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Research

The Effects of ILeVO and VOTiVO on Root Penetration and Behavior of the Soybean Cyst Nematode, *Heterodera glycines*

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Abstract

The objective of this study was to determine the effects of ILeVO (fluopyram) and VOTiVO (*Bacillus firmus* I-1582) seed treatments on *Heterodera glycines* second-stage juvenile (J2) root penetration and behavior. In a growth chamber experiment, roots of soybeans grown from treated or untreated seeds were inoculated with *H. glycines* J2s at soil depths of 2.5, 5, or 7.5 cm. ILeVO significantly reduced *H. glycines* root penetration compared with the untreated control, but only when J2s were inoculated at a soil depth of 2.5 cm, which was near the seed. Changes in nematode behavior were assessed by collecting 60-s videos of J2s after 2 h of exposure to exudates from treated seeds or radicles from treated seeds or from soil leachates in which treated seeds were planted. X- and y-coordinates of each of the 13 reference points were recorded every hour for 24 h. A custom program analyzed and transformed the coordinates into nematode motion parameters (speed and total change in curvature). ILeVO, but not VOTiVO, seed exudates significantly reduced J2 speed relative to the untreated control. Soil leachates from ILeVO or VOTiVO treatments had no consistent effect on *H. glycines* speed or total change in curvature compared with the untreated control. In another experiment, treated or untreated seeds were incubated in wells of 6-well tissue culture plates containing 11.5% Pluronic gel. Seeds were removed after 2 h, and approximately 50 J2s then were pipetted into each well. The plates were scanned every 60 min for 24 h, and the number of J2s in each well that moved a minimum distance of ≥300 μm was determined using another custom software program. ILeVO, but not VOTiVO, significantly reduced the movement of J2 populations relative to control wells in which no seeds were added. And wells that had seeds, treated or not, yielded significantly less J2 movement compared with the no-seed control. The results of these experiments indicate that ILeVO reduces activity on *H. glycines* J2s but may not affect nematodes beyond a limited area surrounding the treated seed.

The soybean cyst nematode, *Heterodera glycines* Ichinohe, is the most damaging pathogen of soybean, *Glycine max* (L.) Merr., in the United States and Canada (Allen et al. 2017). Host resistance and growing nonhost crops in rotation with soybeans have been the principal *H. glycines* management strategies for decades (Niblack et al. 2006). However, both management options have limitations. *H. glycines* eggs can survive for a decade or more in soil without a host (Inagaki and Tsutsumi 1971); thus growing nonhost crops in rotations with soybean will not eliminate infestations. And *H. glycines* populations capable of reproducing on soybeans containing the most common source of resistance, PI 88788, have become more prevalent in recent years (McCarville et al. 2017; Mitchum et al. 2007; Niblack et al. 2008). These issues have stirred interest in alternative management strategies, including using nematode-protectant seed treatments.

Seed treatment is a method in which seeds are coated with formulated products containing active ingredients (AIs) designed to protect young plants against pests and diseases (Munkvold et al. 2014). Seed treatment options for soybeans include products with AIs that target fungi, oomycetes, insects, and nematodes. Although nematode-protectant seed treatments have been studied since at least 1960 (O'Bannon and Reynolds 1960), they only have been available for soybeans since the mid-2000s. Both chemical nematicides and antagonistic microorganisms have been developed into commercially available seed treatments. There are several seed treatments for *H. glycines* control on the market, and a number of new products are expected in the near future.

Seed treatments are a promising management tool for *H. glycines* and other plant-parasitic nematodes because the products deliver AIs directly to the seed and root zones, reducing the total amount of AI used per hectare relative to other soil application methods (Munkvold et al. 2014). However, the performance of nematode-protectant seed treatments, as measured by increased crop yields or reduced nematode reproduction, has been inconsistent in field trials (Bissonnette et al. 2018; Gaspar et al. 2014; Wheeler et al. 2013). One possible explanation for observed performance variability is interactions of the seed treatments with the environment (Wheeler et al. 2013).

VOTiVO (AI *Bacillus firmus* I-1582; BASF) and ILeVO (AI fluopyram; BASF) are two seed treatment products that contain AIs with documented effects on plant-parasitic nematodes. *B. firmus* I-1582 stimulates plant defenses and inhibits *H. glycines* hatching and motility (Schrimsher 2013). Fluopyram is both fungicidal (Avenot and Michailides 2010) and nematistatic (Faske and Hurd 2015) and affects both *H. glycines* and the causal pathogen of soybean sudden death syndrome, *Fusarium virguliforme* (Kandel et al. 2016; Zaworski 2014). Our understanding of the effects of both AIs on *H. glycines* as formulated seed treatments is limited. For example, it is unclear how VOTiVO and ILeVO affect nematodes in different parts of the soil profile or if the AIs move reliably on or through the roots in quantities sufficient for nematode control in different sections of the root.

The objectives of this research were to examine i) root protection conferred by ILeVO and VOTiVO at different soil depths, ii) the effects of exudates (seed and radicle) from treated seeds and soil leachates from soil in which treated seeds were planted on *H. glycines* motion, and iii) the effects of treated seeds on movement of J2 populations.

Materials and Methods

Seed treatments and source of *H. glycines* inoculum. Seeds of an *H. glycines*–susceptible soybean cultivar (cv. Williams 82) were

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treated by Bayer CropScience personnel (Research Triangle Park, NC) at commercial rates of AI: 0.15 mg fluopyram/seed for ILeVO and 5×10^6 *B. firmus* spores/seed for VOTiVO. No other fungicides or insecticides were applied to the seeds used in the experiments. The population of *H. glycines* used in this study was from field soil (Fruitfield coarse sand) collected in Muscatine, Iowa. The *H. glycines* population was determined to be HG type 2.5.7 (Niblack et al. 2002). The nematodes were cultured on *H. glycines*–susceptible soybean (cv. Williams 82) under greenhouse conditions. Females and cysts (dead females) were collected from 4- to 8-week-old *H. glycines*–infected soybeans by dislodging the nematodes on the surface of the roots with a stream of water and collecting the nematodes and any soil and root debris on a 250-μm-pore sieve. Females and cysts then were crushed with a motorized rubber stopper to release the eggs (Faghihi and Ferris 2000), and the *H. glycines* eggs were separated from the debris using sucrose centrifugation (Jenkins 1964). Microsieves (30-μm pore) were constructed from nylon mesh (Elko Filtering Co., Switzerland) and test tube caps (Wong et al. 1993). Eggs were pipetted on the microsieves and incubated in a dish of sterile distilled water at 25°C. Second-stage juveniles (J2s) that hatched and moved through the sieves and into the dishes within 3 days were used for all experiments. Prior to experimental setup, the J2s were pipetted onto clean microsieves in dishes of sterile distilled water, and only the nematodes that moved through the sieves and into the dish within a few hours were used for experiments.

Root penetration experiment. The conical bottoms of 50-ml centrifuge tubes (Thermo Fisher Scientific Inc., Waltham, MA) were removed with a saw. Three holes (approximately 2 mm in diameter) were drilled in a straight line down the length of the tubes with a power drill. The capped tubes were inverted and filled with a pasteurized 2:1 sand:soil mixture (pH 7.6, organic matter 3.9%). The experiment was organized as a two-factor factorial with three levels of seed treatment (ILeVO, VOTiVO, and untreated) and three inoculation depths (2.5, 5, and 7.5 cm below the soil surface). Treated (ILeVO and VOTiVO) and untreated soybean seeds were planted approximately 2.5 cm deep, one seed per tube. The tubes were arranged in a growth chamber (25°C, 16-h photoperiod) in a randomized complete block design with four replications per experimental run. The soybeans were watered with tap water daily for 5 days before *H. glycines* inoculation. Roots of soybeans were inoculated with approximately 300 J2s at depths of 2.5, 5, and 7.5 cm below the soil line by pipetting nematodes in one of the holes drilled down the length of the tube. Two days after inoculation, the J2s that had penetrated the soybean roots were extracted. First, the roots were cut into 1- to 2-cm-long pieces and homogenized using a Farberware kitchen blender (Meyer Corp., Vallejo, CA) for approximately 30 s (de Boer et al. 1999). The homogenate then was poured over a 250-μm-pore sieve on top of a 25-μm-pore sieve, and a motorized rubber stopper was used to grind root fragments on the top sieve (Faghihi and Ferris 2000). The J2s were collected on the 25-μm-pore sieve and quantified visually using a Nikon AZ100 microscope (Nikon Instruments Inc., Tokyo, Japan) at 20× magnification. The experiment was repeated once.

Seed and radicle exudate collection. Seed and radicle exudates were collected using the method reported by Beeman and Tylka (2018). Seeds were incubated in sterile distilled water at a ratio of one seed per 5 ml water for 1 h on a platform shaker (100 rpm). Radicle exudates were collected similarly; first seeds were germinated on 1.5% water agar, and radicles (5 to 7 cm length) of intact, 3-day-old soybean seedlings were incubated on a platform shaker at 100 rpm for 1 h in sterile distilled water at a ratio of one radicle per 5 ml water. During radicle exudate collection, the shoots of the seedlings (including the seed coat) were suspended on cheesecloth and were not in contact with the sterile water. All exudates were poured over 30-μm-pore nylon mesh to remove debris and stored at approximately 4°C before experimental setup.

Soil leachate collection. Microsieves (30-μm pore, 2 cm height, and 2 cm diameter) were filled with the pasteurized, 2:1 sand:soil mixture described previously and watered with sterile distilled water to approximate field capacity (2.5 ml per microsieve). Treated (VOTiVO and ILeVO) and untreated Williams 82 seeds were planted in the microsieves at a ratio of one seed per microsieve and incubated at 25°C for 24 h. Another 2.5 ml of sterile distilled water then was added to each microsieve, and the leachates escaping from the bottom of each microsieve were collected in wells of 6-well tissue culture plates (Corning Inc., Corning, NY). Solutions collected from microsieves with no seeds, hereafter referred to as the blank, and sterile distilled water served as controls. Each soil leachate treatment had three replications that subsequently were pooled. The leachates then were centrifuged at $2{,}200 \times g$ for 2 min to remove soil debris, and the supernatant was stored at approximately 4°C before experimental setup.

Effect of exudates and leachates on *H. glycines* motion. Changes in speed and curvature of individual *H. glycines* J2s were studied using a method developed by Jensen et al. (2018). Exudates (seed and radicle) and soil leachates were tested in separate experiments. *H. glycines* J2s were pipetted into 1.5-ml centrifuge tubes containing exudates or leachates for 2 h (25°C). The J2s then were rinsed three times with sterile distilled water and then suspended in water; 10 to 15 μl of the suspension was pipetted onto glass slides with a 20-mm-diameter coverslip and imaged with a QICAM 12-bit Color Fast 1394 camera (Ziosoft Inc., Tokyo, Japan) connected to a Leica M205-C microscope (Leica Microsystems Inc., Buffalo Grove, IL) at 63× magnification (6.3× zoom, 10× lens, 1× camera) with overhead lighting. Each nematode was imaged for 1 min with a rate of 10 frames per second. In both experiments, water served as a positive control and heat-killed nematodes as a negative control. The heat-killed nematodes were generated by placing live nematodes in sterile distilled water in a tube and immersing the tube in boiling water for 2 min. The videos were processed using two custom software programs written in MATLAB (MathWorks Inc., Natick,

Table 1. Total number of *Heterodera glycines* juveniles (J2s) analyzed for exudate (seed and radicle) and soil leachate experiments over two experimental runs

	Total number of J2s analyzed		
Treatment	**Seed exudates**	**Radicle exudates**	**Soil leachates**
ILeVO	15	10	10
VOTiVO	8	9	14
Untreated	12	12	12
Controls	**Seed and radicle exudate experiments**		**Soil leachates**
Heat killed	11		13
Water	8		9
Blank	N/A		12

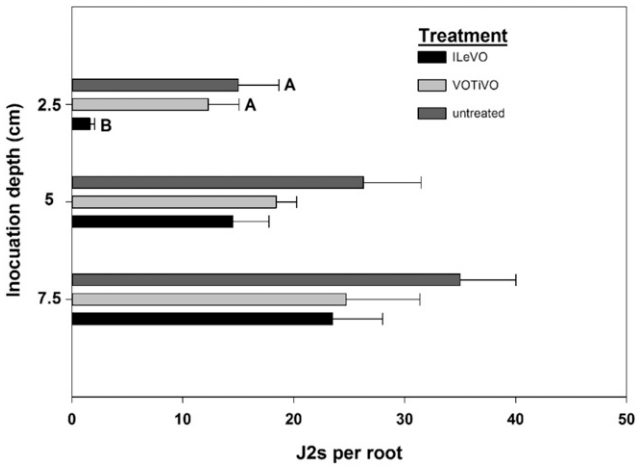

Fig. 1. Number of *Heterodera glycines* second-stage juveniles (J2s) recovered from roots of soybeans grown from treated seeds after inoculation with approximately 300 J2s at three different soil depths in a growth chamber experiment. Error bars are standard errors of the means. Different letters to the right of the bars in the same inoculation depth indicate significant differences according to Tukey's honest significant difference test ($\alpha = 0.05$).

MA) to measure changes in nematode behavior (Jensen et al. 2018). Briefly, the software assigned 13 tracking points along the length of a nematode body and recorded the x- and y-coordinates of each point. The *H. glycines* motion parameters that were calculated from the x- and y-coordinates were speed (13 discrete body points) and total change in curvature (whole body). Average speed of each body point over the 1-min video was determined by averaging the instantaneous speeds, in μm/s, of the body points at each of the frames over the 60-s video (Jensen et al. 2018; Njus et al. 2015). The speed of the anterior-most body point, referred hereafter as the head point, was analyzed for each nematode and considered the representative speed for the whole body. Curvature was assessed by calculating the radius of a hypothetical circle tangent to two lines connecting three continuous tracking points on a nematode body (Jensen et al. 2018; Likitlersuang et al. 2012), and values were expressed in 1/μm. Total change in curvature was calculated by summing the instantaneous change in curvature at each of the body points in successive frames and averaging the value over the length of the video. Eight to 15 nematode juveniles were analyzed per treatment over two experimental runs (Table 1).

Effect of seed treatments on movement of *H. glycines* populations. Movement of populations of *H. glycines* J2s after exposure to seed treatments was measured using a scanner assay described by Jensen et al. (2018). Wells of 24-well tissue culture plates (Corning Inc., Corning, NY) were filled with 500 μl of 11.5% Pluronic F-127 gel (Sigma Life Science, St. Louis, MO). A single treated (ILeVO or VOTiVO) or untreated Williams 82 seed was inserted into each well. Wells without seeds, referred to hereafter as the blank, served as a control. Seeds were incubated in the wells at 25°C for 2 h before being removed with clean forceps. Then, 54 ± 14 J2s (mean ± standard deviation) were pipetted into each well. The 24-well plate was incubated on an EPSON Perfection V750-M Pro scanner (Seiko Epson Corp., Japan) housed inside a temperature-controlled poly(methyl methacrylate) box (25°C). The plate was scanned (2,400 dpi) automatically every hour for 24 h (Jensen et al. 2018). After image collection, the J2s in each image were marked with a cursor in a custom program written in MATLAB, and the x- and y-coordinates of the centroids of the J2s in each of the wells at every time point was determined by the program. The x- and y-coordinates of all J2s were analyzed in another custom MATLAB program to calculate the percent of the *H. glycines* population that had moved 300 μm or more during the hour between when each pair of images was captured (Jensen et al. 2018). Percent movement data were collected for each time point in the experiment. There were three to four replications per run, and the experiment was repeated once.

Statistical analyses. The data from all experiments were analyzed in SAS Version 9.4 (SAS Institute, Cary, NC). The combined data from each experiment were subjected to analysis of variance (ANOVA) using the normal distribution, and residual plots were examined before additional analysis. Main effects (seed treatment and inoculation depth) and interactive effects were analyzed for significance in the root penetration experiment using PROC GLIMMIX. For the scanner experiment, repeated measures analysis was conducted using PROC MIXED. Results from the scanner experiment were then averaged for 0–6 h, 7–16 h, and 17–24 h, and treatments were compared for each of these time periods. For all experiments, least-squared means were separated using Tukey's honest significant difference (HSD) test (PROC GLIMMIX, $\alpha = 0.05$).

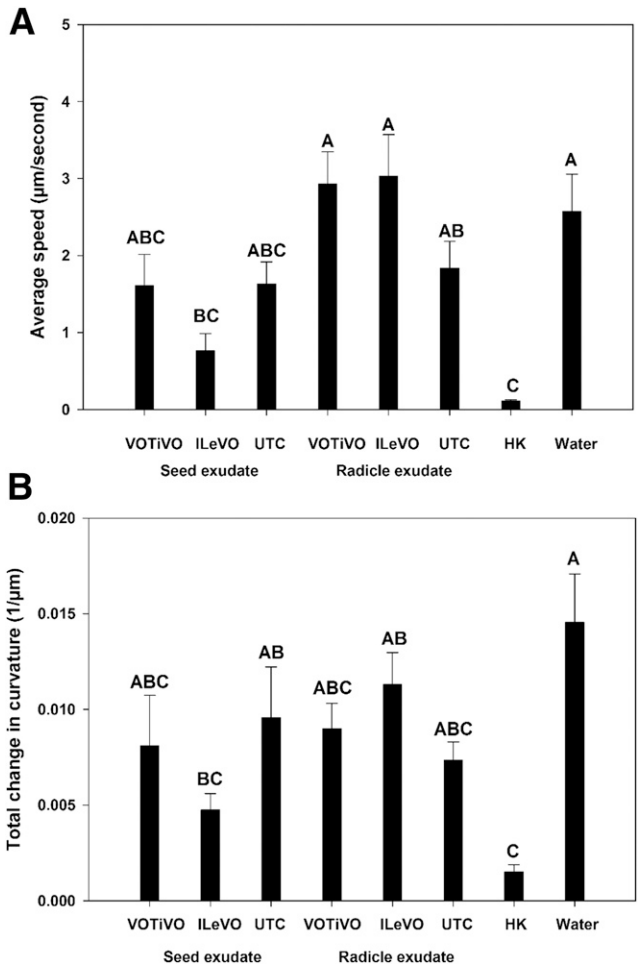

Fig. 2. Mean (± standard error of the mean) head speed (**A**) and total change in curvature (**B**) of *Heterodera glycines* second-stage juveniles (J2s) exposed to seed and radicle exudates for 2 h. HK = heat-killed J2s, UTC = untreated control. Different letters indicate significant differences according to Tukey's honest significant difference test ($\alpha = 0.05$).

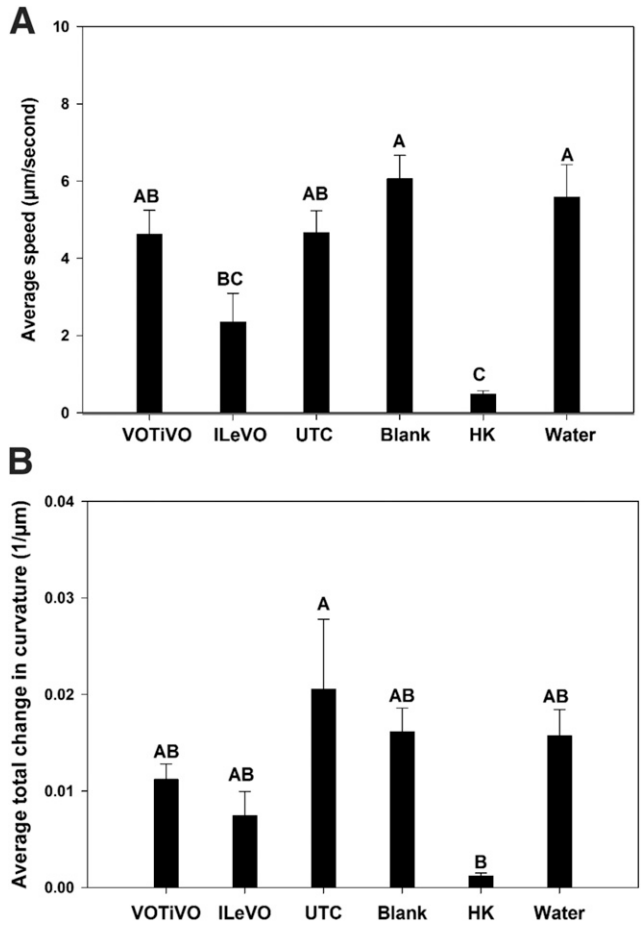

Fig. 3. Mean (± standard error of the mean) head speed (**A**) and total change in curvature (**B**) of *Heterodera glycines* second-stage juveniles (J2s) exposed to soil leachates for 2 h. HK = heat-killed J2s, UTC = untreated control. Different letters indicate significant differences according to Tukey's honest significant difference test ($\alpha = 0.05$).

Results

Root penetration experiment. When analyzed as a two-factor factorial experiment, significant main effects were detected for seed treatment and inoculation depth. There was no interaction between the main factors. Data then were analyzed by seed treatment as a single factor experiment. Across all inoculation depths combined, ILeVO significantly reduced root penetration relative to the untreated control (data not shown). However, when the data were analyzed for effects of seed treatment at different inoculation depths, there was a significant, 87% reduction in the number of J2s that had penetrated roots at the 2.5-cm inoculation depth compared with the untreated control (Fig. 1). There were no significant effects of ILeVO at the 5- or 7.5-cm inoculation depths (Fig. 1). VOTiVO did not affect root penetration at any inoculation depth (Fig. 1) in our experiments.

Effect of exudates and leachates on *H. glycines* motion. For each experiment, the number of J2s analyzed in each treatment is listed in Table 1. The experiments studying the effects of seed and radicle exudates were conducted at different times, and the data were analyzed separately (Figs. 2 and 3). For seed exudates, motion of the heads of nematodes incubated in the ILeVO treatment was numerically lower than in the VOTiVO and untreated seed exudates, but not significantly different. ILeVO and VOTiVO radicle exudates did not affect the head speed of J2 compared with that of untreated radicle exudates (Fig. 2A). The speed of J2s exposed to ILeVO seed exudate was significantly less than that of J2s exposed to the water control and VOTiVO and ILeVO radicle exudates (Fig. 2A). ILeVO or VOTiVO seed and radicle exudates did not significantly affect average total change in curvature of the nematodes relative to the untreated seed or radicle exudate control (Fig. 2B). However, J2s exposed to ILeVO seed exudates had significantly less total change in curvature relative to the water control.

In the soil leachate experiment, there were no significant differences in the speed of head motion (Fig. 3A) or change in curvature (Fig. 3B) among the seed treatments and the untreated control. However, ILeVO soil leachates significantly reduced the speed of motion of the head of *H. glycines* J2s relative to the blank control (Fig. 3A).

Limited levels of motion and change in curvature were detected in the heat-killed control nematodes in both experiments. This motion was due to small vibrations or drift of the dead nematodes (Figs. 2 and 3).

Effect of seed treatments on movement of *H. glycines* populations. In general, populations of *H. glycines* in the treatments had similar levels of movement in the first few hours, but amounts of movement diverged according to treatment at later time points (Fig. 4A). The movement of the nematode populations in the ILeVO treatment was less than in the untreated control at most of the time points (Fig. 4A). The results of the repeated measures analysis revealed significant interactive effects of treatment and time ($P < 0.0001$). Consequently, average percent movement over three time periods (0–6 h, 7–16 h, 17–24 h) was calculated and compared. From 0 to 6 h, movement of the *H. glycines* populations was similar across all treatments, with the exception of a significant difference between the blank and ILeVO treatments (Fig. 4B). Between 7 and 16 h, nematodes in the ILeVO treatment moved at a significantly lower rate compared with the untreated, the VOTiVO, and the blank control treatments (Fig. 4C). From 17 to 24 h, movement across all treatments was reduced to less than 25%, with the blank treatment having significantly greater movement than the other three

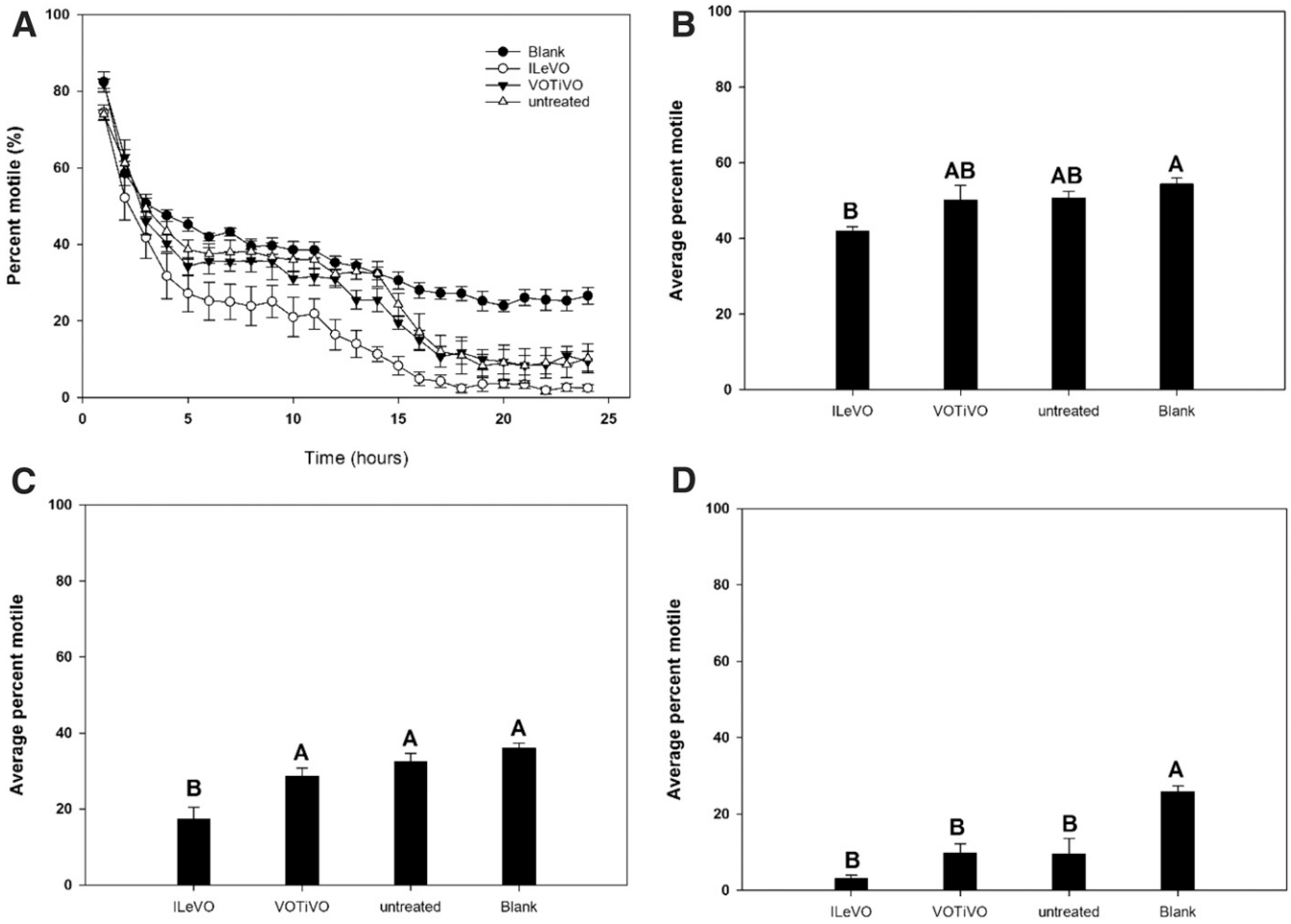

Fig. 4. Movement of *Heterodera glycines* second-stage juveniles (J2s) in 3-cm wells that previously contained treated or untreated seeds. (**A**) Average percent movement (mean ± standard error of the mean) at each 1 h time point, (**B**) movement of *H. glycines* J2 populations averaged from 0 to 6 h, (**C**) movement of *H. glycines* J2s from 7 to 16 h, (**D**) movement of *H. glycines* J2s from 17 to 24 h. Error bars indicate standard errors of the mean. Letters above bars in **B**–**D** indicate significant differences according to Tukey's honest significant difference test ($\alpha = 0.05$).

treatments that contained a seed prior to introduction of the nematode (Fig. 4D).

Discussion

The seed treatment ILeVO affected most of the *H. glycines* parameters studied in our experiments. The effects were evident when the J2s were in close proximity to ILeVO-treated seeds or in ILeVO seed exudates but not when the nematodes were exposed to ILeVO radicle exudates or leachates from soil containing ILeVO-treated seeds. In contrast, VOTiVO had no effect on the *H. glycines* parameters we measured in our experiments.

ILeVO conferred protection to soybean roots when *H. glycines* J2s were inoculated at a 2.5 cm soil depth near the planted seeds. However, root protection from ILeVO was not observed at 5 and 7.5 cm nematode inoculation depths, which was 2.5 and 5 cm below the seed. This result may indicate that ILeVO protects soybean roots against *H. glycines* infection in a limited area near the treated seed. Similar results were reported for roots of cotton plants grown from abamectin-treated seeds, where initial *Meloidogyne incognita* galls occurred further down the cotton taproot grown from abamectin-treated seed compared with untreated check plants (Faske and Starr 2007). Using an assay described by Jensen et al. (2018), we also found exudates from ILeVO-treated seeds significantly reduced the speed of motion of the *H. glycines* head and curvature of the body, relative to a water-only control. But ILeVO and VOTiVO seed and radicle exudates and leachates from soil in which treated seeds were planted had no consistent effect on the motion of the head of *H. glycines* J2s relative to that of J2 in the untreated seed controls. The lack of effects of radicle exudates and soil leachates from seeds treated with *B. firmus* I-1582 (AI of VOTiVO) and fluopyram (AI of ILeVO) on the nematode J2s may indicate that the seed treatment AIs did not travel in large quantities on roots or in the soil in our experiments. However, it is possible that both seed treatments could have greater mobility in soils with different properties (pH, organic matter, etc.) or under different watering regimes. We did not test leachates from different soil types to assess this hypothesis.

The scanner assay, also described in Jensen et al. (2018), estimated the percentage of *H. glycines* J2s in a population that traveled a critical threshold distance ($\geq$300 μm) over 1 h in wells that contained treated or untreated seeds for 2 h prior to the addition of the nematodes. Regardless of treatment, movement of the J2 populations decreased over time. This decreased movement trend of the J2s was possibly due to lack of external chemical stimuli or the gradual depletion of oxygen in the wells over time. ILeVO-treated seeds significantly reduced the movement of *H. glycines* populations between 7 and 16 h after the start of the experiment, relative to an untreated seed control (Fig. 4C). This result indicates that the amount of fluopyram that moved from the treated seed into the Pluronic gel after 2 h was enough to affect *H. glycines* movement. Thus, it is possible that an ILeVO-treated seed would leach fluopyram into the soil surrounding the seed zone and inhibit movement of nearby J2s, potentially resulting in reduced nematode infection and plant damage.

VOTiVO did not affect any aspect of *H. glycines* biology that was measured in our experiments. Schrimsher (2013) reported that *H. glycines* J2s were immobilized when incubated with *B. firmus* concentrations ranging from 1×10^6 to 1×10^7 cfu/ml. However, in another study, *B. firmus* did not immobilize J2s or reduce nematode root penetration (Beeman and Tylka 2018). There are a few possible explanations for the lack of activity of VOTiVO on *H. glycines* in our study. The seeds were treated at a commercial rate of 5×10^6 spores/seed, and it is conceivable that the number of spores coming off the seed and into the soil would be less than the effective concentrations reported by Schrimsher (2013). Additionally, the spores of the bacteria likely need to germinate and reproduce to be effective, and the duration of our experiments may have been too short for *B. firmus* to reach densities necessary for *H. glycines* control. VOTiVO is reported to induce plant defenses (Schrimsher 2013), potentially leading to increased plant biomass relative to an untreated control. We did not measure any plant growth parameters in our experiments. Lastly, the *B. firmus* I-1582 used in our experiments may not have been viable, something that we did not check. Repeating the same studies with seeds treated with new and different batches of *B. firmus* I-1582 would be necessary to explore this possibility. Additional research with altered experimental conditions and measured plant growth variables is necessary to fully understand the nematode-protectant capabilities of VOTiVO.

There are a few challenges in measuring behavioral differences in plant-parasitic nematodes compared with other soil-dwelling nematodes. In general, plant-parasitic nematodes are less active than other nematodes inhabiting the soil, such as *Caenorhabditis elegans*. For example, in our experiments, *H. glycines* J2s in control treatments had average head motion speeds of approximately 2–6 μm/s, whereas centroid points on the body of *C. elegans* have been reported to move at speeds around 100 μm/s or more when measured with the same or similar software and image-capturing techniques (Njus et al. 2015; Saldanha et al. 2013). Also, the movement of the nematode populations in the scanner was below 50% for most of the experiment and below 15% by the end of the experiment for the treatments that had a seed in the well prior to placement of the nematodes in the wells. Because *H. glycines* activity under ambient conditions started at a relatively low baseline in our studies, it may have been more difficult to discern treatment effects than it would be using more active nematodes such as *C. elegans*. It is also unclear whether other, unmeasured factors in the studies had an impact on the baseline activity of the nematodes that further challenged comparing treatments. ILeVO reduced root penetration and movement of J2s in some experiments, but it is unclear whether such a reduction would result in reduced nematode reproduction under less controlled conditions. It is important to note that any effects observed in vitro may not be relevant in the field environment (Spence et al. 2008). Similarly, lack of effects in our laboratory studies may not mean that such effects do not occur in the field. In our experiments, we attempted to study the effects of these seed treatments on specific aspects of *H. glycines* biology under controlled experimental conditions. Additional work examining the fitness of nematodes treated with sublethal amounts of ILeVO and field efficacy trials are necessary to determine whether behavioral effects of the compound on *H. glycines* result in meaningful control in the context of the field.

Our results suggest that ILeVO has activity against *H. glycines*, but its effects on the nematode may dissipate as the roots grow away from the treated seed. However, significant plant biomass increases have been reported by delaying nematode inoculation by several days (Huang and Ploeg 2001). Brief periods of soybean root protection conferred by ILeVO (and possibly VOTiVO) therefore could result in higher soybean biomass accumulation and yields. Field studies to understand the interactions of seed treatments with environmental factors and nematode densities, as well as economic cost-benefit analyses of the different seed treatment options, could enhance the utility of this management strategy.

Acknowledgments

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