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Fluopyram as a rescue nematicide for managing sting nematode (*Belonolaimus longicaudatus*) on commercial strawberry in Florida

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ABSTRACT

The sting nematode (*Belonolaimus longicaudatus*) is one of the most economically damaging pests of strawberry in Florida. In this study we evaluated in-season application of fluopyram for management of *B. longicaudatus* populations at nine commercial strawberry farms. In-crop application of fluopyram reduced *B. longicaudatus* population densities in soil by the end of the growing season in four out of six of the commercial strawberry farms where *B. longicaudatus* was detected during the growing season. At the four sites, relative suppression of *B. longicaudatus* by fluopyram ranged from 54 to 96%. Despite significant suppression of *B. longicaudatus* population densities in soil, in-crop application of fluopyram did not result in greater strawberry plant vigour by the end of the growing season. Overall, in-crop application of fluopyram shows considerable potential to reduce *B. longicaudatus* populations on strawberry in Florida.

1. Introduction

In Florida, strawberry (*Fragaria* X *ananassa* Dusch.) is grown as an annual crop on raised beds covered with polyethylene mulch (Whitaker et al., 2016). Transplant material includes bare root and plug plants produced in nurseries located in more northern states and Canada and are typically planted in early October. Most commonly, one drip tape irrigation line is placed in the middle of each bed, and overhead irrigation is used to aid in the initial establishment of young transplants. Plant-parasitic nematodes are a major limiting factor for successful growth and yield of this crop (Abu-Gharbieh and Perry, 1970; Watson and Desaeger, 2019). The sting nematode (*Belonolaimus longicaudatus* Rau) is the most serious nematode pest of strawberry in this production region (Kokalis-Burelle, 2003). Infested plants show severe stunting of growth, yield decline, as well as symptoms characteristic of nutrient deficiency (Olson and Santos, 2010). Root symptoms include reduced root biomass as well as the formation of a tight mat of short roots that often assume a swollen appearance. Symptomatic plants usually occur in circular patches of poor plant growth within an infested field (Fig. 1). Due to the severe damage caused by sting nematode a low population densities, finding a single sting nematode in soil from a strawberry field early or mid-season often warrants management action.

For many decades, pre-plant application of methyl bromide in combination with chloropicrin was the fumigant regime of choice for strawberry growers (Overman and Martin, 1978); however, methyl bromide has now been phased out (Watson et al., 1992). Many alternative soil fumigants have been explored for use in strawberry production in Florida, including various combinations of 1,3-dichloropropene with chloropicrin, metam sodium, metam potassium, dimethyl disulfide, and dazomet (Locascio et al., 1999; Noling, 2002; Kokalis-Burelle, 2003). Problems associated with many alternative soil fumigants include: (1) performance inconsistencies relative to methyl bromide, (2) label restrictions regarding use, (3) the need for personal protection equipment during application, and (4) incompatibility for post-plant nematode management during the growing season. In Florida, much of the commercial strawberry production is located adjacent to urban developments and dwellings. As a result, if effective alternatives were available, many growers would prefer to move away from soil fumigants. Current research on *B. longicaudatus* management on strawberry has focused on evaluating non-fumigant chemical nematicides (fluensulfone, fluopyram, and fluazaindolizine) as well as various biological nematicides (Watson and Desaeger, 2019). In addition to being safer to apply than soil fumigants, many non-fumigant nematicides

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Fig. 1. Characteristic circular patch of poor plant growth caused by sting nematode (*Belonolaimus longicuadatus*) at a commercial strawberry farm in Thonotosassa, Florida on February 15, 2019.

have an added benefit in that they can be applied as a post-plant nematode management option during the growing season.

In a recently conducted field trial, Watson and Desaeger (2019) showed that pre-plant application of fluopyram followed by reapplication 40 days after transplanting reduced *B. longicaudatus* populations on strawberry, improved yield, as well as showed a strong trend towards enhanced plant vigour. In the current study, fluopyram was applied as a post-plant nematicide to manage *B. longicaudatus* at nine commercial strawberry fields with a history of nematode infestation. The objectives of this study were to evaluate the effects of in-crop application of fluopyram on *B. longicaudatus* population densities and plant vigour throughout the remainder of the growing season.

2. Methods

2.1. Site description

The study was conducted at nine commercial farms located in Hillsborough County, Florida, United States during the 2018–2019 winter strawberry production season. Commercial farms were selected based on a previous history of *B. longicaudatus* infestation, or sites where the nematode had subsequently been detected during the growing season. Soil at each site was sandy in texture, low in organic matter (<1.3% organic matter), and had a pH ranging from 6.3 to 7.6 (Table 1). Irrigation, fertilization, insecticide, and fungicide applications were performed by the grower according to commercial production practices for Florida strawberry (Whitaker et al., 2016). All fields were fumigation with 1,3-dichloropropene and chloropicrin during bed formation, except site SK which was fumigated with metam potassium through the drip line prior to three weeks prior planting.

2.2. Experimental design

The experimental design at each of the nine commercial farms was a paired-plot design, with a varying number of replicates depending on the size of the strawberry farm and nematode infested area. Treatments consisted of: (1) fluopyram (Velum Prime®; Bayer CropScience, Research Triangle Park, NC) applied at 0.48 L of product per hectare, or (2) no treatment (control). Plots consisted of entire planting rows which ranged from 76- to 122-m long, with two rows of plants per bed and a 38-cm spacing between plants. Fluopyram was applied to each strawberry farm through the drip tape irrigation system using the grower's commercial irrigation pump. A valve was installed at the start of each row receiving the control treatment to prevent fluopyram from entering into the untreated plots. After the nematicide was injected (38- to 90-min duration), valves were opened, and all beds were irrigated for a 1-h cycle. Nematicides were applied at varying stages of crop development: early in the harvest (December 12, 2019; 2 farms), mid-harvest (January 10 – 18, 2019; 5 farms), and late in the harvest (February 12, 2019; 2 farms).

2.3. Crop growth

At each of the nine commercial farms, plant vigour was monitored using a GreenSeeker™ hand-held crop sensor (Trimble, Sunnyvale, CA) one month after nematicide application, as well as every two weeks until the end of the growing season (mid-March) in the farms that received early and mid-harvest nematicide application. Normalized difference vegetation index (NDVI) values were determined by taking the average of two measurements along both sides of the length of each plot at a height of approximately 80 cm above the bed.

In March 2019, row greenness at sites LG, MG-NW, MG-NE, MG-SE, AM, ML, and RG was assessed using low-altitude aerial imagery. Aerial imaging surveys were conducted using a DJI™ Phantom 4 Pro unmanned aerial system (DJI Innovation Company Inc., Shenzhen, China) equipped with a DJI 24 mm 20-megapixel camera with an Exmor-R complementary metal oxide semiconductor sensor. Image orthomosaics were created using DroneDeploy™ cloud software platform (DroneDeploy, San Francisco, USA), with an image resolution of 10–20 mm per pixel. Processed RGB maps were analyzed using ESRI™ ArcGIS v10.33 (ESRI, Redlands, USA), and percent greenness in each row was calculated using the following formula: (green pixels/total pixels) x 100.

Table 1
Site locations, soil characteristics, nematicide application dates, and initial sting nematode (*Belonolaimus longicaudatus*) pressure at the time of nematicide application.

Site	Location	Application Date [a]	Replications	Soil Texture	Organic Matter (%)	Soil pH	*Belonolaimus longicaudatus*/200 mL soil
FF	Thonotosassa, FL	Dec. 12, 2018 (E)	10	Sand	0.8	7.6	1
LG	Dover, FL	Dec. 12, 2018 (E)	8	Sand	0.7	7.3	6
MG-NW	Dover, FL	Jan. 10, 2019 (M)	8	Sand	0.6	7.3	0
MG-NE	Dover, FL	Jan. 10, 2019 (M)	8	Sand	0.7	6.9	0
MG-SE	Dover, FL	Jan. 10, 2019 (M)	8	Sand	0.7	6.3	0
SK	Durant, FL	Jan. 11, 2019 (M)	9	Sand	0.8	6.9	2
AM	Thonotosassa, FL	Jan. 18, 2019 (M)	10	Sand	0.8	7.2	89
ML	Seffner, FL	Feb. 12, 2019 (L)	8	Sand	0.6	7.1	3
RG	Dover, FL	Feb. 12, 2019 (L)	4	Sand	1.3	7.0	285

[a] (E) refers to early-harvest application, (M) refers to mid-harvest application, and (L) refers to late-harvest application.

2.4. Nematode populations

For each of the nine commercial farms, initial populations of soil nematodes in the untreated control were determined one day after nematicide application. Eight soil cores (20 cm in length, 2.5 cm in diameter) were obtained from each plot. A soil core was taken directly from the rooting zone of eight randomly selected plants in each plot. Soil samples were placed into plastic bags and stored at 4 °C for a maximum of 48 h prior to subsequent processing. Nematodes were extracted from a 200-mL subsample of soil form each plot using the Baermann pan technique (Forge and Kimpinski, 2007), with a two-day incubation period. After collecting the nematodes over a 25-μm sieve, the nematode samples were transferred in water into plastic scintillation vials and stored at 4 °C for a maximum of 48 h prior to counting. The abundance of *B. longicaudatus* in samples was determined using an inverted compound microscope. For each of the nine commercial farms, soil nematodes were monitored in all plots one month after nematicide application as well as at the end of the growing season (mid-March), as described previously.

2.5. Statistical analysis

Data were subjected to a one-way ANOVA in SAS Studio (SAS University Edition; version 3.3; SAS Institute Inc., Cary, NC, USA) using the PROC GLM procedure. Differences between means were examined using Tukey's HSD test ($P < 0.05$).

3. Results

3.1. Crop growth

Early-harvest application of fluopyram to sites FF and LG had a significant effect on plant vigour (Table 2). At FF, plants were more vigorous in the fluopyram treatment relative to that of the untreated control at 6 weeks post application (WPA); however, no significant differences in plant vigour were observed on any subsequent measurement date during the growing season. At LG, plants were more vigorous in the fluopyram treatment relative to that of the untreated control at 10 WPA and showed a strong trend ($P = 0.051$) toward greater plant vigour in the fluopyram treatment at 12 WPA as well.

Mid-harvest application of fluopyram had no effect on plant vigour relative to that of the untreated control at sites MG-NW, MG-SE, SK, and AM; however, plant vigour was lower in the fluopyram treatment relative to that of the control in site MG-NE at 6 WPA.

Late-harvest application of fluopyram to sites ML and RG had no effect on plant vigour relative to that of the untreated control.

For the majority of the sites that drone imagery was utilized, no differences in end-of-season row greenness were observed between the fluopyram-treated and untreated control rows (Table 3). However, at site ML, row greenness was significantly greater in the fluopyram-treated rows relative to that of the control.

3.2. Nematode populations

Early-harvest application of fluopyram at site FF had no effect on population densities of *B. longicaudatus* in soil at 4 WPA or by the end of the growing season (12 WPA) (Table 4). At LG, application of fluopyram reduced population densities of *B. longicaudatus* in soil by 74% relative to that of the untreated control by the end of the growing season (12 WPA).

At sites MG-NW, MG-NE, and MG-SE, *B. longicaudatus* was not recovered from soil throughout the growing season despite a history of infestation with this nematode in previous seasons. At SK, mid-harvest application of fluopyram showed a strong trend ($P = 0.06$) towards lower population densities of *B. longicaudatus* in soil relative to that of the untreated control at 4 WPA (89% reduction); however, population densities of *B. longicaudatus* were low (2 nematodes per 200 mL soil) in both treatments by the end of the growing season and thus did not differ significantly. At AM, mid-harvest application of fluopyram reduced population densities of *B. longicaudatus* in soil relative to that of the untreated control at 4 WPA (42% reduction) as well as at the end of the growing season (8 WPA; 54% reduction).

Late-harvest application of fluopyram reduced soil population densities of *B. longicaudatus* by the end of the growing season (4 WPA) at sites ML (96% reduction) and RG (83% reduction).

Table 2
Effect of nematicide application on plant vigour at nine commercial strawberry farms in Florida. NDVI refers to normalized difference vegetation index and WPA refers to weeks post-application. Soil treatments within a site sharing the same letter do not differ significantly ($P > 0.05$), according to Tukey's HSD test.

Site	Treatment	N	Plant Vigour (NDVI)				
			4 WPA	6 WPA	8 WPA	10 WPA	12 WPA
FF	Control	10	0.704 a	0.733 b	0.692 a	0.700 a	0.695 a
	Fluopyram	10	0.707 a	0.762 a	0.721 a	0.703 a	0.709 a
	P-value	-	*0.798*	*0.038*	*0.241*	*0.752*	*0.100*
LG	Control	8	0.696 a	0.711 a	0.713 a	0.716 b	0.686 a
	Fluopyram	8	0.710 a	0.726 a	0.725 a	0.756 a	0.723 a
	P-value	-	*0.267*	*0.255*	*0.240*	*0.012*	*0.051*
MG-NW	Control	8	0.722 a	0.700 a	0.740 a	–	–
	Fluopyram	8	0.723 a	0.717 a	0.733 a	–	–
	P-value	-	*0.913*	*0.324*	*0.734*	-	-
MG-NE	Control	8	0.824 a	0.806 a	0.816 a	–	–
	Fluopyram	8	0.804 a	0.783 b	0.802 a	–	–
	P-value	-	*0.146*	*0.011*	*0.083*	-	-
MG-SE	Control	8	0.796 a	0.771 a	0.801 a	–	–
	Fluopyram	8	0.789 a	0.779 a	0.810 a	–	–
	P-value	-	*0.686*	*0.590*	*0.477*	-	-
SK	Control	9	0.668 a	0.681 a	0.675 a	–	–
	Fluopyram	9	0.659 a	0.686 a	0.673 a	–	–
	P-value	-	*0.623*	*0.815*	*0.904*	-	-
AM	Control	10	0.505 a	0.496 a	0.548 a	–	–
	Fluopyram	10	0.555 a	0.529 a	0.574 a	–	–
	P-value	-	*0.129*	*0.236*	*0.252*	-	-
ML	Control	8	0.776 a	–	–	–	–
	Fluopyram	8	0.800 a	–	–	–	–
	P-value	-	*0.254*	-	-	-	-
RG	Control	4	0.546 a	–	–	–	–
	Fluopyram	4	0.538 a	–	–	–	–
	P-value	-	*0.932*	-	-	-	-

Table 3
Effect of nematicide application on end-of-season row greenness at nine commercial strawberry farms in Florida. Soil treatments within a site sharing the same letter do not differ significantly (P > 0.05), according to Tukey's HSD test.

Site	Treatment	N	Row Greenness (%)
LG	Control	8	74.2 a
	Fluopyram	8	75.6 a
	P-value	-	*0.359*
MG-NW	Control	8	58.3 a
	Fluopyram	8	60.4 a
	P-value	-	*0.403*
MG-NE	Control	8	69.1 a
	Fluopyram	8	67.3 a
	P-value	-	*0.190*
MG-SE	Control	8	64.3 a
	Fluopyram	8	65.4 a
	P-value	-	*0.689*
AM	Control	10	45.4 a
	Fluopyram	10	48.5 a
	P-value	-	*0.359*
ML	Control	8	59.1 b
	Fluopyram	8	63.6 a
	P-value	-	*0.027*
RG	Control	4	40.1 a
	Fluopyram	4	49.5 a
	P-value	-	*0.215*

Table 4
Effect of nematicide application on sting nematode (*Belonolaimus longicaudatus*) population densities in soil at nine commercial strawberry farms in Florida. WPA refers to weeks post-application. Soil treatments within a site sharing the same letter do not differ significantly (P > 0.05), according to Tukey's HSD test.

Site	Treatment	N	*Belonolaimus longicaudatus*/200 mL soil	
			4 WPA	End-of-season
FF	Control	10	1 a	13 a
	Fluopyram	10	2 a	16 a
	P-value	-	*0.181*	*0.704*
LG	Control	8	12 a	31 a
	Fluopyram	8	11 a	8 b
	P-value	-	*0.603*	*0.039*
MG-NW	Control	8	0	0
	Fluopyram	8	0	0
	P-value	-	-	-
MG-NE	Control	8	0	0
	Fluopyram	8	0	0
	P-value	-	-	-
MG-SE	Control	8	0	0
	Fluopyram	8	0	0
	P-value	-	-	-
SK	Control	9	9 a	2 a
	Fluopyram	9	1 a	2 a
	P-value	-	*0.060*	*0.842*
AM	Control	10	154 a	94 a
	Fluopyram	10	89 b	43 b
	P-value	-	*0.043*	*0.011*
ML	Control	8	–	26 a
	Fluopyram	8	–	1 b
	P-value	-	-	*0.021*
RG	Control	4	–	206 a
	Fluopyram	4	–	34 b
	P-value	-	-	*0.032*

4. Discussion

Effective in-crop nematicides could provide strawberry growers with a valuable alternative to soil fumigants for nematode management throughout the growing season. In this study, in-crop application of fluopyram reduced *B. longicaudatus* population densities in soil by the end of the growing season in four out of the six commercial strawberry farms where this nematode was detected during the growing season. Fluopyram reduced *B. longicaudatus* population densities 50% of the time when applied as an early-harvest or mid-harvest rescue nematicide, and 100% of the time when applied late in the harvest. At site SK, soil population densities were low (i.e. 2 *B. longicaudatus* per 200 mL soil) at the end of the growing season in both the fluopyram and untreated control plots, which likely contributed to why no significant differences were observed between the two treatments at this farm. The relative suppression of *B. longicaudatus* by fluopyram ranged from 54 to 96% suppression at the four farms that showed significant reductions in nematode population densities. Our data are in agreement with a previously conducted field trial (Watson and Desaeger, 2019), where pre-plant application of fluopyram followed by reapplication 40 days after transplant reduced end-of-season *B. longicaudatus* population densities in soil by 97% relative to that of the untreated control. On turfgrass, fluopyram has also shown considerable potential to reduce *B. longicaudatus* populations in North Carolina (Kerns and Butler, 2018). Overall, fluopyram shows substantial nematicidal activity towards *B. longicaudatus*, and has good potential to be a component of an integrated management strategy for this nematode on strawberry in Florida.

Despite significant suppression of *B. longicaudatus* soil population densities, in-crop application of fluopyram often did not result in improvements in the two different measures of plant growth by the end of the growing season (i.e. NDVI values and row greenness); parameters which have previously shown to be effective measures of relative fruit yield (Noling, 2011). Many of the fluopyram treated plots had numerically greater NDVI values relative to that of the untreated control, as measured with the handheld crop sensor; however, only the LG site approached statistical significance by the end of the growing season. The sites that were surveyed using aerial image analysis showed a similar trend, with only site ML showing greater row greenness in the fluopyram treated plots relative to that of the untreated control. Lack of improvements in plant vigour despite successful nematode suppression may have been a result of the presence of other pests and diseases in the field that soil application of fluopyram does not control, including weeds (Gilreath et al., 2006), insects and mites (Decou, 1994), as well as oomycete and bacterial diseases (Rebollar-Alviter et al., 2007; Turechek and Peres, 2009). Although there were some minor differences observed between the two different techniques used to monitor plant growth, both techniques generally showed a similar trend. Interestingly, at site MG-NE, which had no *B. longicaudatus*, reduced plant vigour was observed in the fluopyram treated rows at six weeks post application. This finding may have been a result of suppressing beneficial fungi in the soil that contribute to improved plant growth due to the broad-spectrum fungicidal activity of fluopyram (Avenot and Michailides, 2010), suggesting that this product should only be applied to sites that are known to have nematode or fungal disease issues. Overall, minimal improvements in plant growth were observed on the current strawberry crop through in-crop application of fluopyram at commercial strawberry farms in Florida, suggesting minimal yield improvements would be obtained by the farmer.

Although there were no apparent improvements in plant growth through in-crop application of fluopyram on the current season's strawberry crop, there may have been longer term benefits to nematicide application. Many strawberry growers double-crop with a vegetable crop on the same plastic beds at the end of the strawberry growing season, and many of the vegetable crops that are planted (e.g. peppers and cucurbits) are highly susceptible to *B. longicaudatus* (Bekal and Becker, 2000). Reducing *B. longicaudatus* population densities in soil prior to planting the double-cropped vegetable could significantly reduce nematode damage on the subsequent crop. Moreover, use of in-crop rescue nematicides could provide effective nematode management options for relay-intercropping systems (Duval, 2005), and thereby contribute to increased overall farm profitability throughout the year. Our future work will evaluate the longer-term economic benefits of in-crop application of fluopyram as a rescue nematicide in strawberry production systems.

Several new non-fumigant nematicides are entering the market, and some of these products will have potential to be used as in-crop rescue nematicides. Using rescue nematicides as a reactive approach to nematode issues as opposed to the traditional prophylactic approach of applying soil fumigants to strawberry fields, regardless of nematode pressure, has the potential to reduce fumigant usage in commercial strawberry production in Florida. In-crop rescue nematicides could also provide growers with additional economic benefits because the growers could reuse the raised plastic beds for more than one strawberry growing season while maintaining low population densities of *B. longicaudatus* in the field throughout the year. Overall, in-crop rescue nematicides may provide strawberry growers with a valuable nematode management tool to be used alongside other integrated management practices in a non-fumigant soil management regime.

5. Conclusion

In this study, in-crop soil application of fluopyram reduced *B. longicaudatus* population densities by the end of the growing season in four out of six of the commercial farms where the nematode was detected during the growing season. No significant differences in plant vigour were detected by the handheld crop sensor by the end of the growing season; however, aerial image analysis revealed greater row greenness in the fluopyram treated rows at one farm. Overall, in-crop application of fluopyram shows considerable potential to reduce *B. longicaudatus* populations on strawberry in Florida.

CRediT authorship contribution statement

Tristan T. Watson: Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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