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Nematicide effects on arthropods in bermudagrass

Benjamin Waldo[1], Felipe Soto-Adames[2], and William Crow[1,]*

Abstract

In turfgrass systems, nematicides are a valuable tool for managing plant-parasitic nematode populations, but few studies have examined non-target nematicide effects on arthropods. Our study evaluated effects of turfgrass nematicide formulations of abamectin (Divanem SC), fluopyram (Indemnify), furfural (MultiGuard Protect EC), and fluensulfone (Nimitz Pro G) on arthropod populations in bermudagrass (*Cynodon* spp.; Poaceae). A randomized block design was used with 5 replications of the 4 nematicide treatments and an untreated control. Plots were 6 m^2 with 0.6 m untreated borders between adjacent plots. Data were collected from 1.5 m^2 subplots located in the center of the treatment plots. Nematicides were applied at labeled rates every 4 wk as a summer treatment program from 7 Jun to 30 Aug 2016 and from 24 Apr to 18 Jul 2017 at the University of Florida Plant Science Research and Education Unit in Citra, Florida, USA. Samples were collected before treatment and at 2 d, 14 d, 56 d, and 238 d after the final treatment. Data from each nematicide treatment were compared to the untreated data at each sample date using analysis of covariance with initial population counts serving as the covariate. Abamectin treatments significantly increased fungivore mite abundance relative to the untreated control at 2 sampling dates in 2016 and 3 sampling dates in 2017. Abamectin also decreased collembolan abundance significantly at 2 sampling dates in 2017. Fluopyram increased predatory mite abundance significantly at 3 sampling dates in 2016; decreased collembolan abundance significantly at 4 sampling dates in 2017; and significantly increased phytophagous insect abundance at 2 dates in 2017. Furfural and fluensulfone had low impacts on arthropod abundance. The results of this study suggest nematicides can impact arthropods in bermudagrass, which could affect organic matter decomposition and nutrient cycling processes.

Key Words: bioindicator; mites; collembola; turfgrass

Resumen

En los sistemas de césped, los nematicidas son una herramienta valiosa para controlar las poblaciones de nematodos parásitos de plantas, pero pocos estudios han examinado los efectos de los nematicidas no objetivo en los artrópodos. Nuestro estudio evaluó los efectos de las formulaciones de nematicidas de césped de abamectina (Divanem SC), fluopiram (Indemnify), furfural (MultiGuard Protect EC), y fluensulfona (Nimitz Pro G) en poblaciones de artrópodos en bermudagrass (*Cynodon* spp.; Poaceae). Se utilizó un diseño de bloques al azar con 5 repeticiones de los 4 tratamientos con nematicidas y un control sin tratar. Las parcelas eran de 6 m^2 con 0,6 m de bordes sin tratar entre parcelas adyacentes. Los datos se recolectaron de subparcelas de 1,5 m^2 ubicadas en el centro de las parcelas de tratamiento. Los nematicidas se aplicaron a las dosis indicadas cada 4 semanas como un programa de tratamiento de verano desde el 7 de junio al 30 de agosto del 2016 y desde el 24 de abril al 18 de julio del 2017 en el Unidad de Educación e Investigación en Ciencias Vegetales de la Universidad de Florida en Citra, Florida, EE. UU. Las muestras se recolectaron antes del tratamiento y a los 2 días, 14 días, 56 días, y 238 días después del tratamiento final. Los datos de cada tratamiento con nematicida se compararon con los datos no tratados en cada fecha de muestra utilizando un análisis de covarianza con los recuentos de población iniciales que sirven como covariables. Los tratamientos con abamectina aumentaron significativamente la abundancia de ácaros fungívoros en relación con el control no tratado en 2 fechas de muestreo en el 2016 y 3 fechas de muestreo en el 2017. La abamectina también disminuyó significativamente la abundancia de colémbolas en 2 fechas de muestreo en el 2017. Fluopiram aumentó significativamente la abundancia de ácaros depredadores en 3 fechas de muestreo en el 2016; disminuyó significativamente la abundancia de colémbolos en 4 fechas de muestreo en el 2017; y aumentó significativamente la abundancia de insectos fitófagos en 2 fechas en el 2017. El furfural y la fluensulfona tuvieron un bajo impacto en la abundancia de artrópodos. Los resultados de este estudio sugieren que los nematicidas pueden afectar a los artrópodos en el pasto bermuda, lo que podría afectar la descomposición de la materia orgánica y los procesos de ciclo de nutrientes.

Palabras Clave: bioindicador; ácaros colémbola; césped

Bermudagrass (*Cynodon* sp.) (Poaceae) is an important groundcover used in golf courses, lawns, and athletic fields in the southeastern US. In Florida, the turfgrass industry generates over a billion dollars in revenue annually (Haydu et al. 2006). Plant-parasitic nematodes (Nematoda) are important pathogens that may cause decline or death in turfgrass by feeding on roots (Crow et al. 2003). Golf courses commonly utilize nematicides as a form of chemical control to reduce population densities of plant-parasitic nematodes (Crow 2014). Whereas plant-parasitic nematodes are the intended target of nematicide applications, beneficial non-target soil biota such as bacteria, fungi, protists, oligochaetes, and arthropods could be exposed to potentially harmful pesticides. These valuable organisms contribute to soil health through complex multitrophic food web interactions and assist in organic matter breakdown, nutrient cycling, and may act as antagonists to plant pests and pathogens (Brussaard 1997; Altieri 1999; Barrios 2007). In ecological studies, organisms may be placed into functional groups based on their role in the ecosystem (Paoletti et al. 1996). These groupings are based on feeding preferences and general life history (Cross-

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ley et al. 1992). Due to the redundancy and overlap of feeding groups in the soil, analyzing the composition and abundance of invertebrate functional groups in the soil over time may reveal changes in ecosystem function after a disturbance event such as a pesticide application (Paoletti et al. 1996; Yeates 2003).

Mites (Acari) and collembola (Collembola) are among the most dominant invertebrate groups in soil (Hopkin 1997a; Kranz & Walter 2009). Mites may feed as saprophages, microbivores, algivores, fungivores, predators, or phytophages (Kranz & Walter 2009). Most collembola feed on decaying vegetation, fungi, or microbes, but some are predators of arthropods and nematodes (Hopkin 1997a). Mites and collembola indirectly increase decomposition rates by mechanically breaking down detritus and increasing surface area that can be used by microbes (Seastedt 1984; Dindal 1990; Heneghan et al. 1998; Kampichler & Bruckner 2009). Oribatid mites also have been proposed as a bioindicator for assessing succession stages in agroecosystems due to their selective sensitivity to environmental disturbance (Behan-Pelletier 1999). Collembola have been used in toxicology laboratory and field studies of pesticides and pollutants as a non-target organism due to their sensitivity to pesticides (Frampton 1994; Hopkin 1997b).

Field studies evaluating non-target effects of insecticides on soil arthropods have shown oribatid mite populations generally lack sensitivity to insecticide applications whereas predatory non-oribatid mite, collembola, and insect populations often are suppressed by insecticides (Hopkin 1997b; Frampton 1999; Förster 2011). In turfgrass systems, chlorpyrifos, bendiocarb, and isofenphos have been shown to reduce population densities of gamasid (Mesostigmata) and actinedida (Prostigmata) mites while having low impacts on oribatids (Cockfield & Potter 1983). Chlorothalonil fungicide treatments have been shown to reduce oribatid and collembola abundance in turfgrass, and chlopyrifos and imidacloprid also reduced collembola (Peck 2009; Gan & Wickings 2017). In some turfgrass studies, hister and carabid beetles and staphylinid larvae have been shown to be susceptible to imidacloprid and bendiocarb, but ants and spiders were not suppressed (Kunkel et al. 1999, 2001).

While nematicide effects have been studied on plant-parasitic nematodes, very little research has been devoted to the impact of nematicides on non-target arthropods in turfgrass. Alterations of soil arthropod abundance and feeding group composition from nematicide applications could reduce soil ecosystem function (Crossley et al. 1992; Altieri 1999). The objective of our study was to apply nematicides to turfgrass plots and analyze changes in arthropod population densities over time. Since the selected pesticides were formulated for plant-parasitic nematode management, we predicted low impacts on soil arthropod abundance after exposure to nematicide applications over short- and long-term sampling dates.

Materials and Methods

The study was performed at the University of Florida Plant Science Research and Education Unit in Citra, Florida, USA. 'Tifdwarf' bermudagrass (*Cynodon dactylon* [L.] Pers.) (Poaceae) was the turfgrass cultivar planted in research plots and was managed with common turfgrass management practices by staff at the Plant Science Research and Education Unit. Fertilizer, plant growth regulator, and herbicides were the only chemical tools used to maintain plots during the study. Thiencarbazone-methyl, foramsulfuron, and halosulfuron-methyl (Tribute; Bayer CropScience, St. Louis, Missouri, USA), sulfentrazone (Dismiss; FMC Corporation, Philadelphia, Pennsylvania, USA), and trinexapac-ethyl (Primo; Syngenta Crop Protection, Basel, Switzerland) were applied as needed for weed control and turf management. Herbicide treatments were applied to reduce contamination of weeds in turfgrass plots. Since herbicides were applied to all plots, any treatment differences should be due to nematicide applications. Plots were fertilized with Harrell's 13-4-13 controlled release golf course green fertilizer during the growing season. Soil texture was comprised of 97% sand, 2% clay, and 1% silt as determined by the hydrometer method (Bouyoucos 1962). Organic matter was 4% according to the dry-loss-on-ignition method (Nelson & Sommers 1996) and pH was 7.1 as measured from soil slurry using a soil pH meter (Beckman Coulter Life Sciences, Indianapolis, Indiana, USA).

EXPERIMENTAL DESIGN

A randomized-block design with 5 treatments and 5 replicates was used. In addition to an untreated control, the experimental treatments used were: abamectin (Divanem; Syngenta Crop Protection, Basel, Switzerland), furfural (MultiGuard EC; Agriguard Company LLC, Windsor, Colorado, USA), fluopyram (Indemnify; Bayer CropScience, St. Louis, Missouri, USA), and fluensulfone, (Nimitz Pro G; ADAMA Group Company, Raleigh, North Carolina, USA). Rates were based on the maximum allowable rate as listed on each label (Table 1).

Applications of liquid treatments were made using a CO_2-powered backpack sprayer (Weed Systems, Hawthorne, Florida, USA) with TJ-08 nozzles delivering 1,222 liter solution per ha. Nimitz Pro G was applied using a walk-behind Gandy (Owatonna, Minnesota, USA) drop-spreader. Treatment plots were 6 m^2 in area and samples were collected from smaller 1.5 m^2 data collection plots located in the center of treatment plots to minimize any cross contamination between plots. All plots were separated by an untreated 0.6 m border on each side. After each application, all treated and untreated plots were immediately irrigated with 0.64 cm of water. Treatments were applied every 4 wk replicating a summer treatment program from 7 Jun to 30 Aug 2016 (4 applications) and 24 Apr to 17 Jul 2017 (4 applications).

SAMPLE COLLECTION

Samples were collected prior to the initial treatment, and at 2 d, 14 d, 56 d, and 238 d after the final treatment application each yr. Plugs were collected using a 3.81-cm diam ball mark plugger (Turf-Tec International, Tallahassee, Florida, USA) to a depth of 6.35 cm. Eight plugs were randomly collected from the data collection subplots and combined in plastic sampling bags (International Plastics, Inc.; Greenville, South Carolina, USA) for analysis. Excess soil was gently shaken from the plugs and invertebrates were extracted from thatch

Table 1. Nematicide formulations used in the field study and their per-application labeled application rates, EC = Emulsifiable Concentrate and G = granular.

Active ingredient (a.i.)	Trade name (manufacturer)	Application rate	Formulation
Abamectin	Divanem (Syngenta)	0.89 liters product/ha (70g a.i. per ha)	EC
Fluopyram	Indemnify (Bayer)	1.25 liters product/ha (500 g a.i. per ha)	EC
Furfural	MultiGuard Protect (Agriguard)	56 liters product/ha (60 kg a.i. per ha) 2016 74 liters product/ha (77 kg a.i. per ha) 2017	EC
Fluensulfone	Nimitiz Pro G (ADAMA)	62.25 kg product/ha (1 kg a.i. per ha)	G

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and roots of 4 plugs by Berlese funnel extraction (Berlese 1905). The other 4 plugs were used for nematode extraction in a separate study (Waldo et al. 2019). Berlese samples were left for 120 h in a growth chamber with 400 watt bulbs. Distance between turfgrass plug and light bulb was 61 cm to expose turfgrass to 35 °C heat from the light source to slow the rate of drying. Specimens were collected and preserved in 100% ethanol and stored in scintillation vials (Wheaton Industries, Millville, New Jersey, USA). Mites, collembola, and insects were identified from Berlese extracted samples with a dissecting microscope (Olympus Corporation, Shinjuku, Tokyo, Japan). Slide mounted voucher mite specimens were identified using a compound phase microscope (Olympus Corporation, Shinjuku, Tokyo, Japan) at 100× magnification under oil immersion. Arthropods were identified using Stehr (1987, 1991), Dindal (1990), Christiansen and Bellinger (1998), Daly et al. (1998), and Kranz and Walter (2009) guides. Mites representative of 1% or greater of all mites counted were identified to family level and assigned to a feeding group based on Kranz and Walter (2009). All other arthropods were identified to family level using a dissecting scope at 40× and specimens were assigned to feeding groups based on Daly et al. (1998).

STATISTICAL ANALYSIS

Population counts were analyzed using analysis of covariance (ANCOVA) using R software version 3.3.2 (R Core Team 2016). Data were log transformed to improve normality and homogeneity of variance. Population means of the different sampling dates were compared to the initial sample means using the untreated control as a covariate. ANCOVA was chosen to help account for natural seasonable variation.

Results

Two-hundred arthropod samples were collected and specimens were extracted using Berlese funnels. Two samples were excluded from data analysis due to storage contamination. A total of 64,907 arthropods were counted and identified in the study.

Acari, Collembola, and Insecta were the major taxa identified within Arthropoda. A total of 27,270 mites were counted and made up 42% of arthropods in the study. Detritivores were the dominant group comprising 89% of mites identified. The most abundant detritivores were oribatid families Tectocepheidae (16,876 total specimens; 26% of total arthropods), Liacaridae (3,895 total specimens; 6% of total arthropods), and Tokunocepheidae (1,948 total specimens; 3% of total arthropods. The most abundant predatory mites belonged to the mesostigmatid family Acaridae which represented 2% (1,299 total specimens) of total arthropods.

Collembola made up 29% of arthropods in the study with 18,936 individuals counted. Isotomidae (97% of total collembola) and Tullbergiidae (3%) were the only 2 collembola families encountered. One species encountered was a new record for the state of Florida (*Mesaphorura yosiii* Rusek) (Collembola: Tullbergiidae) and 1 a new record to Florida and the continental US (*Folsomides centralis* Denis) (Collembola: Isotomidae) (FDACS 2017a, b). All collembola identified belonged to the detritivore feeding group.

Insects were the third most abundant taxon with 18,699 specimens identified representing 28% of total arthropods. Mealybugs (Hemiptera: Pseudococcidae) were the dominant insect group representing 93% of insects and were placed in the phytophagous feeding group. Less than 0.1% of arthropods identified belonged to Diplura. These arthropods were not included in statistical analysis because only a few individuals were encountered.

MITES

Detritivore mite abundance increased relative to the untreated control in abamectin plots ($P \leq 0.1$) at 2 d after final treatment and 56 d after final treatment in 2016 (Fig. 1). Abamectin treated plots had greater detritivore mite abundance relative to the untreated control ($P \leq 0.05$) at 2, 14, and 56 d after final treatment in 2017 (Fig. 1). Abamectin plots had greater predatory mite abundance relative to the untreated control ($P \leq 0.1$) at 14 d after final treatment in 2016 (Fig. 2). Fluopyram treated plots had increased predatory mite abundance relative to the untreated control ($P \leq 0.05$) in 2016 at 2, 14, and 56 d after final treatment (Fig. 2). Fluopyram plots had increased predatory mite abundance relative to the untreated control ($P \leq 0.1$) at 238 d after final treatment in 2017 (Fig. 2). Furfural and fluensulfone treatments did not have a significant effect on fungivore or predatory mite abundances relative to the untreated control ($P > 0.1$).

COLLEMBOLA

Abamectin had no significant effect on the abundance of collembola relative to the untreated control in 2016 ($P > 0.1$), but population numbers in 2017 were reduced relative to the untreated control ($P \leq 0.01$) at 2 and 14 d after final treatment (Fig. 3). Fluopyram treatments significantly reduced collembola relative to the untreated control ($P \leq 0.1$) at 2 d after final treatment in 2016 (Fig. 3). Fluopyram reduced collembola counts relative to the untreated control ($P \leq 0.01$) at 2, 14, 56, and 238 d after final treatment in 2017 (Fig. 3). Furfural reduced collembola relative to the untreated control ($P \leq 0.01$) at 2 d after final treatment in 2017 (Fig. 3). Fluensulfone treatments did not have a significant effect on collembola abundance relative to the untreated control ($P > 0.1$).

INSECTS

Abamectin reduced phytophagous insect counts relative to the untreated control ($P \leq 0.1$) at 2 d after final treatment in 2016 (Fig. 4). Fluopyram treated plots had greater phytophagous insect abundance relative to the untreated control ($P \leq 0.05$) at 14 d after final treatment 2016 (Fig. 4) and greater phytophagous insect abundance relative to the untreated control ($P \leq 0.1$) at 2 and 14 d after final treatment in 2017 (Fig. 4). Furfural significantly decreased phytophagous insect abundance relative to the untreated control ($P \leq 0.05$) at 238 d after final treatment in 2017 (Fig. 4). Fluensulfone treatments did not have a significant effect on phytophagous insect abundance relative to the untreated control ($P > 0.1$).

Detritivore and predatory insect abundances were very low in both yr. Total counts often were below 5 individuals per plot. Due to the low counts, data analysis and figures were not included for detritivore or predatory insects.

Discussion

The results of this study indicate nematicides may impact population densities of non-target arthropods. Soil food webs are intricate and complex. Drastic changes in biodiversity could have unintended consequences to ecosystem services (Gessner et al. 2010; Handa et al. 2014; Soliveres et al. 2016). Abamectin caused a reduction in collembola, but an increase in mites and insects compared to untreated control densities. Decline in collembola abundance occurred in early sampling dates but effects were short-lived as populations recovered later in the season. This is common for *r* strategists like collembola (Hopkin 1997b). The increase in mites and insects was unexpected due to sus-

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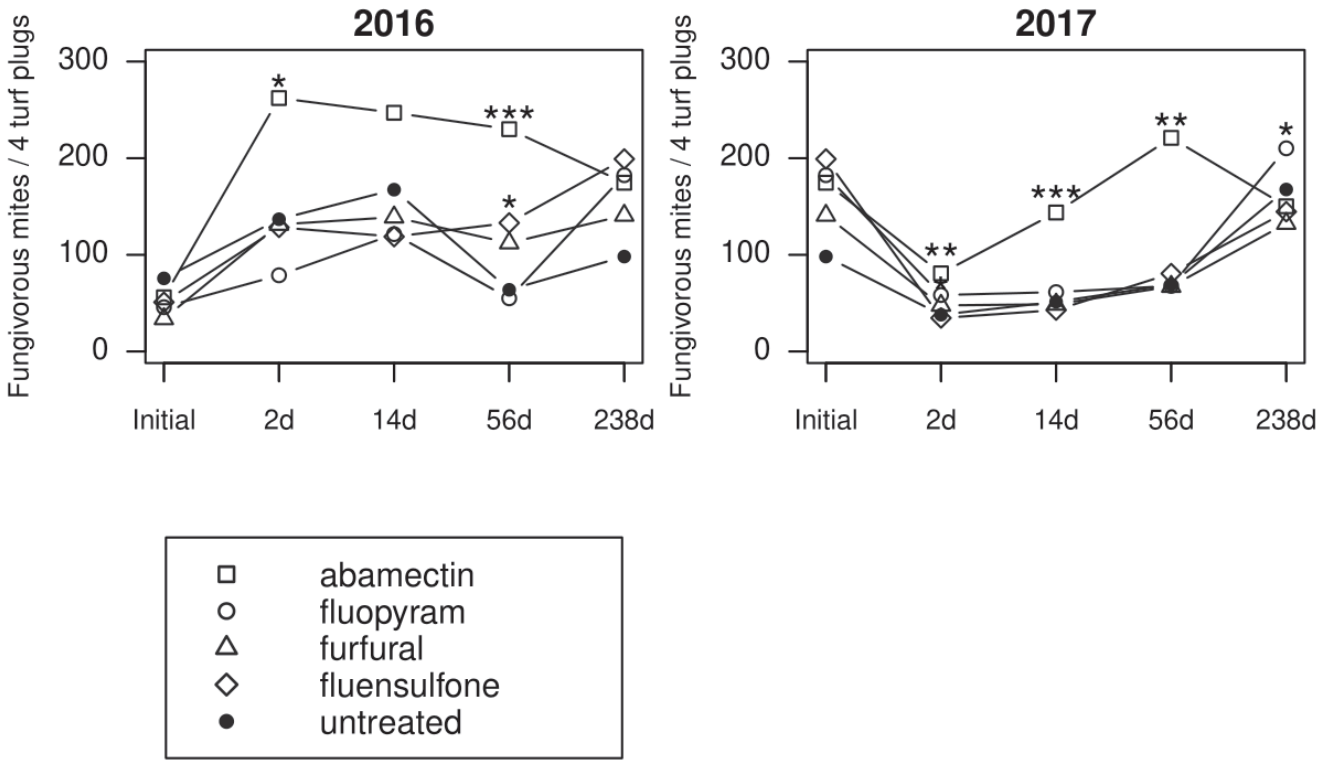


Fig. 1. Population densities of fungivorous mites from Berlese extraction as affected by different nematicide applications at all sampling dates. Sample collection dates before the first treatment application (initial) and d after final treatment application (2 d, 14 d, 56 d, 238 d) are presented on the x-axis. *, **, ***Different from the untreated according to analysis of covariance ($P < 0.1, 0.05, 0.01$, respectively).

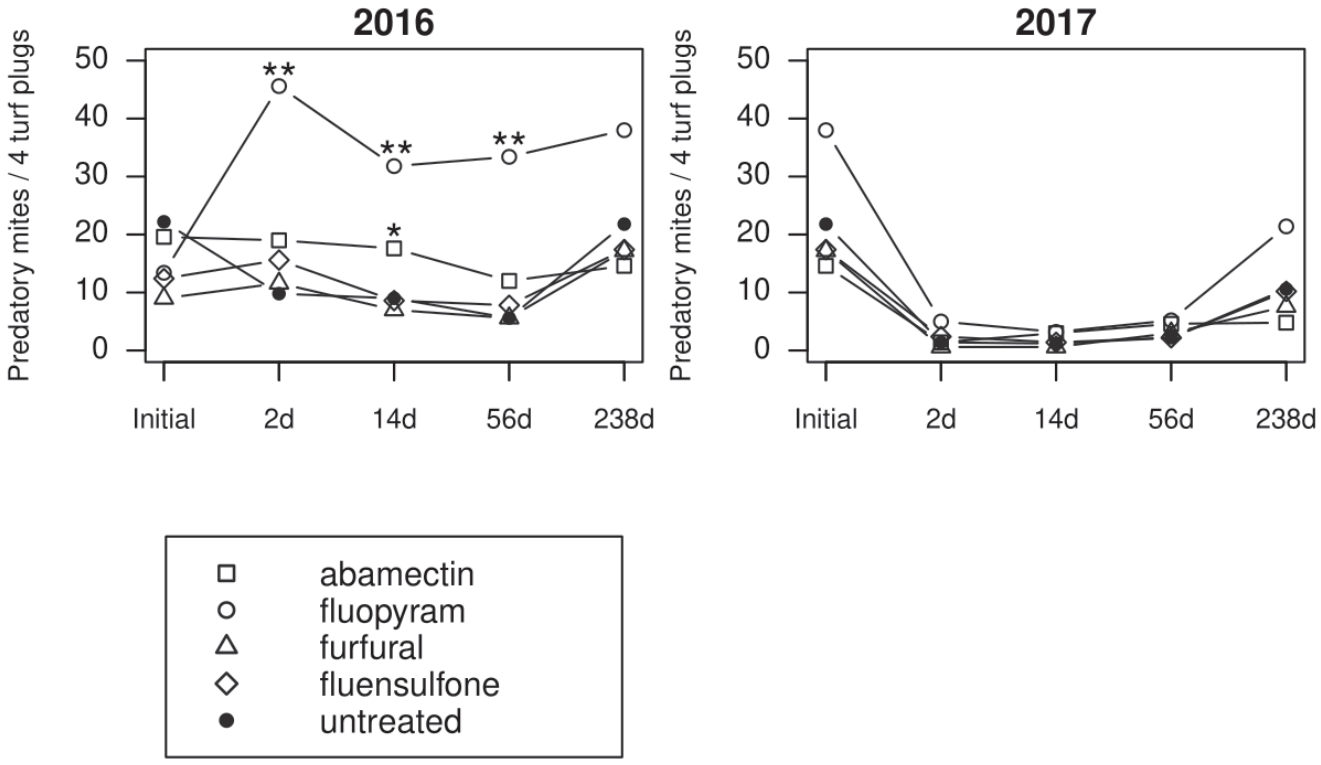


Fig. 2. Population densities of predatory mites from Berlese extraction as affected by different nematicide applications at all sampling dates. Sample collection dates before the first treatment application (initial) and d after final treatment application (2 d, 14 d, 56 d, 238 d) are presented on the x-axis. *, **, ***Different from the untreated according to analysis of covariance ($P < 0.1, 0.05, 0.01$, respectively).

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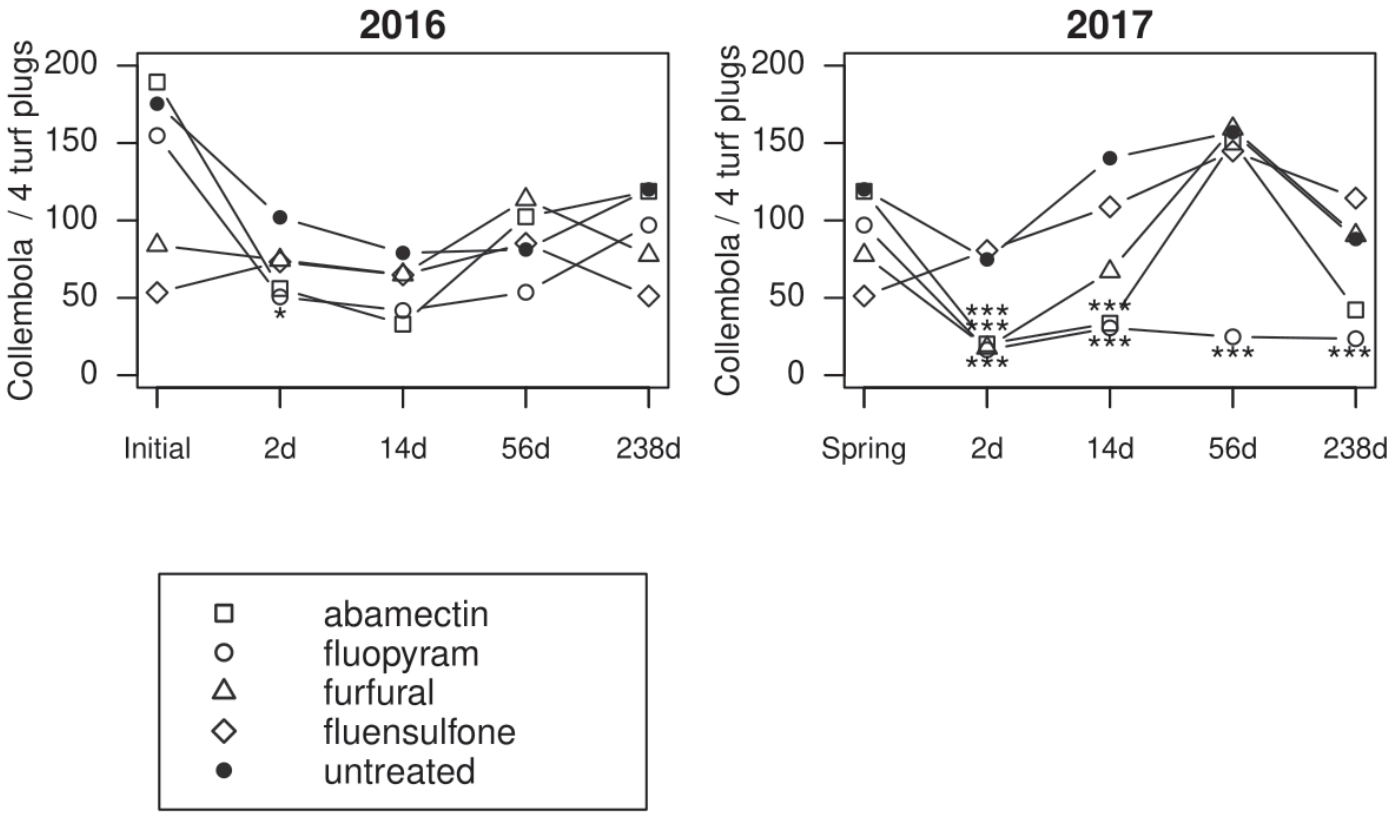


Fig. 3. Population densities of collembola from Berlese extraction as affected by different nematicide applications at all sampling dates. Sample collection dates before the first treatment application (initial) and d after final treatment application (2 d, 14 d, 56d, 238d) are presented on the x-axis. *, **, ***Different from the untreated according to analysis of covariance ($P < 0.1$, 0.05, 0.01, respectively).

ceptibility of mites and insects to abamectin (Putter et al. 1981; Lasota & Dybas 1991; Copping & Duke 2007; Lumaret et al. 2012). Acaricides often contain abamectin or related avermectin compounds to control mites in agricultural settings (Lumaret et al. 2012). Abamectin is recommended in integrated pest management programs for its reported low toxicity on beneficial arthropods and rapid degradation in sunlight (Wilson 1993). However, avermectins have been documented with selectivity within mite and insect groups (Römbke et al. 2010; Förster et al. 2011). Förster et al. (2011) found significant reduction in collembola densities and no significant effect on mite densities in pasture soil exposed to ivermectin. Soil mites exposed to ivermectins in laboratory and field settings were not significantly affected except under very high nematicide concentrations. Other studies reported abamectin having low toxicity or sub-lethal effects on non-target mites (Zhang & Sanderson 1990). In our study it is possible dead fungal feeding nematodes and collembola could have served as a food source for microbes that enabled the fungal feeding mites to flourish during the mo treatments were applied with less competition. While the increase in fungal feeding mites could be favorable for organic matter decay, it could indicate a disturbed food web that has lower ecological resilience due to fewer fungal feeders in other taxa.

Fluopyram also had notable impacts on soil arthropods. Collembola were the most affected group of arthropods. In addition to use as a nematicide, fluopyram also has fungicidal effects. Collembola can be killed by direct exposure to chemical application or from indirect effects such as reduced food source (Frampton 1994). In our study, it is possible collembola were affected by direct pesticide contact, reduction in fungal food sources, or a combination of both factors. The decrease in collembolan abundance could slow decomposition occurring in the thatch layer. Slowed decomposition could contribute to increased fertilizer requirements and thatch buildup, but could reduce nutrient losses from leaching (Ineson et al. 1982; Arnold & Potter 1987).

The other arthropod groups largely were unaffected by fluopyram, except for increases in predatory mites and phytophagous insects. The reduction in predatory nematodes could have allowed predatory mites to increase with reduced competition over shared food resources. Put et al. (2016) found no reductions of predatory mite *Euseius gallicus* Kreiter and Tixier (Acari: Phytoseidae) treated with fluopyram + tebucinazole in greenhouse assays on vegetables. Low toxicity of fluopyram has been reported for mites by the European Food Safety Authority (EFSA 2013, 2018).

Furfural had low impacts on arthropods with reduction in collembola and phytophagous insects at 1 date each. No published data on furfural toxicity to arthropods are currently available for comparison. Furfural is a byproduct from sugarcane processing and is readily broken down by microbes, possibly reducing the amount of time available for the compound to contact arthropods. Botanical products like furfural are used in integrated pest management systems and can have repellant and sublethal effects on non-target arthropods (Desneux et al. 2007). Selectivity of botanical products found in other studies have demonstrated low impacts on non-target soil arthropods (De Souza Tavares et al. 2009; Elias et al. 2013).

Mite, collembola, and insect abundances in the fluensulfone treated plots largely were unaffected, suggesting low ecological risk to soil microarthropods. The effect of fluensulfone has not been studied in arthropods in other published studies at this time. In nematodes, this compound has a mode of action distinct from the more traditional organophosphate, carbamate, and ivermectin active ingredients, which could result in lower impacts on the soil ecosystem (Kearn 2014).

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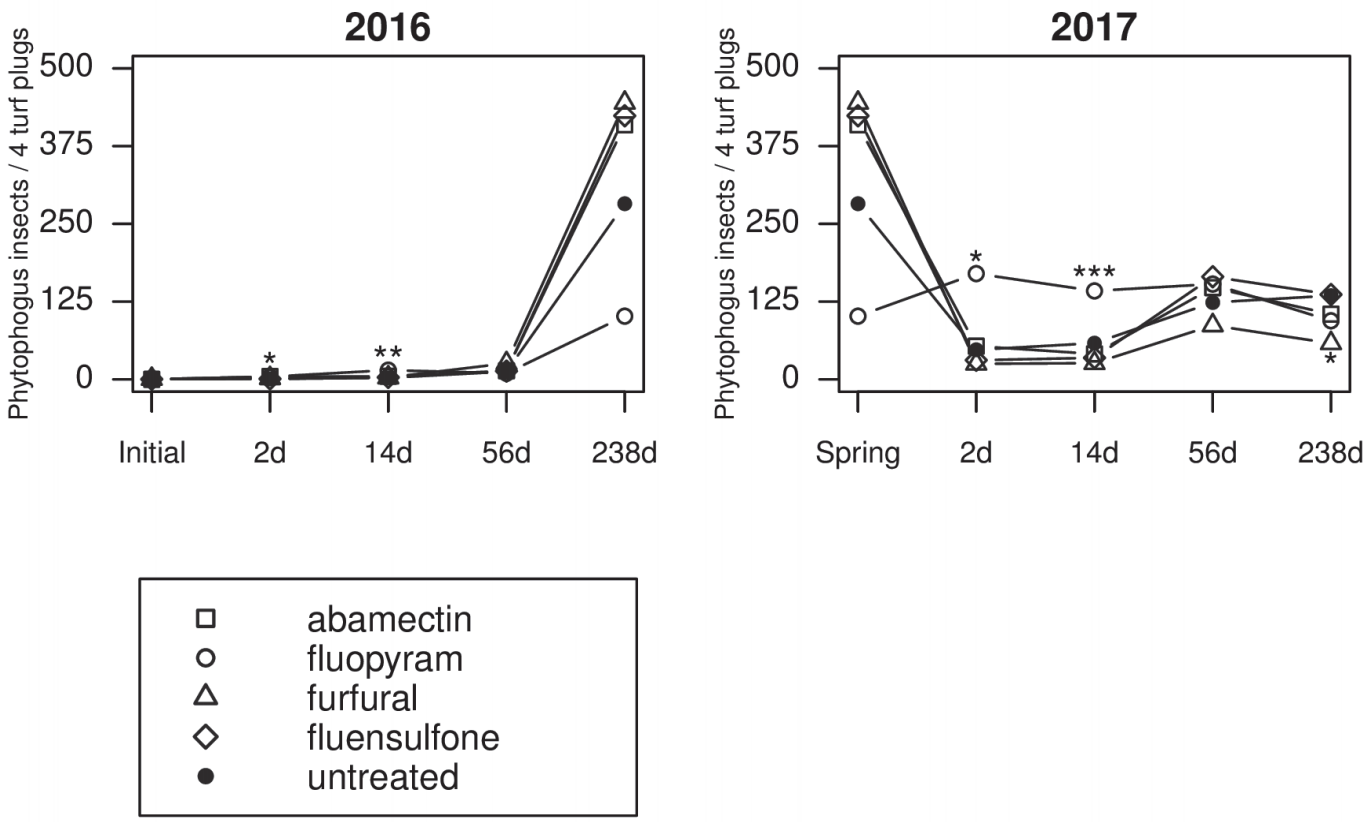


Fig. 4. Population densities of phytophagous insects from Berlese extraction as affected by different nematicide applications at all sampling dates. Sample collection dates before the first treatment application (initial) and d after final treatment application (2 d, 14 d, 56 d, 238 d) are presented on the x-axis. *, **, ***Different from the untreated according to analysis of covariance ($P < 0.1$, 0.05, 0.01, respectively).

SUMMARY

In summary, nematicide products are capable of impacting soil arthropod densities in turfgrass systems. Abamectin and fluopyram had the largest impact on soil arthropod densities of the nematicides tested in our study. Furfural and fluensulfone had low impacts on arthropod densities. Whereas there were significant impacts from the treatments, it should not be assumed that all changes in community structure were due to direct effects of the chemicals. Treatments may affect nematodes, fungi, bacteria, etc., that are predators, pathogens, or food for different types of arthropods that influence the arthropod community structure. Whereas all the treatments were applied according to their labels, their application rates and timing may not reflect their recommended use in the field. For research purposes, they all were applied on the same schedule using the maximum allowable labeled rate. However, a golf course might make fewer applications, space out treatments at different time intervals, apply lower rates than the maximum, or rotate chemistries. The objective of this research was not to determine the effectiveness of certain nematicides. Rather, the intent was to introduce the concept of soil health into the discussion of golf course nematode management and to promote further research into integrated pest management.

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