Molecular gut-content analysis of a predator assemblage reveals the effect of habitat manipulation on biological control in the field

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Received 24 March 2009; accepted 21 October 2009

Abstract

Habitat manipulation in agroecosystems can influence predator–prey interactions. In this study, we collected foliar predators from field potato plots with different mulch treatments and assayed them for DNA of the target prey, Leptinotarsa decemlineata (Say), using species-specific primers. Concurrently, L. decemlineata larval abundance and plant damage were recorded from the same plots. Predator species abundance and diversity were not influenced by habitat manipulation, while prey density was highest in plots without mulch. Gut-content analysis revealed that the highest incidence of predators positive for L. decemlineata DNA was in plots without mulch, where target prey abundance was highest. Therefore, the lower prey abundance in mulched plots was not due to predation. The most abundant species in the predator assemblage was Coleomegilla maculata, which had the lowest proportion of L. decemlineata DNA in the gut. Podisus maculiventris, Perillus bioculatus, and Lebia grandis were less abundant but had a higher incidence of target prey DNA in the gut. DNA detectability half-lives were used to adjust for inter-specific variation in DNA digestive rates of the four predator species. Using this information to adjust actual number of positives for prey DNA, we compared proportions positive for L. decemlineata and found that P. maculiventris is the most effective predator species in the complex.

Zusammenfassung


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Published by Elsevier GmbH on behalf of Gesellschaft für Ökologie.

**Keywords:** PCR; DNA detectability half-life; Target prey; Cover crop; Mulch; Coleoptera; Hemiptera; Heteroptera; Coccinellidae; Carabidae

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**Introduction**

Habitat structural complexity is thought to increase the accumulation and conservation of natural enemies in managed ecosystems through a reduction in predator interference by competition and intra-guild predation, and via increased availability of alternative food, shelter, and favourable microclimate (Andow 1991; Landis, Wratten, & Gurr 2000; Sunderland & Samu 2000). On the other hand, predator foraging efficiency may vary inversely with habitat heterogeneity, because of the negative effects of structural complexity, such as a decrease in predator–prey encounter rates due to increased surface area for searching (Hughes & Grabowski 2006; O’Neil 1997). Visually searching predators may be more successful in locating herbivores in less complex habitats (Uetz 1979). Measuring prey suppression by an indigenous predator assemblage in field experiments is further complicated by the fact that prey abundances vary with changes in biotic and abiotic features of their environment (e.g. Baggen & Gurr 1998), thus modifying the net outcome of biological control.

Information on predation rates can be gathered by direct observation (Brust 1994; Chang & Snyder 2004; Nyffeler, Sterling, & Dean 1994), but molecular gut-content analysis of specimens from the field is a more effective way of collecting accurate information on predation rates without altering the environment with experimental equipment or investigator intrusion (Harwood & Greenstone 2008). This method is also able to provide meaningful information on predation without manipulating prey abundance in the field (e.g. Kuusk, Cassel-Lundhagen, Kvarnheden, & Ekborn 2008). Molecular gut-content analysis relies on species-specific markers for fragments of prey DNA that are able to reliably detect the presence of prey remains in the predator’s gut. Since predator species may differ in digestive physiology, a prior knowledge of the prey DNA detectability time-course is required to compare and rank predator species (Greenstone, Rowley, Weber, Payton, & Hawthorne 2007). These rankings could then be used to inform manipulations of the predator assemblage so as to increase the abundance of the most effective predator species.

The objective of this study was to determine if habitat management might enhance the ability of the indigenous predator assemblage to lower Leptinotarsa decemlineata populations. We investigated the effect of two types of mulches on the resident predator complex in experimental potato fields and measured L. decemlineata predation with molecular gut-content analysis. Using information on differential L. decemlineata DNA digestion rates to normalize raw data from molecular gut content analysis, we compared the relative impact of four predator species on this pest. In addition, we measured changes in two biotic factors, plant damage and L. decemlineata abundance. This research aims to advance our knowledge of the impact of native predator assemblages to improve the outcome of biological control.

**Methods**

Field experiments were conducted in 2006 and 2007 at the Beltsville Agricultural Research Center in Beltsville, MD, USA. Potatoes were grown in rotation; the fields used in the 2 years were ca. 1 km apart. Three treatments were randomized in two blocks in 2006 and three in 2007. Two were mulch treatments of winter rye (Secale cereale L., 100 kg/ha) and hairy vetch (Vicia villosa Roth., 56 kg/ha). The third treatment was a tilled control, where S. cereale L. was also seeded as a winter cover crop, but plots in this treatment were tilled prior to potato seeding; potatoes were then grown without mulch. Plants to create the mulch treatments were seeded on 2 September 2005 and 21 September 2006. Potatoes (Solanum tuberosum L. ‘Kennebec,’ 2027 kg/ha, 76 cm × 30 cm spacing) were seeded on 27 April 2006 and 25 April 2007, in 1 ha fields divided into 12 m × 30 m plots for each treatment. Pre-emergent herbicides (S-metolachlor at 1.78 kg AI/ha, linuron at 981 g AI/ha, and paraquat dichloride at 1.71 kg AI/ha)
were applied within 1 week after planting. The fields were not treated with herbicides during the experiment, but low rates of insecticides (spinosad at 16 g AI/ha on 16 June 2006, and permethrin at 27 g AI/ha on 15 July 2006, and at 54 g AI/ha on 15 and 30 June 2007) and a fungicide (azoxystrobin at 161 g AI/ha on 15 July 2006 and at 96 g AI/ha on 30 June 2007) were applied to protect plants from severe damage.

Each plot (representing a single treatment) was subdivided into three 6 m × 6 m subplots, which was the unit of replication, and weekly predator samples were taken in single subplots, which were rotated weekly to minimize depletion of predators from a specific part of the field. Samples were taken between 26 May and 25 July 2006, and between 4 June and 30 July 2007. Potato foliage was carefully searched for predators for 30 min between 7:30 and 11:00 h; as soon as a predator was detected, it was captured by hand or with an aspirator (once detected, predators rarely evaded capture). The collected predators were placed immediately in 75% ethanol in individual 5 ml glass vials and stored at 4 °C until DNA extraction (max. 2 months storage).

Each habitat treatment replicate contained a plot of 12 potato plants arranged in 4 rows that were sampled for pest numbers and plant damage on the same dates that predator collections were conducted. Leaf damage was quantified visually on a scale based on the percentage of leaf area removed by chewing: 0 (0–5%), 1 (6–24%), 2 (25–49%), 3 (50–74%), and 4 (75–100%). The numbers of immature life stages of *L. decemlineata* were recorded from each plant. Three categories were used for different life stages: egg masses; small larvae, comprising first and second instars; and large larvae, composed of third and fourth instars. This categorization was necessary because not all the predator species consume every life stage of the prey, and because of the considerable differences in behaviors and sizes of the different stages.

Field-collected predators were analyzed for the presence of *L. decemlineata* DNA. Methods of molecular gut-content analysis, including PCR protocols and description of the *L. decemlineata* species-specific primers are in Greenstone et al. (2007). Each PCR reaction included starved negative controls for predators, a *L. decemlineata*-fed predator, a *L. decemlineata*-positive control (a single egg), and a no-DNA control.

In our analyses, we focused on those predator life stages and species that composed >5% of all the predators collected and were consistently found in our samples (Fig. 1). The numbers of adult predators were analyzed with a mixed model (Proc MIXED, SAS Institute Inc., Cary, NC, USA); using date of collection as a covariate, mulch treatment and predator species as fixed factors, and block and year as random factors. Significant treatment effects were further analyzed with SLICE and DIFF options in an LSMEANS statement.

![Fig. 1. Mean (±SE) number of insect predators per plot collected from potato foliage in field plots with rye-, vetch-, or no-mulch treatments in Beltsville, Maryland (data combined from 2006 and 2007). Letters above bars represent significant differences among predator species within a cover treatment (P < 0.05). Species names: C7 – *Coccinella septempunctata*, Cmca – *Coleomegilla maculata*, Harm – *Harmonia axyridis*, Hipp – *Hippodamia convergens*, Lebia – *Lebia grandis*, Per – *Perillus bioculatus*, and Pod – *Podisus maculiventris*.](image-url)

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Significant treatment effects were further analyzed with SLICE and DIFF options in an LSMEANS statement. *L. decemlineata* count data were square root-transformed and percent plant damage was arcsine-transformed prior to performing the analyses to meet the assumptions of analysis of variance. Analysis of variance procedures for *L. decemlineata* density and percent plant damage were performed with PROC MIXED (SAS Institute, Cary, NC, USA). A repeated measures model with an autoregressive period 1 covariance structure was utilized to model the variation over time and within plot. Data were combined over the 2 years, and year was included as a random effect in the model. The effects of treatment-by-week interactions were assessed with SLICE option in an LSMEANS statement and denominator degrees of freedom were estimated with the Kenward–Roger method.

Pearson’s correlations were tested between the proportions of adult predators found positive for *L. decemlineata* DNA and prey abundance over time (SAS Institute, Cary, NC, USA).

Raw proportions of predators positive for prey DNA are misleading, because meals consumed by predators with rapid DNA digestion rates are less likely to be detected than those from slower-digesting predators with identical feeding histories (Chen, Giles, Payton, & Greenstone 2000). To correct for this bias, we first determined the half-lives of *L. decemlineata* DNA detectability in the laboratory for all predator species under simulated field temperatures by the protocol of Greenstone et al. (2007). The percent positives for
L. decemlineata DNA of field-collected animals of a given species was then entered into the half-life regression for that species, which was solved to obtain the time since feeding, or the expected length of time necessary to obtain that percentage. This quantity was then entered as the explanatory variable into the regression equation for that species displaying the middle-most half-life among all the species to arrive at an adjusted percentage. The ranks of adjusted predator proportions for the eligible species were compared with a test for proportions followed by pair-wise comparisons of species at \( \alpha = 0.05 \) (Payton, Greenstone, & Schenker 2003). Comparisons were made separately for years and mulch treatments.

Results

A total of 1648 predators were collected from our experimental potato plots in 2006 and 2007. Seven (2006) and eight (2007) species of predators comprised more than 5% of all predators collected from potato foliage (Table 1). Seventy-two individuals were collected from less common taxa of foliar predators (e.g. Cycloneda munda Say, Nabidae, and Pardosa milvina (Hentz)). Among the three mulch treatments, the individual predator species abundances were not statistically different \( (F_{2, 70} = 1.77, P = 0.18) \); see Appendix A, Table 1). Within mulch treatments, the abundances of the seven predator species were significantly different in rye \( (F_{6, 70} = 5.47, P < 0.01) \) and vetch \( (F_{6, 70} = 3.34, P < 0.01) \), but not in tilled treatment \( (F_{6, 70} = 1.73, P = 0.13) \). Overall, the most abundant species was Coleomegilla maculata De Geer (Coleoptera: Coccinellidae); it was significantly more abundant in rye and vetch treatments than the other species \( (t_{70} > 3.06, P < 0.01, \text{Fig. 1}) \).

L. decemlineata abundance among mulch treatments was not different in the case of egg masses and small larvae \( (F_{2, 28} = 1.48, P = 0.28) \), but mulch treatments had

<table>
<thead>
<tr>
<th>Species</th>
<th>Life stage</th>
<th>Mulch treatment</th>
<th>Total</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td>Tilled</td>
<td>Rye</td>
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<tr>
<td>2006</td>
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<td>161 (7)</td>
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<td>16 (0)</td>
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<td>20 (2)</td>
</tr>
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<td>23 (2)</td>
<td>4 (1)</td>
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<td>27 (14)</td>
<td>1 (1)</td>
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<td>3 (1)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>303 (95)</td>
<td>270 (18)</td>
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<td>2007</td>
<td></td>
<td></td>
<td></td>
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<td>9 (7)</td>
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<td>Geocoris sp.</td>
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<td>16 (2)</td>
</tr>
<tr>
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<td></td>
<td>325 (88)</td>
<td>230 (72)</td>
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</table>

aColeoptera: Coccinellidae.

bColeoptera: Carabidae.

cHemiptera, Heteroptera: Pentatomidae.

dHemiptera, Heteroptera: Lygaeidae.

a significant effect on large larvae ($F_{2, 28} = 3.78, P = 0.02$; Fig. 2). The effect of mulch treatments on prey abundance remained consistent over time for all prey life stages ($P \geq 0.09$ for all interaction terms; see Appendix A: Table 2). Regardless of life stage, tilled plots had the highest prey abundance relative to the other two treatments, and this was significant in the case of large larvae (till vs. rye: $t_{28} = 2.75, P < 0.01$).

Percent defoliation of the potato plants increased over time, with pronounced difference among treatments in the last part of the growing season (Fig. 3). Cover treatments significantly differed in the level of defoliation ($F_{2, 28} = 19.27, P < 0.01$), and trends over time were different for the three treatments as indicated by the significant interaction term ($F_{16, 28} = 12.15, P < 0.01$), but the highest level of defoliation was generally in plots without mulch (Fig. 3).

In 2006, 19%, and in 2007, 29% of the predators collected from the potato plots were positive for L. decemlineata DNA (Table 1). There was a significant difference in the total numbers of L. decemlineata DNA-positive predators among mulch treatments in 2006 ($G_{adj} = 65.64, P < 0.01$), but not in 2007 ($G_{adj} = 1.64, P = 0.44$) with the highest proportion of positives in the tilled treatment in both years (Table 1).

Using the adjusted proportions of adult predators found positive for L. decemlineata DNA, Podisus maculiventris (Say) (Hemiptera, Heteroptera: Pentatomidae) was ranked first in rye-mulched plots in both years, in tilled plots in 2006, and in vetch in 2007. Lebia grandis Hentz (Coleoptera: Carabidae) was ranked first in vetch mulched plots in 2006 and second or third in the two other treatments and years. The ladybeetle C. maculata was ranked last in all treatments and years (Fig. 4).

The correlation between the adjusted proportion of adult predators positive for L. decemlineata DNA and
the number of prey was significant in the case of large larvae in tilled ($r^2=0.9$, $P<0.01$) and vetch-mulched treatments ($r^2=0.7$, $P=0.03$) in 2007 (Fig. 5).

Discussion

In our study, increasing habitat structural diversity did not improve conservation biological control of the target prey. In a meta-analysis by Langellotto and Denno (2004), the only taxa that did not respond positively to increased habitat structural complexity were coccinellids and carabids, and five species in our assemblage belonged to these two groups. Some possible explanations for this outcome are that, (1) all the predator species in the examined assemblage rely to a great extent on their mobility in seeking prey and are therefore hindered in their movements in a habitat with increased surface area; or (2) the predators are able to move between plots to obtain more easily accessible prey in the tilled (simpler) habitat. McNett and Rypstra (2000) suggested that habitat complexity increases abundance of those groups of predators that are either heavily reliant on complex spatial structures, such as web-building spiders, or are evading intraguild predation, and none of these factors were expected to be major influences for any of the predator species in this study, with the possible exception of $C. maculata$, which has a high raw incidence of $Harmonia axyridis$ DNA in the gut (Greenstone unpublished). While predator abundances were not significantly different among our habitat treatments, $L. decemlineata$ density was generally lower in the mulched treatments, especially in plots with rye mulch. This suggests that the majority of the species in the examined predator assemblage are not specialist predators of $L. decemlineata$, because they did not aggregate in the treatment with the highest $L. decemlineata$ density, and this means that it would be worthwhile to study alternate prey identity and availability.

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We found that predator impact, represented by the gut content analysis, is greater in the simple habitat, where prey abundance was also relatively high compared with the complex ones. This suggests that vegetation complexity did not provide a refuge from predation for the prey, which is contrary to the notion that more complex habitats provide enemy-free space (Messina, Jones, & Nielson 1997; Murdoch, Luck, Walde, Reeve, & Yu 1989). It is also clear from the gut content analysis that the relatively lower prey abundance in rye-mulched plots was not due to higher predator efficacy, because in this case we would have expected to find a high proportion of predators positive for the target prey. Suppression of L. decemlineata populations in straw-mulched potato has been reported by others (Brust 1994; Stoner 1993; Stoner, Fernandino, Gent, Elmer, & Lamondia 1996), but the magnitude of the effects attributed to top-down vs. bottom-up process were not studied in detail. Our results taken together with findings of Szendrei, Kramer, & Weber (2009) indicate that lower L. decemlineata abundance in rye-mulched plots is due to the lower colonization rates by L. decemlineata adults early in the growing season. Previous studies that examined the effects of straw mulch on L. decemlineata abundance in potatoes used varied schemes for mulch application, which makes our results difficult to compare with previous ones. Plant-mediated effects on oviposition and larval development of L. decemlineata could also be responsible, since manure-fertilized potato plants altered L. decemlineata population and individual response, compared with synthetic fertilization (Alyokhin & Atlihan 2005). It has been suggested that predator–prey and predator–predator relationships are mediated by both the density and the shape of the habitat structure (e.g. Warfe & Barmuta 2004), but we did not find conclusive evidence for predators responding to the shape of vetch and rye mulches differently.

Our study is the first to integrate information on a predator assemblage and molecular gut content data with differential DNA detectabilities to adjust proportions of predators positive for prey DNA. About a quarter of the specimens collected were positive for L. decemlineata DNA. This suggests that many generalist predators are either frequently in a state of starvation (Bilde & Toft 1998) or are feeding on alternate prey. Since most of the examined species in the assemblage are not L. decemlineata-specific, this may simply reflect undetected feeding on alternate prey or plant material (Heimpel 1991; Valicente & O’Neil 1995; Cottrell & Yeurgan 1998).

C. maculata was the most abundant predator species in our plots, which is in accordance with previous data from potato fields (Hazzard, Ferro, van Driesche, & Tuttle 1991; Brust 1994; Hilbeck & Kennedy 1995). Based on the abundance data, this species was suggested by Hazzard et al. (1991) as a valuable predator of L. decemlineata that should be the focus of conservation control efforts. Our findings show that C. maculata could not compensate for its low target prey consumption by relatively high abundance (Fig. 4). This counters the perception that C. maculata is an important predator of L. decemlineata (e.g. Groden, Drummond, Casagrande, & Haynes 1990; Hazzard et al. 1991) and demonstrates that density data are only useful in combination with a measure of functional response in determining the contribution of a predator to biological control of a target prey (Lester & Harmsen 2002).

Similarly to Chang and Snyder (2004), our study was not able to point to a single-most-effective predator species of L. decemlineata, but our results give us a better idea of the relative importance of some of the common predator species in this context. Based on the adjusted gut-content analysis values, P. maculiventris seems to be the most important predator of L. decemlineata on a per-capita basis, closely followed by P. bioculatus and L. grandis. This is somewhat surprising given that P. bioculatus and L. grandis are specialist predators (Saint-Cyr & Cloutier 1996; Weber, Rowley, Greenstone, & Athanas 2006) of L. decemlineata and therefore would be expected to perform better than the generalist P. maculiventris.

Relationships over time between prey abundance and proportion of predators positive for L. decemlineata DNA did not show consistent statistical correlation, although the peaks of small L. decemlineata larvae were often followed by relatively high peaks of proportion predators positive for prey DNA (Fig. 5).

The mediating effect of habitat complexity on prey density through predation depends on the foraging behavior and microhabitat use of individual predator species (Finke & Denno 2006; Schmitz, Krivan, & Ovadia 2004). Methods such as molecular gut content analysis are important aids in determining which predators are consuming the target prey and whether these species are significantly influenced by habitat manipulation. While plant structural diversity in agroecosystems can be changed, it is important to first understand the benefit derived from such manipulations. Incorporation of actual prey consumption measures is needed for the meaningful interpretation of field data in conservation biological control programs.

Acknowledgements

John Teasdale (USDA, ARS) gave us guidance in cover crop mulch choice and management. Dan Rowley (USDA, ARS, IIBBL) provided valuable advice on all aspects of the molecular work. The authors thank Andrew Hempstead and Leanna Kelly for technical assistance in the experiments. Thanks also to the BARC...
farm crew for maintenance of the fields. Z.S. was supported by an ARS Headquarters Postdoctoral Research Associateship to D.C.W.

Appendix A. Supplementary material

The online version of this article contains additional supplementary data. Please visit doi:10.1016/j.baae.2009.10.006.

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