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ORIGINAL PAPER

Differential impact of adults and nymphs of a generalist predator on an exotic invasive pest demonstrated by molecular gut-content analysis

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Abstract Generalist predators have the capacity to regulate herbivore populations through a variety of mechanisms, but food webs are complex and defining the strength of trophic linkages can be difficult. Molecular gut-content analysis has revolutionized our understanding of these systems. Utilizing this technology, we examined the structure of a soybean food web, identified the potential for adult and immature *Orius insidiosus* (Hemiptera: Anthocoridae) to suppress *Aphis glycines* (Hemiptera: Aphididae), and tested the hypotheses that foraging behaviour would vary between life stages, but that both adults and immatures would exert significant predation pressure upon this invasive pest. We also identified the strength of trophic pathways with two additional food items: an alternative prey item, *Neohydatothrips variabilis* (Thysanoptera: Thripidae), and an intraguild predator, *Harmonia axyridis* (Coleoptera: Coccinellidae). *A. glycines* constituted a greater proportion of the diet of immature *O. insidiosus*, but *N. variabilis* DNA was found in greater frequency in adults. However, both life stages were important early-season predators of this invasive pest, a phenomenon predicted as having the greatest impact on herbivore population dynamics and establishment success. No adult *O. insidiosus* screened positive for *H. axyridis* DNA, but a low proportion (2.5%) of immature individuals contained DNA of this intraguild predator, thus indicating the existence of this trophic pathway, albeit a relatively minor one in the context of biological control. Interestingly, approximately two-thirds of predators contained no detectable prey and fewer than 3% contained more than one prey item, suggesting the possibility for food limitation in the field. This research implicates *O. insidiosus* as a valuable natural enemy for the suppression of early-season *A. glycines* populations.

Keywords *Aphis glycines* · Food web ecology · Immature generalist predators · Molecular gut-content analysis · Predator-prey interactions

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Introduction

Theory predicts that predator and prey populations oscillate, with predators lagging behind prey over time, and such events have been demonstrated many times (Bonsall and Hassell 2007). It is this disparity between population sizes that is purported to translate into ineffective and unsustainable regulation of herbivorous pests by specific predators. However, the community of natural enemies within a system can exert pressure on pest populations (Sunderland et al. 1997) and early-season predation has been suggested as a mechanism delaying herbivore establishment (Landis and Van der Werf 1997; Brosius et al. 2007). The capacity of generalist natural enemies to "sit and wait" for prey to arrive (Settle et al. 1996) and/or build up their populations early in the year (Butler and O'Neil 2007a) enables them to feed upon target pests immediately after colonization (Sunderland et al. 1987; Harwood et al. 2004, 2007). Notwithstanding, there is a large body of evidence suggesting that alternative prey are often of high nutritional quality (Marcussen et al. 1999; Bilde et al. 2000; Butler and O'Neil 2007b) compared to pests (Toft 2005), and ultimately disrupt the capacity of generalist predators in biological control (Madsen et al. 2004; Koss and Snyder 2005; Prasad and Snyder 2006). Furthermore, enhanced biodiversity, despite translating into improved growth and reproductive success (Bilde and Toft 2000; Fawki and Toft 2005), can reduce the impact of a predator population at regulating pest densities (Symondson et al. 2006) through preferential consumption of non-pest prey. Interactions with intraguild predators have also been reported as reducing biological control by predator populations due to competition for shared prey and/or foraging sites (Snyder and Wise 2001; Rosenheim 2005). Although not a universal phenomenon (Colfer and Rosenheim 2001), further research is required to identify the consequence of predator–predator interactions in the field.

One invasive species of considerable concern is the soybean aphid, *Aphis glycines* Matsumura (Homoptera: Aphididae), whose populations are capable of doubling every 6.8 days in the field (Ragsdale et al. 2007). This aphid, first reported in North America in 2000, has subsequently spread throughout the US Midwest and eastern Canada (Ragsdale et al. 2004; Venette and Ragsdale 2004; Mignault et al. 2006) and resulted in the first insecticide application to soybeans, *Glycine max* (L. Merrill), in many regions (Rodas and O'Neil 2006). The potential economic losses have facilitated interest in the utilization of natural enemies from both North America (Ragsdale et al. 2004; Nielsen and Hajek 2005; Butler and O'Neil 2007a; Costamagna et al. 2008; Kaiser et al. 2007) and the native host range in Asia (Heimpel et al. 2004; Liu et al. 2004; Miao et al. 2007), the effects of juvenile hormone on growth and mortality (Richardson and Lagos 2007), and adoption of alternative habitat management approaches (Schmidt et al. 2007). However, it is the density and diversity of predatory arthropods within North American soybean crops (Rutledge et al. 2004) that has received most attention, and the anthocorid, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), can account for up to 85% of the predator community in some regions (Rutledge et al. 2004; Desneux et al. 2006). This natural enemy is also abundant early in the season, potentially impacting pests during colonization and thereby limiting population growth (Rutledge and O'Neil 2005; Desneux et al. 2006). This is further supported by Brosius et al. (2007) who suggested that *O. insidiosus* are most effective if present before aphid colonization. However, adult and immature predators are likely to respond differently to prey availability, and the structure of trophic connections between an immature predator and its prey have only been studied occasionally (e.g., Traugott 2003; Juen and Traugott 2007). Therefore in the context of invasive species biology, it is essential to characterize the structure of arthropod food webs, elucidate the role of generalist predators in conservation biological control and identify potential disruptive mechanisms reducing invasive species suppression.

The strength and structure of predator-prey (Symondson 2002; Sheppard and Harwood 2005) and parasitoid-host (Greenstone 2006) interactions have been defined using molecular techniques, initially through antibody-based approaches and, more recently, specific PCR. The principal limitation of custom-developed monoclonal antibodies (MAbs) has been their capacity to examine only single interactions but, once developed, they can be used to screen large populations against individual target (typically pest) prey (e.g., Hagler and Naranjo 2005). In contrast, field-based analysis of predation using specific PCR has often focused on examining the dynamics of predation upon multiple prey, including pest (Harper et al. 2005; Harwood et al. 2007; Zhang et al. 2007) and non-pest (Agustí et al. 2003; Harper et al. 2005; Juen and Traugott 2007; Harwood et al. 2007) species. Undoubtedly, where specific interactions are of interest, MAbs will continue to be used but the ability of PCR to decipher multiple trophic interactions makes it the assay of choice when examining predator-multiple prey food webs.

In this study, we adopt a multi-disciplinary approach to elucidate the strength of trophic interactions between adult and immature *O. insidiosus* and three categories of prey: an invasive pest (*A. glycines*); a non-pest prey (*Neohydatothrips variabilis* (Beach) (Thysanoptera: Thripidae)) and a late-season predator (*Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae)). These predators and prey represent the most important fauna in soybean fields in Indiana (Rutledge et al. 2004, Desneux et al. 2006, H.J.S.Y. & R.J.O. unpublished data). By integrating field population surveys with molecular gut-content analyses, we will test the hypothesis that early-season predation will be exhibited in both adult and immature generalist predators, but significant differences in foraging behaviour will be evident between life stages. We predict that the relatively immobile immature *O. insidiosus* will consume *A. glycines* more frequently than the adults, which can access prey over a broader spatial scale. This study will also examine the importance of alternative prey in *O. insidiosus* food webs and the likelihood for foraging upon *H. axyridis* by these anthocorid predators. Understanding the mechanisms of foraging behaviour, in relation to invasive pests, alternative prey and intraguild predators, is crucial to understanding the dynamic and changing structure of food webs involving invasive species.

Materials and methods

Field sampling techniques

In 2006, adult and immature *O. insidiosus* were hand-collected weekly (~20 adult and 20 immature predators per week) from two or three 0.16 ha plots within a 7.5 ha soybean field (Beck's 274NRR soybean, Beck's Hybrids, Atlanta, Indiana, USA) located at the Purdue University Agronomy Center for Research and Education, Tippecanoe County, Indiana, USA. During the research study, crops were planted in 76.2 cm rows under standard agronomic practices for soybeans in Indiana. No pesticides were applied during 2006. Following collection by aspirator, predators were transferred into 1.5 ml microcentrifuge tubes filled with 95% EtOH before being stored at −20°C until DNA extraction (see below).

Throughout the sampling period (June–September), predator and prey populations were sampled to identify relationships that exist between field population densities and foraging behaviour. Soybean plants were located by stratified random sampling within field plots and destructive whole-plant counts ($n > 30$ plants per week) taken to estimate densities of *A. glycines*, *N. variabilis* and coccinellid eggs.

Sample preparation and molecular screening protocols

Prior to analysis, all predators were homogenized whole in 0.5 ml mortar-and-pestle microcentrifuge tubes in 100 µl of high salt extraction buffer (Aljanabi and Martinez 1997), supplemented with SDS to 2% and Proteinase K to 400 µg/ml. Following overnight digestion at 60°C, DNA precipitates were resuspended in 100 µl of 0.1× TE, pH 8.0. Polymerase chain reaction (PCR) protocols utilized primers designed by Harwood et al. (2007) for *A. glycines* (Genbank Accession Number EF467229), *N. variabilis* (EF523586), *H. axyridis* (EF192083) and *O. insidiosus* (EF467230). Full molecular screening protocols are described in detail by Harwood et al. (2007). All predators were screened against the primers of all three prey items and against *O. insidiosus*. The screening against predator primer pairs was done to ensure that DNA could be successfully extracted from all specimens.

Data analysis

The number of adult and immature predators screening positive for prey DNA was compared using χ^2 analysis. The relationship between availability of prey resources (monitored by destructive whole-plant sampling) and the proportion of predators screening positive was correlated following arcsine transformation of gut-content data. All data were analyzed using

Minitab® v. 14.1 (Minitab Inc., State College, Pennsylvania, USA).

Results

Previous characterization of this molecular detection system was undertaken by Harwood et al. (2007). In all instances, primer pairs were specific to target prey and elicited no amplification of DNA from 85 closely related or non-target organisms common at this field site. Similarly, previous research optimizing detection limits of prey DNA (Harwood et al. 2007) revealed highly significant correlations between arcsine transformed percentages of predators screening positive for prey DNA and time after feeding.

All field-collected predators were screened against primers for *O. insidiosus* (F: ACACATTATTAGAA AAGAAAGAGGA; R: TAAATAGAAATACGAAT CCTAATG; Genbank Accession Number EF467230, size 281 bp) to ensure successful extraction of DNA. In all cases, 100% of predators screened positive, thus validating the extraction procedures used during this study.

Soybean aphid numbers increased exponentially over time (Fig. 1a), although population densities were low compared to 2005 (Harwood et al. 2007). Similarly, the proportion of *O. insidiosus* screening positive for soybean aphid DNA (Fig. 1b) increased over time, but at a linear rate (adults: arcsine % positive = 7.65 + 2.05 Week number, $r^2 = 0.68$, $F_{1,7} = 12.45$, $P = 0.012$; nymphs: arcsine % positive = 14.5 + 3.70 Week number, $r^2 = 0.79$, $F_{1,4} = 11.57$, $P = 0.042$) and there was no significant correlation between aphid density and consumption of these prey in the field (adults: $r^2 = 0.48$, $F_{1,7} = 5.65$, $P = 0.056$; nymphs: $r^2 = 0.51$, $F_{1,4} = 3.18$, $P = 0.173$). However, significantly fewer adult (13.4%) compared to immature (25.0%) *O. insidiosus* screened positive for soybean aphid DNA ($\chi^2 = 4.226$, $df = 1$, $P = 0.040$).

The density of *N. variabilis* on soybean plants remained low throughout the season (Fig. 2a) but significantly more adult *O. insidiosus* (21.7%) contained *N. variabilis* DNA than the immatures (5.0%) ($\chi^2 = 9.718$, $df = 1$, $P = 0.002$) (Fig. 2b). Interestingly, the feeding frequency of adult *O. insidiosus* increased over time (arcsine % positive = 12.4 + 1.59 week number; $r^2 = 0.63$, $F_{1,7} = 10.09$, $P = 0.019$) but, as with aphid consumption, there was no comparable relationship between the presence of *N. variabilis* DNA in *O. insidiosus* gut samples and their availability in the field ($r^2 = 0.01$, $F_{1,7} = 0.08$, $P = 0.793$). The very low number of immature *O. insidiosus* screening positive for soybean thrips DNA prevented correlative analysis.

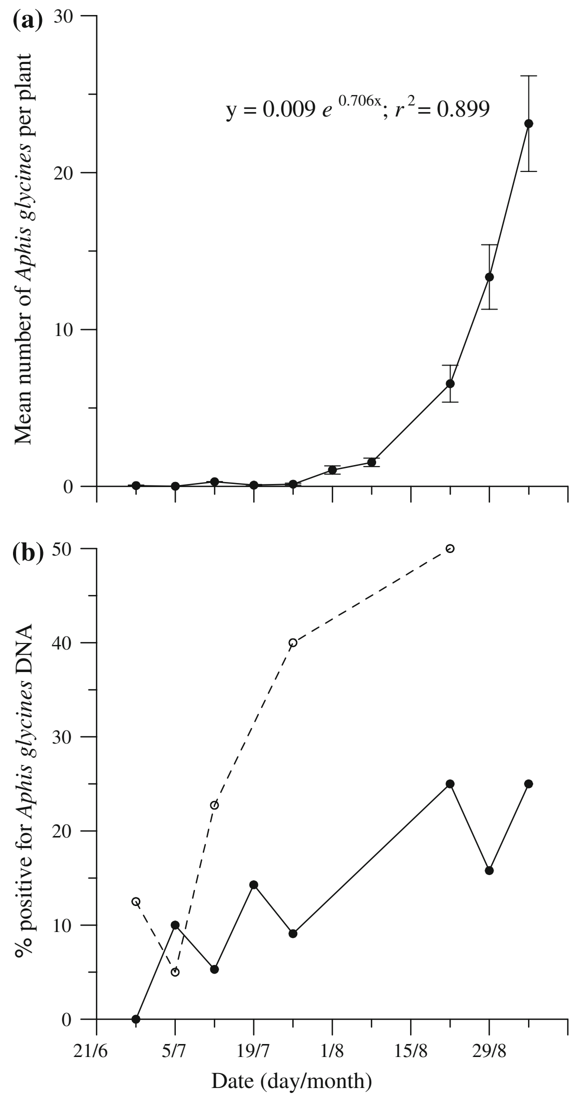

Fig. 1 (**a**) Mean number (±SE) of *Aphis glycines* captured per plant in 2006; (**b**) the percentage of adult (solid line) and immature (dashed line) *Orius insidiosus* screening positive for *A. glycines* DNA on these sampling dates

Coccinellid eggs were scarce throughout 2006, with only 5 eggs collected from 640 destructively sampled soybean plants. Despite this scarcity, we screened *O. insidiosus* against *H. axyridis* primers to indicate the presence or absence of these trophic

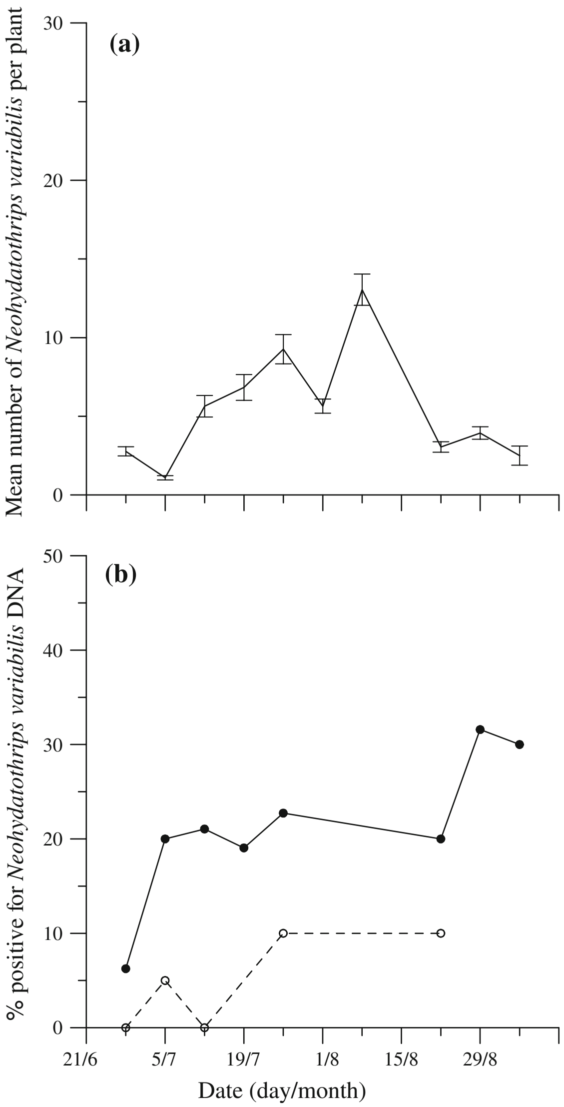

Fig. 2 (**a**) Mean number (±SE) of *Neohydatothrips variabilis* captured per plant in 2006; (**b**) the percentage of adult (solid line) and immature (dashed line) *Orius insidiosus* screening positive for *N. variabilis* DNA on these sampling dates

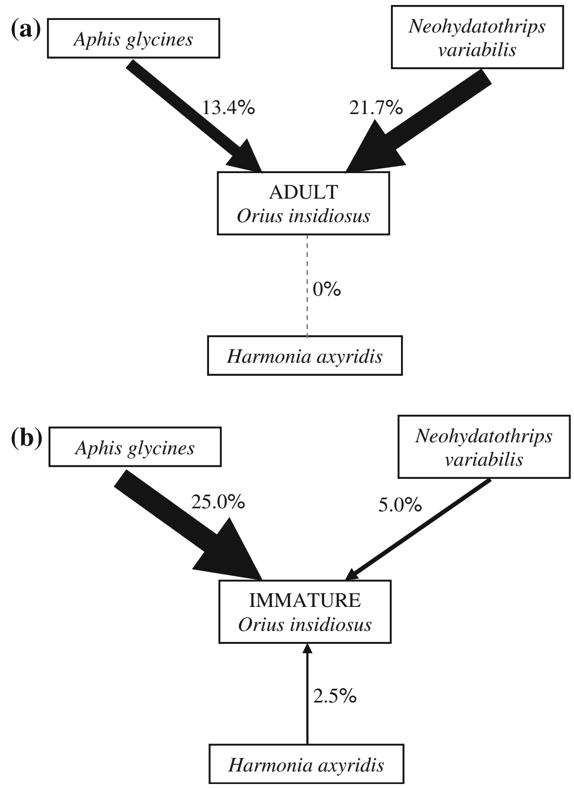

Fig. 3 Structure of *Aphis glycines*, *Neohydatothrips variabilis*, *Harmonia axyridis* and (**a**) adult and (**b**) immature *Orius insidiosus* food web in the soybean agroecosystem. The sizes of arrows and value corresponds to the relative strength of the pathway and the proportion of *O. insidiosus* screening positive for prey DNA

linkages in the field. Interestingly, even though no adult *Orius* screened positive, 2.5% of immatures amplified *H. axyridis* DNA, indicating consumption of intraguild predators in the field. Thus, the overall foraging diets of different life stages of *O. insidiosus* varied significantly (Fig. 3).

Therefore, all predators were probed simultaneously for all three prey items but only 1.9% of adults and 2.5% of immature *Orius* contained more than one prey item. A large proportion (66.9% of adults and 60.0% of immatures) contained none of these three prey items.

Discussion

Invasive species have a major impact on agricultural commodities throughout the world, and since 2000 *A. glycines* has become the most important insect pest of soybeans in North America (Ragsdale et al. 2004; Venette and Ragsdale 2004; Mignault et al. 2006). Thus, there has been considerable focus on identifying the role of natural enemies in suppression of these herbivores (Ragsdale et al. 2004; Nielsen and Hajek 2005; Butler and O'Neil 2007a; Kaiser et al. 2007; Donaldson et al. 2007). Although coccinellids undoubtedly play an important role in *A. glycines* population dynamics (Fox et al. 2004, 2005; Costamagna and Landis 2006; Costamagna et al. 2008), molecular analysis has identified *O. insidiosus* as an important natural enemy in some soybean food webs (Harwood et al. 2007).

Our data indicate that trophic linkages between *O. insidiosus* and *A. glycines* are strong (Fig. 3), especially early in the season when pest densities were low. In addition to deciphering trophic connectedness between natural enemy and pest populations, we tested the hypothesis that foraging behaviour of adult and immature generalist predators (*O. insidiosus*) would differ. Indeed, predation pressure was not consistent between life stages, with significantly more immature *O. insidiosus* screening positive for soybean aphid DNA. Given that predator communities in agroecosystems are dominated by non-adults, this research emphasizes the need for comprehensive examination of food web structure in the context of all life stages. The pressure exerted on *A. glycines* by *O. insidiosus* appears to be primarily attributable to immature predators.

In contrast, significantly more adult *O. insidiosus* screened positive for *N. variabilis* DNA. Despite very low population densities (adult *N. variabilis* peaked at just 1.3 individuals per plant), over 20% of adult *O. insidiosus* had recently consumed these prey items in the field. These proportions screening positive were similar to data from 2005 (Harwood et al. 2007) even though *N. variabilis* populations were significantly lower, thereby exposing predators to very few prey. Given the scarcity of these food resources in 2006, and the high rate of predation upon them, it is likely that some degree of preference for non-target prey was exhibited. Therefore, although there was no relationship between prey availability and consumption in the field, preferential foraging may have led to some degree of prey (thrips) depletion. Despite this unusually high predation on such a scarce prey item, almost 70% of adult *O. insidiosus* did not contain either *A. glycines* or *N. variabilis* prey, species that constituted the majority of food resources in this agroecosystem (H.J.S.Y., unpublished data), leading to the likelihood for resource supplementation through plant feeding, a trait common in these hemipteran omnivores (Lundgren et al. 2008). Even with plant feeding, it can be assumed that, in common with other predators (e.g., Anderson 1974; Bilde and Toft 1998), *O. insidiosus* were experiencing some degree of food limitation in the field.

Despite their nutritive quality (Butler and O'Neil 2007b), *N. variabilis* DNA was found in only 5% of immature *Orius*. In laboratory trials, access to highly mobile prey (e.g., thrips) is artificially increased within the experimental arena; in the field, this variation in foraging activity may reflect behavioural differences and the inability of immature *O. insidiosus* to catch highly active prey. Therefore, when prey is in limiting supply, such as during 2006, growth and development will probably be reduced given that 60% of immature predators contained no recognizable prey. Although trophic linkages may be missed (only three prey items were examined), this is unlikely given the dominance of these food items in the field.

The final trophic connection examined was between *O. insidiosus* and *H. axyridis*. No adult *O. insidiosus* screened positive for DNA of this intraguild predator, but 2.5% of immature specimens had consumed *H. axyridis*. Although such interactions occur in the laboratory, it is thought that these linkages are unlikely to significantly impact biological control in the field. The small number of immature hemipterans testing positive, coupled with the very fast breakdown of *H. axyridis* DNA in *O. insidiosus* (Harwood et al. 2007), indicate that some disruptive effects may occur in the field. However, prey was extremely scarce in 2006, with only 5 coccinellid eggs observed. Further research is required to examine this trophic pathway and test the hypothesis that despite low levels of predation, late season suppression of *A. glycines* by *H. axyridis* is unaffected by these infrequent foraging events.

It is widely recognized that dietary diversification can promote growth and development of generalist predator populations (Bilde and Toft 2000; Fawki and Toft 2005). While alternative prey help maintain predator populations when pests are absent or scarce (Murdoch et al. 1985), they may also contribute to the balancing of amino acid (Greenstone 1989) and protein/lipid (Mayntz et al. 2005) intake. This research demonstrated that, if *A. glycines* and *N. variabilis* constituted the primary prey resources available, *O. insidiosus* were food-limited with only 1.9% of adults and 2.5% of immature containing more than one detectable prey. During these periods, when prey is in short supply, it can therefore be predicted that these predator populations may be reduced through mortality, competitive interactions and/or emigration.

Our molecular elucidation of trophic connections has enabled accurate delineation of an important food web with regard to suppression of a key invasive pest in North America. Although levels of predation upon *A. glycines* were low, early-season foraging was evident, as previously observed in this (Harwood et al. 2007), and other (Sunderland et al. 1987; Harwood et al. 2004), food webs. The phenomenon of early-season suppression has been the subject of intense study because of the effectiveness of generalist predators at this time of year (Chiverton 1986; Chang and Kareiva 1999). This study demonstrated that *O. insidiosus* can impact *A. glycines* early in the season, and that immature stages may be particularly important in biological control. However, the high degree of predation upon scarce alternative food items implies possible disruption of this predator-pest linkage. Furthermore, given that predator biodiversity positively affects herbivore suppression (Snyder et al. 2006, 2008), additional research is required to decipher all interactions within the soybean food web, and formulate a framework for management of *A. glycines* by the natural enemy complex. Not only do other trophic pathways need examining, but the identification of trophic pathways between regions should also be considered given the variable effects and predator assemblages reported across sites.

Finally, despite limited predation upon *H. axyridis* by immature *O. insidiosus*, possibly consumed due to hunger, the *A. glycines* → *H. axyridis* pathways are probably unaffected by these intraguild interactions. Nevertheless, the role of late-season foraging by coccinellid (and other) biocontrol agents needs further examination for season-long control of *A. glycines* to be realized.

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