Short-Term Effects of Sublethal Doses of Nematicides on *Meloidogyne incognita*

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ABSTRACT

Meloidogyne species are one of the most important groups of plant-parasitic nematodes globally because of their ability to damage most cultivated plants. Although they are widespread and economically important, there are limited control measures to combat these nematodes. New nonfumigant nematicides have been discovered that have the potential to be widely utilized for the management of plant-parasitic nematodes. Because of the longer half-lives in soil and lower toxicity of new nematicides compared with traditional fumigant and nonfumigant nematicides, understanding how nematodes respond to sublethal doses of nematicides is imperative to understanding whether nematicide resistance has the potential to develop. Characterizing responses of nematodes to sublethal doses will provide the foundation for future work, such as gene expression studies. In this study, the nematicides oxamyl (Vydate), fluzaindolizine (Salibro), fluensulfone (Nimitz), and fluopyram (Velum), were evaluated to understand how sublethal doses affect the fecundity and mobility of *Meloidogyne incognita* second-stage juveniles (J2). Using a microwell assay system, dose-response curves for each nematicide were established for *M. incognita* J2. Fluopyram was the most toxic nematicide, with effective doses up to 230 times lower than that of other nematicides. The other nematicides had

predicted ED₅₀ values (effective doses that resulted in 50% of the population becoming inactive) of 89.4, 131.7, and 180.6 ppm for oxamyl, fluensulfone, and fluzaindolizine, respectively. The 24-h ED₅₀ of each nematicide was then used in both motility and infectivity assays. The motility and activity of *M. incognita* J2 exposed to ED₅₀ doses of fluzaindolizine and fluensulfone was significantly reduced, with nematodes initially being motile but eventually becoming inactive. However, the motility of *M. incognita* J2 exposed to ED₅₀ doses of fluopyram and oxamyl was not different from a water control. In a pot assay, *M. incognita* J2 exposed to ED₅₀ doses of fluzaindolizine, oxamyl, and fluensulfone were unable to reproduce on tomato, with reproduction factors (RF = final population density/initial population density) of 0 to 0.03. Fluopyram did not reduce reproduction of *M. incognita*, with a mean RF of 38.7 ± 4.5, which was similar to the RF of 46.3 ± 4.6 for the water control. This study is the first comprehensive evaluation of *M. incognita* activity, motility, and fecundity after exposure to the traditional nematicide, oxamyl, as well as three new nematicides, fluzaindolizine, fluopyram, and fluensulfone.

Keywords: disease control, pest management

Plant-parasitic nematodes (PPNs) cause damage to a wide variety of agricultural crops, resulting in yield and quality losses of up to \$118 billion yearly (Bernard et al. 2017). Of the PPNs, root-knot nematodes of the *Meloidogyne* species are the most important genera (Jones et al. 2013). The permanent feeding sites established in roots by these sedentary endoparasitic species during the parasitism process reduces the plant's ability to uptake nutrients and water from the soil. This leads to stunting of plant growth, wilting during mild water stress, and chlorosis (Bernard, et al. 2017; Perry and Moens 2013). The significant economic impact of *Meloidogyne* spp. is attributable to their ability to infect almost all cultivated plants and to their worldwide distribution. Of the species in this genus, *M. incognita* is the most prevalent and destructive (Mitkowski and Abawi 2003; Perry and Moens 2013; Trudgill and Blok 2001). *M. incognita* is found in 29 states in the United States, is widespread in 20 countries in Africa, and is endemic in subtropical and tropical regions around the globe (CABI 2018).

To manage PPNs, common control methods like plant resistance and trap crops can be used, but the most reliable methods of control are often chemical, including fumigates and nonfumigant nematicides (Zasada et al. 2010). Almost half of the \$1 billion global nematicide market is used to control *Meloidogyne* species (Perry and Moens 2013). Traditionally, fumigants such as methyl bromide, chloropicrin, and 1,3-dichloropropene have been heavily relied on to manage PPNs, soilborne pathogens, and weeds, owing to their broad-spectrum activity and efficacy. Other conventionally used nonfumigant nematicides include organophosphates like fosthiazate and fenamiphos and carbamates like oxamyl (Zasada et al. 2010). Both organophosphates and carbamates act as acetylcholinesterase inhibitors; their toxicity is not limited to nematodes and can be hazardous to humans and insects (Costa et al. 2008). Both methyl bromide and oxamyl have relatively short soil half-lives of 5 and 30 days, respectively (Osman et al. 2009; Yates et al. 2002). Because of harmful environmental effects, potential health hazards to workers, and/or limited supply, many of these conventional chemical products have been eliminated or are restricted (Duniway 2002; Martin 2003; U.S. Environmental Protection Agency 2008; Zasada et al. 2010). There have been few new nematicides to fill this gap in PPN management until recently in the past 5 years, with the development of fluopyram (Velum Prime), fluensulfone (Nimitz), and fluzaindolizine (Salibro).

Of these new nonfumigant nematicides, only one has a known mode of action, fluopyram. Fluopyram, also a Fungicide Resistance Action Committee code I1 fungicide, is an inhibitor of the enzyme succinate dehydrogenase and was registered for use in cotton and peanut for the control of PPNs in 2015 (Faske and Hurd 2015). Fluopyram persists much longer in the environment than traditional

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nematicides, with a half-life of up to 746 days depending on soil type (Ludlow 2015a).

Fluensulfone, a heterocyclic fluoroalkenyl sulfone, has an unknown but novel mode of action (Kearn et al. 2014). Fluensulfone is registered for use on a variety of crops from leafy and fruiting vegetables to root vegetables and tree nuts to suppress PPNs such as *Meloidogyne* spp., *Globodera* spp., and *Pratylenchus* spp. Fluensulfone suppressed *G. pallida* and *Meloidogyne* spp. in a variety of crop species, including potatoes, tomatoes, lima beans, and other vegetable crops (Castillo et al. 2017; Jones et al. 2017; Kearn et al. 2017; Morris et al. 2015, 2016). *Caenorhabditis elegans* and *M. incognita* responded similarly when exposed to similar doses of fluensulfone, with the compound resulting in immobility and death. However, *C. elegans* mutants resistant to organophosphates and carbamates were susceptible to fluensulfone, indicating that cholinesterase inhibition is not the target for this compound (Kearn et al. 2014). Fluensulfone has a half-life in soil up to 36 days (Ludlow 2015b).

Fluazaindolizine, an imidazopyridine that is still in the process of registration, is a new nematicide with a novel mode of action different from that of carbamates and organophosphates (Lahm et al. 2017). Inhibition of motility and mortality of *C. elegans* adults and *Drosophila melanogaster* egg and adult stages was not observed at concentrations of 200 and 300 ppm, respectively. However, *M. incognita* exposed to 5 to 50 ppm of fluazaindolizine were increasingly immobile and dead at 24 to 96 h after exposure (Lahm et al. 2017). This indicates that the target of fluazaindolizine is PPN specific.

Nonfumigant nematicides are applied in granular or liquid form and move through the soil via percolation. Effective PPN control with nematicides is only accomplished if a lethal dose of the compound is maintained for the right exposure period. There are many factors that affect nematicide movement and retention in soil, including soil type, affinity of the nematicide to organic matter, mobility of the nematicide, the amount of water applied with the nematicide, speed of microbial degradation, and half-life of the nematicide in the soil (Noling 1997). These variables create a soil environment where sublethal doses are likely to occur and therefore, PPNs will be exposed to a range of concentrations of a compound. This variable dose exposure of nematicides to PPNs has been demonstrated for oxamyl (Haydock et al. 2012). Oxamyl degraded at different rates depending on soil temperature, soil moisture, and soil type. In this study, an increase in soil temperature and loamy sand soil type led to an increase in oxamyl degradation, whereas low soil moisture led to oxamyl retention. The soil dynamics listed above and the longer half-lives of newer nematicides (ranging from 32 to >700 days) combine to create an environment where PPNs have the potential to be exposed to nematicides at sublethal concentrations. Therefore, the goal of this research was to characterize the short-term effects of sublethal concentrations of nematicides on *M. incognita* in order to create a foundation for further work aimed at understanding the underpinnings of how these nematicides affect nematode biology. The objectives of this study were as follows: (i) to develop dose-response curves for the nematicides using *M. incognita*, (ii) to evaluate the effect that sublethal doses of nematicides have on *M. incognita* motility, and (iii) to assess the effect of sublethal doses of nematicides on *M. incognita* fecundity. The nematicides oxamyl (Vydate), fluensulfone (Nimitz), fluopyram (Velum Prime), and fluazaindolizine (Salibro) were evaluated.

MATERIALS AND METHODS

Nematode inoculum. An *M. incognita* population originally collected from grape (*Vitis vinifera*) in Parlier, California, was used in all experiments. To establish the population in culture, a single egg mass was placed on the roots of tomato (*Solanum lycopersicum* 'Rutgers'). After 6 to 8 weeks, additional tomato plants were

inoculated with egg masses hand-picked from the plant inoculated with a single egg mass. *M. incognita* second-stage juveniles (J2) were collected by hand picking egg masses from established culture tomato roots and placing the masses on a 1.5-cm-diameter plastic hatching chamber with a 30- μ m nylon mesh in a 40-ml beaker containing water (Nitao et al. 1999; Zasada et al. 2006). Hatched *M. incognita* J2 were collected after 3 days and stored at 4°C until used (no more than 2 days). *M. incognita* eggs were obtained by covering infected tomato roots with 0.6% sodium hypochlorite and agitating for 3 min at 300 rpm. Working in a laminar flow hood, the roots were rinsed with autoclaved deionized water over sterilized, nested 250- and 25.4- μ m sieves, with eggs collected from the latter.

Microwell dose-response assays. Formulated product was used in all assays. Therefore, all concentrations shown are those of the formulated product, not the active ingredient (a.i.). The percentage of active ingredient is as follows for each product tested: Vydate-L, 24.0% oxamyl; Nimitz, 40.0% fluensulfone; Velum Prime, 41.5% fluopyram; and Salibro, 50.0% fluazaindolizine. To establish dose-response curves of the short-term effects on *M. incognita* J2 for each nematicide, microwell assays were conducted (Meyer et al. 2008; Zasada et al. 2006). In each well of a 24-well cell culture plate (Falcon; Corning, Corning, NY), 50 to 60 *M. incognita* J2 suspended in 100 μ l of water were pipetted into 900 μ l of nematicide solution. Each microwell plate contained a single nematicide at five concentrations, with a water control, replicated four times. Preliminary studies identified an effective range of concentrations for each compound, which were then used to identify nematicide concentrations to test to generate dose-response curves (data not shown). The plates were sealed with parafilm (Parafilm; Bemis Company, Neenah, WI), covered with foil, and incubated at room temperature for 24 h, a time point used in previous studies to represent short-term effects (Faske and Hurd 2015; Oka 2014). After the incubation period, the plate was placed under 100 $\times$ magnification on an inverted microscope (Leica DM IL; Leica Microsystems, Wetzlar, Germany) and the number of active versus inactive *M. incognita* was determined. Inactive nematodes were straight and not moving, whereas moving nematodes were considered active even if they exhibited abnormal movements (sluggishness, twitching, etc.). The nematicide solution was then removed from each well using a pipette and replaced with 1 ml of water to establish whether the effects of the nematicides were reversible. After another 24-h incubation at room temperature, the number of active versus inactive nematodes was determined as described above. Each microwell assay was repeated at least two times encompassing a range of concentrations for each nematicide. The concentrations tested for each nematicide were as follows: fluazaindolizine, 210 to 460 ppm; fluopyram, 0.5 to 4 ppm; fluensulfone, 25 to 700 ppm; and oxamyl, 60 to 140 ppm.

Experiments to evaluate the effects of nematicides on egg hatch of *M. incognita* were also conducted using the same microwell assay system described above. To each well, 100 eggs suspended in 100 μ l of water were added to nematicide solutions. The concentrations tested varied for each nematicide but represented a range of effective concentrations for each nematicide as established by the *M. incognita* J2 dose-response assays. The 24-well plates were sealed with parafilm, covered, and incubated at room temperature for 7 days. The number of hatched J2 and the percentage active J2 were then determined using the same methodology described above. Each assay was conducted at least twice and concentrations were replicated four times.

Motility assays. To determine the effects of sublethal doses of fluazaindolizine, oxamyl, fluensulfone, and fluopyram on the movement of *M. incognita* J2, motility assays were conducted. In a 1.7-ml microcentrifuge tube, 500 *M. incognita* J2 were suspended in 100 μ l of water and treated with 900 μ l of a nematicide solution. The nematicide solution was equivalent to an ED₅₀ dose (effective dose needed to cause 50% of the population to become inactive) of each nematicide established in the *M. incognita* J2 dose-response

assays (fluazaindolizine, 230 ppm; oxamyl, 100 ppm; fluopyram, 1 ppm; and fluensulfone, 95 ppm). After 2 and 24 h of exposure, 500 µl from each tube was placed on a 30-µm mesh hatching sieve, rinsed with water for 5 to 10 s, and placed in a 60- × 15-mm Petri dish containing enough water to just cover the mesh (Nitao et al. 1999). The dishes were covered and incubated for 24 h at room temperature, after which the sieve was removed, the Petri dish was placed under a dissecting microscope (Leica M205 C) at 2× magnification, and active versus inactive *M. incognita* J2 were enumerated. Each time point was replicated six times per nematicide and the assay was conducted three times.

Infectivity assays. To test the effects of sublethal doses of nematicides on reproduction of *M. incognita*, potted tomato Rutgers plants were treated with ED₅₀ doses of nematicides. Two- to three-week-old tomato seedlings were transplanted into a sand/loam mix (1:1) in 10-cm pots (Anderson Pots no. 1007; Anderson Die & Manufacturing Co., Portland, OR). Pots were then drenched with 145 ml of nematicide solution (fluazaindolizine, fluensulfone, fluopyram, or oxamyl at an ED₅₀ dose indicated above for motility assay) or water. Immediately after drenching, plants were inoculated with 2,500 *M. incognita* J2 suspended in 3 ml of water, by pipetting the solution containing nematodes into two holes 4.5 cm deep on either side of the plant into the root zone. Each nematicide treatment and the water control were replicated six times and the experiment was conducted twice. Inoculated plants were arranged in a randomized design in a greenhouse and grown under long-day conditions (16-h photoperiod) with 26/18°C day/night temperatures. After 1 week, plants were fertilized using a 9N-45P-15K plant starter fertilizer at the labeled rate (Jack's Professional Plant Starter; JR Peters Inc., Allentown, PA). Thereafter, plants were watered twice a week with 20N-20P-20K fertilizer at the labeled rate (JR Peters Inc.). After 6 to 7 weeks, plants were destructively harvested. The tops were removed and discarded, and roots were gently washed with water to remove adhering soil. Eggs were extracted by placing rinsed roots in 0.6% sodium hypochlorite and agitating at 300 rpm for 3 min. Roots were then rinsed over nested 250- and 25.4-µm sieves, with eggs collected from the latter. Eggs were enumerated using an inverted microscope as described above. Roots were dried in a 65°C oven for 7 days, after which dry roots were weighed.

Statistical analyses. All statistical analyses were conducted using R software (R Core Team 2017). A dose-response curve model was created for *M. incognita* J2 using the R package *drc* (Ritz et al. 2015); specifically, a three-parameter log-logistic model was

used for the dose-response curves of oxamyl and fluensulfone, and a four parameter log-logistic model was used for fluopyram and fluazaindolizine. The microwell assay data were transformed using a Box-Cox transformation to correct for variance heterogeneity and nonnormality. Each model was then utilized to determine the ED₅₀ concentration in the *drc* package following the methodology outlined in Ritz et al. (2015). Data from the egg hatch dose-response, motility, and infectivity assays were analyzed using the Kruskal-Wallis test and mean separation using the Wilcoxon rank sum comparisons test with a Bonferroni multiple comparison correction. The reproduction factor (RF) was calculated by dividing the final egg density (*Pf*) by the initial density of *M. incognita* J2 (*Pi*) (Windham and Williams 1987). All values are presented as the mean ± standard error.

RESULTS

Microwell dose-response assays. The proportion of active *M. incognita* J2 after 24-h exposure and after the compound was removed and replaced with water was modeled using a log-logistic regression model with four potential parameters (Table 1). The parameters were as follows: *b*, the coefficient denoting steepness of the dose-response curve; *c* and *d*, the lower and upper asymptotes of the response curve, respectively; and *e*, the effective dose needed to cause 50% of the population to become inactive (ED₅₀) (Ritz et al. 2015). The models for fluazaindolizine and fluopyram were modeled using all four parameters. However, the models for fluensulfone and oxamyl were made using a three-parameter model, with *c* (the lower asymptote parameter) fixed at 0.

Fluopyram had the smallest range of concentrations in the dose-response curves (Table 2), 100 times smaller than that of the other compounds, making fluopyram the most toxic of the nematicides evaluated. However, this nematicide, behaved like a nemastatic compound in the short term (Fig. 1A). When the compound was removed and replaced with water, the proportion of *M. incognita* J2 that recovered was between 12.5 and 275% with increasing concentrations.

The dose-response curve for fluensulfone had the shallowest slope of the four curves (Fig. 1B). Fluensulfone behaved like a true nematicide in the short term, with <20% of *M. incognita* J2 recovering after exposure to the nematicide. Fluensulfone had a predicted ED₅₀ intermediate to oxamyl and fluazaindolizine (Table 2).

Of the four nematicides, oxamyl had the steepest slope parameter, approximately four times larger than the other three compounds

TABLE 1. Dose-response curve log-logistic equation parameters for *Meloidogyne incognita* second-stage juveniles exposed to nematicides

Nematicide	Exposure period	Model parameter			
		<i>b</i> ^w	<i>c</i> ^x	<i>d</i>	<i>e</i> ^y
Fluopyram	24 h	1.68 (0.42) ^z <i>P</i> < 0.0001	7.64 (12.98) <i>P</i> < 0.0001	98.53 (2.99) <i>P</i> < 0.0001	1.31 (0.29) <i>P</i> < 0.0001
	24 h after replacement	3.80 (3.17) <i>P</i> = 0.233	65.24 (18.07) <i>P</i> = 0.00045	93.41 (2.19) <i>P</i> < 0.0001	2.63 (1.19) <i>P</i> = 0.029
Oxamyl	24 h	8.24 (1.45) <i>P</i> < 0.0001	Fixed	80.42(6.69) <i>P</i> < 0.0001	89.34 (4.56) <i>P</i> < 0.0001
	24 h after replacement	10.16 (1.50) <i>P</i> < 0.0001	Fixed	79.81(5.58) <i>P</i> < 0.0001	86.71 (2.96) <i>P</i> < 0.0001
Fluensulfone	24 h	0.90 (0.07) <i>P</i> < 0.0001	Fixed	94.14 (3.03) <i>P</i> < 0.0001	131.72 (12.81) <i>P</i> < 0.0001
	24 h after replacement	2.26 (0.21) <i>P</i> < 0.0001	Fixed	94.92 (3.06) <i>P</i> < 0.0001	13.54 (1.75) <i>P</i> < 0.0001
Fluazaindolizine	24 h	2.57 (1.07) <i>P</i> = 0.0176	41.78 (5.88) <i>P</i> < 0.0001	91.80 (2.82) <i>P</i> < 0.0001	180.59 (20.18) <i>P</i> < 0.0001
	24 h after replacement	1.76 (0.45) <i>P</i> < 0.0001	-20.61 (16.86) <i>P</i> = 0.223	89.19 (2.76) <i>P</i> < 0.0001	245.33 (45.89) <i>P</i> < 0.0001

^w *b* is the coefficient denoting steepness of the dose-response curve.

^x *c* and *d* are the lower and upper asymptotes of the response curve, respectively, fixed to zero in some models.

^y *e* is the effective dose needed to cause 50% of the population to become inactive.

^z Model parameter, followed by the standard error in parentheses and the *P* value for the parameter.

(Table 1). Once past 60 ppm, even a small increase in dosage resulted in a sharp decrease in the proportion of active *M. incognita* J2 after 24-h exposure. Oxamyl was nematocidal in the short-term assay (Fig. 1C), with no recovery of *M. incognita* J2 even after the compound was removed and replaced with water.

Fluazaindolizine also acted as a true nematocide (Fig. 1D). *M. incognita* J2 continued to die after the compound was replaced

TABLE 2. Comparison of nematicide doses that resulted in 50% of the population of *Meloidogyne incognita* second-stage juveniles becoming inactive (ED₅₀) in microwell assays

Nematicide	% a.i. ^y	Model predicted ED ₅₀ (ppm) ^z	ED ₅₀ used in mobility and infectivity assays (ppm)
Fluopyram	41.5	1.30 (0.74–1.88)	1
Oxamyl	24.0	89.39 (80.33–98.45)	100
Fluensulfone	40.0	131.72 (106.52–156.92)	95
Fluazaindolizine	50.0	180.59 (140.81–220.37)	230

^y Percentage of active ingredient of formulated nematicide.
^z The 95% confidence interval for each ED₅₀ is enclosed in parentheses.

with water, with the proportion of active *M. incognita* J2 decreasing 18.8 to 100% with increasing concentrations. The model predicted the highest 24-h ED₅₀ for fluazaindolizine of the nematicides tested (Table 2).

The effects of fluopyram, oxamyl, fluensulfone, and fluazaindolizine on *M. incognita* egg hatch and activity of hatched J2 are shown in Figure 2. Only the highest concentration of fluopyram tested (2.5 ppm) reduced *M. incognita* egg hatch compared with the water control (Fig. 2A). Of the *M. incognita* J2 that hatched, fluopyram concentrations ≥ 1.0 ppm had significantly fewer active *M. incognita* J2 compared with the water control (Fig. 2E). At the concentration of fluopyram identified as the 24-h ED₅₀ (1 ppm), there was no difference from the control in the mean percentage of *M. incognita* eggs that hatched; however, the mean percentage of active hatched *M. incognita* J2 was 44% lower than the water control (*P* = 0.017).

Oxamyl reduced *M. incognita* egg hatch at concentrations ≥ 50 ppm compared with the water control (Fig. 2B). The same trend was observed in the mean percentage of active hatched *M. incognita* J2 (Fig. 2F). The 24-h ED₅₀ for oxamyl (100 ppm) reduced egg hatch

Fig. 1. Dose-response curves of *Meloidogyne incognita* second-stage juveniles (J2) exposed to nematicides. *M. incognita* J2 were exposed to **A**, fluopyram, **B**, fluensulfone, **C**, oxamyl, and **D**, fluazaindolizine at a range of concentrations for 24 h. After 24 h, the percentage of active J2 was determined and the nematicide solution was replaced with water. After another 24 h, the percentage of active J2 was determined to ascertain whether the effects of the compounds on *M. incognita* J2 were reversible. Dose-response curves were determined for Box-Cox transformed data using a log-logistic model with three parameters for fluensulfone and oxamyl and four parameters for fluazaindolizine and fluopyram. The solid line is the curve representing the first 24-h exposure period, with the solid circles indicating each data point. The dotted line is the model for the period after the nematicide was removed, with the individual data points represented as triangles. In both models, the 95% confidence interval is represented by the shaded region around the curve.

compared with the water control by 86% ($P = 0.014$) and reduced the mean percentage of active hatched *M. incognita* by 93% ($P = 0.005$).

At all tested concentrations, fluensulfone reduced *M. incognita* egg hatch and percentage of active hatched *M. incognita* J2 compared with the water control ($P < 0.0002$; Fig. 2C and G). Across concentrations, fluensulfone reduced egg hatch by at least

86% compared with the water control. A 100% reduction in the mean percentage of active hatched *M. incognita* J2 was observed at all concentrations compared with the water control ($P < 0.0001$). The fluensulfone 24-h ED_{50} (95 ppm) followed these same trends.

Fluazaindolizine was a poor ovicide with effects on *M. incognita* egg hatch only at the two highest concentrations tested, 390 and

Fig. 2. *Meloidogyne incognita* egg hatch after exposure to nematicides. *M. incognita* eggs were exposed to **A and E**, fluopyram, **B and F**, oxamyl, **C and G**, fluensulfone, and **D and H**, fluazaindolizine for 7 days. The percentage of hatched second-stage juveniles (J2) (graphs on the left) and the percentage of the hatched J2 that were still active calculated for each compound (graphs on the right) are shown. Columns are the mean of eight replicates; error bars represent the standard error. Data within a graph that share a letter are not significantly different ($P \leq 0.05$) based on the Kruskal-Wallis test and mean separation using the Wilcoxon rank sum comparisons test with a Bonferroni multiple comparison correction.

460 ppm ($P < 0.0001$; Fig. 2D). At these two highest concentrations, egg hatch was reduced by 51 and 59%, respectively, compared with the water control. Fluazaindolizine had more of an effect on the mean percentage of active hatched *M. incognita* J2, with significant differences from the water control at all concentrations tested, except at 150 ppm ($P = 0.034$; Fig. 2H).

Motility assays. Motility of *M. incognita* J2 exposed to ED₅₀ doses of oxamyl, fluopyram, fluazaindolizine, and fluensulfone did not differ from the water control at 2 and 24 h of exposure (Figs. 3A and C). However, differences were observed with fluensulfone and fluazaindolizine in the percentage of active *M. incognita* J2 recovered (Fig. 3B and D). At both 2 and 24 h of exposure to fluensulfone, there was a 61 and 98% reduction in the percentage of active *M. incognita* J2, respectively, compared with the water control ($P < 0.0001$). Fluazaindolizine resulted in a 42 and 91%

reduction in the percentage of active *M. incognita* J2 at 2 and 24 h of exposure compared with the water control, respectively ($P < 0.0001$). Oxamyl and fluopyram had no effect on the percentage of active *M. incognita* J2 compared with the water control at either exposure period (Fig. 3B and D).

Infectivity assays. When nematicides were applied at sublethal doses, only fluopyram was unable to prevent reproduction of *M. incognita* (Fig. 4A), with rates of reproduction similar to the untreated control ($P = 1.0$). The other nematicides had a mean RF of 0 and were significantly different from the untreated control ($P < 0.0001$).

Plants treated with fluensulfone had the lowest mean root weight (0.31 ± 0.03 g) compared with root weights of the untreated control and fluopyram ($P < 0.001$; Fig. 4B). Fluopyram was the only treatment that had similar root weights to that of the untreated control ($P = 0.94$). Root weights were reduced by 56, 51, and 35%

Fig. 3. *Meloidogyne incognita* second-stage juvenile (J2) motility after exposure to nematicides. *M. incognita* J2 were exposed to fluensulfone, fluazaindolizine, fluopyram, and oxamyl at 95, 230, 1, and 100 ppm, respectively, for 2 and 24 h. **A and C**, Recovered *M. incognita* J2 that were able to swim through a 25- μ m sieve after exposure for 2 and 24 h are shown, respectively. **B and D**, The percentage of recovered *M. incognita* J2 that were active after 2- and 24-h exposure are shown, respectively. Columns are the mean of 12 replicates; error bars represent the standard error. Data within a graph that share a letter are not significantly different ($P \leq 0.05$) based on the Kruskal-Wallis test and mean separation using the Wilcoxon rank sum comparisons test with a Bonferroni multiple comparison correction.

for fluensulfone, fluazaindolizine, and oxamyl, respectively, compared with the untreated control.

DISCUSSION

M. incognita is a major global pest, with few effective chemical controls available. Of the new nematicides to come onto the market, there have been few published side-by-side comparisons as well as comparisons to industry standards. This study is the first comprehensive evaluation of *M. incognita* activity, motility, and fecundity after exposure to the traditional nematicide, oxamyl, as well as three new nematicides, fluazaindolizine, fluopyram, and fluensulfone. There are many factors that affect persistence and effective concentrations of nematicides in soil, leading to an environment where PPNs are often exposed to sublethal doses of nematicides. Continual exposure to sublethal doses of nematicides could lead to selection of PPNs that are resistant to nematicides, limiting the resources available to manage these pests, which is a profound problem in other phytopathogen systems. Identifying sublethal doses of new nonfumigant nematicides will set the stage for future studies to identify modes of action, cellular effects, effects on gene expression, and possible insights into resistance development.

Oxamyl, a compound used to effectively manage *M. incognita* on a variety of hosts, has traditionally been thought to be a nematostatic compound (McGarvey et al. 1984). *M. incognita* J2 were able to recover from exposure to 4,000 ppm of oxamyl for 10 min, when placed in water for 24 h after exposure (McGarvey et al. 1984). In this study, the effect of oxamyl on *M. incognita* activity, motility, and fecundity was variable. Oxamyl was nematocidal as shown in the dose-response and fecundity assays. When exposed to a range of oxamyl concentrations (50 to 140 ppm) for 24 h and then placed in water for an additional 24 h, *M. incognita* J2 were unable to recover. Moreover, oxamyl behaved as a true nematicide, with *M. incognita* exposed to a sublethal dose of oxamyl unable to reproduce on

tomato. However, in the motility assays, *M. incognita* J2 were able to move and survive after exposure to oxamyl for 2 or 24 h, indicating that oxamyl was nematostatic similar to what was observed by McGarvey et al. (1984). A future experiment looking at rates of movement after nematicide exposure could be beneficial to determine when impact on movement/motility occurs. Oxamyl was also a good ovicide at higher doses, with an 86% reduction of hatch when eggs were exposed to 100 ppm of oxamyl.

Fluensulfone, a nematicide registered in 2014, behaved like a true nematicide in our short-term exposure assays. *M. incognita* J2 inactivity continued to increase after fluensulfone was removed and replaced with water even at low concentrations. At 25 ppm, inactivity increased by 60% once fluensulfone was removed and replaced with water. Fluensulfone was also an effective ovicide, with a 90% reduction in hatch at the lowest concentration of fluensulfone tested. Oxamyl and fluensulfone had similar ranges of effective concentrations with similar ED₅₀ values of 100 and 95 ppm, respectively. However, unlike oxamyl, fluensulfone had no effect on nematode motility at 2 and 24 h of exposure, but activity of motile nematodes was severely affected. This pattern of inactivity followed that observed in the microwell assays; once *M. incognita* J2 were exposed to this nematicide even at low doses, they were unable to recover. This was further exemplified when *M. incognita* J2 were exposed to a sublethal dose of fluensulfone in soil and were unable to reproduce on a susceptible host. It is important to note that fluensulfone may be phytotoxic. Fluensulfone had the lowest root biomass of all treatments. This could be an indication of phytotoxicity of this compound or root damage caused by early nematode feeding. Castillo et al. (2017) did not find tomato vigor or yield to be affected when fluensulfone was drip applied at a midlabel rate of 5.85 liter/ha. Further evaluation of the effects of fluensulfone on plant vigor are required.

Fluazaindolizine, a nematicide not yet registered by the U.S. Environmental Protection Agency, has no literature available about its efficacy in a greenhouse or field setting against *M. incognita* or

Fig. 4. *Meloidogyne incognita* fecundity on tomato (*Solanum lycopersicum* ‘Rutgers’) treated with nematicides. Tomato seedlings were drenched with the nematicides fluensulfone, fluazaindolizine, fluopyram, and oxamyl at 95, 230, 1, and 100 ppm, respectively. After drench, *M. incognita* J2 were inoculated onto the tomatoes. Reproduction factor values were calculated by dividing the final population density of *M. incognita* by the initial *M. incognita* population density. **A**, Reproduction factor values for *M. incognita* for each nematicide and **B**, root weight are shown. Columns are the mean of 12 replicates; error bars represent the standard error. Data within a graph that share a letter are not significantly different ($P \leq 0.05$) based on the Kruskal-Wallis test and mean separation using the Wilcoxon rank sum comparisons test with a Bonferroni multiple comparison correction.

other PPNs. In our short-term assays, it behaved as a true nematicide against *M. incognita*. *M. incognita* J2 were unable to recover after exposure to fluazaindolizine. Of all the nematicides tested, fluazaindolizine required the highest concentration to be effective. Fluazaindolizine had a 24-h ED₅₀ of 230 ppm, which was more than twice that of oxamyl. *M. incognita* J2 exposed to even high doses of fluazaindolizine did not have high rates of inactivity at 24 h of exposure. However, removal of the compound did not result in an increase in active J2; rather, there was an increase in inactivity between 13 to 31% for all concentrations tested. Unlike fluensulfone, fluazaindolizine had little effect on egg hatch. Only at 390 and 460 ppm of fluazaindolizine was egg hatch significantly reduced. Similar to the other nematicides tested, *M. incognita* J2 motility was not affected by fluazaindolizine; but like fluensulfone, activity of the motile *M. incognita* J2 was significantly affected. Motile *M. incognita* J2 exposed to fluazaindolizine for 2 and 24 h had inactivity rates of 50 and 94%, respectively. Combined with the effects of fluazaindolizine shown in the dose-response curves, fluazaindolizine is a slower-acting nematicide compared with fluensulfone. This is consistent with results by Lahm et al. (2017), in which *M. incognita* J2 exposed to low doses (5 to 50 ppm a.i.) of fluazaindolizine showed no symptoms in the first hours after exposure but ultimately died 24 to 96 h later. Fluazaindolizine was very effective at preventing reproduction of *M. incognita* on tomato even when soil was treated with a sublethal dose.

Fluopyram was the only nematicide found to be nematostatic in these short-term exposure assays. At all concentrations evaluated (0.5 to 4 ppm), at least 80% of *M. incognita* J2 were able to recover from exposure. Faske and Hurd (2015) demonstrated that *M. incognita* J2 exposed to fluopyram at 5.58 ppm for 2 h reduced mobility by 80%. However, their results also showed a 58% recovery of mobility after removal of the nematicide, consistent with *M. incognita* J2 recovery shown in this study. Faske and Hurd (2015) also determined a 24-h ED₅₀ of 1.18 ppm and a 24-h ED₉₀ of 5.31 ppm compared with the predicted ED₅₀ and ED₉₀ of 1.30 and 4.85 ppm found in this study, respectively. Egg hatch was unaffected by all but one concentration tested, making fluopyram a poor ovicide against *M. incognita*. Fluopyram had no effect on the motility of nematodes and little impact on the activity of nematodes that were motile. This again reflects the nematostatic nature of this compound. Fluopyram also did not suppress reproduction of *M. incognita* on tomato when soil was drenched with a sublethal dose. This is in contrast to a 31% reduction in root galling of tomato compared with the water control when *M. incognita* J2 were exposed to 1.3 ppm of fluopyram for 2 h and inoculated onto tomato (Faske and Hurd 2015).

Several studies have evaluated these new nematicides in greenhouse, microplot, and field trials. Fluopyram, oxamyl, and fluensulfone have been reported as effective nematicides for controlling *M. incognita* in lima beans, both in greenhouse trials and microplots (Jones et al. 2017). Lima beans treated with oxamyl at a rate of 2.25 liter a.i./ha reduced *M. incognita* galling by 10 to 70%. In this same trial, fluopyram seemed to perform just as well as oxamyl at a rate of 0.22 liter a.i./ha, with a reduction in *M. incognita* galling on lima beans of 55 and 64%. Fluensulfone performed the best, with a 91 and 81% reduction in *M. incognita* galling on lima beans when applied at 2.34 liter a.i./ha. These results (Jones et al. 2017) support the findings of this study, in that oxamyl and fluensulfone are both strong nematicides and effective treatments for *M. incognita*. The results of Jones et al. (2017) also indicate that in order for fluopyram to be an effective nematicide, dosage is important, with control observed in the lima bean study, but none found in this study when a sublethal dose of fluopyram was used. Jones et al. (2017) also indicated that fluensulfone had potentially phytotoxic effects at the 2.34 liter a.i./ha rate of application in a microplot trial where there was a 14% reduction in lima bean emergence, consistent with the low tomato root weights shown in this study.

When fluensulfone was applied at a rate of 3.0 kg a.i./ha through drip-pulse irrigation to cucumber, there was a 73% reduction in *M. incognita* J2 population densities along with a reduction in root galling (Morris et al. 2016). In growth chamber trials conducted in the same study (Morris et al. 2016), fluensulfone was applied foliarly to tomato at rates of 3,000, 6,000 and 12,000 ppm; an additional treatment in the study was foliar applied oxamyl at a rate of 4,800 ppm. All of the treatments reduced root galling by *M. incognita* and the number of females in the root system compared with an untreated control, but the treatments were unable to eliminate *M. incognita* reproduction. Oxamyl was the most effective treatment in reducing *M. incognita* females and galling except in comparison with the highest dosage of fluensulfone. In this study, when drenching the soil at rates a fraction of those used by Morris et al. (2016), oxamyl at 100 ppm and fluensulfone at 95 ppm both eliminated reproduction of *M. incognita*. This finding, along with the success of drip application of fluensulfone in another study (Morris et al. 2016), suggests that fluensulfone works best when applied to the root system.

Fluopyram applied at the labeled rate (249 g a.i./ha) to the surface of tomato plants 2 days postinoculation with *M. incognita* eggs had no effect on the number of *M. incognita* eggs per gram of root compared with an untreated control (Heiken 2017). These results support those found in this study; fluopyram was a poor nematicide when applied as a soil drench at low doses. However, Heiken (2017) found that when fluopyram was applied at the labeled rate 2 weeks after tomato plants were inoculated with *M. incognita* eggs, fluopyram was able to reduce the number of eggs per gram of root from 16,000 to <1,500 compared with the untreated control. In the present study, fluopyram was a poor ovicide, with the only effects on *M. incognita* egg hatch observed at the highest concentration tested. The timing and stage of *M. incognita* present when treatment is applied may be critical to the efficacy of this nematicide.

Overall, the newly developed nematicides, fluensulfone and fluazaindolizine were comparable in their efficaciousness against *M. incognita* to the traditionally used nematicide oxamyl. Even at sublethal doses, these three nematicides suppressed *M. incognita* in fecundity assays. However, the effects of these compounds on plant vigor remain unclear and warrant further exploration. All of the nematicides tested had limited effects on short-term *M. incognita* J2 motility, but this did not affect the ability of these compounds to protect against nematode infection. In this study, fluopyram was the least effective nematicide against *M. incognita*. Fluensulfone proved to be the only ovicidal compound at sublethal doses, with oxamyl having ovicidal activity at higher concentrations. The dose-response curves developed in this study will be beneficial in future research, as the ED₅₀ doses determined could be used to explore the broader effects of nematicides on *M. incognita* biology and potentially understanding the modes of action of fluazaindolizine and fluensulfone.

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