Investigating the Suppressive Potential of Generalist Predators and Entomopathogenic Nematodes Against *Resseliella maxima* (Diptera: Cecidomyiidae) in South Dakota[1]

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J. Entomol. Sci. 61(2): 000–000 (Month 2025)
DOI: 10.18474/JES25-20

Abstract The soybean gall midge, *Resseliella maxima* Gagné, emerged as a new species and pest of soybean in the northern Great Plains in 2018. Management tools such as insecticides are largely ineffective because the damaging larval stages, which occur inside a plant stem, are hidden and protected; thus, alternative approaches such as biological control need to be investigated. The purpose of this research was to assess the biological control potential of generalist predators that had not been previously exposed to soybean gall midge and of entomopathogenic nematodes (EPN), which have been shown to infect and kill other gall midge species but have not been evaluated for soybean gall midge. The objectives were (a) to compare soybean gall midge predation rates of three ground-dwelling and three foliar predator species collected from a soybean field without soybean gall midge in bioassays with and without soil and (b) to evaluate the susceptibility of soybean gall midge larvae to four commercially available EPN isolates in the laboratory, each at three inoculation rates. Generalist predators not previously exposed to soybean gall midge consumed 39–98% of larvae within 24 h, although the addition of soil to the bioassay significantly reduced consumption rates for species evaluated in both bioassays. We confirmed that all four EPN species successfully infected and killed larvae, with similar survival rates among EPN species and inoculation rates tested. These results suggest that biological control agents may be a vital component for integrated pest management of soybean gall midge.

Key Words soybean gall midge, *Heterorhabditis*, *Steinernema*, Carabidae, Coccinellidae

The soybean gall midge, *Resseliella maxima* Gagné (Diptera: Cecidomyiidae), was identified as a new species (Gagné et al. 2019) and pest of soybean in the midwestern United States in 2018 in Iowa (Hodgson 2018), Minnesota (Potter and Koch 2018), Nebraska (McMechan et al. 2021b), and South Dakota (Varenhorst and Strunk 2020). By the end of 2024 soybean gall midge was reported from 178 counties in seven states (Soybean Gall Midge Alert Network 2025), including

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Kansas (Zuckoff 2024), Missouri (Lucas et al. 2021), and North Dakota (Knodel 2023). The adult lays eggs in fissures that naturally form below cotyledons at the base of V2 to V3 soybeans (McMechan et al. 2021a) or develop when plants are damaged by weather events such as hail or wind (Varenhorst and Strunk 2020), which can result in a simple gall (Gagné and Jaschof 2021). The larvae feed and develop between the living and dead plant tissue in the stem, reducing the uptake of water and nutrients by the plant. Infested plants wilt, abort pod development, and ultimately die when gall midge population is too high (McMechan et al. 2021a).

Gall midges (Diptera: Cecidomyiidae) are difficult to control because the larval stages, which cause the damage, are hidden and protected within plant tissue. Soybean gall midge management relies heavily on chemical insecticides, yet these treatments are largely ineffective because they cannot directly target the larvae (Hodgson and Helton 2021, McMechan 2021, Montenegro et al. 2022, Hodgson and Kolbe 2023). As a result, farmers are left with significant plant stand and yield losses as high as 100% near field edges (McMechan et al. 2021a). Alternative management approaches such as biological control (e.g., predators, parasitoids, and entomopathogens) are being investigated (Melotto et al. 2023a, 2023b; von Gries et al 2025).

Biological control agents may be more advantageous than insecticides because these agents can detect and seek out larvae and pupae occurring within plant galls or in the soil (Evans et al. 2015). Of particular interest are generalist predators such as lady beetles (Coleoptera: Coccinellidae) and ground beetles (Coleoptera: Carabidae), which actively orient toward prey. Melotto et al. (2023c) investigated the predation behavior of foliar and ground-dwelling predators collected from Minnesota fields infested with soybean gall midge. In petri dishes, the predators consumed larvae within 1 h, although prey consumption rates differed among predators after 24 h. Von Gries et al. (2025) found that *Pterostichus melanarius* (Illiger) (Coleoptera: Carabidae) preferred soybean gall midge larvae over soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae). It is unclear whether generalist predators that have not been previously exposed to soybean gall midge are preadapted to consuming the midge larvae and whether the inclusion of soil would affect predation rates.

Entomopathogenic nematodes (EPNs) of the families Heterorhabditidae and Steinernematidae (Nematoda: Rhabditida) are another group of biological control agents that are naturally occurring soil-borne parasites of insects (Kaya and Gaugler 1993). The infective juvenile (IJ) stage is free living, occurs in a state of arrested development, and must identify potential insect hosts to infect for food, development, and reproduction (Ishibashi and Kondo 1990). IJs must enter a host through a natural opening (i.e., mouth, anus, or spiracles) or through the insect cuticle and bypass the insect's immune response (Castillo et al. 2011, Hazir et al. 2022). Once in the host, the IJ releases a mutualistic bacteria that causes sepsis or toxemia and results in quick host death within 72 h (Li et al. 2007). Once established inside the host, the IJs begin feeding, develop, and reproduce, completing one to three generations (Lewis and Clarke 2012). When food resources are depleted, new IJs are formed and emerge from the host into the soil to seek out new hosts, completing the life cycle. EPNs are attractive for biological control because they naturally persist in the soil (Shields et al. 2018), rapidly kill insect hosts after infection (Li et al. 2007), significantly proliferate in the host (Wang and Grewal 2002), and are compatible with other management tools (Shapiro-Ilan

et al. 2019). Recent advancements in rearing technology have made it feasible to mass produce EPNs for biological control, and at least 13 EPN species have been used for commercial applications (Shapiro-Ilan et al. 2023).

EPNs have infected and killed larvae and pupae of other gall midge species in laboratory and field trials. For example, *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) caused 90–100% mortality of larvae of the swede midge, *Contarinia nasturtii* (Kieffer) (Diptera: Cecidomyiidae) in various soil types in the laboratory (Corlay et al. 2007). In another study, *Steinernema feltiae* (Filipjev, 1934) Wouts, Mráček, Gerdin & Bedding, 1982 (Rhabditida: Steinernematidae), and *H. bacteriophora* significantly suppressed swede midge adult emergence in broccoli fields (Evans et al. 2015). Soybean gall midge spends a portion of its life cycle in the soil—late instars move into the soil for pupation and overwintering—where they may naturally encounter EPNs (McMechan et al. 2021a). Evans et al. (2015) also observed that EPNs applied to cauliflower meristems infected with swede midge moved into the gall within 72 h. EPNs have not yet been evaluated against the soybean gall midge but may provide sustainable and long-term management of this pest in soybean.

The overall goal of this study was to assess the biological control potential of generalist predators and EPNs for soybean gall midge in South Dakota. The objectives of this study were (a) to compare soybean gall midge predation rates of ground-dwelling and foliar predators collected from an area that has not experienced soybean gall midge infestations and (b) to evaluate the susceptibility of soybean gall midge larvae to four commercially available EPN isolates in the laboratory, each at three inoculation rates. These experiments will determine whether biological controls may be useful for controlling soybean gall midge populations and, thus, whether additional research is warranted.

Materials and Methods

Soybean gall midge. Larvae for the nematode rate bioassay were collected from two soybean fields in Minnehaha County, SD, on 11 August and 16 August 2023. Additional larvae for the predator bioassay were collected once weekly from one soybean field between 17 July and 7 August 2024. Entire plants showing symptoms of soybean gall midge infestation (i.e., wilting foliage and/or dark simple gall near base of plant) were collected and brought to the USDA, Agricultural Research Service (ARS), North Central Agricultural Research Laboratory (NCARL) in Brookings, SD. Entire plants were stored in a dark cold room at 9°C, and the larvae were used within 72 h.

Predators. Three species of abundant soil-dwelling arthropods (Table 1), *Arctosa rubicunda* (Keyserling) (Araneae: Lycosidae), *Bembidion quadrimaculatum oppositum* Say (Coleoptera: Carabidae), and *Harpalus pensylvanicus* (Degeer) (Coleoptera: Carabidae), were collected weekly using dry pan traps from 18 July to 9 August 2024 from a corn field and soybean field in Brookings, SD. Each pan trap consisted of a plastic sweater box (26 $\times$ 32 $\times$ 9 cm) (EN 631-1; Rubbermaid Commercial Products Inc., Winchester, VA) that was set into the ground so the upper lip was flush with the soil and was filled with a shallow layer ($\sim$1.3 cm) of soil containing residues in the bottom of each trap to resemble the soil surface environment. After 48 h, we brought traps into the lab and sorted and identified

Table 1. Ground-dwelling and foliar predator species evaluated in the soybean gall midge predation bioassay.

Predator Type	Order: Family	Species	Medium	Life Stage (n)
Ground	Araneae: Lycosidae	*Arctosa rubicunda*	Petri	Adult (11)
	Coleoptera: Carabidae	*Bembidion quadrimaculatum oppositum*	Petri	Adult (20)
			Soil	Adult (20)
	Coleoptera: Carabidae	*Harpalus pensylvanicus*	Petri	Adult (10)
Foliar	Coleoptera: Coccinellidae	*Coccinella septempunctata*	Petri	Larva (14), adult (20)
			Soil	Adult (20)
	Coleoptera: Coccinellidae	*Harmonia axyridis*	Petri	Adult (21)
	Coleoptera: Coccinellidae	*Hippodamia convergens*	Petri	Larva (18), adult (22)
			Soil	Larva (10), adult (31)
Control			Petri	(20)
			Soil	(20)

predators before starting the bioassay. Three foliar predator species (Table 1), *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), and *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), were hand collected weekly from 25 July to 9 August 2024 from a soybean field in Brookings, SD that has not experienced soybean gall midge infestations, and predators were starved for 24 h in the lab before the start of the experiment.

Predation bioassay. Following the protocol of Melotto et al. (2023c), we used a paintbrush to carefully transfer seven third-instar soybean gall midges to individual Petri dishes (9 × 1.5 cm). We then placed one predator in each Petri dish and sealed the dish with parafilm to prevent insects from escaping. We also included a subset of control dishes with no predators to ensure that larvae did not escape or die. All dishes were randomly placed in a walk-in growth chamber at 25°C with 50% relative humidity. We counted the number of larvae consumed or killed per Petri dish 1 h after inoculation and again at 24 h. We then assessed predation rates for three of the predators (Table 1) in soil using the aforementioned experimental setup but with a small layer of autoclaved field-collected soil (30 ml) placed into each Petri dish before adding seven third instars and a single predator; a subset of control dishes did not include a predator. We evaluated the number of larvae

consumed after 24 h by manually sifting through the soil and counting the number of larvae.

EPNs. Cultures of four EPN species were obtained from the USDA-ARS Fruit and Tree Nut Research Laboratory in Byron, GA: *H. bacteriophora* (VS strain), *Heterorhabditis indica* Poinar, Karunakar & David, 1992 (HOM1 strain), *Steinernema carpocapsae* Weiser, 1995 (All strain), and *Steinernema riobrave* Cabanillas, Poinar, & Raulston, 1994 (355 strain). All four of the EPN species are commercially available. Before the experiment, EPN populations were cultured by infecting larvae of the greater wax moth, *Galleria mellonella* (L.), and isolating them with a modified White trap (White 1927, Hazir et al. 2022). Isolated EPNs were maintained in culture flasks with water in a dark room at 14°C and used within 2 weeks of emergence. A native isolate of *S. carpocapsae* (strain SD) was obtained from agricultural soils in Brookings, SD and similarly reared and maintained as the other EPNs. Before experimentation, we used serial dilution to adjust EPN concentrations for all species.

Nematode rate bioassay. The virulence of different EPNs applied at three different rates were evaluated against soybean gall midge. Each experimental replicate consisted of a 118-ml Mason jar (5.0 cm diameter) with a screw top lid (Ball® Corporation, Broomfield, CO) that was punctured once to allow airflow and lined with a filter paper (no. 1, Whatman, Clifton, NJ). IJs from each EPN strain were applied to individual filter papers in 1 ml of water; water controls did not receive EPNs. Three inoculation rates were tested for each EPN strain: 50 IJs/larva (low rate) (i.e., $\sim$25 IJs/cm^2), which is recommended for agricultural field applications (Hazir et al. 2022); 200 IJs/larva (intermediate rate); and 500 IJs/larva (high rate). Soybean plant galls were longitudinally dissected, and 10 presumed third instars—indicated by their orange coloration—were carefully transferred with a paintbrush to each Mason jar. Larvae were checked for EPN infection every 3 d for 30 d by probing them with a paintbrush; larvae were recorded as dead, alive, or missing. Dead larvae were removed from the Mason jar and immediately dissected for the presence of EPNs to assess whether the infection progressed past death. Two experimental sets were conducted consecutively with four and five replicates in each set, respectively, for a total of nine replicates per treatment–rate combination. We then repeated this experiment but with only *S. carpocapsae* (SD) at the three inoculation rates and a water control.

Statistical analysis. For the predation study, we first calculated mortality rates for each predator by dividing the number of larvae remaining at 1 and 24 h for the Petri dish bioassay and at 24 h for the soil bioassay by the starting number of larvae ($n = 7$). We did not include the control treatment in the analyses because none of the larvae died or escaped during the study. In separate analyses by bioassay and time, we compared mortality rates among predator species and life stages by using a generalized linear mixed model in PROC GLIMMIX in SAS 9.4 (SAS Institute Inc., Cary, NC). We designated life stage nested within predator as the fixed effect and the residual as a random effect. Multiple comparisons were conducted using a simulated adjustment with LSMeans at $\alpha < 0.05$. We similarly compared mortality rates among predator species and life stages that were evaluated in both the Petri dish and soil bioassays by using PROC GLIMMIX but with the predator*bioassay interaction as a fixed effect.

For the nematode rate bioassay, we first calculated soybean gall midge survival and infection rates per replicate after exposure for 30 d. Survival rates were calculated by dividing the number of live larvae by the starting total number of larvae. Infection rates were calculated for each replicate by dividing the number of dead larvae with confirmed EPN infection (at the time of breakdown) by the starting total number of larvae tested. Both survival and infection percentage data were subjected to arcsine square root transformation to satisfy normality criterion (Warton and Hui 2011). Cumulative percentage survival and percentage infection among EPN treatments were determined in separate analyses with a nested analysis of variance (ANOVA) with PROC GLIMMIX in SAS 9.4. Main effects were EPN (i.e., *H. bacteriophora*, VS strain; *H. indica,* HOM1 strain; *S. carpocapsae,* All strain; *S. riobrave,* 355 strain; and water control), rate (i.e., 50, 200, and 500 IJs/larva), and rate nested within each EPN strain. Experimental set was designated as a random effect. Mean comparisons were conducted using the simulated adjustment (Edwards and Berry 1987) with LSMeans at $P \leq 0.05$.

We also compared both percentage survival and percentage infection over time in separate analyses by inoculation rate (50, 200, and 500 IJs/larva) with a repeated measures ANOVA in PROC GLIMMIX. Fixed effects were EPN, time, and the EPN*time interaction. Experimental set and time were designated as random effects. Mean comparisons were conducted separately for each evaluation time with the slicediff option and a simulated adjustment with LSMeans at $P \leq 0.05$. We subsequently compared the regression coefficients stemming from survival and infection over time among inoculation rates in separate analyses for each EPN species using PROC GLIMMIX to determine whether the strength of the relationship differed among inoculation rates.

Results

Predation bioassay. In the Petri dish bioassay, soybean gall midge larvae consumption rates significantly differed among predators. After 1 h of exposure to soybean gall midge larvae, *H. axyridis* and adult *H. convergens* consumed more prey than did the spider *A. rubicunda* and the ground beetle *B. quadrimaculatum* ($F = 7.04$; df $= 7, 128$; $P < 0.0001$) (Fig. 1A). After 24 h, *A. rubicunda* and *B. quadrimaculatum* consumed fewer prey than did all other predators including lady beetle larvae and adults and the ground beetle *H. pensylvanicus* ($F = 26.45$; df $= 7, 128$; $P < 0.0001$) (Fig. 1B). Consumption rates did not differ among predator species in the soil bioassay ($F = 2.39$; df $= 3, 77$; $P = 0.07$) (Fig. 2). For the predator species and life stages evaluated in both bioassays, the addition of soil to Petri dishes significantly reduced consumption of soybean gall midge larvae ($F = 20.61$; df $= 6, 153$; $P < 0.0001$) (Fig. 2).

Nematode rate bioassay. After 30 d of exposure to EPN treatments, soybean gall midge larval survival was 57–66%, and the nested ANOVA analysis indicated survival was similar among EPN treatments, including the water control (Table 2). Survival rates were also not affected by inoculation rate, and the EPN*rate interaction was not significant (Table 2). Repeated measures analysis revealed that larval survival decreased over time for all three inoculation rates (Table 3; Fig. 3). However, the regression coefficients for the four EPN species were similar to those of the control (0 IJs/larva) at the intermediate (200 IJs/larva) and high (500 IJs/larva)

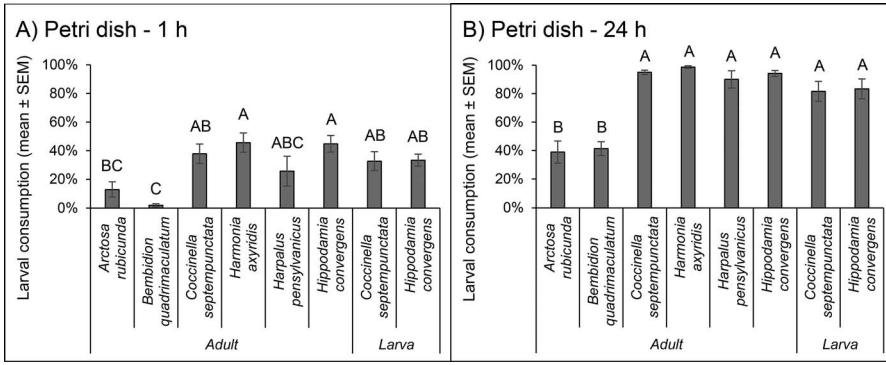

Fig. 1. Soybean gall midge larval consumption rates by predator species evaluated after 1 h (A) and 24 h (B) in Petri dishes. For each graph, different uppercase letters represent significant differences ($P < 0.05$) from multiple comparisons of consumption rates among predator species and life stages.

inoculation rates. At the low rate (50 IJs/larva), only larvae exposed to *H. indica* were less likely to survive compared with the control (data not shown).

Soybean gall midge larval infection rates differed among EPN treatments after 30 d of exposure (Table 2). The four EPN treatments infected 13–20% of larvae,

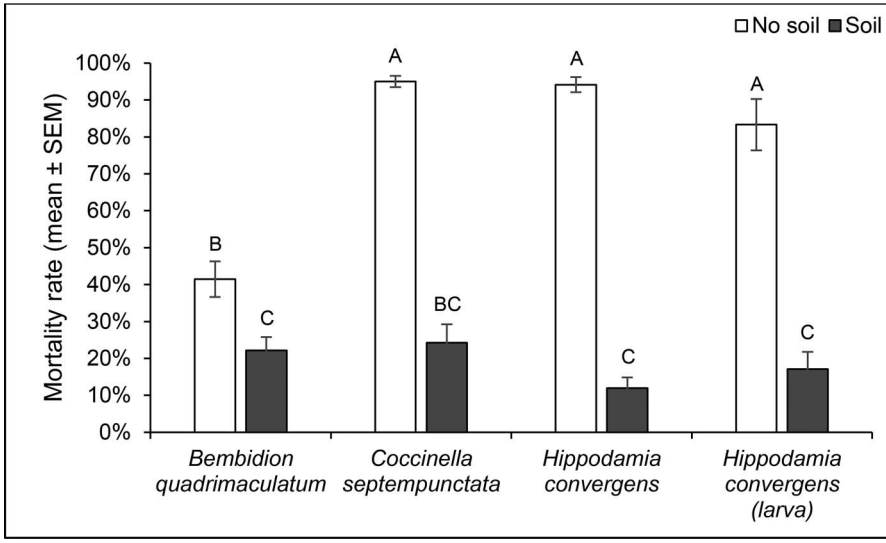

Fig. 2. Soybean gall midge larvae consumption rates among predators after 24 h in Petri dishes with (solid bars) and without (open bars) the addition of a soil substrate. Different uppercase letters represent significant differences ($P < 0.05$) from multiple comparisons analyses comparing consumption rates among predator species and substrates.

Table 2. Nested analysis of variance results for effects of entomopathogenic nematode (EPN) treatment, inoculation rate, and rate nested within treatments on the survival and infection of soybean gall midge larvae after 30 d in the nematode rate bioassay. EPNs were *Heterorhabditis bacteriophora, H. indica, Steinernema carpocapsae,* and *S. riobrave,* each at three inoculation rates (50, 200, and 500 IJs/larva). A water control was included.

Analysis	Main Effect(s)	df	*F*	*P*
Survival rate	EPN	4, 120	0.89	0.4734
	Inoculation rate	2, 120	0.63	0.5354
	EPN*inoculation rate	8, 120	0.73	0.6657
Infection rate	EPN	4, 120	15.90	<0.0001
	Inoculation rate	2, 120	2.49	0.0868
	EPN*inoculation rate	8, 120	0.97	0.4645

which was a higher percentage compared with the water control with zero infected larvae, although the EPN treatments did not significantly differ from one another. Overall infection rates were not influenced by inoculation rate, and the EPN*rate interaction was not significant (Table 2). When evaluating infection rates among treatments across inoculation rates, *H. bacteriophora, H. indica,* and *S. riobrave* infected more larvae than did the water control and *S. carpocapsae* treatments at the low rate (Fig. 4). At the intermediate rate, both heterorhabditid treatments infected more larvae than did the control and steinernematid treatments (Fig. 3). At the high rate, all four EPN species infected more larvae than did the water control (Fig. 3).

Repeated measures analysis indicated that infection rates also increased over time for the four EPN species for each inoculation rate (Table 3; Fig. 5). At the low rate, *H. bacteriophora, H. indica,* and *S. riobrave* infected more larvae than did the control starting at 15 d; *S. carpocapsae* was similar to the control at every time point. At the intermediate rate, *H. indica* infected more larvae than did the control starting at 9 d, *H. bacteriophora* at 15 d, and *S. carpocapsae* at 27 d; *S. riobrave* was similar to the control at every time point. At the high rate, *S. riobrave* infected more larvae than did the control starting at 12 d, *H. bacteriophora* and *S. carpocapsae* at 18 d, and *H. indica* at 21 d. Comparisons of regression coefficients indicated that the rate of increase in infectivity over time for both *S. carpocapsae* (Fig. 6C) and *S. riobrave* (Fig. 6D) was significantly greater at the high rate compared with the intermediate and low rates. *Heterorhabditis bacteriophora* (Fig. 6A) larvae were infected more quickly over time at the high rate than at the low rate, whereas *H. indica* (Fig. 6B) was most affected by the intermediate rate.

Discussion

The protective gall in which gall midge larvae develop makes management of these species difficult, and insecticides currently have low efficacy against

Table 3. Repeated measures analysis of variance results for effects of entomopathogenic nematodes (EPN) treatment, time, and their interaction on the survival and infection of soybean gall midge larvae throughout the experiment. EPN treatments were *Heterorhabditis bacteriophora, H. indica, Steinernema carpocapsae,* and *S. riobrave*. A water control was included.

Analysis	Inoculation Rate (IJs/larva)	Main Effect(s)	df	*F*	*P*
Survival rate	50	EPN	4, 360	0.55	0.7018
		Time	9, 360	73.83	<0.0001
		EPN*time	36, 360	0.96	0.5377
	200	EPN	4, 360	1.10	0.3565
		Time	9, 360	70.40	<0.0001
		EPN*time	36, 360	0.67	0.9266
	500	EPN	4, 360	0.13	0.9723
		Time	9, 360	85.48	<0.0001
		EPN*time	36, 360	1.00	0.4772
Infection rate	50	EPN	4, 360	2.94	0.0207
		Time	9, 360	22.85	<0.0001
		EPN*time	36, 360	3.07	<0.0001
	200	EPN	4, 360	5.61	0.0002
		Time	9, 360	20.89	<0.0001
		EPN*time	36, 360	2.05	0.0005
	500	EPN	4, 360	5.26	0.0004
		Time	9, 360	35.73	<0.0001
		EPN*time	36, 360	2.86	<0.0001

soybean gall midge in soybean (Hodgson and Helton 2021, McMechan 2021, Montenegro et al. 2022, Hodgson and Kolbe 2023). Biological control agents may be advantageous because they are naturally occurring in the soil environment and can actively seek out or respond to prey. In this study we evaluated soybean gall midge predation by several generalist predator species that have not been previously investigated: the ground-dwelling spider *A. rubicunda,* adults and larvae of the lady beetles *C. septempunctata* and *H. convergens,* and the ground beetle *H. pensylvanicus.* We confirmed that specimens collected from a field with no history of soybean gall midge are preadapted to consume soybean gall midge larvae; they began consuming larvae within 1 h of exposure. We also observed consumption rates similar to those reported by

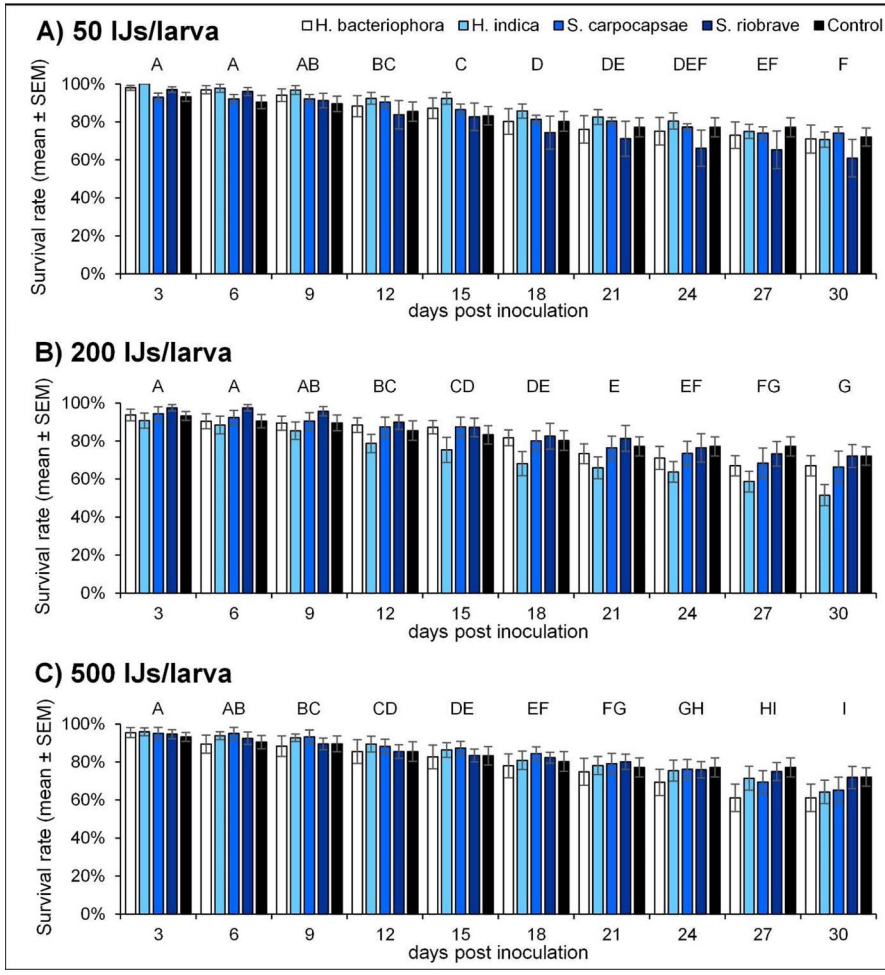

Fig. 3. Mean percentage of soybean gall midge larvae that survived nematode treatments over time for the (A) low (50 IJs/larva), (B) intermediate (200 IJs/larva), and (C) high (500 IJs/larva) inoculation rates. Different uppercase letters represent significant differences ($P < 0.05$) from multiple comparisons analyses comparing time periods.

Melotto et al. (2023c). In both studies, *H. axyridis* consumed approximately 50% of larvae within 1 h, and *B. quadrimaculatum* consumed approximately 40% of larvae after 24 h. Von Gries et al. (2025) found that the ground beetle *P. melanarius* preferred soybean gall midge larvae over soybean aphid. It is unknown whether lady beetle species, which are not ground dwelling but appear in response to abundant prey such as soybean aphid in soybean (which co-occurs with soybean gall midge), would show preference for soybean gall midge or be satiated by soybean aphid.

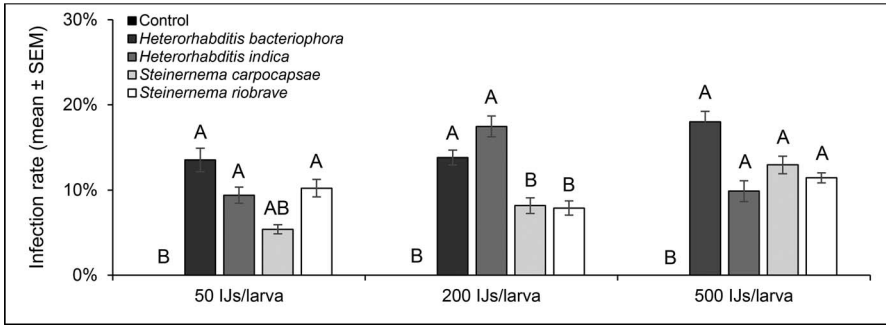

Fig. 4. Mean percentage of soybean gall midge larvae infected by different entomopathogenic nematode (EPN) treatments at low (50 IJs/larva), intermediate (200 IJs/larva), and high (500 IJs/larva) inoculation rates. Different uppercase letters represent significant differences ($P < 0.05$) from multiple comparisons analyses comparing percentage infection among EPN treatments within each inoculation rate.

Harpalus pensylvanicus, the most abundant ground beetle in Brookings, SD (A.J.P. pers. obs.), consumed the most larvae in this study despite being predominately a granivore (Menalled et al. 2007). Larochelle and Larivière (2003) reported that *H. pensylvanicus* consumed soft-bodied insects, including larvae of beetles and aphids. Eskelson et al. (2011) used molecular techniques to verify the presence of slugs in *H. pensylvanicus* guts, and Kirk (1972) observed *H. pensylvanicus* preying on corn rootworm larvae and European corn borer larvae in the field. One limitation for generalist predators is the discrete nature of soybean gall midge larvae because most of their life cycle is spent inside the plant stem. There is a short window of time in which larvae are present in the soil, when they exit the plant and bury themselves for pupation (McMechan et al. 2021a). However, soybean gall midge consumption rates significantly declined for all predators evaluated when soil was added to the experiment. More research is needed to determine the timing and the extent to which generalist predators suppress soybean gall midge populations (including different life stages) in the field and whether these predators can actively identify and consume larvae present in infested plant stems or late-instar larvae and pupae in the soil.

EPNs are among the most promising biological control agents used against soil-dwelling insects because EPNs occur naturally in soil, cause rapid host mortality, and are compatible with integrated pest management practices (Shapiro-Ilan et al. 2019, 2023). Soybean gall midges spend part of their life cycle in the soil environment for pupation and overwintering, where they are likely to encounter EPNs. In this study, we confirmed the ability of four EPNs, *H. bacteriophora* (VS strain), *H. indica* (HOM1 strain), *S. carpocapsae* (All strain), and *S. riobrave* (355 strain), to infect and kill soybean gall midge larvae in the laboratory, albeit at relatively low rates. Infection rates were determined by dissecting dead larvae at the time of breakdown (approximately 1–3 d postinfection), which may be a conservative estimate of EPN-induced death. EPN progeny typically emerge 1 week after

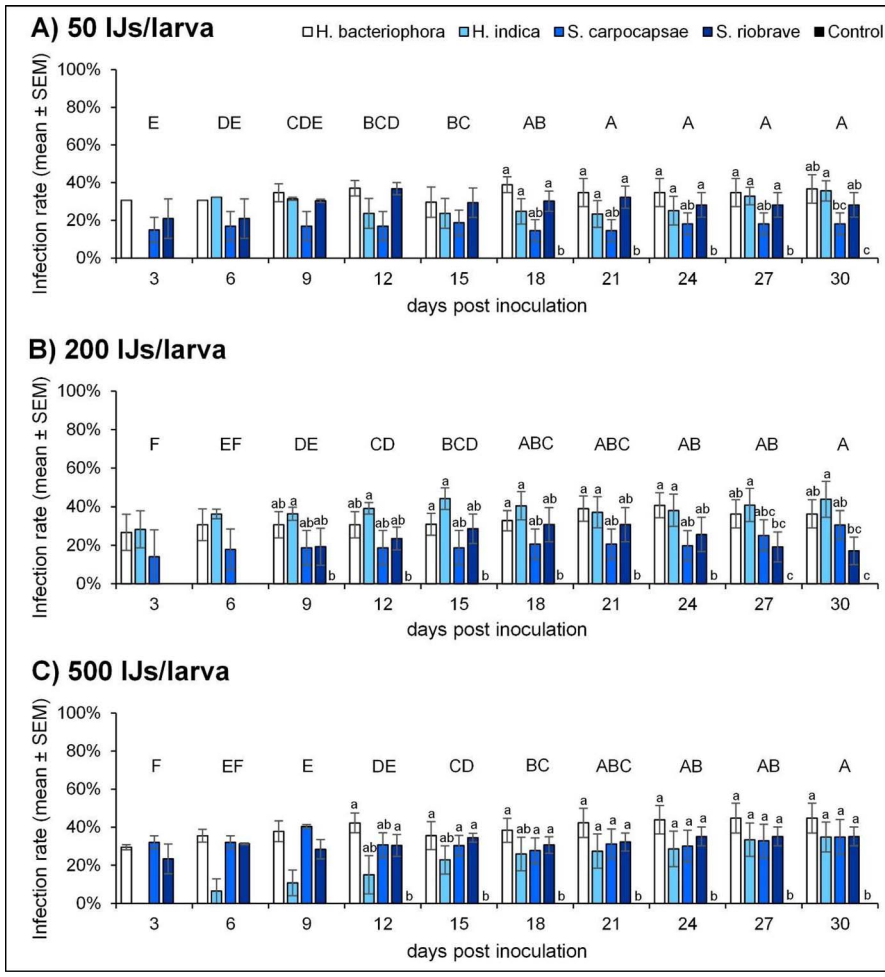

Fig. 5. Mean percentage of dead soybean gall midge larvae that were infected by different entomopathogenic nematode (EPN) treatments overtime for the (A) low (50 IJs/larva), (B) intermediate (200 IJs/larva), and (C) high (500 IJs/larva) inoculation rates. Different uppercase letters represent significant differences ($P < 0.05$) from multiple comparisons analyses comparing time periods. Different lowercase letters represent significant differences from multiple comparisons analyses comparing EPN treatments within each time period.

infection (Wang and Grewal 2002, Li et al. 2007). Otherwise, it is possible for EPNs and/or their bacteria to cause host death but not result in progeny due to other factors (Alonso et al. 2018, Chantab et al. 2024). Regardless, these findings indicate that EPNs are capable of successfully infecting and killing soybean gall midge.

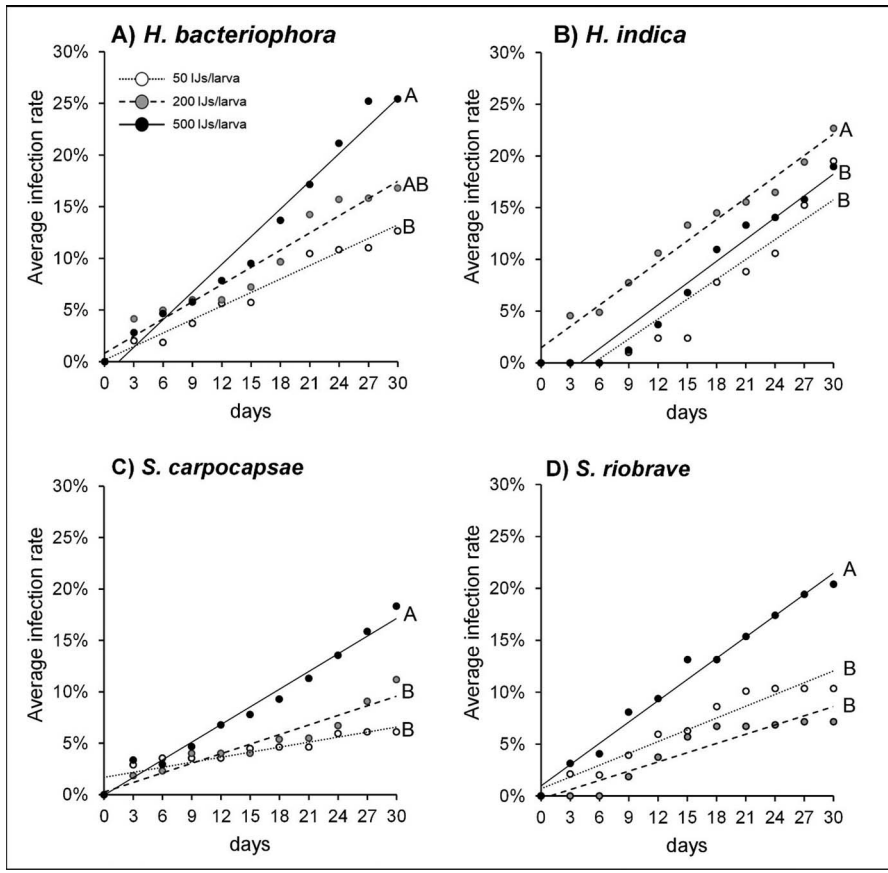

Fig. 6. Simple linear regressions assessing the relationship between the average soybean gall midge infection rate per replicate over time among the low (50 IJs/larva; open circles), intermediate (200 IJs/larva; shaded circles), and high (500 IJs/larva; solid circles) inoculation rates for the four entomopathogenic nematodes (EPNs): *Heterorhabditis bacteriophora* (A), *H. indica* (B), *Steinernema carpocapsae* (C), and *S. indica* (D). Different uppercase letters represent significant differences ($P < 0.05$) from multiple comparisons analyses comparing the regression coefficients of the inoculation rates for each EPN.

Gall midge species (Diptera: Cecidomyiidae) generally differ in their susceptibility to EPNs. For example, the brassica pod midge, *Dasyneura brassicae* (Winnertz, 1853) (Diptera: Cecidomyiidae), was largely unaffected by EPNs in laboratory studies (Nielsen and Philipsen 2004, 2005). The swede midge, *C. nasturtii* was highly susceptible to *H. bacteriophora* but not to *S. carpocapsae* or *S. feltiae.* We found that both *H. bacteriophora* and *H. indica* infected more larvae than did the water control at all three inoculation rates, whereas *S. carpocapsae*

and *S. riobrave* were effective at only the highest rate. *Heterorhabditis indica* was the only species to infect more larvae at the intermediate rate than at the high rate. Previous research indicates that high inoculation rates may result in lower mortality and infection rates due to intraspecific competition (Denno et al. 2008). Corlay et al. (2007) reported that *H. bacteriophora* caused >80% mortality to swede midge larvae *C. nasturtii,* at a rate of 1,000 IJs/larva, whereas *S. carpocapsae* and *S. feltiae* were more efficacious at low (50 IJs/larva) and high (500 IJs/larva) rates, respectively. Majić et al. (2019) also observed a reduction in midge mortality at 1,000 IJs/larva. Future research is needed to evaluate additional EPN species and strains, various inoculation rates and application methods (e.g., to soil and soybean plants), and their interactions with other management tools such as chemical insecticides (Özdemir et al. 2021) and other biological control agents (Půža and Tarasco 2023) such as ground beetles and lady beetles to determine whether soybean gall midge mortality and infection can be improved. Laboratory and field trials in various systems conducted to evaluate EPNs for controlling western corn rootworm in corn, Japanese beetle *Popillia japonica* Newman, and tawny mole cricket *Scapteriscus vicinus* Scudder in turfgrass have revealed that predators such as lady beetles and ground beetles are generally compatible with EPN applications (Georgis et al. 1991, Shapiro-Ilan and Cottrell 2005, Koppenhöfer and Foye 2024).

Soybean gall midge continues to spread geographically throughout the U.S. Great Plains region. As a new species, there was initially no information regarding management of this pest in soybean. The biological control agents evaluated in this study naturally occur in soybean fields in South Dakota and have potential for reducing larval populations in the field. Generalist predators and EPNs are promising as a management tools for soybean gall midge larvae because this pest pupates and overwinters in the soil where nematodes naturally occur. Further research is needed to evaluate the persistence and efficacy of a variety of EPNs in a field setting for soybean gall midge following soil or foliar application. The nontarget effects of EPNs in a field setting also need to be assessed.

Acknowledgments

The authors thank Brad Ferrell for identifying soybean fields and two anonymous farmers for allowing us to sample soybeans containing soybean gall midge larvae for this study. We thank Emma Von Hagel and Jonah Worden for their assistance with data collection. We also thank Stacy Byrd for providing EPNs and rearing expertise for this research. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA, which is an equal opportunity provider and employer.

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