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Research article

Biocontrol on the edge: Field margin habitats in asparagus fields influence natural enemy-pest interactions

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ABSTRACT

We evaluated pest and predator spatial distributions in relation to asparagus field margins, developed molecular gut content analysis methods for two key asparagus pests, and determined trophic links between the two pests and arthropod predators. Our results indicated that the abundance of natural enemies is higher outside asparagus fields than inside, and fields bordered by forests had higher numbers of predators compared to other types of field margins. We screened 3646 field-collected predators from 10 commercial asparagus fields using molecular gut content analysis in 2014 and 2015, and found that 29 arthropod families feed on the two key pests. Significantly more predators positive for the two key pests' DNA were found in field margins in both years than inside the asparagus field. We highlight the potential significance of unmanaged field margins, particularly forested ones, in providing biocontrol services in agricultural fields.

1. Introduction

Agricultural field margins are important sources of ecosystem services, but their beneficial contributions to pest management are not well understood (Bell et al., 2002; Dennis and Fry, 1992; O'Rourke and Jones, 2011; Vickery et al., 2009). Field margins represent crop field edges that interface areas of managed or unmanaged natural vegetation, crop fields, or anthropogenic structures, such as roads (Marshall and Moonen, 2002). Generally, higher arthropod abundance and diversity is observed in field edges than in the field interior (Botero-Garcés and Isaacs, 2004; Denys and Tscharntke, 2002). One proposed explanation for this is that intensively managed agroecosystems are frequently sprayed with insecticides, thus creating temporal arthropod deserts, and field margins can provide habitat for shelter and recolonization (Ramsden et al., 2015). Therefore, promoting the development of alternative non-cropped habitats outside fields could contribute to ecosystem friendly pest management if they provide biological control services (O'Rourke and Jones, 2011; Tschumi et al., 2016). However, there is concern about the effects of field margin habitat on pest control because they may harbor harmful arthropods (Duelli et al., 1990; O'Rourke and Jones,2011).

Increasing plant diversity in field margins may lead to an improvement in resources for beneficial arthropods which in turn can enhance the magnitude and outcome of biocontrol (Dennis and Fry, 1992; Fiedler and Landis, 2007; Isaacs et al., 2009; Walton and Isaacs, 2011a, 2011b). Conversely, some plant species may be disproportionately attractive to pests, which would defeat the purpose of providing such habitat. For example, some arthropod pests find and develop on alternate hosts, which would sustain pest populations in agricultural landscapes (Blitzer et al., 2012; Schellhorn et al., 2008). Encouragingly, studies show consensus that natural enemies are more commonly attracted to diverse high quality field margins and non-cropping areas in agricultural landscapes than pests and this leads to enhancing conservation biocontrol programs for key pests (Fielder and Landis, 2007; Isaacs et al., 2009; Letourneau et al., 2011; Thies and Tscharntke, 1999; Tscharntke et al., 2005).

Commonly, pest management is focused on a few key pests that are the top priorities for securing economically profitable yields (e.g., Reitz et al., 1999). The efficacy of habitat enhancement programs for key pest control hinges on whether pests and natural enemies spatially and temporally overlap (e.g., Woodcock et al., 2016). For instance, arthropod natural enemies may move into agricultural fields from field margins during periods of abundant prey, while others may only randomly disperse into the field looking for prey using margins as permanent homes. To advance our understanding of biocontrol in agricultural landscapes, we need to better understand the interactions that occur between pests and natural enemies across crop to field margin interfaces.

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Characterizing interactions between arthropod herbivores and predators has been revolutionized by the use of molecular gut content analysis (Furlong, 2015; King et al., 2008; Sheppard and Harwood, 2005; Symondson and Harwood, 2014). This method provides a qualitative approach to unraveling food webs and determining which field-collected predators are providing biocontrol services. Studying trophic interactions with this approach has become increasingly used in agricultural systems; however, the primary focus previously has been on interactions taking place within managed fields (e.g., González-Chang et al., 2016; Szendrei et al., 2010). With a growing recognition of the importance of agricultural landscape structure on pest management, research is needed on the effects of margin habitat and landscape elements on biocontrol services using molecular gut content analysis as a tool.

In this study, we focus on the interface between field margins and agricultural fields to aid in the development of a conservation biocontrol program for two key asparagus pests, the asparagus miner (*Ophiomyia simplex* Loew; Diptera: Agromyzidae) and common asparagus beetle (*Crioceris asparagi* L.; Coleoptera: Chrysomelidae) (Barnes, 1937; LeSage et al., 2008). Past studies in asparagus have determined asparagus miner to be spatially aggregated at field edges, providing the possibility for overlap with natural enemies preferring field margin habitat and the opportunity of designing habitat management programs to improve biological control (Morrison and Szendrei, 2013). Our specific goals were to: 1) evaluate pest and predator spatial distributions in relation to field margin types, 2) develop molecular gut content analysis methods for both key pests, 3) determine the predators of these key pests using molecular gut content analysis, and 4) investigate the impact of field margin type and spatial location (i.e., within field or near field margin) on the incidence of predation.

2. Materials and methods

2.1. Arthropod collections

We collected predators and pests weekly in 10 postharvest commercial asparagus fields in Oceana County, Michigan, USA, from July to August 2014 (five sampling dates), and June–August 2015 (nine sampling dates; Table S1). Two margin regions per field were designated as collection sites. For all fields, vegetation outside the field edge consisted of a ~5 m wide drive row that typically consisted of mowed weeds or grass, and is a common feature of agricultural fields in the US to allow the movement of farm equipment. Beyond the drive row, we classified the margins as one of four types: asparagus, crop (alfalfa, cherry, or corn), forest (unmanaged areas with mixtures of deciduous hardwoods and coniferous evergreen softwoods, e.g., maple (*Acer* spp.), pine (*Pinus* spp.), beech (*Fagus* spp.), and hemlock (*Tsuga* spp.)) and non-crop (infrequently managed areas with mixtures of grasses, e.g., *Poa* spp., *Lolium* spp., *Festuca* spp., and *Agrostis* spp., and weeds, e.g., *Plantago* spp., *Amaranthus* spp., *Anthemis* spp., and *Taraxacum* spp., that were often adjacent to an anthropogenic structure, such as a building or road). Each sampled margin region was divided into three transects, each consisting of a 10 m × 1 m sampling area running parallel to the field margin. One sampling area was located 10 m away from the asparagus field in the margin habitat, another at the asparagus field edge, and the third was 20 m into the asparagus field (Fig. S1).

Collections of live pest and predatory arthropods were done using a sweep net for canopy-dwelling arthropods and a field vacuum (Toro® Power Vac, Bloomington, MN, USA) modified with a fitted mesh bag over an 11 cm diameter inlet for soil-dwelling arthropods. Five vacuum samples were taken at random within each transect's 10 m × 1 m sampling area for 10 s per sample and was consistent between all margin habitats. Sweep net sampling in asparagus fields was comprised of 40 sweeps in each sampling area from ~100 to 150 cm canopy height. In forested margins, sweep net samples were taken from low tree branches and understory flora ~100–150 cm from the soil surface. However, in crop (alfalfa and cherry) and non-crop habitats plant material below 100 cm in height were sampled because these plants are kept short with management by farmers. Arthropods were sorted in the field immediately after collection, predatory specimens were then placed individually into chilled vials containing 75% ethanol, and stored on ice until they were frozen in the lab at −20 °C. Only those predatory arthropods were retained that were in a life-stage that was feeding on other arthropods; for example, only larval stages of Chrysopidae were collected for further processing since adults are not predatory.

2.2. Molecular gut content analysis

2.2.1. Primer design for asparagus miner and common asparagus beetle DNA

Primers designed to amplify asparagus miner and common asparagus beetle DNA were developed to establish predatory linkages. Sequences for primer design were obtained using cytochrome c oxidase subunit I (COI) primers Nancy (5′ − CCC GGT AAA ATT AAA ATA TAA ACT TC − 3′) and Ron (5′ − GGA TCA CCT GAT ATA GCA TTC CC − 3′) (Simon et al., 1994). PCRs (50 µl) were comprised of 36.25 µl PCR certified H_2O (Teknova, Hollister, CA, USA), 5 µl 10× PCR buffer, 1.5 µl (50 mM $MgCl_2$), 1 µl (0.2 µM) dNTP, 1 µl (0.2 µM) of each general primer, 0.25 µl Taq (ThermoFisher Scientific Inc., Waltham, MA, USA), and 4 µl of asparagus miner or asparagus beetle DNA. PCR was conducted with an Eppendorf Mastercycler® Pro (Eppendorf, Hauppauge, NY, USA) thermal cycler using the PCR protocol of 94.5 °C for 3 min, followed by 40 cycles of 94.5 °C for 45 s, 41 °C for 1 min, 72 °C for 2 min, and a final extension period of 72 °C for 5 min. Gel electrophoresis (60 V for 3 h) confirmed amplification using 6 µl of PCR product in 3% agarose gel (Invitrogen UltraPure® Agarose, ThermoFisher Scientific Inc.) stained with 7.5 µl GelRed nucleic acid stain (Phenix Research Products, Candler, NC, USA). Reactions with sufficient PCR product were purified and sequenced at the Michigan State University Genomics Core Facility (East Lansing, MI, USA).

Sequences for all available Agromyzidae and Chrysomelidae were downloaded from GenBank and aligned with asparagus miner and common asparagus beetle COI sequences using MUSCLE (Edgar, 2004). Primers for asparagus miner and common asparagus beetle were selected following testing in *Primer 3* (Rozen and Skaletsky, 2000). Primers selected for asparagus miner had sequences of 5′ − CTT CAT TTA GCT GGA ATT TCT TCT ATT − 3′ (AM_F, T_m = 59 °C) and 5′ − ATA GGG TCT CCC CCT CCA G − 3′ (AM_R, T_m = 60 °C) and produced a 238 bp amplicon product. Primers selected for the common asparagus beetle had sequences of 5′ − TCA CAG TTG GTG GTT TAA CAG GA − 3′ (AB_F, T_m = 62 °C) and 5′ − TGC AAA CAC TGC CCC TAT TG − 3′ (AB_R, T_m = 62 °C) and produced a 122 bp amplicon product. Primer specificity was screened against a non-target library of 100 arthropods representing 44 families from 12 orders (Schmidt et al., 2016) and there was no amplification with any of the non-target species.

2.2.2. Predator gut content extraction

To establish trophic linkages to asparagus miner and common asparagus beetle, molecular gut content analysis was conducted on the field-collected predators. Predators were identified to family, genus or species prior to DNA extraction (Arnett, 2000; Arnett and Thomas, 2000; Arnett et al., 2002; Bradley, 2012; Stehr, 1987; Ubick et al., 2009). Specimens were then removed from their respective collection vials, rinsed with double-distilled H_2O and 95% ethanol, dried, and placed in autoclaved 1.7 ml centrifuge vials. The whole predator was pulverized with a pestle and total DNA was extracted and purified using a QIAGEN DNeasy® Blood and Tissue kit using the protocol outlined by the manufacturer for animal tissue extraction (QIAGEN Inc., Chatsworth, CA, USA).

2.2.3. Predator gut content screening

Predatory linkages were established by screening extracted predator DNA for the presence of asparagus miner and common asparagus beetle DNA using multiplex PCR and gel electrophoresis. The PCR mix contained 4.33 µl PCR certified H_2O (Teknova, Hollister, CA, USA), 6.25 µl 2× PCRBIO HS Taq Mix Red (PCR Biosystems Ltd., London, UK), 0.50 µl (10 mM) asparagus miner primer, and 0.42 µl (10 mM) common asparagus beetle primer were mixed with 1 µl of extracted whole predator DNA. Asparagus miner and common asparagus beetle DNA were used as positive controls. PCR was conducted using an Eppendorf Mastercycler® Pro thermal cycler using the protocol of 95 °C for 2 min, followed by 30 cycles of 95 °C for 30 s, 56.5 °C for 30 s, 72 °C for 45 s, and a final extension period of 72 °C for 5 min. Gel electrophoresis (2%, Invitrogen UltraPure® Agarose; 7.5 µl GelRed nucleic acid stain) was conducted using 6 µl of PCR product at 90 V for 1.5 h. A reference (1.5 µl, GeneRuler LR, 25–700 bp, ThermoFisher Scientific Inc.) was used to verify correct product sizes.

2.3. Statistical analysis

Spatial autocorrelation between collection sites was checked using a Mantel's test (package = "ADE4") to ensure independence between collection sites for pests and predators prior to analysis (R Core Development Team, 2015). Asparagus miner and common asparagus beetle abundances were determined from sweep net samples only, as vacuum sampling resulted in few asparagus miners and no asparagus beetles, and predator abundances were the sum of vacuum and sweep net collections. All data were analyzed using a mixed effects model with a Poisson distribution GLMER (package = "LME4") with margin type and transect sampling location as fixed effects, and collection date and field as random effects. We compared reduced and full models using Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) to select the model with the best fit for the data. Collection years were analyzed separately because 2014 represented a five-year low in degree days accumulated over the growing season, 18% below the five-year average, and 2015 represented an above-average degree day accumulation at 2% above the five-year average (MSU Enviro-weather, 2016). A post-hoc least squares means comparison with Bonferroni correction was made on fixed factors detected as significant using generalized linear hypothesis test ($\alpha = 0.05$; package = "MULTCOMP").

We created food webs using the proportion of predators testing positive for pest DNA, corrected for overall predator abundance, which allowed visualization of predatory linkages and the relative strength of those links (package = "BIPARTITE"). To test for predation differences, we compared the total number of predators testing positive for asparagus miner and common asparagus beetle DNA by margin habitat type and collection transect sampling location using a Pearson's chi square test with post-hoc multiple pairwise comparisons ($\alpha = 0.05$; package = "STATS").

Predator community composition was analyzed by collection type (vacuum or sweep) and by transect sampling location. To meet acceptable stress levels for community analysis, predator totals from the field edge and 20 m sampling locations were summed (Clarke, 1993). Analysis was done at the family taxonomic level (except for Opiliones) with non-metric multidimensional scaling (NMDS) and analysis of similarity (ANOSIM; package = "VEGAN"; $\alpha = 0.05$). However, sweep net data had no convergent solutions; therefore, only vacuum samples were analyzed with NMDS. The exception was 2015 sweep net data from field margins, which produced convergent solutions with acceptable stress values for NMDS (Clarke, 1993).

3. Results

3.1. Pest abundance

We confirmed for asparagus miners and beetles that collection sites were independent (Mantel's test: $r = -0.12$, $p = 0.92$). We collected 809 and 2102 asparagus miners in 2014 and 2015, respectively. In 2014, there was no significant margin effect on pest abundance; however, in 2015, a significant effect was detected (2014: $\chi^2 = 4.94$, df = 3, $p = 0.18$; 2015: $\chi^2 = 12.34$, df = 3, $p < 0.01$). For both years, significant transect sampling location (2014: $\chi^2 = 75.44$, df = 2, $p < 0.01$; 2015: $\chi^2 = 250.60$, df = 2, $p < 0.01$) and margin × transect sampling location interaction (2014: $\chi^2 = 172.34$, df = 6, $p < 0.01$; 2015: $\chi^2 = 170.53$, df = 6, $p < 0.01$) were found (Table S2a). In 2015, the abundance of asparagus miners was statistically higher in sites adjacent to asparagus borders than those bordered by crops and non-crop borders ($z > 3.15$, df = 3, $p < 0.01$; Fig. 1a). Asparagus miners were significantly more abundant in both years at the field edges when compared to the margins and inside the field (2014: $z > 8.92$, df = 2, $p < 0.01$; 2015: $z > 9.06$, df = 2, $p < 0.01$; Fig. 1b).

We collected 40 and 95 common asparagus beetles in 2014 and 2015, respectively. The effect of margin type on the number of asparagus beetles in either year was not significant (2014: $\chi^2 = 2.25$, df = 3, $p = 0.81$; 2015: $\chi^2 = 2.70$, df = 3, $p = 0.75$; Fig. 1c). In 2014, transect sampling location was not a significant predictor of asparagus beetle abundance ($\chi^2 = 7.32$, df = 2, $p = 0.12$); however, in 2015, significantly more asparagus beetles were found at the field edge when compared to the other sampling locations ($\chi^2 = 11.92$, df = 2, $p = 0.02$; Fig. 1d). No interaction between margin and transect sampling locations were detected in either year (2014: $\chi^2 = 1.61$, df = 6, $p = 0.95$; 2015: $\chi^2 = 8.91$, df = 6, $p = 0.18$; Table S2b).

3.2. Predators of asparagus miner and common asparagus beetle

Spatial autocorrelation was not found among our sites for predators (Mantel's Test: $r = -0.01$, $p = 0.47$). In 2014 and 2015, there were significant differences in arthropod predator abundance across margin types (2014: $\chi^2 = 17.88$, df = 3, $p < 0.01$; 2015: $\chi^2 = 9.43$, df = 2, $p = 0.02$) and transect sampling locations (2014: $\chi^2 = 60.54$, df = 2, $p < 0.01$; 2015: $\chi^2 = 1167.31$, df = 2, $p < 0.01$). Significant interactions between margin and transect sampling locations were also detected in both years (2014: $\chi^2 = 36.60$, df = 6, $p < 0.01$; 2015: $\chi^2 = 71.47$, df = 6, $p < 0.01$; Table S2c).

Predator abundance was significantly higher in fields with forested margins than fields with asparagus or crop margins in 2014 ($z > 2.61$, df = 3, $p < 0.04$). In 2015, forested margins also had the highest predator abundance of all margin types and was significantly higher than fields with asparagus margins ($z = 3.61$, df = 3, $p < 0.01$; Fig. 1e). Significant differences in predator abundance relative to transect sampling location was found in both years with significantly more predators collected from the field margins than at the field edge or within the field (2014: $z > 2.85$, df = 2, $p < 0.01$; 2015: $z > 25.00$, df = 2, $p < 0.01$; Fig. 1f).

3.3. Predator communities

Predator communities collected from inside the asparagus fields by vacuum relative to margin vegetation type in both years were similar to each other (2014: ANOSIM $R = -0.05$, $p = 0.77$, NMDS stress = 0.11; 2015: $R = -0.10$, $p = 0.94$, NMDS stress = 0.17; Fig. S2). In 2014,

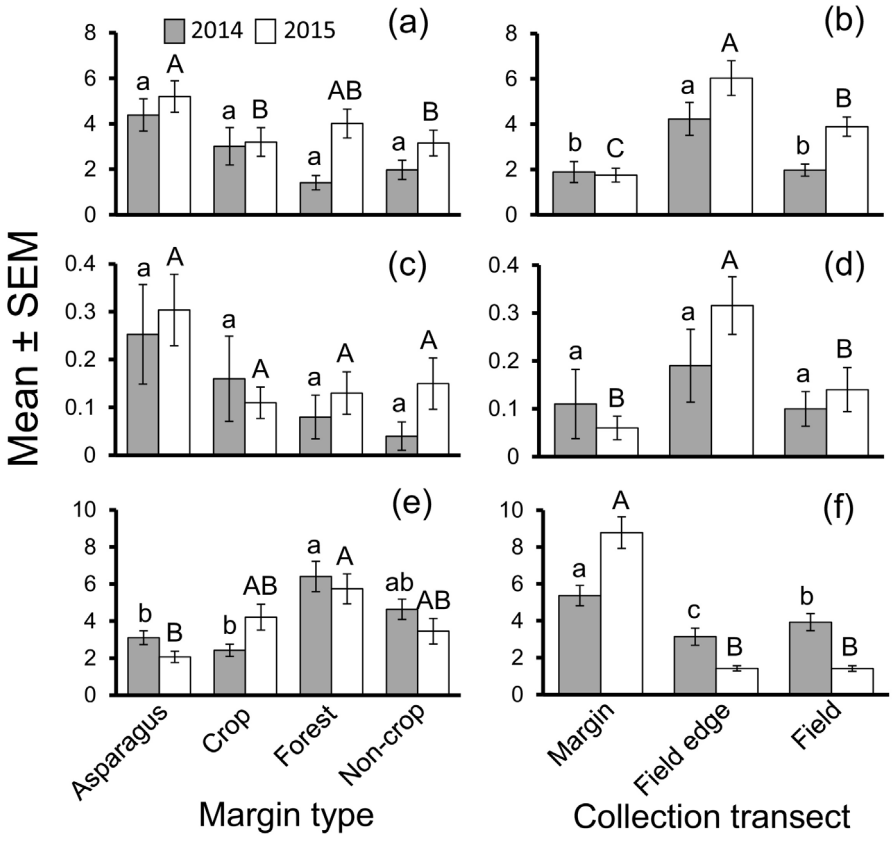

Fig. 1. Mean ± SEM number of asparagus miner, common asparagus beetle, and predators collected in asparagus fields in 2014 (grey bars) and 2015 (white bars). Asparagus miner abundance by margin type (a) and transect (b) and asparagus beetle abundance by margin type (c) and transect (d). Both pests were collected by sweep nets. Predator abundance by margin (e) and transect (f). Predators were collected with vacuum and sweep nets.

predators in forested margins had a distinct community compared to the other margin types (ANOSIM $R = 0.21$, $p < 0.05$, NMDS stress = 0.15; Fig. S3a). However, this pattern did not continue in 2015, when all margin predator communities were similar to each other ($R = -0.02$, $p = 0.56$, NMDS stress = 0.13; Fig. S3b). Sweep net-collected samples from inside asparagus fields gave no convergent solutions in either year, and therefore could not be analyzed with NMDS. However, in 2015, sweep net collections from forested margins had a significantly different predator community composition than all other margin vegetation types ($R = 0.27$, $p < 0.01$, NMDS stress = 0.14; Fig. S4).

3.4. Molecular gut content analysis summary: food webs of key asparagus pests

Of the 1456 predators we screened in 2014, 80 (6%) tested positive for asparagus miner DNA and 16 (1%) tested positive for asparagus beetle DNA. The arthropods that tested positive for asparagus miner represented 22 groups (13 spider groups and 9 insect families; Fig. 2a). In total, we collected 1244 individuals that belonged to these taxonomic groups (Table S3a). We found 400 individuals that came from six taxonomic groups (two spider groups and four insect families; Fig. 2a), which tested positive for asparagus beetle DNA (Table S4a). In 2014, two individuals tested positive for DNA of both pests; a *Nabis americoferus* Carayon (Hemiptera: Nabidae) and a rove beetle from the subfamily Aleocharinae (Coleoptera: Staphylinidae).

In 2015, we screened 2190 predators and had 307 individuals (14%) test positive for asparagus miner DNA, and 64 individuals (3%) positive for asparagus beetle DNA in gut contents. These predators represented 24 predatory groups for asparagus miner (12 spider groups and 12 insect families; Fig. 2b; Table S3b), and 12 predatory groups (6 spider groups and 6 insect families) that tested positive for asparagus beetle DNA (Fig. 2b; Table S4b). We had 2091 and 1419 individuals that came from families that tested positive for asparagus miner and asparagus beetle, respectively. Similar to 2014, we only had a few individual predators that tested positive for both prey. Staphylinids were positive for both pests in 2015, with one individual from the subfamily Aleocharinae and seven individuals from the genus *Tachyporus*.

3.4.1. Asparagus miner predators

Overall, spiders from the Linyphiidae family had the most individuals testing positive for asparagus miner in 2014, and Thomisidae had the most positive individuals in 2015 (Fig. 2; Table S3). Among the Insecta predators testing positive for asparagus miner DNA, we found that in both years staphylinids and ground beetles (Carabidae) were prominent predatory groups for asparagus miner (Fig. 2; Table S3).

Margin type significantly influenced the number of individuals positive for asparagus miner DNA in both years (2014: $\chi^2 = 38.70$, df = 3, $p < 0.01$; 2015: $\chi^2 = 80.69$, df = 3, $p < 0.01$; Fig. 3a). In 2014, in the presence of forested margins, the number of positive samples increased by 3-fold, compared to all other margin types ($\chi^2 > 15.52$, df = 1, $p < 0.01$). In 2015, fields with crop and forested margins had significantly more predators testing positive for asparagus miner compared to the other margin types ($\chi^2 > 20.86$, df = 1, $p < 0.01$; Fig. 3a). When comparing sampling locations (transects in relation to margin habitat), we observed significant differences in the total number of predators positive for asparagus miner DNA in 2014 and 2015 (2014: $\chi^2 = 10.00$, df = 2, $p < 0.01$; 2015: $\chi^2 = 252.71$, df = 2, $p < 0.01$; Fig. 3b). Field margins in 2014 had double the number of predators testing positive for asparagus miner DNA compared to the other sampling locations ($\chi^2 > 6.67$, df = 1, $p < 0.01$).

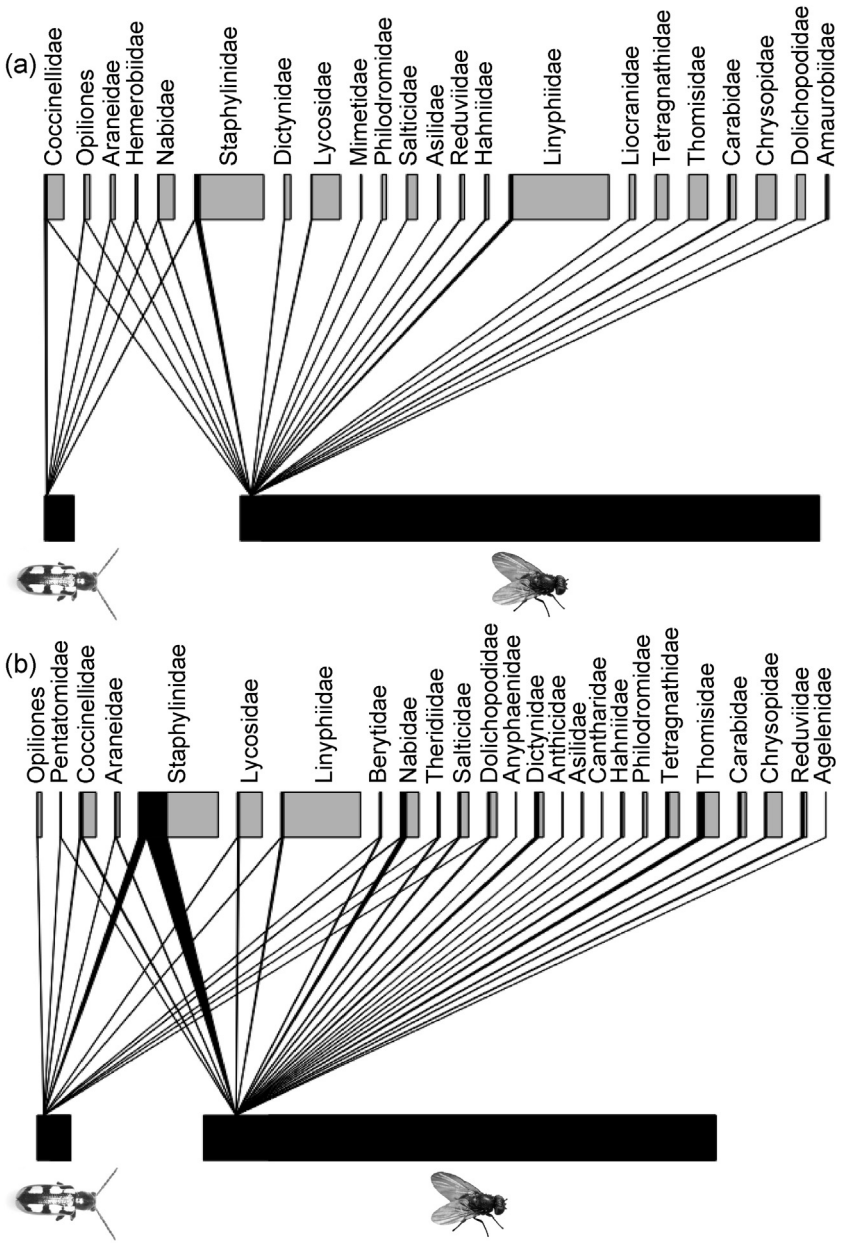

Fig. 2. Predatory linkages visualized using food webs for common asparagus beetle and asparagus miner for 2014 (a) and 2015 (b). In each year, the width of upper and lower horizontal bars represents total abundance of the arthropod groups. Lower horizontal bars represent the relative abundance of asparagus beetle and asparagus miner. Upper horizontal bars represent relative abundance of predators. Lines connecting the upper and lower axes, and the corresponding black area of upper horizontal bars indicate the proportion of each predatory group that were positive for asparagus miner and/or asparagus beetle DNA determined by molecular gut content analysis.

In 2015, we found more than a 5-fold greater abundance of predators outside asparagus fields than inside that tested positive for asparagus miner DNA ($\chi^2 > 131.70$, df = 1, $p < 0.01$; Fig. 3b).

3.4.2. *Asparagus beetle predators*

Comparatively fewer predators tested positive for asparagus beetle in 2014 (Fig. 2; Table S4), likely related to low abundance of this pest (Fig. 1c and d). In both years, a diversity of Insecta and Arachnida predators tested positive for asparagus beetle DNA (Fig. 2; Table S4). Insects from Coccinellidae and Staphylinidae were prominent predatory families for asparagus beetle, with coccinellids making up 50% of the predators testing positive in 2014 and staphylinids accounting for 59% of the predators testing positive in 2015 (Fig. 2; Table S4).

Margin habitat type had no significant effect on the total number of predators positive for asparagus beetles in 2014 ($\chi^2 = 1.50$, df = 3, $p = 0.68$), but significantly affected the number of predators testing positive in 2015 ($\chi^2 = 77.00$, df = 3, $p < 0.01$; Fig. 3c). In 2015, 87% of predators testing positive for asparagus beetles came from crop margins ($\chi^2 > 25.14$, df = 1, $p < 0.01$). Forested margins accounted for 16% of the total number of predators testing positive, which was significantly more than in non-crop margins ($\chi^2 = 5.33$, df = 1, $p = 0.02$; Fig. 3c). The effect of transect sampling location on predators testing positive for beetle DNA was significant in both years (2014: $\chi^2 = 6.13$, df = 2, $p < 0.05$; 2015: $\chi^2 = 70.72$, df = 2, $p < 0.01$). In 2014, 56% predators testing positive for beetle DNA came from the margin, which was significantly more than from the field ($\chi^2 = 6.4$, df = 1, $p = 0.01$). Margin transects had 83% of the predators positive in 2015, which was significantly more than the other transect sampling locations ($\chi^2 > 35.27$, df = 1, $p < 0.01$; Fig. 3d).

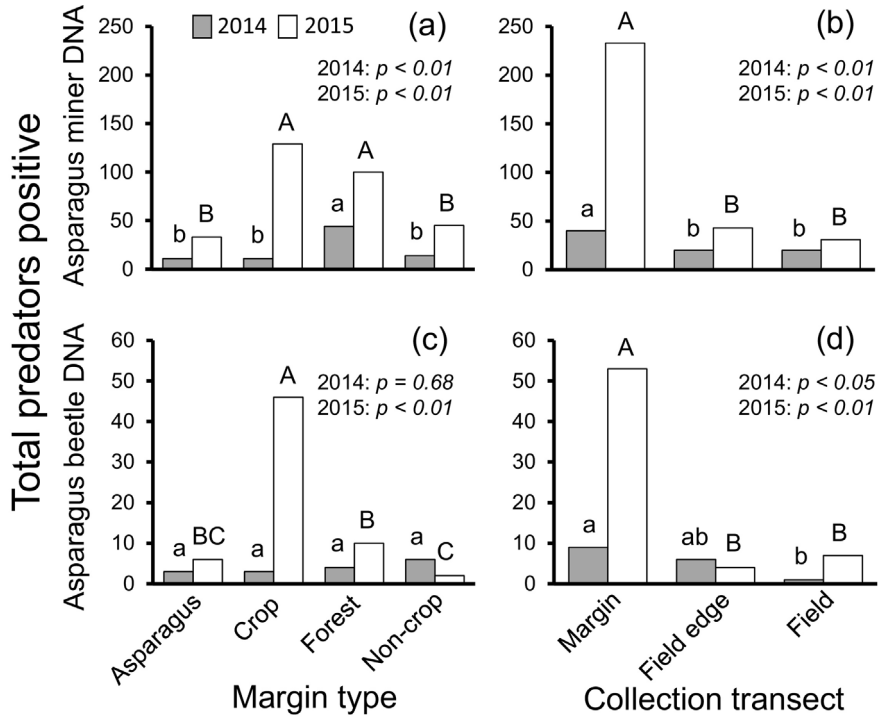

Fig. 3. Total number of predators collected from commercial asparagus fields that tested positive for asparagus miner DNA (a, b) and common asparagus beetle (c, d) with molecular gut content analysis in 2014 and 2015. Significant differences among bars of the same color, within years, were determined with a Pearson's chi square test with post-hoc multiple pairwise comparisons ($\alpha = 0.05$).

4. Discussion

Our analysis of asparagus food webs is among the first studies to characterize predatory communities in a landscape context using molecular gut content analysis (Hagler et al., 2004; Sheppard et al., 2004). In general, our results indicated that the abundance of natural enemies is higher outside asparagus fields than inside, and this coincided with higher predation levels on two key pest species. Furthermore, we found that margin habitat type shapes predator communities; asparagus fields bordered by forests contained more abundant predator communities as compared to other types of field margins. However, overall incidents of predation were relatively low in both years which makes establishing key predators as potential targets for biological control programs difficult. A diversity of predators was found to have fed on the two key pests, indicating that predator community diversity may be important for biological control in this system. This supports the growing consensus in the literature about the importance of biodiversity for ecosystem functions, such as biological control (Cardinale et al., 2006), and emphasizes that agroecosystem function depends on sustaining biodiversity in field margins to help maintain biocontrol agents in agricultural landscapes (Wratten, 1988; Wratten et al., 1998).

Although asparagus is a commonly grown crop around the world, few studies have documented the predatory communities of these systems (Angalet and Stevens, 1977; Capinera and Lilly, 1975; Drake and Harris, 1932; Starý, 1990; Watts, 1938). Only three studies have documented predators of asparagus beetle (Capinera and Lilly, 1975; Drake and Harris, 1932; Watts, 1938), while none have described predators of asparagus miner. The predators we collected, especially arachnids, had higher incidences of predation in asparagus fields with forested borders as compared to other margin types, suggesting that increasing vegetation structural complexity, especially vegetation cover, may be an important factor for these groups of arthropods (Bell et al., 2002; Dennis and Fry, 1992; White and Hassall, 1994;). Many of the predators in our study were flightless and soil-dwelling with a diffuse distribution relative to the field margin, indicating that these species are habitat generalists, moving between field margins and agricultural fields in search of prey ("soft-edge" species, Duelli et al., 1990). Forested field margins seem to be an important source of refugia, likely increasing the number of predator immigrants into asparagus fields.

Field margins may be sources of pests, and in our system, the abundances of the two herbivorous pests were generally lower outside asparagus fields than inside. This was expected since both pests are obligate asparagus feeders (Barnes, 1937; Drake and Harris, 1932; LeSage et al., 2008) and are most likely visiting volunteer asparagus plants in field margins, although asparagus miner adults (the life stage we collected) feed on nectar and can be seen visiting many species of flowers (*Z.S. pers. obs.*). Furthermore, the forested margins had a favorable effect on predators and predation, with a correspondingly low abundance of asparagus miners and beetles. This result suggests that the interaction between predators and these pests is particularly high on the forested margins of fields, and the efficacy of biological control in this system may be related to the amount of forested area in the landscape.

Arachnids testing positive for asparagus miner DNA were a mixture of soil-dwelling, arboreal, web-building, and wandering spiders. In 2014, linyphiids were the most abundant predator inside asparagus fields, with 67% of all linyphiids testing positive for asparagus miner coming from inside the fields. Linyphiids are a particularly interesting arachnid family as a potential target for conservation biocontrol as they seem to tolerate disturbance and can make up 93–99% of the total spiders in many different field and vegetable crops (reviewed in Nyffeler and Sunderland (2003)). Interactions between the miners and web-building linyphiids is most likely to occur when adults are captured as they move on and between plants. In 2015, Thomisidae spiders had relatively high abundance in forested borders and frequently tested positive for asparagus miners. These predators sit-and-wait for their prey, often at flowers. Therefore, it is possible that they could capture miner adults visiting flowers outside the asparagus fields. In both years, arachnids made up less than 25% of the total predators testing positive for asparagus beetle DNA with no clearly dominate

predatory taxa. However, those that did test positive represented taxa that utilize the same hunting modes and occupy the same spatial niches within the landscape as those described for the asparagus miner.

Among the insect predators, staphylinids represented one of the numerically dominant groups and frequently tested positive for the two key pests. Many staphylinids are known facultative predators (Frank and Thomas, 1999) and, as omnivores, they can establish early in crop fields before pest populations are high and can feed on plants when prey are unavailable, mitigating mortality (Capinera, 2008). Staphylinids are also known scavengers and can test positive for prey DNA after feeding on carrion (von Berg et al., 2012). Therefore, the roles of staphylinids and other generalist predators in these food webs are complex, and we may have overestimated predation on live pests due to secondary predation (Mansfield and Hagler, 2016; Sheppard and Harwood, 2005). False positives for predation can also occur when secondary predators (hyperpredators) feed on primary predators, creating food chain errors in molecular predation studies (Hagler, 2016; Harwood et al., 2001; Sheppard and Harwood, 2005). We hypothesized, that if staphylinids feed on live prey, they are most likely to feed on the immobile pupal stages of the two pests due to spatial separation and differences in mobility during the other prey life-stages. It is also difficult to discern if the positive occurrences we found for the two pests were not simply the result of staphylinids scavenging on dead or dying prey on the ground. Considering the propensity of staphylinids to feed on carrion and the potential of secondary predation it is difficult to verify our results without direct observations. Further studies on the roles of staphylinids in terrestrial food webs are clearly needed to better understand these issues.

We hesitate to make comparisons among predator groups for effectiveness as biocontrol agents because there is known variability in prey DNA detectability caused by differences in biotic conditions, the size, type and frequency of meals consumed, and the life stage of the predator (Greenstone et al., 2014). This is a challenge and out of the scope for the current study given the diversity of predator taxa observed. While our current analysis of the system provides the first food web characterizing the communities of predators feeding on key asparagus pests, and their relationship to landscape characteristics, future work will clarify the importance of individual predatory taxa (e.g., Szendrei et al., 2010).

5. Conclusions

In summary, our study contributes to filling the knowledge gap in linking predators and prey through direct trophic linkages. We also highlight the importance of unmanaged field margins, particularly forested ones, in providing biocontrol services in agricultural fields. Many of the predator taxa that we confirmed to feed on key pests are not pollen and nectar feeders; therefore, in this system, predation and margin management with flowers may not be positively correlated. In the absence of forested borders, floral resources in margins may provide habitat for predators and attract parasitoids which could synergize with predators for more efficient biocontrol. While forested field margins tend to be only a small part of agricultural landscapes, their conservation should be promoted for increasing ecosystem services and biodiversity, and their benefits should be integrated into pest management programs.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2017.04.011.

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