LINKING HABITAT DIVERSITY WITH SPATIAL ECOLOGY FOR AGRICULTURAL PEST MANAGEMENT

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Diverse agricultural landscapes have been shown to support many ecosystem services including clean water, species conservation, and carbon sequestration. However, far less is known about the role of diverse agricultural landscapes in agricultural pest control. This research investigated how diverse agricultural landscapes affect insect pest dynamics and evolution. A comprehensive literature review of this subject revealed that direct effects of landscape diversity on insect pest control have been largely ignored. Nevertheless, increases in pest mortality and decreases in fecundity are likely with increasing landscape diversification. Field surveys of insect populations in agricultural landscapes of varying complexity across New York further illuminated landscape-insect relationships. Surveys showed that populations of insect pests in field corn were generally lower and that natural enemy populations were generally higher as agricultural landscapes increased in their diversity. Spatially-explicit modeling further explored landscape-insect relationships and considered pests with varied life histories and the influence of crop rotation and economic thresholds. Model results suggested that insect diet breadth and regional crop management play pivotal roles in landscape-insect relationships. Furthermore, pest management may be more intense in highly agricultural than in diverse landscapes, which may reduce apparent pest control benefits of landscape diversification. Finally, the role of diverse landscapes in slowing the evolution of insect resistance to Bt crops was explored. A detailed study of the European corn borer's host utilization, fecundity, and U.S. distribution indicated that diverse agricultural landscapes are not likely to substantively slow the evolution of resistance to Bt corn for this pest. Together, this research supports pest control as an important ecosystem service of diverse agricultural landscapes. Additionally, it highlights the roles that insect life history and regional pest management play in shaping landscape-insect relationships.

BIOGRAPHICAL SKETCH

Megan O’Rourke grew up in Blairstown, NJ, where she completed all of her primary education. Following high school, she became a Rotary Exchange student in Japan, where she discovered her interests in sustainable agriculture. She began college at Brigham Young University in Utah as an agronomy major. There, she soon met and married her husband, Aaron Rust, and moved to Long Island, NY to attend the State University of New York at Stony Brook. At Stony Brook, Megan pursued her honors thesis in the Department of Ecology and Evolutionary biology studying the effects of atmospheric nitrogen deposition on plant growth. She also had the opportunity to take an entomology class from Dr. Doug Futuyma, which was highly influential in her choice of future studies. Meanwhile, Megan’s first daughter was born. In 2002, Megan finished her B.S. in Biology with an environmental emphasis. She then moved with her family to pursue a dual M.S. in Entomology and Ecology from Iowa State University. Upon arrival in Iowa, Megan’s son was born. Studying under Dr. Matt Liebman and Dr. Marlin Rice, Megan’s M.S. focused on how cropping systems affect insect communities and the biological control of agricultural weeds. After completing her M.S. in 2005, Megan moved to New York to pursue her Ph.D. at Cornell University with Dr. Alison Power. Before beginning her Ph.D. studies, Megan’s second daughter was born. Megan spent four and a half years at Cornell completing her research on agricultural pest control as an ecosystem service of diverse landscapes. Throughout her education, Megan has maintained a focus on sustainable agriculture and environmental stewardship and has strived to balance work and family.

This work is dedicated to my husband, Aaron, with gratitude for his unwavering faith in my work.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

Research Statement and Justification

This research advances our knowledge of plant-insect interactions in agricultural systems. I apply basic ecological and evolutionary theories to questions of applied pest management. Specifically, I investigate the hypotheses that diverse plant communities support lower densities of specialist herbivorous insects and that plant diversity can slow insect adaptation to plant defenses. I apply these ideas to agriculture by asking whether diverse landscapes suppress crop pests and whether landscape diversity can substitute for structured Bt corn refuges to slow the evolution of European corn borer resistance to transgenic corn.

There are fundamental differences between natural and agricultural systems that warrant investigation into whether ecological theories are applicable to the latter. One such difference, examined here, is the spatial scale of plant diversity. In natural systems, it is common that plant species and genotypes are well mixed. In industrial agricultural systems, large monocultures of a single plant genotype are the norm. However, beyond individual fields, agricultural landscapes can be quite diverse with many crops and non-crop habitat. Therefore, it is necessary to ask whether plant-insect interactions that occur in natural systems translate to agricultural systems, despite differences in the spatial scales of plant diversity.

Study System

In my research, I have focused on how landscape diversity affects insects in corn (*Zea mays*). Corn systems offer a number of advantages for agro-ecological research: corn is very economically important, it is prevalent across the globe, and there is already an extensive background literature. The economic import and prevalence of corn increase the applied impacts of research while the detailed knowledge available for several corn insects is invaluable for experimental design and interpretation. Corn is also particularly amenable to landscape-scale ecological research. Since farmers maintain relatively uniform monocultures of corn across diverse natural environments, this creates pseudo-experimental landscapes that are useful for isolating the effects of landscape-scale habitat features.

Research Approach

I used field surveys to determine patterns of plant-insect interactions and a variety of analytical techniques to determine the mechanisms underlying those patterns and to generalize field observations. These techniques included GIS, stable carbon isotope analysis, and simulation modeling. GIS was used to quantify agricultural landscapes to relate to surveyed insect densities. Stable carbon isotope analysis was used to determine the host utilization of corn pests. Simulation modeling was used to make general predictions about how agricultural landscapes affect insect pests with varying life histories.

Thesis Organization

This thesis contains four research papers that were written for publication in ecological journals. The first three focus on the relationships between landscape-scale habitat diversity and agricultural insect populations. The fourth paper, which was accepted for publication into the journal Ecological Applications in September 2009, explores the influence of landscape-scale habitat diversity on insect pest adaptation to

Bt crops. The objectives of each paper and the data they contain are summarized below and are labeled by their thesis chapter.

Chapter 2: Agricultural diversification at local and landscape scales: a review of the direct and indirect effects on insect pests *by Megan E. O'Rourke***.** This chapter provides an introduction to the literature on habitat diversity-insect relationships. In it, I review the evidence for pest suppression by agricultural diversification at local and landscape scales. I focus the review on the direct effects of plant diversity on herbivores as well as the indirect effects via natural enemies. I explore how mechanisms underlying direct and indirect effects differ when habitat diversity is incorporated at local or landscape scales. I identify mechanisms of pest suppression in diverse landscapes that deserve further investigation.

Chapter 3: The landscape context of insect pest and predator densities in field corn *by Megan E. O'Rourke, Kaitlin Rienzo-Stack, and Alison G. Power*. In this chapter, we document patterns of landscape-insect relationships. We quantify relative densities of the most economically important pests of corn in North America, the western corn rootworm (WCR), the northern corn rootworm (NCR), and the European corn borer (ECB), and two generalist predator lady-beetles, *Coleomegilla maculata* (Cmac), and *Propylea quatuordecimpunctata* (P14) in corn fields throughout upstate New York. We also quantify habitat types on the perimeters, in 1km radius areas, and in 20km radius areas surrounding sampled fields. We then determine the correlations between land-use at different scales around corn fields and insect densities within fields.

Chapter 4: Diverse agricultural landscapes and pest management: an empirical and modeling approach to understanding ecosystem services *by Megan E. O'Rourke and Laura E. Jones***.** We develop general predictions of landscape-herbivore relationships based on insect life history. We begin by contrasting empirical landscape-herbivore relationships detailed for *Diabrotica virgifera* and *Ostrinia nubilalis* in chapter 2. We attempt to explain these different landscape-herbivore relationships by examining host specialization and nation-wide management of the two pests. We seek to support the empirical data and to generalize landscape-insect relationships with a spatially explicit model of insect population dynamics. The model predicts spatially explicit insecticide use and population dynamics of herbivores that vary in their reproductive capacities, utilization of alternative habitats/hosts, and maximum dispersal distances.

Chapter 5: Managing resistance to Bt crops in a genetically variable herbivore, *Ostrinia nubilalis* *by Megan E. O'Rourke, Tom W. Sappington, and Shelby J. Fleischer*. We investigate the importance of unstructured refuges for slowing the evolution of resistance to Bt corn by *O. nubilalis* (ECB). We first investigate host utilization by the two pheromone races of ECB in New York to determine the proportion of each that develops on non-corn hosts. We also look at how developing on non-corn hosts affects their body mass and fecundity. Finally, we compile data on the geographic distributions of ECB pheromone races in the US. These data are used to recommend whether non-corn habitat can adequately substitute for non-Bt corn as refuge for production of ECB susceptible to Bt corn.

Co-Author Contributions

Each paper has involved different sets of collaborators. Alison ("Sunny") Power is Professor of Ecology and Evolutionary Biology at Cornell University and has served as my major advisor. She has provided intellectual support throughout this thesis, particularly with regard to the design and analysis of my field work. Kaitlin Rienzo-Stack was an undergraduate research fellow at Cornell who helped to quantify landscape-natural enemy relationships for chapter three. Laura Jones is a Senior

Research Associate of Ecology and Evolutionary Biology and field member of Applied Mathematics at Cornell University. Laura drafted the simulation model and provided extensive guidance for chapter four. Tom Sappington is a Research Entomologist with the USDA-ARS Corn Insects and Crop Genetics Research Group in Ames, Iowa. Tom supported the intellectual development of Chapter five and provided guidance for stable carbon isotope analysis of ECB. Shelby Fleischer is Professor of Entomology at Penn State University. He provided extensive, unpublished data on distributions of ECB pheromone races in the United States for chapter five.

CHAPTER 2

AGRICULTURAL DIVERSIFICATION AT LOCAL AND LANDSDCAPE SCALES: EXPLORING THE DIRECT AND INDIRECT EFFECTS ON INSECT PESTS

Megan E. O'Rourke

Abstract

The 'resource concentration hypothesis' and the 'enemies hypothesis' are well studied mechanisms by which local plant diversity (i.e., growing polycultures) can reduce insect pests in agriculture both directly and indirectly, respectively. Despite the potential benefits of polycultures for pest control, they are not widely utilized in industrial agricultural countries, and it is unclear whether the pest control benefits of observed in small-scale polyculture experiments would scale up across a landscape. Consequently, increasing attention is being paid to the potential benefits of landscape-scale agricultural diversity for pest management. Here, I compare the evidence for direct and indirect pest control when habitat diversity is incorporated into agriculture at local and landscape scales. Diversity at either scale can have similar effects in reducing herbivory indirectly by enhancing natural enemy populations. However, the direct effects of plant diversity on herbivores differ at local and landscape scales. Whereas local plant diversity tends to disrupt host plant cues and reduce herbivore residency, landscape-scale agricultural diversity tends to increase herbivore mortality and to reduce herbivore fitness. To date, the direct effects of landscape-scale agricultural diversity on insect pests have largely been ignored and clarifying these mechanisms will reveal fruitful directions for future landscape-herbivore research. By

understanding the mechanisms behind landscape-herbivore relationships, we can better assess and predict pest control as an ecosystem service provided by diverse agricultural landscapes.

Introduction

In his 1973 monograph, Root classically proposed the ‘resource concentration hypothesis’ and the ‘enemies hypothesis’ to explain how some herbivores can dominate insect communities and reach higher densities in monocultures than polycultures (Root 1973). The resource concentration hypothesis posits that plant diversity directly reduces densities of specialist herbivores by reducing immigration into and increasing emigration out of polycultures. Polycultures can reduce immigration by obscuring the visual and volatile chemical cues of hosts that herbivores use for host location. The morphology and chemistry of plants, which affects host location and acceptance, also may differ in polyculture and monoculture. As insects forage in polycultures, low mobility larvae may die while searching for a host, while more mobile pests will encounter non-hosts, which can stimulate emigration (Bernays and Chapman 1994, Altieri and Nicholls 2004).

Root’s ‘enemies hypothesis’ describes how plant diversity indirectly suppresses herbivore populations by increasing natural enemy populations (Root 1973). More natural enemies presumably exert greater top-down control on herbivores through increased predation and parasitism rates. Polycultures benefit natural enemies by providing a greater diversity of food resources, which may be available for more of the year than is typical in monocultures. Also, the physical habitat of polycultures is often more favorable than monocultures, with milder microclimates and shorter periods of bare soil (Altieri and Nicholls 2004).

Both the ‘resource concentration hypothesis’ and the ‘enemies hypothesis’ can simultaneously act to suppress pests in diverse plant assemblages. To date, there have been hundreds of studies examining the effects of local plant diversity on insect communities and agricultural pests (Risch et al. 1983, Sheehan 1986, Andow 1991, Bommarco and Banks 2003). These studies are directly applicable to pest control in polyculture agriculture, which is quite common in some places (Altieri 1995). However, their applicability to industrial agricultural systems dominated by monocultures is questionable.

As an alternative to polycultures, diversity can be incorporated into agricultural systems at landscape scales. With landscape-scale agricultural diversity, individual fields are still monocultures of one species, but there is a greater diversity of crops, more leys and fallows, and/or more non-cropped habitat in a region. The possibility that landscape-scale agricultural diversity can suppress insect pests has received increasing attention over the last decade (Bianchi et al. 2006). However, landscape-herbivore research is still an emerging area of study compared with polyculture-herbivore research. Most landscape-herbivore studies have documented relationships between landscapes and insects with relatively little focus on the mechanisms underlying those patterns. Where mechanisms have been investigated, studies have focused almost exclusively on the indirect effects of landscape diversity on herbivores via effects on natural enemies. In other words, most studies have essentially extended the ‘enemies hypothesis’ to the landscape scale. In contrast, investigations into the direct effects of landscape diversity on herbivore pests, a landscape-scale extension of the ‘resource concentration hypothesis,’ are virtually missing from the literature.

In this paper, I examine how the ‘resource concentration hypothesis’ and ‘enemies hypothesis’ scale up with landscape-scale agricultural diversity (Figure 2.1).

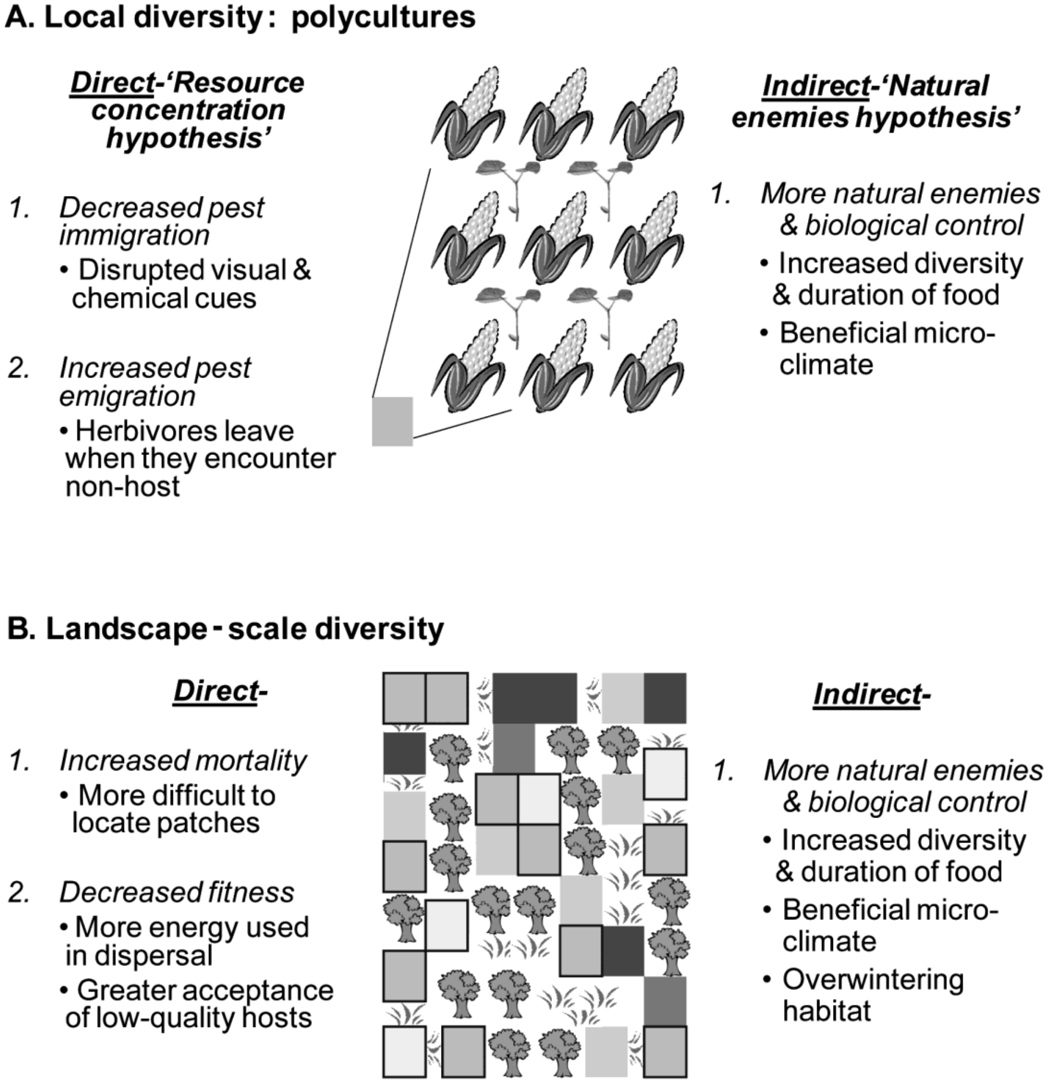


Figure 2.1. A Comparison of the direct and indirect mechanisms by which habitat diversity at local and landscape scales may affect agricultural herbivores. A) An example of a locally diverse agricultural habitat with multiple plant species planted together as a polyculture within one field. B) An example of a diverse agricultural landscape where individual fields may be monocultures, but many different crops (represented by different colored rectangles) and natural habitats (represented by trees and grass) are integrated in the landscape.

I begin by briefly summarizing the polyculture research and the relative importance of the ‘resource concentration hypothesis’ and ‘enemies hypothesis’ for pest control in polycultures. I then summarize the literature from landscape-scale diversity studies and compare research emphases within the polyculture and landscape-scale diversity literatures. This provides the background for a detailed analysis of the direct and indirect effects of landscape-scale agricultural diversity on insect pests. While the indirect effects of diverse agricultural landscapes on insect pests appear to follow closely the ‘enemies hypothesis,’ the direct effects differ from those of the ‘resource concentration hypothsis.’ From empirical, modeling, and evolutionary studies, I propose that landscape-scale diversity directly suppresses specialist agricultural pests by increasing mortality during dispersal among host patches and by reducing fitness (Figure 2.1 B).

Local Diversity

Reviews of the polyculture literature generally support suppression of herbivore pests by polycultures. In 1983, Risch et al. reviewed 150 studies of 198 herbivores (Risch et al. 1983) and found that 53% of the species were found to be less abundant, 29% showed no or variable response, and 18% had increased densities in polyculture. In 1991, Andow expanded on the previous review and summarized results from 209 polyculture studies that included 287 herbivore species (Andow 1991). The additional studies confirmed the previous review, and he found that herbivore densities were lower, variable or unchanged, or increased in 52%, 33%, or 15% of species, respectively. In 1994, Tonhasca & Byrne conducted a meta-analysis on 21 studies from 1984-1994 and found that herbivore densities were significantly reduced in polycultures, with an average effect size of 0.35 (Tonhasca and Byrne

1994). In many instances when pest suppression was not detected in polycultures, the pests were polyphagous (Andow 1991).

The presumption of larger natural enemy populations and greater biological control in polycultures than monocultures is generally supported by the literature. In 1989, Russell (1989) reviewed mortality rates of herbivores by natural enemies in polycultures and found nine studies reporting increased mortality, two reporting decreased mortality, and two reporting no change. In 1991, Andow summarized research covering 130 natural enemies species and found that 53%, 39%, or 9% of natural enemies reached higher, variable or unchanged, or lower densities in polycultures, respectively (Andow 1991). In 2004, Langellotto and Denno (2004) conducted a meta-analysis on 32 studies where habitat complexity was increased through polyculture, intercropping, and no-till mowing and found a significant average increase in natural enemy densities with an effect size of 0.69.

The numerous polyculture studies beg the question as to which mechanisms are most important for suppressing pest populations in agriculture: direct effects described by the ‘resource concentration hypthesis’ or indirect effects described by the ‘enemies hypothesis’. Reviews by Risch et al. and Andow, which share many but not all the same references, both suggest that direct effects of plant habitat on herbivores are more important than natural enemies for lowering herbivore populations in polycultures (Risch et al. 1983, Andow 1991). The strongest support for this conclusion is that monophagous herbivores are suppressed in polycultures more often than polyphagous herbivores. We would expect the direct effects of habitat diversification to suppress monophagous herbivores more than polyphagous herbivores that can feed on many plants within a polyculture. However, if plant diversity causes a large increase in enemies that exert top-down control of herbivores, then monophagous and polyphagous herbivores should be equally suppressed in polycultures. Since Risch et al. (1983) found that 61% of monophagous and 27% of polyphagous herbivores were suppressed in polycultures, and Andow (1991) similarly found that 61% of monophagous and 24% of polyphagous herbivores were suppressed in polycultures, the mechanisms associated with the ‘resource concentration hypothesis’ seem to offer the best explanation for reduced herbivore densities in polycultures.

Landscape Diversity

Despite the promise of polycultures for pest suppression, there are important practical and theoretical questions concerning its relevance for industrial agricultural systems. Socio-economic and agronomic factors limit the adoption of polycultures, despite pest control benefits (Vasey 1992). Furthermore, it is not clear whether pest control would still occur if polycultures were scaled up over large tracts of land (Bergelson and Kareiva 1987, Bommarco and Banks 2003, Prasifka et al. 2005). Bommarco & Banks (2003) reviewed 66 polyculture experiments based on plot size and found that pest suppression and natural enemy benefits were reduced with increasing plot size and that no effects of polycultures were detected in plots larger the $256m^2$. If the scale of experimental treatments is small relative to insect dispersal, then insects may simply be exhibiting habitat preferences in small-plot polyculture experiments. In general, polyculture studies have been hampered by ignoring long-term population dynamics and the dispersal scales of insects.

Increasing landscape-scale agricultural diversity offers a possible solution to the practical barriers and issues of scale associated with the adoption of widespread polycultures. With landscape-scale diversity, individual fields can still be monocultures, which suits agronomic practices typical of industrial agriculture. Also, the positive effects of landscape-scale diversity on natural enemy populations are likely to reflect demographic processes, while polycultures may simply act to concentrate local natural enemies in specific locations. By providing undisturbed overwintering habitat, diverse landscapes support multi-generational conservation of natural enemies (Figure 1B) (Landis et al. 2000). Therefore, increasing attention has been paid to the potential pest control benefits of diverse agricultural landscapes.

Trends in Landscape-Insect Studies

In contrast to the polyculture literature, there have been far fewer investigations into the effects of landscape-scale diversity on agricultural pests and natural enemies. The emphasis on pattern rather than process also differs from the polyculture literature in which Root's (1973) proposed 'resource concentration hypothesis' and 'enemies hypothesis' helped to launch the field. When mechanisms are examined, there is a general assumption in the landscape-insect literature that diverse landscapes indirectly suppress pests by enhancing natural enemy populations (Bianchi et al. 2006). This is effectively an extension of the 'enemies hypothesis' to the landscape scale. However, we do not see the 'resource concentration hypothesis' extended to the landscape scale to examine possible direct effects of landscape diversity on herbivores.

Despite the limited number of studies, landscape-insect research is beginning to show that pest suppression is characteristic of diverse agricultural landscapes. In 2006, Bianchi et al. published the first analytical review of data from landscape-scale diversity studies, which showed that natural enemies were generally enhanced and herbivores were generally suppressed in complex landscapes (Bianchi et al. 2006). Their review included 28 studies published from 1966 through 2005 with 18 studies reporting only on natural enemies, 6 reporting on both pests and natural enemies, and 4 reporting solely on pests. They found that pest pressures were reduced, unaffected, or enhanced by landscape diversity in 45%, 40% and 15% of cases. Natural enemies were enhanced, unaffected, or reduced by landscape diversity in 74%, 21%, and 5% of cases. Studies published since the review continue to support ideas of pest suppression and enhanced natural enemy communities in diverse landscapes. In cases when pests were not suppressed in diverse landscapes, this was often associated with species that utilize multiple habitats (Bianchi et al. 2006, Zaller et al. 2008).

Mechanisms of Pest Suppression in Diverse Agricultural Landscapes

While the data are promising that landscape-scale diversity can suppress agricultural pests, the continued emphasis on landscape-natural enemy relationships and the limited number of systems studied limits the generality of results. Nearly three quarters of the studies reviewed in 2006 focused exclusively on landscape-natural enemy relationships and half of the landscape-herbivore studies were on aphids (Bianchi et al. 2006). Since then, the emphasis on landscape-natural enemy relationships, rather than on landscape-herbivore relationships relevant to pest management decisions, has continued. Of the additional studies published since the Bianchi et al. (2006) review, only 2 quantify herbivore populations (Geiger et al. 2005, Zaller et al. 2008).

Pest control, as an ecosystem service of diverse landscapes, can be better understood by applying ideas from polyculture research to landscape-insect research (i.e. from small-scale to large-scale diversity studies). In particular, focusing on the mechanisms underlying landscape-insect relationships will increase our ability to predict the effects of landscape-scale agricultural diversification on pest management. Whereas polyculture studies indicate that both direct and indirect effects of habitat diversity help to suppress herbivore pests and that direct effects might be most important, only the possibility of indirect effects has received attention in landscape-scale studies.

If landscape-scale diversity, indeed, only affects herbivores indirectly through its effects on natural enemies, this has important implications for landscape-herbivore relationships. For example, we would predict that pests with depauperate natural enemy communities, common with invasive pests (Barbosa and Schultz 1987), are not strongly affected by landscape diversification. Also, pests with relatively sessile natural enemies, such as nematodes (Toepfer et al. 2009), would be expected to respond more to within-field management than to landscape characteristics. On the other hand, if diverse landscapes also directly suppress herbivore pests, these predictions do not hold. Therefore, understanding the processes that create landscape-herbivore relationships is necessary to correctly predict those relationships. In the following sections, I conceptually extend the ‘enemies hypthesis’ and ‘resource concentration hypothesis’ to the landscape scale and specify the mechanisms by which landscape diversity may indirectly and directly affect herbivore populations and provide pest control services.

Indirect Effects of Landscapes

With landscape-scale diversification, the mechanisms whereby natural enemy populations are increased are probably similar to those underlying the ‘enemies hypothesis’ at the polyculture scale. Namely, diverse landscapes offer diverse food resources and habitats with superior microclimates. An added benefit of landscape-scale habitat diversity is the increased availability of overwintering sites for natural enemies. However, in contrast to polycultures, resources are spatially segregated in diverse landscapes. Therefore, in order for diverse landscapes to enhance biological control, natural enemies must be able to access these segregated resources through dispersal. Examining natural enemy dispersal and distributions in different habitats can provide insight into the indirect effects of diverse landscape on agricultural pests.

Natural enemies can be classified as species that: 1) are restricted to undisturbed, or natural, habitats, 2) favor natural areas but spill over into disturbed habitats, 3) utilize both disturbed and undisturbed habitats, often at different life stages, or 4) are restricted to disturbed habitats (Duelli et al. 1990, Burel et al. 2000). For biological control purposes, it is optimal for natural enemies to be in categories three or four. These types of natural enemies are dispersive enough to locate and prey upon herbivore pests where infestations begin (Wiedenmann and Smith 1997).

However, landscape-scale diversification is likely to benefit natural enemies in categories two and three. These types of insects use both undisturbed and disturbed habitats, which are available in diverse landscapes. Therefore, knowing which natural enemies fall into these two categories would be useful for predicting the effects of landscape diversification. Category three natural enemies are most of the biological control species commonly studied in annual cropping systems and include many spiders, predatory insect species of Heteroptera, Homoptera, Neuroptera, and Coleoptera predatory insects, as well as Hymenopteran and Dipteran parasitoids (Wissinger 1997, Marino et al. 2006). Natural enemies that disperse between annual crops and more stable habitats are common. In pre-agricultural times, these species probably inhabited grassland-forest ecotones and were already adapted to utilizing resources from habitat mosaics (Wissinger 1997).

Insects with limited propensity to disperse into crop fields, category two natural enemies, are expected to be more sensitive to the particular configuration of diverse landscapes than category three natural enemies. Their presence in disturbed habitats would presumably increase in diverse landscape with small fields and high perimeter to area ratios. However, predicting which natural enemies fall into this category is complicated. Similar to category three natural enemies, which are more likely to disperse into crop fields, category two natural enemies include a wide array of arthropod taxa with varied life histories. In general, spiders appear more restricted to undisturbed habitat boundaries than insects (Duelli et al. 1990, Samu et al. 1999), although there are exceptions to this rule (Lemke and Poehling 2002). Beyond contrasting spiders and insects, comparing different functional groups such as predators or parasitoids, dispersal modes such as aerial or cursorial, or size provides no obvious trends in natural enemy distributions in agricultural fields (Duelli et al. 1990, Dennis and Fry 1992, Marino and Landis 1996, Fournier and Loreau 1999, Holland et al. 2005). For example, some large, epigeal carabids are increasingly abundant near field edges while others are ubiquitous throughout agricultural fields (Holland et al. 2005). Likewise, some small, aerially dispersing parasitoids show edge effects while others do not (Marino and Landis 1996). If there are predictive profiles for natural enemies that are fairly restricted to the proximity of natural habitats, they are likely based on complex suites of factors.

Despite the difficulties in predicting which natural enemy species most benefit from landscape-level diversification, it seems clear that extra-field habitat diversity reliably increases the number of natural enemy species within annual crop fields. In his studies of natural enemies in cereals versus less disturbed grasslands and wetlands, Duelli (1990) determined that 60% of spider, 16% of carabid, and 22% of staphylinid species present in wheat would have been absent without the surrounding semi-natural areas. Similarly, Dennis and Fry (1992) found that 45% of the natural enemy groups they collected in pitfall traps showed edge effects within 50m of field boundaries. In irrigated wheat fields surrounded by desert in Israel, 60% of spider species in the wheat were thought to have spilled over from the surrounding landscape (Gavish-Regev et al. 2008). In general, species richness should increase with landscape-level diversification, and small fields with high perimeter-area ratios are more likely to receive dispersing natural enemies throughout fields than are large fields with low ratios.

Direct Effects of Landscapes

Whereas the mechanisms of the ‘enemies hypothesis’ seem to apply to diverse landscapes so long as natural enemies can disperse among habitat patches, the major mechanisms of the ‘resource concentration hypothesis’ do not. The structural and chemical complexities of plant mixes that directly suppress herbivores by decreasing pest immigration and increasing pest emigration into and out of polycultures do not exist in diverse landscapes that still harbor large monocultures of hosts. However, in diverse landscapes, the distances among suitable crop fields and the number of habitat boundaries between host crops is increased, on average. This can lead to direct effects on herbivore populations by increasing mortality during dispersal and by reducing fitness. In the following sections, I combine evidence from empirical, modeling and evolutionary studies to support the thesis that diverse landscapes directly increase herbivore mortality and decrease fitness.

Direct Effects: Dispersal Mortality

Insect mortality can be high during dispersal and may increase in diverse landscapes with fewer host patches. This is especially true for passively dispersed insects. Insects dispersing above their flight boundary layer in the atmosphere have little control over their displacement and are carried downwind. In aircraft-net surveys, 18 orders of insects have been collected at altitudes between 200-5000 feet (Gatehouse 1997). Even wingless arthropods, such as gypsy moth larvae (*Lymantria dispar*), Douglas-fir tussock moth larvae (*Orgyia pseudotsugata*), eastern spruce budworm larvae (*Choristoneura fuminferana*), and many spiders and mites engage in aerial movement with adaptive behaviors for passive wind dispersal on silks (Pedgley 1982). After downwind displacement, passive dispersers are then faced with the challenge of locating hosts. Of the many aspects of insect dispersal, "The termination of migration is the least understood" (Reynolds et al. 2006). However, mortality during descent and host location can be quite high. There are various accounts of small insects landing in inappropriate places such as lawns (Kisimoto and Sogawa 1995) and mountaintop snowfields (Edwards 1986). Many insects also die after being blown out to sea (Chapman et al. 2004), or over the Great Lakes (Reynolds et al. 2006). Also, when airborne insects are deposited by rain, small insects probably drown in large numbers (Reynolds et al. 2006).

While observations indicate that many insects die during dispersal, there are few examples of precise dispersal mortality estimates. However, the existing estimates are high. Mortality of the bird cherry-oat aphid leaving grasses and searching for overwintering hosts in England was calculated at 99.4% between 1984-1992 (Ward et al. 1998). Others have even speculated the percentage of aphids dying during dispersal to be as high as 99.999% (Johnson 1969). Recent estimates of western spruce budworm dispersal mortality in a Douglas-fir region in southern British Columbia, Canada varied between 35-63% over 5 years of observation (Nealis and Regniere 2009). Notwithstanding the dearth of specific estimates of insect mortality during dispersal, there is reason to suspect that it is high, especially for passively dispersed insects, and could be increased by reducing the prevalence of suitable habitat in diversified agricultural landscapes.

For strong flyers that can actively direct their search for hosts, it is less clear how landscape diversification would affect mortality during the search for new host patches. Many insects utilize visual and olfactory cues in host discovery (Bernays and Chapman 1994). However, even for strong flyers, these cues may be more difficult to detect in diverse landscapes with less hosts and more habitat boundaries. For example, some butterflies turn around upon encountering habitat boundaries (Charrier et al. 1997, Ries and Debinski 2001). More habitat boundaries in diversified landscapes may, therefore, make it more difficult for these species to find hosts. Even if changes in landscape structure do not completely prohibit host location, they likely will result in greater search times. More time searching for hosts means a greater chance of being carried away by weather fronts (Sparks et al. 1985). Increased host searching time may also increase the probability that herbivore pests will be attacked by natural enemies. Thus, while mortality of strong flyers during dispersal is far from being understood, there is reason to believe it will increase, to some degree, with landscape diversification.

Direct Effects: Reduced Fecundity & Host Discrimination

In addition to the likelihood of increased mortality during dispersal, there would likely be fitness consequences for successful dispersers if their hosts became less prevalent with landscape diversification. Fueling dispersal is energetically costly and can reduce the energy reserves available for reproduction. Zera & Denno (1997) reviewed the trade-offs between dispersal and fitness in wing polymorphic insects and highlighted the high energetic costs of triglyceride biosynthesis to fuel extended dispersal. During dispersal, the metabolic rates of insects can be 20-100 times higher than when resting (Rankin and Burchsted 1992). Energy devoted to dispersal may mean energy unavailable for reproduction. Work on soybean aphids supports dispersal-fecundity trade-offs. Zhang et al. (2009) found that soybean aphids that flew more than 0.5km in flight mill studies had significantly lower fecundity and longevity than aphids that flew less than 0.5km. Interestingly, this fitness reduction was also transferred to the next generation and offspring of long distance dispersers also had reduced fecundity. Presumably, lipid depletion during long-distance dispersal affected provisioning of the developing oocyte, resulting in reduced fitness a generation removed from the dispersal event (Zhang et al. 2009).

Additional fitness consequences may be incurred in diverse landscapes if herbivores accept lower quality hosts than they would in simple landscapes. Various studies have shown that increased dispersal or depleted energy reserves are related to decreased host discrimination. For example, fruit flies that were starved for 24hrs were more likely than fruit flies that had not been starved to accept rotting oranges, despite the fact that successful progeny development on oranges was only half that on apples (Hoffmann and Turelli 1985). Similarly, Kareiva (1982) observed that flea beetle discrimination of collard host quality decreased as distances between host patches increased. Theoretical investigation into animal movement and host discrimination also predicts reduced discrimination with increasing distances between patches (Walsh 1996). If herbivore dispersal increases with landscape diversification, more herbivores may accept low-quality hosts, which would reduce their fitness.

Direct Effects: Modeling Evidence

Another line of evidence that herbivore mortality may be higher in diverse landscapes, with increased dispersal distances among patches, than in simple landscapes comes from population models of butterflies. Models that incorporate the assumption that mortality increases with dispersal distance have proven accurate in predicting insect population dynamics. In 2000, Ilka Hanski and colleagues (Hanski et al. 2000) published their Virtual Migration Model in which the probability of dispersal mortality decreases with the connectedness of habitat patches. Connectedness, in turn, depends on the distances among habitat patches and insect dispersal behavior. In other words, when habitats are not connected, there is high mortality. When the Virtual Migration Model was fitted to mark-recapture data on the false heath fritillary butterfly, *Melitaea diamina*, in Finland approximately 17% were estimated to have died during dispersal (Hanski et al. 2000). The model was also fitted to mark-recapture data on the bog fritillary butterfly, *Proclossiana eunomia*, in Belgium where 12% were estimated to have died during dispersal (Petit et al. 2001). When demographic parameters of *P. eunomia* were derived for populations inhabiting four landscapes across Finland and Belgium that varied in the percentage of suitable habitat, there were large differences in the estimates of dispersal mortality. Estimates varied from approximately 5% in a landscape with 48% suitable habitat to over 30% in landscapes with either 13% or 0.4% suitable habitat (Mennechez et al. 2003). While these studies provide only indirect evidence for insect mortality during dispersal, they do appear to describe butterfly dynamics in patchy landscapes. These models describing empirical data indicate that dispersal mortality would increase for insect pests whose hosts are less prevalent in diverse agricultural landscapes.

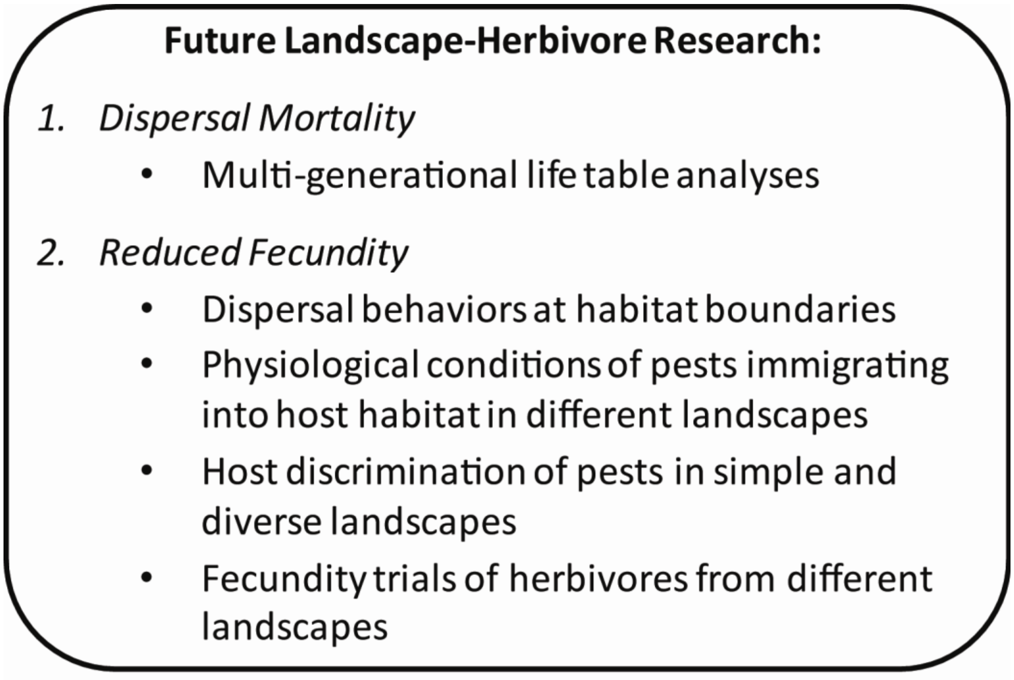
Direct Effects: Evolutionary Evidence

Evolutionary trends also indicate the risks to insects during dispersal; risks that may increase if agricultural landscapes were diversified so that suitable habitat was reduced. First, there is evidence that dispersal ability is lost in stable habitats. Roff (1990) found that in stable habitats such as woodlands, the ocean, various freshwater habitats, and caves, flightlessness was more prevalent than expected within many insect orders. Furthermore, studies of wing polymorphism within species, where some individuals are winged and some are not, also confirm that winglessness is associated with habitat stability (Zera and Denno 1997). This has also been observed with species of spider mites, in which populations in orchards were less dispersive than populations in corn (Margolies 1995). Reduced dispersal in stable habitats appears to be an adaptation for the risks associated with dispersal.

A second evolutionary indication that dispersal is risky is that dispersive species of insects often possess greater reproductive capacity than less dispersive species. In a comparison of life history strategies of congeneric insects, Dingle (1981) found that the more dispersive species consistently exhibited higher intrinsic rates of increase. Higher reproductive rates in dispersers indicate a possible compensation for mortality during dispersal. It is worth noting that evolutionary evidence initially appears to contradict empirical evidence concerning dispersal-fecundity relationships in insects. Evolutionary evidence indicates that more dispersive species have higher fecundities while empirical evidence indicates that dispersal reduces herbivore fecundity. However, there are likely differences between an evolved adaptation for dispersal and the costs to a species whose habitat is rapidly diversified. If landscape diversification occurs over short time periods, agricultural pest are likely to experience fitness costs associated with increased energy expended to disperse among disparate host patches. However, over evolutionary time, an insect may evolve higher fecundity in response to increased risks associated with dispersal in diverse landscapes.

Future Research

To better understand the direct effects of landscape-scale diversity on agricultural pests, it will be fruitful to design studies that compare herbivore mortality and fitness in different landscapes (Figure 2.2). Life table analyses of pests in different landscapes could be conducted to estimate differences in dispersal mortality and fecundity. Behavioral studies of pest dispersal at habitat boundaries could provide useful information about whether locating new habitat patches is more difficult in diverse landscapes. Field studies comparing pest populations in simple and diverse



Future Landscape-Herbivore Research:

1. *Dispersal Mortality*
    - Multi-generational life table analyses
2. *Reduced Fecundity*
    - Dispersal behaviors at habitat boundaries
    - Physiological conditions of pests immigrating into host habitat in different landscapes
    - Host discrimination of pests in simple and diverse landscapes
    - Fecundity trials of herbivores from different landscapes

Figure 2.2. Proposed research agenda to further understand possible mechanisms, namely increased dispersal mortality and reduced fitness, by which diverse agricultural landscapes may directly affect herbivore populations. The bulleted list describes methods for quantifying these direct effects.

landscapes could be expanded to also measure indicators of pest fitness. For instance, one could compare the biomass, lipid and carbohydrate reserves, and/or egg loads of pest immigrants in different landscapes.   Differences in host discrimination by pests in simple and diverse landscapes could also be measured to assess fitness consequences of landscape diversification. Together, these types of studies will help to create a mechanistic understanding of the direct effects of landscape diversification on agricultural insect pests.

Summary / Conclusions

Diversifying agriculture at both local- and landscape-scales can help to suppress pest herbivores in agriculture.   Much of the polyculture literature has followed from Roots 1973 monograph in which he proposed the ‘resource concentration hypothesis’ and ‘enemies hypothesis’, which outline how plant diversity both directly and indirectly contributes to reduced pest populations. Polycultures can directly reduce herbivore loads by masking the visual and chemical cues involved in host location and by changing properties of the host plant. Polycultures can indirectly reduce herbivore loads by enhancing natural enemy populations that benefit from the increased diversity and duration of food as well as an improved microclimate. Landscape-scale diversity studies are far fewer than polyculture studies and there has been less emphasis on parsing out the mechanisms underlying patterns of pest suppression. In particular, there has been little exploration of the direct effects of landscape diversity on pest populations. Whereas mechanisms of the ‘enemies hypothesis’ appear applicable to landscape-scale diversification so long as natural enemies can disperse among segregated resources, the mechanisms underlying the ‘resource concentration hypothesis’ appear somewhat less applicable. However, direct effects of landscape diversification seem likely and include increased mortality and reduced fitness as insects expend more energy dispersing among habitat patches and possibly accept hosts of inferior quality. Future research into the mechanisms controlling landscape-herbivore relationships will hone our predictions of the pest control services supported by diverse landscapes.

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CHAPTER 3

THE LANDSCAPE CONTEXT OF INSECT HERBIVORE AND NATURAL ENEMY DENSITIES: A MULTI-SCALE APPROACH

Megan E. O'Rourke, Kaitlin Rienzo-Stack, and Alison G. Power

Abstract

Landscapes are important regulators of ecosystems services, including agricultural pest management. However, relationships between land-use and agricultural insect pests are not well understood and investigators have not considered many possible complexities in the relationships. We tested the hypothesis that landscape-insect relationships can be better understood by considering multiple scales of land-use simultaneously, and by considering land-use at scales much larger than are typical. We tracked densities of three specialist herbivore pests, the European corn borer (*Ostrinia nubilalis*), the western corn rootworm (*Diabrotica virgifera*), and the northern corn rootworm (*Diabrotica barberi*), and two generalist predator lady beetles, *Coleomegilla maculata*, and *Propylea quatuordecimpunctata*, in field corn and determined their relationships to three scales of land-use: perimeter, 1km-, and 20km-radius areas. Predicted relationships between pest densities and scales of land-use were quite different when scales were considered individually or together in analyses. A mutli-scale approach indicated that pest densities were either higher (*D. virgifera* and *D. barberi*) or unchanged (*O. nubilalis*) in landscapes with more corn while natural enemy densities were either lower (*C. maculata*) or unchanged (*P. quatuordecimpunctata*). These relationships were not always straightforward and 1km-radius or perimeter scale land-use had more effect on *D. virgifera* and *C.*

maculata densities, respectively, when the larger landscape had high amounts of corn. The 20km radius land-use scale was a significant predictor of *D. virgifera* densities and our data indicate that pests may respond to land-use at larger scales than natural enemies. Interactions among scales and the significance of 20km radius areas for predicting insect densities underscore the complexities of landscape-insect relationships. Government policies that affect habitat diversity on agricultural lands may have unexpected effects on insect pest control, depending on the scales of land-use they influence.

Introduction

Evidence suggests that increasing agricultural diversity can enhance ecosystem services including biological control and insect pest suppression (Bianchi et al. 2006, Gardiner et al. 2009). Diverse landscapes can enhance natural enemy densities and species richness by providing more overwintering sites (Sarthou et al. 2005) and prey (Landis et al. 2000). These natural enemies, in turn, prey upon pests. Additionally, diverse landscape may directly suppress herbivore populations. While direct mechanisms are far less studied than indirect mechanisms, there is evidence that herbivore mortality may increase and fecundity may decrease in diverse landscapes because pests must disperse farther than in simple landscapes to find suitable habitat (Fahrig and Paloheimo 1988, Schneider 1999, den Belder et al. 2002, O'Rourke 2010).

While most studies support the idea that natural enemy populations are enhanced and that herbivore pests are suppressed in diverse agricultural landscapes, there are exceptions. Usually, these exceptions can be related to insect life history. For example, herbivore pests that use multiple hosts or that overwinter in non-crop habitat may actually benefit from diverse landscapes (Bianchi et al. 2006, Zaller et al. 2008). However, landscape-insect studies may also produce confusing results due to particulars of experimental design. Specifically, considering only one scale of land-use at a time and not considering large enough scales may confound landscape-insect research.

A "concentric circle" approach is commonly used to explore landscape-insect relationships. In these types of studies, insect densities within sample fields are related to land-uses in circular regions of increasing radii around fields (Tscharntke et al. 2005, Westphal et al. 2006). This can create statistical issues. If land-uses at different spatial scales are correlated, examining individual scales of land-use may indicate that insects are positively correlated to a landscape scale when they are, in fact, negatively correlated (Figure 3.1). Indeed, while these results were not discussed, Bianchi et al. (2005) found that linear regression estimates between land-use at different scales and biological control of *Mamestra brassicae* eggs in cabbage could be quite different and could even change sign when scales of land-use were tested individually or together.

Empirical evidence also indicates that land-use at multiple scales concurrently affects insects. For example, Tscharntke et al. (2002) found that parasitism of rape pollen beetles was lower at the centers than the edges of fields only in simple landscapes dominated by agriculture and not in diverse landscapes. Brewer et al. (2008) found that the effects of field-scale landscape modifications depended on the regional landscape context. In their study, adding sunflower strips to wheat–fallow strip cropping systems increased rates of wheat aphid parasitism more in simple regional landscapes dominated by grasses than in diverse landscapes with more non-grass habitat. Both of these examples support the idea that the regional landscape context determines the effectiveness of local landscape manipulations and that local land-use matters more when the regional landscape is not already diverse (Tscharntke et al. 2005).

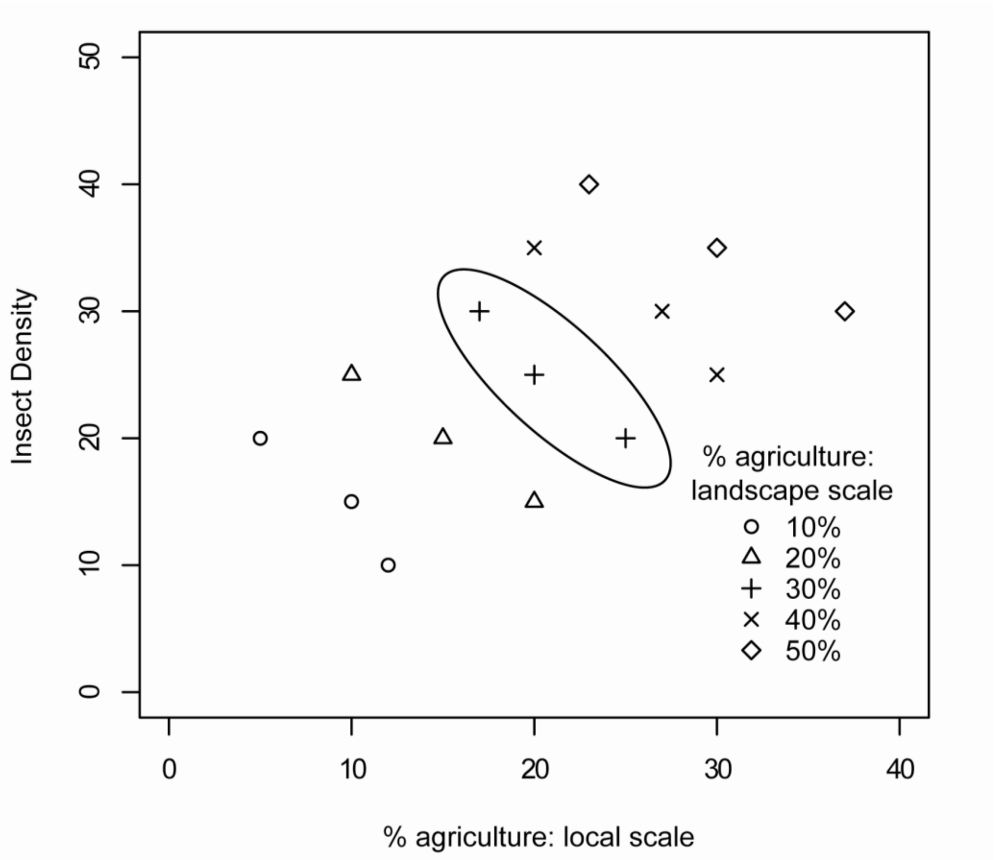


Figure 3.1. Relationships among insect densities, local-, and landscape-scale agricultural land use with hypothetical data to illustrate how multivariate analyses should be used when two landscape factors are correlated. Univariate regression between insect density and local agricultural land use indicates a significant positive relationship (slope = 0.50; $P = 0.046$) while multivariate analysis reveals a significant negative relationship (slope = -0.93; $P < 0.0001$).

Insect dispersal behavior provides further support for examining the effects of multiple scales of land-use concurrently in landscape-insect studies. Insects typically have different dispersal behaviors and habitat requirements at different life stages, such as reproduction, overwintering, and foraging (Johnson 1969). For example, many insects, including species of aphids and lady-beetles, rely on non-crop habitats for overwintering while relying on agricultural habitats for food (Moran 1992, Hodek and Honek 1996). They may undergo relatively long-distance migrations traveling to and from overwintering sites. However, when searching for food, the same insects may typically take shorter "appetitive" flights and will settle in agricultural areas with abundant food (Johnson 1969). Thus, when insects require natural areas to overwinter, their populations may correlate positively to natural habitat at large scales, but their distributions when sampled during growing seasons may be positively correlated to agricultural food sources (i.e. negatively correlated to natural habitat) at local scales. Even insects that are habitat specialists may fly long distances to lay eggs while making shorter flights to search for food (Johnson 1969, Spencer et al. 2005). In this case, the larger landscape may determine population dynamics while local distributions may result from interactions of local land-use and species-specific foraging behaviors.

Increasing the scale at which we measure land-use may also be an important step towards better understanding landscape-insect relationships. In previous studies, the largest landscapes usually did not exceed 5km radius areas, (den Belder et al. 2002, Bianchi et al. 2005, Bianchi et al. 2008), and were usually much smaller. These small scales may suffice for insects that disperse over short distances (Tscharntke et al. 2005). However, insect dispersal is poorly understood, and often underestimated (Johnson 1969). Furthermore, larger landscapes are probably important to strong dispersers, including many agriculturally adapted species

(Peterson and Denno 1998). Indeed, the few studies that have examined regional land-use suggest it may be an important predictor of agricultural insect populations. Elliott et al. (1999) measured landscapes in 24 x 24 km areas in South Dakota and found that patterns of land-use at large scales may affect insect predator abundances. In Germany, Clough et al. (2005) studied spiders in three different regions measuring 400-500km^2, and found that abundances of the majority of species were variable among the regions. However, they did not attempt to discern attributes of the regions possibly responsible for these differences.

In this study, we designed field surveys to test the relationships between land-use and densities of insects in field corn (*Zea mays*) in upstate New York. Surveys were specifically designed to include large, regional scales of land-use, which were 20km radius circular areas around sample fields (1257km^2). They were also designed to test for simultaneous effects of multiple scales of land-use on insect densities. Therefore, we measured land-use at the 1km radius (3km^2), and perimeter (land-use bordering sample fields) scales around sample fields, in addition to the 20km radius scale.

We sampled both herbivore and natural enemy species of insects in corn. The herbivores were three of the most economically important corn pests in the United States: the northern corn rootworm (*Diabrotica barberi*, Coleoptera: Chrysomelidae), the western corn rootworm (*Diabrotica virgifera*, Coleoptera: Chrysomelidae), and the European corn borer (*Ostrinia nubilalis*, Lepidoptera: Pyralidae). In New York, rootworm adults typically lay eggs during autumn at the base of corn plants. In spring, eggs hatch and larvae feed on corn roots if corn has been planted there again (Levine and Oloumi-Sadeghi 1991). European corn borer adults lay eggs on corn during summer and their larvae burrow into leaves and stalks (Mason et al. 1996). In addition to their economic importance in agriculture, *D. barberi* and *D. virgifera* herbivores were specifically included in this study because insect natural enemies are not thought to play important roles in their population dynamics (Levine and Oloumi-Sadeghi 1991). Thus, relationships between landscapes and *Diabrotica* densities are likely due to the direct effects of habitat configuration rather than to indirect effects via changes in natural enemy populations. We also sampled two generalist predator lady-beetles, *Coleomegilla maculata* (Coleoptera: Coccinellidae), and *Propylea quatuordecimpunctata* (Coleoptera, Coccinellidae). *C. maculata* and *P. quatuordecimpunctata* larvae and adults feed on a variety of soft-bodied insects, and corn pollen is an important food source for *C. maculata*. Both species can live multiple years and overwinter as adults in perennial habitat. *C. maculata* is native to the United States while *P. quatuordecimpunctata* is a non-native, introduced species (Hodek and Honek 1996).

In concurrence with the prevailing views of landscape-insect relationships, we hypothesized that herbivore densities would be higher in simple agricultural landscapes with more corn and that, conversely, natural enemy densities would be lower in simple landscapes with less alternative prey and overwintering sites. However, we further hypothesized that multiple scales of land-use affect insect densities. Specifically, we expected that herbivore and natural enemy populations would be more affected by local land-use where the regional landscape was dominated by corn than where it was already diverse. We also expected that insect densities could exhibit opposite relationships with local and regional scales of land-use.

Materials & Methods

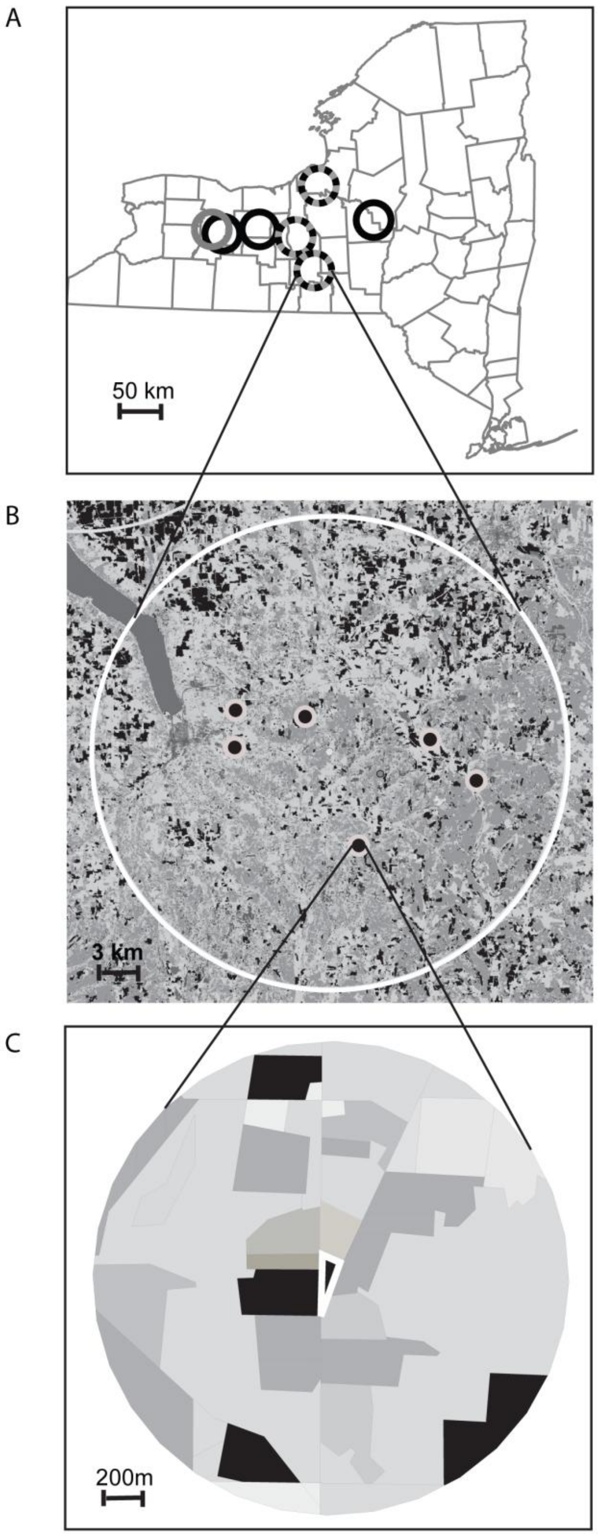
Study Region and Landscapes

Sampling was conducted in 2006 and 2007 in upstate New York, which contains a range of agricultural land-use intensities. All sampling was conducted in first-year corn (*Zea mays*) fields that were planted with non-Bt varieties, and were rotated from a variety of different crops grown the previous year. Sampling only first-year corn ensured that any rootworm and European corn borer adults in those fields were immigrants from the surrounding landscape. Furthermore, sampling only non-Bt corn ensured that the offspring of European corn borer immigrants could survive and cause detectable damage in sampled fields. Additionally, no aerial insecticides were applied to sample fields. However, in some fields, clothianidin insecticidal seed treatments or tefluthrin soil insecticides were applied to control for various seed and seedling insect pests, not including the insects we sampled. Weeds were managed independently by farmers in each field and were controlled through conventional herbicides. Planting dates ranged from May 15-June 15.

Thirty corn fields were sampled in 2006 and 36 different corn fields were sampled in 2007. The centers of fields were at least 2km apart. In 2006, the 30 fields were distributed within four regions with four to ten fields per region. In 2007, the 36 fields were evenly distributed within 6 circular regions. Each region was a circular, 20km radius area and the centers of regions were located at least 40km apart in a given year of study (Figure 3.2).

Landscapes surrounding sample fields were measured at three spatial scales: perimeter, 1km radius, and 20km radius circular areas. At the 20km scale, the percentage of land in row crops was determined from the 2001 National Land Cover Database with 30m^2 resolution raster data (http://www.mrlc.gov/) using ArcGIS 9.0 software. Direct field observations were used to determine the percentage of land in corn at the 1km and perimeter scales surrounding sample fields. Observed land-use was then digitized into vector maps over TerraServer aerial photographs (http://terraserver-usa.com/) using Manifold 8.0 software. Percent corn at the 1km scale was calculated using all observed corn except the sample field. Percent corn at

Figure 3.2. Three scales of land-use measured around first-year corn fields where insects were sampled in 2006 and 2007. A) Twenty kilometer radius regions in upstate New York. Grey, black, and grey/black dashed circles indicate regions sampled in 2006, 2007, and in both 2006 and 2007, respectively. Magnified view of one large region showing how multiple sample fields were nested within each 20km radius region. The six black circles represent sample fields surrounded by 1km radius regions. Black pixels represent field crops. C) Magnified view of one corn field with its perimeter highlighted in white and the surrounding land-use within a 1km radius region. Black polygons represent corn fields.


A
50 km
B
3 km
C
200m

the perimeter scale was calculated as the percentage of a sample field's edges abutting a corn field or a road that was bordered by a corn field.

Insect Sampling

Densities of two herbivores (western corn rootworm, *Diabrotica virgifera,* and northern corn rootworm, *Diabrotica barberi*) and two generalist predator lady beetles (*Coleomegilla maculata* and *Propylea quatuordecimpunctata)* were monitored in corn fields using sticky cards. . *Ostrinia nubilalis* (European corn borer) was monitored by measuring percentages of plants with holes in the stalks caused by larvae.

Insects were monitored at 6 sub-sample locations per field, along two transects. Transects were at least 30m from each other and 30m from the parallel edges of fields. Insects were sampled along transects at the third corn row, at 75m, and at 150m into fields. Beetle densities were monitored using Olson yellow sticky traps wrapped around and nailed to corn stalks at ear height according to Hein & Tollefson (1984). Sticky cards were monitored and replaced approximately once a week and beetle densities were recorded four times per field between July 31 and Sept 1 in 2006, and six times per field between July 24 and Sept 6 in 2007. ECB densities were estimated once per year, concurrent with the last sticky-card sampling, by recording the number of plants with holes in the stalks of 10 plants at each of the six sub-sample locations per field (60 plants per field).

Statistics

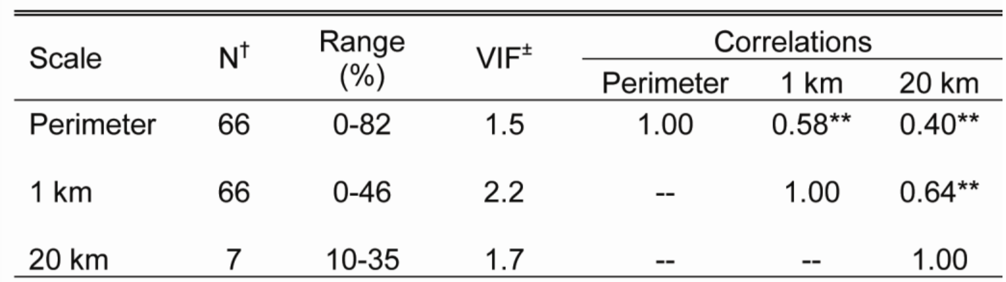
Prior to analyses, the appropriateness of using all landscape variables together in multiple regression models was explored by calculating correlations between landscape variables and their Variance Inflation Factors (VIF) proc REG in SAS 9.1 (SAS/STAT 2003). The effects of land-use patterns on insect densities were tested by manual reverse step-wise regression using proc GLMMIX in SAS 9.1. Each insect species was analyzed separately and the models began with the fixed effects: time (year; week for some species), 20km, 1km, and perimeter landscape variables, and all two and three way interactions between landscape variables. Interactions with the largest *P* values above 0.05 were sequentially removed from the models. Random effects partitioned the model variance so that fields within 20km regions were pooled to test for effects of 20km scale landscapes. Models for *D. virgifera* and *C. maculata* included week as categorical variables and data from the same fields over weeks were treated as repeated measures. For *P. quatuordecimpunctata,* due to low sample size, data were averaged across weeks per year prior to analysis. For *D. barberi*, due to even lower sample size, data were averaged across weeks and then transformed to three categories (*D. barberi* densities card^{-1} field^{-1} year^{-1} = 0; = 0 to .1; and > .1), which each included approximately 1/3 of the data. Data for *D. virgifera*, *C. maculata*, and *P. quatuordecimpunctata* densities as well as % plants damaged by *O. nubilalis* were transformed (ln[species+1]) prior to analyses and were analyzed using normal distributions. *D. barberi* data were analyzed using a multinomial distribution. Partial regression plots were used to illustrate the relationships between land-use and insect densities in Figures 3.3 & 3.5 (Kutner et al. 2005).

Results

Landscapes

Landscapes surrounding sample fields were highly variable. However, land-uses at different scales were correlated, indicating that a field with little corn on its perimeter was likely to have little corn surrounding it at the 1km and 20km scales. Despite these correlations, multiple regression analyses between insect densities and

Table 3.1. Summary of the scales of agricultural land-use surrounding first-year corn fields where insects were scouted in 2006 and 2007.



Scale	N[†]	Range (%)	VIF[±]	Correlations		
				Perimeter	1 km	20 km
Perimeter	66	0-82	1.5	1.00	0.58**	0.40**
1 km	66	0-46	2.2	--	1.00	0.64**
20 km	7	10-35	1.7	--	--	1.00

[†] Number of independent sampling units at different scale over 2006 and 2007

[±] Variance Inflation Factors < 10 indicate acceptable collinearity for multiple regression (Philippi 1993)

[**] Pearson's pairwise correlations significant at $P \leq 0.001$

Table 3.2. Relationships between insect densities and land-use highlighting significant scales of land-use (statistics in bold), and land-use parameters that differ in significance when examining multiple scales of land-use simultaneously or singly in general linear mixed models (parameter names in bold).

Trophic Level	Order	Species[ŧ]	Mean[β]	Parameter	Multiple LP[¥]			Single LP[€]		
					Est.[¤]	F	*P*	Est.	F	*P*
Herbivore	Coleoptera	*D. barberi*[†]	0.2	year[±]	0.42	0.42	0.52	v[£]	v	v
				perimeter	0.41	0.08	0.78	**2.56**	**5.39***	**0.02**
				1 km	**8.14**	**6.71**	**0.01**	**5.55**	**8.44**	**0.0051**
				20 km	-3.55	0.36	0.58	2.53	0.27	0.63
	Coleoptera	*D. virgifera*	11.2	year	0.21	1.47	0.23	v	v	v
				week	m[Ŧ]	45.83	<.0001	v	v	v
				perimeter	**-0.81**	**3.88**	**0.05**	0.76	3	0.09
				1 km	-2.21	1.08	0.3	**3.33**	**28.05**	**<.0001**
				20 km	1.56	0.93	0.36	**4.63**	**11.02**	**0.02**
				1 km x 20 km	**15.77**	**5.07**	**0.03**	na[§]	na	na
	Lepidoptera	*O. nubilalis*	18%	year	-0.12	0.72	0.4	v	v	v
				perimeter	0.29	0.49	0.49	0.04	0.01	0.91
				1 km	-0.76	0.76	0.39	-0.4	0.59	0.45
				20 km	0.03	0.002	0.97	-0.3	0.21	0.65
Generalist predator	Coleoptera	*C. maculata*	6.8	year	-0.003	0.001	0.98	v	v	v
				week	m	76.68	<.0001	v	v	v
				perimeter	-0.09	0.05	0.82	-0.32	1.32	0.25
				1 km	**-2.34**	**17.98**	**<.0001**	**-1.87**	**21.17**	**<.0001**
				20 km	-1.95	2.55	0.16	**-2.9**	**6.87**	**0.05**
				perimeter x 1 km	**3.03**	**5.46**	**0.02**	na	na	na
	Coleoptera	*P. 14*	0.13; 0.42	year	0.25	19.43	<.0001	v	v	v
				perimeter	0.2	2.21	0.14	**0.24**	**5.49**	**0.02**
				1 km	-0.24	0.8	0.37	0.25	1.95	0.17
				20 km	0.38	1.16	0.32	0.31	0.84	0.41

[¥] Multiple landscape predictors were tested simultaneously in general linear mixed (GLMMIX) models.

[β] Untransformed means of # beetles sticky card^{-1} week^{-1} year $^{-1}$; P. quatuordecimpunctata data are the "mean for 2006; mean for 2007". O. nubilalis mean is the untransformed percentage of plants damaged year^{-1}.

[€] GLMMIX models tested one landscape measurement at a time along with year and/or week predictor variables.

[ŧ] Insect density data (except for *D. barberi)* were natural log transformed prior to analyses (i.e. ln[insect s+ 1]).

[†] GLMMIX models for *D. barberi* used multinomial distributions; prior to analyses data were transformed: 0=2; 0 to .1=1; >.1=0.

[±] Positive estimates indicate that insect populations were larger in 2007 than 2006.

[£] Statistics were variable in different models.

[Ŧ] Multiple estimates were generated because week was a categorical variable.

[§] Not applicable-interactions could not be tested.

[¤] Beta estimates of GLMMIX models that predict transformed values of insect data. Land-use parameters are scaled as proportions corn or row crops (range = 0 to 1) in the models.

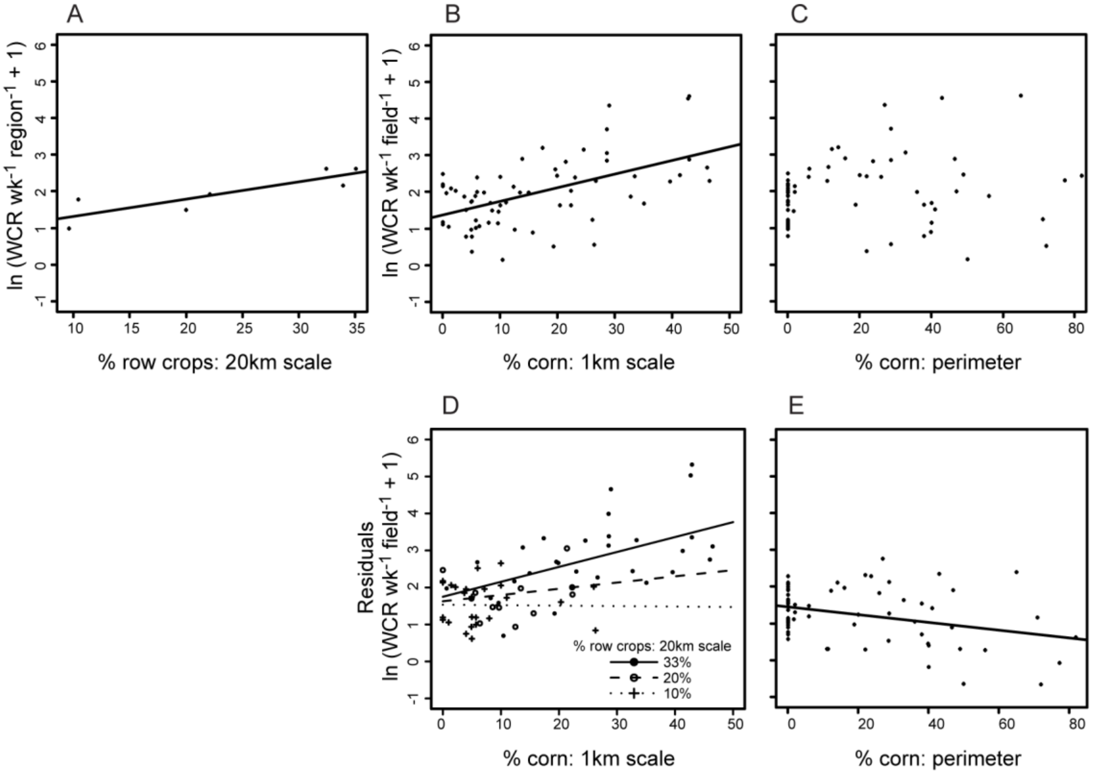


Figure 3.3. Contrasting relationships between *D. virgifera* (WCR) densities and land-use when land-use scales are tested individually (A,B,C) or together (D,E) in regression analyses. Relationships between *D. virgifera* densities in field corn and land-use at the 20km radius (A), the 1km radius (B), and the perimeter (C) scales. D) Interaction between 1km corn and 20km row crops and *D. virgifera* densities with the predicted effects of the 20km scale depicted by three regression lines (solid=33% row crops, dashed=20%, dotted=10%). Data points represent residual *D. virgifera* densities when perimeter and year estimates are taken into account. Filled circles, open circles, and crosses show data where row crops within the 20km radius surrounding regions were 32-35%, 20-22%, or 9.5 -10.5% of total land-use, respectively. E) Regression of *D. virgifera* residual densities against the percentages of sample fields' perimeters bordered by other corn fields accounting for sampling year and agricultural land-use at 20km, 1km, and 20km x 1km scales.

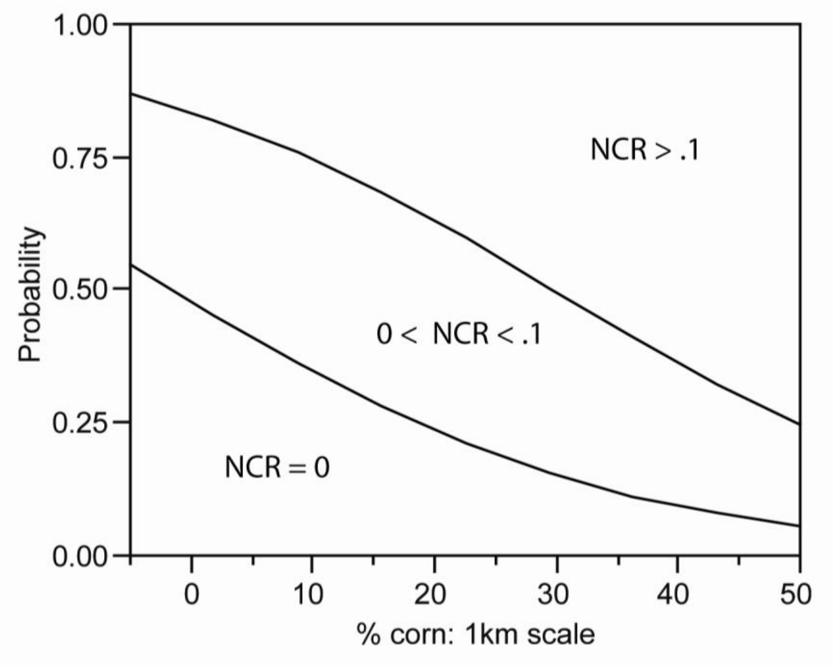


Figure 3.4. Ordinal logistic regression plot showing how the probability that *D. barberi* densities will fall within three different categories (0; 0 to .1; >.1) varies with the amount of corn within 1km radius regions surrounding sample fields.

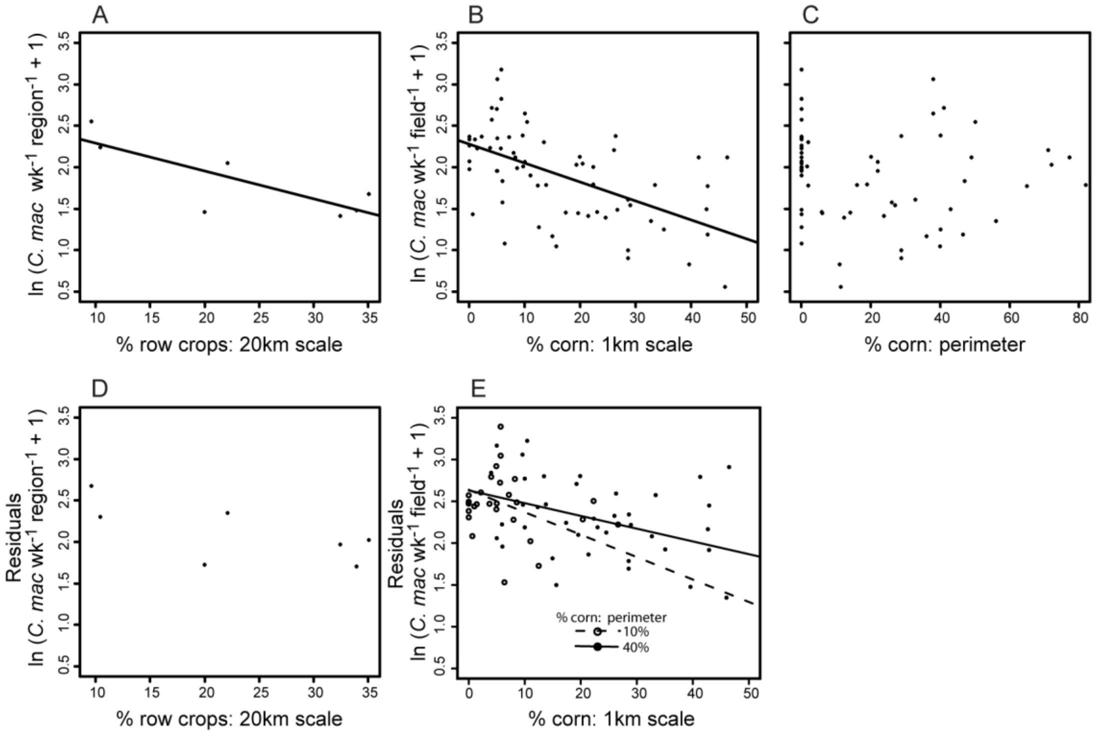


Figure 3.5. Contrasting relationships between *C. maculata* (*C. mac*) densities and land-use when land-use scales are tested individually (A,B,C) or together (D,E) in regression analyses. Relationships between *C. maculata* densities in field corn and land-use at the 20km radius (A), the 1km radius (B), and the perimeter (C) scales. D) Regression between residual *C. maculata* densities and row crops within 20km radius regions around sample fields with sampling year, 1km, perimeter, and 1km x perimeter scales of agricultural land-use taken into account. E) Interaction between 1km and perimeter scales of corn and *C. maculata* densities with the predicted effects of the perimeter scale depicted by two regression lines (solid=40% corn, dashed=10% corn). Data points represent residual *C. maculata* densities when 20km scale row crops and sampling year are taken into account. Filled and open circles represent sample points where corn was >20-46% or 0-20%, respectively, of total land use.

multiple scales of land-use were still appropriate, as indicated by low variance inflation factors for each land-use scale (Table 3.1).

Landscapes and Herbivores

Densities of two herbivores, *D. barberi* and *D. virgifera* were significantly related to land-use surrounding first-year corn fields. In contrast, the percentage of plants damaged by *O. nubilalis* was unrelated to land-use. Densities of *D. virgifera* immigrants ranged from 0.2 to 101 insects week^{-1} sticky card^{-1}. *D. barberi* abundance was much lower in both years of study and ranged from 0 to 2.2 insects week^{-1} sticky card^{-1} and no individuals of this species were trapped in 30% of surveyed fields. *O. nubilalis* damage was detected in 94% of fields surveyed and affected up to 56% of plants.

For *D. virgifera*, linear regressions between insect densities and individual scales of land-use showed significant relationships at the 1km and 20km scales, but no relationship at the perimeter scale (Table 3.2; Figure 3.3 A, B, & C). More row crops at the 20km scale and more corn at the 1km scale were related to higher densities of *D. virgifera* adults immigrating into corn fields. Analysis of the simultaneous effects of multiple scales of land-use on *D. virgifera* densities showed that the effect of corn at the 1km scale depended on land-use at the 20km scale (Figure 3.3 D). *D. virgifera* densities showed no predicted increase with increasing corn at the 1km scale where only 10% of land was planted to field crops at the 20km scale. However, with 33% row crops at the 20km scale, fields surrounded by 50% corn at the 1km scale were predicted to have nearly nine times higher densities of *D. virgifera* immigrants than fields with no corn in the surrounding 1km scale landscape. When accounting for corn at the 1km scale and row crops at the 20km scale, increasing amounts of corn on the perimeter of first year corn fields was related to lower densities of *D. virgifera* (Figure

3.3 E). Regressions between *D. virgifera* densities and individual or multiple scales of land-use suggested different relationships at every scale of land-use (Table 3.2; Figure 3.3).

For *D. barberi*, logistic regression that included only one scale of land-use indicated that at the 1km scale, the percentage of corn significantly predicted the probability of *D. barberi* immigrants. Additionally, more corn bordering sample fields was related to higher densities of *D. barberi* (Table 3.2). Logistic regressions that included more than one scale of land-use showed that more corn was related to higher densities of *D. barberi* only at the 1km scale. In fields with no corn in the surrounding 1km radius areas, there was approximately a 50% probability of *D. barberi* immigrants. In contrast, there was greater than 90% probability of *D. barberi* immigrants into fields where the surrounding 1km radius areas were comprised of 50% corn (Figure 3.4).

Landscapes and Ladybeetles

In contrast to the herbivorous beetles, more corn in the landscape was related to lower densities of *C. maculata*. *P. quatuordecimpunctata* lady-beetles were less abundant than *C. maculata* and exhibited weaker relationships with land-use. *C. maculata* were trapped in every field sampled and ranged from 0.75 to 23 insects week^{-1} sticky card^{-1}. *P. quatuordecimpunctata* were less abundant, ranging from 0 to 1.8 insects week^{-1} sticky card^{-1}, and were not trapped in 6% of fields.

Like *D. virgifera*, predictions of *C. maculata* densities based on land-use varied when land-use scales were considered singly or together (Table 3.2). With regressions between *C. maculata* and individual scales of land-use, more row crops at the 20km scale or more corn at the 1km scale was consistently related to lower *C. maculata* densities (Table 3.2; Figure 3.5 A & B). Corn bordering sample fields had no effect on *C. maculata* densities when the effect of perimeter scale corn was considered in isolation from other scales of land-use (Table 3.2; Figure 3.5). When multiple scales of land-use were used as predictor variables in a single analysis of *C. maculata* densities, there was a significant interaction between 1km scale and perimeter scale corn. Where there was more corn bordering sample fields, increasing corn at the 1km scale was less detrimental to *C. maculata* populations than where non-corn habitat predominated on field borders (Figure 3.5 E). Accounting for 1km and perimeter corn, more row crops in the landscape at the 20km scale was not significantly related to lower *C. maculata* densities (Figure 3.5 D).

P. quatuordecimpunctata densities were only weakly, if at all, related to surrounding landscapes. In regression analyses with only single scales of land-use as predictors of *P. quatuordecimpunctata* densities, only the perimeter-scale was significant with more corn bordering sample fields related to higher densities (Table 3.2). When multiple scales of land-use were considered together in analysis, no scale was predictive of *P. quatuordecimpunctata* densities.

Discussion

Our data indicate direct suppression of specialist herbivore densities in diverse landscapes. In agreement with our original hypothesis, we found that densities of both corn pests, *Diabrotica barberi* and *Diabrotica virgifera,* were generally higher in corn fields surrounded by simple agricultural landscapes with more corn than by diverse landscapes with less corn. . Pest suppression in diverse landscapes typically has been attributed to increased natural enemy densities and biological control (Thies and Tscharntke 1999, Thies et al. 2003). However, for *D. barberi* and *D. virgifera*, which are not expected to be controlled by natural enemies (Levine and Oloumi-Sadeghi 1991), the direct effects of habitat fragmentation in regions with low corn acreage may suppress their populations. Den Beler et al. (2002) also found evidence for direct suppression of onion thrips in diverse landscapes and hypothesized that woodlots acted as dispersal barriers for the pest. While there are many examples where habitat fragmentation reduces abundances of species in natural habitats (Debinski and Holt 2000), this research suggests that fragmentation of agricultural habitat, through landscape diversification, can also directly suppress pests. Therefore, habitat diversification could be exploited as a cultural management tool for pests, even in the absence of strong control by natural enemies.

While land-use diversity was correlated with lower densities of *D. barberi* and *D. virgifera*, we did not find any relationship between land-use and corn damage by *O. nubilalis*. We consider dispersal and diet breadth to be likely reasons for this result. There is evidence that *O. nubilalis* can disperse long distances (Showers et al. 2001, Krumm et al. 2008, Kim et al. 2009) and populations may respond to land-use at scales larger than 20km radius. Alternatively, *O. nubilalis* may not be as much of a habitat specialist as *D. barberi* and *D. virgifera*. There is evidence that *O. nubilalis* has a wide diet breadth (Hodgson 1928) and may use alternative hosts in regions where corn is not abundant (Losey et al. 2001, O'Rourke et al. in press).

In contrast to corn herbivores, abundance of the lady beetle *Coleomegilla maculata* was generally lower in simple landscapes with more corn than in diverse landscapes with less corn. This is consistent with our original hypothesis that densities of natural enemies that rely on diverse food sources or habitats are lower in simple landscapes with more corn. *C. maculata* forages in many crops besides corn and prefers to overwinter at the base of dominant trees (Hodek and Honek 1996). In contrast, we did not see an effect of landscape on densities of the lady beetle *Propylea quatuordecimpunctata* when multiple scale of land-use were considered together in analysis, even though it is also a generalist predator that overwinters in natural habitats. Low densities of *P. quatuordecimpunctata* may have made it difficult to detect relationships with land-use. Alternatively, the fact that *P. quatuordecimpunctata* is invasive in the U.S. may explain these results.   Previous studies have shown that another invasive lady beetle, *C. semptempunctata*, showed no relationship (Elliott et al. 2002), or even a negative relationship (Kriz et al. 2006) to landscape diversity. Since most introductions of non-native lady beetles for biological control do not result in successful establishment (Obrycki and Kring 1998), the non-native species that have become established are presumably well-adapted to agricultural habitat.

Investigating relationships between insects and land-use at multiple scales concurrently is a novel aspect of this research and has provided new insights. We hypothesized that farm-scale land-use has a greater effect on insect densities where the regional landscape is simple and dominated by agriculture (Tscharntke et al. 2002, Brewer et al. 2008). Data for *C. maculata* confirm this hypothesis (Fig 5b). Where there was little corn at the 1km scale, *C. maculata* densities tended to be high in all fields, regardless of the habitat on field borders. However, where the 1km scale landscape was simplified with high amounts of corn, field borders mattered. In these cases, corn fields with lots of bordering corn supported higher densities of *C. maculata* than fields with alternative habitats on their perimeters. If we assume that a homogenous, single-crop habitat is detrimental to generalist predator densities, this result initially seems non-intuitive. However, corn pollen constitutes an important part of *C. maculata's* diet. Furthermore, clusters of corn fields may foster high densities of aphids, which can concentrate *C. maculata* populations near their prey (Hodek and Honek 1996). Previous studies also showed that *C. maculata* densities increased with more row crops and alfalfa at the 0.5km radius scale (Kriz et al. 2006) and with more corn in 0.5 x 0.5km square landscapes (ColungaGarcia et al. 1997). However, their densities increased with more woods at the 4.8 x 4.8km scale in South Dakota (Elliott et al. 2002).

These earlier studies suggest that clusters of corn fields at very local scales can concentrate *C. maculata* populations but that diverse land-use at broader scales supports larger *C. maculata* populations. Our research expands on this previous work by suggesting, in a single study, that medium and regional scale landscapes dominated by corn suppress *C. maculata* populations, but that these lady beetles tend to congregate in corn fields that are clustered near one another. This type of interaction between multiple scales of land-use would have been missed in a single-scale study.

Examining multiple scales of land-use has also revealed the complexity of landscape relationships with herbivore pests. We provide the first data showing that farm-level land-use matters more to pest populations where the regional landscape is dominated by agriculture. *D. virgifera* densities were low everywhere the 20km scale landscape was already diverse. However, with one third of the 20km scale landscape in row crops, diverse 1km scale landscapes had lower *D. virgifera* densities than simple 1km scale landscapes (Fig 3a). This indicates that farm-scale diversification efforts to control pests may only have a measurable effect if there is a large regional pest population supported by a simple, agricultural regional landscape. However, the scale of local diversification matters. Fields with less corn on their borders actually had higher *D. virgifera* densities than fields with more neighboring corn (Figure 3E). Meanwhile, fields surrounded by less corn at the 1km scale had lower densities of *D. virgifera* (Figure 3D). These seeming contradictions of scale may by understood by considering *D. virgifera* dispersal and foraging behaviors. For *D. virgifera*, regional landscapes with high percentages of corn may facilitate successful dispersal among habitat patches (Onstad et al. 2003), supporting large pest populations, while neighboring corn fields may dilute populations at the field scale. Adult *D. virgifera* feed on corn pollen and silks and pollinating corn fields attract *D. virgifera* adults away from adjacent, non-pollinating fields (Darnell et al. 2000). As with *C. maculata*, these complex relationships between land-use and *D. virgifera* could only be pieced together by considering multiple scales of land-use simultaneously.

Beyond the complexities of landscape-insect relationships, our data also show some simple differences between the land-use scales relevant to insect pests and natural enemies. In multiple regression analyses with multiple scales of land-use, the two pest species, *D. virgifera* and *D. barberi*, were related to larger scales of land-use than the generalist lady-beetles, *C. maculata*. *D. virgifera* densities were related to landscapes at all scales up to 20km, and *D. barberi* densities were related to 1km scale corn, while *C. maculata* densities were related to 1km and perimeter scales of corn. Specialist herbivores become pests in agriculture specifically because of their high success in tracking continuously shifting habitat mosaics. On the other hand, generalist natural enemies such as lady beetles do not need to precisely track the shifting agricultural landscape because they can survive in many habitats. Thus, agricultural pests that are habitat specialists, by definition, may be expected to be highly dispersive and respond to landscapes at large scales compared to generalist natural enemies. This pattern, where pests are affected by larger landscape scales than natural enemies has also been observed in studies in clover and wheat with aphid, midge, and weevil pests and parasitoids (Kruess and Tscharntke 1994, Thies et al. 2005). However, this research strengthens the prediction that pests respond to landscapes at larger scales than natural enemies by providing evidence from a new system with new insect guilds.

This study also provides a novel opportunity to examine the assumption put forth by many landscape-insect studies that scales of land-use most correlated to insect densities are indicative of their dispersal abilities (Tscharntke et al. 2005, Westphal et al. 2006). Due to the economic importance of corn systems, there are population genetic (Krafsur et al. 1993, Krafsur et al. 1995, Kim and Sappington 2005, Krafsur et al. 2005, Krumm et al. 2008), mark-recapture (Showers et al. 2001, Qureshi et al. 2006, Toepfer et al. 2006), and flight mill studies (Naranjo 1990) from which we can infer the dispersal abilities of our study species. These studies indicate their relative dispersal abilities to be: *O. nubilalis* > *D. virgifera*, > *D. barberi*, ≥ *C. maculata*, ≥ *P. quatuordecimpunctata*. If we assume that no relationship was found between land-use and corn damage by *O. nubilalis* because *O. nubilalis* responds to landscapes at scales larger than 20km radius, then the scales at which these insects most strongly correlate to the landscape (Table 3.2) generally follow the same rank-order as their dispersal abilities. Thus, while this study highlights that landscape-insect relationships are complex, it also suggests that general insights into insect dispersal can be obtained through these types of studies.

In conclusion, our data suggest that diverse agricultural landscapes support pest management as an ecosystem service by directly suppressing pests and by enhancing natural enemy populations. However, these relationships are not simple. Densities of some pests and natural enemies are more related to land-use than others. Insect densities and land-use may be positively related at one scale and negatively related at another scale. Furthermore, landscape-insect relationships at one scale may depend on land-use at another scale. Detailed information about insect life history, including diet breadth, overwintering behavior, dispersal ability, and invasiveness may help to interpret and predict landscape-insect relationships. In general, farm-scale changes in land-use appear to have more effect on insect populations where the larger landscape is very agricultural than where it is diverse. Also, it appears that pests respond to land-use at larger scales than natural enemies.

Farm policies aimed at enhancing agricultural diversity at local scales, by encouraging such things as beetle bank construction and hedge row conservation, may be most important for enhancing natural enemies and biological control. Moreover, farmers adopting these practices may see the largest effect on insect communities in regions that are dominated by agriculture. On the other hand, farm policies that transform regional cropping patterns, by encouraging integrated crop-livestock systems, cover cropping, and land fallowing may also contribute to pest control through direct suppression of pests.

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CHAPTER 4

A SPATIALLY EXPLICIT MODEL OF INSECT PEST DYNAMICS IN AGRICULTURAL LANDSCAPES

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Abstract

Diverse agricultural landscapes help to support ecosystem services, including pest management. However, due to the intrinsic difficulties of landscape-scale research, landscape-insect relationships are still poorly understood. Here we combine empirical data with a spatially explicit model of herbivore population dynamics to clarify the role of landscapes in agricultural pest management. We found that densities of the western corn rootworm, *Diabrotica virgifera,* were lower in diverse landscapes whereas damage to maize by the European corn borer, *Ostrinia nubilalis,* was unrelated to land-use. Stable carbon isotope analysis showed that *D. virgifera* developed exclusively on maize whereas 11% of *O. nubilalis* developed on non-maize hosts. We also found that in states with less corn, a smaller percentage of corn was treated with insecticide and a smaller percentage of corn was Bt to control these pests. Given these data, we hypothesized that landscape diversification affects populations of specialist insects more than generalists. We also hypothesized that to maintain similar pest densities, a smaller percentage of corn fields need to be treated with insecticides in diverse than in simple landscapes. We designed a spatially-explicit model of herbivore dynamics in agricultural landscapes to see if we could predict our empirical observations and support our hypotheses. Our model predicted that diet breadth was, indeed, a strong factor in whether landscape diversification suppressed pests. The

model also predicted less insecticide use in diverse landscapes, which reduced apparent pest suppression. We also predicted that populations of specialist herbivores with relatively low reproductive rates are very sensitive to landscape diversification. These predictions can be used to guide future landscape-insect research and can provide insights into pest management as an ecosystem service of diverse agricultural landscapes.

Introduction

Habitat diversity in agricultural landscapes affects the ecosystem services they can support (Burel et al. 1998, Tscharntke et al. 2005). Agricultural landscapes are dynamic, and habitat diversity within those landscapes can increase or decrease (Primdahl et al. 2003, Landis et al. 2008). For example, we have seen a general increase in farm and field sizes and concurrent decrease in unmanaged habitat on farms in the United States and Europe since World War II (Vasey 1992). However, in some regions, we have also seen increases in natural habitat. In the northeastern United States, farm abandonment has increased old field and forest areas (Flinn and Vellend 2005), and agri-environmental legislation has increased hedge rows, fallows, and conservation land on some European farms (Kleijn and Sutherland 2003). In the future, we expect agricultural landscapes will continue to change with advances in technology, population growth, rising demand for biofuels, and climate change (Nelson 2005). Therefore, it is imperative that we understand the ecosystem services provided by diverse agricultural landscapes, which could be lost as landscapes continue to change.

Diverse agricultural landscapes have been linked to a variety of ecosystem services including insect pest suppression and reduced insecticide use (Tscharntke et al. 2005, Landis et al. 2008). However, the link between agricultural landscape diversity and pest suppression remains an emerging area of research and a general synthesis of how changes in agricultural landscapes affect the spectrum of agricultural insect pests is still lacking. In fact, few studies have directly linked agricultural landscape diversity with insect pest control. Most landscape scale studies have focused on natural enemies, and when pests are directly measured, results have been mixed. A 2006 review of the literature cited 24 studies relating agricultural landscapes to natural enemies, which are assumed to exert top-down control on insect pests, while only 10 studies directly quantified relationships between agricultural landscapes and insect pests (Bianchi et al. 2006). Of these ten studies, half were on aphids and the results were highly variable. In diverse landscapes, 45% of herbivore observations indicated suppression, 40% indicated no effect, and 15% indicated increased pest pressures (Bianchi et al. 2006). Studies published thereafter have continued to emphasize landscape-natural enemy relationships over landscape-herbivore relationships, with the few relevant studies showing varied relationships (Geiger et al. 2005, Zaller et al. 2008).

When diverse agricultural landscapes fail to suppress insect pests, the results have often been attributed to herbivore life history characteristics, especially wide diet breadth and utilizing multiple habitats. For example, Thies et al. (2005) found that densities of cereal aphids were similar in wheat surrounded by diverse and simple landscapes. Underlying this neutral effect was higher aphid immigration accompanied by higher parasitism rates of aphids in wheat fields surrounded by diverse than by simple landscapes. It was hypothesized that higher immigration rates of aphids into wheat surrounded by diverse landscapes may be due to overwintering on perennial grasses or trees common in diverse agricultural landscapes. Similarly, Roschewitz et al. (2005) found that densities of the same aphid species were higher during wheat ripening in diverse than in simple landscapes in the same region of Germany studied by Thies et al. (2003). When Zaller et al. (2008) found that densities of stem weevils and pollen beetles were higher and that densities of pod midge were the same in winter oilseed rape fields surrounded by diverse than simple landscapes, they also hypothesized that feeding or overwintering on alternative hosts may explain why pest suppression was not detected in diverse landscapes.

In addition to considering herbivore life histories when trying to predict when landscape diversification will suppress pests, it is also important to consider how regional pest management affects herbivore population dynamics. Insecticides are very effective at reducing pest densities and area-wide management programs suppress regional insect pest populations (Koul et al. 2009). If more insecticides are applied in simple than diverse landscapes, herbivore densities may be artificially suppressed in simple landscapes. Thus, insecticide use that varies with landscape diversity may confound results of landscape-scale research, obscuring natural pest suppression in diverse agricultural landscapes. However, landscape-level studies rarely include data for regional insecticide use or discuss its influence on research results.

A modeling approach coupled with empirical investigations can help to clarify when diverse agricultural landscapes serve to suppress insect pests (Levins 2006). By systematically manipulating insect life-history attributes in models, we can tease apart which factors make agricultural pests most susceptible to control by land-use diversification. Furthermore, models can be used to control for pest management practices and to understand how regional insecticide use affects empirical measures of natural pest management as an ecosystem service of diverse agricultural landscapes. Landscape-scale studies are also particularly amenable to model investigations because of the intrinsic hurdles in the scale of research. Unlike plot-scale plant diversity studies, which can be carefully controlled in research facilities, landscape-scale studies usually consist of observational correlations between existing landscapes and insects. Furthermore, landscape-scale studies are challenging because of the coordination typically required between researchers and many private land-owners. Models can be used to synthesize our knowledge of landscape-herbivore systems and to make predictions about how changes in land-use will affect a variety of agricultural pests. These predictions can then be compared with current research findings and can guide future empirical studies.

In this research, we combined empirical and modeling approaches to develop predictions about which types of agricultural insect pests may be controlled by landscape diversification. We monitored two of the most economically important maize pests (Tollefson and Calvin 1994), the western corn rootworm (WCR), *Diabrotica virgifera*, and the European corn borer (ECB), *Ostrinia nubilalis*, in landscapes of varying habitat diversity. We then interpreted the insect surveys in light of data on the insects' host utilization and regional management for the pests. We developed a spatially explicit, agent-based model of herbivore population dynamics to test whether our assumptions about herbivore-landscape interactions and pest management could predict trends in our empirical data. The model was further used to make general predictions about which types of insects, which varied in host specificity, dispersal, and reproduction, may be suppressed by diverse agricultural landscapes.

We began with a number of hypotheses for our empirical work and assumptions for our model of landscape-herbivore interactions. In the field, we expected densities of some herbivore pests to be more related to land-use than others. We further expected these differences to be due to insect life history attributes, especially host specialization. Specialist insects should be more suppressed in diverse landscapes than generalists. We also expected management for corn pests to vary with land-use and that less insecticide is used per field in diverse landscape. For our model, we assumed that we could predict trends in our empirical studies by incorporating simple pest management rules (crop rotations and economic thresholds) and basic insect life history attributes (dispersal, reproduction, and diet breadth). We expected our model to yield insights into complex interactions between land-use, insect life history, and pest management.

Materials and Methods

Empirical Herbivore-Landscape Survey

Western corn rootworm (WCR), *Diabrotica virgifera virgifera*, densities, and European corn borer (ECB), *Ostrinia nubilalis*, damage to maize was measured in 66 1st year maize fields in upstate New York in 2006 and 2007. Fields were located in seven, non-overlapping, 20km radius circular regions. The proportion of each region planted to row crops was determined from the 2001 National Land Cover Database (http://www.mrlc.gov/). The relationships between proportions of row crops within 20km regions and maize pests were analyzed using standard least squares regressions in JMP 7.0 (JMP 2007). Details about the management of maize fields, insect sampling, landscape analysis, and statistical procedures can be found in chapter three of O'Rourke (2010). In this paper, we present averaged densities of insects from all fields within the 20km scale regions.

Observed Host Specificity

Stable carbon isotope analysis of ECB and WCR adults was conducted to determine their host utilization of C4 and C3 plants (Deniro and Epstein 1978, Tallamy and Pesek 1996, Ponsard et al. 2004b). Since few plants besides maize are

C4 in New York, stable carbon isotope content ($\delta^{13}C$) of ECB and WCR can largely determine whether they have developed on maize or alternative hosts (Uva et al. 1997, Ponsard et al. 2004a). ECB sampling methods can be found in chapter five of O'Rourke (2010). WCR were collected from a single, first-year maize field in Cayuga County, NY in 2007 using a Pherocon CRW kairomone trap baited with the western/northern lure and stun bait. Insects were dried at 55°C for at least one week prior to analysis. The wings of 68 E and 71 Z race ECB (two distinct pheromone strains of ECB), randomly sampled from collections, and the wings of 20 WCR were analyzed. The $\delta^{13}C$ contents of samples were determined using a Thermo Delta Plus isotope ratio mass spectrometer (IRMS) interfaced to a NC2500 elemental analyzer at the Cornell Isotope Laboratory (COIL). $\delta^{13}C$ signatures were converted to categories of host history by scoring individuals with $\delta^{13}C$ values < -20‰ as having a C3 host history, while $\delta^{13}C$ values > -15‰ were scored as having a C4 host history (Tallamy and Pesek 1996, Ponsard et al. 2004a). Differences in diet between ECB and WCR were determined using loglinear ratio tests implemented in JMP 7.0 (JMP 2007).

National Maize Pest Management

Insecticide usage and maize acreage were determined from research reports published by the United States Department of Agriculture. The percentage of maize per state treated with insecticide, and the percentage of maize per state rotated with other crops came from the 2004 and 2006 Agricultural Chemical Usage Reports (USDA 2004, 2006a). The only insecticide used in maize that was not registered for use against rootworms was propargite, which was applied to less than 0.5% of maize acreage in 2004 and 2006 (NPIRS 2009). Therefore, insecticide data were considered as effective against rootworms. Data on the percentage of maize per state that was Bt targeting ECB and/or WCR came from Acreage reports (USDA 2008). Maize acreage grown per state in 2003, 2005, 2007, and 2008 came from crop production summary reports and included the areas planted to corn for grain and for silage (USDA 2006b, 2009). The proportion of non-maize habitat per state was derived by dividing maize acreage per state by the total area of each state and subtracting from one. Data were analyzed with JMP 7.0 software using standard least squares regression procedures (JMP 2007). Specifically, our statistical models related the proportion of maize treated with insecticide per state to the predictor variables: year, maize rotation rate, and the percentage of the state in non-maize habitat. For the proportion of maize planted to Bt varieties per state, we tested the predictor variables: year, and the percentage of the state in non-maize habitat.

Herbivore-Landscape Model

We created a single-species, spatially explicit population dynamics model using the basic package of R version 2.8.1 (R 2008). The organisms modeled represent generic, univoltine, herbivorous insects that specialize on a preferred crop but can accept hosts in alternative habitats. Insect populations were controlled using integrated pest management (IPM) methods that included crop rotation and economic thresholds (Norris et al. 2003). The input parameters of interest in the model were: proportion of alternative habitat in the landscape (H_A), maximum insect dispersal distance (*Disp.*), ability to reproduce in alternative habitat (*Diet*), and reproductive rate in preferred habitat (r_0) (Table 4.1).

Landscape

The simulated landscape was designed as a 128 x 128 matrix of habitat patches with each patch measuring 1km^2. There were three types of patches: preferred habitat, agricultural alternative habitat, and non-agricultural alternative habitat. Initial locations of patch types were randomly assigned. Crop rotation was simulated with preferred habitat and agricultural alternative habitat patches randomly redistributing amongst themselves while keeping their proportions equal each year. This created a crop rotation scenario where preferred habitat had a 50% probability of rotating to another crop each year. Non-agricultural alternative habitat remained fixed in space throughout simulations. At the beginning of simulations, herbivores were randomly seeded onto 5% of the landscape with seeded patches receiving a mean and standard deviation of 100 and 30 insects, respectively.

Dispersal

During each year of the model, all insects dispersed. Insects did not discriminate between patch types during dispersal. Dispersal distances were chosen from a probability distribution calculated from an equation describing female ECB dispersal (Qureshi et al. 2005):

$$N = \frac{17}{(1 + 8.4x)^{0.5}}$$

where N represents the numbers of insects and x represents dispersal distances in meters. This equation was converted to a discrete probability function by dividing the vector N as a function of all x (meters) by the sum of all values of N. The maximum value of x in each simulation was the parameter *Disp.* (Table 4.1). There was equal probability of dispersing in all directions. The boundaries of the landscape were absorbing and all insects dispersing beyond the landscape matrix were assumed to have dispersed out of it.

Reproduction

After dispersal, insects reproduced according to input parameters r_0, if individuals landed in preferred habitat, or *Diet*, if individuals landed in alternative habitat (Table 4.1). Reproduction in preferred habitat followed the logistic growth equation:

$$N_{t+1} = r_0 * N_t * \left(1 - \frac{N_t}{K}\right)$$

where N_t represents the population of insects in a preferred habitat patches at time t, and K is the carrying capacity of preferred habitat patches, set at 5000. Insects in alternative habitat patches reproduced according to:

$$N_{t+1} = Diet * N_t$$

where *Diet* = 0 means that no offspring were produced, and *Diet* = 0.75 means that N_{t+1} will be only 75% the size of N_t.

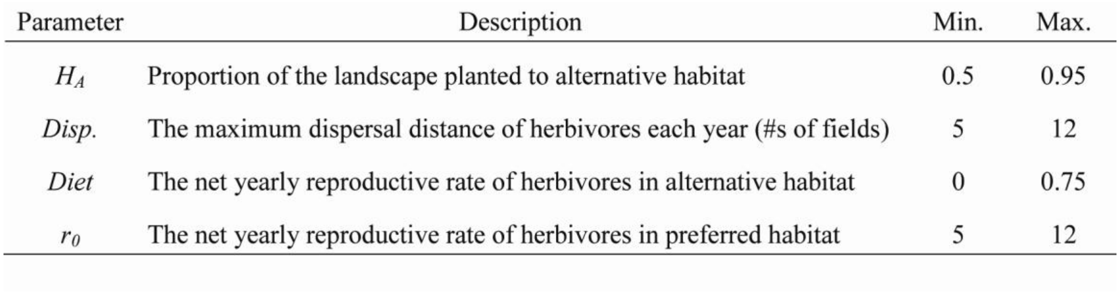
Pest Management and Economic Threshold

Preferred habitat patches with populations above an economic threshold (Higley and Pedigo 1996), were treated with insecticide. The insecticide efficacy in any given patch was chosen from a uniform distribution ranging from 0.8 to 0.99. Pesticides reduced populations from their post-reproduction size. The economic threshold was set at 500 insects per preferred habitat patch. As the density at which farmers are supposed to gain more from saved yields than they spend on insecticides to control pests, an economic threshold of 500 insects per farm field is obviously not a realistic economic threshold. However, it represented a compromise between capturing spatial dimensions of insect behavior and running the model within a reasonable amount of time.

Sensitivity Analysis

Sensitivity analysis was conducted to determine the influence of model parameters on simulated metrics of insect density (*Density*) and insecticide use (*Insecticide*), defined below. Analyses were conducted on 750 model simulations using a latin hypercube sampling scheme over the ranges of input parameters (Ellner and Guckenheimer 2006). *Density* was calculated in a two-step process. First, the numbers of insects in preferred habitat patches at the beginning of each year, scaled as a proportion of the economic threshold, were averaged over space. Spatially averaged insect densities were then averaged over time for the last 10 years of model simulations to create a single *Density* metric for each set of model parameters. *Insecticide* was calculated similarly. However, in this case, we were interested in the proportions of preferred habitat patches receiving insecticide. The sensitivity of *Density* and *Insecticide* to model parameters was determined using standard least squares multiple regression. Specifically, we tested the sensitivity of *Density* or *Insecticide* to changes in the proportion of alternative habitat in the landscape (H_A), yearly net reproductive rate in non-maize patches (*Diet*), maximum insect dispersal distance (*Disp.*), the yearly net reproductive rate in alternative habitat (r_0), and all two-way interactions with H_A. We reported scaled regression estimates, which are regression coefficients derived from analyses with the input parameters scaled to means of zero and ranges of two (JMP 2007). The main parameters in our sensitivity analyses can be interpreted as the predicted increase (positive estimate) or decrease (negative estimate) in *Density* or *Insecticide* when H_A or an insect life history characteristic was increased by one half its range (Table 4.1). H_A^2 indicates non-

Table 4.1. Descriptions and ranges of input parameters to the model.



Parameter	Description	Min.	Max.
H_A	Proportion of the landscape planted to alternative habitat	0.5	0.95
Disp.	The maximum dispersal distance of herbivores each year (#s of fields)	5	12
Diet	The net yearly reproductive rate of herbivores in alternative habitat	0	0.75
r_0	The net yearly reproductive rate of herbivores in preferred habitat	5	12

linearity between *Insecticide* or *Density* and H_A. Interaction terms between H_A and insect life history characteristics mean that an increase in the life history parameter results in H_A having a stronger (estimate has same the sign as the H_A estimate) or weaker (estimate has the opposite sign as the H_A estimate) effect on *Insecticide* or *Density* than average.

Results

Empirical Herbivore-Landscape Survey

WCR densities were correlated with land-use in upstate New York while ECB damage to maize was not. WCR densities ranged from 1.7 to 12.9 WCR sticky card^{-1} week^{-1} region^{-1} and the proportion of maize damaged by ECB varied from 0.08 to 0.23 of maize per region. Increasing amounts of non-row crop habitat was strongly related to suppressed WCR densities ($R^2 = 0.75$; $F_{1,5} = 14.9$; $P = 0.01$) (Figure 4.1B). In contrast, regional land-use was not related to ECB damage to maize ($R^2 = 0.0$; $F_{1,5} = 0.01$; $P = 0.9$) (Figure 4.1A).

Observed Host Specificity

More ECB developed on non-maize hosts than WCR ($\chi^2_{1,159} = 4.3$; $P = 0.04$) (Figure 4.2). According to δ^{13}C analysis, 11% percent of the ECB tested developed on C3 plants whereas none of the WCR developed on C3 plants. The range of δ^{13}C values for ECB designated as developing on C4 hosts was -10.25 to -13.98‰ and on C3 hosts was -23.75 to -31.04‰. The δ^{13}C content for all WCR ranged from -10.38 to -14.69‰, indicative of development on C4 hosts.

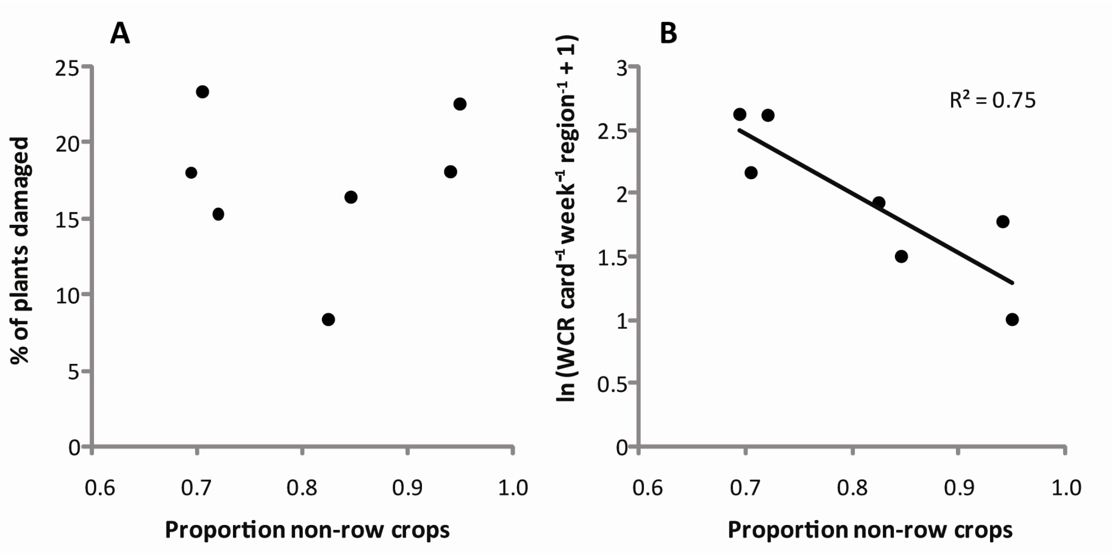


Figure 4.1. Relationships between insects sampled in maize and the proportions of 20km radius regions in non-row crop habitat in upstate New York. A) No relationship was found between the percentages of plants damaged by European corn borer larvae and non-row crop areas ($F_{1,5} = 0.009$; $P = 0.9$). B) Diversification away from row crops was highly predictive of reduced WCR adults immigrating into first-year maize fields ($F_{1,5} = 14.9$; $P = 0.01$).

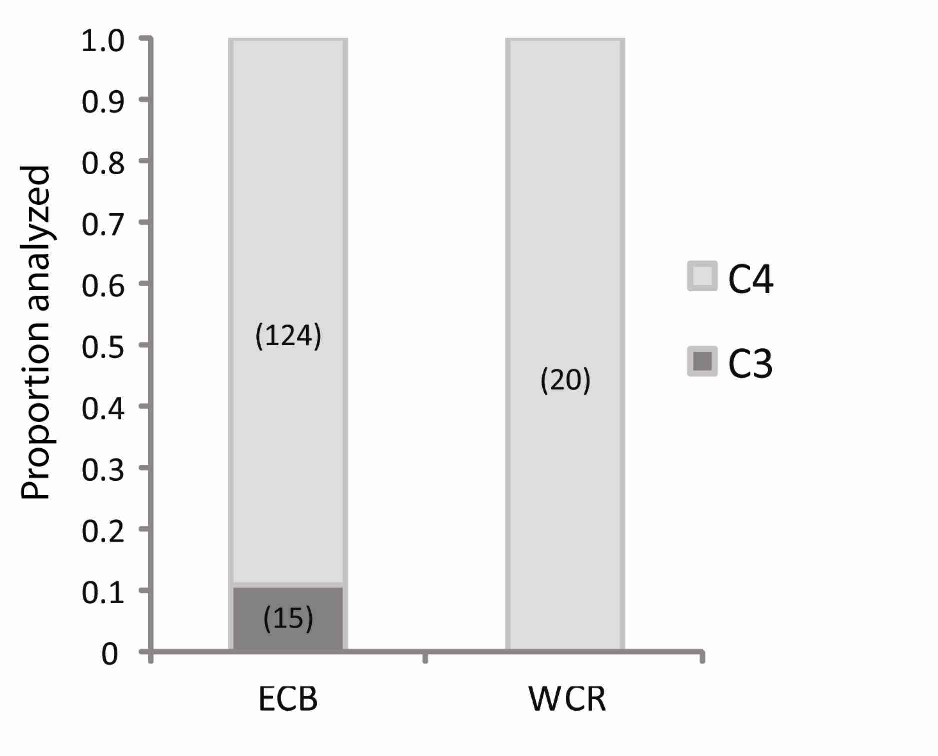


Figure 4.2. The proportions of ECB and WCR collected in New York that developed on C3 or C4 species of plants, as determined from δ^{13}C analysis. Numbers in parenthesis are sample sizes.

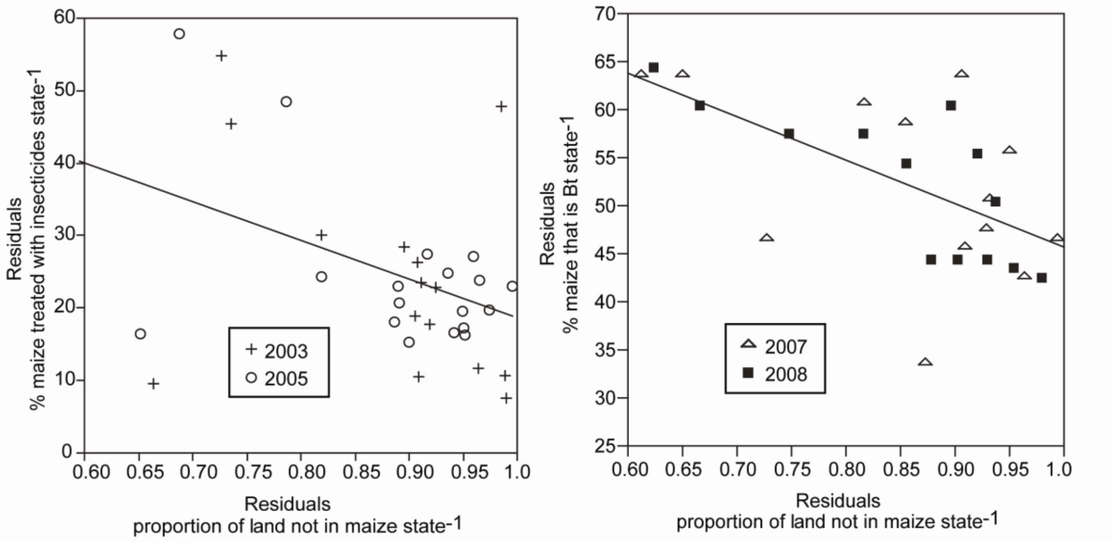


Figure 4.3. Partial residual plots between insecticide use and Bt maize acreage and the proportions of states in non-maize habitat. A) In states with more non-maize land use, a smaller proportion of maize is treated with conventional insecticides (not including transgenic Bt maize)($F_{1,30}=6.2$; $P = 0.02$). B) In states with more non-maize land use, a smaller proportion of maize is a Bt transgenic variety ($F_{1,23} = 13.7$; $P = 0.001$).

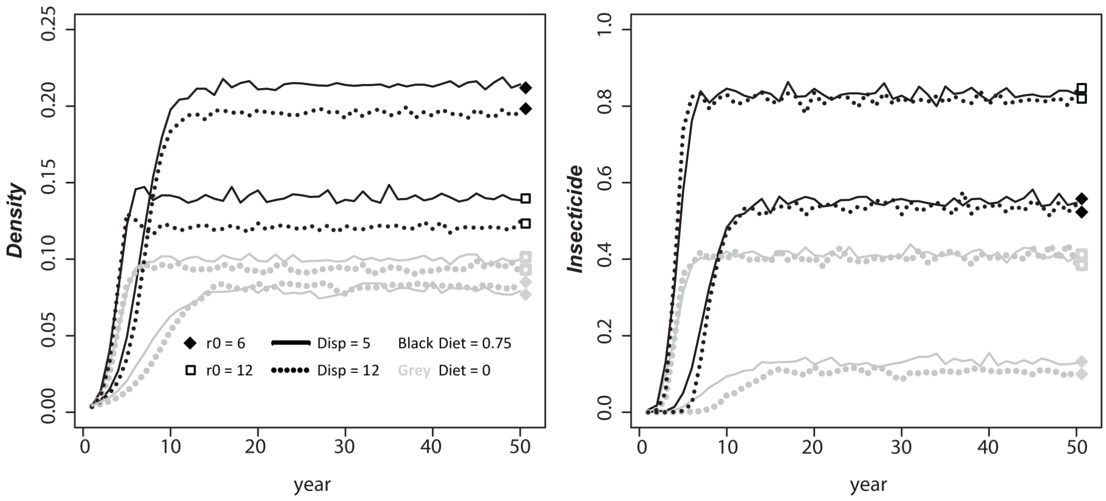


Figure 4.4. Characteristic simulations through time of A) insect densities measured as proportions of economic threshold (*Density*) and B) proportions of preferred habitat receiving insecticide (*Insecticide*). Eight different insects are modeled, with life histories described in the figure legend. *Diet* is yearly net reproductive rate in alternative habitat, *Disp.* is maximum dispersal distance, and r_0 is yearly net reproductive rate in preferred habitat.

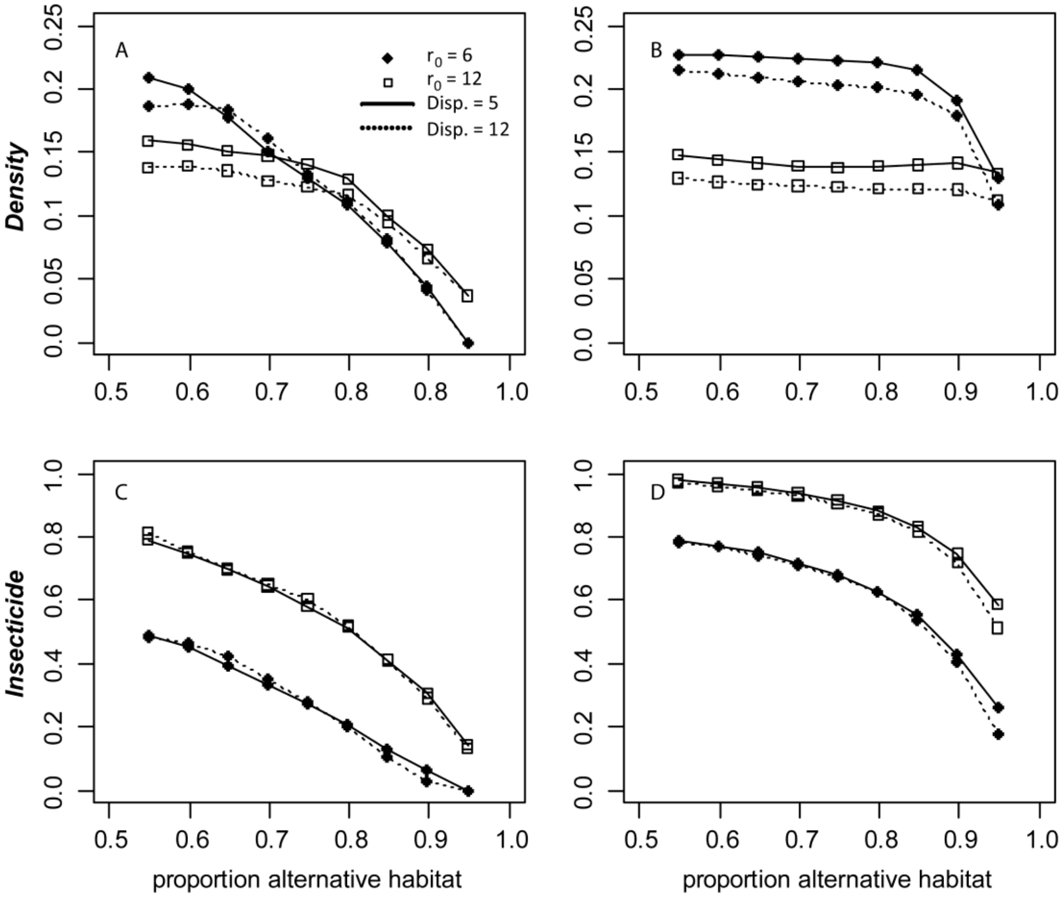


Figure 4.5. Simulations of eight insect pests that vary in *Diet*, *Disp.*, and r_0, in landscapes with increasing amounts of non-crop habitat. *Diet* is net yearly reproduction in alternative habitat, *Disp.* is maximum dispersal distance, and r_0 is yearly net reproductive rate in preferred habitat. A) *Density* predictions for four habitat specialist insects that can only reproduce in maize (*Diet* = 0) and that vary in r_0 and *Disp.* as indicated in the legend B) *Density* predictions for four insects that are relative habitat generalist utilizing alternative habitats for marginal reproduction (*Diet* = 0.75) and that vary in r_0 and *Disp.* as indicated in the legend C) Predicted *Insecticide*, proportions of preferred habitat treated with insecticide, against the four habitat specialist insects modeled in panel A. D) Predicted *Insecticide*, proportions of preferred habitat treated with insecticide, against the four habitat generalist insects modeled in panel B.

National Maize Pest Management

In the U.S., lower proportions of maize fields were treated with insecticides and lower proportions of maize were Bt varieties in states with more non-maize habitat. In 2003 and 2005, the proportions of maize treated with insecticides decreased as non-maize habitat per state increased ($F_{1,30} = 6.2$; $P = 0.02$) (Figure 4.3A). Crop rotation and year of study were also marginally predictive of insecticide use; more corn acreage rotated to other crops was related to lower proportions of maize treated with insecticides ($F_{1,30} = 3.6$; $P = 0.07$), and more corn was treated with insecticides in 2003 than 2005 ($F_{1,30} = 4.0$; $P = 0.05$). In 2007 and 2008, the proportions of maize planted to Bt varieties decreased with increased non-maize habitat per state ($F_{1,30} = 6.2$; $P = 0.02$) (Figure 4.3B). Also, more Bt maize was planted in 2008 (57%) than 2007 (49%) in states surveyed by USDA ($F_{1,30} = 6.2$; $P = 0.02$).

Herbivore-Landscape Model

During the first 10 to 20 years of model simulations, insects dispersed to all their preferred habitat patches and characteristic *Density* and *Insecticide* levels were reached (Figure 4.4). Despite stochasticity in the precise locations of preferred and alternative habitats, *Density* and *Insecticide* were highly predictable based on input parameters. For example, we replicated 32 model scenarios four times each, and found that the average standard deviation of *Density* across replicates was only 0.1%. Model predictions of *Density* and *Insecticide* could be summarized quite well through linear regressions with model parameters and all two-way interactions with alternative habitat (H_A), capturing 91% and 99% of the variance in predictions, respectively.

Specific scenarios illustrate the behaviors of our model and relationships between *Density* and *Insecticide* (Figure 4.5). We simulated *Density* and *Insecticide* in increasingly diverse landscapes that were infested with eight insects that had contrasting life histories. Four insects were habitat specialists that only reproduced in preferred habitat (*Diet* = 0; Figure 4.5A & C) and four could reproduce in alternative habitat but at very low rates (*Diet* = 0.75; Figure 4.5B & D). The insects also differed in their maximum dispersal distances (*Disp.* = 5 or 12) and reproductive rates in preferred habitat (r_0 = 6 or 12). Simulations showed that *Disp.* had little effect on model predictions. Increasing *Diet* decreased pest suppression in diverse landscapes and increased *Insecticide*. Higher r_0 generally resulted in lower *Density* because of correspondingly high values of *Insecticide*. *Density* of a model insect that subsisted in alternative habitat (Diet = 0.75) and had a high reproductive rate in its preferred habitat (r_0 = 12) (Figure 4.5B, squares), changed little with landscape diversification. However, *Insecticide* for this same pest was predicted to decrease with landscape diversification (Figure 4.5D, squares).

Model Sensitivity Analysis

Sensitivity analysis shows how model parameters affected simulated insect densities (Figure 4.6). It also shows that not all types of insects were equally suppressed by landscape diversification in our model. In simulations, herbivores that subsisted in multiple habitats (high *Diet*) and had high reproductive rates in preferred habitat (r_0) were less affected by habitat diversification than habitat specialists (low *Diet*) with low reproductive rates (r_0). In Figure 4.6, this is shown by estimates for the interaction terms H_A * *Diet* and $H_A * r_0$ having opposite signs than H_A. Alternative habitat (H_A) had a strong influence on model predictions and, on average, increasing H_A resulted in a non-linear decrease in *Density*. This is shown by the large negative estimates for H_A^2. Insect life history parameters from most to least important in the model, as illustrated by the relative sizes of their estimates, are r_0, *Diet*, and *Disp*. On

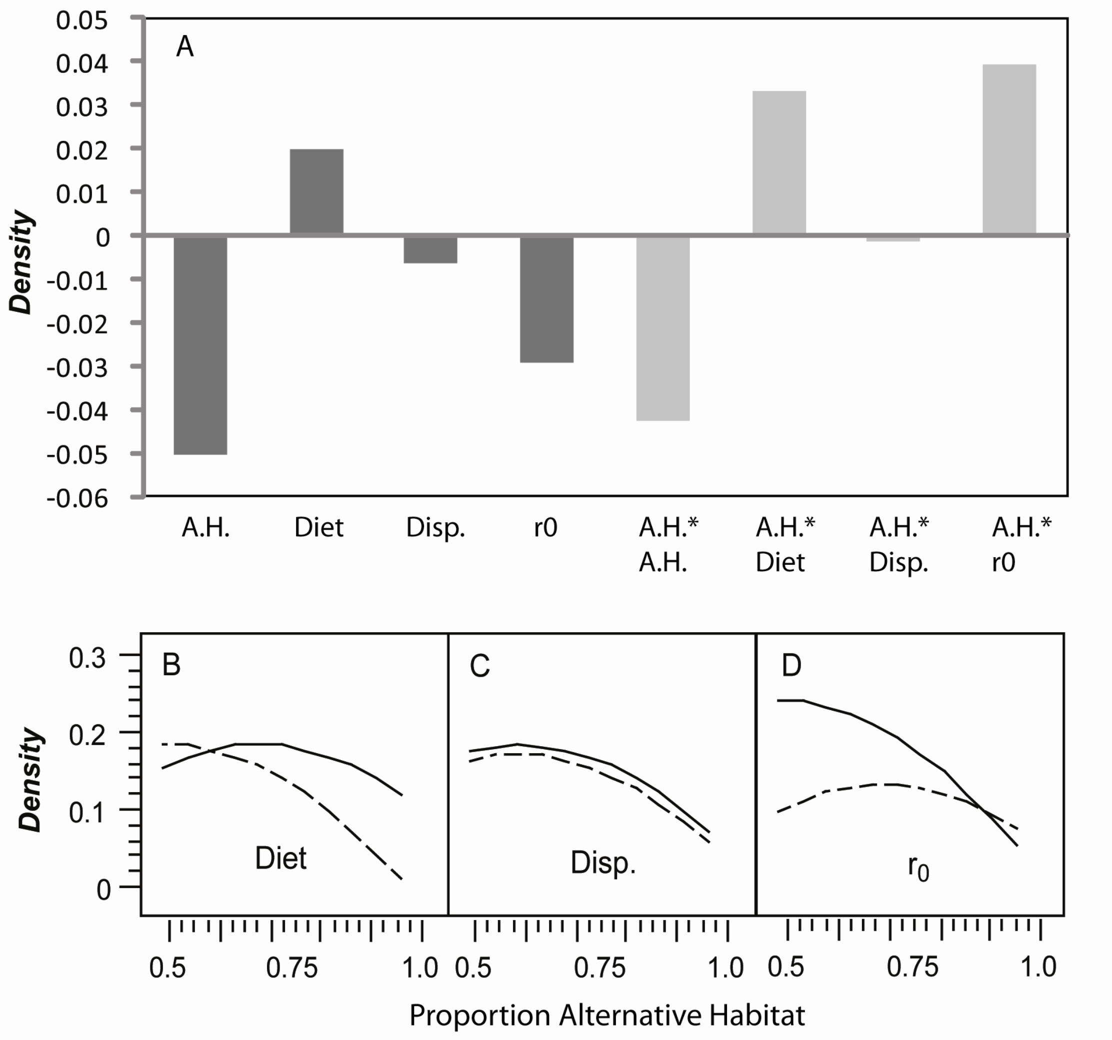


Figure 4.6. Sensitivity analysis of insect densities at the beginning of the growing season (*Density*) to model input parameters. A) Scaled estimates from multiple regression analysis of model parameters H_A, alternative habitat, *Diet*, yearly net reproductive rate in alternative habitat, *Disp.*, maximum dispersal distance, r_0, yearly net reproductive rate in preferred habitat, and two-way interactions between H_A and model parameters. B,C,D) Interaction plots of the relationship between alternative habitat, H_A, and insect density, *Density*, with minimum (solid lines) and maximum (dashed lines) values of *Diet, Disp.* and r_0, as noted in Table 4.1.

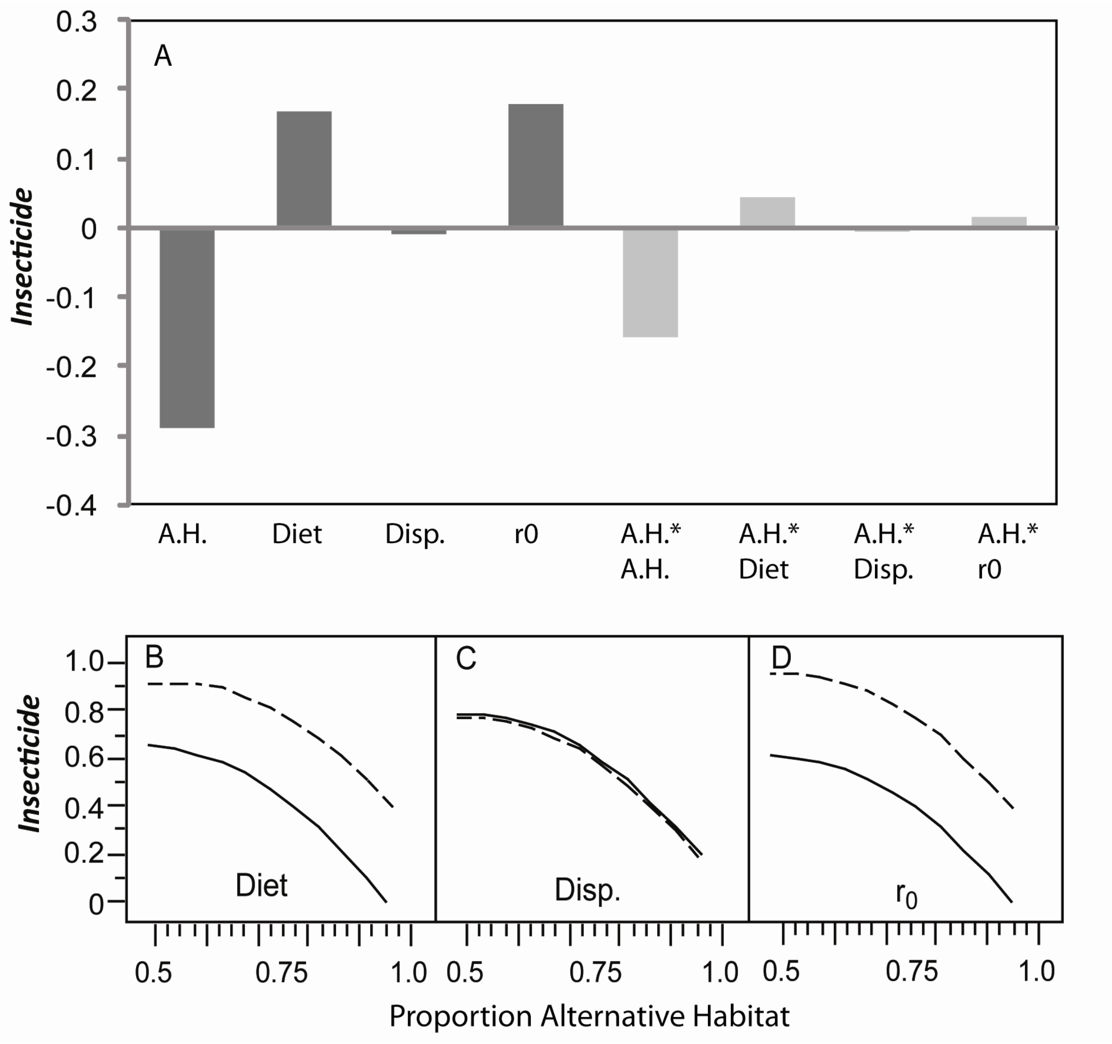


Figure 4.7. Multiple regression analysis between the proportions of preferred habitat sprayed with insecticide and model parameters for 750 model simulations. A) Scaled estimates of model parameters *H_A*, alternative habitat, *Diet*, yearly net reproductive rate in alternative habitat, *Disp.*, maximum dispersal distance, *r_0*, yearly net reproductive rate in preferred habitat, and two-way interactions between *H_A* and model parameters. B,C,D) Interaction plots of the relationship between alternative habitat, *H_A*, and proportions of preferred habitat treated with insecticide, *Insecticide*, against insects with minimum (solid lines) and maximum (dashed lines) values of *Diet*, *Disp.* and *r_0*, as noted in Table 4.1.

average, increasing r_0 decreased *Density* because of high insecticide use. Increasing *Diet* increased *Density* because alternative habitat supplied immigrants into preferred habitat.

Figure 4.7 shows how model parameters affected simulated proportions of preferred habitat receiving insecticide (*Insecticide*). The relatively large negative estimate for H_A and the relatively weak interactions between H_A and life history parameters show that our model predicted *Insecticide* to decrease with landscape diversification for all types of insects (Figure 4.7B, C, & D). The negative H_A^2 estimate shows that *Insecticide* leveled-off at relatively high rates in simple landscapes and fell non-linearly with landscape diversification. The effects of insect life history parameters on *Insecticide* are shown by estimates for *Diet*, *Disp.* and r_0. Changes in insect *Diet* and r_0 strongly affected *Insecticide* while *Disp.* had little effect. Increasing *Diet* or r_0 over the modeled parameter ranges resulted in similar amounts of *Insecticide* increases.

Discussion

Our field work has shown that some agricultural pests, such as the western corn rootworm (WCR), are suppressed in diverse landscapes while others, such as the European corn borer (ECB) are not. We further determined that WCR is more specialized on maize than ECB. This supports the findings of others who have shown that in many cases where diverse landscapes did not suppress agricultural pests, the pests use multiple habitats either for overwintering or as alternative food (Thies et al. 2005, Bianchi et al. 2006, Fiedler et al. 2008, Zaller et al. 2008). While only 11% of ECB developed on non-maize hosts, our model suggests that even habitats that are demographic sinks and cannot support populations on their own can greatly increase pest pressures in diverse agricultural landscapes. In diverse landscapes, the prevalence of habitats that are poor quality to specialist herbivores may still play an important role in their population dynamics because even small numbers of dispersers from alternative to preferred habitats early in the growing season can greatly increase pest pressures throughout the growing season (Speight et al. 2008).

Our data showing how regional pest management may affect herbivore-landscape relationships is a novel aspect of this research. Data on management practices across the United States to control WCR and ECB show that fewer maize fields are managed with insecticide or Bt in diverse landscapes where maize is not prevalent. Our model also predicted that fewer maize fields would be treated with insecticides in diverse agricultural landscapes. Less insecticide use in diverse landscapes will tend to homogenize pest densities in simple and diverse landscapes. Therefore, regional patterns of insecticide use may provide a hypothesis, along with host specialization, for why some pests are not suppressed in diverse landscapes. While regional pest management has not been invoked in previous studies as a mechanism behind the poor suppression of some pests in diverse landscapes, our research shows that herbivore-landscape relationships cannot be properly understood without considering pest management. Rather than focusing solely on suppression as the key ecosystem service of diverse landscapes in relation to agricultural pests, scientists should also address the potential ecosystem service of reduced insecticide use even in the absence of measured pest suppression.

Another novel aspect of this research is the combination of empirical and theoretical approaches to understanding herbivore-landscape relationships. Due to the large scale of this type of research, the preponderance of studies document observed correlations between insect densities and surrounding land-use (Bianchi et al. 2006). However, with observational studies, it is difficult to isolate the processes underlying the patterns. By coupling modeling to observed herbivore-landscape relationships, we can test how well our assumptions about a system predict dynamics in the field. In this study, we were able to model very simple assumptions about how agricultural pests behave and are managed to predict how densities of different insects and levels of insecticide use might change if agricultural landscapes become more or less diverse. We were able to correctly predict trends in the effects of land-use patterns on two of the most important maize pests, WCR and ECB, based on differences in diet breadth. We were also able to predict trends in regional pest management in maize. Thus, coupling landscape-herbivore observations with modeling can provide compelling support for empirical data and can clarify the mechanisms underlying observations.

In addition to supporting our empirical data, our model makes general predictions about which types of insect pests will be suppressed by landscape diversification. Insects that are habitat specialists with relatively low reproductive rates in their preferred crop will be better suppressed by landscape diversification than insects that use multiple habitats and have high reproductive rates in their preferred habitat. However, the maximum dispersal distance of pests does not appear to be an important factor in regional pest dynamics. While these predictions may not be intuitive, they can be understood by carefully considering the insect behaviors and agricultural system modeled.

Our model prediction that maximum dispersal distance (*Disp.*) is not an important parameter affecting insect densities differs from other models (King and With 2002). In our model, the range of *Disp.* and structure of the landscapes, together, made *Disp.* a relatively unimportant model parameter. We assumed that even relatively poor dispersers could travel many fields from their natal site. This allowed insects to quickly occupy all available patches in simulated landscapes, which were fine-grained relative to insect dispersal abilities. The model then behaved like a well-mixed model, which made *Disp*. relatively unimportant. Since most agricultural pests are adapted to ephemeral habitats and tend to be quite dispersive (Johnson 1969), assuming that pests can disperse at least a few fields from their natal habitat is justifiable. If pests are able to reach unoccupied habitat, increasing their maximum dispersal distance may, indeed, have little effect on equilibrium population dynamics.

Subsisting in alternative habitats (*Diet*) played a strong role in boosting modeled pest pressures for two major reasons: large differences in reproduction between preferred and alternative habitat, and pest management. Unlike most source-sink systems where source populations are always large and sink populations contribute relatively little to sources (Dias 1996), pesticides severely reduce insect populations in otherwise good source habitat in agricultural systems. Therefore, even though we modeled alternative habitats as demographic sinks, they could provide refuges from insecticide and contributed immigrants back into preferred habitat where reproductive rates were high. Even when the ratio of sink to source dispersers was small, high reproductive rates in preferred habitat translated to large pest increases. Likewise, because of high reproductive rates in preferred source habitats, alternative sink habitats could be constantly replenished, which perpetuated the population cycling between demographic sources and sinks. This potential for non-crop habitat to act as a reservoir for pests is why many agronomists have recommended managing field edges (Ruberson 1999). However, since many studies have shown the benefits of non-crop habitat for conserving natural enemies of pests and enhancing biological control (Bianchi et al. 2006), the balance between non-crop habitat as a pest reservoir and as a natural enemy refuge deserves further attention.

The prediction that pests with lower reproductive rates are more suppressed by landscape diversification than pests with higher reproductive rates may be surprising until pest management in the model is carefully considered. In our model, insecticides severely reduced insect populations, but they were only used where pest densities exceeded the economic threshold. For insects with high reproductive rates, nearly all the preferred habitat in crop intensive landscapes exceeded the economic threshold and received insecticides. This is equivalent to an area-wide pest management program, which can be effective at regionally suppressing pests (Koul et al. 2009). Thus, insects with high r_0 are suppressed by insecticides in simple landscapes, leaving little potential for landscape diversification to suppress densities further. However, insecticide use against insects with high r_0 is still predicted to decrease with landscape diversification. Again, this work supports reduced insecticide use as an ecosystem service of diverse landscape, even where pest control is not detected.

A final assumption in our model, which deserves attention because of the large effect it has on predictions, concerns how insect dispersal behavior was modeled. We made the simplifying assumption that insects are passive dispersers that do not “home in” on preferred habitat. This means that the numbers of dispersers that located preferred habitat decreased with landscape diversification. Of course, we know that insects discriminate among hosts (Bernays and Chapman 1994), but there is surprisingly little data on quantitative relationships between land-use and dispersal mortality. However, we do know that insect mortality can increase, and that fitness and host specialization can decrease in diverse landscapes (Moeser and Vidal 2005, O'Rourke 2010). Numerous studies also show that biological control increases in diverse landscapes (Bianchi et al. 2006, O'Rourke 2010). Therefore, while we know our assumption of passive dispersal is not entirely correct, the negative relationship it created between the proportion of dispersers locating preferred hosts and landscape diversification roughly incorporates many known biological realities. In modeling dispersal, we accept simplicity over reality (Levins 2006). Inasmuch as our simple model correctly predicted observed WCR and ECB populations and pest management in different landscapes, we think that its simplicity is defensible.

Our combined empirical and modeling approach to herbivore-landscape research provides compelling evidence that pest suppression as an ecosystem service of diverse agricultural landscapes can be predicted by insect life history. It also highlights that herbivore-landscape relationships in agro-ecosystems can only be understood in the context of regional pest management. In addition to researching pest suppression, scientists should examine pesticide reduction as a possible ecosystem service provided by diverse agricultural landscapes. Our work indicates that diverse agricultural landscapes support pest suppression in addition to a variety of other ecosystem services including soil and water, biodiversity, and pollinator conservation (Tscharntke et al. 2005, Swinton et al. 2006, Jackson et al. 2007, Power et al. 2009).

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CHAPTER 5

MANAGING RESISTANCE TO BT CROPS IN A GENETICALLY VARIABLE INSECT HERBIVORE, *OSTRINIA NUBILALIS*

Megan E. O'Rourke, Thomas W. Sappington, and Shelby J. Fleischer

Abstract

To slow the resistance evolution of the European corn borer (ECB) to Cry proteins expressed in transgenic *Bacillus thuringensis* (Bt) corn, the United States Environmental Protection Agency (EPA) has adopted an insect resistance management (IRM) plan that relies on a "high dose/refuge" strategy. However, this IRM plan does not consider possible ecological differences between the two ECB pheromone races (E and Z). Using carbon isotope analysis, we found that unstructured (non-corn) refuges contribute more to E race (18%) than to Z race (4%) populations of ECB in upstate New York. Furthermore, feeding on non-corn hosts is associated with decreased body mass and reduced fecundity. We also show that the geographic range of E race ECB is restricted within the range of the Z race and that E race ECB are increasingly dominant in regions with increasing non-corn habitat. While the proportion of E race ECB developing in unstructured refuges is higher than previously assumed, low rates of unstructured refuge use by the Z race, evidence for reduced fecundity when reared on non-corn hosts, and complete sympatry within the E race range all argue against a relaxation of current IRM refuge standards in corn based on alternative host use. We also discuss implications of this research for integrated pest management in vegetables and IRM in Bt cotton.

Introduction

Insect races within a single species may have very different ecologies despite being morphologically indistinguishable (Ferrari et al. 2006, Bickford et al. 2007). This can have important implications for managing agricultural pest species that are composed of multiple races. The European corn borer (ECB *Ostinia nubilalis*) is a classic example of an economically important agricultural pest for which management strategies and regulatory policies have largely ignored the potential differences between known host races.

Genetically modified corn (*Zea mays*) varieties that produce *Bacillus thuringensis* (Bt) derived protein toxins *in vivo* have proven very effective in controlling ECB feeding damage (EPA 2001) and are planted on nearly 20% of the world's corn acreage, and 50% of US corn acreage (James 2007, USDA. 2008). To maintain the efficacy of transgenic Bt corn, and *B. thuringensis* insecticide sprays used by organic growers against ECB, an insect resistance management (IRM) plan has been adopted by the United States Environmental Protection Agency (EPA) to slow the evolution of resistance in ECB to Bt products (EPA 2001, Andow 2002). The key component of the IRM plan is the "high-dose/refuge strategy." Models and data indicate that a high-dose of insecticide that is capable of killing any heterozygous resistant insects, combined with a non-Bt refuge that will produce homozygous susceptible insects, can significantly slow the evolution of resistance to Bt (Gould 2000, Andow 2002, Qiao 2008). The expectation is that homozygous susceptible insects that develop in the refuge will mate with the rare homozygous resistant individual that survives in the Bt corn, and that the resultant heterozygous offspring will be killed by the high-dose of Bt toxin in transgenic corn. As of 2006, the EPA requires farmers using Bt corn in non-cotton growing regions to plant a 20% non-Bt corn refuge within 0.8km of Bt corn as a "nursery" for Bt susceptible ECB (EPA 2006b).

For polyphagous insects, unstructured refuges (including non-corn crops and non-cropped areas) potentially could substitute for the structured refuges (non-Bt corn) mandated by EPA (Bontemps et al. 2004, Andow and Zwahlen 2006, EPA 2008). In the case of ECB, there is uncertainty concerning its degree of polyphagy. Historical records of ECB indicate that the species is polyphagous, consuming over 200 plant species (Hodgson 1928), and it is currently considered a pest on other crops including wheat, cotton, and numerous vegetables (Mason et al. 1996). A recent laboratory study of ECB also supports a wide diet breadth, with neonates preferentially feeding on various weed species over corn (Tate et al. 2006). However, studies commissioned by the EPA concluded that adult ECB could be produced from weeds and some grain crops, but in insufficient numbers to replace structured refuge mandates (EPA 2001). Losey et al. (2001, 2002) also concluded that non-corn plants probably contribute little to adult populations of ECB.

Differences in reported feeding behaviors of ECB may be related to different races. There are two distinct pheromone races of ECB in the U.S that utilize different pheromone blends of Z and E isomers of 11-tetradecyl-acetate (Carde et al. 1978). Since the two ECB pheromone races are partially isolated genetically (Dopman et al. 2005), Bt resistance may develop at different rates in the two races (Bontemps et al. 2004). It is possible that they differ in host use patterns, and it is imperative to know how the two races utilize unstructured refuges for IRM. Research from France indicated that the E race 'hop-mugwort' ECB indeed had very different host utilization than Z race ECB and generally segregated to non-corn hosts (Thomas et al. 2003, Bontemps et al. 2004). However, recent genetic (Malausa et al. 2007) and taxonomic (Frolov et al. 2007) studies indicate that the French 'hop-mugwort' race is better characterized as another species, *Ostrinia scapulalis,* and that it is distinct from E race *Ostrinia nubilalis*. Refuge utilization by E race ECB in the United States remains largely unknown and previous research assessing ECB development in unstructured refuges did not distinguish pheromone races (EPA 2001, Losey et al. 2001). However, there is circumstantial evidence supporting differences in host utilization between races in the U.S. with reports from North Carolina suggesting that the E race is more likely to infest cotton and potato than the Z race (Sorenson et al. 2005).

Here we use carbon stable isotope analysis to assess the contribution of unstructured refuges to populations of E and Z race ECB. Plants that utilize the C4 photosynthetic pathway sequester a significantly higher proportion of ^{13}C to ^{12}C, a ratio designated as $\delta^{13}C$, than plants utilizing the C3 pathway. The $\delta^{13}C$ levels of an organism's tissues largely reflect the carbon signatures of its food, and thus can provide information on the feeding history of that individual (Deniro and Epstein 1978). Observations of ECB trapped in France and reared on diet derived from different plant material confirm that $\delta^{13}C$ analysis of adult ECB can distinguish unambiguously between a C3 or C4 larval host history (Ponsard et al. 2004, Malausa et al. 2007). In the northeastern United States, corn is by far the most common C4 plant, though a small number of other C4 agricultural crop and plant species also exist (Uva et al. 1997, Ponsard et al. 2004). Any ECB with a C3 host history must have developed on a non-corn host, and therefore in an unstructured refuge.

In addition to providing data on proportions of E and Z race ECB development in unstructured refuges, we explore other ecological factors essential to the goals of IRM. We have identified physiological consequences for ECB developing in unstructured refuges that may reduce their fecundity relative to ECB developing in structured non-Bt corn refuges. Reduced fecundity would indicate that unstructured refuges may be less efficient than structured refuges in producing Bt susceptible adults, which could increase the rate of resistance evolution (Ives and Andow 2002). We also have compiled extensive data on the geographic ranges of the two ECB pheromone races. If the two races exhibit different utilization of unstructured refuges and are not entirely sympatric, then adjusting IRM refuge requirements in different regions may be warranted.

Materials & Methods

Isotope Experiments

ECB were collected weekly in upstate New York along the borders of sweet corn fields during 2006 in Scentry Heliothis traps baited with either E or Z race-specific Pherocon pheromone lures. While there is a small error rate in E and Z race males responding to the pheromone blend typical of the opposite race (Linn et al. 1997), we assume that trap captures reflect the true race of the responding ECB. Random sub-samples of moths from each sampling location were dried at 55^{o}C for at least one week, and were weighed individually. The wings of 68 E and 71 Z race ECB were each analyzed for δ^{13}C content using a Thermo Delta Plus isotope ratio mass spectrometer (IRMS) interfaced to a NC2500 elemental analyzer at the Cornell Isotope Laboratory (COIL) (Table S1 in Appendix).

A second δ^{13}C stable isotope experiment was conducted on all ECB (n=138 E; 206 Z) collected on one farm in Penn Yan, NY in 2006 to determine the relationships between adult weight, pheromone race, and host history. Moths were dried and weighed individually. Moths from the 'large' and 'small' tails of the weight distributions of each pheromone race were selected for isotope analysis. The weights of individuals of each race were matched within size classes so that the mean weights of each category would be as similar as possible. Eleven 'large' E (mean $\underline{+}$ SD weight = 9.83 $\underline{+}$ 3.66mg), eleven 'large' Z (10.92 $\underline{+}$ 1.97mg), seven 'small' E (3.92 $\underline{+}$

0.24mg), and 9 'small' Z moths (3.78 ± 0.37mg) were analyzed. Additionally, the four smallest moths collected (2.05 ± 0.13mg), which were all E race, were analyzed for $\delta^{13}C$ content. Isotope analysis was conducted as described above.

$\delta^{13}C$ signatures were converted to categories of host history by scoring individuals with $\delta^{13}C$ values < -20‰ as having a C3 host history, while $\delta^{13}C$ values > -15‰ were scored as having a C4 host history (Deniro and Epstein 1978, Ponsard et al. 2004). For the first isotope experiment, a Fischer exact test was used to assess the relationship between pheromone race and ECB host history. The relationship between sampling location and ECB host history was assessed for the E race, using likelihood ratio tests of chi square estimates. The effects of host history and pheromone race on moth dry weight were tested using a standard least squares analysis of variance (ANOVA) of a full factorial model. Contrasts were made to test the specific hypotheses that E and Z race ECB weigh the same when they develop on C4 plants, and that each race weighs less when they develop on C3 plants (JMP 2007). For the second isotope experiment, the effects of moth size category ('large' and 'small') and pheromone race on host history were analyzed with an exact logistic regression using Stata™ 10.0 software (STATA 2008). The relationship between pheromone race and host history within the different size categories was further investigated using Fischer exact tests of two-by-two contingency tables (JMP 2007).

Female Size and Fecundity

We examined the relationship between size and lifetime egg production of Z race ECB reared at the USDA-ARS Corn Insects and Crop Genetics Research Unit, Ames, IA. The ECB colony was established in June 2007 from wild adults captured in light traps in central Iowa, and three cohorts representing three generations (N=66, 37, and 60 females) were tested during January-April 2008. Insects were reared at 27°C, 16:8 (L:D), and 80% relative humidity, following standard procedures (Guthrie et al. 1985). Eggs were heat-treated to ensure a disease-free colony (Raun 1961).

Female pupae were weighed to the nearest mg on the second day after pupation. Pupae were held separately in small plastic cups and checked daily for adult emergence. On the day of eclosion, each female was transferred to a small wire-mesh cage (8.5 cm dia, 6 cm tall) containing two, 2-5 days old males for mating (Kira et al. 1969). The males remained in the cage throughout the life of the female, and were replaced with fresh males if the former died. The mesh on the lid was wide enough to allow females to oviposit through it onto a wax paper disc held in place by the upper half of a glass petri dish. The wax sheet was changed daily and the eggs were allowed to develop for two days. Fertile and infertile eggs were distinguished by color change associated with embryo development and were counted under a dissecting microscope. Total eggs laid, including both fertile and non-fertile from females laying greater than 50% fertile eggs, was used in analysis. The effect of female pupal size on fecundity was assessed by linear regression of total lifetime eggs laid on female pupal weight (Analytical-Software 2000).

Geographic Distributions

ECB data from New England and Mid-Atlantic States were obtained from the Pestwatch database (Fleischer 2008). Data from North Carolina were obtained from Sorenson et al. (Sorenson et al. 2005), data from South Carolina were obtained from Durant et al. (Durant et al. 1986), data from Ohio were contributed by C. Welty (unpublished data), and data from Iowa were contributed by D. Sumerford and T. Sappington (unpublished data). ECB data were consolidated by summing all E and Z moths collected over all years of record per county before further analyses (Table S2 in Appendix).

Spatial information about ECB distributions and agronomic data were compiled and maps were created in Manifold®Systems 8.0 Geographic Information Systems (GIS) software (Manifold 2008). Data on corn and vegetable acreages (including sweet corn) per county in the Pestwatch database and North Carolina, Ohio, and South Carolina were obtained from the 2002, 1992, and 1987 Census of Agriculture, respectively (USDA 2008). The relationships between the proportion of E moths and longitude, field corn acreage, and vegetable acreage were analyzed using multiple regression (JMP 2007). Prior to analysis, corn and vegetable acreages per county were normalized by the total area of each county. Data from Iowa were excluded from this analysis to avoid skewing the results with an outlier data point. ANOVA was used to test whether E race ECB were significantly more abundant in counties in the eastern (east of the Hudson River: Maine, New Hampshire, Vermont, Massachusetts, Connecticut, and Long Island, NY), than western (west of the Hudson River: Pennsylvania, Maryland, Delaware, upstate New York) regions of the Pestwatch dataset (JMP 2007).

Results

Isotope Experiments

All moths sampled had either a $\delta^{13}C$ isotope signature reflecting larval feeding on C4 (N = 160, mean = -12.198‰, max = -10.254‰, min = -14.638‰) or C3 (N = 26, mean = -27.901‰, max = -23.752‰, min = -31.041‰) plants. Among the moths sampled, there was no evidence that any had a mixed diet of C3 and C4 type plants as larvae (Figure S1 in Appendix).

E and Z race ECB utilize non-corn hosts at different rates (Fischer's exact 2-tail test, N = 139, P = 0.013). Approximately 18% of the E race adults captured in pheromone traps throughout upstate New York developed as larvae on C3 plants (12 of 68) compared to only 4% of captured Z race moths (3 of 71). However, rates of utilizing unstructured refuges were spatially very variable for the E race, ranging from 0% to 40%. Indeed, location significantly affected the rate that E race moths developed in unstructured refuges ($\chi^2 = 16.496$, df = 4, P = 0.0024) (Figure 5.1).

Larval host plant had a significant effect on adult weight of E race ECB. There was no significant difference in adult weight between the E and Z race when they developed on C4 plants (F = 3.69, df = 1,91, P = 0.06). However, weights of E race ECB were significantly less when they developed on C3 (mean ± SD weight = 4.72 ± 2.33mg) than C4 hosts (6.16 ± 1.53mg) (F = 6.33, df = 1,91, P = 0.01). In contrast, there did not appear to be a decrease in weight for Z race ECB that developed in unstructured refuges (F = 0.59, df = 1,91, P > 0.5), but there were only two Z race ECB to test in this category (Figure 5.2).

The second isotope experiment further supports a relationship between ECB pheromone race, adult weight, and host history (Table 5.1). Small moths were significantly more likely to have developed on a C3 plant than large moths (odds ratio = 92.577, 95% C.I. = 3.5 to infinity, P < 0.0001). Also, E moths were significantly more likely to have developed on a C3 plant than Z moths (odds ratio = 34.168, 95% C.I. = 8.8 to infinity, P = 0.0014). Differences in host history between the two ECB races are especially apparent in small moths where the E race is much more likely than the Z race to have developed on C3 plants (Fischer's exact 1-tail test, N = 16, P = 0.0007). The four smallest E race ECB, which were the smallest of all the moths sampled at Penn Yan, NY, all had $\delta^{13}C$ signatures indicative of a C3 host history.

Female Size and Fecundity

Lifetime egg production was positively related to female size, as indexed by pupal weight (F = 2.56, df = 1,161, P < 0.0001). Female pupal weight explained 25%

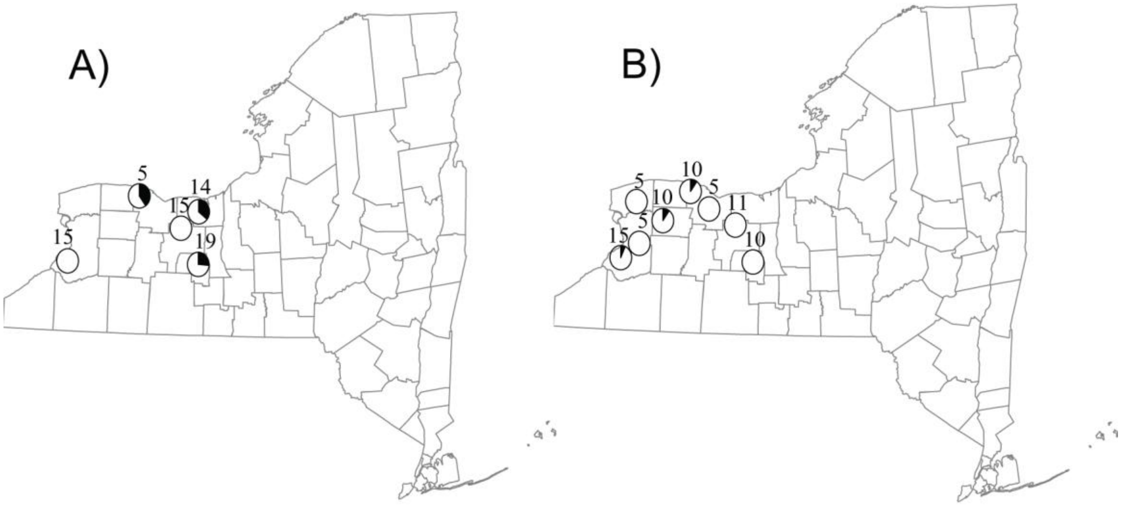


Figure 5.1. Proportions of adult ECB collected in pheromone traps in upstate New York specific to the A) E race or B) Z race whose $\delta^{13}C$ signatures reflect larval feeding on C3 (black shading) and C4 (white shading) plants in upstate New York. Numbers above pie charts correspond to sample sizes.

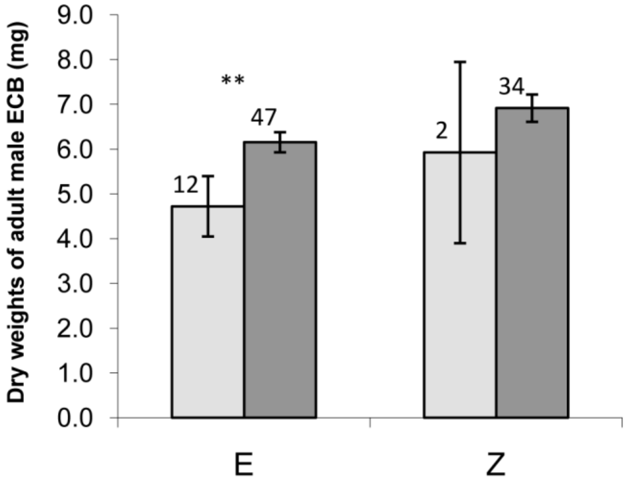
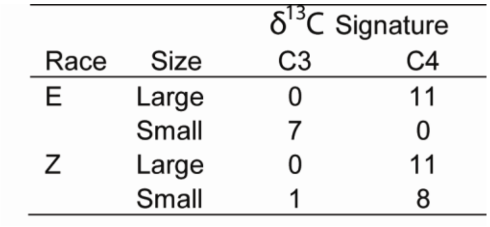


Figure 5.2. Dry weights (mg) of adult male ECB collected in E and Z pheromone baited traps in upstate New York. Dark gray bars show mean weights of ECB that developed on C4 hosts. Light gray bars show mean weights of ECB that developed on C3 hosts. Error bars represent standard errors of sample means. Numbers above bars indicate sample sizes. Asterisks denote a significant difference (*P* = 0.01) in the weights of E moths that fed on C3 or C4 plants.



		$\delta^{13}C$ Signature	
Race	Size	C3	C4
E	Large	0	11
	Small	7	0
Z	Large	0	11
	Small	1	8

Table 5.1. Numbers of E and Z race adult male ECB of two size categories collected in pheromone traps in Penn Yan, NY that had $\delta^{13}C$ signatures characteristic of either C3 or C4 larval host plants.

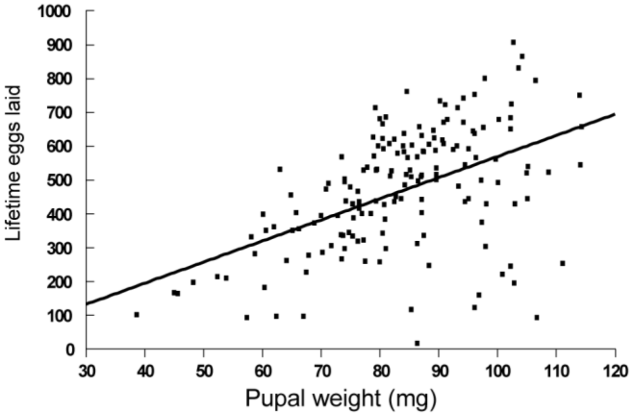


Figure 5.3. Linear regression of lifetime eggs laid against female pupal weight for laboratory-reared Z-race European corn borers.

Figure 5.4. Proportions of E (black shading) and Z (white shading) pheromone races of ECB in the Eastern and Central United States based on captures in pheromone traps. See Appendix 5.1 for sources and details of samples.

of the variation in lifetime egg production. Within the range of pupal weights examined, our regression model predicts that a threefold increase in pupal weight corresponds to a 3.5 fold increase in the number of eggs an ECB female will lay (Figure 5.3). Lifetime percent fertile eggs laid per female averaged 86.6 (S.E. = 0.08).

Geographic Distributions

On a county-wide scale, Z and E race ECB always co-occurred at the locations sampled in this study, except in Iowa where moths responded only to the Z pheromone (Figure 5.4). However, the proportion of E moths expected in a county is significantly related to longitude, increasing from west to east (F = 21.04, df = 1,83, P < 0.0001). Counties east of Pennsylvania and upstate New York reported an average of 59% E moths, which is significantly more than the average 23% E race reported in the western Pestwatch region (F = 54.61, df = 1,73, P < 0.0001). In addition to longitude, land-use also appears to play a role in the distribution of ECB races. The proportion of E race ECB per county is positively related to non-corn acreage (F = 4.98, df = 1,83, P = 0.028). Vegetable acreage was not significantly related to the distribution of E moths (F = 0.15, df = 1,83, P > 0.5).

Discussion

The differences in host use between E and Z race ECB found in this study have important implications for IRM in Bt-corn (Gould 1998). Relatively high proportions of E race ECB developing on species other than corn indicate that unstructured refuges may contribute to slowing the development of resistance to Bt in the E race. In contrast, our results indicate that only a very low proportion of Z race ECB develop in unstructured refuges in upstate New York, and that unstructured refuges probably contribute relatively little to preventing Bt resistance evolution in this race. The average 4% of Z race ECB that developed in unstructured refuges in this study concurs with data from France where only 3% of Z race *O. nubilalis* collected in pheromone traps had developed on C3 hosts (Bontemps et al. 2004). Furthermore, since "mistaken" attraction to the lure of the opposite race can happen, especially in the case of E males responding to the Z pheromone blend (Linn et al. 1997), the proportion of Z race ECB developing in unstructured refuges determined in our study could be an overestimate.

Previous research concluding that unstructured refuges are unlikely to contribute to adult populations of ECB may have been working primarily with Z race ECB, even though the race was not identified. This is certainly true for studies in the Midwestern United States where the E race presumably is not present (EPA 2001) (Figure 5.3). Additionally, research by Losey et al. (2001, 2002) on unstructured refuges and ECB was conducted in only one region in upstate New York and one in Pennsylvania. While both pheromone races are present in these regions (Figure 5.3), farm to farm variability in the proportional abundance of E moths can be quite high (Sorenson et al. 2005), and the contribution by the E race was undetermined.

Taken as a whole, our results do not support a relaxing of structured refuge requirements for ECB in Bt corn. While our isotope survey shows that E race ECB develop in unstructured refuges at relatively high rates in New York, and correlations between non-corn habitat and E race ECB confirm that wide host utilization may occur throughout its range, other results indicate that adjusting structured refuge requirements for Bt corn would be unwarranted. First, there seems to be a large amount of spatial variability in the proportions of E race ECB feeding on non-corn hosts. Second, adult weights of ECB appear to be reduced by feeding on C3 hosts. Reduced weight of ECB females, in turn, was related to lower fecundity. Because low weight moths produce fewer lifetime eggs, the effective refuge size of non-corn hosts likewise is reduced and could increase rates of resistance evolution (Gould 1998, Ives and Andow 2002, Gustafson et al. 2006). Finally, our compilation of E and Z race ECB distributions indicates that the Z race's range fully overlaps with that of the E race in the United States at a county-wide scale. Where there is sympatry, IRM strategies should be conservatively based on the Z race, for which non-corn hosts appear to produce insufficient numbers of susceptible adults to serve as reliable unstructured refuges.

Our research showing different rates of unstructured refuge utilization between ECB pheromone races also has important implications for IRM in Bt cotton. Surveys of the major U.S. cotton pests, *Heliothis virescens* and *Helicoverpa zea,* indicate that both species utilize unstructured refuges at higher rates than we observed for ECB (Gould et al. 2002, Gustafson et al. 2006, Orth et al. 2007, Jackson et al. 2008). These data have played important roles in the 2007 approval by EPA to eliminate structured refuge requirements in IRM plans for Bt cotton varieties pyramided with Cry1Ac and Cry2Ab2 genes in parts of the U.S. (EPA 2006a, 2008). However, as with IRM plans for *O. nubilalis*, differences in host utilization between possible races of cotton pests have not been carefully considered. Research indeed points to the possibility of pheromone races within *H. armigera*, the Old World sister species of *H. zea* (Tamhankar et al. 2003, Cho et al. 2008), and to variation in pheromone composition and male response in *H. virescens* (Groot et al. 2009). Given the large differences we observed for unstructured refuge utilization between *O. nubilalis* pheromone races and the potential impacts on Bt resistance evolution and IRM, more research into possible races of cotton pests appears warranted.

In addition to its implications for IRM, differences in feeding behaviors between E and Z race ECB are important for integrated pest management (IPM) in vegetable crops. Since vegetable processors have very low tolerances for ECB contamination, insecticides targeting ECB are often applied preventively based on ECB flight activity and plant growth stage (Mason et al. 1996). However, our data indicate that adult ECB that have fed on C3 plants, including vegetables, are predominantly E race. This result raises questions about the role of Z race ECB in vegetable systems other than sweet corn. It is unclear whether Z race ECB lay eggs on vegetables but their larvae have low survival to adulthood, or whether the small percentage of Z race ECB that feed on C3 plants is sufficient to cause economic losses in vegetables. Alternatively, the E race ECB may constitute the major contributor to vegetable damage. If so, IPM programs for managing ECB in vegetables other than sweet corn should be focused on the flight activity of E moths, which is not necessarily in phase with that of Z moths (Sorenson et al. 2005). An increased focus on the biology and ecology of the E race could improve pest management and reduce insecticide use in vegetable crops.

This research also provides general insight into the ecological differences between the ECB races. We have shown through isotope analysis that E and Z moths have different host use patterns in New York. The large-scale geographic relationship that we found between non-corn habitat and proportions of E race ECB further indicates that development in unstructured refuges by E race ECB may be a general phenomenon throughout its range. Our distribution map of ECB pheromone races also shows clear differences in their ranges. The underlying reasons for the strong longitudinal trend are unknown, but a variety of hypotheses can be posited. The E race was not recognized until 1972 (Roelofs et al. 1972) and its reduced range may simply be the result of its introduction after the Z race, since multiple introductions of ECB into the United States have been acknowledged (Brindley and Dicke 1963). Alternatively, the E race may be more ecologically constrained in North America than the Z race. They may be poorer dispersers resulting in slower range expansion, or less adapted to the climate in the Midwestern United States.

Ecological differences between the pheromone races, along with evidence for restricted gene flow and independent evolution (Dopman et al. 2005) emphasize that ECB races should be clearly identified in future research. This is especially true in the New England states where we found that the E race often predominates. In addition to pheromone races, there are genetically distinct voltinism races that utilize the Z pheromone blend and differ in post-diapause development times (Coates et al. 2004, Dopman et al. 2005). Although diapause response has been extensively studied among voltinism races (Calvin and Song 1994), little is known about the distributions or behaviors of the univoltine race. As with the pheromone races, they likely have unique evolutionary trajectories and should not be ignored. In our research, since uni- and bivoltine Z race ECB are known to exist sympatrically in New York (Glover et al. 1991), $\delta^{13}C$ samples likely included both voltinism races. However, their proportions in samples are unknown and we cannot definitively conclude that both Z voltinism races use unstructured refuges at very low rates.

All the different races of ECB make the taxonomy of the species difficult. Until recently, studies from France indicated that E race ECB infested mainly hop and mugwort (Thomas et al. 2003, Bontemps et al. 2004). However, Malausa et al. (2007) suggested the degree of reproductive isolation between the Z and E race ECB in France was high enough to justify species status. Frolov et al. (2007) concluded that the E race populations in France actually belong to the sibling species *Ostrinia scapulalis*, though the existence of E race *O. nubilalis* in other parts of Europe is still recognized. While we found that E race ECB from New York consumed non-corn plants at an average rate of 18%, this is very different from the E race 'hop-mugwort' species (*O. scapulalis*) in France that infests C3 plants at a rate of 86% (Bontemps et al. 2004). Thus, our $\delta^{13}C$ data on the host history of E race ECB in New York support the idea that they are E race *O. nubilalis* rather than E race 'hop-mugwort' *O. scapulalis* studied in France. However, the possibility remains that there is assortative mating within the E race between those that feed on corn and non-corn hosts, or that there is a combination of E races of *O. nubilalis* and *O. scapulalis* within the United States.

This research has shown that utilization of unstructured refuges differs between the E and Z pheromone races of ECB in the United States, with important implications for IRM and IPM plans. Where multiple races of a species have overlapping distributions, IRM strategies should be conservatively based on the race most likely to develop resistance, in this case the Z race of ECB. This, of course, requires not only that we can identify the cryptic races, but also that we understand the basic ecological, behavioral, and evolutionary differences between them. While unraveling the differences among members of race- or species-complexes can be difficult, we have shown how understanding them can have important applied economic and policy implications.

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CHAPTER 6

CONCLUSIONS

Agro-ecosystems are much more complex than simply a collection of crop fields. Rather, agro-ecological processes of key import, including insect population dynamics, are regulated by non-crop as well as by crop habitats. In this thesis, we have demonstrated the importance of non-crop habitat in the population dynamics and evolution of key insects in corn fields. The major conclusions from each research paper and the relationships between the chapters are summarized below.

In **chapter two**, I focused attention on the gaps in landscape-insect research. To do this, I examined the literature on local plant diversity-insect relationships that proliferated following Dick Root's Ecological Monograph, "Organization of a plant-arthropod association in simple and diverse habitats – fauna of Collards (Brassica-Oleracea)." I then compared that research to the relatively small and recent body of landscape-insect literature. This revealed that most of the landscape-insect studies have pursued the 'natural enemies hypothesis' put forth by Dr. Root, which predicts that diverse habitats reduce herbivore densities by increasing natural enemy densities. However, most landscape-insect studies ignore Dr. Root's 'resource concentration hypothesis,' which predicts that specialist herbivore populations are directly reduced by diverse habitats. I posited mechanisms by which diverse agricultural landscapes may directly reduce specialist herbivore populations. Specifically, I argued that greater dispersal distances between suitable habitat patches in diverse landscapes decrease herbivore fecundity and increase mortality.

In addition to a lack of research on the direct effects of agricultural landscapes on herbivores, research documenting relationships between agricultural landscapes and herbivore densities is scarce. Most research to date has focused on relationships between agricultural landscapes and natural enemies. Therefore, in **chapter three**, we reported relationships between land-use patterns at multiple scales and densities of three herbivore pests of field corn. We also quantified lady beetle densities in the same landscapes to test the hypothesis that diverse agricultural landscapes differentially affect specialist herbivore pests and generalist predator natural enemies. Our data showed that specialist pest densities were generally suppressed while generalist natural enemy densities were enhanced in diverse landscapes. However, these relationships were complicated. For example, relationships between landscape diversity and insect densities differed when diversity on the perimeter or in the county surrounding corn fields was considered. Also, the strength of pest suppression or natural enemy enhancement in diverse agricultural landscapes varied from strong to undetectable, depending on the insect. This research highlighted how multiple scales of land-use may affect insect populations and how insect life history may influence landscape-insect relationships.

In **chapter four**, we attempted to address the issue of predictability in landscape-herbivore relationships and generated hypotheses about which types of herbivores, based on life history characteristics, are most suppressed by diverse landscapes. We also introduced the issue of regional pest management and its impacts on landscape-herbivore relationships. This chapter borrowed data about relationships between landscapes and herbivore populations from the previous chapter, and combined it with laboratory data on host specialization, literature data on national management of the pests, and predictions from a spatially-explicit simulation model of landscape-herbivore population dynamics. We showed that insects that use alternative hosts, even at low rates, may not be suppressed by landscape diversification. We also showed that insecticide use may be correlated to land use with less insecticide used in diverse landscapes. Within the context of regional pest management, we proposed that specialist herbivores with relatively low reproductive rates are more suppressed by landscape diversification than herbivores that use multiple hosts and have high reproductive rates.

In the final research chapter of this thesis, **chapter five**, we examined the effects of landscape-scale habitat diversity on pest evolution. Having quantified host utilization by corn pests for the previous thesis chapter, we began to think about the implications of host utilization for insect resistance management (IRM) to Bt crops. Specifically, in cases where pests utilize multiple hosts, we wondered whether diverse agricultural landscapes could substitute for structured, non-Bt refuges mandated by the US EPA to slow the evolution of resistance to Bt crops. As a case study, we examined host utilization by the European corn borer (ECB) and detailed other aspects of ECB ecology that should be examined when considering relaxing Bt refuge policies. We looked at differences in host utilization between the two ECB pheromone races, the effects of non-corn host utilization on ECB fitness, and the ranges of the two pheromone races. We concluded that habitat diversity cannot substitute for Bt refuge requirements in IRM plans for Bt corn and that other factors besides host utilization by pests are important. Possible cryptic insect races with different host utilization behaviors, and fitness on alternative hosts should also be considered along with host utilization when developing IRM refuge strategies.

Together, this research has implications for both basic ecology and evolutionary biology as well as for applied pest management. On the basic side, it helps to clarify the spatial context of plant-insect interactions and to explain why insect communities vary in different locations. Land-use patterns may also help to explain why certain insect genotypes have evolved. On the applied side, this research helps to predict pest pressures under government policies that promote land-use change. For example, we might expect densities of corn specialist pests and insecticide use to increase with expanding corn acreage to meet US government biofuel mandates. Conversely, we may see suppression of specialist herbivores and more generalist herbivores in annual crops if perennial habitat expands with presumed advances in cellulosic ethanol technology. Land-use patterns may also help to predict where insects will first evolve resistance to Bt crops and other pest management strategies.

Nearly 40% of the world's inhabited countries are devoted to agriculture, which has huge impacts on global ecosystems. In this research, we have shown the pest control benefits of integrating habitat diversity into agricultural landscape. This is in addition to other known ecosystem services supported by diverse agricultural landscapes including wildlife, water, and soil conservation. However, changing agriculture to incorporate more diversity is not a simple challenge. Agricultural landscapes result from social and economic factors in addition to physical and biological constraints, and any attempts to change agriculture must consider these diverse variables. Nevertheless, while directed changes may be difficult to catalyze, diversifying agricultural landscapes could provide huge ecosystem services benefits.

APPENDIX

Table S1. Proportions of E and Z race ECB sampled from upstate New York that developed on C3 hosts according to $\delta^{13}C$ analyses.

	Sample Size		Geographic Coordinates		Proportion C3	
Nearby Town	E	Z	X	Y	E	Z
Batavia	--	10	-78.2605	43.0148	--	0.10
E. Aurora	--	5	-78.6136	42.7678	--	0.0
Eden	15	15	-78.8592	42.6917	0.0	0.07
Farmington	15	11	-77.2730	42.9671	0.0	0.0
Hamlin	5	10	-78.0341	43.3500	0.40	0.0
Lockport	--	5	-78.8439	43.2151	--	0.0
Penn Yan	19	10	-76.9826	42.6266	0.26	0.0
Spencerport	--	5	-77.7500	43.1667	--	0.0
Williamson	14	--	-77.2318	43.2228	0.36	--

Table S2. Total numbers of E and Z race moths trapped, the number of trap locations monitored, and the duration of trapping for each county mapped in Fig. 4.*

State[a]	County	Trap pairs[b]	Beginning	End	Total e	Total z
CT	Hartford	6	2002	2002	214	196
CT	New Haven	3	2002	2002	122	43
CT	Tolland	2	2002	2002	42	189
DE	Kent	1	2002	2002	12	290
DE	Sussex	1	2002	2002	15	178
IA[c]	Story	3	2007	2007	0	439
MA	Berkshire	8	2002	2007	135	314
MA	Bristol	8	2002	2006	403	385
MA	Essex	6	2002	2006	508	299
MA	Franklin	10	2002	2007	2849	1093
MA	Hampden	7	2002	2007	357	452
MA	Hampshire	14	2002	2007	2760	2501
MA	Middlesex	11	2002	2007	550	453
MA	Norfolk	5	2002	2007	639	494
MA	Worcester	14	2002	2006	785	536
MD	Frederick	4	2007	2007	87	125
ME	Androscoggin	15	2002	2006	1759	872
ME	Cumberland	15	2002	2006	2112	572
ME	Franklin	5	2002	2006	276	136
ME	Kennebec	13	2002	2006	753	539
ME	Knox	3	2002	2004	165	29
ME	Lincoln	15	2002	2006	731	904
ME	Oxford	7	2002	2006	904	263
ME	Penobscot	11	2002	2006	81	625
ME	York	20	2002	2006	1524	377
NC[d]	Chatham	6	1998	2000	88	378
NC	Franklin	6	1998	2000	378	687
NC	Henderson	3	1998	2000	2	87
NC	Randolph	6	1998	2000	172	961
NC	Wake	9	1998	2000	676	340
NC	Wayne	3	1998	2000	99	26
NH	Cheshire	3	2002	2007	271	49
NH	Hillsborough	13	2006	2007	863	105
NH	Sullivan	2	2002	2003	96	199
NY	Cattaraugus	3	2004	2006	13	183
NY	Cayuga	10	2001	2007	67	467
NY	Chautauqua	9	1999	2007	110	1057
NY	Erie	14	1999	2007	226	5273
NY	Genesee	18	1999	2007	251	2720
NY	Madison	8	1999	2007	426	226
NY	Monroe	18	1999	2007	1303	2849
NY	Niagara	9	1999	2007	218	1734
NY	Onondaga	7	2001	2007	979	550
NY	Ontario	18	1999	2007	3888	3235
NY	Orleans	11	1999	2007	361	1589

NY	Suffolk	27	2000	2006	15731	931
NY	Tioga	13	1999	2007	260	1645
NY	Wayne	10	1999	2007	434	730
NY	Yates	10	1999	2007	1089	2490
OH[e]	Adams	2	1991	1991	5	52
OH	Clark	3	1989	1989	2	45
OH	Pickaway	3	1989	1989	13	440
OH	Washington	4	1990	1991	11	153
PA	Adams	8	1999	2007	69	211
PA	Bedford	9	1999	2005	53	100
PA	Berks	3	1999	2007	247	69
PA	Blair	20	1999	2007	266	697
PA	Bucks	26	1999	2007	781	4177
PA	Butler	2	2007	2007	80	75
PA	Centre	19	1999	2007	1128	2336
PA	Clinton	8	2000	2007	170	812
PA	Cumberland	9	1999	2006	146	1105
PA	Dauphin	11	2000	2007	247	651
PA	Erie	13	1999	2006	126	650
PA	Fayette	7	2000	2006	38	117
PA	Franklin	18	2000	2006	235	872
PA	Huntingdon	2	2002	2007	144	410
PA	Indiana	26	1998	2007	45	3553
PA	Lancaster	34	1999	2007	2799	7642
PA	Lebanon	5	1999	2007	252	837
PA	Lehigh	9	1999	2007	371	3737
PA	Luzerne	22	1999	2007	1610	3813
PA	Lycoming	13	2000	2007	145	1351
PA	Mifflin	5	2003	2007	93	237
PA	Monroe	6	2000	2001	21	219
PA	Montour	3	2002	2007	12	247
PA	Northampton	8	2000	2007	164	1608
PA	Potter	4	2000	2001	3	92
PA	Schuylkill	22	1999	2007	418	2019
PA	Somerset	1	2007	2007	63	57
PA	Union	15	2000	2007	326	1792
PA	Washington	5	2002	2006	23	173
PA	Westmoreland	11	1999	2007	110	1211
PA	York	5	2003	2007	180	861
SC[f]	Florence	4	1984	1985	108	7
SC	Pickens	2	1984	1985	14	33
SC	Sumter	2	1984	1985	85	18
VT	Bennington	2	2002	2003	67	372
VT	Windham	4	2002	2005	554	188

(Table S2 continued)

[a] All data are from the Pestwatch database unless otherwise noted
[b] One trap pair is equivalent to one E plus one Z race pheromone trap
[c] Iowa data are unpublished contributions from D. Sumerford and T. Sappington
[d] North Carolina data are from Sorenson et al. (2005)
[e] Ohio data are unplublished contributions from C. Welty
[f] South Carolina data are from Durant et al. (1986)
[*] Only farms in the Pestwatch database (Fleischer 2008) where both E and Z race ECB pheromone lures were deployed, and where cumulative catch exceeded 50 moths, were included in analyses. Extension educators have contributed data to Pestwatch since 1999 on ECB collected on vegetable farms using Scentry Heliothis nylon traps baited with Pherocon lures, or wire cone traps of the same size (TP-75-50) baited with lures from Hercon. North Carolina data from Sorensen et al. (2005) include 1998-2000 ECB sampling. South Carolina data are from table 1 in Durant et al. (1986). The Ohio data spans 1989-1991 and was collected using Scentry Heliothis traps baited with E and Z race Scentry lures that were placed at least 30m apart along the edges of sweet corn fields.

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Figure S1. Histogram of δ^{13}C values of all ECB analyzed.