

## **Ants Increase Cloverworm Herbivory Via Nonconsumptive Pathways**

Authors: Penn, Hannah J., and Athey, Kacie J.

Source: Florida Entomologist, 103(2) : 160-167

Published By: Florida Entomological Society

URL: <https://doi.org/10.1653/024.103.0202>

---

BioOne Complete ([complete.BioOne.org](https://complete.BioOne.org)) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/terms-of-use](https://www.bioone.org/terms-of-use).

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

---

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

# Ants increase cloverworm herbivory via nonconsumptive pathways

Hannah J. Penn<sup>1,\*</sup>, and Kacie J. Athey<sup>2</sup>

---

## Abstract

Generalist arthropod predators often exhibit a range of intraguild interactions reducing their potential efficacy as biological control agents. These interactions may include consumptive or nonconsumptive effects that mediate the impacts of herbivores. We examined interactions among 2 generalist predators, the striped lynx spider (*Oxyopes salticus* Hentz; Araneae: Oxyopidae) and an ant (*Lasius neoniger* Emery; Hymenoptera: Formicidae), and a herbivore, the green cloverworm (*Hypena scabra* F.; Lepidoptera: Erebidae), all prevalent in central Kentucky soybean agroecosystems. We hypothesized that individual predator treatments would reduce green cloverworm survival and resultant leaf damage, but that predators would interfere with each other when both were present. To study these interactions, field cages containing potted soybeans were used to examine 8 treatment combinations (control, cloverworm, spider, ant, spider + cloverworm, ant + cloverworm, ant + spider, and ant + spider + cloverworm). When proportional leaf damage was compared, spider, ant + spider, and spider + cloverworm treatments had significantly less damage than the ant, ant + cloverworm, and ant + spider + cloverworm treatments. Spider presence tended to decrease plant damage while ant presence significantly increased damage. No differences among treatments were found for either spider or cloverworm recovery, indicating potential nonconsumptive effects of ants that may include compensatory feeding. We found that generalist predators, such as *L. neoniger*, can inhibit biological control due to nonconsumptive interactions even when the same species in a different system provides substantial levels of pest suppression.

Key Words: *Hypena scabra*; field cage; food web; *Lasius neoniger*; *Oxyopes salticus*

## Resumen

Los depredadores de artrópodos generalistas a menudo exhiben un rango de interacciones intra-gremial (entre el grupo depredador) reduciendo su eficacia potencial como agentes de control biológico. Estas interacciones pueden incluir efectos consuntivos o no consuntivos que median los impactos de los herbívoros. Examinamos las interacciones entre dos depredadores generalistas, la araña lince rayada (*Oxyopes salticus* Hentz; Araneae: Oxyopidae) y una hormiga (*Lasius neoniger* Emery; Hymenoptera: Formicidae), y un herbívoro, el gusano verde de trébol (*Hypena scabra* F.; Lepidoptera: Erebidae), todos prevalentes en los agroecosistemas de soja del centro del estado de Kentucky. Presumimos que los tratamientos de depredadores individuales reducirían la sobrevivencia del gusano verde de trébol y el daño resultante en las hojas, pero que los depredadores interferirían entre sí cuando ambos estuvieran presentes. Para estudiar estas interacciones, se utilizaron jaulas de campo que contenían soja en maceta para examinar 8 combinaciones de tratamiento (el control, el gusano verde de trébol, araña, hormiga, araña + el gusano verde de trébol, hormiga + el gusano verde de trébol, hormiga + araña, y hormiga + araña + el gusano verde de trébol). Cuando se comparó el daño proporcional de las hojas, los tratamientos de araña, hormiga + araña y araña + el gusano verde de trébol tuvieron un daño significativamente menor que los tratamientos de hormiga, hormiga + el gusano verde de trébol y hormiga + araña + el gusano verde de trébol. La presencia de las arañas tiende a disminuir el daño a las plantas, mientras que la presencia de las hormigas aumenta significativamente el daño. No se encontraron diferencias entre los tratamientos para la recuperación de la araña o el gusano verde de trébol, lo que indica posibles efectos no consuntivos de las hormigas que pueden incluir la alimentación compensatoria. Descubrimos que los depredadores generalistas, como *L. neoniger*, pueden inhibir el control biológico debido a interacciones no consumidoras, incluso cuando la misma especie en un sistema diferente proporciona niveles sustanciales de supresión de plagas.

Palabras Claves: *Hypena scabra*; jaula de campo; red alimentaria; *Lasius neoniger*; *Oxyopes salticus*

---

Biological control to prevent plant damage and disease transmission depends on natural enemies found in agroecosystems to actively prey upon and suppress pest populations (Landis et al. 2000; Welch & Harwood 2014). The characteristics and disturbances of agricultural habitats influence natural enemy species differently, altering natural enemy community composition and abundance in cropping systems (Schmidt et al. 2005; Gardiner et al. 2010; Jonsson et al. 2015). By enhancing populations of already occurring natural enemies, through conservation biological control, pest suppression can be intensified via non-chemical means while benefiting non-target species (i.e., pol-

linators, detritivores, and predators) through potential reduction of insecticide input (Landis et al. 2000). Additionally, increased species richness, diversity, and evenness of natural enemies within agroecosystems can lead to greater biological control effectiveness (Losey & Denno 1998; Straub & Snyder 2008; Finke & Snyder 2010).

The additive effects of multiple generalist predator species on biological control are confounded by intraguild interactions, i.e., competition or predation among predators (Polis et al. 1989; Rosenheim et al. 1995; Snyder & Wise 1999). Predators have the potential to actively interfere with each other, releasing pests from predation (Prasad & Sny-

---

<sup>1</sup>School of Earth, Environmental, and Marine Sciences, University of Texas Rio Grande Valley, Edinburg, Texas 78539, USA; E-mail: Hannah.penn@utrgv.edu (H. J. P.)

<sup>2</sup>Crop Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA; E-mail: kacie.athey@gmail.com (K. J. A.)

\*Corresponding author; Email: Hannah.penn@utrgv.edu

der 2006). This interaction hinges on the identity of and environmental conditions surrounding the predators (Straub & Snyder 2006). The presence of 2 predators with a tendency to interfere with each other could increase crop damage by either inducing behavioral changes (nonconsumptive effects) or directly attacking each other. For instance, a predator could consume another in place of the target pest species (Traugott et al. 2012; Messelink et al. 2013), or an aggressive predator could interfere with the normal behaviors of other predators, preventing the threatened individuals from consuming their food items in a typical fashion (Eubanks et al. 2002; Thaler & Griffin 2008; Blubaugh et al. 2017). Similarly, the presence of 1 predator might later change the behaviors of the prey item so that the latter is at greater risk as a food item or the prey item moves to habitats out of predator reach (Sih et al. 1998; Griffin et al. 2011; Greyson-Gaito et al. 2016).

Ants and spiders are abundant generalist predators in many systems including agroecosystems and exhibit many types of intraguild interactions including bi-directional predation and nonconsumptive behavioral shifts. Over 100 species of spiders have been found to consume ants regularly (Edwards et al. 1974; Cushing 2012). *Callilepis nocturna* (L.) (Araneae: Gnaphosidae) has been found to feed facultatively on *Formica* spp. and *Lasius* spp. (Hymenoptera: Formicidae) (Heller 1974). Another previously documented effect of spiders on ants was reported by Gastreich (1999) where *Pheidole bicornis* Forel (Hymenoptera: Formicidae) did not forage on leaves where the silk of *Dipoena banksi* Chickering (Araneae: Theridiidae) was present, indicating potential predation risk to the ant.

Alternatively, several ant species affect the behavior and survival of spiders. For instance, the ant species *Azteca sericeasur* Longino (Hymenoptera: Formicidae) within coffee agroecosystems (Marín et al. 2015) and *Lasius niger* L. (Hymenoptera: Formicidae) in grasslands (Schuch et al. 2008) have been found to positively influence spider populations, thereby increasing overall predation levels. *Formica cunicularia* (Latreille) (Hymenoptera: Formicidae) altered densities of Linyphiid (Araneae) spiders; i.e., where ants were excluded, spider density increased 3-fold. Additionally, where *F. cunicularia* was not excluded, Lepidopteran larval populations were reduced (Sanders & Platner 2007). These data taken in conjunction with a study on fire ants (Seagraves & McPherson 2006), indicate that ants could disrupt spider predation while also maintaining high predation rates on herbivorous prey items.

Little is known about the intraguild interactions between ants and spiders within simplified and highly disturbed habitats even though they both have been shown to be excellent predators under such circumstances. The ant, *Lasius neoniger* Emery (Hymenoptera: Formicidae), has been found to prey upon green cloverworm, *Hypona scabra* Fabricius (Lepidoptera: Erebididae) in soybeans and black cutworm larvae, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), and Japanese beetle eggs, *Popilla japonica* Newman (Coleoptera: Scarabaeidae), in turfgrass (López & Potter 2000; Penn et al. 2017). The striped lynx spider, *Oxyopes salticus* (Hentz) (Araneae: Oxyopidae), has been well documented as a predator of several pest species in cotton (Breene et al. 1990; Nyffeler et al. 1992; Nyffeler & Sunderland 2003) and is common in soybeans (Penn 2018). However, the addition of both predators within the same area, as is common in agricultural field settings, could exhibit any combination of intraguild interactions including changes in behavior unrelated to consumption (Bucher et al. 2014; Mestre et al. 2014).

The objective of this study was to determine the consequences of intraguild interactions between ants and spiders within the context of soybean production. Specifically, we evaluated the influence of ants (*L. neoniger*) and striped lynx spiders (*O. salticus*) on a soybean pest that does not produce incentives for ant-protection (honeydew), i.e., the green cloverworm (*H. scabra*). Moreover, we predicted that both

predators would interfere with each other resulting in increased cloverworm recovery and subsequent leaf damage.

## Materials and Methods

### EXPERIMENTAL CAGE SET-UP

The experiment was conducted in Jun 2015 (a single temporal replicate) at the University of Kentucky Spindletop Research Farm in Lexington, Kentucky, USA (38.1254722°N, 84.5153889°W). Field cages were composed of nylon mesh screening (52 × 52 mesh count) fine enough to exclude arthropod entry or exit but permit sunlight and rainfall (Athey et al. 2017). The screened cages (Lumite Inc., Alto, Georgia, USA) measured 1.83 m × 1.83 m × 1.83 m, were secured to the ground with tent stakes and permitted researcher access via a side zipper. Cages were then buried 20 cm to prevent movement of arthropods in the top layer of soil. All cages were placed based on the observed presence of *L. neoniger* colonies with 1 colony per cage in a grassy field that had not been in crop production for 1 yr. Plant material within cages was killed via 2 sequential applications of the herbicide Killzall® (Voluntary Purchasing Groups, Inc., Bonham, Texas, USA), per label recommendations, with all plant material removed manually 1 wk before the study. Removal of pre-existing arthropods within cages was completed using a leaf blower (Poulan Pro 25cc Gas Blower/Vac, Poulan, Charlotte, North Carolina, USA) set to reverse with an insect net attached to the air intake. All arthropods captured in this way were released outside of cages. Yellow sticky cards (15.00 cm × 5.00 cm, 1 per cage) were deployed from the top of each field cage the wk before and during the study to capture the remaining non-ground dwelling, non-target arthropods. To ensure that ants remained in cages after removal of other arthropods and plant material, pitfall traps (9.5 cm diam, 12 cm deep) with Styrofoam rain guards (22 cm diam) containing ethylene glycol were installed in the center of each cage. Pitfall traps were set the wk before, as well as during, the cage study.

### EXPERIMENTAL TREATMENTS

Eight treatments were used to evaluate the interactions of ants, spiders, and green cloverworms, with 4 replicates of each treatment assigned randomly to cages ( $n = 32$  cages total). Soybeans ('Viking 2265' organic soybeans, Johnny's Selected Seed, Winslow, Maine, USA) were sown at a rate of 1 seed per cm within plastic pots (15.24 cm × 60.33 cm × 20.00 cm) in the greenhouse at  $25 \pm 1$  °C, 16:8 h (L:D) photoperiod, and  $65 \pm 5\%$  RH until plants reached a growth stage of R1. Randomly selected pots ( $n = 2$ ) were placed into each cage with foliage overlapping to allow for between-plant movement of organisms 3 d before the start of the experiment. Pots within ant-exclusion treatments were painted with Fluon® (INSECT-a-SLIP, BioQuip Products, Inc., Rancho Dominguez, California, USA). Spiders and cloverworms were obtained via sweep nets from fields of grass and alfalfa at the Spindletop Research Farm and immediately placed into field cages to mimic arthropod hunger during field conditions (Breene et al. 1990). These organisms were selected based on their overall prevalence within Kentucky soybean fields during the preceding 3 yr (Penn et al. 2017; Penn 2018). Organisms were placed simultaneously on the soybean foliage at a rate of 4 adult or sub-adult spiders and 15 second instar cloverworms per cage, similar to rates observed in similar fields in the area (Stone & Pedigo 1972). All organisms were allowed to interact with soybean plants and each other for 4 d following spider and cloverworm addition in order to allow accurate assessment of spider gut contents (Macías-Hernández et al. 2018).

## ARTHROPOD OBSERVATIONS

Once daily observations (20 min each, starting at 10:00 AM each d) of spider sex/life stage, and counts of observed ants, spiders, cloverworms, and non-target arthropods within cages were made by a single researcher throughout the experiment. Non-cloverworm herbivores causing chewing damage were removed from the cage by hand when found. The within-cage locations (i.e., top of leaf, bottom of leaf, stem, ground, or cage wall or ceiling) of the focal species were recorded daily for each individual observed. At the end of the exposure time, all cloverworm larvae and spiders (live and dead combined) and any active ant foragers were recovered, counted, and stored individually at  $-20^{\circ}\text{C}$  in autoclaved 1.5 mL microcentrifuge tubes containing 95% ethanol. Cloverworms found as precocious pupae were placed in sealed (59 mL) plastic condiment containers for observation of emerging parasitoids and were stored similarly upon emergence.

## MOLECULAR GUT CONTENT ANALYSIS

Following collection, spider samples were homogenized in 180  $\mu\text{L}$  of tissue lysis buffer. Total DNA was extracted from all samples using DNeasy Blood and Tissue Kits<sup>®</sup> (Qiagen Inc., Valencia, California, USA) following the animal tissue protocol provided by manufacturer. DNA was stored in autoclaved 1.5 mL microcentrifuge tubes at  $-20^{\circ}\text{C}$  until PCR analysis. The total DNA in all samples was amplified with cloverworm (*H. scabra*) primers (HS517F and HS598R) (Penn et al. 2017). All PCRs (12.5  $\mu\text{L}$  total volume) consisted of 1X Takara buffer (Takara Bio Inc., Shiga, Japan), 0.2 mM of each dNTP, 0.2 mM of each primer, 0.31 U Takara Ex Taq<sup>™</sup> and template DNA (1  $\mu\text{L}$  of total DNA). To determine the viability of extractions not tested positive for cloverworm DNA, samples were screened using general COI primers Jerry (Simon et al. 1994) and Ben3R (Villesen et al. 2004). All reactions were carried out using Bio-Rad PTC-200 and C1,000 thermal cyclers (Bio-Rad Laboratories, Hercules, California, USA). The PCR cycling protocol for the cloverworm primers (with Takara reagents as above) was  $94^{\circ}\text{C}$  for 1 min followed by 40 cycles of  $94^{\circ}\text{C}$  for 45 s,  $62^{\circ}\text{C}$  for 45 s, and  $72^{\circ}\text{C}$  for 30 s. The PCR cycling protocol for the general COI primers Jerry and Ben3R (with Takara reagents as above) was  $94^{\circ}\text{C}$  for 1 min followed by 35 cycles of  $95^{\circ}\text{C}$  for 60 s,  $47^{\circ}\text{C}$  for 60 s, and  $72^{\circ}\text{C}$  for 90 s. Following amplification, reaction success was determined by electrophoresis of 5  $\mu\text{L}$  PCR product pre-stained with GelRed nucleic acid gel stain (1X Biotium, Hayward, California, USA) on 2% SeaKem agarose (Lonza, Rockland, Maine, USA). In all cases, sets of PCRs contained 1 positive control of cloverworm DNA and 1 negative control without the addition of DNA. Any reactions that did not test positive with the general COI primers ( $n = 4$ ) were assumed to be unreliable and were not included in results.

## FINAL PLANT DAMAGE ANALYSIS

The leaf damage produced by the green cloverworms was analyzed in addition to the recovery of cloverworms and spiders to assess the nature of ant-spider intraguild interactions. Following arthropod removal, all soybean stems were snipped at soil level, placed into trash bags (1 per cage), transported to the lab, and stored in a cold room ( $15^{\circ}\text{C}$ ) for 12 h until processing (Breene et al. 1990). A 5 plant sub-sample was blindly and randomly selected out of the bag by hand for every cage. Leaves of each sub-sample were removed from the stem, flattened, placed onto a white background with a scale, and photographed at the same distance and zoom using a Canon EOS digital camera (Canon Inc., Tokyo, Japan). Plant damage was assessed via ImageJ (National Institutes of Health, Bethesda, Maryland, USA) (Rasband 2016), where each photo was scaled globally using an in-

photograph scale. Missing leaf area was measured and calculated as a proportion of the entire leaf area (Schneider et al. 2012; Schindelin et al. 2015).

## STATISTICAL ANALYSES

All analyses were completed in R vers. 3.5.2 (R Core Team 2019). The within-cage location of observed spiders in relation to treatment (8 categories, factorial arrangement), d (to account for weather changes), and sex/life stage was analyzed using a multinomial logistic regression using the polr function in the MASS software package (Venables & Ripley 2002). Not enough data were gathered on ant and cloverworm locations to allow for statistical analyses. Recovery of spiders and cloverworms (not including precocious pupae), as well as spider gut contents (presence or absence of cloverworm DNA), were compared among all relevant treatments using a general linear regression model (GLM) with a binomial distribution. Marginal effects, statistics indicating changes in the dependent variable associated with a single unit change in the independent variable (Onukwugha et al. 2015), were calculated with the mfx software package (Fernihough & Henningsen 2019); predicted probabilities were calculated using the Effects package (Fox et al. 2018). Main effects were modeled similarly, using the presence and absence of the relevant organisms and the interaction term. Total leaf area measured and total area consumed were compared among treatments using a Tukey-Kramer HSD. For further analysis, the area of leaf damage ( $\text{cm}^2$ ) was standardized using the average area damaged in all soybean only treatments. The standardized leaf area damaged was analyzed using GLM. Main effects were modeled similarly using presence and absence of relevant organisms and interaction term. To determine the overall impacts of cloverworm presence, ant presence, spider presence, and any predator presence on leaf area damaged, 5 contrasts were conducted with a Sidak correction for multiple contrast (Saha et al. 2012; Mangiafico 2015) using the car package (Fox & Hong 2009; Fox & Weisberg 2019). All figures were constructed using ggplot2 (Wickam 2016). For all analyses, differences were considered significant at  $P < 0.05$ .

## Results

### DAILY ARTHROPOD OBSERVATIONS

All cages contained non-target arthropods that had emerged either during the observation period or were unable to be removed during cage preparation, the most common of which included Acrididae (Orthoptera), Cicadellidae (Hemiptera), and *Colaspis brunnea* F. (Coleoptera: Chrysomelidae). Ants were found via visual observations and pitfall traps in all treatments the wk before and the wk of the experiment. Additionally, Fluon application appeared to be effective in preventing ant access to the pots of soybeans throughout this study in ant-exclusion treatments. Daily observations of cloverworms and ants provided insufficient data for location analyses because both were difficult to observe without disturbing the system. But cloverworms generally were located on the bottom of soybean leaves when discovered. When we analyzed the location of spiders in relation to treatment, d, and spider sex/stage we found the final model had an Akaike information criterion (AIC) of 389.72. Neither d ( $P = 0.06$ ) nor spider sex/stage (male:female  $P = 0.37$ ; sub-adult:female  $P = 0.20$ ) were significant variables for explaining spider location. The treatment variable was found to be a significant indicator of spider location (Table 1). The spider-only treatment increased the number of observations made of spiders on the cage structure but decreased the probability of spiders on top of leaves (Fig. 1).

**Table 1.** Marginal effects of treatment (when compared against spider-only treatment, Os), sex (compared against female), and d on spider location. Effect is listed followed by *P*-value in parentheses. Ln indicates the presence of ants, *Lasius neoniger* Emery; Os indicates the presence of striped lynx spiders, *Oxyopes salticus* (Hentz); and Hs indicates the presence of green cloverworms, *Hypena scabra* Fabricius.

Category	Variable	Leaf top	Leaf bottom	Stem	Cage	Ground
Treatment	Ln + Os	0.28 (< 0.01)	-0.02 (0.77)	-0.07 (0.01)	-0.17 (< 0.01)	-0.02 (0.07)
	Os + Hs	0.27 (< 0.01)	0.01 (0.89)	-0.07 (0.01)	-0.19 (< 0.01)	-0.03 (0.07)
	Ln + Os + Hs	0.22 (0.01)	0.01 (0.91)	-0.06 (0.02)	-0.15 (< 0.01)	-0.02 (0.09)
Sex	Male	-0.04 (0.39)	-0.002 (0.35)	0.01 (0.39)	0.04 (0.37)	0.01 (0.42)
	Sub-adult	-0.08 (0.12)	-0.09 (0.35)	0.03 (0.13)	0.13 (0.27)	0.01 (0.25)
Day	Day	-0.03 (0.07)	-0.02 (0.14)	0.01 (0.11)	0.04 (0.07)	0.01 (0.18)

## RECOVERY OF CLOVERWORMS AND SPIDERS

When the final proportions of recovered spiders were compared (Table 2), there were no differences among treatments based on sex ratio (male:female  $P = 0.51$ ) or stage (sub-adult:female  $P = 0.11$ ). Main effects analyses indicated similar results, i.e., ant ( $P = 0.27$ ) and cloverworm presence ( $P = 0.45$ ) as well as their interaction term ( $P = 0.42$ ) were not significant. The same was found for cloverworms among treatments containing cloverworms (Fig. 2; Table 2). Main effects analyses again indicated similar results, i.e., ant ( $P = 0.23$ ) and spider presence ( $P = 0.50$ ) as well as the interaction term ( $P = 0.87$ ) were not significant. No statistical differences were found in the proportion of precocious cloverworm pupae (parasitism) between treatments ( $F = 1.89$ ;  $df = 3,12$ ;  $P = 0.19$ ).

## MOLECULAR GUT CONTENT ANALYSIS

Of all spiders placed into field cages ( $n = 64$ ), 39 were recovered after 4 d and tested for cloverworm DNA. Of these samples, 11 tested positive in spider + cloverworm and ant + spider + cloverworm treatments combined, but 15 spiders tested positive in spider-only and ant + spider treatments. Regression results indicate that there was no difference between treatments in gut contents (spider + cloverworms  $P = 0.19$ ; spider + ant  $P = 0.70$ ; ant + spider + cloverworm  $P = 0.714$ ),

**Table 2.** Marginal effects of treatment for cloverworm and spider recovery models (when compared against cloverworm-only treatment and spider-only treatment, respectively). Effect is listed followed by *P*-value in parentheses. Ln indicates the presence of ants, *Lasius neoniger* Emery; Os indicates the presence of striped lynx spiders, *Oxyopes salticus* (Hentz); and Hs indicates the presence of green cloverworms, *Hypena scabra* Fabricius.

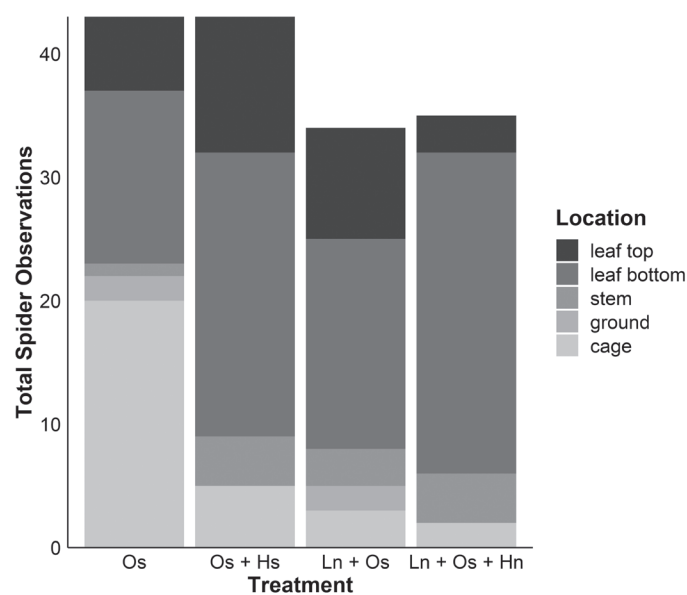
Category	Variable	Cloverworm	Spider
Treatment	Ln + Hs	0.10 (0.25)	NA
	Ln + Os	NA	-0.17 (0.38)
	Os + Hs	-0.05 (0.48)	-0.10 (0.64)
	Ln + Os + Hs	0.02 (0.83)	-0.10 (0.64)
Sex	Male	NA	-0.10 (0.50)
	Sub-adult	NA	-0.35 (0.10)

NA = not available

with marginal effects indicating no differences when compared with spider-only treatment (Table 3). Main effects also indicated no impact of ant ( $P = 0.70$ ) or cloverworm ( $P = 0.19$ ) presence or their interaction ( $P = 0.72$ ) on gut content positives. Given that cloverworm-positive gut contents should be only in treatments with cloverworm addition (and further testing with COI primers), results indicated that the samples were found but the molecular gut content analyses were inconclusive. Therefore, it remains unknown whether spiders were preying upon cloverworms rather than only harassing them. However, given the final recovery number of cloverworms, it appears that even if spider predation was occurring, it was not significant.

## FINAL PLANT DAMAGE

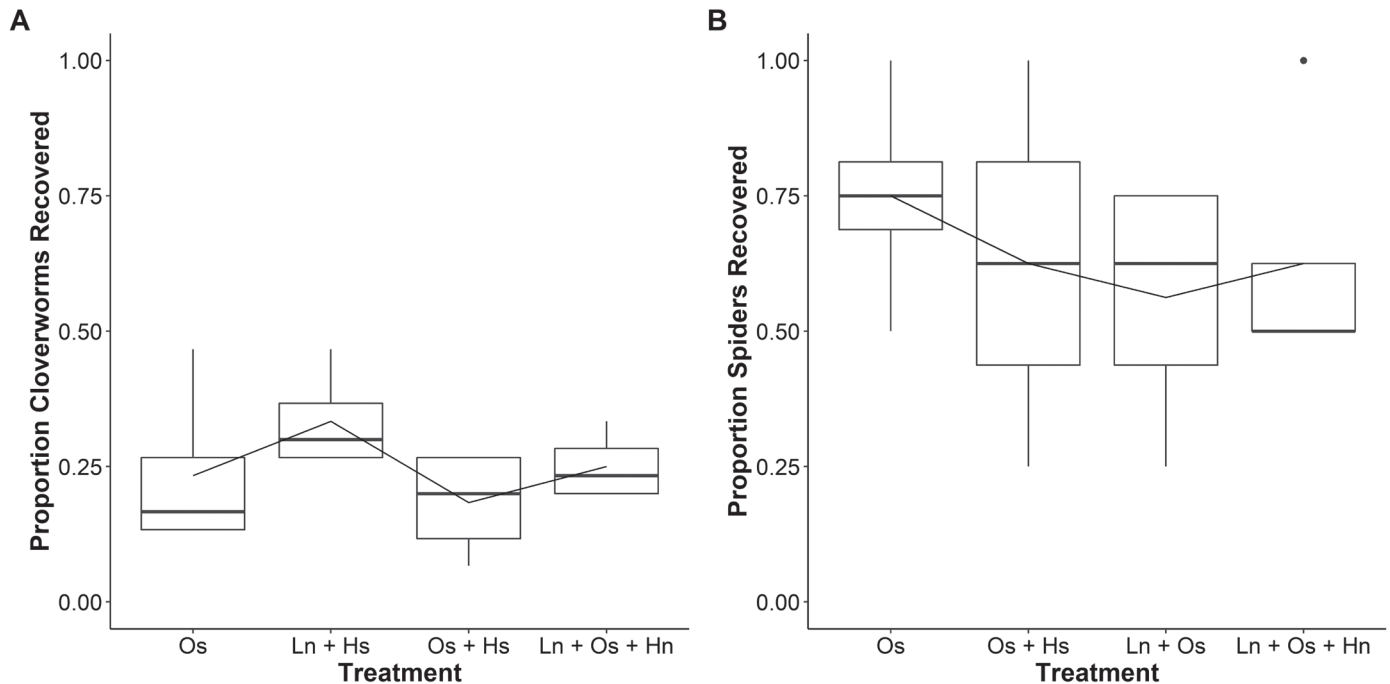
When plant damage (Table 4) was analyzed with GLM (Table 5, Fig. 3), the ant + spider + cloverworm treatment was the only significant treatment ( $P < 0.05$ ). We found that plants did not experience more damage when cloverworms alone were present than when cloverworms were present with any predator + cloverworm treatment ( $P = 0.25$ ). Similarly, plant damage was not different between ant only and spider only treatments ( $P = 0.16$ ). However, ant + cloverworm and spider + cloverworm treatments experienced less damage when compared with the ant + spider + cloverworm treatment ( $P < 0.05$ ) as did spider + cloverworm when compared alone with ant + spider + cloverworm ( $P < 0.05$ ). Main effects indicated that the presence of ants had a significant impact ( $P < 0.05$ ) on reducing leaf damage, though the presence of cloverworms ( $P = 0.37$ ) and spiders ( $P = 0.73$ ) and their respective ant interaction terms (ant  $\times$  cloverworm [ $P = 0.73$ ], ant  $\times$  spider [ $P = 0.32$ ]) did not.



**Fig. 1.** The total number of striped lynx spiders, *Oxyopes salticus* (Hentz), observed over 4 d at each location (legend) by treatment containing spiders. Ln indicates the presence of ants, *Lasius neoniger* Emery; Os indicates the presence of striped lynx spiders, *Oxyopes salticus* (Hentz); and Hs indicates the presence of green cloverworms, *Hypena scabra* Fabricius.

## Discussion

Given that few ants were observed actively foraging on plants in non-exclusion treatments, it might be surprising that a strong ant ef-



**Fig. 2.** (A) The proportion of the initial 15 second instar green cloverworms including precocious pupa, *Hypena scabra* Fabricius (Hs), and (B) lynx spiders, *Oxyopes salticus* (Hentz) (Os), recovered. Ln indicates the presence of the ant, *Lasius neoniger* Emery. The solid line indicated calculated predicted probabilities based on the final model.

**Table 3.** Marginal effects of treatment (when compared against spider-only treatment, Os) on whether spider gut contents tested positive for cloverworm DNA. Ln indicates the presence of ants, *Lasius neoniger* Emery; Os indicates the presence of striped lynx spiders, *Oxyopes salticus* (Hentz); and Hs indicates the presence of green cloverworms, *Hypena scabra* Fabricius.

Treatment	Marginal effect	P-value
Ln + Os	-0.09	0.69
Os + Hs	0.28	0.15
Ln + Os + Hs	0.08	0.71

fect was seen in leaf damage data. The transient nature of ant scouts could mean that more ants would have been observed had a greater length of daily observation time been deployed (Wenninger et al. 2016). Also, Buckley (1990) has shown some ant species are more apt to protect herbivores during nocturnal predation events, which were not observed in our study. The recovery of spiders and cloverworms

**Table 4.** Mean  $\pm$  SE total area and consumed area ( $\text{cm}^2$ ) of each leaf ( $n = 20$  per treatment) over the 4 d study. Ln indicates the presence of ants, *Lasius neoniger* Emery; Os indicates the presence of striped lynx spiders, *Oxyopes salticus* (Hentz); and Hs indicates the presence of green cloverworms, *Hypena scabra* Fabricius.\*

Treatment	Total area ( $\text{cm}^2$ )	Consumed area ( $\text{cm}^2$ )
Soy	181.87 $\pm$ 11.64 a	1.02 $\pm$ 0.23 a
Hs	191.42 $\pm$ 9.96 a	1.18 $\pm$ 0.49 a
Ln	187.33 $\pm$ 7.05 a	1.75 $\pm$ 0.38 a
Os	217.61 $\pm$ 12.08 a	0.31 $\pm$ 0.09 a
Ln + Hs	169.94 $\pm$ 7.48 a	2.56 $\pm$ 0.77 ab
Ln + Os	195.83 $\pm$ 12.81 a	0.43 $\pm$ 0.09 a
Os + Hs	189.06 $\pm$ 9.85 a	0.68 $\pm$ 0.14 a
Ln + Os + Hs	207.66 $\pm$ 12.69 a	4.48 $\pm$ 1.34 b

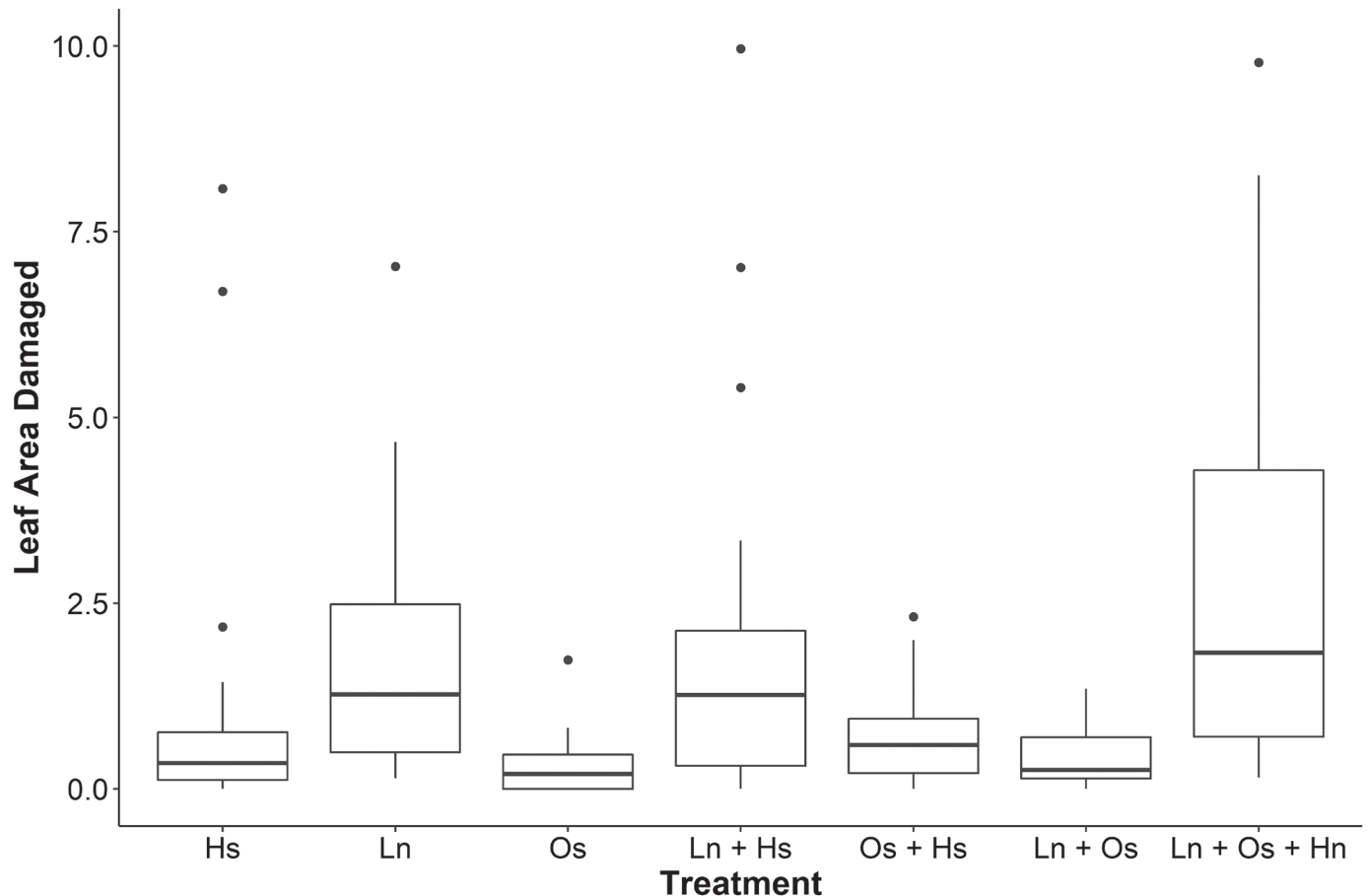
\*Means within each treatment with different letters are significantly different ( $P < 0.05$ ) Tukey-Kramer HSD tests.

**Table 5.** Generalized linear model results for total area consumed area ( $\text{cm}^2$ ) standardized with the average damage of the soybean only treatment. Ln indicates the presence of ants, *Lasius neoniger* Emery; Os indicates the presence of striped lynx spiders, *Oxyopes salticus* (Hentz); and Hs indicates the presence of green cloverworms, *Hypena scabra* Fabricius.

Treatment	Parameter estimate	Standard error	P-value
Intercept	0.16	0.64	0.81
Ln	0.57	0.90	0.53
Os	-0.87	0.90	0.34
Ln + Hs	1.41	0.90	0.12
Ln + Os	-0.74	0.90	0.41
Os + Hs	-0.50	0.89	0.58
Ln + Os + Hs	3.30	0.90	> 0.05

was not influenced by ant presence, indicating that this interaction was not predacious but a nonconsumptive interaction that altered cloverworm behavior in the presence of ants (Schmitz et al. 1997; Mestre et al. 2016). This is consistent with a previous study where *Solenopsis invicta* Buren (Hymenoptera: Formicidae) failed to reduce either predator or pest abundance in cotton fields through predation (Sterling et al. 1979). Ants also have been shown to benefit pest species even if said species does not intentionally solicit ant protection via honeydew production, similar to the non-honeydew producing cloverworms used here (James et al. 1997). Furthermore, the increase in plant damage may be due to compensatory feeding by cloverworms in the presence of ants (Fraser & Gilliam 1987; Stachowicz & Hay 1999; Thaler et al. 2012; Walzer et al. 2015).

Nonconsumptive intraguild interactions between other arthropod predators have been frequently observed; for example, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) are repelled from aphid consumption by the presence of *Tetramorium caespitum* L. (Hymenoptera: Formicidae) and *L. niger* via harassment but not predation (Katayama & Suzuki 2003). Spiders could have sensed chemical cues from the ants



**Fig. 3.** The leaf area damaged (cm<sup>2</sup>) after 4 d. Ln indicates the presence of ants, *Lasius neoniger* Emery; Os indicates the presence of striped lynx spiders, *Oxyopes salticus* (Hentz); and Hs indicates the presence of green cloverworms, *Hypena scabra* Fabricius.

present and arrested normal predation behaviors for fear of antagonism (Clark et al. 2000). Furthermore, ant harassment of spiders in that experiment could be due, in part, to the relative simplicity of the system presented to them, because a similar effect has been observed in laboratory studies examining the intraguild interactions of mirids (*Tytthus vagus* [Knight] [Hemiptera: Miridae]) and wolf spiders (*Pardosa littoralis* Banks [Araneae: Lycosidae]) (Finke & Denno 2002).

We found that cloverworm recovery did not diminish in spider treatments (without ants present) and indicated that spiders also could have induced nonconsumptive behavioral shifts in cloverworms (Whitehouse et al. 2011; Rypstra & Buddle 2013). The consumption habits of cloverworms could have decreased simply because of spider presence (spiders were present and observed preying on other small arthropods, i.e., Hemiptera: Cicadellidae), resulting in less leaf damage (Thaler & Griffin 2008). These nonconsumptive effects were further substantiated by spider location trends when treatments included cloverworms, i.e., spiders were found where cloverworms were likely to be present (e.g., undersides of leaves). Similar results have been observed in other systems such as damsel bug on aphid populations despite the prevention of predation (Nelson et al. 2004). Such influential nonconsumptive effects are an important consideration for biological control using generalist predators (Ohgushi 2008), because even in the absence of predation on a pest species, the presence of a predator such as our studies with spiders could alter food web interactions of the pest (Kéfi et al. 2012; Eubanks & Finke 2014; Majdi et al. 2014), cascading through the system to the benefit of the plants (Preisser et al. 2005; Preisser & Bolnick 2008).

We observed that overall levels of cloverworm-attributed damage were low, but not unreasonable for the life stage (second instar) and exposure period of plants (4 d). Previous studies have indicated that larvae at this life stage consume 0.64 to 1.6 cm<sup>2</sup> per d and consumption rates vary greatly with environmental conditions (Stone & Pedigo 1972; Hammond et al. 1979). Our results indicated that intraguild interactions occurred between ants and spiders within the soybean system as we predicted; when both predators were present simultaneously, plant damage by the pest increased. However, the interactions between predators and between predators and pests probably were not mediated by direct predation as we had supposed, but via nonconsumptive interactions between predators and between predators and pests. We concluded this based on increased plant damage in ant-containing treatments despite no significant differences in spider or cloverworm recovery. Furthermore, trophic interactions between predators and pests are based on environmental conditions as exhibited by our ant species that inhibited biological control services in this study, even though the same species has been shown to provide substantial levels of pest suppression in other highly disturbed systems.

## Acknowledgments

We thank Katelyn A. Kesheimer and Andrew Dale for assistance with experiments, as well as Jamin M. Dreyer, Michael I. Sitvarin, Andre Perez, and Leslie Potts for cage set-up and tear-down. Support was received

from the University of Kentucky Agricultural Experiment Station State Project KY008063 granted to James D. Harwood and an NSF Graduate Research Fellowship 2011124868 to HJP. The information reported in this paper (No. 17-08-111) is part of a project of the Kentucky Agricultural Experiment Station and is published with the approval of the Director.

## References Cited

- Athey KJ, Sitvarin MI, Harwood JD. 2017. Laboratory and field investigation of biological control for brown marmorated stink bug (*Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae)). *Journal of the Kansas Entomological Society* 90: 341–352.
- Blubaugh CK, Widick IV, Kaplan I. 2017. Does fear beget fear? Risk-mediated habitat selection triggers predator avoidance at lower trophic levels. *Oecologia* 185: 1–11.
- Breene RG, Sterling WL, Nyffeler M. 1990. Efficacy of spider and ant predators on the cotton fleahopper (Hemiptera: Miridae). *Entomophaga* 35: 393–401.
- Bucher R, Binz H, Menzel F, Entling MH. 2014. Effects of spider chemotactile cues on arthropod behavior. *Journal of Insect Behavior* 27: 567–580.
- Buckley R. 1990. Ants protect tropical Homoptera against nocturnal spider predation. *Biotropica* 22: 207–209.
- Clark RJ, Jackson RR, Cutler B. 2000. Chemical cues from ants influence predatory behavior in *Habrocestum pulex*, an ant-eating jumping spider (Araneae, Salticidae). *Journal of Arachnology* 28: 309–318.
- Cushing PE. 2012. Spider-ant associations: an updated review of myrmecomorphy, myrmecophily, and myrmecophagy in spiders. *Psyche: A Journal of Entomology* 2012: 151989. doi:10.1155/2012/151989
- Edwards GB, Carroll JF, Whitcomb WH. 1974. *Stoidis aurata* (Araneae: Salticidae), a spider predator of ants. *Florida Entomologist* 57: 337–346.
- Eubanks MD, Blackwell SA, Parrish CJ, Delamar ZD, Hull-Sanders H. 2002. Intraguild predation of beneficial arthropods by red imported fire ants in cotton. *Environmental Entomology* 31: 1168–1174.
- Eubanks MD, Finke DL. 2014. Interaction webs in agroecosystems: beyond who eats whom. *Current Opinion in Insect Science* 2: 1–6.
- Fernihough A, Henningsen A. 2019. mfx: Marginal effects, odds ratios and incidence rate ratios for GLMs. The Comprehensive R Archive Network, Queen's University, Belfast, Northern Ireland.
- Finke DL, Snyder WE. 2010. Conserving the benefits of predator biodiversity. *Biological Conservation* 143: 2260–2269.
- Fox J, Hong J. 2009. Effect displays in R for multinomial and proportional-odds logit models: extensions to the effects package. *Journal of Statistical Software* 32: 1–24.
- Fox J, Weisberg S. 2019. An R companion to applied regression, 3rd edition. Thousand Oaks, California, USA.
- Fox J, Weisberg S, Friendly M, Hong J, Andersen R, Firth D, Taylor S, R Core Team. 2018. effects: Effect displays for linear, generalized linear, and other models. <https://CRAN.R-project.org/package=effects> (last accessed 28 Feb 2020).
- Fraser DF, Gilliam JF. 1987. Feeding under predation hazard: response of the guppy and Hart's rivulus from sites with contrasting predation hazard. *Behavioral Ecology and Sociobiology* 21: 203–209.
- Gardiner M, Landis D, Gratton C, Schmidt N, O'Neal M, Mueller E, Chacon J, Heimpel G. 2010. Landscape composition influences the activity density of carabidae and arachnida in soybean fields. *Biological Control* 55: 11–19.
- Gastreich K. 1999. Trait-mediated indirect effects of a theridiid spider on an ant-plant mutualism. *Ecology* 80: 1066–1070.
- Greyson-Gaito CJ, Barbour MA, Rodriguez-Cabal MA, Crutsinger GM, Henry GHR. 2016. Freedom to move: Arctic caterpillar (Lepidoptera) growth rate increases with access to new willows (Salicaceae). *The Canadian Entomologist* 148: 673–682.
- Griffin JN, Soomdat NN, Brun KE, Chejanovski ZA, Silliman BR. 2011. Top predators suppress rather than facilitate plants in a trait-mediated tri-trophic cascade. *Biology Letters* 7: 710–713.
- Hammond RB, Pedigo LP, Poston FL. 1979. Green cloverworm leaf consumption on greenhouse and field soybean leaves and development of a leaf-consumption model. *Journal of Economic Entomology* 72: 714–717.
- Heller G. 1974. Zur Biologie der ameisenfressenden Spinne *Callilepis nocturna* Linnaeus 1758 (Aranea, Drassodidae). Johannes Gutenberg-Universität, Mainz, Germany.
- James D, Stevens M, O'Malley K. 1997. The impact of foraging ants on populations of *Coccus hesperidum* L. (Hem., Coccidae) and *Aonidiella aurantii* (Maskell) (Hem., Diaspididae) in an Australian citrus grove. *Journal of Applied Entomology* 121: 257–259.
- Jonsson M, Straub CS, Didham RK, Buckley HL, Case BS, Hale RJ, Gratton C, Wratten SD. 2015. Experimental evidence that the effectiveness of conservation biological control depends on landscape complexity. *Journal of Applied Ecology* 52: 1274–1282.
- Katayama N, Suzuki N. 2003. Changes in the use of extrafloral nectaries of *Vicia faba* (Leguminosae) and honeydew of aphids by ants with increasing aphid density. *Annals of the Entomological Society of America* 96: 579–584.
- Kéfi S, Berlow EL, Wieters EA, Navarrete SA, Petchey OL, Wood SA, Boit A, Joppa LN, Lafferty KD, Williams RJ. 2012. More than a meal . . . integrating non-feeding interactions into food webs. *Ecology Letters* 15: 291–300.
- Landis DA, Wratten SD, Gurr GM. 2000. Habitat management to conserve natural enemies of arthropod pests in agriculture. *Annual Review of Entomology* 45: 175–201.
- López R, Potter DA. 2000. Ant predation on eggs and larvae of the black cutworm (Lepidoptera: Noctuidae) and Japanese beetle (Coleoptera: Scarabaeidae) in turfgrass. *Environmental Entomology* 29: 116–125.
- Losey JE, Denno RF. 1998. Positive predator–predator interactions: enhanced predation rates and synergistic suppression of aphid populations. *Ecology* 79: 2143–2152.
- Macías-Hernández N, Athey K, Tonzo V, Wangenstein OS, Arnedo M, Harwood JD. 2018. Molecular gut content analysis of different spider body parts. *PLOS ONE* 13: e0196589. DOI: 10.1371/journal.pone.0196589
- Majdi N, Boiche A, Traunspurger W, Lecerf A. 2014. Predator effects on a detritus-based food web are primarily mediated by non-trophic interactions. *Journal of Animal Ecology* 83: 953–962.
- Mangiafico SS. 2015. R companion: contrasts in linear models, pp. 264–275 *In* An R Companion for the Handbook of Biological Statistics, vers. 1.3.3. Rutgers Cooperative Extension, New Brunswick, New Jersey, USA.
- Marín L, Jackson D, Perfecto I. 2015. A positive association between ants and spiders and potential mechanisms driving the pattern. *Oikos* 124: 1078–1088.
- Messelink GJ, Bloemhard CMJ, Sabelis MW, Janssen A. 2013. Biological control of aphids in the presence of thrips and their enemies. *BioControl* 58: 45–55.
- Mestre L, Bucher R, Entling MH. 2014. Trait-mediated effects between predators: ant chemical cues induce spider dispersal. *Journal of Zoology* 293: 119–125.
- Nelson EH, Matthews CE, Rosenheim JA. 2004. Predators reduce prey population growth by inducing changes in prey behavior. *Ecology* 85: 1853–1858.
- Nyffeler M, Dean DA, Sterling WL. 1992. Diets, feeding specialization, and predatory role of two lynx spiders, *Oxyopes salticus* and *Peucetia viridans* (Araneae: Oxyopidae), in a Texas cotton agroecosystem. *Environmental Entomology* 21: 1457–1465.
- Nyffeler M, Sunderland KD. 2003. Composition, abundance and pest control potential of spider communities in agroecosystems: a comparison of European and US studies. *Agriculture, Ecosystems & Environment* 95: 579–612.
- Ohgushi T. 2008. Herbivore-induced indirect interaction webs on terrestrial plants: the importance of non-trophic, indirect, and facilitative interactions. *Entomologia Experimentalis et Applicata* 128: 217–229.
- Onukwughu E, Bergtold J, Jain R. 2015. A primer on marginal effects—Part I: Theory and formulae. *PharmacoEconomics* 33: 25–30.
- Penn HJ. 2018. Wooded field margins increase potential for cultural and biological control of soybean pests. *Agriculture, Ecosystems & Environment* 255: 45–51.
- Penn HJ, Athey KJ, Lee BD. 2017. Land cover diversity increases predator spatial aggregation to, and consumption of, prey. *Ecology Letters* 20: 609–618.
- Polis GA, Myers CA, Holt RD. 1989. The ecology and evolution of intraguild predation: potential competitors that eat each other. *Annual Review of Ecology and Systematics* 20: 297–330.
- Prasad RP, Snyder WE. 2006. Polyphagy complicates conservation biological control that targets generalist predators. *Journal of Applied Ecology* 43: 343–352.
- Preisser EL, Bolnick DI. 2008. The many faces of fear: comparing the pathways and impacts of nonconsumptive predator effects on prey populations. *PLoS One* 3: e2465. doi: 10.1371/journal.pone.0002465
- Preisser EL, Bolnick DI, Benard MF. 2005. Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology* 86: 501–509.
- R Core Team. 2019. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rasband WS. 2016. ImageJ. US National Institutes of Health, Bethesda, Maryland, USA.
- Rosenheim JA, Kaya HK, Ehler LE, Marois JJ, Jaffee BA. 1995. Intraguild predation among biological-control agents - theory and evidence. *Biological Control* 5: 303–335.
- Rypstra AL, Buddle CM. 2013. Spider silk reduces insect herbivory. *Biology Letters* 9: 20120948. doi: 10.1098/rsbl.2012.0948



- Saha N, Aditya G, Banerjee S, Saha GK. 2012. Predation potential of odonates on mosquito larvae: implications for biological control. *Biological Control* 63: 1–8.
- Sanders D, Platner C. 2007. Intraguild interactions between spiders and ants and top-down control in a grassland food web. *Oecologia* 150: 611–624.
- Schindelin J, Rueden CT, Hiner MC, Eliceiri KW. 2015. The ImageJ ecosystem: an open platform for biomedical image analysis. *Molecular Reproduction and Development* 82: 518–529.
- Schmidt MH, Roschewitz I, Thies C, Tscharrntke T. 2005. Differential effects of landscape and management on diversity and density of ground-dwelling farmland spiders. *Journal of Applied Ecology* 42: 281–287.
- Schmitz OJ, Beckerman AP, O'Brien KM. 1997. Behaviorally mediated trophic cascades: effects of predation risk on food web interactions. *Ecology* 78: 1388–1399.
- Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH image to ImageJ: 25 years of image analysis. *Nature Methods* 9: 671–675.
- Schuch S, Platner C, Sanders D. 2008. Potential positive effect of the ant species *Lasius niger* on linyphiid spiders. *Journal of Applied Entomology* 132: 375–381.
- Seagraves MP, McPherson RM. 2006. Monitoring red imported fire ant (Hymenoptera: Formicidae) foraging behavior and impact on foliage-dwelling arthropods on soybean produced under three ant suppression regimes. *Journal of Entomological Science* 41: 374–384.
- Sih A, Englund G, Wooster D. 1998. Emergent impacts of multiple predators on prey. *Trends in Ecology & Evolution* 13: 350–355.
- Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene-sequences and a compilation of conserved polymerase chain-reaction primers. *Annals of the Entomological Society of America* 87: 651–701.
- Snyder WE, Wise DH. 1999. Predator interference and the establishment of generalist predator populations for biocontrol. *Biological Control* 15: 283–292.
- Stachowicz JJ, Hay M. 1999. Reduced mobility is associated with compensatory feeding and increased diet breadth of marine crabs. *Marine Ecology Progress Series* 188: 169–178.
- Sterling WL, Jones D, Dean DA. 1979. Failure of the red imported fire ant to reduce entomophagous insect and spider abundance in a cotton agroecosystem. *Environmental Entomology* 8: 976–981.
- Stone JD, Pedigo LP. 1972. Selected bibliography of the green cloverworm, *Plathypena scabra* (Lepidoptera: Noctuidae). *Bulletin of the Entomological Society of America* 18: 24–26.
- Straub CS, Snyder WE. 2006. Species identity dominates the relationship between predator biodiversity and herbivore suppression. *Ecology* 87: 277–282.
- Straub CS, Snyder WE. 2008. Increasing enemy biodiversity strengthens herbivore suppression on two plant species. *Ecology* 89: 1605–1615.
- Thaler JS, Griffin CAM. 2008. Relative importance of consumptive and non-consumptive effects of predators on prey and plant damage: the influence of herbivore ontogeny. *Entomologia Experimentalis et Applicata* 128: 34–40.
- Thaler JS, McArt SH, Kaplan I. 2012. Compensatory mechanisms for ameliorating the fundamental trade-off between predator avoidance and foraging. *Proceedings of the National Academy of Sciences* 109: 12075–12080.
- Traugott M, Bell J, Raso L, Sint D, Symondson WO. 2012. Generalist predators disrupt parasitoid aphid control by direct and coincidental intraguild predation. *Bulletin of Entomological Research* 102: 239–247.
- Venables WM, Ripley BD. 2002. *Modern applied statistics with S*, 4th edition. Springer, New York, USA.
- Villesen P, Mueller UG, Schultz TR, Adams RM, Bouck AC. 2004. Evolution of ant-cultivar specialization and cultivar switching in *Apterostigma* fungus-growing ants. *Evolution* 58: 2252–2265.
- Walzer A, Lepp N, Schausberger P. 2015. Compensatory growth following transient intraguild predation risk in predatory mites. *Oikos* 124: 603–609.
- Welch KD, Harwood JD. 2014. Temporal dynamics of natural enemy–pest interactions in a changing environment. *Biological Control* 75: 18–27.
- Wenninger A, Kim TN, Spiesman BJ, Gratton C. 2016. Contrasting foraging patterns: testing resource-concentration and dilution effects with pollinators and seed predators. *Insects* 7: 23. doi: 10.3390/insects7020023
- Whitehouse MEA, Mansfield S, Barnett MC, Broughton K. 2011. From lynx spiders to cotton: behaviourally mediated predator effects over four trophic levels. *Austral Ecology* 36: 687–697.
- Wickam H. 2016. *ggplot2: Elegant graphics for data analysis*. Springer-Verlag, New York, USA.