Fluopyram Suppresses Population Densities of *Heterodera glycines* in Field and Greenhouse Studies in Michigan

M. G. Roth,^{1,2} J. L. Jacobs,¹ S. Napieralski,³ A. M. Byrne,¹ A. Stouffer-Hopkins,¹ F. Warner,¹ and M. I. Chilvers^{1,2,†}

¹ Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI 48824, U.S.A.

² Genetics Graduate Program, Michigan State University, East Lansing, MI 48824, U.S.A.

³ Department of Geoscience, University of Wisconsin-Madison, Madison, WI 53706, U.S.A.

Abstract

The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, causes significant damage to soybean production annually. Fluopyram is a fungicide commonly used in soybean seed treatments intended to control soilborne fungal pathogens; however, recent studies have also suggested inhibitory effects on SCN. We examined the effects of a fluopyram seed treatment, ILeVO, on SCN reproduction, sudden death syndrome (SDS) development, and yield in a 3-year field study. Overall, fluopyram had a significant effect on yield ($P = 0.046$) and end-of-season SCN eggs and second-stage juveniles (Pf, $P = 0.033$) but no significant effect on SCN reproduction (Rf) or SDS disease index ($P > 0.05$). Post hoc tests indicated that fluopyram increased yield and suppressed SCN quantities. However, Rf was

consistently greater than 1 whether or not the seed was treated with fluopyram, indicating that SCN populations were still increasing in the presence of fluopyram. A follow-up greenhouse study indicated that fluopyram reduced SCN relative to nontreated controls, as observed in the field, but only reduced SCN DNA within roots of a susceptible cultivar. These results indicate that fluopyram can suppress SCN quantities relative to nontreated seed but may not successfully reduce nematode populations without the use of additional management strategies.

Keywords: chemical, disease management, field crops, fungi, nematodes, oilseeds and legumes

Soybean crops are significantly affected by many pests and pathogens throughout North America. According to yield loss estimates by field crop pathologists, the soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, consistently ranks as the top threat to soybean production in the United States (Allen et al. 2017; Koenning and Wrather 2010). Soybean cyst nematodes are obligate parasites that require a compatible plant host to complete their life cycle. Hatching, development, and reproduction can be adversely affected by low temperatures ($<15^{\circ}\text{C}$) and low pH (≤ 5.5) but SCN is generally able to complete its life cycle in 3 to 4 weeks under field conditions (Alston and Schmitt 1988; Anand et al. 1995). Egg hatching and root penetration by infective juveniles (J2s) are influenced by root exudates (Riga et al. 2001). The J2 nematodes physically pierce the root with a stylet and release cell-wall-degrading enzymes through saliva (Wang et al. 1999). The J2s manipulate plant physiology in order to prevent an immune response and to initiate the development of a feeding site, or syncytium, by secreting effector proteins into the root from their esophageal glands (Mitchum et al. 2013; Noon et al. 2015). Both male and female J2s that successfully

develop a syncytium will begin to swell. Females continue to swell and produce eggs inside of their inflating body, while males regain musculature, becoming mobile again. Females produce most eggs internally before dying, using their dead body as a protective casing for the eggs, although some eggs emerge in a gelatinous matrix outside of the cyst. These cysts allow eggs to survive in the soil for 10 years or more, and a cyst can contain up to 600 eggs, making SCN difficult to manage long term (Niblack et al. 2006; Sipes et al. 1992).

Genetic resistance has been a popular choice for SCN management due to its early success. Genetic resistance to SCN is quantitative and originates from multiple parental lines but growers have historically selected cultivars with resistance derived from PI88788 (McCarville et al. 2017). Genetic resistance in PI88788 is attributed to a quantitative trait locus (QTL) on chromosome 18 (linkage group G) with an increased copy number of three unrelated genes (Concibido et al. 2004; Cook et al. 2012). Additional QTLs associated with SCN resistance have been identified (Concibido et al. 2004; Mitchum et al. 2013) but resistance mechanisms and causal genes remain largely unknown. The strong resistance conferred by the PI88788 background has led to repeated planting of cultivars with this resistance source, providing a significant selection pressure on SCN populations to adapt to and overcome the resistance. Populations of SCN have overcome resistance derived from many resistant sources, including PI88788 (McCarville et al. 2017). Analyzing the SCN genome has revealed that SCN virulence is controlled by multiple recessive loci, with some loci being mutually exclusive in their association for overcoming soybean resistance on specific indicator lines (Gardner et al. 2017; Masonbrink et al. 2019).

Chemical control of nematodes and other soilborne pathogens has been an active area of research for many years (Hartwig et al. 2008; Newhall 1955). Chemical fumigants can reduce nematode populations but often use broad-spectrum modes of action that kill many beneficial microbes, too, and are often quite expensive (Hartwig et al. 2008; Reynolds et al. 2000). However, many soybean growers have shifted away from fumigation and use new chemistries with specific modes of action formulated as seed treatments. Soybean seed treatments provide chemical control of pathogens and pests and have become popular in recent years. One new chemical, fluopyram, is a

Present address of M. G. Roth: Department of Plant Pathology, University of Wisconsin-Madison, Madison, WI 53706, U.S.A.

[†]Corresponding author: M. I. Chilvers; chilvers@msu.edu

Funding: This study was supported by Bayer CropSciences, the Michigan Soybean Promotion Committee, and the North Central Soybean Research Program.

*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary table is published online.

The author(s) declare no conflict of interest.

Accepted for publication 6 January 2020.

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 2020.

succinate dehydrogenase inhibitor (SDHI) fungicide which targets cellular respiration in fungi and has proven efficacy against *Botrytis cinerea*, *Fusarium virguliforme* (which causes sudden death syndrome [SDS]), and *Sclerotinia sclerotiorum* (which causes Sclerotinia stem rot) (Mahoney et al. 2014; Veloukas and Karaoglanidis 2012; Wang et al. 2017). However, recent studies have also shown potential for fluopyram as a nematode control agent, slowing motility and preventing root penetration (Beeman and Tylka 2018; Beeman et al. 2019; Faske and Hurd 2015; Galle et al. 2017; Kandel et al. 2017). The objectives of this study were to determine how fluopyram (ILeVO) as a seed treatment affects end-of-season SCN quantities, SDS severity, and yield across 25 soybean cultivars in Michigan, and examine the combined effects of the seed treatment on SCN and SDS development.

Materials and Methods

Field trials. Field trials were conducted between 2014 and 2016 at a field site in Decatur, MI with natural SCN and *F. virguliforme* populations. The SCN population in these fields was HG Type 2.5.7 (Niblack et al. 2002), indicating that SCN female development exceeds 10% on resistant cultivars derived from PI88788, PI209332, and PI548316. The fields at this location were planted with soybean alternating every other year with corn or wheat. In all years, a randomized complete block design was implemented, and the trials were conducted in two-row plots that were 5.3 m long with 76-cm row spacing, and planted at a density of approximately 29 seeds/m. All cultivar–treatment combinations had five replicate plots in 2014 and 2015 and four replicate plots in 2016. The plots were planted on 1, 6, and 9 May in 2014, 2015, and 2016, respectively. Plots were harvested on 1, 2, and 4 October in 2014, 2015, and 2016, respectively, and converted into kilograms per hectare. All seed was treated by Bayer CropSciences with base seed treatment or base seed treatment with fluopyram (as ILeVO) at a rate of 0.15 mg seed⁻¹. The base seed treatment contained active ingredients pyraclostrobin, metalaxyl, and flupyrroxad (Rossman et al. 2018).

Cultivars planted each year were commercial varieties representative of what was available to growers in Michigan but the cultivars were coded, making it a blind study. However, SCN resistance source and SDS resistance ratings were provided for each cultivar. Cultivars either had no SCN resistance (susceptible), unknown SCN resistance, or SCN resistance derived from Peking or PI88788. Cultivars had SDS ratings of T = tolerant, MT = moderately tolerant, S = sensitive, or NG = not given.

Plots were rated for average SDS disease severity (DS) within each plot using a 0-to-9 disease rating scale developed at Southern Illinois University (J. Bond, *unpublished*). Ratings of 0 = no visible symptoms, 1 = 1 to 10% of leaf surface chlorotic or 1 to 5% necrotic, 2 = 10 to 20% chlorotic or 6 to 10% necrotic, 3 = 20 to 40% chlorotic or 11 to 20% necrotic, 4 = 40 to 60% chlorotic or 21 to 40% necrotic,

5 = greater than 60% chlorotic or greater than 40% necrotic, 6 = premature leaf drop up to one-third defoliation, 7 = premature leaf drop up to two-thirds defoliation, 8 = premature leaf drop greater than two-thirds defoliation, and 9 = premature plant death. Disease incidence (DI) was also collected as the percentage of plants in the plot displaying SDS symptoms. Disease ratings were performed on 26, 20, and 23 August in 2014, 2015, and 2016, respectively, when soybean plants were at the R5 growth stage (Fehr et al. 1971). The DS and DI values were converted to a continuous disease index (DX) by taking $(DI \times DS)/9$, where 9 is the maximum disease severity rating possible.

Soil samples were collected at planting and within 1 week after harvest using a 2.45-cm-diameter, 15.24-cm-deep soil core. Six soil cores were collected throughout the two-row plot using a zig-zag sampling method. The soil was homogenized in a plastic bag and a 100-cm³ subsample was used to isolate SCN using a modified centrifugation and flotation method, and quantified in a counting dish with a dissecting microscope (Jenkins 1964). The SCN reproduction factor (Rf) was calculated as the final egg and J2 counts (Pf) divided by the initial egg and J2 counts (Pi).

Greenhouse experiments. Two cultivars were selected from the 2014 field trial for a controlled study in the greenhouse. Cultivar A was susceptible to HG type 0 (race 3) SCN, with an SDS rating of MT. Cultivar B was resistant to HG type 0 (race 3) SCN, with an SDS rating of T. Bulk soil was collected from the 2014 Decatur, MI field site and homogenized in a cement mixer. Cysts, eggs, and J2s were isolated and quantified from six subsamples of homogenized soil and determined to be sufficiently homogeneous, with 27 ± 1 cysts, $2,280 \pm 105$ eggs, and 247 ± 28 J2s; therefore, at-planting quantities of *F. virguliforme* were also assumed to be equal. Soil was aliquoted into 1-liter pots and 5 seeds were sown for each combination of cultivar (A or B) and treatment (base or base + fluopyram). Each pot was considered a unit, with 5 seeds/pot and six pot replicates. A plant from each pot was culled as needed, 1 week after planting, so that each pot had four germinated seedlings. The greenhouse experiment was performed twice.

After 38 days, the plants were at the V3 growth stage (Fehr et al. 1971) and were harvested by removing the shoots from the roots just above the soil line. Roots were washed clean of soil and gently scrubbed to remove mature cysts and developing females from the root surface. The root scrub and soil were combined and SCN were isolated and quantified using a modified centrifugation and flotation method (Jenkins 1964). The washed roots were dried at 50°C for 48 h and ground using two 1-cm steel balls in a GenoGrinder 2010 (SPEX Sample Prep, Metuchen, NJ, U.S.A.). DNA was extracted using an automated phenol-chloroform protocol using an AutoGen 850 Alpha system (AutoGen Inc., Holliston, MA, U.S.A.), or the same protocol by hand, where each of the six pot replicates served as a biological replicate. DNA was quantified using a Nanodrop 1000 (Thermo

Table 1. Analysis of variance (ANOVA) using yield, end-of-season soybean cyst nematode (SCN) eggs and juveniles (Pf), or sudden death syndrome (SDS) disease severity as dependent variables and cultivar and treatment as the independent variables^a

Dataset	Explanatory variable	Yield	Pf	Rf ^b	R5 SDS disease index ^c
All years	Cultivar	<0.001*	<0.001*	<0.001*	<0.001*
	Treatment	0.046#	0.033†	0.378	0.628
	Cultivar × treatment	0.999	0.998	0.515	0.999
2014	Cultivar	<0.001*	<0.001*	<0.001*	<0.001*
	Treatment	0.214	0.095	0.130	0.345
	Cultivar × treatment	0.999	0.996	0.966	0.745
2015	Cultivar	<0.001*	0.004*	0.002*	<0.001*
	Treatment	0.039#	0.091	0.285	0.959
	Cultivar × treatment	0.778	0.933	0.664	0.991
2016	Cultivar	0.001*	0.038*	0.007*	<0.001*
	Treatment	0.990	0.600	0.001#	0.889
	Cultivar × treatment	0.813	0.493	0.430	0.896

^a Symbols: * = significant cultivar effect ($P < 0.05$), † = significant treatment effect (fluopyram < base), and # = significant treatment effect (fluopyram > base).

^b The logarithm of Rf was used in all ANOVAs in order to meet the assumption of a normal distribution.

^c R5 = soybean reproductive growth stage 5.

qPCR assay was used to quantify SCN using the method described by Ye (2012), with modifications. Briefly, the forward primer (SCNrTf), reverse primer (SCNrTr), and TaqMan probe (SCNrTp) described by Ye (2012) were purchased and 25- μ l reactions were used to quantify SCN, containing 12.5 μ l of TaqMan Universal

PCR Master Mix (Thermo Fisher Scientific); 0.2 μ M each SCNrtF, SCNrtR, and SCNrtP; 9 μ l of nuclease-free water; and 2 μ l of DNA template. The reactions were incubated at 95°C for 3 min, followed by 50 cycles of 95°C for 10 s and 62°C for 45 s in a CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories).

Data analysis. All data were analyzed in R, version 3.5.1 (R Core Team 2018). Basic data manipulations were performed with the ‘reshape2’ package (Wickham 2007). The Rf data were log transformed prior to analysis to generate a normal distribution of values but the raw Rf values were used for presentation purposes. All *t* tests, analyses of variance (ANOVAs), and post hoc

Fig. 2. Cultivar response to fluopyram (ILeVO). Soybean sudden death syndrome (SDS) **A**, disease index and **B**, yield. Treatment effects within cultivar are denoted as ns = not significant or * = significant at $P < 0.05$. Significant differences between years are denoted with a, b, or c. SCN resistance = soybean cyst nematode (SCN) resistance source of the cultivar, where PK = Peking, PI = PI88788, S = susceptible, Un = unknown. SDS rating = SDS resistance rating, where S = susceptible, MT = moderately tolerant, T = tolerant, NG = not given.

tests were performed with the ‘stats’, ‘car’, and ‘emmeans’ packages (Fox and Weisberg 2011; Lenth 2018). All figures were produced with the ‘ggplot2’ and ‘ggpubr’ packages (Kassambara 2018; Wickham 2016). All analyses can be found at https://github.com/rothmi12/SCN_fluopyram.

Results

Field trials. Cultivar had a significant overall effect on yield, Pf, Rf, and SDS (Table 1). Fluopyram treatment also had a significant overall effect on yield and Pf but no significant effect on Rf or SDS (Table 1). A Tukey’s honestly significant difference (HSD) post hoc test indicated that base + fluopyram had greater yield and lower Pf than the base treatment alone. The cultivar–treatment interaction was not significant for any of these response variables. Additional ANOVAs showed that year was a significant factor for the response variables; therefore, the data were separated by year and reanalyzed. Within each year, cultivar consistently had significant effects on yield, Pf, Rf, and SDS DX (Table 1). The only significant effects that treatment had within years was on yield in 2015 and Rf in 2016 (Table 1). A Tukey’s HSD post hoc test indicated that base + fluopyram had higher yield than base alone in 2015, and that base + fluopyram had higher Rf than base alone in 2016.

The Pi, Pf, Rf, SDS DI, and yield were all significantly influenced by year ($P < 0.05$). Because each cultivar differed between years, each response variable was examined within cultivar to determine effects of fluopyram seed treatment and remove the effect of year. By chance, fluopyram-treated cultivar 17 was sown in soil containing significantly lower Pi, on average, than its base-only control ($P < 0.05$) (Fig. 1A). Thirteen other cultivars displayed a similar trend in Pi but it was not significant ($P > 0.05$) (Fig. 1A). Fluopyram-treated cultivar 4 had significantly lower Pf, on average, than its base-only control ($P < 0.05$) (Fig. 1B). Nineteen other cultivars displayed a similar trend in Pf but it was not significant ($P > 0.05$) (Fig. 1B). Rf was significantly greater in cultivars 23 and 25 when they were treated with fluopyram, on average, compared with their base-only controls ($P < 0.05$) (Fig. 1C). Twelve cultivars had a reduction in SDS DX due to the fluopyram treatment but none of these reductions were significant ($P > 0.05$) (Fig. 2A). In all, 19 of the 25 cultivars had a yield increase ($P > 0.05$) when treated with fluopyram but only cultivar 16 had a significant yield increase when treated with fluopyram ($P < 0.05$) (Fig. 2B).

Combining all of the final disease data and viewing each cultivar response to fluopyram, we observed that all but two cultivars

Fig. 3. Soybean cyst nematode (SCN), sudden death syndrome (SDS), and yield response to fluopyram (ILeVO) for each cultivar tested. Negative x-values indicate reduced SCN eggs and juveniles (Pf), negative y-values indicate reduced SDS disease index, and blue colors indicate positive yield response due to the presence of fluopyram seed treatment relative to the base-only treatment of the same cultivar. Numbers within points indicate the cultivar. All but one cultivar saw a benefit from fluopyram with respect to either SDS or SCN (quadrant I), while nine cultivars saw a benefit against both (quadrant III). Values in quadrant III also show a higher yield response when seed-treated with fluopyram.

exhibited some benefit from the fluopyram treatment in either reduced Pf or reduced SDS DI, with nine cultivars showing a reduction in both (Fig. 3). Only cultivar 23 had more SCN and SDS when treated with fluopyram, although it was nonsignificant ($P > 0.05$). Cultivar 12 was the only cultivar that had SCN resistance derived from Peking, and this cultivar performed very well with the highest yield, lowest Pf, and no SDS development whether it was treated with fluopyram or not (Figs. 2 and 3).

Greenhouse experiments. Cultivars A and B used in the greenhouse were exposed to a homogeneous amount of SCN and *F. virguliforme* for 38 days in two experimental replicates. Experimental replicate had a significant effect on all variables of interest, but similar trends and statistical differences were observed in both experiments. When treated with fluopyram, both cultivars showed a significant reduction in SCN females and cysts relative to their base-only cultivar controls (Fig. 4). However, the amount of SCN DNA within roots was only significantly reduced by fluopyram in one variety in one experimental replication (Fig. 4). Both cultivars exhibited a significant reduction in *F. virguliforme* DNA within plant roots when treated with fluopyram (Fig. 4). No foliar SDS symptoms were observed after 38 days. In general, more SCN and *F. virguliforme* were detected in the second greenhouse experiment than the first but the colonization by either pathogen was generally reduced in the presence of fluopyram (Fig. 5).

Discussion

Fluopyram is formulated against diseases caused by pathogenic fungi such as *F. virguliforme*, which causes soybean SDS. Marketed

Fig. 4. Effects of fluopyram (ILeVO) on soybean cyst nematode (SCN) and *Fusarium virguliforme* abundance in soybean roots 38 days post inoculation in **A**, the first greenhouse replicate and **B**, the second greenhouse replicate. Symbols: ns = not significant and *, **, and *** indicate $P < 0.01$, 0.005, and 0.001, respectively.

as ILeVO, fluopyram was shown to significantly reduce SDS DX in multiple locations in 2013 and 2014, including Decatur, MI, the site of these trials (Kandel et al. 2016). In a similar study, fluopyram again reduced SDS but had no effect on SCN reproduction (Kandel et al. 2017). In contrast, our study showed a significant reduction of Pf and no effect on SDS when seed was treated with fluopyram. However, our results showed the same trend as previous reports with respect to yield, where fluopyram is associated with a significant increase in yield (Table 1) (Kandel et al. 2016, 2017).

Many studies, including this study, show that cultivar or SCN resistance source has a significant effect on SCN reproduction and Rf (Supplementary Table S1) (Conley et al. 2011; Donald et al. 2006; Kandel et al. 2017; Niblack et al. 2006). Field trials examining the effects of cultivar or treatment on nematodes commonly use the ratio of eggs and J2 after harvest (Pf) divided by the eggs and J2 at planting (Pi), also called the Rf (Kandel et al. 2017; Li and Chen 2005; Wang et al. 2000). The Rf ratio is meant to be a simple indicator of nematode reproduction success; if Rf is >1, nematode populations are increasing and if Rf < 1, nematode populations are decreasing. However, Rf can be significantly influenced by low Pi, and SCN distributions have been reported to be unevenly distributed throughout fields (Avendaño et al. 2003; Roth et al. 2019). For instance, the significantly lower Pi observed in 2016 led to significantly elevated Rf values, even though Pf values were still significantly lower in 2016 than 2014. If analyzing Rf alone, one might interpret the 2016 cultivars as being very susceptible to SCN, with no relief provided by fluopyram. Even though the 2016 cultivars were susceptible to SCN populations, the Pf indicates that SCN is not as significant of an issue as it may be in other locations. Therefore, it is important to investigate all three factors—Pi, Pf, and Rf—to fully understand cultivar and treatment effects on SCN. Additional environmental and edaphic factors such as temperature, moisture, and soil type can also affect treatment efficacy. In the current study, fluopyram resulted in an overall decrease in Pf but had no significant effect on Rf (Table 1). A reduction in Pf but not Rf suggests that fluopyram may provide some suppression of SCN reproduction relative to nontreated seed but is not sufficient as the only strategy to reduce nematode populations over multiple years.

To examine the effects of fluopyram on SCN more closely, a follow-up study was performed in the greenhouse. One cultivar was listed as resistant to HG type 0, while the other was listed as susceptible. However, the SCN population used in this greenhouse study was HG type 2.5.7, suggesting that neither cultivar would be resistant to these nematodes. When treated with fluopyram, both cultivars had significantly fewer SCN cysts and females compared with the base-

only seed treatment. Fewer SCN cysts and females may indicate that fluopyram can have a positive effect on both resistant and susceptible cultivars, providing additional protection in addition to genetic resistance. Evidence suggests that fluopyram can reduce SCN mobility, speed, and root penetration (Beeman et al. 2019), and perhaps this effect led to the observations in this study. Alternatively, fluopyram may prime resistance to SCN or it may be toxic to SCN in the same way that it is toxic to root-knot and reniform nematodes (Faske and Hurd 2015). Because fluopyram has known growth-inhibiting effects on *F. virguliforme* (Sang et al. 2018; Wang et al. 2017), observing a significant reduction of *F. virguliforme* in fluopyram-treated roots is consistent with previous findings and acts as a positive control for this study (Fig. 4). Examining *F. virguliforme* and SCN DNA quantities within roots simultaneously reveals that fluopyram can reduce the amount of both pathogens inside of soybean roots (Fig. 5).

The mechanism for growth inhibition by fluopyram is currently unknown. Fluopyram is classified as an SDHI fungicide along with other common chemistries such as carboxin, boscalid, and penthiopyrad (FRAC 2018). However, some fungal plant pathogens that are very resistant to boscalid or penthiopyrad are still very sensitive to fluopyram (Avenot and Michailides 2010; Ishii et al. 2011). The lingering sensitivity to fluopyram suggests that fluopyram binds to its target differently than other SDHIs, and different target residues may be involved, as one molecular docking study suggests (Fraaije et al. 2012). SDHI fungicides are advertised as widely beneficial for controlling plant-pathogenic fungi due to a lack of cross-resistance with other chemistries and their efficacy across a broad-spectrum of fungi (Avenot and Michailides 2010). In the current study, we provide evidence that fluopyram may lead to a reduction in nematode abundance relative to nontreated soybean, though fluopyram does not appear to prevent SCN reproduction. The toxic effects of fluopyram on fungi (Wang et al. 2017) and nematodes (Faske and Hurd 2015) indicate that fluopyram has cross-kingdom activity (Sierotzki and Scalliet 2013), which begs the question of what other effects it may be having on soil microbial communities. However, the formulation as a seed treatment likely minimizes exposure and decreases the selection pressure to develop resistance compared with other fungicide applications such as foliar sprays or soil drenches. Additional studies on the mechanism of succinate dehydrogenase inhibition by SDHI chemistries such as fluopyram are likely underway, which may reveal its true spectrum of efficacy in controlling plant pests and pathogens.

Acknowledgments

We thank C. Druskovich for use of fields in Decatur, MI, and J. Riggs for providing base and ILeVO treatments of seed.

Literature Cited

- Allen, T. W., Bradley, C. A., Sisson, A. J., Byamukama, E., Chilvers, M. I., Coker, C. M., Collins, A. A., Damicone, J. P., Dorrance, A. E., Dufault, N. S., Esker, P. D., Faske, T. R., Giesler, L. J., Grybauskas, A. P., Hershman, D. E., Hollier, C. A., Isakeit, T., Jardine, D. J., Kelly, H. M., Kemerait, R. C., Kleczewski, N. M., Koenning, S. R., Kurlle, J. E., Malvick, D. K., Markell, S. G., Mehl, H. L., Mueller, D. S., Mueller, J. D., Mulrooney, R. P., Nelson, B. D., Newman, M. A., Osborne, L., Overstreet, C., Padgett, G. B., Phipps, P. M., Price, P. P., Sikora, E. J., Smith, D. L., Spurlock, T. N., Tande, C. A., Tenuta, A. U., Wise, K. A., and Wrather, J. A. 2017. Soybean yield loss estimates due to diseases in the United States and Ontario, Canada, from 2010 to 2014. *Plant Health Prog.* 18:19-27.
- Alston, D. G., and Schmitt, D. P. 1988. Development of *Heterodera glycines* life stages as influenced by temperature. *J. Nematol.* 20:366-372.
- Anand, S. C., Matson, K. W., and Sharma, S. B. 1995. Effect of soil temperature and pH on resistance of soybean to *Heterodera glycines*. *J. Nematol.* 27:478-482.
- Avendaño, F., Schabenberger, O., Pierce, F. J., and Melakeberhan, H. 2003. Geostatistical analysis of field spatial distribution patterns of soybean cyst nematode. *Agron. J.* 95:936-948.
- Avenot, H. F., and Michailides, T. J. 2010. Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. *Crop Prot.* 29:643-651.
- Beeman, A. Q., Njus, Z. L., Pandey, S., and Tylka, G. L. 2019. The effects of ILeVO and VOTiVO on root penetration and behavior of the soybean cyst nematode, *Heterodera glycines*. *Plant Dis.* 103:392-397.

Fig. 5. Quantities of *Fusarium virguliforme* (Fv) and soybean cyst nematode (SCN) DNA inside of soybean roots 38 days after planting, with 95% confidence intervals based on seed treatment in **A**, the first greenhouse replicate and **B**, the second greenhouse replicate.

- Beeman, A. Q., and Tylka, G. L. 2018. Assessing the effects of ILeVO and VOTiVO seed treatments on reproduction, hatching, motility, and root penetration of the soybean cyst nematode, *Heterodera glycines*. *Plant Dis.* 102:107-113.
- Concibido, V. C., Diers, B. W., and Arelli, P. R. 2004. A decade of QTL mapping for cyst nematode resistance in soybean. *Crop Sci.* 44:1121-1131.
- Conley, S. P., Gaska, J. M., Pedersen, P., and Esker, P. 2011. Soybean yield and *Heterodera glycines* response to rotation, tillage, and genetic resistance. *Agron. J.* 103:1604-1609.
- Cook, D., Lee, T., Guo, X., Melito, S., Wang, K., Bayless, A., Wang, J., Hughes, T., Willis, D., Clemente, T., Diers, B., Jiang, J., Hudson, M., and Bent, A. F. 2012. Copy number variation of multiple genes at *Rhg1* mediates nematode resistance in soybean. *Science* 338:1206-1209.
- Donald, P. A., Pierson, P. E., St. Martin, S. K., Sellers, P. R., Noel, G. R., MacGuidwin, A. E., Faghihi, J., Ferris, V. R., Grau, C. R., Jardine, D. J., Melakeberhan, H., Niblack, T. L., Stienstra, W. C., Tylka, G. L., Wheeler, T. A., and Wysong, D. S. 2006. Assessing *Heterodera glycines*-resistant and susceptible cultivar yield response. *J. Nematol.* 38:76-82.
- Faske, T. R., and Hurd, K. 2015. Sensitivity of *Meloidogyne incognita* and *Rotylenchulus reniformis* to fluopyram. *J. Nematol.* 47:316-321.
- Fehr, W. R., Caviness, C. E., Burmood, D. T., and Pennington, J. S. 1971. Stage of development description for soybeans, *Glycine max* (L.) Merrill. *Crop Sci.* 11:929-931.
- Fox, J., and Weisberg, S. 2011. An R Companion to Applied Regression, 2nd ed. SAGE Publications, Inc., Thousand Oaks, CA, U.S.A.
- Fraaije, B. A., Bayon, C., Atkins, S., Cools, H. J., Lucas, J. A., and Fraaije, M. W. 2012. Risk assessment studies on succinate dehydrogenase inhibitors, the new weapons in the battle to control Septoria leaf blotch in wheat. *Mol. Plant Pathol.* 13:263-275.
- FRAC. 2018. FRAC Code List: Fungicides Sorted by Mode of Action (including FRAC Code Numbering). Fungicide Resistance Action Committee. <https://www.frac.info/publications/downloads>
- Galle, G. H., Nunez, K. H., and Kerns, J. P. 2017. In vitro analysis of nematocides and fungicides on *Belonolaimus longicaudatus*. In: ACS Annu. Meet. P808. Tampa, FL, U.S.A.
- Gardner, M., Heinz, R., Wang, J., and Mitchum, M. G. 2017. Genetics and adaptation of soybean cyst nematode to broad spectrum soybean resistance. G3: Genes Genomes Genet. 7:835-841.
- Hartwig, J., Sommer, H., and Müller, F. 2008. Nematicides. *Encycl. Ind. Chem.* 24:1-35.
- Ishii, H., Miyamoto, T., Ushio, S., and Kakishima, M. 2011. Lack of cross-resistance to a novel succinate dehydrogenase inhibitor, fluopyram, in highly boscalid-resistant isolates of *Corynespora cassicola* and *Podosphaera xanthii*. *Pest Manage. Sci.* 67:474-482.
- Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. *Plant Dis. Rep.* 48:692.
- Kandel, Y. R., Wise, K. A., Bradley, C. A., Chilvers, M. I., Byrne, A. M., Tenuta, A. U., Faghihi, J., Wiggs, S. N., and Mueller, D. S. 2017. Effect of soybean cyst nematode resistance source and seed treatment on population densities of *Heterodera glycines*, sudden death syndrome, and yield of soybean. *Plant Dis.* 101:2137-2143.
- Kandel, Y. R., Wise, K. A., Bradley, C. A., Chilvers, M. I., Tenuta, A. U., and Mueller, D. S. 2016. Fungicide and cultivar effects on sudden death syndrome and yield of soybean. *Plant Dis.* 100:1339-1350.
- Kassambara, A. 2018. ggpubr: 'ggplot2' based publication ready plots. <https://cran.r-project.org/web/packages/ggpubr/index.html>
- Koenning, S., and Wrather, A. 2010. Suppression of soybean yield potential in the continental United States by plant diseases from 2006 to 2009. *Plant Health Prog.* 11. <https://doi.org/10.1094/PHP-2010-1122-01-RS>
- Lenth, R. 2018. emmeans: Estimated marginal means, aka least-squares means. <https://cran.r-project.org/web/packages/emmeans/index.html>
- Li, Y. H., and Chen, S. Y. 2005. Effect of the *rhg1* gene on population development of *Heterodera glycines*. *J. Nematol.* 37:168-177.
- Mahoney, K. J., McCreary, C. M., and Gillard, C. L. 2014. Response of dry bean white mould [*Sclerotinia sclerotiorum* (Lib.) de Bary, causal organism] to fungicides. *Can. J. Plant Sci.* 94:905-910.
- Masonbrink, R. E., Maier, T. R., Muppirala, U., Seetharam, A. S., Lord, E., Juvala, P., Schmutz, J., Johnson, N. T., Korkin, D., Mitchum, M. G., Mimee, B., Eves-van den Akker, S., Hudson, M., Severin, A. J., and Baum, T. J. 2019. The genome of the soybean cyst nematode (*Heterodera glycines*) reveals complex patterns of duplications involved in the evolution of parasitism genes. *BMC Genomics* 20:119.
- McCarville, M. T., Marett, C. C., Mullaney, M. P., Gebhart, G. D., and Tylka, G. L. 2017. Increase in soybean cyst nematode virulence and reproduction on resistant soybean varieties in Iowa from 2001 to 2015 and the effects on soybean yields. *Plant Health Prog.* 18:146-155.
- Mitchum, M. G., Hussey, R. S., Baum, T. J., Wang, X., Elling, A. A., Wubben, M., and Davis, E. L. 2013. Nematode effector proteins: An emerging paradigm of parasitism. *New Phytol.* 199:879-894.
- Newhall, A. G. 1955. Disinfestation of soil by heat, flooding and fumigation. *Bot. Rev.* 21:189-250.
- Niblack, T. L., Arelli, P. R., Noel, G. R., Opperman, C. H., Orf, J. H., Schmitt, D. P., Shannon, J. G., and Tylka, G. L. 2002. A revised classification scheme for genetically diverse populations of *Heterodera glycines*. *J. Nematol.* 34:279-288.
- Niblack, T. L., Lambert, K. N., and Tylka, G. L. 2006. A model plant pathogen from the kingdom Animalia: *Heterodera glycines*, the soybean cyst nematode. *Annu. Rev. Phytopathol.* 44:283-303.
- Noon, J. B., Hewezi, T., Maier, T. R., Simmons, C., Wei, J.-Z., Wu, G., Llaça, V., Deschamps, S., Davis, E. L., Mitchum, M. G., Hussey, R. S., and Baum, T. J. 2015. Eighteen new candidate effectors of the phytonematode *Heterodera glycines* produced specifically in the secretory esophageal gland cells during parasitism. *Phytopathology* 105:1362-1372.
- R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for statistical computing, Vienna, Austria. <https://www.R-project.org>
- Reynolds, L. B., Potter, J. W., and Ball-Coelho, B. R. 2000. Crop rotation with *Tagetes* sp. is an alternative to chemical fumigation for control of root-lesion nematodes. *Agron. J.* 92:957-966.
- Riga, E., Welacky, T., Potter, J., Anderson, T., Topp, E., and Tenuta, A. 2001. The impact of plant residues on the soybean cyst nematode, *Heterodera glycines*. *Can. J. Plant Pathol.* 23:168-173.
- Rossman, D. R., Byrne, A. M., and Chilvers, M. I. 2018. Profitability and efficacy of soybean seed treatment in Michigan. *Crop Prot.* 114:44-52.
- Roth, M. G., Noel, Z. A., Wang, J., Warner, F., Byrne, A. M., and Chilvers, M. I. 2019. Predicting soybean yield and sudden death syndrome development using at-planting risk factors. *Phytopathology* 109:1710-1719.
- Sang, H., Witte, A., Jacobs, J. L., Chang, H.-X., Wang, J., Roth, M. G., and Chilvers, M. I. 2018. Fluopyram sensitivity and functional characterization of SdhB in the *Fusarium solani* species complex causing soybean sudden death syndrome. *Front. Microbiol.* 9:2335.
- Sierotzki, H., and Scalliet, G. 2013. A review of current knowledge of resistance aspects for the next-generation succinate dehydrogenase inhibitor fungicides. *Phytopathology* 103:880-887.
- Sipes, B. S., Schmitt, D. P., and Barker, K. R. 1992. Fertility of three parasitic biotypes of *Heterodera glycines*. *Phytopathology* 82:999-1001.
- Veloukas, T., and Karaoglani, G. S. 2012. Biological activity of the succinate dehydrogenase inhibitor fluopyram against *Botrytis cinerea* and fungal baseline sensitivity. *Pest Manage. Sci.* 68:858-864.
- Wang, J., Bradley, C. A., Stenzel, O., Pedersen, D. K., Reuter-Carlson, U., and Chilvers, M. I. 2017. Baseline sensitivity of *Fusarium virguliforme* to fluopyram fungicide. *Plant Dis.* 101:576-582.
- Wang, J., Donald, P. A., Niblack, T. L., Bird, G. W., Faghihi, J., Ferris, J. M., Grau, C., Jardine, D. J., Lipps, P. E., MacGuidwin, A. E., Melakeberhan, H., Noel, G. R., Pierson, P., Riedel, R. M., Sellers, P. R., Stienstra, W. C., Todd, T. C., Tylka, G. L., Wheeler, T. A., and Wysong, D. S. 2000. Soybean cyst nematode reproduction in the north central United States. *Plant Dis.* 84:77-82.
- Wang, J., Jacobs, J. L., Byrne, J. M., and Chilvers, M. I. 2015. Improved diagnoses and quantification of *Fusarium virguliforme*, causal agent of soybean sudden death syndrome. *Phytopathology* 105:378-387.
- Wang, X., Meyers, D., Yan, Y., Baum, T., Smant, G., Hussey, R., and Davis, E. 1999. *In planta* localization of a β -1,4-endoglucanase secreted by *Heterodera glycines*. *Mol. Plant-Microbe Interact.* 12:64-67.
- Wickham, H. 2007. Reshaping data with the reshape package. *J. Stat. Softw.* 21:1-20.
- Wickham, H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer, New York, NY, U.S.A.
- Ye, W. 2012. Development of primetime-real-time PCR for species identification of soybean cyst nematode (*Heterodera glycines* Ichinohe, 1952) in North Carolina. *J. Nematol.* 44:284-290.