6-2015

Survey of Soybean Insect Pollinators: Community Identification and Sampling Method Analysis

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Abstract
Soybean, *Glycine max* (L.) Merrill, flowers can be a source of nectar and pollen for honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), wild social and solitary bees (Hymenoptera: Apoidea), and flower-visiting flies (Diptera). Our objectives were to describe the pollinator community in soybean fields, determine which sampling method is most appropriate for characterizing their abundance and diversity, and gain insight into which pollinator taxa may contact soybean pollen. We compared modified pan traps (i.e., bee bowls), yellow sticky traps, and sweep nets for trapping pollinators in Iowa soybean fields when soybeans were blooming (i.e., reproductive stages R1–R6) during 2011 and 2012. When all trap type captures were combined, we collected 5,368 individuals and at least 50 species. Per trap type, the most pollinators were captured in bee bowls (3,644 individuals, 44 species), yellow sticky traps (1,652 individuals, 32 species), and sweep nets (66 individuals, 10 species). The most abundant species collected include *Agapostemon virescens* F. and *Lasioglossum (Dialictus)* species (Hymenoptera: Halictidae), *Melissodes bimaculata* Lepeletier (Hymenoptera: Apidae), and *Toxomerus marginatus* Say (Diptera: Syrphidae). To determine if these pollinators were foraging on soybean flowers, we looked for soybean pollen on the most abundant bee species collected that had visible pollen loads. We found soybean pollen alone or intermixed with pollen grains from other plant species on 29 and 38% of the bees examined in 2011 and 2012, respectively. Our data suggest a diverse community of pollinators—composed of mostly native, solitary bees—visit soybean fields and forage on their flowers within Iowa.

Keywords
soybean, native bee, syrphid, Apis mellifera, pollen

Comments
This article is published as Gill, K. A., and M. E. O'Neal. "Survey of soybean insect pollinators: Community identification and sampling method analysis." Environmental entomology 44, no. 3 (2015): 488-498. doi: 10.1093/ee/nvv001. Posted with permission.

Survey of Soybean Insect Pollinators: Community Identification and Sampling Method Analysis

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Environ. Entomol. 44(3): 488–498 (2015); DOI: 10.1093/ee/nvv001

ABSTRACT Soybean, *Glycine max* (L.) Merrill, flowers can be a source of nectar and pollen for honey bees, *Apis mellifera* L. (Hymenoptera: Apidae), wild social and solitary bees (Hymenoptera: Apoidea), and flower-visiting flies (Diptera). Our objectives were to describe the pollinator community in soybean fields, determine which sampling method is most appropriate for characterizing their abundance and diversity, and gain insight into which pollinator taxa may contact soybean pollen. We compared modified pan traps (i.e., bee bowls), yellow sticky traps, and sweep nets for trapping pollinators in Iowa soybean fields when soybeans were blooming (i.e., reproductive stages R1–R6) during 2011 and 2012. When all trap type captures were combined, we collected 5,368 individuals and at least 50 species. Per trap type, the most pollinators were captured in bee bowls (3,644 individuals, 44 species), yellow sticky traps (1,652 individuals, 32 species), and sweep nets (66 individuals, 10 species). The most abundant species collected include *Agapostemon virescens* F. and *Lasioglossum (Dialictus)* species (Hymenoptera: Halictidae), *Melissodes bimaculata* Lepeletier (Hymenoptera: Apidae), and *Toxomerus marginatus* Say (Diptera: Syrphidae). To determine if these pollinators were foraging on soybean flowers, we looked for soybean pollen on the most abundant bee species collected that had visible pollen loads. We found soybean pollen alone or intermixed with pollen grains from other plant species on 29 and 38% of the bees examined in 2011 and 2012, respectively. Our data suggest a diverse community of pollinators—composed of mostly native, solitary bees—visit soybean fields and forage on their flowers within Iowa.

KEY WORDS soybean, native bee, syrphid, *Apis mellifera*, pollen

Insect pollinators provide a critical ecosystem services to many fruit, vegetable, and field crops that depend on pollination for fruit and seed production. The European honey bee, *Apis mellifera* L. (Hymenoptera: Apidae), is an important agricultural pollinator, particularly for monocultures of mass flowering crops. Wild insects, native bees (Hymenoptera: Apoidea: Anthophila) and flower-visiting flies (Diptera), also visit crop flowers and contribute to crop pollination (Delaplane and Mayer 2000, Klein et al. 2007, Winfree et al. 2011). Despite these relationships, descriptions of flower-visiting insect communities and their interactions with crops are lacking for many major productions systems (Klein et al. 2007).

The pollinator community that visits soybean, *Glycine max* (L.) Merrill, is one example of such a production system. Soybean plants can produce as many as a half million florets per acre (Woodcock 2012), but because soybeans are breed to be self-fertile and self-pollinating, it is thought that flowers attract few pollinators. However, considering the limited floral diversity in many agricultural landscapes, pollinators may exploit mass flowering soybean fields to obtain floral resources. For instance, some beekeepers describe soybean as a significant source of nectar for Midwest honey, documenting variability in the attractiveness of flowers and the quality, quantity, and accessibility of floral resources among soybean varieties and field conditions (Oertel 1980, Woodcock 2012). Furthermore, yield improvements have been attributed to insect pollination when soybeans are exposed to pollinators versus caged plants and in hybrid seed production trials (reviewed in McGregor 1976, Klein et al. 2007).

Many of the aforementioned studies are based on *A. mellifera*, but surveys of the flower-visiting insects that visit soybean fields are needed to provide information on wild pollinators that may reside in soybean fields. For instance, flower-visiting flies such as syrphids (Diptera: Syrphidae) visit a variety of flowering plants for nectar and pollen (Kevan and Baker 1983, Tooker et al. 2006, Ssymank et al. 2008). Adults of some syrphid species are documented pollinators of other mass flowering crops including greenhouse sweet peppers, *Capsicum annuum* L., oilseed rape, *Brassica napus* L., and almonds, *Prunus dulcis* (Miller) D.A. Webb (Jarlan et al. 1997, Jauker and Wolters 2008, Klein et al. 2012). The abundance of syrphids in Iowan soybean fields is considered a function of the predation by the larval stages on insect pests, like the soybean aphid, *Aphis glycines* Matsumura, (Hemiptera:

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Aphidiidae) (Schmidt et al. 2008). However, the relationship between adult syrphids and other flower-visiting flies and the floral resources available in soybeans is not well defined.

In a more general context, understanding pollinator communities in row crops such as soybean can inform conservation and management decisions by providing baseline data for assessing pollinator response to landscape changes and pest management practices. Establishing sampling methods to effectively survey pollinators in soybean fields is vital for obtaining data that accurately characterizes the diversity and abundance of pollinators in this cropping system. Methods used to survey insects vary among targeted insect communities and study system characteristics. Several methods for monitoring activity and density of insects in soybean fields have been evaluated (Kogan and Herzog 1980). This includes methods for measuring insect pest populations in soybean to inform the farmer's management decisions (O'Neal et al. 2001, Hodgson et al. 2004) and for describing the natural enemy community of soybean insect pests (Bechinski and Pedigo 1982, Schmidt et al. 2008). Differences in the insect community composition were observed within the same sample area based on the trapping method and protocol. Techniques evaluated for sampling insects in soybean fields include active trapping (e.g., sweep netting and field observations) and passive trapping (e.g., yellow sticky traps and pan traps) methods, many of which are used in insect surveys across different natural and cultivated habitats.

Sweep netting is widely used for sampling insect communities in many different row crops and is capable of capturing high amounts of foliar dwelling insects per sample unit with minimal damage to plants (Kogan and Pitre 1980). Sweep net sampling is an effective tool for monitoring defoliators of soybean such as the bean leaf beetle, *Cerotoma trifurcata* Forster (Coleoptera: Chrysomelidae) (Kogan et al. 1980). For other foliage feeders in soybean, such as leafhoppers (Hemiptera: Cicadellidae), monitoring using sweep nets has shown mixed results, depending on the species (Helm et al. 1980).

Yellow sticky traps are used to monitor insect pests in row crops, including soybean and corn, *Zea mays* L. For example, Hein and Tollefson (1985) recommended yellow sticky traps to monitor adult western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae), in Iowa cornfields. Yellow sticky traps are recommended for detecting rotation-resistant western corn rootworm in soybean fields (O'Neal et al. 2001). Although these recommendations are for the same pest species, the most effective placement of yellow sticky traps differed between corn and soybean fields; yellow sticky traps were placed at ear height on corn, but just above the soybean canopy.

A variety of sampling methods evaluated for describing the natural enemy community in soybean fields indicate significant differences regarding the composition of species captured by trapping methods. Schmidt et al. (2008) observed that foliar dwelling natural enemy communities captured in soybean varied by sampling method. Sedentary species, and certain life stages thereof, were observed or taken directly from plants, while more mobile species were more likely to be captured using sweep nets and yellow sticky traps. For example, yellow sticky traps captured nearly two orders of magnitude more syrphids (*Toxomerus* spp.) than any other sampling method per sample effort; however, other natural enemies such as *Orius insidiosus* Say (Hemiptera: Anthocoridae) were captured infrequently with these traps. Schmidt et al. (2008) suggested that several trapping methods are needed to fully describe a guild of insects that is composed of several insect species with varying life history traits. It is unclear if the same methods used to describe pest and natural enemy populations will be effective in capturing pollinators in soybean and if multiple methods are required to obtain an accurate representation of this community.

Sweep nets are commonly used to collect insects, including pollinators. However, different protocols exist regarding how the net is handled for capturing pollinators in contrast to pest herbivores and natural enemies of insect pests. A deliberate approach is often used to sample pollinators, in which a net is used to hand-net individuals directly from flowers during timed, visual collections (Cane et al. 2000, Roulston et al. 2007, Matteson et al. 2008, Westphal et al. 2003). Hand-netting pollinators from flowers, as well as visual observations, may limit data collection to individuals specifically trained in recognizing different species of insect pollinators. These drawbacks may be especially evident when sampling soybean plants compared with plants that have more conspicuous and larger flowers. Given that soybean flowers are located at stem nodes and often hidden by leaves—especially in closed canopies—it may be more difficult to visually detect and then hand-net insects visiting flowers. A simpler, more general approach is often used when sampling pests or natural enemies in soybean fields. Typically, a larger net is pulled across or through rows soybean plants sweeping the vegetation of multiple plants using a pendulum-type motion. Rust et al. (1980) conducted a survey of pollinators in soybean fields using this approach, but other methods were not evaluated. Therefore, the extent to which a general sweep netting technique describes the pollinator community in soybean fields is not clear.

Traps left in the field in the absence of human disturbance and remain in the field over time may be a more efficient way to collect species with different foraging patterns. Schmidt et al. (2008) observed more syrphids collected on yellow sticky traps than sweep nets when both were used in soybean fields. Drawbacks associated with yellow sticky traps include difficulty in identifying insects, as defining characters can be damaged. Other traps have been optimized for attracting and capturing bees, including a method analogous to sampling with pan traps (hereafter referred to as "bee bowls"). This method has been used in bee surveys across a wide range of geographical regions and different plant communities (Cane et al. 2000, Stephen and Rao 2005, Westphal et al. 2003, Gardiner et al. 2010a, Droege 2011, Grundel et al. 2010). Bee bowls are painted fluorescent colors (Droege 2011) and

mimic flower colors with wavelengths attractive to bees (Kevan and Baker 1983). Because bee bowls are customized for trapping bees, the attractiveness of bee bowls to other flower visitors, such as fly pollinators, is unclear.

In contrast to the benefits of bee bowls, some negative aspects have been reported. Bee bowls may preferentially attract small-bodied bees and may not effectively monitor larger bees such as *A. mellifera*. Tuell and Isaacs (2009) noted that *A. mellifera* was captured when traps were places at canopy height as opposed to directly on the ground. Westphal et al. (2003) suggested bee bowl captures compared favorably to visual observations of flowers, but unless both methods are used, it is difficult to confirm that the same pollinators are foraging on nearby flowers because the bowls themselves are visually attractive. Therefore, pollinator diversity, abundance, and floral relationships may be poorly represented in bee bowls. However, additional evidence, like the presence of pollen from collected specimens can help confirm that foraging took place and bees were not just moving through a habitat in which bee bowls were deployed.

The need to standardize sampling methods for monitoring pollinators to effectively conduct large-scale inventories, compare pollinator communities in different habitats, and determine pollinator relationships to flowering crop species is mentioned among many studies (LeBuhn et al. 2012). It is clear among the documented comparisons of transect walks, visual observations, hand-netting, yellow sticky traps, and pan-trapping that current methods are subject to variation for many reasons. Similar to the conclusion reached by Schmidt et al. (2008) for describing the natural enemy community in soybeans, others have also concluded that multiple sampling methods may be complimentary for describing a pollinator community (Williams et al. 2001, Roulston et al. 2007, Westphal et al. 2003, Wilson et al. 2008, Grundel et al. 2010). Because of these previous observations, comparing the diversity and abundance of pollinator across different trapping methods can provide insight into whether one trapping method is sufficient or if multiple methods are needed.

The objectives of this study were to describe the pollinator community within soybean fields, determine which sampling method is most appropriate for characterizing the abundance and diversity of this community, and gain insight on pollinator taxa that may contact soybean pollen. We hypothesize that the diversity and abundance of pollinators in soybean fields will vary across all sampling methods and that the diversity and abundance of different pollinator guilds (i.e., bees versus flies) may vary within each method. Sampling methods were selected based on common usage in integrated pest management programs for insect pests of soybeans (sweep nets and yellow sticky traps) or recommended for sampling bees (bee bowls). Lastly, we hypothesize pollinators captured in the soybean fields are visiting soybean flowers and the greatest abundance of pollinators will be observed during soybean reproductive stages in which flowers are blooming.

Materials and Methods

Study Sites and Experimental Design. Data were collected from soybean fields across four study sites in central Iowa during the 2011 and 2012 growing seasons (Table 1). Study sites were located ~2–65 km from Iowa State University (ISU), Ames, IA, and a minimum of 17-km separated sites from each other. These fields were managed by two privately owned farms and two ISU research farms during 2011, and one privately owned farm and three ISU research farms during 2012. At all sites, soybeans were grown according to standard production methods (low or no-tillage, glyphosate herbicide, 76 cm row spacing) and in a rotation with corn. Therefore, the same fields could not be surveyed in consecutive years. Foliar pesticides were not applied at any of the fields during the sampling period in either year. Soybean field size among sites ranged from 0.5 to 12 ha, but the sampled area was a standard 50- by 50-m portion within each field.

Pollinators were collected at 30 sampling points along two transects arranged in an "X" formation within soybean fields at each study site. The location of transects in each field was determined by randomly selecting a field edge. At this edge, the origin of each transect (spaced 50 m apart) was placed after the first three rows of soybean plants. Transects extended 50 m toward the center of the field. Along each transect, points were recorded every 3.3 m and designated as "sample points," resulting in 15 sample points per transect. Passive trapping and active trapping methods

Table 1. Soybean fields surveyed in 2011 and 2012

Year	County	Coordinates	Farm name[a]
2011	Dallas	41° 46′39.76″ N, 93° 59′50.50″ W	Private farm
	Polk	41° 45′23.69″ N, 93° 48′44.67″ W	Private farm
	Boone	42° 00′05.69″ N, 93° 47′19.72″ W	Field Extension Education Laboratory
	Story	42° 00′08.54″ N, 93° 39′32.57″ W	Curtiss Research Farm
2012	Hardin	41° 24′13.43″ N, 93° 18′09.27″ W	Private farm
	Boone	42° 00′05.69″N, 93° 47′19.72″ W	Field Extension Education Laboratory
	Story	42° 06′23.65″N, 93°35′23.79″ W	Horticulture Research Station
	Story	41° 58′54.94″N 93°38′38.41″ W	Johnson Research Farm

[a] Farm name also indicates if soybean fields were privately owned farms or an ISU research farms.

were used to survey insects at these sample points throughout the survey period.

Bee bowls, yellow sticky traps, and sweep nets were used to sample pollinators. Bee bowls were 96-ml cups (3.25 oz. SOLO brand white plastic soufflé cups, Food Service Direct, Hampton, VA) painted fluorescent yellow, fluorescent blue (East Coast Guerrra Paint and Pigment, New York, NY), or left white. Unbaited yellow sticky traps were sheets of glue-coated cardboard dyed yellow (Pherocon AM, Trécé Inc. Adair, OK). Yellow sticky traps were folded, creating a double-sided trapping surface with each side measuring 22.86 by 13.97 cm. Sweep nets (BioQuip Products, Rancho Dominguez, Compton, CA) were used to actively sample foliage.

Stands were constructed to hold bee bowl and yellow sticky traps to ensure traps could be left in the field unattended for passive collection and to allow trap height to be adjusted as soybean plants grew. A polyvinyl chloride (PVC) pipe (Silver-Line Plastics, Sch. 40 PVC, Ashville, NC) was cut into 25-cm sections and painted red (Heirloom Red 1010-3, Valspar, Chicago, IL). Red was selected to not interfere with the color of traps and so stands were clearly visible in the field to the investigators. A white 5.08-cm PVC coupling (Charlotte Pipe PVC DWV coupling, Monroe, NC) attached to one end of the PVC section created an inlay for securely holding a single bee bowl trap. The cut PVC with couplings attached was securely fastened to stakes using zip-ties to hold traps in position maintaining just enough slack to allow the PVC to slide up and down the stake. Stands were adjusted each week so that they held bee bowl at canopy height. Stands were positioned along transects at each designated sample point (30 stands per field).

In both 2011 and 2012, data were collected when soybean plants were in reproductive growth stages (R1–R6 per Pederson 2009). In 2011, the sampling period spanned 8 wk (6 July–23 August 2011), and in 2012, the sampling period spanned 6 wk (26 June–2 August 2012). In both years, each farm was visited on two consecutive days per week during the sampling period. During the first visit, bee bowl were randomly assigned to sample points based on color (5 yellow, 5 blue, and 5 white) per transect (10 of each color per site). A single bee bowl was secured on a stand and filled with 50 ml of soapy water solution made from 2% aqueous solution of Dawn brand, original scent dishwashing liquid (Procter & Gamble, Cincinnati, OH). Bee bowls remained in the field for ~24 h, and were only deployed during favorable environmental conditions (<30% cloud cover, no precipitation, and limited wind gusts), conditions in which the majority of pollinator species are considered to be most active. The next day, insects samples within each bowl were collected. Then, yellow sticky traps were attached to the same stands and adjusted to canopy height (i.e., the bottom of the trap was situated just above the plant canopy) and left in the field for ~5 d. The number of yellow sticky traps (1 per sample point) and spacing between traps is the same as described for bee bowls (30 traps per field per week). On one of the two weekly visits, sweep net samples were taken by sweeping foliage while walking along the transects. One sweep net sample consisted of 10 pendulum swings, per the general method described in the introduction and were taken at three locations: near the field edge (3–16 m), middle of the transect (19–33 m), and centermost end of the transect (36–50 m) for a total of three sweep net samples per field per week. Sweep net samples were collected from 11 a.m. to 2 p.m. during all dates. Throughout the sampling period, growth stage, total number of flowers per plant, and plant height (cm) were measured on five randomly selected soybean plants per study site.

Specimen Processing and Identification. Prior to identification, specimens captured by bee bowls were processed according to methods described by Droege (2011). Dichotomous keys and identification guides were used to identify bees (Ascher and Pickering 2012) and flies (McAlpine 1981, Bugg et al. 2008, Triplehorn and Johnson 2005). When possible, individuals were identified to species. When species-level identification could not be resolved, individuals were identified to the lowest taxonomic unit possible and classified to morphospecies. Voucher specimens were deposited in the Department of Entomology, Insectary at ISU, Ames, IA.

Pollen Analysis. Individuals from bee bowl samples with any type of obvious pollen loads visible to the naked eye (referred to as "pollen present" bees) were separated from the rest of the specimens and placed in vials, and stored in a cold chamber for pollen analysis. From the "pollen present" bees, a subset of female bees were selected for examination to determine if they were visiting soybean flowers. These represented the most abundant species present when soybean flowers were blooming. Pollen analysis was restricted to female bees because females retain larger quantities of pollen in scopal hairs and pollen-carrying structures. Pollen was removed from these "pollen present" bees and examined for the presence of soybean pollen.

Pollen grains were removed from soybean flowers from each site to create reference slides. Pollen grains from flowers were degreased and slide-mounted (for methods see Westrich and Schmidt 1986 and Westrich 1990) and stained with Calberla's fluid (for methods see Bernhardt 2005). These slides were examined, cataloged, and photographed using a microscope-mounted camera. These slides were then used as reference images for soybean pollen identification, as well as images found in the literature (U.S. Department of Agriculture [USDA] 2011). Pollen was removed from the selected bees using an insect mounting pin to dislodge pollen from their bodies and then pollen grains were degreased, slide mounted, and stained as described above. Tools used to remove pollen and prepare slides were cleaned between each specimen to reduce cross-contamination and undesired transfer of pollen grains among replications. Slide-mounted pollen grains removed from bees were examined with light microscopy to determine if soybean pollen was present or absent on bees collected in bee bowls. If one or more grains of soybean pollen either alone or

intermixed with pollen from other plant species were detected, then the bee was noted as having "soybean pollen present." When soybean pollen was detected, the slide was photographed using a microscope-mounted camera and the size, shape, and morphological characteristics of the pollen removed from bees was compared with the reference slides and photographs. To avoid a false positive identification of soybean pollen, positive identifications were recorded only when the defining characteristics on one or more grains were clearly visible. If features were obscured, or not clearly visible, the identification was recorded as unresolved and not included in the final summary. Comparisons regarding amount of pollen carried by bees and pollinator efficiency were beyond the scope of this study.

Statistical Analyses. To describe the pollinator community in soybean fields, species richness (number of unique species/morphospecies) and abundance (number of individuals) were calculated using all observations and summarized for combined pollinators (bees and flies) and separately for each. To determine if the pollinator community was sufficiently surveyed, species accumulation curves were generated based on randomized resampling of trap observations (1,000 permutations) and nonparametric bootstrap estimators were used to estimate extrapolated species richness in the survey area (Gotelli and Colwell 2011). These analyses were performed using the "vegan" package version 2.0-8 in R version 3.0.1 (Oksanen et al. 2011, R Development Core Team 2013).

To analyze differences in species richness and abundance of pollinators between years, trap types, and sample point, a linear mixed model analysis of variance (ANOVA) was used. The model included trap type (bee bowls and yellow sticky traps), time (sampling week), sample point (trap location along transects), and all two- and three-way interactions of trap, time, and sample point as fixed effects. Location (site) was included as a random effect. The Kenward–Roger option was used to approximate denominator degrees of freedom. When the overall ANOVA showed an effect, estimate statements were used to generate *t*-tests for specific comparisons between traps and years and least squares means analyses for sampling week. Because of the variation between years, data were analyzed separately for each year. (SAS PROC Mixed, v9.3 SAS Institute 2010, Cary, NC). Data used to describe pollinator species richness for bees and flies was limited to taxonomic units identified to species or classified as morphospecies.

To test for differences in pollinator abundance and species richness among the different colors of bee bowl traps, a general linear ANOVA model was used. These analyses used species richness and abundance data and the model included the main effects of site, time (sampling week), and bee bowl color. The two-way interactions of color and time were also included; however, as the interactions were not significant, only means for the main effect of color were reported. A post hoc mean comparisons test was performed for bees and flies to determine differences in species richness and abundance among bee bowl of different colors using the Tukey's studentized range (honestly significant difference) grouping procedure ($\alpha = 0.05$) (PROC GLM, SAS software version v9.3 SAS Institute 2010).

Results

Pollinator Community. In total, 5,368 pollinators were captured in the Iowan soybean fields, of which bees accounted for 52% and flies for 48% of the total summed across all trapping methods. This pollinator community was composed of > 50 taxonomic units (species or morphospecies), including 32 bee species, 8 syrphid fly species, and 7 other fly families containing several morphospecies (Supp Table 1 [online only]). Significant year-to-year variation in bee and fly abundance was observed. When the content of all traps were combined across all sites, total bee abundance increased by more than half in 2012 than 2011 ($F = 46.52$; df = 1, 297; $P < 0.001$). The opposite was observed for fly abundance, which was five times greater in 2011 than 2012 ($F = 34.97$; df = 1, 297; $P < 0.0001$). Despite the variation between years, *Agapostemon virescens* F. (Hymenoptera: Halictidae), *Lasioglossum* species in the subgenus *Dialictus* (Hymenoptera: Halictidae), *Melissodes bimaculata* Lepeletier (Hymenoptera: Apidae), and *Toxomerus marginatus* Say (Diptera: Syrphidae) were the most abundant pollinators in both years. Very few pollinators were captured using sweep nets. Throughout the study, only 10 species were captured, of which 6 were represented by only one individual (i.e., singletons, Supp Table 1 [online only]). Therefore, further analyses focused on data collected from bee bowls and yellow sticky traps.

In both years, differences in bee and fly abundance were observed between bee bowls and yellow sticky traps. Bee bowls trapped 36 to 24 times more bees than flies in 2011 ($F = 44.53$; df = 1, 239; $P < 0.0001$) and 2012 ($F = 36.08$; df = 1, 239; $P < 0.0001$), respectively. In 2011, almost two times more flies were captured on yellow sticky traps compared with bees ($F = 8.11$; df = 1, 239; $P = 0.0049$). In 2012, no significant difference was observed between fly and bee abundance on yellow sticky traps ($F = 4.10$; df = 1, 239; $P = 0.0672$) (Fig. 1).

Species accumulation curves approached an asymptote for bees (Fig. 2) and flies (Fig. 3). This indicates sufficient sampling effort in both years. Bootstrap estimates of the species pool suggest the species richness observed represent the pollinator community predicted to be present in our survey area. These estimates suggest most of the species predicted to present and captured in each trap type, were observed in our survey (Table 2).

Bee Bowls. In total, 3,644 pollinators (bees and flies combined) and at least 44 species were captured using bee bowls. Overall, bee bowls captured 2,690 bees (~32 taxonomic units) and 954 flies (~12 taxonomic units; Supp Table 1 [online only]). Proportionally, the pollinator community observed in bee bowls was composed of 74% bees and 26% flies. From both years combined, 1,680 bowls were deployed, of which

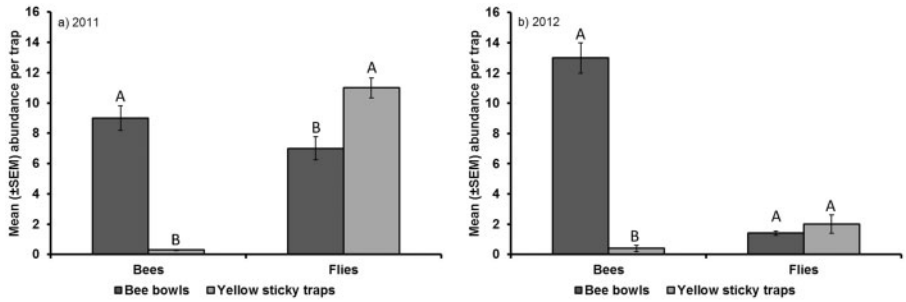

Fig. 1. Mean (± SEM) of bees and flies captured by trapping method in 2011 (a) and 2012 (b) pooled across sites and sampling weeks. Unique letters within a taxa indicate significant differences in the mean abundance between bee bowls and yellow sticky for bees (designated by capital letters) and flies (designated by lowercase letters) at $\alpha = 0.05$.

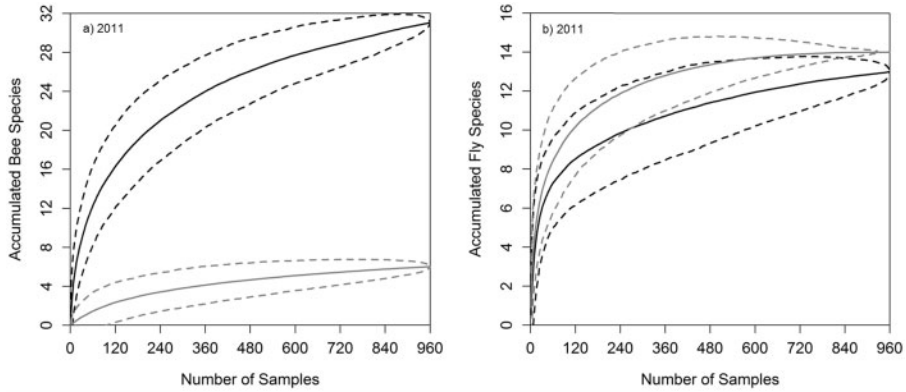

Fig. 2. Species accumulation curves generated from 1,000 permutations for bee (a) and fly (b) species captured by trapping method in 2011. Insects collected with bee bowls and yellow sticky trap are designated with a black and grey lines, respectively. Dotted lines around each solid line indicate 95% confidence levels.

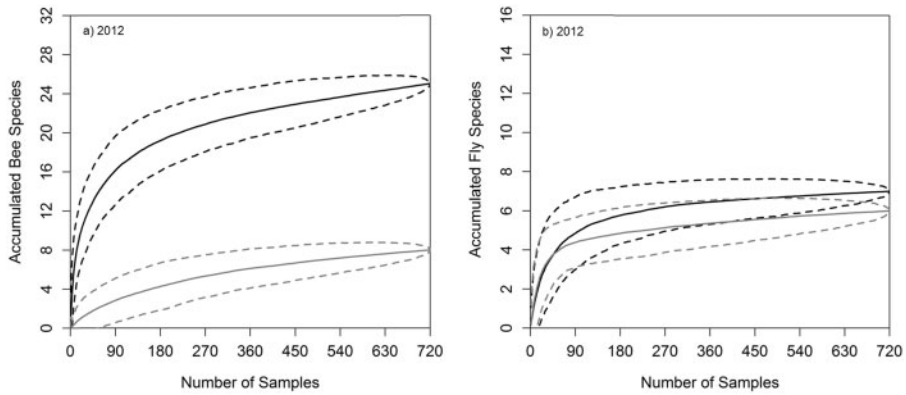

Fig. 3. Species accumulation curves generated from 1,000 permutations for bee (a) and fly (b) species captured by trapping method in 2012. Insects collected with bee bowls and yellow sticky trap are designated with a black and grey lines, respectively. Dotted lines around each solid line indicate 95% CIs.

1,116 contained one or more pollinators resulting in a 66% success rate of pollinator capture.

Bee abundance and species richness varied significantly by color of bee bowl in 2011 (bee abundance: $F = 10.61$; df = 2, 95; $P < 0.0001$; bee species richness: $F = 8.05$; df = 2, 95, $P < 0.0006$) and 2012 (bee abundance: $F = 16.98$; df = 2, 71; $P < 0.0001$; bee species richness: $F = 8.83$; df = 2, 71; $P < 0.0001$). In both years, greater bee abundance was observed in blue bee bowls compared with yellow and white bowls. In 2011, bee species richness was greatest in blue bee bowls, and in 2012, species richness was greater in blue

Table 2. Mean (± SEM) bee and fly species observed and number in Iowan soybean fields by trap type

Year	Pollinator	Bee Bowls		Yellow sticky traps	
		S_{obs} [a]	S_{boot} [b]	S_{obs} [a]	S_{boot} [b]
2011	Bees	31	34 ± 1.5	6	7 ± 0.8
	Flies	13	14 ± 0.9	14	14 ± 0.5
2012	Bees	25	27 ± 1.1	8	9 ± 0.9
	Flies	7	7 ± 0.5	6	6 ± 0.5

[a] S_{obs} = the number of species observed in the species pool.
[b] S_{boot} = extrapolated species richness means based on random resampling of trap observations (1,000) permutations and SE based on variation in sample order among randomizations.

Table 3. Mean (± SEM) abundance and species richness of bee and fly pollinators by bee bowl color

Year	Pollinator	Blue	Yellow	White
2011	Bee abundance	7 ± 1.97a	9 ± 1.58b	7 ± 0.84b
	Bee richness	5 ± 0.34a	3 ± 0.31b	3 ± 0.32b
	Fly abundance	5 ± 1.86b	11 ± 3.41a	9 ± 2.79ab
	Fly richness	2 ± 0.33b	4 ± 0.36ab	3 ± 0.29a
2012	Bee abundance	34 ± 3.80a	21 ± 2.61b	12 ± 1.41c
	Bee richness	7 ± 0.34a	6 ± 0.42ab	4 ± 0.35c
	Fly abundance	2 ± 0.69a	3 ± 0.61a	2 ± 0.47a
	Fly richness	1 ± 0.20a	2 ± 0.26a	1 ± 0.17a

Means within a row followed by unique letters are significantly different at $\alpha = 0.05$.

and yellow compared with white bee bowls (Table 3). No significant differences in fly abundance and species richness by color of bee bowl were observed in either year; 2011 (fly abundance $F = 1.41$; df = 2, 95; $P = 0.2480$; fly species richness: $F = 2.41$; df = 2, 95; $P = 0.0945$) and 2012 (fly abundance $F = 0.88$, df = 2, 71; $P = 0.4190$; fly species richness: $F = 2.52$; df = 2, 71; $P = 0.0878$).

We did not observe significant differences ($P > 0.05$) in pollinator abundance in bee bowls (both bees and flies) at varying distances along the transects. We analyzed separately the abundance and species richness of bees and flies in sample points closest to the field edge (3 m from the edge) to those furthest into the field (50 m from edge). No significant differences were detected in the abundance or species richness for either taxa between traps at the edge compared with the center in 2011 (bee abundance: $t = 1.65$; df = 69; $P = 0.1033$; bee species richness $t = 1.63$; df = 69; $P = 0.1075$; fly abundance: $t = 0.85$; df = 69; $P = 0.4005$; fly species richness: $t = 1.11$; df = 69; $P = 0.2711$) or 2012 (bee abundance: $t = 1.18$; df = 51; $P = 0.0769$; bee species richness: $t = 1.42$; df = 51; $P = 0.1629$; fly abundance: $t = 1.29$; df = 51; $P = 0.2036$; fly species richness: $t = 1.25$; df = 51; $P = 0.1352$).

We expected pollinator abundance in bee bowls to be greatest when the most soybean flowers were present. However, we did not observe significant differences in bee abundance among any point in time in 2011($F = 1.52$; df = 7, 95; $P = 0.1736$) or 2012 ($F = 1.16$; df = 5, 71; $P = 0.3428$). In both years, the abundance of flies captured in bee bowls varied significantly among sampling weeks (2011: $F = 3.04$; df = 7, 95; $P = 0.0075$; 2012: $F = 8.82$; df = 5, 71; $P < 0.0001$) with the greatest abundance of flies observed when soybeans were initially in the full flowering stage (i.e., R2; Fig. 4).

Yellow Sticky Traps. In total, 1,652 pollinators (bees and flies combined) and at least 23 taxonomic units were captured using yellow sticky traps. Overall, yellow sticky traps captured 85 bees (~8 taxonomic units) and 1,567 flies (~15 taxonomic units; Supp Table 1 [online only]). The pollinator community observed in yellow sticky traps was composed of 5% bees and 95% flies. Summed for both years, 1,680 yellow sticky traps were deployed and 744 contained samples with one or more pollinator(s) resulting in a 44% success rate.

We did not observe a significant difference ($P > 0.05$) in the abundance of pollinators (both bees and flies) on yellow sticky traps at varying distances along the transects. The abundance and species richness of bees and flies separately in yellow sticky traps located closest to the edge (3 m from the edge) compared with traps furthest into the field (50 m from edge) was compared. We did not observe significant differences in abundance or species richness of bees at the edge compared with the center in 2011 (abundance: $t = 0.49$; df = 69; $P = 0.6225$; species richness $t = 0.26$; df = 69; $P = 0.7944$) or 2012 (abundance: $t = 0.97$; df = 51; $P = 0.3378$; species richness: $t = 1.67$; df = 51; $P = 0.1018$). However, we observed significantly greater fly abundance and species richness in yellow sticky traps located at the edge of the field compared with those located in the center in 2011(abundance: $t = 1.96$; df = 69; $P = 0.0545$; species richness: $t = 2.35$; df = 69; $P = 0.0.0217$). These differences were not observed in 2012 (abundance: $t = 0.24$; df = 51; $P = 0.8110$; species richness: $t = 1.90$; df = 51; $P = 0.0628$).

In 2011, we did not observe significant differences in bee abundance among any of the weeks in which yellow sticky traps were deployed ($F = 0.83$; df = 7, 95; $P = 0.5640$). However, in 2012, we observed significant differences in bee abundance on yellow sticky traps ($F = 4.89$; df = 5, 71; $P = 0.0010$) with the greatest abundance occurring when soybeans were in the R2 stage (full flowering). In both years, the abundance of flies captured on yellow sticky cards varied among sampling weeks (2011: $F = 6.59$; df = 7, 95; $P < 0.0001$; 2012: $F = 8.75$; df = 5 71; $P < 0.0001$), with the greatest abundance observed after peak bloom (data not shown).

Sweep Netting. In total, 66 pollinators (bees and flies combined) were captured using sweep nets and were comprised of 10 bees (five taxonomic units) and 56 flies (five taxonomic units; Supp Table 1 [online only]). Of the 10 species of pollinators collected with sweep nets, 6 were represented by only one individual (i.e., singletons). Dolichopodidae dominated the pollinator community observed in the sweep net samples, which accounted for 70% of the total abundance observed in sweep nets. None of the species captured in sweep nets were unique to that sampling method, as

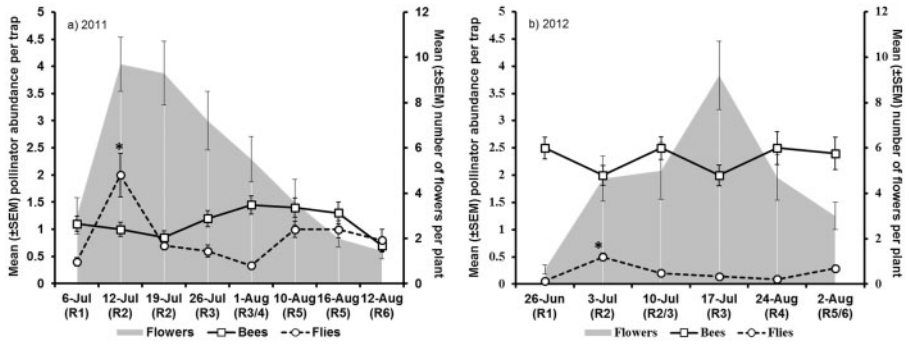

Fig. 4. Mean (± SEM) bees and flies captured in bee bowls per sampling week and the mean (± SEM) number of flower per plant (shaded area) in 2011 (a) and 2012 (b) pooled across sites and reps. An asterix indicates significant differences, which were only observed for fly abundance at one time point in each year. The shaded area shows flowers were present throughout our sampling period.

Table 4. Bee species collected in bee bowls and screened for the presence of soybean pollen

Year	Taxa[a]	Examined for pollen	Pollen present[b]	Soybean pollen present[c]
2011	*Agapostemon texanus*	4	0	0
	Agapostemon virescens	68	51	12
	Augochlorella aurata	10	2	0
	Halictus confusus	14	1	1
	L. (*Dialictus*) spp.	29	16	7
	Melissodes bimaculata	34	13	4
	Sub-Total	160	83	24
2012	*Agapostemon texanus*	51	3	1
	Agapostemon virescens	280	61	24
	Augochlorella aurata	99	4	1
	Halictus confusus	22	0	0
	L. (*Dialictus*) spp.	184	9	6
	Melissodes bimaculata	115	33	10
	Sub-Total	751	110	42

[a] The most abundant taxa of female bees collected in soybean fields were sampled for pollen.
[b] The amount of each species examined for the presence of visible pollen loads.
[c] The amount of examined individuals of each species with any pollen present.
[d] The number of "pollen present" individuals of each species in which soybean pollen was detected.

these species were also observed in bee bowls, yellow sticky traps or both.

Pollen Analysis. We identified the presence of soybean pollen on the most abundant species of female bees collected in bee bowls from each year. Individuals of these species were selected from two different periods during flowering. In 2011, we limited our analysis to those species collected during 2 wk (12 and 19 July 2011) in which we observed the greatest number of soybean plants in the R2 growth stage. Because bees abundance in soybean fields was not greatest at peak flowering (i.e., R2), we expanded the period for pollen analysis across all weeks in 2012.

In total, six species were represented by 911 female bees (Table 4), which accounted for 49% of all bees collected during those dates. In 2011, 29% of the bees with visible pollen loads contained soybean pollen (24 individuals). In 2012, 38% of the bees with visible pollen loads contained soybean pollen (42 individuals). Of those species with pollen, all but one, *M. bimaculata*, were Halictidae. In both years, *A. virescens* were the most abundant species in the subsamples used for pollen analysis. *A. virescens* had the greatest number of individuals carrying soybean pollen, followed by *M. bimaculata* and *Lasioglossum* species in the subgenus *Dialictus*.

Discussion

We hypothesized the abundance and diversity of pollinators in soybean fields would vary across sampling methods, and these results would reveal if sampling methods commonly used within integrated pest management programs for soybean production are effective for surveying pollinators in soybean fields. Bee bowls captured the greatest abundance and diversity of pollinators throughout the study, consistent with modifications designed to attract pollinators. All of the bees and the majority of flies observed on yellow sticky traps were also captured in bee bowls except for four species of syrphids (*Eupodes* sp., *Melanostoma mellinum* L., *Platycherius* sp., and *Syrphus* sp.) that were unique to yellow sticky traps. Yellow sticky traps could be used to compliment bee bowls, especially if there is interest in monitoring the abundance and diversity of flower-visiting flies. For the effort we used, using sweep nets in a general manner were the least effective sampling method, capturing only 1% of the total pollinators observed throughout the study. The few bees and flies captured in sweep nets were also captured in bee bowls or yellow sticky traps. The low capture rates in sweep nets may be explained by the way in which the nets were used to sample foliage. More deliberate sampling may be necessary if a sweep net is to be used to sample pollinators in soybean fields. Although direct observations and targeted sweep-net sampling may eliminate the occurrence of pollinators that are not directly visiting soybean flowers, the amount of sampling effort and

training may limit the usefulness of these methods. These data suggest that bee bowls can provide an estimate of the pollinator community in soybean fields, both in terms of abundance and diversity.

The biodiversity of insects within soybean fields is influenced by the surrounding landscape, as species richness of some insect taxa increases when a field is surrounded by a greater diversity of land-use types (Gardiner et al. 2009, 2010b). Fields within central Iowa were used in those studies, representing landscapes with limited diversity, primarily surrounded by other soybean and cornfields. Although determining the relationship between pollinator diversity in soybeans and the landscape diversity surrounding these fields was not a goal of this study, we observed remarkable pollinator species richness in these soybean fields of central Iowa. Although these data suggest that pollinator communities can be diverse in Iowa's soybean fields, it is unclear if individuals were visiting soybean flowers or were collected because of the attractive nature of the bee bowls and yellow sticky traps. Two lines of evidence suggest that the bees captured were likely using these fields for forage. First, this diversity was not limited to field edges as abundance and diversity was not limited to bee bowls located near field edges. Second, bees were screened for soybean pollen to confirm that they had visited soybean flowers, and were not just present in soybean fields because of the attractive nature of the traps. From a subset of six species, we consistently observed bees with soybean pollen (Table 4). Additional studies are required, however, to determine if pollen is transferred to these plants, as the type or amount of pollen carried on a bees' body is not always a reliable proxy for determining pollinator efficacy. Although our goal was to simply determine the presence or absence of soybean pollen on our samples, we did observed several other types of pollen, including pollen from species belonging to the following plant families Asteraceae, Poaceae, and Fabaceae. This indicates that bees were likely visiting several different plants near our study sites.

Although bee bowls captured a diverse community of bees, with some species especially abundant (e.g., *M. bimaculata*), other important bee species were rarely captured. Although *A. mellifera* colonies were kept on or in close proximity to the farms where soybean fields were sampled (K. Gill, personal observation), only 14 individuals were captured in our bee bowls and even fewer with the other methods. This is consistent with other studies that have used bee bowls. There may be several explanations for this including, the capacity of *A. mellifera* to either avoid or escape from bee bowls because of their size or a general disinterest in soybean as a source of nectar or pollen. The size of the bee bowls likely does not explain the lack of *A. mellifera* captured, as we found many similar sized bees (e.g., *M. bimaculata*) in bee bowls. Furthermore, *A. mellifera* has been reported visiting soybean flowers by entomologists in the United States (Rust et al. 1980) and beekeepers have reported honey crops from soybeans (McGregor 1976).

Another explanation for the low abundance of *A. mellifera*, and possibly other species, is that nearby floral displays may have influenced foraging behavior, detracting from the attractiveness of either the soybean flowers or the traps (Cane et al. 2000, Roulston et al. 2007, Baum and Wallen 2011). As we did not record flower visitation, the extent to which flower abundance affects sampling with bee bowls in soybean is unclear and deserves further investigation. However, we did not observe pollinator abundance in bee bowls to vary across time, even when soybeans were in peak bloom ($\sim$R2). Therefore, we suggest that the abundance of pollinators, especially *A. mellifera*, in bee bowls deployed in soybeans is likely strongly influenced by the occurrence of other flowering resources around the field.

Although a global decline in pollinators has been reported (Potts et al. 2010), a lack of historical and present-day inventories of pollinator communities across different regions and vegetation types (Roubik 2001; LeBuhn et al. 2007, 2012) limit the inferences that can be drawn from these data. For example, it is not clear if the relative low abundance of *A. mellifera* is part of a general decline in their abundance within the United States (Calderone 2012). Although there is evidence that *A. mellifera* visit soybean flowers (Erickson et al. 1978), soybean flowers were thought to attract few bees (McGregor 1976). Rust et al. (1980) observed *A. mellifera* within soybean fields, but did not report their abundance, only noting that species of native bees were more abundant. Overall, Rust et al. (1980) reported 29 species (including unidentified species of *Dialictus*) of native bees from soybean fields in Delaware, Wisconsin, and Missouri, but did not report the occurrence of dipteran pollinators. Unfortunately, comparison of the bee community that we observed in 1978 and 1979 is limited because of differences in sampling methods and the lack of abundance data reported for all species captured. Despite these differences, Rust et al. (1980) reported that *M. bimaculata* was found in all three states, and was observed with soybean pollen. The frequent occurrence of *M. bimaculata* with soybean pollen across soybean fields in multiple U.S. states suggests that this species may use soybean flowers as a source of pollen and nectar. We recommend that future studies of pollinator communities in soybeans uses a standard methodology that rely on bee bowls being used within this study.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

Acknowledgments

We acknowledge funding to conduct this research from DuPont Pioneer Hi-bred International and the Leopold Center for Sustainable Agriculture. We had assistance with fieldwork and bee identification from Michael Joseph Wheelock and Kathryn Russell.

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Received 9 April 2014; accepted 6 January 2015.