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FUNGAL PATHOGENS INFECTING SOYBEAN APHID AND APHIDS ON OTHER CROPS GROWN IN SOYBEAN PRODUCTION AREAS OF MICHIGAN

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ABSTRACT

Seasonal prevalence of fungal pathogens infecting soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), was assessed from 2004 to 2006 in two Michigan soybean production areas. In 2005 and 2006 field-collected soybean aphids were incubated, and fungal infection was detected at both sites early in August 2005 during soybean pod development and high soybean aphid densities. Significantly higher proportions of winged aphid morphs were infected (20 and 90% infection at the two sites) than wingless aphid morphs (1 and 3% infection). All cases of mycosis examined involved one pathogen species, *Pandora neoaphidis* (Remaudiére & Hennebert) Humber (Entomophthorales: Entomophthoraceae). In 2004 and 2005, we surveyed for pathogens of the soybean aphid in soybean as well as pathogens in other aphid species feeding on other crop plants (alfalfa, clover, corn, and wheat) by inspecting for sporulating aphid cadavers every 2 to 3 wk during the soybean growing season. Aphid cadavers were most abundant in alfalfa, especially in August; were less common in clover, corn, and soybean; and were not found in wheat. *Pandora neoaphidis* was associated with cadavers of *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae) in alfalfa and clover during the same period when soybean aphid infection was detected. Overall, mortality of soybean aphid and other aphid species due to fungal infection was confirmed in Michigan. The results also implicate infected winged soybean aphid morphs as potential agents for fungal dispersal, and *A. pisum* in alfalfa and clover as a source of fungal propagules for soybean aphid.

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Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a pest of soybean from Asia, recently established in the soybean production regions of North America (Ragsdale et al. 2004). Heavy infestations are associated with soybean yield loss (Ragsdale et al. 2007). Insecticide use, which was previously uncommon in soybean, became widespread for control of this aphid (Myers et al. 2005). In Michigan, outbreaks of soybean aphid were reported in 2000, 2001, 2003, and 2005 (DiFonzo and Hines 2002, T. N., pers. obs.).

Aphid pathogens, especially certain entomophthoralean fungi, may play a role in soybean aphid suppression (Wu et al. 2004, Nielsen and Hajek 2005). In China, *Neozygites* (=*Entomophthora*) *fresenii* (Nowakowski) Batko (Entomophthorales: Entomophthoraceae) is one of the most common pathogens infecting soybean aphid, and its prevalence was positively correlated with humidity and aphid density (Wu et al. 2004). In North America, fungal disease was found in up to 84% of aphids sampled in New York State in 2003 and 2004 (Nielsen and Hajek 2005), and 3 to 70% of aphids sampled in Minnesota between 2002 and 2006 (K. Koch, personal communication). *Pandora neoaphidis* (Remaudiére & Hennebert) Humber (Entomophthorales: Entomophthoraceae) was the most abundant pathogen detected in both studies.

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Obligate aphid pathogenic fungi from the order Entomophthorales are widely distributed across temperate regions and infect a range of aphid species inhabiting various vegetation (Latgé and Papierok 1988, Nielsen 2002, Shah et al. 2004). Natural epizootics of aphid diseases often occur under favorable biotic and abiotic conditions (Latgé and Papierok 1988, Shah et al. 2004, Zhang et al. 2006). Many crop plants besides soybean that are cultivated in Michigan's soybean production areas harbor aphids, including corn and wheat that are often rotated with soybean, and occasionally alfalfa (Blackman and Eastop 2000, USDA-NASS 2007).

In this study in Michigan, we quantified seasonal prevalence of fungal pathogens infecting soybean aphid, which allowed for regional comparisons with earlier studies in New York (Nielsen and Hajek 2005) and Minnesota (K. Koch, personal communication). In addition, we quantified abundance of sporulating aphid cadavers on other crop plants common in the soybean production region of lower Michigan, allowing for consideration of potential sources of inoculum near soybean fields.

MATERIALS AND METHODS

Study sites. Sampling of aphids and their pathogens were conducted at two Michigan locations: Kellogg Biological Station, Long-Term Ecological Research site, Hickory Corners (42°24'N, 85°23'W) and Entomology Farm, East Lansing (42°41'N, 84°29'W). Sites were managed by Michigan State University and were separated by 80 km. Soybean was sampled for soybean aphid at the Hickory Corners site in 2004 and at both sites in 2005 and 2006. Alfalfa, corn, wheat, and clover were also sampled for other aphids at the Hickory Corners site in 2004 and 2005. All crops sampled at both sites were cultivated under (rain-fed) condition without irrigation. We sampled four replicated plots of each crop through the soybean growing season in Michigan (June through September). The size of different crop plots at Hickory Corners were either 9 by 27 m (soybean [2004, 2005], wheat [2004, 2005], corn [2004, 2005], and clover [2004]) or 1 ha (soybean [2006] and alfalfa [2004, 2005]). All plots were set in the same experimental area devoted to crop rotational experiments, replications were randomized, and no insecticide or fungicide was used. The plot sizes of soybean at the Entomology Farm were 17 × 33 m.

Soybean aphid density. Soybean aphid populations were sampled four to eight times during the soybean growing season from 2004 to 2006 at each site. Soybean growth stages were noted using the scale of Fehr and Caviness (1977). The sampling periods were spread across a) mid-June, during early vegetative growth (V2-V3) when aphids may first migrate to soybean; b) mid-July, during flowering (R1-R2) when aphids may be multiplying on soybean; c) early August, during soybean pod fill (R3-R5) when aphids may be reaching peak densities; and d) late August, during plant senescence (R6) when aphids may be declining in density. Field aphid density was estimated by visually inspecting a random sample of 25 plants per plot (a total of 100 plants per site). On each plant, up to 50 aphids were counted, after which the number of aphids was estimated using a series of count ranges: 51-100, 101-500, 501-1,000, and 1,001-5,000. The high-end range was based on our field observations and past studies (DiFonzo and Hines 2002) that indicated aphid densities varied widely once populations surpassed several hundreds per plant but did not exceed 5,000 per plant during this period.

Latent fungal infections in field-collected soybean aphid. In 2005 and 2006, prevalence of aphid pathogenic fungi infecting soybean aphid at each site on each sampling date was estimated by incubating field-collected live aphids in the laboratory. Aphid-infested leaves were collected from at least 20 soybean plants, taken randomly across the four plots. The aphids were kept cool while being transported to the laboratory. Up to 100 aphids were selected for incubation

from the entire leaf collection representing each site. The selection consisted of all individuals of the winged morph (alate adults), which were always found in small numbers or absent, and all or a portion of the wingless morphs (apterous adults and nymphs that were third instar or older). The selected aphids were dislodged from the leaves and incubated on fresh soybean leaflets encaged in 1-ounce plastic portion cups lined with 2% water agar (Nielsen and Hajek 2005). Aphids, in groups of five individuals per cage, were incubated at 21°C with a photoperiod of 16:8 (L:D) h. Because different rates of fungal infection between aphid morphs have been noted (Nielsen and Hajek 2005), winged morphs were monitored for infection separately from wingless morphs. Aphid mortality was checked daily for 4 d, and aphid cadavers were removed from the cages and positioned in a 5-mm gap between two microscope slides (Nielsen and Hajek 2005). The slides were kept under high humidity at 15°C overnight to promote sporulation of fungal pathogens from the aphid cadavers. Discharged conidia that adhered to the slides were mounted in a drop of 90% lactic acid, and the morphology of conidia was examined under the compound light microscope for identification of aphid pathogens (Balazy 1993, Humber 1997, Keller 1991).

Mean aphid density per plant was calculated for each sampling date and site using the midpoint of the count ranges and soybean plots as replicates (n = 4). Prevalence of fungal infection was estimated for each sampling date and study site as a percentage of aphids from which sporulation by aphid pathogens was observed. The calculation was carried out separately for winged and wingless aphid morphs using aphid collections across the entire study site. Aphids collected from different plots were pooled together to estimate infection rates because of patchy aphid distribution in the soybean field and scarce collection of winged aphids. The chi-square test for independence (Gomez and Gomez 1984) was used to compare percent infection between the two aphid morphs.

Active fungal infection of aphids in soybean and other crops. At the Hickory Corners site in 2004 and 2005, soybean, alfalfa, corn, wheat, and clover were sampled for aphids and sporulating aphid cadavers. Soybean, alfalfa, and corn were sampled from mid-June through early September. Wheat was sampled from mid-June through mid-July until harvest, and clover, which was an under-story crop in wheat plots, was sampled from late July through early September 2004. Clover was not available in 2005 due to frost kill in the spring. For each crop, a random sample of 25 plants was inspected per plot (a total of 100 plants for each crop type per sampling date). The entire plant above the ground was inspected, and counts of aphids and aphid cadavers were recorded for each aphid species. In 2005 (but not in 2004), aphid cadavers found were brought back to the laboratory for identification of any pathogen involved. Fungal sporulation from aphid cadavers was promoted under high humidity, and aphid pathogens were identified using the same methods used for fungal pathogens of soybean aphid (Nielsen and Hajek 2005). Two sampling methods were used to quantify infection in soybean aphid (rearing pathogens from field-collected aphids and cadaver inspection) to maximize ability to detect infection. Infection in all other aphid species was sampled using cadaver inspection to check for similarity of infection patterns in other crop-infesting aphids common to soybean production areas.

Mean densities of aphids and aphid cadavers per plant were calculated for each crop type, sampling date, and aphid species using plots as replicates (n = 4). Numbers of aphid cadavers were compared among crops by year of sampling, using analysis of variance (PROC GLM, SAS Institute 2004). The independent variables included in the model were crop type, sampling date, plot, and the interaction between crop type and sampling date. Crop type was considered as a fixed variable, and sampling date and plot were considered random variables. The cadaver counts (number of aphid cadavers per plant [X]) were transformed into a logarithmic scale ($\log_{10}$ [100X + 1/6]) to satisfy the assumption of normality for analysis of variance

(Mosteller and Tukey 1977). When the crop type or sampling date effect was significant, Tukey's multiple comparisons test ($\alpha = 0.05$) was used to separate means of cadaver abundance.

RESULTS AND DISCUSSION

Latent fungal infections in field-collected soybean aphid. Soybean aphid occurred at relatively high densities in 2005 (Fig. 1) and relatively low densities in 2006 (data not presented graphically, peak aphid densities per plant were 3.6 in Hickory Corners and 0.5 in East Lansing) at both soybean sites. Fungal pathogens infecting soybean aphid were detected on 1 August 2005 at both study sites during soybean pod development (Fig. 1). All cases of infection detected involved one pathogen, *P. neoaphidis*, which was also the dominant pathogen of soybean aphid detected in New York and Minnesota (Nielsen and Hajek 2005; K. Koch, personal communication). Additional aphid pathogens were detected in the New York study, which was likely due to higher sampling intensity, but *P. neoaphidis* was by far the dominant species. Our findings were also similar to those of Nielsen and Hajek (2005) in that the fungal infection was associated with high aphid densities late in the growing season (Fig. 1). In 2006 when aphid densities were very low at both study sites throughout the season, no infection was detected.

When fungal infection was detected on 1 August 2005, significantly greater proportions of alate adults were infected than the wingless morphs at both study sites (N = 100 aphids per site): 20% alate adults and 1% wingless morphs were infected at the Hickory Corners site ($\chi^2 = 67.40$, df = 1, $P < 0.01$; Fig. 1a), and 90% alate adults and 3% wingless morphs were infected at the East Lansing site ($\chi^2 = 203.13$, df = 1, $P < 0.01$; Fig. 1b). We acknowledge that sample sizes were limited for quantifying infection rates among the alate adults compared with wingless aphids. The uneven sample sizes between the two aphid morphs reflected the fact that alate adults were always rare relative to wingless morphs during this study (Fig. 1). Regardless of the uneven sample sizes, our results (significantly higher proportions of alate adults were infected compared with wingless aphids) were consistent at both of our study sites and with the study in New York (Nielsen and Hajek 2005). These results suggest that movement of winged aphids infected by *P. neoaphidis* may play a role in the onset of disease (Zhang et al. 2006).

Active fungal infection of aphids in soybean and other crops. At the Hickory Corners site, sporulating aphid cadavers were found in soybean, alfalfa, clover, corn, but not wheat during the soybean growing season (Fig. 2c,d) (only dates when cadavers were detected are shown; other sampling dates were 14 June and 15 July in 2004 and 14 June, 12 July, 25 July, and 31 August in 2005). Of six aphid species found infesting these five crops during the two year study (Fig. 2a,b), fungal sporulation was most often observed on cadavers of *Acyrthosiphon pisum* (Harris) (69 cadavers, 57 on alfalfa and 12 on clover), followed by cadavers of *Therioaphis trifolii* (Monell) (19 cadavers, 18 on alfalfa and one on clover), *Rhopalosiphum maidis* (Fitch) on corn (7 cadavers), *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae) on corn (1 cadaver), and soybean aphid (1 cadaver). (Fig. 2 c,d). Two aphids, *Rhopalosiphum padi* (L.) on corn and wheat, and *Sitobion avenae* (Fabricius) (Hemiptera: Aphididae) on wheat, were detected but fungus-killed individuals were never found. The interaction between crop type and sampling date was significant in 2004 ($F = 8.47$; df = 13, 66; $P < 0.0001$) and 2005 ($F = 6.09$; df = 10, 54; $P < 0.0001$). In 2004, the numbers of cadavers found on alfalfa were greater than on clover and corn on 16 August, but there were no differences for other dates (Fig. 2c). In 2005, cadavers were only found on alfalfa on 27 June and 15 August (Fig. 2d). There were differences in plot sizes among crops (i.e., large plots for alfalfa and smaller plots for other crops), which could possibly contribute to differences in cadaver densities among crops. But greater numbers of aphid cadavers often
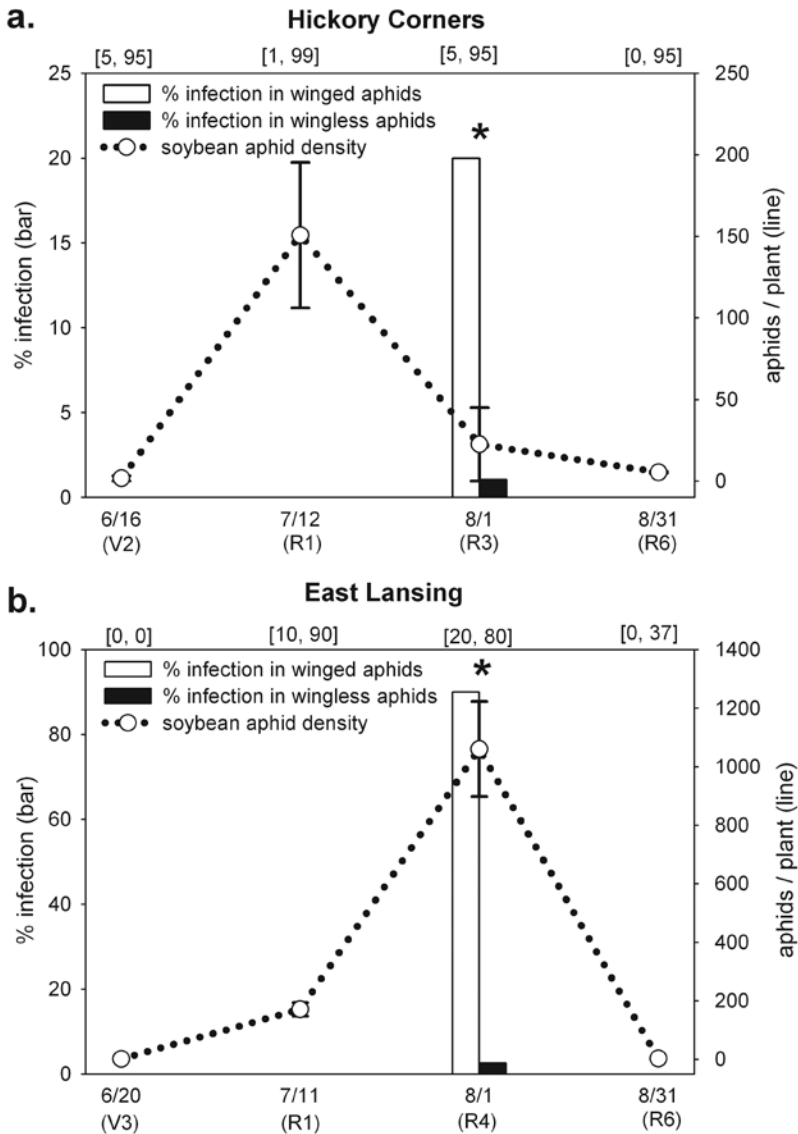
Figure 1. Mean soybean aphid densities (±SE) (lines) and percent infection found in winged (white bars) and wingless (black bars) aphid morphs monitored at two Michigan soybean sites in (a) Hickory Corners and (b) East Lansing during 2005. Percent infection was estimated on four dates. In 2006 (not shown), aphid densities were very low at both study sites, and no infection was detected. Percent infection was measured by incubating field-collected aphids (numbers of winged and wingless aphids, respectively, assessed are listed in brackets on top of graph). An asterisk over bars within a date indicates a significant difference in percent infection between winged and wingless aphids (χ^2 test, $\alpha = 0.05$). Codes below the dates are soybean growth stages (Fehr and Caviness 1977). V, vegetative stages; R, reproductive stages.
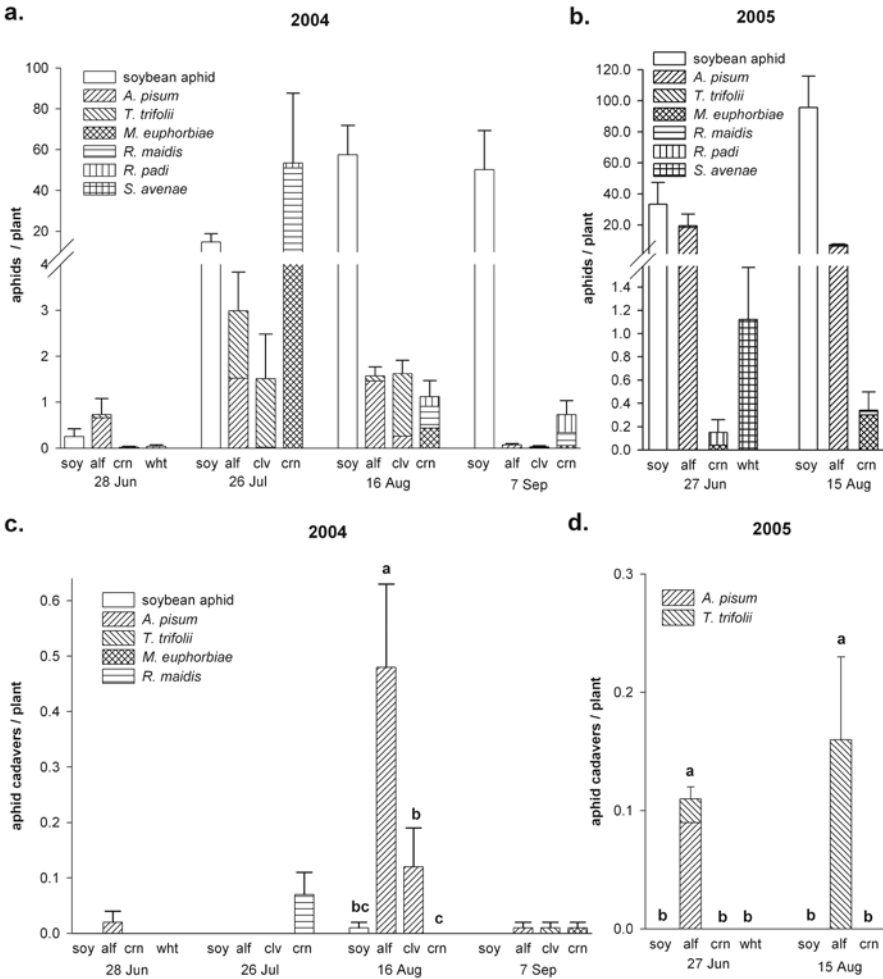
Figure 2. Abundance of aphids (a, b) and sporulating aphid cadavers (c, d) among soybean, alfalfa, clover, corn, and wheat monitored in Hickory Corners, Michigan, in 2004 and 2005. Sampling dates on which cadavers were found on at least one crop are shown. Patterns of bar graphs indicate species composition of aphids (a, b) and aphid cadavers (c, d) in soybean (soy), alfalfa (alf), clover (clv), corn (crn), and wheat (wht). Different letters within a date in (c) and (d) indicate significant differences in cadaver abundance by Tukey's test ($\alpha = 0.05$).

found in alfalfa appeared to be attributed to higher disease incidences associated with aphid species found in alfalfa (*A. pisum* and *T. trifolii*) and not to the larger plot sizes of alfalfa.

In 2005 when disease was identified from aphid cadavers, *P. neoaphidis* was associated with *A. pisum* on alfalfa, and *Zoophthora* sp. was associated with *T. trifolii* on alfalfa. Shah et al. (2004) found that *A. pisum* was highly susceptible to *P. neoaphidis*, and soybean aphid is also a suitable host of *P. neoaphidis* based on observations in this and other studies (Nielsen and Hajek 2005; K. Koch, personal communication). *Zoophthora occidentalis* (Thaxter) Batko (Entomophthorales: Entomophthoraceae) was infrequently detected from soybean aphid in New York (Nielsen and Hajek 2005) but was not observed in Minnesota (K. Koch, personal communication) or during the present study.

A few cadavers of *R. maidis* and *M. euphorbiae* were found on corn in 2004 (associated pathogens not identified) (Fig. 2c). In Idaho, *P. neoaphidis* and *Conidiobolus* spp. have been reported from these aphids (Feng et al. 1990). We did not detect diseased aphids on wheat. Although two aphid species (*R. padi* and *S. avenae*) were found infesting wheat, aphid densities were low (≤ 1.1 aphids per plant) from mid-June to mid-July when plants were mature and drying. Relatively low susceptibility to fungal infection has been previously reported for *R. padi* to *P. neoaphidis* (Shah et al. 2004).

There was a consistent under-reporting of fungal incidence by counting mycotized cadavers encountered in the field versus by the collection of living aphids that were incubated in the laboratory to allow development of any pathogens they carried. For instance, at the Hickory Corners site, infection was detected only by incubating field-collected aphids (Fig. 1a) and not by inspecting aphid cadavers (Fig. 2d). Similarly, Nielsen and Hajek (2005) observed consistently higher infection rates by incubating live aphids than collecting cadavers. In other systems involving different aphid species and crops, relative sensitivities of these sampling techniques varied widely (Nielsen and Hajek 2005). It seems prudent to utilize both methods when first assessing fungal pathogen infections of insect pests.

In conclusion, we observed fungal infection in soybean aphid populations (Fig. 1) with *P. neoaphidis* to be the most dominant aphid pathogen, but soybean aphid cadavers were rarely seen (Fig. 2c). Infections (as measured by percent infection of field-collected and laboratory-incubated aphids) were associated with high aphid densities late in the soybean growing season, and were primarily detected in the winged aphid morph. The same pathogen was the dominant species in other studies reported from the midwestern and eastern US (Nielsen and Hajek 2005; K. Koch, personal communication). The seasonal patterns observed in this and other studies implicate that fungi have the potential to disrupt annual life cycles of soybean aphid by infecting late season aphid populations, especially migratory populations. On the other hand, fungi may have limited potential for within-season control of soybean aphid. In our study, mycoses were also detected in aphids present on other crops common in Michigan soybean production regions. *Pandora neoaphidis* was associated with cadavers of *A. pisum* in alfalfa and clover during the same period when soybean aphid infection was detected. Because aphid-pathogenic fungi can infect a range of aphid species on different plants (Latgé and Papierok 1988, Nielsen 2002, Shah et al. 2004), and dispersal of infective conidia across a variety of habitats is the common pathway through which insect pathogens reach their hosts (Tanada and Kaya 1993), *A. pisum* on alfalfa and clover may be an important source for fungal propagules that infect soybean aphid. Overall, mortality of soybean aphid and other aphid species due to fungal infection was confirmed in Michigan. The results also implicate infected winged aphid morphs as potential agents for fungal dispersal, and *A. pisum* in alfalfa and clover as a source of fungal propagules for soybean aphid.

ACKNOWLEDGMENTS

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