Honey bee landscape ecology: foraging, toxic exposure, and apicultural outcomes

DISSERTATION

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By

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Abstract

The unifying thesis of my dissertation is that the biology of a honey bee colony cannot be understood apart from the landscape in which it lives; this influence of landscape applies especially to honey bee foraging biology and toxic exposure, and consequently to apicultural outcomes. In Chapter 1, I present and elaborate this thesis in the context of existing literature and lay out the scope of my dissertation accordingly.

In Chapter 2, I describe a study in which I collaborated with volunteer beekeepers to measure the success of honey bee colonies surrounded by different types of landscape in Ohio, USA. The results of this study showed that the most successful colonies tended to be those surrounded by agricultural land as opposed to those in forested or urban landscapes, which was contrary to the prevailing opinion that agricultural landscapes are too dominated by crop monocultures and too contaminated with pesticides to support healthy honey bees. This led me to hypothesize that the relationship between honey bee success and landscape is driven mainly by the availability of certain key floral taxa that, in Ohio, occur most abundantly in the interstices of the agricultural landscape.

Chapter 3 further pursues the question of whether honey bees prefer agricultural or urban land use by setting up a foraging choice test between these two landscape types. Using a combination of dance language analysis and pollen identification, I monitored the spatial and taxonomic patterns of honey bee foraging at an apiary located on the interface of urban and agricultural land use. The results indicate a strong and consistent
preference for the agricultural landscape, corroborating the results of Chapter 1 with an independent data set and using different lines of evidence.

In Chapter 4, I turn my attention to the issue of toxic exposure, constructing a critical review of existing approaches to modeling toxic exposure in honey bees. All existing approaches suffer from serious shortcomings in biological realism, largely due to a failure to approach toxic exposure from a spatially explicit landscape perspective. I demonstrate these shortcomings and then present the key biological mechanisms governing the acquisition of pesticide by foraging bees and the subsequent distribution of pesticide within the nest. Then I explore ways in which these mechanisms might be incorporated into honey bee exposure models and ultimately linked with mechanistic effects models to achieve an integrated field of honey bee toxicology.

Chapter 5 applies the modeling principles presented in Chapter 4 to the issue of honey bee exposure to neonicotinoid insecticides during the planting of seed-treated corn. During planting, the neonicotinoid-laden seed treatment material is shed from the seed surface and dispersed in the environment in small particles. This inadvertent release of insecticide has been implicated in honey bee mortality incidents throughout Europe and North America, but the precise route of exposure linking honey bees to the neonicotinoids has remained obscure. I approach the issue from a landscape perspective, using two years of field data on honey bee exposure and mortality during corn planting in central Ohio. By constructing overlapping models of environmental contamination and honey bee foraging, I estimate the degree of exposure potential associated with different components of the landscape, and then use these estimates to test the predictions of several hypothesized routes of exposure. My results indicate that the primary route of
exposure is the contamination of bee-attractive flora within corn fields at the time of planting, implying that exposure could be mitigated effectively by the destruction of in-field weeds prior to planting.

Chapter 6 tests and extends the findings of Chapter 5 using a simulation model of honey bee exposure to corn seed treatment neonicotinoids. The patterns of exposure generated by this model support the conclusions of Chapter 6 while indicating that the preservation or augmentation of flowering weeds in soybean fields may be equally important in suppressing honey bee exposure.
To my parents, Doug and Sue Sponsler, who nurtured and frequently forgave a childhood of entomological enthusiasm. And to my wife, Molly, who has waited so patiently.
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Fields of Study

Major Field: Entomology
Table of Contents

Abstract .......................................................................................................................... ii

Acknowledgments ........................................................................................................ vi

Vita ................................................................................................................................ viii

List of Tables .................................................................................................................. xiii

List of Figures ................................................................................................................ xiv

Chapter 1: Honey bee biology in context ........................................................................ 1

1.1 Introduction ............................................................................................................. 1

1.2 Recent Progress ...................................................................................................... 3

1.3 Scope of the Present Work ..................................................................................... 7

1.4 References ............................................................................................................. 9

Chapter 2: Honey bee success predicted by landscape composition in Ohio, USA........ 13

2.1 Abstract .................................................................................................................. 13

2.2 Introduction .......................................................................................................... 15

2.3 Materials and Methods ......................................................................................... 18

2.4 Results ................................................................................................................... 27
Chapter 3: Spatial and taxonomic patterns of honey bee foraging: a choice test between urban and agricultural landscapes

3.1 Abstract

3.2 Introduction

3.3 Methods

3.4 Results

3.5 Discussion

3.6 References

Chapter 4: Mechanistic modeling of pesticide exposure: the missing keystone of honey bee toxicology

4.1 Abstract

4.2 Introduction

4.3 Primary Exposure

4.4 Secondary Exposure

4.5 Discussion and Conclusions

4.6 References

Chapter 5: Elucidating the route of exposure linking honey bees to seed treatment neonicotinoids released during corn planting
5.1 Abstract .............................................................................................................. 105
5.2 Introduction ....................................................................................................... 107
5.3 Methods ........................................................................................................... 113
5.4 Results ............................................................................................................. 132
5.5 Discussion ....................................................................................................... 143
5.6 References ...................................................................................................... 152

Chapter 6: Simulating the effects of in-field weed suppression on honey bee exposure to seed treatment neonicotinoids during corn planting ................................................. 156
6.1 Abstract ........................................................................................................... 156
6.2 Introduction ..................................................................................................... 158
6.3 Model description ............................................................................................ 159
6.4 Model application ............................................................................................ 165
6.5 Results ............................................................................................................. 166
6.6 Discussion ....................................................................................................... 168
6.7 References ...................................................................................................... 171

Appendix A: Survey questionnaires ....................................................................... 173
Appendix B: Detailed description of dance analysis methods.................................. 184
Appendix C: Complete pollen data set ................................................................... 187
Appendix D: Voucher specimens and reference slides ........................................... 190
Appendix E: Sample R script for landscape processing ........................................ 205
List of Tables

Table 2.1. Summary of model selection statistics for each colony success metric……..24

Table 4.1. Summary of existing quantitative models of primary exposure……………….70

Table 5.1. Toxicity parameters and application rate of corn seed treatment neonicotinoids…………………………………………………………………………………108

Table 5.2. Risk partitioned by landscape component for each site-year………………..127

Table 5.3. Hypothesized routes and sub-routes of exposure with corresponding statistical models………………………………………………………………………………129

Table 6.1. Model parameters, values, and justifications…………………………………164
List of Figures

Figure 1.1. Schematic representation of the honey bee dance language.........................5

Figure 2.1. Landscape composition of study sites at 2 km radius...............................28

Figure 2.2. Principal components biplot of major land cover classes at a radius of 2 km........................................................................................................................................29

Figure 2.3. Food accumulation and wax production were negatively correlated with PC2..........................................................................................................................................................31

Figure 2.4. Adult population was positively correlated with beekeeper years of experience (A) and supplemental syrup feeding (B)........................................................31

Figure 2.5. Colony food accumulation decreased significantly with increasing urban land cover in sites where Urban + Crop > 50%.................................................................32

Figure 3.1. Landscape surrounding research apiary .......................................................46

Figure 3.2. Spatial foraging data (top) aligned with corresponding pollen data (bottom)................................................................................................................................................52

Figure 3.3. Spatial foraging patterns inferred from dance analysis for each sample date..................................................................................................................................................54

Figure 4.1. Relationship between individual-level exposure, individual-level effects, and colony-level effects.................................................................................................67

Figure 4.2. Comparison of contact and dietary exposure estimates.........................81

Figure 4.3. Processing pathways of nectar-associated and pollen-associated pesticide....85

Figure 5.1. Seed treatments are applied to seeds as flowable solids that dry to form a coating........................................................................................................................................110

Figure 5.2. Study sites were distributed throughout central Ohio (left).......................114

Figure 5.3. Seed treatment deposition as a function of distance from field edge........120
Figure 5.4. Visitation probability as a function of distance………………………………122

Figure 5.5. Spatial components of risk…………………………………………………128

Figure 5.6. Exposure plotted against weedy corn risk for 2014 (A), 2015 with Site B included (B), and 2015 with Site B omitted (C)……………………………………133

Figure 5.7. Exposure plotted against drift risk for 2014 (A), 2015 with Site B included (B), and 2015 with Site B omitted (C)……………………………………135

Figure 5.8. Exposure plotted against total forage risk for 2014 (A), 2015 with Site B included (B), and 2015 with Site B omitted (C)……………………………………137

Figure 5.9. Exposure plotted against corn risk for 2014 (A), 2015 with Site B included (B), and 2015 with Site B omitted (C)……………………………………139

Figure 5.10. Exposure plotted against corn area for 2014 (A), 2015 with Site B included (B), and 2015 with Site B omitted (C)……………………………………141

Figure 5.11. Q-Q plot illustrates skew in the right tail of the multiple regression model used to parse the influence of weedy corn risk and drift risk………………143

Figure 5.12. Site B was unusual in that decoded dances indicated peak foraging activity at about 1.5 km from the hive………………………………………………148

Figure 6.1. Landscape geometries generated by voronoi polygon tessellation………165

Figure 6.2. Histograms depicting exposure distributions generated for each landscape geometry under each level of weed prevalence…………………………166

Figure 6.3. Violin plots describing the distributions of exposure levels generated under different weed prevalence conditions (pooled across landscape geometries)……167

Figure B.1. Screenshot showing four tracked waggle runs, together comprising a single decoded dance……………………………………………………………186
Chapter 1: Honey bee biology in context

1.1 Introduction

Austrian zoologist Karl von Frisch famously described the biology of the honey bee (*Apis mellifera* L.) as “a magic well” that inexorably refills as knowledge is drawn from it (von Frisch 1964). Indeed, the honey bee is one of the most well-researched organisms on the planet, having been studied from Aristotle to the present day, at scales ranging from molecular genetics (The Honey Bee Genome Sequencing Consortium 2006) to population biogeography (Whitfield et al. 2006); yet it continues to inform, inspire, and mystify its students with no sign of depletion.

One aspect of honey bee biology that is only beginning to be appreciated is the complex relationship between honey bee colonies and the landscapes in which they live. To satisfy its need for massive and sustained inputs of floral nectar and pollen, a honey bee colony can routinely survey an area of more than 100 km$^2$ in search of floral resources, a challenge that requires not only the discovery and coordinated exploitation of resources but the ongoing monitoring of constant fluctuations in reward (Seeley 1995). Thus, the success of a honey bee colony depends critically not only on its immediate surroundings but on the landscape-scale distribution, abundance, and quality of floral resource patches, the influence of which has been mirrored in the evolution of the
unparalleled sophistication of honey bee foraging behavior (von Frisch 1967; Seeley 1995).

The acquisition of food, however, is not the only way in which a honey bee colony is connected to its landscape. In collecting food, a colony simultaneously exposes itself to the diverse array of toxic hazards that exist outside the relative safety of the nest. For honey bees, as for other terrestrial organisms, food collection and toxic exposure are coupled phenomena, occurring jointly in the process of foraging. While honey bees have faced toxic hazards from natural sources throughout their evolutionary history (Johnson 2015), the proliferation of synthetic pesticides in the last century has profoundly reshaped (and continues to reshape) the profile of toxic exposure that honey bees face (Berenbaum 2016). Just as the foraging success of honey bees depends on the spatiotemporal distribution of resources in the landscape, so the toxic exposure of honey bees depends on the spatiotemporal distribution of toxic hazards in the landscape. Neither honey bee food acquisition nor honey bee toxic exposure can be explored fully without reference to each other and to the landscape context in which they occur.

The close—yet complex—dependency of honey bees on the landscapes they live in becomes an even more compelling perspective on honey bee biology when viewed in light of the dramatic land use changes that have occurred in the last century, due mainly to the expansion and intensification of agriculture and urban development (Vitousek et al. 1998; Barnosky et al. 2012). Both urbanization and agricultural intensification can have profound, though variable, impacts on the composition and phenology of floral communities, with corresponding consequences for organisms that depend on floral
communities for food (McKinney 2008; Staley et al. 2013; Leong and Roderick 2015; Leong et al. 2016; Otto et al. 2016). With respect to toxic exposure, the importance of agricultural pesticide use is obvious, but urban landscapes may pose distinct patterns of toxic hazard, too, both in terms of pesticides (Hopwood et al. 2012) and industrial pollutants (Morón et al. 2011).

1.2 Recent Progress

A landscape-ecological perspective on honey bee biology has gained some traction in recent years, due both to a general trend toward landscape-scale ecological research and to some specific technical advances that have overcome major obstacles to landscape-scale study of honey bees.

Foraging biology

With respect to honey bee foraging biology, the most significant gains have come from a group at the University of Sussex that has reinvented the way the honey bee dance language is employed as a research tool. The basic syntax of the dance language was famously elucidated by Karl von Frisch (1967): a honey bee forager, upon returning to the nest from a rewarding resource patch, performs a characteristic “dance” in which the bearing of the resource patch relative to the position of the sun on the horizon is communicated by the vertical angle of the straight line component of the dance, and the distance of the resource patch from the nest is communicated by the duration of each circuit [specifically the straight line component of the circuit, as later determined by
Michaelson et al. (1992)] (Figure 1.1). Building on the early work of von Frisch and others, Seeley used the dance language to explore the regulation of foraging behavior, developing the theory of the honey bee colony as an integrative hub of information about the surrounding landscape, shared by nestmates to optimize foraging [reviewed in Seeley (1995)]. Despite these insights, though, the honey bee dance language was rarely applied to landscape-ecological questions because of the difficulty of generating large datasets by decoding dances in real time using a stopwatch and a marker. Also, the intrinsic imprecision of the dance language created interpretive difficulties; dances were always decoded as discrete points in the landscape, even though it was acknowledged that doing so masked the considerable uncertainty associated with the decoded distance and bearing. Couvillon et al. (2012) addressed the first of these problems by developing a more efficient protocol for dance decoding that decodes only the minimum number of circuits per dance and uses high-definition video recording to overcome the problem of keeping up with dancing bees in real time. Then, Schürch et al. (2013) developed a Bayesian probabilistic approach that acknowledged the intrinsic uncertainty in the dance language by mapping dances as probability clouds rather than discrete points, an approach that also enables the use of more powerful statistical analysis of dance data. Based on the decoding protocol of Couvillon et al. (2012) and the mapping protocol of Schürch et al. (2013), there has emerged a new sub-field of honey bee ecology that uses the dance language to explore honey bee habitat preferences and other spatial patterns of foraging activity (Couvillon et al. 2014a; Couvillon et al. 2014b; Couvillon et al. 2014c; Couvillon and Ratnieks 2015; Garbuzov et al. 2014; Garbuzov et al. 2015).
Figure 1.1. Schematic representation of the honey bee dance language. The honey bee dance language encodes the direction and distance of a resource patch in the angle and duration, respectively, of dance circuits. Nestmates follow the dancing bee to gain information about the resource patch.

Toxic exposure

The relationship between landscape and toxic exposure in honey bees has also received some research attention in recent years. Field-scale toxicology studies have become more common, and these acknowledge, at least implicitly, the role of landscape in shaping toxic exposure (e.g. Pilling et al. 2013; Rundlöf et al. 2015). More explicit acknowledgement of landscape has emerged in conceptual (Carreck and Ratnieks 2014) and empirical (Garbuzov et al. 2014) work related to the dilution of systemic
neonicotinoid exposure by foraging on alternative, untreated flora. Perhaps the most promising sign that the toxicological role of landscape is beginning to be taken seriously is the development of spatially explicit honey bee risk assessment models that address directly the role of landscape composition in determining exposure (EFSA 2013; Baveco et al. 2016).

Applied apiculture

Aside from its salient role as von Frisch’s “magic well” of basic research, the honey bee has enormous cultural and economic significance as an integral part of human agriculture and society. Thus, any insights gleaned from a landscape-ecological perspective on honey bee biology should be both applied to and informed by the needs and insights of beekeepers.

Perhaps not surprisingly, the appreciation for landscape that seems relatively novel in the world of academic honey bee research has been virtually axiomatic among practicing beekeepers throughout the history of apiculture. The apicultural acknowledgement of landscape is expressed in the emphasis that beekeepers have always put on strategic colony placement to maximize honey yield, obtain varietal honey, achieve crop pollination, or avoid pesticide exposure [discussed in Graham (1992)]. The role of landscape in determining apicultural outcomes, though, has been addressed mainly in anecdotal terms.

Recently, some studies have attempted with greater scientific rigor to relate apicultural endpoints to measurable landscape variables. Their results, however, have
often been inconsistent. Some studies, for example, have suggested a positive influence of forest area on colony health (Sande et al. 2009; Odoux et al. 2014; Clermont 2015) while others find the opposite (Sponsler and Johnson 2015). Similar inconsistencies have been reported with respect to agricultural and urban land use (Donkersley et al. 2014; Clermont et al. 2015; Lecoq et al. 2015; Sponsler and Johnson 2015; Youngsteadt et al. 2015; Danner et al. 2016; Dolezal et al. 2016; Otto et al. 2016; Smart et al. 2016). When it comes to apicultural significance of landscape, perhaps the most important insight to emerge thus far is that there is enormous variation within general landscape categories, and the relationship between landscape composition and apicultural outcomes is highly context-dependent. This underscores the need for increasingly nuanced treatment of landscape classification with respect to honey bee ecology, while perhaps validating the anecdotal tradition of apicultural wisdom that emphasizes descriptive local knowledge over generalizations.

1.3 Scope of the Present Work

The work presented herein explores honey bee landscape ecology from each of the three perspectives discussed above: foraging biology, toxicology, and applied apiculture. Chapter 2 begins with a project I conducted in collaboration with local beekeepers throughout the state of Ohio. The focus of this chapter is on the apicultural outcomes associated with different landscapes, specifically evaluating the popular claim that honey bees are healthier in cities than in agricultural land. Chapter 3 approaches the issue of urban and agricultural land use again but from a more basic honey bee foraging
biology perspective. In this study, I set up a foraging choice test by establishing an apiary along the interface between urban and agricultural land use. Using the dance analysis method developed by the Sussex group (Couvillon et al. 2012; Schürch et al. 2013), combined with microscopic pollen identification, I determine the relative allocation of foraging activity between the urban and agricultural components of the surrounding landscape along with which floral taxa were of principal dietary importance. In Chapter 4, my focus shifts to the toxicological aspect of honey bee landscape ecology, and I construct a landscape-ecological critique of existing approaches to modeling honey bee pesticide exposure. Chapter 5 is an application of the modeling principles I call for in Chapter 4 to the problem of honey bee exposure to neonicotinoid insecticides during the planting of seed-treated corn. In this study, I combine a landscape model of pesticide contamination with a landscape model of honey bee foraging and evaluate the degree of predicted intersection between foraging honey bees and different components of the contaminated landscape. Then, I use these results to evaluate several hypotheses regarding the primary route of exposure linking honey bees to seed treatment neonicotinoids. Finally, Chapter 6 describes a simulation model that I developed to explore honey bee exposure to seed treatment neonicotinoids during corn planting. Following the principles outlined in Chapter 4, my simulation model generates stochastic and distributional patterns of exposure arising from the intersection of individual foraging bees with a heterogeneously contaminated environment. This model is used to test the conclusions of Chapter 5 and elucidate the fundamental patterns of exposure linking honey bees to seed treatment neonicotinoids during corn planting.
The unifying thesis connecting these studies is that the biology of a honey bee colony cannot be understood apart from the landscape in which it lives. The influence of landscape applies equally to both foraging biology and toxic exposure, and consequently to apicultural outcomes.

1.4 References


Sponsler DB, Johnson RM. 2015. Honey bee success predicted by landscape composition in Ohio, USA. PeerJ 3:e838


Chapter 2: Honey bee success predicted by landscape composition in Ohio, USA

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2.1 Abstract

Foraging honey bees (*Apis mellifera* L.) can routinely travel as far as several kilometers from their hive in the process of collecting nectar and pollen from floral patches within the surrounding landscape. Since the availability of floral resources at the landscape scale is a function of landscape composition, apiculturists have long recognized that landscape composition is a critical determinant of honey bee colony success. Nevertheless, very few studies present quantitative data relating colony success metrics to local landscape composition. We employed a beekeeper survey in conjunction with GIS-based landscape analysis to model colony success as a function of landscape composition in the State of Ohio, USA, a region characterized by intensive cropland, urban development, deciduous forest, and grassland. We found that colony food accumulation and wax production were positively related to cropland and negatively related to forest and grassland, a pattern that may be driven by the abundance of dandelion and clovers in agricultural areas compared to forest or mature grassland. Colony food accumulation was also negatively correlated with urban land cover in sites
dominated by urban and agricultural land use, which does not support the popular opinion that the urban environment is more favorable to honey bees than cropland.
2.2 Introduction

Honey bees (*Apis mellifera*, L.) exist in large, eusocial colonies that require massive and sustained inputs of floral nectar and pollen. They meet this demand by foraging at an extremely large spatial scale and with rapid responsiveness to changes in the surrounding floral community (Visscher & Seeley, 1982; Seeley, 1995). Depending on local floral availability, colonies may routinely forage over an area of more than 100 km$^2$ (Seeley, 1995), and much larger ranges have been reported under extreme conditions (Eckert, 1931; Beekman & Ratnieks, 2001).

Because honey bee foraging is a decidedly landscape-scale process, one should expect landscape composition to interact meaningfully with colony nutrition and overall colony success. While the plausibility of such a relationship is widely acknowledged (Steffan-Dewenter & Kuhn, 2003; Naug, 2009; vanEngelsdorp & Meixner, 2010; Härtel & Steffan-Dewenter, 2014), and the importance of apiary location is axiomatic among practicing beekeepers, there are very few published studies that quantitatively measure colony success in response to local landscape variables. As rapid landscape conversion continues as a global phenomenon, and beekeepers in many regions continue to suffer unsustainable losses, the task of refining and expanding our knowledge of honey bee landscape ecology takes on obvious urgency.

Several studies have indirectly explored the relationship between landscape and colony success by analyzing the spatial information encoded in the honey bee dance language (von Frisch, 1967). Waddington et al. (1994) found that colonies located in two suburban landscapes tended to forage over a smaller area and with a less clumped
distribution than a previously studied colony located in a temperate deciduous forest (Visscher and Seeley, 1982), suggesting that suburban landscapes might provide richer and more evenly distributed resource patches. Similarly, Garbuzov et al. (2014) found that colonies in the city of Brighton, UK, concentrated most of their foraging within city limits rather than venturing into surrounding countryside that was well within their foraging range. Conversely, Beekman and Ratnieks (2001) observed remarkably long-distance foraging under conditions of apparently scarce local resources in a suburban landscape and highly rewarding resources in outlying seminatural heather moors. In agricultural landscapes, honey bee foraging patterns suggest that pollen sources can be scarcer and floral patches less spatially and temporally variable in highly simplified cropping systems compared to more structurally complex habitats (Steffan-Dewenter & Kuhn, 2003), while conservation management within farmlands can increase the availability of bee-attractive flora (Couvillon, Schürch & Ratnieks, 2014).

Landscape composition can also influence the type and quality of pollen foraged by honey bees. Donkersley et al. (2014) found that the protein content of “beebread” (processed pollen stored by honey bees) was negatively correlated with agricultural land cover and positively correlated with broad-leaf forest, improved grassland, and urban land cover.

Two recent studies have directly related colony success to local landscape variables (Sande et al., 2009; Odoux et al, 2014). In the dry coastal forest habitat of southeastern Kenya, Sande et al. (2009) found that a colony's honey production was positively correlated with its proximity to forest patches. Odoux et al. (2014) similarly
found that colony size was positively correlated with forest land cover in the intensively agricultural landscape of central-western France.

Among non-peer-reviewed sources, there is a widely circulated opinion that honey bee success is favored by urban/suburban landscapes, especially in comparison to cropland (Graham, 1992; New York Times, 2008; Wilson-Rich, 2012). These claims remain unsubstantiated but plausible given the ostensibly positive effects of suburban land use suggested by Waddington et al. (1994) and the more direct evidence supporting the favorability of suburban land use for bumble bees (Hymenoptera: Bombus, Latreille) living in predominantly agricultural areas (Goulson et al., 2002; 2010).

Here, we present a quantitative study of honey bee colony success in relation to landscape composition in the State of Ohio, USA, a region characterized by a mixture of intensive cropland, deciduous forest, grassland, and urban development. While there are many ways to measure colony success, we focused on four metrics that are highly relevant to beekeepers and easily assessed through simple hive inspection: honey and pollen accumulation, wax production, adult population, and brood population. Using a citizen-science survey, we investigate the relationship between colony success and the landscape as a whole, accounting for all major land cover types and also for the potential influence of hive management variables that vary between beekeepers. Then, we specifically evaluate the putative favorability of urban land use using a subset of sites dominated by urban development and/or cropland.
2.3 Materials and Methods

Survey design

In 2012 and 2013, we used a survey-based, citizen-science approach to measure the productivity of honey bee colonies in the state of Ohio, USA. All participants were beekeepers whose hives were registered with the Ohio Department of Agriculture and who volunteered to participate in our study. Volunteers were enlisted through a combination of email communications, public speaking engagements, and cooperation with local beekeeping organizations; our study was publicized as widely as possible, and we did not attempt to target any particular demographic. Our survey was conducted with written exemption from IRB review by the Ohio State University Office of Responsible Research Practices (Protocol # 2012E0136 and 2013E0012).

In order to standardize the initial strength of the colonies in our study (hereafter “study colonies”) and minimize the influence of parasites and pathogens, we restricted our study to colonies that had been started from artificial swarms, known as “package bees”, in the spring of each study year. Honey bee packages are created by combining a standard quantity of worker bees (usually 1.36 kg) with a newly mated queen. The initial strength of colonies started from package bees is, therefore, less variable than that of over-wintered colonies. Moreover, because they are sold without comb or brood, they tend to have reduced parasite and pathogen loads.

Data for each study colony were gathered using a two-part survey consisting of spring and fall components (hereafter “spring survey” and “fall survey”). The spring survey was made available beginning in early March, and participants were instructed to
complete the survey immediately after installing their honey bee packages. In the spring survey, we gathered the geographic location of each study colony and the years of experience of each participating beekeeper (see Appendix A for full spring survey questionnaire). The fall survey was made available in mid-September and completed by mid-October. To complete the fall survey, each participant performed a frame-by-frame hive inspection and reported the number of frames in the study hive belonging to the following categories: (1) more than half honey/nectar, (2) more than half pollen, (3) more than half brood, (4) more than half empty wax comb, (5) more than half bare foundation (no wax comb). Participants also reported the quantity of sugar syrup that had been given to their hives as supplemental feeding, a common beekeeping practice that could affect colony success. See Appendix A for full fall survey questionnaire.

Survey processing

Each beekeeper was instructed to submit data for only one study hive at one apiary site, and each beekeeper was included in only one of the two years of our study. The data quality of all surveys was carefully vetted prior to analysis, and surveys missing critical data or having irreconcilable inconsistencies were discarded. Fall surveys reporting hives that had died since spring installation were also discarded. The final numbers of surveys included in analyses for 2012 and 2013 were 32 and 18, respectively; these were selected from a pre-processing total of 55 surveys in 2012 and 33 in 2013. The minimum distance between study hives, combing both years, was 2.65 km.
From our survey data, we derived four metrics to represent colony success: *net food accumulation*, *net wax production*, *adult population*, and *brood population*. For consistency, all metrics were recorded in units of standard deep frames.

*Net food accumulation:*

\[
\text{Food} = H + H_{\text{harv}} - H_{\text{add}} + P
\]

where \(H\) = honey/nectar frames in hive at time of inspection, \(H_{\text{harv}}\) = honey frames harvested prior to inspection, \(H_{\text{add}}\) = honey frames added to the hive prior to inspection (beekeepers sometimes transfer honey frames between hives to increase food stores of weak colonies), and \(P\) = frames of pollen in hive at time of inspection. This variable will hereafter be abbreviated \(\text{Food}\).

*Net wax production:*

\[
\text{Wax} = H + H_{\text{harv}} + P + B + B_{\text{rm}} + D - H_{\text{add}} - B_{\text{add}} - D_{\text{add}}
\]

where \(B\) = brood frames in hive at time of inspection, \(B_{\text{rm}}\) = brood frames removed prior to inspection (brood frames may be transferred between colonies to modulate population size), \(D\) = drawn but mostly empty frames in hive at time of inspection, \(B_{\text{add}}\) = brood frames added to the hive prior to inspection, and \(D_{\text{add}}\) = drawn but mostly empty frames (frames with wax comb constructed but no cell contents) added to hive prior to inspection. This variable will hereafter be abbreviated \(\text{Wax}\).
Adult population (hereafter, AdultPop) was measured as the number of frames “more than half covered” with adult bees at time of inspection. Brood population (hereafter, BroodPop) was simply the number of “mostly brood” frames reported by the inspecting beekeeper.

We also measured two hive management variables: years of beekeeping experience of the participating beekeeper (experience) and quantity of sugar syrup fed to the study hive since its installation (syrup).

Landscape analysis

Geographic coordinates for each study hive were determined and mapped using QGIS v. 2.1 (QGIS Development Team, 2014). To encompass a range of spatial scales at which landscape effects on colony success might be seen, we defined the landscape of each hive using six nested buffers having radii of 0.5, 1, 2, 3, 4, and 5 km, respectively. Land cover data for the State of Ohio were obtained from the 2006 dataset provided by the National Land Cover Database (NLCD 2006) (Fry et al., 2011). The NLCD 2006 land cover layer for Ohio is comprised primarily of seven land cover classes: cultivated crops, pasture/hay, deciduous forest, and four levels of urban development (open space, low intensity, medium intensity, high intensity). Minor classes, present only at very low abundance, include evergreen forest, mixed forest, woody wetland, herbaceous wetland, grassland/herbaceous, shrub/scrub, barren land, and open water. To simplify our analysis of landscape composition, we condensed the non-crop land cover classes (ignoring barren land and open water) into three aggregate classes: Forest (deciduous +
evergreen + mixed + woody wetland + shrub/scrub), Grassland (pasture/hay + grassland/herbaceous + herbaceous wetland), and Urban (open space + low intensity + medium intensity + high intensity). The landscape composition of each study site, measured in terms of the total land cover of Crop (cultivated crop) and each aggregate class, was determined at each spatial scale using LECOS (Jung, 2013), a QGIS plugin for calculating patch-based landscape metrics. As a measure of overall landscape heterogeneity, we also calculated Simpson’s Diversity Index \( (D) \) based on the original, non-aggregated land cover classes.

Data Analysis

We first reduced the dimensionality of our landscape data using principal components analysis (PCA) based on the covariance between the variables Crop, Forest, Grassland, and Urban. This step was repeated for each spatial scale. For all scales, the first two principal components \( (PC 1 \text{ and } PC 2) \) explained > 96% of total variance.

To model the relationship between landscape composition and colony success, accounting also for the management variables experience and syrup, we conducted model selection using Akaike’s Information Criterion corrected for small sample size \( (AIC_c) \) (Burnham & Anderson, 2002). Each success metric--Food, Wax, AdultPop, and BroodPop--was modeled separately. Fourteen candidate linear models were constructed for each success metric at each spatial scale; these included all combinations of the landscape variables \( (PC 1, PC 2, D) \) and the coupled management variables experience and syrup, a year-only model, and an intercept-only model. For each success metric, we
present the candidate model having the lowest AICc score at each scale along with any competing models having an AICc difference of < 2 (Table 2.1) (Burnham & Anderson, 2002). We then selected a single best model for each success metric by choosing the model with the lowest AICc score across all spatial scales.

To evaluate the prediction that urban land cover favors honey bee success relative to agricultural land cover, we first extracted the subset of our sites (n = 30 to 33, varying with spatial scale) for which Urban + Crop was greater than 50% of total landcover, a threshold chosen a priori to identify sites that were strongly characterized by urban and/or agricultural land use. Then, we then set up separate linear regression models for Food and Wax with Urban as the explanatory variable. Only Food and Wax were analyzed because the results of the PCA described above indicated that only these two success metrics should be expected to respond to landscape variables. We did not use experience and syrup as covariates because previous analysis showed they were not predictive of Food or Wax. Regression analysis was repeated for each spatial scale.

All analysis was performed in R statistical software (R Core Team, 2014). AICc model selection used the package AICcmodavg (Mazerolle, 2014). Model assumptions were verified by visual assessment using the plot(lm) function in R.
Table 2.1 Summary of model selection statistics for each colony success metric. Only models with AICc < 2 are presented as competing models. Models within each spatial scale are listed in order of increasing AICc value. The best model for each success metric is depicted in bold.

<table>
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<th>METRIC</th>
<th>RADIUS (KM)</th>
<th>MODEL</th>
<th>LOG-LIKELIHOOD</th>
<th>K</th>
<th>AICc</th>
<th>ΔAICc</th>
<th>W</th>
<th>ADJUSTED R²</th>
<th>COEFFICIENTS</th>
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<td>0.233</td>
<td>0.047</td>
<td>-5.9142</td>
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<td>0.055</td>
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<td>0.563</td>
<td>0.131</td>
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<tr>
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Continued
Table 2.1 continued

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<th>PC2 + years + syrup</th>
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<td>331.620</td>
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BOLD = BEST MODEL FOR GIVEN SUCCESS METRIC
2.4 Results

Landscape analysis

The landscapes surrounding the colonies in our survey represented a broad range of landscape composition in terms of the major land cover classes *Crop*, *Forest*, *Grassland*, and *Urban* (Figure 2.1). Principal components analysis of these four variables yielded two readily interpretable axes that explained greater than 96% of total variance (Figure 2.2). *PC 1* was essentially an urban-rural axis, with sites dominated by *Urban* scoring low and sites dominated by combinations of *Crop*, *Forest*, and/or *Grassland* scoring high. *PC 2* partitioned non-urban landscapes into those characterized by *Crop* and those characterized by *Forest* and, to a lesser extent, *Grassland*. 
Figure 2.1. Landscape composition of study sites at 2 km radius. Sites are depicted in order of increasing urban (red) land cover. Other major land cover classes include crop (gold), forest (dark green), and grassland (light green). Remaining land cover (grey) consisted of barren land and open water.
Figure 2.2. Principal components biplot of major land cover classes at a radius of 2 km. Principal component 1 (PC1) comprises an urban-rural axis, with lower scores corresponding to higher urbanness. Principal component 2 (PC2) forms an axis that separates sites characterized by forest/grassland from those characterized by cropland. This pattern was consistent at all spatial scales with only minor variation.
Modelling colony success metrics by landscape principal components

*Food* and *Wax* were best modeled with *PC 2* as the only explanatory variable. Almost all competing models (ΔAIC<sub>c</sub> < 2) included *PC 2* alongside other explanatory variables, further supporting the conclusion that *PC 2* was the single most important predictor (*Table 2.1*). For *Food*, the optimal spatial scale was a 1 km radius, while *Wax* was best predicted at a 2 km radius. In both cases, the relationship was negative and the linear regression models were statistically significant (*Food*: \( F = 4.796, \text{df} = 48, p = 0.033; \) *Wax*: \( F = 6.184, \text{df} = 48, p = 0.016 \)) (*Figure 2.3*). *AdultPop* was best modeled with the coupled management variables *experience* and *syrup* as the only explanatory variables. The relationship was positive and the linear regression model was significant \( (F = 6.128, \text{df} = 47, p = 0.004) \), with significant contributions from both *experience* \( (t = 2.98, \text{df} = 47, p = 0.005) \) and *syrup* \( (t = 2.474, \text{df} = 47, p = 0.017) \) (*Figure 2.4*).

*BroodPop* was best predicted by the intercept-only model, indicating that none of our measured explanatory variables were good predictors of this success metric.

Modeling colony success by urban landcover

We found a significant \( (p < 0.05) \) negative relationship between *Food* and *Urban* (*Figure 2.5*) at all spatial scales except for the two extremes of 0.5 km and 5 km.; the relationship was strongest at the 2 km scale \( (F = 6.041, \text{df} = 29, p = 0.02) \). *Wax* was not significantly related to the *Urban* \( (p > 0.05) \).
Figure 2.3 Food accumulation and wax production were negatively correlated with PC2. This indicates that productivity in terms of food and wax increased in the direction of cropland and decreased in the direction of forest/grassland. A 95% confidence band is shaded in gray.

Figure 2.4 Adult population was positively correlated with beekeeper years of experience (A) and supplemental syrup feeding (B). A 95% confidence band is shaded in gray.
Figure 2.5. Colony food accumulation decreased significantly with increasing urban land cover in sites where Urban + Crop > 50%. This pattern was strongest at a 1 km radius (shown above). A 95% confidence band is shaded in gray.

2.6 Discussion

The negative responses of Food and Wax to PC 2 indicate that food accumulation and wax production increase with surrounding cropland and decrease with forest/grassland. This finding seems to contradict the conventional wisdom that agricultural land conversion threatens honey bee nutrition through the depauperation of floral resources relative to semi-natural environments (De La Rúa et al., 2009), but is
consistent with studies that have found honey bees to be notably resilient to natural habitat loss compared to other bee taxa (Ricketts et al., 2008; Winfree et al., 2009). The productivity of honey bees does not depend so much on the presence of undisturbed natural floral communities as it does on the availability of rich resources that can be exploited efficiently by cooperative foraging (Visscher & Seeley, 1982), and agricultural environments can offer honey bees surprisingly rich floral resources in the form of “weeds” (Odoux et al., 2012; Requier et al., 2014). In Ohio, the largest honey yield is believed to come from non-native clovers (Trifolium spp. L.) (Pellett, 1920; Bailey, 1955; Goltz, 1975); these plants grow abundantly along roadsides, in field margins, and in grassy yards, but they are scarce in habitats shaded by forest canopy or dominated by the dense herbaceous vegetation of unmowed grassland. In addition to the clovers, Erickson (Erickson, 1984) observed that, under some conditions, honey bees will forage very productively on soybean (Glycine max (L.) Merr.), and corn/soybean rotations comprise the vast majority of Ohio cropland. Dandelion (Taraxacum officinale F. H. Wigg.), one of the most important spring flora for honey bees in the Midwest (Jaycox, 1976) during the period of peak wax production, is distributed in much the same pattern as the clovers, thus favoring wax production in cropland over seminatural forest and grassland.

Interestingly, our finding that colony productivity is favored by cropland relative to forest/grassland is strikingly consistent with an anecdotal description of regional honey production in Ohio published nearly forty years ago (Goltz, 1975). In Goltz’ account, the areas of “primary” and “secondary” importance for honey production are in the heavily
cultivated glacial plains that comprise most of the state, while the forest-dominated Appalachian Plateaus in the southeast are described as only “marginally” productive.

The positive response of AdultPop to the management variables experience and syrup is difficult to interpret. In early spring, when new colonies are very small and limited in their foraging ability, it is standard practice to supplement colony nutrition with sugar syrup. All workers produced during the period of spring build-up, though, died long before colonies were inspected in the fall, so any positive effect of the springtime management on adult population at time of inspection would have to be mediated by factors that allow colonies to increase reproduction later in the year. An alternative interpretation is plausible if we allow that significant feeding may have occurred later in the year. While supplemental feeding is normally concentrated in early spring, some Ohio beekeepers also feed their colonies in mid-late summer, a period of perceived dearth in natural forage. Feeding during the summer dearth period might trigger a population increase that would persist until fall inspection. Our survey did not distinguish between feeding at different times during the season. The effect of beekeeper experience on adult population is difficult to parse, as all aspects of hive management would be expected to improve on average with increasing experience. Somewhat ironically, a positive relationship between colony success and beekeeper experience might be explained by the tendency of more experienced beekeepers to perform less colony management; the enthusiasm of new beekeepers can lead to unnecessary interventions that do more to disturb natural colony function than to ameliorate ills (James Tew, pers. comm., 2014).
By late September and early October, when beekeepers were inspecting their colonies for the fall survey, the bees had likely already begun to reduce brood rearing in preparation for winter (Graham, 1992). This would explain the failure of both landscape and management variables in predicting BroodPop.

The negative relationship observed between Food and the ratio of Urban in the subset of our sites strongly characterized by urban and/or agricultural land use does not support the popular opinion that urban landscapes favor honey bee success relative to agricultural landscapes. At least in Ohio, the relationship appears to be the opposite, and the fact that Food was the only success metric to respond to Urban ratio suggests a likely mechanism. The last major nectar and pollen flow in Ohio is usually from goldenrod (Solidago spp. L.) (Morse, 1972; D. B. Sponsler, unpublished data), which blooms prolifically from late summer into fall, roughly the same period during which beekeepers in our study were conducting fall hive inspections and filling out the fall survey. At this time of year, honey bees rarely produce additional wax (Lee & Winston, 1985), and brood rearing has begun to slow down in preparation for winter (Graham, 1992), so incoming food is stored rather than being invested in brood or wax production. Goldenrod occurs abundantly in uncultivated fields and conservation strips throughout agricultural landscapes, but it is relatively scarce in developed areas where vegetation is more often subject to mowing and weed control. This is consistent with the anecdotal observation of Burgett et al. (1978) that urban hives tend to have poor late-season honey production, which the authors attribute to scarcity of late-blooming “weeds”, including goldenrod.
We conclude that both landscape composition and colony management contribute to the success of nascent honey bee colonies in our study region. Due to complexities not explored in this study, the prediction of colony success was partitioned such that landscape predicted food accumulation and wax production, while colony management predicted only adult worker population. We find no support for the opinion that honey bees in urban landscapes are more successful than those in cropland. To the contrary, we find that colony food accumulation responds negatively to urban land cover in landscapes dominated by urban or agricultural land use, a pattern that we attribute to the influence of late-season floral availability, particularly goldenrod.

It is important to note that while model selection identified landscape composition as the best predictor of colony food and wax accumulation, the amount of unexplained variation in our models was high, indicating that factors other than the ones we measured are also at play in the determination of colony success. Such factors may include (1) fine-scale landscape variables that were not measurable using the NLCD dataset, (2) hive management variables not accounted for in our beekeeper survey, and (3) “in-hive” determinants of colony success like queen fertility, disease prevalence, and parasite load. We also suggest caution in generalizing our results beyond our study region. While the landscape of Ohio is broadly similar to much of the American Midwest, it would be premature to extend our findings to other ecoregions that may differ strongly both in natural flora and in agricultural practices.
2.7 References


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Chapter 3: Spatial and taxonomic patterns of honey bee foraging: a choice test between urban and agricultural landscapes

3.1 Abstract

The health of honey bee colonies cannot be understood apart from the landscapes in which they live. Urban and agricultural development are two of the most dramatic and widespread forms of human land use, but their respective effects on honey bees remain poorly understood. Here, we evaluate the relative attractiveness of urban and agricultural land use to honey bees by conducting a foraging choice test. Our study was conducted in the summer and fall, capturing a key portion of the honey bee foraging season that includes both the shift from summer- to fall-blooming flora and the critical period of pre-winter food accumulation. Colonies located at an apiary on the border of urban and agricultural landscapes were allowed to forage freely, and we observed their spatial and taxonomic foraging patterns using a combination of dance language analysis and pollen identification. We found a consistent spatial bias in favor of the agricultural landscape over the urban, a pattern that was corroborated by the prevalence in pollen samples of adventitious taxa common in the agricultural landscape. The strongest bias toward the agricultural environment occurred late in the foraging season, when goldenrod became the principal floral resource. We conclude that, in our study region, the primary honey bee foraging resources are more abundant in agricultural than in urban landscapes, a
pattern that is especially marked at the end of the foraging season as colonies prepare to overwinter. Urban beekeepers in this region should, therefore, consider supplemental feeding when summer-blooming flora begin to decline.
3.2 Introduction

The collective ability to survey a large foraging area and concentrate foraging effort on the most rewarding resources is a hallmark of honey bee foraging biology (Seeley 1995). This ability is conferred by the sophisticated dance language (von Frisch 1967) whereby individual foragers integrate their knowledge of resource availability, scent, and location (Seeley 1995; Grüter and Farina 2009). The intelligibility of the dance language to human observers allows the logic of honey bee foraging to be inverted to yield ecological insight: as a honey bee colony assesses its environment and allocates its foragers to the most rewarding resources, the spatial allocation of foragers revealed by the dance language can be used to infer the types of available habitat most suitable for honey bee foraging (Couvillon et al. 2014a; Garbuzov et al. 2014; Couvillon and Ratnieks 2015; Garbuzov et al. 2015). Spatial habitat inferences from dance language analysis can also be supported by the taxonomic identification of pollen loads collected by honey bees in the same study area (Garbuzov and Ratnieks 2013; Garbuzov et al. 2015).

Understanding the suitability of different habitat types for honey bee foraging is of central importance in the task of improving honey bee health and productivity, and honey bee habitat utilization may also inform the conservation of other pollinator species (Härtel and Steffan-Dewenter 2014). Moreover, the foraging decisions made by a honey bee colony with respect to its surrounding landscape can furnish theoretical insights into how the honey bee foraging system has evolved to optimize the collection of resources in complex environments (e.g. Visscher and Seeley 1982).
Because of the honey bee’s close association with humans, any discussion of honey bee foraging habitat must emphasize the role of human land use in shaping the composition, distribution, and abundance of floral resources (Härtel and Steffan-Dewenter 2014). Two main categories of human land use, urban development and agricultural cultivation, are comparably profound but divergent departures from a natural or semi-natural condition, and it is important to understand their respective effects on honey bee health. Studies directly comparing urban and agricultural landscapes with respect to honey bee health are equivocal, perhaps reflecting the enormous diversity of landscape composition subsumed by the terms “urban” and “agricultural”. In the UK, Garbuzov et al. (2014) found that honey bees located within the city of Brighton foraged almost exclusively within the urban environment rather than extending their flights into the agricultural countryside, and Donkersely et al. (2014) found that the protein content of beebread was correlated positively with urban land use and negatively with agricultural land use. In Denmark, hives located in predominantly urban landscapes were shown to have a higher average weight than those in mixed or predominantly agricultural landscapes (Lecocq et al. 2015). In the Midwestern USA, however, Sponsler and Johnson (2015) found that agricultural landscapes tended to favor honey bee productivity compared to urban or semi-natural (forest) habitat. Similar results were reported from Luxembourg, where Clermont et al. (2015) found frequent positive correlations between various forms of urban land use and honey bee overwintering colony loss, while certain forms of agriculture and rural land use tended to be negatively correlated with colony
loss. Other studies of honey bees in either urban or crop-dominated landscapes (not in direct comparison) have often suggested negative effects of both compared to more diversified landscapes (Steffan-Dewenter and Kuhn 2003; Couvillon et al. 2014a; Odoux et al. 2014; Requier et al. 2015; Danner et al. 2016; Dolezal et al. 2016; Smart et al. 2016; Youngsteadt et al. 2015).

Here, we directly compare the foraging quality of urban and agricultural landscapes by a field-scale choice test using honey bee colonies located at a site along the interface of city and farmland. To determine the relative allocation of foraging activity between these two landscapes, we use a combination of dance language analysis and pollen identification to infer both spatial and taxonomic patterns of foraging.

3.3 Methods

Study site and timeframe

An apiary consisting of five honey bee colonies—two in standard Langstroth hives and three in three-frame observation hives—was established on the grounds of a historic cemetery located on the western edge of the metropolitan area of Columbus, OH (Figure 1). Using QGIS 2.1 software (QGIS Development Team, 2016), we digitized the landscape within a five-kilometer radius of the apiary and classified it using the binary categories of “urban” (predominantly residential and commercial) and “agricultural” (predominantly field crop). Digitization was performed by tracing the boundaries between residential/commercial development (“urban”) and neighboring farmland (“agricultural”) visible in 2013 aerial imagery from the Ohio Statewide Imagery Program,
corroborated by reference to the 2011 National Land Cover Database land use layer (Homer et al., 2015). While the categories of “urban” and “agricultural” represent internally heterogeneous landscapes, parsing these categories into more specific landscape classifications was beyond the scope of this study; our central question was how the general land use syndromes represented by the terms urban and agricultural affect honey bee foraging. Thus, roadways and small residential areas occurring in predominantly agricultural surroundings were classified as part of the larger pattern of agricultural land use; similarly, forest patches and fields occurring in predominantly built-up surroundings were classified as urban.
Our study was conducted in the summer and fall of 2014, beginning in late July and continuing to late September. This time frame encompasses the phenological transition between summer and fall flora along with the critical period of pre-winter food storage.
Dance recording and decoding

From August 7 to September 26, 2014, dance behavior was recorded one day per week from the three observation hives, representing a total of seven days of foraging activity (no dances were recorded on August 21 due to poor weather conditions). On each recording day, a morning (0930-1100 hrs) and an afternoon (1300-1600 hrs) session were recorded, each lasting approximately 45 minutes. During each recording session, all three colonies were recorded simultaneously using three separate cameras. See Appendix B for a description of the camera models used.

Video from each recording session was first split into one-minute segments, and then every fifth segment was subsampled for analysis. Each one-minute analysis segment was imported separately into the FIJI distribution of the image analysis software ImageJ (Schindelin et al. 2012), and dances were decoded using the MTrackJ plugin (Meijering et al. 2012). Following Couvillon et al. (2012), four waggle runs from each dance were decoded, including two right turns and two left turns. See Supplemental Material (Appendix B) for details on the application of ImageJ to dance decoding.

Decoded dances from all three colonies were mapped together using the Bayesian probabilistic method developed by Schürch et al. (2013) in which decoded locations are plotted not as discrete points, but as probability clouds derived by sampling 1000 point location estimates from the posterior probability distribution of each dance. This method acknowledges the intrinsic uncertainty in the dance language and allows for the
computation of credible intervals to test for habitat-based foraging biases (Garbuzov et al. 2014; Garbuzov et al. 2015).

Pollen collection and identification

The two Langstroth hives were fitted with bottom-mounted pollen traps (Sundance I, Ross Rounds, Inc), and pollen was collected in one-week intervals on the same schedule as the dance recordings plus one sample on July 31 prior to the first date of dance recording. To minimize nutritional stress, pollen was alternatively trapped from only one of the two colonies each week while the other was allowed to forage freely. Thus, each pollen sample represents seven consecutive days of pollen trapping for one of the two colonies. Upon return to the laboratory, pollen samples were stored in an airtight container at -20°C.

A pollen reference collection was constructed by collecting floral specimens from the vicinity of the research apiary and from other locations in the region. Approximately 360 voucher specimens were collected, and an additional 74 pollen samples were collected directly from the anthers of plants in curated botanical gardens. All specimens were identified to the lowest possible taxonomic level. For a complete list of voucher specimens and prepared reference slides, see Appendix D.

Trapped pollen was first weighed (wet weight) and subsampled. From samples with a total mass of more than 100 g, a 10 g subsample was taken. All other samples were subsampled at 10% of their total mass. Pollen pellets from each subsample were then sorted by color, texture, and other visual characteristics into preliminary taxonomic
groups, with mixed pollen pellets [i.e. rarely occurring single pellets consisting of visible bands of contrasting pollens, such as described by Percival (1947)] and singletons (groups represented by only one corbicular pollen pellet) being omitted from further analysis. These preliminary taxonomic groups were weighed and then scanned using a flatbed scanner (Canon LiDE 210) to record the visual characteristics of the pollen prior to the destructive process of microscopic preparation.

From each of the preliminary groups, ten pollen pellets (or all pellets for groups having fewer than ten total) were mixed with several drops of water in a microcentrifuge tube to form a homogenous suspension. Then, small aliquots of suspended pollen were mounted on a microscope slide using glycerin jelly stained with basic fuchsin (Kearns and Inouye 1993).

Mounted pollen specimens were examined at 400-1000x and identified by comparison with similarly prepared specimens from the reference collection and by the corbicular characteristics recorded in the scanned images. For slides containing more than one pollen type (due to imperfect sorting or mixed foraging), the relative abundance of each pollen type was estimated by identifying and counting all grains within the microscope field of view and shifting the field of view until a total of approximately 500 grains were counted. Because the grains of different pollens vary widely in size, it is more informative to express the relative abundance of different pollens in terms of volume rather than grain count (O’Rourke and Buchmann 1991). Following O’Rourke and Buchmann (1991), we modeled each pollen type as either a sphere or ellipsoid and estimated its volume by measuring its mean polar and equatorial axis length (based on
five randomly selected grains) and applying the corresponding formula. The proportional volume of each pollen type was then multiplied by the total mass of the sorted group represented by the microscope slide to estimate the proportion of the total mass contributed by each pollen type.

Statistical analysis

Following Garbuzov et al. (2014, 2015), we computed Agresti-Coull 95% credible intervals for the proportion of urban foraging activity, treating the apportionment of point locations between the urban and agricultural landscape classes as a binomial distribution. Pseudoreplication was avoided by dividing the number of urban points (p) and the total number of points (n) by the number of simulations of each dance (1000) (Garbuzov et al. 2014; Garbuzov et al. 2015). Credible intervals not including 0.5 (i.e. an equal allocation of urban and agricultural foraging) were interpreted as indicating a statistically significant positive or negative bias. Credible intervals were computed individually for each day of dance recording and then also for the pooled data set of all days. All analyses were performed in R (R Core Team 2015) using the “prevalence” package (Devleesschauwer et al. 2014).

3.4 Results

Spatial foraging patterns

For all dates, the majority of foraging activity occurred in the agricultural landscape, and this bias was significant in each case (credible interval for the proportion
of urban foraging < 0.5) (Figure 3.2). The proportion of urban foraging rose each sampling date from August 7 (0.16) to September 4 (0.38), began to decline on September 12 (0.20), and then fell sharply on September 19 (0.04) and remained low on September 26 (0.10).
Figure 3.2. Spatial foraging data (top) aligned with corresponding pollen data (bottom). On all dates, a significant majority of foraging activity inferred from dance language analysis occurred in the agricultural landscape (error bars depict 95% Agresti-Coull credible intervals, none of which include 0.50). Color-coded area plot shows the relative abundance of major (≥ 2.5% of at least one sample) pollen taxa; minor taxa are shown in gray. *Chamaecrista* types 1 and 2 differed notably in corbicular color but were both matched microscopically to a *C. fasciulata* reference specimen. This is most likely due intraspecific variation in the pollen or in the honey added to it by the bees, but it is possible that our samples included the closely related *C. nictitans*, which was not represented in our reference collection but is found in Ohio.
Foraging activity was most concentrated near the apiary, as expected from previous studies of honey bee foraging distance (e.g. Couvillon et al. 2014b). When foraging activity ranged more than 1 km from the apiary, it was consistently concentrated in the agricultural landscape to the south and west, though occasional foraging occurred along the urban-agricultural interface to the north (August 7, September 4) and in the urban landscape to the east (September 12) (Figure 3.3). The most distant foraging occurred September 4, when a small amount of activity occurred in the agricultural landscape approximately 4 km southwest of the apiary.
Figure 3.3. Spatial foraging patterns inferred from dance analysis for each sample date. The complete black ring depicts a 3 km radius around the study apiary, and the incomplete black ring in the lower left of each panel represents the southwestern extremity of the 5 km radius to which landscape classification was constrained. The urban-agricultural border is demarcated by a black line, and the urban area (east of the border line) is shaded darker. Foraging activity is represented for each sample date by a probability density cloud (red color ramp) depicting the relative probability that each patch (25 x 25 m bin) was visited by bees whose dances were decoded. The number of decoded dances for sample date is shown in the bottom right of each panel.
Taxonomic foraging patterns

Between July 31 and September 12, pollen samples were comprised mainly of legumes (\textit{Trifolium} and \textit{Chamaecrista} spp.) and wild carrot (\textit{Daucus carota}) (Figure 3.2). These gradually gave way to Canada goldenrod (\textit{Solidago canadensis}), which became the predominant pollen source in the last two weeks of the study period.

Beside these major taxa, many minor pollen types occurred in low abundance. A total of 42 pollen types were identified in our samples, representing at least 11 plant families (Appendix C), and this number almost certainly underestimates the true taxonomic richness due to our omission of singleton pollen pellets.

In addition to these, one sample contained several corbicular pellets of fungal spores, as has been occasionally documented by other observers (Wingfield et al. 1989).

3.5 Discussion

Honey bees in our study exhibited a consistent, and often dramatic, foraging bias in favor of the agricultural over the urban landscape. This pattern, observed directly in our spatial foraging data, was corroborated taxonomically by the overwhelming prevalence in our pollen samples of flora common in Midwest agricultural landscapes (\textit{Daucus carota}, \textit{Trifolium} spp, \textit{Chamaecrista fasciculata}, \textit{Solidago canadensis}). It should be noted that spatial patterns inferred from dance analysis reflect both pollen and nectar foraging, whereas pollen identification reveals only the former. Nevertheless, most of the flora that dominated our pollen samples—particularly the \textit{Trifolium} spp. and \textit{Solidago canadensis}—are also known to be major nectar sources for honey bees (Pellett,
1920; Goltz, 1975; Ayers and Harman, 1992), so it is likely that the spatial foraging patterns we observed largely represent patches of the principle floral taxa found in our pollen samples.

The degree of bias toward agricultural habitat varied, though, across sampling dates, and this variation corresponded to taxonomic shifts in pollen collection (Figure 3.2). The greatest proportion of urban foraging occurred on September 4, when pollen samples were dominated by clovers (Trifolium spp.). The sharp decline of urban foraging activity starting September 12 coincided with the taxonomic shift from clovers to goldenrod, the latter evidently being concentrated in the agricultural landscape to the south and west of the apiary (Figure 3.3). White clover, due to its prostrate growth habit, can tolerate frequent mowing and is common in urban open areas including residential lawns (Frank and Hathaway, 2015) as well as in the field margins and roadsides of the agricultural landscape. In contrast, goldenrod, with its tall growth habit, is restricted to unmowed open areas; in our study region, these include mainly uncultivated fields and conservation strips, consistent with the growth patterns and habitat associations described by Pavek (2011). By mid-September in our study region, the summer-blooming clovers are in decline and goldenrod emerges as the last major pollen and nectar source of the year. In the absence of late blooming urban flora, like the ivy (Hedera helix) common in the UK landscape studied by Garbuzov et al. (2013), strictly urban honey bees without access to other foraging habitat might suffer an early end to their foraging season, as predicted by Burgett et al. (1978) and inferred by Sponsler and Johnson (2015). Urban
beekeepers in our study region should, therefore, consider providing supplemental feeding as soon as the major summer flora begin to decline.

The preference of honey bees for agricultural over urban landscapes observed in this study must be interpreted cautiously. Urban landscapes can differ markedly from one another, both within and between cities. In Ohio, for example, the densely developed landscape of Columbus differs strongly from the landscape of nearby Cleveland, which contains extensive ruderal land colonized by adventitious plants. Similarly, agricultural landscapes, even those with the same major crops, can differ significantly in the composition and prevalence of non-crop vegetation, which may provide the bulk of honey bee foraging resources (Requier et al. 2015; Long and Krupke 2016). Nevertheless, the adventitious plants of the agricultural landscape that supported honey bee foraging in our study—particularly clovers and goldenrod—are widely recognized as major pollen and nectar sources throughout much of the U.S. and Canada (Pellett, 1920; Goltz, 1975; Severson and Parry, 1981; Ayers and Harman, 1992; Stimec et al., 1997; Long and Krupke, 2016), and it is likely that wherever these plants are of prime importance, agricultural landscapes will surpass their urban counterparts in the provision of honey bee foraging resources in the summer and fall. This pattern could potentially be offset by changes in urban land management that would allow flowering plants to grow in areas conventionally maintained as turfgrass, such as residential yards and public greenspaces.
3.6 References


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Chapter 4: Mechanistic modeling of pesticide exposure: the missing keystone of honey bee toxicology

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4.1 Abstract

The role of pesticides in recent honey bee losses is controversial, partly because field studies often fail to detect effects predicted by laboratory studies. This dissonance highlights a critical gap in the field of honey bee toxicology: there exists little mechanistic understanding of the patterns and processes of exposure that link honey bees to pesticides in their environment. We submit that 2 key processes underlie honey bee pesticide exposure: (1) the acquisition of pesticide by foraging bees and (2) the in-hive distribution of pesticide returned by foragers. The acquisition of pesticide by foraging bees must be understood as the spatiotemporal intersection between environmental contamination and honey bee foraging activity. This implies that exposure is distributional, not discrete, and that a subset of foragers may acquire harmful doses of pesticide while the mean colony exposure would appear safe. The in-hive distribution of pesticide is a complex process driven principally by food transfer interactions between colony members, and this process differs importantly between pollen and nectar. High priority should be placed on applying the extensive literature on honey bee biology to the development of more rigorously mechanistic models of honey bee pesticide exposure. In
combination with mechanistic effects modeling, mechanistic exposure modeling has the potential to integrate the field of honey bee toxicology, advancing both risk assessment and basic research.
4.2 Introduction

The potential risk that some pesticides pose to honey bees is universally acknowledged, but the extent to which specific chemistries can be blamed for particular patterns or incidents of colony damage is controversial. Most recently, this controversy has surrounded the neonicotinoid insecticides and their possible role in honey bee losses in Europe and North America (Godfray et al. 2014). While laboratory experiments have clearly established the potential for both lethal and sublethal effects of neonicotinoids on individual bees (Cresswell 2011; Blacquière et al. 2012; Godfray et al. 2014), field studies have often failed to detect colony-level effects (Cutler & Scott-Dupree 2007; Nguyen et al. 2009; Pohorecka et al. 2012; 2013; Pilling et al. 2013; Cutler et al. 2014; Rundlöf et al. 2015), and where colony-level effects have been observed (Sandrock et al. 2014; Alburaki et al. 2015; Budge et al. 2015), their biological significance is unclear.

Any putative link between a toxic compound and a toxic effect is necessarily predicated on some model, whether stated or implied, of toxic exposure. At present, though, there exists little mechanistic understanding of the patterns and processes of honey bee pesticide exposure (USEPA 2012), and this might account for much of the dissonance between laboratory predictions and field observations (Carreck & Ratnieks 2014) and the controversy surrounding the design and interpretation of field studies (Hoppe et al. 2015).

Mechanistic modeling of toxic exposure is not a novel task in the larger field of ecotoxicology (Pastorok et al. 1996; Sample et al. 1997), and sophisticated exposure
models have been developed for many organisms, including humans (Loos et al. 2010b). Honey bees, however, present unique challenges to exposure modeling due to their complex social biology (USEPA 2012; Wisk et al. 2014). A healthy honey bee colony is composed of 3 castes: a single reproductive female (the queen), up to several hundred males (drones), and many thousands of sterile females (workers). The worker caste, which is responsible for all colony tasks except reproduction, is further subdivided into loose, age-based functional guilds: new workers initially clean and cap cells, then progress to brood and queen tending, then to comb construction and food handling, and finally to the outside tasks of ventilation, guarding, and foraging (Winston 1987). As foragers, they may collectively survey over 100 km² of the environment surrounding their hive (Seeley 1995), collecting nectar, pollen, resin, and water from a vast array of sources. Foraged materials are then returned to the hive where they are processed and utilized in various ways by other colony members. In this complex economy, all castes and life stages are vulnerable to toxic exposure from multiple routes, and parsing this system into tractable components for modeling is no trivial challenge.

Constraining our discussion to pesticide exposure initiated by foraging in a contaminated environment (i.e. excluding in-hive pesticide applications), we identify 2 main challenges of honey bee exposure modeling: (1) predicting the acquisition of pesticide by foraging honey bees (primary exposure) and (2) tracing the in-hive distribution of pesticide returned to the hive by foragers (secondary exposure). For concision, we will refer to these 2 challenges, respectively, using Purdy’s (2015) terminology of “primary” and “secondary” exposure. Within this framework, we explore
the biological mechanisms underlying exposure, review existing efforts to capture these mechanisms through quantitative modeling, and discuss ways in which future models can achieve greater predictive and heuristic power. We conclude that both primary and secondary exposure are governed by aspects of honey bee behavior and environmental complexity that have not been adequately addressed in existing models, and that these oversights are manifest principally in the failure to represent exposure as a fundamentally individual-based phenomenon that cannot be subsumed by colony-level approaches to honey bee toxicology (Figure 4.1).
Figure 4.1. Relationship between individual-level exposure, individual-level effects, and colony-level effects. A normal distribution of individual exposure levels (A) clustered tightly around the mean (blue dashed line) results in a very small proportion of the colony experiencing doses above the predicted no effect concentration (PNEC) (red dashed line). Bimodal (B) or lognormal (C) distributions having the same mean as the normal distribution result in a much larger proportion of bees experiencing pesticide doses in excess of the level of concern. The distribution of exposures (depicted by red color intensity) experienced by individual bees causes a distribution of individual effects (depicted by opacity), ranging from mild sublethal impairment to death (upside-down bees). These individual effects may translate into effects on colony-level functions.
4.3 Primary Exposure

Pesticide exposure begins with the foraging of bees in a contaminated environment. This results both in the exposure of the foragers themselves and, perhaps more importantly, the delivery of pesticide to the rest of the colony. Though not considered here, bees may also be exposed to pesticides applied inside the colony by the beekeeper to control parasites and pathogens.

Biological background

Honey bees gather resources from their surrounding landscape within a foraging range that routinely extends a few kilometers from the hive (Couvillon et al. 2014a) and can extend considerably farther under conditions of local scarcity and distant reward (Beekman and Ratnieks 2001). Foragers integrate their individual knowledge of resource patches through a unique “dance language” (von Frisch 1967; Seeley 1995) that communicates, among other things, the odor and location of valuable forage (Grüter & Farina 2009). This generates colony-level knowledge of a vast foraging environment, which, in combination with private information (Biesmeijer and Seeley 2005; Grüter and Ratnieks 2011), enables the colony to focus its foraging effort on the most rewarding resource patches (Seeley, et al. 1991; Seeley 1994).

Because flowering plants are heterogeneous in their spatial distribution and bloom phenology, honey bee foraging is characterized by marked spatiotemporal heterogeneity (Visscher and Seeley 1982; Henry et al. 2012b; Couvillon, et al. 2014a, b). Spatiotemporal heterogeneity similarly characterizes environmental pesticide
contamination, since pesticide application is normally restricted to discrete landscape components during discrete time intervals. Primary exposure, therefore, must be understood as the spatiotemporal intersection of environmental contamination and honey bee foraging activity, jointly determined by environment and behavior. This means that a mechanistic exposure model must consist of 2 basic components: (1) a submodel of environmental contamination, and (2) a submodel of honey bee foraging behavior.

Existing models

Most models of primary exposure have not attempted mechanistic representations of both environmental contamination and honey bee foraging behavior, and some attempt neither. Here, we present a summary of existing models in order of increasing mechanistic realism (Table 4.1).
Table 4.1. Summary of existing quantitative models of primary exposure. Models are described according to the modes of pesticide application they represent, the modes of exposure they estimate, and their approaches to modeling the critical components of environmental contamination and honey bee foraging behavior.

**Contact exposure models for foliar sprays**

A traditional model for estimating contact exposure to foliar sprays is the Atkins model, which uses a simple conversion factor, originally derived from a large empirical data set, to estimate the critical field application rate needed to reach LD50 contact exposure (dose needed to kill 50% of exposed bees) in bees foraging on a treated crop (assuming early morning, pre-foraging application) (Atkins et al. 1981) (Equation 4.1).

$$LD50 \ (\mu g \ a.i/bee) \times 1.12 = \text{critical field application rate} \ (g \ a.i./ha)$$  (Eq. 4.1)
Algebraic conversion yields a prediction of contact exposure per bee given a known application rate (Equation 4.2).

\[
\text{field application rate (g a.i./ha)} / 1.12 = \text{bee exposure (µg a.i/bee)} \quad (\text{Eq. 4.2})
\]

Poquet et al. (2014) propose an approach that estimates per-bee exposure (assuming that bees are foraging in the field at the time of application) by multiplying field application rate (g/ha) by the effective exposure surface area of a honey bee (1.05 cm²). The latter value they calculated by exposing bees to controlled spray applications in the laboratory and taking the average ratio of the application rate (mass/area) and the resulting residues detected on treated bees (mass/bee).

As tools for screening-level risk assessment, these models meet the demand for simplicity and ease of use. They are not, however, designed to model spatial or temporal heterogeneity of environmental contamination or foraging behavior.

*Bee-REX contact and dietary exposure model*

The U.S. Environmental Protection Agency (USEPA) has recently developed the Bee-REX model to predict both contact and dietary exposure of foraging honey bees under a variety of pesticide application scenarios (USEPA 2014). Analogous to the Atkins model, contact exposure for aerial spray is estimated as a simple conversion factor based on the field data of Koch and Weiβer (1997). Dietary exposure (contamination of
nectar and pollen) via foliar spray is estimated using the contamination rate determined for tall grass vegetation in the terrestrial residue exposure model (T-REX), a model based on the work of Hoerger and Kanega (1972) and originally designed to estimate pesticide residues in avian and mammalian food items. Dietary exposure via systemic translocation of seed treatment pesticides is assumed to be the peak estimate (1 ppm) recommended by Alix et al. (2009); dietary exposure via systemic translocation of tree trunk injections is estimated as the mass of injected pesticide divided by the tree’s combined mass of leaves and flowers; and dietary exposure via systemic translocation of soil treatment is estimated using the fugacity model of Briggs et al. (1982, 1983). All dietary exposures are converted from concentration to mass-per-bee doses using estimates of feeding rates for each caste and life stage.

The Bee-REX model is comprehensive in scope while retaining the ease of use needed to be an effective screening-level exposure model. Nevertheless, Bee-REX suffers from the same key shortcomings as the Atkins and Poquet models: all exposure estimates ignore variability in environmental contamination and the behavioral patterns of honey bee foraging.

*Barmaz drift model of dietary exposure*

When a treated crop itself is not attractive to foraging bees or not in bloom at the time of treatment, field application rate is no longer a meaningful determinant of honey bee exposure. To account for this, Barmaz et al. (2010, 2012) model a scenario in which a pesticide sprayed on a crop drifts into off-crop habitat (Barmaz et al. 2010; Barmaz et al.
In this model, environmental contamination is modeled by a function relating active ingredient deposition to distance from crop edge. Temporal dynamics are also accounted for by calculating rates of pesticide movement and decay. Honey bee foraging is assumed to occur only in off-crop vegetation, which is subject to a gradient of pesticide contamination determined by the drift function, and predicted exposure is taken to be the mean of the contamination gradient in the off-crop habitat.

The Barmaz model addresses the issue of variation in environmental contamination by calculating a drift gradient of pesticide deposition. For simplicity, though, this gradient is collapsed into its mean, which effectively removes the element of spatial heterogeneity from the resulting exposure estimates. A unique strength of the Barmaz model, though, is that it incorporates an additional dimension of heterogeneity by modeling pesticide movement and decay through time. As with all the models discussed so far, though, the Barmaz model attempts no mechanistic treatment of honey bee foraging, except to acknowledge that it does not occur in an unattractive/non-blooming crop.

EFSA landscape model of dietary exposure

In its guidance document on bee risk assessment (EFSA 2013), the European Food Safety Authority (EFSA) presents a preliminary model designed to estimate the average concentration of pesticide in nectar and pollen entering a honey bee colony from a heterogeneously contaminated landscape. The model is highly generalized, designed to
accept as input any discrete pattern of environmental contamination and any estimate of pesticide concentration in floral nectar or pollen. Its basic form is given by Equation 4.3.

\[
PEC_{\text{hive}} = \frac{\sum_{n=1}^{N} f_n a_n PEC_n}{\sum_{n=1}^{N} f_n a_n}
\]  

(Eq. 4.3)

where \( PEC_{\text{hive}} \) is the average concentration of pesticide entering the hive in pollen or nectar from \( N \) patches, \( f_n \) is a coefficient representing the attractiveness of patch \( n \), which has surface area \( a_n \) and a nectar/pollen pesticide concentration of \( PEC_n \). By dividing the area- and attractiveness-weighted sum of all patch concentrations by the attractiveness-weighted total patch area, an average concentration of pesticide entering the hive in nectar or pollen is calculated.

The EFSA model is remarkable in its versatility; given an estimate of pesticide concentrations in floral nectar or pollen and an estimate of the relative attractiveness of relevant floral patches, the average concentration entering a honey bee colony can be calculated for any landscape and any application scenario, and this concentration can be converted to a per-bee dose using feeding rate estimates. This design allows the EFSA model to accommodate virtually any degree of complexity and mechanistic realism in the representation of environmental contamination, provided that contamination is assumed to be spatially discrete (i.e. patch-based, not gradient-based). A major weakness of the model, which its authors acknowledge, is that it is extremely sensitive to errors in estimating a colony’s effective foraging range, since this value defines the spatial scale of the model and has a strong effect on the area term in the denominator of the exposure
equation. The model also relies heavily on estimates of the attractiveness of different flora to honey bees, and such estimates are scarce and difficult to verify across different contexts. Perhaps most importantly, though, the EFSA model represents exposure only as a colony-level mean and does not deal with the distributional nature of individual-level exposure.

Baveco dilution model of dietary exposure

The recent model of Baveco et al. (2016) is by far the most mechanistic with respect to honey bee foraging behavior. In the basic (“single optimal”) version of this model, a virtual colony selects a single optimal forage patch, based on the optimization of energetic efficiency (a function of floral properties and patch distance), from within a heterogeneous landscape composed of potentially treated mass-flowering crops and untreated non-crop features. Patch selection is then iterated over hourly time steps to incorporate the effects of nectar depletion on patch selection. The more complex (“recruitment limited”) version of the model adapts a previous model of honey bee foraging (Camazine and Sneyd 1991; Seeley et al. 1991) to simulate the dynamic allocation of foragers across multiple resource patches, regulated by rates of recruitment and abandonment. Both of these mechanistic approaches to simulating patch selection avoid imposing an assumed foraging range as in the EFSA model. The net concentration of pesticide in foraged nectar (pollen is not considered) over the simulation period is determined by the proportion of foragers that collected from treated crop vs. alternative
habitat. Thus, the potential diluting effect of uncontaminated forage are taken into account.

The strengths of the Baveco model are that it (1) explicitly accounts for spatial heterogeneity of environmental contamination, (2) incorporates the mechanistic role of honey bee foraging behavior in determining pesticide exposure, and (3) simulates the pesticide collected on individual foraging trips rather than just the colony average. This last point, while not emphasized by the authors in the paper (since the focus was on the dilution of the colony average by uncontaminated forage), is perhaps the most important, as will be discussed in the Future steps section. A weakness of the Baveco model is that it cannot be expanded to include exposure via contaminated pollen because its energetics-based patch selection mechanism is relevant only to nectar foraging. The authors also acknowledge that the model relies on somewhat speculative parameters related to floral resource properties and landscape composition, but there is no reason why, in principle, the model could not parameterized more rigorously with empirical data from a particular study area.

Future steps

*Modeling environmental contamination*

Real landscapes—even intensively cultivated ones—are composed heterogeneously of treated and untreated habitat, and thus contain a range of pesticide contamination levels. A honey bee colony’s foragers, therefore, are not exposed to a uniform pesticide dose but rather to a distribution of doses, likely ranging all the way from null to some
maximum (USEPA 2012; EFSA 2013). Nevertheless, existing models that acknowledge
the heterogeneity of environmental contamination (EFSA 2013; Baveco et al. 2016) still
present exposure estimates in terms of colony average (though the Baveco model does, in
fact, calculate exposure on an individual basis).

Little is gained and much obscured by collapsing a distribution of exposure levels
into some central tendency, for average exposure and exposure to the average are not
interchangeable concepts (Macintosh et al. 1994; Purucker et al. 2007). Consider the
situation represented in the Baveco model in which a colony forages either on or off a
uniformly contaminated crop, and compare the exposure predictions of the Atkins,
Poquet, Bee-REX, EFSA, and Baveco (“single optimal” version) models, respectively
(excluding the Barmaz model because we are assuming the treated crop is attractive). To
make the models directly comparable, we assume the following: (1) application is by
foliar spray at a uniform rate of 80 g/ha, with no off-field drift, (2) the application rate of
80 g/ha translates into a uniform concentration of 80 ppb in floral nectar, (3) the treated
crop and alternative forage are equally attractive to honey bees, equal in floral density
and nectar concentration, and never depleted, (4) patches of treated crop and alternative
forage are equidistant from the hive, and (5) honey bees choose randomly between
equally suitable forage patches (this is to account for the fact that, in the Baveco model,
patch selection is based on a deterministic evaluation of patch reward, but under our
assumptions foraging patches do not differ in reward).

**Figure 4.2** summarizes this comparison when performed separately for contact
exposure (Atkins, Poquet, and Bee-REX models) and dietary nectar exposure (Bee-REX,
EFSA, and Baveco models), and repeated under 3 scenarios with differing abundance of treated vs. untreated foraging habitat. Comparing predictions of contact exposure underscores the fact that the Atkins, Poquet, and Bee-REX models are similar in that they estimate exposure by applying a simple coefficient to field application rate. None of these models is designed to account for heterogeneity of contamination, so each, respectively, yields the same exposure prediction under all 3 scenarios. Comparing predictions of dietary exposure shows that, in each scenario, the EFSA model estimates exposure to be the mean of the field contamination distribution while the Bee-REX model, not accounting for heterogeneity of contamination, performs just as it did in the prediction of contact exposure. The Baveco model is unique in that it has the potential to represent the field contamination distribution as a distribution. In each of the scenarios presented, the Baveco model would distribute foragers across the 2 levels of contamination in proportion to the abundance of each; so, for example, under Scenario A, 50% of simulated foragers would collect 0 ppb and 50% would collect 80 ppb. It is important to note, though, that if the distribution of exposure levels encountered by foragers is collapsed to its mean, as it is presented in Baveco et al. (2016), then the Baveco model effectively reduces to the EFSA model under the simplifying assumptions of our comparison. The problem with any approach that collapses a distribution of exposure into a mean is that the mean concentration may actually be quite rare in the environment and experienced by few individual bees. For example, in a strongly bimodal distribution of environmental contamination, such as the one depicted in Figure 4.1B, the mean level of exposure is rare, and both lower and higher levels of exposure would be much more
commonly encountered. The mean of a distribution, without both its form and variance, reveals neither the proportion of foragers that acquire a potentially dangerous dose nor the range of doses that enter the hive.
Figure 4.2. Comparison of contact and dietary exposure estimates. Bars represent the proportion of foraging habitat that is untreated or treated with an application rate of 80 g/ha. Scenario A represents equal abundance of contaminated and uncontaminated forage. In Scenarios B and C, the relative abundance is skewed toward either the contaminated forage (Scenario B) or the treated crop (Scenario C). Under contact exposure, axes labeled “Atkins”, “Poquet”, and “Bee-REX” are transformations of the main x-axis (depicting field application rate) using the coefficients by which field application rate is multiplied in each model. The dashed line shows where the exposure predictions of the models fall with respect to the range of environmental contamination levels caused by a range of field application rates. Under dietary exposure, the dotted lines represent the predictions of the EFSA model and Baveco models (when the latter is collapsed to its mean) for each scenario, while the dashed lines represent those of the Bee-REX model. Asterisks, with subscripts representing the percentage of foragers exposed to the indicated dose, show the raw distribution of exposure predicted by the Baveco model.
CONTACT EXPOSURE

Poquet

Bee-REX

DIETARY EXPOSURE
(nectar)

EFSA/Baveco(mean)
Bee-REX
Baveco(distribution)

0 18 35 54 71 89

0 21 42 63 84 105 µg/bee

0 20 40 60 80

0 20 40 60 80 100

0 20 40 60 80 100

0 20 40 60 80 100

proportion of foraging habitat

field application rate (g/ha)

concentration in floral nectar (ppb)

Atkins

Poquet

Bee-REX

0 5 10 14 19 24

0 5 10 14 19 24

0 5 10 14 19 24

0 5 10 14 19 24

0 20 40 60 80

0 20 40 60 80 100

0 20 40 60 80 100

0 20 40 60 80 100

0 20 40 60 80 100

SCENARIO A

SCENARIO B

SCENARIO C
The heterogeneity of contamination and the distributional nature of toxic exposure have been more fully explored outside of honey bee biology (Purucker et al. 2007; Wickwire et al. 2011). For example, Schipper et al. (2008) and Loos et al. (2010) modeled the exposure of terrestrial vertebrates to levels of cadmium contamination that were heterogeneous both in terms of spatial distribution and concentration in different food items. In a model of human pesticide exposure, Leyk et al. (2009) used a “dynamic hazard surface”, a cellular automata model combining land use data with rates of pesticide deposition and decay, to simulate both the spatial and temporal distribution of pesticide levels in a patch-based landscape. In principle, there is no reason why similar models of heterogeneous environmental contamination could not be applied to pesticide exposure in honey bees.

*Modeling honey bee foraging behavior*

Honey bee foraging biology has been studied extensively, and many mechanistic models already exist [reviewed in Becher et al. (2013)]. The challenge for exposure modeling is not to break new theoretical ground but simply to apply existing knowledge to pesticide exposure scenarios.

Two principals of honey bee foraging–neither of which have been seriously discussed in the context of toxicology–should be addressed in future exposure models. First, and most importantly, colony-level foraging is the collective activity of thousands of individual bees, each of which interacts uniquely with the distributions of floral resources and pesticide contamination in the foraging landscape. While dance language
recruitment creates a degree of non-independence between foragers, the spatial
coarseness of recruitment relative to environmental contamination gradients, the constant
temporal fluctuations in both contamination levels and floral reward, and the propensity
of foragers to ignore the information of the dance language and search independently
(Grüter and Farina 2009) ensure that a colony’s thousands of foragers experience a broad
distribution of exposure levels (Koch and Weißber 1997). As discussed already, a
distribution of doses is not toxicologically equivalent to its central tendency (the exposure
of a hypothetical “average bee”), so the distributional nature of exposure must be
acknowledged and represented in exposure models (Macintosh et al. 1994; Purucker et al.
2007). Second, while selective recruitment to resource patches introduces a non-random
element to honey bee foraging behavior (Frisch 1967), there is evidence that the initial
discovery and continual rediscovery of resource patches is governed by stochastic search
behavior (Reynolds et al. 2007; 2009). It is impossible to predict exactly how a honey bee
colony’s foraging force will be distributed across a landscape, which means that the
distribution of pesticide doses encountered by foragers is an effectively stochastic
phenomenon that should ideally be modeled probabilistically (USEPA 2012). This
problem weakens the predictive potential of exposure models that do not explicitly
simulate the stochastic process of patch selection. The EFSA model, for example,
conceptually distributes foragers across all foragable patches in proportion to their
attractiveness, when in reality a colony would be expected to forage from only a
relatively small subset of available patches over any given time interval (Visscher and
Seeley, 1982). This approach is acceptable if the goal is to evaluate the theoretical
“average” exposure risk for a colony in a given landscape, but it will likely not yield good predictions of actual exposure under specific scenarios.

It is also worth noting that no existing model of primary exposure explicitly addresses the collection of contaminated water or resin. Water, in particular, may be an importance route of exposure in some scenarios (e.g. Samson-Robert et al. 2014) and it deserves to be considered alongside nectar and pollen.

4.4 Secondary Exposure

Pesticide exposure begins in the field, but processes that occur inside the nest are at least as important in determining the exposures experienced by individual colony members (Rortais et al. 2005; USEPA 2012; Wisk et al. 2014; Purdy 2015; Berenbaum 2016). The distribution of exposures generated by foraging (i.e. primary exposure) forms the input to secondary exposure, which begins as soon as a contaminated forager returns to the nest.

Biological background

Incoming nectar and pollen can undergo extensive processing and redistribution prior to consumption, which may significantly modify the initial distribution of pesticide concentrations returned to the hive by foragers (Figure 4.3). The key to elucidating the distribution of pesticide inside the hive is modeling the complex processes of in-hive food transmission. To do this, nectar, pollen, and secreted brood food and royal jelly (hereafter referred to collectively as “jelly”) must be discussed separately.
Figure 4.3. Processing pathways of nectar-associated and pollen-associated pesticide. Pesticide-laden nectar (red) undergoes extensive trophallactic transmission prior to consumption, resulting in widespread but dilute ingestion of nectar-associated pesticides. Pesticide-laden pollen (blue) undergoes no mixing or dilution and is consumed almost exclusively by nurse bees, which may, therefore, receive more extreme (higher and lower) pesticide doses than other colony members. Nurses convert pollen-derived nutrients into glandular secretions (jelly) (purple) that they combine with variable amounts of nectar and raw pollen; these mainly nourish the brood and queen but also supply the dietary protein needed by adult workers and drones.
Nectar

A forager returning with nectar transfers her nectar load via trophallaxis to 1 or more “receiver” bees (younger workers tasked with food handling) (Park 1925). A receiver bee, upon accepting a nectar load from a forager, proceeds to store, process, and/or redistribute the nectar according to the needs of the colony. Under typical conditions, the receiver bee initiates a cascade of trophallactic transfers, giving portions of her load to several other bees, which may, in turn, distribute portions of nectar to additional bees (Maurizio 1975; Seeley 1989; Pirez and Farina 2004; Grüter and Farina 2007). This pattern of food transmission may proceed through many iterations before the nectar is ultimately consumed or deposited in cells for storage (Maurizio 1975), and the process is so efficient that labeled sugar syrup gathered by only a few foragers can be detected in many (Nixon & Ribbands 1952) or all (Feigenbaum & Naug 2010) colony members within just a few hours of initial collection in the field. Consequently, nectar from a single contaminated floral patch may be ingested by all or most colony members, potentially causing pervasive intoxication. Such extensive distribution, however, involves thorough mixing with nectar from potentially uncontaminated sources, so a distribution of field concentrations in nectar would become homogenized toward its mean, increasing the likelihood that a pesticide dose consumed by any particular bee will be highly diluted from the concentration of contaminated nectar in the field.

Another important consideration is that honey bees preferentially transfer nectar to nestmates of similar age, resulting in a gradual net flow of incoming food from the older bees (foragers), to the middle-aged bees (receivers, comb builders), and finally to
the younger bees that are tasked with feeding the queen and brood (Nixon and Ribbands 1952; Free 1957; Feigenbaum and Naug 2010). In this way, the younger workers in the colony, along with the queen and brood, may be buffered against toxic exposure arising from contaminated nectar (Feigenbaum and Naug 2010). It must also be noted that foraging honey bees, in addition to receiving nectar/honey from nestmates in the hive, consume some freshly foraged nectar during their return flights from the field (Brandstetter et al. 1988). Thus, they are exposed to undiluted pesticide doses against which hive bees are buffered by the diluting effect of nectar transmission. This may serve as a critical safeguard against severe toxic exposure in the colony, since foragers collecting highly contaminated nectar will likely perish in the field before sharing their toxic payload among nestmates. The potentially adaptive nature of forager mortality is especially interesting in light of the fact that homing impairment is a frequently observed symptom of pesticide exposure (Vandame et al. 1995; Bortolotti et al. 2003; Henry et al. 2012a; Matsumoto 2013).

*Pollen/beebread*

In contrast to nectar, incoming pollen is not mixed or shared among nestmates. Instead, a returning pollen forager searches out a storage cell directly and unloads pollen pellets into it (Parker 1926). The forager does not process the pollen further, but leaves it to be discovered by pollen-packing bees, which add honey and saliva to the fresh pollen and pack it tightly into the bottom of the cell (at which point the pollen can be referred to
as “beebread”) (Parker 1926). Successive pollen loads are packed on top of each other, forming a stratified column.

While nectar is consumed by all colony members, pollen is consumed almost exclusively by the nurse bees, young workers whose principal work is the tending of brood and queen. Pollen consumption peaks in bees between 4 and 9 days old and decreases to negligible amounts in bees over 20 days old (Crailsheim et al. 1992), closely mirroring the age-dependent activity of proteolytic enzymes that enable pollen digestion (Moritz and Crailsheim 1987). Nurse bees convert the nutrients of dietary pollen into protein-rich glandular secretions (jelly) that comprise the primary food of brood and queens and are shared to a lesser extent with adult colony members of all ages (Crailsheim 1992).

Unlike pesticide-laden nectar loads, which may be thoroughly mixed with other nectar sources prior to consumption, pesticide-laden pollen loads remain segregated in the stratified column of each pollen cell. Any mixing of loads can only occur through individual nurse bees’ consuming pollen from more than 1 storage cell or layer during a feeding bout. The extent to which this occurs has never been reported, but even if some mixing occurs by this mechanism, nurse bees feeding on pollen are likely subject to pesticide doses that reflect the distribution of concentrations collected by foragers much more closely than do the extensively homogenized doses arising from nectar/honey transmission.
**Jelly**

Jelly secreted by nurse bees originates from the hypopharyngeal and mandibular glands of the head, and may be mixed with regurgitated honey and/or pollen, depending on the age and caste of the recipient (Haydak 1970; Winston 1987). The extent to which dietary pesticides can be translocated to the hypopharyngeal and mandibular glands and incorporated into their secretions is largely unknown and no doubt varies with the physicochemical properties of the active ingredient involved. While several studies have documented pesticide residues in jelly (Wittman and Engels 1981; Stoner et al. 1985; Davis and Shuel 1988; Johnson and Percel 2013; Dively et al. 2015; though see De Grandi Hoffman et al. 2013), it is possible that this contamination arose through the incorporation of contaminated nectar and/or pollen into secreted jelly rather than pesticide translocation to glandular tissue (Wittman and Engels 1981; Davis and Shuel 1988).

**Grooming**

Apart from the transmission of contaminated food, grooming behavior could be a significant pathway of exposure to pesticides carried on the body surface. Honey bees both self-groom and allogroom (groom nestmates). Self-grooming is performed mainly with the legs, but often targets the mouthparts (especially the glossa) (Linghu et al. 2015), while allogrooming is performed with the mandibles (Haydak 1945; Milum 1947). Both forms of grooming, therefore, create the potential for oral exposure. Allogrooming is performed principally by “grooming specialist” bees, a small minority of the worker
population (Kolmes 1989). Notably, exposure to particulate matter induces both self-grooming and allogrooming (Land and Seeley 2004), suggesting that grooming may be an especially important route of exposure for microencapsulated pesticides and pesticidal dusts.

Existing models and future steps

Existing models of the in-hive distribution of pesticides have focused on beekeeper-applied acaricides that are introduced directly to the colony (Tremolada et al. 2004; 2011; Bonzini et al. 2011). These models have approached the problem of in-hive distribution from the perspective of fugacity, dividing the colony into internally homogeneous compartments (e.g. wax, bees, honey, air) among which a pesticide becomes partitioned according to its physicochemical properties.

Because beekeeper-applied pesticides largely bypass the usual food transmission process, compartment-based fugacity modeling is a reasonable approach to predict their in-hive fate. For pesticides that enter the hive in contaminated nectar and/or pollen, though, the food transmission process is arguably the more important mechanism of in-hive pesticide distribution, at least over short time scales. Moreover, just as in the modeling of primary exposure, it is vital to predict the distribution of doses experienced by individual bees, not just the aggregate partitioning of pesticide to the “bee compartment”.

Unlike honey bee foraging biology, in-hive food transmission has not been studied through mechanistic modeling, and basic work remains to be done before the
food transmission dynamics can be incorporated into a pesticide exposure model. There is, however, a wealth of empirical and theoretical studies on in-hive food transmission [reviewed in Crailsheim (1998), Farina and Grüter (2009)] that supplies ample material for the design and parameterization of models. The fact that in-hive food transmission involves the complex interaction of many autonomous entities immediately recommends an agent-based modeling (ABM) approach (Railsback and Grimm 2011) that could take full advantage of the many detailed studies of the behavioral rules of food transmission. Moreover, ABMs are fundamentally designed to track state variables on an individual basis, enabling the distributional modeling of exposure levels experienced by individual bees. The development of an ABM, though, is typically a long and demanding process, especially when the model is intended to support regulatory decision-making (Topping et al. 2009; Grimm et al. 2014). A simpler, though minimally mechanistic, approach would be to simulate in-hive pesticide distribution by Monte Carlo sampling. Given an input distribution of pesticide concentrations in nectar or pollen loads delivered to the hive by foragers, it would be possible to emulate the food transmission process by conducting repeated random draws (representing individual colony members) from the input distribution (or some transformation thereof) in a fashion similar to the probabilistic approach of Macintosh et al. (1994).

While we emphasize active food transmission as the key mechanism of in-hive pesticide fate, we acknowledge the importance of complementing food transmission models with models of fugacity and pesticide degradation. This is especially important for pesticide exposure via contaminated nectar/honey. Once ripened, honey can be stored
for weeks or months prior to consumption, during which time passive fugacity and degradation processes would be the main mechanisms affecting the dynamics of nectar-associated pesticides.

The relative importance of grooming as a route of exposure is difficult to estimate. Compared to food transmission, grooming behavior has received little research attention aside from its effects on parasitic mites, and more extensive behavioral studies must precede any attempt at quantitative modeling.

4.5 Discussion and Conclusions

While we acknowledge the role of simple and conservative exposure models as a component of risk assessment frameworks, effective modeling requires a cycle of mechanistic insight and strategic simplification. Simple models designed for efficient risk assessment must be informed and continually revised by reference to more complex models that aim both to predict pesticide exposure and to understand the fundamental mechanisms that govern it. Thus, while complex mechanistic exposure models may never be practicable as standard risk assessment tools, they are necessary to evaluate the validity of risk assessment models and, just as importantly, to advance the basic study of honey bee toxicology.

Perhaps the most salient shortcoming of existing models (except the Baveco model) is the failure to estimate exposure as a distribution of individual doses rather than a discreet “colony-level” dose. This is true even of the most nuanced conceptual models of honey bee pesticide exposure (e.g. Purdy 2015), despite the fact that the individual
variability in exposure is empirically evident (Koch and Weißer 1997). What has led to this dubious consensus?

As a eusocial “superorganism”, the collective functions of a honey bee colony are insulated from the death or impairment of individual bees by a complex web of compensatory mechanisms and negative feedback loops (Henry et al. 2015; Berenbaum 2016). Since the endpoints of concern for honey bee risk assessment are usually colony-level functions like honey production, pollination services, and overwintering survival, there has been a trend in research away from individual-level laboratory assays and toward colony-level studies that aim to observe the net effects of toxic exposure after all the mechanisms of social buffering have played their roles.

An unfortunate consequence of this paradigmatic shift from the individual to the colony is that the legitimate notion of colony-level effects has become implicitly conflated with the misconceived notion of colony-level exposure. Toxic effects can be understood as perturbations of patterns or processes at either the individual- or colony-level. For example, sublethal neonicotinoid exposure can induce the physiological effect of impaired homing ability on individual foragers (e.g Henry et al. 2012), but if enough foragers suffer homing failure, colony-level functions like food acquisition and brood rearing could be disrupted, potentially leading to a fatal breakdown of colony homeostasis (Henry et al. 2012). Individual- and colony-level effects are mechanistically linked, albeit in complex ways, and each is amenable to observation and experimentation. Conversely, toxic exposure is scientifically tractable only when it is understood as the spatiotemporal intersection between a toxic agent and a discrete receptor organism. This
makes the concept of “colony-level exposure” highly problematic. If the term “colony” is used to represent a higher-order system defined by patterns, processes, and relationships emerging from interactions between individuals (e.g. information integration, division of labor, genetic structure, collective fitness), then there is no measurable sense in which such a composite of abstractions can be said to intersect in space and time with a toxic agent. If, alternatively, the term “colony” is used simply to represent an aggregation of associated individuals, then “colony-level exposure” is nothing more or less than the set of unique exposure events experienced by the individual members of a colony. Since the first concept of colony-level exposure is not amenable to empirical methods and the latter concept is indistinguishable from individual-level exposure, it must be concluded that toxic exposure can only be studied as a fundamentally individual-based phenomenon, and that this is equally true of both primary and secondary exposure (Figure 4.1).

Such an individual-oriented approach to honey bee exposure modeling poses considerable challenges, but without a mechanistic understanding of exposure, the rapidly proliferating studies of toxic effects in the laboratory and in the field will remain insolubly disjunct. There is ample precedent for the development and application of rigorous exposure models in the larger context of ecotoxicology and ecological risk assessment, and while the honey bee poses some unique challenges to exposure modeling, it is among the world’s most thoroughly studied organisms, and a wealth of empirical and theoretical literature is available for the construction and parameterization of models. Indeed, many aspects of honey bee biology have already been modeled
extensively (Becher et al. 2013), and the main task of exposure modeling is simply to apply existing knowledge to toxicological scenarios.

The future of honey bee exposure modeling is especially compelling in view of recent advances in mechanistic modeling of pesticide effects, particularly using the versatile BEEHAVE model (Becher et al. 2014; Rumke et al. 2015; Thorbek et al. 2016). The conjunction of mechanistic effects modeling and mechanistic exposure modeling will lead to an unprecedented depth of insight into honey bee toxicology, simultaneously advancing the protection of honey bee health and the basic study of ecotoxicology in a social insect model system.

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Chapter 5: Elucidating the route of exposure linking honey bees to seed treatment neonicotinoids released during corn planting

5.1 Abstract

The overwhelming majority of corn planted in the United States is seed-treated with neonicotinoid insecticides. These can be released from the seed surface during planting and disperse as dust throughout the landscape. This phenomenon is well-documented and has been associated with honey bee mortality incidents throughout North America and Europe, but the precise route or routes of exposure that link honey bees to the neonicotinoids released during corn planting have remained difficult to ascertain. Without a clear understanding of how exposure occurs, mitigation efforts may fail to reduce exposure or even exacerbate it. Here, we analyze the results of a two-year study of honey bee exposure to seed treatment neonicotinoids during corn planting in central Ohio, USA. Beginning with the premise that exposure requires the spatial intersection of honey bee foraging activity with environmental contamination, we construct statistical models that partition contamination into different landscape components and weight contamination by the probability of honey bee visitation. These models are then used to evaluate the predictions of several proposed routes of exposure that might account for observed patterns of honey bee mortality and neonicotinoid residue in honey-bee-collected pollen. Our results support the hypothesis that the
primary route of exposure is the contamination of flowering weeds present in corn fields at the time of planting. This suggests that in-field weed control prior to corn planting would be an effective approach to mitigation. Nevertheless, exposure via in-flight contact with airborne dust cannot be ruled out, and we also recommend mitigation approaches aimed at reducing the initial release of neonicotinoid-laden seed treatment particles during routine planting operations.
5.2 Introduction

It is conservatively estimated that at least 79% of corn hectares in the United States are grown from seed treated with neonicotinoid insecticides (Douglas and Tooker 2015). The predominant neonicotinoids used in corn seed treatments are clothianidin (Poncho®) and thiamethoxam (Cruiser®). These compounds are closely related, clothianidin being a metabolite of thiamethoxam (Nauen et al. 2003), and both are potent agonists of insect nicotinic acetylcholine receptors (Tomizawa and Casida 2005). The efficacy of these broad spectrum insecticides extends to many non-target insects, including honey bees, to which they are fatal in nanogram quantities (Decourtye and Devillers 2010) (Table 5.1). The application rate of clothianidin and thiamethoxam as corn seed treatments ranges from 0.25–1.25 mg per seed. If corn is planted at a seeding rate of 81,500 seeds per hectare, which is the approximate rate recommended in Ohio, then the amount of active ingredient applied per hectare ranges from 20.38–101.88 g. Thus, each hectare of treated corn planted contains enough active ingredient to deliver an LD_{50} dose to at least four billion honey bees. Nevertheless, the opportunity for honey bees to be exposed to seed treatment neonicotinoids should be minimal if the active ingredient remains bound to seed; hence, neonicotinoid seed treatments are judged by many to be relatively safe alternatives to other chemistries and application methods (Carreck and Ratnieks 2014).
Table 5.1. Toxicity parameters and application rate of corn seed treatment neonicotinoids.

<table>
<thead>
<tr>
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<th>ACUTE TOXICITY PARAMETERS (µG/bee)*</th>
<th>APPLICATION RATE</th>
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<tbody>
<tr>
<td></td>
<td>Oral LD50 (48 h)</td>
<td>Contact LD50 (48 h)</td>
</tr>
<tr>
<td>CLOTHIANIDIN</td>
<td>0.003</td>
<td>0.022–0.044</td>
</tr>
<tr>
<td>THIAMETHOXAM</td>
<td>0.005</td>
<td>0.024–0.03</td>
</tr>
<tr>
<td></td>
<td>mg a.i./seed**</td>
<td>seeds/ha (field corn)***</td>
</tr>
<tr>
<td></td>
<td>0.25–1.25</td>
<td>81,500</td>
</tr>
<tr>
<td></td>
<td>20.38–101.88</td>
<td>(field corn)</td>
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</table>

* Reproduced from Decourtye and Devillers (2010)
** Range of labeled rates
*** Based on recommendations for Ohio (Thomison 2015)

A relationship between honey bee mortality and the planting of neonicotinoid-treated corn was first suspected in 1999, when Italian researchers noted a rise in colony damage reports coinciding with spring corn planting (Bortolotti et al. 2009). In subsequent years, similar patterns of honey bee mortality were observed in Italy (Schnier et al. 2003, Greatti et al. 2006, Bortolotti et al. 2009), France (Giffard and Dupont 2010), and Slovenia (Alix et al. 2009; van der Geest 2012; Zabar et al. 2012), but evidence for a causal link with seed treatment neonicotinoids remained inconclusive. In 2008, however, a large-scale bee kill in Germany and neighboring parts of France was uncontroversially attributed to the planting of neonicotinoid-treated corn after an extensive investigation found neonicotinoid residues in dead bees, bee bread, and plant samples collected from the affected area (Chauzat et al. 2010, Forster 2010, Nikolakis et al. 2010, Pistorius et al. 2010). Since then, additional incidents of honey bee mortality during corn planting, accompanied by varying degrees of documentation, have been reported in Slovenia and neighboring Hungary (van der Geest 2012), the United States (Krupke et al. 2012; L. Keller, personal communication, 2016) and Canada (Health Canada 2013).
While these reports clearly establish a causal relationship between the planting of neonicotinoid-treated corn and honey bee mortality, the mechanistic basis for this relationship has remained largely speculative, due in part to the multiplicity of potential exposure routes. During the planting process, seed treatment material sloughs off the seed surface in small particles that disperse in the environment (Figure 5.1). Once released, these particles can intersect with bees by multiple routes, including direct floral contamination through dust deposition, indirect floral contamination via root uptake and systemic translocation, contamination of surface water, contamination of guttation fluids, and direct contact between aerial dust and flying bees [reviewed in Krupke and Long (2015)]. While it is likely that all these routes contribute to honey bee exposure to varying degrees, discerning which route (or routes) is of principal importance is crucial in the design of appropriate mitigation schemes. Floral contamination, for example, might be effectively reduced by suppressing flowering weeds in and around corn fields, but this approach could exacerbate aerial contact by forcing bees to fly farther to meet their foraging needs. A modified exhaust system deflecting expelled particles downward into the soil (Nuyttens et al. 2013) might reduce aerial contact but perhaps magnify the contamination of in-field flowering weeds. Both the efficacy and economic feasibility of mitigating honey bee exposure to seed treatment neonicotinoids depend on an accurate understanding of exactly how exposure occurs.
Figure 5.1. Seed treatments are applied to seeds as flowable solids that dry to form a coating. In corn, this coating tends to adhere poorly to the seed surface, resulting in visible patchiness of coverage (left). As the seed treatment coating degrades, it crumbles into particles of varying size, captured here by scanning electron microscopy (SEM) (right). The striated surface visible in the center of the micrograph is the seed surface, uncovered by the broken seed treatment coating. Macrophotography was performed by M. Spring, and SEM preparation by K. Kaszas.

Of the many plausible routes by which honey bees may be exposed to seed treatment neonicotinoids during corn planting, two main routes emerge as being most likely to cause acute effects during the planting period, such as those documented in the incidents cited above: floral contamination and aerial contact.

Floral contamination

The foraging environment of honey bees in the corn agricultural system of Ohio consists of a combination of in-field and off-field flora. Due to variation in tilling and herbicide application practices, some fields contain flowering weeds (e.g. *Taraxacum officinale, Lamium purpurea, Brassica* spp.) at the time of corn planting while others are barren. These in-field flowering weeds are subject to immediate deposition of seed
treatment particles during planting and thus comprise a likely route of exposure for foraging honey bees. The spring season also affords ample foraging outside of fields, though, including both herbaceous (*Taraxacum officinale*, Brassicaceae spp., Lamiaceae spp.) and woody flora (*Acer* spp., *Fraxinus* spp., *Salix* spp., *Lonicera* spp., rosaceous trees) (Lin et al., in prep). While these off-field sources do not receive the immediate seed treatment deposition that in-field flora do, they can become contaminated by seed treatment particles that drift beyond the field edge (Krupke et al. 2012). Importantly, in-field and off-field floral contamination present different management challenges; while in-field flora can be sprayed or tilled by farmers, off-field flora involve multiple stakeholders with different interests.

**Aerial contact**

Initial investigations of honey bee poisoning during corn planting focused on indirect exposure via the contamination of floral resources (e.g. Pistorius et al. 2010), but later studies have suggested that the direct intersection of flying bees with airborne seed treatment particles may pose a greater risk (Girolami et al. 2011, 2013; Tapparo et al. 2012). These studies have focused on the intersection between flying bees and the localized plume of insecticide-laden air that occurs within about 20 m of a running planter (Girolami et al. 2013). Direct intersections of this kind can yield exposure levels 78–1240 ng a.i. per bee (Tapparo et al. 2012), one to three orders of magnitude higher than the contact and oral LD$_{50}$ values for clothianidin and thiamethoxam (Decourtye and Devillers 2010) (**Table 5.1**). It is also possible, though, that in addition to the threat of
localized dust plumes, very fine dust particles may become suspended and widely
distributed in the atmosphere, forming a diffuse hazard that could conceivably become
decoupled from the location and extents of a planted field. This route of exposure
remains largely unexplored, but it is plausible given that the size range of dust particles
generated during corn planting extends as low as < 20 µm in diameter (Foqué et al. 2014,
Devarrewaere et al. 2015).

Any hypothesis regarding a route of toxic exposure is a fundamentally spatial
proposition because the question of how pesticide exposure occurs is essentially the
question of where the critical intersections between a toxic hazard and a receptor
organism exist. This principle is acknowledged in the growing number of spatially
explicit models of exposure in the ecotoxicology literature (Wickwire et al. 2011), and it
is especially salient in the scenario of honey bee exposure to seed treatment
neonicotinoids during corn planting. Contamination in this scenario is driven by the
release of mobile particles in discrete landscape elements–corn fields–and the subsequent
transport of these particles to surfaces and/or airspace both within and outside fields.
Exposure occurs when these spatial patterns of contamination overlap with spatial
patterns of honey bee foraging activity, which, in turn, are constrained both by the
behavioral rules of honey bee foraging and the spatial patterns of floral resource
availability (see chapter 4). Thus, a hypothesized route of exposure linking honey bees to
seed treatment neonicotinoids implies certain predictions about how patterns of exposure
will relate to patterns of overlap between environmental contamination and honey bee
foraging activity. These predictions can be formulated as statistical hypotheses and then tested to evaluate the plausibility of the hypothesized route of exposure.

Here, we analyze two years of data on honey bee exposure to seed treatment neonicotinoids during corn planting in central Ohio, USA, with the goal of identifying the route of exposure most consistent with observed patterns of exposure and mortality. We first construct a model of seed treatment particle drift to estimate the distribution of neonicotinoid contamination in the landscapes surrounding each of our research apiaries. Then, we construct a model of honey bee foraging that predicts patch visitation probability as a function of distance from hive location. By combining these models, we estimate the degree of intersection between foraging bees and different landscape components (with their corresponding contamination levels). By relating these components of “risk” to the distinct predictions of each hypothesized route of exposure, we infer which route of exposure is most supported by our data.

5.3 Methods

Field methods

*Field sites*

In the springs of 2014 and 2015, research apiaries were established at a total of 13 different sites (A-M) located throughout the corn growing region of central Ohio (Figure 5.2). Six different sites were used in 2014 and ten in 2015, with three sites overlapping across the two years. Sites were selected to represent a range of corn-growing intensity, including one suburban site (H) used in 2015 to approximate a negative “control”
landscape with respect to corn. The composition of the landscape surrounding each site within a 2 km radius, a radius that contained the majority of observed foraging activity (see dance analysis section below), was quantified using a combination of visual ground-truthing and aerial image interpretation (Google OpenLayers), and landscapes were digitized using QGIS software (QGIS Development Team 2015). Immediately prior to corn planting each year, fields within 2 km of each site were visually inspected to determine the prevalence of flowering weeds. Bloom levels of 2, 1, and 0 were assigned to fields having abundant, sparse, and absent flowering weeds, respectively.

Figure 5.2. Study sites were distributed throughout central Ohio (left). Sites used only in 2014 are indicated by yellow dots, sites used only 2015 by blue dots, and sites used both years by green dots. Central Ohio landscapes are characterized by intensive field crop (mainly corn/soybean rotation) cultivation, which appears as brown areas in the National Landcover Database map (Homer et al. 2015) (right).

Timing of corn planting

The timing of peak corn planting activity was determined by communicating with local growers. While the exact timing of planting varies from grower to grower, corn
yield in Ohio is maximized by planting early, so the most intense planting occurs early in
the season as soon as conditions are suitable. In 2014, the period of peak corn planting
activity occurred between May 5 and May 10, though unusually rainy weather made the
planting activity in our study region somewhat more protracted than most years, with
some late planting or re-planting continuing through the end of May. In 2015, good
weather allowed growers to complete planting quickly, with the bulk of planting in our
study region occurring between May 2 and May 8.

**Sampling and analysis of pollen and dead bees**

Pollen and dead bees were collected concurrently from each apiary. In 2014,
collections were made every 2 days during the peak corn planting period and every 3-4
days outside of the peak corn planting period; in 2015, collections were made every 3-4
days throughout the whole study period. In both years, the peak corn planting period
included a total of three sampling dates.

Pollen was collected using pollen traps (Sundance I, Ross Rounds, Inc.), and dead
bees were collected and counted using devices known as drop-zone dead bee traps.
Honey bees maintain nest hygiene by removing dead individuals from inside the nest and
dropping them near the entrance. The drop zone dead bee trap is a box that extends in
front of the hive (100 x 50 cm) to catch expelled dead bees, with a coarse top screen to
exclude scavenging birds and a fine bottom screen to exclude other scavengers like ants.
In both years, pollen was collected from two colonies at each apiary per sampling date,
while dead bees were collected from four colonies at each apiary per sampling date. The
allocation of colonies to pollen collection and dead bee collection, though, varied slightly across years. In 2014, four colonies were monitored at each site; pollen was collected from all colonies but in an alternating fashion such that pollen traps were turned “on” for two of four colonies during each sampling interval and then switched for the next sampling interval. All colonies were equipped with dead bee traps, and dead bees were collected from all four colonies on each sampling date. In 2015, a total of six colonies were monitored at each apiary. Two were dedicated pollen sampling colonies with pollen traps “on” throughout the whole sampling period, while the other four colonies were used only for dead bee sampling and were not equipped with pollen traps.

Bulk pollen samples were subsampled and then sent for neonicotinoid residue analysis. In 2014, 5-gram subsamples of each bulk pollen sample were analyzed at the USDA-AMS lab in Gastonia, NC. In 2015, 3-gram subsamples were analyzed at the EPA Ecosystems Research lab in Athen, GA. All residues were reported as mass-mass concentration (parts per billion).

Spatial analysis of dust drift

The spatial pattern of seed treatment neonicotinoid drift during corn planting was studied using a modified version of the protocol developed by Krupke et al. (2012). Dosimeters were constructed of PVC pipe material and mounted with two arrays of five microscope slides. One array was mounted vertically at a height of about 2 m, while the other was mounted horizontally at a height of about 1/3 m. Each array of slides was sprayed with Tangle-Trap® adhesive (The Tanglefoot Company). During each trial,
dosimeters were set up at varying distances from the edge of the test field, in linear series of four, each dosimeter separated from its neighbor by about 30 m. In 2014, the distances were 1, 10, 50, and 100 m, plus one array of slides placed in the field directly under the path of the planter (0 m distance). In 2015, trials included 0, 5, and 10 m distances. Dosimeters were always placed in the approximate downwind direction relative to the planted field, though wind direction was sometimes erratic, as is typical in our study area during spring planting. Planting activity during each trial consisted of multiple planter passes beginning at the field edge and continuing until planting was completed for roughly the first 100 m from the field edge. Off-field dosimeters remained in place for all of these planter passes, but the in-field arrays of slides that were placed directly under the path of the planter’s first pass were retrieved after the first pass. Following each trial, all slide arrays were detached from the dosimeters and stored in a covered container to prevent an additional movement of seed treatment particles. After the conclusion of field activities each day, dosimeter slides were stored at -20°C to await chemical analysis.

To quantify the neonicotinoid residue on each set of slides, three slides from each 5-slide array were subsampled for analysis. Each triplet of slides was placed in a 50 mL tube, with the individual slides separated by pipette tips used as spacers. Then, each tube was filled with 50 mL LCMS-grade acetonitrile along with 20 ng d4-imidacloprid (Sigma) to serve as an internal standard. After 1 h of sonication and 24 h incubation in darkness at room temperature, the acetonitrile solution in each tube was transferred to a new tube. This solution was then dried down under nitrogen and resuspended in 1 mL
acetonitrile, and the concentration of neonicotinoids was determined by LC-MS analysis (Ohio State University’s Campus Chemical Instrument Center, Columbus, Ohio).

Using the pooled data from both years for the horizontal arrays, we modeled the surface concentration (ng a.i. / cm$^2$) of seed treatment neonicotinoids in the landscape as a piecewise function of distance from corn field edge, such that the estimated concentration at each distance was equal to the highest mean concentration observed at or beyond that distance (Equation 5.1, Figure 5.3). To account for the fact that the slides placed under the planter at the 0 m distance were picked up after a single pass, we added the mean concentration for the 10 m distance to that of the 0 m distance; after the initial planter pass, the 0 m slides would have accumulated seed treatment particles at approximately the same rate as the 10 m slides since planters since typical planters are roughly 10 m wide.

\[
f(x) = \begin{cases} 
(4.47 + 1.95), & x < 0 \\
2.08, & 0 < x < 1 \\
1.95, & 1 < x < 10 \\
1.45, & 10 < x < 50 \\
0.98, & 50 < x < 100 \\
0, & x > 100 
\end{cases}
\]

Eq. 5.1

Spatial analysis of honey bee foraging

Observation hives were set up at four of our field sites in 2015 (B, C, G, and K). Dances were recorded every 1-3 days during the peak corn planting period and less frequently (every 1-12 days) as late at May 26, after the conclusion of most planting. The exact schedule of recording varied across sites due to logistical constraints. Each
recording session lasted approximately one hour. Dance videos were then subsett by extracting for analysis one minute of video every five minutes, resulting in one-minute analysis clips separated by four-minute intervals. Videos were decoded following (Couvillon et al. 2012), modified for use with the FIJI image analysis software (Schindelin et al. 2012). Decoded dances were then mapped using the Bayesian probabilistic method of (Schürch et al. 2013).
Figure 5.3. Seed treatment deposition as a function of distance from field edge. Piecewise drift function (red) represents the highest mean contamination level observed at or beyond each sampling distance. Raw data points are plotted below (black circles) to visualize the variation observed.

We first pooled the data from the four observation hive sites across both site and date and plotted the simulated dance locations with respect to an arbitrary common origin. Then, we plotted the visitation probability of each patch within a 10 km radius of the origin against its distance from the origin and fitted the resulting pattern with a...
nonlinear least squares function, following a modified version of the methods described in Couvillon et al (2014) \((\text{Equation 5.2, Figure 5.4})\)

\[ y_i = (0.1204)(e^{-0.001404 x_i}) \] \hspace{1cm} \text{Eq. 5.2}

where \(y_i\) is the probability of visitation for patch \(I\), and \(x_i\) is the distance of patch \(I\) from the hive. This function describes the general relationship between visitation probability and patch distance (Couvillon et al. 2014) and indicates that the 2 km radius used to delimit landscape characterization encompassed roughly 79% of total foraging activity.
Figure 5.4. Visitation probability as a function of distance. Data represent dances decoded from four sites between May 4 and May 26, 2015, pooled across site and date. Each point represents a landscape bin (25 x 25 m) in a 20 x 20 km grid. For each bin, visitation probability (y-axis) is plotted against its distance (x-axis) from the center of the grid (representing the location of the hive). The red line depicts the overall relationship between distance and visitation probability fit with a nonlinear least squares function. Blue and green dashed lines represent the distances that encompassed 50% and 95% of total foraging activity, respectively. A distance of 2000 m encompassed 79% of foraging activity.
Combining drift and foraging models to estimate risk

Risk—the likelihood of adverse effects—is conventionally defined as the product of hazard and exposure, where hazard is the potential for harm and exposure is the degree or likelihood of encountering the hazard. In the context of neonicotinoid-treated corn planting, the hazard for honey bees can be understood as the concentrations of seed treatment neonicotinoids found in each patch of the foraging landscape. The risk posed by a given patch can then be estimated by multiplying its pesticide concentration by the probability that foraging honey bees will traverse it (aerial contact) or collect food from it (floral contamination). The collective risk posed by a contaminated landscape, then, can be defined as follows in Equation 5.3.

\[ \text{Risk} = \sum_i C_i P_i \]  

Eq. 5.3

where \( C_i \) is the concentration of insecticide in patch \( I \) and \( P_i \) is the probability that foraging honey bees will traverse it or collect food from it. “Risk” calculated in this way is the cumulative product of contamination and visitation probability for a given landscape and should be understood as an effectively unitless index. Total risk can then be partitioned across different landscape components, such as all corn fields (corn risk) (Equation 5.4), only weedy corn fields (weedy corn risk) (Equation 5.5), or foragable habitat subject to drifting seed treatment neonicotinoids (drift risk) (Equation 5.6):

\[ \text{Risk} = \sum_c C_c P_c \]  

Eq. 5.4
\[ Risk = \sum_w C_w P_w \quad \text{Eq. 5.5} \]
\[ Risk = \sum_d C_d P_d \quad \text{Eq. 5.6} \]

To estimate the total risk of seed treatment neonicotinoid exposure for honey bee colonies located at each of our 13 field sites, a series of operations was performed on the classified digitizations generated during the initial landscape analysis using QGIS (QGIS Development Team 2015) and R (R Core Team 2015) “raster” (Hijmans 2015) (Table 5.2) (see Appendix E for sample R script).

1. “Foraging habitat” was assigned as a binary variable describing whether a given landscape element might be expected to host floral resources for honey bees. Based on extensive field work in our study system, the landscape classes of non-crop herbaceous areas (roadsides, field margins, uncultivated fields), residential areas, forests, and bloom level 2 fields were classified as foragable (= 1), while fields with bloom level 0 or 1, paved areas, and open water were classified as non-foragable (= 0) (Figure 5.5A).

2. Each digitized landscape was converted to a pair of raster layers (gdal_rasterize, QGIS) representing foraging habitat (forage raster) and crop type, respectively. A corn raster was generated by setting corn patches equal to 1 and non-corn patches to 0. Similarly, an inverse corn raster was generated by setting corn patches equal to 0 and non-corn patches to 1.
3. A \textit{distance raster} was generated (matching the extents of the landscape raster) in which each cell was assigned a value equal to its distance from central origin (\texttt{distanceFromPoints \{raster\}, R}).

4. A \textit{visitation probability raster} was generated by applying \texttt{Eq. 5.2} to each cell of the distance raster (\texttt{Figure 5.5B}).

5. A \textit{corn-distance} raster was generated by calculating the grid-distance of each patch from the nearest corn patch (\texttt{gridDistance \{raster\}, R}).

6. A \textit{contamination raster} was generated by applying \texttt{Eq. 5.1} to the \textit{corn-distance raster} (\texttt{calc \{raster\}, R}) (\texttt{Figure 5.5C}).

7. A \textit{total risk raster} was generated by multiplying the \textit{visitation probability raster} by the \textit{contamination raster}, thus weighting the contamination of each patch by the probability that it was visited or traversed.

8. A \textit{corn risk raster} was generated by multiplying the \textit{total risk raster} by the \textit{corn raster} ($\text{corn} = 1$, non-$\text{corn} = 0$). Thus, all corn patches retained their original values while non-corn patches were set to 0 (\texttt{Figure 5.5D}).

9. A \textit{weedy corn risk raster} was generated by multiplying the \textit{corn raster} by the \textit{forage raster} ($\text{foragable} = 1$, non-$\text{foragable} = 0$). Thus, all corn patches scored as “foragable”—that is, bloom level 2—retained their original values while all other patches were set to 0 (\texttt{Figure 5.5E}).

10. A \textit{drift risk} raster was generated by multiplying the \textit{total risk raster} by the \textit{inverse corn raster} ($\text{corn} = 0$, non-$\text{corn} = 1$) and then multiplying the product by the \textit{forage raster}. Thus all foragable non-corn patches retained their risk value
(representing weighted contamination due to drift) while all corn patches were set to 0 (Figure 5.5F).

11. The cumulative risk for each risk raster—corn risk, weedy corn risk, and drift risk—was calculated by converting the rasters into data frame and summing across rows (colsums {base}, R).
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Table 5.2. Risk partitioned by landscape component for each site-year. Numbers represent the cumulative product of contamination and visitation probability for each component of each landscape and should be understood as a unitless index.
Figure 5.5. **Spatial components of risk.** Honey bee foraging activity is restricted to habitat that supports floral resources (A) and constrained by distance (B). Environmental contamination occurs both directly in corn fields and in nearby habitats subject to drift (C). Risk can be calculated by multiplying contamination by visitation probability for all corn fields (D), only weedy corn fields (E), or only off-field habitats subject to drift (F).
Statistical models relating risk patterns to honey bee exposures

The four hypothesized routes of exposure (plus one combination of two routes) imply distinct predictions about the relationship between exposure and mortality (response variables) and the distribution of risk across different landscape components (explanatory variables) (Eq. 5.3-5.6, Table 5.3).

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<th>Major route</th>
<th>Sub-route</th>
<th>Predicted model</th>
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<td>diffuse cloud</td>
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Table 5.3. Hypothesized routes and sub-routes of exposure with corresponding statistical models.

*Floral contamination: weedy corn*

If the exposure and mortality of honey bees during corn planting is driven principally by the settling of seed treatment particles on flowering weeds in cornfields, then exposure and mortality should be functions of *weedy corn risk* (Eq. 5.5), calculated as described above using the *weedy corn risk* raster.

*Floral contamination: drift*

If the exposure and mortality of honey bees during corn planting is driven principally by off-field drift, then exposure and mortality should be functions of *drift risk* (Eq. 5.6), calculated as described above using *drift risk raster*. 
Floral contamination: total forage

If the exposure and mortality of honey bees during corn planting is driven by total floral contamination—both weedy corn fields and off-field drift—then exposure and mortality should be functions of both weedy corn risk and drift risk (Eq. 5.5, 5.6). Since these metrics have the same units, they can be represented by a single variable, forage risk, equal to their sum.

Aerial contact: discrete plume

If exposure and mortality are driven principally by aerial contact with the discrete plume expelled by a running planter, then exposure and mortality should depend on the degree to which honey bee foragers traverse corn fields, regardless of whether they are weedy. This predicts that exposure and mortality should be functions of corn risk (Eq. 5.4), calculated as described above using the corn risk raster.

Aerial contact: diffuse cloud

If exposure and mortality are driven principally by aerial contact with a diffuse cloud of seed treatment particles, then this process should be largely independent of the exact locations and extents of corn fields. Instead, the amount of seed treatment particles in the air space over an area would depend simply on the total amount of corn in the vicinity. Thus, exposure and mortality should be functions of total corn area, regardless of weediness and visitation probability.
Each of these hypothesized relationships was plotted and then analyzed using linear regression or, for models exhibiting excessive skew or heterogeneity of variance, Spearman's rank correlation coefficient ($\rho$). Data from 2014 and 2015 were analyzed separately, and $exposure$ (pollen neonicotinoid residues) and $mortality$ (dead bee counts) were analyzed as separate response variables. The $exposure$ response variable was generated by summing the concentrations of pollen neonicotinoid residues found in the samples collected from each apiary (pooled across hives) during the corn planting period. Since the number of samples taken during the corn planting period was equal for all sites and years, summing concentrations across samples was equivalent to taking the mean across samples. I opted for the former approach simply to emphasize that cumulative exposure during corn planting is the concept I wish to relate to hypothesized routes of exposure. The $mortality$ response variable was generated by first taking, for each apiary (pooling across hives), the mean dead bee count during peak planting and outside of peak corn planting. Then, to account for the possibility of an extraneous site effect owing to differences in colony strength, the dead bee counts during and not during peak planting, respectively, were divided by the average dead bee count on a per site basis. Then, the ratio of these two standardized counts was log-transformed to yield the final $mortality$ response variable.

For 2015, Site L was excluded from all analyses involving weedy corn risk because a large proportion (40%) of corn fields at that site could not be accessed for bloom level ground-truthing. Site H in 2015 had only one small corn field located nearly
2 km from the apiary; this field could not be accessed for bloom level ground-truthing, but its size and distance rendered its risk negligible regardless of bloom level, so Site H was retained in all analyses.

5.4 Results

No significant relationships were found in any of the models of mortality for either 2014 or 2015. Possible reasons for this are explored in the discussion section. Below, results are reported for models of exposure only.

Floral contamination: weedy corn

In 2014 (n = 6), the relationship between weedy corn risk and exposure trended positively (Figure 5.6A) but was non-significant (F = 3.3, p = 0.14).

In 2015 (n = 9), the relationship between weedy corn risk and exposure was heavily influenced by one site, Site B, that seemed to be a strong outlier. At Site B, exposure was nearly three times higher than it was at the next highest site despite having zero weedy corn risk. With Site B included in the analysis, no significant relationship was found (\(\rho = 0.44, p = 0.24\)) (Figure 5.6B). If, however, Site B is considered an outlier and omitted from analysis, the remaining sites exhibit a strongly positive linear relationship between weedy corn risk and exposure (\(F = 41, p = 0.0007\)) (Figure 5.6C).
Figure 5.6. *Exposure* plotted against *weedy corn risk* for 2014 (A), 2015 with Site B included (B), and 2015 with Site B omitted (C).
Floral contamination: drift

In 2014 (n = 6), no significant relationship was found between drift risk and exposure ($F = 0.15, p = 0.72$), and no distinct trend could be discerned (Figure 5.7A).

In 2015 (n = 10), no significant relationship was found between drift risk and exposure, regardless of whether Site B was omitted ($rho = 0.25, p = 0.52$) (Figure 5.7B) or retained ($rho = 0.21, p = 0.56$), and no distinct trend could be discerned (Figure 5.7C)
Figure 5.7. Exposure plotted against drift risk for 2014 (A), 2015 with Site B included (B), and 2015 with Site B omitted (C).
Floral contamination: total forage

In 2014 (n = 6), the relationship between *total forage risk* and *exposure* trended positively (Figure 5.8A) but was non-significant \((F = 2.68, p = 0.18)\).

In 2015 (n = 9), the relationship between *total forage risk* and *exposure* was strongly influenced by the outlier Site B, which is not surprising because the bulk of *total forage risk* is attributable to weedy corn fields. With Site B included, the relationship was not significant \((\rho = 0.6, p = 0.097)\) (Figure 5.8B). With Site B omitted, a strongly positive linear relationship was found \((F = 42, p = 0.0006)\), with *exposure* log-transformed to reduce skew (Figure 5.8C)
Figure 5.8. Exposure plotted against total forage risk for 2014 (A), 2015 with Site B included (B), and 2015 with Site B omitted (C).
Aerial contact: discrete plume

In 2014 (n = 6), the relationship between corn risk and exposure trended positively but was not significant ($\rho = 0.60$, $p = 0.24$) (Figure 5.9A).

In 2015 (n = 10), the relationship between corn risk and exposure depended strongly on whether Site B was omitted as an outlier. With Site B retained, a significant positive correlation was observed ($\rho = 0.62$, $p = 0.05$) (Figure 5.9B). With Site B omitted, the positive correlation no longer held ($\rho = 0.50$, $p = 0.18$) (Figure 5.9C).
Figure 5.9. Exposure plotted against corn risk for 2014 (A), 2015 with Site B included (B), and 2015 with Site B omitted (C).
Aerial contact: diffuse cloud

In 2014 (n = 6), the relationship between corn area and exposure trended positively but was not significant (\(\rho = 0.54, p = 0.30\)) (Figure 5.10A).

In 2015 (n = 10), the relationship between corn area and exposure was somewhat sensitive to whether Site B was considered an outlier. With Site B retained, a significant positive correlation was observed (\(\rho = 0.77, p = 0.01\)) (Figure 5.10B). With Site B omitted, a significant positive correlation was still observed, but it was weaker (\(\rho = 0.68, p = 0.05\)) (Figure 5.10C).
Figure 5.10. *Exposure* plotted against *corn area* for 2014 (A), 2015 with Site B included (B), and 2015 with Site B omitted (C).
Comparing *weedy corn* and *total forage risk* models of *exposure* for 2015

Both *weedy corn risk* and *total forage risk* were strongly predictive of *exposure* in 2015 when the outlier site, Site B, was omitted. The two models were approximately equal in the amount of variance they explained (each had an adjusted R\(^2\) value of 0.85), but the *total forage risk* model exhibited greater skew, even when the response variable was log-transformed. The *total forage risk* variable is simply the sum of *weedy corn risk* and *drift risk*, and because *weedy corn risk* is by far the larger of these terms for most sites, the statistical strength of the *total forage risk* model may be driven entirely by the *weedy corn risk* term.

To attempt to parse the influence of *weedy corn risk* and *drift risk* in the *total forage risk* model, I constructed a multiple regression model in which *weedy corn risk* and *total forage risk* were covariates. This approach was validated by checking for correlation (Spearman's *rho*) between *weedy corn risk* and *forage risk*, which would create multicollinearity; no significant correlation was found (*rho* = 0.085, *p* = 0.82).

The multiple regression model was significant (*F* = 20.7, *p* = 0.004), though it exhibited strong right-tail skew when visualized with a Q-Q plot (**Figure 5.11**). The *weed corn risk* covariate was significant (*t* = 6.12, *p* = 0.002) while the *drift risk* covariate was not (*t* = 0.96, *p* = 0.38), indicating that the addition of *drift risk* to the *weedy corn risk* model (approximating the *total forage risk* model) did not improve the prediction of *exposure*. 
Figure 5.11. Q-Q plot illustrates skew in the right tail of the multiple regression model used to parse the influence of *weedy corn risk* and *drift risk*.

5.5 Discussion

None of the statistical models tested were significant for the 2014 data set or for *mortality* in either year. The failure to detect significant relationships in the 2014 data set may simply be due to the poorer level of replication in that year (6 sites) compared to 2015 (10 sites). It is also quite possible, though, that there is considerable variation in the significance of different exposure mechanisms across years. These differences could be
driven by the timing of planting in relation to floral phenology, the degree of synchrony in planting between growers in a given area (i.e. how many fields are planted simultaneously), or climatic variables like humidity and wind conditions. It must also be noted that our analytical methods for measuring pesticide residue in pollen differed between 2014 and 2015. We have reason to believe that the 2015 protocol was an improvement on that of 2014, so it is possible that error introduced by the analytical method obscured the relationship between exposure and the various risk components we tested. As for why none of my models in either year was predictive of mortality, this might be explained by the complex relationship between exposure and effects. We measured exposure by analyzing the neonicotinoid residues in bulk pollen samples. These measurements may or may not meaningfully reflect the doses received by individual bees, but it is these individual doses that would cause measurable effects (e.g. dead bee counts). For example, a bulk pollen residue of 20 ppb could reflect a uniform distribution of insecticide such that every bee consuming the pollen would be exposed to a concentration of 20 ppb, or it could reflect one or a few “needles in the haystack”, highly concentrated pollen pellets whose extreme contamination is masked by the low contamination of the rest of the sample. These two distributions of insecticide could result in very different mortality rates, which could create considerable noise in any model attempting to relate mortality to the factors driving exposure. The relationship between mortality and exposure depends also on the basic toxicological parameters of the toxicant in question. Detectable exposure will only yield detectable mortality if the level of exposure is sufficiently high with respect to the intrinsic toxicity of the toxicant.
The question of which route of exposure was of principal importance in our study hinges critically on the interpretation of the putative outlier site (Site B) in the 2015 data set. Excluding, for now, the possibility of some analytical error to account for the level of neonicotinoid residues in the pollen collected from Site B 2015, two interpretations are salient.

First, it is possible that Site B 2015, while it may be appear to be a statistical outlier, simply represents an extreme case of the same general route of exposure that drove the pollen contamination patterns observed at other sites. Under this interpretation Site B 2015 should be retained in the analysis, and this decision would support some form of aerial contact as the route of exposure. The diffuse cloud hypothesis ($\text{exposure } \sim \text{corn area}$) yielded the strongest statistical model, though the statistical model reflecting the discrete plume hypothesis was also significant.

The second interpretation is that Site B 2015 appears as a statistical outlier because something mechanistically different occurred at that site in 2015 and gave rise to exceptionally high pollen neonicotinoid residues. Under this interpretation Site B 2015 should be excluded from analysis, and this leads to a very different conclusion regarding which route of exposure was chiefly responsible for the observed patterns of exposure. With Site B 2015 excluded, floral contamination via in-field settling of seed treatment particles ($\text{exposure } \sim \text{weedy corn risk}$) emerges as by far the most strongly supported exposure hypothesis. While the $\text{total forage risk}$ model that incorporated both $\text{weedy corn risk}$ and off-field $\text{drift risk}$ ($\text{exposure } \sim \text{total forage risk}$) was slightly stronger than the model using only $\text{weedy corn risk}$, multiple regression analysis indicated that the strength
of the total forage risk model was driven by the weedy corn risk component, which is not surprising given that the bulk of total risk at most sites was due to weedy corn fields.

The strength of the weedy corn risk model with Site B 2015 excluded far exceeds that of the corn area model with Site B 2015 included, which makes a compelling case for the second interpretation. This conclusion, however, begs the question of what alternative route of exposure might have caused the exceptionally high pollen neonicotinoid residues detected at Site B 2015. This question is especially intriguing given the fact that Site B 2015 had zero weedy corn risk within a 2 km radius, so it seems that weedy corn fields contributed little, if at all, to the exposure observed there. A few possibilities can be suggested. It is almost certain that the exposure documented at Site B 2015—by far the highest observed at any site—was driven by some form of aerial contact, since Site B 2015 had zero weedy corn risk and represented the median with respect to off-field drift risk. Tapparo et al. (2012) show that remarkably high levels of exposure are possible when flying bees intersect directly with the discrete plume of seed treatment particles emitted by a running planter, but these direct intersection events must be extremely rare given the small size of the roughly 40 m radius of the planter plume (Girolami et al. 2013) compared to the massive area foraged by a honey bee colony. It may be that one or more of these rare events occurred at Site B in 2015, and this gave rise to the high pollen neonicotinoid residues that were detected. It is also possible that the high exposure observed at Site B 2015 was driven not by one or a few extremely high aerial exposure events but by an accumulation of small or moderate aerial exposure events, such as would be predicted by the diffuse cloud hypothesis. In either case, though,
the question remains of why aerial exposure was so much higher at Site B 2015 than at other site-years. A possible explanation is suggested by the dance language analysis performed at Site B 2015. Of the four sites where dance analysis was performed, Site B 2015 was peculiar in that peak visitation probability occurred about 1.5 km from the hive, while at all other sites peak visitation probability occurred in the immediate vicinity the hive (Figure 5.12). This indicates that forage was relatively scarce at Site B 2015, perhaps due to the scarcity of in-field weeds, forcing bees to forage farther from the hive to find suitable resources. This dilation of the foraging radius would greatly increase both the amount of time spent in flight and the amount of airspace traversed by foraging bees. Since aerial contact exposure should be proportional to flight time and/or the amount of airspace traversed, it is reasonable to think that aerial contact exposure may have been exceptionally high at Site B 2015.
Figure 5.12. Site B was unusual in that decoded dances indicated peak foraging activity at about 1.5 km from the hive. For all other sites, peak foraging occurred in the immediate vicinity of the hive, less than 100 m away. Red lines depict nonlinear least squares fits, and blue and green dashed lines represent the distances that encompassed 50% and 95% of foraging activity, respectively.

If floral contamination in weedy corn fields is the principal route of exposure linking honey bees to seed treatment neonicotinoids, then the simplest and most effective approach to mitigation would be to destroy in-field weeds prior to corn planting. The abundance of flowering weeds in corn fields at the time of planting depends on two
agricultural practices: tilling and herbicide application. These practices vary considerably from grower to grower in Ohio (H. Watters, personal communication). Some growers perform deep tillage (> 5”) in the fall, which destroys newly germinated weeds that would emerge the following spring. Deep tillage in the fall results in spring fields virtually devoid of vegetation. For growers who adopt a no-till or reduced-till (light tillage in spring) strategy, herbicide application is necessary to “burn down” in-field weeds. When exactly this burn down occurs with respect to planting is also variable. Some herbicides can be applied after planting without damaging the crop. Because the timing of planting is more sensitive than the timing of burn down, growers tend to plant at the opportune time regardless of whether they have burned down weeds yet. The result is that some fields are burned down and then planted, while other fields are planted and then burned down. In the latter case there is a critical window during which seed treatment neonicotinoids are released into fields that still contain highly attractive flowering weeds. Since in-field weeds are destroyed regardless of whether burn down immediately precedes or immediately follows planting, destroying in-field weeds prior to planting as a means of reducing honey bee exposure would have little effect on overall floral resource availability. It may, however, dramatically reduce exposure to seed treatment neonicotinoids. The case of Site B 2015, though, warns that the dramatic reduction in floral resource availability that results from the destruction of in-field weeds might force honey bees to search farther from the hive and increase the potential for aerial contact exposure. It would be advisable, therefore, to combine the destruction of in-field weeds with the conservation and/or augmentation of off-field foraging habitat.
We found no evidence supporting the hypothesis that honey bee exposure to seed treatment neonicotinoids is due to off-field drift. Mitigation approaches that focus on drift reduction are, therefore, unlikely to be effective. One such approach that has been explored is the modification of planters to direct exhaust down onto the soil surface instead of up into the air [reviewed in Nuyttens et al. (2013)]. If exposure is driven principally by floral contamination within weedy corn fields, then measures that reduce the off-field movement of seed treatment particles—and, hence, concentrate the deposition of seed treatment particles within fields—might actually exacerbate exposure unless coupled with vigilant in-field weed suppression.

An important caveat to the conclusions presented above is that the apparent role of weedy corn field risk in driving exposure does not preclude the possibility that aerial contact could still be the principal mechanism of exposure. If, during planting, the airspace over corn fields becomes contaminated with airborne seed treatment particles, then it is possible that aerial contact exposure could be a function not only of the amount of airspace traversed (as assumed in our corn risk statistical model) but also of the amount of time spent in the contaminated area. The presence of attractive weeds in corn fields would increase the amount of time that foraging bees would spend within the corn field and, therefore, potentially increase exposure. It should be noted, though, that whether the relationship between exposure and weedy corn fields was driven by floral contamination or aerial exposure has little bearing on the recommended mitigation approach: the destruction of in-field weeds prior to planting should be equally effective at reducing exposure in either case. If exposure in weedy corn fields is mediated by aerial
contact, though, mitigation approaches aimed at dust control, like the planter modification discussed above, might be important to explore in addition to in-field weed control.

In addition to yielding mechanistic insight into the process of honey bee exposure to seed treatment neonicotinoids during corn planting and providing evidence-based recommendations for mitigation, our study is the first application of dance language analysis to weight contamination by visitation probability. This technique overcomes the critical problem of having to decide on some fixed radius that captures “enough” honey bee activity to not exclude important information while not casting so wide a net that the volume of unimportant information swamps the signal. By weighting contamination according to a continuous visitation probability function, no arbitrary outer bound is set that might exclude important information, but at the same time potential interactions closer to the nest are rightly treated as more important than potential interactions farther from the nest. Moreover, by using dance analysis data collected from the same study system, risk analysis is made maximally relevant to the context from which measures of exposure and/or effects were collected. It should be acknowledged that in our study an outer bound of 2 km was used, but this was due to the logistical limitations of ground-truthing large areas. These constraints could at least theoretically be overcome, and they are not intrinsic to the technique of weighting contamination by visitation probability.

Perhaps the most important weakness of our technique is that it relies on a form of data—decoded honey bee dances—that is extremely laborious to generate. Since dance data
sets, such as the one used in this study, are generally fairly small, they may misrepresent, due to mere sampling error, the true function relating visitation probability and distance.

In conclusion, our study supports the hypothesis that the exposure of honey bees to seed treatment neonicotinoids during corn planting is driven primarily by the contamination of flowering plants within corn fields. Exposure could, therefore, be effectively mitigated by destroying in-field flora prior to planting. This measure would not, however, resolve the fundamental problem of the accidental release of seed treatment neonicotinoids that occurs during routine corn planting operations, the impacts of which may extend well beyond damage to honey bees (Goulson 2013). Thus, while the destruction of in-field flora prior to planting may be a short-term solution to the problem of honey bee exposure, long-term mitigation must reduce the initial release of seed treatment particles, either through improved seed treatment formulation, modified planting equipment, or the planting of untreated seed.

5.6 References


Chapter 6: Simulating the effects of in-field weed suppression on honey bee exposure to seed treatment neonicotinoids during corn planting

6.1 Abstract

Honey bees are exposed to pesticides when their foraging patterns, interacting with the distribution of floral resources, overlap with the distribution of environmental contamination. Accordingly, mechanistic models of honey bee pesticide exposure must incorporate each of these three elements. Here, I present a simulation model of honey bee exposure to seed treatment neonicotinoids released during corn planting. Honey bee foraging patterns are modeled using dance language analysis data, floral resource distribution is modeled based on field studies in the central Ohio agricultural landscape, and environmental contamination is modeled according to field data on the drift of seed treatment neonicotinoids. The model runs in randomly generated landscapes composed of simulated corn fields, soybean fields, and interstitial regions representing field margins and roadsides. During each simulation run, exposure is calculated as a distribution of concentrations arising from individual foraging trips. The results of running my model with incremental variation of weed prevalence in corn fields corroborate earlier work recommending the suppression of weeds in corn fields as a means of reducing honey bee exposure. Moreover, though, the results of my model suggest that flowering weeds in soybean fields may be equally important in reducing honey bee exposure by drawing
foragers away from the more contaminated flora in and near corn fields. By incorporating random processes in both landscape generation and honey bee foraging simulation, my model also represents the stochasticity that is intrinsic to the phenomenon of honey bee pesticide exposure.
6.2 Introduction

In Chapter 4, I argue that honey bees, like other motile organisms, experience pesticide exposure as the spatiotemporal intersection of environmental contamination and foraging activity. Given the stochasticity and spatiotemporal heterogeneity of both environmental contamination and honey bee foraging activity, the exposure of honey bees to pesticides must be understood as a fundamentally stochastic and distributional phenomenon. Accordingly, models of honey bee pesticide exposure should aim to capture the key mechanisms of pesticide occurrence and honey bee foraging activity to generate stochastic and distributional predictions of exposure.

My statistical modeling approach to the issue of honey bee exposure to seed treatment neonicotinoids, presented in Chapter 5, uses the spatial overlap between neonicotinoid contamination and honey bee foraging activity to estimate the distribution of exposure levels that a honey bee colony would be expected to experience in different landscapes. This approach stops short, though, of actually simulating the exposure process. Only by simulating exposure as a process can the variability between different instantiations of the process, i.e. the stochasticity of the system, be manifest. In other words, any instance of colony exposure is a distribution of individual exposures drawn probabilistically from a “population” of potential exposure distributions, meaning that the potential exposure of a colony in a given landscape can be best described as a “distribution of distributions”.

Here, I present a simulation model of honey bee exposure to seed treatment neonicotinoids via the collection of contaminated pollen. This model is designed to
complement and extend the statistical modeling approach of Chapter 5. First, I aim to “observe” the stochasticity of the system that is hidden in the statistical approach of Chapter 5 in order to better understand the variability of honey bee exposure patterns. Second, a simulation modeling environment allows for \textit{in silico} experimentation that would be impossible to achieve in the field. In Chapter 5, I present evidence that the primary route by which honey bees are exposed to seed treatment neonicotinoids during corn planting is the contamination of flowering weeds located within corn fields, which predicts that the destruction of in-field weeds prior to planting would be an effective way to mitigate exposure. Using my simulation model, I experimentally test this conclusion by incrementally varying the prevalence of weedy cornfields in the simulation environment and evaluating the effects of this parameter on simulated exposure.

6.3 Model description

Landscape submodel

The simulation environment consists of a 4000 x 4000 array of 1 x 1 m patches, representing an idealized corn/soybean rotation system composed of three habitat types: corn fields, soybean fields, and interstitial strips (representing field margins or roadsides). Fields geometries are randomly generated by voronoi polygon tessellation (Appendix F). The number of voronoi seeds is set to 25, yielding an average field size of 64 ha, which is consistent with the range of field sizes found at the apiary sites presented in Chapter 5.

Forty-percent of the fields (= 10 fields) in the simulation environment are randomly assigned to corn and the remaining fields (= 15 fields) to soybean, reflecting
the relative prevalence of corn and soybean in Ohio (USDA-NASS 2015). Then, the one-dimensional borders between fields are laterally expanded into the first five meters of the neighboring fields they divide to create 10-meter-wide strips of interstitial land, approximating the configuration of field margins or roadsides in an intensive field crop landscape.

Weed control practices in corn and soybean cultivation can involve both tilling and herbicide application, and the timing of these events is variable. Based on typical weed control schedules for corn and soybean in Ohio, about 25% of corn fields and about 45% of soybean fields would be expected to contain intact flowering weeds at the time of corn planting (H. Watters, personal communication). I randomly assign 7 soybean fields (47%) as “weedy” under default model settings and varying the number of weedy corn fields incrementally from 0 to 10 (100%). These weedy fields, along with all interstitial strips, are assumed to be potential foraging habitat containing blooming dandelions (*Taraxacum officinale* Wigg), which are among the most abundant and bee-attractive flora blooming at the time of planting in our central Ohio study system.

**Seed treatment drift submodel**

The drift of neonicotinoid-laden seed treatment material during planting is modeled using the dust drift function described in Chapter 5 (Equation 5.1). During the landscape generation phase of the model, this function is used to calculate the contamination level of each patch in the simulation environment based on its distance
from the nearest corn patch. Because the simulation environment is discrete, contamination at each 1 x 1 m patch is determined by its geometric center.

Foraging submodel

Honey bee foraging is modeled as a stochastic process constrained by distance and foraging habitat. Each potentially foragable patch of the simulation environment (interstitial strips and weedy fields) is first assigned an *a priori* visitation probability (APV) based on its distance from a virtual hive located in the center of the simulation environment, using the visitation probability function described in Chapter 5 (Equation 5.2). During the course of a single day, a honey bee colony typically focuses its foraging activity on just a few highly rewarding resource patches, a process mediated by cooperative information exchange and recruitment (Visscher and Seeley 1982). To capture this aspect of honey bee foraging behavior, the virtual colony in my model randomly draws $n$ patches from the foragable subset of the simulation environment using a two-step algorithm. Approximating the high end of the daily number of major foraging patches reported by Visscher and Seeley (1982), we use a default setting of $n = 10$. In the first step if the patch selection algorithm, the foragable subset of the simulation environment (weedy fields and interstitial strips) is sampled using a distance-biased approach. First, the lowest APV is set to 1, and all other APVs are raised proportionally and rounded to the nearest 10; these modified APVs are represented by $P_i$. Thus, the patches of the foragable landscape are binned into categories having integer values of $P_i$ that are approximately proportional to their APV values. From each bin, a number of
patches equal to the bin’s \( P_i \) are then drawn at random and pooled, resulting in a distance-biased subset of the foragable simulation environment. In the second step of the patch selection algorithm, \( n \) patches are drawn randomly from the distance-biased subset. These patches represent the spatial focal points of colony foraging for each simulation run.

Colony foraging activity, measured in total individual trips \( t \), is assumed to be evenly distributed among the \( n \) foraging focal points, resulting in \( t / n \) foraging trips to each foraging focal point. Honey bee foragers recruited to a resource do not arrive to a point location with high precision; instead, they scatter within the vicinity of the communicated location. In my model, this scatter is represented by a radius in which simulated foragers are randomly distributed around each focal point. Based on the patterns of clustering observed in the dance mapping described in Chapter 5, I chose a default radius of 250 m, acknowledging that in reality this parameter is highly variable from patch to patch depending on the actual distribution of floral resources around the area toward which recruitment is directed. During each simulation run, \( t / n \) foragers are assigned random starting patches within 250 m of each foraging focus. Only patches of foragable habitat (weedy fields + interstitial strips) are eligible starting points, so random patches are drawn strictly from the foragable habitat within 250 m of each foraging focus.

Each forager, beginning at its starting patch, then proceeds in a random walk, representing the visitation of multiple flowers within each foraging trip. The length of the random walk is set to 7 steps, each step representing a transition from one 1 x 1 m patch to an adjacent 1 x 1 m patch in the simulation environment. The number 7 is inferred from Ribbands (1949) and Minderhoud (1930); the former reports that honey bees
collecting pollen from dandelions require an average of 18.3 visits to fill their pollen baskets, and the latter provides a diagrammatic depiction of a honey bee foraging for nectar on densely distributed dandelions (~60 blossoms per m$^2$) showing that the bee visited an average of 2.78 blossoms per m$^2$. If it is assumed that the density of dandelions in my simulation environment is similar to that of Minderhoud’s field, and that spatial density of visitation by pollen foragers is similar to that of the nectar forager he followed, the average number of 1 x 1 m patches visited by a honey bee foraging for dandelion pollen can be calculated as: $18.3 / 2.78 = 6.58$. Rounding to the nearest integer gives the value of 7 that I chose for the number of steps in our random walk algorithm. During the random walk, a bee is not permitted to cross into non-foragable habitat types; random walk iterations that take a bee into non-foragable habitat are repeated until a foragable patch is drawn. Similarly, a bee is not permitted to revisit a patch that has just been vacated in the previous step of the random walk, accounting for the tendency of honey bees to move away from recently visited patches (Waddington 1980). The net exposure each forager experiences is recorded as the mean of the concentrations encountered during the steps of the random walk, thus simulating the effective mixing of pollen from multiple blossoms into a single load of pollen.

Time is not simulated explicitly in my model, but each run of the model can be taken to represent one day of foraging. This is an appropriate time frame because dandelion blossoms remain open for only one day, so blossoms contaminated on one day are closed the next and pose no more exposure risk to foraging bees. The output of each model run is a distribution of neonicotinoid concentrations arising from $t$ foraging trips.
The default setting for \( t \) in our model is 1000, which underestimates the number of pollen foraging trips made by a real colony but allows for streamlined computation while achieving extensive sampling of the environment.

Key model parameters and their justifications are presented in Table 6.1. The model is programmed in Python and can be downloaded from its GitHub repository:

https://github.com/MWransky/DustSimulator.git.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Justification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area of simulation environment</td>
<td>4000 x 4000 m</td>
<td>Captures bulk of honey bee foraging activity in our study system (Chapter 5).</td>
</tr>
<tr>
<td>Resolution</td>
<td>1 x 1 m</td>
<td>Necessary to resolve drift function; appropriate scale at which to simulate honey bee foraging movement (Minderhoud 1930).</td>
</tr>
<tr>
<td># voronoi seeds (fields)</td>
<td>25</td>
<td>Yields realistic mean field size of 64 ha.</td>
</tr>
<tr>
<td>Corn : soybean ratio</td>
<td>2:3</td>
<td>USDA-NASS (2015)</td>
</tr>
<tr>
<td># weedy soybean fields</td>
<td>7/10</td>
<td>H. Watters (personal communication)</td>
</tr>
<tr>
<td>Interstitial width</td>
<td>10 m</td>
<td>Reasonable estimate for roadsides and field margins</td>
</tr>
<tr>
<td>Dust drift function</td>
<td>Equation 5.1</td>
<td>Dosimeter data presented in Chapter 5.</td>
</tr>
<tr>
<td>Visitation probability function</td>
<td>Equation 5.2</td>
<td>Dance analysis data presented in Chapter 5.</td>
</tr>
<tr>
<td># foraging foci</td>
<td>10</td>
<td>Consistent with Visscher and Seeley (1982)</td>
</tr>
<tr>
<td># foraging trips per run</td>
<td>1000</td>
<td>Becomes computationally limiting at higher values.</td>
</tr>
<tr>
<td>Scatter radius around foraging foci</td>
<td>250 m</td>
<td>Consistent with the range of cluster sizes found in the dance data described in Chapter 5.</td>
</tr>
<tr>
<td># steps in random walk</td>
<td>7</td>
<td>Derived from the data of Minderhoud (1930) and Ribbands (1949).</td>
</tr>
</tbody>
</table>

Table 6.1. Model parameters, values, and justifications.
6.4 Model application

My model is scripted to allow for modular separation of the landscape and seed treatment drift submodels from the foraging submodel. Using this functionality, I first generated five random landscape geometries (voronoi polygons with interstitial strips) with their respective contamination patterns determined by the locations of corn fields and the seed treatment drift function (Figure 6.1). Six versions of each landscape were then created to represent different rates of weed prevalence in corn fields: 0/10, 1/10, 3/10, 5/10, 8/10, and 10/10 corn fields weedy, respectively. Then, the foraging submodel was run on each of the 30 landscapes (5 geometries x 6 weed prevalence levels), for a total of 30 model runs yielding corresponding exposure distributions.

Figure 6.1. Landscape geometries generated by voronoi polygon tessellation. Yellow and green polygons represent corn and soybean fields, respectively. Lines represent 10 m wide interstitial strips simulating roadsides or field margins.
6.5 Results

When the exposure output is visualized as a set of histograms representing each landscape geometry and each level of weed prevalence, both the distributionality and the stochasticity of the model are evident (Figure 6.2). All runs yielded a range of exposure levels representing the 1000 individual foraging trips simulated in my model. The distribution of exposure encountered under each level of weed prevalence exhibited considerable variability across landscape geometries due both to the differences in landscape configuration and the stochasticity of the foraging algorithm.

![Figure 6.2. Histograms depicting exposure distributions generated for each landscape geometry (A-E) under each level of weed prevalence (0/10-10/10) in corn fields.](image)

The effect of weedy field prevalence can be seen most clearly when the results are visualized as violin plots representing the exposure distributions at each level of weed.
prevalence pooled across landscapes (Figure 6.3). When weedy corn fields were entirely absent from the landscape, simulated foraging trips only yielded lower levels of exposure caused by off-field drift. With a single weedy corn field in the landscape, a small minority of bees encountered the relatively high level exposure found directly in corn fields. As the number of weedy corn fields increased, the number of bees encountering these higher doses expanded accordingly. As more corn fields were set to weedy, the distribution exhibited an increasing mode at the high extreme. At all levels of weed prevalence, though, the strongest mode existed at zero, reflecting the extensive uncontaminated foraging habitat provided by weedy soybean fields beyond the drift range of neighboring corn fields.

Figure 6.3. Violin plots describing the distributions of exposure levels generated under different weed prevalence conditions (pooled across landscape geometries). Width of line represents the number of simulated foraging trips during which each level of exposure (y-axis) was encountered.
6.6 Discussion

The results of my model suggest that honey bee exposure to seed treatment neonicotinoids via contaminated flora is fundamentally tri-modal. A strong mode exists at zero, representing the large amount of virtually uncontaminated habitat beyond the range of drifting seed treatment particles, a component of the landscape that is comprised largely of weedy soybean fields. A second mode exists at an intermediate level of exposure, representing the gradient of contamination found in areas subject to drifting seed treatment particles from neighboring corn fields. While this range of the exposure distribution exhibits multiple modes in Figure 6.3, this is likely an artifact of the piecewise drift function used to generate the contamination gradient, and real world exposures would likely be unimodal in the intermediate range of the distribution. Finally, with the exception of landscapes having zero weedy corn fields, there exists a third mode at the high extreme of the distribution, representing foraging upon contaminated weeds within corn fields. This high mode tapers off in the downward direction because in some foraging trips, the random walk algorithm crosses into or out of a weedy corn field, thus yielding a lower net exposure than would occur if the entire foraging trip took place inside a corn field.

With respect to exposure mitigation, the results of my model suggest that the risk posed to honey bees by seed treatment neonicotinoids released during corn planting is roughly proportional to the prevalence of weedy corn fields, and that the destruction of in-field weeds prior to planting would effectively reduce exposure, thus corroborating the findings of Chapter 5. Agricultural fields are large landscape components compared to
other habitat types, so weedy agricultural fields can dominate the total foraging habitat of a landscape. In my model, the risk posed by weedy corn fields is evidently offset by the safe forage provided by weedy soybean fields, as indicated by the consistent low mode at zero exposure. This suggests that preserving weeds in soybean fields may be as important for the reduction of honey bee exposure as destroying weeds in corn fields. Together, these two measures could exert a push-pull effect on honey bee foraging that would maximize the low mode of exposure and minimize the high mode. It should be noted, though, that soybean seeds are also typically treated with a neonicotinoid insecticide, and corn and soybean are typically grown in annual rotations. Thus, weeds in soybean fields (or even in field margins) could become contaminated by neonicotinoid residues left in the soil from a legacy of seed treatment use (Kupke et al. 2012, Stewart et al. 2014, Bonmatin 2015, Botías et al. 2015, Rundlöf et al. 2015, Botías et al. 2016, Mogren and Lundgren 2016). The magnitude of potential exposure via this route should be determined empirically before it is concluded that weeds in soybean fields offer safe forage for honey bees and other pollinators.

The stochasticity of my model arises from two processes: the random generation of landscape geometries and the random honey bee foraging algorithm. The execution of the model reported herein does not permit these two sources of stochasticity to be parsed, but design of the model enables such parsing in principle. Given sufficient computing time, the foraging algorithm could be iterated over each landscape, and landscape generation could be iterated over multiple set-seed foraging algorithms, thus isolating the effect of each stochastic process. Even without parsing these sources of stochasticity,
though, my model suggests that, in the real world, uncertainty regarding the distribution of floral resources and pesticide contamination in the landscape combined with the stochastic nature of honey bee foraging will undermine the relevancy of any deterministic approach to predicting honey bee pesticide exposure.

More rigorous simulation of honey bee exposure would require at least three types of data to inform model structure and parameterization. First, any simulation of honey bee foraging behavior can only be as accurate as the underlying model of floral resource distribution. In my model, it is implicitly assumed that floral resources are uniformly distributed in weedy fields, field margins, and roadsides. In reality, floral resources exhibit varying degrees of patchiness, in both space and time, making the landscape-scale quantification of resources a persistent problem in the field of pollinator ecology (Frankl et al. 2005). Second, the searching behavior of honey bees that ultimately governs the allocation of foragers amongst resource patches is poorly understood, due mainly to the technical difficulties of observing the behavior of foragers at the spatial scale of honey bee foraging. While empirical insights into this aspect of honey bee biology remain sparse, agent-based modeling approaches may at least allow for some testing of hypothesized behavioral rules (Becher et al. 2016). Finally, exposure estimates rely on an accurate understanding of environmental contamination problems. In my model, I employ a static function to simulate the contamination of a landscape by drifting seed treatment particles during corn planting. In reality, the mechanisms governing this process are probably no less complex and stochastic than those governing honey bee foraging, and
empirical observation no less elusive. Thus, my model likely underestimates the true variation and stochasticity of the seed treatment neonicotinoid exposure scenario.

In conclusion, my simulation model supports my interpretation of the statistical modeling presented in Chapter 5, indicating that the suppression of weeds in corn fields would be a promising approach to reducing honey bee exposure to seed treatment neonicotinoids. The patterns generated by my model further suggest, however, that preserving or augmenting the uncontaminated forage provided by weedy soybean fields may be equally important in suppressing honey bee exposure. While the mechanistic realism of my model is limited by the paucity of landscape-scale spatiotemporal data concerning the distribution of floral resources, the searching patterns of honey bee foragers, and the distribution of environmental contamination, my model explicitly simulates each of these elements with the most relevant data available. The resulting patterns of exposure are consistent with empirical patterns of exposure (Chapter 5), and the stochasticity of these patterns are consistent with theoretical predictions (Chapter 4).

6.7 References


Appendix A: Survey questionnaires

Spring Survey
To be completed after installing a package of bees

This is the first part of a two-part survey examining the success of honey bee colonies in urban, suburban and rural environments. If you complete this survey you will be contacted again in August and asked to complete a survey on the hive’s condition at that time. Your participation in this research survey is completely voluntary and you may withdraw at any time without penalty. Any information you provide through this survey will be considered confidential and will only be presented in a manner in which individual beekeepers are not identifiable. If you have any questions or concerns about the survey please contact Doug Sponsler (sponsler.18@osu.edu, 215-475-7203) or Reed Johnson (johnson.5005@osu.edu, 330-202-3523). For questions about your rights as a participant in this study or to discuss other study-related concerns or complaints with someone who is not part of the research team, you may contact Ms. Sandra Meadows in the Office of Responsible Research Practices at 1-800-678-6251. We thank you for your participation!
I. Eligibility (Please circle Yes or No)

Are you 18 years of age or older? Yes No

Are you starting a colony with a package of bees this spring? Yes No

Is the apiary where your new colony will be placed located in the State of Ohio? Yes No

Is your apiary in compliance with any local laws related to beekeeping? Yes No

If you answered “No” to any of the above questions you are not eligible to participate in this survey.

Is this apiary registered with the Ohio Department of Agriculture? Yes No

If your apiary is not registered, please fill out the attached registration form and send it in to the Ohio Department of Agriculture within 10 days.

II. Location

Where is your apiary located? Please provide one of the following: street address, latitude and longitude, or description of your apiary location so that it can be located on a map.

III. Colony Information

When did you install the package of bees?

Date (mm/dd/yyyy): _________
What race of queen came with your package of bees? (Circle one)

Italian

Carniolan

Russian

I don’t know

Other (please describe below)

How many total hives are in the apiary that contains the study hive?

Number of hives: _____

Provide a brief description of the apiary location (e.g. in a backyard, on a rooftop, next to apple orchard).

Which direction does the study hive face? (Circle one)

North South East West Northeast Southeast

Northwest Southwest

At what time in the morning does the study hive first receive direct sunlight on a sunny day?

Time of day (nearest hour): ________
At what time in the afternoon or evening does the study hive last receive direct sunlight on a sunny day?

**Time of day (nearest hour): ____**

IV. Management Practices

How many years have you been beekeeping?

**Number of years: ____**

What is the largest number of hives you have ever managed in a single year?

**Number of hives: ____**

V. Follow up

Did you place a sticker or leave a mark on the hive so that you know which hive is the “study hive”? (Circle one)

Yes  No

Please provide an e-mail address so that we can contact you again in mid-August to follow up on this hive’s success.

**E-mail:**

If you would prefer to answer the summer follow-up survey by mail, please provide your name and mailing address:

176
Name:
Street address / P.O. Box:
City, State ZIP:
Please return this survey to:

Beekeeper Survey Project
Thorne Hall
The Ohio State University – OARDC
1680 Madison Ave.
Wooster, OH 44691
Summer Survey
To be completed during week of August 19-25

Instructions

Thanks for filling out the survey about your new package this spring. This is the follow-up survey to measure that colony's success up to this point.

I. Hive status

Are you looking at the same study hive that you looked at in the spring survey? (Circle one)

Yes No

Has this hive been moved since the previous survey? (Circle one)

Yes No

Is this hive still alive? (Circle one)

Yes No

II. Queen status

Does the study hive appear to be healthy? (Circle one and write any comments below)

Yes No I’m not sure

Is the hive currently queenless? (Circle one and write any comments below)
Can you see any eggs or open brood in the colony? (Circle one and write any comments below)
Yes No I’m not sure

Can you see any of the capped brood? (Circle one and write any comments below)
Yes No I’m not sure

Did the study hive swarm? (Circle one)
Yes No
If yes, when did it swarm? (Circle one and write any comments below)
Date (mm/dd/yyyy): _______

Was the original queen replaced with a new queen that the bees produced either through emergency queen replacement or queen supersedure? (Circle one)
Yes No
If yes, when do you think the queen was replaced? (Circle one and write any comments below)
Date (mm/dd/yyyy): _______
Did you replace the queen yourself with a new queen or queen cell? (Circle one)

Yes No

If yes, when did you replace the queen? (Fill in date and comment below if possible)

Date (mm/dd/yyyy): __________

III. Hive report

What style of equipment is the hive? (Circle one and write any comments below)

10-frame 8-frame Top-bar Other

What does your hive consist of? (Check appropriate boxes working from the bottom of the hive to the top)

<table>
<thead>
<tr>
<th>Deep</th>
<th>Medium</th>
<th>Shallow</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>(9 5/8”)</td>
<td>(6 5/8”)</td>
<td>(5 11/16”)</td>
<td></td>
</tr>
</tbody>
</table>

Bottom

2nd

3rd

4th

5th

6th
When you were setting up this equipment, before you gave it to the bees, how many frames were drawn comb and how many were just foundation? (Write numbers in table below)

<table>
<thead>
<tr>
<th>Frames with just foundation</th>
<th>Drawn frames</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottom</td>
<td></td>
</tr>
<tr>
<td>2nd</td>
<td></td>
</tr>
<tr>
<td>3rd</td>
<td></td>
</tr>
<tr>
<td>4th</td>
<td></td>
</tr>
<tr>
<td>5th</td>
<td></td>
</tr>
<tr>
<td>6th</td>
<td></td>
</tr>
</tbody>
</table>

How many gallons of sugar syrup have you fed the hive since installing the bees?
Number of gallons: ______

Have you added or removed any frames from the colony? Did you add or remove honey frames? Did you equalize colonies by moving frames of brood and bees? If you are not sure about how many frames you moved, just give your best guess (Write numbers in table below)

<table>
<thead>
<tr>
<th>Honey frames</th>
<th>Honey frames</th>
<th>Brood frames</th>
<th>Brood frames</th>
</tr>
</thead>
<tbody>
<tr>
<td>removed</td>
<td>added</td>
<td>removed</td>
<td>added</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bottom</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd</td>
<td></td>
</tr>
</tbody>
</table>
Categorize all of the frames that are currently in each box in the hive. (Write numbers in table below)

<table>
<thead>
<tr>
<th>Bottom</th>
<th>2nd</th>
<th>3rd</th>
<th>4th</th>
<th>5th</th>
<th>6th</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undrawn</td>
<td>Less</td>
<td>Mostly</td>
<td>Mostly</td>
<td>Mostly</td>
<td>Drawn,</td>
</tr>
<tr>
<td>foundation</td>
<td>than $\frac{1}{2}$</td>
<td>nectar or</td>
<td>pollen</td>
<td>brood</td>
<td>but</td>
</tr>
<tr>
<td>drawn</td>
<td>honey</td>
<td></td>
<td></td>
<td>mostly</td>
<td>empty</td>
</tr>
<tr>
<td>out</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Estimate the number of combs in each box that are more than half covered with bees on both sides. (Write numbers in table below)

Frames more than half covered with bees

182
Have you used any beekeeping drugs since installing the package? (Circle any that apply and write any comments on the right)

Fumagillin B
Terramycin®
Tylan®
Check Mite®
Apistan®
Powdered sugar
ApiLife Var®
Apiguard®
MiteAway Quick Strip®
HopGuard®
Other (please describe below)

IV. Follow up

Are you interested in being contacted to complete another survey related to beekeeping sometime in the future? (Circle one)

Yes No
Appendix B: Detailed description of dance analysis methods

Decoding honey bee dances in ImageJ: an adaptation of Couvillon et al. (2012):

Dance recording
Dance behavior was recorded using three different camera models: Canon Vixia HD, Canon PowerShot ELPH 340, and Canon PowerShot SX 150. These have equal frame rates and so can be processed equivalently in ImageJ.

Video pre-processing
Record your observation hive, being careful to reduce glare and keep the camera squarely pointed at the hive so that distances don’t get distorted. Cut the video into 1 minute segments in AVI format (not mp4).

Step-by-step instructions for dance decoding
Preparing files in the FIJI distribution of ImageJ (v. 2.0) (http://fiji.sc/)

1. Open FIJI and go to File → Import → AVI
2. Check “Convert to Grayscale” and uncheck “Use Virtual Stack”. Click OK.
3. Once video is loaded, click Image on the main toolbar and select Properties. Change the frame interval to match the frame rate of your camera (i.e. seconds per frame) and click OK.
4. Swap the number in “Slices” with the number in "Frames" (by default, FIJI interprets temporal frames as spatial layers, as in a micrograph)
5. Click “Plugins” in the main toolbox, go to “Tracking” and select MtrackJ. A small toolbox should appear.

*Tracking dances using MtrackJ*

1. Watch video and locate a dance having at least two left turns and two right turns.
2. Pause the video and use the wheel on your mouse to rewind the video until the bee is about to begin the dance.
3. Select “add” in the MtrackJ toolbox and click the middle of the thorax of the bee when she starts a waggle run. You can tell when the waggle run starts because the bee becomes distorted or fuzzy on the screen due to its rapid movement. Follow the bee by using the wheel of the mouse to scroll forward until the bee completes the waggle run and place another point on the bee’s thorax.
4. To start a new track, click the “add” button on the MtrackJ toolbox twice.
5. Repeat the steps 3 and 4 four times to decode each dance (Figure B1)
6. After completing the second dance (tracks 5-8), press “Cluster” in the MtrackJ toolbox. Hold control and select run #5. Click the drop down menu in the box and select “new”. This will cluster the first four tracks automatically and change track 5 to 2.1. Hold control and select the following runs in order and add them to the second cluster. You should have 1.1-1.4 in the first cluster and 2.1-2.4 in the second cluster. Repeat for every dance in each video.
8. Enable “Cluster Measurements” by holding “CTRL” and clicking on “Measure”. Check the box “Display Cluster Measurements”. You can uncheck “Display Point Measurements” and “Display Track Measurements” as we’re not interested in these measurements.
9. After all the dances are decoded and clustered, select “measure”. Copy all of the data in the “cluster measurements” window and paste it into a spreadsheet.
Figure B.1: Screenshot showing four tracked waggle runs, together comprising a single decoded dance
Appendix C: Complete pollen data set
<table>
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<th>Aug. 7</th>
<th>Aug. 13</th>
<th>Aug. 21</th>
<th>Aug. 28</th>
<th>Sept. 4</th>
<th>Sept. 12</th>
<th>Sept. 19</th>
<th>Sept. 26</th>
</tr>
</thead>
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<td>Sagittaria sp.</td>
<td></td>
<td>1.039</td>
<td>0.397</td>
<td>3.322</td>
<td>0.434</td>
<td>0.37</td>
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<td>44.27</td>
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<tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
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<td>Aster sp. C</td>
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<td></td>
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<td>0.013</td>
<td>0.018</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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<td>0.367</td>
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<td>Eupatorium sp.</td>
<td>0.054</td>
<td>0.455</td>
<td>1.488</td>
<td>0.09</td>
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<td></td>
<td>0.069</td>
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<td>2.068</td>
<td>11.913</td>
<td>35.297</td>
<td>97.07</td>
<td>80.967</td>
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<tr>
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<td>0.249</td>
<td>0.104</td>
<td>0.015</td>
<td></td>
<td></td>
<td>0.002</td>
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<td>3.103</td>
<td>0.661</td>
<td>0.105</td>
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<td>Impatiens sp.</td>
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<td></td>
<td>0.113</td>
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</table>

Continued
### Appendix C

**Continued**

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**Continued**
### Appendix D Continued

| V129 | Medicago sativa | 9 July 2014 | Clover Cemetery | Sponsler Wallace |
| V130 | Prunella vulgaris | 9 July 2014 | Clover Cemetery | Sponsler Wallace |
| V132 | Sagittaria | 31 July 2014 | Clover Wetland | Sponsler Sponsler |
| V133 | Veronica | 31 July 2014 | Clover Wetland | Sponsler Sponsler |
| V134 | Polygonum pennsylvanicum | 7 August 2014 | Clover Cemetery | Sponsler Wallace |
| V135 | undetermined | 7 August 2014 | Clover Cemetery | Sponsler |
| V136 | Cyperus? | 7 August 2014 | Clover Cemetery | Sponsler Lin |
| V137 | Asclepias incarnata | 7 August 2014 | Clover Cemetery | Sponsler Lin |
| V138 | undetermined | 7 August 2014 | Clover Cemetery | Sponsler |
| V139 | Securigera varia | 7 August 2014 | campus | Sponsler |
| V140 | Erodium cicutarium | 7 August 2014 | campus | Sponsler Lin |
| V141 | Commelina | 7 August 2014 | campus | Sponsler Lin |
| V142 | undetermined | 7 August 2014 | campus | Sponsler |
| V143 | Scrophulariaceae undetermined | 7 August 2014 | campus | Sponsler Lin |
| V144 | Hydrangeae paniculata | 7 August 2014 | campus | Sponsler Lin |
| V145 | Heleneium autumnale | 7 August 2014 | riverside | Sponsler Lin |
| V146 | undetermined | 7 August 2014 | riverside | Sponsler |
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| V149 | Malva | 7 August 2014 | riverside | Sponsler Lin |
| V150 | Lythrum salicaria | 7 August 2014 | riverside | Sponsler Lin |
| V151 | Verbana? | 7 August 2014 | riverside | Sponsler Lin |
| V152 | Lotus corniculatus | 7 August 2014 | riverside | Sponsler Lin |
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| V154 | undetermined | 7 August 2014 | riverside | Sponsler |
| V155 | Oenothera | 7 August 2014 | riverside | Sponsler Lin |
| V156 | Lythrum | 7 August 2014 | riverside | Sponsler Lin |

Continued
<p>| V157 | Asteraceae | undetermined | 7 August 2014 | campus | Sponsler | Lin |
| V158 | Verbesina | alternifolia | 7 August 2014 | Farm Science Review | Sponsler | Sponsler |
| V159 | Oenothera | | 7 August 2014 | Gwynn Reserve | Sponsler | Lin |
| V160 | Primulaceae | Angallis? arvensis? | 7 August 2014 | Farm Science Review | Sponsler | Lin |
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| V162 | Asteraceae | undetermined | 23 July 2014 | Farm Science Review | Sponsler | |
| V163 | Daucus | carota | 23 July 2014 | Farm Science Review | Sponsler | Sponsler |
| V164 | Melilotus | albus | 23 July 2014 | Clover Cemetery | Sponsler | Lin |
| V165 | Melilotus | albus | 23 July 2014 | Clover Cemetery | Sponsler | Sponsler |
| V166 | Glycine | max | 23 July 2014 | Farm Science Review | Sponsler | Lin |
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| V168 | undetermined | | 7 August 2014 | Gwynn Reserve | Sponsler | |
| V169 | Mimulus | ringens? | 7 August 2014 | Gwynn Reserve | Sponsler | Wallace |
| V170 | Senna? | hebecarpa | 7 August 2014 | Farm Science Review | Sponsler | Wallace |
| V171 | Desmodium | canadense? | 7 August 2014 | Farm Science Review | Sponsler | Lin |
| V172 | Scrophulariaceae | undetermined | 7 August 2014 | Farm Science Review | Sponsler | Wallace |
| V173 | Hibiscus | trionum | 7 August 2014 | Farm Science Review | Sponsler | Lin |
| V174 | Poaceae | Setaria | 7 August 2014 | Farm Science Review | Sponsler | Wallace |
| V175 | Lamiaceae | Mentha | arvensis | 7 August 2014 | Gwynn Reserve | Sponsler | Wallace |
| V176 | Prunella | vulgaris | 23 July 2014 | Clover Cemetery | Sponsler | Lin |
| V177 | Apiaceae | undetermined | 23 July 2014 | Clover Cemetery | Sponsler | |
| V178 | Sagittaria | | 23 July 2014 | Clover Wetland | Sponsler | Sponsler |
| V179 | Monarda | fistulosa | 23 July 2014 | Farm Science Review | Sponsler | Lin |
| V180 | Asteraceae | Rudbeckia | 23 July 2014 | Farm Science Review | Sponsler | Wallace |
| V181 | Fabaceae | Medicago | lupulina? | 23 July 2014 | Farm Science Review | Sponsler | Sponsler |
| V182 | Hibiscus | moscheutos | 23 July 2014 | Clover Wetland | Sponsler | Wallace |
| V183 | Lamiaceae | Prunella? | 22 July 2014 | OARDC | Sponsler | Lin |</p>
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<td>4 September 2014</td>
<td>Clover Wetland</td>
<td>Sponsler</td>
<td>Wallace</td>
</tr>
<tr>
<td>V327</td>
<td>Lobelia siphilitica</td>
<td>4 September 2014</td>
<td>Clover Wetland</td>
<td>Sponsler</td>
<td>Wallace</td>
</tr>
<tr>
<td>V328</td>
<td>Asteraceae Helianthus? tuberosus?</td>
<td>4 September 2014</td>
<td>Clover Wetland</td>
<td>Sponsler</td>
<td>Wallace</td>
</tr>
<tr>
<td>V329</td>
<td>Asteraceae undetermined</td>
<td>4 September 2014</td>
<td>Clover Wetland</td>
<td>Sponsler</td>
<td>Wallace</td>
</tr>
<tr>
<td>V330</td>
<td>Euthamia graminifolia</td>
<td>4 September 2014</td>
<td>Clover Wetland</td>
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<td>Wallace</td>
</tr>
<tr>
<td>V331</td>
<td>Asteraceae Symphyotrichum novae-angliae</td>
<td>4 September 2014</td>
<td>Clover Wetland</td>
<td>Sponsler</td>
<td>Wallace</td>
</tr>
<tr>
<td>V332</td>
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<td>Clover Wetland</td>
<td>Sponsler</td>
<td>Wallace</td>
</tr>
<tr>
<td>V333</td>
<td>Lotus</td>
<td>4 September 2014</td>
<td>riverside</td>
<td>Sponsler</td>
<td>Wallace</td>
</tr>
<tr>
<td>V334</td>
<td>Duchesnea or Potentilla</td>
<td>4 September 2014</td>
<td>Bob’s house</td>
<td>Sponsler</td>
<td>Wallace</td>
</tr>
<tr>
<td>V335</td>
<td>Asteraceae Aster ericoides or pilosus</td>
<td>4 September 2014</td>
<td>riverside</td>
<td>Sponsler</td>
<td>Wallace</td>
</tr>
<tr>
<td>V336</td>
<td>Asteraceae Symphyotrichum novae-angliae</td>
<td>4 September 2014</td>
<td>riverside</td>
<td>Sponsler</td>
<td>Wallace</td>
</tr>
<tr>
<td>V337</td>
<td>Asteraceae Aster?</td>
<td>4 September 2014</td>
<td>riverside</td>
<td>Sponsler</td>
<td>Wallace</td>
</tr>
<tr>
<td>V338</td>
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<td>campus</td>
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<tr>
<td>V339</td>
<td>Solanum dulcamara</td>
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<td>campus</td>
<td>Sponsler</td>
<td>Wallace</td>
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<tr>
<td>V340</td>
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<td>campus</td>
<td>Sponsler</td>
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<tr>
<td>V341</td>
<td>Solanaceae Petunia?</td>
<td>4 September 2014</td>
<td>campus</td>
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<tr>
<td>V342</td>
<td>Setaria</td>
<td>21 August 2014</td>
<td>FSR</td>
<td>Sponsler</td>
<td>Wallace</td>
</tr>
<tr>
<td>V343</td>
<td>Agrostis gigantea</td>
<td>21 August 2014</td>
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<tr>
<td>V344</td>
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<tr>
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<td>Sponsler</td>
<td>Wallace</td>
</tr>
<tr>
<td>Page</td>
<td>Plant Name</td>
<td>Species</td>
<td>Date</td>
<td>Location</td>
<td>Author</td>
</tr>
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<td>Senna</td>
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<td>208</td>
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<td>riverside</td>
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<tr>
<td>210</td>
<td>Conyza</td>
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<td>21 August 2014</td>
<td>riverside</td>
<td>Sponsler Wallace</td>
</tr>
<tr>
<td>211</td>
<td>Eupatorium serotinum or altissimum</td>
<td></td>
<td>21 August 2014</td>
<td>campus</td>
<td>Sponsler Wallace</td>
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<tr>
<td>212</td>
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<td>campus</td>
<td>Sponsler Wallace</td>
</tr>
<tr>
<td>213</td>
<td>Poa?</td>
<td></td>
<td>21 August 2014</td>
<td>campus</td>
<td>Sponsler Wallace</td>
</tr>
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<td>214</td>
<td>Convolvulus</td>
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<td>campus</td>
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<tr>
<td>215</td>
<td>Cirsium</td>
<td>discolor</td>
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<td>Clover Wetland</td>
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</tr>
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<td>216</td>
<td>Eupatorium</td>
<td>serotinum or altissimum</td>
<td>28 August 2014</td>
<td>riverside</td>
<td>Sponsler Wallace</td>
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<td>coelestinum</td>
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<td>Sponsler Wallace</td>
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<td>218</td>
<td>Ambrosia</td>
<td>artemisifolia</td>
<td>28 August 2014</td>
<td>riverside</td>
<td>Sponsler Wallace</td>
</tr>
</tbody>
</table>
library(raster)
library(rgdal)
library(rgeos)

setwd("/Users/dougsponsler/Documents/Research/Risk_Index_Analysis/new_analysis_stepwise_drift_MEAN")

####################################################
######### Dust and Visitation Parameters ############
####################################################

### prepare dust drift data
dust_sub <- subset(dust, Location != "H" & Location != "P") # subset out the high detector and the planter-mounted detector
tapply(dust_sub$CLO_THX, dust_sub$Distance, mean) # get mean concentration per distance

peaks_x <- c(0, 1, 10, 50, 100) # distances for representative means; 5m omitted because mean was lower than 10m
peaks_y <- c((4.47 + 1.95), 2.08, 1.95, 1.45, 0.98) # means at representative distances (10m concentration added to 0 m concentration to account for under-planter detector being picked up after first run)

### plot stepwise drift function: estimated concentration at each distance = max mean concentration at or beyond that distance
pdf("dust_drift.pdf", width = 6, height = 6)
plot(CLO_THX ~ Distance, data = dust_sub,
     ylab = "Neonicotinoid concentration (ng/cm^2)",
     xlab = "Distance from field edge (m)"
    )
lines(peaks_x, peaks_y,
      type = "S",
      col = "red",
      lwd = 2)
#dev.off()

### Define stepwise function for representative peaks (not generalizable to unprocessed data)
dust_drift_function <- function(x) {
    ifelse (x == 0, y <- (4.47 + 1.95),

ifelse (x > 0 & x <= 1, y <- 2.08,
  ifelse (x > 1 & x <= 10, y <- 1.95,
    ifelse (x > 10 & x <= 50, y <- 1.45,
      ifelse (x > 50 & x <= 100, y <- 0.98, y <- 0))))
}

### Dance data: parameters of \( p(\text{visitation}) \sim \text{distance} \) NLS fit

\[
B_0_{\text{vis}} \leftarrow -0.1204
\]

\[
B_1_{\text{vis}} \leftarrow -0.001404
\]

### Western Branch 2015

### set hive coordinates (EPSG:26917) and create 2km buffer

\[
w_b_{\text{easting}} \leftarrow 271629.4143
\]

\[
w_b_{\text{northing}} \leftarrow 4415735.8504
\]

\[
w_b_{\text{coords}} \leftarrow \text{SpatialPoints(cbind(w_b_{\text{easting}}, w_b_{\text{northing}}))}
\]

\[
\text{proj4string}(w_b_{\text{coords}}) \leftarrow \text{CRS("+init=epsg:26917")}
\]

\[
w_b_{\text{2km_buffer}} \leftarrow \text{buffer}(w_b_{\text{coords}}, \text{width} = 2000, \text{quadsegs} = 50)
\]

### load landscape data (rasterizations of our vector data)

\[
w_b_{\text{2015 class}} \leftarrow \text{raster("/Users/dougsponsler/Documents/Research/Risk_Index_Analysis/new_analysis_stepwise_drift_MEAN/wb_2015_class.tif")}
\]

\[
\text{projection}(w_b_{\text{2015 class}}) \leftarrow \text{CRS("+init=epsg:26917")}
\]

\[
w_b_{\text{2015 crop}} \leftarrow \text{raster("/Users/dougsponsler/Documents/Research/Risk_Index_Analysis/new_analysis_stepwise_drift_MEAN/wb_2015_crop.tif")}
\]

\[
\text{projection}(w_b_{\text{2015 crop}}) \leftarrow \text{CRS("+init=epsg:26917")}
\]

\[
w_b_{\text{2015 bloom}} \leftarrow \text{raster("/Users/dougsponsler/Documents/Research/Risk_Index_Analysis/new_analysis_stepwise_drift_MEAN/wb_2015_bloom.tif")}
\]

\[
\text{projection}(w_b_{\text{2015 bloom}}) \leftarrow \text{CRS("+init=epsg:26917")}
\]

\[
w_b_{\text{2015 forage}} \leftarrow \text{raster("/Users/dougsponsler/Documents/Research/Risk_Index_Analysis/new_analysis_stepwise_drift_MEAN/wb_2015_forage.tif")}
\]

\[
\text{projection}(w_b_{\text{2015 forage}}) \leftarrow \text{CRS("+init=epsg:26917")}
\]

\[
w_b_{\text{2015 distance}} \leftarrow \text{distanceFromPoints(w_b_{\text{2015 class}}, c(w_b_{\text{easting}}, w_b_{\text{northing}}))} \quad \# \text{a raster in which each cell is assigned a value equal to its distance in meters from the origin (i.e. from the hive)}
\]

\[
\text{projection}(w_b_{\text{2015 distance}}) \leftarrow \text{CRS("+init=epsg:26917")}
\]

### create raster of visitation probability based on dance language analysis

\[
w_b_{\text{2015 pVis}} \leftarrow B_0_{\text{vis}} * \exp(B_1_{\text{vis}} * w_b_{\text{2015 distance}})
\]

\[
\text{projection}(w_b_{\text{2015 pVis}}) \leftarrow \text{CRS("+init=epsg:26917")}
\]

### create corn-only and not-corn-only rasters

\[
w_b_{\text{2015 corn}} \leftarrow w_b_{\text{2015 crop}}
\]

\[
w_b_{\text{2015 corn}}[w_b_{\text{2015 corn }} != 1] \leftarrow \text{NA}
\]

\[
w_b_{\text{2015 notCorn}} \leftarrow w_b_{\text{2015 crop}}
\]
### create distance-from-corn raster (this will take about 30 min)

```r
wb_2015_corn_distance <- gridDistance(wb_2015_corn, origin = 1, doEdge = T)
```

writeRaster(wb_2015_corn_distance, "wb_2015_corn_distance.tif") # write raster to avoid repeating this long step

```r
wb_2015_corn_distance <- raster("wb_2015_corn_distance.tif") # uncomment to reload saved raster
```

### convert-distance-from-corn to contamination level

```r
wb_2015_contam <- calc(wb_2015_corn_distance, dust_drift_function)
```

### create distance-weighted risk index

```r
wb_2015_distance_weighted_RI <- wb_2015_pVis * wb_2015_contam
```

### create distance-weighted risk index restricted to foragable patches

```r
wb_2015_distance_weighted_RI_forage <- wb_2015_pVis * wb_2015_contam * wb_2015_forage
```

### create distance-weighted risk index restricted to corn fields

```r
wb_2015_distance_weighted_RI_corn <- wb_2015_pVis * wb_2015_contam * wb_2015_corn
```

### create distance-weighted risk index restricted to WEEDY corn fields

```r
```

### create distance-weighted risk index restricted to off-field drift

```r
```

### raster brick of risk index layers

```r
projection(wb_2015_risk_index) <- CRS("+init=epsg:26917")
```

### mask and trim risk index brick raster to 2km buffer

```r
wb_2015_risk_index_mask <- mask(wb_2015_risk_index, wb_2km_buffer) ### this step takes a while
```

```r
wb_2015_risk_index_trim <- trim(wb_2015_risk_index_mask, values = NA)
```

writeRaster(wb_2015_risk_index_trim[[1]], "wb_2015_RI.tif") # risk index map

writeRaster(wb_2015_risk_index_trim[[2]], "wb_2015_RI_forage.tif") # risk index map, restricted by forage

writeRaster(wb_2015_risk_index_trim[[3]], "wb_2015_RI_corn.tif") # risk index map, restricted to corn

writeRaster(wb_2015_risk_index_trim[[4]], "wb_2015_RI_weedyCorn.tif") # risk index map, restricted to weedy corn

writeRaster(wb_2015_risk_index_trim[[5]], "wb_2015_RI_drift.tif") # risk index map, restricted to weedy corn
writeRaster(wb_2015_risk_index_trim, "wb_2015_risk_index_trim.tif") # trimmed raster brick saved to avoid repeating long masking and trimming process
  # wb_2015_risk_index_trim <- brick("wb_2015_risk_index_trim.tif") # uncomment to reload saved raster

### convert to data frame for analysis
wb_2015_risk_index_df <- data.frame(as.matrix(wb_2015_risk_index_trim))
  colnames(wb_2015_risk_index_df) <- c("distance_weighted_RI", "distance_weighted_RI_forage", "distance_weighted_RI_corn", "distance_weighted_RI_weedyCorn", "distance_weighted_RI_drift")

### histograms
pdf("wb_2015_RI_hist.pdf", height = 4, width = 5)
  hist(wb_2015_risk_index_df$distance_weighted_RI, col = "gray")
  dev.off()

pdf("wb_2015_RI_forage_hist.pdf", height = 4, width = 5)
  hist(wb_2015_risk_index_df$distance_weighted_RI_forage, col = "gray")
  dev.off()

pdf("wb_2015_RI_corn_hist.pdf", height = 4, width = 5)
  hist(wb_2015_risk_index_df$distance_weighted_RI_corn, col = "gray")
  dev.off()

pdf("wb_2015_RI_weedyCorn_hist.pdf", height = 4, width = 5)
  hist(wb_2015_risk_index_df$distance_weighted_RI_weedyCorn, col = "gray")
  dev.off()

pdf("wb_2015_RI_drift_hist.pdf", height = 4, width = 5)
  hist(wb_2015_risk_index_df$distance_weighted_RI_drift, col = "gray")
  dev.off()

### cumulative risk index
  colSums(wb_2015_risk_index_df, na.rm = T)

208