Research

Effects of Clariva Complete Beans Seed Treatment on *Heterodera glycines* Reproduction and Soybean Yield in Iowa

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Abstract

In recent years, nematode-protectant seed treatments have become available to supplement resistant soybean cultivars to manage soybean cyst nematode (*Heterodera glycines*; SCN). Twenty-seven small-plot and 18 strip-trial experiments were conducted comparing the effects of Clariva Complete Beans (CCB) and CruiserMaxx Advanced plus Vibrance (CMV) on SCN reproduction and soybean yield on a moderately resistant (2014) and resistant (2015 to 2016) soybean cultivar. Yield data were collected, and an SCN reproductive factor was calculated by dividing final (at harvest) SCN egg population densities by initial (at planting) population densities from soil samples collected in each small plot or sampled area in the strip trials. Relative to the CMV treatment, CCB significantly decreased SCN reproductive factor in two small-plot experiments (one each in 2014 and 2015) but not in any of the strip trials in any year. Soybean yields were significantly greater with CCB versus CMV in 5 of the 18 strip trials but not in any of the small-plot experiments, even when there were significant decreases in SCN reproduction. For unknown reasons, CCB significantly decreased yields in two small-plot experiments and at one strip-trial location. In summary, the effects of CCB seed treatment on SCN reproduction and soybean yields were variable in the years that these experiments were conducted in Iowa.

The soybean cyst nematode (*Heterodera glycines* Ichinohe; SCN) is a devastating pest of soybeans (*Glycine max* [L.] Merr.) responsible for upward of one billion dollars in yield losses every year in the United States. Between 2010 and 2014, annual soybean yield loss associated with SCN was estimated to average 108 million bushels (2.94 million metric tons) in the United States (Allen et al. 2017). The nematode is widespread throughout the soybean-growing regions of the country (Tylka and Marett 2017) and is considered the most damaging disease of soybeans in the United States, causing more than twice the yield losses attributed to sudden death syndrome each year, the second most damaging soybean disease (Allen et al. 2017).

Since the late 1990s, the use of seed treatments to control and manage soil-inhabiting pathogens and pests has increased dramatically (Munkvold 2009). Use of biological control agents as seed treatments has become the focus of many research efforts as the cost and difficulty associated with developing novel chemicals for pest control has increased (Marrone 2014). The availability of biological and chemical seed treatments with nematicidal action targeting SCN has increased in recent years. Such seed treatments are intended to supplement the current integrated pest management strategies of rotating to nonhost crops and SCN-resistant soybean cultivars.

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Several species of bacteria in the genus *Pasteuria* have been studied for use in biological control of nematodes. These organisms are described as "mycelial and endospore-forming [bacteria] that [are] parasitic on plant-parasitic nematodes" (Starr and Sayre 1988). There are four known species in the genus *Pasteuria* that infect plant-parasitic nematodes, each having a narrow host range (Giblin-Davis et al. 2003; Noel et al. 2005; Sayre and Starr 1985; Sayre et al. 1991a; Starr and Sayre 1988). The most widely studied species, *P. penetrans*, parasitizes root-knot nematodes, *Meloidogyne* spp. (Sayre and Starr 1985), whereas *P. nishizawae* parasitizes cyst nematodes in the genera *Heterodera* and *Globodera* (Nishizawa 1987; Noel et al. 2005; Sayre et al. 1991b). In the United States, Noel and Stanger (1994) were the first to report *P. nishizawae* parasitizing *H. glycines* second-stage juveniles; they observed a decrease in SCN population density in microplots where the bacterium was established, a result that had previously been reported by Nishizawa (1987) in Japan.

Reductions in SCN population density in the presence of *P. nishizawae*, coupled with the production of endospores capable of withstanding environmental extremes (Siddiqui and Mahmood 1999), make this bacterium desirable for use as a biological control organism for SCN. However, producing *P. nishizawae* endospores on an industrial scale historically has been difficult (Parnell et al. 2016). In the past decade, researchers at Pasteuria Bioscience, Inc. (later part of Syngenta, Greensboro, NC) were able to successfully produce these bacteria in vitro. Following this success, Syngenta developed Clariva Complete Beans (CCB), a nematode-protectant seed treatment that includes *P. nishizawae* as one of the active ingredients (Ireland et al. 2017).

Mourtzinis et al. (2017) conducted a 3-year study on the effects of 1 year of Avicta Complete Beans (active ingredient abamectin, by Syngenta) and 2 years of CCB, analyzed as a single seed-treatment factor, on soybean yields and on SCN population densities in the spring after the 3-year study was completed. They found no significant effect of the seed treatment factor on soybean yields or SCN egg population densities at the conclusion of the study. However, the effects of the individual seed treatments were not analyzed, nor were SCN populations determined and compared in each year of the study. The effects of CCB on SCN reproduction and soybean yield under field conditions in individual growing seasons and across multiple locations within a state have, to date, not been reported in the scientific literature. The objectives of our work reported herein were to determine the effects of CCB on (i) SCN reproduction and (ii) soybean yield in field experiments conducted in Iowa.

Small-Plot Experiments

Studies were conducted by scientists at Iowa State University from 2014 to 2016 at nine locations that differed in each year across the state of Iowa (Fig. 1A), one in each of the nine crop-reporting districts used for reporting agricultural statistics in the state (https://www.nass.usda.gov/). All experiments were conducted in fields that had corn (*Zea mays* L.) grown in the previous year. Nematode-protectant seed treatments for SCN are intended to supplement current management practices, so soybean cultivars with the PI 88788 source of SCN resistance were used in all of the experiments. A cultivar with moderate resistance (Asgrow 2433; Monsanto, St. Louis, MO) was used in 2014, and a resistant cultivar (NK S22-S1 and NK S25-L9; Syngenta) was grown in 2015 and 2016, respectively. Prior to planting, one of two seed treatments was applied: CruiserMaxx Advanced (0.0756 mg of thiamethoxam, 0.0113 mg of mefenoxam, and 0.0038 mg of fludioxonil per seed; Syngenta) plus Vibrance (0.0038 mg of sedaxane per seed; Syngenta) (CMV) or CCB (CMV plus 10^7 *P. nishizawae* spores per seed; Syngenta). Plots were planted with a four-row Almaco CTS planter in May of each year, and the experiments were arranged in a randomized

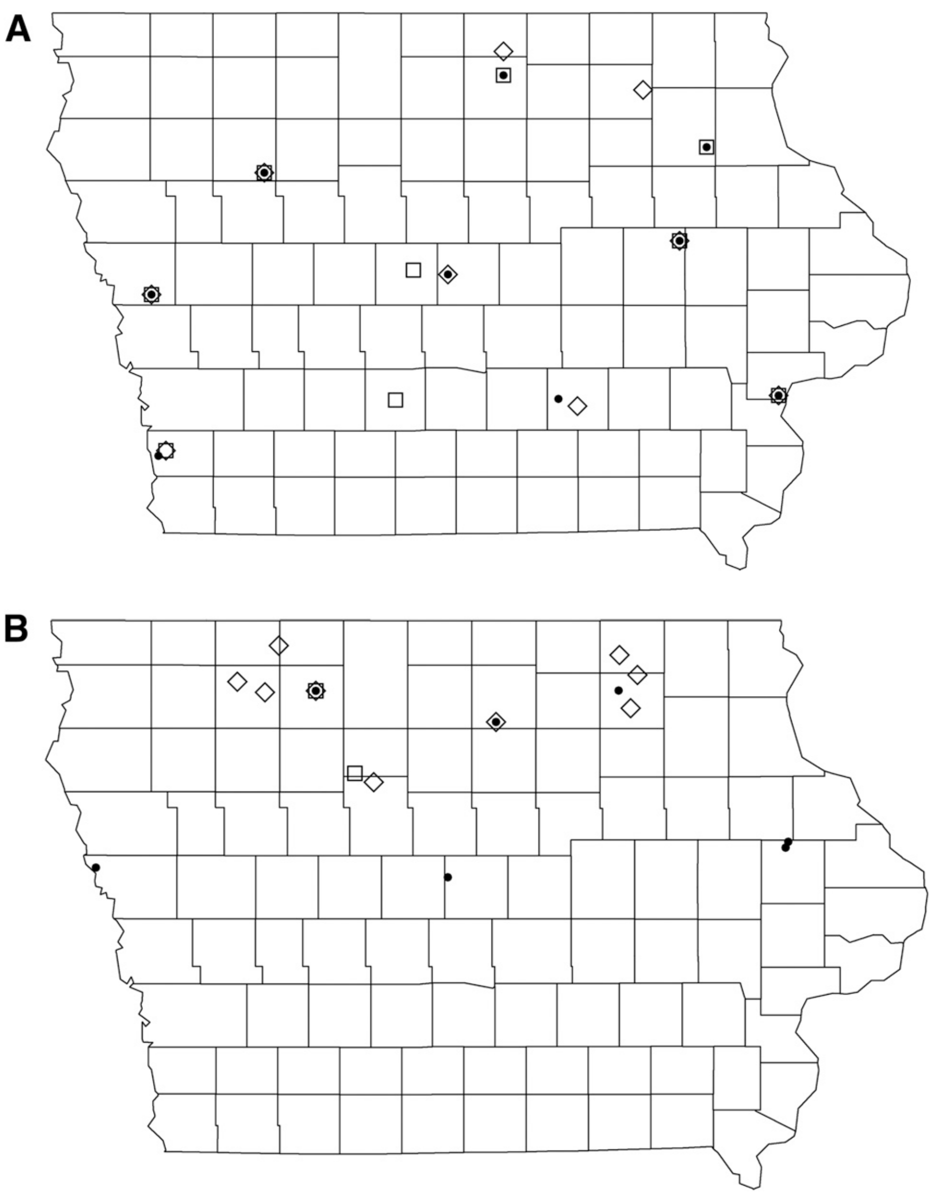

FIGURE 1

Locations of the 27 small-plot experiments (**A**) and 18 replicated strip trials (**B**) across Iowa in each year of the study. Solid circles represent sites used in 2014, diamonds represent sites used in 2015, and squares represent sites used in 2016; multiple symbols are combined for the same location.

complete block design with 12 replications per treatment (Fig. 2). Each plot was 17 ft (5.18 m) long and four rows wide with a 30-in. (0.76-m) row spacing. Plots were harvested in late September or October each year with an Almaco SPC-20 two-row plot combine. Yield was calculated for each plot.

Nematode quantification. To quantify initial and final SCN population densities, 10 1-in. (2.5-cm) diameter and 8-in. (20-cm) deep soil cores were collected in a zig-zag pattern from the root zone of the center two rows of each plot (Fig. 2), and the cores were thoroughly mixed to constitute a sample for the plot. Samples were collected from each plot every year at each location at the time of planting and immediately following harvest. The numbers of SCN eggs in samples collected at planting were considered the initial population densities (P_i) (Table 1), and numbers from samples collected immediately following harvest represented the final population densities (P_f). SCN cysts were extracted from each soil sample on a 250-µm-pore sieve using a modified wet-sieving and decanting method (Gerdemann 1955). SCN eggs then were extracted from the cysts with a motorized rubber stopper (Faghihi and Ferris 2000), collected on a 25-µm-pore sieve, stained, and counted (Niblack et al. 1993). A reproductive factor (RF) was calculated for each plot by dividing the P_f by the P_i . When RF = 1, there was no change in population density from the beginning of the season to the end of the season. However, when RF > 1, P_f was greater than P_i, meaning that the population density increased over the growing season, and when RF < 1, P_i was greater than P_f, indicating the population density decreased over the growing season.

An HG type test (Niblack et al. 2002) was conducted on the SCN population present in each field in which a small-plot experiment was conducted. The HG type of the SCN populations and the percentage of the SCN population that reproduced on PI 88788 for all 27 small-plot experiments are shown in Table 1.

Measuring the effects of CCB in small plots. Analyses of the data from the small-plot experiments were completed using SAS Statistical Software (Rockville, MD) with the mixed model procedure (PROC MIXED). Seed treatments were considered fixed effects and year, location (nested in year), and block (nested in year and location) were treated as random effects. RF and yield data were analyzed by individual location for each year and pooled over all locations for each year and over all years. Best linear unbiased predictors were constructed to estimate random effects. Significant differences were determined at $P \leq 0.10$.

TABLE 1
Initial soybean cyst nematode (SCN) population densities for each small-plot field experiment in each year and the HG type test results of the SCN populations in each field. Percentage of the SCN population that can reproduce on PI 88788 in each field also is shown because the cultivars used in the experiments possessed SCN resistance genes from PI 88788.

Year	Location	P_i[a]	SCN HG type[b]	Percent reproduction on PI 88788[c]
2014	Northwest	5,460	2.5.7	58
	North Central	1,610	1.2.5.7	51
	Northeast	7,140	2.5.7	42
	West Central	14,880	2.5.7	13
	Central	3,777	2.5.7	44
	East Central	3,101	2.7	10
	South West	5,345	2.5.7	63
	South Central	742	1.2.5.7	37
	Southeast	1,320	7	8
2015	Northwest	4,894	2.5.7	22
	North Central	11,992	2.7	19
	Northeast	1,763	2.5.7	29
	West Central	769	2.5.7	33
	Central	5,016	2.5.7	32
	East Central	760	2.7	14
	South West	1,902	2.5.7	16
	South Central	6,208	2.5.7	38
	Southeast	6,082	2.5.7	22
2016	Northwest	732	1.2.5.7	41
	North Central	4,060	2.5.7	26
	Northeast	1,097	2.5.7	40
	West Central	2,298	2.5.7	20
	Central	300	1.2.5.7	55
	East Central	242	2.5.7	47
	South West	1,850	1.2.5.7	37
	South Central	322	2.7	15
	Southeast	3,500	2.5.7	66

[a] P_i = initial SCN population density reported as the number of SCN eggs per 100 cm^3 of soil.
[b] SCN HG type test results are indicated as >10% reproduction on Peking or PI 548402 (1), PI 88788 (2), PI 209332 (5), and/or PI 548316 (7).
[c] Percent reproduction of SCN on PI 88788 relative to reproduction on a susceptible soybean cultivar.

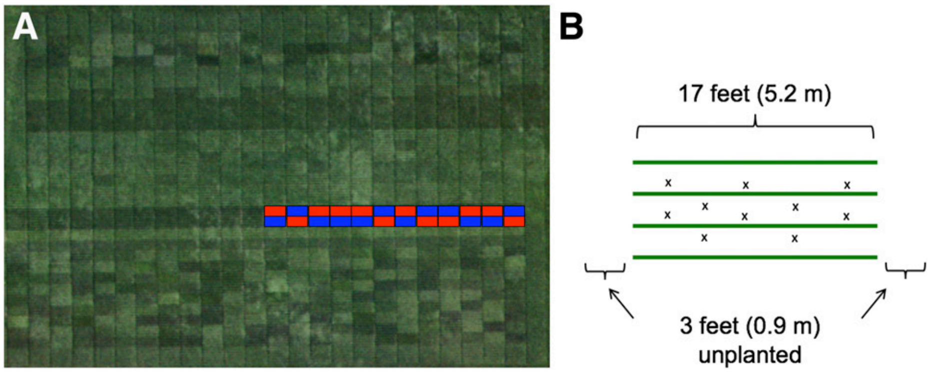

FIGURE 2
Aerial image of a small-plot experiment positioned among other research plots (**A**) and a diagram of soil sampling within individual small plots (**B**). In the aerial image, the 12 pairs of replicate plots of the two seed treatments are shown as red and blue rectangles. In the diagram, the arbitrarily selected locations where soil cores were collected are represented by "x" in the center two of four rows (shown as green lines) within an individual plot. The center 14 ft (4.3 m) of the center two rows of each plot were harvested for yield. The total area of the small-plot seed treatment experiment was 20 ft (6.1 m) by 240 ft (73.1 m).

Effects of CCB on SCN Reproduction and Yield in Small-Plot Experiments

Differences in SCN reproduction and yield between the seed treatments were not consistently observed in the small-plot experiments (Table 2). Significant reductions in RF were observed with CCB relative to CMV in some locations and years, but the reduced RF values were not accompanied by significant increases in yield in the experiments. Specifically, significant differences in SCN RF between the treatments were detected at the East Central location in 2014 (P = 0.04) and the South Central location in 2015 (P = 0.10) but at no locations in 2016 (Table 2). The CCB treatment at the East Central location in 2014 had an RF of less than 1 (RF = 0.72), significantly less than the CMV treatment, which almost doubled the initial population density of SCN (RF = 1.85). For 2015, RF values of less than 1 at the South Central location indicate that SCN population densities declined in plots grown from seeds with both seed treatments, with RF value of 0.39 in the CCB treatment and 0.60 in the CMV treatment. Despite both RF values being less than 1 in this experiment, the difference in RF between the two treatments was statistically significant (P = 0.10).

Significant differences in yield in the small-plot experiments were observed only in 2015 between treatments at the North Central (P = 0.06) and South West (P = 0.08) locations (Table 2). A significant yield reduction of 1.4 bu/acre (94.2 kg/ha) was observed in the CCB treatment at each of these locations compared with the CMV treatment. The yield of the CCB-treated plots averaged 75.4 bu/acre (5,070 kg/ha) at the North Central location and 70.8 bu/acre (4,761 kg/ha) at the South West location.

When data from all nine experimental locations in the state were pooled for analyses for each year individually, the RF significantly differed (P = 0.09) between treatments in 2014, and yield significantly differed (P = 0.04) between treatments in 2015 (Table 2). In 2014, a half-fold reduction in SCN RF was observed in the CCB-treated plots (RF = 2.26) compared with the plots with CMV overall (RF = 2.75). Although SCN reproduction was significantly reduced in the CCB treatment overall in 2014, the final SCN density in the soil was more than double the initial SCN population density in both treatments, perhaps because the cultivar used in all of the small-plot experiments that year was moderately resistant to SCN, not fully resistant. In 2015, the CCB-treated plots had significantly lower yield

TABLE 2
Mean reproduction of soybean cyst nematode (SCN) and mean soybean yield in small-plot experiments with Clariva Complete Beans (CCB) and with CruiserMaxx Advanced plus Vibrance (CMV). The differences between treatment means are listed for each experiment in each year. Also, data from all locations were pooled within years and over all years for analyses. Statistical differences were determined at $P \leq 0.10$. Experimental locations were unique to each year.

		SCN reproductive factor[a]			Yield (bu/acre)		
Year	Location	CCB	CMV	Difference[b]	CCB	CMV	Difference[b]
2014	Northwest	1.4	2.1	–0.7	49.2	48.8	0.4
	North Central	6.8	7.4	–0.6	58.7	57.7	1.0
	Northeast	0.7	0.5	0.2	53.2	51.1	2.1
	West Central	1.1	1.8	–0.7	73.0	71.9	1.1
	Central	2.2	2.1	0.1	63.4	64.0	–0.6
	East Central	0.7	1.9	–1.2*	65.2	67.5	–2.3
	South West	0.6	0.4	0.2	68.1	68.7	–0.6
	South Central	1.0	0.6	0.4	46.4	46.2	0.2
	Southeast	5.9	8.1	–2.1	62.2	61.3	0.9
	All locations	2.3	2.8	–0.5*	59.9	57.9	0.2
2015	Northwest	4.1	5.1	–1.0	79.6	79.5	0.1
	North Central	0.5	0.3	0.2	75.4	76.8	–1.4*
	Northeast	0.7	1.3	–0.4	56.9	58.0	–1.1
	West Central	1.6	1.7	–0.1	74.0	73.5	0.5
	Central	0.6	0.8	–0.2	61.3	61.5	–0.2
	East Central	1.3	1.8	–0.5	71.5	72.8	–1.3
	South West	4.0	5.0	–1.0	70.8	72.2	–1.4*
	South Central	0.4	0.6	–0.2*	55.0	57.3	–2.3
	Southeast	0.6	0.7	–0.1	59.4	59.0	0.4
	All locations	1.5	1.9	–0.4	67.1	67.8	–0.7*
2016	Northwest	25.3	31.1	–5.8	73.0	71.5	1.5
	North Central	3.1	2.5	0.6	80.5	79.5	1.0
	Northeast	1.0	0.7	0.3	53.9	50.6	3.3
	West Central	2.4	3.1	–0.8	75.8	77.7	–1.9
	Central	10.1	11.6	–1.5	78.4	77.8	0.6
	East Central	4.4	5.5	–1.1	68.0	68.7	–0.7
	South West	1.4	1.2	0.1	72.0	71.7	0.3
	South Central	2.1	1.0	1.1	78.0	76.7	1.3
	Southeast	0.2	0.2	0.0	42.4	41.4	1.0
	All locations	5.5	6.3	–0.8	69.1	68.4	0.7
All years	All locations	3.1	3.7	–0.6	65.4	65.3	0.1

[a] Reproductive factor was calculated by dividing the number of SCN eggs in each plot at harvest (P_f) by the number in each plot at planting (P_i).

[b] Difference was calculated by subtracting the mean SCN reproductive factor or mean yield for the CMV treatment from the CCB treatment. Asterisk (*) indicates difference between treatments was statistically significant ($P \leq 0.10$).

(67.1 bu/acre; 4,513 kg/ha) than the plots with CMV (68.7 bu/acre; 4,620 kg/ha). Significant differences between treatments were not observed for RF or yield when locations were pooled for 2014 or 2016 or over all years.

Strip Trials

The Iowa Soybean Association On-Farm Network conducted replicated strip trials comparing CCB and CMV at seven locations in 2014, at nine locations in 2015, and at two locations in 2016. All locations were in the northern half of the state (Fig. 1B). For each year, the soybean cultivar used in the strip trial experiments was the cultivar used in the small-plot experiments. More specifically, the seed treatments were applied to a large amount of the same cultivar of seed from a single seed lot each year, and then the treated seeds were used in both the strip trials (described below) and the small-plot experiments (described above). Strip trials were conducted in cooperation with Iowa farmers, who planted and harvested the strips using their own equipment. Therefore, planting and harvesting equipment, dates, and methods varied by strip trial and are not mentioned in detail here. At each strip trial, field-length strips ranging from 820 to 1,600 ft long (250 to 488 m long) were planted for each treatment (Fig. 3). Treatment strips were

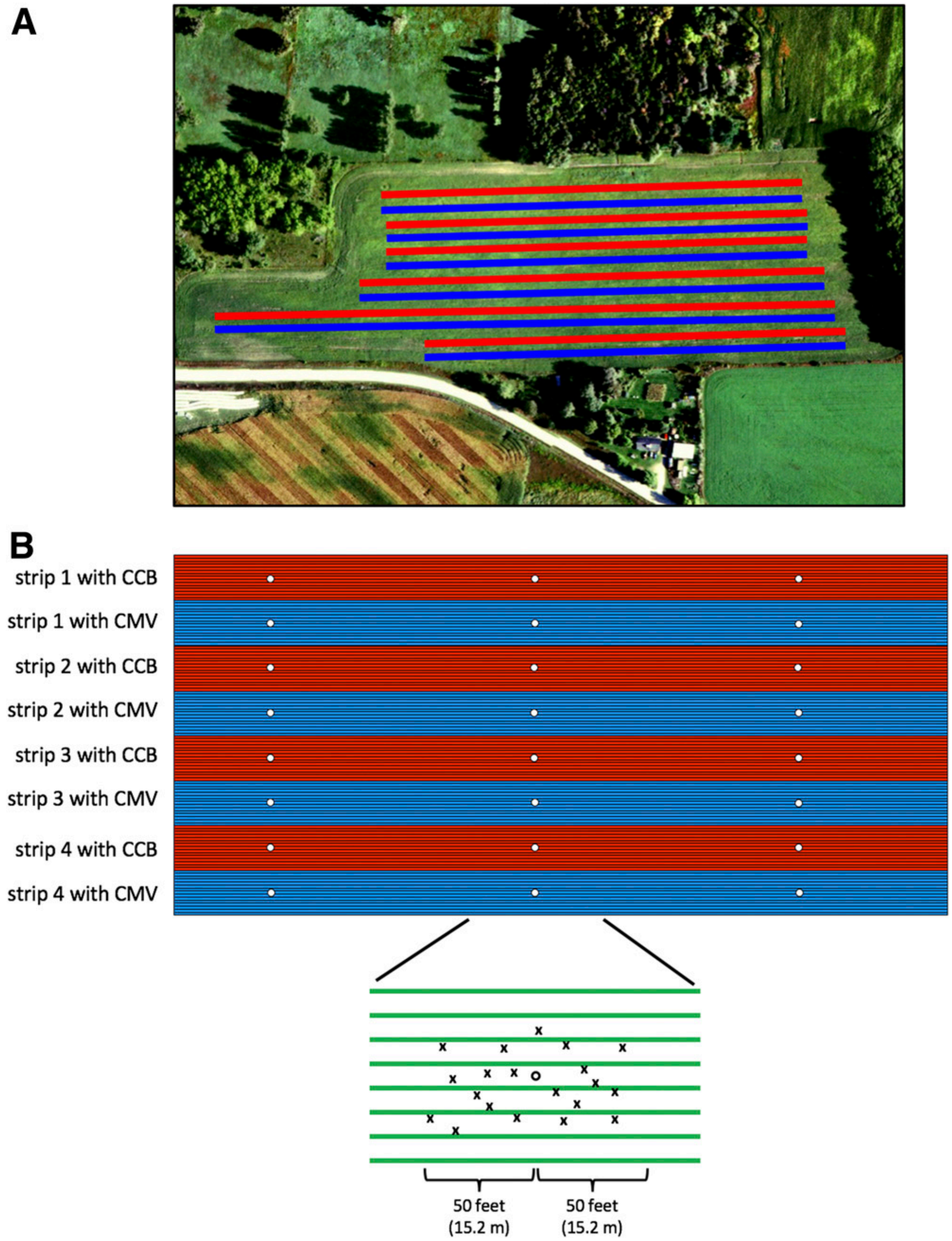

FIGURE 3

Aerial image of an example of a strip-trial experiment (**A**) and diagram of soil sampling within the strips (**B**). In the aerial image, the six pairs of strips with the two seed treatments are depicted in red and blue. In the diagram, the arbitrarily selected locations where soil cores were collected are represented by "x" around an arbitrarily selected center point (circle) in the center four strips of eight rows of soybeans (green lines) within a strip. The entire lengths of all rows of each strip were harvested for yield. Individual treated strips in the aerial image were 15 ft (4.6 m) wide, and the longest of the strips was 1,600 ft (488 m) long.

replicated four or more times at each location. Yield was calculated for each strip using a yield monitor equipped with GPS technology.

Nematode quantification. Soil samples consisting of 20 soil cores were collected in mid-June (representing initial population densities, P_i) and after harvest (representing final population densities, P_f) from three paired but arbitrarily selected sampling locations within each strip (Fig. 3), and the soil cores were combined and thoroughly mixed into a single sample for each sampling location. Soil samples were not collected from all strip trials. The soil samples were processed and SCN egg population densities determined using the same methods previously described for the small-plot experiments.

Measuring the effects of CCB in strip trials. Analysis of the strip-trial data was conducted using R statistical software (R Foundation, Vienna, Austria) with the lme4 or nlme packages. Year, location (nested in year), and block (nested within year and location) were random effects, and seed treatment was a fixed effect. RF and yield data were analyzed by year for each strip trial, and data from strip trials also were pooled and analyzed by year and over all years.

Effects of CCB on SCN Reproduction and Yield in Strip Trials

Differences in SCN RF and yield between the two seed treatments varied among locations and years in the strip trials. In contrast to the results of the small-plot experiments, significantly lower RF values were not observed in the CCB treatment versus CMV in any of the strip trials (Table 3), but significant yield differences were observed between treatments in six strip trials (Table 4).

TABLE 3
Mean reproduction of soybean cyst nematode (SCN) in strips treated with Clariva Complete Beans (CCB) and with CruiserMaxx Advanced plus Vibrance (CMV) and the difference between treatment means for each location in each year. Statistical differences are reported and significance was determined at $P \leq 0.10$. Locations were unique in each year.

		Reproductive factor[a]			
Year	Location	CCB	CMV	Difference[b]	ANOVA *P* =
2014	Chickasaw	3.0	4.3	–1.3	n.s.[c]
	Monona	0.4	0.8	–0.4	n.s.
	Jones 1	0.9	0.0	0.9	n.s.
	Jones 2	1.0	0.9	0.1	n.s.
	All locations	1.3	1.7	–0.4	n.s.
2015	Clay 1	21.4	21.8	–0.4	n.s.
	Chickasaw 1	0.7	1.4	–0.7	n.s.
	Dickinson	2.2	2.0	0.2	n.s.
	Webster	2.8	4.0	–1.2	n.s.
	Clay 2	4.2	4.3	–0.1	n.s.
	Palo Alto	3.9	1.9	2.0	n.s.
	Chickasaw 2	3.5	3.5	0.0	n.s.
	All locations	5.5	5.6	–0.1	n.s.
2016	Humboldt	6.1	8.7	–2.6	n.s.
	Palo Alto	0.2	0.1	0.1	n.s.
	All locations	3.1	4.5	–1.3	n.s.
All years	All locations	3.9	4.3	–0.4	n.s.

[a] Reproductive factor was calculated by dividing the number of SCN eggs in each plot at harvest (P_f) by the number in each plot at planting (P_i).
[b] Difference was calculated by subtracting the yield for the treatment with CMV from the treatment with CCB.
[c] Not significant; *P* value > 0.10.

The SCN RF was calculated for four of the seven strip trials in 2014, seven of nine in 2015, and for both trials in 2016 (Table 3). A significant difference in SCN RF between treatments was not observed for any location in any year. Furthermore, no significant differences between treatments were observed for RF when locations were pooled in each year or over all years (Table 3).

For yield, significant differences between treatments were observed at two strip trials in 2014 and four strip trials in 2015 but no strip trials in 2016 (Table 4). In 2014, a significant yield increase ($P = 0.03$) of 4.6 bu/acre (309 kg/ha) was observed in the CCB treatment versus the treatment with CMV at the strip trial in Story County. In the same year, at one of the two strip trials in Jones County, the strips with CCB yielded 50.8 bu/acre (3,416 kg/ha), which was significantly greater ($P = 0.10$) than the strips with CMV, which yielded 49.5 bu/acre (3,329 kg/ha).

In 2015, significant yield increases were detected with CCB relative to CMV in three strip trials. There was an increase of 1.2 bu/acre (81 kg/ha) with CCB in one of the strip trials in Clay County ($P = 0.01$), an increase of 1.0 bu/acre (67 kg/ha) at the Dickinson County strip trial ($P = 0.1$), and an increase of 1.2 bu/acre (81 kg/ha) in the experiment in Palo Alto County ($P = 0.07$) (Table 4). A significant ($P = 0.006$) yield reduction of 2.8 bu/acre (188 kg/ha) with the CCB treatment occurred in the strip trial in Cerro Gordo County. The seed treatments significantly affected yield when data from all strip trials were pooled for analysis in 2014 ($P = 0.01$), with a 1.3 bu/acre (87

TABLE 4
Mean soybean yield in strips treated with Clariva Complete Beans (CCB) and with CruiserMaxx Advanced plus Vibrance (CMV) and the difference between treatment means for each location in each year. Statistical differences are reported and significance was determined at $P \leq 0.10$. Locations were unique in each year.

		Yield[a]			
Year	Location	CCB	CMV	Difference[b]	ANOVA *P* =
2014	Palo Alto	54.3	54.3	0.0	n.s.[c]
	Cerro Gordo	55.0	54.8	0.1	n.s.
	Chickasaw	50.0	48.8	1.2	n.s.
	Monona	78.7	78.9	–0.2	n.s.
	Story	46.4	41.8	4.6	0.03
	Jones 1	50.8	49.5	1.3	0.10
	Jones 2	36.5	37.2	–0.7	n.s.
	All locations	53.3	50.0	1.3	0.01
2015	Clay 1	67.8	66.6	1.2	0.01
	Chickasaw 1	63.0	62.8	0.2	n.s.
	Dickinson	62.4	61.4	1.0	0.10
	Webster	62.3	62.7	–0.4	n.s.
	Clay 2	63.0	62.3	0.7	n.s.
	Palo Alto	64.3	63.1	1.2	0.07
	Chickasaw 2	61.2	61.0	0.2	n.s.
	Cerro Gordo	62.4	65.2	–2.8	0.006
	Howard	37.1	39.1	–2.0	n.s.
	All locations	58.0	58.4	–0.4	n.s.
2016	Humboldt	48.3	48.7	–0.4	n.s.
	Palo Alto	80.5	79.5	1.0	n.s.
	All locations	56.7	56.6	0.1	n.s.
All years	All locations	57.1	56.5	0.7	0.05

[a] Yield and yield difference are reported in bushels per acre.
[b] Difference was calculated by subtracting the yield for the treatment with CMV from the treatment with CCB.
[c] Not significant; *P* value > 0.10.

kg/ha) yield increase with CCB versus CMV (Table 4). Similarly, when data from all strip trials in all years were pooled for analysis, CCB significantly (P = 0.05) increased yield by 0.7 bu/acre (47 kg/ha) relative to CMV.

Comparison of Results from Small-Plot Experiments and Strip Trials

Numerous bags of seed of the same cultivars from the same lots of seed were treated and then used in small-plot experiments and strip trials every year to allow for comparison of results from both types of experiments without the possibility of variation in plant genetics, quality of seed lots, or applications of seed treatments. Overall, similar trends were observed and the same conclusions were drawn from the results of the small-plot and strip-trial experiments, with SCN reproduction and yield for each treatment varying across environments. Our variable results are consistent with the findings of Gaspar et al. (2014) and Mourtzinis et al. (2017), who reported that seed treatments, whether chemical or biological, can be inconsistent in their effects across environments.

SCN reproduction was similar in both types of experiments, with few significant differences between treatments detected within or across locations. In the small-plot experiments, SCN reproduction was reduced at some locations in the CCB-treated plots to the point that SCN population densities were below initial population densities (RF < 1). However, in a majority of the small-plot experiments (17 of 27) and strip trials (9 of 13), SCN reproduction increased (RF > 1) regardless of the seed treatment applied, indicating that neither treatment prevented SCN population densities from increasing throughout the season. But overall, SCN RF values in the small plots and strips grown from seeds treated with CCB plots were consistently (but not often significantly) lower than RF values from small plots or strips grown from seeds treated with CMV.

The average yield in each experiment followed a pattern similar to the SCN RF results, although the yield differences between treatments varied by location in both types of experiments. More yield decreases were associated with CCB relative to CMV in the small-plot experiments than in the strip trials, for which such a reduction was only detected at one experiment in 2015. Significant yield increases of 1.0 to 4.6 bu/acre (67 to 309 kg/ha) were observed in the strips treated with CCB versus the CMV-treated strips in both 2014 and 2015, but in no years and at no locations in the small-plot experiments. It is unknown why significant yield increases were observed when using CCB in the strip trials but not in the small-plot experiments.

Management Implications

Soil ecology plays a large role in the effectiveness of biological control organisms in the rhizosphere (Cumagun and Moosavi 2015). *P. nishizawae* is an obligate parasite of SCN and, like other *Pasteuria* species, requires a sufficient SCN population density to parasitize and proliferate (Ciancio 1995). In turn, SCN survival relies on a complex interaction among soil ecological components (i.e., bacteria, other nematodes, fungi, etc.) along with soil physical structure, soil pH, and soil temperature to establish itself (Stirling 1991, 2014). For *P. nishizawae* to be an effective biological control organism for SCN, it must come in direct contact with SCN juveniles. Only then is there the potential for significant reductions in SCN and increases in yield.

Because the endospores of *P. nishizawae* coating the seed in the CCB treatment are nonmotile, variability in the initial SCN population density in the soil immediately surrounding the planted seeds likely plays a role in the resulting SCN reproduction and yield response. Avendaño et al. (2004) reported that SCN population densities can differ dramatically within a field at different sampling locations and over time, even in the same experimental plot. Low initial SCN population density, environmental factors, or other soil characteristics may account for the variability of CCB in reducing SCN population density in the soil and increasing soybean yields that occurred in our experiments. Despite the variability and wide range of SCN RF values that were observed in both the small-plot and the strip-trial experiments, CCB had an effect on SCN reproduction in some of our experiments.

To be of economic benefit, the use of a seed treatment must increase yield enough to offset the cost of the treatment. A recent study by Syngenta reported a 4.5% increase in soybean yields when using CCB versus using only the fungicide plus insecticide base (CMV) (Ireland et al. 2017). In our experiments, significant yield increases of almost 5 bu/acre were observed in two-strip trial experiments in 2014, indicating that in some environments, CCB has the potential to increase yield significantly. A 5 bu/acre yield increase would likely offset the cost of the CCB seed treatment at almost any soybean price that has occurred in the past decade. However, in the small-plot experiments, significant yield increases were not observed, but significant yield reductions were observed in some cases in the plots treated with CCB versus those with CMV. Yield differences between the small-plot and strip-trial experiments could be explained by difficulties that arise from calculating yield in small-plot experiments. However, the extra replications in the small-plot trials should help to correct for this error (Kandel et al. *in press*).

Early on, researchers working with *P. nishizawae* found this bacterium to be associated with SCN-suppressive soils, although its suppressive effects often took years to become evident under field conditions (Atibalentja et al. 1998; Nishizawa 1987; Noel et al. 2010; Noel and Stanger 1994). Therefore, the most significant impacts of CCB seed treatment on SCN reproduction may not occur for several years. Because we used different locations in each year of our studies, the lack of widespread significant differences in SCN reproduction between the two treatments may be owing to the insufficient buildup of *P. nishizawae* population densities in the soil for an SCN-suppressive effect to be easily observed. In our studies, the long-term effects of CCB were not studied at the same locations over years.

Although there are many factors compounding the already complex problem of nematode control, the application of *P. nishizawae* to the rhizosphere has the potential to reduce *H. glycines* population densities in the soil (Atibalentja et al. 1998; Noel et al. 2010). Integrating the use of CCB with SCN-resistant soybean cultivars and nonhost crops may, in some cases, reduce SCN population densities in the soil. However, more work is needed to determine the effects of edaphic factors, environmental factors, and the long-term use of CCB as a seed treatment on SCN reproduction and soybean yield.

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