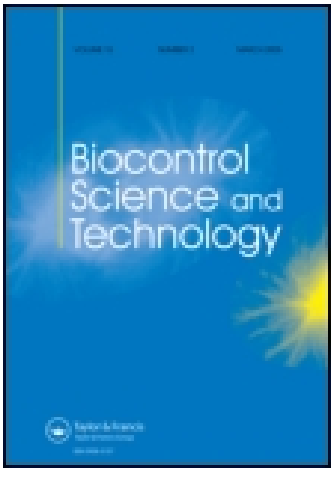

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Protein-marking-based assessment of infield predator dispersal

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SHORT COMMUNICATION

Protein-marking-based assessment of infield predator dispersal

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Understanding infield predator dispersal is crucial for designing predator conservation programmes. A study aimed at evaluating methods of collecting insects in protein-marking studies and monitoring predator movement was conducted. Results indicate that collection by sweep net does not result in false positives and predator groups displayed distinct dispersal patterns.

Keywords: predator movement; indirect ELISA; collection technique

Movement of natural enemies throughout agricultural landscapes contributes to their effectiveness in controlling pest insect populations (Snyder, Chang, & Prasad, 2005). Several studies demonstrate that diversifying vegetation increases numbers of natural enemies in agroecosystems (Bowie, Gurr, Hossain, Baggen, & Frampton, 1999; Nicholls, Parrella, & Altieri, 2001); however, the distance and frequency with which insects move from areas with abundant resources remain largely unknown. Natural enemy movement has been evaluated using diffusion models (Bommarco & Fagan, 2002; Corbett & Plant, 1993; Rudd & Gandour, 1985). Establishing dispersal distances for beneficial insects is important for incorporating diverse vegetation, specifically determining size, number and distance of refuge strips (Carmona & Landis, 1999; Corbett & Plant, 1993).

The recent development of inexpensive protein-markers allows for the detection of movement between fields (Hagler & Naranjo, 2004; Jones, Hagler, Brunner, Baker, & Wilburn, 2006) and crop/non-crop vegetation (Horton, Jones, & Unruh, 2009; Swezey et al., 2013). Protein markers are a valuable tool in assessing dispersal distances for generalist, predatory insects within agroecosystems (Sivakoff, Rosenheim, & Hagler, 2012). However, Hagler and Jones (2010) caution that care must be taken when collecting insects in protein-marking studies. The marker can easily be transferred among specimens, creating false positives (Hagler & Jones, 2010). Many studies employ sticky cards (Krugner et al., 2012) and d-vacs (Sivakoff et al., 2012) to collect insects. The use of sweep nets in protein-marking studies has not previously been evaluated. The objectives of this study were to determine if collecting insects with sweep nets is a viable option in protein-marking experiments and conduct preliminary analysis of infield dispersal distances for generalist predators within alfalfa.

Movement was evaluated in three Pioneer 54V54 variety alfalfa fields on the South Dakota Soil and Water Conservation Research Farm operated by USDA-ARS near Brookings, SD (44.349722, –96.803056). Fields were between 2.3 ha and

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3.2 ha in size and were separated by 225–470 m. Fertilizers and pesticides had not been applied to fields. Alfalfa height was approximately 15 cm. Protein was applied once on 26 September 2011 within a 5.5 × 5.5 m (30.25 m^2) centralised area of each field. Wind speed averaged 30 km/h from the SSE-SSW over the study period. Two fields were treated with milk protein (20% solution; Great Value Nonfat Instant Dry Milk, Walmart, Bentonville, AR) and one field with egg protein (10% solution; Great Value 100% Liquid Egg Whites, Walmart, Bentonville, AR). To aid in marker stability, ethylenediaminetetraacetic (EDTA) acid was added to each solution at a rate of 0.3 g/L. Both protein solutions were applied at a rate of 1 L/3.8 m^2 for a total of 8 L of protein sprayed per area. Protein was applied using a CO_2-powered sprayer, using Teejet XR 8002VS nozzles, and spray pressure of 180 kPa. Contamination of the field outside the area of spray was prevented by the applicator wearing protective footwear and gloves that were removed prior to exiting the area of application.

Predators were sampled by hand collection and sweep net 24 h and 48 h after protein application. Samples were collected at 3 m, 15 m, 30 m and 100 m along four transects radiating NE, NW, SE and SW from the marked area. On the NW transect, a 4-m transect was established centrally and perpendicular to the main transect at each sample distance. Predators were collected using forceps within the 4-m transect, which was sampled for 10 min. Forceps used to collect predators were cleaned with 70% ethanol after every collection. On the remaining transects, arthropods were sampled with sweep nets (38-cm diameter). At each sample point (and starting furthest from the marked area), a centralised 10-m perpendicular transect was established, from which 50 sweeps were collected. A single sweep was considered movement of the net in one direction. Sweep nets were designated for each distance from the area of protein application and each protein marker. Forceps were used to remove predators from the sweep nets and were cleaned with 70% ethanol after every collection. Individual insects were placed in 1.5-mL vials and placed on ice immediately.

In the laboratory, each insect was placed in a 1.0-mL vial within 750 μL of 1 × phosphate buffer saline (PBS) on an orbital shaker (Daigger, Vernon Hills, IL) for 1 h. The insect was then removed from the buffer and the resulting solution was frozen at –20 °C. Insect specimens were identified to species. Thomas J. Henry (USDA-ARS, Washington , D C) identified all Nabidae and Miridae. All other insects were identified by the authors. Araneae were placed in a single grouping.

Egg albumin was detected on insects in the field sprayed with egg protein and bovine casein was detected on insects in fields sprayed with milk protein using the indirect enzyme-linked immunosorbent assay (ELISA) methodology published by Hagler and Jones (2010). Antibody dilutions and dilution agents varied from Hagler and Jones (2010). To detect the egg albumin, the primary antibody, rabbit anti-chicken egg albumin (No. C-6534, Sigma Chemical Company, St. Louis, MO, USA), was diluted 1:6400 in 1 × PBS. The secondary antibody, goat anti-rabbit IgG (whole molecule) conjugated to horseradish peroxidase (Sigma No. A-6154), was diluted 1:8000 in 1% non -fat milk. To detect the bovine casein, the primary antibody, sheep anti-bovine casein (No. K01336S; Meridian Life Sciences, Saco, ME, USA), was diluted 1:400 in 1 × PBS. The secondary antibody, mouse anti-goat/sheep IgG conjugated to horseradish peroxidase (Sigma No. A-9452), was diluted 1:2000 in a 25% egg white solution. Each plate had a series of negative controls, eight wells with PBS only and eight wells with all antibodies added. The mean and

standard deviation of that series were calculated for each plate to establish a baseline absorbance threshold. A sample was considered positive if the absorbance was three times the standard deviation of the negative control series.

An analysis of variance (ANOVA) was conducted to determine if proportion positive varied by day, direction or distance. Because direction was not significant, sweep net samples were combined and a single proportion positive value per transect was calculated to compare to the hand collected samples. An ANOVA was conducted to evaluate the variation between the proportion of positive samples collected by hand and by sweep nets. Collection method was the independent variable and the arc sin transformation of the proportion of positives was the dependent variable. Mean distance dispersed was calculated as described by Sivakoff et al. (2012) to determine the average distance travelled by all predators combined, as well as individual groups comprising >10% of the community sampled.

Proportion of total positive samples did not vary significantly by day, direction or distance. There were no significant differences between the sampling method and proportions of positive and negative samples ($F = 0.06$, df = 1, $P = 0.81$). A total of 422 positives were collected by sweep nets with a total of 1097 insects collected using this method (average proportion = 0.40 ± 0.08 per direction per plot). A total of 25 positive samples were collected by hand with a total of 52 insects collected (average proportion = 0.48 ± 0.24 per plot). Thus, the data indicate contamination of samples is minimal when carefully collecting specimens with sweep nets. Hand collections took about three times longer than sweep net collections and resulted in only 4.5% of the total predators collected. Thus, collecting by sweep net is more time and labour efficient and increases the amount of information that can be gained from protein-marking studies.

Of the 1149 insects and arachnids collected in alfalfa during a 48-hour period, 447 were positive for a protein marker. When all predators were combined into a single analysis, the composite mean distance dispersed per day was estimated to be 75.10 m (±3.42). For those groups that comprised greater than 10% of the sample, an individual mean distance dispersed was calculated. *Orius insidiosus* (Say; Hemiptera: Anthocoridae) was collected in greatest abundance comprising 30% of the total arthropods sampled over two days. *Nabis americoferus* (Carayon; Hemiptera: Nabidae) and *Lygus lineolaris* (Palisot; Hemiptera: Miridae) comprised 21.5% and 11% of the community, respectively. The role of *Lygus* sp. throughout ecosystems varies, serving as a pest, predator or omnivore (Pfannenstiel and Yeargan, 2002). For this study, the species was included in the analysis due to its potential as a generalist predator, although some regard *L. lineolaris* as primarily herbivorous in alfalfa. The Araneae as a group comprised 13.6% of the entire sample.

Predators had distinct but different within-field dispersal patterns over time. Figure 1 demonstrates dispersal of these groups one and two days after protein application. *O. insidiosus* and *L. lineolaris* were collected over a broader area 48 h after protein application than 24 h after protein-marking. In contrast, *N. americoferus* and the Araneae seemed to have more directed movement, such that marked insects were found to incrementally disperse farther from the marked area over the two-day study.

Protein marking to evaluate insect movement is a valuable technique when establishing management recommendations for planting refuges and strip crops to attract natural enemies in agroecosystems. This study demonstrates that collection of

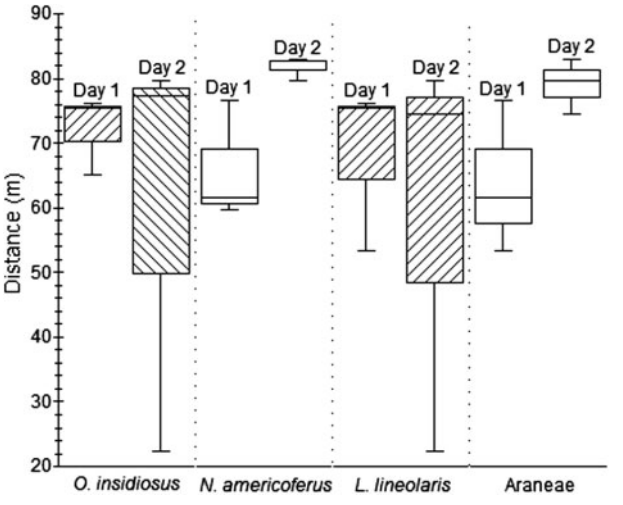

Figure 1. The distance dispersed in alfalfa fields at 24 h and 48 h by *O. insidiosus* (number collected = 348, number marked = 97), *N. americoferus* (number collected = 247, number marked =103), *L. lineolaris* (number collected = 125, number marked = 53) and the Araneae (number collected = 156, number marked = 66).

insects when employing protein marking is not limited to time consuming and inefficient methods such as hand collection or sticky cards. A great number of questions remain to be answered with regard to the distances that insect predators are moving throughout fields. Because our analysis of distance dispersed was limited by the number and size of fields, future studies should evaluate movement on a larger scale. Additionally, the evaluation of movement distance within and between non-crop and crop vegetation will aid in answering the larger question of how to most efficiently incorporate non-crop vegetation with the aim of increasing predator abundance and effectiveness.

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