

## ORIGINAL CONTRIBUTION

**Comparison of sampling methods of *Aphis glycines* predators across the diel cycle**

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**Keywords**

nocturnal predators, observation, vacuum sampling, video surveillance

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**Abstract**

Nocturnal predators are often overlooked in biological control studies, despite evidence that they can make important contributions to insect pest suppression in agroecosystems. Many sampling methods are only employed during the daytime hours due to limitations of time and labour. Additionally, different sampling methods can provide contrasting information about natural enemy community composition and relative abundance. Here, we use *Aphis glycines* and its arthropod predators as a model system to compare natural enemy community composition described by vacuum samples, direct observations and video observations across the diel cycle in soybean. All sampling methods identified several common taxa. Anthocorids were dominant in vacuum samples and direct observations, and both methods indicated that this taxa may be more active in the afternoon. In contrast, anthocorids were recorded infrequently on video, possibly due to their small size. On video samples, lacewing larvae were the most active taxa during the day and lacewing larvae, spiders, opiliones and carabids were the most active taxa at night. We directly observed 22 predation events on soybean aphid: 17 by anthocorids, two by chrysopid larvae, and one each by a coccinellid, spider and predatory mite. The differences between the sample methods suggest that vacuum samples may represent predator abundance more accurately, while video data may miss small predators, but can be used to better assess relative time spent foraging.

**Introduction**

The soybean aphid, *Aphis glycines*, is an invasive insect pest from China with the potential to cause significant yield losses in soybean (DiFonzo and Hines 2002). Since *A. glycines* was first discovered in the United States in 2000, it has been the subject of much study (Ragsdale et al. 2011). A number of studies have focused on identifying the natural enemies that attack *A. glycines* (Rutledge et al. 2004; Allard and Yeargan 2005; Mignault et al. 2006; Hajek et al. 2007; Kaiser et al. 2007; Pike et al. 2007; Hannam et al. 2008; Noma and Brewer 2008) and their relative efficacy (Fox et al. 2005; Desneux et al. 2006; Costamagna and Landis 2007). However, most of these studies have focused on the diurnal predator community

within soybean fields. For example, Costamagna and Landis (2007) conducted direct observations of soybean aphid predation within soybean fields, between 10:00 and 21:00 h.

In contrast, little work has examined the effect of nocturnal natural enemies on soybean aphid populations, despite the fact that these taxa have been demonstrated to make important contributions to pest suppression in soybean fields. For example, nocturnal nabids were the most abundant predator and were responsible for up to half of *Helicoverpa zea* egg predation observed in soybean fields in Kentucky (Pfannenstiel and Yeargan 2002). Similarly, nocturnal spiders were responsible for 98% of lepidopteran egg predation in soybean fields in Texas (Pfannenstiel 2008) and have been observed foraging at similar

frequencies as diurnal coccinellid beetles in Michigan soybean fields (L. Petersen and J.M. Woltz unpublished data). Spiders can be effective natural enemies, and as an important component of the natural enemy guild (Riechert and Lockley 1984; Sunderland 1999), they are capable of suppressing aphid populations (Gavish-Regev et al. 2009). Additionally, spiders can cause trophic cascades, with higher spider density leading to more pest consumption and lower leaf damage in soybean (Carter and Rypstra 1995; Rypstra and Carter 1995). Taken together, these studies suggest the potential importance of nocturnal predators to biological control of pest insects, and the need to examine this potential with nocturnal sampling.

Multiple sampling methods are available for assessing natural enemy communities. The efficacy of different methods varies with the vagility and mode of dispersal of taxa sampled (Schmidt et al. 2008). Additionally, various trade-offs exist between spatial and temporal coverage of sampling methods and the type of information each can generate. Pedigo and Buntin (1993) provide a thorough review of comparisons between various arthropod sampling methods. Briefly, relative sampling methods such as pitfall traps, sweep netting, vacuum sampling and sticky traps can be employed repeatedly over large areas and can provide information about natural enemy community composition and relative density for multiple locations. However, these methods provide no information about the actual density or foraging behaviour of various taxa. On the other hand, absolute sampling methods like visual examination of a given number of leaves, whole plants or a specific area provide absolute density information and can elucidate specific interactions between natural enemies and pests. However, these methods are time-consuming and thus are typically limited in the length of observation periods and the number of observations possible. In contrast to these temporal limitations, video observations can be made continuously, although equipment costs usually limit spatial coverage (Grieshop et al. 2012). Grieshop et al. (2012) provide a side-by-side comparison of the strengths and weaknesses of camera observations versus visual examinations.

Given the potential for soybean aphid suppression from the nocturnal arthropod community and relative strengths and weaknesses of different sampling methods, our objective was to compare the predator communities described by vacuum sampling, direct observations, and video observations across the diel cycle.

## Materials and Methods

### Study locations

Direct predator observations were conducted between 6 June and 13 July 2012 in three V1-V5 stage soybean fields in southern Michigan (table 1). The study was conducted at this time because early- to mid-season predator/prey dynamics are thought to be particularly important for preventing outbreaks of soybean aphid (Rutledge et al. 2004). Direct predator observations were carried out over 24-h period and repeated on three separate occasions in each field, for a total of  $n = 9$  observation surveys. Fields were conventionally managed without systemic insecticide-treated soybean seed. Agriculture in this region consists primarily of annual field crops (corn, soy and wheat) and perennial forage crops (pasture and alfalfa), while non-crop areas are primarily deciduous forest. Video observations were conducted around the clock on eight soybean plants in a fourth 1 ha soybean field on the Michigan State University Department of Crop and Soil Sciences Research Farm from 22 to 29 June 2012 (table 1). Vacuum sampling was conducted in all four fields.

### Aphid infestation

Predators were observed on aphid-infested soybean plants in both direct and video observations. Ten aphids of mixed age classes, including adults and variously sized nymphs, were transferred to a single leaf in the uppermost fully opened trifoliolate of selected plants using a fine-haired paint brush. Aphids were placed in clip cages according to the methods of Fox et al. (2005), on the underside of the selected leaf, as is typical of aphid colonies on small soybean plants. Aphids were allowed to settle for at least 1 h before cages were removed. *Aphis glycines* used in this study were reared under conditions of predator exclusion in  $1.8 \times 3.7 \times 1.8$  m field cages (Lumite Inc, Baldwin, GA) in soybean fields at the Michigan State University Department of Entomology Research Farm, East Lansing, MI. Soybean leaves containing aphids were removed from rearing cages and transferred to experimental fields in coolers. Before infestation, plants were searched for naturally occurring soybean aphids, and any found were removed.

### Predator vacuum sampling

Predators were sampled on the foliage and ground beneath soybean plants using a leaf vacuum (BG 56

**Table 1** Field locations and characteristics for direct and video observation of *Aphis glycines* predator communities and predation events

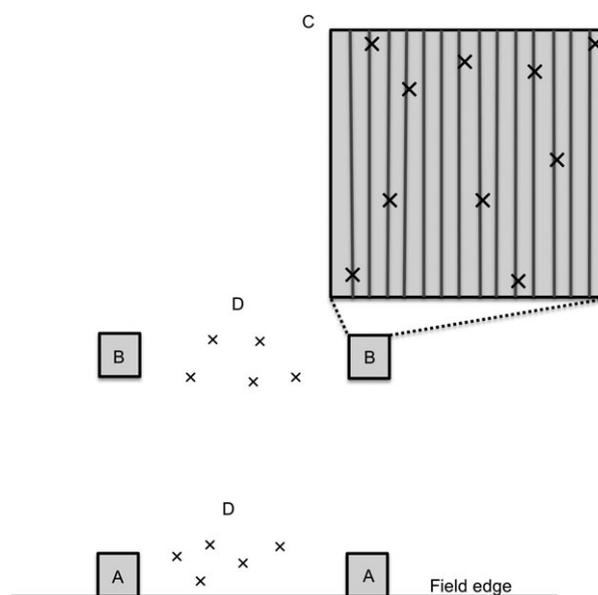
Methods	Location	Coordinates	Field size (ha)	Survey dates
Direct observation, vacuum	Frankenmuth, MI	43 24' 37.77" N 83 42' 06.90" W	10	6–12 July
Direct observation, vacuum	Westphalia, MI	42 51' 57.77" N 84 48' 04.87" W	14	16–20 July
Direct observation, vacuum	Mason, MI	42 37' 38.58" N 84 25' 50.15" W	32	10–13 July
Video observation, vacuum	East Lansing, MI	42 42' 51.86" N 84 27' 52.31" W	1	22–29 July

C-E; Stihl, Waiblingen, Germany) to suck arthropods off of plants into fine mesh collection bags. Vacuum samples were collected in the morning (9:00–10:00), afternoon (15:00–16:00) and at night (22:00–0:00) on each sample date of observations. Workers walked along transects with the vacuum tube placed at the tops of the soybean plants. Arthropods were sucked into the tube and were captured in 1-gallon mesh paint strainer bags placed inside the mouth of the tube and secured with rubber bands. After each collection, the mesh bags were secured with rubber bands and placed into a 18.9-l bucket with a lid, containing Plaster of Paris soaked in ethyl acetate to kill the insects. Predatory arthropods were identified to family or order in the laboratory. Voucher specimens were deposited in the Albert J. Cook Arthropod Research Collection at Michigan State University.

In the three fields in which direct observations were conducted, vacuum samples were collected from ten 20 m transects. In the smaller field in which video observations were conducted, vacuum samples were collected from five 20 m transects. All vacuum samples were collected at least 50 m away from plants under observation to avoid removing predators that might have otherwise colonized the plants during subsequent sampling periods.

### Direct predator observations

In the three fields in which direct observations were conducted, four 10 × 10 m plots were established for soybean aphid predator observations (fig. 1). Each plot encompassed 14 rows of soybean planted on a row spacing of 76.2 cm. Two 'edge' plots were situated between 0 and 10 m into the field, while two 'interior' plots were situated between 50 and 60 m into the field. The plots were at least 50 m from other field edges in all other directions. Within each plot, ten plants were randomly selected for aphid infestation (fig. 1). Observations were conducted for three days in each field, and a different set of soybean plants was randomly selected and infested on each day.



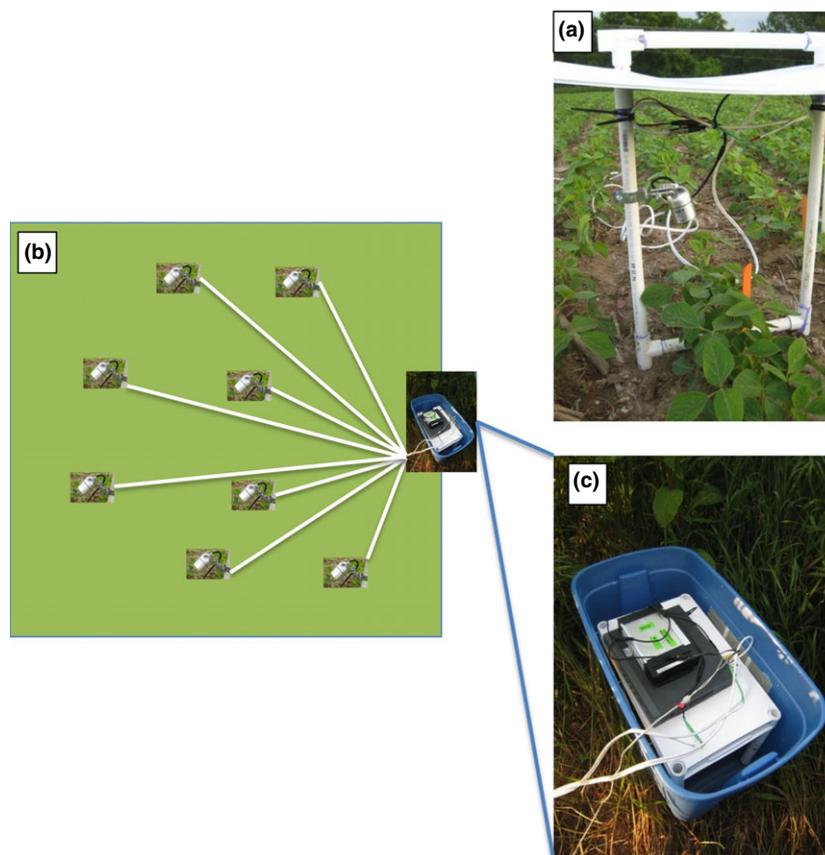
**Fig. 1** Diagram of plot set-up for direct observations. (a) Two edge plots were situated from 0–10 m into the field. (b) Two interior plots were situated from 50–60 m into the field. All plots were 50 m away from all other field edges. (c) Expanded view of plot demonstrated random selection of plants for aphid infestation. (d) Caged plants located in between plots were used to account for mortality in the absence of predation.

To study nocturnal predators, one interior and one edge plot were infested between 18:00 and 20:00 h, with clip cages removed at dusk (approximately 21:00–22:00 for each of our surveys). To study diurnal predators, a second set of interior and edge plots were infested between 3:00 and 5:00, with clip cages removed at sunrise (approximately 6:00 for each of our surveys). This assured that time since aphid colony establishment was held constant for nocturnal and diurnal observations. Aphid-infested plants were surveyed for predators every 3 h for 24 h, at 0:00, 3:00, 6:00, 9:00, 12:00, 15:00, 18:00 and 21:00. Each plant was visually inspected for one to 3 min, while aphids were counted on all plant surfaces. Any predatory arthropods observed on the infested leaves were

recorded as well as whether or not they were observed feeding on *A. glycines*. Whenever possible, observers angled their heads to make observations on the undersides of leaves without disturbing arthropods. Leaves that were too close to the ground to observe in this manner were turned over, in which case predation may have been disrupted and only the presence of the predator could be recorded. Ten plants were observed in two separate sampling locations (edge and interior) for each of the two infestation periods (pre-dusk and pre-dawn) within each of the three replicate surveys conducted in each of the three fields, resulting in 360 plants surveyed at each time period ( $10 \times 2 \times 2 \times 3 \times 3 = 360$ ). The 0:00 and 3:00 time periods were not replicated in pre-dawn infested plots, however, so 180 plants were observed at these time periods ( $3 \times 3 \times 2 \times 10 = 180$ ). The 6:00 samples approximately coincided with dawn, and the 21:00 samples coincided with dusk. At night, predators were counted using Tactikka Plus LED headlamps (Petzl, Crolles, France) with red filters to minimize disturbance to nocturnal insects, according to the methods of Allard and Yeorgan (2005).

### Video observations

Digital surveillance equipment was used to record predators foraging on soybean plants 24 h/day, adapting the methods of Grieshop et al. (2012). Eight waterproof outdoor video surveillance cameras (QOCD36, Q-See.com, Anaheim, CA) were deployed in a soybean field. The cameras were distributed in a semi-circle around the central digital video recorder (DVR; fig. 2). Due to the small size of the field in which video observations were conducted, no distinction was made between observations conducted near the field edge or interior. Cameras were suspended 10 cm above the tops of focal soybean plants from PVC frames placed perpendicular to soybean rows and anchored with rebar (fig. 2). The cameras were connected by coaxial cables to a multichannel H.264 Network DVR (Security Hardware Store, LLC, Boise, ID), where video was recorded onto a 500GB Seagate SV35.5 internal hard drive. The cameras and DVR were powered by a pair of marine batteries linked in parallel, by replacing the DVR's AC/DC adapter with positive and negative terminal connectors. The DVR and batteries were housed



**Fig. 2** Video observation set-up. (a) Aphids and natural enemies were recorded with video surveillance cameras suspended from PVC frames. (b) Eight cameras were arranged radially around a DVR. (c) The DVR was powered by two marine batteries and connected to a removable hard drive rack that allowed video files to be taken to the laboratory for uploading and viewing.

in a 75.7 l plastic storage tub with lid to keep the equipment dry. Holes were cut in the sides of the tub to feed power and coaxial cables through and to allow for ventilation to prevent the DVR from over-heating. Cameras were moved up the frames as needed to maintain a height of 10 cm above the soybean canopy. Cameras were pointed at the uppermost trifoliate and adjusted twice daily to account for changes in the plant's position. Video could be viewed as it was being recorded on a 18-cm digital LCD TV (Haier, New York, NY) connected to the output channel of the DVR. This allowed us to confirm that we recorded the desired part of the plant.

### Video processing

After 7 day of recording, the video files were transferred from the hard drive in the DVR to an external hard drive (Fantom GreenDrive, MicroNet, Torrance, CA) and converted to AVI files for viewing. Laboratory assistants watched four videos at a time at 4× speed to identify frames with arthropod activity, after which arthropod activity was viewed one video at a time at 1× speed. The taxonomic identity (Family or Order), arrival time and departure time of each arthropod visible on any part of the focal plant was recorded. Arthropods of the same taxonomic group that left and re-entered the screen within 5 min were considered a single individual. An arthropod of the same taxonomic group that re-entered the screen after a period of >5 min was recorded as a separate individual.

### Statistical analysis

Analysis of Similarity (ANOSIM, PRIMER 6.1.3; Primer Enterprises Ltd., Ivybridge, Devon, UK) was used to describe differences in the predator community for two data sets: the vacuum samples and predator observations from video, according to the methods of Clarke and Warwick (2001). First, a Bray–Curtis Similarity Index was calculated for each pair of samples, indicating the degree of similarity in community composition between the samples. A value of zero indicates complete dissimilarity (no common species) and a value of 100 represents completely similar samples (both samples have the same species in the exact same abundances). The similarity between a given sample and every other sample is ranked, and this is repeated for each sample. ANOSIM is conducted on the rank similarities, with the null hypothesis that site and time of day have no effect on the rank similarities between samples. An *R* statistic is calculated (analo-

gous to an *F* statistic in ANOVA). The significance of the *R* statistic is calculated by randomly reassigning samples to different sites and times of day and recalculating the *R* statistic. This is repeated 999 times, and the number of times that an *R* statistic greater than or equal to the one calculated for the actual data is achieved by chance is divided by the number of permutations to achieve a significance level. The vacuum samples from all four fields were analysed by time of day (morning, afternoon, evening) and site after preliminary analyses indicated no community differences between edge and interior samples in direct observation fields. For the video data, the predator community observed during the day (6:00–21:00) was compared with the predator community observed at night (21:00–6:00). When differences were found between predator communities, data were assessed visually to determine taxa likely responsible for the differences. The abundance of these taxa during different times of day was then compared using ANOVA (PROC GLIMMIX, SAS 9.2; SAS Institute Inc., Cary, NC).

### Results

Across all methods, 10 families of predatory insects from four orders and two orders of predatory arachnids were sampled (table 2). Most taxa were sampled via all collection methods. However, cantharids and syrphids were only sampled via direct observations and elaterids, and staphylinids were only sampled with vacuuming. Nabids were viewed on video and collected in vacuum samples but were not directly observed.

**Table 2** Predatory taxa captured with each sampling method

	Video	Vacuum sampling	Direct observations
Arachnids			
Araneae	X	X	X
Opiliones	X	X	X
Coleopterans			
Cantharidae			X
Carabidae	X	X	X
Coccinellidae	X	X	X
Elateridae		X	
Lampyridae	X	X	X
Staphylinidae		X	
Hemipterans			
Anthocoridae	X	X	X
Nabidae	X	X	
Other			
Chrysopidae	X	X	X
Syrphidae			X

### Direct predator observations

Predators were observed infrequently during our direct observations. We directly observed 68 predator individuals foraging on our infested soybean plants during a total of 2520 observations (table 3). Anthocorid nymphs and adults were the most abundant predator observed (n = 44 individuals), followed by spider adults and immatures (eight individuals) and coccinellid adults (six individuals). With the exception of anthocorids, which were most frequently observed in the afternoon hours, other predators were not observed at sufficient frequency to ascertain diel patterns.

Additionally, we directly observed 22 predation events on soybean aphid. Of these events, seventeen were by anthocorids, two by chrysopid larvae and one each by a coccinellid, spider and predatory mite. All directly observed predation events occurred during daylight hours, except for one predation event by a spider which occurred at 0:00 hours.

### Vacuum samples

Spiders and anthocorids were the most abundant predatory arthropods captured in vacuum samples. In 67 vacuum samples, we collected 923 anthocorids and 618 spiders and 14 coccinellids. ANOSIM

indicated that the predator community sampled differed significantly among fields ( $R = 0.409$ ,  $P = 0.001$ ) and by time of day ( $R = 0.191$ ,  $P = 0.001$ ). Predator communities were not different between the morning (10:00) and afternoon (16:00) samples ( $R = 0.107$ ,  $P = 0.08$ ) but were significantly different between night samples (22:00) and morning or afternoon samples ( $R = 0.206$ ,  $P = 0.007$ ;  $R = 0.265$ ,  $P = 0.001$ , respectively). More anthocorids and nabids were collected in the afternoon (ANOVA; table 4). Elaterids were found only at night, and coccinellids and staphylinids were found only during the day (table 5).

### Video data

We recorded 792 h of video of aphid-infested soybean plants, including three to six replicate days of video data following each of the eight plants for each 3-h time period (tables 6 and 7). Intermittent technical difficulties with our video-recording equipment prevented continuous recording for the full seven-day period. Aphid colonies persisted on seven of the plants for the duration of the study. The colony on the 8th plant went to 0 for 27 h near the end of the study. After initial infestation, aphid colonies on plants fluctuated over the 7 day of recording. On average, there were 15.8 aphids on a plant at any given time, with a

**Table 3** : Predators directly observed during 24 h aphid surveys. Observations were made 180 times at 0:00 and 3:00 h, and 360 times at all other time periods

	6:00	9:00	12:00	15:00	18:00	Day total	21:00	0:00	3:00	Night total
Araneae	1	0	1	1	0	3	0	4	1	5
Opiliones	1	0	0	0	0	1	0	0	0	0
Cantharidae	0	1	0	0	0	1	0	0	1	1
Carabidae	2	0	0	0	0	2	0	0	1	1
Coccinellidae	0	1	1	0	2	4	0	1	1	2
Lamyridae	1	0	0	0	0	1	0	0	0	0
Anthocoridae	5	5	9	11	7	37	1	3	3	7
Chrysopidae larvae	0	0	0	0	2	2	0	0	0	0
Syrphidae larvae	1	0	0	0	0	1	0	0	0	0

Dataset	Taxon	Diel comparison	
Vacuum samples	Anthocoridae	Afternoon > morning	$t_{64} = 2.51$ , $P = 0.01$
		Afternoon > night	$t_{64} = 3.12$ , $P = 0.003$
	Nabidae	Afternoon > morning	$t_{64} = 2.54$ , $P = 0.01$
		Afternoon > night	$t_{64} = 2.98$ , $P = 0.004$
Video samples	Carabidae	Night > day	$t_{262} = 3.00$ , $P = 0.003$

**Table 4** Diel comparisons (ANOVA) in predatory taxa from vacuum and video samples

**Table 5** Mean  $\pm$  SE number of predator individuals collected in vacuum samples at morning (9:00–10:00), afternoon (15:00–16:00), and night (22:00–0:00)

	Sample times		
	Morning	Afternoon	Night
Araneae	10.53 $\pm$ 2.77	8.88 $\pm$ 1.84	8.52 $\pm$ 1.12
Opiliones	1.11 $\pm$ 0.48	2.00 $\pm$ 0.84	1.04 $\pm$ 0.48
Carabidae	0.05 $\pm$ 0.05	0.28 $\pm$ 0.17	0.17 $\pm$ 0.14
Coccinellidae	0.37 $\pm$ 0.14	0.28 $\pm$ 0.15	0 $\pm$ 0
Elateridae	0 $\pm$ 0	0 $\pm$ 0	0.48 $\pm$ 0.15
Lampyriade	0.11 $\pm$ 0.07	0.04 $\pm$ 0.04	0.17 $\pm$ 0.17
Staphylinidae	0.47 $\pm$ 0.32	0.20 $\pm$ 0.10	0 $\pm$ 0
Anthocoridae	10.00 $\pm$ 2.49	21.96 $\pm$ 4.32	8.00 $\pm$ 1.78
Nabidae	0.53 $\pm$ 0.19	3.68 $\pm$ 1.30	0.48 $\pm$ 0.22
Chrysopidae larvae	0.53 $\pm$ 0.30	0.52 $\pm$ 0.20	0.78 $\pm$ 0.38

maximum of 93 aphids on one plant. The change of aphid numbers on a plant over a 3-h period ranged from a decrease in 29 aphids to an increase in 47

aphids, with a mean change of  $-0.04$ . No alatae were observed and soybean plants were small and not touching, making it likely that changes in aphid colonies were due to birth and mortality rather than aphids dispersing off of the focal soybean plants.

Chrysopid larvae were the most common predator observed on video ( $n = 41$ ), followed by spiders ( $n = 22$ ), carabids ( $n = 29$ ) and coccinellids ( $n = 8$ ). Additionally, 35 coleopterans that could not be identified to family level were observed. The predator community observed on video did not differ significantly between time periods ( $R = 0.032$ ,  $P = 0.31$ ), or broadly between night and day ( $R = 0.029$ ,  $P = 0.29$ ), although there were more carabids observed foraging on soybean plants during the night (ANOVA; table 4). Additionally, opiliones were only present at night, and coccinellids were only present during the day (tables 6 and 7). Lacewing larvae were the most abundant predators observed during the day. Carabids, spiders, opiliones and lacewing larvae were the most abundant predators observed at night.

**Table 6** Mean  $\pm$  SE number of predator individuals observed in daylight hours during each 3-h period of video surveillance

	6:00–9:00	9:00–12:00	12:00–15:00	15:00–18:00	18:00–21:00	Day total
Replicate days video	3	3	4	6	5	
Araneae	0 $\pm$ 0	0.21 $\pm$ 0.10	0.13 $\pm$ 0.13	0.04 $\pm$ 0.03	0.03 $\pm$ 0.03	0.07 $\pm$ 0.03
Opiliones	0 $\pm$ 0					
Carabidae	0.08 $\pm$ 0.06	0.08 $\pm$ 0.06	0.06 $\pm$ 0.04	0 $\pm$ 0	0.05 $\pm$ 0.03	0.05 $\pm$ 0.02
Coccinellidae	0 $\pm$ 0	0.21 $\pm$ 0.10	0 $\pm$ 0	0.02 $\pm$ 0.02	0.05 $\pm$ 0.03	0.05 $\pm$ 0.02
Coccinellidae larvae	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0.03 $\pm$ 0.03	0.01 $\pm$ 0.01
Lampyridae	0.04 $\pm$ 0.04	0 $\pm$ 0	0.06 $\pm$ 0.04	0.04 $\pm$ 0.03	0 $\pm$ 0	0.03 $\pm$ 0.01
Anthocoridae	0.04 $\pm$ 0.04	0 $\pm$ 0	0 $\pm$ 0	0.06 $\pm$ 0.04	0.15 $\pm$ 0.09	0.06 $\pm$ 0.02
Nabidae	0 $\pm$ 0	0.08 $\pm$ 0.06	0 $\pm$ 0	0.02 $\pm$ 0.02	0 $\pm$ 0	0.02 $\pm$ 0.01
Chrysopidae	0 $\pm$ 0	0.04 $\pm$ 0.04	0 $\pm$ 0	0 $\pm$ 0	0.03 $\pm$ 0.03	0.01 $\pm$ 0.01
Chrysopidae larvae	0.04 $\pm$ 0.04	0.38 $\pm$ 0.19	0.13 $\pm$ 0.07	0.13 $\pm$ 0.06	0.25 $\pm$ 0.10	0.18 $\pm$ 0.04

**Table 7** Mean  $\pm$  SE number of predator individuals observed in night hours during each 3-h period of video surveillance

	21:00–0:00	0:00–3:00	3:00–6:00	Night total
Replicate days video	5	4	3	
Araneae	0.10 $\pm$ 0.05	0.06 $\pm$ 0.04	0.17 $\pm$ 0.10	0.10 $\pm$ 0.03
Opiliones	0 $\pm$ 0	0.19 $\pm$ 0.08	0.21 $\pm$ 0.10	0.11 $\pm$ 0.04
Carabidae	0.20 $\pm$ 0.13	0.31 $\pm$ 0.13	0.13 $\pm$ 0.07	0.22 $\pm$ 0.07
Coccinellidae	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Coccinellidae larvae	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Lampyridae	0.03 $\pm$ 0.03	0 $\pm$ 0	0 $\pm$ 0	0.01 $\pm$ 0.01
Anthocoridae	0 $\pm$ 0	0 $\pm$ 0	0.04 $\pm$ 0.04	0.01 $\pm$ 0.01
Nabidae	0.10 $\pm$ 0.05	0.03 $\pm$ 0.03	0 $\pm$ 0	0.05 $\pm$ 0.02
Chrysopidae	0.05 $\pm$ 0.03	0 $\pm$ 0	0.04 $\pm$ 0.04	0.03 $\pm$ 0.02
Chrysopidae larvae	0.08 $\pm$ 0.04	0.22 $\pm$ 0.12	0.04 $\pm$ 0.04	0.11 $\pm$ 0.04

## Discussion

Our study allows us to compare methods of predator sampling over the diel cycle. Data generated by vacuum samples, direct observations and video observations show both broad similarities and key differences. Several predatory taxa were identified via all three methods: Araneae, Opiliones, Carabidae, Coccinellidae, Lampyridae, Anthocoridae, and Chrysopidae. However, a few taxa were only recorded via one sampling method. Staphylinids and elaterids were collected in vacuum samples but not observed directly or on video. The absence of these taxa on video may be due to difficulty in identifying them. In fact, several dark beetles were grouped as 'unknown coleopterans' because it was impossible to make a more specific identification. However, the fact that these taxa were also not observed in direct observations suggests that they may forage infrequently on soybean plants. We sampled during the early- to mid-season when the soybean plants were relatively small, making it likely that vacuums collected dirt, plant debris and arthropods from the ground as well from plants. In contrast, syrphid larvae were directly observed but were not observed on video or collected in vacuum samples. These small larvae were very difficult to identify clearly on video. Furthermore, soft-bodied insects like larvae can be damaged in vacuum collections, making them difficult to locate in the samples.

Additionally, although several taxa were identified in all three methods, vacuum sampling and video recordings sometimes gave different information about the relative abundance of these taxa. For example, spiders and anthocorids were the most abundant predatory arthropods collected in vacuum samples, while chrysopid larvae, carabids, spiders and opiliones were the most abundant taxa observed on video. These differences could be due to multiple factors. Anthocorids, being small (1–5 mm) were likely difficult to identify on video and under-recorded via this method. Direct observations provided corroborating evidence that anthocorids are numerically dominant predators on soybean plants. For the larger, easier to identify taxa, the differences could reflect differences in relative abundance versus time spent foraging. For example, chrysopid larvae may have had lower overall abundances within the field, but would be captured more frequently on video if they spent more time foraging than more abundant taxa. Similar differences between overall abundance and foraging have been found for ground-foraging predators in other systems. In a study in which carabids and chilopods represented the majority of captures in pit-

fall traps, these taxa were infrequently recorded consuming sentinel prey (Grieshop et al. 2012). Additionally, many insects including carabids and chrysopids remain longer and search more intensively after discovering prey, increasing their chances of encountering additional prey in that area (Bond 1980; Lövei and Sunderland 1996). Carabids and chrysopids encountering the aphid colonies being recorded likely initiated such intensive search behaviours, possibly explaining why these taxa were captured on video more often than would be expected by their overall abundances in the soybean fields.

In addition to differences in abundance, some taxa showed different diel patterns across different methods. More carabids were observed on video at night, while no difference was found in carabids collected in vacuum samples at different times of day. The vacuum samples may have collected carabids that were present in fields but not actively foraging at the time, while the video would have only recorded actively foraging carabids. On the other hand, anthocorids were more abundant in afternoon vacuum samples. Anthocorids may have been more active at mid-day when temperatures were warmer (Zhang and Shipp 1998). Although it would be expected that anthocorids would rest in fields when not foraging, they avoid resting in exposed locations (Shields and Watson 1980). It is possible that they are less susceptible to vacuum sampling when in more protected plant crevices. Taken together, these differences suggest that the accuracy of a given sampling method may change throughout the diel cycle as activity levels and the location of taxa change. In general, vacuum sampling may provide more accurate information about presence and overall abundance of predators, while video data may better reflect foraging activity. The foraging-activity data generated by video sampling may be especially relevant for biological control as it could better reflect the likelihood that a given natural enemy taxon will encounter a prey item.

We were unable to reliably detect aphid predation on the videos because the light green aphids did not contrast well with the soybean leaves. Additionally, in some cases, aphids dispersed from the focal point of the camera to other parts of the plant. In these cases, predation could have happened off-screen. Given these limitations, video surveillance may have limited usefulness for recording predation of mobile prey. In contrast, predation events on immobile prey including coleopteran, dipteran and lepidopteran eggs, lepidopteran pupae, and overwintering cocooned lepidopteran larvae have been successfully recorded (Merfield et al. 2004; Grieshop et al. 2012; Walton 2013).

Others have immobilized mobile prey like lepidopteran larvae by gluing or pinning them to a surface (Frank et al. 2007; Grieshop et al. 2012). Cameras in these studies are often aimed at Petri dishes on the ground (Merfield et al. 2004; Frank et al. 2007; Grieshop et al. 2012) but have also been focused on tree limbs (Walton 2013). Additionally, Phillips (2013) was able to successfully monitor the pollinator community visiting pumpkin flowers by immobilizing the flowers with pins for the duration of the video. Furthermore, the studies cited above have used video observations in blueberry and apple orchards, pumpkin and corn fields, vineyards, prairies and hedgerows. When considering video sampling in a given system, it will be important to consider the mobility of both the target prey and the habitat and the degree of difficulty in immobilizing both for video surveillance. With these considerations in mind, the wide variety of focal objects and habitats successfully observed indicate the flexibility of video observation methods.

Sampling methods used in this study each have strengths and weaknesses. Arthropods collected in vacuum samples are easier to identify than those observed on video, because they can be viewed under a microscope in the laboratory and keyed out if necessary. Vacuum samples may also collect more small insects that are not clearly identifiable on video and may be repeated over large areas. However, vacuum samples can collect organisms present in the field, regardless of whether or not those organisms are actively foraging or resting at the time. Video and direct observation data, on the other hand, can provide valuable information about which predatory taxa are actually interacting with prey and how often. While direct observations can allow clear identifications and provide information about predator-prey interactions, these are time and labour-intensive and the number of direct observations possible in a given study will often be limited. Video cameras can be run continuously with infrequent labour to check connections and replace batteries, providing around the clock information about natural enemy activity. Although our analyses focused on broad differences between nocturnal and diurnal activity, these methods could easily be adapted, for example, to examining behaviour of crepuscular insects. Although sorting through video data can be quite time-consuming, it can be performed in the laboratory at a later date and does not require labour in the field at specific times. Given the complementary nature of the strengths and weakness of the insect sampling methods discussed, using multiple methods in concert can provide a more detailed understanding of the insect community.

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