

THE DIVERSITY OF APHID PARASITOIDS (HYMENOPTERA: BRACONIDAE: APHIDIINAE) IN SOYBEAN FIELDS OF EASTERN SOUTH DAKOTA

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ABSTRACT

Aphid parasitoids provide natural and implemented biological control of numerous pest and native aphid species worldwide. A classical biological control program involving the non-native aphid parasitoid *Binodoxys communis* (Gahan) began in the midwestern United States in 2007 in an attempt to provide some natural control of soybean aphid, *Aphis glycines* Matsumura, populations. No pre-release surveys of native aphid parasitoids in soybean were conducted prior to the beginning of this program. Two field seasons of sampling for aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) in soybean fields of eastern South Dakota were conducted during 2017 and 2018. Sampling was completed using a variety of standard techniques including sweep net and whole plant sampling for parasitized aphids in 2017. In 2018 sampling techniques were expanded to include modified Malaise traps, V-shaped flight intercept traps (V-FIT), and yellow bowl traps, during 12 weeks of continuous sampling. A total of 1,540 wasp specimens were collected. *Lysiphlebus testaceipes* was the only wasp species reared from soybean aphid and comprised 63.6% of the parasitoid diversity in all samples. Species of seven additional genera were represented: *Aphidius* [2-3] spp. (23.7%), *Binodoxys* sp. (0.32%), *Diaretiella rapae* (10.3%), *Praon* sp. (1.03%), *Trioxys* sp. (0.13%), *Ephedrus* sp. (0.13%), and an *Adialytus* sp. (0.78%). None of the latter seven taxa were reared from soybean aphid. Nearby plant diversity likely impacted aphidiine diversity in soybean fields. Malaise traps with non-target insect excluders of mesh fabric were the most effective for estimating species diversity and relative abundance.

Keywords

Taxonomic diversity, identification key, multitrophic associations, *Aphis glycines*, soybean aphid parasitoids, sampling protocol

INTRODUCTION

In the midwestern United States (U.S.) one of the primary insect pests of soybean (*Glycine max* (L.) Merr.) is the soybean aphid, *Aphis glycines* Matsumura (Ragsdale et al. 2004). *Aphis glycines* was first described from *G. max* in Sapporo, Hokkaido, Japan (Matsumura 1917) and is occasionally a significant pest of soybean in its native range of far eastern Asia (Kogan and Turnipseed 1987). In the U.S., the aphid was first detected in Wisconsin during 2000 (Allemann et al. 2002). Since this initial confirmation *A. glycines* is now documented in 22 U.S. states and three Canadian provinces (Hunt et al. 2003; Venette and Ragsdale 2004; Ragsdale et al. 2011). *Aphis glycines* maintains a holocyclic and heteroecious life cycle whereby it supposedly uses the widely-distributed and invasive common buckthorn, *Rhamnus cathartica* L., as a primary and overwintering host, and soybean as a secondary host (Ragsdale et al. 2004). *Rhamnus cathartica* was historically used in shelterbelt plantings in the midwestern U.S. that are often adjacent to soybean fields, which likely exacerbates the soybean aphid pest problem by allowing the aphid immediate and unhindered access to its primary host (Ragsdale et al. 2004).

The primary method of management for *A. glycines* is the use of broad-spectrum insecticides (Olson et al. 2008). Previous research by Ohnesorg et al. (2009) determined that the prophylactic use of insecticides in soybean has a negative impact on predator and parasitoid insect populations and diversity. In addition, such use of insecticides increases the likelihood of insecticide resistance and seasonal pest resurgence (Ohnesorg et al. 2009)

In 2007, a classical biological control program involving the introduction of the non-native aphid parasitoid *Binodoxys communis* (Gahan) was attempted in nine U.S. states (Micijevic et al. 2009; Wyckhuys et al. 2009). This wasp has not been recovered since these initial releases, indicating that the wasp did not successfully establish (Martens and Johnson 2017, 2018). This failure of establishment is likely due to a variety of factors including poor knowledge of the taxonomy, biology, and natural history of the species released, equivocal identification of the wasp species involved, the apparent absence of pre-release surveys for effective native parasitoids, and a questionable release and follow-up protocol that resulted in little to no sampling or post-release monitoring. Intraguild competition between the introduced and native aphid parasitoid species likely also played a role in the biocontrol failure. Additionally, few voucher specimens are available for the *B. communis* that were released in 2007, making accurate confirmation of identity impossible (C. Brabant, Univ. Wisconsin-Madison, pers. comm. 2017; T. McElrath, Illinois Natural History Survey, pers. comm. 2017; L. Musetti, Ohio St. Univ., pers. comm. 2017; G. Fauske, North Dakota St. Univ., pers. comm. 2018; J. Zaspel, Purdue Univ., pers. comm. 2018; R. Thomson, Univ. Minnesota, pers. comm. 2018; G. Parsons, Michigan St. Univ., pers. comm., 2018).

In the U.S., several species of Aphidiinae are recorded as parasitoids of soybean aphid, and most are present at very low densities. These parasitoids include *Aphidius colemani* (Viereck) (Lin and Ives 2003; Kaiser et al. 2007), *Binodoxys communis* (Gahan) (Wyckhuys et al. 2007a, 2007b, 2008, 2009), *B. kelloggensis*

Pike, Starý, and Brewer (Kaiser et al. 2007; Pike et al. 2007), and *Lysiphlebus testaceipes* Cresson (Kaiser et al. 2007; Martens and Johnson 2017, 2018). Some of these species only parasitized *A. glycines* in no-choice laboratory settings and have apparently never been recovered from *A. glycines* in the field. For some species, parasitism occurs, but the wasp larva never fully develops and so prevents the wasp from being an effective biological control agent. Additionally, Nielsen and Hajek (2005) reported two undetermined species of *Praon* Haliday that were reared from *A. glycines* from a field setting. However, few to no voucher specimens exist from the majority of these studies, making identification confirmations of nearly all involved parasitoids impossible. In our area, only the widely polyphagous *L. testaceipes* was reared from mummies in eastern South Dakota as the primary aphidiine parasitoid of *A. glycines* (Martens and Johnson 2017, 2018).

Despite being of great importance to the foundation of knowledge for biological control programs, diversity surveys of the resident aphid parasitoid fauna in soybean have not been conducted or published (Ragsdale et al. 2004; Tilmon et al. 2011). No efficient sampling protocol is published for the explicit and passive sampling of aphid parasitoids generally, or in soybean and other monoculture crops. Knowledge of aphidiine parasitoids native or previously introduced to the U.S., including those that parasitize pest aphid species, is largely unavailable. Modern identification tools, taxonomy, and updated host and biological information for most Nearctic genera in the Aphidiinae are exceptionally outdated and typically inaccurate for species identification, with the exception of the more contemporary revisions and reviews of *Acanthocaudus* Smith (Kula et al. 2017), *Ephedrus* Haliday (Pike et al. 1999), *Praon* (Johnson 1987), and *Trioxys* Haliday (Fulbright et al. 2007). This lack of knowledge is further exacerbated by the introduction of non-native species for biological control and the discovery of new native species described in the past 70 years, since the last revision of the group by Smith (1944). A survey of aphidiine parasitoids and their aphid hosts present in the Pacific Northwest was conducted by Pike et al. (2000), and a list of 42 species representing 13 genera of aphidiine parasitoids recorded and likely present in South Dakota was given by Martens and Johnson (2018). Similar lists and surveys are needed from other regions of the U.S. and crops to improve the knowledge available regarding these parasitoids.

The primary goal of this project was to investigate and document the aphidiine parasitoids present in eastern South Dakota soybean fields. We also developed observations on the biology and host associations of those species through a survey of parasitoids and mummies collected from soybean in eastern South Dakota. Additionally, this project evaluated and determined the most effective and practical sampling protocol for aphidiine parasitoids in soybean using standard and available wasp sampling methods.

METHODS

Parasitoid Sampling—Aphid parasitoid sampling efforts were conducted in 2017 and 2018 at five South Dakota State University experimental research farms

in eastern South Dakota (SD): Brookings County, Aurora Farm Experimental Station 2.7 km northeast of Aurora, 44°18'23"N, 96°40'16"W; Felt Family Farm 5.6 km north of Brookings, 44°22'09"N, 96°47'40"W; Volga Farm Agricultural Experiment Station 3.5 km south of Volga, 44°17'59"N, 96°55'11"W; Union County, the Southeast South Dakota Research Farm 14.8 km southwest of Beresford, 43°3'13"N, 96°53'37"W; and Codington County, South Dakota Agricultural Experiment Station, Northeast Research Station 13.2 km west of South Shore, 45°06'16"N, 97°06'01"W. Soybean plots that were not sprayed with insecticides were selected for sampling. All of the research farms were composed mostly of and surrounded by corn (*Zea mays* L.) and soybean monocultures, with some small grain and native grass plots nearby.

The primary composition of plantings at the research stations was observed and recorded for the 2018 sampling season in an effort to elucidate host and host plant associations of the transient aphid parasitoids collected. The Northeast Research Experimental Station was composed primarily of corn and soybean plots, but the sampled soybean plot was flanked by a small patch of *Silphium perfoliatum* L. and was along a treeline of *Picea pungens* Engelmann. Nearby (within 500 m) was a small mixed native forb and grass planting and a large plot of an oilseed cultivar of sunflower, *Helianthus annuus* L. The Aurora Farm Experimental Station soybean plot was immediately adjacent to plots of winter and spring wheat, *Triticum aestivum* L., cultivars, as well as small patches of *S. perfoliatum*, *Panicum virgatum* L., and *Schizachyrium scoparium* (Michaux) Nash within 500 m of the trapping setup. The plot at Felt Family Farm was located near small patches of *S. perfoliatum* and larger plots of *P. virgatum* and *S. scoparium*, as well as corn and soybean plots. The plot sampled at the Volga Farm Agricultural Experiment Station was adjacent to a shelterbelt that contained mostly eastern redcedar, *Juniperus virginiana* L., green ash, *Fraxinus pennsylvanica* Marshall, and *R. cathartica* and was also adjacent to a plot of *H. annuus*. The plot sampled at the Southeast South Dakota Research Farm was surrounded by corn and soybean plots and was immediately adjacent to a gravel road.

The 2017 sampling season consisted of whole plant samples for *A. glycines* counts, and parasitoid rearing and sweep net sampling for adults. Multiple insect sampling methods were utilized and modified during the 2018 sampling season in an effort to develop an efficient trapping protocol for aphidiine parasitoids in soybean. Modified Townes-style Malaise traps (Figure 1), V-shaped flight intercept traps (V-FIT) (Warner 2017) (Figure 1), and yellow bowl traps (Figure 4) were paired with whole plant samples and sweep net samples. One Malaise trap, one V-FIT trap, and five yellow bowl traps were placed within each soybean plot and maintained weekly from mid-July through mid-September 2018, the primary activity period of the target taxa. A total of 28 sampling periods were conducted with a total of 84 samples recovered from the traps over the course of the 12-week sampling period in 2018. The V-FIT's were anchored using baling wire, tent stakes, concrete wall blocks, and guy-lines to prevent traps from being blown away in summer rainstorms. Yellow bowls were dug into the soil so that the lip of the bowl was at or just above the level of the ground surface in an attempt to examine parasitoid activity beneath the soybean canopy. Initially, standard



Figure 1. V-FIT trap (left) and Malaise trap (right); yellow bowl traps not visible.

Malaise traps were used but collection bottles rapidly filled with non-target taxa like calliphorid and muscid flies which created sorting difficulty and a paucity of wasps. Subsequently, Malaise traps were modified to exclude insects over 2.0 mm in diameter, but allow collection of small insects, particularly parasitoid wasps and aphids (Figure 2). This was done using fine mesh fabric, commonly used for garlic at grocery markets, that was doubled over and sewn by hand into the trap near the collecting head using 10 lb. braided fishing line and crafting needles (Figure 3). All traps were additionally reinforced at stress points, and with guy-lines as done with V-FIT traps, to withstand high winds and rain.

Malaise sample bottles were partially filled with 80% ethyl alcohol, while V-FIT pans and yellow bowls were filled with a 1:1 blend of propylene glycol and water with a small amount of dish detergent added to reduce surface tension. During trap tending, Malaise collecting heads were removed and replaced with new collection bottles and fresh preservative. Yellow bowl and V-FIT samples were decanted into gallon-sized Ziploc® bags; collecting reservoirs were then replenished with the propylene glycol-water mixture.

Five whole plant samples were collected during each site visit. Plants were harvested at ground level, and aphids and parasitoid mummies were counted the same day. Intact mummies and obviously parasitized aphids were collected for specimen rearing. Individual mummies were placed into 1.5 mL microcentrifuge tubes and kept at room temperature until parasitoid emergence, usually within seven days of collection. After emergence, parasitoids were preserved in

80% ethyl alcohol, chemically dehydrated using hexamethyldisilazane (HMDS) per Heraty and Hawks (1998) and point-mounted for identification. Aphidiine specimens were identified to genus using van Achterberg (1997) and to species when possible with original descriptions, or with reference specimens from the University of Minnesota Insect Collection, St. Paul, MN (UMSP), the National Museum of Natural History, Washington, D.C. (USNM), and the Severin-McDaniel Insect Research Collection, Brookings, SD (SMIRC). Due to the current state of aphidiine taxonomy, specimens were identified to genus level only and sorted into morphospecies when possible as species-level determinations for most aphidiines are impossible to make without modern and updated identification tools. Voucher specimens of aphidiines collected throughout this study will be deposited in the SMIRC.



Figure 2. Example of a Malaise sample after the excluder has been installed in the trap. This sample lacks the larger non-target flies, beetles, moths, and bees typically found in a standard Malaise sample.



Figure 3. Garlic mesh exclusion modification after installation in a Malaise trap near the collection head opening.

RESULTS AND DISCUSSION

Despite the potential presence of 42 aphidiine species (Martens and Johnson 2018) and the *B. communis* biocontrol releases, the only aphidiine parasitoid species recovered or reared from soybean aphid mummies and sweep samples during the 2017 sampling season was *L. testaceipes*. This generalist parasitoid was also reared from several other non-pest, native aphid species in South Dakota (Martens and Johnson 2018) and is associated by Mackauer and Starý (1979) with upwards of 20 aphid genera and upwards of 50 species within the Aphidinae throughout the world. Populations of *A. glycines* were well below the economic threshold in plots sampled during 2017, and parasitism occurred only at low levels; aphids and aphid mummies were only encountered occasionally in plots sampled.

Throughout the 2018 sampling season a total of 1,540 aphidiine specimens from eight genera were recovered from modified Malaise trap samples. The genera represented were *Adialytus* Foerster, *Aphidius* Nees, *Binodoxys* Mackauer, *Diaretiella* Starý, *Ephedrus* Haliday, *Lysiphlebus* Foerster, and *Praon* Haliday. Species collected and comments on potential biological associations are presented in Table 1. As with the 2017 sampling, only *L. testaceipes* was reared from aphid mummies on soybean and comprised 63.6% of all aphidiines recovered in Malaise samples. Of the remaining seven genera, *Aphidius* spp. and *Diaretiella rapae*



Figure 4. An example of a yellow bowl trap filled with non-target taxa, rainwater, and mud.

accounted for 23.7% and 10.3% of specimens, respectively. All other genera were recorded at very low levels (<1.0%) of fewer than 16 specimens recovered per genus. Five specimens of a *Binodoxys* species were recovered; these are not morphologically similar to *B. communis* and may represent a species new to science. The number of specimens collected per genus at each site and the percent each genus composes of the total number of specimens are presented in Table 2.

Each of the genera collected was defined as either purposeful (being reared from *A. glycines*) or transient (not being reared from *A. glycines* and serendipitously collected) within soybean fields. The only species reared directly from *A. glycines*, and thus the only species defined as purposeful, was *L. testaceipes*. All other taxa were defined as transient within soybean fields because they were not reared from *A. glycines* throughout the course of this study or reported so elsewhere and provided no apparent biological control of soybean aphid.

Table 1. Notes and biological comments on aphidiine parasitoids collected from soybean fields in eastern South Dakota.

| Taxon | Purposeful/Transient | Biological comments |
|--------------------------------|----------------------|---|
| <i>Lysiphlebus testaceipes</i> | Purposeful | The only known parasitoid of soybean aphid in eastern South Dakota; consistently reared from soybean aphid for several seasons |
| <i>Aphidius</i> spp. (2-3) | Transient | Likely associated with aphids in adjacent wheat plots. |
| <i>Diaretiella rapae</i> | Transient | Widely polyphagous and known from other <i>Aphis</i> spp. but not reared from soybean aphid; likely associated with aphids in adjacent wheat plots. |
| <i>Praon</i> sp. | Transient | Collected in low levels. Associations unknown. |
| <i>Adialytus</i> sp. | Transient | Collected in low levels. Associations unknown. |
| <i>Binodoxys</i> sp. | Transient | Collected in low levels. Associations unknown. |
| <i>Trioxys</i> sp. | Transient | Collected in low levels. Associations unknown. |
| <i>Ephedrus</i> sp. | Transient | Collected in low levels. Associations unknown. |

Table 2. 2018 sampling specimen totals by site and season-long and percent composition each genus represented.

| Site | Total specimens | <i>Lysiphlebus testaceipes</i> | | <i>Diaretiella rapae</i> | | <i>Praon</i> total | <i>Adialytus</i> total | <i>Binodoxys</i> total | <i>Trioxys</i> total | <i>Ephedrus</i> total |
|---------------------|-----------------|--------------------------------|------------|--------------------------|-----------|--------------------|------------------------|------------------------|----------------------|-----------------------|
| | | total | total | total | total | | | | | |
| Aurora | 471 | 133 | 323 | 5 | 10 | 0 | 0 | 0 | 0 | |
| Beresford | 810 | 790 | 12 | 1 | 4 | 0 | 2 | 1 | 0 | |
| Felt Farm | 19 | 13 | 3 | 2 | 0 | 0 | 1 | 0 | 0 | |
| South Shore | 65 | 22 | 14 | 10 | 2 | 12 | 2 | 1 | 2 | |
| Volga | 175 | 22 | 13 | 140 | 0 | 0 | 0 | 0 | 0 | |
| Total | 1540 | 980 | 365 | 158 | 16 | 12 | 5 | 2 | 2 | |
| Percent composition | | 63.6 | 23.7 | 10.3 | 1.03 | 0.78 | 0.32 | 0.13 | 0.13 | |

The Northeast Research Experimental Station site possessed the highest relative aphidiine diversity of all sites sampled with eight genera recovered, and two genera, *Adialytus* and *Ephedrus*, were unique to that site. The high aphidiine diversity at this site is likely correlated with the greater plant community diversity at this site compared to other sites sampled as well as the presence of known aphid parasitoid reservoir plants like creeping thistle, a.k.a. field thistle or Canada thistle (*Cirsium arvense* (L.) Scopoli) (Starý 1986).

The high number of *Aphidius* spp. collected at the Aurora Research Experiment Station site is attributed to the presence of winter and spring wheat plots planted near the trapping setup as none of these wasps were reared from soybean aphid mummies. The *Aphidius* spp. collected were likely parasitizing *Rhopalosiphum padi* L. and *Sitobion avenae* Fabricius aphids that are commonly observed in wheat in South Dakota. This indicates that these species of parasitoids were transient in soybean fields and provided no biological control of *A. glycines*. *Diaretiella rapae*, another widely polyphagous parasitoid, was recovered in relatively high numbers at the Volga Research Experimental Station site, but was never reared from soybean aphid mummies, and the local host remains undetermined.

CONCLUSIONS

Trapping protocol—This study found that modified Malaise traps were far superior to other trapping methods for efficiency and effectiveness at collecting aphidiine parasitoid diversity and relative abundance. Damage to small parasitoids, especially to aphidiines, is common in traditional bulk Malaise samples where small insects become entangled in the tarsi, setae, and wings of larger insects. After access to the intake opening near the collecting head was restricted to only small insects, sample sorting became much simpler and took substantially less time than sorting a traditional Malaise trap sample and was thus more cost effective on a per wasp basis. Restricting the sample uptake by size in the Malaise trap prevented specimen damage from occurring, resulting in a cleaner sample, greatly reduced sample sorting time, and greatly improved specimen and data quality. Dilution of preservatives in the collecting bottle was not an issue with modified Malaise samples as the bulk non-target specimens were much reduced.

We found that V-FIT traps and yellow bowl traps were ineffective for collecting aphidiine parasitoids given the constant weather pattern shifts observed in South Dakota during the soybean growing season. Both yellow bowl traps and the V-FIT collecting pans were often completely inundated with water and mud after a rain event, rendering 56 of the 84 total samples unusable and a complete loss. Yellow bowl traps were susceptible to falling leaves from soybean plants and primarily collected flies and ground beetles, rove beetles, and microphorine burying beetles (Figure 4). The V-FIT trap was highly susceptible to wind and would seldom remain standing for more than a few days. No parasitoid wasps in the samples were collected from V-FIT or yellow bowl traps due to the high level of sediment and the general decay of organisms due to the dilution of the collecting liquid by the rainwater and mud. In large open monoculture crops, these traps are ineffective in providing consistent and repeatable samples.

Implications of taxa collected—Despite the biological control efforts in 2007 in the midwestern U.S., including release sites in eastern areas of the state, *B. communis* was not collected during sampling periods in eastern South Dakota. The only wasp species continuously reared from soybean aphid at all sites was the ostensibly native and widely polyphagous *L. testaceipes*. None of the other aphidiine genera or species collected in soybean fields were reared from soybean aphid mummies, despite some species of all aphidiine genera collected having previously been reported as associated with *Aphis* spp., but never *A. glycines*, with the exception of *L. testaceipes*.

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