Differential Response of *Meloidogyne*, *Pratylenchus*, *Globodera*, and *Xiphinema* Species to the Nematicide Fluazaindolizine

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ABSTRACT

This research focused on the effects of fluazaindolizine on a diversity of plant-parasitic nematodes. In microwell assays, 24-h dose-response curves were generated for several species and populations of *Meloidogyne*, *Pratylenchus neglectus*, *P. penetrans*, *Globodera ellingtonae*, and *Xiphinema americanum*. In a greenhouse study, the impact of fluazaindolizine on fecundity of *M. incognita*, *M. hapla*, and *M. chitwoodi* was tested by exposing nematodes for 24 h in solution and inoculating on tomato. The average 24-h ED₅₀s (dose that resulted in the immobility of 50% of exposed nematodes) for *M. hapla*, *M. chitwoodi*, and *M. incognita* were 325.7, 223.4, and 100.7 ppm, respectively. *M. hapla* had the most variation among populations, with 24-h ED₅₀s ranging from 72 to 788 ppm. *G. ellingtonae* had the lowest 24-h ED₅₀ at 30 ppm. *Pratylenchus* spp. were unaffected by

fluazaindolizine. *X. americanum* was the only species where effects of fluazaindolizine were reversible, but had a 24-h ED₅₀ that fell in the range of the *Meloidogyne* spp. In the greenhouse study, *M. chitwoodi* was the least sensitive with reproduction reaching 62% of the untreated control after a pre-exposure to 47 ppm, whereas *M. incognita* and *M. hapla* at the same exposure dose had reproduction rates of 27 and 36% of the untreated control, respectively. Despite varying in vitro responses to fluazaindolizine, reproduction of all *Meloidogyne* spp. was suppressed after only 24 h of exposure. This study expanded our understanding of how *G. ellingtonae*, *P. thornei*, *P. penetrans*, and *X. americanum* respond to fluazaindolizine.

Keywords: disease control and pest management, nematology

Plant-parasitic nematodes are significant agricultural pests due to their vast host ranges and destructive life styles, which have negative effects on yields and quality of annual and perennial crops. Cyst (*Globodera* spp.) and root-knot (*Meloidogyne* spp.) nematodes stand out as two of the most damaging plant-parasitic nematode genera due to their endoparasitic life-style and ability to manipulate host cells (Jones et al. 2013). *Globodera* spp. are considered quarantine pests throughout the world; in the U.K. alone, they are responsible for 9% loss of potato yield each year (Moxnes and Hausken 2007). *G. ellingtonae*, while demonstrated to have limited to no effect on potato yield (Zasada et al. 2019), has a close phylogenetic relationship to the quarantine pests *G. pallida* and *G. rostochiensis* (Skantar et al. 2011), making it a good proxy for understanding the effects of nematicides on this genus of nematodes. *Meloidogyne* spp. are of great significance due to their global distribution and wide host range (Bernard et al. 2017; Perry and Moens 2013). This group of nematodes can cause yield loss in most major agricultural crops, up to 20% loss in solanaceous vegetables and wine grapes (Koenning et al. 1999; Zasada et al. 2012) and up to 10% loss in corn (Koenning et al. 1999). The group of migratory ectoparasites, *Pratylenchus* spp. also impact longevity and lifetime yield of perennial crops (Quero-Garcia et al. 2017) and are destructive

in row crops, where they have been reported to reduce yield of wheat up to 85% (Nicol et al. 2003). Another group of migratory ectoparasites of global importance are *Xiphinema* spp. This group of nematodes cause root damage and inhibits nutrient and water uptake. Some species also vector plant viruses such as *Tobacco ringspot virus* and *Tomato ringspot virus* (Perry and Moens 2013; Wang et al. 2002). Due to the importance of these plant-parasitic nematodes, species and populations within these genera were the focus of this current study.

Management of plant-parasitic nematodes has changed over the past few decades because of constraints placed on chemical controls to reduce potential risks to the environment and human health (Duniway 2002; EPA 2008; Martin 2003; Zasada et al. 2010). This push for more environmentally and applicator friendly management strategies has led to the development of new chemical controls like fluazaindolizine, Salibro (Lahm et al. 2017). Fluazaindolizine, an imidazopyridine is not yet registered for widespread use. The mode-of-action is currently unknown for fluazaindolizine, however, Lahm et al. (2017) found no evidence to support a mode-of-action similar to that of carbamates or organophosphates. Toxicity of this compound seems to be plant-parasitic nematode specific. When *Caenorhabditis elegans* adults and *Drosophila melanogaster* egg and adult stages were exposed to 200 and 300 ppm of fluazaindolizine, immobility or mortality were not observed (Lahm et al. 2017). The bacteria feeding nematode, *Acrobeloides buetschlii* was exposed to concentrations of fluazaindolizine up to 250 ppm and hatching and movement remained unaffected after 5- and 7-days exposure (Thoden and Wiles 2019).

Other studies have shown the short-term sensitivity of *M. incognita*, *M. javanica*, and *M. hapla* to fluazaindolizine in laboratory assays (Lahm et al. 2017; Thoden and Wiles 2019; Thoden et al. 2019; Wram and Zasada 2019). Thoden et al. (2019) observed population response differences in movement in an aqueous environment and ability to move through sand among five populations each of *M. incognita* and *M. javanica* after exposure to fluazaindolizine. In the same study, *M. javanica* was less sensitive to the effects of fluazaindolizine than *M. incognita*. The observance of differential population response is not limited to fluazaindolizine,

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and differential response was also observed when *Meloidogyne* spp. were exposed to oxamyl (Thoden et al. 2019). Differential response of *Heterodera glycines* populations to penetrate soybean roots was also observed after exposure to aldicarb (Gourd et al. 1993). Application of fluzaindolizine in a field setting has yielded results varying from full suppression of *M. incognita* reproduction to no effect on various hosts in many geographical locations (Becker et al. 2019; Hajihassani et al. 2019; Silva et al. 2019; Watson and Desaegeer 2019), indicating population, and therefore genetic background, could potentially play a role in fluzaindolizine efficacy.

To determine if population has an impact on sensitivity to fluzaindolizine, dose-response curves were created for four populations of *M. chitwoodi*, 10 populations of *M. hapla*, and two populations of *M. incognita*. Additionally, a population each of *M. incognita* and *M. chitwoodi* and two populations of *M. hapla* were pre-exposed for 24 h to fluzaindolizine and inoculated onto a susceptible tomato cultivar to assess impacts on fecundity. Additionally, to expand the knowledge of which plant-parasitic nematode species are sensitive to fluzaindolizine, dose-response curves were generated for one population each of *G. ellingtonae*, *X. americanum*, and *P. neglectus*, and two populations of *P. penetrans*.

MATERIALS AND METHODS

Nematode collection. The plant-parasitic nematode populations used in this study are summarized in Table 1. For the *Meloidogyne* spp., they were either obtained directly from the field or from established cultures. To establish populations in culture, field soil was placed in a 1:1 pasteurized loam/sand mix in 184 cm³ clay pots and planted with a tomato 'Rutgers' seedling. If extracted second-stage juveniles (J2) or eggs were received, then the solution containing nematodes was added to the roots of tomato 'Rutgers'. Nematodes were allowed to develop for 10 to 15 weeks. To obtain *Meloidogyne* spp. J2, egg masses were hand-picked from tomato roots and placed on a 1.5 cm diameter plastic

hatching chamber with a 30 µm nylon mesh in a 40-ml beaker containing water (Zasada et al. 2006; Nitao et al. 1999). Hatched *Meloidogyne* spp. J2 were collected after 3 days and stored at 4°C until used, no more than 2 days. Similar to *Meloidogyne* spp., *G. ellingtonae* J2 were also used in assays. Second-stage juveniles were obtained by placing cysts on 30 µm nylon mesh in a 40-ml beaker containing a 10% potato root exudate solution (Kroese et al. 2011). Nematodes were collected after 7 days and stored at 4°C until used, no more than 2 days. For *X. americanum* and *Pratylenchus* spp., mixed stage nematodes were used. *X. americanum* was extracted from soil by decanting and sieving soil followed by sugar centrifugation with 1 M sucrose solution (Ingham 1994). *Pratylenchus* species were extracted from roots under intermittent mist for 3 to 5 days (Ingham 1994). An *M. incognita* population originally collected from grape (*Vitis vinifera*) in Parlier, CA was used in all experiments as a control because dose-response curves have already been established for this population (Wram and Zasada 2019).

Microwell dose-response assays. Formulated Salibro was used in all assays, which is 41.5% of the active ingredient fluzaindolizine. However, all concentrations shown are that of active ingredient. Dose-response curves were generated for each plant-parasitic nematode species/population using a microwell assay system (Zasada et al. 2006). Nematodes were added to wells of a 24-well cell culture plate (Falcon; Corning, Corning, NY) in 100 µl of water; 900 µl of nematicide solution was then added to achieve the desired fluzaindolizine concentration. Number of nematodes added to wells varied based on species: for *Meloidogyne* spp. and *G. ellingtonae*, 50 to 60 J2 were added to each well; for *Pratylenchus* spp., 50 to 60 mixed stage nematodes were added to each well; and for *X. americanum*, 20 to 25 mixed stage nematodes were added to each well. A single plant-parasitic nematode species/population was tested per plate that contained a water control and the *M. incognita* control at 94 ppm. In all assays, plant-parasitic nematodes were exposed to fluzaindolizine at 47, 94, 141, and 189 ppm. These concentrations were determined to provide a dose-response curve

TABLE 1. Plant-parasitic nematode population collection information and predicted fluzaindolizine effective dose that resulted in 50% inactivity (ED₅₀) in a microwell assay system

Species	Population ^a	State	City	Original host	Stage tested	24-h ED ₅₀ ppm (95% CI) ^c	24-h rinse ED ₅₀ ppm (95% CI) ^d	Citation ^e
<i>Meloidogyne chitwoodi</i>	MC-CA-1	CA	Tulelake	Potato	J2 ^b	147 (127–166)	128 (112–145)	Brown et al. 2009
	MC-WA-1	WA	Prosser	Potato		136 (121–152)	113 (98.1–128)	Brown et al. 2009
	MC-WA-2	WA	Prosser	Potato		136 (108–165)	149 (121–177)	Brown et al. 2009
	MC-WA-3	WA	Quincy	Potato		474.1 (196–752)	167 (144–191)	Mojtahedi et al. 1988
<i>M. hapla</i>	MH-CA-1	CA	Davis	Tomato	J2	319 (160–478)	156 (50–263)	Liu and Williamson 2006
	MH-CT-1	CT	Unknown	Ornamentals		132 (93–172)	116 (98–134)	
	MH-MI-1	MI	Grant	Vegetables		491 (50–931)	174 (126–221)	
	MH-NY-1	NY	Batavia	Tomato		283 (199–367)	102 (46–158)	
	MH-OR-1	OR	Veneta	Grape		369 (118–620)	170 (94–247)	
	MH-WA-1	WA	Prosser	Concord grape		789 (35–1543)	54 (25–82)	
	MH-WA-2	WA	Mattawa	Grape		402 (216–588)	57 (32–82)	
	MH-WA-3	WA	Alderdale	Grape		312 (139–485)	45 (30–61)	
<i>M. incognita</i>	MH-WA-4	WA	Paterson	Grape		88 (68–108)	35 (21–48)	
	MH-WA-5	WA	Benton City	Grape		72 (49–96)	137 (110–162)	
	MI-CA-1	CA	Livingston	Grape	J2	150 (79–220)	44 (19–69)	Anwar et al. 2002
	MI-CA-2	CA	San Joaquin Valley	Row crops		52 (36–67)	34 (28–40)	Walker et al. 1994
<i>Pratylenchus neglectus</i>	PN-OR-1	OR	Pendleton	Wheat	Mixed stage	N/A	N/A	
<i>P. penetrans</i>	PP-WA-1	WA	Lynden	Raspberry		N/A	N/A	
<i>P. penetrans</i>	PP-WA-2	WA	Lynden	Raspberry		N/A	N/A	
<i>Globodera ellingtonae</i>	GE-OR-1	OR	Powell Butte	Potato	J2	30 (14–47)	38 (11–65)	Handoo et al. 2012
<i>Xiphinema americanum</i>	XA-WA-1	WA	Woodburn	Grape	Mixed stage	65 (39–91)	147 (51–244)	

^a Population name indicates genus and species with the first two letters, followed by state where population was collected, and finally the isolate number from that state.

^b Second-stage juvenile (J2).

^c ED₅₀, 24-h predicted effective dose of active ingredient that would cause 50% the population exposed to become immobile, followed by the 95% confidence interval for the dose in parentheses.

^d After 24-h exposure, a water rinse, and another 24-h incubation, the effective dose of active ingredient that would cause 50% the population exposed to become immobile (rinse ED₅₀), followed by the 95% confidence interval for the dose in parentheses.

^e Citation if a known population was used.

resulting in 20 to 50% inactivity of *M. incognita* after 24 h of exposure in a previous study (Wram and Zasada 2019). Treatments were replicated four times per plate. The plate was then sealed with parafilm (Parafilm; Bemis Company, Neenah, WI), covered with foil, and incubated at room temperature for 24 h. After 24 h, nematodes were examined using an inverted microscope (Leica DM IL; Leica Microsystems, Wetzlar, Germany) at 100× magnification to enumerate the number of inactive and active nematodes. For all species besides *X. americanum*, active nematodes exhibited movement regardless if it was abnormal (twitching, sluggish, etc.), while straight nematodes that did not move were considered inactive. For *X. americanum*, inactive and active nematodes were determined by probing each nematode with a needle, with active nematodes being ones that responded to the probe and inactive nematodes having no movement when probed. To determine if effects of exposure were reversible, the fluzaindolizine solution was then removed using a pipette and replaced with 1 ml of water. After another 24-h incubation period at room temperature, the number of active versus inactive nematodes were determined as described above. Each microwell assay was repeated at least two times to generate dose-response curve for each plant-parasitic nematode species and population.

Statistical analyses were conducted using the software R (R Core Team 2017). The R package *drc* (Ritz et al. 2015) was used to generate dose-response curve models for each plant-parasitic nematode species and population. All data were Box-Cox transformed to correct for variance heterogeneity and nonnormality. A dose-response curve was fit to the transformed data using a three-parameter log-logistic model. Each model was then utilized to determine the effective dose needed to affect 50% of the treated nematodes 24 h after exposure (24-h ED₅₀) and 24 h after removal of the compound (24-h rinse ED₅₀) in the *drc* package.

Pre-exposure greenhouse assay. To understand if differential responses to fluzaindolizine observed in the microwell assays translated to differential effects on nematode fecundity, two populations of *M. hapla*, MH-WA-3 and MH-OR-1, *M. chitwoodi* MC-WA-3, and *M. incognita* MI-CA-1 were treated with fluzaindolizine and inoculated onto roots of tomato ‘Rutgers’. Eggs were hatched as described above to obtain 1,500 *Meloidogyne* spp. J2 for each experimental replicate. Nematodes were suspended in 100 µl of water and treated with 900 µl of a fluzaindolizine solution in a 1.7- ml microcentrifuge tube for final exposure concentrations of 47, 94, 141, or 189 ppm of fluzaindolizine; a water control was included in all experiments. The microcentrifuge tubes were incubated at room temperature for 24 h. After 24 h, nematodes were rinsed with water on a 30 µm mesh sieve for 5 to 10 s before being rinsed onto the roots of 2- to 3-week-old tomato ‘Rutgers’ seedlings in a 10 cm pot containing a sand/loam mix (1:1) (Anderson Pots #1007; Anderson Die & Manufacturing Co., Portland, OR). Each fluzaindolizine concentration was repeated four times for each nematode species/population combination and the experiment was repeated. 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 one week, plants were fertilized with 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 10 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 on nested 250 and 25 µm sieves, with eggs collected from the latter. Eggs were enumerated using an inverted microscope as described above.

Data were combined and analyzed by species. To examine the effects of fluzaindolizine on fecundity, the reproduction factor (RF) was calculated by dividing the final egg density (*Pf*) by the initial density of *Meloidogyne* spp. J2 (*Pi*) (Windham and Williams 1987). To normalize for variable reproduction rates of *Meloidogyne*

spp., each treatment replicate RF value was divided by the average mean RF of the water control replicates for each species and population for that experimental replicate ([treatment replicate RF/mean control RF for experiment/species/population] × 100 = percentage RF of mean control RF). This ratio (RF ratio) indicated how well the nematodes were able to reach their maximum reproduction potential (control levels) in the experiment. This ratio was arcsine transformed to account for nonhomogeneous variability and nonnormal distribution of the data. A linear model was generated with factored concentrations and experimental replicate as explanatory variables using the R software (R Core Team 2017). To compare the impact of concentration on RF ratio, the previously described linear models were used as input for the R package ‘lsmeans’ (Lenth 2016) to determine least square means for each concentration, which were then Tukey corrected for multiple comparisons. An additional population parameter was added to the linear model for *M. hapla* to determine if the two populations examined in this study were affected differently by a pre-exposure to fluzaindolizine.

RESULTS

Modeling microwell dose-response assay data. A log-logistic regression model with four parameters was used to model the proportion of active *Meloidogyne* spp. J2 or mixed stages of other nematode species after 24-h exposure to fluzaindolizine, and 24 h after fluzaindolizine was removed and replaced with water (Supplementary Table S1). The four parameters in the log-logistic model are as follows: *b*, the coefficient denoting steepness of the dose-response curve; *c* and *d*, the lower and upper asymptotes of the response curve; and *e*, the ED₅₀ (Ritz et al. 2015). In all dose-response curves for all species and populations tested, the three-parameter model was used where the lower asymptote, *c*, was fixed to 0.

Response of *Meloidogyne* species populations to fluzaindolizine. Of the 16 *Meloidogyne* spp. populations tested, there was clear evidence of the nematocidal activity of fluzaindolizine against this genus. Even 24 h after the nematocidal was replaced with water, all *Meloidogyne* spp. populations at each concentration tested had a further reduction in proportion of active J2 compared with proportion of active J2 at the same concentration 24 h earlier (Fig. 1). Of the *Meloidogyne* spp. populations evaluated, the *M. incognita* populations were the most sensitive to fluzaindolizine at 24 h. At the lowest dose (47 ppm), both populations of *M. incognita* had 50% active J2 compared with the *M. chitwoodi* and *M. hapla* populations, which generally had 76% or more active J2 at the same dose. After 24 h of exposure at 189 ppm, the highest dose tested, this trend still held true. Both *M. incognita* populations had >72% less active J2 compared with the control, whereas most *M. hapla* and *M. chitwoodi* populations had roughly >60 and >50% less active J2 compared with the control, respectively (Fig. 1). The average 24-h ED₅₀s for *M. hapla*, *M. chitwoodi*, and *M. incognita* were 326, 223, and 101 ppm, respectively.

Of the four *M. chitwoodi* populations evaluated, three (MC-CA-1, MC-WA-1, and MC-WA-2) had 24-h ED₅₀s that had overlapping 95% confidence intervals; however, the remaining population, MC-WA-3, had a 24-h ED₅₀ that was three times higher than that of the other *M. chitwoodi* populations (Table 1). *M. hapla*, with 10 populations tested, had the most variation in 24-h ED₅₀ and 24-h rinse ED₅₀, with 24-h ED₅₀ ranging from 72 ppm to greater than 789 ppm (Table 1). This over 10-fold variation was observed in populations that were collected from similar geographical locations (south eastern Washington State) and from geographically disparate locations. Of the *M. hapla* populations, MH-WA-4 and MH-WA-5 were the most sensitive, with the two lowest 24-h ED₅₀s. The 24-h ED₅₀ for *M. hapla* MH-WA-5 was less than half that of the 24-h ED₅₀ determined for *M. hapla* populations from MI, NY, CT, OR, and CA.

In a previous study, a *M. incognita* population from Parlier, CA was predicted to have a 24-h ED₅₀ of 74 ppm for fluazaindolizine (Wram and Zasada 2019). This population was used as a control throughout this study. Of the two additional populations of *M. incognita* tested, MI-CA-1 had a 24-h ED₅₀ that was 2× that of the control *M. incognita* population. The other population, MI-CA-2 had a 24-h ED₅₀ only 22 ppm lower than the control population. Despite having the higher 24-h ED₅₀, MI-CA-1 was more sensitive at higher doses of fluazaindolizine than MI-CA-2 (Fig. 1).

Two *Pratylenchus* spp. were evaluated in this study, one population of *P. neglectus* and two populations of *P. penetrans*. All three of these populations were unaffected by fluazaindolizine at the concentrations tested, with the log-logistic regression model unable to determine either a 24-h ED₅₀ of a 24-h rinse ED₅₀ for any of the populations (Table 1). After 24 h of exposure to fluazaindolizine, between 0 to 3% of *Pratylenchus* spp. were less active than the control (Fig. 1). After fluazaindolizine was removed and *P. neglectus* rinsed, there was a slight decrease in the percentage of active mixed stage nematodes compared with the control, 84 to 85% at all concentrations tested. The two *P. penetrans* populations maintained the 0 and 3% less active nematodes compared with the control 24 h after fluazaindolizine was replaced with water (Fig. 1).

One population of *G. ellingtonae* was examined in this study. This nematode had the lowest 24-h ED₅₀ of all the genera tested, 30 ppm (Table 1). This 24-h ED₅₀ was >8× lower than the average for all the *Meloidogyne* spp. (625 ppm). There was a clear trend of true nematocidal activity of fluazaindolizine on *G. ellingtonae* as even after fluazaindolizine was removed, *G. ellingtonae* J2 were still only 27 and 20% as active as the control at 141 and 189 ppm, respectively. However, this was higher than the activity rates compared with the control after just 24 h of exposure, 16 and 14% for 141 and 189 ppm fluazaindolizine, respectfully.

The *X. americanum* population evaluated was the only nematode genera to have some reversibility to the effects of fluazaindolizine exposure. After 24 h of exposure, only 18

and 5% of *X. americanum* were active compared with control at 141 and 189 ppm, respectively. However, once fluazaindolizine was removed and *X. americanum* was incubated in water for another 24 h activity did increase; at 141 and 189 ppm *X. americanum* were 57 and 40% as active as the control, respectively (Fig. 1).

Pre-exposure fecundity assay with *Meloidogyne* spp. A 24-h pretreatment of *Meloidogyne* spp. with fluazaindolizine was effective at reducing fecundity in all tested species. *M. incognita*, *M. hapla*, and *M. chitwoodi* all had the strongest reduction in fecundity at doses greater than 94 ppm (Fig. 2). At the lowest two doses tested (47 and 94 ppm), *M. chitwoodi* was the least sensitive of the three *Meloidogyne* species, with mean reproduction levels still reaching greater than 50% of the untreated control. Reproduction of *M. chitwoodi* still reached 25% of its reproduction potential (mean untreated control) at all doses tested. This was unlike *M. incognita* and *M. hapla* both of which never exceeded 15% of their reproduction potential at three of the four doses tested (94, 141, and 189 ppm). *M. hapla* and *M. incognita* had levels of reproduction compared with the mean of their respective controls >2× less than *M. chitwoodi* at 47 and 94 ppm. *M. incognita* was the most sensitive of the three species tested. At 47 ppm, *M. incognita* had a 3.3-fold reduction in reproduction compared with the control, whereas *M. hapla* and *M. chitwoodi* had a 2.1 and 1.5-fold reduction, respectively.

Two populations of *M. hapla* were examined in the greenhouse assay (MH-OR-1 and MH-WA-3) to determine if population impacted how nematode fecundity was affected by pre-exposure to fluazaindolizine. When an additional parameter for population was added to the linear model for RF ratio, reproduction of MH-OR-1 was not found to be statistically different from that of MH-WA-3 (*P* = 0.106).

DISCUSSION

Plant-parasitic nematodes are a major global agricultural pest responsible for \$118 billion in yield and quality loss each year (Bernard et al. 2017), making their management of paramount importance for global food security. Recent changes in tolerance for

Fig. 1. Heatmap of mixed stage (*Xiphinema americanum* and *Pratylenchus* spp.) or second-stage juveniles (*Meloidogyne* spp. and *Globodera ellingtonae*) activity after exposure to fluazaindolizine for 24 h. Nematodes were exposed to concentrations of fluazaindolizine (ppm of active ingredient not formulated product) in a microwell assay for 24 h and their activity was observed. The fluazaindolizine solution was then removed and replaced with water. After another 24 h, activity was again observed. The heatmap displays a ratio between the mean percentage activity of the treatment to the mean percent activity of the control (*n* = 8). Darker red indicates the plant-parasitic nematode was less active after exposure to fluazaindolizine compared with a water control.

environmental inputs and applicator safety have resulted in the ban or increased regulation of some chemicals, and the creation of new chemicals for the management of plant-parasitic nematodes (Faske and Hurd 2015; Kearn et al. 2014; Zasada et al. 2010). Fumigant and nonfumigant nematicides with warning or danger label designations have been heavily relied on due to their broad-spectrum efficacy against weeds, soilborne pathogens and plant-parasitic nematodes (Zasada et al. 2010). Newer nematicides have lower toxicity than traditional nematicides with caution label designations (Desaeger and Watson 2019). Due to the reduced risk nature of new nematicides, there is the potential for variable effects against a diversity of nematodes as compared with traditional nematicides that were broad spectrum in nature. Therefore, the aim of this study was to expand the knowledge of what plant-parasitic nematode genera and species may be susceptible to fluazaindolizine and to understand if species geographical location (population) impacts susceptibility to fluazaindolizine. We found that within the genus *Meloidogyne* both species and populations within species had a large range of tolerance, up to 10-fold, to a 24-h exposure of fluazaindolizine. Dose-response curves for fluazaindolizine were

also determined for *G. ellingtonae* and *X. americanum*. Both of these species showed susceptibility to fluazaindolizine, while the two species of the *Pratylenchus* spp. evaluated did not.

Fluazaindolizine had clear irreversible effects on all *Meloidogyne* spp. and populations evaluated. This trend of irreversible effects on *Meloidogyne* spp. has been observed in other studies. Movement of *M. incognita* was reduced by 58 to 87% after 24 h of exposure to 250 ppm of fluazaindolizine (Thoden and Wiles 2019). The two populations of *M. incognita* tested in this study were slightly below or within this range after exposure to fluazaindolizine at 94 ppm, with activity ranging from 45 to 60%. In another study, Thoden et al. (2019) observed immobility of five populations of *M. incognita* and *M. javanica* after J2 were exposed to fluazaindolizine at 50 ppm for 24 h, with immobility ranging from 6 to 56% and 23 to 48%, respectively. Although this range in variability was not observed in this study, after 24 h of exposure to fluazaindolizine there was up to 19% difference in activity between the two populations of *M. incognita* at the same dose, with the lowest variability only 1% difference at 188 ppm. A larger range in inconsistency in responses of *Meloidogyne* spp. populations may be

Fig. 2. *Meloidogyne* spp. fecundity on tomato (*Solanum lycopersicum*) ‘Rutgers’ after a 24-h pre-exposure of second-stage juveniles to fluazaindolizine at four different concentrations (ppm of active ingredient not formulated product) and a water control. Reproduction factor (RF) was determined as the ratio of the initial population density of *Meloidogyne* spp. over final egg population density. To allow comparison between species, RFs were normalized for the variable reproduction rates of the different *Meloidogyne* spp. by dividing the RF of the treatment by the mean RF of the control for each species and population within an experiment replicate (RF ratio). **A**, *M. chitwoodi*, **B**, *M. hapla*, and **C**, *M. incognita*. Columns indicate percent RF ratio and error bars represent standard error ($n = 8$). P values were determined using linear model of arcsine RF ratio. Columns within a graph that share letters are not statistically different from each other (least square means test, 95% confidence level).

inherent to fluzaindolizine. This is in comparison with oxamyl with immobility of five *M. incognita* and *M. javanica* populations after 24 h of exposure to 25 ppm ranging from 78 to 98% and 80 to 99%, respectively, a much more consistent response across species than observed for fluzaindolizine (Thoden et al. 2019). In our study, the same inconsistency in response to fluzaindolizine was observed across the three species tested, *M. incognita*, *M. chitwoodi*, and *M. hapla*. At 47 ppm, the lowest dose tested, percentage of active J2 ranged from 48 to 67%, 78 to 98%, and 54 to 98% for *M. incognita*, *M. chitwoodi*, and *M. hapla*, respectively. This variability remained even after the compound was removed and replaced with water for another 24 h with percentage of active J2 ranging from 40 to 37%, 78 to 97%, and 40 to 91% at the 47 ppm dose for *M. incognita*, *M. chitwoodi*, and *M. hapla*, respectively.

Thoden and Wiles (2019) evaluated the effects of a 24-h pre-exposure to 50 and 250 ppm of fluzaindolizine on the ability of *M. incognita* and *M. hapla*, respectively, to invade and cause galling on susceptible hosts. *M. incognita* pre-exposed to 50 ppm of fluzaindolizine had reduced number of nematodes in roots 7 days after inoculation compared with the control (Thoden and Wiles 2019). Although in our study we did not measure nematode invasion, we found that a 24-h pre-exposure to the lowest dose tested (47 ppm) was able to reduce reproduction to only approximately 25 to 30% of that of the control for both *M. hapla* and *M. incognita*. *M. chitwoodi* was less sensitive to fluzaindolizine, with these levels of suppression observed only at the two highest doses tested. Thoden and Wiles (2019) also reported similar sensitivity of *M. hapla* to fluzaindolizine. *Meloidogyne hapla* J2 pre-exposed to 250 ppm of fluzaindolizine for 24 h had reduced ability of to invade roots, along with a reduction in root-galling scores from 5.8 to 3 at 21 days postinfection.

There have been a number of studies recently evaluating the efficacy of fluzaindolizine on various *Meloidogyne* spp. in different cropping systems in field, greenhouse, and microplot studies. There was a greater than twofold reduction in *M. incognita* reproduction compared with the control with both half and full rates of fluzaindolizine on tomato (Silva et al. 2019). In a microplot study with cucumber, even with starting populations of 20,000 *M. incognita* J2, fluzaindolizine was able to reduce the gall index by half (Hajihassani et al. 2019). In our previous study (Wram and Zasada 2019), fluzaindolizine at 94 ppm applied as a drench eliminated *M. incognita* reproduction on susceptible tomato in a greenhouse study. These results are consistent with the greenhouse experiment performed here, in which *M. incognita* reproduction was consistently suppressed by exposure to fluzaindolizine even when exposure was just for 24 h.

Watson and Desaegeer (2019) found that fluzaindolizine at 1.12 kg/ha did not affect population densities of *M. hapla* in several strawberry field trials. This is inconsistent with the results from our study. While not as sensitive as *M. incognita*, *M. hapla* reproduction rates were consistently reduced between 3- and 12-fold of that of the control. Additionally, there was no difference in suppression of reproduction of the two *M. hapla* populations evaluated by a pretreatment with fluzaindolizine. As these results are inconsistent with field evaluation of this compound, further field evaluation of *M. hapla* from multiple geographical locations and in several different cropping systems may provide more clarity as to the usefulness of fluzaindolizine in managing *M. hapla*. Further investigation into the impacts of fluzaindolizine on *M. chitwoodi* outside of greenhouse experiments is also necessary as this species was the least sensitive to the 24-h pre-exposure of fluzaindolizine.

P. penetrans and *P. neglectus* were unaffected by fluzaindolizine at any of the concentrations evaluated, up to 189 ppm. These results are consistent with a field experiment conducted in strawberry where fluzaindolizine drip applied at 1.12 kg/ha at the time of strawberry transplant failed to suppress *P. penetrans* (Watson and Desaegeer 2019). Further evaluation of other important *Pratylenchus*

species and their sensitivity to fluzaindolizine are necessary to determine if this compound could be effective for the management of other species in this genus in other cropping systems.

There have been no reported evaluations of fluzaindolizine either in vitro or in the field for the management for either *G. ellingtonae* or *X. americanum*. Both of these species were very sensitive to fluzaindolizine in the microwell assays, making fluzaindolizine a new potential management strategy for cropping systems impacted by these nematode species. Further experiments exploring how fluzaindolizine might impact nematode reproduction would be an important stepping stone before deploying this compound for use in the field.

Although the populations of the three different *Meloidogyne* spp. examined in this study showed a large range in fluzaindolizine sensitivity in in vitro assays, reproduction on a susceptible host was clearly suppressed in all species. The level of suppression did vary between these species and with the mode-of-action unknown for this compound, it is unclear how this sensitivity is conferred in each species and whether this could lead to some populations of *Meloidogyne* spp. not responding to fluzaindolizine in the field. This study also determined 24-h ED₅₀s for *G. ellingtonae* or *X. americanum*, which were in general more sensitive to fluzaindolizine than any *Meloidogyne* spp. tested. These differing levels of tolerance between species could lead to the need of more tailored application rates after further exploration in greenhouse or field experiments. Knowledge of the variable response of plant-parasitic nematodes to fluzaindolizine could result in economic savings to growers and unnecessary chemical applications.

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