Assessment of Insecticide/Miticide Treatments on Soybean Cyst Nematode Bioassays Under Greenhouse Conditions

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Soybean cyst nematodes (SCNs) (*Heterodera glycines*, Ichinohe) are a plant parasite that impacts soybean (*Glycine max* Merr.) yields in all major soybean producing countries (Wrather et al. 2010). This obligatory parasite feeds on soybean roots, diverting energy away from plant biomass and seed production. After mating and producing eggs, female SCNs turn into hard, durable cysts, which allows their eggs to survive in the soil for many years before infecting a new plant (Porter et al. 2001). Despite the large amount of information known about this pathosystem, research continues with the aim of developing resistance through conventional breeding and biotechnology. An integral part of this research happens in the controlled environment of greenhouses in scaled-down bioassays. Soybeans grown under greenhouse conditions are often burdened with chronic infestations of plant pests such as aphids, spider mites, white flies, and thrips. The use of pesticides is a common method for keeping such pests under control. Unfortunately, rotating through a variety of pesticides with different modes of action (MoAs) is required to prevent pesticide resistance from developing in insect populations. This research examines the possibility of insecticides and miticides interfering with SCN female, cyst, egg, and second-stage juvenile (J2) numbers either through direct contact with runoff or encountering systemic pesticides when feeding.

Previously, Brady et al. (2012) showed several pesticides to be compatible with SCN bioassays. The aim of this study was to broaden that spectrum by evaluating pesticides with three MoAs not previously tested (active ingredients: bifenthrin, pyridaben, and S-kinoprene), a MoA of new chemistry (clofentezine), and a soil-applied, imidacloprid-based insecticide. All pesticides used in this study are given in Table 1. Although a 10A MoA has been previously tested for interactions with SCNs (Brady et al. 2012), molecular targets for chemicals in this group are yet to be defined (Sparks and Nauen 2015). The 10A MoA active ingredient tested here, clofentezine, has a strikingly different chemical structure compared with that of the hexythiazox-based insecticide tested by Brady et al. (2012). Several previously tested pesticides were also included in this study as comparative checks to previous work (abamectin and imidacloprid).

Combinations of S-kinoprene or clofentezine with foliar-applied imidacloprid were also tested. Although none of the pesticides tested here are marketed as having nematocidal effects, abamectin and other avermectins are well known for having inhibitory effects on nematodes, including human parasites and nematodes that are closely related to SCN such as root knot nematodes (*Meloidogyne* sp.) (Cabrera et al. 2009; Sasser and Kirkpatrick 1982). Furthermore, toxicological studies have shown that all the active ingredients used in this study, except kinoprene (not tested), have inhibitory effects on the model nematode *Caenorhabditis elegans* (Boyd et al. 2016). These reports raise concern as to whether these pesticides can be safely used on SCN bioassays without interference to the nematode.

To test for interference of insecticides and miticides on SCN bioassays, three seeds of the susceptible soybean cultivar KS3406 were planted into D40 Deepots (Stuewe and Sons, Tangent, OR) containing 450 cm³ of a sand–soil mixture infested with Hg type 7 population eggs at an initial population density of 2,560 eggs per 100 cm³. The inoculum was obtained by rearing an Hg type 7 field isolate on KS3406 for 8 to 10 weeks. Plants emerging from a single tube were treated as a single observational unit. A randomized complete block design structure was employed using three blocks, with each block having three repeated measures per treatment. Granular imidacloprid was applied once after plant emergence. For other treatments, plants were sprayed with pesticides every 10 days after planting, three times total with a hand-pump sprayer. Between each treatment the sprayer was rinsed and the hose purged with tap water three times. Control plants were sprayed with tap water. Pesticides were applied at the manufacturer's recommended application rate. Plants were sprayed until leaves were uniformly

TABLE 1
Pesticides evaluated for interference with soybean cyst nematode bioassays including trade name, active ingredient, and the Insecticide Resistance Action Committee mode of action classification (MoA)

Trade name	Active ingredient	MoA	Manufacturer
Avid	Abamectin	6	Syngenta (Greensboro, NC)
Sanmite	Pyridaben	21A	Gowan Comp. (Yuma, AZ)
Talstar P	Bifenthrin	3A	FMC Corp. (Philadelphia, PA)
Marathon II	Imidacloprid	4A	OHP, Inc. (Mainland, PA)
Marathon 1% granular	Imidacloprid	4A	OHP, Inc. (Mainland, PA)
Enstar II	S-Kinoprene	7A	Wellmark (Schaumburg, IL)
Ovation SC	Clofentezine	10A	Scotts (Marysville, OH)

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covered. Soil was not protected from overspray or run-off of pesticides. Roots were harvested 35 days after plant emergence. Female nematodes, cysts, eggs, and J2s were harvested as described in Brady et al. (2012). The experiment was conducted under greenhouse conditions in June 2016 and repeated in August of the same year. Greenhouse day/night temperatures were maintained at $27/22 \pm 2^\circ\text{C}$, and natural lighting was augmented with 16 h of sodium growth lamps. Nematode counts were normalized against total grams of dry root weight per pot. Data were analyzed with SAS software, version 9.4 for Windows, using the GLIMMIX procedure. A log-normal distribution was used to reduce heterogeneity of

variance. Post hoc analyses of treatment means were conducted using Dunnett's procedure for comparing multiple treatment means with the control ($P \leq 0.05$).

No significant differences were found in female + cyst counts or egg + J2 production for pesticide treatments against control plants (Fig. 1). The smallest P value for female + cyst counts compared with the control group was that of bifenthrin (Dunnett adjusted P value = 0.237). The smallest P value for egg + J2 counts was that of imidacloprid (Marathon II) (Dunnett adjusted P value = 0.487). Absolute mean densities of SCN females + cysts and eggs + second-stage juveniles for each treatment are given in Table 2. In general, numbers

FIGURE 1

Boxplot of soybean cyst nematode female + cyst and egg + second-stage juvenile (J2) counts per gram of dry soybean root in pots treated with insecticides and miticides encompassing six modes of action. Dunnett's test showed no significant differences when comparing pesticide treatments to water control for female + cyst or egg + J2 counts. Boxes represent first and third quartiles, and lines in boxes represent treatment medians. Whiskers represent the minimum of either 1.5 times the interquartile range or the extreme of the sample range. Circle (○) indicates female + cyst outliers, and x indicates egg + J2 outliers. GDRM = grams dried root mass; Ctrl = negative control; Enst = Enstar; Mara = Marathon; and Ova = Ovation.

Treatment	(Females + cysts)/GDRM		(Eggs + J2s)/GDRM	
	Mean	Standard error	Mean	Standard error
Ovation	3,303	314	300,609	78,510
Enstar II	3,107	537	195,896	44,412
Avid	2,861	181	213,966	28,121
Marathon granular	1,875	196	166,418	29,967
Marathon II	3,188	475	306,773	66,244
Sanmite	2,758	469	228,368	65,951
Talstar	3,521	550	222,756	73,368
Enstar–Marathon II	2,936	373	227,714	45,798
Ovation–Marathon II	2,789	186	233,510	46,442
Control	2,472	393	221,902	48,813

^a GDRM = grams dried root mass.

of females and cysts per gram of dried root mass were higher than that of the control group. The granular imidacloprid treatment group was the only exception to this, averaging 597 fewer females + cysts per gram of dried root mass. There was no particular pattern to pesticide treatment means compared with the control group regarding egg + J2 numbers produced per gram of dried root mass. Because no significant differences were found between pesticide treatments and the control group, pesticides evaluated in this study should be considered safe to use with SCN bioassays at an application frequency of 1.5 weeks, as well as less frequent application rates owing to chemical degradation under greenhouse conditions.

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