

IMPACT OF NEONICOTINOID INSECTICIDE USE ON NORTHERN BOBWHITE
(*COLINUS VIRGINIANUS*) IN TEXAS

A Thesis

by

HANNAH MAE HALIE ERTL

Submitted to the Office of Graduate and Professional Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Chair of Committee,	Miguel A. Mora-Zacarias
Committee Members,	Donald J. Brightsmith
	Dale Rollins
	Diane E. Boellstorff
Head of Department,	Michael P. Masser

May 2016

Major Subject: Wildlife and Fisheries Sciences

Copyright 2016 Hannah Mae Halie Ertl

ABSTRACT

The widespread use of neonicotinoid insecticides in recent years has led to increasing environmental concern, including impacts to avian populations. In Texas and across their range, Northern Bobwhite (*Colinus virginianus*; hereafter bobwhite) habitat frequently overlaps with agricultural areas of known neonicotinoid use. To address the impacts of neonicotinoids on bobwhites in Texas, we developed the following research objectives: (1) Conduct statistical analysis of bobwhite abundance and neonicotinoid use in Texas over the last 35 years, and (2) Analyze bobwhite samples collected from three field sites across the state for neonicotinoid residues and signs of tissue damage.

Generalized linear, generalized additive, mixed-effects, zero-inflated, and hurdle models were used to analyze long-term data on bobwhite abundance, neonicotinoid use, and environmental variables from 1978-2012. Statewide analysis indicates that total neonicotinoid use is negatively correlated with quail abundance in the periods after neonicotinoid introduction (1994-2003) and after their widespread use (2004-2012).

Analysis by ecoregion provided further support for the significant negative relationship between bobwhites and neonicotinoids in areas of high-use (e.g. High Plains, Rolling Plains, and Gulf Coast Prairies and Marshes). Approximately 10 bobwhites were collected from three field sites in Fall 2014 and Spring 2015, for a total of 61 birds.

Neonicotinoid compounds were detected in trace amounts in the livers of seven quail, including samples from all three field sites and both collecting periods. Signs of testicular degeneration (n = 2) and lipid-type hepatocellular vacuolation (n=8) were

consistent with known results of neonicotinoid intoxication. Overall, we identified evidence of bobwhite exposure to neonicotinoid insecticides, and our statistical analysis indicates that neonicotinoid use may be contributing to quail decline in some ecoregions in Texas.

ACKNOWLEDGEMENTS

I would like to thank the landowners, hunters, and game bird advocates that made this study possible. Without their valuable insights, I would have learned very little about the idiosyncrasies of quail and lacked a full appreciation for bobwhites. I would also like to extend no small amount of thanks to the four-legged field technicians who worked tirelessly to assure that I did not go back to the lab birdless. Without them, I am afraid that I would have become an even poorer excuse for a quail hunter. I am very grateful to have had financial support during the first year of this project. Funding was provided through the Reversing the Quail Decline in Texas Initiative and the Upland Game Bird Stamp Fund based on a collaborative effort between Texas Parks and Wildlife Department and Texas A&M AgriLife Extension Service. To Jack and Linda Greenwade, Russell Green, Brady Foreman, Russell Higgins, Homer, Dawn, and Michael Gomez, Norman Dozier, and Brandon Hubert, I cannot say thank you enough.

NOMENCLATURE

AICc	Akaike's Information Criterion (Corrected)
BBS	North American Breeding Bird Survey
GCPM	Gulf Coast Prairies and Marshes
IMI	Imidacloprid
nAChR	Nicotinic Acetylcholine Receptor
ROPL	Rolling Plains
SOTX	South Texas Plains
TPWD	Texas Parks and Wildlife Department
USGS	United States Geological Survey

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ACKNOWLEDGEMENTS	iv
NOMENCLATURE	v
TABLE OF CONTENTS	vi
LIST OF FIGURES	viii
LIST OF TABLES	ix
CHAPTER I INTRODUCTION	1
1.1 Neonicotinoid Insecticides	1
1.2 Northern Bobwhite	4
CHAPTER II HISTORICAL ANALYSIS OF NORTHERN BOBWHITE (<i>COLINUS VIRGINIANUS</i>) ABUNDANCE AND NEONICOTINOID USE IN TEXAS	7
2.1 Introduction	7
2.2 Methods	11
2.3 Results	18
2.4 Discussion	23
CHAPTER III DETECTION OF NEONICOTINOID INSECTICIDES IN NORTHERN BOBWHITE (<i>COLINUS VIRGINIANUS</i>) TISSUES IN TEXAS	28
3.1 Introduction	28
3.2 Methods	31
3.3 Results	38
3.4 Discussion	39
CHAPTER IV CONCLUSIONS	45
LITERATURE CITED	47

APPENDIX A 56

LIST OF FIGURES

	Page
Figure 2.1 Study Areas Included in the Historical Analysis of Bobwhite Abundance and Neonicotinoid Use.....	12
Figure 2.2 Estimated Total Neonicotinoid Use in Texas Counties (2012)	19
Figure 3.1 Total Estimated Neonicotinoid Use and Study Site Locations.....	32
Figure 3.2 Selected Histopathology Slides of Bobwhites Collected from High Neonicotinoid Use Areas in Texas.....	40

LIST OF TABLES

	Page
Table 1.1 Neonicotinoid Compounds and Uses	2
Table 3.1 UPLC-MS/MS Fragmentation of Neonicotinoid Compounds.....	37

CHAPTER I

INTRODUCTION

1.1 Neonicotinoid Insecticides

Neonicotinoids are a relatively new class of insecticide that act as agonists against nicotinic acetylcholine receptors (nAChRs) in the central nervous system, causing paralysis and death (Tomizawa and Casida, 2003). All neonicotinoids exhibit systemic properties which allow them to be absorbed and distributed throughout a plant as it grows, making the plant toxic to insects and protecting it throughout the growing season (Elbert et al., 2008). Since their introduction in the early 1990's, research has demonstrated that neonicotinoids are persistent and highly water soluble, which facilitates their entrance and frequent occurrence in the environment (Fossen, 2006; Hladik et al., 2014; Lewis et al., 2015). It is estimated that their primary application method (seed dressing) releases ~95% of the active ingredient to field margins, soils, surface waters, and ground waters, while only ~5% reaches the target crop (Goulson, 2014). Neonicotinoids are currently registered for over 500 different uses worldwide (Douglas and Tooker, 2015), and compounds belonging to the neonicotinoid class of insecticides include imidacloprid (IMI), acetamiprid, clothianidin, thiamethoxam, dinotefuran, nitenpyram, and thiacloprid. They are used on a variety of crop types and are effective at controlling sucking and chewing insect pests, as well as household pests including cockroaches and fleas (Table 1.1).

Table 1.1 Neonicotinoid Compounds and Uses

Compound	Target Pests	Application	Crop Types	Common Formulations
Imidacloprid	sucking chewing	foliar, seed, soil, veterinary, home and lawn	cereals, cotton, fruit, oilseed rape, ornamentals, rice, sugar, vegetables	Guacho [®] Confidor [®] Admire [®]
Acetamiprid	sucking chewing household	foliar, soil	citrus, cotton, orchards, ornamentals, potato, tea, vegetables, vines	Mospilan [®]
Clothianidin	sucking chewing	foliar, seed, soil	cereals, corn, fruit, oilseed rape, potatoes, rice, sugar beets, vegetables	Poncho [®] Dantotsu [®] Dantop [®]
Thiamethoxam	sucking chewing soil-living	foliar, seed, soil	cereals, cotton, fruit, potatoes, rice, tobacco, vegetables	Actara [®] Cruiser [®] Platinum [®]
Thiacloprid	sucking chewing	foliar	cereals, cotton, oilseed rape, ornamentals, pome fruit, potato, rice, vegetables	Calypso [®]
Nitenpyram	sucking	foliar, soil, veterinary	fruit, rice, tea, vegetables	Bestguard [®]
Dinotefuran	sucking	foliar, soil	apples, cotton, fruit, ornamentals, potato, rice, sugar beets, vegetables	Starkle [®] Safari [®] Venom [®]

Table modified from Elbert et al. (2008)

Since the introduction of neonicotinoids, insecticidal seed treatments have increased exponentially. By 2008, neonicotinoids were valued at over \$2.6 billion and made up 80% of the market for insecticidal seed treatments (Jeschke et al., 2011).

During this same year, imidacloprid was the second most profitable agrochemical behind

the herbicide glyphosate, with sales of \$1.28 billion, and four neonicotinoid formulations (of imidacloprid, thiamethoxam, and clothianidin) ranked in the top 11 of all agrochemical sales (Pollack, 2011).

Neonicotinoids have been implicated in large-scale declines in pollinators (Blacquère et al., 2012) and birds (Mineau and Palmer, 2013), elicit sub-lethal impacts on non-target organisms across taxa (Gibbons et al., 2015), and may even present a risk to ecosystem functioning (Chagnon et al., 2014). Of particular interest in the present study is their impact on avifauna. In laboratory analyses, (1) Tokumoto et al. (2013) demonstrated that clothianidin causes hepatocellular vacuolation and reproductive abnormalities in Japanese quail (*Coturnix japonica*), including delayed embryonic development and fewer and impaired germ cells with single-stranded DNA in the seminiferous tubules of male quail; (2) Lopez-Antia et al. (2013, 2015) found that imidacloprid induced oxidative stress and alterations in biochemical parameters and reproduction, including eggshell thinning, reduced fertilization, delayed egg laying, smaller clutch sizes, and immune suppression in adults and offspring in red-legged partridges (*Alectoris rufa*); (3) Pandey and Mohanty (2015) reported that imidacloprid disrupts the pituitary-thyroid axis of red munia (*Amandava amandava*), potentially affecting thyroid homeostasis and reproduction; and (4) Balani et al. (2011) reported reduced leukocyte count (i.e. immunotoxicity) and degenerative changes in the liver of white leghorn cockerels (*Gallus domestica*) after treating them with imidacloprid. In field analyses, (1) Turaga et al. (2015) confirmed that bobwhites and scaled quail (*Callipepla squamata*) are exposed to neonicotinoids in the Rolling Plains; (2) Hallmann

et al. (2014) found imidacloprid levels in surface waters correlated to the decline of farmland birds in the Netherlands; and (3) Mineau and Palmer (2013) presented numerous cases of poisoning and mortality in wild birds resulting from the ingestion of neonicotinoid treated seeds or contaminated grubs.

1.2 Northern Bobwhite

Northern bobwhites are among the most intensively studied birds, with thousands of publications dedicated to their life history (Scott, 1985). They are found across the southeastern U.S. (Figure A.1), and are frequently associated with agricultural landscapes (Lusk et al., 2002, Janke and Gates, 2013). Adults are predominantly granivorous, but will consume, to a lesser extent, insects and green vegetation. Chicks and breeding females have higher nutritional demands and increase arthropod consumption during breeding and brood-rearing to meet their protein requirements (Hernández et al., 2007; Larson, 2010:4-9). On average, bobwhites produce between 12-15 eggs per clutch and may make multiple nesting attempts per season (Hernández et al., 2007); however, annual survival may be as low as 18% (Hernández et al., 2007). Adequate nesting cover (e.g. warm-season native perennial bunchgrass) is essential to sustain long-term quail populations, which exhibit a phenomenon of irruptions known as boom-and-bust cycles. These irruptions are generally attributed to the influence of precipitation on the amount and quality of bobwhite habitat (Hernández et al., 2005), and can confound efforts to identify short-term population trends and the factors influencing such population changes.

Despite their important social and economic value, bobwhites have experienced range-wide declines since at least the 1960's. Land use changes are commonly proposed as the driver of grassland bird decline (Brennan and Kuvlesky, 2005), although recent analyses indicate that widespread pesticide use may also impact bird populations (Mineau and Whiteside, 2013). Bobwhites collected from agricultural areas have been shown to contain residues of neonicotinoid compounds in their tissues (Turaga et al., 2015), which likely results from the consumption of treated seeds and contaminated insects and vegetation foraged from fields or field margins bordering agricultural crops.

Bobwhites consume, and even prefer, the seeds of many agricultural crops (Michael and Beckwith, 1955). Neonicotinoids are primarily applied as a seed dressing, and bobwhites may be exposed to neonicotinoid-treated crop seeds in cultivated croplands. Many common pests of cotton, wheat, sorghum, sunflower, corn, and other agricultural crops are preferred by bobwhites, including arthropods from the orders of Coleoptera, Hemiptera, Hymenoptera, Lepidoptera, and Orthoptera (Moorman et al., 2013). All orders listed above are included in the suite of crop pests controlled by neonicotinoid insecticides. Bobwhites may be impacted by contaminated insects in two ways: (1) they may consume contaminated insects and become contaminated themselves, and (2) neonicotinoid efficacy could reduce prey abundance sufficiently to limit protein essential in the diets of chicks and breeding females. Reductions in prey abundance due to insecticidal applications have been implicated in grassland and farmland bird decline (Wilson et al., 1999; Benton et al., 2002; Boatman et al., 2004), and include the application of neonicotinoids specifically (Hallmann et al., 2014).

We hypothesize that neonicotinoid use may be contributing to bobwhite decline in some agriculturally-dominated landscapes of Texas, and that bobwhites collected from these areas will contain detectable levels of neonicotinoid compounds in their tissues. To assess the overall impact of neonicotinoid use on bobwhites in Texas, we established the following objectives: (1) analyze historical trends in statewide neonicotinoid use and bobwhite abundance, and (2), analyze quail tissues collected from high-use areas for neonicotinoid residues and evidence of tissue damage consistent with neonicotinoid toxicity.

CHAPTER II

HISTORICAL ANALYSIS OF NORTHERN BOBWHITE (*COLINUS VIRGINIANUS*) ABUNDANCE AND NEONICOTINOID USE IN TEXAS

2.1 Introduction

Neonicotinoids are a relatively new class of insecticide that possess up to 3000-fold higher insecticidal activity than their botanical relative, nicotine (Tomizawa and Casida, 2003). There are seven neonicotinoid compounds currently on the market: imidacloprid (IMI), acetamiprid, clothianidin, thiamethoxam, thiacloprid, dinotefuran, and nitenpyram. All exhibit systemic properties which allow them to be absorbed and distributed throughout a plant as it grows, making the plant toxic to insects and protecting it throughout the growing season (Elbert et al., 2008). Neonicotinoids were introduced to Texas in 1994 and became widely marketed in Texas and the U.S. in the mid 2000's. They act as agonists against postsynaptic nicotinic acetylcholine receptors (nAChRs) in the central nervous system, and variation in the functional structure of vertebrate and insect nAChRs facilitates their selective action towards insects (Tomizawa and Casida, 2003). Their popularity as the most widely used class of insecticide in the world is partially attributable to this selective action, which results in a lower vertebrate toxicity than their predecessors (e.g. organophosphates and carbamates). Neonicotinoids are effective at controlling sucking and chewing insect pests, including household pests such as cockroaches and fleas, and are registered for use on cereals, fruits, ornamentals, vegetables, cotton, vines, potatoes, and for home, lawn,

and veterinary purposes. They also have applications in biological vector control (Elbert et al., 2008), and are frequently formulated with mixtures of other pesticides (e.g. fungicides), especially when applied as a seed dressing (Krohn et al., 2008).

Neonicotinoids are used in a variety of applications (e.g. foliar spray, soil drench, trunk injection, etc.), but are primarily used as a seed dressing. Since their introduction in the mid 1990's, the prophylactic application of insecticidal seeds treatments has increased exponentially. By 2008, neonicotinoids comprised 80% of the insecticidal seed treatment market (Jeschke et al., 2011), and virtually all neonicotinoid use on corn, soybeans, and wheat in the U.S. from 2000 to 2012 was applied as a seed dressing (Douglas and Tooker, 2015). When applied as a seed dressing, only ~5% of the active ingredient reaches the target crop, while the other ~95% is lost to the environment (Goulson, 2014). As neonicotinoids are highly water soluble ($\log K_{ow}$ -0.55 to 1.26; Hladik et al., 2014) and have long half-lives (up to 545 days in soil and 40 days in water; Lewis et al., 2015; Fossen, 2006), seed treatments facilitate their entrance, transport, and persistence the environment. Recently, Morrissey et al., (2015) compiled a list of neonicotinoid detections in surface waters in 29 independent studies of nine countries across the world, including detections made outside of the growing season and outside of cultivated croplands.

Neonicotinoids were initially regarded for their high insect specificity and low vertebrate toxicity, but concerns have emerged in recent years regarding their effects on pollinators (Blacquère et al., 2012; Gill et al., 2012; Henry et al., 2012; Whitehorn et al., 2012; Fairbrother et al., 2014), other non-target organisms (Goulson, 2013; Mineau and

Palmer, 2013; Mason et al., 2013; Hallmann et al., 2014; Gibbons et al., 2015; Morrissey et al., 2015;), and ecosystem functioning (Kreutweiser et al., 2009; Chagnon et al., 2014; van der Sluijs et al., 2015). Adverse and sub-lethal side effects have been reported in pollinators and other non-target invertebrates, birds, mammals, and herpetofauna (Gibbons et al., 2015). These concerns sparked a review and 2-year moratorium on imidacloprid, clothianidin, and thiamethoxam in the European Union (EU, 2013), and prompted the U.S. Environmental Protection Agency to review the impacts neonicotinoids have on pollinators in the U.S. (Johnson and Corn, 2015).

The effects of neonicotinoids on avifauna are of particular interest and concern in the present study. Laboratory analyses demonstrate that birds subjected to various neonicotinoid compounds at field-realistic levels (i.e. dosage consistent with the manufacturer's suggested application rate) elicit signs of oxidative stress, immunotoxicity, degenerative changes in the liver, alterations in biochemical parameters, disruption of the pituitary-thyroid axis, and most importantly, alterations in reproductive ability, including fewer and fragmented germ cells, reduced fertilization, eggshell thinning, delayed embryonic development and egg laying, severely reduced clutch size, and immunosuppression in adults and offspring (Balani et al., 2011; Tokumoto et al., (2013); Lopez-Antia et al., 2013, 2015; Pandey and Mohanty, 2015). Furthermore, neonicotinoids may cause prey-based collapses, as illustrated by studies of neonicotinoids and other insecticides (Wilson et al., 1999; Boatman et al., 2004; Hallmann et al., 2014). Research has confirmed that northern bobwhite (*Colinus virginianus*; hereafter, bobwhite) and scaled quail (*Callipepla squamata*) are exposed to

neonicotinoids in the Rolling Plains of Texas and Oklahoma (Turaga et al., 2015), and cases of wild bird poisoning and mortality have been documented as resulting from the ingestion of neonicotinoid-treated seeds and contaminated grubs (Berny et al., 1999; de Snoo et al., 1999; Bro et al., 2010; Mineau and Palmer, 2013).

Bobwhites are grassland birds frequently associated with agriculture (Lusk et al., 2002, Janke and Gates, 2013), and are known to feed on (and even prefer) the seeds of agricultural crops (Michael and Beckwith, 1955). Adults are predominantly granivorous, but will consume green vegetation and insects during breeding and brood-rearing. Chicks and breeding females have higher nutritional demands and consume more insects to meet their protein requirements (Larson, 2010: 4-9)

Despite their important social and economic value, bobwhites have experienced range-wide declines for decades. North American Breeding Bird Survey (BBS) analyses indicate that Texas bobwhite populations had an overall increase of 3.3% per year from 1966 to 1979, and have decreased 4.7% per year from 1980 to 1996 and 5.8% per year from 2001 to 2011 (Sauer et al., 1997, 2012).

Habitat loss by agricultural intensification and other causes is often proposed as the primary driver of grassland and shrubland bird decline, including bobwhites (Brennan, 1991; Brennan and Kuvlesky, 2005). Other factors have also been implicated in regional quail losses, including drought (Hernández et al., 2005), epizootics and parasites (Dunham et al., 2014), local over-harvest (Tomeček et al., 2015), over-grazing (Lusk et al., 2002), and the advance of red imported fire ants (*Solenopsis invicta*; Allen et al., 1995). Recently, evidence has emerged that broad-spectrum pesticide application

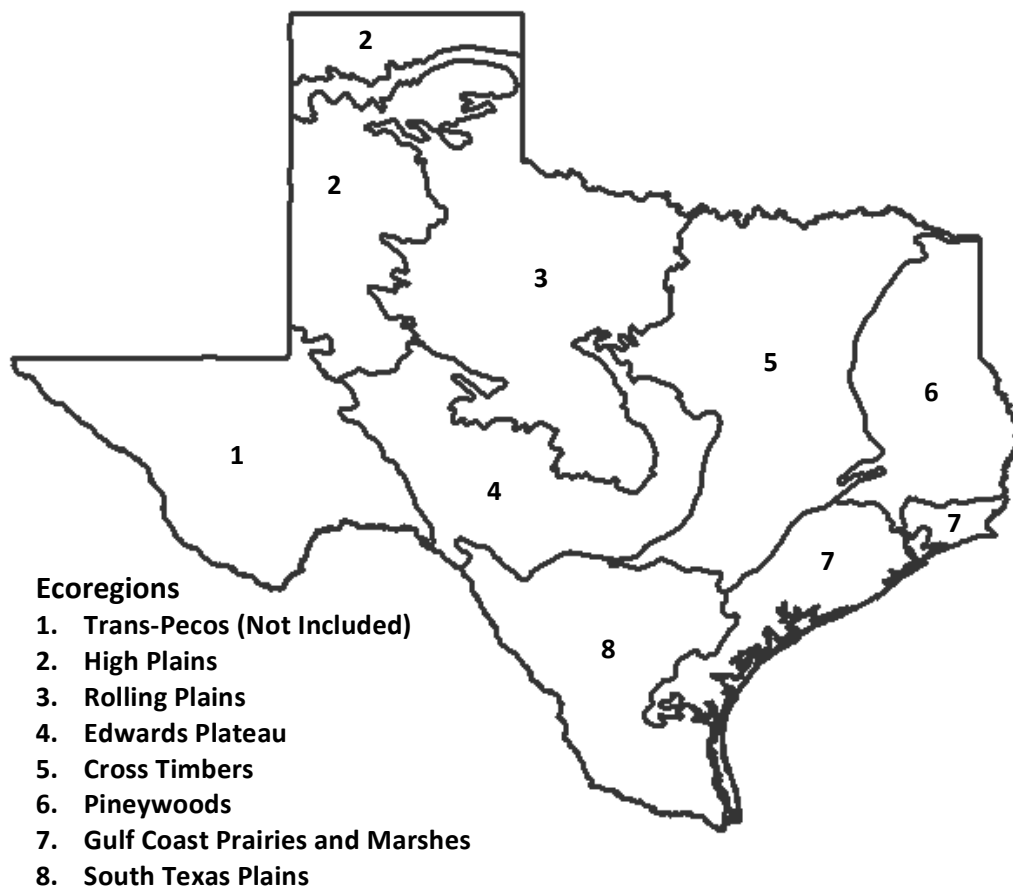
may contribute to grassland bird decline (Wilson et al., 1999; Beecher et al., 2002; Mineau and Whiteside, 2013). There is still much uncertainty regarding the various causes of declining quail populations in Texas, and this study aims to increase our overall understanding of bobwhite decline. As it is likely that quail decline results from a suite of stressors, the widespread and frequent use of neonicotinoid insecticides in bobwhite habitats warrants a thorough analysis of relationship between bobwhite abundance and neonicotinoid use in the state of Texas.

Since bobwhites are frequently associated with agricultural areas where neonicotinoids are used, and may be exposed to neonicotinoids in the form of treated seeds or contaminated insects and vegetation, we hypothesize that the use of neonicotinoids may be negatively affecting Texas bobwhite populations. Our objective is to analyze long-term data at the state- and ecoregion-level to identify any existing correlations between bobwhite abundance and neonicotinoid use in Texas.

2.2 Methods

In order to determine the potential effects of neonicotinoid use on Texas bobwhites, we utilized available data on bobwhite abundance, neonicotinoid use, and environmental predictor variables in a statistical analysis for the years 1978-2012. Our study area encompassed all ecoregions of Texas excluding the Trans-Pecos (Figure 2.1), as this is the western periphery of the bobwhite range. We conducted statistical analyses at both the state- and ecoregion- levels to identify overall state and regional trends. We

Figure 2.1 Study Areas Included in the Historical Analysis of Bobwhite Abundance and Neonicotinoid Use



aggregated the Cross Timbers, Post Oak Savannah, and Blackland Prairies ecoregions into a single region (referred to as “Cross Timbers”).

2.2.1 Construction of Study Plots

We used the Breeding Bird Survey and Texas Parks and Wildlife Department (TPWD) driving transects to develop study plots from which we gathered spatial data for our analysis. Driving transects were obtained online (BBS; ArcGIS, 2011) or directly

from Texas Parks and Wildlife Department (M. Frisbie, TPWD, March 2015). Driving transects were imported into ArcGIS 10.2.1 (ESRI, 2011), and re-projected into NAD 1983 UTM Zone 14 N. Plots were constructed by placing a 0.5 km buffer around driving transects, and a total of 165 BBS and 143 TPWD plots were included in the analysis. Texas Parks and Wildlife Department plots averaged 32.5 km \pm 0.38 km in length with a low of 30.9 km and a high of 33.0 km, and Breeding Bird Survey plots averaged 41.0 km \pm 2.8 km in length with a low of 30.2 km and a high of 49.1 km. For various reasons, the Breeding Bird Survey and Texas Parks and Wildlife Department were unable to consistently survey all plots; therefore, when a plot was not surveyed in a given year, it was omitted from the analysis for that year.

2.2.2 Data Collection

2.2.2.1 Quail Abundance

We obtained quail abundance data directly from Texas Parks and Wildlife Department (M. Frisbie, TPWD, March 2015) and from the USGS Patuxent Wildlife Research Center online database (Pardieck et al., 2014). Survey protocols varied between organizations. Texas Parks and Wildlife Department biologists conduct quail surveys in August by driving at 32.2 km per hour along driving transects and recording visual observations of quail (Peterson and Perez, 2000). Breeding Bird Survey volunteers conduct general avian surveys in June by stopping 50 times for exactly 3 minutes at equal intervals along driving transects and recording visual and auditory observations of all birds (Pardieck et al., 2014).

Breeding Bird Survey routes were more evenly distributed across ecoregions (Figure A.2), and are therefore likely a better representation of general trends in most ecoregions. We present results of our analyses from both datasets (BBS and TPWD), but focus our discussion on BBS results, as they are likely more indicative of overall statewide and ecoregion trends in abundance.

2.2.2.2 Neonicotinoid Use Estimates

In order to estimate the neonicotinoid levels in each plot we obtained USGS ePest values of estimated county-level neonicotinoid use (Thelin and Stone, 2013; Baker and Stone, 2015) for all compounds applied in Texas from 1978-2012. The summed total of all ePest High compounds was used to obtain a single value of estimated annual county-level neonicotinoid use. Total neonicotinoid use within each plot was calculated by multiplying the cumulative county neonicotinoid use by the proportion of county agriculture that fell within each plot.

2.2.2.3 Climate

Research has shown that the Palmer Modified Drought Index (hereafter, drought index) may be used as a good predictor of quail abundance (Bridges et al., 2001), while breeding season (April through August) precipitation and summer (June through August) mean maximum daily temperature are predictive of quail productivity (i.e. age ratios; Tri et al., 2012). In order to determine the climatic conditions within each plot, we obtained the following data for each year of the study period: (1) raster images of precipitation for

each month of the breeding season and (2) monthly summer mean maximum monthly temperature (daily values were not available) from the Parameter-elevation Regressions on Independent Slopes Model online databank (Daly et al., 2008), and (3) monthly summer drought index values, obtained from the National Ocean and Atmospheric Administration (NOAA, 2015). Precipitation and temperature data were statistically modeled raster graphics and are the U.S. Department of Agriculture's official spatial climate data. Drought index values range from -5.0 (severe drought conditions) to +5.0 (extreme wet conditions) and are calculated using precipitation, temperature, evapotranspiration rates, and other climatic variables (Heddinghaus and Sabol, 1991).

Monthly precipitation and mean maximum monthly temperature rasters were imported into ArcGIS 10.2.1 (ESRI, 2011) and re-projected into NAD 1983 UTM Zone 14 N. Zonal Statistics was used to identify mean precipitation across each plot for each month of the breeding season. These values were then summed, yielding total breeding precipitation. Summer mean maximum monthly temperature was calculated by averaging the maximum temperature in each plot for each of the summer months using Zonal Statistics. Drought index values are available regionally in areas closely resembling Gould's (1975) ecoregions (Figure A.3). Drought index values were averaged over summer months for each ecoregion, resulting in a single value representing the summer drought index.

2.2.2.4 Land Use

As habitat fragmentation by agricultural intensification and urbanization is frequently cited as a major contributor to quail decline, we used total agricultural (i.e. cultivated cropland) area and total developed area in our analysis. In order to identify these land use variables in our plots, we used statistically modeled land cover raster images obtained from the USGS Earth Resources Observation Systems lab (EROS, 2013). Land use rasters were imported into ArcGIS 10.2.1 (ESRI, 2011) and re-projected into NAD 1983 UTM Zone 14 N. We reclassified land use into two separate binary raster images for each year of the study period: (1) agriculture-non agriculture and (2) developed-undeveloped. Tabulate Area was then used to calculate the total agricultural area and total developed area falling within each plot.

2.2.2.5 Supporting Shapefiles

Supporting boundary layers including, state, ecoregion, and county boundaries were obtained online from Texas Natural Resources Information Systems (TNRIS, 2015). These vector files were imported into ArcGIS 10.2.1 and re-projected into NAD 1983 UTM Zone 14 N prior to their use in any operations.

2.2.3 Statistical Analysis

Because survey protocols varied drastically between BBS and TPWD (e.g. driving transect lengths and observation procedures), and could influence model outcome, datasets from both organizations were analyzed separately. Analyses were

divided into three time periods: (1) Before neonicotinoid introduction (1978-1993; i.e. Pre-Neonic); (2) After neonicotinoid introduction, but before their widespread use (1994-2003; i.e. Light-Neonic) and (3) After the widespread use of neonicotinoids in Texas (2004-2012; i.e. Heavy-Neonic). Computational analyses were conducted using R Statistical Programming Language, version 3.2.3 (R Core Team, 2015). A list of variables included in statistical analysis is provided in Table A.1.

2.2.3.1 Data Exploration

In order to identify distribution patterns in the data, we constructed histograms and q-q plots of all variables. It was apparent that quail abundance was zero-inflated (i.e. overdispersed). Specialized statistical analyses (e.g. generalized linear, zero-inflated, hurdle, generalized additive, and generalized linear mixed-effects models) with a negative binomial distribution were employed for their ability to accommodate non-parametric and zero-inflated count data (Zuur, 2009:209, 261, 323). We also defined ecoregions of low-, moderate-, and high- neonicotinoid use by summing the estimated use values of each ecoregion.

2.2.3.2 Model Selection

Six different models were generated to describe trends in the statewide analysis and for each of the ecoregion-level analyses: one model explaining each dataset (BBS and TPWD) for each of the three time periods (Pre-, Light -, and Heavy -Neonic).

In order to identify the most parsimonious model for each of our analyses, we used the corrected Akaike Information Criterion (AICc; corrected for finite sample sizes) weight of evidence approach in model selection (Burnham and Anderson, 2002). Variables included in the model selection process included drought index, total breeding season precipitation, summer mean maximum monthly temperature, agricultural area, developed area, and neonicotinoid use (Table A.1). In the statewide analysis, ecoregion was included as a categorical variable and was a random effect in all mixed-effects models.

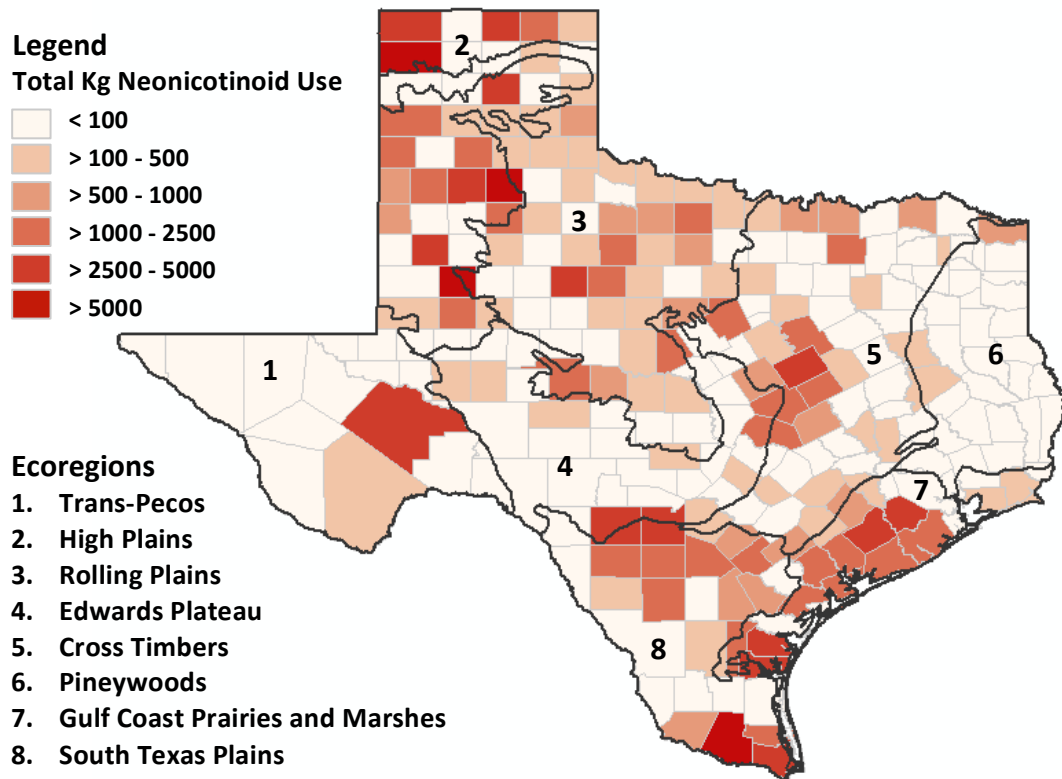
First, we fit all predictor variables to a generalized linear model with a negative binomial distribution, and used stepwise regression in both forward- and backward- directions to identify the combination of variables that yielded the lowest AICc value. Next, the resulting model was subjected to generalized linear, zero-inflated, hurdle, generalized additive, and generalized linear mixed models, all with negative binomial distributions. Finally, we calculated AICc weights (which quantify the weight of evidence in favor of a given model) for all candidate models, and the best-fit model was selected. See Figure A.4 for sample script of model selection.

2.3 Results

2.3.1 Use Areas

The High Plains, South Texas Plains, Gulf Coast Prairies and Marshes, and Rolling Plains each received over 20,000 kg of neonicotinoid use in 2012 alone and were considered high-use areas (Figure 2.2). We designated the Cross Timbers and Edwards

Figure 2.2 Estimated Total Neonicotinoid Use in Texas Counties (2012)



Plateau moderate-use areas, as each of these ecoregions received between 10,000 and 20,000 kg of neonicotinoid use in 2012. Finally, the Pineywoods ecoregion received less than 2,000 kg of neonicotinoid use and was considered low-use (the Trans-Pecos was not included in our analysis, but was found to be a low-use region as well, with less than 3,000 kg of neonicotinoids applied in 2012).

2.3.2 Statewide Results

All neonicotinoid count model correlation coefficients and p-values are detailed in Table A.2. The drought index was significantly ($p < 0.05$) positively correlated with bobwhite abundance in four out of six statewide models. It was not included in the best-fit Pre-BBS model, and was not significant in Light-BBS. Breeding season precipitation was significantly ($p < 0.001$) negatively correlated with bobwhite abundance in the Heavy-TPWD model, but was not included or not significant in other models. Summer mean maximum monthly temperature was significantly positively correlated with bobwhite abundance in all Pre- and Light-BBS and TPWD models, but was not included in the Heavy-BBS or Heavy-TPWD best-fit models. Agricultural area was significantly ($p < 0.01$) negatively correlated with bobwhite abundance in the Pre-BBS and Pre-TPWD models, but was not significant in models explaining the other two time periods. Developed area was included in only one of the best-fit models (Pre-TPWD), and was significantly ($p < 0.04$) negatively correlated with bobwhite abundance. Neonicotinoid use was significantly ($p < 0.03$) negatively correlated with quail abundance in the Light-BBS and TPWD models and in the Heavy-BBS model, but was positively correlated (although not significant) in the Heavy-TPWD model.

2.3.3 Ecoregion Results

All variables included in our analysis (i.e. summer drought index, total breeding season precipitation, summer mean maximum monthly temperature, agricultural area, developed area, and total neonicotinoid use) exhibited both positive and negative

correlations with bobwhite abundance in at least 1 of the 39 models generated in our ecoregion analysis, indicating that variables influenced bobwhite populations differently across time and space.

Agricultural area was the strongest predictor of quail abundance in the Cross Timbers in terms of consistency and significance across time periods, and was significantly ($p < 0.008$) negatively correlated with bobwhite abundance in 4 out of 6 models (Pre-BBS, Light-BBS, Light-TPWD, Heavy-BBS). Neonicotinoid use was not significantly associated with bobwhite abundance in any of the 6 best-fit Cross Timbers models.

None of the variables in our analysis had consistent, significant associations with bobwhite abundance across all three time periods in the Edwards Plateau ecoregion, but neonicotinoid use was significantly ($p < 0.02$) negatively associated with bobwhite abundance in the Light-BBS and Heavy-BBS models.

Similarly, there were no consistent, significant relationships between predictor variables and bobwhite abundance across all three time periods in the Gulf Coast Prairies and Marshes analyses, but neonicotinoid use was significantly ($p < 0.02$) negatively associated with bobwhite abundance in the Light-BBS and Heavy-BBS models.

Summer mean maximum monthly temperature was the strongest predictor of quail abundance in our analysis of the High Plains ecoregion, and was significantly ($p < 0.02$) positively correlated with bobwhite abundance in 4 of the 6 best-fit models (Pre-TPWD, Light-BBS, Light-TPWD, Heavy-TPWD). Neonicotinoid use was significantly

($p < 0.001$) negatively associated with bobwhite abundance in both the Heavy-BBS and Heavy-TPWD models.

Drought index was the strongest predictor of quail abundance in the Rolling Plains ecoregion, with 5 out of 6 models (all but Pre-BBS) exhibiting a significant ($p < 0.03$) positive relationship between bobwhite abundance and drought index.

Neonicotinoid use in this ecoregion was significantly ($p < 0.02$) negatively associated with bobwhite abundance in both time periods following their introduction (Light-BBS, Heavy-BBS, Heavy-TPWD).

None of the predictor variables in the South Texas Plains analysis were both significant and consistent across time periods. Neonicotinoid use was significantly ($p < 0.001$) negatively associated with bobwhite abundance in both the Light-BBS and Light-TPWD models, but was not significant in any of the Heavy-models.

The Pineywoods ecoregion is only surveyed by the BBS, and none of our attempted models successfully converged for the Heavy-BBS dataset, indicating that the variables we used were not able to describe patterns in bobwhite abundance in this ecoregion during the Heavy-Neonic time period. Agricultural and developed areas were equally strong predictor variables in terms of significance and consistency in the Pre-BBS and Light-BBS models. Agricultural area was significantly ($p < 0.001$) positively associated with bobwhite abundance, while developed area was significantly ($p < 0.002$) negatively associated with bobwhite abundance. Neonicotinoid use was not included in any of the best-fit models describing the Pineywoods ecoregion.

2.4 Discussion

The results of our analyses suggest that neonicotinoids may contribute to bobwhite decline in some parts of the state. Outcome from the statewide Breeding Bird Survey dataset illustrated that use of neonicotinoids corresponded to declines in bobwhite populations in the time periods after the introduction and widespread use of these insecticides. Similar trends were identified in some ecoregions receiving moderate- or high- levels of neonicotinoid use. Specifically, bobwhites in the Gulf Coast Prairies and Marshes, High Plains, Rolling Plains, Edwards Plateau, and possibly South Texas Plains, may be negatively impacted by the use of neonicotinoid insecticides.

In areas where neonicotinoids may contribute to bobwhite decline, we would expect to see an inverse relationship between these two variables during the time period after the widespread use of neonicotinoids (2004-2012), and possibly the time period directly following their introduction (1994-2003). The High Plains, Rolling Plains, Gulf Coast Prairies and Marshes, South Texas Plains, and Edwards Plateau all exhibited a negative relationship between bobwhite abundance and neonicotinoid use during at least one of these two time periods. Neonicotinoid use was inversely related with bobwhite abundance in the High Plains following the widespread use of these pesticides, and in the Rolling Plains, Gulf Coast Prairies and Marshes, and Edwards Plateau in both time periods after the introduction of neonicotinoids. In the South Texas Plains, neonicotinoid use inversely related to bobwhite abundance during the light-neonic time period, but not in the heavy-neonic time period, indicating that other factors may have

been responsible for bobwhite decline in the South Texas Plains after the widespread use of neonicotinoids.

The Cross Timbers and Pineywoods ecoregions received moderate and low levels of neonicotinoid use respectively, but neonicotinoid use was not a significant predictor of bobwhite abundance in either ecoregion for any of the two time periods following the introduction of neonicotinoids to Texas. Land use variables were the strongest predictors of bobwhite abundance in the Cross Timbers and Pineywoods, indicating that agricultural or developed areas may have the strongest impacts on bobwhites in these ecoregions.

Our findings indicate that bobwhites may be negatively affected by neonicotinoid use in the High Plains, Rolling Plains, Gulf Coast Prairies and Marshes, Edwards Plateau, and possibly the South Texas Plains. All of these ecoregions produce crops that are beneficial for bobwhites, including winter wheat, upland cotton, corn, sorghum, sunflower, and soybeans (USDA, 2010). Bobwhites are known to consume (and even prefer) seeds of farm crops (Michael and Beckwith, 1995), and forage from field margins bordering cultivated cropland (Best et al., 1990; Brennan, 1991; Brennan and Kuvlesky, 2005; Moorman et al., 2013). In 2014, Texas growers harvested 2.2 million acres of corn and 2.3 million acres of sorghum from the High Plains, South Texas Plains, Gulf Coast Prairies and Marshes, Cross Timbers, and Edwards Plateau. In the same year, 2.2 million acres of winter wheat and 4.6 million acres of cotton were harvested from these regions as well as the Rolling Plains, 92 thousand acres of sunflower were harvested mainly from the South Texas Plains, and 140 thousand acres of soybeans were

harvested from the Gulf Coast Prairies and Marshes (USDA, 2010; Texas Almanac, 2014).

Neonicotinoid-treated seeds probably present the biggest hazard to granivorous species because they likely deliver higher concentrations of active ingredient than other sources (i.e. contaminated insects or vegetation; Gibbons et al., 2015). Bobwhites' susceptibility to neonicotinoid compounds is well established, and the LD₅₀ (dose causing mortality in 50% of individuals) of bobwhites to imidacloprid is 152 mg/kg bodyweight (Toll, 1990). At the manufacturer's suggested application rate of a common formulation of imidacloprid (Gaucho® 600 Flowable), an average-sized (170 g) bobwhite consuming 20 IMI- treated corn kernels, 211 IMI- treated sorghum seeds, 112 IMI-treated soybean seeds, or 304 IMI-treated sunflower seeds will reach the LD₅₀. Onset of severe incapacitation resulting from exposure to imidacloprid is seen in bobwhites at levels between 30-60% of the LD₅₀, and neurotoxic effects are usually exhibited within minutes of ingestion (Thyssen and Machemer, 1999). Bobwhites consuming 4 IMI-treated corn kernels, 164 IMI-treated wheat seeds, 8 IMI-treated sorghum seeds, 39 IMI-treated soybean seeds, or 11 IMI-treated sunflower seeds are expected to undergo oxidative stress, immune suppression, liver degeneration, and impaired reproduction (Lopez-Antia et al., 2013, 2015; Tokumoto et al., 2013). Neonicotinoid seed treatment is a common practice for many crops planted in moderate- and high-use areas, and bobwhites may be exposed to treated seeds not properly stored, shallowly sown, or spilled during planting. Based on the recommended application

rates, neonicotinoid-treated seeds have the potential to negatively impact bobwhites by causing direct mortality or adverse, sub-lethal effects.

Neonicotinoids may be limiting bobwhite populations in Texas in several ways. First, the neurotoxic effects of neonicotinoids may increase bobwhites' susceptibility to predation (Walker, 2003), as is seen in studies of other acetylcholine-inhibiting insecticides (Galindo et al., 1985; Buerger et al., 1991). Second, neonicotinoids may impair bobwhite reproduction, and therefore limit their ability to recruit a sufficient number of individuals each year to maintain populations. Many Texas crops are planted in the spring (e.g. corn, sorghum, soybeans, sunflower, cotton), and as a result, neonicotinoid applications often coincide with the development of sex organs as bobwhites physiologically prepare for the breeding season. Neonicotinoids are also persistent in the environment (Goulson, 2013), and have been detected in field margin plants (Greatti et al., 2006; Krupke et al., 2012) and outside of the growing season (Main et al., 2014), potentially making them available to bobwhites throughout the year. Their adverse effects on reproduction (Balani et al., 2011, Lopez-Antia et al., 2013, 2015; Tokumoto et al., 2013; Pandey and Mohanty, 2015) could directly limit the number of offspring produced, or even predispose hens to clutch abandonment or reduced chances of re-nesting (Mineau, 2005). Third, immune suppression, a common side effect of neonicotinoid exposure (Balani et al., 2011; Badgular et al., 2013; Lopez-Antia et al., 2013, 2015), could increase bobwhites' susceptibility to epizootic and parasitic infestation (Köhler and Triebkorn, 2013). Parasite infestation by eyeworms (*Oxyspiruso petrowi*) has been documented in bobwhites in the Rolling Plains, and

although their effects are largely unknown, there is concern that they negatively impact bobwhites (Dunham et al., 2014). Finally, neonicotinoid use may limit prey abundance during critical periods (i.e. breeding, brood-rearing, and over-wintering), which has previously been linked to declines in farmland birds (Wilson et al., 1999; Benton et al., 2002; Boatman et al., 2004);Hallmann et al., 2014)

Turaga et al. (2015) recently analyzed 98 bobwhite and scaled quail in the Rolling Plains and determined that they are not directly impacted by the use of neonicotinoids based on two lines of evidence: a lack of treated seeds in their crops and low concentrations (≤ 62.29 ng/g) of neonicotinoids in their livers. These authors suggest that quail may circumvent neonicotinoid poisoning due to repellent effects of treated seeds, avoidance of treated seeds, and seed husking. However, research indicates that neonicotinoids do not elicit any initial repellent effects (USEPA, 2007), and birds are unlikely to be able to avoid treated seeds in field-realistic conditions (USEPA, 2007; Mineau and Palmer, 2013; Lopez-Antia et al., 2014). Additionally, analysis of crop contents has suggested that bobwhites do not husk seeds (Madison and Robel, 2001; See Chapter 3) Like other birds (Berny et al., 1999; de Snoo et al., 1999; Bro et al., 2010; Mineau and Palmer, 2013), bobwhites are likely to consume treated seeds when available, potentially subjecting them to lethal or sub-lethal doses of neonicotinoids.

CHAPTER III

DETECTION OF NEONICOTINOID INSECTICIDES IN NORTHERN BOBWHITE

(*COLINUS VIRGINIANUS*) TISSUES IN TEXAS

3.1 Introduction

Neonicotinoids are a relatively new class of insecticide and were registered for use in Texas as early as 1994. Imidacloprid (IMI) is the most frequently applied neonicotinoid, and other compounds belonging to the neonicotinoid class of insecticides include clothianidin, thiamethoxam, acetamiprid, dinotefuran, thiacloprid, and nitenpyram. Neonicotinoids are effective at controlling many common sucking and chewing insect pests, and are used on cereals, fruits, ornamentals, vegetables, cotton, vines, potatoes, and for home, lawn, and veterinary purposes. All compounds act as agonists against nicotinic acetylcholine receptors (nAChRs) in the central nervous system, causing insect paralysis and death (Tomizawa and Casida, 2003). They are also systemic, meaning that once they are applied, they are distributed throughout a plant as it grows, making the plant toxic to feeding insects (Elbert et al., 2008).

Because of their systemic properties, neonicotinoids are most frequently applied as an insecticidal seed treatment, and since their registration, the prophylactic use of insecticidal seed treatments has increased dramatically (Douglas and Tooker, 2015). Because neonicotinoids are highly water soluble (Hladik et al., 2014) and have long half-lives (Lewis et al., 2015; Fossen, 2006), their application as a seed treatment facilitates their entrance, transport, and persistence in the environment. When applied as a seed

dressing, it is estimated that only ~5% of the active ingredient reaches the target crop, while the remaining ~95% is lost to the environment (Goulson, 2014). As a result, neonicotinoids are frequently found in surface waters (Morrissey et al., 2015) and are detected during and outside of the growing season (Main et al., 2014).

The use of neonicotinoids has increased dramatically since their introduction in the mid 1990's, and they are now the most widely used class of insecticide in the world (Jeschke et al., 2011). In 2012, over 160,000 kg of neonicotinoids were applied to Texas crops (Baker and Stone, 2015). Neonicotinoids gained popularity partly because of their high insect specificity and presumably low vertebrate toxicity; however, concerns about their adverse impacts on birds and other non-target organisms have led to scrutiny in recent years (Balani et al., 2011; Blacquère et al., 2012; Lopez-Antia et al., 2013, 2015; Tokumoto et al., 2013; Gibbons et al., 2015; Pandey and Mohanty, 2015).

The use of pesticides in modern agriculture has been linked to declines in bird populations across the globe (Wilson et al., 1999; Benton et al., 2002; Boatman et al., 2004; Mineau and Whiteside, 2013). In the Netherlands, neonicotinoid levels in surface waters were correlated with declines in farmland birds (Hallmann et al., 2014), and exposure of wild birds to neonicotinoids has been documented in cases of poisoning and mortality resulting from the ingestion of neonicotinoid-treated seeds or contaminated insects (Berny et al., 1999; de Snoo et al., 1999; Bro et al., 2010; Mineau and Palmer, 2013). In Texas, neonicotinoid residues were detected in Northern bobwhite (*Colinus virginianus*; hereafter, bobwhite) and scaled quail (*Callipepla squamata*) in the Rolling Plains ecoregion during the planting season for winter wheat (Turaga et al., 2015).

Bobwhites are grassland birds frequently associated with agriculture (Lusk et al., 2002; Janke and Gates, 2013), and feed on (and even prefer) the seeds of agricultural crops (Michael and Beckwith, 1955). Adults are predominantly granivorous, but will consume green vegetation and insects. Chicks and breeding females have higher nutritional demands and increase arthropod consumption during breeding and brood rearing to meet their protein requirements (Larson, 2010: 4-9).

Agricultural crops and field margins used by bobwhites for feeding and foraging may be contaminated with neonicotinoid insecticides. Neonicotinoids are applied to corn, wheat, sorghum, sunflower, and soybeans, typically in the form of treated seeds (Jeschke et al., 2011). Arthropods preferred by bobwhites, such as those from the orders of Coleoptera, Hemiptera, Hymenoptera, Lepidoptera, and Orthoptera, (Moorman et al., 2013) are targeted by neonicotinoids and bobwhites could potentially consume these insects after they become contaminated. Additionally, neonicotinoid residues can persist in field margin vegetation for over a year after a field has been planted (Greatti et al., 2006; Krupke et al., 2012).

Bobwhites are a socially and economically important game bird and have thousands of publications dedicated to their life history (Scott, 1985). Despite their importance, bobwhites have experienced range-wide declines for decades. Breeding Bird Survey analyses indicate that Texas bobwhite populations had an overall increase of 3.3% per year from 1966 to 1979, and have decreased 4.7% per year from 1980 to 1996 and 5.8% per year from 2001 to 2011 (Sauer et al., 1997, 2012). Accelerating reductions in bobwhite populations have prompted wide-spread research efforts into the

mechanism of their decline. Although there is still much uncertainty regarding the reason for bobwhite decline, land-use changes resulting from agricultural intensification and urbanization are often proposed as the primary driver of grassland bird decline (Brennan, 1991; Brennan and Kuvlesky, 2005). Other potential factors include drought (Hernandez et al., 2005), epizootics and parasites (Dunham et al., 2014), local over-harvest (Tomeček et al., 2015), over-grazing (Lusk et al., 2002), and the advance of red imported fire ants (*Solenopsis invicta*; Allen et al., 1995).

Since bobwhites are frequently associated with agricultural areas, they may be impacted by neonicotinoid use via the ingestion of treated seeds, contaminated insects, contaminated vegetation, or reduced prey abundance during critical periods (e.g. breeding, brood rearing, and over-wintering). In order to evaluate the association between neonicotinoid use and Texas bobwhites, we identified the following objectives: Collect quail from areas of high neonicotinoid use (i.e. the Rolling Plains, Gulf Coast Prairies and Marshes, and South Texas Plains) and (1) Perform chemical analysis to determine whether they are exposed to neonicotinoid insecticides and (2) Perform histopathological analysis to determine if bobwhites exhibit evidence of tissue damage consistent with known effects of neonicotinoid poisoning.

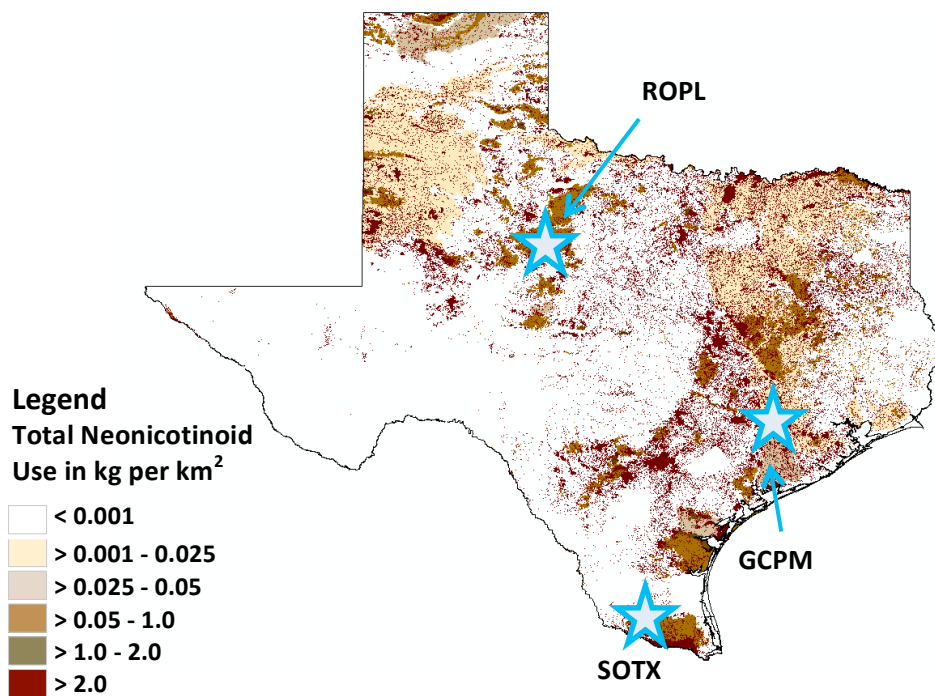
3.2 Methods

3.2.1 Study Areas

To assess the impact of neonicotinoids to bobwhites in Texas, samples were collected from three locations across the state of Texas chosen to represent areas of high

neonicotinoid use (Figure 3.1). Non-disclosure agreements were requested by landowners at all field areas, so specific locations are withheld from this document; rather, site descriptions and approximate locations are provided.

Figure 3. 1 Total Estimated Neonicotinoid Use and Study Site Locations



The Rolling Plains field site (hereafter, ROPL) was located near Abilene, Texas, in the central Rolling Plains. This region is dominated by cotton and winter wheat production and cattle ranching (USDA, 2010). No ranching activities occurred at the ROPL field site, but the land was managed for white-tailed deer (*Odocoileus virginianus*) production and hunting, with pairs of deer feeders and hunting blinds

occurring frequently. Sandy soils dominate this field site, and brush to tree- sized shinnery oak (*Quercus havardii*) and various tall grasses grow in abundance, providing excellent cover for bobwhites. Winter wheat treated with Gaucho® (a formulation of imidacloprid) was planted in mid-September along sandy roadways and in patches of varying size as supplemental deer feed. We collected bobwhites within approximately 150 m of treated winter wheat. Patches often co-occurred with deer feeders containing protein pellets and whole corn, which can be attractive to bobwhites. We used the corn provided as supplemental feed as bait in trapping efforts.

The South Texas Plains field site (hereafter, SOTX) was located near Edinburg, Texas. This region is dominated by agricultural production, including cotton, citrus, sorghum, corn, sugarcane, sunflower, and vegetables. Huisache (*Acacia farnesiana*) and grasses grow in abundance, providing excellent bobwhite habitat. The SOTX study site was managed for trophy white-tailed deer, exotic antelope, upland game bird hunting, and cattle ranching. This site bordered vast fields of annual sorghum and sunflower rotation crop. Hunting and trapping efforts were focused solely on patches of woody cover within approximately 50 m of agricultural fields. We used locally grown sorghum as bait in our traps, which was provided by the landowner who uses it as supplemental feed for bobwhites.

The Gulf Coast Prairies and Marshes field site (hereafter, GCPM) was located near Sealy, Texas, in the coastal prairies. The land at the GCPM field site is managed for cattle ranching and upland game bird hunting. Although neonicotinoids were not used by the property owner, cultivated cropland is located within ~ 1km of the pastures we

hunted. Hunting efforts focused on un-grazed pastures and did not target agricultural areas like the other two field sites. Bobwhites do not receive supplementary feed at the GCPM field site and therefore hunting was the only method used to collect samples.

3.2.2 Sample Collection

Sample collection and analyses were approved by Texas Parks and Wildlife Department (SPR-0493-605) and the Texas A&M University Institutional Animal Care and Use Committee (IACUC 2014-0183). Approximately 10 bobwhite carcasses were collected from each field site during fall 2014 and spring 2015, for a total of 61 samples. Quail were collected by hunting over bird dogs at all locations, and were also trapped at sites where supplemental feed was provided (i.e. ROPL and SOTX). Wire funnel traps baited with supplemental feed (corn and/or sorghum) were placed under shrubs or trees bordering agricultural plots or at roost sites, and trapped quail were euthanized via asphyxiation in a CO₂ chamber.

We conducted all necropsies in the field. Quail were placed on a hard, foil-covered surface and age (adult or juvenile) was determined by coloration of primary coverts. Bobwhites are sexually dimorphic and sex was recorded based on facial coloration. Body condition was recorded on a scale from 1-5 using the pectoral muscle and weight was recorded. External and internal abnormalities were recorded throughout the necropsy. Liver, kidney, spleen, and gonadal tissue were extracted for analysis and the remaining carcass was stored in the freezer at -18° C. One mm cross section of liver, whole kidneys, whole testes or ovaries, and whole spleen, incised to allow formalin

fixation, were stored in 90 mL plastic screw-top containers filled with approximately 60 mL of 10% neutral buffered formalin (VWR International, Radnor, Pennsylvania, USA) and stored at ambient temperature. The remaining liver tissue and whole crop were stored in Level I EPA quality-assured 60 mL glass screw-top containers (VWR International, Radnor, Pennsylvania, USA) on dry ice in the field prior to laboratory storage at -80° C, until chemical analysis. Foil was replaced and utensils were sequentially sterilized with boiling water and acetone between each necropsy.

3.2.3 Chemical Analysis

In order to determine if bobwhites are exposed to neonicotinoids in the field, we performed chemical analysis on all liver and crop content samples we collected. Chemical analysis was conducted using sample preparation methods of Xiao et al. (2011) and UPLC-MS/MS methods of Galeano et al. (2013). Liver and crop samples were extracted using an accelerated solvent extractor (ASE, Dionex, USA) equipped with 11 mL and 33 mL stainless steel cells (for liver and crop samples, respectively). Liver tissue was weighed and homogenized with diatomaceous earth (DE; 1:1.25, tissue/DE; Agilent Technologies, Santa Clara, California, USA) in a 100 mL mortar. Crop tissue was weighed and loosely ground (but not homogenized) with diatomaceous earth (1:1.25, tissue/DE) in a 100 mL mortar to prevent samples from clogging the cells. Mixtures were loaded into 11 mL or 33 mL cells fitted with a cellulose filter disk (Environmental Express, Atlanta, Georgia, USA) and 10 µm frit at the bottom. Caps, also fitted with a 10 µm frit, were screwed into placement, and cells were loaded onto

the extractor. Extracts were collected in 60 mL glass vials. Extraction conditions included: pure water as extraction solvent, static extraction time of 5 minutes, two static cycles, extraction temperature at 80° C, and extraction pressure at 10 MPa.

Extracts were placed in the refrigerator at 2° C for a minimum of 15 minutes prior to cleanup. The supernatant was then loaded into a 500 mL Oasis HLB cartridge (Waters Corp., Milford, Massachusetts, USA) previously conditioned with 5 mL methanol and 5 mL pure water (Fisher Scientific, Pittsburgh, Pennsylvania, USA). Sample extracts were passed through the columns under a vacuum and columns were rinsed with 5 mL of water and 5 mL of methanol-water (20:80, v/v). The analytes were eluted with 3 mL of methanol and the eluate was evaporated under a gentle stream of nitrogen at 40° C. Remaining residue was topped to 1 mL with methanol-water (30:70 v/v) and syringe filtered using a 0.2 µm nylon filter (VWR International, Radnor, Pennsylvania, USA) into an auto sampler vial.

A Waters ACQUITY UPLC/MS system (Waters Corp., Milford, Massachusetts, USA) was used. The UPLC was equipped with a binary solvent manager, sample manager, column heater, with a tandem quadrupole (TQD) mass spectrometer equipped with an ESI source. The column used was an Acquity UPLC BEH Shield RP18 column, 150 mm × 2.1 mm, 1.7 µm (Waters Corp., Milford, MA). Nitrogen was used both as a drying gas and as nebulizing gas, while argon was used as the collision gas (Praxair, Bryan, TX). The nitrogen gas flow conditions were 450 and 50 L/h for desolvation and at the cone, respectively. The source block temperature and desolvation temperature were set at 120 and 250 °C, respectively. The solvents were 0.05% formic acid in H₂O

(solvent A) and acetonitrile (solvent B). The gradient was 10% B from 0 to 3 min, 95% B from 2 to 3 min, 10% B isocratic from 4-5 minutes to allow for column equilibration before the next injection. The flow rate was 0.2 mL/min. Neonicotinoids were detected in MS/MS conditions, programming the chromatographic run in the SRM mode (selected reaction monitoring) as reported in Table 3.1.

The limits of detection and quantification were determined by signal-to-noise approach as demonstrated by Galeano et al. (2013). Limits of quantification are as follows: acetamiprid, 0.12 µg/kg; clothianidin, 3.20 µg/kg; imidacloprid, 2.80 µg/kg; and thiamethoxam, 0.50 µg/kg, and limits of detection were approximately 30% of the limits of quantification.

Table 3.1 UPLC-MS/MS Fragmentation of Neonicotinoid Compounds

Compound	Precursor ion (m/z)	Product Ion (m/z)	Cone Voltage (V)	Collision energy (eV)
		126.2	25	18
Acetamiprid	223.0	56.0	25	18
		168.9	20	15
Clothianidin	250.0	131.8	20	15
		129.0	20	17
Dinotefuran	203.1	113.0	20	17
		209	25	25
Imidacloprid	256.0	175	25	25
		211.1	23	17
Thiamethoxam	292.0	131.9	23	17

3.2.4 Histopathology Analysis

To determine if bobwhites exhibited evidence of tissue damage consistent with the known results of neonicotinoid intoxication, we performed histopathological analysis on various tissues. Liver, kidney, spleen, and gonadal tissues were stored in 10% neutral buffered formalin and delivered to the necropsy lab in the Department of Veterinary Pathobiology at Texas A&M University. Samples were routinely processed for paraffin embedding and slides (5 µm sections) were stained with hematoxylin and eosin prior to careful examination under a microscope. Histological and pathological abnormalities were recorded.

3.3 Results

3.3.1 Chemical Analysis

Of the 61 liver samples analyzed, at least one neonicotinoid compound was detected in 11% (n = 7) of samples. In fall 2014, we detected imidacloprid in one bobwhite and acetamiprid, clothianidin, and thiamethoxam in another bobwhite at the SOTX field site. We also detected acetamiprid in 2 bobwhites at the ROPL field site during fall 2014. In spring 2015 at the GCPM field site, we detected acetamiprid in the liver of one bobwhite and acetamiprid, clothianidin, and thiamethoxam in the liver of another. We also detected imidacloprid in the liver of one bobwhite at the SOTX field site during spring 2015. The detection levels, however, were below the limit of quantitation in all cases. We did not detect neonicotinoid compounds in any of the 53 crop samples analyzed.

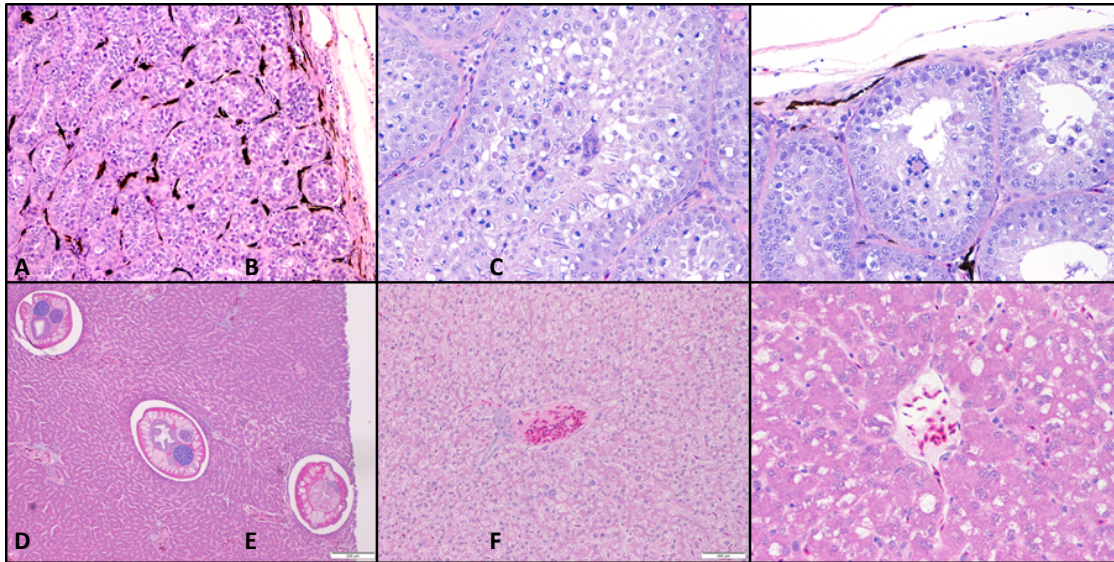
3.3.2 Histopathology Analysis

Foci of lymphoplasmacytic inflammatory infiltrate and granulomas were present in the livers and kidneys of 4 bobwhites. No infectious agents were detected, but in one bird an intralesional nematode was observed in the liver. Mild tubulitis with intratubular crystals in the kidney was present in 8 bobwhites. Mild to moderate hepatocellular vacuolation was a frequent finding (lipid- and glycogen-type; n = 10), and mild bile duct hyperplasia was present in 2 bobwhites. Multinucleated germ cells (n = 2) were also identified in the testes. The spleen of one bird had mild follicular lymphoid hyperplasia. Histological findings in the 7 birds containing neonicotinoid residues include: lipid-type hepatocellular vacuolation (n = 1), autolysis (n = 1), and hematopoiesis (n = 2) in the liver, few intratubular crystals and granulomatous tubulitis in the kidney (n = 1), and no unusual histopathological findings (n = 2). Images of selected histopathology findings are available in Figure 3.2.

3.4 Discussion

Our analyses indicate that 11% of the bobwhites we collected were recently exposed to neonicotinoids in the environment. Because neonicotinoid compounds are quickly metabolized *in vivo* and do not accumulate in animal tissues (Thyssen and Macheimer, 1999), it is likely that some of the birds we analyzed were exposed at some

Figure 3.2 Selected Histopathology Slides of Bobwhites Collected from High-Neonicotinoid Use Areas in Texas



(A) Immature testes; (B, C) Multinucleated cells in seminiferous tubules; (D) Intralesional nematode; (E) Glycogen-type hepatocellular vacuolation; (F) Lipid-type hepatocellular vacuolation.

point, but had broken down the parent compounds prior to collection, making them undetectable in our analysis. Additionally, it is possible that the almost immediate neurotoxic effect of neonicotinoids may have increased bobwhites' susceptibility to predation (Walker, 2003), as seen in the effects of methyl parathion, another acetylcholine inhibiting insecticide (Galindo et al., 1985; Buerger et al., 1991). This would have limited the number of exposed bobwhites we were able to collect.

We detected neonicotinoid compounds at levels below our limits of quantification, suggesting that bobwhites were exposed to neonicotinoids at some point before they were collected. The absence of neonicotinoids in the crop contents of all the

samples we analyzed indicates that bobwhites were not exposed at the time of collection, and the bait we used in trapping efforts was not contaminated.

Neonicotinoids are known to have synergistic effects when they occur together (Morrissey et al., 2015), and our analysis indicated that 2 bobwhites collected from the GCPM and SOTX field sites each contained 3 different neonicotinoid compounds in their livers. In spring 2015, 3 bobwhites in our analysis contained neonicotinoid compounds in their tissues, coinciding with the development of reproductive organs as bobwhites (and birds in general) physiologically prepare for the breeding season. Neonicotinoids are known to elicit sub-lethal reproductive effects including fewer and fragmented germ cells, reduced fertilization, lower body condition and survival in chicks, impaired embryonic development, reduced clutch size, and delayed egg laying (Balani et al., 2011; Lopez-Antia et al., 2013, 2015; Tokumoto et al., 2013; Pandey and Mohanty, 2015). R-selected species, such as bobwhites, invest energy in maximizing their reproductive capacity, but have limited longevity. Adult bobwhite annual survival is estimated to be as low as 18-30% (Hernández et al., 2007), and productivity and recruitment are therefore important for maintaining bobwhite populations. Exposure to neonicotinoids during the breeding season could affect reproduction, potentially having negative effects on bobwhite populations by limiting productivity.

Conversely, it is possible that pesticide application during the fall could limit over-wintering survival of birds, either directly or indirectly (Benton et al., 2002; Lopez-Antia et al., 2015). In fall 2014, 4 bobwhites in our analysis contained detectable levels of neonicotinoids in their livers. Body condition was considered ideal (score of 3) in all

4 bobwhites, and they all fell within one standard deviation of the mean body weight observed in our samples ($168.1 \text{ g} \pm 15.8 \text{ g}$), suggesting that food was not limited during the time of collection.

Neonicotinoid detections in bobwhites collected from the Rolling Plains and Gulf Coast Prairies and Marshes field sites coincided with the planting of main agricultural crops in each region (e.g. winter wheat is planted in the fall at ROPL while rice and cotton are planted in the spring at GCPM); however, at the South Texas Plains field site, we identified compounds in bobwhite livers in both the fall and spring. This site is bordered by an annual rotation crop of sunflower and sorghum, both of which are planted in the spring. Neonicotinoids are persistent in the environment, and the detection of compounds at this location in the fall (i.e. outside of the growing season) suggest that bobwhites may be exposed to these compounds throughout the year.

Despite the use of Gaucho® on winter wheat at the Rolling Plains field site, acetamiprid was the only neonicotinoid detected in the tissues of 2 quail collected in the fall at this site. Acetamiprid is also used on winter wheat, and it is therefore likely that collected birds were exposed to wheat treated with acetamiprid nearby. Similarly, there was no neonicotinoid use at the GCPM field site, but bobwhites may have been exposed to these compounds in cultivated cropland located ~ 1 km away.

The liver and testes are the secondary targets of neonicotinoids (Thyssen and Machamer, 1999), which damage tissues through oxidative stress (Tokumoto et al., 2013). Approximately 20% ($n = 12$) of the bobwhites we collected exhibited evidence of liver or testicular degeneration; however, only one bobwhite containing detectable

levels of neonicotinoids displayed tissue degeneration (lipid-type hepatocellular vacuolation) consistent with known results of neonicotinoid toxicity. Neonicotinoids metabolize quickly *in vivo*, thus, the absence of detectable compounds in bobwhites exhibiting evidence of neonicotinoid-induced tissue damage is not surprising. Lipid-type hepatocellular vacuolation identified in 8 bobwhites was similar to reported results of Japanese quail (*Coturnix japonica*), that were administered clothianidin and exhibited dose-dependent increases in the number and size of lipid droplets in liver hepatocytes (Tokumoto et al., 2013). Bile duct hyperplasia may be caused by oxidative stress (Bottari et al., 2015) resulting from neonicotinoid toxicity, and was identified in 2 bobwhites in our analysis. In previous studies, the administration of clothianidin to rats has resulted in abnormalities in male germ cells (Bal et al., 2012); we found multinucleated germ cells in the seminiferous tubules of 2 bobwhites in our analysis. The lesions we identified that corresponded to known results of neonicotinoid toxicity were likely a secondary toxic result of oxidative stress. Senescence, other pesticides, and various other causes may also induce oxidative stress and could elicit signs of tissue damage similar to what we observed.

We identified an intralesional nematode in the liver of one bobwhite. Immunosuppression has been linked to a higher susceptibility to parasite infestation (Köhler and Triebkorn, 2013) and was reported in birds exposed to neonicotinoid compounds (Balani et al., 2011; Lopez-Antia et al., 2013, 2015). In the last few years, parasitic eyeworms (*Oxyspirusa petrowi*) have been found in bobwhites in the Rolling Plains of Texas, and there is still much uncertainty regarding their effects (if any) on

bobwhite populations (Dunham et al., 2014). It is possible that exposure to neonicotinoids could increase bobwhites' susceptibility to parasite infestation, and further analysis investigating the relationship between neonicotinoid exposure and eyeworm prevalence could enhance our understanding of quail decline in the Rolling Plains and possibly elsewhere.

Our results indicate that 30% (n = 18) of the bobwhites we collected were either exposed to neonicotinoids in the environment or exhibited evidence of tissue damage corresponding to the known results of neonicotinoid toxicity. Based on our findings, we conclude that neonicotinoids may potentially negatively impact bobwhite populations that frequent cultivated croplands. Bobwhites may experience increased susceptibility to predation as a result of neonicotinoids' neurotoxic effects, and may undergo reproductive impairment, limiting their ability to maintain populations. They may also be predisposed to parasitic infestations (e.g. eyeworms) as a result of neonicotinoid-induced immune suppression. Our results are in agreement with similar findings from various other studies that have linked pesticide use with bird declines (Wilson et al., 1999; Beecher et al., 2002; Boatman et al., 2004; Mineau and Whiteside, 2013; Hallmann et al., 2014). Based on their potential harmful effects, a thorough ecological risk assessment is warranted to verify this relationship and quantify the risks neonicotinoids may pose to quail in Texas.

CHAPTER IV

CONCLUSIONS

The present study attempted to enhance our overall understanding of the mechanisms responsible for quail decline by investigating the impact of neonicotinoid insecticides on Northern bobwhites in Texas. We performed a historical analysis (1978-2012) to identify trends in bobwhite abundance and neonicotinoid use, as well as a field analysis to determine if bobwhites are exposed to neonicotinoids under natural circumstances, and if they exhibit evidence of tissue damage consistent with known results of neonicotinoid toxicity.

Results from our historical analysis, which encompassed the time periods prior to neonicotinoid introduction (1978-1993), after their introduction (1994-2003), and after their widespread use (2004-2012), suggest that neonicotinoid use may contribute to bobwhite decline in the High Plains, Rolling Plains, Gulf Coast Prairies and Marshes, Edwards Plateau, and possibly the South Texas Plains ecoregions. Each of these ecoregions receive moderate to high levels of neonicotinoid use and produce a variety of crops used by bobwhites for feeding or foraging (e.g. corn, soybeans, sorghum, winter wheat, sunflower, and cotton). All of these crops are frequently protected by neonicotinoids, which are usually applied as a seed dressing. Bobwhites may be exposed to neonicotinoids in the form of neonicotinoid-treated seeds or contaminated insects and vegetation foraged from field margins bordering cropland treated with neonicotinoids. The negative relationships we identified between bobwhite abundance

and neonicotinoid use may result from direct, adverse, sub-lethal (and lethal) effects elicited by neonicotinoids, including immune suppression, impaired reproduction, and neurotoxicity, and possibly the indirect effect of prey-collapse.

We detected at least one neonicotinoid compound (imidacloprid, acetamiprid, clothianidin, or thiamethoxam) in 11% of bobwhites collected from the Rolling Plains, Gulf Coast Prairies and Marshes, and South Texas Plains. All neonicotinoid detections were made in liver samples, and none of the crop contents we analyzed contained neonicotinoid residues. Additionally, neonicotinoids were detected in bobwhite tissues during and outside of growing seasons, suggesting that bobwhites may be exposed throughout the year.

Neonicotinoids elicit adverse effects on the liver and testes through oxidative stress. We identified evidence of liver and testicular tissue damage corresponding to the known results of neonicotinoid toxicity in 20% of collected bobwhites. These included hepatocellular vacuolation and bile duct hyperplasia (indicative of liver degeneration), and multinucleated germ cells (indicative of testicular degeneration).

In 2012, twenty Texas counties each applied over 2500 kg of neonicotinoids, and 12 of those applied greater than 5000 kg. Their potential effects on bobwhites and other grassland birds cannot be ignored. To better understand the mechanisms behind quail decline, we suggest a thorough ecological risk assessment to evaluate the risks neonicotinoids pose to Texas bobwhites.

LITERATURE CITED

- Allen, C.R., Lutz, R.S., Demarais, S., 1995. Red imported fire ant impacts on northern bobwhite populations. *Ecol. Appl.* 632-638.
- ArcGIS, 2011. Breeding Bird Survey Route Locations for Lower 48 States (Data Basin Dataset).
- Badgujar, P.C., Jain, S.K., Singh, A., Punia, J.S., Gupta, R.P., Chandratre, G.A., 2013. Immunotoxic effects of imidacloprid following 28 days of oral exposure in BALB/c mice. *Environ. Toxicol. Pharmacol.* 35, 408-418 doi: 10.1016/j.etap.2013.01.012.
- Baker, N.T., Stone, W.W., 2015. Estimated annual agricultural pesticide use for counties of the conterminous United States, 2008-2012: U.S. Geological Survey Data Series. Estimated annual agricultural pesticide use for counties of the conterminous United States, 2008-12 907, 9 p doi: <http://dx.doi.org/10.3133/ds907>.
- Bal, R., Türk, G., Yılmaz, Ö., Etem, E., Kuloğlu, T., Baydaş, G., Naziroğlu, M., 2012. Effects of clothianidin exposure on sperm quality, testicular apoptosis and fatty acid composition in developing male rats. *Cell Biol. Toxicol.* 28, 187-200.
- Balani, T., Agrawal, S., Thaker, A.M., 2011. Hematological and biochemical changes due to short-term oral administration of imidacloprid. *Toxicology International* 18, 2-4 doi: 10.4103/0971-6580.75843.
- Beecher, N.A., Johnson, R.J., Brandle, J.R., Case, R.M., Young, L.J., 2002. Agroecology of birds in organic and nonorganic farmland. *Conserv. Biol.* 16, 1620-1631 doi: 10.1046/j.1523-1739.2002.01228.x.
- Benton, T.G., Bryant, D.M., Cole, L., Crick, H.Q.P., 2002. Linking agricultural practice to insect and bird populations: a historical study over three decades. *J. Appl. Ecol.* 39, 673-687 doi: 10.1046/j.1365-2664.2002.00745.x.
- Berny, P.J., Buronfosse, F., Videmann, B., Buronfosse, T., 1999. Evaluation of the toxicity of imidacloprid in wild birds. A new high performance thin layer chromatography (HPTLC) method for the analysis of liver and crop samples in suspected poisoning cases. *J. Liq. Chromatogr. Rel. Technol.* 22, 1547-1559 doi: 10.1081/JLC-100101750.
- Best, L.B., Whitmore, R.C., Booth, G.M., 1990. Use of cornfields by birds during the breeding season: the importance of edge habitat. *Am. Midl. Nat.* 123, 84-99.

- Blacqui re, T., Smagge, G., van Gestel, C. A. M., Mommaerts, V., 2012. Neonicotinoids in bees: a review on concentrations, side-effects and risk assessment. *Ecotoxicology* 21, 973-992 doi: 10.1007/s10646-012-0863-x.
- Boatman, N.D., Brickle, N.W., Hart, J.D., Milsom, T.P., Morris, A.J., Murray, A.W.A., Murray, K.A., Robertson, P.A., 2004. Evidence for the indirect effects of pesticides on farmland birds. *Ibis* 146, 131-143.
- Bottari, N.B., Mendes, R.E., Lucca, N.J., Schwertz, C.I., Henker, L.C., Olsson, D.C., Piva, M.M., Sangoi, M., Campos, L.P., Moresco, R.N., 2015. Oxidative stress associated with pathological lesions in the liver of rats experimentally infected by *Fasciola hepatica*. *Exp. Parasitol.* 159, 24-28.
- Brennan, L.A., 1991. How can we reverse the Northern bobwhite population decline? *Wildl. Soc. Bull.* 19, 544-555.
- Brennan, L.A., Kuvlesky Jr., W.P., 2005. North American grassland birds: an unfolding conservation crisis? *J. Wildl. Manage.* 69, 1-13 doi: [http://dx.doi.org/10.2193/0022-541X\(2005\)069<0001:NAGBAU>2.0.CO;2](http://dx.doi.org/10.2193/0022-541X(2005)069<0001:NAGBAU>2.0.CO;2).
- Bridges, A.S., Peterson, M.J., Silvy, N.J., Smeins, F.E., Wu, X.B., 2001. Differential influence of weather on regional quail abundance in Texas. *J. Wildl. Manage.* 65, 10-18 doi: 10.2307/3803270.
- Buerger, T.T., Kendall, R.J., Mueller, B.S., Devos, T., Williams, B.A., 1991. Effects of methyl parathion on northern bobwhite survivability. *Environmental Toxicology and Chemistry* 10, 527-532 doi: 10.1002/etc.5620100413.
- Burnham, K.P., Anderson, D.R., 2002. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*, 2nd ed. Springer Science & Business Media, New York, New York, U.S.A.
- Bro, E., Decors, A., Millot, F., Soyez, D., Moinet, M., Berny, P., Mastain, O., 2010. Intoxications des perdrix grises en nature. Nouveau bilan de la surveillance. *Faune Sauvage* 289, 26-32.
- Chagnon, M., Kreutweizer, D., Mitchell, E.A.D., Morrissey, C.A., Noome, D.A., Van der Sluijs, J. P., 2014. Risks of large-scale use of systemic insecticides to ecosystem functioning and services. *Environ. Sci. Pollut. Res. Int.* 22, 119-134 doi: 10.1007/s11356-014-3277-x.
- Daly, C., Halbleib, M., Smith, J.I., Gibson, W.P., Doggett, M.K., Taylor, G.H., Curtis, J., Pasteris, P.P., 2008. Physiographically sensitive mapping of climatological

temperature and precipitation across the conterminous United States. *Int. J. Climatol.* 28, 2031-2064 doi: 10.1002/joc.1688.

de Snoo, G.R., Scheidegger, N.M.I., de Jong, Fr. M. W., 1999. Vertebrate wildlife incidents with pesticides: a European survey. *Pestic. Sci.* 55, 47-54 doi: 10.1002/(SICI)1096-9063(199901)55:1<47::AID-PS859>3.0.CO;2-#.

Douglas, M.R., Tooker, J.F., 2015. Large-scale deployment of seed treatments has driven rapid increase in use of neonicotinoid insecticides and preemptive pest management in U.S. field crops. *Environ. Sci. Technol.* 49, 5088-5097 doi: 10.1021/es506141g.

Dunham, N.R., Soliz, L.A., Fedynich, A.M., Rollins, D., Kendall, R.J., 2014. Evidence of an *Oxyspirura petrowi* epizootic in Northern Bobwhites (*Colinus virginianus*), Texas, USA. *J. Wildl. Dis.* 50, 552-558 doi: 10.7589/2013-10-275.

Elbert, A., Haas, M., Springer, B., Thielert, W., Nauen, R., 2008. Mini-review: Applied aspects of neonicotinoid uses in crop protection. *Pest Manag. Sci.* 64, 1099-1105 doi: 10.1002/ps.1616.

EROS, 2013. Landcover Modeling. US Geological Survey Earth Resources Observation and Science Center. 2015.

ESRI, 2011. ArcGIS Desktop. Environmental Systems Research Institute, Redlands, CA, USA. Release 10.

EU, 2013. European Union Regulation 485/2013.

Fairbrother, A., Purdy, J., Anderson, T., Fell, R., 2014. Risks of neonicotinoid insecticides to honeybees. *Environmental Toxicology and Chemistry* 33, 719-731 doi: 10.1002/etc.2527.

Fossen, M., 2006. Environmental fate of imidacloprid. California Department of Pesticide Regulation.

Galeano, P.M., Scordino, M., Sabatino, L., Panto, V., Morabito, G., Chiappara, E., Traulo, P., Gagliano, G., 2013. UHPLC/MS-MS analysis of six neonicotinoids in honey by modified QuEChERS: Method development, validation, and uncertainty measurement. *International Journal of Food Science* 2013, 1-7 doi: 10.1155/2013/863904.

Galindo, J.C., Kendall, R.J., Driver, C.J., Lacher Jr., T.E., 1985. The effect of methyl parathion on susceptibility of bobwhite quail (*Colinus virginianus*) to domestic cat predation. *Behav. Neural Biol.* 43, 21-36.

- Gibbons, D., Morrissey, C., Mineau, P., 2015. A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. *Environ. Sci. Pollut. Res. Int.* 22, 103-118 doi: 10.1007/s11356-014-3180-5.
- Gill, R.J., Ramos-Rodriguez, O., Raine, N.E., 2012. Combined pesticide exposure severely affects individual-and colony-level traits in bees. *Nature* 491, 105-108 doi: 10.1038/nature11585.
- Gould, F.W., 1975. Texas plants- a checklist and ecological summary. Texas A&M University, Agricultural Experiment Station.
- Goulson, D., 2013. Review: An overview of the environmental risks posed by neonicotinoid insecticides. *J. Appl. Ecol.* 50, 977-987 doi: 10.1111/1365-2664.12111.
- Goulson, D., 2014. Ecology: Pesticides linked to bird declines. *Nature* 511, 295-296.
- Greatti, M., Barbattini, R., Stravisi, A., Sabatini, A.G., Rossi, S., 2006. Presence of the a.i. imidacloprid on vegetation near corn fields sown with Gaucho ® dressed seeds. *Bulletin of Insectology* 59, 99-103.
- Hallmann, C.A., Foppen, R.P.B., van Turnhout, C. A. M., de Kroon, H., Jongejans, E., 2014. Declines in insectivorous birds are associated with high neonicotinoid concentrations. *Nature* doi: doi:10.1038/nature13531.
- Heddinghaus, T.R., Sabol, P., 1991. A review of the Palmer Drought Severity Index and where do we go from here? *Proceedings of the 7th Conference on Applied Climatology*, 242-246.
- Henry, M., Beguin, M., Requier, F., Rollin, O., Odoux, J.F., Aupinel, P., Aptel, J., Tchamitchian, S., Decourtye, A., 2012. A common pesticide decreases foraging success and survival in honey bees. *Science* 336, 348-350 doi: 10.1126/science.1215039.
- Hernández, F., Hernández, F., Arredondo, J.A., Bryant, F.C., Brennan, L.A., Bingham, R.L., 2005. Influence of precipitation on demographics of northern bobwhites in southern Texas. *Wildl. Soc. Bull.* 33, 1071-1079.
- Hernández, F., Peterson, M.J., Brennan, L.A., 2007. Northern bobwhite ecology and life history. *Texas Quails: Ecology and Management* , 40-64.
- Hladik, M.L., Kolpin, D.W., Kuivila, K.M., 2014. Widespread occurrence of neonicotinoid insecticides in streams in a high corn and soybean producing region, USA. *Env. Poll.* 193, 189-196 doi: 10.1016/j.envpol.2014.06.033.

- Janke, A.K., Gates, R.J., 2013. Home range and habitat selection of Northern bobwhite coveys in an agricultural landscape. *J. Wildl. Manage.* 77, 405-413 doi: 10.1002/jwmg.461.
- Jeschke, P., Nauen, R., Schindler, M., Elbert, A., 2011. Overview of the status and global strategy for neonicotinoids. *J. Agric. Food Chem.* 59, 2897-2908 doi: 10.1021/jf101303g.
- Johnson, R., Corn, M.L., 2015. Bee health: the role of pesticides. Congressional research service report. Congressional Research Service: Report R43900, 4-8.
- Köhler, H.R., Triebkorn, R., 2013. Wildlife ecotoxicology of pesticides: can we track effects to the population level and beyond? *Science* 341, 759-765 doi: 10.1126/science.1237591.
- Kreutzweiser, D.P., Thompson, D.G., Scarr, T.A., 2009. Imidacloprid in leaves from systemically treated trees may inhibit litter breakdown by non-target invertebrates. *Ecotoxicol. Environ. Saf.* 72, 1053-1057 doi: 10.1016/j.ecoenv.2008.09.017.
- Krohn, P.W., Becker, R.C., Hungenberg, H., 2008. Synergistic mixtures exhibiting insecticidal and fungicidal action. 2008, US Patent 20,080,261,811.
- Krupke, C.H., Hunt, G.J., Eitzer, B.D., Andino, G., Given, K., 2012. Multiple routes of pesticide exposure for honey bees living near agricultural fields. *PLoS one* 7, e29268 doi: 10.1371/journal.pone.0029268.
- Larson, J.A., Fulbright, T.E., Brennan, L.A., Hernández, F., Bryant, F.C., 2010. *Texas Bobwhites: A Guide to their Foods and Habitat Management*. University of Texas Press, Austin, Texas, USA.
- Lewis, K.A., Green, A., Tzilivakis, J., Warner, D., 2015. The Pesticide Properties DataBase (PPDB) developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, 2006-2015.
- Lopez-Antia, A., Ortiz-Santaliestra, M.E., Mougeot, F., Mateo, R., 2013. Experimental exposure of red-legged partridges (*Alectoris rufa*) to seeds coated with imidacloprid, thiram and difenoconazole. *Ecotoxicology* 22, 125-138 doi: 10.1007/s10646-012-1009-x.
- Lopez-Antia, A., Ortiz-Santaliestra, M.E., Mateo, R., 2014. Experimental approaches to test pesticide-treated seed avoidance by birds under a simulated diversification of food sources. *Sci. Total Environ.* 496, 179-187 doi: <http://dx.doi.org/10.1016/j.scitotenv.2014.07.031>.

- Lopez-Antia, A., Ortiz-Santaliestra, M.E., Mougeot, F., Mateo, R., 2015. Imidacloprid-treated seed ingestion has lethal effect on adult partridges and reduces both breeding investment and offspring immunity. *Environ. Res.* 136, 97-107 doi: 10.1016/j.envres.2014.10.023.
- Lusk, J.J., Guthery, F.S., George, R.R., Peterson, M.J., DeMaso, S.J., 2002. Relative abundance of bobwhites in relation to weather and land use. *The Journal of Wildlife Management* 66, 1040-1051 doi: 10.2307/3802936.
- Madison, L.A., Robel, R.J., 2001. Energy Characteristics and Consumption of Several Seeds Recommended for Northern Bobwhite Food Plantings. *Wildl. Soc. Bull.* 29, 1219-1227.
- Main, A.R., Headley, J.V., Peru, K.M., Michel, N.L., Cessna, A.J., Morrissey, C.A., 2014. Widespread Use and Frequent Detection of Neonicotinoid Insecticides in Wetlands of Canada's Prairie Pothole Region. *PloS One* 9, e92821 doi: 10.1371/journal.pone.0092821.
- fossarum. *Pestic. Biochem. Physiol.* 104, 178-186 doi: 10.1016/j.pestbp.2012.07.008.
- Mason, R., Tennekes, H., Sánchez-Bayo, F., Jepsen, P.U., 2013. Immune suppression by neonicotinoid insecticides at the root of global wildlife declines. *Journal of Environmental Immunology and Toxicology* 1, 3-12 doi: 10.7178/jeit.1.
- Michael, V.C., Beckwith, S.L., 1955. Quail preference for seed of farm crops. *The Journal of Wildlife Management* 19, 281-296 doi: 10.2307/3796864.
- Mineau, P., 2005. A review and analysis of study endpoints relevant to the assessment of "long term" pesticide toxicity in avian and mammalian wildlife. *Ecotoxicology* 14, 775-799 doi: 10.1007/s10646-005-0028-2.
- Mineau, P., Palmer, C., 2013. The impact of the nation's most widely used insecticides on birds. *American Bird Conservancy, U.S.A.*
- Mineau, P., Whiteside, M., 2013. Pesticide acute toxicity is a better correlate of US grassland bird declines than agricultural intensification. *PLoS One* 8, e57457 doi: 10.1371/journal.pone.0057457.
- Moorman, C.E., Plush, C.J., Orr, D.B., Reberg-Horton, C., 2013. Beneficial Insect Borders Provide Northern Bobwhite Brood Habitat. *PloS one* 8, e83815 doi: 10.1371/journal.pone.0083815.
- Morrissey, C.A., Mineau, P., Devries, J.H., Sanchez-Bayo, F., Liess, M., Cavallaro, M.C., Liber, K., 2015. Neonicotinoid contamination of global surface waters and

associated risk to aquatic invertebrates: A review. *Environ. Int.* 74, 291-303 doi: 10.1016/j.envint.2014.10.024.

NOAA, 2015. Palmer drought severity index divisional data. National Ocean and Atmospheric Administration.

Pandey, S.P., Mohanty, B., 2015. The neonicotinoid pesticide imidacloprid and the dithiocarbamate fungicide mancozeb disrupt the pituitary–thyroid axis of a wildlife bird. *Chemosphere* 122, 227-234
doi:<http://dx.doi.org/10.1016/j.chemosphere.2014.11.061>.

Pardieck, K.L., Ziolkowski Jr., D.J., Hudson, M.A.R., 2015. North American Breeding Bird Survey Dataset 1966 - 2014, version 2014.0. U.S. Geological Survey, Patuxent Wildlife Research Center.

Peterson, M.J., Perez, R.M., 2000. Is quail hunting self-regulatory? Northern bobwhite and scaled quail abundance and quail hunting in Texas. 4, 85-91.

Pollack, P., 2011. *Fine Chemicals: The Industry and the Business*. 2nd ed. John Wiley & Sons Inc., Hoboken, New Jersey, USA.

R Core Team, 2015. *R: A Language and Environment for Statistical Computing*.

Sauer, J.R., Hines, J.E., Gough, G., Thomas, I., Peterjohn, B.G., 1997. *The North American Breeding Bird Survey Results and Analysis*. Version 96.4. Patuxent Wildlife Research Center, Laurel, MD.

Sauer, J.R., Hines, J.E., Fallon, J.E., Pardieck, K.L., Ziolkowski, D.J.J., Link, W.A., 2012. *The North American Breeding Bird Survey, Results and Analysis 1966 - 2011*. Version 12.13.2011 USGS Patuxent Wildlife Research Center, Laurel, MD.

Scott, T.G., 1985. *Bobwhite Thesaurus*, 1 ed. International Quail Foundation.

Texas Almanac, 2014. Texas crop production: acres, yield, value. Texas State Historical Association.

Thelin, G.P., Stone, W.W., 2013. Estimation of annual agricultural pesticide use for counties of the conterminous United States, 1992–2009: U.S. Geological Survey Scientific Investigations Report . 2013-5009, 54 p.

Thyssen, J., Machemer, L., 1999. Imidacloprid: toxicology and metabolism, in: *Nicotinoid insecticides and the nicotinic acetylcholine receptor*, Yamamoto et al., Springer, Japan, pp. 213-222.

- TNRIS, 2015. Data Catalog. Texas Natural Resources Information System.
[htTPWD://tnris.org](http://tpwd://tnris.org).
- Tokumoto, J., Danjo, M., Kobayashi, Y., Kinoshita, K., Omotherara, T., Tatsumi, A., Hashiguchi, M., Sekijima, T., Kamisoyama, H., Yokoyama, T., Kitawgawa, H., Hoshi, N., 2013. Effects of exposure to clothianidin on the reproductive system of male quails. *Journal of Veterinary Medical Science* 75, 755-760 doi: 10.1292/jvms.12-0544.
- Toll, P.A., 1990. An Acute Oral LD50 with Bobwhite Quail. Technical NTN 33893, 25p.
- Tomeček, J.M., Pierce, B.L., Peterson, M.J., 2015. Quail abundance, hunter effort, and harvest of two Texas quail species: implications for hunting management. *Wildl. Biol.* 21, 303-311 doi: 10.2981/wlb.00075.
- Tomizawa, M., Casida, J.E., 2003. Selective toxicity of neonicotinoids attributable to specificity of insect and mammalian nicotinic receptors. *Annu. Rev. Entomol.* 48, 339-364 doi: 10.1146/annurev.ento.48.091801.112731.
- Tri, A.N., Sands, J.P., Buelow, M.C., Williford, D., Wehland, E.M., Larson, J.A., Hernández, F., Hardin, J.B., Hernández, F., Brennan, L.A., 2012. Impacts of weather on Northern bobwhite sex ratios, body mass, and annual production in South Texas. *J. Wildl. Manage.* 77, 579-586 doi: 10.1002/jwmg.490.
- Turaga, U., Peper, S.T., Dunham, N.R., Kumar, N., Kistler, W., Almas, S., Presley, S.M., Kendall, R.J., 2015. A survey of neonicotinoid use and potential exposure to Northern Bobwhite (*Colinus virginianus*) and Scaled quail (*Callipepla squamata*) in the rolling plains of Texas and Oklahoma. *Environ. Toxicol. Chem.* doi: 10.1002/etc.3305.
- USDA, 2010. Field crops: Usual planting and harvesting dates. Agricultural Handbook. USDA National Agricultural Statistics Service, Agricultural Handbook 628.
- USEPA, 2007. EFED Section 3 and IR-4 risk assessment for imidacloprid for use on soybeans, peanuts, kava, millet, oats, artichoke, wild raspberry, and caneberry subgroup 13A. PC code 129099. 72 pp.
- van der Sluijs, J. P., Amaral-Rogers, V., Belzunces, L.P., Bijleveld van Lexmond, M. F. I. J., Bonmatin, J.M., Chagnon, M., Downs, C.A., Furlan, L., Gibbons, D.W., et al., 2015. Conclusions of the Worldwide Integrated Assessment on the risks of neonicotinoids and fipronil to biodiversity and ecosystem functioning. *Environmental Science and Pollution Research* 22, 148-154 doi: 10.1007/s11356-014-3229-5.

- Walker, C.H., 2003. Neurotoxic pesticides and behavioural effects upon birds. *Ecotoxicology* 12, 307-316 doi: 10.1023/A:1022523331343.
- Whitehorn, P.R., O'Connor, S., Wackers, F.L., Goulson, D., 2012. Neonicotinoid Pesticide Reduces Bumble Bee Colony Growth and Queen Production. *Science* 336, 351-352 doi: 10.1126/science.1215025.
- Wilson, J.D., Morris, A.J., Arroyo, B.E., Clark, S.C., Bradbury, R.B., 1999. A review of the abundance and diversity of invertebrate and plant foods of granivorous birds in northern Europe in relation to agricultural change. *Agric., Ecosyst. Environ.* 75, 13-30.
- Xiao, Z., Li, X., Wang, X., Shen, J., Ding, S., 2011. Determination of neonicotinoid insecticides residues in bovine tissues by pressurized solvent extraction and liquid chromatography–tandem mass spectrometry. *Journal of chromatography. B* 879, 117-122 doi: 10.1016/j.jchromb.2010.11.008.
- Zuur, A., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., 2009. *Mixed Effects Models and Extensions in Ecology with R*. Springer Science & Business Media.

APPENDIX A

Figure A.1 Northern Bobwhite Distribution

1990's

2000's

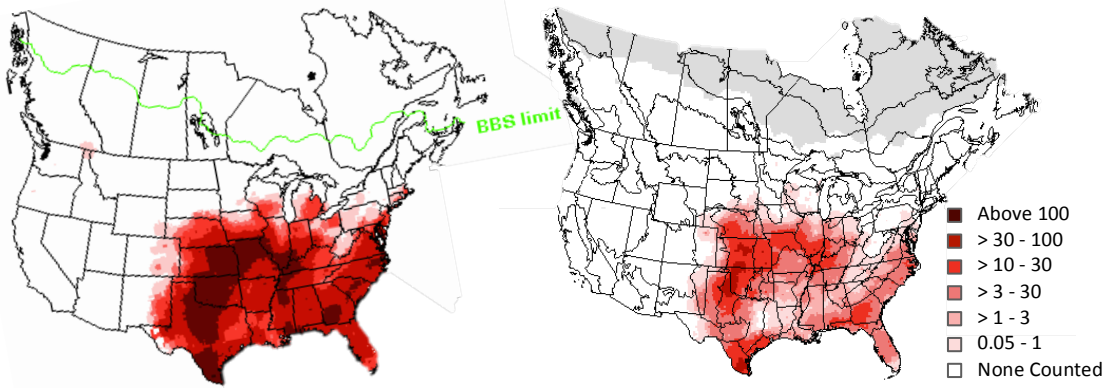


Figure modified from Sauer et al. (1997, 2012).

Figure A.2 Distribution of Breeding Bird Survey and Texas Parks and Wildlife Department Study Plots Included in the Historical Analysis

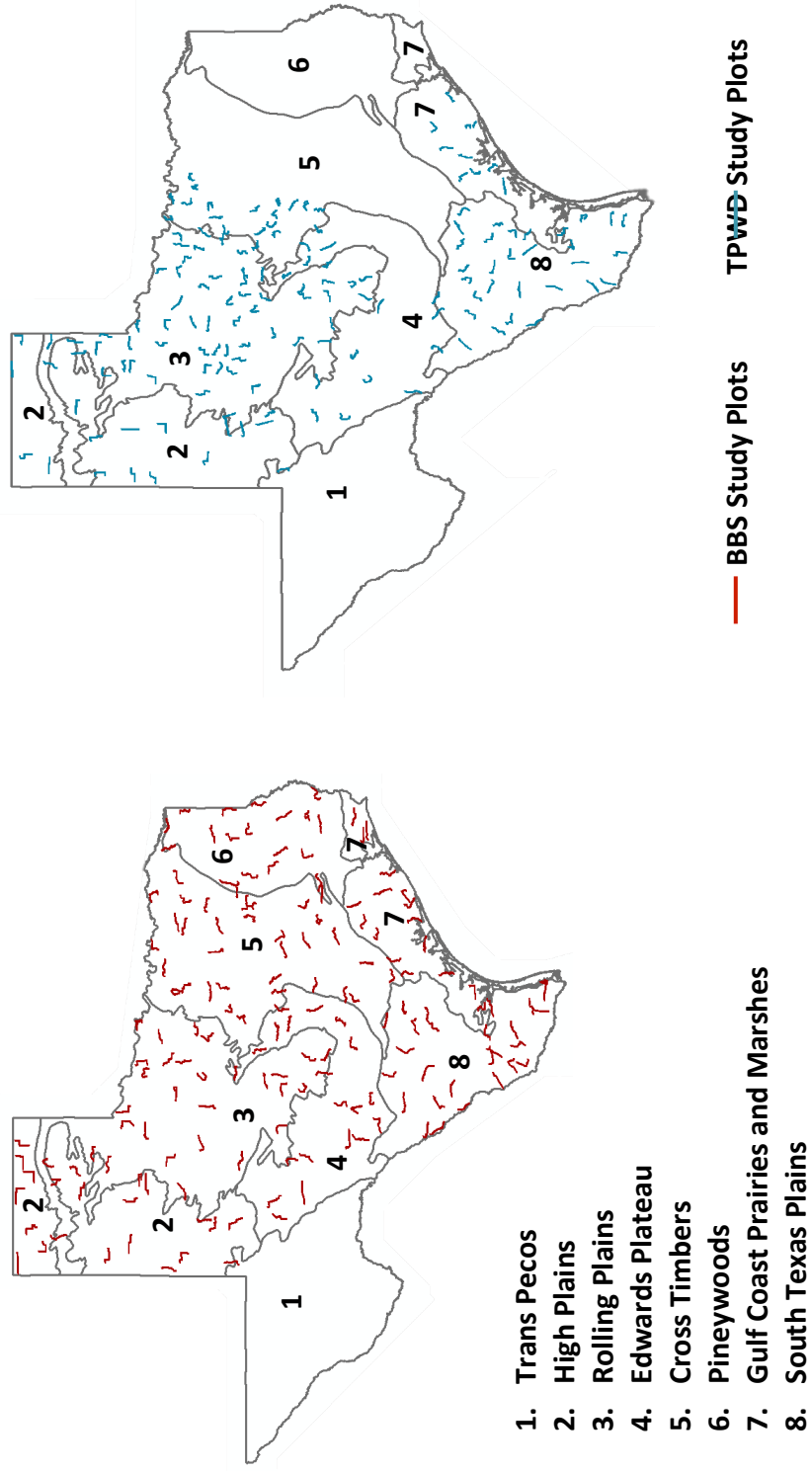
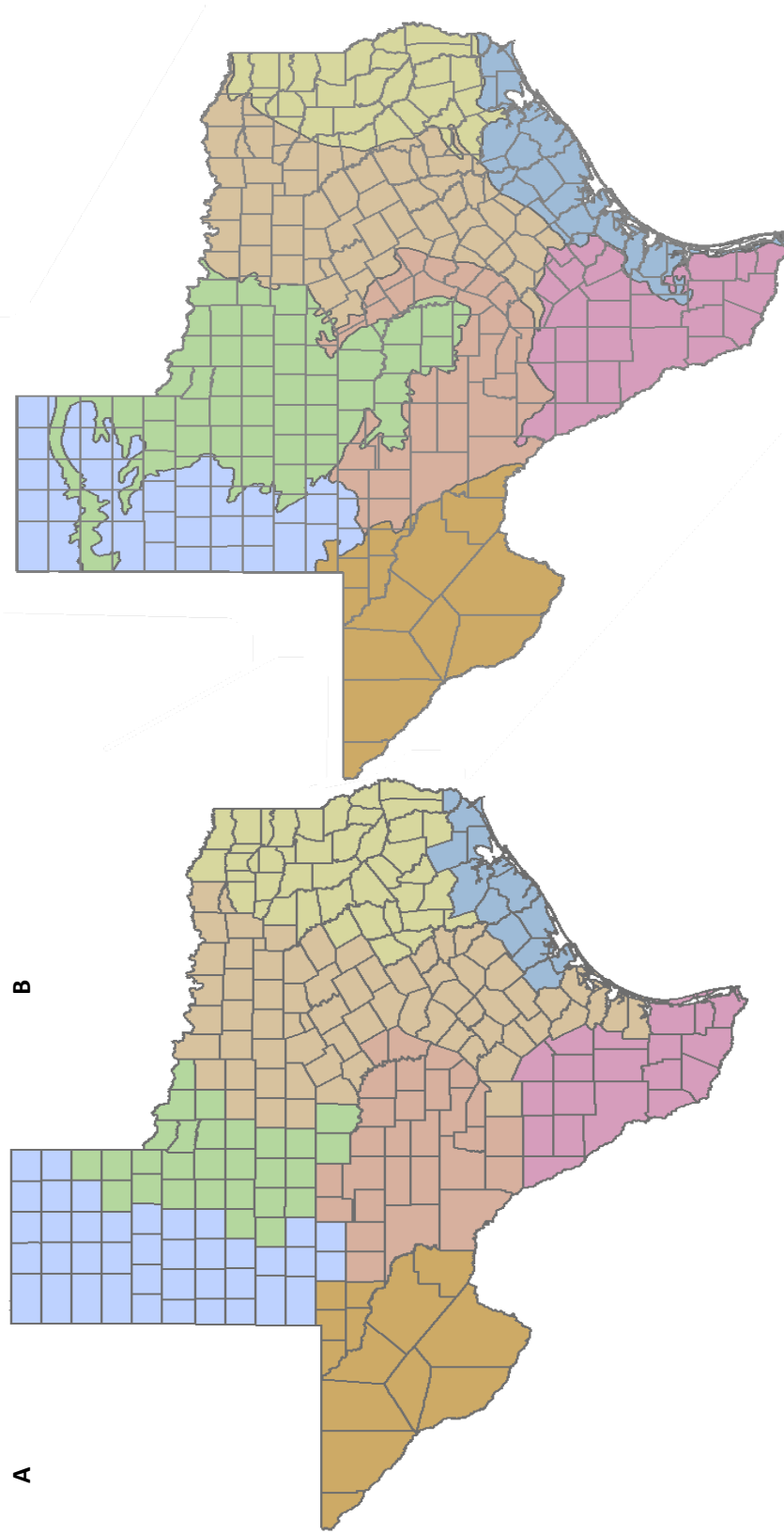


Figure A.3 Palmer Modified Drought Index Regions Used in the Historical Analysis



(A) Palmer Modified Drought Index Regions and (B) Texas Ecoregions

Table A.1 Description Of Variables Used In the Historical Analysis.

Variable	Category	Description	Source
Abundance	Response	Quail abundance within route.	TPWD 2015, Pardieck et al. 2014
Temperature	Climate	Summer mean maximum monthly temperature within route.	Daly et al. 2008
Precipitation	Climate	Total breeding season precipitation within route.	Daly et al. 2008
Drought Index	Climate	Summer Palmer Modified Drought Index within route.	NOAA 2015
Developed Area	Land Use	Total developed area within route.	EROS 2015
Agricultural Area	Land Use	Total agricultural area within route.	EROS 2015
Neonicotinoid Use	Pesticide	Total neonicotinoid application within route (ePest High estimate).	Thelin and Stone 2013, Baker and Stone 2015
*Ecoregion	Boundary	Gould ecoregions.	TNRIS 2015

* denotes a random effect variable when included in mixed-model analysis

Figure A.4 Sample Script of Historical Analysis

```
# Statewide Heavy-BBS
#####
library(MASS)
library(psc1)
library(car)
library(mgcv)
library(AICcmodavg)

# Check Null Model and Select Variables
mod0 <- glm.nb(abun ~ 1, data = heavy.bbs)
AIC(mod0)

mod1 <- glm.nb(abun ~ pmdi + precip + temp + ag + dev + neonic + eco, data
               = heavy.bbs)
stepAIC(mod1, direction = "both")
AIC(mod1)

mod2 <- glm.nb(abun ~ pmdi + ag + neonic + eco, data = heavy.bbs)
stepAIC(mod2, direction = "both")
AIC(mod2)
vif(mod2)

# Fit Candidate Models
glm <- glm.nb(abun ~ pmdi + ag + neonic + eco, data = heavy.bbs)
gam <- gam(abun ~ pmdi + ag + neonic + eco, data = heavy.bbs, family =
          negbin(1), optimizer = "perf")
zinb <- zeroinfl(abun ~ pmdi + ag + neonic + eco, data = heavy.bbs, dist =
             "negbin")
hurdle <- hurdle(abun ~ pmdi + ag + neonic + eco, data = heavy.bbs, dist =
              "negbin")
library(glmmADMB)
glmm <- glmmadmb(abun ~ pmdi + ag + neonic + (1|eco), data = heavy.bbs,
                family = "nbinom", admb.opts = admbControl(noinit=FALSE,
                  shess=FALSE))

# Calculate AICc Weights
LL <- c(logLik(glm), logLik(gam), logLik(zinb), logLik(hurdle),
        logLik(glmm))
K <- c(length(coef(glm)), length(coef(gam)), length(coef(zinb)),
        length(coef(hurdle)), length(coef(glmm)))
Modnames <- c("GLM", "GAM", "ZINB", "Hurdle", "GLMM")
aictabCustom(LL, K, modnames = Modnames, nobs = 1098)

# AICc Weight: ZINB 0.98

heavybbs.mod <- zinb
save(heavybbs.mod, file = paste0(wd, "/SelectedModels/heavybbs.mod.rda"))
rm(mod0, mod1, mod2, glm, gam, zinb, hurdle, glmm, LL, K, Modnames)
```

Table A.2 Count Model Correlation Coefficients and p Values

Statewide Results						
	Drought Index	Precip.	Temp.	Ag.	Dev.	Neonic. Use
Pre - BBS		0.003 p<0.09	0.07 p<0.002	-0.02 p<0.001		
Pre - TPWD	0.12 p<0.001	0.0005 p<0.1	0.09 p<0.003	-0.01 p<0.008	-0.26 p<0.04	
Light- BBS	0.04 p<0.12		0.07 p<0.02	-0.006 p<0.12		-0.009 p<0.02
Light- TPWD	0.06 p<0.003		0.06 p<0.012	0.005 p<0.09		-0.007 p<0.03
Heavy-BBS	0.06 p<0.001			0.005 p<0.27		-0.02 p<0.001
Heavy-TPWD	0.19 p<0.001	-0.002 p<0.001		-0.003 p<0.71		0.007 p<0.34
Cross Timbers Results						
	Drought Index	Precip.	Temp.	Ag.	Dev.	Neonic. Use
Pre - BBS	-0.05 p<0.014			-0.03 p<0.001	-0.03 p<0.03	
Pre - TPWD						
Light- BBS	0.13 p<0.02	0.002 p<0.006	-0.16 p<0.13	-0.07 p<0.001		
Light- TPWD	0.14 p<0.013	0.001 p<0.03		-0.02 p<0.008		
Heavy-BBS	-0.2 p<0.03		-0.29 p<0.008	-0.11 p<0.001	-0.07 p<0.11	
Heavy-TPWD			-0.22 p<0.16	0.003 p<0.97		0.18 p<0.61
Edwards Plateau Results						
	Drought Index	Precip.	Temp.	Ag.	Dev.	Neonic. Use
Pre - BBS		0.002 p<0.002	0.12 p<0.08	0.1 p<0.001	-0.12 p<0.30	
Pre - TPWD		0.002 p<0.004		0.02 p<0.13	-1.08 p<0.008	
Light- BBS					-0.27 p<0.014	-0.75 p<0.001
Light- TPWD		0.0002 p<0.59	-0.09 p<0.22			
Heavy-BBS			-0.21 p<0.001			-0.04 p<0.02
Heavy-TPWD		0.005 p<0.03	0.41 p<0.02			

Count Model Correlation Coefficients and p-Values Continued

Gulf Coast Prairies and Marshes Results

	Drought Index	Precip.	Temp.	Ag.	Dev.	Neonic. Use
Pre- BBS					0.18 p<0.001	
Pre - TPWD	0.14 p< 0.008		0.25 p<0.004		-2.41 p<0.06	
Light- BBS		-0.0009 p<0.0075	0.1 p<0.07		-0.09 p<0.04	-0.07 p<0.0014
Light- TPWD		-0.0008 p<0.03		0.004 P<0.5		-0.03 p<0.22
Heavy- BBS	0.12 p<0.013		0.17 p<0.011			-0.01 p<0.02
Heavy- TPWD	0.19 p<0.08		0.30 p<0.02		0.36 p<0.070	

High Plains Results

	Drought Index	Precip.	Temp.	Ag.	Dev.	Neonic. Use
Pre - BBS				-0.02 p<0.03	0.37 p<0.001	
Pre - TPWD	0.10 p<0.31	0.007 p<0.0047	0.42 p<0.02	-0.0005 p<0.98	1.06 p<0.52	
Light- BBS			0.38 p <0.001			
Light- TPWD			0.25 p<0.001	-0.01 p<0.03		
Heavy- BBS						-0.03 p<0.001
Heavy- TPWD	0.39 p<0.001		0.47 p<0.001			-0.07 p<0.001

Rolling Plains Results

	Drought Index	Precip.	Temp.	Ag.	Dev.	Neonic. Use
Pre - BBS		0.002 p<0.005	0.14 p<0.001	-0.017 p<0.001	-0.19 p<0.10	
Pre - TPWD	0.14 p<0.001		0.06 p<0.07	-0.01 p<0.054	-0.84 p<0.001	
Light- BBS	0.09 p<0.03	0.0006 p<0.14	0.2 p<0.001	0.01 p<0.011	-0.4 p<0.003	-0.85 p<0.001
Light- TPWD	0.11 p<0.0018	0.0006 p<0.11	0.11 p<0.02	0.02 p<0.001		-0.34 p<0.09
Heavy- BBS	0.11 p<0.004	-0.0007 p<0.42		0.03 p<0.001	0.14 p<0.012	-0.06 p<0.001
Heavy- TPWD	0.21 p<0.001	-0.004 p<0.001		0.02 p<0.07	0.03 p<0.79	-0.06 p<0.02

Count Model Correlation Coefficients and p-Values Continued

South Texas Plains Results						
	Drought Index	Precip.	Temp.	Ag.	Dev.	Neonic. Use
Pre - BBS				-0.04 p<0.001		
Pre - TPWD	0.17 p<0.001					
Light- BBS			-0.09 p<0.11			-0.01 p<0.001
Light- TPWD		-0.001 p<0.012		0.02 p<0.001		-0.01 p<0.001
Heavy- BBS	0.08 p<0.02	-0.0008 p<0.15			-0.05 p<0.20	-0.008 p<0.09
Heavy- TPWD	0.16 p<0.08		0.29 p<0.01		0.23 p<0.23	
Pineywoods Results						
	Drought Index	Precip.	Temp.	Ag.	Dev.	Neonic. Use
Pre - BBS	-0.12 p<0.003			0.05 p<0.001	-0.24 p<0.001	
Light- BBS	0.19 p<0.04	0.003 p<0.007	-0.23 p<0.04	0.07 p<0.001	-0.35 p<0.0014	
Heavy- BBS	na	na	na	na	na	na