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**For Submission to Crop Protection**

**Title**: Invertebrate communities in spring wheat and the identification of cereal aphid predators through molecular gut content analysis.

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**Abstract**

Cereal aphid complexes are responsible for reducing spring wheat production worldwide. Generalist predators may contribute to reducing cereal aphid numbers and preventing significant damage to crops. A two-year survey identifying the arthropod community on wheat vegetation, at the soil surface and within the soil of wheat fields was conducted to better guide conservation efforts. The arthropod complex in wheat was diverse with 103 taxa identified. The soil-dwelling arthropod community had the greatest abundance and diversity when compared with the foliar-dwelling community. Sentinel *Rhopalosiphum padi* L. (bird cherry-oat aphid, BCOA) were placed on wheat plants and predator gut-content analysis employed to identify specific species actively consuming cereal aphids. Twenty five percent of collected predators tested positive for *R. padi* DNA in their guts. The diverse and abundant predatory arthropod community reduced cereal aphid numbers, which remained at low densities throughout the duration of the study.

**Keywords:** wheat, cereal aphids, *Rhopalosiphum padi*, gut content analysis, bioinventory

## 1. Introduction

Wheat is the fourth most widely planted agricultural crop in the U.S. with 18.7 million ha harvested in 2014, with South Dakota producing 1.14 million metric tons of spring wheat in 2012 (NASS, 2012; 2014). Insecticidal treatments within spring wheat are rare, with only 12% of fields nationally treated with insecticide sprays in 2012 (NASS, 2012). Although insecticides are not common in many regions, cereal yields can be reduced by a suite of aphid species throughout the Northern Great Plains (Kieckhefer et al., 1994; Riedell et al., 2007). The cereal aphid complex in South Dakota wheat includes *Rhopalosiphum padi* L. (bird cherry-oat aphid, BCOA), *Schizaphis graminum* (Rondani) (greenbug), *Sitobion avenae* (F.) (English grain aphid) and *Diuraphis noxia* Kurdjumov (Russian wheat aphid) (Kieckhefer and Kantack, 1980; Hesler et al., 2005; Riedell et al., 2007). During the seedling and boot stages, densities of 30-40 aphids per plant reduce wheat yields significantly (Kieckhefer and Kantack, 1980). Yield loss is the result of feeding damage, as well as the transmission of Barley yellow dwarf virus by cereal aphid populations (Riedell et al., 2003). Despite the fact that the literature reports significant losses from cereal aphids, attempts during this 2-year study to infest wheat plots with one of the most abundant pests of wheat in our region, *R. padi* (Kieckhefer and Kantack, 1980; Riedell et al., 2003), repeatedly failed, possibly due to natural enemy abundance.

A variety of generalist predators inhabit cereal crops, including spring wheat, and contribute to reducing cereal aphid numbers below economically damaging population levels (Kuusk et al., 2008; Brewer and Elliott, 2004; Schmidt et al., 2003; Sunderland et al., 1987). Reported predators in wheat include spiders, specifically lycosids (Kuusk et al. 2008) and linyphiids (Sunderland et al., 1986), lacewing larvae, carabids, staphylinids (Schmidt et al., 2003), and several species of adult and larval coccinellids (Chen et al., 2000; Schmidt et al., 2003; Brewer and Elliott, 2004; Hesler et al., 2004; Hesler and Kiekhefer, 2008). Fuente et al. (2003) identified 19 beneficial species inhabiting Argentinean wheat fields. Exclusion experiments demonstrate significant increases in aphid populations when both ground-dwelling and flying predators were excluded from aphid populations (Schmidt et al., 2003). Populations of polyphagous predators vary by year (Chambers et al., 1983), tillage treatments (Rice and Wilde, 1991), and seed treatment prevalence (Seagraves and Lundgren, 2012). Additionally, the ability of species to control aphid populations may be negatively impacted by seed treatments (Bredeson et al., 2015). Direct observations, ELISA (enzyme-linked immunosorbent assay), gut dissections and PCR-based gut-content analysis have been employed to understand cereal aphid consumption by generalist predators. Sunderland et al. (1987) report 62% of predatory species collected had consumed aphids in winter wheat as determined by gut dissections and ELISA. Knowledge regarding the relative contributions of the predator community to aphid suppression allows for further conservation research.

Previous surveys of wheat insect communities employ a single method of sampling such as sweepnets or pitfall trapping (Elliott et al., 1998; Hesler et al., 2000; Fuente et al., 2003; Schmidt et al., 2003). Additional studies focus on how diversification of cropping systems influences predator and/or pest communities (Elliott et al., 1998). Comprehensive surveys of the complete arthropod community throughout agroecosystems are rare, but necessary for establishing an understanding of the food webs throughout these systems. A search of the peer-reviewed literature indicates that a system-wide bioinventory of the arthropods in a North American wheat field has not previously been published. In conjunction with a general bioinventory of arthropods, direct observations of predation events (Chang and Snyder, 2004) combined with molecular gut content analysis of predators (Harwood and Obrycki, 2005) further our understanding of insect community dynamics throughout wheat systems. The objectives of this study are to establish a comprehensive record of the insect communities in South Dakota spring wheat, and identify key predators that reduce aphid population numbers.

## 2. Methods

### *2.1. Wheat fields*

Sixteen untreated spring wheat fields were established within four 169 × 34 m alfalfa fields (Pioneer 54V54 variety; a 3 year stand) during the summer of 2011 and 2012 on the South Dakota Soil and Water Conservation Research Farm operated by USDA-ARS near Brookings, SD (44.349722, -96.803056). Brigg's Hard Red spring wheat plots (37 × 24 m) surrounded by a 4.5 m border of alfalfa were planted at a rate of 117 kg/ha with 19 cm spacing on May 4, 2011 and April 10, 2012. A starter fertilizer (NPK: 14-36-13) was applied with the drill at planting at a rate of 106 kg/ha. Immediately after planting, 3.25 L/ha glyphosate (RoundUp®, Monsanto, St. Louis, MO) was applied to kill the alfalfa and prevent it from competing with the spring wheat crop. Three weeks after planting, 295 ml/ha dicamba (Clarity®, BASF, Triangle Park, NC) plus 12.5 ml/ha thifensulfuron (Harmony®, DuPont™, Wilmington, DE) were applied for additional weed control. Within each plot, a sampling grid of 35 numbered points (5 × 7) was established with each point separated by 5 m from all other points.

*2.2. Invertebrate community assessment*

Invertebrates were sampled at randomly selected grid points. Sampling was conducted in 16 fields in 2011 weekly from June 9 to July 26 (seven sampling dates), and in eight fields in 2012 bi-weekly from May 31 to July 11 (four sampling dates).

Soil-dwelling invertebrate communities (predators and pests) were assessed using two methods on each sample date. Surface-dwelling invertebrates were sampled using a quadrat comprised of a sheet metal frame (0.5 × 0.5 m; 15 cm tall) that was inserted into the ground, and all visible insects within the top 1 cm of soil were collected with a mouth aspirator (Lundgren and Fergen, 2010). In year one (2011), two quadrats were sampled per plot during three sampling dates (9, 30, June, 21 July), and in year two four quadrats were sampled per plot during all four sampling dates (30 May, 12, 21 June, 11 July 2012). In addition to the quadrats, soil cores (10 cm diameter and 10 cm deep) were used to sample invertebrates in the soil column in 2011. These cores were collected on 16 June, 7, 26 July, and four cores were taken from randomly selected grid points in each plot on each sampling date; invertebrates were extracted from cores into 70% ethanol in Berlese funnels.

Foliar-dwelling insect communities (predatory invertebrates and pests) were assessed using two methods on each sample date. First herbivore and predator populations were recorded from whole plant counts conducted during both years. Wheat plants within a 30 × 30 cm quadrat were observed for 5 min around four randomly selected grid points within each plot. All invertebrates were collected from the entire wheat plant using a mouth aspirator during five sampling events in year one (9, 16, 30 June, 21, 26 July 2011), and four sampling events in year two (31 May, 12, 21 June, 11 July 2012). In 2011, foliar communities were also sampled with sweepnets. Three 9 m long transects were established down the rows of wheat and swept with a 38-cm diameter net during two sampling dates (16 June, 7 July 2011). These transects were centered along the long sides of each plot. Two of the three transects were 3 m into the wheat from the alfalfa border and the third was 12 m into the plot.

All samples were placed on ice in the field and returned to the laboratory, where they were preserved for identification. Invertebrates were identified to species level when possible, using appropriate keys (carabid beetles: Lindroth, 1966; ants: Fisher and Cover, 2007) or the authors' extensive taxonomic experience in working with arthropod communities with cropland of Eastern South Dakota.

*2.3. Predation on cereal aphids*

In 2012, five exclusion cages were placed in eight plots for a 10 d period (5 June -15 June and 22 June -2 July). Cages were placed over two wheat plants (cleaned of endemic insects) at Zadoks' stage 45 (approximately when the boot head was swollen within the sheath; Zadoks et al., 1974). Cages were placed at varying distances from the alfalfa border and along a 26 m transect that began in the center of the long side of the plots and extended to the back plot corner. Cages measured 0.4 m high and 0.15 m in diameter and were covered with a fine mesh that restricted aphid movement and excluded predators. Barley clippings with 20 laboratory-reared *R. padi* (13:11 L:D; 19.0ºC, 18.0ºC) were placed at the base of caged wheat plants. Soil from the edges of the plots was mounded around the cage base to prevent predators from entering. Aphids remaining in the cages were counted after 10 d.

Sentinel aphids were placed near all five cages in each plot. At each cage location, aphids were placed on individual wheat plants 0.3 m to the N, S and E of the cage on 6 June and 2 July (Gardiner et al., 2009; Blaauw and Isaacs, 2012). A total of 15 sentinel locations were within each plot. Ten *R. padi* from the same laboratory colony were gently placed in 1.5 mL capsules. One open capsule was wired to each wheat plant and the aphids allowed to climb onto plants for 60 min. These resulting sentinel aphids were monitored for the first 24 h post-establishment, with observations conducted every 3 h (0000, 0300, 0600, 0900, 1200, 1500, 1800, 2100 h). During this monitoring, sentinel aphids were observed and any predators near the aphids were collected using a mouth aspirator. If predators could not be collected, the identity was recorded. At the end of 24 h, the total number of sentinel aphids on each wheat plant within each plot was recorded, as well as the number of aphids that remained within the capsule on the plant. Predators collected from these plants were immediately frozen at -20° C in 70% ethanol.

The DNA of each predator was extracted using DNeasy® Blood and Tissue Extraction Kit (QIAGEN, Valencia, California, USA). Prior to extraction, all predators were surface washed for 10 s in 10% aqueous sodium hypochlorite. All extractions were stored at -20ºC. Primers identified by Chen et al. (2000) were optimized to amplify a *R. padi*-specific DNA sequence and screened against nontarget arthropods (forward- TTCGACTCTTAATTTCATCA; reverse- GGATTGCATCAATTTTAATAGCTAAA). PCR (25 µL) reactions were composed of 9.5 µL of molecular-grade water (Sigma-Aldrich, St. Louis, Missouri, USA), 12.5 µL 2× Brilliant SYBR Green qPCR Master Mix (Qiagen), 225 nmol/L of each primer, and 1 µL template DNA. Extractions were amplified using a MX3000P qPCR system (Strategene, La Jolla, California, USA) with the following thermal cycles: 15 min at 95º C, 15 s at 94º C, 30 s at 53º C (annealing temperature), 30 s at 72º C. Fluorescence was recorded at 492 nm to quantify SYBR Green during the annealing step of each cycle. Five positive (*R. padi* DNA) and three negative (water) controls were run with each PCR. The unique melting temperature of the *R. padi* amplicon was used to verify the specificity of the results, and the primer sets were tested for cross reactivity against 48 other non-target species (including five other aphid species) from this study system.

### *2.4. Data Analysis*

#### *2.4.1. Predator Community*

All sampling dates were combined to establish a season-long estimate of the community captured with each sampling method and the mean number of each arthropod group collected per plot calculated. Rarefaction analyses were conducted to determine the ability of each sample method (soil cores, quadrats, sweeps, and whole plant dissections) to fully capture the predicted arthropod communities (Analytic Rarefaction 1.3; Holland, 2003). Data for the rarefaction analysis were based on pooled community assessments across all replicate fields (Holland, 2003). The Chao 1 metric (S) was used to predict how many taxa were expected from each plot given the community assemblage (Chao, 1984).

#### *2.4.2. Aphid Predation*

The mean number of remaining aphids exposed to and protected from predators was calculated per plot. For sentinels, this included aphids remaining on the plant and in the original capsule. The mean percentage of aphids consumed was calculated by using the number of aphids available for predation (e.g., those that moved onto the plant out of the capsule). A one-way ANOVA was used to determine if the number of predators observed varied by observation times. Tukey's HSD tests were conducted to separate means. All statistical comparisons were conducted using Systat 13 (SYSTAT Software Inc., Richmond, CA, USA).

## 3. Results

A total of 43,435 invertebrates representing 103 taxa were collected over the 2-yr sampling period. Nine insect orders were represented by more than three individuals: Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Neuroptera, Orthoptera, Plecoptera and Thysanoptera. Non-insect arthropod orders collected include: Acari, Annelida, Araneae, Chilopoda, Collembola, Diplopoda, Diplura, Opiliones, and Pseudoscorpionida. Mean numbers of individuals collected per field and taxonomic groups varied among sampling methods (Tables 1-4). Soil-dwelling invertebrates were more abundant than foliar communities. Soil cores had the greatest number of individuals collected per field (Table 1); which is most likely due to the large number of mites within the soil. When mites are excluded from the core samples, the mean number of individuals collected per $m^2$ extrapolates out to 7,059 (± 601) in the top 10 cm of soil; this is twenty times greater than the mean number of individuals collected per $m^2$ on the soil surface (Table 2). The mean number of foliar-dwelling insects collected per field by sweeping (Table 3) and whole plant counts (Table 4) was approximately 10% of the total specimens collected.

Sixty nine taxa were represented in quadrats and 59 taxa in soil cores. Rarefaction analysis (Figure 1) and Chao 1 estimators (± SD) predict greater species diversity in soil cores (64.0 ± 26.5) than quadrats (50.6 ± 18.1). Chao 1 estimates indicate slightly less diversity per plot for soil-dwelling arthropod species than rarefaction analysis for the entire community (pooled across plots) (Figure 1). Foliar-dwelling invertebrates were represented by 44 taxa observed during whole plant counts and 28 taxa collected with sweep nets. Similar to estimates for soil-dwelling taxa, rarefaction curve (Figure 2) indicate greater species diversity for whole plant counts and sweep net samples than Chao 1 estimates 35.5 (± 12.1) and 26.7 (± 12.9), respectively. Rarefaction estimates that species diversity in whole plant counts should be similar to that of quadrat samples, suggesting that additional sampling of foliar-dwelling communities is necessary to gain a greater understanding of the diversity.

Sampling efforts aimed at collecting soil-dwelling invertebrates resulted in greater diversity of taxa per field than foliar-dwelling collection methods. Despite demonstrating the greatest overall diversity of taxa, quadrat sampling resulted in fewer species per field than core samples, 31.7 ± 1.9 and 38.0 ± 1.2, respectively. The foliar-dwelling communities were less diverse with a mean of 19.9 ± 0.8 taxa identified per field during whole plant counts and 17.7 (± 0.7) taxa identified in sweep net samples.

Soil-dwelling species varied between the two sampling methods. Mites (770.4 ± 67.3) and Collembola (275.4 ± 32.8 per field) were collected in greatest abundance in soil core samples in 2011. *Lasius neoniger* had the third greatest abundance in cores (664.5 ± 125.3 per $m^2$) and it was the most abundant taxon in quadrats on the soil surface (3.0 ± 0.4 per $m^2$). Collembola were the second most abundant taxon in quadrats (Table 2), but were much less prevalent on the soil surface when compared to within the soil. Additionally, two other Formicidae groups were common throughout plots within soil cores, *Solenopsis* sp. and an unknown species (57.9 (± 43.2), 30.0 (± 3.3)). Spiders were one of the most abundant predators collected in quadrat samples (Table 2); individuals were less frequently collected in soil cores.

Abundances of the foliage-dwelling community varied with sampling type, with sweep net collections being dominated by Thysanoptera (Table 3). Spiders and *L. neoniger* individuals were the second and third most abundant taxa in sweep net collections. *Lasius neoniger* was the most common species collected during whole plant counts with a mean of 7.7 (± 1.3) individuals collected within 30 by 30 cm areas of wheat. A greater number were collected with sweep net sampling (8.9 ± 2.1); however, a much greater area of the plot was sampled. Aphids were detected on wheat plants in both sample types and were the third most abundant taxon in sweep net samples (Tables 3 and 4). Predators on wheat plants included *L. neoniger* discussed previously, as well as an unknown ant species, *Nabis americoferus* and Araneae. Collembola were one of the most abundant groups in both whole plant and sweep net observations.

*3.1. Aphid Predation:*

Of the 20 original aphids placed within each cage, an average of 45.00 ± 14.35% of aphids per cage per date remained when predators were excluded. The mean percent of aphids within cages after the 10 d periods beginning 5 June 2012 and 22 June 2012 were 51.00 ± 15.45 and 38.50 ± 15.45%, respectively, and did not vary significantly.

Of the 10 original aphids that were exposed to predators (i.e., outside of the cages), an average of 24 ± 12% aphids per location per date remained on wheat plants after 24 h, and this was consistent across sample dates. After the 10 d period no aphids were recovered from infested plants. Seventy seven predators were observed or collected near sentinel *R. padi* during the two 24 h observation periods. Predators were collected in all plots with 3 being the fewest collected in a plot and 19 the greatest. The within-plot location of sentinels had no observable influence on the presence of predators. Forty four of the specimens were collected and analyzed, with 25.0% containing *R. padi* DNA within their stomachs (Table 5). Four *Hippodamia convergens* and two mites were collected during sentinel observations, all of which tested positive for aphid DNA in their stomachs. The most abundant predators observed were members of the group Araneae; however, only a single specimen tested positive for *R. padi* DNA.

Mean number of predators observed varied significantly by time of day ($F = 3.73$, $df = 7$, $P = 0.042$). The greatest number of predators were observed and collected at 6:00 am (0.10 ±0.02 predators per plant), which was statistically more than observed at 12:00 am and 3:00 pm (0.01 ± 0.01 and 0.02 ± 0.01 per plant, respectively). Numbers of predators observed during all other collection times were not statistically different from these times or one another. The most common predators at 6:00 am were *L. neoniger* (N = 6), phalangiids (N = 5) and spiders (N = 5). Mites were only observed at 6:00 pm and at least one spider was collected or observed during each sampling time.

## 4. Discussion

This study reveals a diverse wheat community containing multiple beneficial species that contributed to pest management. Bioinventories of agroecosystems are an important tool for conservation and pest management research. Similar, thorough diversity studies are rare, making comparisons between agricultural production systems difficult. The majority of the 103 identified taxa were not pest insects of wheat, with great diversity of formicid and coccinellid species and an abundance of mites and collembola. The bioinventories presented here are well replicated, but only within a narrow geographic region. More extensive species inventories throughout wheat production areas would aid in understanding how well this inventory represents wheat communities under other conditions. Also, examining different field sizes from the ones selected may affect the resident arthropod community, and we recommend including larger fields in future bioinventories. Agroecosystems currently represent up to 40% of the terrestrial land surface of the planet (FAO, 2011), and this necessitates that biodiversity promotion efforts work closely with farmers and land managers. As climates, land use, farm management practices, etc. change, understanding how these changes affect local arthropod communities and their interactions in key crops will aid the resiliency of our food production systems.

Foliar-dwelling predator communities were dominated by *L. neoniger* ants, (common in both sweepnet and whole plant counts), Nabidae, Opiliones, and spiders. Spiders frequently have high abundance in wheat foliage (Rice and Wilde, 1991; Hesler et al., 2000; Schmidt et al., 2004; von Berg et al., 2009) and have been documented preying on cereal aphids in the field (Sunderland et al., 1987; Kuusk et al., 2008;). Oelbermann and Scheu (2009) report improved wheat growth in microcosms when spiders were present in high (10) and low (5) densities. Hesler et al. (2000) report that nabids were the most abundant aphidophagous insect group collected in a 4-year study throughout spring wheat-alfalfa plots in South Dakota. Similar to our surveys, other studies found lacewings (Chambers et al., 1983; Hesler et al., 2000) and a variety of coccinellid species such as *H. convergens, C. septempunctata* and *H. parenthesis* (Hesler et al., 2000) to be abundant predators.

Although invertebrates were abundant and diverse in spring wheat foliage, soil arthropod communities were much more diverse. Ninety percent of total specimens and 85% of species collected were represented in quadrat and core samples. A similar comprehensive study in soybeans demonstrates greater diversity in pitfall (13 families) and quadrat (17 families) samples than in sweepnet samples (5 families) (Lundgren et al., 2013). Common ground-dwelling and soil-dwelling predators include spiders, mites, *L. neoniger* and *Soleonpsis* sp. Ant-aphid mutualisms can result in an increase in aphid abundance; however, aphid-tending was not observed at any time throughout this 2-year study, and aphid consumption by *L. neoniger* was demonstrated through molecular gut content analysis (Table 5). Other studies recognize the role of ants as aphid predators (Sakata, 1994, 1996; Offenberg, 2001). *Lasius neoniger* individuals were the most abundant ground-dwelling species and the third most abundant soil-dwelling species; thus, their role in controlling aphid pest populations in wheat is worth further investigation. Spiders, also one of the most abundant predators on wheat foliage, were collected in even greater numbers on the soil surface. Snyder and Ives (2003) observed an immediate decrease in pea aphid populations when cages were open to ground-dwelling generalist predators, demonstrating that soil predators can be important sources of pest management within the plant canopy. The tremendous diversity of soil invertebrates in agroecosystems and their potential in providing pest management services necessitates that we better identify and understand the linkages between soil and foliar communities.

Despite variation in abundance, both foliar and soil-dwelling predators have been observed to reduce aphid population numbers in winter wheat (Holland et al., 2012). It is likely with the diversity of predators observed, that they contributed to the rapid decline in incipient aphid populations.  However, previous studies demonstrate that natural enemies may not be solely responsible for reduction in aphid numbers (Carter et al., 1982), and additional factors such as weather (Carter and Dixon, 1981) and species specificity of predators (Macfadyen et al., 2009) influence establishment of pests. Natural cereal aphid populations were undetectable throughout plots, individuals disappeared quickly when sentinel aphids were placed onto vegetation, and gut analysis of predators identified a suite of species that consumed the sentinel aphids in the field. It is important to note that aphid numbers inside and outside the cages differed initially, and aphids were placed on the plants with slightly different approaches (in both cases the aphids were allowed to walk onto the infested plants on their own), which may have influenced their relative survival rates in addition to predation. Results from cage studies presented here and elsewhere (Chambers et al., 1983; Snyder and Ives, 2003; Holland et al., 2012) demonstrate the importance of predators in reducing aphid population numbers. Cage studies conducted by Schmidt et al. (2003) showed no variation in aphid population numbers as a result of predator presence or absence during wheat flowering; however, during milk-ripening a 44% increase in cereal aphid populations was observed when ground-dwelling predators were excluded and a 102% increase when flying predators were excluded. Wheat stages varied from wheat flowering to ripening (Zadoks et al., 1974) between the two sentinel sample dates, with the greatest number of predators positive for cereal aphid DNA collected during wheat flowering. *Rhopalosiphum padi* DNA was detected in 25.0% of predators analyzed and seven species.  Rates of prey DNA detection in the guts of field collected generalist predators vary among studies with 11% of collected predators containing western corn rootworm DNA (Lundgren et al., 2009) and 26% of lycosid spiders containing *R. padi* DNA (Kuusk et al., 2008). *Hippodamia convergens* most frequently tested positive for aphids, with all individuals collected testing positive for aphid DNA in their guts. Results of this research also demonstrate that the predator community in spring wheat operates throughout the 24 h diel cycle. Thus, studies that focus only on the photophase may be missing important predation events (e.g., Brust et al., 1986; Pfannenstiel and Yeargan, 2002; Lundgren et al., 2010). Our study demonstrates the diversity of invertebrates throughout wheat agroecosystems and identifies some of the key predators within this system.

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**Table 1.** Invertebrates collected in 2011 soil cores. The mean number of each taxonomic group collected per field in a total of 192 soil cores (each core representing 0.0078 $m^2$) over the season. Groups infrequently collected (< 3 specimens) are presented as a footnote. A total of 16 fields were sampled. Shaded species represent the five most commonly collected taxonomic groups.

| Taxonomy | Common name | Mean ± SEM per field (number of fields collected) |
|---|---|---|
| Coleoptera | Unknown beetle | 8.44 ± 1.58 (16) |
| | Unknown beetle larva | 4.69 ± 1.34 (15) |
| | Little brown beetle A | 0.44 ± 0.26 (4) |
| | Little brown beetle B | 1.56 ± 0.49 (8) |
| Coleoptera: Anthicidae | Unknown anthicid | 4.31 ± 1.09 (14) |
| Coleoptera: Cucujidae | Unknown cucujid | 1.38 ± 0.41 (10) |
| Coleoptera: Curculionidae | Weevil | 0.25 ± 0.14 (3) |
| Coleoptera: Dytiscidae | Diving water beetle | 0.25 ± 0.11 (4) |
| Coleoptera: Carabidae | Unknown carabid adult | 2.75 ± 0.71 (13) |
| | Unknown carabid larva | 5.63 ± 0.89 (15) |
| | *Bembidion* sp. | 1.44 ± 0.51 (11) |
| | *Bembidion rapidum* | 1.00 ± 0.35 (7) |
| | *Bembidion quadrimaculatum* | 0.44 ± 0.27 (3) |
| | *Elaphropus* sp. | 0.25 ± 0.14 (3) |
| | *Polyderus* sp. | 0.56 ± 0.18 (7) |
| Coleoptera: Staphylinidae | Unknown rove beetle | 10.44 ± 1.38 (16) |
| Coleoptera: Coccinellidae | Unknown lady beetle | 0.44 ± 0.32 (3) |
| | *Scymnus* sp. | 0.56 ± 0.24 (5) |
| Hymenoptera: Parasitica | Parasitoid wasp | 1.25 ± 0.31 (11) |
| Hymenoptera: Formicidae | Unknown ant adult | 30.00 ± 3.25 (16) |
| | Unknown ant larva | 1.00 ± 0.81 (3) |
| | Unknown ant pupae | 6.81 ± 6.42 (3) |
| | *Crematogaster* sp. | 3.75 ± 3.75 (1) |
| | *Ponera* sp. | 4.56 ± 1.48 (15) |
| | *Ponera exotica* | 0.31 ± 0.12 (5) |
| | *Myrmica sculptilis* | 0.31 ± 0.15 (4) |
| | *Lasius* sp. | 6.50 ± 4.12 (5) |
| | *Lasius alienus* | 1.25 ± 0.78 (5) |
| | *Lasius neoniger* | 62.63 ± 11.81 (16) |
| | *Solenopsis* sp. (subgenus *Diplorhoptrum*) | 57.88 ± 43.23 (12) |
| | *Solenopsis molesta* | 0.50 ± 0.33 (3) |
| Hemiptera | Unknown Hemiptera adult | 4.06 ± 0.85 (15) |
| | Unknown Hemiptera nymph | 16.00 ± 3.76 (15) |
| | Unknown Homoptera adult | 0.56 ± 0.44 (3) |
| | Unknown Homoptera nymph | 0.31 ± 0.20 (3) |
| Hemiptera: Aphididae | Unknown aphid | 1.19 ± 0.44 (8) |
| Hemiptera: Cicadellidae | Leafhopper | 19.06 ± 4.08 (16) |
| Hemiptera: Membracidae | Treehopper | 1.13 ± 0.56 (6) |
| Hemiptera: Nabidae | *Nabis americoferus* | 0.81 ± 0.21 (10) |
| Hemiptera: Miridae | Unknown mirid | 0.56 ± 0.22 (6) |
| Hemiptera: Corixidae | Water bug | 0.38 ± 0.27 (2) |
| Diptera | Unknown Diptera adult | 22.00 ± 3.24 (16) |
| | Unknown Diptera larva | 7.25 ± 1.53 (14) |

| | | |
|---|---|---|
| Diptera: Culicidae | Unknown mosquito | 16.88 ± 3.07 (16) |
| Diptera: Syrphidae | Hoverfly | 1.62 ± 0.56 (8) |
| Neuroptera: Chrysopidae | Unknown lacewing larva | 0.69 ± 0.62 (2) |
| Plecoptera | Unknown stonefly adult | 2.25 ± 0.70 (10) |
| Lepidoptera | Unknown Lepidoptera adult | 0.25 ± 0.11 (4) |
| | Unknown Lepidoptera larva | 3.38 ± 2.47 (8) |
| Thysanoptera | Thrips | 17.81 ± 2.72 (16) |
| | | |
| Araneae | Spider | 7.63 ± 0.79 (16) |
| Acari | Mite | 770.44 ± 67.28 (16) |
| Collembola | Springtail | 275.44 ± 32.76 (16) |
| Diplopoda | Millipede | 5.94 ± 1.39 (12) |
| Chilopoda | Centipede | 0.38 ± 0.15 (5) |
| Protura + Diplura | Unknown proturan + dipluran | 26.18 ± 4.14 (16) |
| Annelida | Earthworm | 3.19 ± 1.44 (12) |
| Pseudoscorpionida | Unknown pseudoscorpion | 0.31 ± 0.25 (2) |
| | | |
| Total invertebrates | | 1435.88 ± 93.11 (16) |

Specimens represented by three or fewer specimens collected included:

Coleoptera: Water beetle (Dytiscidae), Weevil (Curculionidae), Little Brown Beetle B, Flea beetle (Chrysomelidae), Tortoise beetle (Chrysomelidae), click beetle (Elateridae), picnic beetle (Nitidulidae), *Agonum placidum* (Carabidae), *Harpalus* (Carabidae), *Harpalus pensylvanicus* (Carabidae), *Poecilus lucublandus* (Carabidae), *Stenolophus comma* (Carabidae), *Hippodamia convergens* (Coccinellidae), *Hippodamia tredecimpunctata* (Coccinellidae), Hymenoptera: *Formica fusca* grp (Formicidae), *Myrmica* sp. (Formicidae), *Myrmica americana* (Formicidae), Winged formicidae, Hemiptera: water boatman (Corixidae), *Lygus lineolaris* (Miridae), stink bug (Pentatomidae), Trichoptera: caddisfly, Ephemeroptera: mayfly, Orthoptera: grasshopper (Acrididae), *Allonemobius* (Gryllidae), Psocoptera: Book louse, Isopodae: sow bug.

**Table 2.** Invertebrates collected in 2011 and 2012 in quadrats. The mean number per plot of each taxonomic group collected in 224 quadrats (each quadrat representing 0.25 m$^2$) over the season. Groups infrequently collected (< 3 specimens) are presented as a footnote. A total of 24 fields were sampled over both years of study. Shaded species represent the five most commonly collected taxonomic groups.

| Taxonomy | Common name | Mean ± SEM per plot (number of plots collected) |
|---|---|---|
| Coleoptera | Unknown beetle | 0.75 ± 0.29 (6) |
| | Unknown beetle larva | 0.13 ± 0.09 (2) |
| | Little brown beetle (C) | 0.88 ± 0.24 (11) |
| Coleoptera: Anthicidae | *Leptoremus* sp. | 0.21 ± 0.10 (4) |
| Coleoptera: Curculionidae | Weevils | 1.54 ± 0.49 (14) |
| Coleoptera: Lampyridae | *Pleotomodes* sp. | 0.58 ± 0.15 (11) |
| Coleoptera: Meloidae | *Epicauta* sp. | 0.38 ± 0.19 (4) |
| Coleoptara: Carabidae | Unknown carabid adult | 0.63 ± 0.19 (9) |
| | Unknown carabid larva | 0.21 ± 0.10 (4) |
| | *Bembidion* sp. | 0.25 ± 0.14 (4) |
| | *Bembidion quadrimaculatum* | 1.67 ± 0.56 (8) |
| | *Elaphropus* sp. | 0.38 ± 0.15 (8) |
| Coleoptera: Staphylinidae | Unknown rove beetle | 0.50 ± 0.17 (8) |
| Coleoptera: Coccinellidae | Unknown lady beetle adult | 0.33 ± 0.16 (3) |
| | Unknown lady beetle larva | 0.42 ± 0.16 (7) |
| | Unknown lady beetle pupa | 0.21 ± 0.10 (4) |
| | *Coccinella septempunctata* | 0.29 ± 0.11 (6) |
| | *Hippodamia parenthesis* | 0.25 ± 0.12 (4) |
| | *Hippodamia convergens* | 0.33 ± 0.18 (5) |
| | *Scymnus rubricaudus* | 0.51 ± 0.15 (10) |
| Hymenoptera: Parasitica | Parasitoid wasp | 2.67 ± 0.63 (17) |
| Hymenoptera: Formicidae | Unknown ant | 1.88 ± 1.04 (9) |
| | *Ponera* sp. | 0.21 ± 0.13 (3) |
| | *Formica* sp. | 0.17 ± 0.10 (3) |
| | *Formica* sp. *fusca* grp. | 0.42 ± 0.29 (4) |
| | *Formica subintegra* | 0.58 ± 0.25 (5) |
| | *Myrmica* sp. | 0.29 ± 0.15 (4) |
| | *Myrmica americana* | 3.88 ± 1.41 (13) |
| | *Myrmica sculptilis* | 8.00 ± 1.97 (23) |
| | *Myrmica detritinodes* | 8.54 ± 2.28 (14) |
| | *Lasius* sp. A | 3.83 ± 1.84 (7) |
| | *Lasius* sp. C | 0.83 ± 0.40 (4) |
| | *Lasius* sp. E | 0.38 ± 0.38 (1) |
| | *Lasius alienus* | 6.50 ± 2.40 (13) |
| | *Lasius neoniger* | 65.29 ± 8.69 (24) |
| | *Solenopsis* (subgenus *Diplorhotrum*) | 0.29 ± 0.11 (6) |
| Hemiptera | Unknown adult | 0.38 ± 0.12 (8) |
| | Unknown nymph | 11.04 ± 1.13 (24) |
| | Unknown Homoptera nymph | 0.46 ± 0.24 |
| Hemiptera: Aphididae | Unknown aphid | 3.67 ± 0.53 (23) |
| | *Schizaphis graminum* | 0.21 ± 0.10 (4) |
| | *Sitobion avenae* | 0.33 ± 0.17 (4) |
| Hemiptera: Cicadellidae | Leafhopper adult | 41.71 ± 7.44 (24) |
| | Leafhopper immature | 1.42 ± 1.12 (2) |
| Hemiptera: Membracidae | Treehopper adult | 5.17 ± 1.09 (22) |

| | | |
|---|---|---|
| Hemiptera: Nabidae | *Nabis americoferus* adult | 5.13 ± 0.82 (24) |
| Hemiptera: Miridae | Unknown mirid | 0.29 ± 0.11 (6) |
| | *Lygus lineolaris* | 2.96 ± 0.87 (10) |
| | *Trigonotylus coelestialium* (Kirkaldy) | 3.63 ± 0.76 (20) |
| Hemiptera: Pentatomidae | Stink bug adult | 1.46 ± 0.76 (20) |
| Hemiptera: Geocoridae | *Geocoris* sp. | 2.71 ± 0.91 (9) |
| Hemiptera: Anthocoridae | *Orius insidiosus* adult | 0.17 ± 0.12 (2) |
| Diptera | Unknown flies | 7.17 ± 1.28 (24) |
| Neuroptera: Chrysopidae | *Chrysoperla* sp. larva | 0.33 ± 0.12 (7) |
| Neuroptera: Hemerobiidae | Unknown adult | 0.17 ± 0.08 (4) |
| Lepidoptera | Unknown adult | 0.17 ± 0.08 (4) |
| | Unknown larva | 11.96 ± 3.77 (18) |
| Thysanoptera | Thrips | 1.42 ± 0.63 (11) |
| Orthoptera: Acrididae | Grasshopper | 0.33 ± 0.13 (6) |
| Orthoptera: Gryllidae | *Allonemobius* sp. | 6.88 ± 1.59 (21) |
| | *Gryllus* sp. | 0.58 ± 0.18 (8) |
| | Unknown nymph | 0.88 ± 0.38 (8) |
| | Unknown adult | 1.54 ± 0.64 (6) |
| Diplopoda | Unknown millipede | 1.33 ± 0.47 (9) |
| Chilopoda | Unknown centipede | 0.42 ± 0.15 (8) |
| Araneae | Spider | 38.50 ± 5.68 (24) |
| Opiliones: Phalangiidae | *Phalangium opilio* | 8.75 ± 2.67 (19) |
| Acari | Mite | 15.71 ± 2.81 (24) |
| Collembola | Springtail | 52.00 ± 9.21 (24) |
| Total invertebrates | | 346.46 ± 22.92 (24) |

Specimens represented by three or fewer specimens collected included:

Coleoptera: Larvae (Coleoptera), Little Brown Beetle A (Coleoptera), Little Brown Beetle B (Coleoptera), *Diabrotica undecimpunctata* (Chrysomelidae), *Collops* sp. (Melyridae), Unknown mordellid (Mordellidae), *Amara angustata* (Carabidae), *Bembidion rapidum* (Carabidae), *Harpalus herbivagus* (Carabidae), *Poecilus lucublandus* (Carabidae), *Pterostichus femoralis* (Carabidae), *Brachiacantha* sp. (Coccinellidae), *Coleomegilla maculata* (Coccinellidae), Hymenoptera: *Formica montana* (Formicidae), *Lasius* sp. D (Formicidae), Homoptera: *Rhopalosiphum padi* (Aphididae), Hemiptera: Fulgoroidea, *Adelphocoris lineolatus* (Miridae), Diptera: Diptera larvae, Syrphidae, Odonata: Damselfly, Neuroptera: *Chrysoperla carnea* adult, Protura/Diplura.

**Table 3.** Invertebrates collected in 2011 sweepnet samples. The mean number of each taxonomic group collected in 96 sweeps over the season. Groups infrequently collected (< 3 specimens) are presented as a footnote. A total of 16 fields were sampled over both years of study. Shaded species represent the five most commonly collected taxonomic groups.

| Taxonomy | Common name | Mean ± SEM per field (number of fields collected) |
|---|---|---|
| Coleoptera | Unknown beetle | 0.44 ± 0.16 (6) |
| Coleoptera: Curculionidae | Weevil | 1.81 ± 0.36 (12) |
| Coleoptera: Lampyridae | *Pleotomodes* sp. | 1.63 ± 0.62 (10) |
| Coleoptera: Coccinellidae | *Brachiacantha ursina* | 0.31 ± 0.25 (2) |
| | *Hippodamia convergens* | 0.31 ± 0.22 (2) |
| | *Scymnus rubricaudus* | 0.31 ± 0.18 (3) |
| Hymenoptera: Formicidae | Unknown ant | 0.44 ± 0.27 (3) |
| | *Formica subintegra* | 0.50 ± 0.16 (7) |
| | *Lasius neoniger* | 8.94 ± 2.08 (16) |
| Hemiptera | Unknown Hemiptera immature | 0.69 ± 0.22 (7) |
| Hemiptera: Aphididae | Unknown aphid | 4.25 ± 0.67 (14) |
| | *Rhopalosiphum padi* | 0.50 ± 0.18 (6) |
| Hemiptera: Cicadellidae | Unknown leafhopper | 0.31 ± 0.12 (5) |
| Hemiptera: Nabidae | *Nabis americoferus* | 6.56 ± 0.81 (16) |
| Hemiptera: Miridae | Unknown mirid | 0.63 ± 0.18 (8) |
| | *Lygus lineolaris* | 1.81 ± 0.37 (13) |
| | *Trigonotylus coelestialium* | 4.13 ± 0.85 (13) |
| Hemiptera: Pentatomidae | Unknown stink bugs | 3.38 ± 0.66 (16) |
| Hemiptera: Geocoridae | *Geocoris* sp. | 0.50 ± 0.32 (4) |
| Hemiptera: Anthocoridae | *Orius insidiosus* | 0.56 ± 0.27 (5) |
| Diptera | Unknown Diptera adult | 1.44 ± 0.30 (13) |
| Diptera: Syrphidae | Hoverfly | 0.44 ± 0.16 (6) |
| Neuroptera: Chrysopidae | Lacewing larva | 0.69 ± 0.18 (9) |
| Lepidoptera | Unknown caterpillar | 1.06 ± 0.42 (7) |
| Thysanoptera | Thrips | 78.44 ± 15.02 (16) |
| Araneae | Spider | 9.25 ± 1.02 (16) |
| Opiliones: Phalangiidae | *Phalangium opilio* | 1.25 ± 0.32 (9) |
| Collembola | Springtail | 4.44 ± 1.71 (12) |
| Total arthropod | | 136.88 ± 14.93 (16) |

Specimens represented by three or fewer specimens collected included:

Coleoptera: Picnic beetle (Nitidulidae), Tiger beetle (Cicindellidae), *Harpalus herbivagus* (Carabidae), rove beetle (Staphylidae), lady beetle (Coccinellidae), *Hippodamia tredecimpunctata* (Coccinellidae), *Scymnus rubricaudus* (Coccinellidae), Hymenoptera: *Myrmica* sp. (Formicidae), *Myrmica sculptilis* (Formica), *Lasius alienus* (Formicidae), Hemiptera, Homoptera: Treehopper (Membracidae), Diptera: Culicidae, Odonata: dragonfly, Neuroptera: *Chrysoperla* sp. (Chrysopidae), Acari: Mite, Diplopoda: Millipede.

**Table 4.** Invertebrates collected in 2011 and 2012 on whole plant counts. The mean number of each taxonomic group collected per 448 observations of a 30 × 30 $cm^2$ area. Groups infrequently collected (< 3 specimens total) are presented as a footnote. A total of 24 fields were sampled over both years of study. Shaded species represent the five most commonly collected taxonomic groups.

| Taxonomy | Common name | Mean ± SEM per field (number of fields collected) |
|---|---|---|
| Coleoptera: Coccinellidae | Unknown egg | 0.88 ± 0.88 (1) |
| | Unknown larvae | 0.21 ± 0.08 (5) |
| | Unknown pupae | 0.17 ± 0.10 (3) |
| | *Brachiacantha ursina* (Hatch) | 0.54 ± 0.22 (6) |
| | *Coccinella septempunctata* (L.) | 0.17 ± 0.10 (3) |
| | *Hippodamia convergens* Guérin-Méneville | 0.25 ± 0.14 (4) |
| | *Hippodamia parenthesis* (Say) | 0.21 ± 0.10 (4) |
| Coleoptera: Curculionidae | Weevil | 0.21 ± 0.10 (4) |
| Coleoptera: Lampyridae | *Pletomodes* sp. | 0.96 ± 0.26 (13) |
| Hymenoptera: Formicidae | Unknown ant | 4.88 ± 1.14 (16) |
| | *Formica subintegra* Wheeler (Formicidae) | 0.17 ± 0.08 (4) |
| | *Lasius neoniger* Emery | 7.67 ± 1.32 (24) |
| | *Myrmica Americana* Weber | 0.29 ± 0.11 (6) |
| | *Myrmica detritinodis* Emery | 0.58 ± 0.24 (8) |
| | *Myrmica sculptilis* Francoeur | 0.92 ± 0.26 (11) |
| Hymenoptera: Parasitica | Parasitoid wasp | 0.46 ± 0.16 (7) |
| Hemiptera | Immature Hemiptera | 1.04 ± 0.20 (16) |
| Hemiptera: Anthocoridae | *Orius insidiosus* Say | 0.17 ± 0.08 (4) |
| Hemiptera: Aphididae | Unknown aphid | 6.54 ± 1.06 (23) |
| | *Acyrthosiphon pisum* Harris | 0.17 ± 0.08 (4) |
| | *Rhopalosiphum padi* L. | 4.75 ± 1.19 (15) |
| | *Schizaphis graminum* (Rondani) | 0.25 ± 0.15 (3) |
| | *Sitobion avenae* (Fabricius) | 1.83 ± 0.41 (14) |
| Hemiptera: Cercopidae | Unknown spittlebug | 0.25 ± 0.14 (3) |
| Hemiptera: Cicadellidae | Unknown leafhopper | 4.21 ± 0.70 (22) |
| Hemiptera: Geocoridae | Big-eyed bug | 0.17 ± 0.08 (4) |
| Hemiptera: Membracidae | Unknown treehopper | 3.38 ± 1.10 (12) |
| Hemiptera: Miridae | Unknown plant bug | 0.38 ± 0.13 (7) |
| | *Lygus lineolaris* (Palisot de Beauvois) | 0.25 ± 0.11 (5) |
| | *Trigonotylus coelestialium* (Kirkaldy) | 0.21 ± 0.10 (4) |
| Hemiptera: Nabidae | *Nabis americoferus* Carayon | 1.17 ± 0.21 (17) |
| Hemiptera: Pentatomidae | Unknown stink bug | 0.83 ± 0.18 (14) |
| Diptera | Unknown dipteran adult | 2.21 ± 0.39 (19) |
| Diptera: Culicidae | Mosquito | 0.17 ± 0.10 (3) |
| Diptera: Syrphidae | Hoverfly | 0.29 ± 0.13 (5) |
| Lepidoptera | Caterpillar | 0.54 ± 0.16 (10) |
| Thysanoptera | Thrips | 2.96 ± 0.35 (23) |
| Orthoptera: Acrididae | Grasshopper | 0.42 ± 0.15 (7) |
| Acari | Mite | 1.29 ± 0.29 (15) |
| Araneae | Spider | 5.04 ± 0.55 (23) |

| | | |
|---|---|---|
| Collembola | Springtail | 7.17 ± 1.82 (21) |
| Diplopoda | Millipede | 0.25 ± 0.21 (2) |
| Opiliones: Phalangiidae | *Phalangium opilio* L. | 0.5 ± 0.18 (9) |
| Total invertebrates | | 67.38 ± 5.82 |

Specimens represented by three or fewer specimens collected included:

Coleoptera: Little brown beetle A, Little brown beetle B, Little black beetle, *Bembidion quadrimaculatum* (L.) (Carabidae), *Harpalus* sp. (Carabidae), Unknown lady beetle (Coccinellidae), *Cycloneda munda* Say (Coccinellidae), *Scymnus* sp. (Coccinellidae), *Epicauta* sp. (Meloidae), Unknown melyrid (Melyridae), Sap beetle (Nitidulidae), Unknown rove beetle (Staphylinidae). Hymenoptera: *Formica* sp. A (Formicidae), *Formica* sp. B (Formicidae), *Formica* sp. C (Formicidae), *Formica* sp. E (Formicidae), *Lasius* sp. A (Formicidae), *Lasius* sp. B (Formicidae), *Myrmica* sp. A (Formicidae), *Ponera* sp. (Formicidae). Hemiptera: Unknown waterboatman (Corixidae), *Adelphocoris lineolatus* (Goeze) (Miridae). Diptera: Unknown fly larva. Neuroptera: Green lacewing (Chrysopidae), Lacewing egg (Chrysopidae). Chilopoda: Centipede. Gastropoda: Snail.

**Table 5.** Taxa of predators collected during 24 h observation period of sentinel *R. padi.* The total number of each group collected and the total number that tested positive for *R. padi* DNA.

| Taxonomy | Total Number Collected | Total Number Positive |
|---|---|---|
| Arachnida | | |
| Acari (mites) | 2 | 2 |
| Araneae | 13 | 1 |
| *Phalangium opilio* | 5 | 1 |
| Coleoptera | | |
| Anthicidae | 2 | 1 |
| *Coccinella septempunctata* | 1 | 0 |
| Coccinellidae larvae | 2 | 1 |
| *Hippodamia convergens* | 4 | 4 |
| Staphylinidae | 1 | 0 |
| Hemiptera | | |
| *Geocoris sp.* | 2 | 0 |
| *Nabis americoferus* | 3 | 0 |
| Hymenoptera | | |
| *Lasius neoniger* | 8 | 1 |
| Orthoptera | | |
| *Gryllus sp.* | 1 | 0 |

**Figure Legends**

**Figure 1.** Sample based rarefaction curve for soil core and quadrat sampling methods plotting the cumulative number of species observed versus sampling effort. Error bars represent the 95% confidence interval.

**Figure 2.** Sample based rarefaction curve for whole plant counts and sweepnet sampling methods plotting the cumulative number of species observed versus sampling effort. Error bars represent the 95% confidence interval.

Figure(s)
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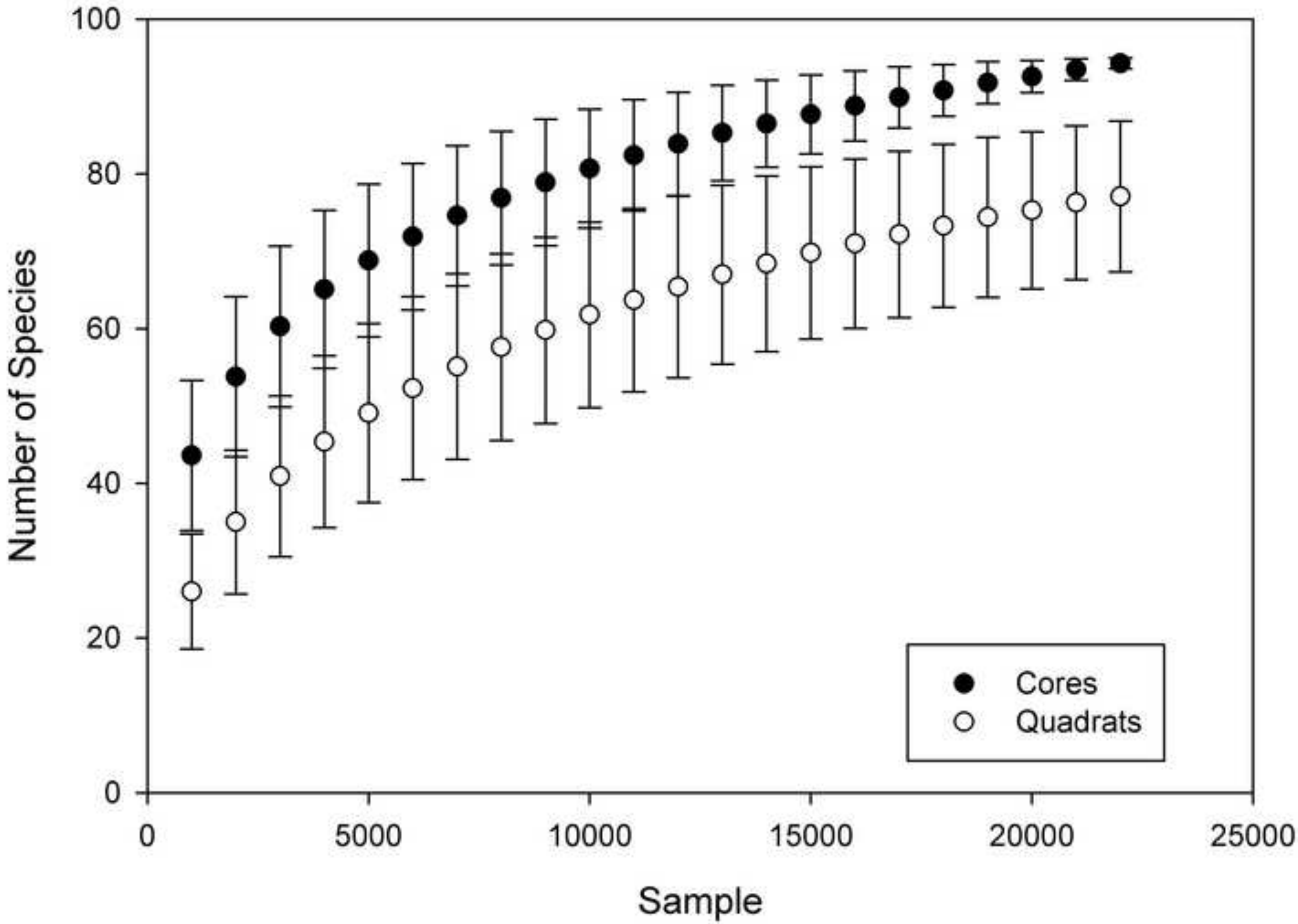

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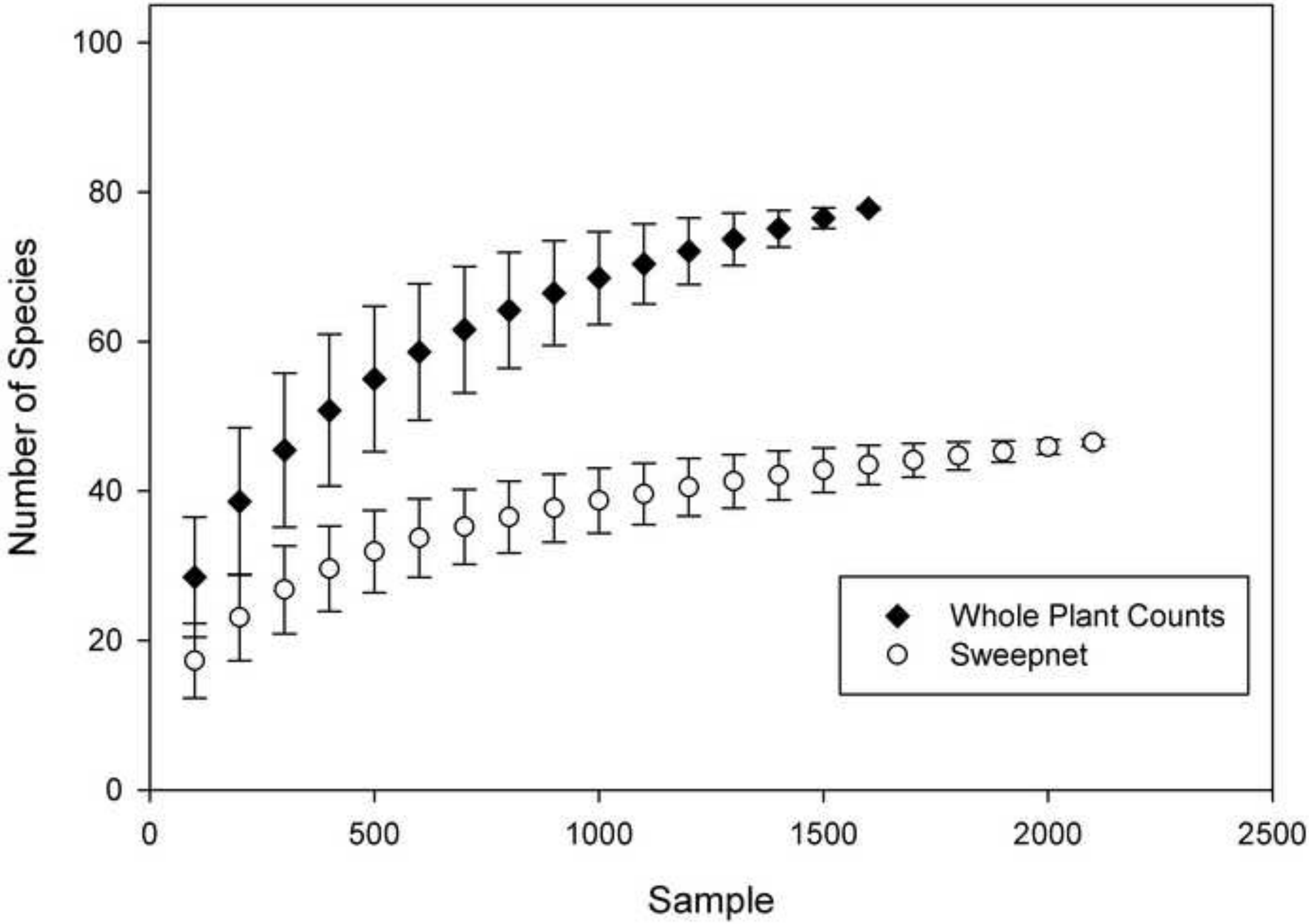

Highlights

- A two-year survey of the arthropod community throughout wheat fields was conducted.
- One hundred and three taxa were identified on wheat vegetation, on the soil surface and in the soil.
- Soil-dwelling arthropods were more abundant and diverse than foliar-dwelling arthropods.
- Predator gut-content analysis was employed to identify predators of *Rhopalosiphum padi*.
- Twenty five percent of collected predators tested positive for *R. padi* DNA in their guts.