ABSTRACT

How above- and belowground plant pests interact with each other and how these interactions affect productivity is a relatively understudied aspect of crop production. Soybean cyst nematode, *Heterodera glycines* Ichinohe, a root parasite of soybean, *Glycine max* (L.) Merr., is the most threatening pathogen in soybean production and soybean aphid, *Aphis glycines* Matsumura, an aboveground phloem-feeding insect that appeared in North America in 2000, is the key aboveground herbivore of soybean in the midwestern United States. Now, both soybean aphid and soybean cyst nematode co-occur in soybean-growing areas in the Upper Midwest. The objectives of this study were to examine aphid colonization patterns and population growth on soybean across a natural gradient of nematode density (range, \(11015\) 900 and 27,000 eggs per 100 cm\(^3\) soil), and to investigate the effect of this pest complex on soybean productivity. Alate (winged) soybean aphid colonization of soybean was negatively correlated to soybean cyst nematode egg density (\(r = -0.363, P = 0.0095\)) at the end of July, at the onset of peak alate colonization. However, both a manipulative cage study and openly colonized plants showed that soybean cyst nematode density below ground was unrelated to variation in aphid population growth (\(r = 0.01\)). Based on regression analyses, soybean aphids and cyst nematodes had independent effects on soybean yield through effects on different yield components. High soybean cyst nematode density was associated with a decline in soybean yield (kg ha\(^{-1}\)), whereas increasing soybean aphid density (both alate and apterous) significantly decreased seed weight (g 100 seeds\(^{-1}\)).

KEY WORDS *Aphis glycines*, *Heterodera glycines*, above and belowground interactions, path analysis, aphid colonization

Agricultural crops are frequently exposed to multiple pests and pathogens during their development. Soybean, *Glycine max* (L.) Merr., a major agricultural crop in the north central United States, is host to two main pests that individually have significant effects on yields. Since its first report in 2000 (Alleman et al. 2002), the soybean aphid, *Aphis glycines* Matsumura, native to Asia, has caused serious damage to soybean in the United States and Canada. Soybean aphids feed on phloem sap and can cause significant reduction in soybean yield (Ostlie 2001, Powell et al. 2006, Ragsdale et al. 2007). Yield loss caused by soybean aphids averages \(\approx 7\%\) per 10,000 cumulative aphid days (Ragsdale et al. 2007) and reached as high as \(19\%\) in a controlled field experiment (Myers et al. 2005). Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, is an endoparasitic obligate pathogen which infects and reproduces in the roots of soybean and other leguminous hosts. The second-stage juvenile (J2) infects roots and migrates intracellularly through the root within or near the vascular tissues establishing a specialized feeding site, the syncytium (Niblack et al. 2006). Soybean that is severely infected with SCN can become stunted, chlorotic, and in cases of severe infestations can die (Riggs and Schmitt 1989, Niblack et al. 2004). Foliar symptoms of chlorosis and stunting may or may not occur; yield losses of 15\% have been documented in the absence of foliar symptoms (Wang et al. 2003). SCN overlaps the distribution of soybean aphid in the north central region of the United States.

Recent studies on the physiological and molecular basis of plant responses to herbivory suggest that multiple herbivores sharing a host plant can indirectly interact through their shared host via induced, often systemic, plant responses (Karban and Baldwin 1997, Bezemer and van Dam 2005). Herbivory on different plant parts or above- and belowground plant structures can also result in systemic effects (van Dam et al. 2003, 2005; Kaplan et al. 2008, 2009; Wang et al. 2009). SCNs are present in the soil year-round and can infect soybean early in the season just after germination and in advance of aphid colonization. This infection results in changes in production of secondary metabolites, such as flavonoids (Ithal et al. 2007a, Jones et al. 2007), and in root structure (Kim et al. 1987). Based on these observations, we hypothesized that prior infection of soybean by SCN could alter the
colonization and population growth of later arriving soybean aphid. A prior laboratory study suggested that under controlled conditions alate soybean aphids preferred noninfected plants over SCN-infected plants, but that aphid population growth of apterae was generally unaffected by SCN infection (Hong et al. 2010). The goal of this study was to examine whether SCN and aphids interact in similar ways in the field. Our objectives were to examine colonization patterns of soybean aphid in the field on soybean naturally infected by SCN, and to measure population growth of aphids. We also examined the effect of soybean aphid and SCN, and their combination on soybean yield and yield components.

Materials and Methods

Study Site. In 2008, a soybean field (Plainfield loamy sand soil) was established at the University of Wisconsin, Hancock Agricultural Research Station (ARS) at Hancock, WI (Waushara Co.), on a site used previously to screen soybean genotypes for their response to SCN and thus known to have a range of SCN abundance (of HG Type 2). A soybean cultivar with the PI 88788 source of resistance to SCN was planted on 22 May with 0.9-m row spacing and 35 seeds per m. A mildly nematode resistant variety was used in this study because of the potentially high nematode pressure expected in this field. Fifty plots (one row of 1.5 m of soybean) were established in this field in a 5 by 10 array. Plots were separated by 4.6 m between rows and 12.2 m within a row. The entire study area was 122 by 45.7 m. Soybean was Roundup Ready, grown using standard agronomic procedures, and plants were not treated with any insecticides or nematicides during this study.

SCN Densities. Population densities of the SCN were heterogeneous in the field due to prior cropping history. To determine SCN density, soil samples were collected from each plot on 29 May, 1 wk after planting soybean. SCN egg density was used to determine the relationship with soybean yield and aphids because nematode eggs are more highly correlated with yield than cyst counts (Niblack et al. 1993). Approximately five to six individual soil cores (2 cm in diameter by 20 cm in depth) per plot were collected from the areas immediately adjacent to planted soybean, combined into one composite sample and stored at 5°C until processing. Soil was mixed thoroughly and a 100-cm³ sample was assayed (Jenkins 1964) by using nested screens (32-74 μm) to collect cysts. All cysts were crushed to recover eggs by using a motorized pestle (Faghihi and Ferris 2000) and suspended in a known volume of water. The total number of SCN eggs per 100 cm³ soil was determined by first estimating the eggs per ml of water and then multiplying by the total volume of water in which eggs were suspended. The mean of egg numbers per milliliter was determined by taking a subsample (100 μl) of solution from 1 ml of water and counting nematode eggs on a microscope slide under a microscope. Data collected close to the time of planting were used in the analysis because yield is most strongly influenced by the first nematode generation (Schmitt and Ferris 1998), particularly for SCN-resistant varieties that limit reproduction by nematodes.

Soybean Aphid Sampling. Soybean aphid sampling was conducted every week from 20 June to 11 September. Within each plot (i.e., one row of 1.5 m), five soybean plants were randomly selected, and number of alate and alate aphids was (nondestructively) counted and recorded. For each plot, cumulative abundance per plot of alate soybean aphids over the season was calculated using the formula cumulative aphid days (CAD) = \( \sum_{i=1}^{n} \left( \frac{x_i + x_{i-1}}{2} \right)^2 t_i \), where \( n \) is the number of sampling dates, \( x_i \) is the average number of aphids per plot on sampling date \( i \), and \( t_i \) is the number of days since the previous sampling date (Hanafi et al. 1989). Average aphid weekly abundance was used (rather than cumulative abundance) for alate aphids. Soybean plant vegetative and reproductive stages (Ritchie et al. 1994) were recorded at sampling time.

Estimation of Aphid Population Growth. Aphid data collected weekly from plants in naturally infested plots were used to calculate the relative rate of growth of the aphid population. We estimated the slope of a line fitted to the average aphid abundance/plot (log \( x + 1 \) transformed) versus sampling date, using all data spanning the range from when aphids were first detected up to peak aphid abundance, giving an average 7.4 sample points over time per plot from which relative growth rates were calculated.

Aphid population growth was also estimated using a cage experiment. Two or three plants were randomly selected in each plot and on 22 July, and as plants were starting to flower (plants at R1 stage), three mixed-age adult apterous females were carefully placed on newest fully expanded trifoliate. Plants were bagged using fine-mesh organza sleeves, with one bag on each selected plant. All aphids used for this experiment came from field-collected colony at the University of Wisconsin Arlington Agricultural Research Center (Columbia Co., WI) and maintained on soybean plants grown in a greenhouse at the University of Wisconsin (Madison, WI). The total number of soybean aphids in each cage was counted 15 (plants at R3 developmental stage) and 29 (plants at R5 stage) days after the release of aphids into cages. Relative growth rate of aphids in a plot \( i \) was calculated as follows: relative growth rate \( r_i = \left| \frac{N_{29} - N_{15}}{N_{15}} \right| / 14 \), where \( N_i \) is the average number of aphids per bag in each plot on day \( x \).

Soybean Yield. Soybean yields and yield parameters were measured by harvesting the entire experimental plot (i.e., one row of 1.5 m) on 6 October. After harvest, total grain weight (g plot⁻¹), seed mass (g 100 seeds⁻¹), and number of plants plot⁻¹ were determined. From these data, we estimated average total grain mass per plant (g per plant). Grain moisture content was determined and grain weight was adjusted to 13% moisture content to estimate the grain yield (kg/ha, by scaling up from the size of each plot).
Data Analysis. Data obtained from these studies consisted of SCN egg density at the time of soybean planting, weekly soybean aphid counts from 20 June through 11 September, soybean aphid relative growth estimated from 1) natural field populations, and 2) cage assay, and soybean yield (kg ha$^{-1}$) and yield components (seed mass 100 seeds$^{-1}$, plant number plot$^{-1}$, total seed mass plot$^{-1}$). Correlation analysis was used to examine the relationship between SCN density per plot and soybean aphid abundance (alate on different dates) and apterous relative growth rates (measured in open field plants and cages). Statistical comparisons using both unadjusted P values and sequential Bonferroni corrected values are reported.

Multiple regression analyses were performed to examine how soybean yield (kg ha$^{-1}$ or g 100 seeds$^{-1}$) was related to SCN egg density and soybean apterous aphid abundance (using CAD). We report the relationship between soybean aphid abundance measured on 31 July (plants at R2) and soybean yield from those same plots at the end of the season because this relationship was the strongest ($r = -0.47, P < 0.001$). All statistical tests were performed in R (R Development Core Team 2009).

Results

SCN Density in Field. SCN egg density (average ± SD) in a field plot was 7,678 ± 5,169 eggs/100 cm$^3$ soil, with 50% of plots (25/50) having between 5,000 and 10,000 eggs per 100 cm$^3$ soil, respectively. In other studies, average SCN egg densities in soybean fields surveyed varied from ≈1,700 to 33,600 eggs per 100 cm$^3$, depending on soybean maturity group and location (Riggs et al. 2000, Wang et al. 2000, Mitchum et al. 2007).

Soybean Aphid Abundance. The first occurrence of soybean aphids in the field was 2 July. By 17 July, 64% of plots were infested with soybean aphids, and 100% of plots were infested by 22 July. Colonization of soybean by soybean aphid started in mid-late July when soybean plants where beginning to flower (R1) and develop pods (R3–5), at which time both alate and apterous aphids were observed (Fig. 1). Peak apterous aphid populations (≈325 aphids per plant) were observed on 5 September (R6, average plant growth stage), ≈2 wk after alate populations peaked (≈7 aphids per plant in 20 August).

The relationship between SCN egg density (measured at planting) and soybean aphid density was examined relative to alate and CAD apterous abundance at each date over the course of the season (Fig. 2). Soybean aphid alates were significantly negatively correlated ($r = -0.363, P < 0.01$) with SCN egg density at the onset of peak aphid colonization of plots (31 July).

Soybean Aphid Growth Rate. Aphid populations naturally colonizing soybean plots showed no consistent differences in growth rates across the range of SCN egg density present in the soil ($r = -0.01, P = 0.56$; Fig. 3, open circles). Similarly, there was no relationship between aphid growth rates measured in mesh bags and SCN egg density ($r = 0.01, P = 0.16$; Fig. 3, closed circles).

Soybean Yields. Average soybean yield in this study was 2,622 ± 526 kg ha$^{-1}$, (mean ± SD, ≈39 bu/acre). Simple and multiple regressions found that only SCN egg abundance early in the season was negatively related to total soybean yield (Table 1). The relationship between aphid abundance and soybean yield parameters was significant between 31 July (R2–3) and 28 August (R6) (univariate or multiple regression, $P < 0.05$, only the analysis from 31 July shown for
simplicity; Table 1). There was no association between SCN and soybean aphid on number of plants per plot.

**Discussion**

As plants are subject to both aboveground and belowground herbivory, it has been hypothesized that herbivores feeding on different parts of the plant can indirectly influence each other via their effects on plant resource allocation or defense responses (Kaplan et al. 2008). In this study, we found mixed evidence of interactions between soybean aphid (an aboveground herbivore) and SCN (a belowground herbivore) jointly exploiting soybean in the field. The population growth of soybean aphid apterae (as measured in both open plots and experimental cages) was

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**Fig. 2.** Correlation (r) between SCN egg density (measured at planting) and CAD for apterae (closed circles) and average weekly abundance per plant for alatae aphids (open circles) measured at different times during the growing season. ***,** significant at α < 0.01 (unadjusted P value).

**Fig. 3.** Relationship between SCN density in soil (eggs per 100 cm³ of soil) and aphid population growth rate as measured from open plots (open circles), and mesh cages (closed circles).
not related to early-season SCN density in plots. At the densities of pests observed in this study, SCN had significant effects on soybean yield, whereas soybean aphid only influenced seed mass. The range and extent of yield loss was consistent with the yield performance of SCN-resistant cultivars in the same field during a previous study (MacGuidwin et al. 1995). However, there was a response of early-season colonizing adults to egg densities of SCN with aphids being less common in soybean plots heavily infested with nematodes.

The extent of interactions in the field between nematodes and aphids in soybean varies depending on the aphid morph. Aphid alates found in plots in early in the season are probably colonizing from other fields in the landscape because aphid populations were low or nonexistent within our experimental field before this time. These colonizing aphids seem to discriminate between plants with varying levels of nematode infestations below ground. This finding is consistent with laboratory preference assays (Hong et al. 2010) that found alate aphids preferentially colonize uninfected soybean plants at the expense of infected plants in a two-choice assay. After landing on soybean plants, however, there was no evidence that variation in nematode egg abundance in the soil affected performance of apterous aphids feeding on soybean leaves. This finding is also consistent with laboratory studies using potted soybean plants with belowground SCN infestations that in general found few differences in aphid population growth on SCN-infected compared with control (noninfested) plants (Hong et al. 2010). Various studies indicate that soybean can respond to a variety of external factors such as insects, pathogens, or climate changes (McDonald and Cahill 1999; Cheong et al. 2000; Wegulo et al. 2005; Diaz-Montano et al. 2006; Kelley et al. 2006; Ithal et al. 2007a,b; Mazarei et al. 2007; Li et al. 2008; Zhang et al. 2008). Root infection by SCN causes changes in biochemical (e.g., flavonoids) and morphological components in soybean (Kim et al. 1987, Ithal et al. 2007a, Jones et al. 2007).

Juvenile nematodes infect and begin to establish feeding sites in resistant soybean cultivars before host defense responses are expressed and some nematodes obtain sufficient nutrition to mature, so it is likely that the plants were expressing a variety of changes when aphids began to feed. Given the ability of soybean to respond to a diverse array of stressors, we expected soybean aphid preference and performance to be negatively impacted by the presence of root pathogens (Stout et al. 2006, Wurst and van der Putten 2007, Kaplan et al. 2009). The density of SCN eggs measured in this study was well within the range of natural infestations and areas of the field were considered very heavily infested. Decreases in soybean yield further show that soybean plants were physiologically impacted by the belowground parasitism suggesting a season-long effect of the SCN. Other areas of the field however had very light infestations (<1,000 eggs per 100 cm³ soil). Given that this experiment relied on natural infestations of SCN, there were no plots that had no SCN offering “unimpacted” soybean against which to examine aphid performance. Nevertheless, we hypothesize that given the wide range of nematode egg densities used in this experiment, the flat response of aphids to nematode infestation in both caged and open field experiments (Fig. 3) and that aphid population growth rate (0.10) was within the range of observed responses from a study of soybean aphid population growth rates measured across the Midwest (mean r = 0.13; range, 0.052–0.2610; Ragsdale et al. 2007), plant response to belowground feeding nematodes does not affect soybean aphids.

Allocation to seed production was significantly affected by both SCN and soybean aphids. At the densities of aphids observed in this study (average 7,000 CAD, 325 peak aphid density at R6 average plant growth stage), soybean aphid influenced soybean seed mass did not translate to decreases in total yield (Table 1), potentially due to increased seed production. Beckendorf et al. (2008) similarly found that soybean aphids decreased average seed mass, although at the densities they and others studied (Myers et al. 2005, Ragsdale et al. 2007) effects of soybean aphids on overall soybean yield are widespread. Ragsdale et al. (2007) reported 273 and 674 aphids per plant for economic threshold and economic injury level for soybean, respectively, at ≥R1–R5 plant growth stage. Densities observed in this study are below those generally observed to have an important effect on total yield. However, SCN infestations were

### Table 1. Simple (1 and 2) and multiple regression model (2 and 3) coefficients (b ± SE) and significance values (P) of soybean yield parameters as a function of peak soybean aphid apterae (CAD) and cyst nematode (SCN) abundance from a field experiment at Hancock Agricultural Experiment Station (Waushara Co., WI) in 2008

<table>
<thead>
<tr>
<th>Model/variable</th>
<th>Yield (kg ha⁻¹)</th>
<th>Seed mass (g 100 seed⁻¹)</th>
<th>Plant density (no. row⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R²</td>
<td>b ± SE</td>
<td>P</td>
</tr>
<tr>
<td>1. Intercept</td>
<td>0.09</td>
<td>3.75 ± 0.14</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SCN eggs</td>
<td>-0.09 ± 0.04</td>
<td>0.02</td>
<td>0.19</td>
</tr>
<tr>
<td>2. Intercept</td>
<td>-0.01</td>
<td>3.57 ± 0.23</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Aphid apterae*</td>
<td>0.09</td>
<td>3.95 ± 0.26</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>3. Intercept</td>
<td>-0.08 ± 0.09</td>
<td>0.375</td>
<td>-0.08 ± 0.02</td>
</tr>
</tbody>
</table>

Data were log₁₀(x) transformed before analysis.

* Soybean aphid abundance measured on July 31 (plants at R2 stage) was used for the analysis.
significantly associated with yield declines, probably due to decreases in total seed production. Because of the generally low aphid densities in the year of this study it was not possible to quantitatively determine whether co-infestations of aphids and nematodes had interactive effects on soybean yield. Because apterae abundance was uncorrelated to nematode abundance, and soybean aphid effects on total soybean yield were not significant, the largest effects on yield in this study were primarily attributable to nematodes. In field studies, SCN resistant cultivars produced ~28% more yield average than susceptible cultivars (Chen et al. 2001) suggesting that nematode damage is important in influencing soybean yield. Soybean responses to nematode infection suggest that reduced allocation to reproductive structures occurs, whereas allocation to growth and defense may not be as impacted (Wang et al. 2003). Compensatory growth by soybean may in part explain why aphid feeding on leaves and phloem sap may not be sensitive to nematode feeding.

In summary, alate aphids were sensitive to variation in belowground infestations of SCN and were found less often on plants in soil with higher nematode egg populations. However, these initial alighting patterns did not translate to differences in overall aphid abundance differences nor did aphid population growth rates vary as a function of nematode abundance. This lack of response by aboveground herbivores given belowground stress by nematodes on their shared host plant comes with mounting evidence of indirect interactions between herbivore species by way of induced plant responses (Kaplan et al. 2009). At the relatively low aphid densities present in the year of this study, there was no evidence that aphids and nematodes interacted to affect soybean yield: aphids and nematodes influenced different yield components with only nematodes abundant enough to affect total yield. Moreover, studies of caterpillar-\textit{Heterodera} interactions also have shown the possibility of aboveground to belowground effects (Alston et al. 1993) and additive effects on soybean yield. Thus it is possible that under conditions of high aphid pressure, when aphids are known to have impacts on yield through decreases in seed production, the joint effects of aphids and nematodes may be realized. Additional studies that directly manipulate nematode abundance or use larger field plots could give additional perspectives on aphid–nematode interactions.

Acknowledgments

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