Density-dependent responses of soybean aphid (*Aphis glycines* Matsumura) populations to generalist predators in mid to late season soybean fields

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Received 30 March 2007; accepted 12 June 2007
Available online 21 June 2007

Abstract

Since the arrival of the soybean aphid, *Aphis glycines*, to the U.S. in 2000, year to year abundances have varied substantially. Although, field studies have suggested that several resident generalist predators have the ability to suppress *A. glycines* populations, factors responsible for its population dynamics remain unclear. For natural enemies to regulate herbivore populations, predation rates must increase as prey population densities increase (i.e., density dependence). Experiments were conducted in 2004–2006 to assess the ability of the existing natural enemy community to exert density-dependent effects on *A. glycines* populations. Aphid densities were manipulated on individual plants (transplanted to the field) or in small plots to contain a range of aphid densities from low (10 per plant) to high (>1000 per plant). Populations were then monitored weekly. Caged controls were included to measure population growth in the absence of predators. In 2006, an additional treatment was included to quantify the proportion of aphids emigrating from plants. In all experiments, a strong density-dependent decline in population abundance was observed as aphid populations on plants with low initial densities (<50 aphids per plant) tended to increase while plants with high initial densities (>100 aphids per plant) decreased by as much as two orders of magnitude over a 1–2 week period. We estimate that fewer than 5% of aphids emigrated from plants when aphid densities were below 4000 per plant. This suggests that the extreme declines in aphid populations over these brief experiments were not due to aphids leaving plants, but rather, due to an aggregative response of predators to high density prey populations. Given the rapidity and degree of density-dependent population decline in three consecutive years of study, we suggest the resident community of natural enemies in Midwestern soybean may have great potential to regulate soybean aphid populations.

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Keywords: Generalist natural enemies; *Aphis glycines*; Emigration; Mortality; Soybean; Predators

1. Introduction

Natural enemies can regulate herbivore populations when mortality is affected in a spatially or temporally density-dependent manner. Traditional models of biological control have suggested that generalist predators are not well-suited to successfully control pests (i.e., achieve lower stable equilibria in pest populations) because their population dynamics are not synchronous to the pest and they often lack the ability to track rapidly increasing pest populations (Murdoch et al., 1985). However, others have suggested generalists, because of their polyphagous habit, are ideally suited to regulating pest populations, particularly for those with potential for high population growth rates (Murdoch et al., 1985, 2006). Because generalists can feed on a variety of prey, some of which have populations that do not spatially or temporally overlap with that of a target pest within a crop, they can move between available resources, thereby persisting regardless of the target prey’s abundance (Symondson et al., 2002). Thus, aggregation to patches of high densities of a prey species can be a feature
of generalist predators that make them capable of suppressing pest populations.

Research in biological control and other fields of ecology is increasingly recognizing the movement of organisms within or among habitats as being critically important for interactions within ecological communities and agricultural systems. In fact, the ability of a natural enemy to aggregate to areas of high prey density (behavioral/aggregative response) can be as important as its numerical response (i.e., reproduction and survival), and may even compensate for inverse density dependence typical of a type 2 functional response common in many predators (i.e., lower proportion of prey consumed as prey density increases) (Schellhorn and Andow, 2005). In this study, we examined how soybean aphid (Aphis glycines Matsumura) population growth may be affected by predation by generalist natural enemies as a function of aphid density.

Prior to 2000, before A. glycine, an exotic pest of soybean (Glycine max L.), was detected in the U.S., soybean was relatively free of serious pests in the upper Midwest. Likewise, because soybean lacked major pests, the crop did not support diverse or abundant natural enemies (Kogan and Turnipseed, 1987). Upon arrival from Asia, populations of A. glycines exploded and spread quickly throughout much of the region, causing serious economic damage (Ragsdale et al., 2004). While its distribution continued to expand in subsequent years and now corresponds to all major soybean growing areas in the U.S. and Canada, populations have been variable with widespread damage (Ragsdale et al., 2004). Population growth may be affected by predation by generalist natural enemies as a function of aphid density.

The natural enemy complex of soybean is dominated by generalist predators, which can exert strong top-down effects on A. glycines populations (Fox et al., 2005; Costamagna and Landis, 2006; Desneux et al., 2006). The most important predators in this system, coccinellids including the exotic Harmonia axyridis Pallas, and the anthocorid, Orius insidiosus (Say) (Costamagna and Landis, 2006), have been shown to suppress populations of A. glycines in soybean even when overall field densities were low (Fox et al., 2005; Desneux et al., 2006) and these effects were more important than how crops were managed (e.g., no-till vs. conventional, Costamagna and Landis, 2006). Yet, how natural enemies respond to variation in aphid densities, a necessary condition for population regulation (Colfer and Rosenheim, 2001) has not been demonstrated. In this study, we examined the density-dependent responses of A. glycines populations to the exclusion of natural enemies.

Because natural enemies in this system are predominately highly mobile generalist predators, we predicted that aphid population growth rate would decrease as density increased due to the ability of natural enemies to aggregate to high aphid density. In addition, studies were conducted to distinguish the effects of predators from other factors (e.g., intraspecific competition, emigration) that could also cause density-dependent reductions in aphids.

2. Materials and methods

2.1. Aphids and establishment of density treatments

We conducted five experiments at the University of Wisconsin Agricultural Research Stations (ARS) at Arlington (August 2004, July and August 2005) and West Madison (July 2006) to examine how aphid density influences the importance of predation on aphid population growth. The general approach was to establish a range of soybean aphid densities, from low (<10 per plant) to high (>1000 per plant) either (1) on individually potted plants subsequently transplanted to the field, or (2) in 2 × 2 m field plots. Aphid populations were then measured to determine growth as a function of initial population density. Bagged plants served as predator exclusion controls whereby we could infer the effects of natural enemies on aphid mortality.

A greenhouse A. glycines colony was maintained for the duration of the study. Aphids originated from field collected populations at West Madison and Arlington ARS in South-central Wisconsin and were replenished each year by adding additional aphids collected from the field. Experimental, potted soybean plants were infested with aphids either by transferring aphids by hand (low density treatments), or by allowing colonization from leaves excised from colony plants (high density treatments). Aphids were allowed to establish for several hours after transferring by hand, or for 1–3 days for high density treatments. Plants were then transported to the field and recounted prior to being transplanted.

Soybean (cv. Colfax) plants used in field experiments were grown in the greenhouse to the V2 growth stage (3rd expanded trifoliate) (Ritchie et al., 1994) in 4 in. pots with a 1:1 peat: Metro-Mix® growth medium (Scotts-Sierra Horticultural Products Company, Marysville, OH). We fertilized plants twice weekly by watering with a 4 ppm solution of 10:10:10 N:P:K fertilizer.


For experiments in 2004 and 2005, 10 replicate soybean plants were established in the laboratory at each of six densities (10, 25, 50, 100, 500, and 1000 aphids per plant).
Within 3–6 h of aphid densities being established (and confirmed), plants were brought to the field where they were transplanted into a large (>3 ha) soybean field. The field was planted in early May with agronomic varieties of RoundUp-Ready® soybean, in rows with 0.46 m spacing. Replicate blocks of treatments were arranged within existing rows, with >3 m separation between randomized experimental units. A small clearing was made around the transplants to prevent above-ground contact with neighboring plants. Caged controls were included in each study with 50 aphids per plant. Aphid populations on controls were protected from predators by covering the plant with a wire cage enclosed in a fine mesh sleeve, buried into the soil at the bottom and tied off at the top. Experiments began on 25 August in 2004, and on 8 July and 10 August, respectively, in 2005. The total number of aphids per plant was recounted after 11 days in 2004, and after 7 and 14 days in 2005. Natural enemies present on plants during aphid counts were counted and identified to at least the family level.

2.3. Small-plot density manipulation (2005)

A small-plot experiment was conducted in 2005 to determine if aphid population growth would be similarly affected when densities were manipulated at a plot scale compared with density manipulations at a plant scale. Steel-framed field cages (2 × 2 × 2 m, n = 24) covered with fine mesh nylon material were placed within a soybean field 3 weeks after planting (30 May, 2005) when emerging soybeans were in the early V0 (unifoliate) growth stage. Some aphid colonization had already occurred naturally by this time. Thus initial aphid densities were not randomly assigned to cages, but rather were the result of natural colonization. After 6 weeks (July 14), per plant aphid density inside cages ranged from <10 to 2500. On July 14, the mesh screens were removed from 18 cages, while screens were left on six cages (with low aphid densities) as a no-predator treatment. The average number of aphids per plant was calculated when cages were removed and 7 and 14 days after cages were removed, respectively, by counting the total number of aphids on five randomly selected plants per 2 × 2 m plot. Abundance of natural enemies was also determined on the same five plants.

2.4. Factorial density-dependent predation/emigration experiment (2006)

Density-dependent changes in aphid populations can be due to factors other than predators, such as changes in emigration or intraspecific competition (Dixon, 1998). In addition, high population growth rates measured in cages may be an artifact of emigrants (alates) returning to plants. To address these issues, a fully factorial 3 × 3 design was employed in 2006, with three aphid densities (10, 100, 1000 per plant), and three predator exclusion treatments. In addition to the un-caged and caged treatments used in prior experiments, a second cage design was employed that allowed aphids to emigrate from plants into collection cups filled with propylene glycol (Fig. 1). Wire tomato cages were inverted and staked into the ground over transplanted soybeans, and a mesh sleeve, sewn into a funnel shape was fit over the tomato cage. The bottom of the sleeve was buried, while the top of the sleeve was threaded through and then folded down around a 10 cm long × 10 cm diameter section of PVC pipe. The PVC pipe was supported by the tomato cage and held in place with rubber bands. Emigration traps were constructed out of 2-l plastic bottles, cut off at the base, and a 100 ml plastic collection cup affixed to the threaded lip of the bottle. The collection trap was placed over the section of PVC pipe (and mesh sleeve) and compressed with large rubber bands to hold it in place (Fig. 1). Collection cups were then filled with propylene glycol to just below the rim of the bottle top. By constructing cages in this manner, aphids that walk or fly off of the plant to the sides of the cage migrate up the sleeve into the collection trap, finally ending up in the liquid preservative in the top collection chamber. The numbers of aphids caught in the collection device serves as an index of aphid emigration from plants as a function of density.

Experimental plants were established as described above (Single-plant density manipulations). Fields were established
early in May and soybean plants (V4–V5 growth stage) were transplanted on 14 July. The total number of aphids and natural enemies per plant were counted after 7 and 12 days as were the total number of altoid and apterous aphids in emigration cups and on the inside surfaces of the cage.

2.5. Analyses

Aphid population growth (or decline) was calculated as log(density at time t/initial density). For experiments conducted in 2004 and 2005, relationships between initial aphid density and population change were assessed using linear regression. Initial density was log transformed prior to analyses. In 2006, the 3 × 3 factorial experiment was analyzed using two-way analysis of variance (ANOVA) with treatment (cages, open, emigration) and density (10, 100, 1000) and their interaction as factors. We found no significant effect of block (random effect), therefore block was not included in our final statistical analyses. Post hoc means comparisons for main effects were made using a Tukey’s test. The relationship between total number of aphids captured in emigration traps and on the side of the cage, a relative measure of emigration, and final aphid density were evaluated using linear regression. The number of alatae and apterae aphids captured in collection cups were tallied for each density treatment and percent emigration was calculated as a function of final aphid density (aphids counted on plant + aphids collected in the emigration cups). To assess how initial aphid density affected natural enemy abundance on plants or in plots, we used one-way ANOVA calculated separately for each sampling date of an experiment (day 7, 11, 12, or 14, depending on the experiment). All analyses were performed with JMP 5.01 (SAS Institute, 2003).

3. Results


Soybean aphid population dynamics were significantly linearly density dependent across a wide range of initial starting aphid densities in all of the five experiments conducted between 2004 and 2006. In 2004 and 2005, density-dependent effects on population growth were apparent on a per-plant scale after 11 and 14 days, respectively. By the end of each experiment, aphid colonies in the low density treatments (10–50 per plant) either remained constant or tended to increase approximately one order of magnitude, while plants containing initial densities over 100 per plant declined (Fig. 2). In July and August 2005, density-dependent effects were evident after only 7 days (r = −0.72, P < 0.001, respectively), and by day 14 (both July and August, 2005, Fig. 2B and C), populations that began at densities over 1000 aphids per plant had decreased by about two orders of magnitude, or from 1000 to 10 aphids. In each of the three experiments conducted at the plant scale (Fig. 2), caged aphid populations starting at 50 aphids per plant grew between 500% to almost 1000% during the same period of time.

3.2. Small-plot density manipulation (2005)

Experiments where aphid densities were manipulated at a plot scale (2 × 2 m) showed the same pattern of density dependence as observed with individual plants (Fig. 3).

Fig. 2. *Aphis glycines* population growth as a function of initial population density (Log scale). Each point indicates aphid population change on an individual soybean plant 11 days in August 2004 (A), and 14 days in July and August 2005 (B and C), respectively, after being transplanted into existing soybean fields. Points above “0” indicate population growth, whereas those below “0” indicate population decline. Shaded triangles indicate mean population growth for bagged plants (predators excluded) ±1 SEM.
When protected from potential effects of natural enemies, aphid population growth in 2×2 m field cages was variable, but increased by an average of almost 1.5 orders of magnitude by day 7 (10–398 ± 360 SEM), and almost 2 orders of magnitude by day 14 (Fig. 3A). In un-caged plots with low aphid abundances (<10 per plant), populations also grew, albeit less than in cages (Fig. 3B). However, aphid disappearance was dependent on initial density; a pattern that was evident by day 7 (r = −0.62, P < 0.001) and even more pronounced by day 14 (Fig. 3B). Aphid populations consistently declined when average initial aphid densities in 2×2 m plots were ≥100 per plant.

3.3. Factorial density-dependent predation/emigration experiment (2006)

A fully crossed 3×3 factorial experiment (3 densities, 3 cage designs) was used to determine the relative importance of emigration and predation in explaining population changes. Aphid populations showed strong density dependent declines (Fig. 4, main effect of density treatment: F2,49 = 41.02, P < 0.001). The main effect of cage was also highly significant (Fig. 4, F2,49 = 7.82, P = 0.001). After 12 days, un-caged plants that began the experiment with greater than 1000 aphids had fewer than 10 aphids, a 2 orders of magnitude decrease, while colonies with low initial density (10 aphids per plant) had positive growth rates. When protected from natural enemies within cages, aphid populations grew regardless of initial density (significant cage × density interaction; F4,49 = 17.02, P < 0.001). There was no difference in aphid population growth rate on plants covered in mesh cages compared with that measured in emigration cages (Tukey’s test: P > 0.05). For caged plants only, there was no difference in growth rates as a function of density (Tukey’s test: P > 0.05).

Emigration cages allowed us to measure the relative movement of aphids off of soybean plants as a function of aphid density. Both apterae and alatae emigrated from plants in all three density treatments, however apterae were significantly more abundant in collection cups (91% of 5968 total emigrants) than were alatae. Moreover, the percentage of alates emigrating did not differ between density treatments (F2,12 = 0.82, P = 0.46) and remained consistently low across all density treatments (mean = 3.9 ± 2%). The total numbers of emigrants (apterous and altoids) varied from 0 to over 2000 individuals in collection traps. Emigration rates (percent of final density) increased with increasing final aphid density (Fig. 5). This relationship was highly significant, driven mostly, however, by two plants with extremely high final aphid densities (6550 and 10,100, respectively) that exhibited emigration rates close to 20%. In fact, excluding the two plants above, emigration rates did not show an overall increase with aphid density (around 4% for densities <4000 per plant; r = 0.19, P = 0.55).

3.4. Natural enemies

The vast majority of natural enemies observed foraging on plants infested with A. glycines were generalist preda-
tors. The most abundant taxa we observed on plants and often feeding on aphids were larvae of predatory midges (Aphidoletes sp., Cecidomyiidae; 1484 total obs.), followed by larval coccinellids (472 obs.; including H. axyridis), O. insidiosus (198 obs.), lacewing larvae (45 obs.), adult coccinellids (25 obs.; mostly H. axyridis), and syrphid fly larvae (23 obs.). We observed a total of 10 parasitized aphid mummies out of over 200,000 aphids counted on experimental plants.

The total number of predators observed on soybean plants increased with increasing initial aphid density in each experiment (Fig. 6, P < 0.02 for the effect of treatment density on predator abundance for all experiments). After 14 days, however, aphid densities had declined on high density plants relative to low density plants (P > 0.10 for all experiments). By day 14 the only predators observed on plants with previously high densities of aphids were larvae of predatory midges (Aphidoletes sp.) and ladybeetles (H. axyridis), whose numbers were higher on plants with high initial aphid densities in two of four experiments (P < 0.05).

4. Discussion

Since 2000, when it was first discovered in the upper Midwest, A. glycines abundance has varied tremendously among years and much research has been conducted to determine the factors that influence the population dynamics of this important pest. In this set of field experiments conducted in mid-summer over a three year period, resident predators of A. glycines were capable of reducing high density aphid patches at both a single plant and a plot scale as inferred by comparing aphids on caged and open plants and visual observations of predators. The density-dependent effect of predation on aphid populations was clearly evident in as little as 1 week. By 2 weeks after small-scale outbreaks were established in the field, populations of aphids were reduced by as much as two orders of magnitude and the size of the effect was dependent on initial aphid densities. Moreover, our data strongly suggest that the dramatic reductions in aphid populations were not due to other factors such as density-dependent emigration from high density plants (Fig. 5) or intraspecific interactions (Fig. 4). Our results are consistent with other studies that suggest resident natural enemy communities are capable of significantly depressing populations of A. glycines (Fox et al., 2005; Nielsen and Hajek, 2005; Costamagna and Landis, 2006; Desneux et al., 2006).

Density-dependent effects of natural enemies may lead to population regulation of pest populations and can be a result of both functional (increased foraging efficiency) and numerical (behavioral and reproductive) responses of predators. Our data suggest that generalist predators respond numerically, in a behavioral (aggregative) manner, to high density aphid colonies. We found an overall average of five predators per plant, 66% of those predators being larval cecidomyiids (Aphidoletes sp.), and 21% being larval coccinellids. The high relative abundance of predators in general (Fig. 6), and of larval predators specifically, on plants with high aphid densities relative to plants with low initial aphid densities suggests a direct behavioral response (e.g., increased reproduction by aggregating adults) of predators to high aphid densities.
Despite their relatively high abundance on plants with the greatest aphid density at the end of the experiments, it is unlikely that larval cecidomyiids were directly responsible for the rapid decline of large aphid colonies observed in this study. Although, they were the most abundant predators observed on plants at the end of our experiments, this is likely a result of the lag between oviposition by female midges in high density aphid patches and larval emergence several days later. This time lag, coupled with the fact that cecidomyiids are small and generally have low aphid consumption rates (Sell, 1985), diminishes the likelihood that they played a dominant role in the large decreases in aphid numbers observed in this study. More likely, the 7 day sampling frequency used in these studies was too coarse for us to observe directly an aggregative response of more mobile predators such as adult *H. axyridis* or *O. insidiosus*, which are implicated as most important for soybean aphid control (Fox et al., 2004; Rutledge et al., 2004; Rutledge and O’neil, 2005; Costamagna and Landis, 2006; Desneux et al., 2006). Several studies have previously shown these predators to inflict significant aphid mortality in this system. For example, Desneux et al. (2006) found that *O. insidiosus* may be effective at regulating soybean aphid populations early in the season if aphids are distributed in a clumped manner, but not when aphids are dispersed. A study by (Fox et al., 2004), in which aphid populations were allowed to build within field cages, showed that natural enemies, predominately *H. axyridis* and *O. insidiosus*, quickly decreased populations to background levels upon removal of cages.

Studies in other systems examining the effects of predators on aphids suggest coccinellids can show behavioral responses to aggregations of prey and that these behavioral responses may lead to density-dependent regulation of prey populations. For example, in a study by Colfer and Rosenheim (2001), the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville inflicted density-dependent mortality on cotton aphids (*Aphis gossypii* Glover). Similar to the pattern we found in soybean, when predators were allowed access, aphid populations increased when initial densities were less than approx. 50 aphids per cage (3–7 seedlings per cage), and declined in a density-dependent manner when aphid densities were higher. Similarly, Schellhorn and Andow (2005) have demonstrated that four different species of coccinellids readily aggregate to high densities of corn leaf aphid, *Rhopalosiphum maidis* (Fitch), but at different spatial scales. Some species aggregate more readily to a single plant, while others respond on a 10 × 10 m plot scale. In this study, we found that density-dependent responses were comparable whether densities varied at a plot (2 × 2 m) or single plant scale.

It is important to note that spatial (behavioral) responses of natural enemies resulting in density-dependent population processes are more likely to occur at small than at large spatial scales (Harrison and Cappuccino, 1995). For example, the studies reported here were conducted when background aphid densities were generally low (in all 3 years). While natural enemies responded strongly to isolated patches of densely populated plants in these studies or even small plots, differences in aphid density among fields and larger scale density dependence may result in different qualitative patterns and may require different mechanisms such as numerical responses, in order to control pest populations. As a result, although small-scale density dependence as shown in this study may affect local outbreaks, it is unclear how these results scale to entire management units such as farmer’s fields.

Finally, other factors, such as emigration and intraspecific competition, also may affect aphid population dynamics in a density-dependent manner. For example, alate production increases with increasing population density both for *A. glycines* (Hodgson et al., 2005) and for aphids in general (Baugh and Phillips, 1991; Ebert and Cartwright, 1997; Dixon, 1998), a response that could explain some portion of the population declines on densely populated plants. However, data from the emigration experiment suggest that over the course of our experiments only a small fraction of aphids leave plants (<5%) as a result of density-dependent emigration. Higher rates of emigration occurred only when densities were >4000 aphids per plant suggesting that the decline in high density populations is unlikely due to emigration from crowded plants. In fact, even if we prevent emigrants from returning to plants (by way of collection chamber), aphid densities on cages are no different than those where potential emigrants have no option to escape the bag (i.e., normal cages). Another potential density-dependent effect on aphid populations may result from intraspecific competition, where aphid population growth is reduced because of reduction in resources (space or nutrients in plant phloem) because of crowding (Colfer and Rosenheim, 2001). The observation that aphid growth rates in cages (Caged and Emigration cases, Fig. 4) were comparable whether aphid populations started at 10, 100 or 1000 aphids per plant suggests that, at least across these ranges of densities, there are no obvious direct negative interactions between aphids. Finally, although other potential cage-related effects on aphid populations (e.g., temperature) were not monitored within field cages in this study, others have found that similar cages have only subtle effects on micro-environmental conditions (Fox et al., 2004). Nevertheless, because the design of this study does not control for all potential cage effects, we cannot exclude the possibility that some occurred. However, even if cages caused subtle differences in temperature or some other factor that affected aphid population growth rate, it is unlikely that these could account for differences in growth rates as dramatic as we observed here. In short, over the relatively short duration of these experiments (≤14 days) the rapid declines in aphid populations as a function of density is best explained by the action of natural enemies.

In summary, we suggest that density dependent decreases in soybean aphid populations is likely the result of predation by generalist natural enemies, and may play
an important role in regulating aphid populations at least at small spatial scales. Other factors are certainly important, and clearly, natural enemies are not always sufficient to suppress aphid populations. For example, it is worth exploring how bottom-up factors such as plant stress (e.g., drought), or environmental conditions, allow \textit{A. glycines} populations to escape natural enemy control in some years. Nevertheless, the rapidity with which the resident natural enemy community responded to isolated high density \textit{A. glycines} colonies is noteworthy, and suggests the need for further study of their potential for effective biological control in US soybean fields.

Acknowledgments

We thank the University of Wisconsin ARS field crews at Arlington and West Madison, and in particular Scott Chapman for maintaining the soybean fields in which these experiments were conducted. Also, the work was manageable in large part due to the efforts of undergraduate summer field crews (Anna Soper, Joe Kaiser, Michelle Becker, Aaron Carpiaux, Laura Dyer, Genya Erling, Laurie Gerber). This project was supported by the National Research Initiative and Risk-Assessment and Mitigation Programs of the USDA Cooperative State Research, Education and Extension Service (CSREES), Grants 2004-35302-14726 and 2004-51101-02210 to C. Grattan.

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