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Molecular gut-content analysis reveals high frequency of *Helicoverpa zea* (Lepidoptera: Noctuidae) consumption by *Orius insidiosus* (Hemiptera: Anthocoridae) in sweet corn

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Molecular gut-content analysis reveals high frequency of *Helicoverpa zea* (Lepidoptera: Noctuidae) consumption by *Orius insidiosus* (Hemiptera: Anthocoridae) in sweet corn

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Abstract

Management of corn earworm *Helicoverpa zea* in sweet corn grown for processing can be challenging due to the lack of effective transgenic and chemical control options. However, biological control by generalist predators can provide a significant impact on pests in this cropping system. One of the most ubiquitous predators of *H. zea* and other lepidopterans is the insidious flower bug, *Orius insidiosus*. This small hemipteran has been observed as an important mortality agent of *H. zea* in several cropping systems, but the strength of the trophic connection between these species has not been documented in sweet corn. Molecular gut-content analysis was conducted to test field-collected *O. insidiosus* for the presence of *H. zea* DNA using species-specific PCR primers developed and optimized for this project. Controlled feeding trials determined that the detectability half-life of this technique was 2.32 h. At peak predation in late August, 32% of *O. insidiosus* tested

positive for *H. zea* DNA. The date of peak predation also corresponded with peak silking of sweet corn plants, which is the most attractive crop growth stage to both *H. zea* and *O. insidiosus*. These results indicate that within a short window prior to collection from the field, on the peak date of predation, approximately one third of *O. insidiosus* in sweet corn had consumed one to two *H. zea* eggs and/or first instar larvae. The demonstration of this high frequency of predation allows for the assertion that *O. insidiosus* is a critical mortality agent of *H. zea* in sweet corn, and conservation biological control practices should be explored to protect and promote this key predator.

Keywords: Predator-prey interactions, Gut-content analysis, PCR, Biological control, Corn earworm, Insidious flower bug

1. Introduction

Corn earworm *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) can be a damaging pest in field crops and vegetables, such as sweet corn, field corn, cotton, soybean, sorghum, tomato, and beans grown across the United States (Capinera, 2004). Minnesota is the top producer of sweet corn, *Zea mays* L., harvested for processing, a crop valued between $73–120 million annually over the past three years (USDA-NASS, 2016). The most important insect pests of sweet corn in the upper Midwest are *H. zea* and *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), which can consistently be found at economically damaging levels (Flood et al., 2005; Hutchison et al., 2004). *Helicoverpa zea* moths migrate from the southern United States into Minnesota and other parts of the northern Corn Belt each year (Lingren et al., 1994). Females are attracted to newly emerged corn silks and are capable of ovipositing >2000 eggs within a two-week period (Hutchison et al. 2004; McLeod, 1988). When eggs hatch, early instar larvae move down the silks to the tip of the corn ear, where they cause damage by feeding on developing corn kernels (O'Rourke and Hutchison, 2004).

Management of *H. zea* in sweet corn can be challenging. Transgenic sweet corn expressing *Bacillus thuringiensis* (Bt) proteins provides significant suppression of *H. zea* (Burkness et al., 2001, 2010; Shelton et al., 2013), but is not widely adopted in sweet corn produced for the processing market due to restrictions on genetically-modified crops in international markets (Flood and Rabaey, 2007). The efficacy of chemical control of *H. zea* with pyrethroids and other conventional insecticides may also be reduced due to the protected feeding location of larvae within corn ears, difficulty in determining the optimum application timing, and low to moderate levels of insecticide resistance within pest populations (Hutchison et al., 2007; Jacobson et al., 2009). Spray applications of "reduced risk" insecticides, such as

spinosad, are effective against *H. zea* (Farrar et al., 2009), but timing of applications to treat larvae before they enter corn ears is a significant challenge, and they are often more expensive. Due to these challenges in the implementation of transgenic and chemical control methods, biological control is an important consideration for *H. zea* management; this is particularly evident for producers of organic-certified sweet corn.

The potential impact of biological control on *H. zea* populations has been explored utilizing a variety of natural enemies attacking various pest life stages, such as entomopathogenic nematodes attacking late instar larvae, prepupae, or pupae in the soil (Feaster and Steinkraus, 1996) or bats feeding on large numbers of moths (Maine and Boyles, 2015). However, a large portion of the literature has focused on mortality of the egg and early instar stages of *H. zea*, due to the vulnerability of these stages and their importance in population management at the individual field and season scale. The primary mortality agents for these stages include parasitoid wasps (Manandhar and Wright, 2015; Smith, 1996) and arthropod generalist predators (Sansone and Smith, 2001a; Seagraves and Yeargan, 2009).

In particular, minute pirate bugs (Hemiptera: Anthocoridae) can be of distinct importance as predators of crop pests and are an important component of the natural enemy community in many agroecosystems. Within the Anthocoridae, the insidious flower bug, *Orius insidiosus* (Say), is a common species in agroecosystems and various unmanaged habitats in the eastern and Midwestern United States (Lattin, 1999). *Orius insidiosus* is a polyphagous predator, generally preferring thrips, aphids, whiteflies, and lepidopteran eggs, and often supplements its diet with plant-provided resources, such as pollen and plant sap (Barber, 1936; Lattin, 1999; Lundgren, 2009). *Orius insidiosus* is one of the most common predatory arthropods found in sweet corn (Musser and Shelton, 2003a,b; Musser et al., 2004), including in Minnesota (Wold et al., 2001).

Orius insidiosus has long been reported as a natural enemy of *H. zea* eggs and/or early larval instars in corn (Barber, 1936; Pfannenstiel and Yeargan, 2002; Winburn and Painter, 1932), cotton (Nuessly and Sterling, 1994; Quaintance and Brues, 1905), soybean (Anderson and Yeargan, 1998) and sorghum (Jacobson and Kring, 1995; Tillman, 2006). These studies have sought to describe predation on *H. zea* life stages due to *O. insidiosus* and other predators through detailed observations of sentinel prey in the field, the construction of life tables, and the use of predator exclusion techniques. However, field observation methods may be inadequate for teasing apart the contribution of individual predatory species; using visual observation alone, it is often only possible to classify a predation event as caused by a predator with either piercing-sucking or chewing mouthparts (e.g., Nuessly and Sterling, 1994). In addition, predated eggs are often disrupted from the

plant or completely desiccated, leading to an inability to account for all predation when constructing life table analyses. Indeed, life table analyses often report very high levels of "unknown" mortality, and are left to assume that much of this is due to predation that has been unobservable in the field (e.g., Tillman, 2006). However, molecular gut-content analysis allows for non-disruptive determination of trophic relationships without the need to observe predation events in the field, which can be essential for predators like *O. insidiosus* that are small in size, move rapidly, and engage in cryptic liquid feeding (Harwood and Greenstone, 2008). The use of DNA-based techniques, such as polymerase chain reaction (PCR) with prey-specific primers can provide a better technique that allows for the determination of trophic relationships without interference in ecological interactions occurring in the field, and has been successfully used with *Orius* spp. (Gomez-Polo et al., 2016; Harwood et al., 2007; Simmons et al., 2015). Therefore, in this study, PCR molecular gut-content analysis was used to determine the frequency of predation of a critical pest, corn earworm *H. zea*, by a predator, the insidious flower bug *O. insidiosus*, in sweet corn agroecosystems in Minnesota.

2. Methods

2.1. Field site

A 0.4 ha field of 'Passion' and 'Passion II' sweet corn (Seminis Vegetable Seeds, Inc., St. Louis, Missouri, USA) was planted 27 June 2011 at the University of Minnesota Outreach, Research and Education (UMORE) Park in Rosemount, Minnesota, USA (GPS coordinates at center of field: 44.70557° N, 93.11028° W). The research plot was seeded at a rate of 64,467 seeds/ha at a depth of 4.4 cm and maintained under standard agronomic practices for sweet corn in Minnesota, with no insecticidal applications.

2.2. Prey population monitoring

A Texas style Hartstack pheromone trap (Hartstack et al., 1979) was established on the field edge and male *H. zea* moth populations were monitored using Hercon Luretape (Hercon Environmental, Emigsville, Pennsylvania, USA). Trapping was initiated on 23 May 2011 with lures changed weekly and traps checked at regular intervals until 19 September 2011. All counts were smoothed by calculating a double, three-day moving average (Hartstack and Hollingsworth, 1974; Lopez et al., 1979). The number of male moths captured in pheromone traps can be correlated with egg laying in adjacent silking corn fields (Latheef et al., 1991). Therefore, male moth catches

were used as an estimate of *H. zea* prey availability to *O. insidiosus*. In addition, following the methods of Burkness et al. (2001), late instar larval populations of *H. zea* were estimated via sampling a total of 80 ears (20 ears each from four randomized plots that were not treated with insecticides) when ears had reached maturity (□75% moisture) on 12 September 2011.

2.3. Field collection of predators

Once a week, 25 adult *O. insidiosus* were located using visual searching and collection in the morning hours between 0800 and 1000 CDT with a handheld aspirator from 4 August 2011 (whorl stage sweet corn) to 15 September 2011 (brown silk, 3 days after ear harvest). As adult *O. insidiosus* became scarce on the September collection dates, nymphs were also collected to yield a total of 25 *Orius* per date. Collected predators were stored individually in 0.67 mL microcentrifuge tubes containing chilled >95% ethanol and stored at −20 °C for molecular screening. Samples were identified as *O. insidiosus* based on species distribution (Herring, 1966) and reference specimens in the University of Minnesota Insect Collection (UMSP).

2.4. Molecular gut-content analysis

2.4.1. Primer design and optimization

To obtain sequences for primer design, total DNA was extracted from both field-collected (UMORE Park, Rosemount, Minnesota, USA) and colony-sourced (French Agricultural Research, Inc., Lamberton, Minnesota, USA) *H. zea* using QIAGEN® DNeasy Blood & Tissue Kits (QIAGEN Inc., Chatsworth, California, USA) following the animal tissue protocol. PCR was used to amplify a 658 bp region of the cytochrome *c* oxidase I (COI) mitochondrial DNA using LCO-1490 and HCO-700dy primers (Folmer et al., 1994). PCR mixes (50 μL) contained 1× Takara PCR buffer (Takara Bio Inc., Shiga, Japan), 0.2mM of each dNTP, 0.2mM of each primer, 1 U Takara *Ex Taq*™ and 5 μL of template DNA. PCR was performed in Bio-Rad PTC-100 or PTC-200 thermal cyclers (Bio-Rad Laboratories, Inc., Hercules, California, USA). PCR cycling protocol began with an initial denaturing step of 94 °C for 1 min, followed by 50 cycles of 94 °C for 45 s (denaturation), 40 °C for 45 s (annealing), and 72 °C for 45 s (elongation), and a final extension step of 72 °C for 10 min. Reactions were visualized to ensure successful amplification by electrophoresis of 10 μL of PCR product in 2% SeaKem® agarose (Lonza, Rockland, ME, USA) stained with GelRed™ Nucleic Acid Gel Stain (15 μL GelRed per 150 mL agarose gel solution, Biotium, Hayward, California, USA). PCR products that yielded positive results were purified with the QIAGEN Min Elute PCR purification kit according to the manufacturer's

guidelines. The ABI Big-Dye Terminator mix (v. 3.0) was used to cycle sequence in both the forward and reverse directions in an ABI 9700 thermal cycler, and the sequence data were acquired on an ABI 3730xl sequencer (Applied Biosystems, Foster City, CA, USA). Forward and reverse sequences from the same individuals were assembled using Geneious Pro (Drummond et al., 2011). These sequences, plus COI sequences of related non-target arthropods accessed from GenBank were aligned using multiple sequence alignments performed using MUltiple Sequence Comparison by Log-Expectation (MUSCLE; Edgar, 2004). Determination of species to be included in the primer design matrix was based on published phylogenies of the sub-family to which the target, *H. zea*, belongs (Lepidoptera: Noctuidae: Heliothinae) (Cho et al., 2008; Hardwick, 1970; Matthews, 1991). The matrix included sequences from the target *Helicoverpa zea* obtained from three sources: Minnesota field site, lab colony, and GenBank, plus sequences from 5 other *Helicoverpa* species, 24 *Schinia* spp., 15 *Heliocheilus* spp., 10 *Heliothis* spp., 3 *Adisura* spp., 2 *Heliolonche* spp., 2 *Australothis* spp., 2 *Pyrrhia* spp., 1 *Erythroecia* sp., 1 *Rhodoecia* sp., 1 *Eutricopis* sp., and 1 *Heliothodes* sp. obtained from GenBank. The aligned sequences of target and non-target organisms were viewed in BioEdit v 7.1.3 (Ibis Biosciences, Carlsbad, California, USA) to facilitate primer design. A species-specific primer pair was designed to amplify *H. zea* and analyzed using the Primer3 website (Rozen and Skaletsky, 2000). Optimal annealing temperature for the primer pair was determined using a Bio-Rad PTC-200 gradient-block heat thermal cycler. To confirm that the primers did not amplify non-target organisms, they were screened for their ability to amplify DNA from a diverse array of non-target invertebrates (140 species from 89 families and 14 orders of Arthropoda, Mullusca, and Nematoda), including those most common in fields used for the current research (Supplementary Table 1). To confirm that primers did not amplify predator DNA, resulting in false positives, primers were also screened against starved *O. insidiosus* (n=12) collected from soybean fields at UMORE Park (Rosemount, Minnesota, USA).

2.4.2. DNA extraction and PCR of field-collected *Orius insidiosus*

Total DNA was extracted from whole bodies of field-collected *O. insidiosus* using QIAGEN® DNeasy Blood & Tissue Kits as described above. PCR (25 µL per reaction) was carried out as described above, except that designed *H. zea*-specific primers were used with the following PCR protocol: initial denaturing step of 94 °C for 1 min, followed by 45 cycles of 94 °C for 45 s (denaturation), 62 °C for 45 s (annealing), and 72 °C for 30 s (elongation), and a final extension step of 72 °C for 10 min. Reaction success was determined by electrophoresis of 10 µL of PCR product in 2% SeaKem® agarose

stained with GelRed. Finally, field-collected *O. insidiosus* that screened negative against the *H. zea* primer pair were screened with the general COI primers (LCO-1490 and HCO-700dy) to ensure that DNA extraction had been completed successfully and eliminate any potential false negative results.

2.4.3. Feeding trial to determine primer detection period

Adult *O. insidiosus* were collected by hand-held aspirator from soybean fields at UMORE Park (Rosemount, Minnesota, USA). Predators were transferred to the laboratory and maintained individually in Petri dishes with moistened floral foam (Oasis Floral Products, Kent, Ohio, USA) at 25 °C under a 16 h:8h (light:dark) cycle. Following protocols described in Harwood et al. (2007), *Orius* were starved for 24 h prior to feeding trials but provided moisture, then provided a single *H. zea* egg (French Agricultural Research, Inc., Lamberton, Minnesota, USA). Predators were allowed to feed for 2 h and feeding events of each *O. insidiosus* were recorded every 15 min. After 2 h, *O. insidiosus* that had been observed to feed were transferred to new, clean Petri dishes and provided 1 soybean aphid *Aphis glycines* Matsumura (Hemiptera: Aphididae) as "chaser prey" to mitigate the effects of starvation on rates of prey digestion and impact on gut-content detection limits (Chen et al., 2000; Greenstone and Hunt, 1993). Following 1 h of feeding time on chaser prey, 8 *O. insidiosus* were immediately transferred into 1.5 mL microcentrifuge tubes containing>95% ethanol (i.e., t=1 h postfeeding) and stored at −20 °C until molecular gut-content analysis. The remaining *O. insidiosus* were maintained as described above and 8 individuals were transferred into>95% ethanol and stored at −20 °C at 2, 3, 5, 9, 17, 25, and 37 h. The longest time periods were chosen based on previous results indicating that *O. insidiosus* can test positive for prey DNA using other primer pairs after as many as 24 h post consumption (Harwood et al., 2007). DNA was extracted from all *Orius* in the feeding trials and screened using PCR and gel electrophoresis as described above.

2.5. Statistical analyses

The relationship between *O. insidiosus* testing position for *H. zea* DNA and prey availability was determined by running a Pearson's product-moment correlation using SAS software (v. 9.4; SAS Institute Inc., Cary, NC, USA). To determine the rate of prey DNA decay in the digestive tract of the predator and the half-life for detectability, feeding trial data were analyzed by fitting a four parameter logistic curve (dose-response) regression equation to the proportion positive at each time period using SigmaPlot (v. 13; Systat Software Inc., San Jose, California, USA) (after Greenstone et al., 2007; Payton et al., 2003).

3. Results

3.1. Field collection of prey and predators

During the period of *O. insidiosus* collection (4 August to 15 September 2011) the average *H. zea* moth catch per night was 7.05 with a one night peak of 63 moths on 1 September 2011. *Helicoverpa zea* larval population per ear on 12 September averaged 0.58 with 50% of ears infested. *Orius insidiosus* collected on dates from 4 August 2011 to 31 August 2011 consisted entirely of adult specimens. However, on 8 September 2011, 40% (10 of 25) were nymphs and on 15 September 2011, 24% (6 of 25) were nymphs.

3.2. Molecular gut-content analysis

3.2.1. Primer design and optimization

Primers were successfully designed and optimized to detect *H. zea* DNA by amplifying a 201 bp region of the COI gene: Forward (Hzea-154F): TCTTTAATTGGAGATGATCAAATTTAC and Reverse (Hzea-307R): AAGTAAAGTTAGGGAAGGGGGG. When screened for cross-reactivity against 140 non-target DNA extractions (see Supplementary Table 1), all primers yielded no bands. Additionally, when screened against 12 starved *O. insidiosus* extractions, no reactivity was observed.

3.2.2. PCR screening of field-collected *Orius insidiosus*

All 175 field-collected *O. insidiosus* were screened against the *H. zea* primers. For all predators combined, 7.43% screened positive for *H. zea* and percent positive for *H. zea* varied significantly by date, with the highest percent positive (32%) on 24 August 2011 (**Fig. 1**). When *Orius* that had screened negative for *H. zea* were screened against the general COI primers, all yielded positive results, indicating there were zero false negatives due to failure of the DNA extraction process. There was not a significant correlation between prey availability (double three-day moving average of male moths collected in pheromone traps) and percent of *O. insidiosus* testing positive for *H. zea* DNA ($r=0.54$, $p=0.35$).

3.2.3. Feeding trial to determine primer detection period

Analysis of the feeding trial specimens yielded a nonlinear regression decay curve ($r2 = 0.87$, $F3,7 = 17.2$, $P=0.0094$) with a DNA detectability half-life of 2.32 h (**Fig. 2**).

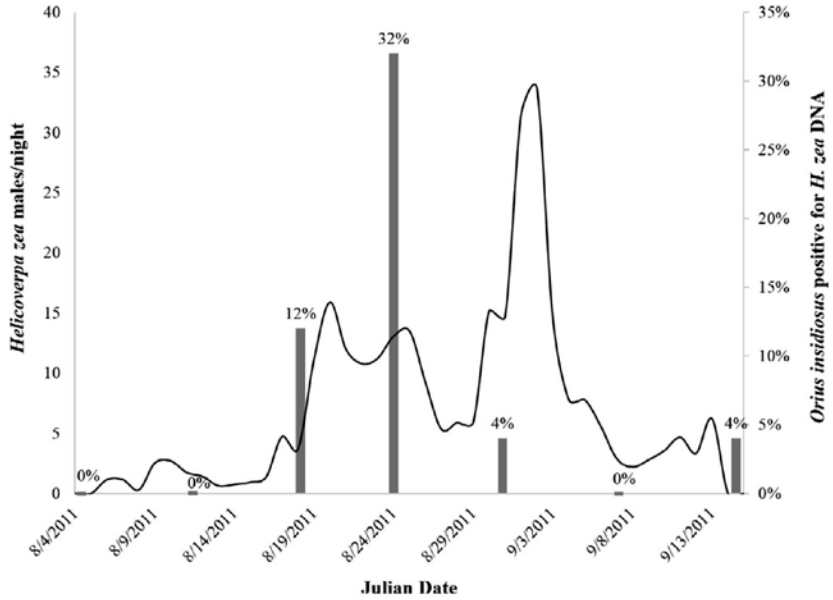

Fig. 1. Double three-day moving average of male *Helicoverpa zea* moths caught in pheromone traps (as an indicator of prey availability) during study period, black line on the left-hand axis; percent of *Orius insidiosus* testing positive by PCR for *H. zea* DNA during the study period, grey bars on the right-hand axis.

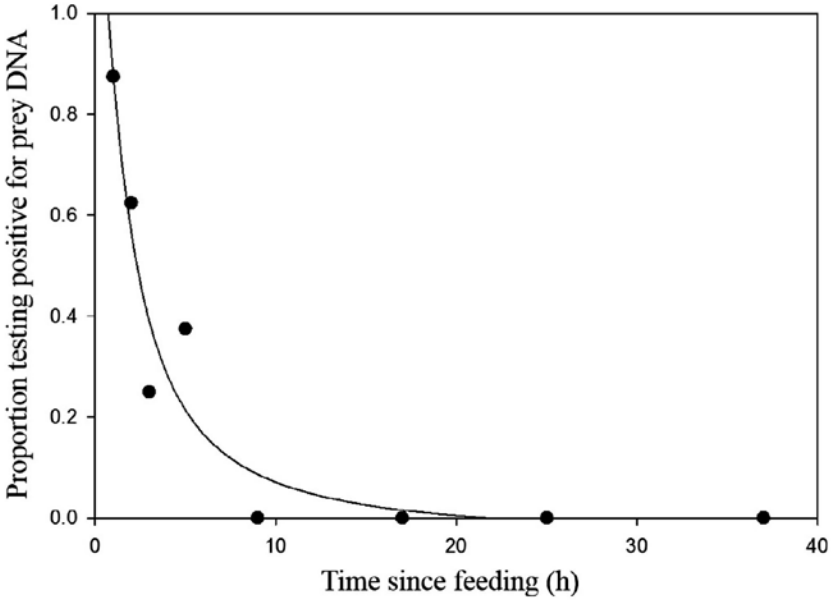

Fig. 2. Detection of DNA of *Helicoverpa zea* over time following consumption by *Orius insidiosus*.

4. Discussion

Feeding trials to determine the detection period of *H. zea* DNA in the gut-contents of *O. insidiosus* revealed a half-life of only 2.32 h. This is a shorter detection window than those reported for *Orius* spp. screened against primer pairs specific to species of aphids, thrips, whitefly, and ladybeetle, which ranged from 2.7 to 21.8 h (Gomez-Polo et al., 2016; Harwood et al., 2007; Simmons et al., 2015). Predatory hemipterans are one of the taxa found to have a wide range of detectability half-lives (Greenstone et al., 2014). It has been hypothesized that predators feeding by liquid ingestion, such as hemipterans and spiders, have longer detectability half-lives than other taxa (Greenstone et al., 2007); however, more recent studies have not supported this hypothesis (Greenstone et al., 2014; Simmons et al., 2015), instead proposing that the wide range in prey detectability half-lives across arthropod predator taxa are likely caused by variations between prey taxa, prey item size, feeding protocols, PCR protocols, and amplicon size. The short detection window of the molecular tool developed for this study should affect our interpretation of the results: any positives are indicative of very recent feeding (most likely within the past 5 h prior to predator collection from the field). In addition, by having a short detectability half-life, false positives due to secondary predation are even less likely to occur (Harwood et al., 2001), allowing accurate interpretation of positive results as evidence of direct predatory behavior.

Predation of *H. zea* by *O. insidiosus* peaked in late August, with 32% of field-collected predators testing positive. Given the narrow detection window, these results indicate a high frequency of predation on *H. zea* by *O. insidiosus* at this time during the growing season. In reality, our results may even be underestimating predation due to the diurnal activity pattern of *O. insidiosus*. In sweet corn in Kentucky, over 85% of predation events between *O. insidiosus* and *H. zea* occurred during daylight hours; two approximately equal peaks in predation occurred between 0900–1200 and 1500–1800 EDT (Pfannenstiel and Yeargan, 2002). Our predator sampling period was between 0800–1000 CDT, and likely captured the first diurnal peak in predation activity; however, a second peak in predation activity in the mid-afternoon could have been undocumented.

One potential challenge in interpreting DNA-based gut-content analysis studies is the lack of stage-specificity using these techniques (Harwood and Greenstone, 2008). However, extensive work has been conducted to examine the acceptability of various life stages of *H. zea* as prey for *O. insidiosus*. Isenhour et al. (1989) reported that *O. insidiosus* will consume fewer 4-day-old larvae than 2-day-old larvae, and 8-dayold larvae were completely unacceptable. Similarly, Jacobson and Kring (1994) demonstrated that predation by *O. insidiosus* on *H. zea* on sorghum in the greenhouse dropped

significantly between the first and second instar, concluding that second instar larvae are too large for *Orius* predation. When *H. zea* eggs are laid on corn silks, first instar larvae proceed almost immediately down the silk channel and into the ear tip after hatching (Hardwick, 1965). Therefore, predation by *O. insidiosus* must occur during the egg stage or immediately after hatching. Previous studies have reported that *O. insidiosus* are likely to consume approximately 1.2–1.8 *H. zea* eggs, or 0.5–2.1 first instar larvae, per day (Barber, 1936; Jacobson and Kring, 1994; Parajulee et al., 2006; Sansone and Smith, 2001b); we can therefore assume that each positive result from our study is the result of *O. insidiosus* consuming one or two *H. zea* eggs or first instar larvae within 5 h of collection.

Our results indicate a higher frequency of predation than revealed by the only other published study using gut-content analysis to examine *O. insidiosus-H. zea* predation in corn. Corey et al. (1998) used polyacrylamide gel electrophoresis (PAGE) to detect the presence of *H. zea* and other food proteins in *O. insidiosus* collected from field corn in Kansas. They reported a peak of ~20% of *O. insidiosus* testing positive for *H. zea* during late July (1992) and mid-August (1993). However, the authors interpreted these results as being indicative of insignificant predation, as detection rates were lower for *H. zea* than for thrips, corn pollen, and an undetermined protein hypothesized to be from corn kernels (Corey et al. 1998). Differences from our results may be due to the detection method used (PAGE vs. PCR), geographic location of the studies (Kansas vs. Minnesota), and ecological differences and prey population dynamics in the study systems (field corn vs. sweet corn). In addition, Corey et al. (1998) acknowledged that their collection methods may have led to under-reporting of predation events due to collection of predators earlier in the morning (0700 CDT), further emphasizing the importance of the insidious flower bug's diurnal activity as discussed above.

Despite some differences between our results and those of Corey et al. (1998), the majority of literature supports the result that *O. insidiosus* has a significant predatory relationship to *H. zea*. In Texas cotton fields, enzyme-linked immunosorbent assays (ELISA) sensitive to heliothine egg-specific proteins were used to screen the *Orius* community composed of *O. insidiosus* and *O. tristicolor* (Sansone and Smith, 2001a,b). Predation of heliothine eggs peaked in late June with 59% of *Orius* spp. testing positive, although predation rates were affected by plant physiology (reduced plant feeding due to lower quality during drought conditions increased prey feeding) and availability of alternative prey (presence of thrips in cotton bolls decreased predation of heliothine eggs) (Sansone and Smith, 2001b). In the same system, predation by *Orius* spp. accounted for up to 84% of *H. zea* egg mortality (Sansone and Smith, 2001a). Similar results have been found for *H. zea* in other crops, with up to 77% of eggs predated by *O. insidiosus* in field corn (Barber 1936), 62% of eggs in sorghum shriveled or missing (assuming

predation, with *O. insidiosus* accounting for 94% of the predator complex) (Jacobson and Kring, 1995), and 70% of egg mortality in soybean ascribed to predation by the natural enemy community (Anderson and Yeargan, 1998).

The biology and behavior of *O. insidiosus* facilitates its predatory relationship with *H. zea* in sweet corn. Both predator and prey are attracted to corn at the same phenological stage, when tassels and silks have first emerged and silks are still fresh and moist (growth stage: VTR1) (Barber, 1936; Reid and Lampman, 1989; Reid 1991). Our study demonstrates this fact, as the first *O. insidiosus* samples were collected on 4 August 2011, which coincided with the corn being at the whorl stage (Abendroth et al., 2011) and 0% of samples being positive for *H. zea* DNA (Fig. 1). As sample collection progressed, 10% of plants had exerted fresh silk on 11 August, 50% on 18 August and 100% of plants had exerted fresh silk on 24 August. Subsequent samples on 31 August (silk turning brown) and 8 and 15 September (brown silk) were collected during periods of reduced attractiveness of corn silks to *H. zea* oviposition (Hardwick, 1965) and likely led to reduced numbers of *O. insidiosus* adults being present in the field samples on later collection dates.

Using data from this study and other publications, it is possible to extrapolate the ratio between the number of pests consumed by *O. insidiosus* and the number of new *H. zea* eggs being laid in the field (**Table 1**). Published estimates of the abundance of *O. insidiosus* in sweet corn fields range between 0.35 and 5.89 individuals per plant (Musser and Shelton 2003b;

Table 1. Estimates of *Helicoverpa zea* oviposition and consumption rates by *Orius insidiosus* in Minnesota sweet corn, extrapolated from data provided by this study and others (Latheef et al. 1991; Musser and Shelton 2003b; Musser et al. 2004; Wold et al. 2001).

Date	*Mean male moths /night*	*% of ears with fresh silk*	*H. zea eggs laid /plant*[a]	*% Orius positive by PCR*	*H. zea eggs or larvae consumed /plant*[b]	*Ratio of pests consumed to new eggs laid*
8/4/2011	0	0%	–	0%	0.00	–
8/11/2011	1.7	10%	0.014	0%	0.00	0.0
8/18/2011	3.7	50%	0.017	12%	0.04–0.71	2.4–41.0
8/24/2011	13.1	100%	0.050	32%	0.11–1.88	2.3–38.0
8/31/2011	14.7	100%	0.056	4%	0.01–0.24	0.3–4.2
9/08/2011	2.2	0%	–	0%	0.00	–
9/15/2011	0	0%	–	4%	0.01	–

a. Calculated from Latheef et al. (1991): log(eggs/ha+1) = 3.71 + 0.22 * 1og (S+1) * log(T+1) − 0.3 * 1og(S+1), where S=number of corn ears with new silks per hectare, and T=mean *Helicoverpa zea* male moths per pheromone trap. To calculate S, a plant population of 61,244 plants per hectare was used.

b. Calculated using the range of 0.35–5.89 *Orius insidiosus* per plant (Musser and Shelton 2003b; Musser et al. 2004; Wold et al. 2001).

Musser et al. 2004; Wold et al. 2001). This wide range is likely due to many ecological factors, including geographic location. In Minnesota, Wold et al. (2001) reported a mean of 0.41 *O. insidiosus* per sweet corn plant. Using the regression equation published by Latheef et al. (1991), an estimate of the number of new *H. zea* eggs laid per night during silking can be made if the number of male moths per night and number of corn ears with new silks is known. Using these estimates, we see that predation rates on *H. zea* eggs and larvae on 18 and 24 August are at least more than double the number for new eggs being laid (Table 1). However, as moth flights increase and percent positive for *H. zea* DNA decreases, the *O. insidiosus* consumption rate at the low end of population estimates is only 30% for the newly laid eggs on 31 August (Table 1). This shift in the relative contribution of *O. insidiosus* predation as the season progresses corresponds with other studies of generalist predators that have found that early season predation of pests when they are first colonizing crop fields and/or before the pests have reached the exponential growth phase is critical (Harwood et al. 2007; Settle et al. 1996; Sunderland et al. 1997; Welch et al. 2012).

Due to the high frequency of *H. zea* predation by *O. insidiosus*, biological control should be considered as a reliable component of IPM for this key pest of sweet corn. As a native species that is already found at relatively high levels in agroecosystems, *O. insidiosus* lends itself to the practice of conservation biological control (Gurr et al., 2017; Landis et al., 2000). The conservation and promotion of *O. insidiosus* and other predatory hemipterans in crop fields can be achieved via a reduction in insecticide use and/or the selection of less toxic insecticides (Vasileiadis et al., 2017) and the enhancement of the agricultural landscape via intercropping (Bickerton and Hamilton, 2012; Manandhar and Wright, 2015) and diverse, non-crop host plants to support predator persistence and emigration into nearby crops (Perdikis et al., 2011; Veres et al., 2012). Moreover, with increasing reports of *H. zea* resistance to pyrethroid insecticides (e.g., Jacobson et al., 2009) and selected Bt corn events (Dively et al., 2016), a renewed effort to encourage biological control is warranted and timely.

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Appendix A. Supplementary data — Supplementary Table 1 follows the References.

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Supplementary Table 1. Identity of non-target DNA extractions used for primer optimization.

Phylum	Class	Order	Family	Species
Arthropoda	Arachnida	Araneae	Araneidae	*Araneus diadematus*
			Linyphiidae	*Florinda coccinea*
				Frontinella communis
				Tennesseellum formica
			Lycosidae	*Pardosa* sp.
			Salticidae	*Phidippus* sp.
			Tetragnathidae	*Leucauge venustra*
				Tetragnatha sp.
	Chilopoda	Lithobiomorpha	Lithobiidae	*Lithobius* sp.
	Insecta	Coleoptera	Aderidae	Aderidae sp.
			Bruchidae	*Stator limbatus*
			Cantharidae	*Chauliognathus pensylvanicus*
			Chrysomelidae	*Acalymma* sp.
				Chaetocnema denticulata
				Chaetocnema pulicaria
				Diabrotica undecimpunctata
				Diabrotica virgifera virgifera
				Leptinotarsa decimlineata
				Phyllotreta striolata
				Systena blanda
			Coccinellidae	*Coleomegilla maculata*
				Harmonia axyridis
				Hippodamia sp.
				Sasaiiscymnus tsugae
				Scymnus sp.
			Curculionidae	*Otiorhynchus* sp.
			Lampyridae	*Lucidota atra*
			Latridiidae	Latridiidae sp.
			Meloidae	*Epicauta* sp.
			Nitidulidae	*Carpophilus* sp.

	Ptilodactylidae	*Anchycteis velutina*
	Scarabaeidae	*Popillia japonica*
	Scolytidae	*Hypothenemus hampei*
	Staphylinidae	*Atheta* sp.
		Platydracus sp.
Collembola	Isotomidae	*Folsomia candida*
	Entomobryidae	*Sinella curviseta*
Diptera	Agromyzidae	Agromyzidae sp.
	Anthomyzidae	Anthomyzidae sp.
	Cecidomyiidae	Cecidomyiidae sp.
	Ceratopogonidae	Ceratopogonidae sp.
	Chironomidae	Chironomidae sp.
	Chloropidae	Chloropidae sp.
		Elachiptera sp.
		Thaumatomyia sp.
	Culicidae	*Aedes aegypti*
		Aedes albopictus
		Aedes polynesiensis
	Dolichopodidae	*Condylostylus* sp.
		Dolichopus comatus
	Drosophilidae	*Scaptomyza* sp.
	Empididae	Empididae sp.
	Ephydridae	Ephydridae sp.
	Lonochopteridae	Lonochopteridae sp.
	Milichiidae	Milichiidae sp.
	Muscidae	Muscidae sp.
	Mycetophilidae	Mycetophilidae sp.
	Phoridae	Phoridae sp.
	Psychodidae	Psychodidae sp.
	Sciaridae	Sciaridae sp.
	Sciomyzidae	*Sepedomerus macropus*
		Sepedonea isthmi

	Syrphidae	Syrphidae sp.
	Tephritidae	*Euaresta aequalis*
Hemiptera	Acanaloniidae	*Acanalonia conica*
		Acanaloniidae sp.
	Adelgidae	*Adelges tsugae*
		Pineus strobi
	Aleyrodidae	Aleyrodidae sp.
		Bemisia tabaci biotype B
		Bemisia tabaci biotype O
	Anthocoridae	*Orius albidipennis*
		Orius insidiosus
		Orius laevigatus
	Aphididae	*Aphis craccivora*
		Myzus persicae
		Rhopalosiphum maidis
	Cicadellidae	*Circulifer tenellus*
		Cuerna striata
		Empoasca fabae
		Graphocephala coccinea
	Psyllidae	*Bactericerca cockerelli*
		Cacopsylla pyricola
	Coccidae	*Coccus hesperidium*
		Eulecanium cerasorum
		Neolecanium cornuparvum
		Pulvinaria innumerabilis
		Parthenolecanium quercifex
	Psuedococcidae	*Pseudococcus maritimus*
	Cydnidae	*Melanaethus* sp.
	Geocoridae	*Geocoris bullatus*
		Geocoris punctipes
	Lygaeidae	*Lygus lineolaris*
	Nabidae	*Nabis alternatus*
	Pentatomidae	*Acrosternum hilare*

		Euschistus servus
	Reduviidae	Reduviidae sp.
	Rhopalidae	*Boisea trivittata*
	Rhyparochromidae	*Myodocha serripes*
Hymenoptera	Aphelinidae	*Encarsia inaron*
	Apidae	*Apis mellifera*
		Bombus sp.
	Bethylidae	*Prorops nasuta*
	Braconidae	*Alyciini* sp.
		Alysiinae sp.
		Aphidiinae sp.
		Bracon sp.
		Meteorus sp.
		Microgastrinae sp.
	Ceraphronidae	*Aphanogmus* sp.
	Chalcidae	Chalcidae sp.
	Crabronidae	*Mimesa* sp.
	Eulophidae	*Phymastichus coffea*
	Figitdae	Eucoilinae sp.
	Formicidae	Formicidae sp.
		Tapinoma sp.
	Halictidae	*Agapostemon* sp.
	Ichneumonidae	*Campoletis* sp.
		Ichneumonidae sp.
	Mutillidae	*Pseudomethoca* sp.
	Platygastridae	*Trimorus* sp.
	Pompilidae	Pompilidae sp.
	Sphecidae	*Ammophila procera*
	Vespidae	*Polistes* sp.
Lepidoptera	Noctuidae	*Heliothis virescens*
		Pseudaletia unipuncta
		Trichoplusia ni
	Nymphalidae	*Danaus plexippus*

			Pyralidae	*Galleria mellonella* *Ostrinia nubilalis*
		Neuroptera	Chrysopidae	*Chrysopa oculata*
			Hemerobiidae	Hemerobiidae sp.
		Orthoptera	Gryllidae	*Gryllus pennsylvanicus*
		Thysanoptera	Phlaeothripidae	*Karnyothrips flavipes*
			Thripidae	*Frankliniella occidentalis* *Thrips tabaci*
Mollusca	Gastropoda	Stylommatophora	Discidae	*Anguispira alternata*
			Polygyridae	*Mesodon zaletus*
Nematoda	Chromadorea	Rhabditida	Steinernematidae	*Steinernema carpocapsae*
	Secernentea	Tylenchida	Allantonematidae	*Thripinema* sp.