Interactions of host-plant-resistance and seed treatments on soybean aphid (*Aphis glycines* Matsumura) and soybean cyst nematode (*Heterodera glycines* Ichinohe)

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BACKGROUND: Soybean cyst nematode, *Heterodera glycines,* and soybean aphid, *Aphis glycines,* are invasive, widespread and economically important pests of soybean, *Glycines max*, in North America. Management of these pests relies primarily on use of pesticides and soybean germplasm with genetic resistance. A three-year field study and complementary greenhouse experiment were conducted to determine the benefits of host-plant resistance (HPR) and pesticidal seed treatments for managing pest populations and preserving soybean yield.
RESULTS: Host-plant resistance significantly decreased the abundance of *A. glycines* and, in most study sites, suppressed *H. glycines*. Neonicotinoid seed treatment reduced *A. glycines* abundance on the cultivar that was susceptible to both aphids and nematodes, but abamectin nematicide seed treatment had no effect on *H. glycines* populations in the field or greenhouse.

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CONCLUSION: These results suggest that the seed treatments included in our experiments may suppress pests, but not consistently for all soybean cultivars or study sites. Ultimately, HPR more consistently reduced pest numbers compared to the use of pesticidal seed treatments. The planting of HPR cultivars should be a primary tool for integrated pest management of both soybean pests.

Keywords: abamectin, invasive pests, integrated pest management, soybean aphid, soybean cyst nematode, thiamethoxam

1. INTRODUCTION

Soybean cyst nematode, *Heterodera glycines* Ichinohe (Tylenchida: Heteroderidae), and soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), are both invasive pests that can reduce soybean yield.[1, 2] *Heterodera glycines* and *A. glycines* co-occur in many soybean-producing regions of the United States, where 34 million hectares of soybeans were grown in 2015 at a value of over $37 billion.[1, 3, 4] *Heterodera glycines* has been present in North America for more than 60 years and continues to be one of the leading suppressors of soybean yield among plant diseases and pests.[5] *Aphis glycines* has been present in North America since 2000 and yield losses from this pest can be as high as 40% when populations are not managed.[6]

One tool that can help manage crop pests is host-plant-resistance (HPR), which for insects, functions through mechanisms of antibiosis, antixenosis, tolerance, or combinations of these mechanisms, and is conferred through genetically heritable traits.[7] Since the discovery of *H. glycines* in North America in 1954, management of this pest has relied on soybean germplasm with genetic resistance.[8, 9] Some of the main sources of *H. glycines*-resistant germplasm include Peking, PI88788, and PI90763, with PI88788 found in the vast majority of soybean cultivars that are currently labeled as resistant to *H. glycines*.[10, 11]

Management of *H. glycines* with HPR has been complicated in recent years by the evolution of virulent biotypes to one of the most commonly used resistance genes, PI88788.[11] This trend likely resulted from farmers relying exclusively on a single form of HPR. For growers in the North Central United States, >95% of the soybean cultivars with *H. glycines* resistance use the trait from PI88788.[11, 12] Populations of *H. glycines* virulent to PI88788, according to the *H. glycines* HG type test[13], have been observed in Iowa, Illinois, Minnesota, Missouri and Ontario.[14] Rotation schemes that include susceptible soybean varieties (with or

without nematicides) and soybeans with different sources of resistance can help to prevent the buildup of virulent biotypes, which have increased reproduction on resistant cultivars.[9, 11] If the trend of increasing virulence is not stalled, the yield benefit derived from growing resistant cultivars could be diminished.

Host-plant-resistance also can be used to manage *A. glycines*.[15] To date, four genes conferring resistance to *A. glycines* have been identified, specifically *Rag*1, *Rag*2, *Rag*3, and *Rag*4.[16, 17, 18] The first soybean cultivars with *A. glycines* resistance used a single resistance gene, *Rag1*, and were available in 2010.[19] Aphid-resistant cultivars are not completely devoid of aphid populations, but *A. glycines* populations feeding on those cultivars typically have overall lower fecundity.[20] However, virulent biotypes of *A. glycines* that can overcome HPR have been discovered in North America.[21] While these biotypes may be problematic for the planting of single-gene *Rag* cultivars, recent studies suggest that two and three-gene *Rag* pyramids can reduce the reproduction of virulent *A. glycines* populations, and these cultivars with *Rag* pyramids should be more durable for aphid management.[22, 23]

Soybean producers have the option to manage *A. glycines* and *H. glycines* with insecticides and nematicides applied as seed treatments. Compared to conventional pesticides used on soil or plant foliage, seed treatments are desirable for their percieved reduced risks to the environment.[24] The most widely used class of insecticides that are registered for seed treatments are the neonicotinoids, including clothianidin, imidacloprid, and thiamethoxam, with neonicotinoids making up one-third of the world's insecticide market.[25, 26] Many neonicotinoid seed treatments on soybean are aimed at early season pests such as bean leaf beetle, *Cerotoma trifurcata* Forster (Coleoptera: Chrysomelidae), and wireworm, *Melanotus* spp., *Agriotes* spp.,

and *Limonius* spp. (Coleoptera: Elateridae), which usually injure soybean prior to the reproductive stage of the plant.[27, 28, 29]

Some labels for neonicotinoid seed treatments on soybean include *A. glycines* as a target pest. However, *A. glycines* populations typically colonize soybean fields after the time period when insecticidal seed treatments are most effective, which is typically the first 55 days after planting.[15] Neonicotinoid seed treatments may help manage *A. glycines* populations early in the growing season, but there is little evidence that they can prevent economic damage to soybeans because *A. glycines* populations typically increase at a rapid pace in later months of the growing season, such as in late July and August.[30, 31] Furthermore, outbreaks of *A. glycines* populations are unpredictable in a given year and location, which makes it difficult to predict the potential benefits of using an insecticidal seed treatment on soybean, a decision that must be made prior to planting.[31, 32]

Seed treatments containing fungicides, insecticides, nematicides, or different combinations of these chemicals have been shown to increase soybean stand density in fields containing pest populations, including nematodes like *H. glycines*.[33] Abamectin, a nematicide used in some seed treatment products, is not systemic but it moves along the channels of developing roots and interferes with the nervous system of nematodes.[34] Many of the new nematicide seed treatments labeled for *H. glycines* management utilize pathogenic microorganisms and/or their toxic metabolites, including strains of *Pasteuria nishizawae* (Pn1) and the bacterium *Bacillus firmus* (I-1582).[35, 36]

Integrated pest management (IPM) strategies that include growing soybean cultivars with HPR to manage both *H. glycines* belowground and *A. glycines* aboveground can protect crops with low environmental risk.[3, 37] The co-occurrence of both *H. glycines* and *A. glycines* in the

North Central region of the United States also is of importance because there is evidence that *A. glycines* feeding may enhance the quality of soybean as a host plant for *H. glycines*.[37] If feeding of both pests has a non-additive, negative impact on soybean yield, the planting of soybean cultivars with stacked resistance genes for both pests would be a good strategy for preserving yield. For soybean cultivars that lack resistance genes, a pesticidal seed treatment may help to provide some pest suppression and consequently preserve yield, however, the question remains as to whether or not this gain in yield would be economical. Under high pest pressure, the addition of a pesticidal seed treatment to resistant cultivars may be profitable. However, in other situations, pesticidal seed treatments may add unnecessary costs. Among soybean growers, 47-65% use pesticidal seed treatments without considering the target pest for which the seed treatment is intended.[26]

To address the effects of HPR and seed treatments on soybean pests, and to understand the value of these tactics for integrated pest management in soybean production, we conducted field and greenhouse studies to address the following hypotheses: 1) HPR cultivars will support lower populations of *A. glycines* and *H. glycines*; 2) a pesticidal seed treatment containing an insecticide and nematicide will provide added suppression of *A. glycines* and *H. glycines*; 3) soybean cultivars with HPR will promote superior yield; and 4) soybean with pesticidal seed treatment will have increased stand density and greater yield.

2. MATERIALS AND METHODS

2.1 Field plot experiments and pest sampling

Small-plot soybean field experiments were conducted for three years (2013, 2014, and 2015) at two different locations each year for a total of six unique study sites. The northeast study sites were located at the Iowa State University's Northeast Research and Demonstration

Farm near Nashua, Iowa. The central and northwest study sites were located in Ames and Newell, Iowa, respectively, and were on privately owned farms. The study sites within a location were changed annually. The northeast study site was planted on May 16, 2013; May 20, 2014; and May 12, 2015. The central study site was planted on June 7, 2013, and May 23, 2015. The northwest study site was planted on May 17, 2014.

At each study site, plots were established in a randomized complete block design with four blocks, 12 treatments, replicated eight times, for a total of 96 plots per study site. Plots contained one of 12 treatments from a complete factorial design with four soybean cultivars and three levels of seed treatment. Originally, we intended to include a foliar insecticide application for *A. glycines* as an additional factor in the experiments; however, *A. glycines* populations never reached the economic threshold of 250 aphids plant^{-1} before the R5 developmental stage in soybean. Consequently, no insecticides were sprayed in the field experiments. As a result, the factor of an insecticide application was removed from the experimental design and each block contained two replications per treatment.

We used four Syngenta soybean cultivars for the experiment with four combinations (2 by 2 fully crossed design) of aphid resistance (*Rag1* vs none) and SCN resistance (PI88788 vs none) (Table 1; Syngenta AG, Greensboro, North Carolina, U.S.A.). No other single seed company offered cultivars with these combinations of pest resistance genes at the time. The seed treatments used in this study were 1) ApronMaxx®, which contained the fungicides mefenoxam (0.0113 mg AI seed^{-1}) and fludioxonil (0.0038 mg AI seed^{-1}), 2) Avicta Complete®, which contained the same fungicides in ApronMaxx® at the same rates plus the nematicide abamectin (0.15 mg AI seed^{-1}) and the neonicotinoid insecticide thiamethoxam (0.0907 mg AI seed^{-1}), and 3) seeds that were left untreated (Syngenta AG, Greensboro, North Carolina, U.S.A.). Plots were

planted at a rate of 411,400 seeds hectare^{-1} using standard farming practices. Plots were 3.04 m wide with four 5.18 m rows (15.75 m^2 area). The plots contained 41 seeds per m^2 (ca. 31 seeds per linear m) and there was 0.91 m of space between different plots.

Plots were harvested when at least 95 percent of soybean pods reached maturity. All study sites were harvested in the first two weeks of October. The center two rows of plots were harvested using a plot combine. The values for moisture and total seed weight in each plot were used to determine yield expressed as kilograms hectare^{-1} at 13% moisture.

Heterodera glycines sampling was done at both the time of planting and at the time of harvest by collecting ten soil cores (19 mm in diameter, 15-20 cm in length) from both sides of the two center rows of each soybean plot. *Heterodera glycines* cysts and eggs were extracted from 100 cm^3 subsamples of dried soil with a modified wet sieving and decanting technique.[4, 38] Cysts are dead *H. glycines* females that contain eggs, and they can be found on roots or in the soil. Suspensions of *H. glycines* cysts extracted from soil were put on a 250-µm-pore sieve and crushed using water and a motorized rubber stopper to release the *H. glycines* eggs, which were collected on a 25-µm-pore sieve.[39] Eggs were suspended in 100 mL water and stained with acid fuchsin before a 1 mL sample representative of the suspension was counted using a dissecting microscope. Egg counts were performed twice for each sample, and the average density of *H. glycines* eggs per plot was calculated.

Aphis glycines populations (both alate and apterous) per plant were counted once per week from early June (i.e., June 7-11) to early September (i.e., September 6-10). Plants were sampled at random within each plot. We started with 20 randomly selected plants per plot. However, we were forced to reduce the number of plants sampled per plot per week later in the season because the proportion of plants with aphids increased, the plants became larger, and the

number of aphids per plant increased; all of which increased the time required to sample a plot. The number of plants sampled per plot (average ± standard deviation) were: 18.90 ± 2.14 in June, 11.67 ± 2.41 in July, 5.42 ± 2.30 in August, and 3.00 ± 0.00 in September. The average number of aphids per plant counted in each plot on the day of data collection was summed over the growing season to calculate cumulative aphid days (CAD), which serves as an estimate of the season-long aphid abundance.[40]

The HG type of *H. glycines* populations in each study site was determined with the HG type test described by Niblack et al.[13] using a standardized set of soybean cultivars with different genetic sources of *H. glycines* resistance. If the average number of *H. glycines* females per resistant cultivar was more than 10% of the number of females on the SCN-susceptible cultivar (Williams 82), the population was labeled with the appropriate HG type (Supp. Tables 1 & 3). For each combination of study site by year, the female index values on PI88788 was calculated by dividing the number of females per plant on a PI88788 cultivar by the number of females per plant on Williams 82, which is a nematode-susceptible cultivar, and then multiplying the value by 100. The protocol for HG type tests was similar to the greenhouse experiment described below that measures one generation of *H. glycines* reproduction.

2.2 Greenhouse experiments on H. glycines populations

Because field conditions can greatly impact the reproduction of *H. glycines*, particularly soil temperature and rainfall, we wanted to study the *H. glycines* populations and treatments within a more controlled environment. We performed 30-day greenhouse experiments modified from the Standard Cyst Evaluation-2008 (SCE-08) protocol to measure effects of treatments, used in our field study, on a single generation of *H. glycines* reproduction, using the *H. glycines* populations from our study sites.[41] The experimental design followed McCarville et al.[37] with

some slight modifications. *Heterodera glycines* typically completes one generation every 25 to 32 days at 27 to 30 ºC, thus the 30-day experiment estimates the amount of *H. glycines* reproduction in one generation.[42] After completing *H. glycines* egg counts for soil samples collected at planting, all remaining soil from one study site was combined, mixed together and used for the greenhouse experiment. We performed five separate greenhouse experiments, with one experiment per study site. We could not conduct a greenhouse experiment for the northeast study site in 2015 because it had a very low density of *H. glycines* eggs. Depending on the density of *H. glycines* eggs, field soil from study sites was diluted with the appropriate amount of construction sand to adjust the *H. glycines* egg densities to ca. 10 eggs per cm^3 soil. The greenhouse experiments used the same 12 treatments of soybean cultivar by seed treatment that were used in the field experiments. Single soybean plants were grown in 125-mL cone-shaped containers (Stuewe & Sons, Tangent, OR) containing 100 mL field soil.

One container for each treatment was placed in a random arrangement inside a 7.5 L plastic bucket with the bottom sealed that was filled with construction sand up to the level of the soil inside each container. In each experiment, six replications (i.e., buckets of single plants) were established, with each bucket containing one container per treatment, for a total of 12 containers per bucket. Buckets were placed in a temperature-controlled water bath to stabilize soil temperatures between 27 and 30 ºC. Water baths were housed in a greenhouse and placed on benches under artificial light (16:8 (L:D)), and plants were watered as needed throughout the experiment. At the end of the experiment, containers with plants were removed from buckets and roots were placed on a 600-µm–pore sieve over a 250-µm-pore sieve and sprayed with a faucet to dislodge *H. glycines* females (cysts). The cysts were then washed from the 250-µm-

pore sieve into individual 100 mL beakers and later counted using a microscope (Leica S6 E, Leica Microsystems, Wetzlar, Germany).

2.3 Data analysis

Unless otherwise stated, we analyzed data with a mixed-model analysis of variance (ANOVA) using the PROC MIXED procedure in SAS 9.4.[43] Random effects were tested using a log-likelihood ratio statistic (-2 RES log likelihood) based on a one-tailed χ^2 test assuming one degree of freedom.[44] Random factors were removed from the model to increase the statistical power when these factors were not significant at a level of $\alpha < 0.25$.[45]

For the field study, we analyzed cumulative aphid days, density of *H. glycines* eggs at the time of harvest, soybean stand density, and soybean yield with an ANOVA that had the fixed factors of soybean cultivar, seed treatment, and their interactions. Random factors were study site, block (nested within study site), and all interactions of study site and block (nested within study site) with the fixed factors. When significant fixed effects were present, pairwise comparisons were made using the PDIFF statement in PROC MIXED. Pairwise comparisons were based on least-square means with an experiment-wise significance level of $P < 0.05$ based on a Bonferroni adjustment for multiple comparisons. For analyses of individual study sites (see Supplemental Tables), we performed a mixed-model ANOVA with the same fixed factors and the random factors of block and all interactions of block with the fixed factors. When a significant fixed effect was present, pairwise comparisons were made using Tukey's Honest Significant Difference (HSD) Test with a 95% confidence level.

For the greenhouse experiment, the number of *H. glycines* females (cysts) per soybean plant was analyzed with a mixed-model ANOVA that included the fixed factors of cultivar, seed treatment, and their interactions. Because all of the study sites had *H. glycines* populations

identified as HG type 2 (Supp. Table 1), meaning that the *H. glycines* population in each experimental field had ≥10% reproduction on PI88788, study sites were combined for the overall analysis with that included the random factors of study site and all interactions of study site with the fixed factors in the model.

3. RESULTS

3.1 Field plot experiments

For cumulative aphid days (CAD), the interaction of the factors of cultivar and seed treatment was significant (Table 2, Fig. 1). The soybean cultivar that was susceptible to *A. glycines* and *H. glycines* (NK S24-K2) had significantly lower CAD on plants that were grown from thiamethoxam-treated seeds compared to untreated ones and from those only treated with fungicides (Fig. 1). However, for all other soybean cultivars there were no significant differences in CAD among plants with or without seed treatment (Fig. 1). Cumulative aphid days on *Rag1* cultivars (NK S25-F2 and NK S21-Q3) were significantly lower than CAD on aphid-susceptible cultivars (NK S24-K2 and NK S23-P8) (Table 2, Fig. 1). Between the two aphid-susceptible cultivars, we observed that the cultivar with *H. glycines* resistance (NK S23-P8) had significantly lower CAD than the cultivar without *H. glycines* resistance (NK 24-K2) (Fig. 1).

For data on density of *H. glycines* eggs in the soil at the end of the field season, we found a significant effect of cultivar, but no significant effect of seed treatment or the interaction of these factors (Table 3). We also found that nematode-resistant cultivars (i.e., those with PI88788) had significantly fewer *H. glycines* at harvest compared to susceptible cultivars (Table 3, Fig. 2). However, analyses of *H. glycines* population densities separated by study site

revealed that there was not a difference between resistant and susceptible cultivars in the central study site in 2015 (Supp. Table 2).

We observed significant differences in stand density of soybeans at the V1 stage for the factors of cultivar, seed treatment, and the interaction of these factors (Table 4). The significant interaction arose because *H. glycines*-susceptible cultivars S24-K2 and S25-F2 that received seed treatments displayed a greater stand density, compared to the untreated control; and this difference was larger than that observed for the *H. glycines*-resistant cultivars (S23-P8 and S21-Q3) (Fig. 3). However, there were no significant differences in soybean yield for the factors of cultivar (df = 3, 69; F-value = 2.53; $P = 0.0646$), seed treatment (df = 2, 46; F-value = 1.38; $P = 0.2614$), or their interaction (P = df = 6, 138; F-value = 0.75; $P = 0.6070$).

All study sites had overall averages of >500 *H. glycines* eggs per 100 cm^3 soil before planting except for the northeast study site in 2015, which averaged <150 eggs per 100 cm^3 soil (Supp. Table 2). Because of the low number of *H. glycines* eggs in the northeast study site in 2015, an HG type could not be determined. All study sites contained *H. glycines* populations with a biotype considered to be virulent against PI88788 (HG type 2), which is the source of resistance that was in our *H. glycines*-resistant cultivars (S23-P8 and S21-Q3; Supp. Tables 1 and 3). The female index values on PI88788, which measures the percent virulence of a population against PI88788, ranged from 12 to 43% (Supp. Table 1).

3.2 Greenhouse study with H. glycines

There was a significant effect of cultivar on the numbers of *H. glycines* females (cysts) per plant, but no significant effect of seed treatment or the interaction of these factors (Table 5, Fig. 4) in the greenhouse experiment. Overall, there were approximately 20-30% fewer females on the *H. glycines*-resistant cultivars relative to the susceptible cultivars. Considering the

greenhouse results of each study site and year separately, the numbers of *H. glycines* females per plant were significantly lower on resistant cultivars, except in the experiments representing the northeast and northwest study sites in 2014 (Supp. Table 1).

4. DISCUSSION

In this study, we found that HPR consistently suppressed both *A. glycines* and *H. glycines* populations (Figures 1 and 2). We also found that the neonicotinoid thiamethoxam in the fungicide, insecticide, and nematicide (FIN) seed treatment reduced the cumulative aphid days (CAD) on one of the aphid-susceptible cultivars (NK S24-K2; Fig. 1). However, thiamethoxam did not significantly reduce *A. glycines* populations on the other cultivars. The fungicide and FIN seed treatments improved stand densities for some cultivars (Fig. 3), but there was no effect of either seed treatment on yield. Additionally, the nematicide abamectin in the FIN seed treatment did not reduce population densities of *H. glycines* in field or greenhouse experiments (Figures 2 and 4).

Our results with *A. glycines* populations on *Rag1* cultivars are consistent with previous studies, which also measured lower *A. glycines* populations on these resistant cultivars. In one field experiment, *A. glycines* populations peaked at a few hundred aphids per plant on *Rag1* cultivars while susceptible cultivars had thousands of aphids per plant.[46] We did not have "aphid-free" control plots, which are routinely sprayed with insecticides, to compare the inherent differences in yield potential in our different cultivars. Although the *Rag1* cultivars were shorter than aphid-susceptible cultivars around the time of harvest (data not shown), they appeared to have more nodes and pods that could compensate for overall yield. The *Rag1* gene or a pyramid of *Rag* genes does not cause a "yield drag" when they are introduced into soybean cultivars.[22] Another field experiment found that there were no significant differences in yield between

resistant cultivars and susceptible cultivars in the absence of *A. glycines* populations.[19] Despite our results with these HPR cultivars, it is still possible for virulent biotypes of *A. glycines* to colonize a soybean field and to reduce yield on single-gene *Rag* soybeans.[21] There are now soybean cultivars with two- or three-gene pyramids of different *Rag* genes which can suppress *A. glycines* populations more than single-gene cultivars alone.[20] These soybean cultivars with pyramided *Rag* genes could be more durable from an insect resistance management (IRM) standpoint and help to stall the buildup of virulent biotypes.[20, 22, 23]

The results for *H. glycines* population densities in the field experiment demonstrate that *H. glycines*-resistant soybeans can slow the buildup of *H. glycines* populations, even when those populations may have some virulence to a source of *H. glycines* resistance. *Heterodera glycines* injury does not always cause obvious symptoms in the aboveground biomass[47], thus many growers may not know that *H. glycines* are present without soil or root sampling. *Heterodera glycines* can cause up to a 30% reduction in yield and losses may increase in drier growing seasons.[9] All of our study sites contained virulent *H. glycines* biotypes identified as HG type 2 (Supp. Table 3), and yet we measured lower *H. glycines* reproduction on resistant (PI88788) cultivars compared to the *A. glycines*- and *H. glycines*-susceptible cultivar in the field and greenhouse experiments (Figures 2 and 4). Virulent *H. glycines* biotypes may be more widespread in North America than they were 10 years ago and this trend of increasing virulence could negatively impact soybean production, since HPR cultivars rely heavily on the PI88788 genes.[11, 12, 48] Unfortunately, there are no available soybean cultivars a pyramid of resistance genes to manage *H. glycines*. In order to delay the buildup of *H. glycines* populations and virulent biotypes, researchers have outlined rotation schemes for growers that utilize different

sources of *H. glycines* resistance and by planting non-host crops such as corn, alfalfa, wheat, and cotton.[1]

Although the thiamethoxam seed treatment reduced *A. glycines* populations on the soybean cultivar that was susceptible to both *A. glycines* and *H. glycines* (Figure 1), it is difficult to extrapolate from our results whether the same degree of pest suppression would occur if *A. glycines* populations had reached overall greater numbers. In >95% of our field plots, the aphid populations did not reach the economic injury level of 674 aphids plant^{-1}.[6] Compared to studies that observed *A. glycines* populations with >20,000 CAD on soybean[20, 30], the *A. glycines* populations in our study sites were relatively small and remained <5,000 CAD in most study sites (Fig. 1 and Supp. Table 2). Previous studies found that neonicotinoid seed treatments like thiamethoxam do not consistently prevent *A. glycines* populations from reaching economically damaging levels.[49, 50] This inconsistent reduction in *A. glycines* populations with neonicotinoid seed treatments often is due to the timing of *A. glycines* colonization, which typically occurs when the concentration of the neonicotinoid compounds in the soybean tissues has diminished.[51] Furthermore, thiamethoxam did not provide additional suppression of *A. glycines* populations on *Rag1* soybeans in our plots, which may have been due, in part, to the overall low *A. glycines* populations on those *Rag1* cultivars. In these situations under low pest pressure where HPR is already being used, an insecticidal seed treatment may not be needed to suppress pests.

The abamectin seed treatment had no impact on numbers of *H. glycines* in either the field or greenhouse experiments (Figures 2 and 4). Abamectin has been reported to suppress root-knot nematodes (*Meloidogyne* spp.) in cotton and vegetable crops, but otherwise there is little published evidence that it can suppress cyst-forming nematodes including *H. glycines*.[24, 52, 53] In the course of completing this three-year field experiment, new seed treatment products have

become available for management of *H. glycines*, including the bacterium *Pasteuria nishizawae* (Pn1), the bacterium *Bacillus firmus* (I-1582), and the fungicide fluopyram, all of which have nematicidal activity.[35, 36] Future studies should test how these nematicides affect *H. glycines* and may fit with soybean IPM.

The lower populations of *A. glycines* on the *H. glycines*-resistant cultivar (NK S23-P8) may represent an indirect interaction between *A. glycines* and *H. glycines* (Figure 1). Constitutive plant defenses in resistant cultivars that suppress *H. glycines* might confer similar effects on aboveground pests like *A. glycines*, as occurs in other systems.[54] However, it is currently unknown why this effect occurred. Hong et al.[55] measured a greater intrinsic growth rate of *A. glycines* on soybeans that were attacked by *H. glycines*, implying that feeding by *H. glycines* may increase host-plant quality for *A. glycines*. Because *H. glycines* and *A. glycines* co-occur in many soybean-producing regions, there could be an added benefit to planting *H. glycines*-resistant soybeans if suppression of the belowground nematode pest could lead to lower populations of *A. glycines* and vice-versa.[37]

For some soybean cultivars, we measured significantly greater stand density for soybeans with either fungicidal seed treatment or FIN seed treatment compared to untreated soybeans (Fig. 3, Table 4 and Supp. Table 2). Previous field studies also have found that seed treatments with fungicides, insecticides, and/or nematicides increased the germination and establishment of soybeans in the field.[33] Despite our result of greater stand density, seed treatments did not significantly improve soybean yield. Soybean plots with lower stand density appeared to be able to compensate for yield, with greater canopy space per plant likely increasing the yield of individual plants. Previous studies conducted in the Midwest also have found inconsistent benefits of these seed treatments on soybean for managing pest populations.[30, 33, 50, 51] In contrast,

another study found that neonicotinoid seed treatments reduced insect pest injury and increased soybean yield in the Mid-South growing region of the United States (e.g., Arkansas, Louisiana, Mississippi, and Tennessee)[57]. The southern region of the United States harbors a greater diversity of insect pests than the Midwest and overall has more pest activity early in the soybean-growing season than in the Midwest. In regions like the Mid-Southern United States, insecticidal seed treatments may be a more useful tool for soybean IPM than in the North Central region.

5. CONCLUSION

Compared to pesticidal seed treatments, HPR is a more reliable tool for soybean IPM that can consistently suppress pest populations, including those that are difficult to monitor, such as nematodes in the soil, or those that can rapidly increase in population size, such as aphids.[58] Another benefit of growing *H. glycines*-resistant soybean is that there is no premium for purchasing the seed compared to *H. glycines*-susceptible soybean. Suppression of *H. glycines* with resistant cultivars may indirectly affect *A. glycines* reproduction through host-plant defenses or alterations to host-plant quality, but further studies are needed on the interactions between these pests.[37, 55] Thiamethoxam seed treatment may help reduce *A. glycines* populations on susceptible cultivars, but HPR cultivars appear to not benefit from thiamethoxam when they have substantially reduced *A. glycines* populations. Additionally, abamectin seed treatment did not reduce *H. glycines* populations (Fig. 3). In the North Central region, soybean growers should consider using cultivars with HPR to manage *A. glycines* and *H. glycines*, but using seed treatments with the nematicide abamectin or the insecticide thiamethoxam for these pests likely will not be profitable, at least under conditions of low pest pressure similar to those that occurred in our field experiments. If a grower does not have access to *A. glycines*-resistant cultivars, the most cost effective tool would be scouting and application of foliar insecticides if *A. glycines*

populations reach the economic threshold.[51, 58, 59] Soybean production in North America relies heavily on cultivars with the PI88788 resistance gene. Thus, soybean producers should implement rotation schemes with non-host crops and different sources of resistance to help delay the buildup of virulence by *H. glycines* to PI88788 genes.

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TABLES AND FIGURES

Table 1: Soybean cultivars used in the field and greenhouse experiments

NK Brand[a]	RM[b]	EM[c]	SCN gen.[d]	Aphid gen.[e]
S21-Q3	2.1	3	Resistant	Resistant
S23-P8	2.3	3	Resistant	Susceptible
S24-K2	2.4	3	Susceptible	Susceptible
S25-F2	2.5	3	Susceptible	Resistant

[a] Syngenta® product
[b] RM: Relative maturity number indicates maturity group, second number indicates within-group maturity rating on a 0-9 scale (0 = early, 9 = late)
[c] EM: Emergence rating on a 1-9 scale (1 = best, 9 = worst)
[d] SCN gen: Genetics for soybean cyst nematode, *Heterodera glycines* (resistant = PI88788)
[e] Aphid gen: Genetics for soybean aphid, *Aphis glycines* (resistant = *Rag1*)

Table 2: Mixed model analysis for cumulative aphid days in the field experiments.

Fixed effect	d.f.	F-value	*P*-value
Cultivar	3, 69	19.36	<0.0001
Seed treatment	2, 46	3.54	0.0371
Cultivar × seed treatment	6, 138	4.68	0.0002
Random effect	**d.f.**	**χ^2**	***P*-value**
Study site	1	126.0	<0.0001
Study site (block)	1	0.4	0.2635
Study site (block) × cultivar	1	99.3	<0.0001
Study site (block) × seed treatment	1	2.3	0.0647
Study site (block) × cultivar × seed treatment	1	3.0	0.0416

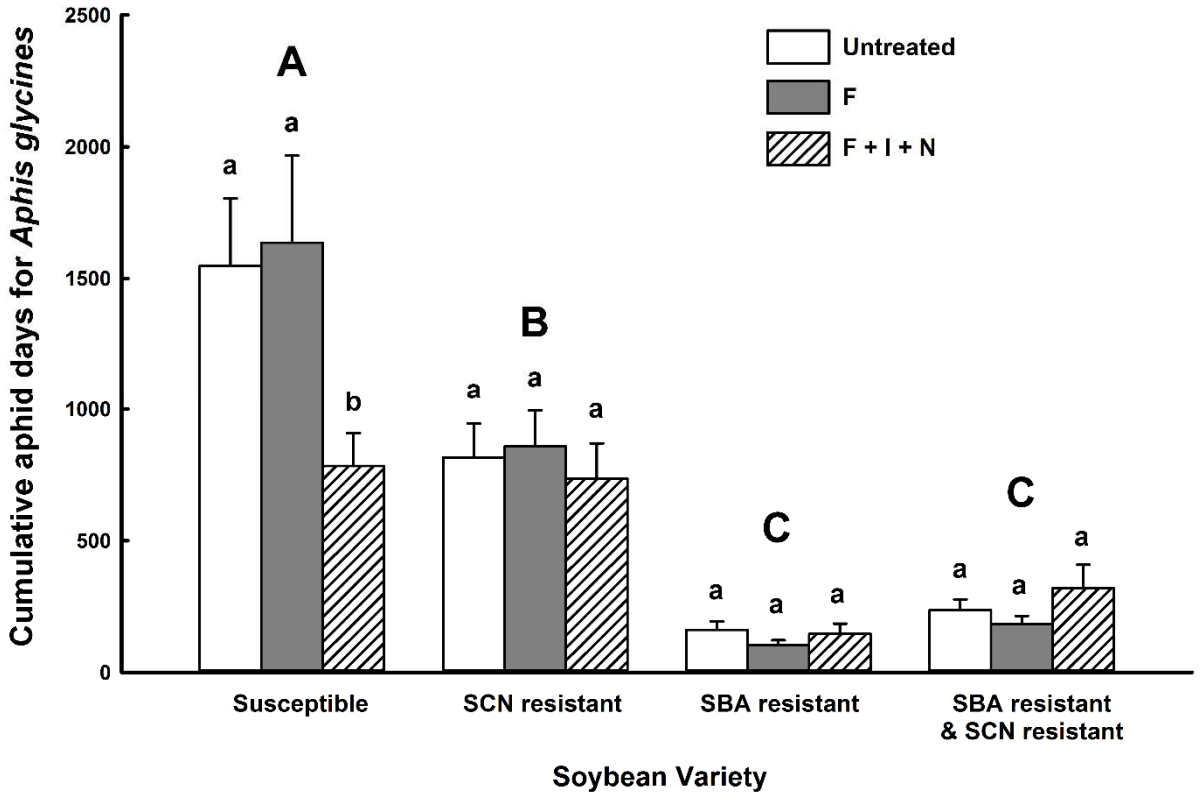

Figure 1: Average cumulative aphid days in the field experiments. Bars represent combinations of cultivar and seed treatment. Bar heights are the mean, and error bars represent the standard error of the mean. Bar groups are separated by soybean cultivar. SCN = soybean cyst nematode. SBA = soybean aphid. Shading or patterns in the bars represent seed treatment. F = fungicides; I = insecticide; N = nematicide. Capital letters above the bars represent significant differences between soybean cultivars. Lowercase letters above the bars denote a significant difference between means for seed treatments within a soybean cultivar.

Table 3: Mixed model analysis for soybean cyst nematode (*Heterodera glycines*) population densities at the time of harvest in the field experiments.

Fixed effect	d.f.	F-value	*P*-value
Cultivar	3, 69	11.96	<0.0001
Seed treatment	2, 46	1.24	0.2996
Cultivar × seed treatment	6, 426	0.72	0.6307
Random effect	**d.f.**	**χ^2**	***P*-value**
Study site	1	483.8	<0.0001
Study site (block)	1	240.3	<0.0001
Study site (block) × cultivar	1	3.9	0.0241
Study site (block) × seed treatment	1	4.9	0.0134

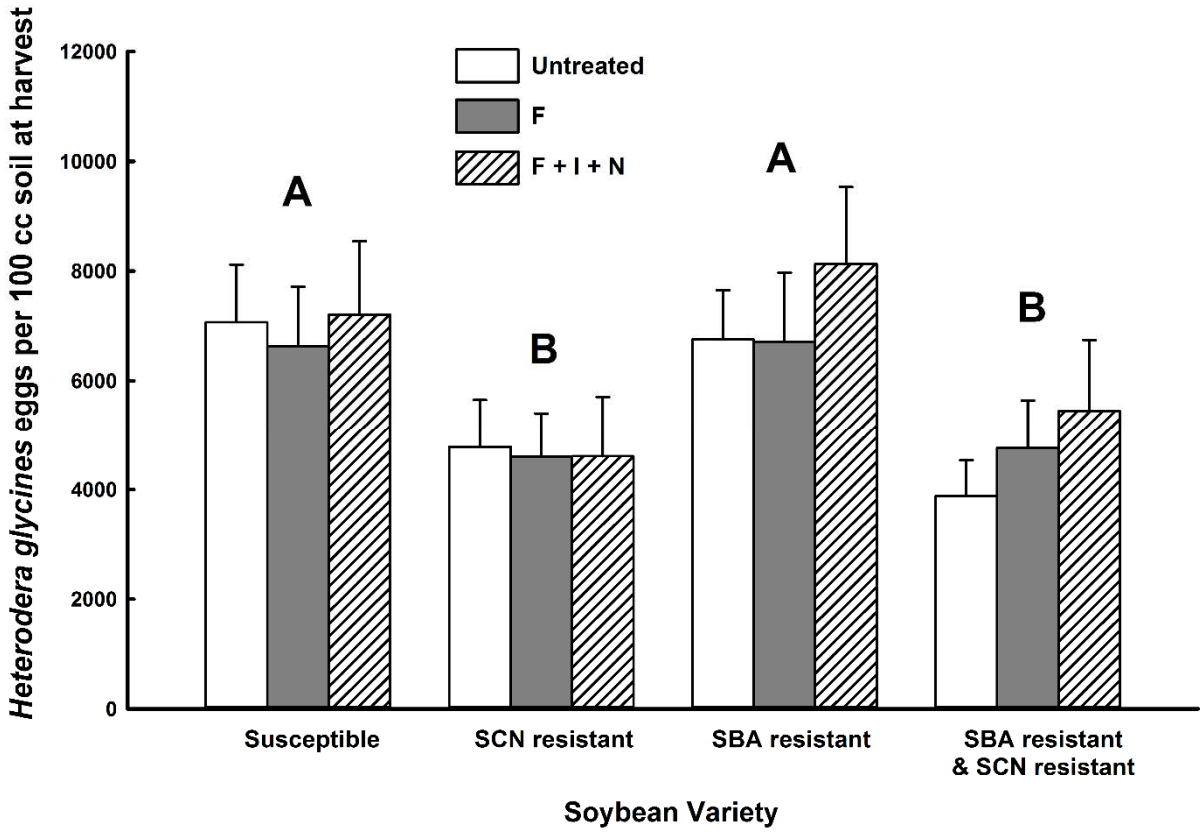

Figure 2: Average number of *H. glycines* eggs per 100 cm^3 soil at the time of harvest in the field experiments. Bars represent combinations of cultivar and seed treatment. Bar heights are the mean, and error bars represent the standard error of the mean. Bar groups are separated by soybean cultivar. SCN = soybean cyst nematode. SBA = soybean aphid. Shading or patterns in the bars represent seed treatment. F = fungicides; I = insecticide; N = nematicide. Capital letters above the bars represent significant differences between soybean cultivars.

Table 4: Mixed model analysis for soybean stand density in the field experiments.

Fixed effect	d.f.	F-value	*P*-value
Cultivar	3, 69	8.78	<0.0001
Seed treatment	2, 46	14.86	<0.0001
Cultivar × seed treatment	6, 138	6.00	<0.0001
Random effect	**d.f.**	**χ^2**	***P*-value**
Study site	1	217.0	<0.0001
Study site (block)	1	8.4	0.0019
Study site (block) × cultivar	1	7.3	0.0034
Study site (block) × seed treatment	1	7.6	0.0029
Study site (block) × cultivar × seed treatment	1	0.1	0.3759

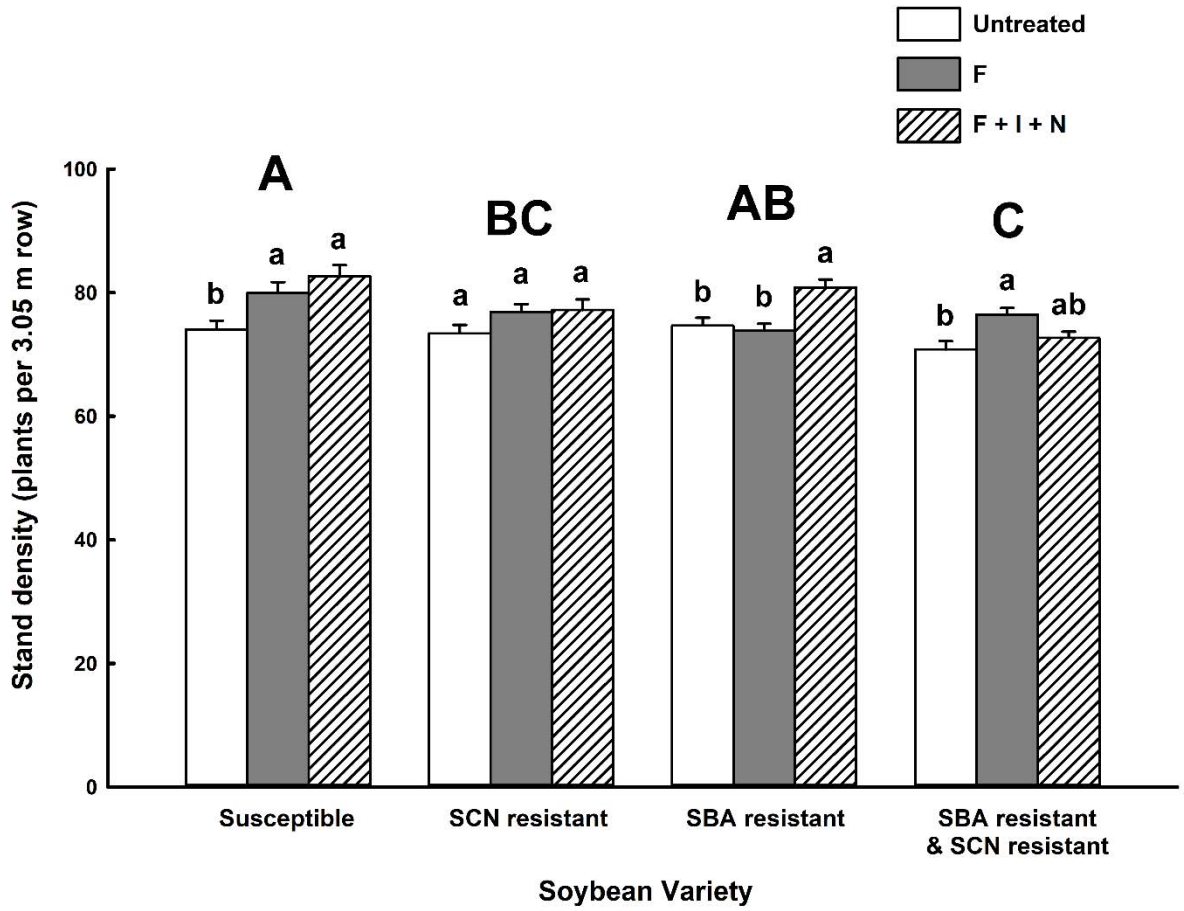

Figure 3: Average soybean stand density in the field experiments. Bars represent combinations of cultivar and seed treatment. Bar heights are the mean, and error bars represent the standard error of the mean. Bar groups are separated by soybean cultivar. SCN = soybean cyst nematode. SBA = soybean aphid. Shading or patterns in the bars represent seed treatment. F = fungicides; I = insecticide; N = nematicide. Capital letters above the bars represent significant differences between soybean cultivars. Lowercase letters above the bars denote a significant difference between means for seed treatments within a soybean cultivar.

Table 5: Mixed model analysis for soybean cyst nematode (*Heterodera glycines*) females per soybean plant in the greenhouse experiment.

Fixed effect	d.f.	F-value	*P*-value
Cultivar	3, 12	5.54	0.0127
Seed treatment	2, 8	0.29	0.7555
Cultivar × seed treatment	6, 24	1.44	0.2392
Random effect	**d.f.**	**χ^2**	***P*-value**
Study site	1	44.4	<0.0001
Study site × cultivar	1	2.2	0.0690
Study site × seed treatment	1	4.2	0.020

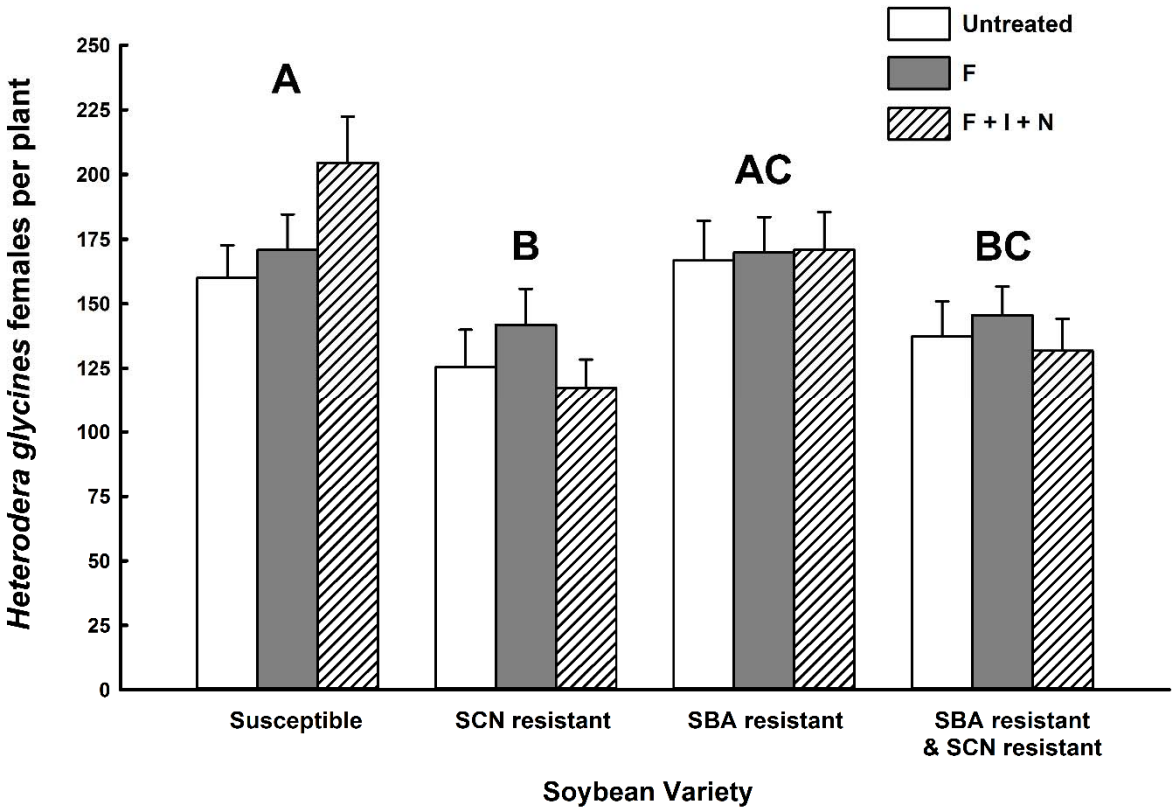

Figure 4: Average number of *H. glycines* females per plant after 30 days in the greenhouse experiment. Bars represent combinations of cultivar and seed treatment. Bar heights are the mean, and error bars represent the standard error of the mean. Bar groups are separated by soybean cultivar. SCN = soybean cyst nematode. SBA = soybean aphid. Shading or patterns in the bars represent seed treatment. F = fungicides; I = insecticide; N = nematicide. Capital letters above the bars represent significant differences between soybean cultivars.