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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).