



Efficacy of succinate dehydrogenase inhibitor nematicides fluopyram and cyclobutrifluram against *Hoplolaimus galeatus* in Golf Turf

Research Article

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Abstract: Following the cancellation of fenamiphos, lance nematodes (*Hoplolaimus* spp.) have become one of the most challenging plant-parasitic nematodes to manage in turfgrass systems. Succinate dehydrogenase inhibitor nematicides are among the most used nematode management tools on golf courses in the United States. Currently, there are two SDHI turfgrass nematicides registered in the United States: the fluopyram nematicide Indemnify, which was launched in 2016, and the cyclobutrifluram nematicide Trefinti, which was launched in 2025. The objective of this research was to compare the efficacy of these two nematicides against the lance nematode *Hoplolaimus galeatus* in microwell bioassays, greenhouse, and field experiments. Microwell bioassays compared the percent mortality of *H. galeatus* among the nematicides following exposure to different concentrations of the nematicides at different time intervals. Greenhouse experiments compared the efficacy of the nematicides when applied at field-relevant rates to *H. galeatus*-infested turfgrass in pots. Finally, the efficacy of the two nematicides for management of *H. galeatus* was compared in turfgrass field trials. In the bioassay experiment, exposure of *H. galeatus* to cyclobutrifluram resulted in higher nematode mortality than exposure to fluopyram. The greenhouse experiment demonstrated that cyclobutrifluram at 250 g a.i./ha and above significantly suppressed *H. galeatus* population density, whereas fluopyram did not. Finally, results from field trials indicate that cyclobutrifluram is an effective treatment for enhancing the health of golf turf infested by *H. galeatus*. These findings suggest that the cyclobutrifluram nematicide Trefinti exhibits strong efficacy against *H. galeatus* and could serve as an effective treatment for management of *H. galeatus* on golf turf.

Keywords: Cyclobutrifluram • Fluopyram • *Hoplolaimus galeatus* • Lance nematode • Nematicide • Succinate dehydrogenase Inhibitor • Turfgrass

Lance nematodes (*Hoplolaimus* spp.) rank among the three most damaging nematode pests of warm-season golf turf (Crow, 2021). While several *Hoplolaimus* spp. are known to parasitize turfgrasses (Holguin et al., 2015; McCurdy et al., 2024), *H. galeatus* is the most common species of lance nematode infecting warm-season turfgrasses in Florida (Florida Nematode Assay Lab, unpublished). These nematodes tunnel in and out of the root system as they feed, resulting in damage to tissue structure and poor root function (Giblin-Davis et al. 1995; Settle et al., 2007), contributing to turfgrass decline (Todd and Tisserat, 1990; Crow, 2021).

Management of lance nematode is challenging due to its migratory endoparasitic lifestyle, which limits exposure to nematicides and hampers early detection (Crow, 2021). In past decades, fenamiphos provided excellent results against lance nematodes on golf turfgrass due to its systemic activity, but it is no longer available for use in the United States. Among the new generation of nematicides are the succinate dehydrogenase inhibitors (SDHIs). While this group of pesticides includes many fungicides, currently there are two known to have high efficacy against nematodes: fluopyram and cyclobutrifluram. The 34.5% fluopyram turfgrass nematicide Indemnify (Envu, Cary, NC) was launched commercially in 2016, and it quickly became one of the most commonly used nematicides by the golf turf industry. A second SDHI turfgrass

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nematicide, cyclobutrifluram, received US EPA registration in 2025, and the 38.5% cyclobutrifluram turfgrass fungicide/nematicide Trefinti® T&O (Syngenta, Greensboro, NC) was launched in 2026. These nematicides suppress nematode activity by inhibiting succinate dehydrogenase, thereby disrupting mitochondrial respiration (Li et al., 2021).

Effective management of lance nematodes on golf courses is essential for maintaining turfgrass aesthetics and playability. Consequently, evaluating the efficacy of fluopyram and cyclobutrifluram is an important step toward achieving more effective and durable control of this pest. This study evaluates the efficacy of the currently available SDHI nematicides for suppressing lance nematode on golf course turfgrass.

1. Materials and methods

1.1 Microwell mortality bioassay

The effects of fluopyram and cyclobutrifluram on lance nematode were evaluated using mortality bioassays in 5 mL Falcon® 12-well clear multi-well plates (Corning, Rochester, NY) and were repeated non-concurrently in 2025. Nematodes were exposed to cyclobutrifluram and fluopyram at concentrations of 0, 10, and 50 ppm a.i.; for fluopyram, the commercial nematicide formulation Indemnify was used, and for cyclobutrifluram, the commercial nematicide formulation Trefinti was used.

Lance nematode inocula were collected from cultures maintained on 'Latitude 36' bermudagrass (*Cynodon dactylon* × *C. transvaalensis*) grown in 1-liter clay pots filled with golf course top-dressing sand under greenhouse conditions. Nematode inocula were extracted using a modified Baermann method, as described by McSorley and Frederick (1991), to ensure healthy nematodes were used. Nematode solutions were prepared to contain at least 25 nematodes/mL, and 1 mL of nematode solution was pipetted into each of thirty microwells. Fluopyram and cyclobutrifluram solutions were prepared at three concentrations (0 ppm, 20 ppm, and 100 ppm a.i.), double the desired final concentrations. One milliliter of nematicide solution was then pipetted into the microwells containing the nematode solution to achieve final exposure concentrations of 0, 10, and 50 ppm a.i.

Nematode activity was assessed after 24 and 72 hr of exposure using an Olympus CK30 microscope. Following the addition of 70 µL of 1 N NaOH (Fisher Chemical, Pittsburgh, PA), as described by Chen and Dickson (2000),

the numbers of active (moving) and inactive (non-moving) nematodes were recorded. This stimulant induced a response in living nematodes, causing them to twist their bodies, while dead nematodes remained rigid and straight. Following the application of the stimulant, a thirty-second interval was allowed for the nematodes to react before counting, ensuring sufficient time to elicit a response but not long enough for the NaOH to kill the nematodes. The percent mortality was calculated by dividing the number of active nematodes by the total number of nematodes and multiplying by 100. Because NaOH is toxic to nematodes, separate untreated control treatments were used for each exposure time, so there were a total of six treatments (three concentrations and two observation times). The experiment was set up in a randomized complete block design with five replications for each treatment.

1.2 Greenhouse experiment

A greenhouse trial compared the efficacy of cyclobutrifluram to fluopyram using field-labeled application rates for the management of lance nematode on bermudagrass turf. The experiment was originally conducted from June to October 2021 and repeated during the same period in 2025. The experiment was conducted in "pipe pots" constructed of PVC pipes measuring 5-cm diameter by 20 cm deep. The bottom of each pot was covered with screen and landscape fabric to allow drainage, and each was filled with 440 cm³ of sterilized sand. Each pot was sprigged with Latitude 36 bermudagrass and allowed to establish root systems for 30 days before inoculation. Lance nematodes were extracted from Latitude 36 bermudagrass as described above using a modified Baermann funnel technique (McSorley and Frederick, 1991) to ensure healthy inoculum was used. Inoculum solution was concentrated to approximately 400 nematodes per mL. Following root establishment, each pot was inoculated with 400 mixed life stages of lance nematodes by pipetting 1 mL of nematode solution into a 2-cm-deep hole made in the sand of each pot that was closed after inoculation. 2 weeks after inoculation, treatment applications commenced.

The experiment was arranged in a randomized complete block design with 5 blocks of 5 treatments. The treatments included fluopyram at 500 g a.i./ha, cyclobutrifluram applied at 125, 250, and 500 g a.i./ha, and untreated control. Treatments were applied 4 times at 4-week intervals as described by the curative spot-treatment rates on the Indemnify (fluopyram) and Trefinti (cyclobutrifluram) labels. Treatment solutions were prepared to deliver the

desired amount of nematicide in 15 mL of solution, while untreated control pots received 15 mL of water. Treatments in the 2021 greenhouse trial were applied using the same spray equipment described for the field trial, whereas treatments in the repeated 2025 greenhouse trial were applied as a soil drench. All pots were maintained under consistent greenhouse conditions (18–33°C), received biweekly applications of Miracle-Gro® (Scotts Miracle-Gro, Marysville, OH), and were routinely trimmed to promote uniform turf growth.

4 weeks after the final treatment application, all pots were taken down for nematode extraction. Lance nematodes were extracted from the soil using centrifugal flotation (Jenkins, 1964). Lance nematode per 100 cm³ soil were compared among treatments to determine the efficacy of each treatment in suppressing nematode populations.

1.3 Field trials

Turfgrass field trials were conducted in 2021 and 2022 to evaluate the cyclobutrifluram nematicide Trefinti as a treatment for management of lance nematodes on golf greens compared to the standard fluopyram nematicide Indemnify. The 2021 field trial was conducted on ‘Sunday’ bermudagrass infested with *H. galeatus*, and the 2022 trial on ‘TifEagle’ bermudagrass infested with *H. galeatus* along with the grass root-knot nematode *Meloidogyne graminis*. Both sites were located at the University of Florida’s Plant Science Unit in Citra, Florida, and were maintained as golf greens. Plots were 1.5 m² with 0.62 m untreated borders between adjacent plots.

Treatments in both trials were (i) untreated control, (ii) four monthly applications of 500 g fluopyram/ha as per the curative spot-treatment rate for turfgrass use allowed by the Indemnify label, (iii) four monthly applications of cyclobutrifluram at 250 g a.i./ha, and (iv) four monthly applications of cyclobutrifluram at 500 g a.i./ha as allowed by the spot-treatment rate on the Trefinti label. Applications of all treatments were made using a CO₂-powered backpack sprayer with TJ-08 nozzles delivering 2445 l/ha of solution. All plots, including the untreated controls, were treated with a modified alkylated polyol soil penetrant (Revolution®, Aquatrols, Paulsboro, NJ) at each treatment application date, a standard golf management practice to facilitate even movement of water into the soil profile. After each application, treated and untreated plots were irrigated with 0.32 cm of irrigation to move the nematicides off of the turf surface and into the soil. The experiments used a randomized block design with five replications, with blocking based on the initial *H. galeatus* population density in individual plots.

Measurements evaluated were (i) population density of *H. galeatus* in soil, (ii) root health, (iii) turf quality, and (iv) normalized difference vegetative index (NDVI). Nematode samples consisted of nine 1.9-cm-diam. plugs taken 9- to 10-cm-deep from each plot. Nematodes were extracted from a 100 cm³ soil subsample using the centrifugal-flotation method (Jenkins, 1964). Nematode samples were collected before the initial treatment application (Pi), 2 weeks after the second treatment application (Pm), and 2 weeks after the final treatment application (Pf). Root samples were two 3.8-cm-diam. cores taken 15.25 cm deep from each plot (175 cm³ of soil from each core). Soil was washed from the roots, inspected visually, and rated for root depth, root density, and root quality. Each root parameter used a 1-5 scale, with 1 being the worst and 5 being the best. The three individual root measurements were used to calculate percentage overall root health using the formula $[(\text{length} \times 20) + (\text{density} \times 20) + (\text{quality} \times 20)]/3 \times 100$. Root samples were collected before the initial treatment applications (initial), 2 weeks after the second treatment application (mid), and 2 weeks after the final treatment application (final). Turf quality is a subjective 1-9 scale widely used by the turfgrass industry, with 9 being the best quality and 6 the minimum acceptable quality color (Krans and Morris, 2007). NDVI was measured using a hand-held GreenSeeker (Trimble, Westminster CO). Turf quality and NDVI were measured at approximately 2-week intervals starting the day of the initial treatment application and ending 2 weeks after the final treatment application.

1.4 Statistical analysis

Statistical analyses for microwell and greenhouse experiments were conducted using R version 4.5.1. All microwell mortality bioassays had the response variable represented as counts of dead and alive nematodes. A generalized linear model (GLM) with a logit link was fitted using the `glm()` function. Variables such as nematicide, concentration, and time were converted to factors when needed due to their categorical nature. For greenhouse trials, a generalized linear mixed model (GLMM) was fitted using the `glmmTMB` package in R (version 4.5.1; Brooks et al., 2017) to analyze the effect of treatment on final nematode counts.

The model included treatment as a fixed effect and block as a random effect to account for variability among experimental blocks. Data were modeled using a negative binomial distribution (`nbino2`) with a log-link function. Model assumptions were assessed with DHARMA residual diagnostics, including QQ plots (KS test) and dispersion tests. A Type II Wald Chi-square analysis of deviance

was used to test for the significance of predictors (exposure time, concentration, and nematicide) on nematode mortality in the *in vitro* assay and on final nematode counts in the greenhouse experiments. Data from the two greenhouse trials were tested for heterogeneity and were not different from each other. Therefore, data from both trials were combined into a single dataset for mean separation according to Tukey's HSD test ($P \leq 0.05$).

For the field experiments, data were subjected to analysis of variance, and treatment means were separated according to Tukey's HSD test ($P \leq 0.05$) in SAS 9.4 (SAS Institute, Cary NC).

2. Results

2.1 *In vitro* mortality bioassay

A GLM model with main effects terms was tested, and concentration 0 was removed to prevent complete separation

in the model, which manifested as convergence warnings. Diagnostic plots showed no evidence of overdispersion (first experiment $P = 0.688$, repeated experiment $P = 0.632$), outliers (first experiment $P = 1.0$, repeated experiment $P = 1.0$), and non-uniform residual patterns (KS test for first experiment $P = 0.894$, repeated experiment $P = 0.905$).

Tests of significance revealed that nematicide had the greatest effect on mortality in both experiments ($P < 0.001$), followed by exposure time ($P < 0.001$) and concentration ($P < 0.01$). No significant two-way or three-way interactions were observed in either experiment. Estimated marginal means indicated that cyclobutrifluram consistently caused higher mortality in lance nematodes than fluopyram. In the first experiment, at 24 hours, exposure to 10 ppm resulted in about 11% mortality with cyclobutrifluram compared to less than 1% with fluopyram. At 50 ppm, cyclobutrifluram caused 20% mortality, whereas fluopyram resulted in only 1% mortality (Figure 1a). At 72 hours, the difference became more pronounced. Cyclobutrifluram caused 54% mortality at 10 ppm and nearly 69% at 50 ppm, while fluopyram produced only 4% and 8% mortality at 10 ppm and 50 ppm, respectively (Figure 1a).

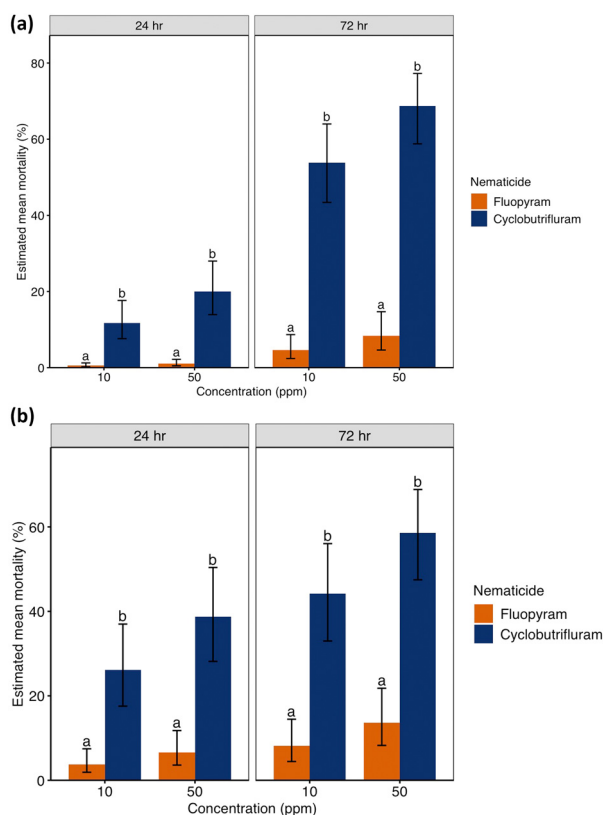


Figure 1. Estimated mean mortality of *Hoplolaimus galeatus* exposed to fluopyram and cyclobutrifluram at 10 and 50 ppm in microwells in (a) the first experiment and (b) the second experiment. Bars with common letters are not different according to Tukey's HSD test ($P \leq 0.05$).

The repeated experiment showed a similar pattern. At 24 hours, exposure to 10 ppm resulted in approximately 26% mortality with cyclobutirfluram compared to 3% with fluopyram. At 50 ppm, cyclobutirfluram caused 38% mortality, whereas fluopyram resulted in only 6% (Figure 1b). By 72 hours, the difference was more pronounced. Cyclobutirfluram caused 44% mortality at 10 ppm and nearly 58% at 50 ppm, while fluopyram produced only 8 and 13% mortality at 10 ppm and 50 ppm, respectively (Figure 1b).

2.2 Greenhouse experiment

DHARMA residual diagnostic plot showed that the model fit the data well; hence, no major assumption was violated (KS test $P = 0.76$; dispersion $P = 0.43$; outlier $P = 1$). Final nematode counts differed significantly among treatments (Type II Wald $\chi^2 = 107.81$, $P < 0.001$). Population densities of *H. galeatus* in the fluopyram treatment (500 g/ha) and the lowest rate of cyclobutirfluram (125 g/ha) did not differ from the untreated control. However, significantly ($P \leq 0.05$) lower population densities were observed at the higher cyclobutirfluram rates (250 and 500 g/ha; Figure 2).

2.3 Field trials

Neither fluopyram nor cyclobutirfluram reduced population densities of *H. galeatus* in either field trial ($P > 0.05$);

Table 1. Effects of treatments on the population density of *Hoplolaimus galeatus*/100 cm³ of soil in field trials on 'Sunday' bermudagrass in 2021 and TifEagle' bermudagrass in 2022

Treatment	a.i./ha	Pi	Pm	Pf
Sunday 2021				
Untreated	0.00 g	323	158	219
Fluopyram	500 g	325	218	178
Cyclobutirfluram	250 g	346	235	272
Cyclobutirfluram	500 g	359	269	299
TifEagle 2022				
Untreated	0.00 g	246	168	93
Fluopyram	500 g	246	159	161*
Cyclobutirfluram	250 g	274	184	150
Cyclobutirfluram	500 g	247	131	141

*Different from the untreated according to Tukey's HSD test ($P > 0.05$).

Table 1). However, in 2022, the fluopyram treatment had greater *H. galeatus* Pf than the untreated control. Both SDHI nematicides had positive impacts on turf health in both trials. Percent root health was improved compared to the untreated ($P \leq 0.05$) by the highest rate of cyclobutirfluram in 2021, and by all SDHI nematicide treatments in 2022 (Table 2). NDVI was improved ($P \leq 0.05$) compared to the untreated by fluopyram and the lower rate of cyclobutirfluram on 1 date and by the higher rate of

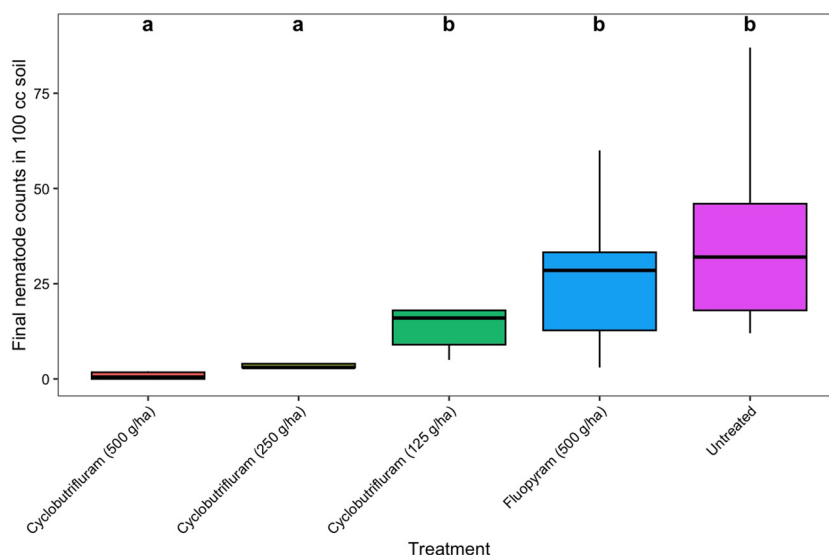


Figure 2. Final nematode counts of *Hoplolaimus galeatus*/100 cm³ of soil following exposure to cyclobutirfluram and fluopyram in a greenhouse experiment in 2022 and 2023. Data from both repetitions are combined for analysis. Bars with common letters are not different according to Tukey's HSD test ($P \leq 0.05$).

Table 2. Effects of treatments on percent root health (0-100) in field trials in field trials on *Hoplolaimus galeatus*-infested ‘Sunday’ bermudagrass in 2021 and TifEagle’ bermudagrass in 2022

Treatment	a.i./ha	Initial	Mid	Final
Sunday 2021				
Untreated	0.00 g	36	43	35
Fluopyram	500 g	34	47	47
Cyclobutrifluram	250 g	45	45	44
Cyclobutrifluram	500 g	45	64**	59***
TifEagle 2022				
Untreated	0.00 g	60	51	46
Fluopyram	500 g	60	67**	69***
Cyclobutrifluram	250 g	60	73***	67***
Cyclobutrifluram	500 g	63	73***	75***

*, **, ***Different from the untreated according to Tukey’s HSD test ($P \leq 0.05, 0.01, 0.001$, respectively).

cyclobutrifluram on 4 dates in 2021 (Table 3). In 2022, fluopyram improved NDVI compared to the untreated on 4 dates, and compared to both cyclobutrifluram rates on 7 dates (Table 3). In 2021, turf quality was improved compared to the untreated only by the higher rate of cyclobutrifluram (Table 4). In 2022, fluopyram improved turf quality on 5 dates and the cyclobutrifluram treatments on 6 dates (Table 4).

Table 3. Effects of treatments on NDVI (0-1) measurements in field trials on *Hoplolaimus galeatus*-infested ‘Sunday’ bermudagrass in 2021 and TifEagle’ bermudagrass in 2022

Treatment	a.i./ha	Measurement date							
Sunday 2021									
		2/10	2/24	3/9	3/26	4/8	4/23	5/11	5/20
Untreated	0.00 g	0.44	0.39	0.45	0.52	0.61	0.58	0.68	0.64
Fluopyram	500 g	0.37	0.37	0.49**	0.54	0.64	0.60	0.70	0.66
Cyclobutrifluram	250 g	0.41	0.42*	0.53***	0.57	0.66	0.63	0.74	0.70
Cyclobutrifluram	500 g	0.40	0.42**	0.54***	0.61	0.67**	0.66**	0.79**	0.71
TifEagle 2022									
		3/3	3/30	4/14	4/28	5/12	5/24	6/8	6/23
Untreated	0.00 g	0.68	0.61	0.65	0.64	0.69	0.70	0.76	0.77
Fluopyram	500 g	0.68	0.64*	0.69**	0.65	0.72	0.72	0.80***	0.81***
Cyclobutrifluram	250 g	0.67	0.65**	0.71***	0.69*	0.76***	0.76***	0.81***	0.81***
Cyclobutrifluram	500 g	0.68	0.66**	0.71***	0.70**	0.76***	0.76***	0.81***	0.81***

*, **, ***Different from the untreated according to Tukey’s HSD test ($P \leq 0.05, 0.01, 0.001$, respectively).

3. Discussion

Fluopyram had little to no effect on *H. galeatus* in micro-well and greenhouse experiments, supporting field observations that this nematicide is not effective against lance nematodes (Crow, 2021). In contrast, cyclobutrifluram exhibited strong nematicidal activity against *H. galeatus* in both experiments, indicating its potential as an effective management tool. These results show that cyclobutrifluram has higher activity against lance nematodes than the currently available SDHI nematicide, fluopyram. Mortality of lance nematodes from cyclobutrifluram in micro-wells increased with concentration and exposure times, indicating a dose-dependent toxic effect. In greenhouse trials, the strongest suppression occurred at rates of 250 g/ha and above, whereas 125 g/ha had no effect.

While microwell and greenhouse trials indicate that cyclobutrifluram is efficacious on *H. galeatus*, no reductions in population density of *H. galeatus* were observed in field trials. This was not unexpected, as Dant et al. (2025) reported that population densities of lance nematodes often do not decline following application of an effective nematicide, despite clear improvements in turfgrass health. Lance nematodes move in and out of turfgrass roots (Giblin-Davis et al., 1995; Settle et al., 2007), so their soil counts increase and decrease throughout the year, explaining why Pm counts from soil were sometimes lower than the Pi and Pf numbers. The primary objective

Table 4. Effects of treatments on turf quality (1-9) in field trials on *Hoplolaimus galeatus*-infested 'Sunday' bermudagrass in 2021 and TifEagle' bermudagrass in 2022

Treatment	a.i./ha	Measurement date							
Sunday 2021									
		2/10	2/24	3/9	3/26	4/8	4/23	5/11	5/20
Untreated	0.00 g	4.0	3.2	3.8	4.0	4.0	3.8	5.2	4.2
Fluopyram	500 g	3.0	2.8	4.0	4.6	4.6	4.6	5.4	5.0
Cyclobutrifluram	250 g	3.6	3.4	4.4	4.4	4.8	4.6	5.2	5.2
Cyclobutrifluram	500 g	3.4	3.0	4.4	5.4**	5.4**	5.6**	6.0	6.6***
TifEagle 2022									
		3/3	3/30	4/14	4/28	5/12	5/24	6/8	6/23
Untreated	0.00 g	6.4	5.0	5.0	6.0	5.4	4.8	5.2	5.4
Fluopyram	500 g	6.2	5.0	5.6*	6.2	6.4*	6.0**	6.6**	7.8***
Cyclobutrifluram	250 g	6.2	5.2	6.0**	6.6**	7.2**	6.8***	7.6***	8.0***
Cyclobutrifluram	500 g	6.6	5.4	6.4***	6.8**	7.4***	7.0***	7.4***	8.0***

*, **, ***Different from the untreated according to Tukey's HSD test ($P \leq 0.05, 0.01, 0.001$, respectively).

of a nematicide treatment to golf turf is to promote healthy grass and not to reduce measured nematode counts (Crow et al., 2017). In the 2021 field trial, *H. galeatus* was the only plant-parasitic nematode detected, and only the highest rate of cyclobutrifluram improved root health, NDVI, and turf quality. However, in the 2022 trial, root-knot nematode juveniles were present in the soil along with the lance nematodes, and fluopyram and both rates of cyclobutrifluram improved root health, NDVI, and turf quality. Given that fluopyram has excellent activity on root-knot nematodes infecting golf turf (Crow et al., 2017), as does cyclobutrifluram (Dant et al., 2025), we hypothesize that the turf health improvements from fluopyram in 2022 may have been due to effects on root-knot nematodes rather than lance nematodes. Because accurate assessment of root-knot nematodes on turfgrasses necessitates different sampling and extraction methods than those used for lance nematodes (Crow et al., 2020), their abundance was not analyzed in this trial, although their presence was noted.

Overall, the findings of this study show that cyclobutrifluram is effective on lance nematodes compared to fluopyram, demonstrating its potential for use in integrated nematode management programs in turfgrass systems. Our field trials indicate consistent turf improvement following cyclobutrifluram applications, whether lance nematodes are the sole nematode of concern or if other plant-parasitic nematodes are also present. This is a significant breakthrough for management of lance nematodes given the history of poor efficacy of other nematicides against this pest in warm-season turf (Crow et al., 2017).

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Author contributions

Erica B. Guri: Investigation, data collection, statistical analysis, writing – original draft. William T. Crow: Conceptualization, methodology, statistical analysis, funding acquisition, supervision, resources, writing – review & editing.

Conflict of interest statement

Authors state no conflict of interest.

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