Lack of strong refuges allows top-down control of soybean aphid by generalist natural enemies

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ABSTRACT

Refuges have been shown to be important mediators of predator–prey interactions, and in particular, have been proposed as a potential mechanism allowing herbivore populations to reach outbreak levels. However, very little research on the role of refuges has been conducted in systems dominated by generalist predators. We investigated the existence of refuges from predation for the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae) at multiple scales. This species invaded North America and in spite of previous studies demonstrating strong suppression by generalist natural enemies, its populations periodically cause significant economic losses. Using naturally occurring populations of soybean aphid and its natural enemies, we tested for the presence of *A. glycines* spatial and dynamic refuges at the within-field, single plant, and within-plant scale. At the within-field level, we found only weak and transient spatial patterns in aphid populations suggesting the lack of spatial refuges at this scale. Similarly, at the plant level we found no individual colonies that escaped predation and aphid suppression was 9- to 28-fold greater in comparison with caged controls regardless of initial aphid density. When high aphid populations were exposed to predation they were rapidly reduced to levels close to the average field density and showed reduced per capita growth rates, indicating an absence of dilution of predation risk at increased aphid density. Finally, we found a significant shift in the distribution of aphids to the lower portions of the plant in the presence of generalist predators, suggesting a partial refuge from predation at the within-plant scale. Overall, we found the naturally occurring community of generalist predators to exert strong top-down suppression of soybean aphid populations at multiple scales, and no evidence that the presence of refuges at the scales studied can lead to outbreak populations. The partial refuge from predation at the within-plant scale revealed in our study may have important consequences for the within-season population dynamics of *A. glycines*, since it may be associated with low plant quality trade-offs, and therefore warrants further research.

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1. Introduction

The existence of refuges from predation has been postulated as an important factor mediating top-down herbivore regulation (Berdegue et al., 1996; Berryman and Hawkins, 2006; Hawkins et al., 1993). Several theoretical studies suggest that prey refuges serve as a stabilizing factor in predator–prey dynamics (Berryman and Hawkins, 2006; Hochberg and Holt, 1995; Murdoch et al., 2005). However, it has also been suggested that refuges can be sources for herbivore increase and dispersal that eventually may reduce top-down suppression and increase the chance of herbivore escape (Berryman and Hawkins, 2006; Bommarco et al., 2007; Hawkins et al., 1993). To date, most theoretical work has focused on the role of prey refuges from specialist predators (i.e.,

parasitoids), with systems dominated by generalist predators receiving far less attention (Murdoch et al., 2006). Nevertheless, the ability of generalist predators to survive with alternative resources has been suggested to result in more effective long term herbivore control when refuges are present (Chang and Kareiva, 1999). Moreover, the varied feeding habits and temporal occurrence of generalist predators are predicted to result in narrow prey refuges (Symondson et al., 2002). In addition, prey fitness in refuges may be lower, creating trade-offs that can result in varied predator–prey dynamics (Lima, 1998). Therefore, to understand the outcome of predator–prey interactions in systems dominated by generalist predators, the presence and role of prey refuges must be elucidated.

A variety of phenomena that mediate predator–prey interactions can result in refuges from predation, including physical shelter, competition for resources, predator functional responses, enemy-free space, and mimicry, among others (Berdegue et al., 1996; Berryman and Hawkins, 2006; Bommarco et al., 2007; Hawkins et al., 1993). Spatial refuges consist of habitats,

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microhabitats or structures in which mortality is diminished, and could be the result of physical shelter from predators (e.g., Finke and Denno, 2006), prey behaviors that reduce predation risks, such as predator avoidance (e.g., Schmitz, 1998), or different patterns of predator and parasitoid foraging (e.g., Hacker and Bertness, 1995; Hopkins and Dixon, 1997). In contrast, dynamic refuges are variable in time and result from changes in prey density, alternative prey density or invulnerable prey stages (Berryman and Hawkins, 2006). Clusters with high prey numbers are likely to be attractive to predators, and therefore more frequently located than low density patches (Ives et al., 1993). However, prey can still escape control if predator numerical (aggregative) and functional responses are counteracted by high reproduction rates that result in lower net per capita predation risks or "dilution" effects (Bommarco et al., 2007; Cappuccino, 1987, 1988; Kareiva and Odell, 1987; Turchin and Kareiva, 1989).

Aphids are considered relatively unprotected prey but have developed several strategies to persist under strong predation pressure (Hopkins and Dixon, 1997). Several types of refuges have been documented for aphids, including spatial refuges such as the upper surface of leaves (Hopkins and Dixon, 1997), plant structures (Gonzales et al., 2001), shorter plants (Hacker and Bertness, 1995), and dynamic refuges, such as small size instars (Lin and Ives, 2003), and numerical escapes (Cappuccino, 1987, 1988; Turchin and Kareiva, 1989).

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is an invasive pest of soybean in North America (Ragsdale et al., 2011). Outbreak populations of *A. glycines* have led to significant soybean yield reductions in about one half of the years since its first detection in the US in 2000 (Gardiner et al., 2009). Recent studies demonstrate that *A. glycines* is frequently suppressed by generalist natural enemies (Costamagna and Landis, 2006; Donaldson et al., 2007; Fox et al., 2004; Gardiner et al., 2009), although the causes of aphid escape during outbreak years remain poorly understood (Costamagna and Landis, 2006). We hypothesized that one contributing factor in the escape of *A. glycines* from natural enemy control may be the existence of refuges from predation. We tested this hypothesis at three spatial scales: within-field, single plant, and within-plant scale, using naturally increasing aphid populations. In addition, we tested for the presence of dynamic refuges assessing predator impacts at different aphid densities. At the within-field level, we indirectly assessed the existence of refuges by contrasting the spatial distribution of aphid patches (clusters of aphid density above the field average) and gaps (clusters of aphid density below the field average) over seven consecutive weeks. We hypothesized that persistent high density patches within a field indicate a lack of predator responses to aphid increase, suggesting the presence of spatial refuges. At the plant and within-plant scales, we used predator exclusion cages to directly assess the effects of predation on aphid population growth on plants naturally colonized at different aphid densities. We hypothesized that the presence of plants, plant parts, or high aphid densities that escape predation indicate the presence of spatial or dynamic refuges for soybean aphid.

2. Materials and methods

2.1. Field site and sampling of natural populations

Field studies were conducted in a conventional soybean field (50 × 100 m) located on the Michigan State University, Entomology Farm in East Lansing, Michigan, USA (N 42.690674W 84.496239) during 2003. Soybeans (Pioneer 92B38) were planted on 05/22/2003, and maintained with conventional agricultural techniques. We characterized the spatio-temporal dynamics of

soybean aphid by sampling weekly in a 5 × 10 grid of points equally spaced at 10 m intervals, starting at 5 m from the field border ($n = 49$ samples per week, one sample missing at the NW corner due to field irregularity, Fig. 1). Each sample consisted of destructively sampling 5–10 random plants within a 3 m radius of each grid point, and determining the average number of aphids per plant. We did not observe any aphid dropping behaviors that may affect our counts, and this technique has been successfully used to estimate *A. glycines* density in previous studies (i.e., McCornack et al., 2008). In addition, we conducted visual sampling of natural enemies by inspecting 3 samples of 1 linear meter of soybean during 3 min (Costamagna and Landis, 2006) in each grid sector ($n = 49$ samples per week, expressed as individuals per 3 linear meters). Sampling started on 06/27/2003, when 45.8% of the plants were naturally colonized by *A. glycines*.

2.2. Natural enemy manipulations

To test for the presence of refuges at the plant and within-plant scales, naturally occurring colonies of *A. glycines* were caged at different initial densities on 07/03/2003. Using *A. glycines* density obtained in the grid sampling we classified field patches into three categories of initial aphid density: low (<5 aphids/plant), medium (5–10 aphids/plant) and high (>10 aphids/plant). Within each category, 6 patches were randomly chosen to receive predator manipulation treatments consisting of, a predator exclusion cage, a sham cage, or an un-caged control. The exclusion cage consisted in a cylindrical framework of wire of 0.4 × 1.0 m, covered by a fine mesh white no-see-um netting (Kaplan Simon Co., Braintree, MA), buried 15 cm in the soil and tied at the top. The sham cage was identical except the mesh was held 5 cm above ground surface by four wooden stakes, and had eight rectangular openings (2 × 20 cm) arranged in 3 circular patterns alternating such that there were openings in all directions. These rings of openings were located from 10 to 30 cm above the soil, encompassing the height range of the canopy. Finally, the un-caged control ("open") consisted of three contiguous uncovered plants marked by wire flags. Each cage enclosed the focal plant containing a naturally occurring aphid colony within the range desired and two additional soybean plants initially free of aphids. Plants were at the V3–V4 stage (Ritchie et al., 1994) at the beginning of the experiment. After two weeks, the plants were reduced to one per cage (typically the central one), to allow un-crowded growth. One exclusion cage was eliminated from the analysis due to failure to exclude natural enemies.

The total number of aphids and their location within plants was assessed by direct counts on each plant node once a week. For this study we considered as part of the node, any aphids on the trifoliate arising from that node, any lateral growth arising from the same node (i.e., axillary trifoliates), and the portion of the main stem below the node of interest (McCornack et al., 2008). The first two leaves in soybean are unifoliates and since they arise from the first node, they were considered together as part of one node. To test predator impacts on the increased aphid populations observed within the exclusion cages, as well as to rule out potential local effects of the soybean plants on aphid growth, exclusion and sham cages were switched in a subset of the cages after two weeks (Fox et al., 2004; Turchin and Kareiva, 1989). On 07/17/2003 exclusion cages were matched in pairs according to aphid density, and one member of each of pair was randomly switched with its sham counterpart, whereas the other was left as a control, for a total of 6 plots switched and 6 control plots. The experiment was conducted for three additional weeks, but sampling of the control plots stopped at week 4 due to high numbers of aphids and the potential for alate retention as a factor artificially inflating aphid numbers in exclusion cages.
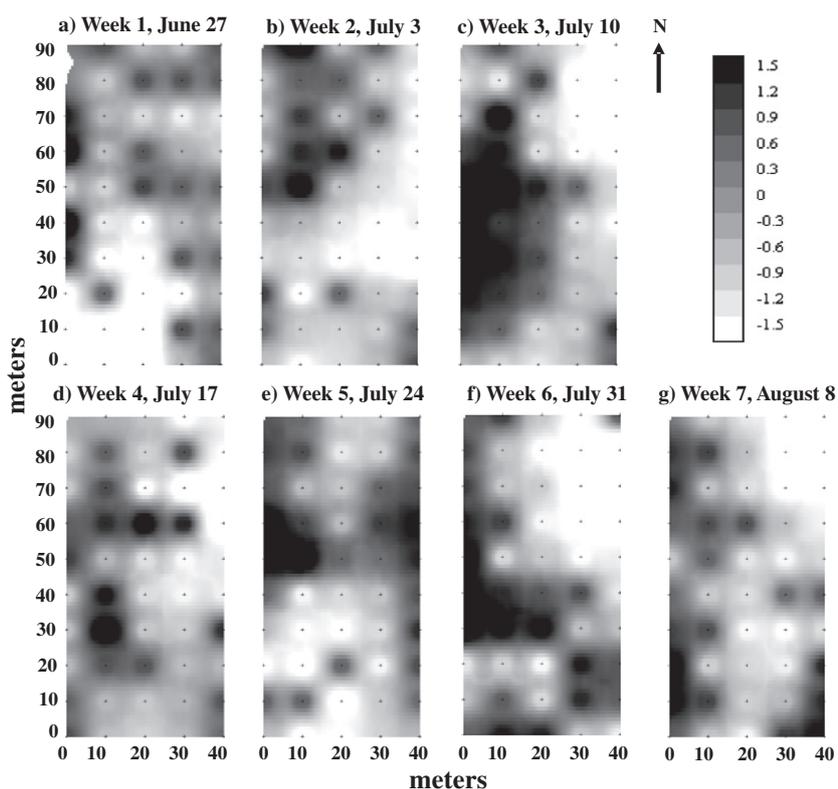
Fig. 1. Spatial distribution of *A. glycines* natural population in a soybean field in Michigan over seven consecutive weeks in 2003. Cluster values of 1.5 or higher indicate aphid aggregations (in black), whereas values equal or lower than −1.5 indicate gaps in aphid distribution (in white). The black dots represent the sampling coordinates of the grid sampling (*n* = 49 per date). Spatial statistics associated with these maps are presented in Table 1.

2.3. Statistical analysis

The within-field spatio-temporal aphid dynamics were analyzed using Spatial Analysis by Distance Indices (SADIE). This method was specifically developed for ecological data utilizing counts to characterize the spatial pattern of organisms, and has been frequently used to study the spatial pattern of aphid populations (Perry, 1998; Winder et al., 2001, 1999). To characterize the overall spatial pattern of aphids within the field, we used the Index of Aggregation (I_a), that tests spatial association among the counts obtained in the grid. At the 5% level of probability, an I_a significantly higher than 1 ($P_a < 0.025$), indicates spatial aggregation, and an I_a significantly lower than 1 ($P_a > 0.975$), indicates a regular distribution. Finally, if I_a does not differ significantly from 1, then organisms are randomly distributed within the grid of counts (Perry, 1998). An expansion of this analysis allows testing for the presence of aggregations over the field average (patches), versus clusters with significantly lower abundance than the field average (gaps). Thus, the clustering index V_i, specifically tests for the presence of significant patches of high abundance ($V_i > 1.5$, $P_i < 0.05$), while the gap index V_j, tests for the presence of significant gaps in abundance in the field ($V_j < -1.5$, $P_j < 0.05$). We summarized the results of these analyses using "red-blue" (cluster-gap) plots representing threshold cluster values of 1.5 and −1.5, respectively (Perry et al., 1999). The individual clustering indices obtained for each sample on the grid were used to test for spatial associations of aphids during consecutive weeks (Winder et al., 2001). To perform this test, an overall spatial association index X_p can be calculated allowing for small-scale spatial autocorrelations, and indicates positive associations if $X_p > 0$ ($P < 0.025$) or negative dissociations if $X_p < 0$ ($P > 0.975$) (Perry and Dixon, 2002). Using these methods we assessed the overall pattern of aphid distribu-

tion in the field, and tested for the presence of persistent patches or gaps across time, which would indicate the presence of persistent aphid aggregations and a consequent failure of predators to suppress these populations. Spatial analyses were conducted using the program SADIEShell (version 1.22), freely available at http://www.rothamsted.bbsrc.ac.uk/pie/sadie. Plots of clustering indexes were constructed using linear interpolation with the software QuikGrid (Coulthard, 2007, freely available at http://www.galiander.ca/quikgrid/).

To test for the presence of refuges at the plant scale, we analyzed two variables in the cage experiment, aphid abundance and per capita rate of increase. Per capita rate of increase (λ^T) was calculated as $\lambda^T = N_t/N_0$, where N_0 is initial aphid density (i.e., day 0 before cages were switched and day 14 after switching), and N_t is the density 14 days after the beginning of the manipulation (day 14 before cages were switched, day 28 after switching). If increased aphid density provides a dynamic refuge from predation by diluting predation risk, we expect to obtain higher λ^T at higher initial aphid densities in the presence of predators (Turchin and Kareiva, 1989). Before cage switching, the effects of initial aphid density and natural enemy manipulation on *A. glycines* population growth and per capita rate of increase were assessed by ANOVA using a split-plot design, with aphid density as the whole-plot factor and natural enemy as the sub-plot factor (Proc MIXED, SAS Institute, 2001). After cage switching, data were analyzed by ANOVA using a factorial design with cage switching and predator manipulation as the main effects (Proc MIXED, SAS Institute, 2001). Significant differences between predator manipulation treatments indicate an absence of spatial refuges, suggesting that predators are able to reduce aphid populations in all plants exposed (i.e., all colonies are eventually impacted by predation). In contrast, a significant effect of initial aphid density indicates numerical escape (i.e., the

impact of predation is lower at high aphid densities). For the aphid population increase analysis, sampling date was analyzed as a repeated measures factor. When significant interactions were found, main effects were explored by slicing, i.e., performing single factor ANOVAs at each level of the other factor, using the mean square residual from the original factorial ANOVA in the denominator of the F tests (Quinn and Keough, 2002). Means were compared using Least Square Mean Difference tests (LSMD) and were adjusted for multiple comparisons using the sequential Bonferroni test (Rice, 1989).

To test for the presence of refuges at the within-plant scale, aphid distribution was analyzed by MANOVA on a split-plot design with initial aphid density as the whole-plot factor and natural enemy as the subplot factor (Proc GLM, SAS Institute, 2001). The proportions of aphids present on each node were analyzed as the dependent variable of the MANOVA, and separate analysis were conducted for each date due to the variable number of nodes among dates. Roy's greatest root was reported as the statistic for the MANOVA due to its power to detect differences among treatments (Scheiner, 2001). When significant differences were detected by MANOVA, differences among predator manipulation treatments within each node were assessed using univariate ANOVA tests (protected ANOVA, *sensu* Scheiner, 2001). To further assess changes in aphid distribution, we tested if groups of nodes – hereafter termed zones and corresponding to the upper, middle, and lower third of the plant – differed in the proportion of aphids present by conducting separate ANOVAs for each zone (Proc Mixed, SAS Institute, 2001). We hypothesized that if predators randomly attacked aphids on any part of the plant, then the proportion of aphids present in each node or plant zone will be similar between treatments exposed versus protected from predation. In contrast, increased proportional number of aphids on some nodes in plants exposed to predators would indicate a refuge from predation. For all analyses, data were log or arcsine-transformed prior to statistical analysis when necessary to meet normality and homocedasticity assumptions.

3. Results

3.1. Aphid spatio-temporal dynamics

Our spatial analysis revealed that high aphid density patches did not escape predation at the field scale. Aphid populations remained low for the first three weeks of study, but then steadily increased exceeding the economic threshold level of 250 aphids/plant (Ragsdale et al., 2007) on weeks 6–7 (Table 1). A reduced sampling regime conducted for two additional weeks revealed that aphid populations were still increasing at the end of August (week 8: 826.4 ± 424.2; week 9: 854.1 ± 713.6, mean aphids/plant ± SD, n = 30 plants/week). The SADIE analyses revealed only weak spatial patterns (Fig. 1). There was a trend for marginally significant

overall aggregation during week 6 of the study (P_a = 0.039), with the rest of the sampling dates showing distributions not significantly different from random (Table 1 and Fig. 1). Similarly, the more refined analysis revealed only marginally significant patches during weeks 3 and 6, and marginally significant gaps during weeks 1, 3, 6, and 7 (Table 1 and Fig. 1). Despite the weak spatial patterns observed, significant spatial associations were observed between some consecutive weeks, although these associations did not persist more than three weeks (Table 1). Altogether, these results suggest that predators were effective at suppressing high aphid density patches to levels that were close or below field average levels during the duration of this study.

Alate immigration may have also affected aphid spatial patterns at the field scale. During week 5, an increase of an order of magnitude in aphid density (Table 1), was associated with a sudden increase in alates, suggesting a migration event. Specifically, we observed a dramatic increase in the number of alates per plant (4.7 ± 4.5 versus 0.7 ± 0.3 alates/plant in the previous week), the percentage of plants with alates (89% versus 5% in the previous week), and the percentage of alates of the total aphid population (4.4% versus less than 0.7% in weeks 1–4 and 6). Therefore, the lack of association observed between week 5 and the previous week could also be a consequence of altered aphid within-field distribution due to alate immigration.

3.2. Natural enemy abundance

A total of 294 natural enemies were sampled during the season, and their abundance and identity were consistent with previous reports for Michigan (Costamagna and Landis, 2006, 2007; Costamagna et al., 2008; Fox et al., 2004). Initially, natural enemies were very scarce (0.4 ± 0.8 individuals per sample), but densities increased gradually, reaching levels seven times higher than initial densities by mid August (2.8 ± 1.6 individuals per sample). Natural enemies were dominated by the coccinellids *Coccinella septempunctata* L. (21%), and *Harmonia axyridis* Pallas (20%), followed by first and second instar coccinellid larvae that were not identified to species (10%), and *Hippodamia convergens* Guérin-Méneville and *Coleomegilla maculata* De Geer adults (2% combined). Other numerically important natural enemies included the anthocorid *Orius insidiosus* (Say) (24%), aphidophagous larvae of Syrphidae (14%) and Chrysopidae (3%), Nabidae (3%), and Braconidae mummies (3%).

3.3. Natural enemy impacts at the plant scale

Predators exerted strong suppression on soybean aphid that resulted in an absence of spatial or dynamic refuges from predation at the plant scale. During the first two weeks of manipulation, both aphid density and λ^T (Fig. 2) were significantly affected by natural enemies (density: F = 90.87; df = 2, 30.2; P < 0.0001; λ^T: F = 129.75;

Table 1
Results of the spatio-temporal analysis of *A. glycines* field populations in Michigan, during 2003. Mean aphid abundance per plant (±SE), and SADIE statistics for overall pattern of association (I_a), presence of patches (V_i) and gaps (V_j), and association index calculated between consecutive weeks (X_p), are presented with their respective probabilities (P; in bold, P < 0.10).

| Week	Date	Mean			Patches		Gaps		Association	
		Aphids/plant	I_a	P_a	V_i	P_i	V_j	P_j	X_p	P_{X_p}
1	6/27	3.7 ± 0.6	1.40	0.077	1.10	0.256	−**1.44**	**0.051**	–	–
2	7/3	6.3 ± 0.9	1.17	0.231	1.07	0.256	−1.11	0.282	−0.005	0.450
3	7/10	5.7 ± 0.4	1.22	0.205	**1.32**	**0.077**	−**1.24**	**0.051**	**0.449**	**0.002**
4	7/17	17.7 ± 1.5	0.99	0.410	1.17	0.192	−1.08	0.321	**0.415**	**0.002**
5	7/24	106.8 ± 4.6	1.05	0.321	1.09	0.180	−1.13	0.192	−0.077	0.695
6	7/31	287.8 ± 18.3	**1.55**	**0.039**	**1.34**	**0.051**	−**1.36**	**0.077**	−0.119	0.790
7	8/8	380.1 ± 32.7	1.20	0.128	1.17	0.103	−**1.22**	**0.077**	**0.278**	**0.027**
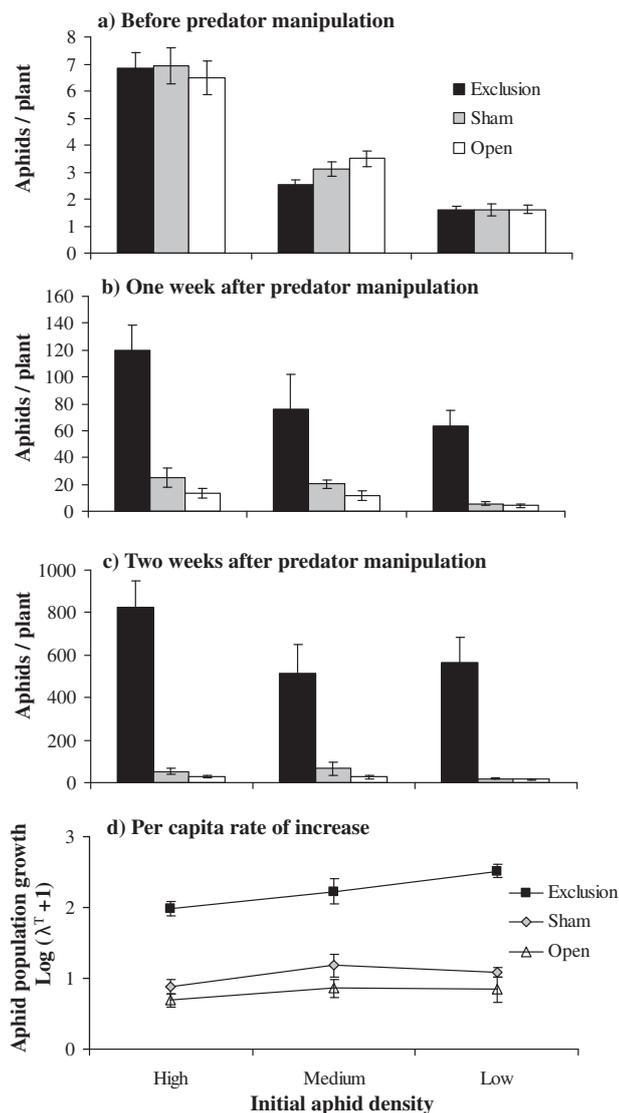
Fig. 2. *A. glycines* population growth from three initial densities under three predator manipulation treatments. Mean (±SE) aphid abundance is shown at (a) before natural enemy manipulation, and (b) one week, (c) two weeks after manipulation, and (d) mean (±SE) per capita rate of increase after 14 days. Statistics for these results are presented in the text.

df = 2, 29; $P < 0.0001$), and significantly or marginally significantly affected by initial aphid density (density: $F = 12.41$; df = 2, 15; $P = 0.0007$; λ^T: $F = 3.48$; df = 2, 15; $P = 0.0573$). We observed no significant aphid density by natural enemy interaction (density: $F = 1.63$; df = 4, 30.2; $P = 0.1922$; λ^T: $F = 1.05$; df = 4, 29; $P = 0.3934$), indicating that predator manipulations had consistent effects over the range of initial aphid densities present in the field. Predator exclusion resulted in significantly higher aphid densities, ranging from 9 to 28 times above the sham and open controls after two weeks of manipulation (Fig. 2; $P < 0.0001$, LSMD tests). Open cages had significantly lower aphid densities than sham cages ($P < 0.05$, LSMD test), although the difference was less than 40 aphids/plant (Fig. 2), suggesting only minor cage effects. Comparing the means of the natural enemy treatments revealed a 16-fold decrease in λ^T on sham and open treatments in comparison with the predator exclusion treatment (LSMD tests, $Ps < 0.0001$), and no significant differences between the sham and open treatments (LSMD test, $P > 0.05$, Fig. 2d), confirming the strong impact of predation on aphid growth.

Initial aphid density also affected aphid abundance, with significantly higher aphid abundance at the high and medium versus the low initial aphid density treatments ($P < 0.05$, LSMD tests). However, this effect was only marginally significant on λ^T (Fig. 2d). Thus, although both factors were significant, predator impacts were of much higher magnitude (14- to 27-fold reduction, sham and open treatment, respectively), than initial aphid density effects (1.5-fold reduction, for medium and low density treatments) in determining aphid abundance.

The strong impacts of predation on aphid population growth observed during the two initial weeks of the experiment were dramatically confirmed when cages were switched. As we found a change in population trajectories due to the cage switching, predator manipulation treatments showed a marginally significant interaction with switching treatment and date ($F = 3.09$; df = 2, 30; $P = 0.0602$); and therefore we analyzed this response within each of these combinations (Fig. 3). Within the control treatment, exclusion cages continued to have significantly higher aphid populations than open and sham controls ($P < 0.05$, LSMD tests, Fig. 3a and b). Due to the extraordinarily high aphid populations, observations on the control cages were stopped after week 4. We observed a complete reversal in that trend in the switched cages, with initially higher populations in the sham cages (formerly exclusion cages), that were suppressed by predation to the same levels as the open cages in week 4 (Fig. 3a and b). Finally, during week 5, we observed significantly higher aphid densities in the exclusion than the open treatment, with a similar trend in the sham treatment ($F = 3.98$; df = 2, 10; $P = 0.0534$; Fig. 3c). We also observed a significant difference in the impact of natural enemies on switched versus control treatments for aphid λ^T (significant predator manipulation × switching interaction, $F = 9.70$, df = 2, 24; $P = 0.0008$). Aphid λ^T did not differ among predator manipulations for control treatments (slicing test $F = 1.18$; df = 2, 24; $P = 0.3230$). However, we observed significantly lower λ^T in the sham versus the exclusion and open treatments in the switched treatment (slicing test $F = 30.02$; df = 2, 24; $P < 0.0001$, Fig. 3d). As a whole, these results indicate an absence of spatial and dynamic refuges at the plant level, even when initial aphid densities exposed to predation are very high, suggesting that predator responses (functional or aggregation) overcome any potential dilution of predation risks at high aphid density.

3.4. Natural enemy impacts on aphid within-plant distribution

Aphid distribution was not biased before manipulation, with no significant differences due to initial aphid densities ($F = 1.37$, df = 6, 26, $P = 0.2619$), or predator manipulation treatment assigned ($F = 2.12$, df = 6, 26, $P = 0.0847$, data not shown). We observed significant effects of aphid initial density on aphid within-plant distribution (MANOVA: week 1: $F = 4.32$, df = 9, 21, $P = 0.0028$; week 2: $F = 2.35$, df = 12, 18, $P = 0.0493$). However, at the individual node level, initial density treatments only differed in node 3 after one week of manipulation (univariate test: $F = 6.22$, df = 2, 14, $P = 0.0117$), due to low proportion of aphids in the low density treatment on that node (data not shown). Furthermore, initial aphid density did not alter the response to predator manipulation (initial aphid density × predator manipulation interaction, week 1: $F = 2.14$, df = 9, 23, $P = 0.0687$; week 2: $F = 1.71$, df = 12, 20, $P = 0.1383$). These results suggest that initial aphid density had only minor effects on the within-plant distribution of the aphids and hereafter we present the results for the predator manipulation treatments combining all initial aphid densities.

Predation had significant impacts on aphid within-plant distribution during the first two weeks of manipulation (MANOVA: week 1: $F = 2.37$, df = 9, 21, $P = 0.0499$, week 2: $F = 6.64$, df = 12, 18, $P = 0.0002$), affecting the proportion of aphids on several
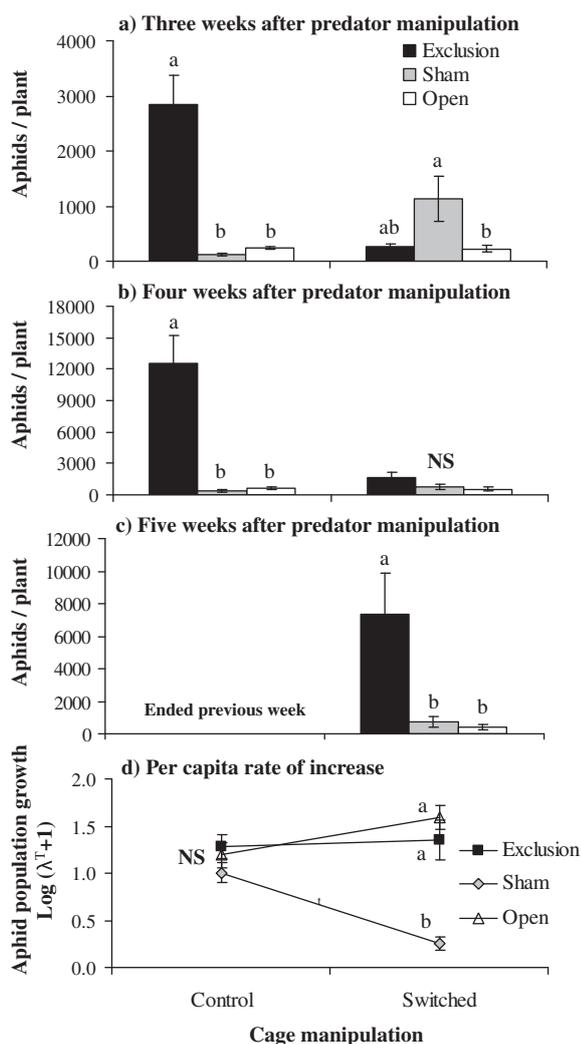
Fig. 3. *A. glycines* population growth under three predator manipulation treatments after cage switching treatments. During week 2, six plots were paired according to aphid density in the exclusion treatment and were randomly chosen to switch exclusion and sham cages (switched) or remained un-switched (control). Mean (±SE) aphid abundance is shown at (a) three, (c) four, (c) five weeks after predator manipulation, and (d) mean (±SE) per capita rate of increase after 14 days. Control treatments were interrupted at week 4 due to the extraordinary high aphid densities achieved, and therefore week 5 was analyzed separately (see methods). Different letters represent significant differences among natural enemy treatments ($P < 0.05$, LSMD adjusted by the sequential Bonferroni method for multiple comparisons).

individual nodes (Fig. 4a and c). When nodes were grouped by plant zone, after two weeks we observed significantly lower proportions of aphids on the upper third of the plant in treatments exposed to predation (ANOVA: $F = 12.65$, df = 2, 28, $P = 0.0001$), but significantly higher proportions on the lower third of the plant (ANOVA: $F = 10.54$, df = 2, 28, $P = 0.0004$) (Fig. 4b and d). There were no significant effects of predator manipulation on the proportion of aphids in different zones during the first week of the experiment and in the middle third of the plant during the second week (ANOVA: all $P > 0.05$).

Un-switched controls showed significant impacts of predation on aphid within-plant distribution for the next two weeks (MANO-VA: week 3: $F = 6.87$; df = 13, 4; $P = 0.0382$; and week 4: $F = 5.36$; df = 12, 5; $P = 0.0378$, Fig. 5a and c). However, these differences were due to a small subset of nodes differing in the proportion of aphids, and were not observed when the nodes were grouped by zone (ANOVA: all $P > 0.05$, Fig. 5b and d). Finally, switching

exclusion and sham cages completely diluted the effect of predation on aphid within-plant distribution obtained during the first part of the experiment, which resulted in an absence of significant differences among treatments (MANOVA: week 3: $F = 1.63$; df = 12, 5; $P = 0.3091$, and week 4: $F = 1.61$; df = 12, 5; $P = 0.3119$, data not shown). In summary, our results indicate a significant shift in aphid distribution towards lower plant nodes for colonies exposed to predators, during the first two weeks of the study, when aphid populations were below outbreak levels (Figs. 2 and 4), but the trend faded as aphid populations subsequently increased (Figs. 3 and 5).

4. Discussion

Our results demonstrate that predators are highly effective at suppressing *A. glycines* at the field and plant scales, resulting in an absence of spatial refuges at these scales. However, predators showed less effectiveness in suppressing aphids on the lower part of the plant, indicating the presence of a partial refuge at the within-plant scale. Finally, our plant manipulations revealed strong impacts of natural enemies on *A. glycines* abundance across different initial aphid densities, suggesting an absence of numerical escapes due to dilution of predation.

At the within-field scale, we found weak spatial patterns which never persisted for more than three consecutive weeks. Previous studies on other aphid species as well as in *A. glycines* have shown variable spatio-temporal dynamics at similar scales as in our study. Significant spatial patterns were detected for the English grain aphid *Sitobion avenae* (Perry et al., 1999; Winder et al., 1999), the corn leaf aphid, *Rhopalosiphum maidis* (Park and Obrycki, 2004), and for *A. glycines* (Hodgson, 2005; Rhainds et al., 2008) at sample grids varying between 7.5 and 50 m distance intervals, suggesting the scale we selected was likely adequate to detect spatial patterns in aphids. Previous studies on *A. glycines* spatial patterns found a lack of significant aggregations of aphids during the initial weeks of aphid colonization, but significant aggregations were observed after aphid densities reached outbreak levels (Hodgson, 2005; Rhainds et al., 2008). In our seven-week study we did not detect consistent areas of high aphid densities that escaped predation, although aphid populations were slowly increasing above economic threshold, and significant spatial patterns may have developed after reaching higher densities. However, our study demonstrates that during the preceding as well as the initial weeks of outbreak aphid populations, high density patches that serve as refuges from predation are absent, and therefore the development of outbreak aphid populations in this study cannot be attributed to the presence of refuges at the within-field or plant scales.

Several phenomena could be responsible for the absence of persistent high density patches, including, aphid movement between plants, alate migration, and the impact of natural enemies. Both alate emigration and apterous *A. glycines* movement have been shown to be much reduced until aphids reach densities of around 4000 aphids/plant (Donaldson et al., 2007), suggesting that these factors likely played only a minor role at the densities observed in our study. In contrast, a large peak of immigrating alate aphids observed during week 5 could be responsible for a dilution of aphid patches. As this event was coincident with the initial phase of aphid escape, it suggests that the role of aphid migration in disrupting predator control requires further research. Finally, the predator manipulation cages in our experiment demonstrated that predation is largely responsible for suppressing aphid growth to the relatively low levels observed in the un-manipulated populations in the field at large, confirming previous findings (Costamagna and Landis, 2006; Donaldson et al., 2007; Fox et al., 2004). This is aligned with the behavior of various ladybeetle species, that
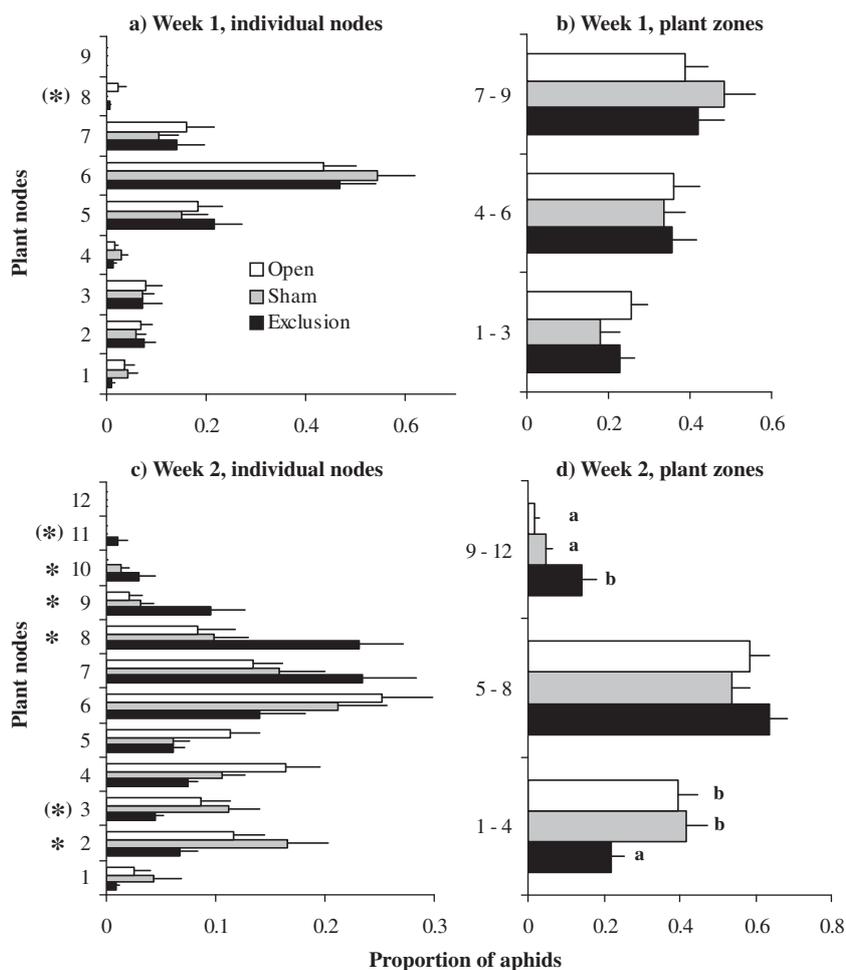
Fig. 4. Within–plant distribution of naturally occurring *A. glycines* (mean ± SE proportion of total aphids) after one (a and b) and two (c and d) weeks of manipulation on individual nodes (a and c) or plant zones (b and d). Asterisks at the right of each node in (a) and (c) present the results of univariate tests among predator manipulation treatments (∗ = $P < 0.05$, and $^{(*)}$ = $P < 0.10$, non significant results not indicated). In (b) and (d), different letters represent significant differences among natural enemy treatments ($P < 0.05$, LSMD adjusted by the sequential Bonferroni method for multiple comparisons), after significant separate ANOVAs for each plant zone. Additional statistics are presented in the text.

show density-dependent responses to patches of high aphid density (Ives et al., 1993; Turchin and Kareiva, 1989), including those attacking *A. glycines* (Costamagna and Landis, 2007; Donaldson et al., 2007). Therefore, our results suggest that natural enemy impacts are the most likely explanation for the lack of spatial patterns of aphid field distribution.

At the plant scale, predators significantly reduced aphid populations on all plants exposed to predation (i.e., sham and open treatments) in comparison with predator exclusion controls, suggesting the absence of individual colonies escaping predation. After we switched cages, aphid populations were again significantly higher in the predator exclusion cages (former sham treatment) after three weeks of manipulation, despite 9-fold lower initial aphid densities than in the predator exclusion treatment. These results dramatically demonstrate a density-dependent response of predators that is triggered for high density colonies. Interestingly, we observed that *A. glycines* populations under predator exclusion conditions showed a trend of lower λ^T at higher aphid densities, suggesting a potential self-limitation in population growth, confirming previous findings (Costamagna et al., 2007). Similar self-limitation processes of population growth have been shown for the cotton aphid, *A. gossypii* (Colfer and Rosenheim, 2001). However, the complete reversal of aphid abundance during week 5, and the absence of difference in the λ^T between switched and con-

trol exclusion cages, suggest that the main factor reducing the λ^T in a density-dependent fashion was predation. Similar results were obtained by Donaldson et al. (2007) using a wider range of *A. glycines* densities and quantifying predator response to increased aphid densities. In summary, our results show that predators exerted strong suppression at the various initial aphid densities and are capable of suppressing colonies that increase significantly over the field average. Furthermore, these results indicate an absence of 'dilution effects' at high aphid densities (Turchin and Kareiva, 1989), suggesting that *A. glycines* do not have dynamic refuges from predation at the plant level under a wide range of aphid densities.

At the within-plant scale, we found that initial exposure to predation significantly shifted aphid distribution towards lower nodes on the plant. However, as aphid densities increased, high proportions were also found on the lower nodes of the predator exclusion treatment, resulting in an absence of differences between treatments when nodes were grouped by plant zone. This lack of difference could be the result of active aphid redistribution, increased reproduction in plant zones with lower predation risk, or a combination, and additional studies are necessary to elucidate the exact mechanism(s).

Predator foraging patterns are consistent with the changes in aphid within-plant distribution observed in our study. In a prior
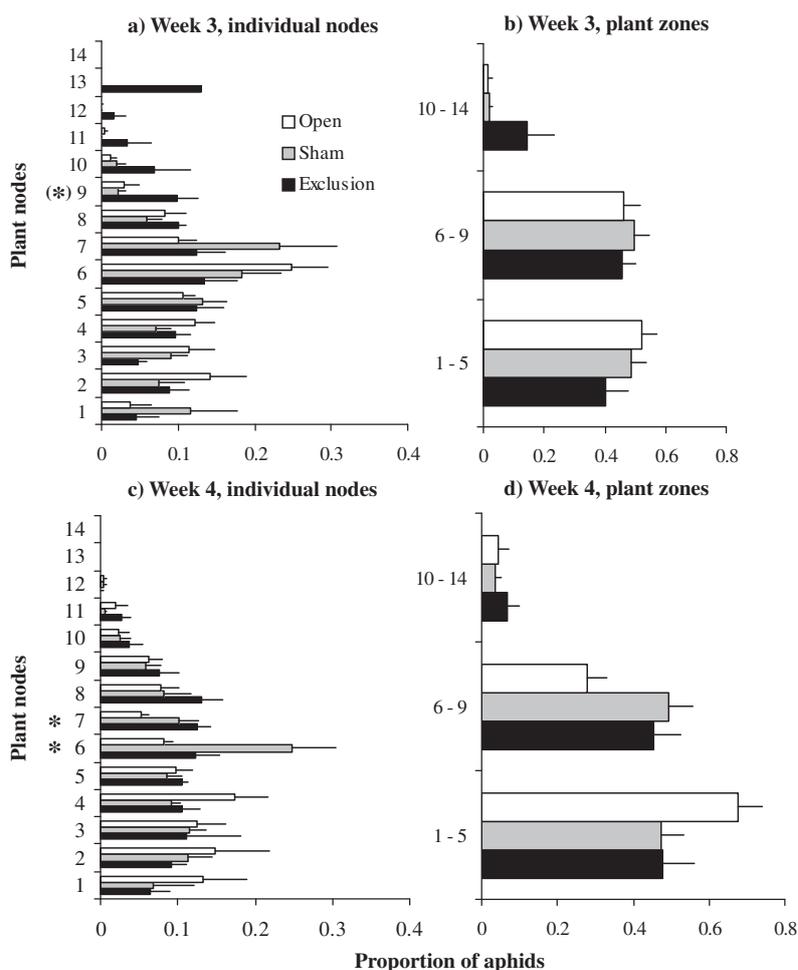
Fig. 5. Within-plant distribution of naturally occurring *A. glycines* (mean ± SE proportion of total aphids) after three (a and b) and four (c and d) weeks of manipulation on individual nodes (a and c) or plant zones (b and d), in control replicates that were not switched (switched treatments did not differed, see text for details). See Fig. 4 legend for additional details.

study, direct observation of predation in the *A. glycines* system revealed significantly more predation events on the upper portion of the plants, suggesting reduced predation risk on lower portions of the plant (Costamagna and Landis, 2007). In the current study, the most abundant predators were coccinellids, which have also been shown to be the most effective group suppressing *A. glycines* (Costamagna and Landis, 2007; Costamagna et al., 2008). Several species of coccinellids have been observed foraging more frequently on the upper portion of plants, resulting in restriction of aphids to short plants (Hacker and Bertness, 1995; Hodek, 1996). Similarly, studies on the within-plant distribution of the second most important predator in the system, *O. insidious*, indicate that this predator is more abundant in the middle third of soybean plants, which also suggests lower predation pressure in the lower third of the plant (Clements and Yeargan, 1997). Finally, aphidophagous Syrphidae larvae tend to forage in areas of high aphid aggregation and do not show a strong preference for plant height (Schneider, 1969), being therefore less likely to affect the vertical distribution of the aphids. In summary, the foraging patterns of the most important natural enemies present in the system strongly suggest that the lower part of the soybean plant is under reduced predation pressure and functions as a partial refuge from predation. However, as we did not directly measure aphid responses to predators, additional research is needed to determine whether this shift in distribution is a result of differential removal by predators or active predator avoidance behaviors in *A. glycines*.

The presence of refuges at the within-plant level can have important direct and indirect effects on the predator – *A. glycines* interaction. A direct, positive effect of the refuge consists of allowing prey to subsist at low population levels, avoiding extinction, and thus providing stability to the system (Berryman and Hawkins, 2006; Hochberg and Holt, 1995; Murdoch et al., 2005, 2006). However, the use of refuges from predation is usually associated with tradeoffs in prey reproduction or other fitness components (Berdegue et al., 1996; Hacker and Bertness, 1995; Hopkins and Dixon, 1997). Thus, changes in prey within-plant distribution may result in an indirect, negative effect of further reduction in prey population growth than expected by direct mortality. In aphids, the nutritional quality of plants differs with leaf age, with younger leaves being more suitable due to the concentration of metabolites (Dixon, 1998). Thus, it is likely that the observed predation effect of shifting *A. glycines* towards lower nodes that bear mostly older leaves result in indirect negative effects on aphid population growth. Further studies should be conducted to address the importance of this potential indirect effect of predation and its consequences in *A. glycines* population regulation.

In conclusion, our findings provide some of the first empirical evidence in support on theoretical expectations of a reduced refuge for herbivores exposed to generalist natural enemies (Symondson et al., 2002). Furthermore, these results demonstrate for the first time that existing natural enemies are effective controls for *A. glycines* at the various scales. It is interesting to note that, despite the

variety of different natural enemies attacking *A. glycines* (Costamagna and Landis, 2007), and the strong predation observed, aphids still have a spatial refuge at the within-plant level. The role of this novel partial refuge in aphid persistence under strong predation and the existence of tradeoffs with plant quality remain unclear and needs further research. From an applied perspective, our findings suggest two benefits of selecting biological control agents with a foraging pattern that includes the lower part of the plant. First, they are more likely to have additive effects and increase aphid suppression by exploiting this partial refuge from generalist predators. Second, natural enemies that forage in this partial enemy free space will be exposed to reduced levels of intraguild predation. These benefits should be considered in any plans directed to add biological control agents in the *A. glycines* system.

Acknowledgments

References

Berdegue, M., Trumble, J.T., Hare, J.D., Redak, R.A., 1996. Is it enemy-free space? The evidence for terrestrial insects and freshwater arthropods. Ecological Entomology 21, 203–217.

Berryman, A.A., Hawkins, B.A., 2006. The refuge as an integrating concept in ecology and evolution. Oikos 115, 192–196.

Bommarco, R., Firle, S.O., Ekbom, B., 2007. Outbreak suppression by predators depends on spatial distribution of prey. Ecological Modelling 201, 163–170.

Cappuccino, N., 1987. Comparative population dynamics of two goldenrod aphids – spatial patterns and temporal constancy. Ecology 68, 1634–1646.

Cappuccino, N., 1988. Spatial patterns of goldenrod aphids and the response of enemies to patch density. Oecologia 76, 607–610.

Chang, G.C., Kareiva, P., 1999. The case of indigenous generalists in biological control. In: Cornell, H.V. (Ed.), Theoretical Approaches to Biological Control. Cambridge University Press, Cambridge.

Clements, D.J., Yeargan, K.V., 1997. Seasonal and intraplant distribution of *Orius insidiosus* (Hemiptera: Anthocoridae) on soybean and possible interactions with *Nabis roseipennis* (Hemiptera: Nabidae). Journal of the Kansas Entomological Society 70, 94–99.

Colfer, R.G., Rosenheim, J.A., 2001. Predation on immature parasitoids and its impact on aphid suppression. Oecologia 126, 292–304.

Costamagna, A.C., Landis, D.A., 2006. Predators exert top-down control of soybean aphid across a gradient of agricultural management systems. Ecological Applications 16, 1619–1628.

Costamagna, A.C., Landis, D.A., 2007. Quantifying predation on soybean aphid through direct field observations. Biological Control 42, 16–24.

Costamagna, A.C., Landis, D.A., Brewer, M.J., 2008. The role of natural enemy guilds in *Aphis glycines* suppression. Biological Control 45, 368–379.

Costamagna, A.C., Van der Werf, W., Bianchi, F.J.J.A., Landis, D.A., 2007. An exponential growth model with decreasing r captures bottom-up effects on the population growth of *Aphis glycines* Matsumura (Hemiptera: Aphididae). Agricultural and Forest Entomology 9, 297–305.

Coulthard, J., 2007. QuikGrid: 3-D rendering of a surface represented by scattered data points. Available from: <http://www.galiander.ca/quikgrid/>.

Dixon, A.F.G., 1998. Aphid ecology. An optimization approach. Chapman and Hall, London, UK.

Donaldson, J.R., Myers, S.W., Gratton, C., 2007. Density dependent responses of soybean aphid (*Aphis glycines* Matsumura) populations to generalist predators in mid to late season soybean fields. Biological Control 43, 111–118.

Finke, D.L., Denno, R.F., 2006. Spatial refuge from intraguild predation: implications for prey suppression and trophic cascades. Oecologia 149, 265–275.

Fox, T.B., Landis, D.A., Cardoso, F.F., DiFonzo, C.D., 2004. Predators suppress *Aphis glycines* Matsumura population growth in soybean. Environmental Entomology 33, 608–618.

Gardiner, M.M., Landis, D.A., Gratton, C., DiFonzo, C.D., O'Neal, M., Chacon, J.M., Wayo, M.T., Schmidt, N.P., Mueller, E.E., Heimpel, G.E., 2009. Landscape diversity enhances biological control of an introduced crop pest in the north-central USA. Ecological Applications 19, 143–154.

Gonzales, W.L., Gianoli, E., Niemeyer, H.M., 2001. Plant quality vs. risk of parasitism: within-plant distribution and performance of the corn leaf aphid, *Rhopalosiphum maidis*. Agricultural and Forest Entomology 3, 29–33.

Hacker, S.D., Bertness, M.D., 1995. A herbivore paradox: why salt-marsh aphids live on poor-quality plants? American Naturalist 145, 192–210.

Hawkins, B.A., Thomas, M.B., Hochberg, M.E., 1993. Refuge theory and biological control. Science 262, 1429–1432.

Hochberg, M.E., Holt, R.D., 1995. Refuge evolution and the population dynamics of coupled host–parasitoid associations. Evolutionary Ecology 9, 633–661.

Hodek, I., 1996. Food relations. In: Hodek, I., Honěk, A. (Eds.), Ecology of Coccinellidae. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 143–238.

Hodgson, E.W., 2005. Population dynamics and sampling of the soybean aphid. Department of Entomology. University of Minnesota, Saint Paul. pp. 147.

Hopkins, G., Dixon, A.F.G., 1997. Enemy-free space and the feeding niche of an aphid. Ecological Entomology 22, 271–274.

Ives, A.R., Kareiva, P., Perry, R., 1993. Response of a predator to variation in prey density at three hierarchical scales – lady beetles feeding on aphids. Ecology 74, 1929–1938.

Kareiva, P., Odell, G., 1987. Swarms of predators exhibit preytaxis if individual predators use area-restricted search. American Naturalist 130, 233–270.

Lima, S.L., 1998. Nonlethal effects in the ecology of predator–prey interactions. What are the ecological effects of anti-predator decision-making? Bioscience 48, 25–34.

Lin, L.A., Ives, A.R., 2003. The effect of parasitoid host-size preference on host population growth rates: an example of *Aphidius colemani* and *Aphis glycines*. Ecological Entomology 28, 542–550.

McCornack, B.P., Costamagna, A.C., Ragsdale, D.W., 2008. Within-plant distribution of soybean aphid (Hemiptera: Aphididae) and development of node-based sample units for estimating whole-plant densities in soybean. Journal of Economic Entomology 101, 1488–1500.

Murdoch, W., Briggs, C.J., Swarbrick, S., 2005. Host suppression and stability in a parasitoid–host system: experimental demonstration. Science 309, 610–613.

Murdoch, W.W., Swarbrick, S.L., Briggs, C.J., 2006. Biological control: lessons from a study of California red scale. Population Ecology V48, 297–305.

Park, Y.L., Obrycki, J.J., 2004. Spatio-temporal distribution of corn leaf Aphids (Homoptera: Aphididae) and lady beetles (Coleoptera: Coccinellidae) in Iowa cornfields. Biological Control 31, 210–217.

Perry, J.N., 1998. Measures of spatial pattern for counts. Ecology 79, 1008–1017.

Perry, J.N., Dixon, P.M., 2002. A new method to measure spatial association for ecological count data. Ecoscience 9, 133–141.

Perry, J.N., Winder, L., Holland, J.M., Alston, R.D., 1999. Red-blue plots for detecting clusters in count data. Ecology Letters 2, 106–113.

Quinn, G.P., Keough, M.J., 2002. Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, UK.

Ragsdale, D.W., Landis, D.A., Brodeur, J., Heimpel, G.E., Desneux, N., 2011. Ecology and management of the soybean aphid in North America. Annu Rev Entomol 56, 375–399.

Ragsdale, D.W., McCornack, B.P., Venette, R.C., Potter, B.D., MacRae, I.V., Hodgson, E.W., O'Neal, M.E., Johnson, K.D., O'Neil, R.J., Difonzo, C.D., Hunt, T.E., Glogoza, P., Cullen, E.M., 2007. Economic threshold for soybean aphid (Hemiptera: Aphididae). Journal of Economic Entomology 100, 1258–1267.

Rhainds, M., Brodeur, J., Borcard, D., Legendre, P., 2008. Toward management guidelines for soybean aphid, *Aphis glycines*, in Quebec. II. Spatial distribution of aphid populations in commercial soybean fields. Canadian Entomologist 140, 219–234.

Rice, W.R., 1989. Analyzing tables of statistical tests. Evolution 43, 223–225.

Ritchie, S.W., Hanway, J.J., Thompson, H.E., Benson, G.O., 1994. How a Soybean Plant Develops? Iowa State Cooperative Extension. Iowa State University, Ames.

SAS Institute, 2001. SAS/STAT User's manual. Version 8.2. SAS Institute Inc., Cary, NC.

Scheiner, S.M., 2001. Manova. Multiple response variables and multispecies interactions. In: Scheiner, S.M., Gurevitch, J. (Eds.), Design and Analysis of Ecological Experiments. Oxford University Press, New York, pp. 99–115.

Schmitz, O.J., 1998. Direct and indirect effects of predation and predation risk in old-field interaction webs. American Naturalist 151, 327–342.

Schneider, F., 1969. Bionomics and physiology of aphidophagous Syrphidae. Annual Review of Entomology 14, 103–124.

Symondson, W.O.C., Sunderland, K.D., Greenstone, M.H., 2002. Can generalist predators be effective biocontrol agents? Annual Review of Entomology 47, 561–594.

Turchin, P., Kareiva, P., 1989. Aggregation in *Aphis varians*, an effective strategy for reducing predation risk. Ecology 70, 1008–1016.

Winder, L., Alexander, C.J., Holland, J.M., Woolley, C., Perry, J.N., 2001. Modelling the dynamic spatio-temporal response of predators to transient prey patches in the field. Ecology Letters 4, 568–576.

Winder, L., Perry, J.N., Holland, J.M., 1999. The spatial and temporal distribution of the grain aphid *Sitobion avenae* in winter wheat. Entomologia Experimentalis Et Applicata 93, 277–290.