as cereal straw, brans, and sugarcane bagasse via hydrolysis (Burger, 2005). The nematicidal potential of furfural was recognized in the early 1990s (Walter and Rodriguez-Kabana 1992; Rodriguez-Kabana et al., 1993). Two furfural nematicides, CropGuard® and Protect®, are currently being used in parts of Africa, and Multi-Guard Protect was approved in the United States in 2011. Luc and Crow (2013) conducted greenhouse trials with the Multiguard Protect formulation, and they demonstrated that it was efficacious against *B. longicaudatus*. The material also showed potential for use as a turfgrass nematicide. The objective of this study was to determine if furfural is an effective turfgrass nematicide in field situations. The criteria used to make this determination are (i) improvement in turf health, and/or (ii) reduction in population density of target nematodes.

Materials and Methods

General field trials: Three field trials were conducted to evaluate a commercial formulation of furfural (Multiguard Protect EC, 90% a.i.; Agriguard, Cranford, NJ) as a turfgrass nematicide. These trials were all conducted on dwarf bermudagrass grown under putting green conditions at the University of Florida Plant Science Research and Education Unit (PSREU) at Citra, FL. In these trials, population density of plant-parasitic nematodes and turf percent green cover in treated plots to untreated plots were compared. Plots were 1.5 m^2 with 0.6-m untreated borders between adjacent plots. The experimental design was a randomized block design with five replications of two treatments. In each trial, three applications of furfural were made at either 2- or 4-wk intervals at the maximum labeled rate of 75 liters formulation/ha (67.5 liters furfural/ha). The furfural formulation was mixed in water and sprayed onto the research plots with a CO_2-powered backpack sprayer (Weed Systems, Hawthorne, FL). Immediately before and after each application, the trial sites were irrigated with 0.64 cm of water per label recommendations.

Nematode data was collected 2 wk before the first treatment application (Pi) and 2 wk after the third

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Professor and Postdoctoral Research Associate, respectively, Entomology and Nematology Department, University of Florida, Gainesville, FL 32611.
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E-mail: wtcr@ufl.edu
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application (Pf). Nematode samples consisted of nine 1.9-cm-diam. × 10-cm-deep cores from each plot. The top layer of the cores, consisting of leaves, stolons, and rhizomes, along with the associated organic thatch layer, 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 percent green cover data was collected six times at approximately 2-wk intervals beginning with the day of the initial treatment application. Turf percent green cover is the percentage of the plot surface covered by green turf and is a measurement of turf health. To determine turf percent green cover, a digital photo was taken of the center 1.0 m^2 of each plot. The percentage of the pixels in each photo that were "green" was determined using a macro developed by faculty at the University of Arkansas (Karcher and Richardson, 2005) for use with SigmaScan Pro 5 software (SPSS, Inc., Chicago). Percentage of the total pixels in the image that were green (hue 55 to 105, saturation 15 to 100) was the measure of turf percent green cover.

Nematode and turf green cover data were subjected to analysis of covariance using SAS 9.2 software (SAS Institute, Cary, NC) with the initial nematode population density as the covariate. The treated was compared with the untreated and the P-value for the comparison was used to determine treatment differences.

The Tifdwarf trial was conducted on 'Tifdwarf' bermudagrass naturally infested with damaging numbers of *M. graminis* and sting nematode *B. longicaudatus*. In this trial, treatments were applied at 4-wk intervals beginning 1 April 2011.

The Jonesdwarf trial was conducted on 'Jonesdwarf' bermudagrass naturally infested with damaging numbers of *B. longicaudatus* and moderate numbers of *M. ornatum*. In this trial, treatments were applied at 2-wk intervals beginning 28 February 2012.

The Champion trial was conducted on 'Champion' bermudagrass naturally infested with damaging numbers of *M. graminis* and moderate numbers of *H. pseudorobustus*. In this trial, treatments were applied at 2-wk intervals beginning 29 March 2013.

Sampling depth trials: In fall 2011, two trials were conducted to determine the effects of application amount on the efficacy of furfural against *B. longicaudatus* at different soil depths. The Palatka trial was conducted at the Palatka Golf Club at Palatka, FL, on three 'Tifdwarf' bermudagrass tees, whereas the Celebration trial was conducted at the PSREU at Citra, FL, on a 'Celebration' bermudagrass tee. Both sites were naturally infested with *B. longicaudatus*. Plots were 28 m^2 with 0.6-m untreated borders between plots. The experimental design was a randomized complete block with five replications. Furfural treatments were applied using a commercial turf sprayer (Toro, Bloomington, MN) for the Palatka trial and a CO_2-powered sprayer for the Celebration trial. Treatments were (i) two doses of furfural at 67.5 liters/ha 1 hr apart (135 liters/ha total), each followed by 0.6 cm of irrigation; (ii) a single dose of furfural at 67.5 liters/ha preceded and followed by 0.6 cm of irrigation; and (iii) untreated control irrigated twice with 0.6 cm of irrigation 1 hr apart. Treatments were applied three times at 2-wk intervals. Nematode samples consisted of eight soil cores (4-cm-diam. × 20-cm-depth) taken from each plot that were then sectioned into soil depths of 0 to 5 cm, 5 to 10 cm, and 10 to 15 cm after removing the turf and thatch layers. Nematode samples were collected 1 wk before the initial application, and then 2 wk after each treatment application. Nematodes were extracted from 100-cm^3 subsamples by centrifugal-flotation and counted. Nematode data at each depth, and for all depths combined, were subjected to analysis of covariance with the initial nematode population density as the covariate. The treated was compared with the untreated and the P-value for the comparison was used to determine treatment differences.

Results

General field trials: Improvement in turf green cover ($P \leq 0.1$) resulted from furfural treatment in all three trials (Table 1). However, reduction in Pf of target plant-parasitic nematodes was not observed ($P > 0.1$) in any trial (Table 2). In the Champion trial, Pf of *M. graminis* and *H. pseudorobustus* were higher in furfural-treated plots than in untreated plots.

Sampling depth study: Population densities of *B. longicaudatus* were lower in furfural-treated plots than in untreated plots at several depths and dates during both trials (Table 3). There were no differences ($P > 0.1$)

Table 1. Effects of treatment with furfural on percent green cover (0–100) of bermudagrass in three field trials. Furfural was applied three times at 67.5 liters/ha each at either 4-wk (Tifdwarf) or 2-wk (Jonesdwarf and Champion) intervals. Percent green cover was measured six times at approximately 2-wk intervals beginning on the day of the initial treatment application. Data are means of five replications.

	Tifdwarf trial						Jonesdwarf trial						Champion trial					
Treatment	1 Apr	15 Apr	29 Apr	13 May	26 May	9 Jun	28 Feb	13 Mar	26 Mar	10 Apr	24 Apr	8 May	29 Mar	10 Apr	24 Apr	13 May	22 May	5 Jun
Untreated	11	7	27	35	73	60	31	12	15	13	32	70	8	40	86	55	79	88
Furfural	12	11	58*	51	88	73*	40	22**	35***	24***	41	70	10	41	89	72***	87**	90**

Treated different from untreated according to analysis of covariance, * $P \leq 0.10$, ** $P \leq 0.05$, *** $P \leq 0.01$.

TABLE 2. Effects of treatment with furfural on population density of plant-parasitic nematodes/100 cm^3 of soil on bermudagrass in three field trials. Furfural was applied three times at 67.5 liters/ha each at either 4-wk (Tifdwarf) or 2-wk (Jonesdwarf and Champion) intervals. Nematode population density was assayed 2 wk before the initial application (Pi) and 2 wk after the third application (Pf). Data are means of five replications.

	Tifdwarf trial				Jonesdwarf trial				Champion trial			
Treatment	R-k Pi	R-k Pf	Sting Pi	Sting Pf	Sting Pi	Sting Pf	Ring Pi	Ring Pf	R-k Pi	R-k Pf	Spiral Pi	Spiral Pf
Untreated	282[b]	226	9	23	36	17	94	123	37	222	20	39
Furfural	279	182	12	12	37	27	113	207	22	387*	57	126*

R-k = *Meloidogyne graminis* J2, Sting = *Belonolaimus longicaudatus*, Ring = *Mesocriconema ornatum*, Spiral = *Helicotylenchus pseudorobustus*. Treated different from untreated according to analysis of covariance, * $P \leq 0.10$.

among treatments in the top 5 cm of soil profile in either trial. At 5-cm-deep and greater, *B. longicaudatus* population density reductions ($P \leq 0.1$) were observed from 135 liters/ha furfural after one, two, and three applications in the Palatka trial, and after two and three applications in the Celebration trial. At 5-cm-deep and greater, *B. longicaudatus* population density reductions ($P \leq 0.1$) were observed from 67.5 liters/ha furfural after two and three applications in the Palatka trial and after two applications in the Celebration trial. For combined depths, reduction in population ($P \leq 0.1$) density of *B. longicaudatus* only occurred for the 135 liter/ha rate in the Palatka trial.

DISCUSSION

The results from the general efficacy trials indicated consistent turf improvement from applications of furfural to nematode-infested bermudagrass turf. However, this turf benefit was difficult to relate to nematode effects because no nematode reductions were observed. Therefore, one of the two efficacy criteria, that of improvement in turf health, was met. However, because of the lack of nematode reductions observed, the sampling depth study was conducted to gain more in-depth information on nematode effects.

Results from the sampling depth study showed that repeat application of furfural at the current maximum labeled rate reduced population density of *B. longicaudatus*, but only at soil depths of 5 cm or greater. Therefore, the second efficacy criteria, reduction in nematode population density, was met by furfural. When collecting nematode samples as in the general efficacy trials or for typical turf diagnostic purposes, cores are generally collected to a depth of 10 cm. In those samples half of the sample came from the portion of soil profile where furfural was not effective (0 to 5 cm), a fact that diluted the overall effect. This explained why significant reductions in nematode population density were rarely observed without conducting more detailed depth

TABLE 3. Effects of furfural on population density of *Belonolaimus longicaudatus*/100 cm^3 of soil at different soil depths in bermudagrass field trials at two locations. Furfural was applied three times at 2-wk intervals with 0.0, 67.5, or 135 liters/ha at each application. Nematode samples were collected 2 wk before the initial application, and 2 wk after each of the three applications. Data are means of five replications.

		Treatments					
		Palatka trial			Celebration trial		
Number of applications	Depth (cm)	0.0 liter/ha	67.5 liter/ha	135 liter/ha	0.0 liter/ha	67.5 liter/ha	135 liter/ha
0	0 to 5	50	45	52	16	29	18
	5 to 10	43	40	33	76	80	73
	10 to 15	41	48	50	80	67	82
	0 to 15	48	44	42	57	59	57
1	0 to 5	62	116	83	22	40	26
	5 to 10	74	63	37**	119	138	71
	10 to 15	74	74	44*	147	116	77
	0 to 15	70	84	54	96	98	58
2	0 to 5	41	46	26	20	10	30
	5 to 10	51	30**	22**	111	57***	47***
	10 to 15	60	25**	27**	101	58	66
	0 to 15	51	34	25**	78	42**	48*
3	0 to 5	19	29	26	12	15	20
	5 to 10	30	20*	14**	74	66	42**
	10 to 15	37	23*	16**	63	58	46
	0 to 15	29	24	18**	50	46	36

Treatment different from untreated at a specific depth and time during each trail, * $P \leq 0.10$, ** $P \leq 0.05$, *** $P \leq 0.01$.

sampling. Because the sampling depth trial evaluated only *B. longicaudatus*, similar trials evaluating effects on other plant-parasitic nematodes such as *M. graminis*, *M. ornatum*, and *H. pseudorobustus* should be conducted in the future to determine if similar effects occur for these species.

The reason for lack of efficacy in the top 5 cm of soil profile is unknown at this time, but two possibilities have been suggested. Unpublished data (G. L. Burger, Ilovo Sugar) indicated that furfural is rapidly biodegraded, particularly under aerobic conditions such as found in the top few cm of the turf soil profile. It could be that biodegradation in the top portion of the soil profile happened so quickly that the furfural did not have enough time to affect the nematodes. A second possible explanation was that furfural moves into the soil profile as a band with irrigation and was deposited below 5-cm-depth by irrigation or rainfall. Further studies are required to adequately explain this phenomenon.

The increase in population density of *M. graminis* second-stage juveniles (J2) following application of furfural in the Champion trial was not unexpected and has been observed in other, unpublished, trials (W. T. Crow, University of Florida). One hypothesis for this was that furfural, a solvent, may dissolve the gelatinous matrix surrounding *Meloidogyne* eggs and thereby stimulates rapid egg hatch and leads to a short-term increase in number of J2. Dissolution of *Meloidogyne javanica* egg masses by furfural has been reported previously (Steyn and Van Vuuren, 2006). On dwarf bermudagrass, *M. graminis* is known to be most abundant in the top 5 cm of soil profile (Laughlin and Williams, 1971), the depth where our results showed furfural to be ineffective. It should be noted that in other, ongoing, trials with ornamental crops we have observed reduction in *Meloidogyne* spp. from furfural over the long term (Baidoo et al., 2013).

Luc and Crow (2013) found that exposure of *B. longicaudatus* in soil to furfural in the general concentration range expected from a single spray application of the current maximum labeled rate (67.5 liters/ha), caused only a slight reduction in nematode population density. In the sampling depth study, significant reductions in population density of *B. longicaudatus* from furfural at the maximum labeled rate were not observed until after two applications were made. This indicated that a single furfural application was not likely sufficient to provide adequate turf improvement and nematode reductions required by turf managers. Rather, a series of several applications made at regular intervals, each lowering nematode population densities an increment may be required for optimal results.

These results indicated that multiple applications of furfural to turf reduced population density of plant-parasitic nematodes and improved turf health. Although direct correlation between turf improvement and nematode reduction at soil depths below 5 cm was difficult to ascertain, furfural appeared to be a good tool for inclusion in turf nematode integrated pest management (IPM) programs. Field trials to develop such turf nematode IPM programs that include furfural as a component are currently underway (Crow and Kenworthy, 2013).

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