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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.

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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

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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; 19.0 °C, 18.0 °C) 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