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Effect of Rotation Crops on *Heterodera glycines* Population Density in a Greenhouse Screening Study

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Abstract: Crop rotation is a common means of reducing pathogen populations in soil. Several rotation crops have been shown to reduce soybean cyst nematode (*Heterodera glycines*) populations, but a comprehensive study of the optimal crops is needed. A greenhouse study was conducted to determine the effect of growth and decomposition of 46 crops on population density of *H. glycines*. Crops were sown in soil infested with *H. glycines*. Plants were maintained until 75 days after planting, when the soil was mixed, a sample of the soil removed to determine egg density, and shoots and roots chopped and mixed into the soil. After 56 days, soil samples were again taken for egg counts, and a susceptible soybean ('Sturdy') was planted in the soil as a bioassay to determine egg viability. Sunn hemp (*Crotalaria juncea*), forage pea (*Pisum sativum*), lab-lab bean (*Lablab purpureus*), Illinois bundleflower (*Desmanthus illinoensis*), and alfalfa (*Medicago sativa*) generally resulted in smaller egg population density in soil or number of cysts formed on soybean in the bioassay than the fallow control. Sunn hemp most consistently showed the lowest numbers of eggs and cysts. As a group, legumes resulted in lower egg population densities than monocots, *Brassica* species, and other dicots.

Key words: *Brassica*, *Crotalaria juncea*, crop rotation, *Desmanthus illinoensis*, *Glycine max*, *Heterodera glycines*, *Lablab purpureus*, management, *Medicago sativum*, *Pisum sativum*, population, soybean cyst nematode.

The soybean cyst nematode, *Heterodera glycines* Ichinohe, is the most damaging pest of soybeans (*Glycine max* (L.) Merr.) in the US (Wrather et al., 2003; Monson and Schmitt, 2004). Rotation with resistant soybean cultivars and nonhost plants are the principle tactics for management of *H. glycines* (Niblack and Chen, 2004; Niblack, 2005). However, effectiveness of management using resistant cultivars and crop rotation depends on numerous factors: mainly the availability of cultivars resistant to the various *H. glycines* HG Types or races; variability of *H. glycines* populations in pathogenicity on different soybean genotypes; species of rotation crops; number of years rotation crops used; and nematode survival ability in different geographical locations.

A number of crops have been evaluated in greenhouse and field studies for their effectiveness in lowering *H. glycines* population densities. Some of the crops effectively reduced *H. glycines* population densities when grown in rotation or as cover crops with soybean. A cover crop is any crop grown to provide soil cover, either inter-seeded or in rotation with other crops. Studies in the southern US demonstrated that American jointvetch (*Aeschynomene americana*), bahiagrass (*Paspalum notatum*), cotton (*Gossypium hirsutum*), sorghum (*Sorghum bicolor*), hairy indigo (*Indigofera hirsuta*), velvetbean (*Mucuna pruiens*), and wheat (*Triticum aestivum*) used as rotation crops or as winter/summer cover crops effectively lowered *H. glycines* population densities and in most cases increased soybean yield (Dabney et al., 1988; Rodriguez-Kabana et al., 1988, 1989, 1990, 1991a, 1991b; Weaver et al., 1993; Dillon et al., 1997; Weaver et al., 1998; Vargas-Ayala and Rodriguez-Kabana, 2001; Hague and Overstreet, 2002). In Japan, Nishizawa (1978) reported that millet (*Pennisetum glaucum*), rape (*Brassica napus*), and potato (*Solanum tuberosum*) were also effective in lowering *H. glycines* population density.

In the North Central region of the US, soybean is commonly rotated annually with corn (*Zea mays*), and this cropping system is conducive to *H. glycines* population development when soybean is grown. A diversified cropping system including additional crops in rotation or using cover or trap crops is needed for long-term, effective management of the nematode in the region. Several studies have been carried out to evaluate other crops for their potential as rotation crops in managing *H. glycines* populations and crop yields. A Kansas study showed that crop rotation with nonhosts grain sorghum and wheat was effective in reducing pre-plant populations of *H. glycines*, but high levels of nematode reproduction when soybean was planted resulted in serious damage to the susceptible cultivar during the first year back into soybean production (Long and Todd, 2001). Double cropping of soybean and winter wheat in rotation with grain sorghum did not help in cyst management, as yield suppression in the susceptible cultivar was comparable to that seen in full-season soybean (Long and Todd, 2001). Jackson et al. (2005) found that nonhost crops oat (*Avena sativa*), canola (*Brassica napus*), sesame (*Sesamum indicum*), corn, sorghum, and red clover (*Trifolium pratense*) did not appear to decrease the ability of *H. glycines* to infect and develop on subsequent soybean crops in Missouri, so the benefits of rotation with these nonhost crops are limited to reducing *H. glycines* populations and the frequent increase in yield in subsequent soybean crops. Under Minnesota conditions, a 5-yr rotation of nonhost crops or rotation of resistant soybean with a nonhost crop was

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needed to lower *H. glycines* population densities to below damaging level (e.g., 200–500 eggs/100 cm^3 soil) (Chen et al., 2001b).

A few studies have been conducted in Minnesota to determine the effectiveness of diverse crops in the greenhouse and in fields as rotation, trap, or cover crops in lowering *H. glycines* population densities. Sortland and MacDonald (1987) found in a greenhouse study that crop rotation to decrease population of *H. glycines* race 5 must extend through two growing periods and preferably through three. They found that adzuki bean (*Phaseolus angularis* cv. Manoka) and pea (*Pisum sativum*) allowed some development of *H. glycines* females on roots, but could work as rotation crops; corn led to the lowest *H. glycines* population levels and was the most effective rotation crop of the study, and lambsquarters (*Chenopodium album*) and pigweed (*Amaranthus retroflexus*) are not hosts but may detrimentally affect the growth of the subsequent soybean crop (Sortland and MacDonald, 1987). In another Minnesota field study, Miller et al. (2006) evaluated 16 crops commonly produced in Minnesota or having potential for use in the state as rotation crops: barley (*Hordeum vulgare*), flax (*Linum usitatissimum*), oats, sorghum, wheat, buckwheat (*Fagopyrum sagittatum*), canola, corn, rye (*Secale cereale*), sugar beet (*Beta vulgaris*), potato, sunflower (*Helianthus annuus*), alfalfa, hairy vetch (*Vicia villosa*), red clover, and pea. They found that legumes that are not or are poor hosts appeared to be the best crops for reducing the *H. glycines* population density, while monocots including corn appeared to be the least effective. Hairy vetch, a leguminous crop, supported the development of *H. glycines* females on its roots in the field and was probably a moderate host of *H. glycines* (Miller et al., 2006). Chen et al. (2006) studied the effects of alfalfa, red clover, and perennial ryegrass as cover crops inter-seeded with soybean on *H. glycines* and soybean and corn yields. Their results were inconsistent among three sites, and any reductions in *H. glycines* populations were minimal. Pea, when interseeded with corn as a trap crop, reduced *H. glycines* population density as compared with the corn-only control, but it was not cost-effective to manage *H. glycines* in this way (Chen et al., 2001a).

The objective of this screening study was to measure the changes in *H. glycines* egg population density in soils planted and subsequently incorporated with 46 crops and the changes in number of cysts formed on subsequent susceptible soybeans planted in these soils. The research was designed to better evaluate rotation crops that could be used to manage *H. glycines* in Minnesota, such as additional untested crops and the mechanisms involved in control of *H. glycines*. The results of this study, along with those of field experiments and previous greenhouse studies (Sortland and MacDonald, 1987; Miller et. al., 2006), may help determine which crops could be chosen for use as alternative rotation or cover crops to manage soybean cyst nematode in the region.

Materials and Methods

Forty-six crops were evaluated in the greenhouse for their potential as rotation or cover crops in managing for *H. glycines* by lowering soil egg population densities. The plant species chosen for this study are either common crops or have potential as alternative crops in Minnesota. Some of them, such as sunn hemp, have been shown to have potential in managing plant-parasitic nematodes including *H. glycines* (Wang et al., 2002; Kushida et al., 2003). Several of the crops, namely black oat (*Avena strigosa*), brassica smother crop (*Brassica campestris*), camelina (*Camelina* sp.), crown vetch (*Coronilla varia*), foxtail hay millet (*Setaria italica* cv. Manta), and triticale (*Triticosecale rimpaui* cv. Spring), included in this study had apparently not been included in any *H. glycines* host range, hatch, or rotation tests previously. Some of the other crops needed to be re-tested due to mixed results in other research.

Soil infested with *H. glycines* race 3 (HG Type 0-) was collected from field plots of a crop rotation experiment (Chen et. al., 2001b) on a commercial farm in Waseca County, MN, on November 6, 2003 (Assay 1), and in spring 2004 (Assay 2). The soil was a Webster clay loam (fine-loamy, mixed, mesic Typic Endoaquoll) (38.7% sand; 29.8% silt; 31.5% clay; pH 7.9). The soil was mixed thoroughly and supplemented with egg-free field soil to obtain an even distribution of cysts at a density of approximately 20,000 (Assay 1) or 23,000 (Assay 2) eggs/100 cm^3 soil. Approximately 1,250 g of this soil was placed in 16-cm-diam. clay pots and planted with the selected crops (Table 1). Planting was done by pouring most of each 1,250 g soil portion into its pot, except for a ~2-cm layer, which was added after the seeds were scattered on the soil surface. Seeding rate was determined by estimating how many plants of each species would appropriately fit in a 16-cm-diam. pot as they developed over the course of the experiment. There was also a fallow (no plant) control. Six replicates of each crop were used.

Pots were arranged in randomized blocks by replicate and were maintained in a greenhouse (with temperatures estimated at 20–33°C). They were watered every day to keep the soil moist. Plants were given N, P, K fertilizer in the irrigation water (1.2 g N, 1.2 g P, and 1.2 g K/liter) after about 30 d of growth. After 75 d, the plants were cut at the soil surface, and the fresh weight of aerial material was recorded. Soil in each pot was mixed thoroughly, and a sub-sample of 50 cm^3 soil in Assay 1 and 100 cm^3 soil in Assay 2 was taken to determine *H. glycines* egg densities. Cysts were extracted using hand decanting, sieving (850-µm aperture for the top sieve, 250-µm aperture for the bottom sieve), and 63% sucrose solution flotation, and eggs were released

TABLE 1. Crops selected and seeding rates for screening study.

Crop group[a]	Selected crops	Scientific name	Seeds/plot
2	Alfalfa	*Medicago sativa* L. cv. Travois	40
1	Annual ryegrass	*Lolium perenne* L. ssp. *multiflorum* (Lam.) Husnot	50
2	Annual white sweet clover	*Melilotus albus* Medikus cv. Hubam	30
1	Barley	*Hordeum vulgare* L. cv. Robust	30
2	Barrel medic	*Medicago truncatula* Gaertner	30
2	Berseem clover	*Trifolium alexandrinum* L.	40
2	Birdsfoot trefoil	*Lotus corniculatus* L. or *Lotus tenuis* Waldst. & Kit. ex Willd.	50
1	Black oat	*Avena strigosa* Schreb.	25
3	Brassica smother crop	*Brassica rapa* L. var. *rapa* (L.) Thell.	40
4	Buckwheat	*Fagopyrum esculentum* Moench	15
3	Camelina	*Camelina* sp. Crantz	50
3	Canola	*Brassica napus* L. cv. Hyola 357RR	30
1	Corn	*Zea mays* L. cv. DKC 48-83	12
1	Corn-BT	*Zea mays* L. cv. DKC 44-46	12
2	Crimson clover	*Trifolium incarnatum* L.	40
2	Crown vetch	*Coronilla varia* L.	30
4	Flax	*Linum usitatissimum* L. cv. Rehab	25
2	Forage pea	*Pisum sativum* L.	20
3	Forage turnip	*Brassica* sp. L.	30
1	Foxtail hay millet	*Setaria italica* (L.) Beauv. cv. Manta	50
1	Grain sorghum	*Sorghum bicolor* (L.) Moench ssp. *bicolor*	20
3	Green cabbage[b]	*Brassica oleracea* L. (hybrid) cv. Green Rocket	30
2	Hairy vetch[b]	*Vicia villosa* Roth	20
2	Illinois bundleflower	*Desmanthus illinoensis* (Michx.) MacM. ex B.L. Robins. & Fern.	40
2	Lab-Lab	*Lablab purpureus* (L.) Sweet	12
1	Millet	*Pennisetum glaucum* (L.) R. Br. cv. Proso	25
2	Mung bean	*Vigna radiata* (L.) R. Wilczek cv. Filsan	25
1	Oat	*Avena sativa* L. cv. Reeves	25
3	Oilseed radish	*Raphanus sativus* L.	30
3	Oilseed rape	*Brassica napus* L. cv. Dwarf Essex	30
2	Partridge pea	*Chamaecrista fasciculata* Michx. or *Chamaecrista nictitans* Michx.	20
2	Pea	*Pisum sativum* L. cv. Polar	12
1	Perennial ryegrass	*Lolium perenne* L.	50
4	Potato	*Solanum tuberosum* L.	3 eyes
3	Red cabbage[b]	*Brassica oleracea* L. (hybrid) cv. Ruby Perfection	30
2	Red clover	*Trifolium pratense* L. cv. Marathon	40
1	Rye	*Secale cereale* L. cv. Homil 21	25
–	*H. glycines*-resistant soybean	*Glycine max* (L.) Merr. cv. Pioneer 9234	12
2	Subterranean clover	*Trifolium subterraneum* L. cv. Mt. Barker	30
4	Sugar beet	*Beta vulgaris* L. cv. Crystal 820	10
4	Sunflower	*Helianthus annuus* L. cv. Dahlgren 9711	12
2	Sunn hemp	*Crotalaria juncea* L.	15
1	Triticale	× *Triticosecale rimpaui* Wittm. [*Triticum_aestivum* × *Secale cereale*] cv. Spring	25
1	Wheat	*Triticum aestivum* L. cv. Oxen	40
2	White clover	*Trifolium repens* L. cv. Dutch	50
2	Wild/perennial lupine	*Lupinus perrenis* L.	20
–	Control	(No plant)	0

[a] Crop groups are as follows: 1) monocots, 2) leguminous non/poor hosts, 3) Brassicaceae, 4) other dicots.
[b] Planted for Assay 2 only.

using a cyst crusher (Faghihi and Ferris, 2000). Eggs were then collected on nested sieves (75-µm aperture for the top sieve, 25-µm aperture for the bottom sieve), cleaned using centrifugation in a 38% sucrose solution, and counted.

The aerial material of each crop was cut into 3-cm sections and evenly distributed among the six pots in which it was grown. Plant materials were mixed with the remaining soil and roots in a container and then returned to the same pot. These pots were maintained (watered daily and hand-weeded when necessary) in the greenhouse for 56 d, and then the soil was again mixed thoroughly and 100 cm^3 (190 g) samples were taken from each pot to determine egg population density following the procedures described above.

Approximately 150 cm^3 of the remaining soil of each pot was placed in a 7-cm-diam. clay pot. 'Sturdy' soybean seeds were soaked in water for 3.25 hr and planted two per pot. After 7 d, the seedlings were thinned to one plant per pot. The pots were maintained in the greenhouse and watered every day. After 32 d, the soybeans with the soil were removed from their pots, each placed in a 1-liter beaker, soaked in tap water for at least 30 min, then gently washed to remove soil. Cysts (females) were dislodged from the roots with a high-pressure water jet over a set of nested sieves (850-µm

aperture for the top sieve, 250-µm aperture for the bottom sieve). Cysts caught on the bottom sieve were collected and counted.

Data analysis: To determine nematode population change during the rotation crop period (first 75 d) and the following fallow period (the following 56 d), egg population change factors (PCF) during these two periods were computed: PCF1 = nematode egg population density at the end of the rotation crop growing period/initial egg population density; PCF2 = egg population density at the end of fallow period/egg population density at the end of crop-growing period; and PCF1+2 = egg population density at the end of fallow period/initial egg population density at planting the rotation crops. Nematode reproduction factor (Rf) on the susceptible soybean following the fallow period was computed: Rf = cyst counts × 1,000/initial eggs per pot, which represents the number of females produced per 1,000 eggs. Initially, data were tested for normality using the Shapiro-Wilk test (Shapiro and Wilk, 1965) using the Statistix 8.0 program (Analytical Software, Tallahassee, FL). PCF1 and PCF1+2 were not transformed for statistical analysis. PCF2, cysts formed per plant, and Rf in Assay 1 were transformed with $\mathrm{Ln}(x)$, $x^{0.65}$, and $x^{0.63}$, respectively, and PCF2, cysts formed per plant, and Rf in Assay 2 were transformed with $x^{0.2}$, and $x^{0.1}$, and $\mathrm{Ln}(x)$, respectively, to improve homogeneity of variance before being subjected to analysis of variance. Means of individual crops were compared using Tukey's Studentized Range (HSD) test at $\alpha = 0.05$. To determine differences among groups of crops, the data were averaged by four groups: 1) monocots; 2) leguminous, non/poor hosts; 3) Brassicaceae; and 4) "other dicots" besides the leguminous, non/poor hosts and Brassicaceae (Table 1). Orthogonal contrasts among the groups were performed.

Results

Assay 1

Egg population 75 d after planting (PCF1): After the crops had been growing in the infested soil for 75 d, soil samples were taken to determine soil egg population density. Of all the crops evaluated, only the brassica smother crop soil had a mean egg count which was higher (61%) than that of the fallow control. The egg count in the brassica smother crop was also higher than many other crops (Table 2). As a group, leguminous non/poor hosts led to the lowest egg counts. The Brassicaceae family group resulted in greater egg population density than monocots, "other dicots," and leguminous non/poor hosts (Table 2).

Egg population 56 d after harvesting crops (PCF2 and PCF1+2): The egg population change factor during the 56 d of fallow period after harvesting the crop (PCF2) did not differ among the individual crops and the crop groups (Table 2). However, the population change between the time of planting the crops and the end of fallow period (PCF1+2) differed among the individual crops (Table 2). Brassica smother crop did not show greater egg populations than the control at this point of time. Sunn hemp lowered egg population densities (38% lower) compared with the fallow control. When compared with sunn hemp, PCF1+2 values were greater for the following crops: annual ryegrass, barrel medic, berseem clover, brassica smother crop, buckwheat, canola, corn-BT, forage turnip, oat, oilseed radish, partridge pea, and triticale. As a group, the leguminous non/poor hosts again led to the lowest PCF1+2 values compared with all other groups. The Brassicaceae group also resulted in slightly greater PCF1+2 than monocots (Table 2).

Female number on soybean roots (Cysts and Rf): When the susceptible soybean cultivar 'Sturdy' was planted in the soils as a bioassay, there was no difference in cyst number and Rf among individual crops or the crop groups. However, sunn hemp resulted in the numerically lowest mean cyst number, 55% lower than the mean of the fallow control.

Assay 2

Egg population 75 d after planting (PCF1): The results were slightly different for Assay 2 compared with Assay 1. Seventy-five days after planting the crops in the infested soil, the following crops showed decreases in egg population density as compared with the fallow control: lab-lab (44% lower), pea (56% lower), and sunn hemp (60% lower) (Table 3). Compared with sunn hemp, all crops except crimson clover, crown vetch, forage pea, green cabbage, lab-lab, mung bean, pea, resistant soybean, and white clover had higher PCF1 values (Table 3). As a group, the leguminous non/poor hosts led to the lowest egg counts. Unlike the result in Assay 1 (Table 2), plants in the Brassicaceae family in Assay 2 resulted in smaller egg population densities than the monocots and the "other dicots" (Table 3).

Egg population 56 d after harvesting (PCF2 and PCF1+2): After plant materials were mixed into the soil and were maintained with no plant growth for 56 d in the greenhouse, lab-lab (53% lower) and sunn hemp (58% lower) showed differences in PCF1+2 as compared with the fallow control (Table 3). Compared with sunn hemp, the following crops led to greater PCF1+2: annual ryegrass, barley, black oat, brassica smother crop, buckwheat, canola, corn-BT, flax, foxtail hay millet, grain sorghum, hairy vetch, Illinois bundleflower, millet, oilseed radish, oilseed rape, partridge pea, perennial ryegrass, potato, sunflower, and triticale. As a group, leguminous non/poor hosts had a smaller PCF1+2 than any of other three groups (Table 3). Plants in the Brassicaceae family also resulted in smaller PCF1+2 than the group of "other dicots." There were no significant differences in PCF2 among the crops.

Number of females on soybean roots (Cysts and Rf): The results from Assay 2 showed some differences in *H. glycines* cyst counts among the crops (Table 3). The lowest

TABLE 2. Population density of *Heterodera glycines* in response to rotation crops in Assay 1 (fall soil) of a greenhouse study.[a]

Crop[b]	Crop group	PCF1		PCF2		PCF1+2		Cysts		Rf	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Alfalfa	2	0.67	0.04 b	1.21	0.15	0.78	0.06 b–g	532	77	35.0	7.2
Annual ryegrass	1	0.82	0.08 ab	1.41	0.15	1.10	0.05 a–d	242	51	11.3	2.7
Ann. wt. sweet clover	2	0.71	0.04 b	1.34	0.08	0.95	0.08 a–g	341	101	19.1	6.5
Barley	1	0.80	0.10 ab	1.29	0.12	0.99	0.08 a–g	382	100	20.1	6.1
Barrel medic	2	0.88	0.03 ab	1.23	0.06	1.08	0.08 a–e	434	152	21.8	8.4
Berseem clover	2	0.78	0.10 b	1.54	0.22	1.10	0.11 a–d	510	129	24.3	5.8
Birdsfoot trefoil	2	0.68	0.09 b	1.29	0.17	0.81	0.05 b–g	356	105	21.9	6.5
Black oat	1	0.76	0.05 b	1.33	0.15	0.99	0.10 a–g	415	75	22.1	4.0
Brassica smother crop	3	1.12	0.05 a	1.10	0.08	1.22	0.08 a	367	105	16.3	5.1
Buckwheat	4	0.86	0.07 ab	1.35	0.09	1.13	0.03 ab	384	120	16.4	5.0
Camelina	3	0.90	0.05 ab	1.11	0.13	0.98	0.10 a–g	351	97	17.4	4.2
Canola	3	0.65	0.06 b	1.65	0.16	1.04	0.07 a–f	526	134	26.5	6.6
Corn	1	0.73	0.09 b	1.40	0.18	0.95	0.06 a–g	371	100	19.6	5.3
Corn-BT	1	0.82	0.09 ab	1.29	0.14	0.99	0.04 a–f	427	126	22.1	6.6
Crimson clover	2	0.72	0.06 b	1.09	0.16	0.75	0.05 d–g	229	76	14.9	4.4
Crown vetch	2	0.69	0.05 b	1.33	0.07	0.91	0.05 a–g	422	124	23.0	6.5
Flax	4	0.85	0.08 ab	1.13	0.10	0.94	0.08 a–g	362	82	20.6	4.8
Forage pea	2	0.68	0.03 b	1.06	0.11	0.71	0.06 fg	491	72	34.6	3.5
Forage turnip	3	0.89	0.05 ab	1.28	0.11	1.11	0.04 a–c	487	151	21.3	6.3
Foxtail hay millet	1	0.74	0.06 b	1.36	0.11	0.98	0.05 a–g	259	57	13.3	2.8
Grain sorghum	1	0.77	0.08 b	1.23	0.16	0.89	0.06 a–g	507	129	27.2	6.2
Illinois bundleflower	2	0.69	0.07 b	1.23	0.22	0.78	0.06 b–g	455	585	5.7	2.0
Lab-lab	2	0.75	0.06 b	1.12	0.09	0.84	0.09 b–g	439	167	26.8	8.3
Millet	1	0.76	0.05 b	1.31	0.17	0.97	0.09 a–g	306	110	16.6	6.6
Mung bean	2	0.68	0.03 b	1.16	0.14	0.77	0.07 c–g	384	136	24.2	8.4
Oat	1	0.79	0.07 ab	1.34	0.15	1.01	0.08 a–f	249	90	13.4	5.4
Oilseed radish	3	0.88	0.07 ab	1.16	0.09	1.01	0.09 a–f	478	88	25.2	5.1
Oilseed rape	3	0.87	0.04 ab	1.00	0.04	0.87	0.04 b–g	261	61	15.2	3.5
Partridge pea	2	0.67	0.04 b	1.64	0.13	1.07	0.05 a–e	448	97	20.9	4.4
Pea	2	0.64	0.09 b	1.25	0.18	0.73	0.05 e–g	288	92	20.3	6.2
Perennial ryegrass	1	0.83	0.03 ab	1.10	0.12	0.91	0.11 a–g	362	90	20.2	5.2
Potato	4	0.77	0.04 b	1.18	0.14	0.89	0.06 a–g	412	112	24.2	7.2
Red clover	2	0.62	0.07 b	1.36	0.24	0.78	0.05 b–g	405	73	25.8	3.6
Rye	1	0.78	0.11 b	1.42	0.28	0.98	0.06 a–g	398	92	19.4	3.8
H. glycines-resistant soybean	–	0.68	0.08 b	1.14	0.16	0.73	0.06 e–g	442	147	31.2	10.3
Subterranean clover	2	0.82	0.05 ab	1.06	0.04	0.86	0.02 b–g	379	58	22.2	3.6
Sugar beet	4	0.71	0.03 b	1.37	0.13	0.97	0.09 a–g	452	116	23.3	6.5
Sunflower	4	0.72	0.06 b	1.40	0.20	0.95	0.07 a–g	262	120	12.4	5.0
Sunn hemp	2	0.64	0.10 b	1.07	0.15	0.64	0.06 g	205	43	17.6	3.9
Triticale	1	0.77	0.03 b	1.30	0.10	1.00	0.09 a–f	300	82	15.0	3.9
Wheat	1	0.77	0.05 b	1.16	0.10	0.88	0.04 a–g	335	95	18.5	5.1
White clover	2	0.64	0.05 b	1.28	0.12	0.79	0.04 b–g	414	82	25.5	4.4
Wild/perennial lupine	2	0.67	0.05 b	1.41	0.11	0.93	0.06 a–g	492	127	25.4	5.3
Control (no plant)	–	0.70	0.04 b	1.48	0.08	1.02	0.03 a–f	457	78	22.8	4.0
Average by group:											
Monocots (1):		0.78	0.07	1.30	0.15	0.97	0.07	350	92	18.4	4.9
Leguminous non/poor hosts (2):		0.70	0.06	1.26	0.13	0.85	0.06	382	95	22.7	5.5
Brassicaceae (3):		0.88	0.05	1.22	0.10	1.04	0.07	412	106	20.3	5.1
Other dicots (4):		0.78	0.06	1.29	0.13	0.97	0.07	374	110	19.4	5.7
Contrast:	1 vs. 2	***		NS		***		NS		NS	
	1 vs. 3	***		NS		*		NS		NS	
	1 vs. 4	NS		NS		NS		NS		NS	
	2 vs. 3	***		NS		***		NS		NS	
	2 vs. 4	**		NS		***		NS		NS	
	3 vs. 4	**		NS		NS		NS		NS	

[a] PCF1 = nematode egg population density at the end of the rotation crop-growing period/initial egg population density; PCF2 = egg population density at the end of fallow period/egg population density at the end of crop-growing period; PCF1+2 = egg population density at the end of fallow period/egg population density at planting the rotation crops; cysts = nematode cyst population density/soybean plant in bioassay; SE = standard error. Rf is nematode reproduction factor on the *H. glycines*-susceptible soybean following the fallow period: Rf = cyst counts × 1,000/initial eggs per pot, which represents the number of females produced per 1,000 eggs. Data transformations: PCF1 (none), PCF2 (Ln(x)), PCF1+2 (none), cysts ($x^{0.65}$), Rf ($x^{0.63}$) before being subjected to analysis of variance (ANOVA). The values are means of six replicates. The values followed by the same letter or no letter in the column are not different according to Tukey's Studentized Range (HSD) Test at $P \geq 0.05$. *, **, and *** represent significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. NS = not significant at $P \geq 0.05$.

[b] Some crops are not shown in the table due to too many missing data points.

TABLE 3. Population density of *Heterodera glycines* in response to rotation crops in Assay 2 (spring soil) of a greenhouse study.[a]

Crop[b]	Crop group	PCF1		PCF2		PCF1+2		Cysts		Rf	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Alfalfa	2	0.79	0.09 a–g	0.79	0.11	0.59	0.06 a–i	11	3 cd	1.0	0.3 a–c
Annual ryegrass	1	1.02	0.07 a	0.89	0.12	0.87	0.05 a–c	36	10 a–d	1.9	0.6 a–c
Ann. wt. sweet clover	2	–[b]	–[b]	0.82	0.06	0.53	0.10 a–i	30	18 a–d	2.7	1.5 a–c
Barley	1	0.93	0.06 a–d	1.01	0.10	0.91	0.06 ab	70	11 a	3.4	0.5 ab
Barrel medic	2	0.91	0.08 a–d	0.78	0.11	0.68	0.07 a–i	49	16 a–d	3.3	1.0 a–c
Berseem clover	2	0.90	0.06 a–d	0.78	0.08	0.69	0.05 a–i	35	6 a–d	2.1	0.3 a–c
Birdsfoot trefoil	2	0.77	0.08 a–g	0.88	0.13	0.65	0.09 a–i	73	27 a–c	4.6	1.7 ab
Black oat	1	0.89	0.06 a–d	0.82	0.12	0.71	0.10 a–h	51	11 a–d	3.6	1.1 a–c
Brassica smother crop	3	0.95	0.07 a–c	0.97	0.07	0.91	0.06 ab	46	12 a–d	2.4	0.8 a–c
Buckwheat	4	1.00	0.05 ab	0.88	0.04	0.87	0.04 a–c	28	4 a–d	1.4	0.2 a–c
Camelina	3	0.69	0.07 a–h	0.97	0.12	0.64	0.06 a–i	40	9 a–d	2.8	0.6 a–c
Canola	3	0.85	0.06 a–d	0.99	0.09	0.81	0.03 a–d	43	10 a–d	2.4	0.6 a–c
Corn	1	0.94	0.08 a–c	0.74	0.08	0.68	0.06 a–i	57	12 a–c	3.9	0.9 ab
Corn-BT	1	0.92	0.06 a–d	1.03	0.12	0.92	0.09 a	43	7 a–d	2.1	0.4 a–c
Crimson clover	2	0.48	0.05 f–i	0.83	0.06	0.40	0.05 f–i	22	a–d	2.2	0.5 a–c
Crown vetch	2	0.64	0.08 c–i	0.84	0.09	0.53	0.08 b–i	71	24 a–c	5.5	1.8 a
Flax	4	0.89	0.04 a–d	0.94	0.07	0.83	0.04 a–d	37	6 a–d	2.0	0.4 a–c
Forage pea	2	0.48	0.08 f–i	0.85	0.09	0.41	0.09 e–i	23	10 a–d	2.2	0.9 a–c
Foxtail hay millet	1	0.75	0.04 a–g	0.97	0.14	0.71	0.10 a–h	39	4 a–d	2.6	0.4 a–c
Grain sorghum	1	0.86	0.09 a–d	0.97	0.12	0.79	0.05 a–e	35	7 a–d	2.0	0.4 a–c
Green cabbage	3	0.60	0.06 d–i	1.10	0.19	0.64	0.11 a–i	29	9 a–d	2.0	0.5 a–c
Hairy vetch	2	–[b]	–[b]	0.73	0.16	0.79	0.14 a–e	55	18 a–d	3.7	1.5 a–c
Illinois bundleflower	2	1.01	0.04 a	0.82	0.05	0.82	0.03 a–d	11	3 cd	0.6	0.1 c
Lab-lab	2	0.46	0.04 g–i	0.76	0.11	0.35	0.06 hi	30	5 a–d	3.8	0.3 a
Millet	1	0.79	0.06 a–g	0.89	0.08	0.70	0.08 a–h	55	10 a–c	3.7	0.9 ab
Mung bean	2	0.49	0.05 f–i	0.88	0.14	0.41	0.05 e–i	26	9 a–d	2.9	1.1 a–c
Oat	1	0.87	0.06 a–d	0.83	0.13	0.69	0.08 a–i	67	42 a–d	3.9	2.2 a–c
Oilseed radish	3	0.81	0.11 a–f	1.20	0.31	0.83	0.09 a–d	43	15 a–d	2.4	0.9 a–c
Oilseed rape	3	0.94	0.06 a–c	0.79	0.10	0.73	0.08 a–h	27	6 a–d	1.8	0.4 a–c
Partridge pea	2	0.90	0.05 a–d	1.02	0.13	0.89	0.07 ab	29	5 a–d	1.5	0.3 a–c
Pea	2	0.36	0.05 hi	1.12	0.11	0.38	0.03 g–i	14	3 a–d	1.6	0.2 a–c
Perennial ryegrass	1	0.95	0.06 a–c	0.94	0.14	0.85	0.10 a–d	30	7 a–d	1.7	0.5 a–c
Potato	4	0.77	0.05 a–g	1.04	0.08	0.79	0.04 a–f	24	4 a–d	1.3	0.2 a–c
Red cabbage	3	0.75	0.08 a–g	0.90	0.14	0.66	0.09 a–i	25	8 a–d	1.6	0.4 a–c
Red clover	2	0.68	0.11 a–h	0.97	0.24	0.58	0.09 a–i	38	18 a–d	2.6	1.0 a–c
Rye	1	0.84	0.06 a–e	0.82	0.06	0.68	0.05 a–i	27	10 a–d	1.9	0.8 a–c
H. glycines-resistant soybean	–	0.50	0.05 e–i	0.83	0.08	0.40	0.03 e–i	15	1 a–d	1.7	0.2 a–c
Subterranean clover	2	–[b]	–[b]	0.84	0.05	0.65	0.07 a–i	47	14 a–d	3.0	0.8 a–c
Sunflower	4	0.91	0.07 a–d	0.97	0.09	0.86	0.04 a–d	15	5 b–d	0.8	0.3 bc
Sunn hemp	2	0.33	0.07 i	0.99	0.12	0.31	0.06 i	8	1 d	1.4	0.3 a–c
Triticale	1	0.86	0.07 a–d	0.92	0.10	0.76	0.04 a–g	41	11 a–d	2.3	0.6 a–c
Wheat	1	0.87	0.05 a–d	0.79	0.09	0.68	0.08 a–i	42	10 a–d	2.7	0.7 a–c
White clover	2	0.65	0.07 b–i	0.75	0.04	0.49	0.06 c–i	44	23 a–d	3.3	1.3 a–c
Wild/perennial lupine	2	–[b]	–[b]	0.76	0.14	0.42	0.04 d–i	22	4 a–d	2.5	0.6 a–c
Control (no plant)	–	0.82	0.06 a–f	0.90	0.03	0.74	0.08 a–g	75	21 ab	4.7	1.5 a
Average by group:											
Monocots (1):		0.88	0.06	0.89	0.11	0.77	0.07	45.6	11.7	2.7	0.8
Leguminous non/poor hosts (2):		0.66	0.07	0.86	0.10	0.54	0.06	32.4	10.8	2.6	0.8
Brassicaceae (3):		0.80	0.07	0.99	0.14	0.75	0.07	36.0	9.9	2.2	0.6
Other dicots (4):		0.89	0.05	0.96	0.07	0.84	0.04	25.7	4.7	1.4	0.3
Contrast:	1 vs. 2	***		NS		***		***		NS	
	1 vs. 3	**		NS		NS		NS		NS	
	1 vs. 4	NS		NS		NS		**		**	
	2 vs. 3	***		NS		***		$P = 0.056$		NS	
	2 vs. 4	***		NS		***		NS		**	
	3 vs. 4	*		NS		*		NS		$P = 0.051$	

[a] PCF1 = nematode egg population density at the end of the rotation crop-growing period/initial egg population density; PCF2 = egg population density at the end of fallow period/egg population density at the end of crop-growing period; PCF1+2 = egg population density at the end of fallow period/egg population density at planting the rotation crops; cysts = nematode cyst population density/soybean plant in bioassay; SE = standard error. Rf is nematode reproduction factor on the *H. glycines*-susceptible soybean following the fallow period: Rf = cyst counts × 1,000/initial eggs per pot, which represents the number of females produced per 1,000 eggs. Data transformations: PCF1 (none), PCF2 ($x^{0.2}$), PCF1+2 (none), cysts ($x^{0.1}$), Rf (Ln(x)) before being subjected to analysis of variance (ANOVA). The values are means of six replicates. The values followed by the same letter or no letter in the column are not different according to Tukey's Studentized Range (HSD) Test at $P \geq 0.05$. *, **, and *** represent significance at $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively. NS = not significant at $P \geq 0.05$.

[b] Some crops are not shown in the table due to too many missing data points.

cyst count was again from the sunn hemp soil, which was 89% lower than the mean cyst count in the fallow control soil. Illinois bundleflower (86% lower) and alfalfa (85% lower) soils also led to cyst numbers that were lower than the fallow control. Compared with sunn hemp, the number of cysts was higher for the following crops: barley, birdsfoot trefoil, corn, crown vetch, and millet. As groups, the leguminous non/poor hosts and other dicots not belonging to the Brassicaceae family resulted in lower cyst counts than the monocots (Table 3). The Rf values for Assay 2 also showed some differences among crops. Illinois bundleflower and sunflower led to lower Rf values than the fallow control. As a group, the "other dicots" led to the lowest average Rf value (Table 3).

Discussion

The results of this study are generally consistent with results from recent field experiments by Miller et al. (2006), which also found that, compared with monocots and nonleguminous dicots, rotation with leguminous crops as a group led to lower *H. glycines* populations in field soil. Perhaps because this study was carried out under controlled greenhouse conditions, there were greater differences among the individual crops and groups of crops as compared with the previous field studies. This study not only confirmed the results from the previous field study, but also identified additional effective plant species as rotation or cover crops for managing *H. glycines*. Some of the crops such as sunn hemp and lab-lab are highly effective in reducing *H. glycines* population density and may have great potential in managing *H. glycines* and increasing soybean productivity in the North Central region.

As noted above, several crops produced significantly lower *H. glycines* numbers than the fallow control in various parts of the experiment. However, the crop in which *H. glycines* population density was most consistently the lowest was sunn hemp. As a group, the legumes, to which sunn hemp belongs, supported the lowest egg or cyst numbers. However, heterogeneity existed among species within a group, and selection of crops for *H. glycines* management should be based on the data from individual species rather than the groups.

There was some variation between Assay 1 and Assay 2. Fewer of the measurements in Assay 1 were statistically significant. In general, the trends in both assays were similar, with the crops falling in roughly the same order when ranked by egg or cyst counts. The variation may be due to the fact that the soil in Assay 1 was collected in fall, and soil for Assay 2 was collected in spring. Therefore, the eggs in the two Assays would be at different stages of diapause and might respond differently (i.e., they may or may not hatch) to the crops grown in the soil.

In Assay 1, an unexpected result was that PCF1 values were generally greater than PCF1+2 values. This may have been due to lower cyst extraction efficiency of egg population at the end of the rotation crop growing period. Theoretically, PCF should be less than 1 if there is no nematode reproduction. However, many PCF values, especially in Assay 1, were greater than 1 even for the crops that are nonhosts. This doesn't mean that the nematode population increased in these crops, but rather, the higher PCF values were perhaps due to experimental error in soil sampling and sample processing.

The mechanism involved in reducing *H. glycines* populations during the crop-growing period and the fallow period is not fully understood. Because PCF1 and PCF1+2 varied among crops more than PCF2 did, this suggests that the effect of the crops on *H. glycines* egg population density was mainly from the crop-growing period. Because PCF2 values did not differ significantly, *H. glycines* death by plant residue (egg death or hatch stimulation followed by J2 death in the absence of a living host) may not be the main mechanism in lowering *H. glycines* population density. Inducing egg hatch was probably the main mechanism in lowering the egg population density during the crop-growing period. The Rf (number of cysts formed on susceptible soybean per 1,000 eggs) may indicate the viability and infectivity of the eggs. While there was no difference in Rf in Assay 1, differences in Rf were observed in Assay 2. Whether the lower Rf in the "other dicots" group was due to lower viability or infectivity as compared with the other groups of crops could not be determined without further study and additional data.

Crop growth and the nematode population response may differ in different environments. We used the natural field soil rather than sterilized soil to provide soil microbial communities as similar as possible to those under field conditions. The clay loam soil used in this study is common in southern Minnesota and the North Central region. This screening study provided a basis for selecting crops to manage *H. glycines* in the region. However, further studies are needed under different field conditions to ascertain the effectiveness.

In conclusion, leguminous non/poor hosts as a group resulted in the greatest reduction in *H. glycines* egg population densities and cyst numbers, and sunn hemp was the crop which consistently led to the lowest *H. glycines* populations overall. The mechanisms involved in reduction of the *H. glycines* populations are unclear, but the results suggest that the effect occurs during the crop-growing period and is perhaps due to induced egg hatch.

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