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Spatial Processes and Ecotoxicological Risk Assessment Modeling

Steven Thomas Purucker

University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a dissertation written by Steven Thomas Purucker entitled "Spatial Processes and Ecotoxicological Risk Assessment Modeling." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Ecology and Evolutionary Biology.

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Thomas G. Hallam, Major Professor

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We have read this dissertation and recommend its acceptance:

Louis J. Gross, Gary F. McCracken, Suzanne Lenhart

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Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

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SPATIAL PROCESSES AND ECOTOXICOLOGICAL
RISK ASSESSMENT MODELING

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Steven Thomas Purucker
August, 2006

DEDICATION

I dedicate this dissertation to my wife Sharon and our three children, Matthew, William, and Anna.

ABSTRACT

PURUCKER, STEVEN THOMAS.   Spatial Processes and Ecotoxicological Risk Assessment Modeling. (Advisor Thomas G. Hallam).

This dissertation considers the importance of spatial processes in the application of models for environmental risk assessment. The appropriate incorporation of space as an ecological variable to construct spatially delineated risk estimates can improve estimates of the risk outcomes and improve resulting risk management decisions. Three environmental problems are considered, corresponding to three different case studies measuring an ecotoxicological effect at progressively broader spatial scales. The first application addresses how the presence of spatial auto-correlation can affect the exposure of ecological receptors in the environment. The presence of significant spatial correlation can impact methods for adequately determining summary concentrations, hypothesis testing, and for identifying zones in need of remediation at contaminated sites. The second case study addresses how treating habitat as a spatial variable can impact estimates of population doses. Calculated dose estimates are dependent on the distributions of spatial habitat and the toxin. Risk estimates can differ considerably when compared to the more common use of summary statistics to represent an exposure concentration for risk purposes. Measuring the spatial correlation of habitat quality and

contamination distribution is recommended as a means for evaluating whether incorporating habitat and spatial movement models are useful for an environmental assessment. Finally, the risk of insects developing resistance to genetically modified crops is evaluated within a spatial milieu of varying bat predation rates and changing genetically modified crop types and percentages. This model is developed at a broad spatial scale and considers large-scale insect migrations and varying spatial pressure from foraging bats. Incorporation of these larger scale processes significantly impacts temporal estimates of the evolution of insect resistance to toxins in the environment.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. Introduction

This dissertation will consider the importance of space in environmental risk assessment processes. Due to advances in hardware, software, and model development and analysis, tools that assist in the explicit consideration of spatial effects are becoming more common in environmental assessments of risk in a variety of applications. Consideration of space and use of these tools in the assessment process has the potential to improve the estimates of the risk outcomes and therefore can influence the resulting risk management decisions. These improved risk estimates can then be used in optimally designing the risk management decisions that are ultimately implemented. This dissertation is devoted to the topic of *spatial processes and ecotoxicological risk assessment modeling*.

Three main applications will be considered, corresponding to three different case studies measuring a different risk variable at progressively broader spatial scales. The first application is at the relatively local scale, a contaminated site in Oak Ridge, Tennessee, and addresses how the presence of spatial auto-correlation can affect how ecological receptors are exposed in the environment. The presence of significant spatial correlation can impact methods for adequately determining summary concentrations, hypothesis testing, and for identifying zones in need of remediation at contaminated sites.

The second application is a fluoride contaminated site in the western United States, and considers the incorporation of habitat as a spatial variable and its impact on estimates of population doses. The results are dependent on the distribution of spatial habitat and risk estimates can differ considerably when compared to the more common use of summary statistics to represent an exposure concentration for risk purposes. The third application is centered in the Winter Garden region of Texas. This section will evaluate the risk of insects developing resistance to genetically modified (GM) crops within a spatial milieu of varying bat predation rates and changing genetically modified crop types and percentages. This application can be developed at an even broader spatial scale by considering how the Winter Garden region is affected by long distance moth migrations from Mexico; and how subsequent migrations of moths to the northeast from the Winter Garden region is affected by bat predation and GM crops in the Winter Garden region. Chapter 4 is an overview tying changes in approaches for modeling resistance evolution to risk management regulations. Chapter 5 documents the implementation of the *Bacillus thuringiensis (Bt)* resistance model in the presence of bat predation.

1.1 Organization

The dissertation will be organized into an introduction (Chapter 1), four main chapters of the dissertation concerning each of the applications (Chapters 2, 3, 4, and 5), and will close with a conclusions chapter (Chapter 6). Each of the first three application chapters will be written with a target journal in mind and will be submitted in the

appropriate format for that journal.

Chapter 2 examines how methods from spatial statistics can assist in terrestrial ecological risk assessment. The practice of ecology is giving more consideration to spatial structure and heterogeneity in the construction of theory, interpretation of processes, and the collection of data (Fortin and Dale 2005). In ecological site assessment, depositional processes that lead to anthropogenic contamination in the terrestrial environment often cause the spatial distribution of data to be auto-correlated. However, in lieu of efficient and practical methods to consider spatial aspects of exposure in budget-constrained ecological risk assessments (Hope 2005), the Environmental Protection Agency (EPA) and other regulatory agencies have often implicitly assumed homogeneity of contaminant concentrations by estimating exposures with a non-spatial summary statistic. The presence of spatial auto-correlation can affect methods for determining summary concentrations, hypothesis testing, and for identifying zones in need of remediation at contaminated sites. Efficient selective remediation of contaminated sites, the process of remediating sub-areas of a contaminated site to achieve quantitative cleanup goals and specified error tolerances for a larger area (Blacker and Goodman 1994; Brakewood and Grasso 2000), requires knowing the spatial distribution of contamination and of exposures. Therefore, the application of spatial statistical tools to environmental risk assessment is a potentially fruitful area for improving decision-making processes that consider the spatial distribution of contamination at a site.

Chapter 3 considers the exposures of a population of pronghorn to fluoride in Idaho. Anthropogenic contamination is typically distributed heterogeneously through space. This spatial structure can have differing effects on the cumulative doses of individuals exposed to contamination within the environment. These effects are exacerbated when individuals pursue different movement strategies, and movement strategies can be affected by how individuals and species value habitat. Habitat quality is often neglected when ecological risk assessments are performed, despite evidence that inclusion of a quantitative habitat measure can have a significant effect on the overall exposure estimate. This paper couples an exposure model with habitat data to examine the interactions between habitat preferences, the spatial distribution of contamination, and the resulting impact on dose estimates. I have constructed dose distributions for pronghorn exposed to fluoride when foraging on desert sagebrush. The results show the magnitude of the difference between doses when foraging concentrations are positively or negatively correlated to varying degrees with different spatial distributions of habitat preferences. Differences between exposures obtained from the non-spatial versus the spatial methods with different habitat types and variation within the movement model due to different habitat preferences are developed as part of this effort. These results are generally applicable to other contaminants and receptors. Examining the strength of spatial correlation between habitat and contaminant data can be used as a proxy to determine when the implementation of a spatially-explicit ecological risk assessment is

useful. This model has been programmed so that it can be added to Spatial Analysis and Decision Assistance (SADA), which will enhance the exposure of this model due to the overall utility and popularity of the SADA code. The next version of SADA will contain the algorithms I have programmed to generate these exposure distributions and will also have statistical correlation capabilities to help assessors evaluate for themselves potential positive or negative correlations between their contaminant concentration data and quantitative estimates of habitat quality.

The application of spatial methods in terrestrial ecological risk assessment can minimize decision errors and maximize remediation effectiveness and can easily be implemented within the context of a geographical information system (GIS). However, ecological risk assessment methods and guidance do not promote methods to search for zones and strengths of auto-correlation; instead, methods for criterion compliance are promulgated that assume spatial independence. The use of these methods at contaminated sites with spatially correlated contaminant distributions results in a loss of spatial information, which can then lead to higher than acceptable decision error rates and lost opportunities to efficiently identify areas of concern for selective remediation. The majority of typical ecological risk assessments are conducted under assumptions of data independence. Data management capabilities, descriptive statistics for summarizing contaminated areas, screening benchmarks, terrestrial exposure methods, and toxicity reference values are combined to produce tabular output and hypothesis tests sufficient to

document the majority of ecological risk assessments. A GIS provides a platform for additional assessment methods that account for spatial dependence, including spatially relevant descriptive statistics, moving window spatial statistics, modified hypothesis tests, correlation modeling, and interpolation methods. These tools provide a number of methods that can minimize remedial action decision errors, provide spatial designs for efficient remedial design under selective remediation conditions, and provide a rationale and context for additional sampling efforts at contaminated sites. This section summarizes the terrestrial assessment practices under spatially independent and dependent assumptions; further it demonstrates how methods for testing and accounting for spatial correlation can improve the practice of ecological risk assessment.

Chapter 4 is entitled "Resistance is Futile?: Risk Assessment and Modeling for Insect Resistance to Plant-Incorporated Protectants". Plant incorporated protectants in agricultural crops via genetic sequences from *Bt* bacteria were first commercially introduced in 1996. Early modeling results and expectations prior to the technology being introduced were that insect resistance would quickly evolve due to the high selection pressure being placed on the exposed populations. Resistance modeling efforts influenced the risk management steps that were taken to reduce the chances of resistance evolving. Even with these steps, the consensus was that resistance was inevitable and would quickly evolve; somewhat surprisingly, ten years after introduction, insect resistance has yet to be detected in the field. This has occurred despite consistent

increases in the number of licensed crops containing *Bt* genetic material and steady increases in acreage devoted to transgenic crops. This paper reviews the history of risk assessment and resistance modeling dating from the first insect resistance evolution models from the 1970s and tracks the influence of these models on risk management regulations designed to minimize the risk of resistance.

Chapter 5 addresses the implementation of genetically modified crops in the Winter Garden region of Texas and possible effects on the bat-insect co-dynamics in the region. The principal strategy for delaying resistance in insects to *Bt* modified crops is a combination of a high *Bt* dose to the insect in the crop and the creation of a spatial refuge in close proximity so that susceptible insects will mate with insects that develop resistance. This section of the dissertation focuses on the effects that foraging Mexican free-tailed bats may have on the implementation of this strategy in *Bt* fields. A model is developed that examines the resistance population dynamics of the pest insects in the presence of genetically-modified crops and refuges, the mass migration of susceptible moths from Mexico, and then explores the impacts that differential foraging of Mexican free-tailed bats on the insects can have on the dynamics of time to resistance.

In conclusion, the dissertation emphasizes the importance of incorporating space as a variable in environmental assessments of risk and summarizes the models and methods that have been presented. Use of these approaches allow for the incorporation

of different forms of spatial information at different scales in a manner that directly informs risk management decision-making processes. Space is the bridge that connects closed models with mainly heuristic application to the open systems that are being modeled and impacts on decision-making processes. The degree to which space is successfully incorporated can often directly influence the usefulness of the model for risk management purposes.

The format of the dissertation is designed to produce at least three published papers. The spatial population dose of pronghorn to fluoride will be submitted to *Ecological Modelling*. There have been a number of papers recently published pertaining to spatially explicit ecological risk assessment in *Ecological Modelling* (Chow *et al.* 2005; Jager *et al.* 2005). The methods presented in this chapter extend their application while also presenting a means for "production-line" implementations (Hope 2005) of spatial dose assessment since the methods will be incorporated into the SADA freeware.

The spatial statistics application for terrestrial ecological risk assessment will be submitted to *Integrated Environmental Assessment and Management*. The methods presented in this paper will be used in future versions of the SADA and the Environmental Protection Agency's (EPA) Fully Integrated Environmental Decision Support (FIELDS) software for ecological risk assessment.

The target journal for the resistance evolution overview is *Risk Analysis*. This journal publishes a wide variety of papers in the risk assessment field, yet surprisingly has not had many publications examining risks associated with the introduction of biotechnology in food crops, other than surveys of people's perceptions towards genetically modified foods. A paper is anticipated on the insect availability in the Winter Garden region with differing levels of *Bt* implementation in the area. This type of information could be used as an input to a bat energetics model to look at indirect higher level trophic effects of *Bt* implementation on bat colonies in the area. The target journal for the resistance application is the *Journal of Economic Entomology*, where a number of interesting papers with similar types of models have been published (*e.g.*, Carriere *et al.* 2005; Guse *et al.* 2002; Onstad *et al.* 2002; Storer *et al.* 2003a, 2003b). However, more field data (currently being collected) and analysis is needed to parameterize this model before final publication, particularly for a spatial implementation of this system.

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2. Selective remediation and decision support for terrestrial ecological risk assessment

Remediation decisions at contaminated sites typically involve manifold data sources, complex environmental and ecological processes with associated models, risk assessment and cost-benefit considerations, as well as competing stakeholder interests reflecting different decision objectives (Linkov *et al*. 2004). There is also an explicit geographical context to most remedial decisions that can further complicate selection of remedial alternatives. During the remedial alternative selection process, decision makers are called upon to balance these different types of technical input and produce an objectively correct, defendable decision. Given the wealth of data inputs and decision options, it is no surprise that there have been a number of spatial support systems designed to assist decision-making that have evolved over the past decade to meet these needs in a geographic information system (GIS) environment (Ascough *et al*. 2002).

Decision-making for soil remediation sites driven by risk assessment-based cleanup criteria are amenable to spatial decision support approaches (Nyerges *et al*. 1997; Thayer *et al*. 2003), though not without their limitations (Woodbury 2003). These problems contain an explicit spatial context for the distribution of contamination and for exposures to terrestrial receptors. Risk assessment and management for such sites is further complicated by the fact that they contain multiple contaminants present in

multiple media with each combination differing in their spatial distributions of high concentrations. Also, there can be a number of different regulatory decision criteria depending on cleanup context and receptor that need to be met and many sites have multiple contaminants at actionable levels. The advent of GIS programs integrated with environmental assessment features have produced a capability for spatial analyses at sites with multiple contaminants that previously had been impeded by inadequate characterization capabilities (Preston 2002). GIS tools with embedded spatial analysis and risk assessment features allow for efficient organization of data and prioritization of areas and toxicants. The continued development of these tools are improving the implementation of data collection and analysis methods to solve ecological (Chow *et al.* 2005; Gaines *et al.* 2005) and human health (Cech and Montera 2000; Hooker and Nathanail 2006) risk assessment problems.

2.1 Application

A brief application is used to illustrate the capabilities of a decision support system with respect to terrestrial ecological risk assessment and selective remediation. The K-770 Scrap Metal Yard is less than 8 hectares in size and is located on the western end of the Powerhouse peninsula at East Tennessee Technology Park (ETTP) in Roane County, Tennessee. The area operated during the 1940s as an oil storage area, and has operated from the 1960s to the present as a scrap yard, although it is currently inactive. Tens of thousands of tons of metal were stored in piles at the site, but these piles have

been removed. The bulk of the contamination at the site originated from the scrap piles. Considerable sampling has been conducted at this site for a full suite of analytes including metals and PCBs.

Extensive and frequent disturbance of soils from construction and remediation activities has resulted in a plant community highly adapted to disturbance. Areas near the Clinch River, if not paved or graveled, include shrubs and small trees, many of which are nonnative and invasive. The short-tailed shrew is considered one of the receptors of interest for the site conceptual model. Shrews are small insectivorous mammals that inhabit most regions of the United States. They have high metabolic rates and can eat approximately their body weight in food each day. Short-tailed shrews inhabit a wide variety of habitats and are common in areas with abundant vegetative cover (Miller and Getz 1977). Short-tailed shrews are primarily insectivores although they may eat some plant material and small mammals. Soil invertebrates make up a large component of the diet (USEPA 1993). For this assessment it is assumed shrews eat 100% soil invertebrates associated with soil at ETTP sites.

Selective remediation for this site based on a terrestrial ecological risk assessment for the shrew is performed with Spatial Analysis and Decision Assistance (SADA) (Stewart and Purucker 2006) freeware. SADA is Windows-based software that integrates an independent GIS system, statistical analysis capabilities, and risk assessment. While

decision support tools exist that link environmental process models to risk assessment (Babendreier and Castleton 2005; Dortsch 2001; USDOE 2004), SADA is unique in being a publicly available GIS system that integrates data analysis capabilities, with an emphasis on spatial analysis, with risk assessment. The software provides environmental contamination characterization capabilities in support of decision-making within a risk assessment context for multiple media. An overview of primary SADA modules relevant to ecological risk assessment is presented in Figure 2.1 (all tables and figures are in the appendix of chapter 2). SADA is developed at The Institute for Environmental Modeling at the University of Tennessee with funding from the Department of Energy, the Environmental Protection Agency, and the Nuclear Regulatory Commission. The capabilities of SADA can be used independently or collectively to address site specific concerns when characterizing a contaminated site, assessing risk, determining the location of future samples, and when designating areas of concern.

2.2 Environmental decision support software toolkits

An environmental decision support software toolkit consists of a collection of integrated modules that assist environmental assessors and decision makers in a variety of arenas. This includes the analysis and documentation of environmental data and models, identification of environmental problems and uncertainties, promotion of interaction among participants in the decision-making process, allow easy querying of databases, and model results with the capability of producing clear, visually-based outputs. Although

the primary purpose of an environmental toolkit is to run the underlying statistical and environmental science models, features that aid in entering parameters, executing the models, accessing data, consensus building among participants, and presenting results intelligibly are essential for acceptance of a toolkit by assessors and decision-makers. SADA provides environmental contamination characterization capabilities and additional amenities in support of decision-making in a manner that can be considered an environmental software toolkit (Bartell 2004; Holland *et al*. 2004). Emphasis has been applied throughout the software design process to ensure that the user-interface and contained algorithms are easy to implement, that the assumptions behind the models are transparent and easily exportable, and that site information is collected in one SADA file (*.sda) that can be easily transported between different machines and shared with different assessors and decision-makers.

Every environmental model contains a degree of subjectivity that ensues from the selection of a specific quantitative model and of parameter constants and variables used to represent the system of interest. This selection process makes models subject to criticism, particularly in a decision-making context. Validation and verification of a closed mathematical model versus the open environmental system being studied is difficult, if not technically impossible (Oreskes *et al*. 1994). Given the inherent inability to objectively select a uniquely applicable model for the system being modeled, especially under decision-making conditions with various principals representing

different interests, acceptance of a quantitative decision support tool that implements environmental models becomes a negotiated process with different or additional criteria used for model evaluation. Consequently, modeling approaches and evaluation criteria for decision-making purposes can differ from standard theoretical scientific approaches (Haag and Kaupenjohann 2001). In particular, acceptance of a model can be based as much on comprehensibility and model relevance to decision-maker concerns as on traditional measures of scientific representation.

These additional evaluation criteria are pertinent to support systems for environmental remediation since the output is often used to mediate decisions among multiple decision-makers. Successful decision support software will be accessible to the participants and easily generate full sub-model documentation to maximize model transparency. There are a variety of methods to allow accessibility to decision support software, from intensive interaction between modelers and decision-makers to web-enabled applications. SADA accomplishes accessibility by an emphasis on an easy to use graphical user interface, being freely distributed on the operating system used by most environmental decision-makers (Windows), and encompassing all site information in one file that can be easily distributed between different machines. Programs that require significant financial investment, multiple input files, or programs that are limited to implementation on uncommonly used operating systems can impede portability and therefore use by decision-makers, which can therefore complicate acceptance of a

decision support tool in a number of environmental applications.

The primary modules and functions of a decision support toolkit are encapsulated as objects that can be accessed for a variety of decision-informing output (Rizzoli and Davis 1999). For SADA these include methods from simple statistical output to risk-based area of concern maps based on ecological exposure models and geostatistical algorithms. Some forms of output are complex and require a number of parameters to implement for successive models. SADA takes advantage of the linear nature of inputs for these sequential models and classifies the most common types of output, representing the primary forms of environmental decision support provided by the software, as step-by-step interview processes. These interview processes contain all steps necessary to parameterize a particular type of decision support tool and are hyperlinked to the appropriate parameter windows for site-specific data entry (Figure 2.2). This allows the user to sequentially input all the assumptions and parameters necessary to run the desired tool. Once the input has been verified, the user can run the requisite models to produce numerical output and/or a spatial result within the GIS window.

Model transparency, the full reporting of model assumptions, inputs, and outputs, is critical for facilitating communication between decision participants and for meeting minimum documentation standards for environmental model implementation (Jakeman *et al.* 2006). SADA provides extensive information on sub-modules and default parameters

in a help file distributed with the program and in a comprehensive user's guide (University of Tennessee 2005). In addition, each module of the program contains self-documentation procedures that allow for the user to generate complete, customizable reports for decision or modeling results in HTML format. This feature documents all data, models, parameters, and other inputs that were used in generating a graphical or tabular input. This feature allows for full transparency of decision support processes and assists in model evaluation (Oreskes 1998), algorithmic verification, and report generation.

2.3 SADA database management and GIS

The key for successful decision support systems that combine different models is integrated data management that passes information between models without extensive manipulation by the user. Many current support systems employ a "null integration strategy" that shifts responsibility for data transfer between models to the user (Denzer 2005). While this may be acceptable for technical scientific users, it often unnecessarily limits access to the support system for other participants in the environmental assessment process. SADA requires an initial import of laboratory analytical data as either a comma-delimited text file or as a Microsoft Access file and data must be preprocessed to handle laboratory qualifiers and to convert to standard metric units for risk assessment purposes (mg/kg and mg/L). However, once analytical data is imported into the program, no further data manipulation outside the software is needed to run all the module

functionality. Table 2.1 describes the fields that can be imported.

Once the analytical data are imported, it can be displayed in the program's native GIS. GIS software provides a number of well-known advantages in conducting environmental assessments. Individual sites can be defined using the polygon and layer tools, while sampling locations and analytical results can be displayed with GIS layers imported from ESRI shape files or AutoCAD DXF files. Data can be displayed and queried according to separate sampling events and options are available for handling non-detects that are imported at the analytical detection limit and methods for resolving duplicate data are available. Many common statistical methods are available in the software from USEPA guidance (1998) for evaluating contaminated sites, including univariate measures of relative standing, central tendency, and dispersion. Graphical displays for posting plots, histograms, and ranked data plots, and non-parametric hypothesis tests versus decision criteria or reference concentrations are also available.

2.4 Ecological risk assessment

Ecological risk assessment estimates the probability of adverse ecological effects based on exposure to a stressor (USEPA 1992). Ecological risk assessments are increasingly factoring into remediation decisions as the process has become more formalized and recognized as integral in adequately completing site cleanups. Ecological risk assessment is a tiered process that begins with screening steps and proceeds to full

characterization of the ecological risks as necessary to support remediation decisions at contaminated sites. If ecological problems are present at the site, providing support for the design and evaluation of remedial alternatives often entails producing contaminant-specific concentration goals for identifying site-specific contaminants of concern (Screening Values- SVs) and clean-up levels (Preliminary Remediation Goals- PRGs). SVs allow for a quick determination of whether additional site assessment is needed and allows for the contaminant list to be pared down to a manageable size. PRGs allow for the calculation of site- and receptor-specific cleanup goals. There are many sources of SVs and PRGs due to numerous regulatory agencies creating them for application within their domain. Under these circumstances, a decision support tool with access to the variety of SVs and the ability to calculate PRGs are very useful for site assessment purposes as a typical implementation may require access to multiple data sources. The SADA software supports many of the steps in a typical ecological risk assessment. The screening and ecological risk modules are integrated into the environmental information system and allow users to perform comparisons of summary statistics for sub-areas of the site to a variety of SVs (Figure 2.3) and provide the ability to calculate doses and risks for a number of terrestrial receptors (Figure 2.4).

An early step in ecological risk assessment is hazard identification, this process consists of comparing (or screening) environmental measurements to compilations of SVs. Contaminants at the site that exceed these SVs are kept for further examination. If

no exceedances of relevant SVs are observed then this can be used as justification for a no further action determination. SADA contains one of the most complete, publicly available compilations of SV sources and its screening module allows users to perform site comparisons against a large array of SVs in a hierarchical manner. SVs are available for over 50 benchmark sources for sediment, surface water, tissue residue, and soil; they are functions of environmental variables where appropriate (pH, hardness, organic carbon content). Screen results, ratios, and the SVs themselves can be viewed in tabular or histogram form. Terrestrial SVs included with the software distribution are the Dutch Intervention and Target (Crommentuijn 2000a,b; Swartjes 1999), EPA Eco ESLs (USEPA 2003), EPA Region IV (USEPA 2001a), EPA Region 5 ESLs (USEPA 1999), ORNL invertebrates, microbes (Efroymson *et al.* 1997b), and plants (Efroymson *et al.* 1997a). Barron and Wharton (2005) provide a comprehensive overview of many SV sources and a discussion of how they are derived.

The ecological exposure and risk models follow USEPA (1992, 1997, 1998, 2001b) guidance and can be customized to fit site specific exposure conditions for generating risk results and calculating PRGs. Exposure and risk assessment calculations are based on an initial conceptual model for the site that includes the contaminated media, routes of contaminant transport, important and representative ecological receptors, and pathways of exposure for these receptors. Calculations are limited to those contaminants that exceeded relevant SVs in the screening process. The next step is

determining the bioavailability of these chemicals to exposed receptors, this can be done through physiological or food chain models that model the bioaccumulation and biomagnification of the contaminant in different trophic levels of the food chain. The magnitude of exposures to individuals can then be conducted using exposure models for the relevant exposure pathways. A toxicity reference value (TRV) from a dose-response model is then needed, which may call for inter-species extrapolation of the contaminant effects from a species in a laboratory study to a species of interest at the contaminated site. The results of the exposure modeling are then compared to decision criterion (the TRV), and perhaps the model is modified for a number of different exposure scenarios/remedial actions to determine the feasibility of available actions.

The SADA software provides terrestrial dose exposure models to assist in modeling dose body burdens at a contaminated site. Modeling dose to wildlife receptors requires a number of chemical-specific and species-specific exposure parameters. Ecological dose modeling capabilities are present in the ecological risk module and default exposure parameters are distributed with the software for over 20 terrestrial species (Table 2.2) that represent a variety of wildlife receptors, including representative herbivores, insectivores, and carnivores. These species can be individually parameterized for males, females, and juveniles as well as EPA Soil Screening Level (SSL) defaults (EPA 2003) that combine male and female parameters. Default values for males and females are provided based on available literature sources (*e.g.*, USEPA 1993, 2003), but

can be modified to reflect site-specific conditions.

Routines are available for calculating daily intake rates for each location at the site. The routines in the ecological risk module access the contaminant matching and data management functions and model parameters and toxicity information are fully parameterized from USEPA guidance when available. SADA calculates dose (mg intake per kg body weight per day) from food ingestion, soil ingestion, dermal contact, and inhalation for terrestrial exposures as well as total dose summed over all pathways selected. These results are directly comparable to TRVs for risk assessment purposes and are presented in tabular form commonly used to document risk assessment results (Figure 2.4).

For the application, exposures are calculated for polychlorinated biphenyls (PCBs) at the ETTP site. PCBs are a family of man-made chemicals consisting of 209 individual compounds with varying toxicity (ATSDR 1989). Because of their insulating and nonflammable properties, PCBs were widely used in industrial applications such as coolants and lubricants in transformers, capacitors, and electrical equipment prior to 1977 (ATSDR 1989). PCBs are known to bioaccumulate and biomagnify to toxic concentrations in animals (Eisler 1986; ATSDR 1989), and PCBs with higher chlorine content such as 1254 or 1260 tend to persist in the environment longer than those with lower Cl content. Chronic exposures are of particular concern. Although relatively

insoluble in water, PCBs are generally freely soluble in nonpolar organic solvents and in biological lipids (EPA 1980-PCBs).

Mink appear to be among the most sensitive mammals with dietary levels as low as 0.1 ppm wet weight having caused death and reproductive toxicity (Eisler 1986). Ringer *et al.* (1981) reported an LC50 for chronic exposures of 6.65 ppm PCB-1254 for mink exposed over an 8-month period. Exposure of mink for 6 months to 1 ppm PCB-1254 resulted in no significant difference from controls in number of offspring or offspring mortality (Wren *et al.* 1987). Halbrook *et al.* (1999) investigated toxicity of Poplar Creek fish to mink by feeding mink five different diets over a 7-month period. No adverse effects on mink reproduction were observed at dietary concentrations of 1 ppm or a dose of 0.12 mg/kg/d, but dietary concentrations of 1.36 ppm (0.23 mg/kg/d dose) resulted in increased liver EROD activity and a trend toward decreases in adult and kit body weights and litter size at birth. Aulerich and Ringer (1977) exposed mink to 1, 5, and 15 ppm PCB-1254 in their diet over a 4.5 month period. Number of offspring born alive was significantly reduced at the 5 and 15 ppm levels, but not at 1 ppm. Because the study considered exposure over 4.5 months including critical life stages (reproduction), Sample *et al.* (1996) considered the 1 and 5 ppm doses (0.14 and 0.69 mg/kg/d) to be chronic NOAELs and LOAELs.

2.5 Spatial estimation and interpolation

The practice of ecology is giving more consideration to spatial structure and heterogeneity in the construction of theory, interpretation of processes, and the collection of data (Fortin and Dale 2005). Of particular interest for ecological site assessment and management are spatial autocorrelations present in stressor distributions, ecological exposures, or effects. The presence of spatial autocorrelation in observed data indicates that values taken from data pairs a given distance apart are more (or less) similar than expected for random selections of data. In ecological site assessment, depositional processes that lead to anthropogenic contamination in the terrestrial environment often cause the spatial distribution of data to be autocorrelated. In addition, ecological data can be autocorrelated due to a dependence on an underlying variable that is spatially correlated, for example a plant species distribution may be spatially correlated due to a functional dependence on a soil nutrient that is itself autocorrelated; or the presence of spatial correlation may have an underlying biological mechanism, for example a plant or animal that has lower dispersal may be strongly spatially correlated to a central location where the species was first present in a landscape. Therefore, terrestrial ecological risk assessment is conducive to spatial approaches for both the calculation of exposure and the evaluation of alternatives that ameliorate ecological risk. However, in lieu of efficient and practical methods to consider spatial aspects of exposure in budget-constrained ecological risk assessments (Hope 2005), the U.S. Environmental Protection Agency (USEPA) and other regulatory agencies have often implicitly assumed

homogeneity of contaminant concentrations and exposures by estimating ecological risk with a non-spatial summary statistic.

Dating roughly to early papers on the importance of scaling considerations (Levin 1992), the importance of autocorrelation (Legendre 1993), and the application of geostatistical methods for characterizing spatial dependence (Rossi 1992), a growing recognition of the importance of space as a dependent variable led to an appraisal that methods traditionally used to statistically analyze ecological data were in many cases found to be wanting due to underlying assumptions based on independence. Spatial statistical methods account for this by modifying existing methods, implementing common methods within local moving windows, or sometimes creating all new methods to account for the presence of autocorrelation in what has been a burgeoning field in ecology (Fortin and Dale 2005). In many cases, what has held back their more general risk assessment implementation is access to software that performs these algorithms within an environmental assessment context. The spatial functions relevant for risk assessment include methods for measuring spatial correlation among data, hypothesis tests that account for autocorrelation, methods for evaluating stationarity assumptions, modeling spatial correlation, and a variety of spatial interpolants for mapping contamination distributions. These tools then can be integrated with ecological criteria to determine whether action is necessary at a site and identify specific areas of concern. In this manner, the consideration of space and use of these tools in the assessment process

can reduce decision errors, improve the estimates of the risk outcomes, and therefore influence the resulting risk management decisions. These improved risk estimates can then be used to optimally design the risk management decisions that are ultimately implemented.

Initial efforts at describing the degree of spatial autocorrelation in a data set generally focuses on simply determining if it is present. If no spatial correlation is present in the data, then typical assumptions regarding independence of the data and methods used to determine exposure are relevant for determining remediation. However, if correlation is observed or can be determined using a statistical test, then it may be possible to adequately model the distribution of contamination and use that model to inform the remedial design. The two most common methods for investigating spatial autocorrelation are correlograms and variograms. These methods graph the data partitioned into distance classes on the x-axis and a measure of autocorrelation on the y-axis. Correlograms plot either Geary's C (Geary 1954) or Moran's I (Moran 1950) and variograms plot the semivariance as the measure of autocorrelation. For each of these statistics, there is a significance test available that can determine if there is a significant amount of spatial structure present in the data for the distance classes selected. These tests require that some form of a condition of stationarity can be satisfied for the area of study. The form usually defined for these tests is a "weak" stationarity where the expected value of the mean and variance for the contaminant do not change

systematically over the site. To meet this condition, it is important to select site boundaries properly with respect to depositional processes and contaminant distributions. Local Indexes of Spatial Association (LISA) are useful in this regard as they can assist in identifying sub-areas of the site with marked differences in these expected values (Anselin 1995).

If autocorrelation is present for a contaminant, this can cause significant problems for hypothesis testing and sample size calculations. Since samples collected within a correlation range are expected to be more similar, they are unlikely to be independent samples and each sample cannot be considered one full degree of freedom for the purpose of significance testing (Griffith 2005). Methods commonly used for testing and estimating sample sizes necessary to characterize a decision curve, such as those promoted in Data Quality Objectives (DQO) guidance (USEPA 2000) and implemented by SADA, are affected by this pseudoreplication issue and must therefore be corrected for the appropriate number of degrees of freedom. There are a few choices available to makeup for the presence of spatial correlation in data. One is to actually remove data from sample locations such that there are none at the site within the correlation range of each other, but this has the obvious negative drawbacks of lowering power and wasting valuable collected data. There are also methods that can modify (reduce) the degrees of freedom for a test implementation, but again this has the effect of less power in the test implementation, perhaps less than desired (or mandated) by decision-makers in a DQO

type process. This leads to a third alternative of simply collecting more data to compensate for the degree of autocorrelation observed in the initial data collection.

Spatial estimation procedures that account for autocorrelation and allow for the estimation of contaminant concentrations at unsampled locations have clear value for estimating terrestrial risks and designing remedial actions. There are a variety of interpolation procedures that have the desirable properties (for mapping contamination) of being local, in that they are weighted averages of sample locations near to the point being estimated, and of being exact, in that they preserve sample values when the point being estimated is a sampled location. Popular deterministic interpolation methods have these properties and require little parameterization; examples include natural neighbor (Sibson 1981; Watson 1995), which geometrically determines weights based on distance and direction relative to other nearby samples, inverse distance (Shepard 1968), which weights samples based on a power of the distance to the nearby samples, and a form of k-nearest neighbor which simply averages the k nearest neighbor samples. The k-nearest neighbor interpolant is not exact for k>1.

Kriging (Krige 1966) is another interpolation method that can produce maps that are more adaptable to the observed correlation, though they require additional parameters when compared to the above interpolants. It has the additional advantage of having a kriging variance associated with each estimate and can therefore be implemented

stochastically. The kriging method makes use of the variogram to fit a model to the observed spatial autocorrelation and this model is used to derive the weights for nearby samples for each location to be estimated. The variogram provides a graphical measure of the average degree of similarity (measured with the semivariance; half the average squared difference) for different groups of sample pairs as a function of predefined separation distances. Models fit to variogram plots that reflect little continuity have a slope of zero at a y-value similar to the overall variance for the data set. A positive slope in the semivariance values for increasing distance classes indicates the presence of significant spatial autocorrelation. Separate variograms can be calculated for differences in spatial continuity as a function of direction (anisotropy). If no anisotropy is observed then one "omnidirectional" variogram is typically used to summarize the autocorrelation. Kriging uses a continuity model fit to the experimental variogram, then at locations to be estimated this model is used to find weights for nearby samples by solving a system of linear equations. The solution is the best combination of weights that sum to 1 and minimizes the variance while accounting for the structure of the spatial continuity model.

There are a number of different models that can be fitted to a given experimental variogram, and no consensus on best fit approaches for selecting a model. Early fits were often visual but that yielded to regression type approaches such as Cressie (1992); algorithmic approaches to model fitting are important for environmental assessment due to their reproducibility. Crossvalidation approaches are also popular, these remove each

33

sampled data point one at a time, interpolate, and then compare different interpolation approaches. Maximum likelihood methods are now growing in popularity for evaluating interpolation models. There is much more to the application of interpolation in an environmental context than discussed here, and additional sources of information can be found in Isaaks and Srivastava (1989), Goovaerts (1997), Webster and Oliver (2001).

SADA provides a variety of functionality for performing a geospatial analysis in an environmental context. LISA statistics, variography, interpolation methods, and cross-validation procedures are all well integrated with its database and GIS functions to provide a coherent approach for determining if spatial correlation exists in a contaminated area and modeling the spatial distribution of contamination at a site and making remedial decisions based on the output.

Geary's C and Moran's I spatial statistics for local neighborhoods can be mapped in SADA and are useful for evaluating stationarity assumptions for defined areas. For larger sites, variography is insufficient for evaluation of stationarity since it calculates average values for spatial subsets of the distance and therefore may mask local patterns (Anselin 1995). Plotting and analyzing these LISA maps in SADA may identify areas of distinct spatial behavior, therefore causing additional subdivision or grouping of defined contaminated areas at the site before variography and interpolation procedures are performed.

SADA also provides an interface for performing exploratory variography. Variogram plots are conducted to plot semivariance versus distance classes as is typically done. If spatial correlation is present than semivariance levels are discernibly lower for closer distance classes, rising to overall site semivariance levels at further distance classes. Input parameters for controlling distance classes and subdividing search areas to account for anisotropy can easily be modified within the interface. Absence of significant spatial correlation, indicated by constant semivariance for all distance classes on the variogram, allows for traditional hypothesis testing approaches and data independence assumptions inline with EPA (2006) guidance. However, the presence of discernible spatial structure in the variogram provides an impetus for modeling the spatial correlation. SADA provides three different models (spherical, exponential, Gaussian) to fit the exploratory variography results. The user can construct spatial correlation functions by parameterizing these models and visually assessing goodness of fit or using the autofit routines (Pardo-Igúzquiza 1999) encapsulated in SADA to fit the theoretical variogram models to the data.

SADA provides the aforementioned interpolants to model the site distribution of contamination when spatial dependence is assumed (inverse distance, natural neighbor, and nearest neighbor) or the spatial dependence is modeled (ordinary and indicator kriging; based on Deutsch and Journel, 1998). The site is discretized as a two- or three-

dimensional grid, and an interpolation method is applied to predict the concentration value in the center of each block. Each of the interpolants will preserve the original sample values at their locations and produce weighted estimates of contaminant concentration at unsampled locations. All of the interpolants can be used to produce concentration, risk, and area of concern maps for the site. Risk and area of concern maps can show the spatial distribution of risk and determine the area of the site requiring remediation based on a given decision criterion, these methods are discussed in subsequent sections. In addition, kriging can be used to produce probability and variance maps. Probability maps show the probability that each location exceeds a specified concentration. Variance maps display the estimated kriging variance at each estimation location.

Cross-validation methods are also available in SADA to compare different interpolation models versus the data. Evaluation criteria include mean error, absolute mean error, and mean squared error and these errors are plotted in the display window for each sample locations in addition to the statistics for the interpolation being summarized.

For the ETTP application, interpolations were performed for PCB-1254 at the site using the nearest neighbor, natural neighbor, inverse distance (with three different powers), and ordinary kriging (with fitted exponential, spherical, and gaussian theoretical variograms). Cross-validation results for the nine methods evaluated are presented in

Table 2.3 and are sorted by mean squared error. The ordinary kriging results had a larger correlation range and had a tendency to underestimate the high sample locations in the south and were the only three interpolants with negative errors. Inverse distance, though they also performed relatively well on the mean squared error, tended to overestimate the influence of the high sample locations due to the smaller effective correlation range. Natural neighbor and nearest neighbor, which do not have defined static correlation relationships over the site, performed poorly relative to the inverse distance and ordinary kriging implementations. PCB-1254 mass estimates at the site are also presented based on assumptions of 1500 kg/m^3 and a 1 meter contaminated zone thickness; although the cross-validation errors underestimate the concentration at the high sample locations due to smoothing, the ordinary kriging results have some of the highest estimates of total mass at the site for PCB-1254 since the effects of these high samples influence a large portion of the site. Mass estimates of PCB-1254 at the site range from 58 to 71 grams.

2.6 Mapping exposure and risk

The mapping of potential exposure and risk continues to grow in scope for a variety of ecological applications. Maps of contamination provide assistance for generating site conceptual models that detail contaminant pathways for transport and exposure when conducting risk assessments, and can show locations of hazards as well as provide a context for designing methods to reduce exposures (Mayer and Greenberg 2005). Combinations of contaminants, receptors and locations of concern for ecological

risk assessment can quickly be assessed within a software implementation allowing for efficient site characterization.

SADA has a strong emphasis on the spatial distribution of contaminant data and will plot risk results within a spatial context. Original sample concentration or interpolation results can be used to provide a risk map of the entire site. This technique adds a needed spatial component to risk assessment and assists in identifying which areas of concern with respect to risk. SADA integrates a number of ecological risk assessment capabilities with algorithms for spatial estimation and statistical analysis of contaminant distributions to allow for risk based decision-making. This allows remedial decisions to be made in a risk context while explicitly considering the spatial distribution of the data. For example, SADA can screen concentrations against ecological PRGs or benchmarks and display sample points or areas that exceed these values. In addition, one can use risk models in conjunction with geostatistical analyses to produce contoured risk maps, probability maps, remedial design alternatives, cost/benefit analyses, and secondary sampling plans within a risk context. The user can also enter Toxicity Reference Values (TRVs) for each contaminant to produce maps of terrestrial ecological risk through the intermediate calculation of a PRG.

Figure 2.5 presents spatial dose estimate results at the site for the short-tailed shrew. Mapping exposure in this way can also be used as a point of departure for

producing individual-based movement models that simulate realistic movement patterns for individuals in populations in an effort to model the distribution of the exposed population dose (Chow *et al.* 2005).

2.7 Selective remediation

The presence of spatial auto-correlation can enhance methods for identifying zones in need of remediation at contaminated sites. The selective remediation of contaminated sites, the process of remediation of sub-areas of a contaminated site to achieve quantitative cleanup goals and specified error tolerances for a larger area (Blacker and Goodman 1994; Brakewood and Grasso 2000), requires estimating the spatial distribution of contamination and of exposures. These methods make use of interpolation estimates and associated uncertainty to specify the minimal (optimal) amount and locations of soil in need of remediation to achieve a local or site-wide objective. Therefore, the application of spatial statistical tools to environmental risk assessment is a potentially fruitful area for improving decision-making processes that consider the spatial distribution of contamination at a site.

Selective remediation activities proceed by discretizing the site into a grid and identifying cleanup area by remediating individual grid blocks in inverse order of magnitude (worst to least) until an objective is met. For implementation of a not to exceed concentration (NTEC), the interpolation estimate at every grid node is compared

to a cleanup criterion and remediation is conducted if the estimate exceeds the criterion. The NTEC in ecological risk applications is typically a low observed adverse effects level or other less conservative effects estimate. This approach can then be used in conjunction with a more conservative effects estimate, such as a no observed adverse effects level, to implement remediation at a site so that a representative statistic for the entire site is below this effects level. The grid blocks are again remediated in inverse order of magnitude (worst to least) until the site-wide objective is met. This statistic can then be recalculated as subsequent blocks are cleaned and compared to the effects level until the decision criterion is reached.

Implementation of selective remediation in SADA is referred to as area of concern maps. These maps are based on the type of interpolation maps previously discussed in conjunction with an ecological decision threshold value for an ecological PRG, benchmark, or calculated dose level. These maps can be implemented as a NTEC, site-wide statistic, or combination of both statistics and result in a spatially-explicit design with an associated area or volume of remediation.

Figure 2.6 presents area of concern to consider for remedial design based on the two previously discussed daily dose cleanup criteria, 0.14 and 0.69 mg/kg/d. The 0.14 mg/kg/day value is quite low compared to our modeled doses at the site and results in most of the site being declared as an area of concern.

2.8 Conclusions

The application of spatial methods in terrestrial ecological risk assessment can minimize decision errors and maximize remedial effectiveness and can easily be implemented within the context of a geographical information system (GIS). However, ecological risk assessment methods and guidance do not promote methods to search for zones and strengths of auto-correlation. Instead, methods for criterion compliance are promulgated that assume spatial independence. The use of these methods at contaminated sites with spatially correlated contaminant distributions results in a loss of spatial information, which can then lead to higher than acceptable decision error rates and lost opportunities to efficiently identify areas of concern for selective remediation. The majority of typical ecological risk assessments are conducted under assumptions of data independence. Data management capabilities, descriptive statistics for summarizing contaminated areas, screening benchmarks, terrestrial exposure methods, and toxicity reference values are combined to produce tabular output and hypotheses tests sufficient to document the majority of ecological risk assessments. The GIS provides a platform for additional assessment methods that account for spatial dependence; including spatially relevant descriptive statistics, moving window spatial statistics, modified hypotheses tests, correlation modeling, and interpolation methods. These tools provide a number of methods that can minimize remedial action decision errors, provide spatial designs for efficient remedial design under selective remediation conditions, and provide a rationale

and context for additional sampling efforts at contaminated sites. The case study presented here illustrates these properties, by utilizing the spatial correlation present in the data and flexibly applying the decision criteria based on the spatial distribution of the contamination. The result is a map of area in need of selective remediation rather than a simple clean or not clean decision that contains significant possible error due to forced assumptions concerning data independence and the parametric distribution of the data.

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2.10 Appendix

SADA Graphical User Interface & Functionality

Initial Site Information

Sample Design
Subjective
Random
Gridded
Hard data-based
Soft data-based

Database
Import procedures
Polygonal queries
Date queries
Attribute display
Query exports

GIS/GUI
Mapping window
Interview process
dxf, shp files
2D, 3D data display
Vector data, grids

Statistical Analysis
Descriptive stats
Hypothesis tests
Sample size calcs
Interpolation
Geostatistics

N

Adequate characterization?

Y

Selective Remediation
RU/Site scale
Area of concern
Area-risk curves
Volume estimation
Probability maps

Ecological Risk
Chemical benchmarks
Exposure parameters
Benchmark screens
Exposure models
Risk mapping

Remedial Decision

Figure 2.1. The figure displays primary components of the graphical user interface and the functionality of the SADA software. Displayed functionality is limited to SADA components that are germane to the terrestrial ecological risk assessment and the remedial decision-making process.
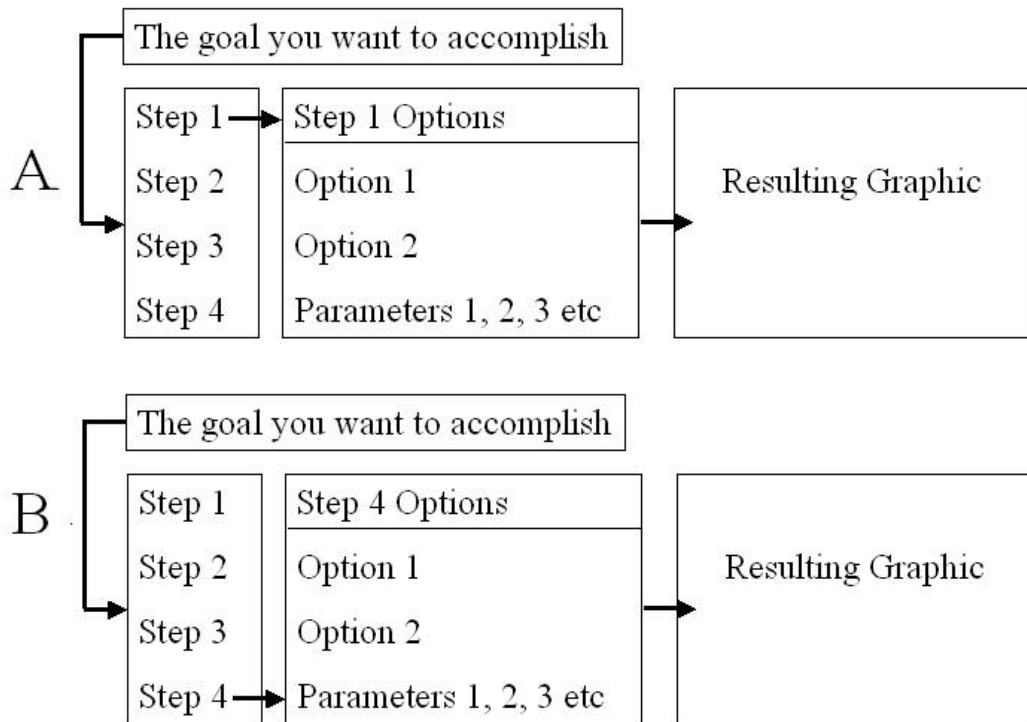
Figure 2.2. Generalized decision support interview process in SADA. This scalable structure allows for new assessment capabilities to be added to the software in a consistent manner and for existing modules to be recombined in useful ways to provide support for a variety of decision support processes (Figure from Stewart and Purucker 2006).

Statistics

- [ ] Detect Frequency
- [x] Mean
- [ ] UCL 95
- [ ] Max Detect
- [ ] Median
- [ ] Variance

Benchmark information is from eco_toxdata.mdb version 4.1. Dated 6/22/2005.

Soil Benchmarks

- [x] Dutch Intervention
- [x] Dutch Target
- [ ] Eco-SSL Avian
- [ ] Eco-SSL Inverts
- [ ] Eco-SSL Mammalian
- [ ] Eco-SSL Plants
- [x] EPA R4
- [x] EPA R5 ESL
- [ ] EPA R6 Earthworms
- [ ] EPA R6 Plants
- [ ] ORNL Invertebrates
- [ ] ORNL Microbes
- [ ] ORNL Plants

Sort (Click on column)
- (•) Ascending
- ( ) Descending

Help

Analyte	Concentration	Units	Mean	Dutch Target	Dutch Intervention	EPA R4	EPA R5 ESL
Anthracene	23	mg/kg	0.8656			0.1	1480
Antimony	10	mg/kg	0.529787	3	2900	3.5	0.142
Arsenic	260	mg/kg	14.54125	29	40	10	5.7
Benzo[a]pyrene	36	mg/kg	1.33205			0.1	1.52
Cadmium	45	mg/kg	4.005862	0.8	12	1.6	0.00222
Copper	5050	mg/kg	234.9775	36	190	40	5.4
Lead	830	mg/kg	59.8275	85	290	50	0.0537
Mercury	9.1	mg/kg	0.650593	0.3	10	0.1	0.1
Nickel	1900	mg/kg	94.26375	35	210	30	13.6
PCB-1254	16	mg/kg	0.957134				
PCB-1260	4.6	mg/kg	0.230203				
Selenium	31	mg/kg	1.203062	0.7	5	0.81	0.0276
Uranium	54000	mg/kg	737.9108			5	
Zinc	1310	mg/kg	163.3937	140	720	50	6.62

(a)

Soil Benchmarks for Arsenic

(b)

Figure 2.3. Ecological Screening Values present in the SADA decision support tool. (a) Tabular view of summary statistics for identified contaminants of concern at the K-770 site and a selectable list of SVs. (b) Histogram of SVs for all available sources for a given contaminant (Arsenic).
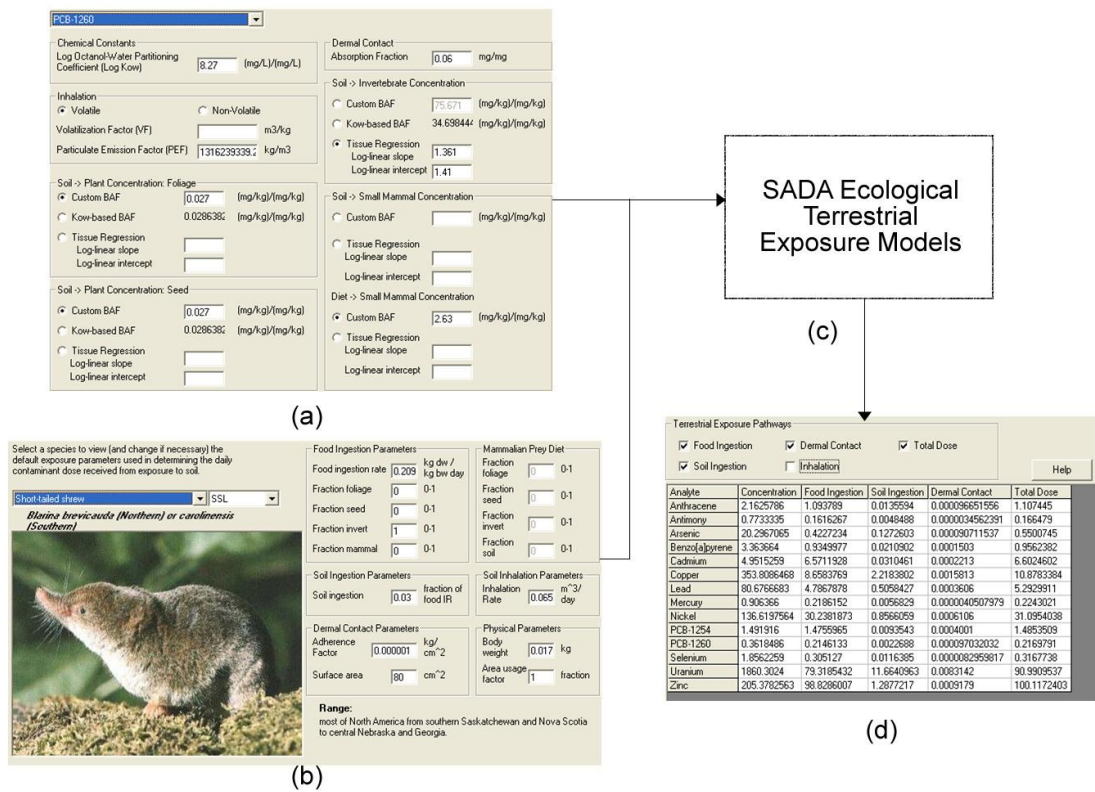
Figure 2.4. Terrestrial exposure modeling dose results for the short-tailed shrew. Contaminant-specific inputs for physical constants and bioconcentration model selection and parameters (a) are combined with species exposure inputs (b) to parameterize the dose exposure models (c). Tabular output (d) for all contaminants of concern at the K-770 site is generated in formats that can be exported to popular word processors and spreadsheet programs.

Analyte	Concentration	Food Ingestion	Soil Ingestion	Dermal Contact	Total Dose
Anthracene	2.1625786	1.093789	0.0135594	0.000096651556	1.107445
Antimony	0.7733335	0.1616267	0.0048488	0.0000034562391	0.166479
Arsenic	20.2967065	0.4227234	0.1272603	0.000090711537	0.5500745
Benzo[a]pyrene	3.363664	0.9349977	0.0210902	0.0001503	0.9562382
Cadmium	4.9515259	6.5711928	0.0310461	0.0002213	6.6024602
Copper	353.8086468	8.6583769	2.2183802	0.0015813	10.8783384
Lead	80.6766683	4.7867878	0.5058427	0.0003606	5.2929911
Mercury	0.906366	0.2186152	0.0056829	0.0000040507979	0.2243021
Nickel	136.6197564	30.2381873	0.8566059	0.0006106	31.0954038
PCB-1254	1.491916	1.4755965	0.0093543	0.0004001	1.4853509
PCB-1260	0.3618486	0.2146133	0.0022688	0.0000097032032	0.2169791
Selenium	1.8562259	0.305127	0.0116385	0.0000082959817	0.3167738
Uranium	1860.3024	79.3185432	11.6640963	0.0083142	90.9909537
Zinc	205.3782563	98.8286007	1.2877217	0.0009179	100.1172403

52

PCB-1254 Correlation Model

(a)

(b)

Figure 2.5.   Semivariogram and ordinary kriging interpolation for PCB-1254 concentrations at the ETTP site. The semivariogram (a) shows a Gaussian theoretical model fitted to the experimental variography using the SADA software. The ordinary kriging results based on this fitted variogram are then shown as spatially modeled daily dose (mg/kg/day) to PCB-1254 for short-tailed shrew inhabiting different portions of the site in (b) using the spatial continuity model. Legend on right shows the scale, light areas have lower dose levels and darker areas have higher dose levels, up to 102 mg/kg/day.

53
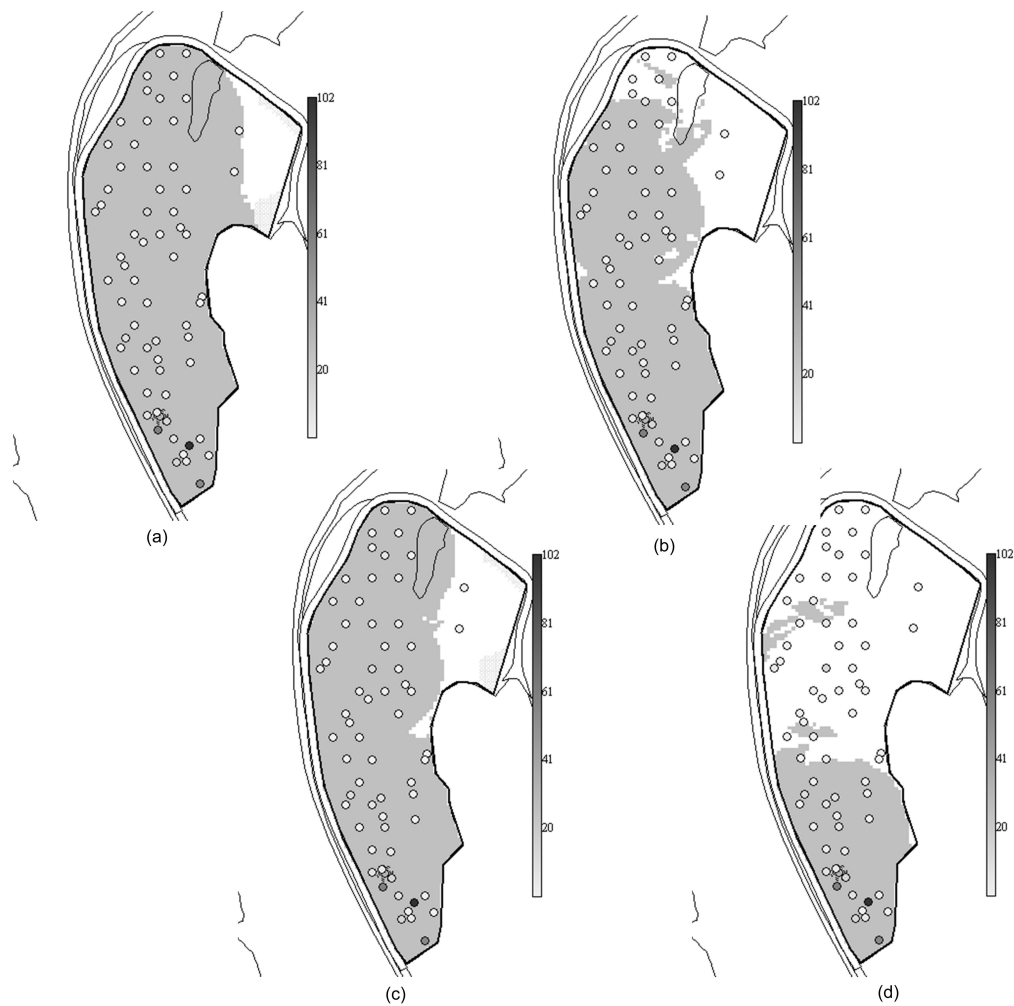
Figure 2.6. Visual depictions of areas of concern (gray areas) that exceed a NTEC or site average for LOAEL and NOAEL. Legend shows calculated dose levels at locations where concentration data were collected, up to a maximum of 102 mg/kg/day. (a) NTEC for NOAEL of 0.14 mg/kg/day yields a cleanup area of 64000 m³. (b) Site average for NOAEL of 0.14 mg/kg/day yields an area of concern of 52000 m³. (c) NTEC representative of a NOEAL for a dose of 0.69 mg/kg/day gives an area of 59000 m³. (d) Site average for LOAEL yields an area of concern of 25000 m³.

Table 2.1. Import fields for creating a SADA file.

Field	Type	Required?	Description
Easting	Double	Yes	Coordinates
Northing	Double	Yes	Coordinates
Depth	Double	No	Coordinates
CAS number	Integer	No	Chemical Abstract System #
Value	Double	Yes	Analytical result
Detect	Binary (0,1)	No	1 = detect, 0 = non-detect
Media ID	Constant	No	Predefined constants for media
Date	Date	No	mo/da/year
Metadata	Any	No	Additional information

Table 2.2. Terrestrial species with default exposure parameters.

Common Name	Scientific name	Receptor Group
American kestrel	Falco sparverius	Avian Carnivore
American robin	Turdus migratorius	Avian Insectivore
American woodcock	Scolopax minor	Avian Ground Insectivore
Black-tailed jackrabbit	Lepus californicus	Mammalian Herbivore
Burrowing owl	Speotyto cunicularia	Avian Carnivore
Deer mouse	Peromyscus maniculatus	Mammalian omnivore
Eastern cottontail	Sylvilagus floridanus	Mammalian Herbivore
Great Basin pocket mouse	Perognathus parvus	Mammalian granivore
Kit fox	Vulpes macrotis	Mammalian Carnivore
Little brown bat	Myotis lucifugus	Mammalian insectivore
Long-tailed weasel	Mustela frenata	Mammalian Carnivore
Meadow vole	Microtus pennsylvanicus	Mammalian Herbivore
Mexican free-tailed bat	Tadarida brasiliensis	Mammalian insectivore
Mourning dove	Zenaida macroura	Avian Granivore
Northern bobwhite	Colinus virginianus	Avian Granivore
Prairie vole	Microtus ochrogaster	Mammalian Herbivore
Red fox	Vulpes vulpes	Mammalian Carnivore
Red-tailed hawk	Buteo jamaicensis	Avian Carnivore
Short-tailed shrew	Blarina brevicauda	Mammalian Ground insectivore

Table 2.3. Cross-validation and PCB-1254 mass estimates for PCB-1254 interpolation.

Interpolation Method	Error	Absolute Error	MSE	PCB-1254 Mass (g)
Ordinary Kriging (Gaussian)	-7.70E-03	1.09	5.6	70.82
Inverse Distance (power = 1)	1.48E-02	1.14	6.11	71.25
Ordinary Kriging (Spherical)	-1.68E-03	1.16	6.37	67.60
Ordinary Kriging (Exponential)	-1.67E-03	1.17	6.44	67.28
Inverse Distance (power = 2)	2.60E-02	1.22	7.06	68.89
Inverse Distance (power = 3)	3.06E-02	1.3	8.12	66.63
Natural Neighbor	6.00E-02	1.41	9.5	62.98
Nearest Neighbor	3.18E-03	1.48	14.1	57.93

3. Habitat-contamination spatial correlation and determining when to perform a spatially explicit ecological risk assessment

The magnitude and effect of contaminant exposure on populations are influenced by the spatial distribution of contaminants, organisms, and habitat (Bell *et al.* 1993; Clifford *et al.* 1995). Even so, the use of non-spatial approaches is still common when characterizing exposures and effects of contaminant stresses. Many current ecological risk assessment methodologies essentially assume that habitat is homogeneous and high quality with the expectation that this results in conservative estimates of exposure.

The presence of spatial autocorrelation in available habitat is known to affect population dynamics and extinction risk (Gonzalez-Megias *et al.* 2005). Higher amounts of spatial environmental autocorrelation can increase the amount of spatial synchrony in the population, which in turn can increase the risk of local extinction (Engen *et al.* 2002). This trend can either be increased or ameliorated by high positive or negative correlations between quality habitat and contaminant concentrations.

The incorporation of landscape features into the ecological risk assessment process holds promise for increasing the accuracy of exposure assessments and ultimately supporting more informed management decisions (Kapustka, 2003; Linkov *et al.* 2002). Screening estimates of exposures to measured contaminant concentrations requires that

multiple data points (measured over space and time) be reduced to a single exposure concentration. The most common approach used in EPA ecological risk assessments for determining an exposure concentration for an area involves the use of a summary statistic (*e.g.*, mean, percentile, confidence limit). Clearly, the use of a summary statistic does not address the potential for variability in exposure due to spatial and/or temporal differences among individuals over time and, while appropriate for screening purposes, may not capture bias in the exposures that are due to non ideal-free use of the habitat. Risks to wildlife are partly a function of this variability in addition to differences in the foraging behavior of organisms being exposed. Species and individuals with foraging areas of different sizes and locations will experience different exposure levels, even at the same contaminated site. While this may be a well-known factor in determining exposure (Suter 1993), methods that account for this uncertainty are seldom incorporated into risk assessments (Freshman and Menzie 1996).

Geographical Information Systems (GIS) are more regularly being coupled with traditional ecological exposure techniques to incorporate spatial variability into ecological risk assessments. The use of GIS tools makes it more feasible to conduct spatially explicit modeling. The additional inclusion of movement models that simulate foraging and other behaviors allows for a more comprehensive assessment of population exposures and risks. Inter- or intra- species differences in movement strategies and habitat valuation can have substantial effects on cumulative exposures, even when they

share a common range. Movement models conducted at an appropriate scale allow for important receptor information such as foraging area/home range, relative desirability of habitat areas, contaminant distribution, and individual behavior to be more realistically captured in the assessment, and their effects on the cumulative exposure distribution explored.

An individual-based model is implemented for this ecotoxicological assessment application and the parameter of interest is the population distribution of the daily dose for a mobile receptor. The elements that are needed to conduct the analysis include habitat quality information, a wildlife exposure model that includes movement, and a spatial distribution of chemical concentrations so that exposures can be calculated for all portions of the site.

The majority of direct terrestrial exposures to contamination in soil occur via foraging activities (EPA 2005). There is already an extensive literature concerning the methods and applications of different foraging strategies, so the incorporation of contaminant exposures into already existing foraging models does not require substantial theoretical development. Successive implementations of a foraging model that accounts for the movement of an individual across an underlying concentration grid and tracks its unique cumulative exposures allows one to build a distribution of the potential population level distribution of exposure at a contaminated site. This can be combined with forage

quality information in the form of the likelihood of feeding at a particular location to more accurately reflect the movement and feeding activities of wildlife.

The application used in this paper concerns the hypothetical exposure of pronghorn to fluoride in desert sagebrush and soil aerially deposited in the vicinity of a fluoride manufacturing plant at an undisclosed location in the western United States.

3.1 Habitat

Habitat is defined as areas that provide resources necessary for survival and reproduction. Habitat preference can be species-specific and also age-dependent. Different species are preferentially drawn to some habitat features due to higher food availability, better cover for hiding, or improved temperature regulation. The resulting process where individuals use available habitats in a nonrandom manner is termed habitat selection (Morris 2003). The foraging area is the area where the species most often finds food resources. Since the majority of the exposure in terrestrial systems comes from ingestion (for most contaminants) (EPA 2005), these sub-areas with high browse quality for the pronghorn are the areas most likely to contribute to total dose. Underestimating the time that the individual spends in these areas can result in an under- or over-estimation of dose and risk depending on whether the concentrations in these foraging areas are high or low relative to average contaminant concentrations within the habitat.

Habitat suitability index (HSI) models provide species-specific habitat information that can be used for impact assessment and habitat management. The model synthesizes available information on habitat quality and is scaled so that each location is assigned a value between 0 for unsuitable habitat and 1 for optimal habitat (Allen *et al.* 1984).

There are a number of instances where strong positive or negative correlations between contaminant distributions and habitat quality (as measured by HSI) may be present. Obviously, there can be strong negative correlations between areas of optimum habitat and contaminant concentrations as a result of anthropogenic disturbance. Foraging habitat may be less desirable at some locations for the pronghorn compared to others due to presence of invasive plant species, human disturbance, pesticide use (Maurer and Holt 1996), and environmental contamination. Other species may thrive under conditions of human disturbance, and these may be expected to have positive correlations between high contaminant concentrations and desirable habitat due to contamination being present in disturbed areas preferred by the species.

HSI models, as originally developed, are based on the assumption that there is a positive relationship between the quality of the habitat (as measured by the quantitative index) and habitat carrying capacity. Data that supports the determination of the probability of movement to each available location would have greater value for

implementing a movement model. Some forms of HSI may be inappropriate to use as the basis of a foraging model if they are based on regressions to variables that are not important for foraging. For example, if the majority of time for a species is spent in shelter from predators or from adverse weather, indices based on these values and used as the basis for a foraging model may inaccurately represent the foraging behavior of the species.

3.2 Pronghorn exposure model

This study looks at the uncertainty in habitat type as a primary contributor to uncertainty of the population dose, so deterministic values were used to parameterize the exposure and effects model. Of the common terrestrial exposure pathways, soil and food ingestion are the dominant pathways (EPA 2005). The simple terrestrial exposure equation sums the contribution from the ingestion of forage species and soil ingestion for an individual pronghorn (Equation 1). Table 3.1 (all tables and figures are in the appendix of chapter 3) summarizes the exposure variables.

$$\overline{Dose_i} = \sum_{t=1}^{T} \frac{(C_t I_f + \frac{C_t}{B_v} I_s)A}{T} \qquad \text{Equation 1}$$

Pronghorn prefer browse and forb species as forage items year-round (Hoover 1966) and do not seem to be limited by food availability during the summer months (Smith and Malechek 1974). A body weight of 40 kg is used here as representative of a

typical adult pronghorn (Nowak 1991). Severson *et al.* (1968) estimated an average daily forage intake of 0.85 kg (dry weight)/d for pronghorn. For a body weight of 40 kg, this is a food ingestion rate of 0.021 kg dry matter/kg body weight/day. The pronghorn soil ingestion rate used is based on annual mean soil ingestion rate of 48.7 grams/day, which is 5.4% of total daily dry matter intake (Arthur and Gates 1988). Welch *et al.* (1983) estimates dry matter digestibility for pronghorn at 54%. This is used as an estimate of the gut absorption rate.

The mammalian toxicity reference value (TRV) commonly used for fluoride is 52.8 mg/kg/day. This is the Low Observed Adverse Effects Level (LOAEL) of 52.8 mg/kg/d reported by Aulerich *et al.* (1987) and is based on dietary studies of mink. The cleanup concentration actually used at the site is less conservative, 30000 mg/kg. The concentration has been converted to a TRV of 760 mg/kg/day using the exposure assumptions for the pronghorn listed in Equation 1 and Table 3.1. This 760 mg/kg/day value is used as a dose-based cleanup standard for determining whether remedial action is necessary at the site under different habitat quality scenarios.

The pronghorn has a variable range, dependent upon habitat, location, and year (Yoakum, 1978); but this range is reasonably large compared to the study site considered here. Pronghorn form large mixed herds of up to 1000 individuals in the fall and winter, breaking up into sex-segregated groups during the spring and summer. Foraging

pronghorn can be expected to cover the full extent of the study area during a summer season. Daily movements are dependent on the availability of resources; Yoakum (1978) has reported movements of 0.1-0.8 km per day in spring and summer and 3.2 to 9.7 km per day in fall and winter. For the purposes of this modeling effort a grid with nodes every 0.5 km is defined for the site covering an area of approximately 310 square kilometers. In the movement model, the pronghorn feeds at one grid location per day and is then assumed to move to and feed at any of eight adjacent patches on the subsequent day depending upon the relative desirability of these eight patches. The use of an eight square movement model (versus nine square) forces average movements of 0.5 km per day and prevent simulated individuals from inhabiting the same square for long periods of time, reducing the quality of the habitat. Habitat quality is assumed to be constant over the course of the simulation, independent of foraging pressure. The simulation is run for a 90-day period to simulate the length of the summer season. The individuals are randomly placed on the grid and the simulation is run for one year before the 90-day exposure period so that individuals start in suitable habitat according to the available habitat quality. A distribution of population exposures was generated by iterating the exposure scenario for 1000 individuals; using a random start location for each individual. Summer is the breeding season, and males over 3 years of age become territorial as the summer progresses while females and younger males travel freely. The movement model implemented is representative of female movement patterns during the summer season; males will sometimes cover more ground during the summer and have established

territories by the fall. The variables important for habitat quality shift in late fall and winter, a different habitat suitability index must be used and this area of high fluoride concentrations is not as important for the pronghorn as they seek areas with more topographic relief as shelter from the elements and cover from predators.

Spatially explicit foraging models have been developed and implemented for ecological risk assessment and management purposes (Freshman and Menzie 1996; Linkov *et al.*, 2002; Woodbury, 2003). There are a number of ecological mechanisms that influence the processes that are incorporated into these foraging models. Habitat resource distribution is the most common process incorporated into ecological risk assessments (Gaines *et al.* 2005; Chow *et al.* 2005). Other ecological mechanisms that can influence the use of habitat and resulting estimates of population dose when used in an ecological risk assessment context include interspecific interactions (Connell 1961; Rosenzweig 1981), intraspecific variability and competition (Gill and Wolf 1975), spatial scale (Morris 1992; Pulliam and Danielson 1991), and density-dependence (Bellows 1981).

Other considerations relevant for individual-based implementations include incorporating aspects of the behavior of individuals into the movement model. Uncorrelated random walk models are the simplest models of animal locomotion- they assume that organisms move in "hops" of fixed length and duration. After each jump, the

organism moves in a new randomly chosen direction. These models vary depending on the number of adjacent grid nodes eligible for movement and whether the grid is square or hexagonal. In contrast, with a correlated random walk model (Kareiva and Shigesada 1983), the organism will be more likely to move in a direction that is similar to the previous direction (Holmes 1993), such that the movement direction is correlated to some degree between daily movements. Use of uncorrelated or random walk models, in the absence of other ecological mechanisms, are not expected to significantly change the summary statistics for exposure in the ecological risk assessment process and therefore would not have an impact on remediation decisions resulting from their implementation. This is because the random foraging strategies are reasonably approximated by mean and confidence limit summary statistics commonly used in risk assessments.

However, non-random movement can significantly impact the exposure estimates used for an ecological risk assessment. An additional constraint that can be added to individual-based movement models is the selection of areas to move to through the use of either prior knowledge concerning productive foraging areas or through the evaluation of the suitability of habitat from a distance by some means (Pulliam and Danielson 1991). This selective use of habitat, in conjunction with positive or negative spatial correlation between habitat quality and contaminant concentrations, can impact the exposure portion of an ecological risk assessment and the resulting risk management decisions. Incorporating this type of behavior allows for the more realistic simulation of foraging in

that individuals use available habitat in a nonrandom manner based on their habitat preferences (Morris 2003). The model chosen to represent Pronghorn movement is a nonrandom walk model (8-square) that incorporates the relative value of different habitat patches through a habitat suitability index value that has been assigned to each grid node. At each movement step, the pronghorn can either stay in the same patch or proceed to one of the eight adjoining patch locations. The probability of moving to any one patch is given by dividing the index value of the prospective patch by the sum of the indices of the corresponding eight patches available for movement. This process is illustrated in Figure 3.1.

This is a reasonable approach for simulating non-territorial movement; and therefore is representative for female pronghorn and younger males that spend the summer months searching for high quality foraging patches, as well as more mature males in early summer before they become territorial (Yoakum 1980). This approach is not relevant for males during the breeding season, where they defend defined territories. Females are our receptor of interest, because the toxicity values for fluoride are derived based on reproductive effects in mammals at dose levels similar to those calculated for this site.

This approach can be implemented in a Monte Carlo framework, where individuals are allowed to move over the landscape based on their valuations of nearby

habitat. At this point in the analysis, since the individual valuation of habitat and movement does not incorporate density-dependence, these individuals can be modeled separately. Summary statistics of the resulting population dose distribution of the individuals modeled for the area can then be looked at for risk management purposes when comparing to a toxicity reference value.

In the modeling efforts conducted for this paper, the habitat is varied for each set of Monte Carlo simulations. While the HSI values for each grid node range from 0 to 1, different habitat scenarios have different correlation strengths when compared to the fluoride concentration interpolations conducted on the same grid resolution. All the habitat data in this paper are therefore hypothetical, but they are tied into the actual spatial distribution of fluoride using correlation. Eight different sets of HSI data are generated, with a range of correlation values (0.75, 0.5, 0.25, 0, -0.25, -0.5, -0.75).

3.3 Spatial distribution of contamination and habitat

The distribution of the contaminant in space is another important feature of the landscape, and rarely are there enough resources available to exhaustively collect data for the entire site being considered. For these situations, and in order to support the underlying movement model of the receptor species, it may be necessary to turn to interpolation methods to estimate contaminant concentrations in areas where data are not available.

The contamination data used for estimating exposure consists of regularly spaced

forage samples of fluoride in desert sagebrush collected for several years in the early

1990s over a 400-kilometer square area. A histogram of the data is presented in Figure

3.2. Reference concentrations for soil concentrations of fluoride in the area range from

200-500 mg/kg (Rope *et al.* 1988). The desert sagebrush fluoride concentrations used

here are as reported in Warren-Hicks and Moore (1995) and are all well above reference

levels. The data fail Lilliefors test for normality, Lilliefors test for lognormality, and the

Anderson-Darling and Kolmogorov-Smirnov tests for a gamma distribution, therefore

non-parameteric data techniques must be used.   For conducting non-spatial risk

assessments (and ignoring any possible auto-correlation), current EPA guidance

recommends using a Chebyshev upper confidence limit (UCL) on the mean for an

exposure concentration with non-parametric data sets (EPA 2002).

There are a number of interpolation methods that can quantitatively describe the

spatial distribution of a contaminant, though care must be taken not to overreach with

inadequate data sets (Woodbury 2003). Various geostatistical methods are available

including various forms of kriging (Rossi 1992; Goovaerts 1997) and geometric

interpolants such as natural neighbor (Watson 1995) and inverse distance (Shepard 1968).

These interpolation methods rely on an assumption of spatial continuity; that is, two

sample locations close together are more likely to be similar than two samples separated

by a greater distance.

Ordinary kriging was used to estimate fluoride concentrations for all grid locations at the site. A Gaussian semivariogram with 14 lags is implemented, with a range of 7000m, and a nugget and sill fitted using iteratively reweighted least squares (Cressie 1985).

The fluoride data set was also used to produce the eight simulated habitat data sets for the pronghorn movement model. These simulated data sets introduce strong positive, strong negative, and neutral correlations between habitat quality and contaminant concentrations to evaluate exposures for a range of conditions. A non-linear transformation of the concentrations C at all locations u_i, with a uniformly distributed random element ϵ, and then scaled by the maximum observed transformed value was implemented for all locations so that the HSI values range from 0 to 1 at areas where fluoride data was collected. In particular,

$$HSI(u_i) = \alpha C(u_i)^{\beta + \epsilon} \text{ for i} = 1,..., \text{N s.t. r} = x$$

Values of α and β were determined by trial and error until the desired correlation strengths, r, for a range of correlations from +0.75 to –0.75 was achieved to represent each habitat scenario. This approach essentially maximizes the entropy of the habitat

variable amongst the different scenarios with respect to the fluoride contamination distribution. Since the non-linear transformation of the concentration data to construct a habitat distribution invalidates the spatial correlation function used in kriging the original fluoride data set, inverse distance with a power of 2 was then used to spatially model the different habitats on the movement grid in order to find HSI values for all u_i. The ordinary kriging output for fluoride and the habitat data sets for different correlation strengths with the fluoride data are presented in Figure 3.3. Also included in the figure is a uniform habitat data set, used for the random walk scenario.

3.4 Model results

The simple discrete time habitat selection exposure model was run with the same grid of exposure concentrations and eight different sets of habitat data (corresponding to spatial correlation strengths of 0.75, 0.5, 0.25, 0, -0.25, -0.5, -0.75 between the habitat index values and the fluoride concentrations). In addition, a random walk model was simulated based on the same exposure data set and a non-spatial assessment based on the mean and UCL95 of the sample data are presented. Model code was developed in Visual Basic and used its pseudo-random number generator. Summary statistics for each set of 1000 Monte Carlo simulations are presented in Table 3.2.

The results given are average daily doses and are for a 1000 simulations of a 90 day period; the averages are directly comparable to our site-specific toxicity reference

values for fluoride of 760 mg/kg/day. The mean fluoride concentration in sagebrush was 16500 mg/kg and the Chebyshev UCL is 25700 mg/kg; resulting in doses of 417 mg/kg/day and 649 mg/kg/day, respectively. The dose results for the summary statistics are below our dose-based standard. For the simulation results, the cumulative distribution function of exposures is used to estimate the percent of simulated doses above our action level. The percent of the population exposures for no correlation (17%) and the random walk scenario (18%) are at levels below the 20% that is sometimes used as a remedial action target. However, the three habitat scenarios with positive correlation see increases in the percent of population that exceed a 20% threshold; the +0.25 correlation has an exceedance rate of 23%, the +0.5 correlation rate is 29%, and the high correlation scenario has a 46% exceedance rate. The negative correlation scenarios show the opposite trends; the –0.25 correlation rate is 12%, the –0.5 correlation rate is 3.4%, and the –0.75 correlation scenario has an exceedance rate of the state standard for only 0.5% of the exposed population. These changes in the exposure dose results when compared to aspatial methods and uncorrelated habitat data demonstrate that the presence of significant spatial autocorrelation between habitat quality and concentration distributions can potentially change the remedial decision that should be made at the site. The results for each habitat scenario are further illustrated in the box plot in Figure 3.4.

Sensitivity analyses were performed for other variables in the exposure model to provide a comparison to the variability in the dose results seen for the different habitat

74

scenarios. The one-at-a-time design method (Daniel 1973; Campolongo *et al.* 2000) is a simple and efficient sensitivity method and was used to vary each input variable one at a time from the standard default value. Reasonable lower and upper values are selected for the daily forage ingestion rate, daily soil ingestion rate, soil-plant concentration factor, and the absorption factor variables to examine the effect of the entire range of the input variable on the population dose output. The effects on the output can then be compared to the population dose results for the full range of correlated habitat strengths to determine the relative importance of these variables. The sensitivity results indicated that none of the exposure variables individually contributed as much variation in the output as the range of habitat scenarios with different correlation strengths.

3.5 Remedial design

Efficient remedial design strategies at a site are strongly dependent on understanding the spatial distribution of contaminants. Therefore, risk methods that explicitly factor in space have much potential for assisting in efficient remedial design (Freshman and Menzie 1996). The addition of methods to use habitat information can improve the quality of terrestrial exposure assessments by adding a spatial and temporal component to these existing capabilities. In a remediation context, the goal is often to determine the specific areas of a contaminated site that need remediation while minimizing the volume of remediation necessary to meet the cleanup criteria for the sake of cost efficiency. Exposure and dose models can be used to assist in optimizing the

remedial design when incorporating spatial information into the assessment. Two different methods are examined; an iterative hot spot removal design with modifications based on conservative foraging assumptions and a dose-based spatial remedial design.

The first method, commonly used, is an iterative hot spot removal process (Freshman and Menzie 1996; Schulz and Griffin 2001). This method constructs a remedial design by simulating the cleaning of individual contaminated grid areas of grid nodes at a site in descending order of magnitude. The representative site statistic that is being compared against a decision criterion (mean, percentile) is recalculated as subsequent blocks are cleaned and compared to a target ecological remedial concentration until the decision criterion is reached. This approach is simple to implement, but does not incorporate habitat or home range considerations. The output can be plotted as a curve of remediation area versus post-remediation concentration to illustrate the effect of progressively cleaning more of the site. Alternatively, the curve can be plotted as cost of remediation versus post-remediation concentration (Van Meirvenne and Goovaerts 2001) when cost is a constraint.

This approach can be modified with conservative foraging assumptions that retains the spatial distribution of the contamination and provides exposure estimates consistent with foraging ranges, without explicitly incorporating habitat information (Freshman and Menzie 1996). Based on the home range of the receptor being assessed,

the method assumes that the receptor is exposed to the most contaminated grid cells at the site equivalent to this area, and remediates until the average concentration of the cells constituting this area is below the cleanup standard. This method for generating an exposure curve can be sufficient for a number of remedial decisions. However, it may under- or over-estimate exposures for receptors in some situations. These situations include the combination of a species that non-randomly uses habitat, a landscape with a heterogeneous distribution of habitat, and a fairly significant degree of positive or negative correlation between contaminant concentrations and habitat quality.

Under these circumstances, rather than implementing a post-remedial exposure curve that cleans areas with highest concentrations, an optimal remedial design method would look at areas of the site where the highest levels of population exposure occur by examining both the spatial distribution of contamination and habitat use. This leads to a more realistic dose-based approach that uses the exposure results for a population simulated within a given habitat landscape and optimizes the design based on where the majority of exposure is occurring. Evaluating this cumulative population exposure at each grid cell has the advantage of incorporating the use of habitat in addition to the spatial distribution of contamination and exposure curves. The main drawback of this approach is that it can be computationally intensive as the set of Monte Carlo simulations has to be run iteratively for each block that is determined to need remediation. However, cleanup area curves can be generated that are intuitive and may differ significantly from

cleanup curves that do not account for how the receptor uses habitat.

3.6 Summary

Though it has been suggested that complications introduced by accounting for spatial heterogeneity may be unimportant (Emlen 1989), spatial structure of contamination and habitat can have a profound impact on the exposure of a population to contamination. These impacts can complicate the tasks of understanding, managing, and preventing unacceptable levels of exposure to contamination in terrestrial environments (McLaughlin and Landis 2000). For the pronghorn model developed here, the movement rules for female pronghorn allowed them to utilize the whole site, but generally be attracted to higher quality foraging areas. Exposure distributions for male pronghorn may be quite different during periods of territoriality, as they partition the site into discrete defended areas. In this instance, the dose distributions of the sexes may differ significantly within a species.

A correlation analysis between contaminant concentrations and habitat quality is recommended as a means of determining whether conducting a spatially explicit exposure analysis that incorporates HSI data is potentially informative versus non-spatial implementations. If it is necessary to perform such an assessment, habitat use by potential endpoint species can be compared to identify species that have high correlations of habitat quality and available contaminant concentrations. This may be an important

part of the endpoint species selection process. When significant correlations are present, it may also be more efficient to incorporate assumptions regarding habitat use into the remedial design process in order to optimize the cleanup of the site.

There is a potential disconnect between the importance of cleaning up areas that are of high habitat quality for species of concern versus the impacts of remediation on these same areas. This is a problem when contaminant concentrations and habitat quality for a species are positively correlated, since a number of remediation technologies result in destruction of habitat, which in itself can cause significant ecological risk to the species of concern. The decision to remediate must take into account the local and regional importance of the habitat and the potential for post-remedial recovery. In industrial areas, contamination and habitat quality for a number of typical receptors often are negatively correlated, and endpoints should be considered that are more likely to inhabit these areas.

Ecological risk assessments need to more fully consider landscape characteristics and spatial interactions. Examining possible spatial correlations between contaminant distributions and habitat quality for species of interest at contaminated sites is a simple way to check for the possible significance of landscape when conducting an ecological risk assessment.

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3.8 Appendix

Table 3.1. Pronghorn exposure variables.

Variable	Description	Lower	Default	Upper	Units	Reference
$Dose_i$	Average dose for individual I	-	-	-	(mg fluoride/kg BW)/ day	Calculated
T	Number of days	-	90	-	day	Length of summer
C_t	Fluoride concentration in forage at time t	6000	-	162000	mg/kg	Data
I_f	Daily forage ingestion rate	0.76	0.85	0.97	kg dry forage/day	Severson *et al.* 1968
I_s	Daily soil ingestion rate	0.0215	0.0487	0.084	kg soil/day	Arthur and Gates 1988
B_v	Soil-plant concentration factor	0.024	0.045	0.056	(mg/kg dry plant)/ (mg/kg soil)	Rope *et al.* 1988
BW	Female body weight	34	40	48	kg	Nowak 1991
A	Absorption factor	0.44	0.54	0.63	unitless	Welch *et al.* 1983

Table 3.2. Descriptive statistics for fluoride exposures (mg/kg/day) for different correlation strengths between habitat and fluoride concentrations.

Scenario	Median	Mean	Standard Deviation	Coefficient of Variance	% Exceeding dose standard
r = 0.75	664	958	811	0.85	46
r = 0.5	381	621	558	0.90	29
r = 0.25	319	543	521	0.96	23
r = 0	230	424	408	0.96	17
r = -0.25	221	379	371	0.98	12
r = -0.5	177	251	221	0.88	3.4
r = -0.75	166	194	86	0.44	0.5
Random Walk	275	439	389	0.89	18
Mean concentration	-	417	-	-	-
Chebyshev UCL	-	649	-	-	-

		HSI Data						Divide each HSI value by HSI sum (=4.13)			Movement Probabilities					

17	0.03	0.54	0.55	0.82	0.56	0.4
89	0.71	0.31	0.32	0.51	0.53	0.0
60	0.79	**0.45**	**0.66**	**0.93**	0.28	0.4
62	0.61	**0.26**		**0.76**	0.98	0.
53	0.02	**0.25**	**0.38**	**0.44**	0.63	0.8
87	0.38	0.74	0.63	0.24	0.28	0.0
73	0.51	0.96	0.20	0.30	0.38	0.1

Divide each HSI value by HSI sum (=4.13)

	0	0	0	0	0	
	0	0	0	0	0	
	0	**0.11**	**0.16**	**0.23**	0	
	0	**0.06**		**0.18**	0	
	0	**0.06**	**0.09**	**0.11**	0	
	0	0	0	0	0	
	0	0	0	0	0	
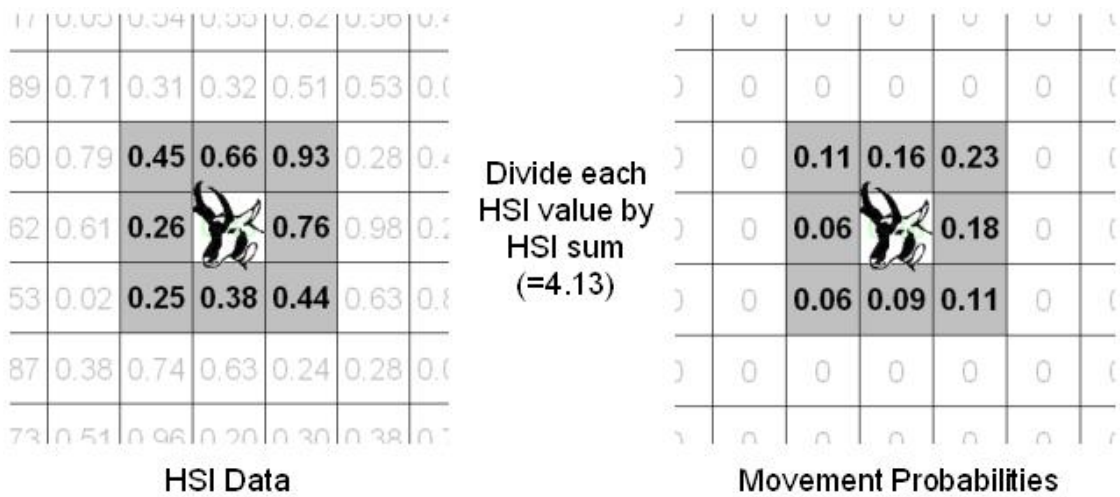
Figure 3.1. Visual description of habitat-based movement model. Pronghorn is eligible to move to one of 8 adjacent grid cells each time step. Movement probabilities for each cell are determined by dividing the HSI value for that cell by the sum of the HSI values for the 8 cells available for movement.

87
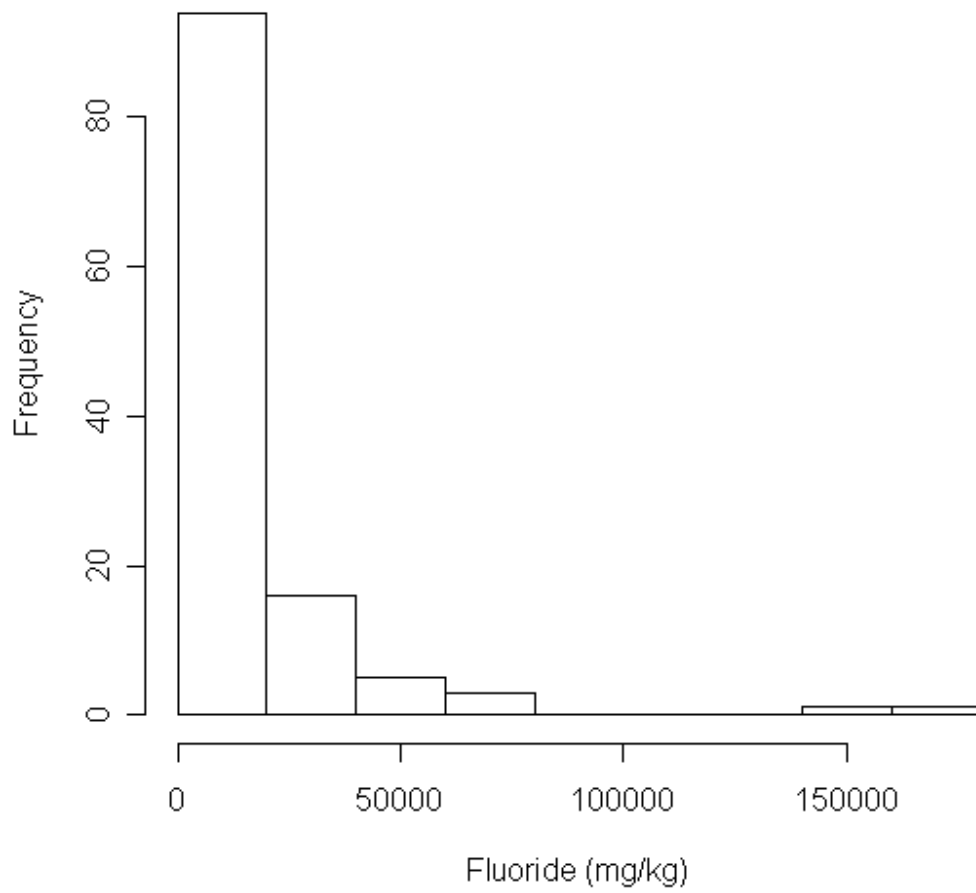
Figure 3.2. Histogram of fluoride sagebrush concentrations in mg/kg. Data fail parametric tests and non-parametric methods are used for confidence limit.
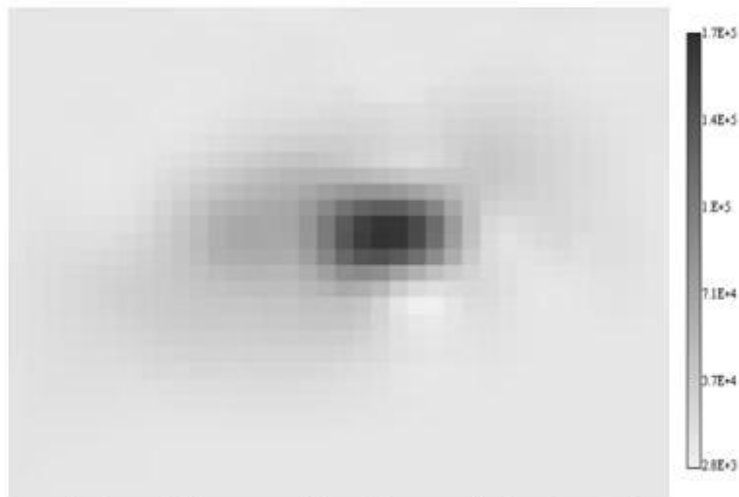
Ordinary kriging map of fluoride concentrations (mg/kg)
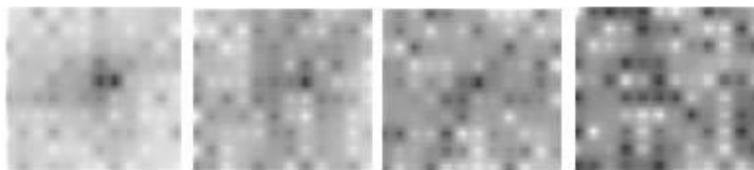
r=0.75   r=0.5   r=0.25   r=0
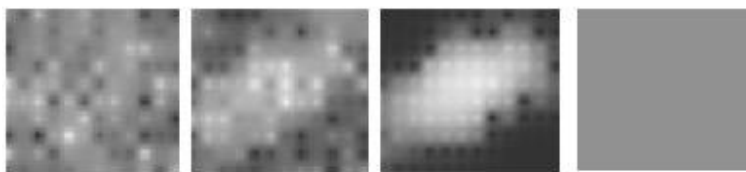
r=-0.25   r=-0.5   r=-0.75   random walk

Habitat maps used for simulations

Figure 3.3. Ordinary kriging results for fluoride concentrations in sagebrush. Smaller figures are simulated HSI data sets with different degrees of correlation to the fluoride data set. The random walk habitat data set has uniform habitat quality.
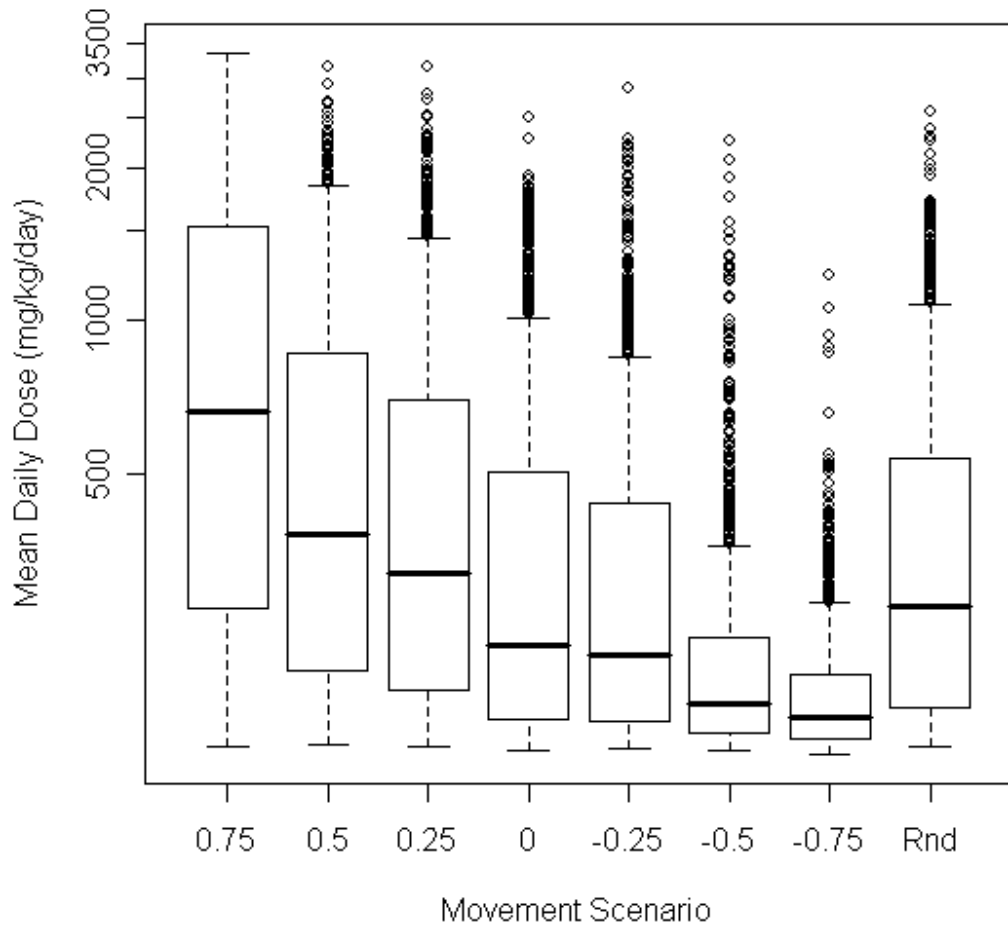
Figure 3.4. The population dose distributions are presented as boxplots for each exposure/movement scenario. Population doses decrease significantly as the degree of correlation between habitat quality and contamination levels decreases from 0.75 to –0.75. The random walk exposure box plot, used for comparison purposes to the correlation scenarios, is similar to the exposures for 0 correlation.

4. Resistance is Futile?: Risk Assessment, Modeling, and Management for Insect Resistance to *Bt* Crops

The commercialization of genetically modified organisms (GMOs) has thus far been focused on agricultural traits, such as herbicide and insect resistance, in economically important crops. A principal form of GM for agriculture is plant-incorporated protectants (PIPs), the incorporation of a genetic sequence from the bacteria *Bacillus thuringiensis* (*Bt*) to express a protoxin with insecticidal activity. *Bt* crops have been commercially successful. The United States plants a significant portion of the world's transgenic crops, accounting for 49.8 of the 90 million total hectares planted internationally in 2005 (James 2006). The area devoted to *Bt* corn and cotton crops has steadily increased since initial commercialization in 1996. In 2005, 35% of corn and 52% of cotton planted in the US was *Bt* modified (USDA 2005). These crops have been successful at minimizing insect damage, reducing the use of broad-spectrum insecticide sprays, and increasing yield and profit for farmers (Shelton *et al.* 2002). Concerns prior to the technology being introduced were that insect resistance would quickly evolve due to the high selection pressure being placed on the exposed populations. These expectations were supported by modeling efforts (Alstad and Andow 1995) and laboratory-selected resistance experiments (McGaughey 1985; Ferré and Van Rie 2002), in addition to the field detection of resistance to *Bt* sprays in the diamondback moth (Tabashnik *et al.* 1990). Resistance modeling efforts influenced the risk management

steps that were taken to reduce the chances of resistance evolving. Even with these proposed measures, the consensus was that resistance was inevitable and would quickly evolve (*e.g.*, Gibbons 1991; Harris 1991). Somewhat surprisingly, ten years after introduction, insect resistance has yet to be detected in the field. This occurred despite consistent increases in the number of licensed crops containing *Bt* genetic material and steadily increasing acreage devoted to transgenic crops. There are millions of hectares planted with transgenic crops now, and selection for resistance in areas with high *Bt* adoption rates can be strong. Resistance management strategies are currently in place (Matten *et al.* 2003), and there have been no accounts of increase in *Bt* resistance frequency due to the use of *Bt* crops (Tabashnik *et al.* 2003). Though significant advances have been made in the last ten years regarding the various components of insect resistance evolution, it is still not possible to say with certainty why resistance has yet to evolve or when it may evolve in the near future.

4.1 Why hasn't resistance evolved yet?

Resistance to pesticides has evolved many times in the past with the application of spray pesticides. Lepidopteran crop pests worldwide have been subjected to carbamate, cyclodiene, organophosphate, and pyrethroid insecticides and have developed resistance to many forms of these insecticide sprays (McCaffery 1998). In the United States, lepidopteran species that cause significant crop damage, despite conventional spraying include the corn earworm/cotton bollworm (*Helicoverpa zea*), the tobacco

budworm (*Heliothis virescens*), and the European corn borer (*Ostrinia nubilalis*), among others. These species are polyphagous, affecting many different agricultural crops, and have a history of resistance to common forms of sprays. These lepidopterans are all targeted by the insecticidal ability of *Bt* plant incorporated protectants. The specificity of *Bt* to these target pest species is a key positive trait ecologically compared to synthetic pesticides, since synthetic forms are generally more broad-spectrum and can have a significant effect on non-target species. Increased use of targeted PIPs can result in lessened use of these synthetic, broad-spectrum pesticides and a correlated reduction (but not elimination) of adverse non-target effects commonly seen in agricultural systems. Due to this reason and the limited number of effective pesticides otherwise available due to the existing resistance in lepidopteran species, government and industry have a significant interest in delaying the onset of *Bt* resistance for as long as possible.

After early expectations that resistance to *Bt* crops would have evolved by now did not materialize, a number of factors were invoked to explain why it has yet to appear. Some of the delay has been attributed to successful management actions intentionally enacted, while other influences may be the genetics of resistance and the attributes of the agroecosystems in which the *Bt* crops are planted. A proper understanding of the genetic basis of resistance is fundamental for constructing strategies to postpone the evolution of resistance. The high dose- spatial refuge strategy implemented by the United States Environmental Protection Agency (EPA) takes into account the spatial configuration of

planted crops and the recessive properties of the resistance allele to minimize resistance evolution.

The high dose portion of the strategy ensures that enough of the toxin is expressed so that high mortality will occur in heterozygous insects carrying one copy of the recessive allele. The dose given is as high as possible, at least 25-fold over the amount needed to kill a homozygous susceptible individual and sufficient to have a high mortality rate for heterozygotes that carry a resistance allele (Gould 2000).

Because large areas of *Bt* crops lead to selection pressures in favor of insects that are not susceptible to *Bt* toxins, for the spatial refuge portion of the strategy, refuges are planted that allow for a sufficient number of susceptible insects to evolve that will mate with any resistant individuals that emerge. The stated goal for these refuges is that non-transgenic hosts must be planted on each farm to produce at least 500 susceptible pest individuals for each resistant individual produced in the *Bt* crop (BANR 2001). The effectiveness of refuges depends on sufficient gene flow between the refuges and the control areas. These dependencies include random mating between susceptible and resistant individuals, dispersal before mating, synchrony of breeding between susceptibles and resistants, resistance is recessive, and that the high dose yields high mortality rates for heterozygotes. Current resistance delay strategies are based on a two allele model of susceptibility. The dominant gene is considered to be susceptible and the

recessive gene is resistant. Further assumptions include that the locus is autosomal, not sex-linked, and there is no epistasis or linkage between the loci. Overall, these guidelines rest on some basic assumptions: that resistance is recessively inherited, that there is a low initial frequency of resistance in the insect populations, and that extensive mating occurs between insects that emerge from the *Bt* crops and the insects that emerge from refuge areas. The first two assumptions are reasonably well established, resistance is recessively inherited in most situations (Tabashnik 2003) and there are low frequencies of resistance currently (*e.g.*, Tabashnik *et al.* 2000). However, the third assumption of extensive random mating between refuge and GM individuals is specific to the spatial configuration of the refuges and GM crops (Carrière *et al.* 2004) as well as other external factors. Assortative mating can and does occur with emerging moths in refuge situations and the extent of assortative mating can minimize the effectiveness of the refuge strategy. Significant non-implementation of GM crops in addition to natural area essentially provides an additional refuge source of susceptible insects, since natural areas can provide host plants for pest insects free of *Bt* selection pressure.

Traditional crop management practices such as tilling and crop rotation are effective in delaying the establishment of resistance alleles, and are therefore used as resistance control strategies in addition to their utility as sound insect management practice. Tilling disturbs development forms of the insects and can enhance overwintering mortality in some species. In addition, crop rotations are often extremely

effective in reducing survival rates of the first generation of emerging insects in the following year. For example, if offspring of a cotton pest emerge in soybean the following year, its survival may be significantly lower. If the offspring are located in fields that were *Bt* the previous year, and if that resistance genome is exposed to a different host plant or a different *Bt* toxin the following year, this can also lower the overall frequency of the resistance allele in the population. In some areas of the world, tilling and rotation are required as part of the formal resistance control plan.

Spatial refuges are a key component of resistance management when a high dose strategy is pursued. The presence of a refuge allows for susceptible insects to survive in the vicinity of *Bt* crops. If emergence from refuge and *Bt* crops occurs simultaneously, then emerging susceptible insects can mate with resistant insects emerging from the *Bt* fields. Dose levels are designed to be high enough that any heterozygous individuals in the next generation will still be selected against with high mortality rates. The uses of refuges are formally documented in a resistance management plan and can vary from 5-50% depending on crop type and toxin type. If a 500:1 ratio of susceptibles to resistants can be maintained, this can be a very effective strategy. In some areas, natural areas and farms that do not implement the technology provide additional sources of susceptible insects. The low overall implementation rates of *Bt* in the US until the last couple of years are thought to be a major factor in why resistance has not yet been observed. Each year more acreage, in the US and worldwide, has the *Bt* transgene. This effectively

reduces the amount of practical refuge and susceptibles available to suppress resistance and therefore increases the selection pressure for resistance.

Another important factor in delaying resistance is the cost of fitness for mutations that allow the lepidopterans to ingest *Bt* without lethal effects. The genetic basis of resistance depends on the mode of action of the insecticide. *Bt* crops produce crystal proteins that are toxic to the insect when ingested. In the alkaline environment of the insect gut the crystal proteins are solubilized and protoxins released. This protoxin eventually passes through the peritrophic membrane and binds to a midgut receptor due to the action of a cadherin gene. Here, the toxin is inserted into the membrane, causing a hydrostatic pressure imbalance, which results in a hole in the gut wall, allowing for spores and gut bacteria to enter the body. The insect becomes immobile and quickly dies as these spores and bacteria multiply in the blood. A number of potential mutations can disrupt the normal activity of the *Bt* toxin in the insect. For example, mutations that do not allow the insect larvae to initially solubilize the toxin or that prevent the toxin from adhering to the midgut wall may confer partial or complete resistance, at a potential cost to fitness of the resistant individual. The most common type of *Bt* toxin resistance is recessively inherited and reduces binding of the toxin to the midgut membrane, allowing for resistance to one (or more) of the *Bt* toxin types (Tabashnik *et al.* 1998). In laboratory-resistant strains of the tobacco budworm, a mutation in the cadherin allele has been identified as conferring resistance to *Bt* toxins. There are many more potential types

of *Bt* resistance and their genetic mechanism and sequence are still undiscovered. These mutations can come at a cost to the insect, as they generally yield pleiotropic effects that cause significant disruptions in the ability of the larvae to process food.

The length of time to resistance is a function of many factors, including the temporal duration/expression/number of applications of the insecticide and the effectiveness/mortality rates of the insecticide and resulting selection pressure. This is due to the high dose strategy that is being pursued. The dosage expressed in *Bt* kills close to 100% of pink bollworms, and greater than 90% of related species such as the corn earworm and tobacco budworm, though field effects may be lower. Selection pressure on other species, such as armyworms are less, at around 70%, to practically 0% for sapsuckers due to the fact that *Bt* is not expressed in phloem. In addition to the high dosage, there are few temporal refuges from *Bt*, as the toxin is designed to be expressed at high levels in all parts of the plant through out the year. In practice, this is not the case as the level of *Bt* decays throughout the growing season and older larval forms of pest species are somewhat less susceptible. These high selection pressures, plus the fact that *Bt* resistance has evolved independently a number of times in the laboratory with pest species, is suggestive of a reasonably high chance of early resistance appearing.

4.2 History

Bt is a natural bacterium that can be used as an organic insecticide in spray form. Foliar insecticides have been made from *Bt* protoxins for over 40 years and *Bt* products dominate the biopesticide market and are heavily used for organic farming. However, *Bt* spray was not a widely used insecticide until its introduction as a genetically modified trait. *Bt* was mostly used in the organic farming industry since it is considered an organic control, and *Bt* spray only held approximately 2% of the pesticide market until the late 1990s. In spray form, *Bt* quickly degrades, and application is manual and organic farming implements a number of methods designed to reduce the number of spray applications. If major pests developed widespread resistance to *Bt* it could be devastating to the organic industry due to their reliance on *Bt* sprays. For organic applications, *Bt* breaks down relatively quickly, making it less likely for resistance to build up in the insect population. However, one insect, the Diamondback moth, has developed field resistance to *Bt* (Tabashnik 1994) from the use of sprays. In addition, the cabbage looper, *Trichoplusia mi*, has evolved resistance in greenhouse applications of the spray (Janmaat and Myers 2003). If common agricultural pests evolve widespread *Bt* resistance, this would not only render the *Bt* transgenic crops much less useful, it could also remove the most effective organic insecticide from the market.

There are more than 100 known types of *Bt* toxins, and each type produces one or more types of crystalline (Cry) proteins and protoxins (Swadener 1994). Therefore, a

number of different *Bt* types have been identified with specific insecticidal activity. They are lethal to certain pests while being harmless to people and the majority of exposed non-target wildlife. *Bt* is considered not to be persistent in the environment but little is known in general about its long-term survival in field ecology. The development of molecular biology techniques to identify, isolate, and clone genes that express specific traits has led to the ability to incorporate these genes into the genomes of unrelated organisms.

Schnepf and Whitely (1981) first cloned and expressed a *Bt* crystal protein gene in *Escherichia coli*. Researchers quickly began to work on incorporating *Bt* genes into crop plants, and the first successes were with the tomato (Fischoff *et al.* 1987), tobacco (Vaeck *et al.* 1987), cotton (Perlak *et al.* 1990), corn (Koziel *et al.* 1993), and potato (Perlak *et al.* 1993). Other *Bt* crops currently approved or under consideration include peanut, broccoli, soybean, and eggplant.

4.3 Risks, Benefits and Management of *Bt* modified crops

Risk assessment and management techniques are used increasingly in the delineation and resolution of complex decisions. One such complex decision is the implementation of genetically modified (GM) crops. Though sometimes expensive for the farmer, sowing GM crops can increase yield while generating cost-savings to the farmer in some areas by efficiently controlling weeds or insect pests. This weed and

insect control is accomplished either directly through the protectant or by conferring traits that allow for increased use of other conventional control products (*e.g.*, glysophate-resistance allows for increased use of glysophate without negative effects on the crop). The quick adoption of approved GM crops in the United States attests to how economically valuable this technology has been perceived to be by a number of farmers. Continued effectiveness of GM traits is connected to preventing the evolution of resistance. Evolution of resistance can completely eliminate the benefits of GM crops or, in order to continue to suppress insect populations, result in harsher applications of pesticides (with associated indirect effects).

There are a number of diverse risks from the release of genetically modified organisms. These risks include the increased risks of invasion from a species with new unintended, undesired properties such as a weed with herbicidal-resistance. Direct effects include toxins acting on non-target receptors. Indirect effects, such as the ecological effects of the diminishment of a pest population on the other species in a community, have been documented and are too numerous to anticipate all of them prior to introduction. *Bt* crops might also exhibit indirect effects in soils. Toxins in naturally-occurring *Bt* bacteria, and the "organic" sprays made from them are in inactive form but become activated once ingested by the target insect. It has been demonstrated in the laboratory that unlike toxins in naturally occurring *Bt* bacteria that require activation, the toxins from crops that express *Bt* genes are already in active form and these toxins

rapidly bind to soil particles and are not broken down by soil microbes (Koskella and Stotzky 1997; Tapp and Stotzky 1998). Hence, the *Bt* genes associated with crops could well be more persistent and more active in the crop soils than their natural analogues. Indirect food chain effects of *Bt* crops, for example, have been documented for insect predators (*e.g.* green lacewing insects (*Chrysopa oculata*) that feed on the European corn borer (*Ostrinia nubilalis*, a pest targeted by *Bt* corn; Hilbeck *et al.* 1998; Romeis *et al.* 2006) or on peripherally affected insect species (*e.g.* Monarch butterflies (*Danaus plexippus*; Conner *et al.* 2003)). Indirect food chain effects of *Bt* crops on avian or mammalian insectivores have not received significant attention in the scientific literature.

The responsibility for regulatory oversight of agricultural biotechnology products is shared by three Federal agencies: the U.S. Department of Agriculture's Animal and Plant Health Inspection Service (USDA-APHIS), the U.S. Environmental Protection Agency (EPA), and the Department of Health and Human Services' Food and Drug Administration (FDA). A food crop plant developed using genetic engineering to produce a pesticide in its own tissue is reviewed by all three regulatory agencies. USDA-APHIS has jurisdiction over the planting of genetically engineered plants. EPA regulates planting and food and feed uses of pesticides engineered into plants. The FDA controls food and feed uses for all foods from plants. The laws that regulate agricultural biotechnology products include: the Plant Protection Act (PPA), the Federal Food, Drug, and Cosmetic Act (FFDCA), the Federal Insecticide, Fungicide, and Rodenticide Act

(FIFRA), and the Toxic Substances Control Act (TSCA).

USDA-APHIS is responsible for protecting agriculture from pests and diseases. Under the Plant Protection Act (PPA), they regulate crops based on the procedures used to incorporate the *Bt* gene. USDA-APHIS oversight begins early in the development of a GM crop and continues until the developer applies for and is granted non-regulated status for the plant. EPA regulates the distribution, sale, use and testing of the GM crop. EPA regulates *Bt* because it is a pesticide and controls the field testing of pesticides through a permit process. In order to sell or distribute the pesticide, the company must register the pesticide with EPA. Through the registration, EPA can establish the conditions of commercial use. EPA is also responsible for setting the amounts or levels of pesticide residue that may safely be in food or feed. EPA may allow an exemption from the requirement to set such a residue level if it can be shown there are no food or feed safety issues associated with the pesticide.

Developers of *Bt* crops also consult with FDA about other possible, unintended, changes to the food or feed. For example, possible changes in nutritional composition or levels of native toxicants. Although this consultation is voluntary, all of the food/feed products commercialized to date have gone through the consultation process. The consultation with FDA serves to ensure that safety or other regulatory issues that fall within the agency's jurisdiction, including appropriate labeling of the food, are resolved

prior to commercial distribution.

Approval and registration processes for genetically modified crops are generally based on the assumption that there are few adverse effects of *Bt* chemicals to agriculturally beneficial species such as birds and mammals. However, these are assumptions derived primarily from toxicological tests performed on individual species, not from an ecological perspective. Indirect impacts on food web structure have been examined for some agro-ecosystems (Romeis *et al*. 2006), but certainly not for all, and the impact of *Bt* implementation in concert with other natural or anthropogenic stressors have not been widely studied. Although ecological approaches to addressing risks from genetically modified crops are being more widely implemented, there remain significant uncertainties in our ability to predict these risks as the use of genetically modified organisms becomes more widespread.

4.4 Risk Management Control Options

The use of *Bt* crops puts very high selection pressures on the pest insects to evolve resistance in the population. Without a refuge, once a small population of resistant individuals gets established, they will quickly spread to become nearly fixed in the population (absent significant cost of fitness for resistance) if all crops were to contain the transgene. It is important to consider the context for the use of a refuge as one of a couple of different control options, and that the use of a refuge is complementary

(and sometimes necessary) based on the use of the other control options. Essentially the choices for resistance control that were considered before implementation of *Bt* crops came down to the following: 1) moderate versus high dose of the toxin; 2) expression of the toxin in all areas of the plant at all times versus selective expression; 3) single versus combinations of toxins; and 4) the production of susceptible insects, involving the use of refuges at one extreme to mixed seed at the other.

An initial control decision was how much of the toxin to express in the plant. There was a school of thought that moderate toxin expression might be preferable to high doses as this would lower the selection pressure on the insects. These lower doses could potentially prevent complete resistance from evolving, by in some cases allowing some susceptibles to survive. Modeling to compare the evolution of resistance for lower doses showed some small delay in resistance, but it was not significant (Gould 1994). A bigger problem was that field trials showed that moderate doses were not effective for pest control, which would limit the implementation of the technology. Therefore, a high dose strategy for implementation of the transgene was decided on early in the process. Modeling of high versus low toxin doses showed that a big driver in the non-success of lower doses for pest control was the survival of heterozygotes. Initial occurrence of the resistance allele is very rare, so growth in allele frequency in the models were driven by survival of heterozygotes, and not homozygous recessives. Lab studies of the dose levels showed that high doses of the toxins were much more effective for controlling

heterozygotes than moderate doses; and therefore were more effective at delaying the growth of the allele in the population.

A second control strategy was a limited expression of the toxin over time or for a particular plant part. The idea is that susceptible insects are allowed to survive temporally during the season but the transgene would be expressed at the fruiting of the crop, knocking out the larval population. This would make the use of a refuge unnecessary because susceptible insects survive at other times of year. This may still a future option but there are technical issues involved in creating limited expression of the transgene for either a part of the plant or for a certain development period of the plant. Current efforts result in partial expression of the transgene, which leads to some of the same problems encountered with the moderate dose strategy. Selective plant part expression also has the potential to allow for partial resistance to evolve, which can jumpstart the resistance problem by providing a population of semi-resistance alleles for future selection. So, it was decided for current implementations to express the *Bt* in all areas of the plant over the entire life history of the plant. This can still be a problem. With the current technology, Bollgard II has shown recent failures in India due to a longer bloom season than in the US, and the *Bt* toxin levels start to decay over time giving the larvae a chance later in the bloom cycle and causing crop damage.

The use of multiple toxins in the crop is another potential technology to control

resistance. There are hundreds of different *Bt* toxins that show activity for different classes of insects. By putting two or more toxins in the crop *Bt* "cocktails" are created that can limit the survival of insects that are resistant to one of the toxins. Jackson *et al.* (2003) showed that bollworm production in North Carolina in normal crops without spraying was on the order of 47000 per hectare, with Bollgard I (one toxin) production was 25000 per hectare, and with Bollgard II (two *Bt* toxins) was less than a 1700 per hectare. Given the stated goal of maintaining a 500:1 ratio of susceptibles:resistants this strategy could diminish the size of the refuge. Currently, Bollgard II is the primary multi-strain toxin available (Widestrike was fully registered by the EPA in 2005), its use has not yet been completely established and it has not factored into official refuge restrictions.

Another control method for delaying resistance evolution is using more than one transgene, assuming the transgenes have different modes of action that limit the possibility of cross-resistance evolving. With multiple transgenes, they can be stacked in the same crop, or utilized in a spatial rotation system combined with monitoring for switching the transgenic crop type when resistance levels reach a certain threshold. The problem with the spatial rotation approach is that, unless there are high costs of fitness associated with the resistance allele, the frequency of the allele does not decay at a significant rate to allow for returning to the original transgene in the future. For this reason, and the fact that stacked varieties have been shown to be more effective in

providing insect control, stacked crops that combine two transgenes are growing in popularity. There are currently three deregulated transgenes commercially available for cotton, and there are two products, Bollgard II by Monsanto and Widestrike by Adventis, available that contain two transgenes each. Any insect that is resistant to one of the transgenes still has a significant probability of mortality because the activity of the other is unrelated, minimizing the chances of cross-resistance. The effect on emerging insects is significant, sometimes providing another order of magnitude impact on the size of populations emerging from *Bt* fields, and this also translates, in theory, to smaller refuges necessary to maintain the 500:1 susceptible to resistant ratio.

The success of these different control strategies is variable, and they are usually used in combination when implementing a resistance control plan. The goal in preventing the evolution of resistance is simply to stay one-step ahead; to put such high, sustained selection pressures on pest insects means that the appearance of resistance is inevitable, and the goal of the agricultural industry is simply to maximize the amount of time until resistance appears and to be prepared with other pest control alternatives for locations where it does appear. So, when deciding on refuge restrictions, the choices are to proceed with a high dose strategy, expressed over the course of the entire year and the entire plant, and because there are mostly single toxins in the field currently multi-toxin strains are not considered.

For the actual distribution of the seed in the field, once a percentage of non-*Bt* was decided upon, a mixed seed approach, where non-*Bt* is randomly or systematically mixed in with *Bt* seed, was considered an inferior strategy for resistance evolution because larvae could spend part of their time feeding on a *Bt* plant, survive but decide it was not to their liking, and move on to a non-*Bt* plant. This could encourage the non-desirable evolution of partial resistance in the field. Therefore it was decided that a separate refuge was best. In this situation, susceptible insects would emerge en masse and mate with resistant insects that were emerging at the same time. This approach is vulnerable to the control of resistance being based on Hardy-Weinberg equilibrium and random mating assumptions. If the crops are treated differently (*e.g.*, non-*Bt* not irrigated, *Bt* irrigated because they are more profitable and water costs money) insects can emerge at different times, leading to assortative mating between susceptible and resistant insects and a higher probability of recessive resistant insects surviving in the next generation. Refuge implementation has also been plagued by non-compliance from farmers, who may not spend the time and money on refuges that are not profitable. For these reasons, a mixed seed approach is being considered in areas like small farms in China where they expect refuge compliance issues to be worse than in the US.

4.5 Resistance Models

Three main classes of resistance models have been used for resistance risk assessment and management. The first resistance models were essentially two patch

models with a treated and untreated patch and varying degrees of realism for handling crop growth and insect dynamics. Discrete space and time models that handle more complex spatial processes on a grid are increasingly used to model real landscapes. Other forms of resistance models include systems of differential equations, generally adapted from other ecological applications, modified to represent crop-insect dynamics.

Analytical models for modeling resistance evolution in a two-patch environment can be traced to Comins (1977) in the context of resistance to spray insecticides. This model demonstrates that random mating between resistance-selected and unselected insect populations can slow the development of pesticide resistance. This deterministic model tracks the three genotypes *ZZ* (susceptible), *ZR* (homozygous), and *RR* (resistant) with two patches, a treated crop and an untreated area (refuge), that allows for gene flow between the two crop types. Egg, larval, and adult forms of the insect are considered. Mating within each region is random and migration occurs between the two regions during the adult phase of the life cycle. Density dependent mortality occurs before spraying, and is given by the following equation (May *et al.* 1974):

$$N_{t+1} = \lambda N_t^{1-b}$$

where *b* can vary between 0 and 2 and represents perfect density dependence when equal to 1, *b* < 1 gives depensatory density dependence (monotonic return to equilibium), and *b* > 1 gives overcompensatory density dependence (oscillatory return to equilibrium).

Application of the insecticide in the treated region yields larval survival rates for the different genotypes of *ZZ=L*, *RR=K*, and the heterozygous *ZR = hL +(1-h)K*. For the heterozygosity parameter *h*, *h*=1 is dominance, *h*=0 is recessive, and values of *h* between 0 and 1 represent partial dominance. Comins (1977) then modeled the migration of adults and the rates between the two patches are proportional to the untreated and treated areas when they have the same population densities. The ratio of the area (untreated/treated) between the two patches is represented by the parameter *G* and the growth rates (in the absence of insecticide) of the two patches are the same. If *T* and *U* are the treated and untreated populations, and *r* is the emigration rate from the treated area and *r/G* the emigration rate from the untreated area then:

$$T' = (1-r)T + \frac{r}{G}U$$

$$U' = (1 - \frac{r}{G})U + rT$$

Though this model was developed for spray insecticides, one can see the basic elements for the high dose- spatial refuge resistance delay strategy implemented for *Bt* crops. The amount of emigration from untreated areas (spatial refuges or external untreated plots or natural insect habitat) can be increased by decreasing the value for *G*; this can then show a delay in the onset of resistance evolution by creating a temporary equilibrium at low resistance allele frequencies. However, these delays are only possible for heterozygosity parameter (*h*) values of below 1/3, representing a recessive resistant allele, and for sufficiently low initial conditions for the initial resistance allele frequency. These

requirements lead to the high dose element of the strategy, which serves to effectively kill heterozygotes and prevent quick increases in resistance allele frequency within the treated population. It is important to recognize that under these conditions, the effect of the high migration rates are not the result of simple dilution; rather these high migration rates allow for a significant portion of the resistant genes to be present in heterozygotes of the next generation, and these heterozygotes have low survival rates due to the combination of a sufficient amount of recessiveness of resistance and a high dose.

As an alternative to the spatial refuge approach, Gould (1986a, 1986b) proposed mixtures of insect-resistant and conventional plant seeds as an effective means for delaying resistance; this strategy has the additional advantage that it could be more likely to be implemented by farmers than spatial refuges. Mallet and Porter (1992) compared these two approaches with a genetic model that examined different stages of the larval life cycle and small-distance movements of insects. In the absence of insect larval movement in mixed seed environments conventional plants act as refuges for susceptible insects and this crop setup can make random mating more likely after emergence. However, for insects that switch host plants during their larval development, mixed seed increases the probability that it splits time between a toxic and a non-toxic host plant. In this situation, the effective *Bt* dose to the larvae is decreased and the fitness of partially resistant larvae is increased, essentially negating some of the aspects of the high dose implementation strategy. Paradoxically, for high rates of mortality for exposures to the

Bt toxin and for low values for heterozygosity (nearly completely recessive), the model demonstrated that the mixed seed strategy could potentially hasten the evolution of resistance when compared to pure transgenic crops. This is due to the promotion of partial resistance under these conditions of mortality and heterozygosity when individual larvae spend development time on both toxic and non-toxic plants. These results stimulated studies on movement rates of insect larvae as they were not well understood at the time for a number of pest species. These results also apply to tissue-specific expression of the *Bt* toxin, for example *Bt* cotton plants that only express *Bt* in the boll could be subject to these same effects on time to resistance for *Heliothis* species that spend part of their larval cycle on the boll and the rest on other parts of the plant. Mallet and Porter (1992) then concluded with a strong argument for legislation mandating refuges along with the commercialization of *Bt* crops due to the low likelihood that refuges would be implemented voluntarily.

A similar model was used to assist EPA in determining a management level for the allowable ratio of resistant insects produced from *Bt* crops to insects produced in the refuge to delay the evolution of resistance (EPA, 1998; Attachment I). The goal was to delay resistance evolution for 10-15 years while allowing for significant use of the technology (USEPA 1997). The model of Gould (1994), a single locus, diallelic model that assumes random mating was used to estimate this ratio. The high dose strategy was assumed to make resistance functionally recessive and it was assumed that the fitness of

all genotypes on the conventional hosts was 1.0. The fitness of ZZ and RR individuals on *Bt* plants was always held at 0.001 and 1.0, respectively. The fitness of RZ individuals on *Bt* plants varied from 0.002 to 0.5 to account for different levels of heterozygosity and an initial resistance allele frequency of 0.005. Refuge size was varied from 4 to 50% and the model simulations resulted in ratios of insects from the refuge and *Bt* crop that ranged from 200:1 to 1715:1 (Gould 1998). From this range, 500:1 was selected as the management level for determining refuge size (EPA 1998).

Alstad and Andow (1995) adapted the Comins model for transgenic *Bt* corn in the US Midwest with the European corn borer (*Ostrinia nubilalis*) as the pest species to evaluate the effect of refuges. This application added a risk management component for temporally and spatially implementing transgenic crops in a manner that delays the onset of resistance. They added features to the Comins model to incorporate the multiple generations within a growing season that most pests experience (and the subsequent overwintering mortality), the moth preference for *Bt* fields based on these plants being more phenologically advanced, and a different form of density dependence for larval mortality based on Hassell (1975). As a management strategy, the farmers were assumed to restrict *Bt* crops to early season planting and in order to maximize the insect suppression effect of the *Bt* crops and to greatly minimize the potential for resistance developing. The model compared patchwork implementation of *Bt* with early and late season planting versus uniform spatial implementation of toxic and non-toxic fields and

calculated the effects on resistance time. *Bt* and conventional fields were the same size in the patch model simulations and indicated that reasonable delays in resistance evolution could be achieved with the use of conventional refuges; the paper further recommended that varying the *Bt* crop percentages and spatial arrangements to optimize *Bt* implementation for local use.

From this point there are a number of resistance models in the literature, spurred by the management implications of the Alstad and Andow (1995) model, examining the evolution of resistance in different physical settings and models. These approaches simulated different resistance risk management and spatial implementation strategies and generalized results versus the planned management strategies. More complex implementations in the two patch models are in Guse *et al.* (2002) and Onstad *et al.* (2002). These authors implemented the two-patch model but with great attention to insect parameters and processes as well as the effects of weather and crop growth. This level of detail allows the authors to examine more detailed processes absent from earlier models such as the effects of irrigation (Guse *et al.* 2002) and the impacts of different levels of insecticide spraying in conjunction with transgenic crops (Onstad *et al.* 2002). These two implementations, essentially the same model parameterized differently, allowed for more detailed assumptions for the timing of insect processes such as survival, dispersal, mating, diapause, and oviposition. Sensitivity analyses were conducted as part of the modeling effort for the parameterization of male dispersal, local or random

oviposition by females, and heterozygosity parameters with associated survival rates. These variables were modified along with control variables such as refuge size and dose level to draw conclusions on the effects of these processes and control variables on time to resistance, as well as the differences in resistance time for the different pest insects evaluated.

Though the two field (patch) models are simple to implement and can give an indication of how important certain control variables are, they are not spatially explicit and are difficult to scale up to larger implementations of *Bt*. Nonspatial analytical approaches, focused on individual farms, are in some ways more conservative compared to the discrete landscape approaches that are becoming more popular. Under current conditions, as scale increases, more areas are incorporated where selection pressures for *Bt* are less (non-adoption areas, natural areas, and immigration/emigration considerations) and there is a larger pool of susceptibles available to suppress and delay a significant onset of resistance alleles. It may be that optimal refuge levels, based on fine-grain implementations of models, may be very conservative when examined at the landscape scale. At the large regional scale of modeling, optimization has not yet been done on the discrete time and space implementations of the time to resistance. Different scenarios have been run at the landscape scale (*e.g.*, Carrière *et al.* 2003; Gutierrez *et al.* 2006a, 2006b), but not generally with the goal of finding the optimal refuge size given some strict guideline on an allowable time to resistance. Changing technologies,

particularly the implementation of stacked genes (2 or more toxins expressed in the same crop) probably render the long-term implications (5+ years) of these sorts of models moot as new technologies that will be placed in the field will change the types of selection pressures that the target insects experience.

Currently, the most common form of modeling for insect resistance consists of variations on a discrete time and discrete space difference model that tracks the growth of multiple insect generations and crops during a growing season. There are many examples of this spatially explicit approach (*e.g.*, Caprio 1998; Caprio 2001; Carrière *et al.* 2003; Gutierrez and Ponsard 2006; Gutierrez *et al.* 2006a, 2006b; Peck *et al.* 1999; Storer *et al.* 2003). Modeling crops, insects, and resistance at this resolution allow for the analysis of the effects of spatial factors such as metapopulation dynamics and migration and can more broadly help understand the role of scale in spatial population dynamics (Kareiva 1990; Levin 1992; Molofsky 1994). Common features of these models are that the landscape is represented by a grid; weather is modeled at a generational, weekly, or daily time step; crop growth proceeds as a function of weather variables; insects develop according to weather and crop development; and three classes of insects (*ZZ, ZR, RR*) are tracked for different stage classes (egg, larva, pupa, adult) with variations in how reproduction and dispersal are accounted for after adult moth emergence. These properties vary widely between different insect species and these models can easily be modified to account for different crop types, different insect pests, and different spatial

layouts of crops and crops rotation strategies. These models also benefit from being easily explainable to managers involved in the regulation of *Bt* crops.

In the early discrete space models, the landscape is modeled on an idealized grid with each grid cell being either *Bt*, conventional, or an unvarying combination. Caprio (1998) and Peck *et al.* (1999) are the earliest spatially explicit *Bt* resistance models. Peck *et al.* (1999) was designed to explore the development of resistance at the regional level. The model utilized a realistic insect age structure, movement of larvae between plants within a field, migration between fields, and daily time steps affecting development time of crops and insects. Factors previously unexamined that were significant in delaying time to resistance at this scale included maintaining the same locations of refuges from year to year, the presence of wild hosts within emergence range in the spring, and maximum rates for migration that no longer delayed the onset of resistance. Caprio (2001) took a similar approach for the model with an emphasis on dispersal effects; he varied the spatial resolution from coarse-grained to fine-grained by decreasing adult dispersal rates, thereby simulating random versus assortative mating. Dispersal was modeled with a variable length random walk and intermediate values of dispersal showed the longest times to resistance, an increase up to a factor of 5 for the range of dispersals simulated and confirming a similar result from Comins (1977) with spray pesticides. Caprio (2001) used these results to suggest minimum refuge sizes based on the dispersal characteristics of the target pest species. He also tested the effects of pre-dispersal and

post-dispersal mating on resistance rates with this model, and concluded that pre-dispersal mating lowers resistance evolution rates due to the increased mating between susceptibles and emerging resistants. Though nonrandom mating always increases the evolution rates, nonrandom mating combined with nonrandom oviposition could cause significant evolution delays under certain conditions. Comparisons of the spatially explicit model with a simpler deterministic model showed similar behavior but longer time to resistance in the deterministic model (Caprio 2001).

More recently, these spatially explicit models are being applied to real landscapes, where the parameterization is based on satellite and USDA data regarding crop types and amount of *Bt* implementation in different areas of the landscape. This allows the model to move from theoretical applications used to derive regulatory guidelines to ecological studies that can be corroborated with field data. Gutierrez *et al.* (2006a, 2006b) implemented a complex, spatially explicit, stochastic model in southern California that includes economic factors and simultaneously evaluates 10 major pests in the area. This model consists of detailed age-structured mass population dynamics models for cotton leaf, stem, root, and fruit that include decline in toxin levels over time; multiple generation insect dynamics parameterized with region-specific data as well as age and genotype-specific effects on reproduction, development time, and mortality; and meteorological data from southern California that drives crop phenology. They use this approach to provide a resistance risk assessment for the different pest species and

120

conclude that resistance is more likely to develop in the pink bollworm and budworm, since these species restrict feeding to cotton more than other polyphagous insect pests in southern California. Increases in pest populations over time was predicted for species that are *Bt* tolerant such as the fall armyworm and *Lygus* species, even with increased pesticide use. Overall, they found that the *Bt* technology in southern California provides short-term economic benefits, but also a significant potential for long-term increased crop loss and economic risk due to loss of predator control when broad spectrum pesticides are used to control the increases in *Bt* tolerant pests. An opposite conclusion is reached for the impacts of *Bt* technology on the pink bollworm in Arizona. Arizona has implemented a regional approach to resistance management and *Bt* crop implementation (Carrière *et al.* 2001) that includes cooperation between growers, universities, and government agencies. Pink bollworm in Arizona was considered a potential hotspot for resistance because of the efficacy of *Bt* on this species, with the resulting selection pressure, and the demonstrated ability of the pink bollworm to evolve resistance to spray pesticides (Henneberry and Naranjo 1998). However, monitoring of pink bollworm levels in this region over a ten-year period has demonstrated a suppression of the pink bollworm population independent of other demographic effects. Carrière *et al.* (2003) use pink bollworm data in conjunction with a spatially explicit model to show this regional suppression effect in areas of Arizona that had higher *Bt* crop acreage at levels not seen before with spray pesticides. The model used actual *Bt* implementation levels for 15 different regions of Arizona and then compared results to insect collection data for these

same regions. These long-term suppression effects can open doors to less restrictive refuge sizes over time and lower the risk of resistance at the regional level. Other examples of spatially explicit landscape applications include resistance in *Helicoverpa zea* in corn and cotton in eastern North Carolina (Storer *et al.* 2003); the bollworm on cotton in China (Ru *et al.* 2002); and Western corn rootworm on maize in the US Midwest (Storer 2003).

There have been a few examples of systems of differential equations used to model resistance evolution, but this approach has not been popular and has not had a significant impact on the risk management of resistance. For example, Hillier and Birch (2002) implemented a modified Lotka-Volterra model with pest-crop dynamics rather than predator-prey. The model has logistic crop growth over time and different functional responses were assigned for the different pest genotypes (*ZZ*, *ZR*, *RR*) in toxic and non-toxic fields. Although limited in realism in terms of crop growth and damage, the main advantages to this model are that it is not limited to diallelic genetic assumptions where resistance is assumed to be constrained to a recessive single gene and costs of fitness for resistant individuals can be incorporated. Another model approach is that of Medvinsky *et al.* (2004), who implement a reaction-diffusion model as a means of simulating an "invasion" of *Bt*-resistant insects. A three-component (plant, susceptible insect, resistant insect) spatio-temporal model is created with zero flux boundary conditions to evaluate nonlinear system dynamics resulting from an invasion. Model

analysis indicates that resistant insects lead to spatially inhomogeneous distributions for both plants and insects and that there is a strong relationship between the insect reproduction period and plant damage as measured by remaining plant biomass in the model. The spatial structure inherent in the plant and insect distribution of this model, even with an initially uniform landscape, have implications for evaluating whether current efforts are adequate for providing spatial coverage when monitoring for resistance, as low sampling efforts could miss areas of significant resistance. The model also had a high sensitivity to small change in environmental parameters, which has negative implications for regulatory control in this nonlinear system. In the Hillier and Birch model, space is not explicitly included. The Medvinsky *et al.* model incorporates space through the use of a reaction-diffusion model, and resistance can spread spatially through the strength of the diffusion coefficient for insects and allows for mating within different cell types. Here space is discretized with two regimes, each corresponding to different growth numbers for the insects representative of *Bt* and non-*Bt* conditions, and the variable ranges where growth of the resistant insects and corresponding effects on plant biomass are determined.

4.6 Spatial Scale

Processes, parameters, and decision-making operate from the finest to the broadest scales in agro-ecosystems. Data on insect populations, nutrient levels, and plant distributions are collected at a fine resolution, many times over in an attempt to

characterize broader spatial distributions. Seeds for crops are planted at this same scale. Decisions concerning agricultural systems are made at the plot-level by farmers concerning when to plant, what crops and strains to grow, when to irrigate and treat with herbicides and pesticides, etc. At the same time other farming parameters operate at the regional level or larger; how much imported irrigated water will be available, local weather patterns, range and dispersal of crop pests and higher trophic levels that feed on crop pests, as well as regulatory restrictions on what is permissible for the farmer. At the same time, temporal effects are at play. Farming is an annual activity controlled by seasonal effects; most types of farming are annual-oriented, where a majority of the plot-level agricultural ecosystem is completely plowed under at the end of each growing season; and growth of higher trophic level populations are controlled by seasonal variables. Farming practices also evolve over the years in response to new technologies and biological/climatological conditions.

The inter-relatedness of the spatial and temporal scales for agricultural assessment, decision-making and any modeling that support these activities can be difficult to untangle. At the same time, data extrapolation or modeling from one scale to another may be somewhat limited due to assumptions concerning the methods or scale of the modeling or data collected. It is necessary to determine if these limitations reduce the applicability of the model or not; and if they do, then additional methods or data need to be collected to adequately represent the processes or decisions being analyzed.

Additional modeling and consideration of the data at a broader spatial scale is then needed, and conducted, in order to characterize this process and support this decision.

The same problems can be seen in the modeling to evaluate the implementation of genetically modified crops in regions of the US. Modeling to support specification of refuge sizes and designs, as well as the escape of transgenes into the environment must be done at the farm level because that is the spatial management scale. Experimental plots used to evaluate the risks of resistance or the probability of transgene escape are often done at finer resolution than the plot level and the most comprehensive studies are done in greenhouses. Additionally, funded studies that evaluate these same risks are on a shorter time frame than these crops have now been implemented in the field. Approval and introduction of GM crops at broader spatial scales and longer time periods within various regions of the US demand that data be collected and models evaluated that are representative for these scales. Therefore, data collection methods and models must change to meet these demands. This means relying on models that account for the spatial scales of higher trophic levels that use these agricultural systems, the longer time scales that these transgenes are in the environment, and the agricultural farming scale now that the introduction of this technology is growing. The presence of GM crops in the environment are increasingly no longer isolated events, rather they are increasingly connected as the probability of neighboring farms implementing the GM technology increases at the regional level. This also means using physical variables of the actual

regional landscape as a platform (for example in a GIS) to determine the effects at these scales as well as analyzing and interpreting data (for example satellite data) to assist in these efforts.

Therefore, the temporal and spatial scales of modeling and data collection efforts are determined by the assessment needs and therefore implementation must be scalable. If the effects on a species in an agroecosystem at a certain spatial scale are being determined then that scale has to be met and if current methods are impractical or unable to meet the scale needs required by the assessment process, then is necessary to look at available methods or create new ones that can support this level of characterization.

4.7 Implications for Monitoring

Molecular methods hold promise for risk assessment (Bishop *et al.* 2001) and the assessment and monitoring of the resistance risk is no exception. There may be issues of optimizing surveillance efforts for looking for resistance at broad-scale implementations of *Bt*. Currently, resistance is very difficult to find in the field, the methods to check for it (bioassays) are very time-intensive, and it is not at all clear where these efforts should be focused. So a further question of significance is where are the real hot spots for resistance and where should USDA/EPA be monitoring so they can catch any emerging resistance issues early?

The gene technology companies are charged with surveillance for resistance. While they have financial incentive to prevent resistance, they also have a financial incentive not to complicate implementation of their technology. The USDA also does monitoring for resistance in insects. Current methods for monitoring resistance are reasonably cheap, but they are time-consuming and there are many areas where *Bt* is being used and many insects exposed. It also takes very high sample sizes to detect low frequencies of the resistance allele where problems will first occur. Therefore, current resistance surveillance is not ubiquitous and it is quite possible that resistance to *Bt* may have evolved locally but the refuges have worked or dispersal characteristics of the insect may have prevented the allele from being established. The methods available for resistance surveillance include bioassays where insects are exposed to differing levels of *Bt* in an artificial diet, F2 screens where insects are crossed based on ability to survive *Bt* artificial doses and the F2 generation is tested for its ability to survive on *Bt*, and molecular methods which are just now appearing. Molecular methods hold great promise as a potential quick screen for resistance, and perhaps testing feces of predators for these same resistance genes may be a way of sampling many insects at once, but are limited based on their ability to identify only resistance genes that are sequenced. While a few genes for resistance have been identified, a number have not, and there is a nearly unlimited potential for unknown resistance variants to be selected for.

Bioassays are not an effective means for determining the spread of resistance in

the field due to the tremendous sample sizes needed to detect meaningful levels of resistance and the number of potential resistance genes. By the time a bioassay can detect the presence of resistant genes at observable levels, it will likely be too late to prevent the spread of resistance or even change tactics.

In-field collection methods are used to provide both direct and indirect estimates of resistance allele frequencies for a variety of insects and crops. Direct estimates are normally conducted by collecting the crop with live larvae attached, then exposing the collected insects to different concentrations of protoxin in the laboratory to determine resistance rates (*e.g.*, Tabashnik *et al.* 2000). Indirect estimates are usually performed by comparing insect densities in *Bt* crops with those in non-*Bt* crops, for example by comparing the mean number of larvae per cotton boll for *Bt* and adjacent non-*Bt* fields. These estimates rest on assumptions that fecundity and non-*Bt* mortality are similar between the two fields and that all survivors in *Bt* fields are homozygous for resistance. These methods can be prone to problems due to non-expression of *Bt* in some plants in the *Bt* field, but there are now field tests that allow a quick check to ensure that the *Bt* is being expressed for each boll or corn ear collected. The rate of homozygous resistant individuals is given by the mean number of larvae per collection in the *Bt* field divided by the mean number of larvae per collection in the non-*Bt* field. Assuming Hardy-Weinberg dynamics, the resistance allele frequency is given by the square root of the homozygous resistance rate.

The direct estimate methods are generally considered more reliable than the indirect methods due to the smaller set of assumptions necessary (Tabashnik *et al.* 2000), but resistance rates based on both direct and indirect methods can be generated from the same set of collected data and compared.

The importance of dispersal in resistance risk estimates indicates that this is an important parameter for reducing uncertainty in resistance models. Estimates of insect movement between GM fields and refuges generally rely on either mark-recapture methods or on genetic variation methods. Mark-recapture methods are possible but are time-intensive and difficult with insects. Gene flow methods such as variation in allozyme frequencies have been used to look for population differentiation in time and space for insect populations. As an example, Han and Caprio (2002) found that *H. zea* had low levels of allozyme differentiation at examined allozyme sites, indicating high levels of spatial flow at local and regional scales.

4.8 Outlook and Conclusion

The number and variety of GM methods are increasing rapidly, and the risks are increasing along with the benefits. Almost all of the current *Bt* crops contain only one of two *Bt* proteins for Lepidoptera control (Cry1Ab for *Bt* corn and Cry1Ac for *Bt* cotton), though a number of different *Bt* crystal proteins are under consideration and some novel

toxins with different modes of action are likely in the near future. The product of the number of transgenic methods, the diversity of ecological settings in which they are applied, and the increasing number of species targeted by transgenic crops are increasing much faster than the number of applications for competently assessing the risks from their presence. It may be an appropriate time to consider regional and situational-specific transgenic risk assessments instead of relying on national standards for *Bt* dose levels and refuge requirements.

The opportunities presented by stacking of multiple toxins will present more control options. An application that may be more common, if expression can be better controlled, is expressing different toxins at different points in the life cycle, depending on when the toxins are most effective. These increased options will mean that spatial and temporal control will become a more important component of resistance management and sustainability in the future.

As *Bt* pressure continues to increase with more transgenic acreage planted, risk assessment modeling for resistance will need to be proactive in supporting insect resistance monitoring efforts; identifying potential hotspots for resistance based on pest insect life history characteristics; determining the spatial deployment of *Bt* in the agro-ecosystem which has only been done for a handful of instances; and current insect monitoring efforts, which are currently stretched thin. The simple, conservative two

patch models are useful when uncertainty is high concerning the introduction of new technology. However, as technologies mature, the conservative assumptions and simple models that have been used to derive early guidelines to delay insect resistance must give way to models that can be applied to landscape scale implementations of the technology.

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5. Bat Predation Impacts on Insect Resistance Evolution

The corn earworm/bollworm (*Helicoverpa zea* (Boddie)) is a major pest of corn and cotton in the Winter Garden region of Texas. Bollworms feed on multiple hosts and are highly mobile allowing for a succession of moth generations over the course of the growing season from spring to late fall. These characteristics have allowed *H. zea* to become a successful pest of agricultural systems (Fitt 1989). It inflicts the most damage in areas where it overwinters, so in the US most damage is in the South, and it typically arrives too late in northern areas to cause significant crop damage. *H. zea* is able to complete 4-5 generations per season in Texas by utilizing a variety of host crops, starting with wild hosts such as Texas bluebonnets and Indian paintbrush, moving to corn, and then to cotton, sorghum, and soybeans for the later generations (Fitt 1989).

Brazilian free-tailed bats (*Tadarida brasiliensis*) provide significant pest control services in Texas (Cleveland *et al.* 2006). They migrate from Mexico for the summer and form enormous breeding colonies in caves and under bridges in the Winter Garden region. McCracken (2003) reviewed estimates that as many as 100 million individuals forage in south-central Texas, each consuming many insects. On average, moths constitute about 30% of the bat diet in this area (Lee and McCracken 2005), including significant quantities of the corn earworm.

Bacillus thuringiensis (*Bt*) is a natural bacterium that has been used for many years as an organic insecticide in spray form. *Bt* is lethal to certain pests while considered to be non-toxic to people and non-target wildlife. A principal form of plant-incorporated protectants (PIPs) for agriculture is the incorporation of a *Bt* genetic sequence to express a protoxin with insecticidal activity. Marketed varieties of *Bt* corn and cotton in the Winter Garden region express the *Bt* toxin to limit or eliminate damage from larval forms of the corn earworm. Studies have shown that susceptibility of *H. zea* to *Bt* is geographically variable. For example, Stone and Sims (1993) demonstrated a 16-fold range in susceptibility to *Bt* in the southern US, and therefore *H. zea* has a natural tolerance to *Bt* in field populations. Due to this existing tolerance and their polyphagous characteristics, *H. zea* is a pest species that is likely to develop resistance to *Bt* crops.

5.1 Resistance Genetics Model

A grid-based model is implemented, displayed in Figure 5.1 (all tables and figures are in the appendix of chapter 5), which examines the relationships between the agro-ecosystem insect populations, the bat populations that prey on these insects, and the effects of biotechnology in the field that targets the insect populations. Processes that are modeled include the evolution of insect resistance in response to *Bt* pressure, the migration of significant numbers of susceptible *H. zea* from Mexico, density-dependent predation by foraging bats, and costs to fitness for resistant insects. The effect of these

processes on resistance evolution rates are examined and quantified.

A two-allele (*Bt*-susceptible (*Z*) and *Bt*-resistant (*R*)) gene frequency model is used to explore the dynamics of time to resistance to *Bt* crops in insect populations. The EPA strategy for delaying the onset of resistance is a high dose/refuge resistance strategy. A high dose of *Bt* toxin is expressed in the crop, designed to kill most insects, with effectiveness against heterozygotes being important to delay resistance. In addition, a non-*Bt* crop refuge is maintained in close proximity where susceptible insects can survive and mate with resistant insects. The model simulates this environment on a 50x50 spatial grid, with differing survival rates for areas identified as refuges and areas of *Bt* crops. Viability selection due to *Bt* exposure operates at each 4 hectare grid area and depends on the genotype of the moths and the type of crop planted at that location. For this model, effectiveness of *Bt* corn and cotton are expected to be similar and 25% of the crop area is considered to be a refuge in a mixed corn-cotton agricultural system. Natural areas are estimated to comprise 40% of the area (based on estimates from aerial photos) and have a lower carrying capacity (10% of crop) for *H. zea*. Natural areas are randomly assigned for the first year and do not vary in location over the simulation, corn-cotton fields and refuges will spatially vary from year to year as the seasonal rotation of refuges is often part of integrated pest management strategies. Parameter values used in this modeling effort are presented in Table 5.1.

Discrete, non-overlapping generations per year are assumed for the insect populations. Primarily a deterministic model, bat predation is the only parameter that varies stochastically over the landscape between generations. Random mating occurs within each grid square according to Hardy-Weinberg dynamics. Pre-dispersal mating occurs in the natal field, and post-dispersal mating can occur either within a given range of the natal field or in a random mating pool anywhere on the grid. *H. zea* females are conservatively assumed to mate and oviposit in natal fields before dispersal, allowing for moderate or long-term dispersal before mating and ovipositioning results in longer estimates of time to resistance (Peck *et al.* 1999). An initial resistance allele frequency (0.0001) is specified for the entire corn earworm population and is uniform throughout the grid (Burd *et al.* 2001). Intensity of foraging mortality due to bat predation is either independent or dependent on moth densities according to the scenario-type. Absolute fitness for each genotype g is given by the product of the fecundity (E), survival probabilities due to exposure to toxicants on the host plant (h), natural mortality (n), overwintering mortality of insects (w), and bat predation (p). Fecundity was estimated at 800 eggs per female, the midpoint of the 600-1000 range given by Sansone *et al.* (2002). Sansone *et al.* (2002) data is also used to estimate natural mortality for the egg-larva stage (0.08) and the larva-adult life stages (0.75). Survival probabilities due to exposure to Bt is estimated at 0.75 for homozygous recessives, 0.05 for heterozygotes due to partial recessive properties of the resistance allele, and 0.001 for susceptible corn earworms (Horner *et al.* 2003, Jackson *et al.* 2004). Overwintering mortality for the last

generation of the growing season is estimated at 0.18 from a range of 0.02 to 0.24 (Benedict 2004). Perfect insect density-dependence is assumed at the larval stage, so if the number of larvae is greater than the number of plants the population is reduced to the carrying capacity (K) of one surviving larva per plant through cannabilism, which conforms with insect behavior (Chillcutt 2006). Fitness for each generation is measured by:

$$\omega_g = Ehnwp$$

Multiple generations are modeled per year, so overwintering mortality is 1 until the last generation of the season. Insect mortality (p) due to bat predation within each grid node (x) is modeled by a genotypic-specific moth survival rate (m_g) raised to a power representative of the average number of bat-moth interactions within that grid square (i). This results in a moth probability of survival that is dependent on the degree of bat pressure in the grid square and on the genotypic ability of the moth to avoid bat predation:

$$p_x = m_g{}^i$$

Acharya and Fenton (1999) found that bats attacking moths that undertake defensive behaviors, such as the corn earworm, were successful in capturing moths up to 50% of the time. Therefore, the parameter m_g was parameterized as a uniform random variable that could vary between 40 to 50% for each grid square. The moth survival probability for each grid square is redrawn from the uniform distribution with each new generation.

142

Eight different scenarios are evaluated to compare and contrast the effects of migration, predation, and costs of resistance fitness on the baseline model. Each of the scenarios is run for no (i=0), moderate (i=1), and high (i=2) bat predation levels. The baseline scenario is the typical resistance evolution model, spatially implemented, that does not include migration or cost of fitness effects and assumes that insect mortality due to bat predation is independent of insect densities. There are three scenarios that incorporate each individual effect in isolation, three scenarios that consider pair-wise effects, and a comprehensive scenario that incorporates all the effects simultaneously.

5.2 Migration

During the Texas growing season, there are significant numbers of immigrating insects from Mexico, where they develop earlier because the climate is warmer. Upon emergence in Mexico these insects migrate north on winds coming out of the south (Westbrook *et al.* 1995a, 1995b). Mexico, for a variety of reasons, including their many native versions of teosinte, does not permit planting of *Bt* crops, because of the potential for transgenic gene flow to endemic crops. Therefore, the insects migrating north from Mexico are presumed to be almost entirely homozygous susceptible because they have not been exposed to *Bt*, this influx may serve to slow the resistance of evolution in the Winter Garden area. The first major migration of corn earworms of the season in the Winter Garden region of Texas originates from northeastern Mexico each June (Raulston

et al. 1992, 1995). While providing significant resources for foraging bats in the area (Lee and McCracken 2005), the first waves of migrants arrive in the area before the local moth populations have developed. The timing of the arrival of the moths is based on moth development variables occurring in northeastern Mexico and wind speeds and directions subsequent to their emergence (Westbrook *et al.* 1997, 1998). Migration processes are simulated in the grid model by allowing a percentage (45%) of emerging adult moths to disperse outside the area after each generation. In addition, adult moths with a susceptible genotype arrive (1000 per hectare) at the end of each generation and contribute to the gene pool for the next generation in the Winter Garden region.

5.3 Density-Dependent Foraging

Due to the different insect selection pressures present in *Bt* and conventional crop fields, there could be significant differences in the amounts of insects available to bats between these types of fields and compared to other natural areas. The preferential spatial feeding of Mexican free-tailed bats on these insects may either enhance or retard the evolution of resistance within moth populations. One possible outcome is that if bats forage heavily in refuges relative to *Bt* fields, then they could reduce the susceptible insect pool and could potentially hasten resistance. Studies have been performed on optimal foraging in bats, but these mostly have pertained to temporal considerations as well as size-based prey selection when foraging (Jones and Rydell 2003), as opposed to spatial considerations regarding where they forage. O'Donnell (2000) showed that

minimum overnight temperature and invertebrate activity in the first hour after sunset, as measured by spotlight transect, are good predictors of bat activity, as measured by calls and feeding buzzes. Anthony *et al.* (1981) also demonstrated high bat activity, as measured by time spent in roost, and correlated this with high minimum temperatures and high levels of insect activity (measured by light traps and transects). Therefore, temporally, one can conclude that foraging is metabolically expensive and bats will only forage if they are reasonably certain of getting an adequate return on their metabolic investment. One is tempted to conclude that spatially, bats preferentially forage in areas with high insect densities, though the record here is not as clear. Lee and McCracken (2002) provide a counter-indicator of this expectation when they assessed towns, ranches and crops assessed for insect activity (using light traps) and bat activity (echolocation). They measured bats with higher foraging activity and attack rates over towns compared to crops and ranchland though measured insect activity in towns was less. However, the efficiency of the light traps in towns may have been compromised by other sources of lights relative to the same traps in fields. In the same Winter Garden region, Lee and McCracken (2005) demonstrated opportunistic foraging with an increase in diet composition of moths from 15% to 43% with large influxes of migratory moths from Mexico (Lee and McCracken 2005). Therefore, the current evidence for optimal spatial foraging as a function of insect densities is mixed, though data collection efforts are underway to address this issue.

The baseline model simulates bat predation assuming density-independent insect mortality. To measure the effect of density-dependent predation, a Type I linear functional response has been employed to simulate the effects of bat predation on *H. zea*. The effect is modeled as low bat predation at low insect densities, linearly climbing to a maximum mortality level for high insect densities; this would mimic a situation where bats could potentially accelerate resistance evolution by feeding preferentially on susceptibles in the refuge as opposed to potential resistants that may be emerging from *Bt* fields. Therefore, with N_x representing the insect population within each grid square x and K_x representing the carrying capacity for x (which varies depending on whether the grid square is a crop, refuge, or a natural area), density-dependent insect predation is modeled by:

$$p_x = \frac{N_x}{K} m^i$$

5.4 Resistance Cost of Fitness

Mutations that allow for the ingestion of Bt without mortality can come at a cost to the fitness of the insect. Therefore, a term is also employed that simulates the cost to fitness of resistance. Fitness costs associated with resistance to insecticides have been observed in the laboratory and the field, including field resistance to *Bt* in the Diamondback moth (Groeters *et al.* 1994), but the type and magnitude of fitness costs can vary considerably for different species and insecticides (Denholm and Rowland 1992).

Resistance costs can include negative effects on survival, fecundity, egg hatch, development time, pupal weight, and behavior (Groeters *et al.* 1994; Gutierrez *et al.* 2006). *H. zea* is known to take avoidance behavior when exposed to ultrasound (Agee 1969). For each insect-bat encounter, there is a probability that the moth will detect the foraging bat and successfully take evasive action to avoid capture. For the cost of fitness scenario, the susceptible genotype is assumed to have the same probability of survival as when there is no cost of fitness (0.4-0.5 raised to the ith power); however, the heterozygous and homozygous recessives have lower probabilities of avoiding capture, and have survival probabilities for the heterozygotes of 0.25-0.35 and for the homozygous recessives 0.15-0.25. Cost of fitness can also impact mating success by affecting mating flight behavior and diminishing reproductive output, but these effects are not accounted for in the current model.

5.5 Model Analysis

Model inputs for the baseline scenario are summarized in Table 5.1. The stated goal for resistance management is that non-transgenic refuges must be planted on each farm to produce at least 500 susceptible pest individuals for each resistant individual produced in the *Bt* crop (BANR 2001; EPA 1998), this ratio is based upon earlier modeling studies (Gould 1994, 1998). Simulations for each bat predation level for the eight scenarios are presented in Figure 5.2, time to resistance is given by the year in which the simulation first exhibits overall populations under this 500:1 ratio. The overall

magnitude of the bat effects on resistance evolution depends on whether migration, cost of fitness, or the preferential feeding on susceptible insects in refuges dominates the process of *Bt*-resistant gene selection in target species. For the baseline scenario, increasing levels of bat predation increases the time to resistance due to an insect suppression effect. Resistance costs show this same pattern, with longer delays accompanying increasing bat predation. Migration rates, in conjunction with density-independent predation, show the biggest delay on time to resistance for the scenarios evaluated. Density-dependent predation does not hasten resistance, but does cancel out the positive benefit of increasing bat predation seen in the other scenarios. The strength of density-dependent predation by the bats is an open question, and fieldwork is currently ongoing to quantify this effect. When all factors are combined, migration and density-dependent predation are equally important in the overall dynamics of time to resistance, with time to a 500:1 susceptible ratio delayed to 13 years (as in the migration only model), but increasing levels of bat predation do not significantly delay or hasten the initial onset of insect resistance above management levels.

Figure 5.3 consists of the spatial dynamics for the scenario that includes all effects of migration, density dependence, and cost of fitness. This figure shows a snapshot if insect densities on the modeled landscape at the point where the susceptible:resistant ratio goes under 500:1 (equivalent to surpassing 0.2% resistance in the insect population). Insect densities for the no bat predation scenario are shown at the point where the

management threshold is passed (Figure 5.3a- 12 years) and later at 20 years (Figure 5.3b). At 20 years, figure 5.3b shows that all locations within the area are asymptotically approaching their carrying capacity for *H. zea* and little variation exists in insect densities when comparing similar habitats. Insect densities for the high bat predation scenario are shown 5.3c and 5.3d. Figure 5.3c again shows the snapshot of insect densities when the ratio goes under 500:1 (14 years). These results are comparable to Figure 5.3a, with significant spatial variation for insect densities within each of the three habitat types (natural areas, refuges, Bt crops). Figure 5.3d shows the insect densities on the landscape at 20 years for the high bat predation scenario, compared to the no bat predation scenario at 20 years (5.3b), this scenario exhibits significant variation in insect densities that is caused by spatial variation in the resistance frequencies. Although the time to resistance for the two bat predation scenarios are similar, after 20 years the stochastic effects of the bat predation have changed the spatial dynamics of the system relative to the no bat predation scenario. Significant variation exists in the spatial distribution of insect densities for the high bat predation scenario, and the overall level of insect resistance is significantly less.

The modeled time to 5% insect resistance allele frequency for 8 different modeling scenarios is presented in Figure 5.4. As in Figure 5.2, each scenario is simulated with no, moderate, and high bat predation levels. The higher resistance rate (5% versus 0.2%) increases the time difference between the bat predation levels. This is

important for the all factors scenario, which demonstrates an increased delay in the time to resistance relative to Figure 5.2 because of the higher resistance level. Therefore, increasing bat predation shows a delay in time to the 5% resistance level for the all factors scenario.

Figure 5.5 presents the no and high bat predation scenario results as a time series instead of snapshots of insect densities on the grid. The figure illustrates the insect population levels for each genotype (*ZZ, ZR, RR*) in refuges and *Bt* crops over a fifty-year period. Comparison of the two scenarios demonstrates that suppression of the homozygous recessives occurs past the management threshold for the high bat predation scenario relative to the no bat predation scenario. The no bat predation simulation allows for homozygous recessives to dominate in *Bt* fields (Refuge RR) with over 20% of the total population. The high bat predation scenario, despite significant density-dependent foraging in refuges (which is expected to encourage the evolution of resistance), maintains homozygous recessives in *Bt* fields at levels below 5% for over 50 years and shows a significantly higher percentage of susceptible insects (*ZZ*) in refuges and *Bt* crops. This is attributed to the combination of bats suppressing insect populations, delaying the growth of the resistant population, and the constant immigration of susceptible moths from Mexico.

Figure 5.6 simulates different levels of bat predation and shows resistance allele

frequencies for *H. zea* populations. Increasing bat predation levels (ranging from i=0 to i=3) levels show lower rates of the resistance alleles over the long-term. Some simulations show brief periods where higher bat predation levels lead to higher resistance levels due to density-dependent predation, but the long term suppression impacts eventually dominate. A histogram of 100 simulations of the time to 5% Bt insect resistance for all factors (density-dependent predation, migration, and cost to fitness) is shown in Figure 5.7. The simulations show a range from the second generation of year 22 to the second generation of year 25, with an average just under 24 years. Differences are due to the stochastic upper bound of moth mortality from bat predation. This mortality is implemented spatially and can vary + or − 5% between grid areas. Overall, this is a low level of variability for the measured time to resistance variable, the introduction of additional distributions for other model parameters will increase the variance for time to resistance.

The ability of bats to suppress pest populations in agricultural settings has been demonstrated (Cleveland et al. 2006, Hallam et al. 2006) and can potentially be a component of integrated pest management approaches, this model demonstrates that the ability of bats to suppress pest populations can also have implications for long-term management of the evolution of resistance to *Bt* crops.

5.6 References

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152

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5.7 Appendix

Table 5.1. Inputs for *Bt* resistance model.

| Parameter	Scenario Baseline	Reference	Range
Generations	4 per year	Fitt 1989	3-5
Initial resistance	0.0001	Burd *et al.* 2001	0.001-0.00001
Initial population size	1000/hectare	Assumed	5-25M
Egg-Larval survival	0.08	Sansone and Smith 2001	0.05-0.19
Larval-Adult survival	0.75	Sansone and Smith 2001	0.6-0.8
Overwintering survival	0.18	Benedict 2004	0.15-0.25
Grid size	50x50	Assumed	--
Grid area	4 hectares	--	--
Host plants per hectare (crops)	67000	Jackson *et al.* 2004	50000-75000
Host plants per hectare (natural area)	6700	Assumed	5000-7500
Number larvae per plant	1	Chillcutt 2006	1-2
Fecundity	800	Sansone *et al.* 2002	600-1000
ZZ Moth *Bt* Survival	0.001	Assumed	0.001-0.01
ZR Moth *Bt* Survival	0.05	Assumed	0.02-0.15
RR Moth *Bt* Survival	0.75	Horner et al. 2003	0.7-0.9
Bat attacks per moth	0,1,2	Assumed	0-2
ZZ Moth- Bat Survival	(0.4-0.5)	Acharya and Fenton 1999	0.2-0.6
ZR Moth- Bat Survival	0.3,0.4	Assumed	0.2-0.6
RR Moth- Bat Survival	0.2,0.4	Assumed	0.2-0.6
Fraction emigrating	0,0.45	Assumed	0.4-0.5
ZZ Immigrants (Mexico)	0,4000/node	Assumed	1M-10M
Natural area fraction	0.2	Assumed	0.1-0.5
Refuge fraction	0.25	EPA guidelines (50% corn, 5% cotton)	0.05-0.5
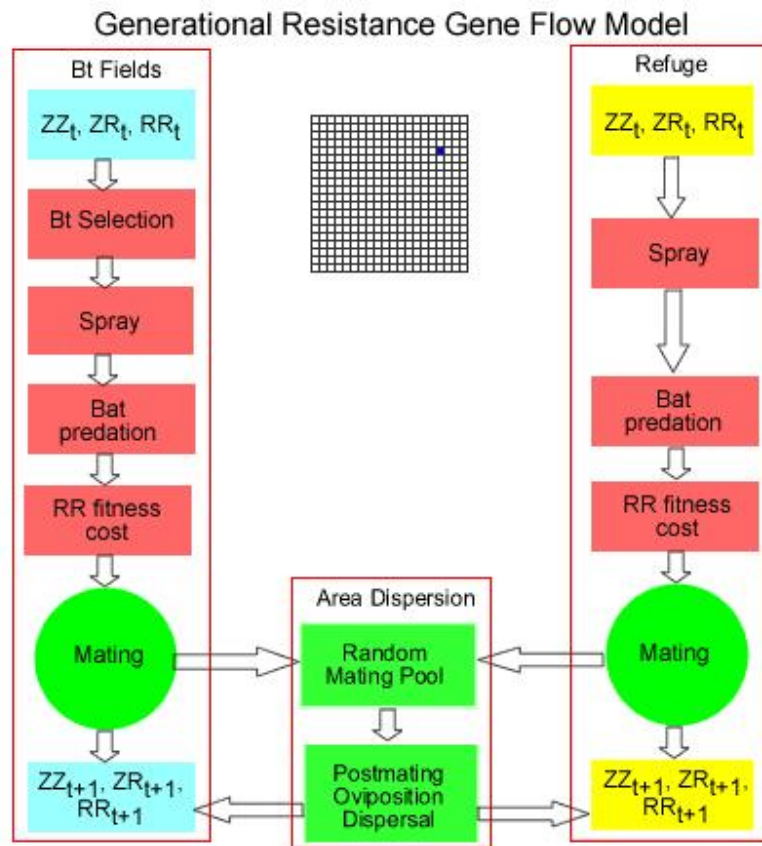
Figure 5.1. A depiction of the general resistance modeling process. This process is conducted for each grid square on a 50x50 grid (inset). The flow chart represents generations of insects that are modeled for important life history processes, with the major difference between *Bt* fields and refuges being the addition of genotypic-specific mortality from exposure to *Bt*. Insects begin as eggs in each generation, experience mortality from natural sources and from pesticide exposure as they transition from egg to larva to adult forms. Mating can occur in the natal field or in a random mating pool anywhere on the grid. Additional modeling processes for bat predation, migration into and out of the grid, and a pleiotropic cost to fitness for resistance can be turned on and off in the model to examine their impacts on the calculated time for resistance to evolve.
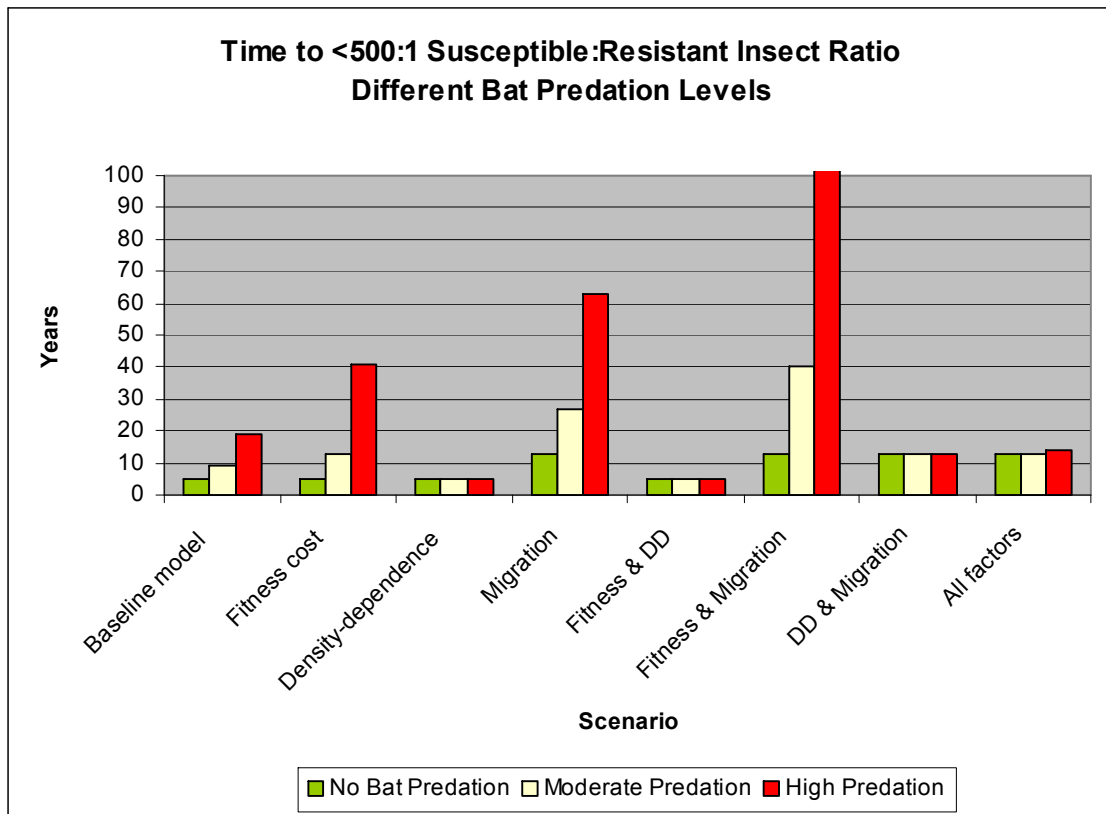
Figure 5.2. Modeled time to susceptible:resistant insect ratios less than 500:1 (equivalent to surpassing 0.2% resistance allele frequency) for 8 different modeling scenarios. Each scenario is simulated with no, moderate, and high bat predation levels. For the baseline scenario, increasing bat predation causes delays to the time to resistance. The further incorporation of pleiotropic cost to fitness of resistance effects and of significant migrations of susceptible moths from Mexico also delays resistance evolution. Incorporation of significant density-dependent predation (bats generally forage more at higher density refuges), causes selection against susceptibles and mitigates delays in resistance from the other examined processes at this low level of resistance in the population.

159

(a) No bat predation- 12 years

(b) No bat predation- 20 years

(c) High bat predation- 14 years
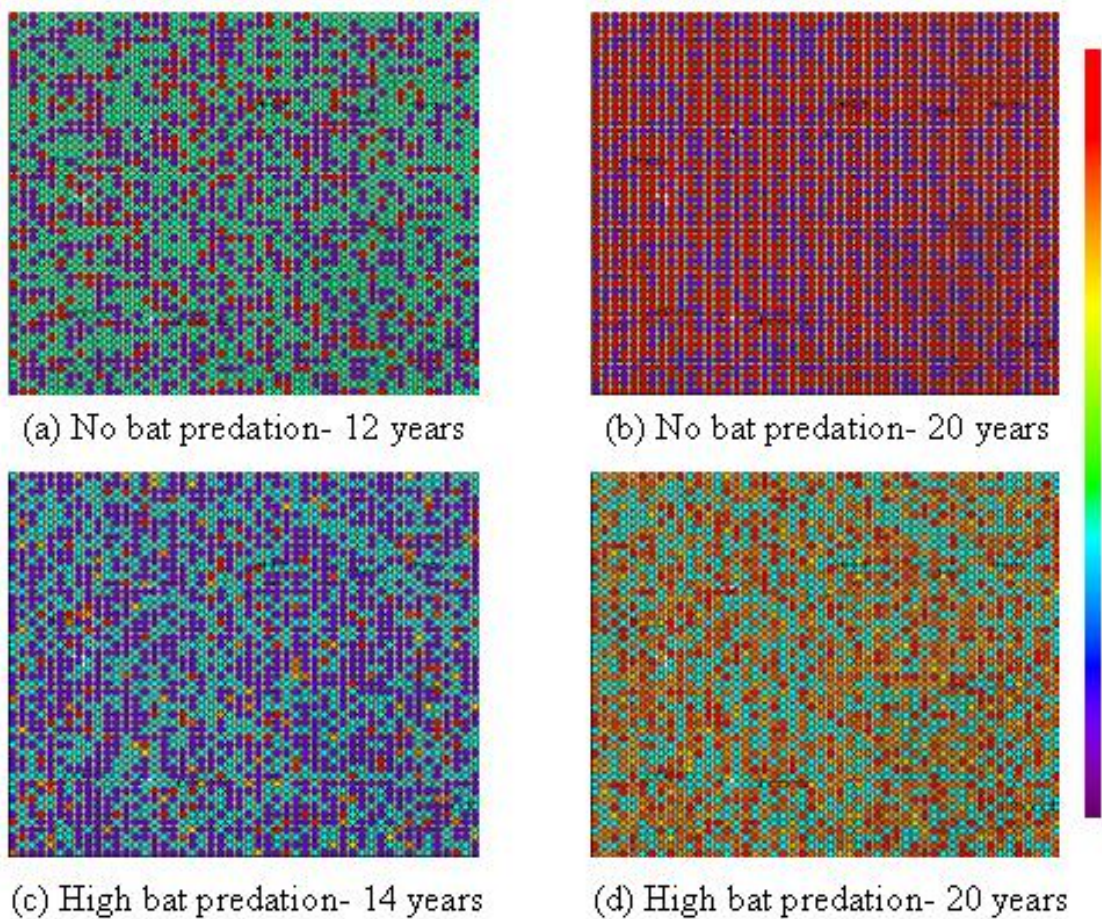
(d) High bat predation- 20 years

Figure 5.3. Four snapshots of insect population levels for the landscape, color indicates population level (red high population, blue low population, legend at right).   The snapshots show spatial dynamics for scenario that includes all effects of migration, cost of fitness, and density-dependent bat predation (for the high bat predation scenario). The top two snapshots depict the no bat predation scenario at the point where the 500:1 susceptible:resistance ratio is no longer met (5.3a-12 years) and at 20 years. (c) and (d) show the same moments for the high bat predation scenario, time to resistance for (c) is 14 years. After 20 years, comparison of (b) and (d) demonstrates that the stochastic effects of the high bat predation scenario have changed the spatial dynamics of the system relative to the no bat predation, which is asymptotically approaching carrying capacity for insects at all grid areas depending on grid cell type.
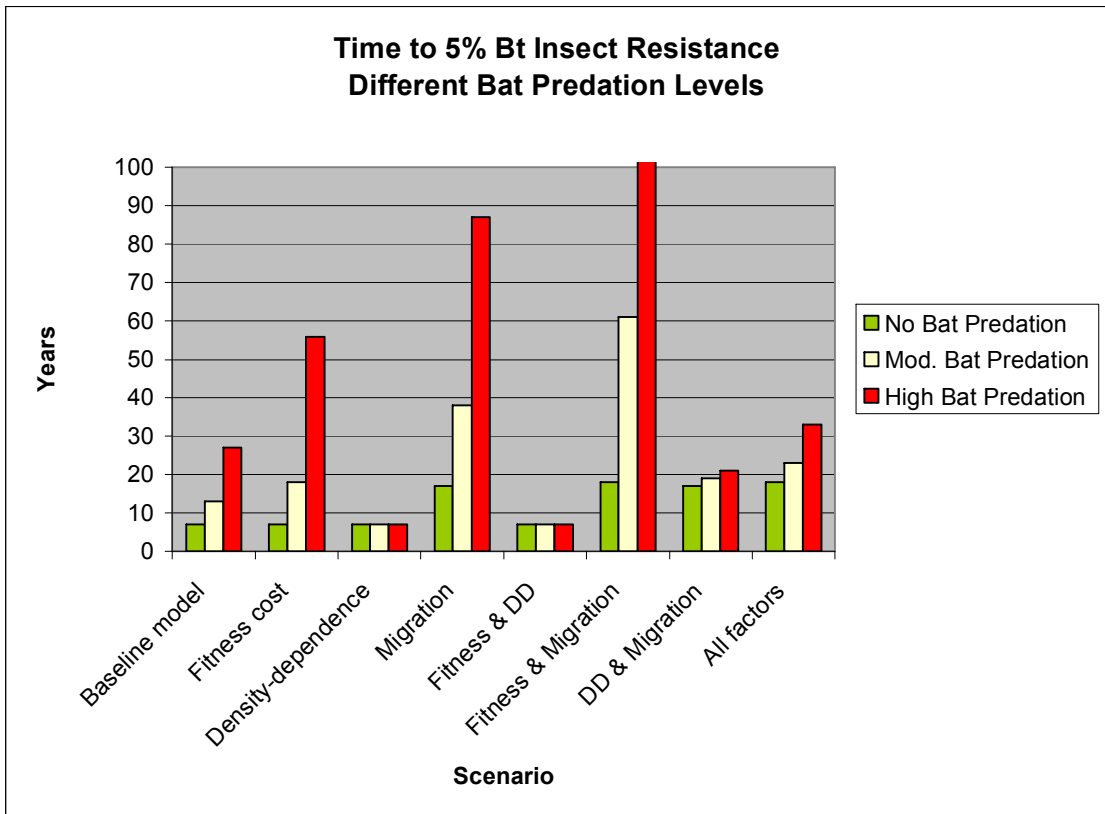
160

Figure 5.4. Modeled time to 5% insect resistance allele frequency for 8 different modeling scenarios. Each scenario is simulated with no, moderate, and high bat predation levels. Results are similar to Figure 5.2, but the higher resistance rate increases the time difference between the bat predation levels. The all factors scenario demonstrates an increased delay in the time to resistance because of the higher resistance level.
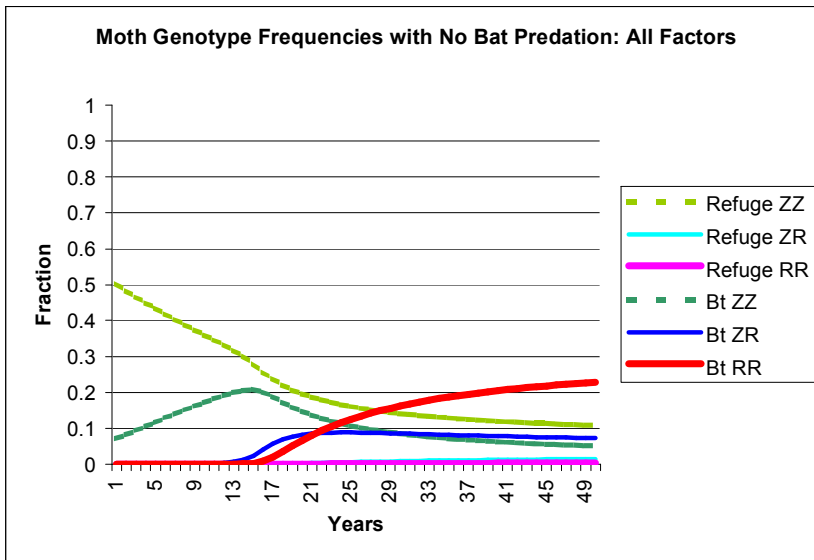
(a)
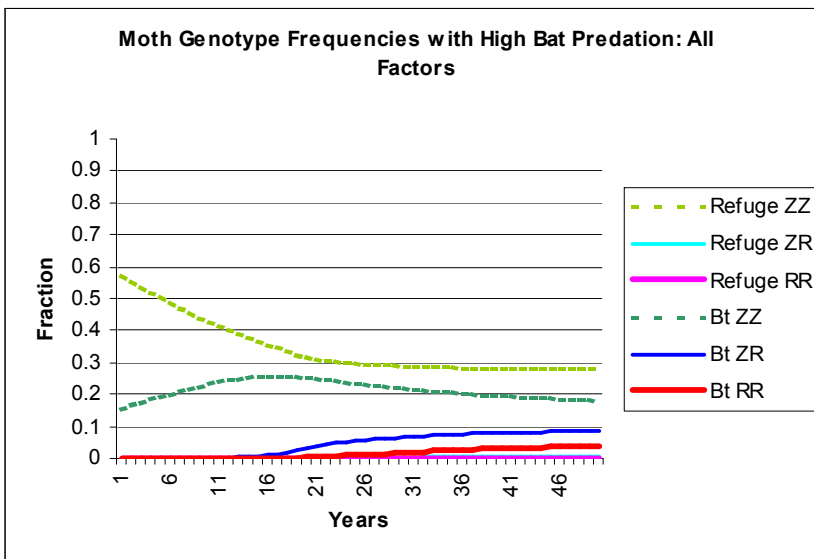
(b)

Figure 5.5. Comparison of insect genotype frequencies for model runs with all factors for no and high bat predation rates. The no bat predation simulation allows for homozygous recessives to dominate in Bt fields with over 20% of the total population. The high bat predation, despite significant density-dependent foraging in refuges, maintains homozygous recessives at levels below 5% for over 50 years. This is attributed to the combination of bats suppressing insect populations in conjunction with the immigration of susceptible moths from Mexico. Even though time to passing the resistance management threshold in figure 5.2 was similar for the different bat predation scenarios, the longer-term dynamics of resistance levels differ considerably between the scenarios.
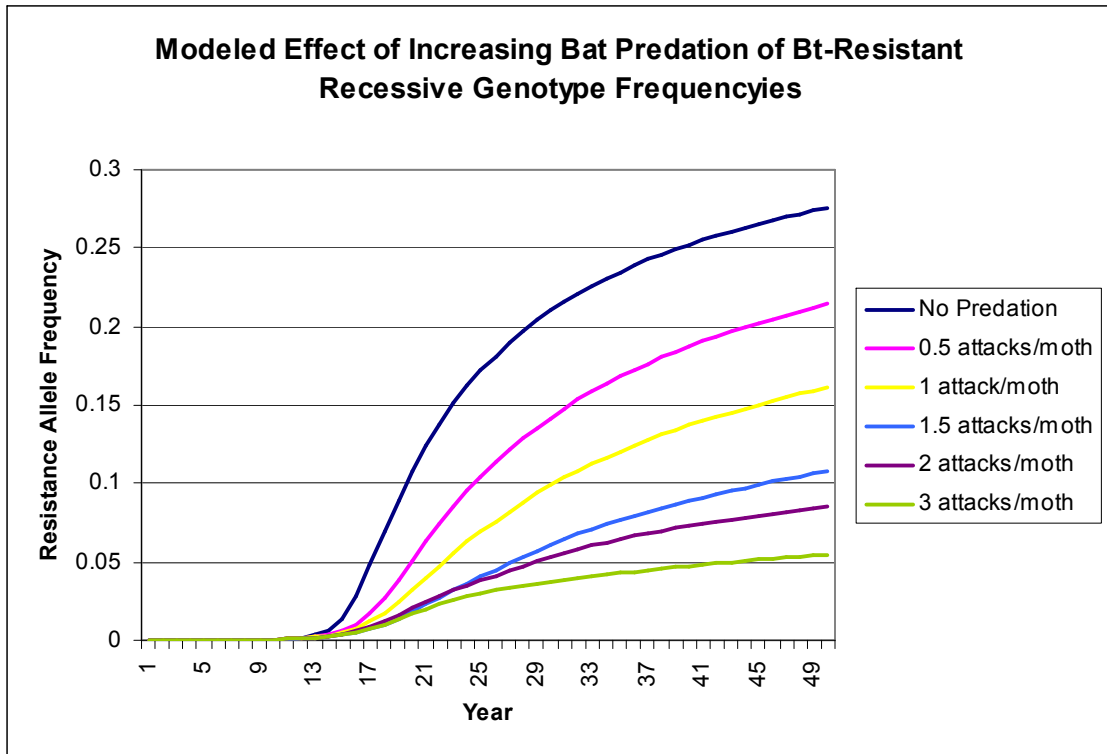
162

Figure 5.6. Simulated resistance allele frequencies for *H. zea* populations in the Winter Garden region of Texas over a 50-year period. Increasing bat predation levels show lower levels of the resistance alleles over the long-term, despite similar times to the 500:1 susceptible:resistant ratio for the different bat predation scenarios (Figure 5.2).

Time to 5% Bt Insect Resistance
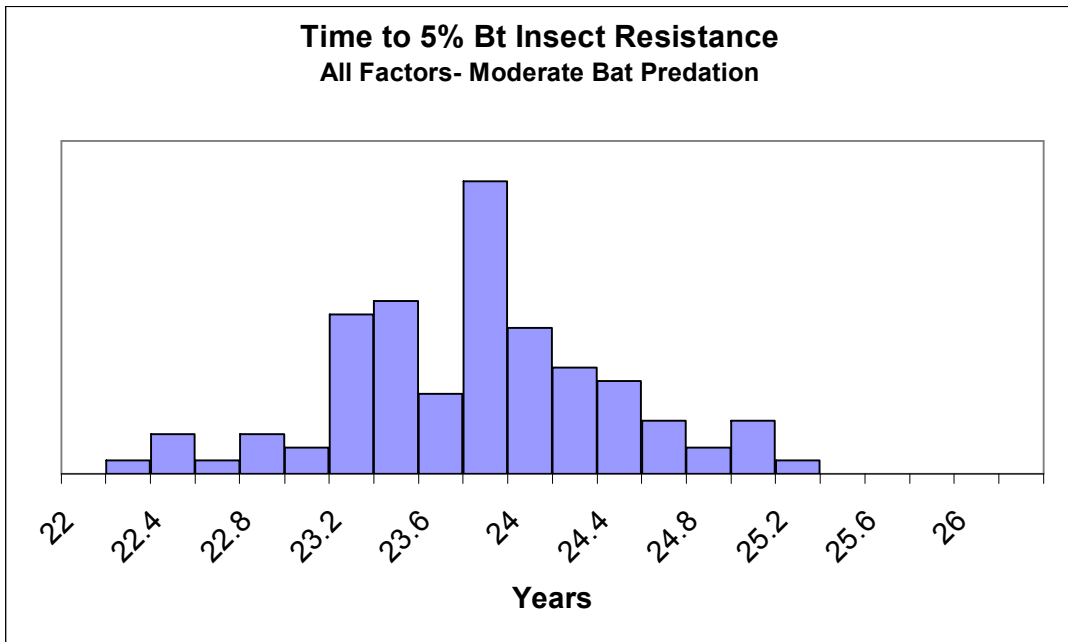**All Factors- Moderate Bat Predation**

Figure 5.7. Time to 5% Bt insect resistance for all factors (density-dependent predation, migration, and cost to fitness). 100 simulations show a range from the second generation of year 22 to the second generation of year 25. Differences are due to the stochastic upper bound of moth mortality from bat predation. This mortality is implemented spatially and can vary + or – 5% between grid areas.

6. Conclusion

This dissertation has considered spatial processes and ecotoxicological risk assessment modeling in the context of three applications. It has demonstrated how the consideration of space and use of these tools in the assessment process has the potential to improve the estimates of the risk outcomes and therefore can influence the resulting risk management decisions. These improved risk estimates can then be used in optimally designing the risk management decisions that are ultimately implemented.

The three applications correspond to three different case studies measuring a different risk variable at progressively broader spatial scales. The first application examined how the presence of spatial auto-correlation can affect how ecological receptors are exposed in the environment. The presence of significant spatial correlation can impact methods for adequately determining summary concentrations, hypothesis testing, and for identifying zones in need of remediation at contaminated sites. The second application was a Fluoride contaminated site in Idaho, and considers the incorporation of habitat as a spatial variable and its impact on estimates of population doses. The results are dependent on the distribution of spatial habitat and risk estimates can differ considerably when compared to the more common use of summary statistics to represent an exposure concentration for risk purposes. The third application was centered

in the Winter Garden region of Texas. This section evaluated the risk of insects developing resistance to genetically modified (GM) crops within a spatial milieu of varying bat predation rates and changing genetically modified crop types and percentages.

Selective remediation for terrestrial ecological risk assessment is a process that achieves a local- and/or site-level concentration level protective of ecological risk while minimizing cleanup volume. Implementation involves inputs of analytical data, a model of the spatial distribution of contamination, ecological exposure information and models, as well as decision-maker cleanup and spatial scale inputs. The resulting design is spatially-explicit and is optimal with respect to model inputs and decision objectives with a goal of minimizing the area of cleanup. A brief application is developed to illustrate the specification of an area of concern for short-tailed shrews exposed to PCB contaminated site in Oak Ridge, Tennessee. Toxicologically-based cleanup criteria are simultaneously applied to determine a spatially explicit remedial design that is protective of shrew exposures. Spatial Analysis and Decision Assistance (SADA) freeware is used to develop the application. SADA contains Geographical Information Systems (GIS) capabilities, ecological exposure and toxicological information, and spatial statistical methods for estimating the location of pollutant in contaminated media and designing remedial alternatives.

Anthropogenic contamination is typically distributed heterogeneously through space. Spatial structure can have differing effects on the cumulative doses of individuals exposed to contamination within the environment. These effects are accentuated when individuals pursue different movement strategies, and movement strategies can be affected by how individuals and species value habitat. Habitat quality is often neglected when ecological risk assessments are performed, despite evidence that inclusion of a quantitative habitat measure can have a significant effect on the overall exposure estimate. An exposure model is coupled with habitat data to examine the interactions between habitat preferences, the spatial distribution of contamination, and the resulting impact on dose estimates. Dose distributions are constructed for pronghorn (*Antilocapra americana*) exposed to fluoride when foraging on desert sagebrush. The results show the magnitude of the difference between doses when foraging concentrations are positively or negatively correlated to varying degrees with different spatial distributions of habitat preferences. Mean exposures obtained from the non-spatial versus the spatial methods can vary by a factor greater than two, and variation within the movement model due to different habitat preferences can vary by an order of magnitude. These differences in the exposure calculation can change the decision from no-action to remediation or vice-versa and impact the remedial design when cleanup is required. Results presented here are generally applicable to other contaminants and receptors. Further model enhancements are planned to account for population interactions within the foraging population and to reflect the impact of foraging on the landscape by modeling the degradation in habitat

168

quality when particular areas are overused. However, the current model demonstrates that examining the strength of spatial correlation between habitat and contaminant data can be used as a quick method to determine when the implementation of a spatially explicit ecological risk assessment is useful.

The evolution of insect resistance to *Bt* modified corn and cotton has been a concern since the commercialization of the technology in 1996. Despite increasing use of *Bt* over the last 10 years, resistance has yet to evolve in insect populations exposed to high selection pressures from transgenic crops. In the US, a resistance management approach consisting of a high dose of the toxicant combined with a spatial refuge is employed to control and delay the onset of resistance. Mathematical models have played an important role in developing the details of the approach and are being used to assist in monitoring for resistance as infield detection methods for meaningful levels of resistance are unwieldy. Bioassays are the common way of monitoring, though molecular methods hold promise. As *Bt* protectants continue to make inroads, high levels of non-adoption will not continue to provide spatial refuges, and selection for resistance will continue to strengthen. Monitoring efforts and modeling methods must keep pace and be integrated with risk management efforts to maximize their effectiveness.

A grid-based model is implemented that examines the relationships between the agro-ecosystem insect populations, the bat populations that prey on these insects, and the

effects of biotechnology in the field that targets the insect populations. Processes that were modeled include the evolution of insect resistance in response to *Bt* pressure, the migration of significant numbers of susceptible *H. zea* from Mexico, density-dependent predation by foraging bats, and costs to fitness for resistant insects. The effect of these processes on resistance evolution rates are examined and quantified. The overall direction and magnitude of the bat effects on resistance evolution depends on whether migration, cost of fitness, or the preferential feeding on susceptible insects in refuges dominates the process of *Bt*-resistant gene selection in target species. For the baseline scenario, increasing levels of bat predation increase the time to resistance due to an insect suppression effect. Resistance costs show this same pattern, with longer delays accompanying increasing bat predation. Migration rates, in conjunction with density-independent predation, show the biggest delay on time to resistance for the scenarios evaluated. Density-dependent predation does not hasten resistance, but does cancel out the positive benefit of increasing bat predation seen in the other scenarios. When all factors are combined, migration and density-dependent predation are equally important in the overall dynamics of time to resistance, but increasing levels of bat predation do not significantly delay or hasten the initial onset of insect resistance. Significant variation exists in the spatial distribution of the insect with higher predation levels due to continued suppression of the homozygous recessives well past the management threshold. This is attributed to the combination of bats suppressing insect populations in conjunction with the immigration of susceptible moths from Mexico. Increasing bat predation levels show

lower levels of the resistance alleles over the long-term. Further enhancements to the model are planned, as field data currently being collected are analyzed to quantify the level of bat pressure insects receive in the different habitats of the Winter garden region in Texas. Also, improvements to the dispersion of insects within the Winter Garden region will be implemented to allow for the model to be applied to a number of different insect species with different dispersal capabilities. The ability of bats to suppress pest populations in agricultural settings is well-known and are a feature of integrated pest management approaches, this model demonstrates that the ability of bats to suppress pest populations also has implications for long-term management of the evolution of resistance to *Bt* crops.

Vita

Steven Thomas Purucker, son of Steven and Nancy Purucker, was born in Baltimore, Maryland on August 22, 1966. He moved to Knoxville, Tennessee in 1981 and graduated from Farragut High School. He attended the University of Tennessee, Knoxville and received a B.A. in Zoology and Anthropology in 1991. He married Sharon Annesi in 1991 and they have three children, Matthew, William, and Anna. He has worked for the University of Tennessee as a Senior Research Associate since 1992 in the School of Information Sciences and then for the Department of Ecology and Evolutionary Biology.

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