CHEMICAL REPELLENTS FOR REDUCING BLACKBIRD DAMAGE: THE IMPORTANCE OF PLANT STRUCTURE AND AVIAN BEHAVIOR IN FIELD APPLICATIONS

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Brandon Amberg Kaiser

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Program:
Environmental and Conservation Sciences

April 2019

Fargo, North Dakota

North Dakota State University
Graduate School

Title

CHEMICAL REPELLENTS FOR REDUCING BLACKBIRD DAMAGE: THE IMPORTANCE OF PLANT STRUCTURE AND AVIAN BEHAVIOR IN FIELD APPLICATIONS

By

Brandon Amberg Kaiser

The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Page Klug
Chair

Ned Dochtermann

Burton Johnson

Approved:

April 8, 2019
Date

Craig Stockwell
Department Chair

ABSTRACT

Across North America, blackbirds (*Icteridae*) depredate high-energy crops, such as sunflower (*Helianthus annuus*), placing an economic burden on producers. Chemically-defended crops, in the form of human-applied repellents, may induce birds to forage elsewhere if a learned aversion can be established. However, repellent deployment must be feasible for producers at the scale of commercial agriculture. Thus, my main objective was to evaluate the efficacy of anthraquinone-based repellents applied to ripening sunflower for reducing blackbird damage. I conducted concentration response (no-choice) and preference tests (two-choice) to evaluate repellent efficacy on captive blackbirds using application strategies practical for agricultural producers. I evaluated field application strategies to assess the potential for broad-scale application using new drop-nozzle technology. Additionally, I describe behavior of captive blackbirds as they interact with ripening sunflower to further inform repellent application. Our results support the conclusion that application of anthraquinone-based repellents is not currently a feasible option for ripening sunflower.

ACKNOWLEDGMENTS

This research was made possible by the assistance and support of countless people. I would first like to thank my thesis advisor, Dr. Page Klug, for her mentorship, wisdom, and optimism through the past few years. I would also like to thank Dr. Scott Werner and Shelagh Deliberto who were instrumental in their consultation and capture of wild red-winged blackbirds. I would like to thank my committee members, Drs. Ned Dochtermann and Burton Johnson, for project assistance and providing an environment where I feel like a scientist with a seat at the table. I thank Dr. George Linz for revisions and project advice. I appreciate the brainstorming which came from lab meetings with all the members of "Team Bird" as it has widened the breadth of my knowledge.

My lab mates, Conor Egan and Michelle Eshleman, as well as the rest of the graduate students within the Biological Sciences department have been instrumental in keeping me sane and happy through graduate school. I thank the Environmental and Conservation Science program and College of Science and Mathematics for providing travel grants. I thank my cohort of technicians, Chelsey Quiring, Jennifer Preuss, Kaitlyn Boteler, Katie Adkins, and Breanna Weber, for their quality work and dedication to the project. A special thank you to Dr. Kirk Howatt and his lab, who provided use and training of the stationary spraying machine which was pivotal in this research. I thank Sally Jacobson at the Red River Zoo for the use of the NDSU Conservation Sciences Aviary; Alison Pokrzyw of Nuseed for contributing sunflower hybrid seeds; Thomas Seamans and Lucas Wandrie for providing recommendations regarding blackbird husbandry; Dr. Michael Ostlie, Pat Beauzay, Dr. Burton Johnson, and Brian Otteson for providing space and planting sunflower plots; and Ken Ballinger of Arkion Life Sciences, LLC for repellent contribution. I thank the NWRC Chemistry Program for conducting chemical residue analyses.

Data collection for this project was approved by the North Dakota State University Institutional Animal Care and Use Committee (Protocol #A17033), North Dakota Game and Fish Department (Scientific Collection Licenses #GNF04326470, GNF04657399), Colorado Department of Natural Resources Parks and Wildlife (Scientific Collection License #17TRb2006), and the United States Fish and Wildlife Service Migratory Bird Permit (#MB019065-2). State of North Dakota, Department of Agriculture, Pesticide Certification (Ground Core and Vertebrate Class) was acquired through NDSU Extension Pesticide Program (Kaiser ID: 10083952). The United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Wildlife Services (WS), National Wildlife Research Center (#7438-0020-CA; QA-2732) and the National Sunflower Association (Project #17-P01) funded this research. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government. Any unused product was destroyed per existing pesticide regulations.

DEDICATION

I dedicate this thesis to my parents, who never cease to amaze me in the amount of support they provide for a career they try so hard to understand! Additionally, for my nephew (Maxim) and niece (Kennedy) who have provided much needed joy in stressful times!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AQ	Anthraquinone
APHIS	Animal and Plant Health Inspection Service
GPA	Gallons per Acre
GMP	Gallons per Minute
GUD	Giving Up Density
NDSU	North Dakota State University
NWRC	National Wildlife Research Center
PPR	Prairie Pothole Region
USDA	United States Department of Agriculture
WS	Wildlife Services

LIST OF SYMBOLS

η_G^2	Generalized Eta Squared
Δ	Delta
$\overline{x}$	Mean
α	Alpha

CHAPTER 1: INTRODUCTION

Foraging theory predicts that animals make foraging decisions to optimize feeding efforts by selecting food resources that best maximize their rate of energy intake (Emlen 1966; MacArthur & Pianka 1966; Krebs et al. 1983). Net rate of energy intake has been shown to impact fitness, thus natural selection should favor animals that make efficient foraging decisions (Lemon 1991). Previous studies have applied components of foraging theory, considering both food quality (caloric value) and food quantity (food density), to explain foraging decisions in animals (Goss-Custard 1977; Krebs et al. 1977; Munger 1984). Numerous studies have provided evidence that animals are capable of evaluating the profitability of prey items (Partridge 1976; Charnov 1976b). According to the marginal value theorem, animals should choose to forage on a particular food resource as long as the resulting energetic gain exceeds the loss (i.e. time or effort) (MacArthur & Pianka 1966; Charnov 1976a). As the food resource or patch is depleted, the energetic intake rate begins to decrease and the energy being gained approaches the cost of foraging efforts.

The density of food remaining in a patch where the costs and benefits are equal is referred to as the giving-up density (GUD) and has been used in previous studies as an effective proxy for evaluating foraging decisions (Hodges & Wolf 1981; Brown 1988; Oyugi & Brown 2003). Although these studies are often conducted in open field settings, a few studies have applied this theory in captive settings (Smith 1995; Dall et al. 2001). Traditionally, GUD have been used to determine how the added cost of predation risk influences foraging decisions (Brown 1988). Additional costs associated with foraging influence the GUD of a particular food source by changing the profitability of the prey items. Sinervo (1997) describes the profitability of prey as the net energetic gain per unit time and is important because it can account for both quality and quantity of the prey items. Profitability of prey decreases as the individual's costs or time required to obtain and process the prey increases. More recent studies have further developed the use of GUD by incorporating additional cost parameters (e.g., food containing toxins) to evaluate foraging decisions (Kirmani et al. 2010; Bedoya-Perez et al. 2013).

As wild animals forage across a landscape, they often encounter chemically-defended food resources such as natural plant defenses (e.g., toxins) (McArthur et al. 2012) or agricultural crops applied with repellents. Chemically-defended food has an added cost of a toxin that reduces the overall profitability of the prey item. Natural chemical defenses are frequently advertised using aposematic cues, which predators can associate with the chemical defenses for future avoidance (Skelhorn & Rowe 2007). Previous studies have shown that some birds and mammals are capable of using aposematic warnings to discriminate between defended and non-defended prey items (Barnett et al. 2012). Although these aposematic cues are thought to be used to avoid defended prey, recent studies have found that predators can continue to ingest toxic prey in the wild using aposematic cues to regulate toxin intake (Barnett et al. 2007; Skelhorn & Rowe 2007). As animals ingest toxic prey, the toxin load within the body increases, forcing individuals to accurately control their intake of toxins (Skelhorn & Rowe 2007; Barnett et al. 2012). Lab experiments have shown controlling toxin intake forces educated predators to balance the costs of ingesting toxic prey with the nutritional benefits (Skelhorn & Rowe 2007).

Barnett et al. (2012) proposed that educated predators, aware of both nutritional and toxic properties of prey, make strategic decisions to forage on toxic prey based on energetic needs. Thus, as energetic needs increase, more chemically-defended prey should be ingested depending on the nutritional value of the prey (Barnett et al. 2007). Previous studies found that animals may endure a higher metabolic cost by increasing the amount of toxin ingested when body condition or energetic state decreases (Skelhorn & Rowe 2007; Barnett et al. 2012). Furthermore, ingestion of toxic prey not only institutes a metabolic cost to fitness, but has also been shown to reduce energy uptake and harvesting rates in both mammals and birds (Kirmani et al. 2010; McArthur et al. 2012). As harvesting rates decrease in the presence of toxic prey, food densities appear lower than actuality and thus have a large impact on the GUD (Kirmani et al. 2010). Although previous studies have found the presence of toxins to negatively impact food profitability, they neglect to relate increased GUD to the displayed behaviors of foraging individuals (Kirmani et al. 2010; Bedoya-Perez et al. 2013).

Understanding foraging behavior is important for evaluating how toxins influence food profitability. A contributing factor in the reduced profitability of prey containing toxins could be a reduced feeding rate (food intake/time) and thus an extended interprey interval or time between prey items (Daneke & Decker 1988; Cresswell et al. 2003; Cowlishaw et al. 2004; Blanchard & Fritz 2007). A reduced feeding rate results from a shift in the foraging time budget, which includes activities such as searching for food, handling food, and scanning for predators (Popp 1988; Cowlishaw et al. 2004). A foraging time budget consists of the total time spent foraging, where increasing the proportion of time allocated to one activity negatively impacts the proportion of time available for another (Popp 1988). For example, an increase in time spent searching for food would decrease handling because handling is dependent on whether a search attempt was successful and food is acquired (Partridge 1976). Thus, if an individual spends more time searching for food, their respective feeding rate decreases. Longer head-down search efforts result in the need for head-up interruptions to scan the surroundings for predators (Lima 1988). Interruptions while searching for food may decrease the probability of successfully acquiring food resulting in a decreased feeding rate (Cresswell et al. 2003).

Foraging theory can be a useful tool for predicting how animals attempt to maximize foraging efforts in the presence of chemically-defended prey. Understanding how foraging behavior changes in the presence of toxins provides insight as to how foragers respond to decreased profitability of prey. This can be useful in the case of agricultural crop fields, which are essentially large and abundant sources of high energy food. Chemical defenses, in the form of avian repellents, can be applied to agricultural crops and may mitigate crop depredation by birds.

Sunflower (*Helianthus annuus*) is an important cash crop worldwide and is subject to wildlife damage wherever grown (Hulke & Kleingartner 2014a). For example, over 84% of the 1.65 million acres of sunflower planted in the United States is localized in North Dakota, South Dakota, and Minnesota and subject to depredation by large flocks of blackbirds, especially within the Prairie Pothole Region (PPR) (Hulke & Kleingartner 2014b; National Agricultural Statistics Service 2018; National Sunflower Association 2018). As sunflower ripens from August to October, local blackbirds are preparing for migration and shifting from an insect-based diet to a seed-based diet, in which sunflower and corn are widely available as sources of nutrition (Hintz & Dyer 1970; Dolbeer 1978; Linz et al. 1984). In the PPR, flocks of blackbirds can number up to 100,000 individuals, resulting in substantial damage to sunflower crops with estimates of single-season damage over $3.5 million in North Dakota (Peer et al. 2003; Linz et al. 2011; Klosterman et al. 2013). Although regional blackbird damage only equates to around 2%, localized damage can reach well over 20% (Klosterman et al. 2013; Shwiff et al. 2017). Continuously high levels of blackbird damage can be financially crippling to individual producers and may cause some to remove sunflowers from their rotation, although other factors such as market price influence this decision as well (Hulke & Kleingartner 2014a).

Various management strategies have been considered to reduce blackbird damage to crops including lethal control, chemical repellents, physical frightening devices (e.g. propane cannons and unmanned aircraft systems), evading strategies (e.g. decoy crops), and habitat management (e.g. cattail management) (Hagy et al. 2008; Werner et al. 2008; Linz & Homan 2011; Linz et al. 2011). Current management methods suffer from a combination of limited extent of effectiveness in space and time, negative cost-benefit ratios, or the habituation of birds to the tool (Gilsdorf et al. 2002; Klug 2017). For example, lethal control has not been deemed cost-effective due to large population sizes, high population turnover rates, and extreme mobility of blackbirds (Blackwell et al. 2003; Linz et al. 2015). Nonlethal chemical repellents hold the potential to be a cost-effective management tool for broad scale agriculture provided application difficulties can be overcome and alternative food is available for foraging birds (Klug 2017).

Anthraquinone (AQ) deters blackbirds from feeding on treated food (e.g., sunflower achenes) in cage and pen trials (Avery et al. 1997; Werner et al. 2009; 2010; 2011) and in small plot field trials (Avery et al. 1998; Werner et al. 2014). Anthraquinone is a secondary repellent, which has been shown to cause distress after ingestion, sometimes leading to vomiting (Avery et al. 1997). Thus, secondary repellents require conditioned learning as they elicit an adverse physiological effect such as pain or sickness that the animal associates with a sensory stimulus (Avery & Mason 1997; Werner & Clark 2003). As a result, use of a secondary repellent is most effective when used on resident pests; and in the case of sunflower damage, resident blackbirds are a major contributor (Dolbeer 1978; DeVault et al. 2013). Anthraquinone-based repellents have shown repellency in laboratory experiments, with the caveat that loose sunflower achenes were consistently coated with repellent using a rotating mixer and offered in a bowl (Werner et al. 2011; 2014; 2015). Although anthraquinone-based repellents have been shown to reduce blackbird consumption in the lab, field studies indicate the growth form of sunflower along with inefficient application strategies (e.g. aerial application) may decrease effectiveness based on how birds interact with the sunflower plant and thus the repellent (Figure 1.1).

A challenge to applying repellent to sunflower in the field is that sunflower heads begin to face down near maturity, making aerial application inefficient as only the back of the head would be treated. Werner et al. (2014) simulated aerial application by spraying an anthraquinone-based repellent on the back of downward-facing sunflower heads without successfully reducing blackbird damage. Additional field trials on foliar sunflower failed to produce sufficient anthraquinone residues on achenes to reduce avian damage to sunflower as ground rigs spraying above the crop do not effectively distribute repellent to the sunflower face (Kandel et al. 2009; Niner et al. 2015). Thus, the use of a repellent on sunflowers requires an improved application strategy to allow the repellent to reach the face of the sunflower (Werner et al. 2014). Application via ground-based sprayers equipped with drop nozzles and upward-oriented spray tips may be used to treat disk flowers and ripening achenes on the downward facing sunflower heads (Figure 1.1A) (Klug 2017). In seminatural field tests, Werner et al. (2011; 2014) was able to successfully reduce blackbird consumption when the repellent was applied directly to the sunflower face using a CO_2 backpack sprayer. This successful reduction in blackbird damage may have been a result of complete and heavy repellent coverage that effectively covered all disk flowers and exposed achenes, a coverage that is not feasible with large-scale spraying equipment. Even if the face of the sunflower could be reached with a drop-nozzle application strategy, the amount of repellent applied would be affected by 1) a realistic cost-effective application rate, 2) achenes being embedded in the sunflower head, and 3) achenes being further protected by disk flowers (Figure 1.1B). Any scenario that requires large amounts of repellent be applied to non-consumed vegetative parts for efficacy is problematic, especially for a repellent that needs to be ingested to be effective. Laboratory experiments have shown that anthraquinone-based repellents are capable of reducing blackbird consumption of sweetcorn kernels when only the husks are saturated (Carlson et al. 2013). Thus, application of a repellent to vegetative parts of the plant may still function to protect the palatable seed, but sufficient repellent will need to cover the part of the plant in which the bird interacts to increase repellent ingestion.

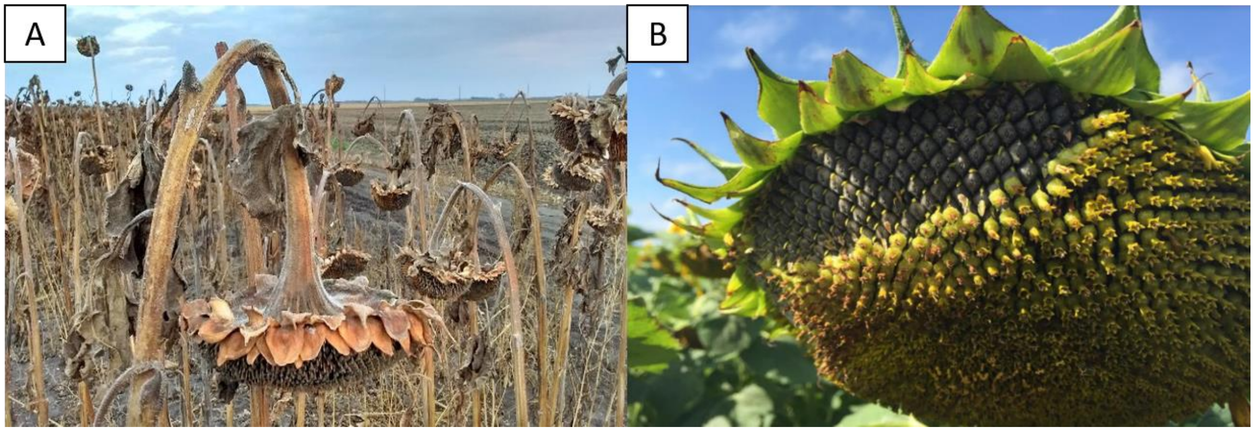

Figure 1.1. A) As sunflowers (*Helianthus annuus*) mature they begin to face downwards, leaving aerially-applied chemicals to land on the back of the head. B) Sunflower achenes are embedded in the sunflower head and protected by disk flowers on the face as well as vegetative bracts covering the outer edges.

Objectives

My main research objective was to evaluate how an avian repellent affects blackbird foraging behavior and resulting consumption of sunflower when applied in a manner that realistically simulates commercial spraying equipment. Although completely saturating sunflower heads with repellent has resulted in reduced blackbird damage (33-34%) in small-plot field tests (Werner et al. 2014), no methods have been tested for effective field application at the broad scale. Ground sprayers equipped with drop-nozzles may be a new technology to overcome the application difficulties for avian repellents at broad scales. Although, complete coverage of the sunflower face and each sunflower plant in a field is improbable, translating effectiveness to a broad scale may be feasible if partial coverage produces anthraquinone residues high enough to alter foraging behavior and reduce blackbird damage. Even partial coverage of the repellent may be effective in changing foraging behavior (e.g., tradeoffs between foraging and vigilance) and thus could increase the effectiveness of other pest management methods such as physical hazing (e.g., unmanned aircraft systems) when deployed in an integrated pest management approach (Gilsdorf et al. 2002; Belant & Martin 2011).

In Chapter 2, I evaluate the efficacy of anthraquinone-based repellents (AV-5055 [13% AQ with visual inert] and Avipel™ [50% AQ without visual inert]) to reduce blackbird damage when applied directly to the face of ripening sunflower in a laboratory setting. I use a concentration response test (no choice) and preference tests (two choice) to evaluate effectiveness of varying repellent concentrations without and with alternative forage, respectively. Previous laboratory studies showing efficacy of anthraquinone-based repellents in reducing sunflower consumption by birds have used loose, dry achenes. It is not known if the formulations optimized for those conditions will be effective when applied to the intact sunflower plant. Thus, my research on avian repellents focuses on the ability of the anthraquinone-based repellent to minimize blackbird consumption when applied to actual the sunflower plant. This work will inform how plant structure influences repellent efficacy and if realistic, best-case spraying scenarios are capable of reducing blackbird feeding in a controlled, laboratory setting. Previous small-plot field studies evaluating anthraquinone-based repellents in sunflower have shown efficacy with a 33-34% reduction in damage when high enough residues (i.e., 385-481 ppm) were achieved on achenes (Werner et al. 2014). Therefore, I also evaluate repellent coverage and residues attainable in the field when the repellent is applied using drop-nozzle equipped ground rigs to reach the downward-facing sunflower head face.

In Chapter 3, I evaluate the general foraging behavior and strategies used by blackbirds foraging on sunflower within a laboratory setting. The face of the ripening sunflowers is covered by disk flowers, which likely receives the majority of repellent and blocks repellent from reaching the achenes. At the same time, achenes are embedded in the sunflower receptacle further limiting anthraquinone residue on achenes. Little is known about how blackbirds interact with disk flowers or how birds remove and handle achenes. These interactions influence the amount of repellent that is ingested and ultimately how crop-specific feeding behavior of birds may affect repellent efficacy. An understanding of how blackbirds interact with disk flowers and depredate sunflower achenes informs effective repellent application by identifying the parts of the plant that need to be targeted for application.

Anthraquinone-based repellents have been found to be effective at reducing blackbird feeding in laboratory experiments. These same repellents have been found to reduce damage to mature sunflowers when applied directly to the sunflower face in amounts that are unrealistic in a real-world spraying scenario. However, it remains unclear how much residue can be deposited on achenes across a broad scale using drop-nozzle application strategies and if practical application strategies can achieve enough residue on sunflowers (i.e., disk flowers or achenes) to reduce blackbird damage. Thus my research fills the gap between laboratory evaluations and practical field application of anthraquinone-based repellents. In Chapter 4, I discuss conclusions and future directions of research focusing on avian repellents for reducing blackbird damage to ripening sunflower.

References

Avery ML, Humphrey JS, Decker DG. 1997. Feeding deterrence of anthraquinone, anthracene, and anthrone to rice-eating birds. *Journal of Wildlife Management* **61**:1359-1365.

Avery ML, Humphrey JS, Primus TM, Decker DG, McGrane AP. 1998. Anthraquinone protects rice seed from birds. *Crop Protection* **17**:225-230.

Avery ML, Mason JR. 1997. Feeding responses of red-winged blackbirds to multisensory repellents. *Crop Protection* **16**:159-164.

Barnett C, Bateson M, Rowe C. 2007. State-dependent decision making: educated predators strategically trade off the costs and benefits of consuming aposematic prey. *Behavioral Ecology* **18**:645-651.

Barnett CA, Skelhorn J, Bateson M, Rowe C. 2012. Educated predators make strategic decisions to eat defended prey according to their toxin content. *Behavioral Ecology* **23**:418-424.

Bedoya-Perez MA, Carthey AJ, Mella VS, McArthur C, Banks PB. 2013. A practical guide to avoid giving up on giving-up densities. *Behavioral Ecology and Sociobiology* **67**:1541-1553.

Belant JL, Martin JA 2011. Bird harassment, repellent, and deterrent techniques for use on and near airports. Transportation Research Board of the National Academies, Washington, DC.

Blackwell BF, Huszar E, Linz GM, Dolbeer RA. 2003. Lethal control of red-winged blackbirds to manage damage to sunflower: an economic evaluation. *Journal of Wildlife Management* **67**:818-828.

Blanchard P, Fritz H. 2007. Induced or routine vigilance while foraging. *Oikos* **116**:1603-1608.

Brown JS. 1988. Patch use as an indicator of habitat preference, predation risk, and competition. *Behavioral Ecology and Sociobiology* **22**:37-47.

Carlson JC, Tupper SK, Werner SJ, Pettit SE, Santer MM, Linz GM. 2013. Laboratory efficacy of an anthraquinone-based repellent for reducing bird damage to ripening corn. *Applied Animal Behaviour Science* **145**:26-31.

Charnov EL. 1976a. Optimal Foraging, Marginal Value Theorem. *Theoretical Population Biology* **9**:129-136.

Charnov EL. 1976b. Optimal foraging: attack strategy of a mantid. *American Naturalist* **110**:141-151.

Cowlishaw G, Lawes MJ, Lightbody M, Martin A, Pettifor R, Rowcliffe JM. 2004. A simple rule for the costs of vigilance: empirical evidence from a social forager. *Proceedings of the Royal Society of London B: Biological Sciences* **271**:27-33.

Cresswell W, Quinn J, Whittingham M, Butler S. 2003. Good foragers can also be good at detecting predators. *Proceedings of the Royal Society of London B: Biological Sciences* **270**:1069-1076.

Dall SR, Kotler BP, Bouskila A. 2001. Attention,'apprehension'and gerbils searching in patches. *Annales Zoologici Fennici* **38**:15-23.

Daneke D, Decker DG. 1988. Prolonged seed handling time deters red-winged blackbirds feeding on rice seed (eds A.C. Crabb, R.E. Marsh). Pages 287-292. In Proceedings of the 13th Vertebrate Pest Conference, University of California, Davis USA.

DeVault TL, Blackwell BF, Belant JL, (eds) 2013. *Wildlife in airport environments: preventing animal–aircraft collisions through science-based management*. Johns Hopkins University Press, Baltimore, MD.

Dolbeer RA. 1978. Movement and migration patterns of red-winged blackbirds: a continental overview. *Bird-Banding* **49**:17-34.

Emlen JM. 1966. The role of time and energy in food preference. *American Naturalist* **100**:611-617.

Gilsdorf JM, Hygnstrom SE, VerCauteren KC. 2002. Use of frightening devices in wildlife damage management. *Integrated Pest Management Reviews* **7**:29-45.

Goss-Custard J. 1977. Optimal foraging and the size selection of worms by redshank, *Tringa totanus*, in the field. *Animal Behaviour* **25**:10-29.

Hagy HM, Linz GM, Bleier WJ. 2008. Optimizing the use of decoy plots for blackbird control in commercial sunflower. *Crop Protection* **27**:1442-1447.

Hintz JV, Dyer MI. 1970. Daily Rhythm and Seasonal Change in Summer Diet of Adult Red-Winged Blackbirds. *Journal of Wildlife Management* **34**:789-799.

Hodges CM, Wolf LL. 1981. Optimal foraging in bumblebees: why is nectar left behind in flowers? *Behavioral Ecology and Sociobiology* **9**:41-44.

Hulke BS, Kleingartner LW. 2014a. Sunflower. Pages 433-458. *Yield gains in major US field crops*. American Society of Agronomy.

Hulke BS, Kleingartner LW. 2014b. Sunflower. *Yield gains in major US field crops*:433-458.

Kandel H, Johnson B, Deplazes C, Linz G, Santer M. 2009. Sunflower treated with Avipel (Anthraquinone) bird repellent. Page 12. In Proceedings of National Sunflower Association Research Forum, Fargo, ND.

Kirmani SN, Banks PB, McArthur C. 2010. Integrating the costs of plant toxins and predation risk in foraging decisions of a mammalian herbivore. *Oecologia* **164**:349-356.

Klosterman ME, Linz GM, Slowik AA, Homan HJ. 2013. Comparisons between blackbird damage to corn and sunflower in North Dakota. *Crop Protection* **53**:1-5.

Klug P. 2017. The future of blackbird management research. Pages 217-234. *Ecology and Management of Blackbirds (Icteridae) in North America.* (eds G.M. Linz, M.L. Avery & R.A. Dolbeer) CRC Press/Taylor & Francis, Boca Raton, Florida, USA.

Krebs JR, Erichsen JT, Webber MI, Charnov EL. 1977. Optimal prey selection in the great tit (*Parus major*). *Animal Behaviour* **25**:30-38.

Krebs JR, Stephens DW, Sutherland WJ. 1983. Perspectives in optimal foraging. Pages 165-221 in Brush AH, and Clark Jr. GA, editors. *Perspectives in Ornithology*.

Lemon WC. 1991. Fitness consequences of foraging behaviour in the zebra finch. *Nature* **352**:153-155.

Lima SL. 1988. Vigilance and diet selection: the classical diet model reconsidered. *Journal of Theoretical Biology* **132**:127-143.

Linz GM, Bucher EH, Canavelli SB, Rodriguez E, Avery ML. 2015. Limitations of population suppression for protecting crops from bird depredation: A review. *Crop Protection* **76**:46-52.

Linz GM, Homan HJ. 2011. Use of glyphosate for managing invasive cattail (*Typha spp*.) to disperse blackbird (*Icteridae*) roosts. *Crop Protection* **30**:98-104.

Linz GM, Homan HJ, Werner SJ, Hagy HM, Bleier WJ. 2011. Assessment of Bird-management Strategies to Protect Sunflowers. *Bioscience* **61**:960-970.

Linz GM, Vakoch DL, Cassel JF, Carlson RB. 1984. Food of Red-Winged Blackbirds, *Agelaius-phoeniceus*, in Sunflower Fields and Corn Fields. *Canadian Field-Naturalist* **98**:38-44.

MacArthur RH, Pianka ER. 1966. On optimal use of a patchy environment. *American Naturalist* **100**:603-609.

McArthur C, Orlando P, Banks PB, Brown JS. 2012. The foraging tightrope between predation risk and plant toxins: a matter of concentration. *Functional Ecology* **26**:74-83.

Munger JC. 1984. Optimal foraging? Patch use by horned lizards (Iguanidae: Phrynosoma). *American Naturalist* **123**:654-680.

National Agricultural Statistics Service. 2018. Quick Stats, Available from https://quickstats.nass.usda.gov/ Accessed February 13th, 2018.

National Sunflower Association. 2018. June Plantings, Available from https://www.sunflowernsa.com/stats/usda-reports/june-plantings/ Accessed February 13th, 2018.

Niner MD, Linz GM, Clark ME. 2015. Evaluation of 9, 10 anthraquinone application to pre-seed set sunflowers for repelling blackbirds. *Human-Wildlife Interactions* **9**:4-13.

Oyugi JO, Brown JS. 2003. Giving-up densities and habitat preferences of European starlings and American robins. *Condor* **105**:130-135.

Partridge L. 1976. Individual differences in feeding efficiencies and feeding preferences of captive great tits. *Animal Behaviour* **24**:230-240.

Peer BD, Homan HJ, Linz GM, Bleier WJ. 2003. Impact of blackbird damage to sunflower: Bioenergetic and economic models. *Ecological Applications* **13**:248-256.

Popp JW. 1988. Effects of food-handling time on scanning rates among American goldfinches. *Auk* **105**:384-385.

Shwiff SA, Ernest KL, Degroot SL, Anderson AM, Shwiff SS. 2017. The economic impact of blackbird damage to crops. *Ecology and Management of Blackbirds (Icteridae) in North America*:207-216.

Sinervo B. 1997. Optimal Foraging Theory: Constraints and Cognitive Processes. Pages 105-130. *Behavioral Ecology*. University of Southern California, Santa Cruz, CA.

Skelhorn J, Rowe C. 2007. Predators' toxin burdens influence their strategic decisions to eat toxic prey. *Current Biology* **17**:1479-1483.

Smith RJ. 1995. Harvest rates and escape speeds in two coexisting species of montane ground squirrels. *Journal of Mammalogy* **76**:189-195.

Werner SJ, Carlson JC, Tupper SK, Santer MM, Linz GM. 2009. Threshold concentrations of an anthraquinone-based repellent for Canada geese, red-winged blackbirds, and ring-necked pheasants. *Applied Animal Behaviour Science* **121**:190-196.

Werner SJ, Clark L. 2003. Understanding blackbird sensory systems and how repellent applications work (ed G.M. Linz). Pages 31-40 in Linz GE, editor. In Management of North American Blackbirds, Proceedings of a special symposium of The Wildlife Society, 9th Annual Conference, USDA-APHIS-Wildlife Services National Wildlife Research Center, Bismarck, North Dakota, USA.

Werner SJ, Cummings JL, Tupper SK, Goldade DA, Beighley D. 2008. Blackbird repellency of selected registered pesticides. *Journal of Wildlife Management* **72**:1007-1011.

Werner SJ, DeLiberto ST, Mangan AM, Pettit SE, Ellis JW, Carlson JC. 2015. Anthraquinone-based repellent for horned larks, great-tailed grackles, American crows and the protection of California's specialty crops. *Crop Protection* **72**:158-162.

Werner SJ, Linz GM, Carlson JC, Pettit SE, Tupper SK, Santer MM. 2011. Anthraquinone-based bird repellent for sunflower crops. *Applied Animal Behaviour Science* **129**:162-169.

Werner SJ, Linz GM, Tupper SK, Carlson JC. 2010. Laboratory Efficacy of Chemical Repellents for Reducing Blackbird Damage in Rice and Sunflower Crops. *Journal of Wildlife Management* **74**:1400-1404.

Werner SJ, Tupper SK, Pettit SE, Ellis JW, Carlson JC, Goldade DA, Hofmann NM, Homan HJ, Linz GM. 2014. Application strategies for an anthraquinone-based repellent to protect oilseed sunflower crops from pest blackbirds. *Crop Protection* **59**:63-70.

CHAPTER 2: IMPACTS OF SUNFLOWER PLANT STRUCTURE ON APPLICATION AND EFFICACY OF ANTHRAQUINONE-BASED AVIAN REPELLENT

Abstract

Blackbirds (*Icteridae*) cause significant damage to sunflower (*Helianthus annuus L.*) crops throughout North America. Chemical repellents may be a cost-effective method for reducing bird damage if application strategies can be developed specific to sunflower. Anthraquinone-based repellents, which have been optimized for loose dry achenes, can reduce feeding on sunflower achenes by more than 80% in the lab. However, efficacy is difficult to replicate in the field due to application issues where vegetative and floral components of sunflower limit repellent contact with achenes. We evaluated an anthraquinone-based repellent applied to mature sunflowers for reducing bird damage. We used captive male red-winged blackbirds (*Agelaius phoeniceus*) to conduct a concentration response experiment and preference experiments using varying concentrations of AV-5055 [13% anthraquinone] and Avipel™ [50% anthraquinone] applied in a manner attainable by sunflower producers. We also conducted a field-based study to assess application methods for increasing repellent coverage and anthraquinone residues when applied using ground rigs equipped with drop-nozzles to reach below the canopy. Anthraquinone-based repellents failed to reduce blackbird feeding and birds did not discriminate between untreated sunflowers and sunflowers treated with varying concentrations of anthraquinone. Disk flowers played a significant role in obstructing repellent from reaching achenes, but even in the absence of disk flowers, repellent failed to significantly reduce blackbird feeding in a laboratory setting. Although residues apparent in field applications can be improved by increasing concentrations of anthraquinone in tank mixtures and decreasing

droplet size through increased pressure, we were unable to sufficiently increase residues to elicit a reduction in bird damage or differences in sunflower yield.

Introduction

Sunflower (*Helianthus annuus*) is an important specialty crop grown worldwide (Linz et al. 2011). In the United States, over 85% of sunflower production occurs in North Dakota, South Dakota, and Minnesota (Hulke & Kleingartner 2014). The ripening sunflower crop is prone to heavy damage from blackbirds (*Icteridae*), a primary agricultural pest in sunflower growing regions (Linz et al. 2017). During the fall, blackbirds use readily-available, highly nutritious crops, such as corn and sunflower, as they form flocks in preparation for southbound migration (Hintz & Dyer 1970; Dolbeer 1978; Linz et al. 1984). These mixed flocks, that can number well over 100,000 individuals, primarily contain red-winged blackbirds (*Agelaius phoeniceus*), but yellow-headed blackbirds (*Xanthocephalus xanthocephalus*) and common grackles (*Quiscalus quiscula*) are also members of the flocks (Linz et al. 2011). Although regional blackbird damage to sunflower production only amounts to roughly 2% crop loss, localized damage can exceed levels where the crop is no longer able to be harvested (Klosterman et al. 2013; Shwiff et al. 2017). After reaching physiological maturity, the crop remains in the field until the achenes dry to about 10% moisture (Larson et al. 2008), creating a long 6-8 week damage window (Peer et al. 2003; Linz et al. 2011; 2017). Continuous avian pest damage may be financially taxing to individual agricultural operations, and thus producers require cost-effective management strategies to combat avian crop depredation (Hulke & Kleingartner 2014).

Nonlethal chemical repellents hold the potential to be a cost-effective management tool for broad scale agriculture, provided application difficulties can be overcome (Linz et al. 2011; Klug 2017). Anthraquinone, (9,10-anthraquinone; The E-Pesticide Manual, Version 3.0 2003) a post-ingestive secondary repellent, has been identified as a candidate for reducing blackbird feeding on corn, rice, sunflower, and other crops (Avery et al. 1997; Werner et al. 2009; Carlson et al. 2013). Anthraquinone acts on the digestive system by inducing water diarrhea and must be ingested for the negative consequence and learned aversion to the repellent to take effect (Avery et al. 1997; Abraham & Sellin 2012).

Anthraquinone is currently registered by United States Environmental Protection Agency (US EPA) to repel Canada geese (*Branta canadensis*) from grass and turf (Flight Control™) and as a seed treatment (Avipel™) for protecting planted seed from birds (Avery 2003). These scenarios, however, do not result in anthraquinone entering the food stream. Foliar-applied repellents must conform to the US EPA food tolerance or maximum residue level for entry into the human or animal food stream. Thus, a major hurdle for expanding repellent registration to include foliar application is developing methods to reduce residues while maintaining repellency (Eisemann et al. 2011). Substantial work on anthraquinone-based repellents has focused on incorporating inert ingredients (e.g., visual components) that act in synergy with anthraquinone to increase efficacy at lower residue levels (Werner et al. 2014a). These repellent formulations are optimized in the lab using loose, harvested achenes. Thus effectiveness when applied to vegetative and floral components of the sunflower are unknown. The method of applying anthraquinone to mature plants is important for both residue levels in the food stream and the amount of residue needed to decrease bird damage. If repellent labels are to be expanded to include foliar application, methods need to be developed that reduce avian feeding while reducing repellent residues on the achene; an achievable goal if residues on vegetative and floral parts are sufficient to reduce feeding.

Although a >80% reduction in consumption has been achieved in lab experiments when sunflower achenes are fully coated with the repellent, field trials struggle to replicate this efficacy due to the complex vegetative growth of unharvested sunflower and shortcomings in application strategies (Werner et al. 2009; 2014b; 2015). After reaching maturity (R_6), sunflower heads begin to droop and face the ground. Repellent deployed above the crop canopy (e.g., aerial crop dusters and high-boy sprayers) results in most of the repellent landing on the back of the sunflower head, which fails to reduce blackbird feeding as insufficient repellent reaches parts of the plant manipulated by the bird (Kandel et al. 2009; Linz et al. 2014; Werner et al. 2014b; Niner et al. 2015). Repellent applied directly to the face of sunflower has been shown to reduce blackbird damage when applied using a CO_2 backpack sprayer that allows extremely high residues on achenes (Werner et al. 2014b). However, intense and direct application in this manner is not feasible at the scale of commercial sunflower fields, which can span up to 250 ha (Egan 2018).

Innovative application strategies, such as using upward-oriented spray nozzles from below the leaf canopy, are needed to improve delivery to the sunflower face and increase repellent contact with the foraging bird (Linz et al. 2011). In the event that the repellent can reach the sunflower face, anthraquinone residue on achenes may still be obstructed by disk flowers. This may be an obstacle by which secondary repellents will be deemed ineffective in sunflower, or depending on how blackbirds interact with the disk flowers, an avenue to limit anthraquinone residues on harvested achenes while simultaneously being an effective repellent. For example, outer vegetative parts, such as corn husks, fully coated in anthraquinone have been shown to reduce blackbird consumption on sweet corn kernels while simultaneously reducing the residue on the edible parts of the crop (Carlson et al. 2013). We aim to evaluate if a repellent applied to sunflower plants with intact disk flowers can reduce feeding on the underlying achenes.

In order to determine the benefit of chemical repellents to sunflower producers, we 1) evaluated if a repellent optimized for harvested achenes is effective when applied to intact sunflower plants, especially in the presence of disk flowers; and 2) if application strategies in the field could be developed to deposit sufficient repellent on the sunflower face to effectively reduce blackbird consumption. We conducted laboratory-based feeding experiments to evaluate efficacy of anthraquinone-based repellents for reducing blackbird consumption on intact sunflower when applied in a best-case scenario (i.e., under cost-effective tank mixtures and simulated commercial spraying operations). We also conducted a field study to assess application methods for increasing repellent coverage and anthraquinone residues when applied using ground rigs equipped with drop-nozzle technology.

Methods

Laboratory Feeding Experiments Using Intact Sunflower Plant

Facilities, Animal Capture, and Care – We captured 130 male red-winged blackbirds (hereafter "blackbirds") in Colorado, USA in February 2017 and transferred them to permanent housing at the Red River Zoo North Dakota State University (NDSU) Conservation Sciences Aviary in Fargo, North Dakota, USA. An additional 43 blackbirds were captured in eastern North Dakota between May and July 2018. We group housed blackbirds in either a 4.8 x 4.8 x 2.4 m cage (<60 birds) or a smaller 2.4 x 2.4 x 2.4 m cage (<20 birds) under a natural light-dark cycle. Birds had free access to a maintenance diet of equal parts millet, milo, sunflower, safflower, and cracked corn with grit and water *ad libitum* and occasional sweet corn, crickets, meal worms, and suet.

Sunflower – Hybrid oilseed sunflower (Daytona, Nuseed®) plots were planted and maintained at NDSU Agriculture Research Experimental Stations including the Prosper, ND, USA site and NDSU Casselton Agronomy Seed Farm in Casselton, ND, USA. The feeding trials occurred over seven weeks in 2017 and three weeks in 2018, thus we had staggered plantings to provide the same stage of sunflower maturity across weeks. We collected sunflowers for feeding trials by cutting approximately 15 cm of stem below the head. For feeding tests, we placed sunflower stems inside a 40-cm long plastic tube and secured the heads to a 90^o bracket on the top end so that the face of each sunflower was perpendicular to the cage floor and secured in the center of each side of the cage (Mason et al. 1989).We visually selected sunflower heads based on sunflower maturity, lack of obvious disease or abnormalities, retention of disk flowers, and size, flatness, and symmetry of the sunflower face. We also used moisture content to approximate physiological maturity of sunflower given color change to the capitula can be subjective (Anderson 1975).

We measured moisture content of achenes every two days throughout each feeding trial by collecting two achene samples (5-8 g each) from 2-3 heads. Achenes were collected in a wedge shape for equal representation of the sunflower. We weighed samples before and after placement in a convection oven at 110°C for 24 hours to determine percent moisture (Kersting et al. 1961). We corrected bird consumption (g) by accounting for differences in weekly moisture estimates by standardizing achene moisture at 10% using Eq. 2.1.

$$\text{Consumption Moisture Correction} = \left(\frac{(100-\text{Moisture Content (\%)})}{(100-10)}\right) * \text{Consumption} \tag{2.1}$$

We measured oil content of the sunflower plots once a week over the course of the feeding trials in 2017. Oil was extracted using n-hexane in an accelerated solvent extraction (conducted by

NDSU Agricultural and Biosystems Engineering Department, Fargo, ND USA). Oil content in 2017 ranged from 6-23% (mean ± SE; 12.5 ± 2.6%).

Repellent Application – We used anthraquinone-based repellent formulations (9,10-anthraquinone; Arkion® Life Sciences, LLC, New Castle, DE, USA at 13% [AV-5055] and 50% [Avipel™]). The formulations were mixed with tap water and R-11® Nonionic Surfactant Spreader Activator (Wilbur-Ellis Company, Fresno, CA, USA) to produce tank mixtures that could be realistically achieved with a commercial sprayer (Table 2.1). We applied all tank mixtures at 126.3 L/ha (13.5 gal/ac) to the sunflower face by placing clipped plants in an automated spraying machine (Control Assemblies Co., Fargo, ND, USA). The machine consisted of one flat-fan nozzle (8001EVS; TeeJet Technologies). We calculated the application rate (Eq. 2.1),

$$\mathrm{L/ha} = \frac{(0.37\ L/min)*166.67}{(0.89\ m/s)*(0.56\ m)} = 126.3 \tag{2.2}$$

where the output is 126 L/ha, the nozzle flow rate is 0.37 L/min, the speed of the sprayer is 0.89 m/s, and the height of the nozzle above the sunflower face is 0.56 m. We quantified percent coverage of repellent on the sunflower using Syngenta Water Sensitive Paper (76.2 x 25.4 mm; Spraying Systems Inc., Wheaton, IL, USA) taped to note cards which were pinned to a sunflower face. We calculated percent coverage using “DepositScan” (Zhu et al. 2011) and conducted a Kruskal-Wallis test in R (version 3.5.2; R Core Team 2019) to compare coverage between treatments.

Table 2.1. Summary of captive red-winged blackbird (*Agelaius phoeniceus*) feeding experiments conducted in 2017-2018 at the Red River Zoo NDSU Conservation Sciences Aviary in Fargo, ND, USA.

	Year[a]	Repellent Formulation[b]	AQ in Formulation[c] (%)	Trt[d]	Sample Size	Application Rate of Formulation (gal/ac)	Formulation in Tank Mix (%)	AQ in Tank Mix (%)	AQ Residues on Achenes (ppm ± SE)	AQ Residues on Disk Flowers (ppm ± SE)
Concentration Response Trial†	2017	AV-5055	13	1	13	0.34	2.5	0.60	0.36 ± 0.08	39.97 ± 2.20
				2	12	0.68	5	0.95	0.77 ± 0.10	78.71 ± 2.31
				3	12	1.35	10	1.64	1.80 ± 0.36	167.71 ± 14.10
				4	13	2.70	20	4.29	2.81 ± 0.38	294.14 ± 9.01
Preference Test Trials‡	2017	AV-5055	13	1	10	0.34	2.5	0.60	0.36 ± 0.08	39.97 ± 2.20
				2	9	0.68	5	0.95	0.77 ± 0.10	78.71 ± 2.31
				3	9	1.35	10	1.64	1.80 ± 0.36	167.71 ± 14.10
				4	10	2.70	20	4.29	2.81 ± 0.38	294.14 ± 9.01
	2018	AV-5055	13	5	9	2.70	20	4.14	4.33 ± 3.08	429.50 ± 50.50
	2018	Avipel	50	6	9	2.70	20	10.21	5.99 ± 2.38	1095.00 ± 95.00
	2018	AV-5055	13	7	8*	2.70	20	4.24	49.35 ± 17.75	N/A

[a] Feeding trials in 2017 occurred over 7 weeks (August to October) and trials in 2018 occurred over 3 weeks (August to September). Feeding trials in 2018 employed control cages (n = 13) to evaluate if the reduction in feeding found in 2017 was due to a cage effect or the introduction of an avian repellent.

[b] AV-5055 (Arkion® Life Sciences, LLC, New Castle, DE, USA) contains a visual inert which has been found to have a synergistic effect with anthraquinone (AQ) to increase efficacy at lower residues (Werner et al. 2014a). Avipel™ (Arkion® Life Sciences, LLC, New Castle, DE, USA) does not contain a visual inert and thus has a higher AQ%.

[c] Remainder of both avian repellents consisted of proprietary ingredients (Arkion® Life Sciences, LLC, New Castle, DE, USA).

[d] In 2017 we tested four AQ concentration levels (Trts 1-4) for both Concentration Response and Preference Tests. In 2018, we repeated the high concentration from 2017 (Trt 5) and added a treatment using Avipel™ to create a tank mix with higher AQ% (Trt 6) and a treatment where we removed disk flowers (Trt 7). Trt 4 and 5 are identical except were conducted in different years.

† Evaluated repellency where blackbirds were provided a single sunflower in a no-choice scenario. Consumption of treated sunflowers during the test days were compared to consumption on untreated sunflowers on pretest days to determine repellency (%).

‡ Evaluated preference and reduction in feeding when blackbirds were provided one treated and one untreated sunflower in a two-choice scenario. Consumption of treated sunflowers was compared to consumption of untreated sunflowers during test days to determine preference. Total consumption on test days was compared to pretest days to determine a reduction in feeding.

* Evaluated preference and reduction in feeding when disk flowers were removed and tank mix was sprayed directly on achenes embedded in the sunflower face.

We collected weekly samples of achenes (20 g) and disk flowers (9-15 g) from two sunflowers per treatment as well as tank mixtures (100 g) to analyze anthraquinone residues (USDA-APHIS-WS National Wildlife Research Center Analytical Chemistry Program, Fort Collins, CO USA conducted the residue analyses). We used gas chromatography with mass spectrometry detection to quantify anthraquinone concentrations from treated and untreated sunflower achenes and disk flowers (±1 ppm anthraquinone). Crushed sunflower achenes and disk flowers were weighed (2.0 g) and extracted with chloroform and hexane. A portion of the extract was cleaned using a dispersive solid phase extraction procedure (dSPE). The samples were filtered through a PTFE filter and analyzed by gas chromatography with tandem mass spectrometry.

Quantification was achieved using calibration standard solutions ranging from 2.55 ng/mL to 511 ng/mL. A deuterated form of anthraquinone (d8-anthraquinone) was used as a surrogate compound to correct for method variability. The method limit of detection (MLOD) was calculated for achenes (62 ppb anthraquinone) and disk flowers (2.3 ppb anthraquinone). The method limit of quantitation (MLOQ) was also calculated for achenes (2.1 ppb anthraquinone) and disk flowers (7.8 ppb anthraquinone). Descriptive statistics ($\bar{x}$ ± SE) were used to summarize anthraquinone residues on sunflower achenes and disk flower samples of repellent-treated and untreated sunflower heads. We calculated a linear regression to evaluate the relationship between residues on disk flowers and achenes in R (version 3.5.2; R Core Team 2019).

Concentration Response – We used a concentration response experiment to evaluate the relationship between the anthraquinone concentration applied to the sunflower face and the reduction in blackbird consumption (Figure 2.1A). We evaluated the efficacy of four treatments with increasing amounts of anthraquinone under an application scenario feasible for large-scale commercial agriculture (Table 2.1). We placed blackbirds, naïve to anthraquinone, in individual feeding cages (1.2 x 0.6 x 0.8 m) over a four day period, including one acclimation day (Day 1), two pretest days (Days 2-3), and one test (Day 4). We provided water *ad libitum*, throughout the experiment. To acclimate the birds to the cage and change in diet, we provided 30 g of maintenance diet and a single sunflower (Day 1). Following acclimation, birds were offered one untreated sunflower during each of the pretest days (Days 2-3) and one sunflower treated with avian repellent on the test day (Day 4). We ranked individual blackbirds based on average pretest consumption and randomly assigned birds to one of four treatment groups such that each group was similarly populated with birds exhibiting high-low daily consumption (Werner et al. 2009; Tupper et al. 2014; 2014b). For all days (Days 1-4), we offered access to the sunflower heads for a 10-hour period (08:00 to 18:00), given this is when red-winged blackbirds are active (Hintz & Dyer 1970). Outside of this time period, birds were offered 30 g of maintenance diet. We measured blackbird damage (change (Δ) in sunflower mass) and consumption (damage minus spillage) at the end of each 10-hour pretest and test day (Days 2-4).

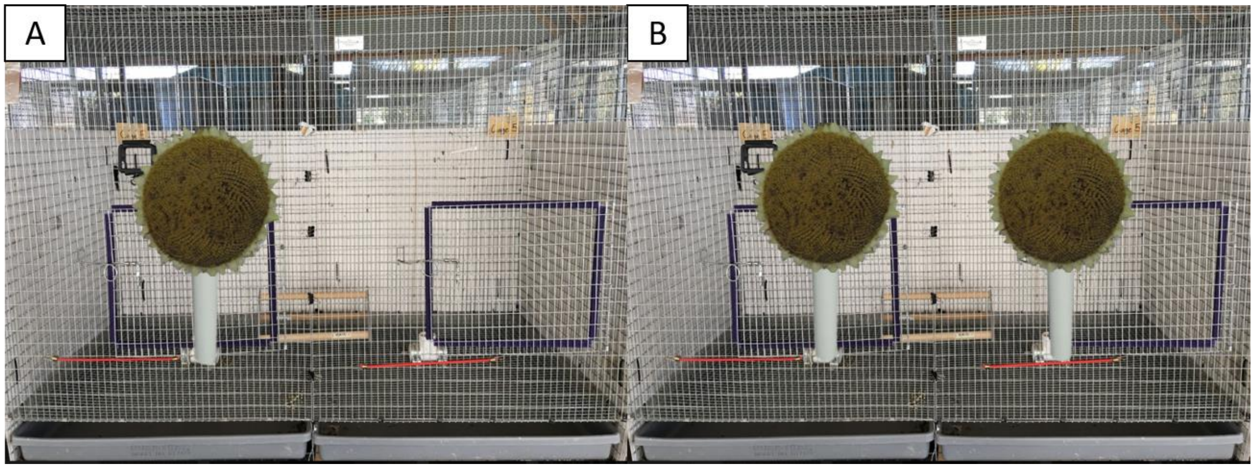

Figure 2.1. A) Cage design for the concentration response experiment (no-choice) with the sunflower consistently offered on one side of the cage. B) Cage design for the preference test experiment (two-choice) with the treated sunflower placed on alternate sides every test day.

Percent repellency was calculated by comparing test day (Day 4) consumption to the average pretest consumption (Days 2-3) (repellency = [1 – (test consumption/average pretest consumption)]*100) (Werner et al. 2014b). We used an analysis of variance (ANOVA) to compare blackbird repellency between four tank mixtures (Table 2.1). We assessed differences in consumption between tank mixtures using a mixed ANOVA via the "ez" package in R (Lawrence 2016) with individual bird as a random effect, our four tank mixture treatments as a between-subject variable, and the day of the experiment as a within-subjects repeated-measures variable. We used pairwise t-tests for multiple comparisons using a Bonferroni correction.

Preference Testing – We used a two-choice, preference design to compare consumption between treated and untreated sunflowers presented at the same time (Figure 2.1B). In 2017 we evaluated four treatments with varying amounts of anthraquinone (Table 2.1). We placed blackbirds naïve to anthraquinone in individual feeding cages (1.2 x 0.6 x 0.8 m) over a five day period including one acclimation day (Day 1), two pretest days (Days 2-3), and two test days (Day 4-5). We provided water *ad libitum* over the course of the experiment. We provided 30 g of maintenance diet and two sunflowers for acclimation (Day 1). On each pretest day (Days 2-3), we offered birds two untreated sunflowers. On test days (Days 4-5) we offered one untreated and one treated sunflower, alternating the side on which the treated sunflower was placed. We paired sunflowers within the same cage according to similar head diameters. We again ranked individual blackbirds based on average pretest consumption and randomly assigned birds to one of four treatment groups (Werner et al. 2009; Tupper et al. 2014; 2014b). For all days (Days 1-5), sunflowers were available for a 10-hour period (08:00 to 18:00) and 30 g of maintenance diet were offered in the remaining hours. We measured blackbird damage and consumption at the end of each 10-hour period for each individual sunflower. Consumption represented daily consumption on either treated or untreated sunflowers separately; total consumption represented daily consumption of both sunflowers in the cage combined.

In 2017, we saw a decline in total consumption (both sunflowers combined) on the final test day (Day 5) of the preference experiment but could not evaluate if the decline was due to the repellent or other factors such as a cage effect. Therefore, in 2018 we conducted additional preference experiments with the inclusion of control cages (i.e., birds were never offered treated sunflowers) and an additional two test days (Days 6-7). We conducted additional testing to evaluate if reduction in total consumption was due to accumulated repellent ingestion or cage effects. We added additional test days to evaluate if blackbirds were having a delayed response to accumulated anthraquinone residues over multiple days of ingestion and if this would continue over time. In 2018, we evaluated three treatments varying in percent anthraquinone including: 1) AV-5055 on intact sunflowers, 2) Avipel™ on intact sunflowers, and 3) AV-5055 applied after disk flowers were removed (Table 2.1). Each week, we tested one treatment on 8-9 blackbirds naïve to anthraquinone along with 4-5 blackbirds in control cages. The feeding trials included one acclimation day (Day 1), two pretest days (Days 2-3), and four test days (Day 4-7). We provided water *ad libitum* over the course of the experiment. We provided 30 g of maintenance diet and two sunflowers for acclimation (Day 1). On each pretest day (Days 2-3), we offered blackbirds two untreated sunflowers. On test days (Days 4-7) we offered blackbirds in treated cages one untreated and one treated sunflower, alternating the side on which the treated sunflower was placed. For birds in control cages, we offered two untreated sunflower on each test day. We ranked individual blackbirds based on average pretest consumption and randomly assigned blackbirds to treatment or control group (Werner et al. 2009; Tupper et al. 2014; Werner et al. 2014b). Sunflowers were available daily (Days 1-7) for a 10-hour period (08:00 to 18:00) and 30 g of maintenance diet were offered in the remaining hours. We measured blackbird damage and consumption at the end of each 10-hour period for each individual sunflower.

We used a two-way mixed ANOVA to evaluate consumption of untreated and treated sunflowers. Consumption was calculated for both untreated and treated sunflowers by averaging consumption on test days (2017: Days 4-5 and 2018: Days 4-7). We used individual bird as a random effect, treatment (repellent concentrations; Table 2.1) and sunflower (untreated and treated) as between-subjects effects, and test day as within-subjects repeated measure effect. We used a one-way mixed ANOVA to evaluate total consumption. Total consumption (both sunflowers combined) was calculated by averaging consumption on test days (2018: Days 4-7). We used individual bird as a random effect, treatment (treated and control cages) as between-subjects effect, and test day as within-subjects repeated-measure effect.

All statistical analyses for concentration response and preference tests were conducted using R (version 3.5.2; R Core Team, 2019). Dependent measures for each mixed ANOVA passed tests concerning assumptions of normality using a Shapiro-Wilk W statistic, equality in variance using Bartlett's Test of Homogeneity of Variance, and sphericity using Mauchly's Tests. When mixed ANOVAs indicated significance, we performed pairwise t-tests for multiple comparisons of means with Bonferroni corrections ($p < 0.05$) to determine which values differed significantly.

Field Application of Repellent Using Drop-Nozzle Equipped Ground Rigs

Study Site – We evaluated foliar application of an avian repellent in an open field experiment at NDSU Carrington Research Extension Center (REC) in Carrington, ND, USA. Personnel at NDSU Carrington REC planted oilseed hybrid sunflower (Pioneer P64ME0) plots on 07 June 2018 and maintained the crop throughout the growing season. We established four replicates of five treatments and a control (no treatment; Table 2.2). Each replicate was 9 m long with 76 cm between sunflower rows for a width of 1.5 m. Each treatment plot covered 0.00138 ha. Each replicate had three rows of treated sunflower, five buffer rows between each treatment, and six buffer rows on each end.

Table 2.2. On 07 September 2018, four plots received no repellent (untreated control) and each of the five treatments had four replicates, each with varying amounts of avian repellent and unique spraying strategies at the NDSU Carrington Research Extension Center in Carrington, ND, USA.

Treatment	**Speed m/s (mph)**	**Pressure PSI**	**Application Rate L/ha (gal/ac)**	**Repellent Application Rate L/ha (gal/ac)†**	**Nozzles***	**Spray Action**	**Residue on Achenes at Application (ppm ± SE)**	**Residue on Disk Flowers at Application (ppm ± SE)**	**Residue on Achenes at Harvest (ppm ± SE)**
F1	1.07 (2.4)	50	187.0 (20.0)	9.35 (1.0)	VK3 XR11001	continuous	7.4 ± 1.2	258.6 ± 14.8	6.1 ± 0.8
F2	1.07 (2.4)	50	187.0 (20.0)	18.71 (2.0)	VK3 XR11001	continuous	27.9 ± 7.4	1,021.9 ± 274.8	12.3 ± 3.7
F3	0.54 (1.2)	50	187.0 (20.0)	9.35 (1.0)	VK3 XR11001	50% pulse	6.0 ± 0.6	342.42 ± 21.4	5.6 ± 1.4
F4	1.07 (2.4)	70	221.0 (23.6)	11.03 (1.2)	VK3 XR11001	continuous	13.3 ± 0.8	404.6 ± 48.3	12.3 ± 1.7
F5	2.2 (4.8)	50	187.0 (20.0)	9.35 (1.0)	VK3 AIXR11002	air induction	4.1 ± 0.5	165.5 ± 12.0	4.9 ± 0.6

*VK3 (n=1) angled backport; XR11001 (n=2) side ports; AIXR11002 (n=2) side ports.

† Tank mixtures had 5% Avipel™ mixed with water, except treatment F2 which had 10% Avipel™

Repellent Application – On 7 September 2018, we used a ground rig equipped with 360 Undercover® drop nozzle sprayers (360 Yield Center, Morton, IL, USA) to apply the anthraquinone-based repellent (Avipel™; 50% anthraquinone; Arkion® Life Sciences, Inc.) when at least 50% of the sunflowers had completed anthesis or the R_6 growth stage (Schneiter & Miller 1981). Prior to repellent application, we pinned Syngenta Water Sensitive Paper (76.2 x 25.4 mm; Spraying Systems Inc., Wheaton, IL, USA) to the faces of five sunflowers per test plot to assess spray coverage. Repellent coverage was calculated for each plot by averaging the percent coverage found on the five spray cards.

We collected disk flower and achene samples for residue analyses immediately after application and prior to harvest. We collected a 100 mL sample of the anthraquinone-based repellent and a 100 mL sample of each repellent tank formulation for residue analyses. Sunflower samples for residue analyses were collected within each of the 24 test plots (4 samples of untreated control and 4 samples of each treatment) upon repellent application (07 September 2018) and at harvest (31 October 2018). At application, we collected 5 g of achenes from four sunflower heads for a total subsample of 20 g of achenes from each plot. Whereas, at harvest, we took 20 g of achenes from the harvested sample. To sample disk flowers, we brushed off the disk flowers from four sunflower heads to collect a subsample of 20 g. We only collected disk flowers during application as most were lost by the time of harvest. Residue analyses were conducted by USDA-APHIS-WS NWRC Analytical Chemistry Unit. We used Kruskal-Wallis tests to evaluate differences in repellent coverage and anthraquinone residue on disk flowers and achenes at time of application between the five treatments (Tables 2.2).

Sunflower Damage and Yield – Although the primary purpose of the field experiment was to collect residue data under different application strategies, free-ranging birds were found within the test plots (based on presence of damage), thus we report damage data. Albeit, the abundance or species of free-ranging birds was not documented. We estimated pretreatment damage within each plot prior to repellent application as virtually null and estimated a final assessment just prior to harvest. Repellent efficacy was based upon comparative bird damage and sunflower yield between repellent-treated and untreated control plots. We estimated seed loss by placing a template with 5 cm^2 grids on the sunflower head (Dolbeer 1975; Klosterman et al. 2013). Percent bird damage on each head was obtained by dividing the total area of damage by the total area potentially available minus the area of the undeveloped center and multiplying by 100. Undamaged heads were measured and 0% damage was recorded. Percent damage for each plot was calculated by sampling every head within the middle row. Sunflower yield was reported in the treated and control plots by weighing and correcting harvested seed to a standardized 10% moisture (Eq. 2.1). Sunflowers were harvested using the NDSU Carrington REC small plot harvester. Any unused product was destroyed per existing pesticide regulations. We used Kruskal-Wallis tests to evaluate our bird damage and sunflower yield between the five treatments and control (Table 2.2).

Results

Laboratory Feeding Experiments Using Intact Sunflower Plants

Repellent Application – When using the automated spray machine, the percent spray coverage ranged from 39-62% with no significant differences between coverage for the six tank mixtures applied to sunflower with intact disk flowers (Trt 1-6; Kruskal-Wallis, $\chi^2 = 8.95$, $p = 0.11$, $df = 5$). As the percent anthraquinone in the tank mixture increased, residues on both disk flowers and achenes increased (Figure 2.2A). We found a significant linear relationship ($p<0.0001$, adjusted $R^2 = 0.64$) between anthraquinone residues on disk flowers and achenes with anthraquinone residues on disk flowers 100 times greater than that found on the achenes of treated sunflowers (y = 0.01(x) – 0.08; Figure 2.2B).

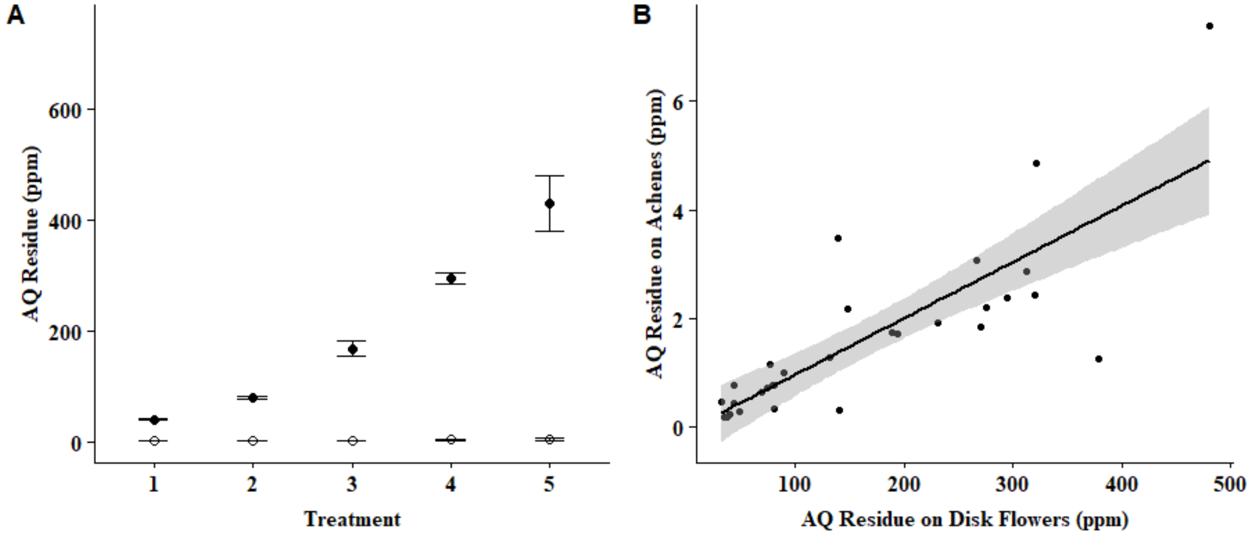

Figure 2.2. A) Anthraquinone (AQ) residue ± SE on disk flowers (range = 40-1,095 ppm; closed circles) and achenes (range = 0-6 ppm; open circles) associated with the five AV-5055 treatments (Trt. 1-5; Table 2.1). AQ residue concentrations increased with increasing AQ% in the tank mixture. B) Relationship between AQ residues observed on disk flowers and achenes ($p < 0.0001$, adjusted $R^2 = 0.64$). For every 100 ppm increase in disk flower residue, there is a 1 ppm increase on the achenes (y = 0.01(x) - 0.08), highlighting that disk flowers act as a barrier for repellent to reach achenes.

Concentration Response – We observed no significant differences in repellency between the four tank mixtures with increasing anthraquinone concentrations (Table 2.1 and Figure 2.3A). Furthermore, repellency failed to approach the target of 80% for any of the tank mixtures tested. We found no significant differences in consumption between tank mixtures ($F_{3,46} = 0.37$, $p = 0.78$, $\eta_G^2 = 0.02$). However, day of the experiment (within-subjects repeated measure) had a significant effect on consumption ($F_{2,92} = 4.92$, $p = 0.009$, $\eta_G^2 = 0.02$; Figure 2.3B). A post-hoc pairwise t-test using the Bonferroni correction showed consumption on pretest Day 2 ($\bar{x} = 12.6 \pm 0.7$ g) to be significantly different than on pretest Day 3 ($\bar{x} = 14.4 \pm 0.7$ g), with test day (Day 4) consumption falling in between ($\bar{x} = 13.7 \pm 0.8$ g).

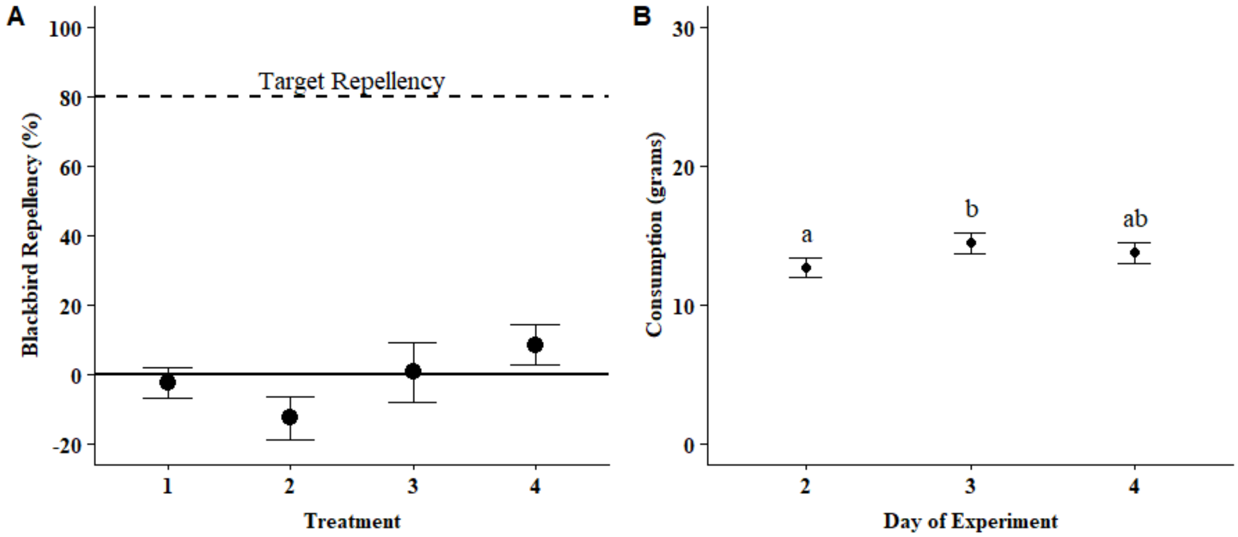

Figure 2.3. A) Blackbird repellency calculated as the consumption of treated sunflowers (Day 4) compared to average consumption of untreated sunflowers (Days 2-3). Dashed line represents target repellency to be considered effective. B) Blackbird consumption on pretest days (Days 2-3) and test day (Day 4). Means with different lowercase letters are significantly different (pairwise comparison with Bonferroni correction, $p < 0.05$).

Preference Testing – In 2017, we found no significant differences in consumption between tank mixture treatments ($F_{3,68} = 0.56$, $p = 0.65$, $\eta_G^2 = 0.017$). Conversely, there was a significant effect of test day ($F_{1,68} = 33.78$, $p < 0.0001$, $\eta_G^2 = 0.128$) and sunflower (untreated vs treated) by day interaction ($F_{1,68} = 4.12$, $p = 0.0464$, $\eta_G^2 = 0.018$) on consumption. Blackbirds did not discriminate between untreated ($\bar{x} = 10.8 \pm 1.0$ g) and treated sunflower ($\bar{x} = 11.4 \pm 0.8$ g) over the test days (Days 4-5). However, a post-hoc pairwise t-test using the Bonferroni correction showed that total consumption (both sunflowers combined) on the final test day (Day 5) was significantly lower than total consumption of the previous three days (Days 2-4), decreasing between 36-39% (Figure 2.4).

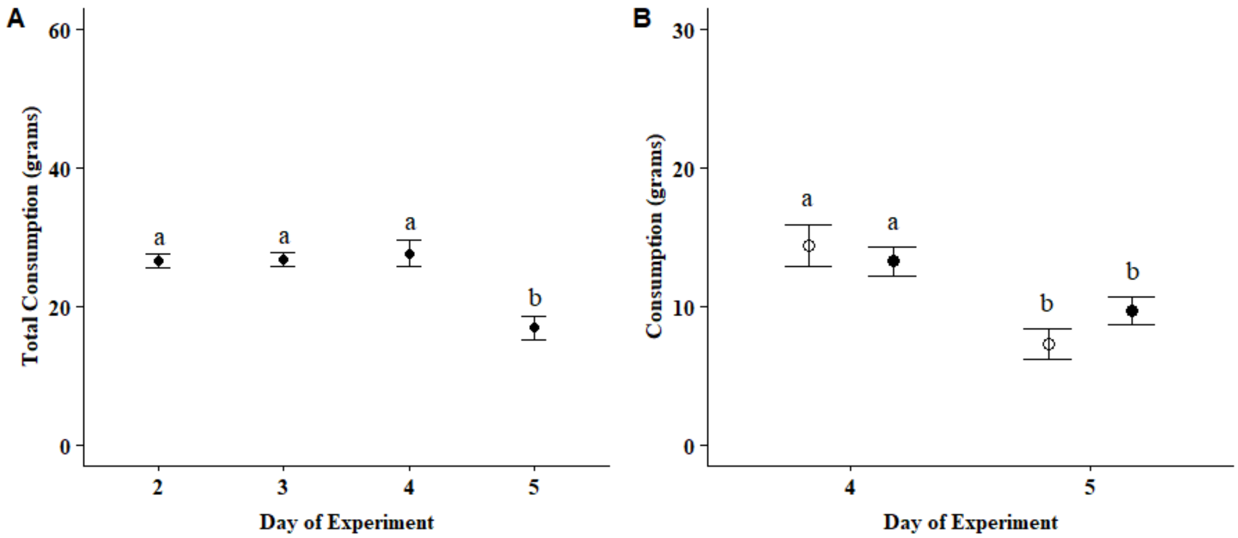

Figure 2.4. A) In 2017, total consumption (mean ± SE) including both treated and untreated sunflowers was less on the second test day (Day 5) than the previous test day (Day 4) and pretest days (Days 2-3). B) Consumption (mean ± SE) on untreated (open circles) and treated sunflowers (closed circles). Means with different lowercase letters are significantly different (pairwise comparison with Bonferroni, $p < 0.05$).

In 2018, we observed no difference in total consumption (both sunflowers combined) between blackbirds in control cages ($\bar{x}$ = 28.9 ± 2.4 g) and blackbirds in treated cages ($\bar{x}$ = 23.2 ± 1.7 g) over the four test days (Days 4-7; Figure 2.5A), but we observed a significant effect of test day ($F_{3,33} = 31.7$, $p < 0.001$, $\eta_G^2 = 0.31$) on total consumption. Blackbirds consumed more on Day 4 (29.8 ± 2.2 g) and Day 5 (31.5 ± 2.9 g) than on Day 6 (20.2 ± 2.5 g) and Day 7 (18.2 ± 2.0 g). Blackbirds in treated cages did not significantly discriminate between untreated sunflower ($\bar{x}$ = 9.6 ± 1.1 g) and sunflower treated with 20% AV-5055 (Trt 5; $\bar{x}$ = 13.6 ± 1.4 g; Figure 2.5B).

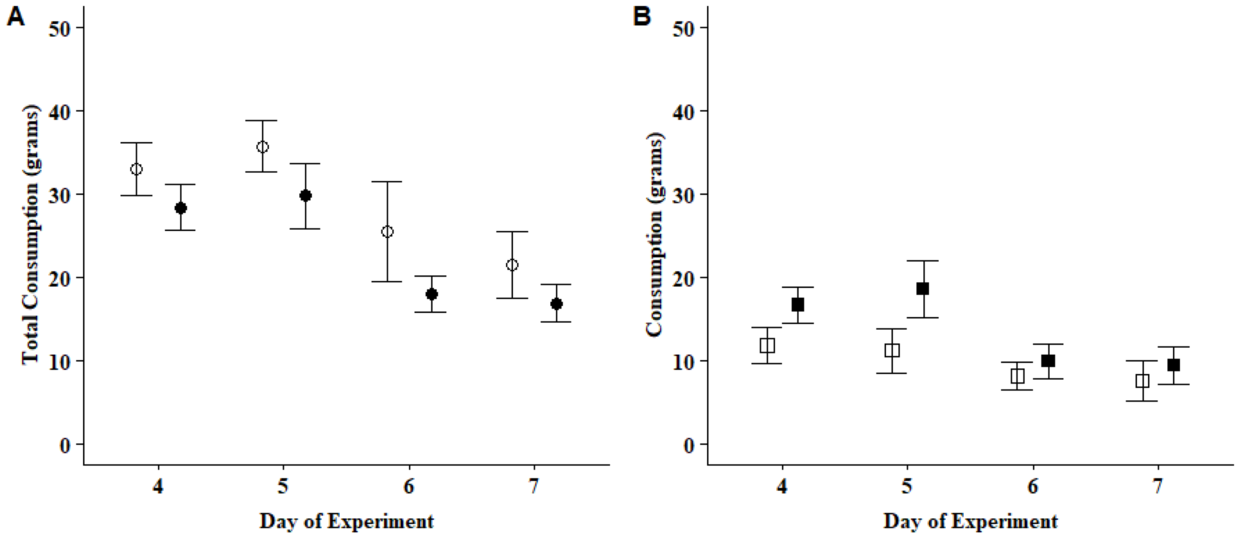

Figure 2.5. A) In 2018, total consumption (both sunflowers combined; mean ± SE) of birds in control cages (open circles) and cages with sunflowers treated with AV-5055 (closed circles; Trt 5 [see Table 2.1]) over the four test days (Days 4-7). B) Consumption of untreated sunflower (open squares) and sunflower treated with AV-5055 (closed squares) in treatment cages over the four test days (Days 4-7).

We observed no significant difference in total consumption (both sunflowers combined) between blackbirds in control cages ($\bar{x}$ = 18.0 ± 1.0 g) and blackbirds in cages treated with Avipel™ containing the highest concentration of anthraquinone (Trt 6 [see Table 2.1]; $\bar{x}$ = 17.8 ± 1.1 g) over the four test days (Days 4-7; Figure 2.6A). However, we observed a significant effect of test day ($F_{3,33} = 7.6$, $p < 0.001$, $\eta_G^2 = 0.22$) on total consumption. Blackbirds consumed more on Day 7 (21.9 ± 0.9 g) than on Day 5 (13.4 ± 1.1 g). We observed no significant difference between consumption of untreated sunflowers ($\bar{x}$ = 9.8 ± 1.1 g) and sunflowers treated with Avipel™ (Trt 6; $\bar{x}$ = 8.0 ± 1.1 g; Figure 2.6B).

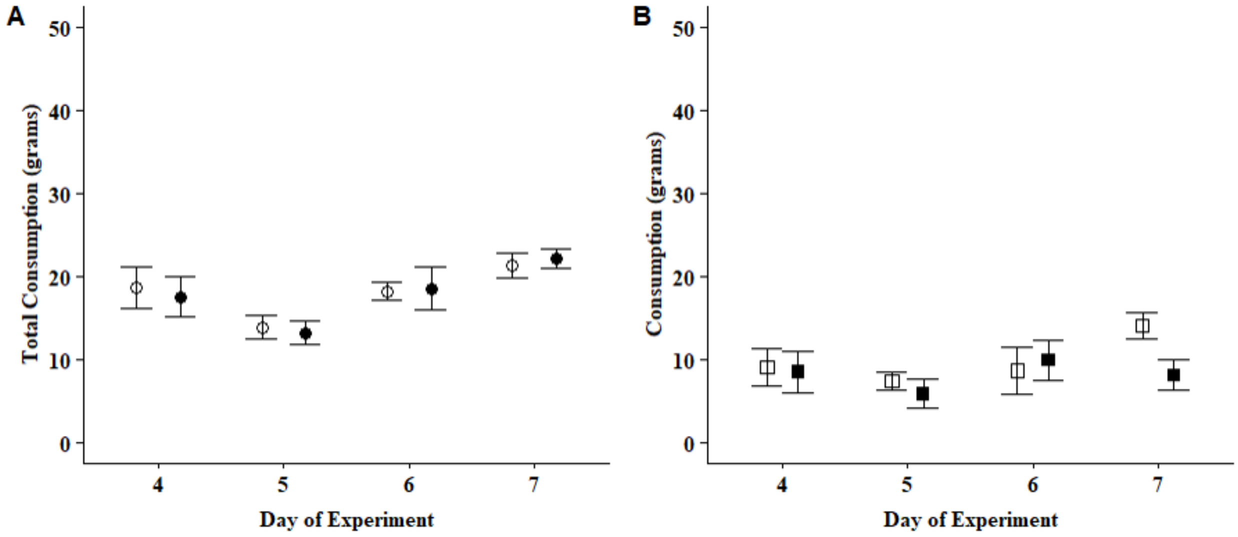

Figure 2.6. A) In 2018, total consumption (both sunflowers combined; mean ± SE) of birds in control cages (open circles) and cages with sunflowers treated with Avipel™, the treatment with the highest concentration of anthraquinone (closed circles; Trt 6 [see Table 2.1]) over the four test days (Days 4-7). B) Consumption of untreated sunflower (open squares) and sunflower treated with Avipel™ (closed squares) in treatment cages over the four test days (Days 4-7).

Total consumption (both sunflowers combined) did not differ between blackbirds in control cages ($\bar{x}$ = 12.7 ± 1.9 g) and blackbirds in cages where sunflowers were treated with AV-5055 after disk flowers were removed (Trt 7 [see Table 2.1]; $\bar{x}$ = 11.1 ± 1.1 g) over the four test days (Days 4-7; Figure 2.7A). However, we observed a significant effect of test day ($F_{3,33} = 4.2$, $p = 0.01$, $\eta_G^2 = 0.17$) with blackbirds consuming significantly more on Day 4 (15.2 ± 3.4 g) and Day 7 (12.6 ± 0.6 g) than on Day 6 (7.7 ± 1.2 g). We observed no significant difference between consumption of untreated sunflower ($\bar{x}$ = 7.0 ± 0.9 g) and sunflowers treated with repellent when the disk flowers were removed ($\bar{x}$ = 4.1 ± 0.7 g; Figure 2.7B).

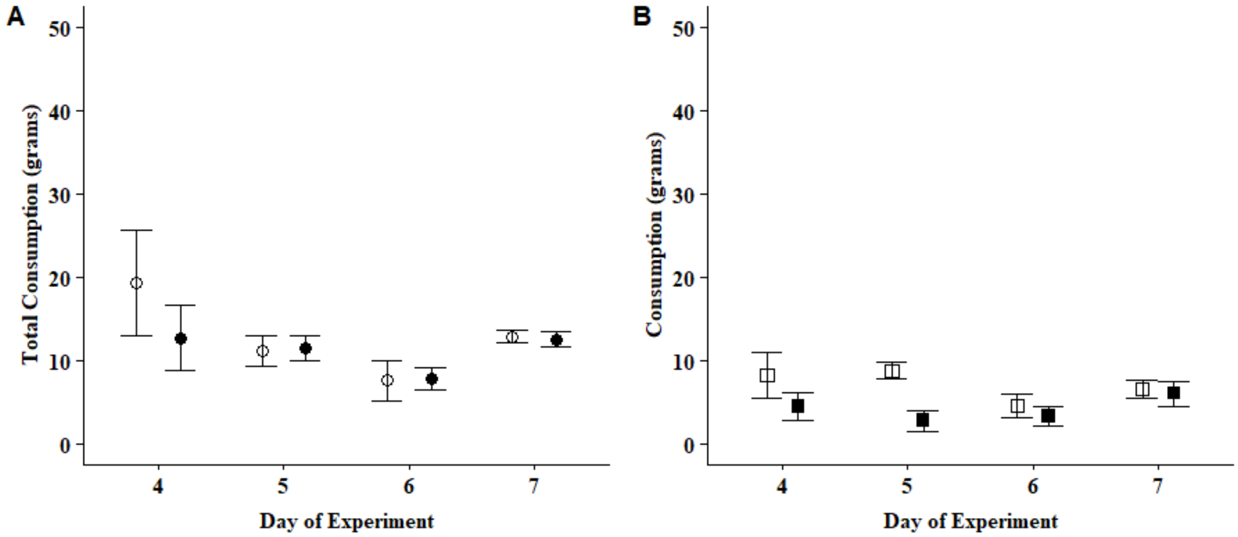

Figure 2.7. A) In 2018, total consumption (both sunflowers combined; mean ± SE) of birds in control cages (open circles) and cages with sunflowers treated with AV-5055 after disk flowers were removed (closed circles; Trt 7 [see Table 2.1]) over the four test days (Days 4-7). B) Consumption of untreated sunflower (open squares) and sunflower treated with AV-5055 after removing disk flowers (closed squares) in treated cages over the four test days (Days 4-7).

Field Application of Repellent Using Drop-Nozzle Equipped Ground Rigs

There were no significant differences in repellent coverage between the five treatments (Trts F1-F5; Kruskal-Wallis, $\chi^2 = 3.1$, p = 0.54, df = 4). Average percent coverage of the five treatments ranged from 0-76% (mean ± SE; 19 ± 2%). Tank mixtures applied in the field ranged from 1.98–2.22 ppm anthraquinone. We found a significant difference in anthraquinone residue on achenes (Figure 2.8A; Kruskal-Wallis, $\chi^2 = 20.7$, p <0.001, df = 5) and disk flowers between treatments (Figure 2.8B; Kruskal-Wallis, $\chi^2 = 18.1$, p <0.003, df = 5). Anthraquinone residues on achenes ranged from 3-49 ppm at application and 2-20 ppm at harvest on the treated plots (Table 2.2). Residues on the disk flowers ranged from 127-1,590 ppm at application for the treated plots (Table 2.2). Disk flowers could not be sampled at harvest due to disk flowers naturally falling off as sunflower phenology progresses.

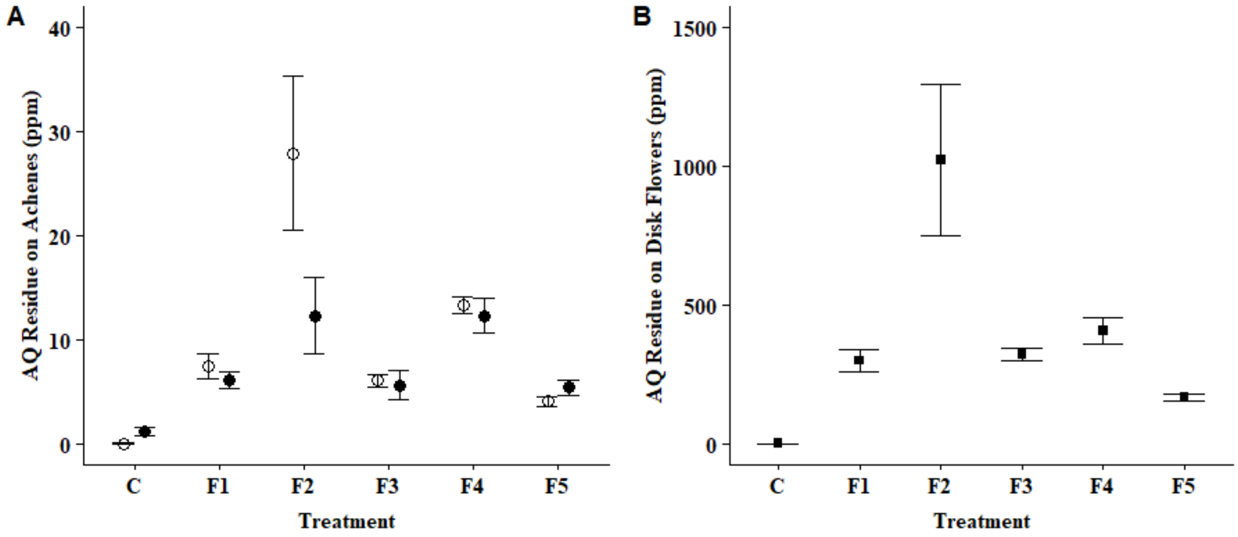

Figure 2.8. A) Anthraquinone (AQ) residue on achenes (mean ± SE) at application (open circles) and after harvest (closed circles) for treatment plots (F1-F5; see Table 2.2) and control plots (C). B) AQ residue on disk flowers (mean ± SE) at application for control and treatment plots.

Average percent damage of the five treatments and control did not statistically differ (Kruskal-Wallis, $\chi^2 = 2.9$, $p = 0.7$, df = 5). Damage was relatively low in both treated plots (mean percent damage ± SE; 5.0 ± 0.4%) and control plots (3.9 ± 0.7%). Average yield did not statistically differ between the five treatments and control (Kruskal-Wallis, $\chi^2 = 5.4$, $p = 0.37$, df = 5). Agronomic differences may influence yield between treated plots (mean yield ± SE; 1,883.0 ± 55.3 kg/ha) and control plots (1,975.9 ± 67.6 kg/ha). However, average area of developed sunflower for treated plots (194.3 ± 10.2 cm^2) and control plots (196.2 ± 16.1 cm^2) were similar as was oil content for control (38.4 ± 0.3%) and treated plots (38.8 ± 0.2%).

Discussion

Repellent optimized for loose, dry achenes does not effectively reduce blackbird consumption when realistically applied to the sunflower plant in laboratory or field settings as necessary residue levels on achenes cannot be achieved due to plant structure. Although anthraquinone residue found on achenes in laboratory and field tests increased as repellent concentration in the tank mixtures increased, residues on achenes (0-49 ppm) were well below biologically relevant amounts (>385 ppm; Werner et al. 2014b). Simply increasing concentrations of anthraquinone in formulations or tank mixtures may increase residues, but only to the disk flowers and exposed portions of the achene. Without disk flowers to obstruct repellent application, achenes would presumably have residues similar to that found on disk flowers. However, when we removed disk flowers prior to repellent application, residues (49 ± 18 ppm) still failed to reach levels necessary to reduce blackbird feeding. Blackbirds also failed to discriminate between untreated and treated sunflowers in the same cage, even though the repellent included a visual inert, which should have aided in reducing consumption when directly applied to achenes. Although not statistically significant, consumption of sunflowers treated with repellent after removing disk flowers ($\bar{x} = 4.1 \pm 0.7$ g) was almost half of untreated sunflowers ($\bar{x} = 7.0 \pm 0.9$ g). These data suggest residues on achenes were approaching, yet still below, the necessary concentration to adequately reduce blackbird feeding.

Our data address an important difference between testing repellents on loose achenes compared to the sunflower plant. Application to the sunflower plant results in, at most, a fraction of each oilseed achene exposed and available for repellent deposition, leaving a majority of the embedded achene untreated. This differs from previous laboratory studies where whole achenes are coated with repellent, resulting in the entire surface area of the hull being treated (Werner et al. 2009; 2014a). Thus, to reach the residue level necessary to reduce avian feeding in the field you would need to achieve the same lab-based residue (~1,000 ppm) on a fraction of the surface area (Werner et al. 2014a). In a field study, Werner et al. (2011; 2014b) found a 33-34% reduction in blackbird feeding at residues around 385 ppm, but this was likely due to heavy repellent application that is unfeasible for producers. Higher application rates paired with increased repellent concentrations may increase residue on achenes, but tank mixtures exceeding 20% repellent applied at >126 L/ha are beyond the concentrations economical and logistically feasible for producers.

Anthraquinone residue on achenes was not only restricted by the limited exposed surface area of embedded achenes, but also by disk flowers obstructing repellent from reaching achenes. Our results are the first to quantitatively measure the role of disk flowers as a barrier for repellent to reach embedded sunflower achenes. Until now, disk flowers have been vastly disregarded in evaluation of repellent application in sunflower given previous studies were focused on application strategies from above the sunflower canopy and mainly interested if the repellent even reached the face. (Linz et al. 2014; Niner et al. 2015). Disk flowers are of greater interest now that drop-nozzle equipped ground rigs can deploy repellent to the sunflower face. Disk flowers cover the face of the sunflower, leaving minimal space for repellent to penetrate through the floral barrier. In our laboratory studies, anthraquinone residue on disk flowers (40-1,095 ppm) was 100 times higher than residue on achenes (0-6 ppm), when repellent was applied to the face of ripening mature sunflower heads. Disk flowers experienced repellent residues that were closer to lab-tested residues on achenes (<1,000 ppm), which have been found to decrease blackbird feeding by >80% (Werner et al. 2014a). However, even disk flowers with higher residues failed to reduce blackbird consumption, suggesting that treating disk flowers plays an insignificant role in reducing blackbird damage on ripening sunflower.

Anthraquinone must be ingested to be effective and thus must be present on parts of the plant that are ingested or come into contact with the mouth. Even though disk flowers were removed by blackbirds in order to reach the underlying achenes, they are not ingested and thus not an efficient avenue for repellent ingestion. Ideally, residues on disk flowers would reduce feeding while keeping the residue on achenes low enough to conform to US EPA food tolerance levels (Eisemann et al. 2011), but high residues on disk flowers did not reduce consumption in our tests. Anthraquinone residue on achenes in the field decreased slightly from application to harvest (9.8 ± 4.0, 7.12 ± 1.8, respectively), but not substantially enough to meet food tolerance restrictions. Similar decreases in anthraquinone residue over time have been observed in field plot studies and were contributed to rainfall (Werner et al. 2014b; Niner et al. 2015). However, achene residues at application were collected from random heads, whereas residues at harvest were collected from threshed sunflower heads. Collecting residues from selected heads in the field may be less consistent as repellent coverage on individual sunflower heads was variable.

For the advancement of application strategies capable of depositing repellent to the face of the sunflower, the issue of plant structure will have to be addressed. For example, as mature sunflower heads approach harvest, disk flowers begin to dry up and shrivel, which would reduce the floral barrier. Reduced disk flowers would allow more repellent to reach the newly exposed achenes. Applying repellent in the field during or after disk flower loss may reduce repellent interception by floral components, but would not protect the crop during the early and most heavily damaged stage of 18 days after petal drop (Cummings et al. 1989). Alternatively, multiple applications across the damage season may allow for more complete protection but would require more product and labor, decreasing cost-effectiveness. At the same time, ground-rig application requires sacrificing two crop rows for each sprayer pass unless extra space is accounted for at planting. Sunflower crops closer to harvest are also more vulnerable to lodging, introducing additional damage to the crop. Thus, the repellent would have to be extremely effective to compensate for this additional loss.

Repellent application in the lab was conducted in a best-case scenario where sunflowers were treated by directly applying repellent to the face using a controlled spraying machine. Field application via drop-nozzles positioned beneath the canopy allow repellent to reach the face of the sunflower, but is subject to variation due to sunflower head position including height and angle of the head relative to the stem. The face of sunflower relative to the spraying nozzles dictates the degree of coverage as well as the amount of repellent that reaches the face. Repellent coverage achieved for the five field-tested application strategies were relatively low (range = 0-76%; mean ± SE = 19 ± 2%), thus scaling up to the field adds further complication in developing an effective repellent for sunflowers.

Anthraquinone residues did not differ with application strategy with the exception of the F2 treatment containing two times (Avipel™ at 10% of tank mixture) the amount of repellent. In this context, the most important parameter for achieving increased residue is the inclusion of higher concentrations of anthraquinone. Increased repellent concentration resulted in higher residues, even with lower overall coverage. When the tank mixture contained twice the repellent formulation, coverage decreased by 33% and variation in coverage increased. This suggests that higher concentrations are needed to reach efficacious residues, but efficacy is still restricted by limited and inconsistent coverage. Other studies have shown that increased concentrations of chemical applications do not increase coverage, but other spraying details, such as droplet size may function to increase coverage (Knoche 1994).

Reducing speed should result in increased coverage if output is similar, but we saw a decrease when speed was reduced by 50% and spraying action was at a 50% pulse. When pressure was increased to 70 PSI, the application rate increased from 20.0-23.6 gal/ac, while resulting in similar coverage and slightly higher residues. Thus, higher output does not necessarily result in increased coverage. Whereas, increased residues may be a result of higher application rates or increased pressure (i.e., smaller droplets) (Nuyttens et al. 2007). The importance of droplet size is not well understood for avian repellents but finer droplets may be required to infiltrate disk flowers. Anthraquinone-based repellents are viscous and higher concentrations of repellent in the tank mixture restricts application to course nozzles (i.e., 0.1 gal/min) to reduce clogging, producing larger droplets, which may be poorly retained due to their weight (Hartley & Brunskill 1958). Our results suggest application of a viscous anthraquinone-based repellent can be achieved under a variety of application parameters, but increased repellent concentration and manipulating droplet size should be further explored. A combination of repellent concentration in the tank mixture and coverage dictate the level of protection and should be the focus to maximize application efficiency at the field scale.

We did not find secondary repellents to currently be a suitable option for sunflower crops as sufficiently high residue levels were not achieved on the achenes due to components of the plant structure. Although lab efficacy has been achieved with anthraquinone-based repellents, field efficacy within the capability of producers to apply the repellent at broad scales remains to be seen. Field application beneath the canopy via drop nozzles provides evidence that chemical application to the face of mature sunflower can be achieved but fails to achieve residues high enough to be effective. However, higher repellent concentrations increased the chemical output and thus the cost, while residues only marginally increased. Even if coverage in the field can be increased to >50%, disk flowers still act as a barrier for repellent to reach the achenes.

The incorporation of sensory cues in chemical repellents has shown potential to increase the cost-effectiveness of avian repellents by reducing residues required to effectively decrease blackbird feeding (Werner et al. 2014a). However, repellents with sensory cues are optimized in the lab on loose, dry achenes offered in a bowl, and it is unknown how red-winged blackbirds perceive the repellent on the vegetative and floral parts of mature crops (Werner et al. 2014a). The complexity of a mature sunflower head may inhibit the visual component of the repellent from providing the same deterrence found in lab studies. For example, sunflowers change from a deep green, to pale yellow, and finally yellowish-brown during the major part of the damage period (Schneiter & Miller 1981). Blackbirds may use visual cues to evaluate crop maturity when choosing a particular field for foraging and the addition of repellent may interfere. For example, Niner (2014) observed more damage to sunflower plots treated with an anthraquinone-based repellent compared to control plots when the repellent was applied above the canopy. Further work on avian repellents should consider how the repellent may change the visual properties of the crop and how this influences the interaction with the foraging pest species.

References

Abraham BP, Sellin JH. 2012. Drug-induced, factitious, & idiopathic diarrhoea. *Best Practice & Research Clinical Gastroenterology* **26**:633-648.

Anderson W. 1975. Maturation of sunflower. *Australian Journal of Experimental Agriculture* **15**:833-838.

Avery ML. 2003. Avian repellants. *Encyclopedia of agrochemicals*.

Avery ML, Humphrey JS, Decker DG. 1997. Feeding deterrence of anthraquinone, anthracene, and anthrone to rice-eating birds. *Journal of Wildlife Management* **61**:1359-1365.

Carlson JC, Tupper SK, Werner SJ, Pettit SE, Santer MM, Linz GM. 2013. Laboratory efficacy of an anthraquinone-based repellent for reducing bird damage to ripening corn. *Applied Animal Behaviour Science* **145**:26-31.

Cummings JL, Guarino JL, Knittle CE. 1989. Chronology of blackbird damage to sunflowers. *Wildlife Society Bulletin (1973-2006)* **17**:50-52.

Dolbeer RA. 1975. Comparison of 2 Methods for Estimating Bird Damage to Sunflowers. *Journal of Wildlife Management* **39**:802-806.

Dolbeer RA. 1978. Movement and migration patterns of red-winged blackbirds: a continental overview. *Bird-Banding* **49**:17-34.

Egan CC. 2018. Evaluating the Potential Utility of Drones to Deter Birds from Areas of Human-Wildlife Conflict. MS Biological Sciences. North Dakota State University, Fargo, ND, USA. p. 93.

Eisemann JD, Werner SJ, O'hare JR. 2011. Registration considerations for chemical bird repellents in fruit crops. *Outlooks on Pest Management* **22**:87-91.

Hartley G, Brunskill R. 1958. Reflection of water drops from surfaces. *Surface Phenomena in Chemistry and Biology* **214**.

Hintz JV, Dyer MI. 1970. Daily Rhythm and Seasonal Change in Summer Diet of Adult Red-Winged Blackbirds. *Journal of Wildlife Management* **34**:789-799.

Hulke BS, Kleingartner LW. 2014. Sunflower. *Yield gains in major US field crops*:433-458.

Kandel H, Johnson B, Deplazes C, Linz G, Santer M. 2009. Sunflower treated with Avipel (Anthraquinone) bird repellent. Page 12. In Proceedings of National Sunflower Association Research Forum, Fargo, ND.

Kersting J, Stickler F, Pauli A. 1961. Grain Sorghum Caryopsis Development. I. Changes in Dry Weight, Moisture Percentage, and Viability 1. *Agronomy Journal* **53**:36-38.

Klosterman ME, Linz GM, Slowik AA, Homan HJ. 2013. Comparisons between blackbird damage to corn and sunflower in North Dakota. *Crop Protection* **53**:1-5.

Klug P. 2017. The future of blackbird management research. Pages 217-234. *Ecology and Management of Blackbirds (Icteridae) in North America.* (eds G.M. Linz, M.L. Avery & R.A. Dolbeer) CRC Press/Taylor & Francis, Boca Raton, Florida, USA.

Knoche M. 1994. Effect of droplet size and carrier volume on performance of foliage-applied herbicides. *Crop Protection* **13**:163-178.

Larson TD, Johnson BL, Henson RA. 2008. Comparison of stay-green and conventional sunflower desiccation in the Northern Great Plains. *Agronomy Journal* **100**:1124-1129.

Lawrence MA. 2016. ez: Analysis of and Visualization of Factorial Experiments. R package version 4.4-0.

Linz G, Klug P, Dolbeer R. 2017. *Ecology and Management of Blackbirds (Icteridae) in North America* (eds G.M. Linz, M.L. Avery & R.A. Dolbeer). Pages 17-41. CRC Press, Boca Raton, Florida, USA.

Linz G, Werner SJ, Niner M, Homan J. 2014. Effectiveness of Foliar Applications of 9, 10-Anthraquinone for Reducing Blackbird Damage to Sunflower (eds R.M. Timm, J.M. O'Brien). Pages 335-337. In Proceedings of the 26th Vertebrate Pest Conference, University of California, Davis.

Linz GM, Homan HJ, Werner SJ, Hagy HM, Bleier WJ. 2011. Assessment of Bird-management Strategies to Protect Sunflowers. *Bioscience* **61**:960-970.

Linz GM, Vakoch DL, Cassel JF, Carlson RB. 1984. Food of Red-Winged Blackbirds, *Agelaius-phoeniceus*, in Sunflower Fields and Corn Fields. *Canadian Field-Naturalist* **98**:38-44.

Mason JR, Dolbeer RA, Woronecki PP, Bullard RW. 1989. Maturational and Varietal Influences on Sunflower Consumption by Red-Winged Blackbirds. *Journal of Wildlife Management* **53**:841-846.

Niner MD. 2014. The effectiveness of 9, 10 anthraquinone as a repellent to protect oilseed sunflower from blackbird depredation. North Dakota State University.

Niner MD, Linz GM, Clark ME. 2015. Evaluation of 9, 10 anthraquinone application to pre-seed set sunflowers for repelling blackbirds. *Human-Wildlife Interactions* **9**:4-13.

Nuyttens D, Baetens K, De Schampheleire M, Sonck B. 2007. Effect of nozzle type, size and pressure on spray droplet characteristics. *Biosystems Engineering* **97**:333-345.

Peer BD, Homan HJ, Linz GM, Bleier WJ. 2003. Impact of blackbird damage to sunflower: Bioenergetic and economic models. *Ecological Applications* **13**:248-256.

Schneiter AA, Miller JF. 1981. Description of Sunflower Growth-Stages. *Crop Science* **21**:901-903.

Shwiff SA, Ernest KL, Degroot SL, Anderson AM, Shwiff SS. 2017. The economic impact of blackbird damage to crops. *Ecology and Management of Blackbirds (Icteridae) in North America*:207-216.

Team RC. 2019. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. *URL:(https://www. R-project. org).*

Tomlin C. 2003. The e-Pesticide Manual, Version 3.0. *The British Crop Protection Council, Hampshire, UK.*

Tupper SK, Werner SJ, Carlson JC, Pettit SE, Wise JC, Lindell CA, Linz GM. 2014. European starling feeding activity on repellent treated crops and pellets. *Crop Protection* **63**:76-82.

Werner SJ, Carlson JC, Tupper SK, Santer MM, Linz GM. 2009. Threshold concentrations of an anthraquinone-based repellent for Canada geese, red-winged blackbirds, and ring-necked pheasants. *Applied Animal Behaviour Science* **121**:190-196.

Werner SJ, DeLiberto ST, Mangan AM, Pettit SE, Ellis JW, Carlson JC. 2015. Anthraquinone-based repellent for horned larks, great-tailed grackles, American crows and the protection of California's specialty crops. *Crop Protection* **72**:158-162.

Werner SJ, DeLiberto ST, Pettit SE, Mangan AM. 2014a. Synergistic effect of an ultraviolet feeding cue for an avian repellent and protection of agricultural crops. *Applied Animal Behaviour Science* **159**:107-113.

Werner SJ, Tupper SK, Pettit SE, Ellis JW, Carlson JC, Goldade DA, Hofmann NM, Homan HJ, Linz GM. 2014b. Application strategies for an anthraquinone-based repellent to protect oilseed sunflower crops from pest blackbirds. *Crop Protection* **59**:63-70.

Zhu H, Salyani M, Fox RD. 2011. A portable scanning system for evaluation of spray deposit distribution. *Computers and Electronics in Agriculture* **76**:38-43.

CHAPTER 3: BLACKBIRD FEEDING BEHAVIOR ON INTACT SUNFLOWER PLANT

Abstract

Agriculture suffers from avian damage and requires effective strategies to reduce the economic burden on producers. However, pest birds and the crops they damage are diverse, often requiring species-specific and crop-specific strategies. Chemical repellents, such as anthraquinone, can be effective for reducing bird damage in situations where direct application is feasible and the pest bird easily consumes the repellent. The ability to reduce avian damage is greatly reduced when the target is difficult to reach with spraying equipment or the birds interact sparingly with the treated plant. We evaluated the feeding behavior of captive male red-winged blackbirds (*Agelaius phoeniceus*) on both untreated sunflowers and sunflowers treated with anthraquinone-based repellents to inform repellent application methods. Blackbird foraging resulted in contact with repellent but handling behavior did not differ in the presence or absence of repellent. We did see changes in handling behavior with sunflower moisture. We saw reduced blackbird gaping of disk flowers with increasing repellent residues. Blackbirds interact with treated parts of the plant but sunflower phenology may influence the degree of these interactions.

Introduction

In North America, the agriculture industry suffers millions of dollars in losses annually due to damage from birds, which readily exploit available crops (Peer et al. 2003; Cummings et al. 2005; Elser et al. 2016). Cereal grains, such as corn, rice, wheat, and sorghum, are often recipients of bird-related damage due to the abundance of these crops across the landscape. When including only a subset of primary-producing states, estimates of economic loss total over $21 million in rice alone (Cummings et al. 2005). Many high-value specialty crops, such as oilseeds, fruits, and nuts also receive devastating avian damage (Emlen 1937; DeHaven & Hothem 1981; Salmon et al. 1986; Tobin et al. 1991; Linz et al. 2011; Anderson et al. 2013). For example, costs associated with bird damage to cherries, including deterrent efforts are estimated at tens of millions of dollars annually across seven states (Lindell et al. 2012). Crops such as sweet corn, fruits, and other produce suffer from direct consumptive loss as well as damage in the form of blemishes, reducing market value and increasing prevalence of disease (Boudreau 1972; Tracey et al. 2006; Carlson et al. 2013). Bird damage is not only limited to consumable crops, but other non-food resources such as turf grazed by Canada geese (*Branta canadensis*) (Conover & Chasko 1985) and wooden telephone poles and buildings used by foraging woodpeckers (*Picidae*) (Tupper et al. 2010). Bird damage is prevalent in numerous agriculture commodities as well as other human and natural resources lending urgency to finding cost-effective solutions.

The extent to which agriculture is impacted by avian damage and the development of potential tools or solutions is complicated by the diversity of species inflicting the damage and the variety of foraging strategies used to exploit the food resource (Tracey et al. 2006). For example, blackbirds (*Icteridae*), ring-necked pheasant (*Phasianus colchicus*), American crows (*Corvus brachyrhynchos*), and greater sandhill cranes (*Grus canadensis tabida*) commonly dig up newly planted crop seeds (Dambach & Leedy 1948; Cummings et al. 2002; Barzen et al. 2018). Woodpeckers (*Picidae*) and corvids (*Corvidae*) take entire nuts (e.g., almonds and pistachios) and cache them off site (Emlen 1937). Various frugivore species including American robins (*Turdus migratorius*) and cedar waxwings (*Bombycilla cedrorum*) damage cherries and berries by consuming the whole fruit or inflicting partial damage through pecking (Boudreau 1972; Lindell et al. 2012). Blackbirds are the main species damaging cereal grains (e.g., corn), and often select early-ripening crops in the milky stage of development, where the inside of kernels are pecked out (Bollinger & Caslick 1985). Therefore, a single, cosmopolitan strategy (e.g., one repellent for multiple crops) is not likely to be effective for every bird pest nor the wide variety of cropping systems.

Species-specific strategies for exploiting crops even differ within the same crop. For example, brown-headed cowbirds (*Molothrus ater*) and European starlings (*Sturnus vulgaris*) exploit ripening corn through the silk channels, whereas red-winged blackbirds (*Agelaius phoeniceus*) and common grackles (*Quiscalus quiscula*) attack through the husk (Bernhardt et al. 1987). Red-winged blackbirds and European starlings also employ gaping to forcibly access kernels through the husk, while grackles peck through the husk (Orians & Angell 1985; Bernhardt et al. 1987). Gaping, or forcibly opening the beak against resistance, is a foraging technique that is unique to blackbirds, starlings and corvids, allowing access to otherwise unavailable food sources. In vineyards, larger birds may consume entire grapes whereas smaller birds peck the grapes leaving partially-eaten fruit (Boudreau 1972). Levey (1987) observed additional strategies such as crushing the fruit pulp in larger-billed birds and the ripping of small pieces by birds with smaller bills. Thus, understanding how birds interact with and manipulate their food resources can be beneficial for optimizing deterrent strategies.

Extensive bird damage to agriculture has resulted in decades of research evaluating strategies for crop protection. Chemical repellents are of particular interest because of their potential to be broadcast across the large spatial extents of modern-day crop fields (Klug 2017). However, chemical repellents are not necessarily a suitable universal solution and what works for one crop or species may not work for another. The optimization of chemical repellents must be informed by the repellent’s mode of action, features of the crop to be protected, and how birds use and manipulate the crop in order to effectively increase crop-specific efficacy.

Chemical repellents exist in two major capacities: primary and secondary repellents (Sayre & Clark 2001). Primary chemical repellents are fast-acting and cause an immediate stimulus, such as pain or irritation. However, the only primary chemical repellent registered by the United States Environmental Protection Agency (US EPA) is methyl anthranilate and evidence of effectiveness at the field scale is lacking, likely due to application difficulties along with the ephemeral nature of the repellent (Vogt 1997). Secondary chemical repellents, such as anthraquinone, require conditioned learning as they elicit an adverse physiological effect such as pain or sickness, which the animal associates with a sensory stimulus (Avery & Mason 1997; Werner & Clark 2003). Thus, secondary repellents require a certain degree of ingestion for aversion to take place. Both primary and secondary repellents show promise of efficacy in lab-based experiments (Avery & Cummings 2003; Werner et al. 2009) with varying results in field-based studies where the details of crop structure, repellent application strategies, and bird foraging behavior converge to influence efficacy (Avery et al. 1996; Werner et al. 2005; Werner et al. 2014; Niner et al. 2015).

Anthraquinone is a secondary repellent that has been shown to effectively reduce Canada goose grazing on turf grass and brown-headed cowbird depredation of millet seeds (Dolbeer et al. 1998). Anthraquinone has also been evaluated as a tool for reducing blackbird damage to sunflower crops, having shown efficacy in the laboratory when tested on loose, dry sunflower achenes (Werner et al. 2009). However, these successful scenarios benefit from direct consumption of the treated food source (i.e., turf grass and millet) or manipulation of seed hulls (i.e., sunflower) fully coated with repellent. In a field situation, sunflower achenes are concealed within the sunflower head and covered with disk flowers, limiting the amount of repellent that reaches the achenes (see Chapter 1; Figure 1.1). Furthermore, achenes are not entirely eaten but are opened to consume the internal seed, further limiting the amount of repellent ingested. Application issues are further intensified by the downward-facing sunflower head not being amenable to the preferred aerial application strategy at the field scale. Thus, results from a variety of field tests indicate that chemical repellents optimized for lab conditions may not work in the context of field application due to application limitations and bird-specific feeding behavior on the sunflower plant (see Chapter 2) (Werner et al. 2014; Niner et al. 2015).

Objectives

A secondary repellent needs to be ingested to be effective, therefore the repellent needs to be deposited on the parts of the plant that are manipulated or ingested by the pest bird. Thus, our main objective was to identify and describe the feeding behavior of captive red-winged blackbirds (*Agelaius phoeniceus*, hereafter blackbirds) on mature sunflower heads. We observed foraging behavior of captive blackbirds that were part of another study evaluating the efficacy of anthraquinone-based repellent to reduce blackbird consumption of sunflower (see Chapter 2). Thus, we were able to test if blackbirds altered their feeding behavior in response to the presence of a repellent, but were mainly interested in how blackbirds interacted with the sunflower plant to inform repellent application.

Our first aim was to evaluate if blackbirds responded to the presence of repellent on the sunflower based on their selection of treated or untreated (unadulterated) sunflower for first perch, peck, and achene removal. We predicted that blackbirds would not show early preference for untreated or treated sunflowers given aversion needs to be learned, but preference may occur if the repellent alters the visual properties of the sunflower in a manner perceptible and biologically important to blackbirds. Given disk flowers cover achenes and intercept the majority of the repellent, our second aim was to describe how blackbirds interact with disk flowers and how this may influence the opportunity for repellent ingestion. Our third aim was to understand the influence of repellent and achene moisture on depredation rates and the amount of wasted achenes. We predicted that treated achenes would have reduced predation rates and increased number of wasted achenes. Finally, we aimed to evaluate handling behavior to identify the parts of the achene that may play a role in increasing repellent ingestion, given only the wide-end of the achene is susceptible to spraying. We were also interested in how handling behavior (i.e., handling rate, number of achenes crushed, and presence of beak wiping) may change with the presence of repellent. We predicted that blackbirds on treated sunflowers would show a decrease in handling rate and number of achenes crushed, but an increase in beak wiping. After observing blackbird behavior, we became interested in the influence of moisture content, which decreases with advancing sunflower phenology. We predicted that beak wiping, handling rate, number of achenes crushed and the number of achenes removed adjacent to depredated achenes would increase in birds foraging on sunflower with increased moisture.

Methods

In 2017 and 2018, we used a two-choice, preference design to evaluate feeding behavior of blackbirds on treated and untreated sunflowers presented simultaneously. We evaluated seven treatments with varying percent anthraquinone (see Chapter 2, Table 2.1). We placed blackbirds naïve to anthraquinone in individual feeding cages (1.2 x 0.6 x 0.8 m) over a four day period including one acclimation day (Day 1), two pretest days (Days 2-3), and one test day (Day 4). We provided water *ad libitum* over the course of the experiment. Although preference tests included additional testing days (see Chapter 2), feeding behavior was only collected on the first test day (Day 4). We provided 30 g of maintenance diet and two sunflowers for acclimation (Day 1). On each pretest day (Days 2-3), we offered birds two untreated sunflowers. On test day (Days 4) we offered one untreated and one treated sunflower. We paired sunflowers within the same cage according to similar head diameters. For all days (Days 1-4), sunflowers were available for a 10-hour period (08:00 to 18:00) and 30 g of maintenance diet were offered in the remaining hours. We collected video of 38 captive blackbirds on the first test day (Day 4) in the preference experiments (see Chapter 2). We positioned a video camera on a single sunflower for a total of 2 cameras per individual cage (i.e., 38 birds; 76 sunflowers). We recorded blackbird feeding behavior using GoPro Hero 5 Black, 1080p at 30 fps and analyzed the video using Behavioral Observation Research Interactive Software (BORIS). All statistical analyses were conducted in R (version 3.5.2; R Core Team 2019).

We recorded the sunflower (i.e., treated or untreated) for which the bird (n = 38) first perched, pecked, and removed an achene to evaluate initial preference, if any (Table 3.1). We used a chi-square test of independence to examine blackbird preference for first perch, peck, and achene removal from untreated and treated sunflowers. We used a Fisher’s exact test to evaluate if treatments differed in the proportion of birds that depredated an achene from the untreated sunflowers first (Trts 1-7; see Chapter 2, Table 2.1).

Table 3.1. Foraging behaviors used in evaluating male red-winged blackbirds (*Agelaius phoeniceus*) on untreated and treated sunflower (*Helianthus annuus*) in a laboratory setting.

Behavior	Description	Metric
Initial Preference[†]		
Perch	Landing on sunflower head	First landing on treated (0) or untreated sunflower (1)
Peck	Pecking sunflower face	First peck on treated (0) or untreated sunflower (1)
Achene	Removing achene	First achene from treated (0) or untreated sunflower (1)
Interaction with Plant[‡]		
Gaping	Forcibly opening bill when inserted into sunflower face	Time gaping on sunflower (minutes)
Handling	Manipulating an achene until achene is dropped or abandoned	Average time spent handling first 10 achenes (seconds/achene)
Crushing	Achene squeezed within beak	Achene crushed (number crushed out of 10)
Crushing Position	Achene position relative to beak	Achene perpendicular, parallel, or both
Crushing Location	Part of the achene crushed by beak	Wide end, narrow end, side, or entire achene
Prying	Achene penetrated with beak	Achene pried (number pried out of 10)
Prying Location	Part of the achene pried by beak	Wide end, narrow end, or side
Seed Depredation	Any part of internal seed eaten	Seed consumed (number of consumed out of 10)
Seed Proximity	Achene location relative to others	Adjacent to other depredated achenes (number of achenes adjacent to pre-existing damage out of 9)
Beak Wiping	Rubbing beak on sunflower	Achene depredation with beak wipe (number of achenes which resulted in beak wiping out of 10)

[†] A total sample of 38 blackbirds. Metrics evaluated as a binary response.

[‡] A total sample of 37 blackbirds: including 18 blackbirds that depredated 10 achenes on both untreated and 10 achenes on treated sunflowers; 26 blackbirds that depredated 10 achenes on treated sunflowers; and 37 blackbirds that depredated 10 achenes on at least one sunflower. Gaping and handling were measured as a continuous variables. Other interactions with the plant were measured as counts.

We evaluated the time spent on both sunflowers to understand if blackbirds (n = 31) were favoring the treated or untreated sunflower over the first 17 minutes of exposure (Table 3.1). To evaluate if exploratory behaviors differed with treated and untreated sunflowers, we recorded the time blackbirds (n = 31) spent gaping (minutes) in the first 17 minutes of exposure, when the disk flowers were still intact (Table 3.1). To record time spent on the sunflower and gaping time, we only included sunflower visits lasting at ≥10 seconds, given minimal foraging behaviors were observed on shorter bouts. We conducted a one-way analysis of variance (ANOVA) to evaluate if time on treated sunflowers differed as a function of treatment (Trts 1-6; see Table 2.1). Additionally, we calculated a linear regression to evaluate the relationship between time on treated sunflowers and anthraquinone residue on achenes (Trts 1-5). A paired-sample t-test was used to assess whether time on sunflower and gaping time differed on treated and untreated sunflowers within the same cage. We calculated a linear regression to evaluate the relationship between gaping time and disk flower residue.

We measured achene handling rates (i.e., average time spent handling 10 achenes) for individual blackbirds when feeding on untreated and treated sunflowers (Table 3.1). Handling began when an achene was removed from the sunflower and concluded when the achene was dropped or abandoned. We restricted analyses to the first 10 achenes depredated by blackbirds on the untreated and treated sunflowers separately and excluded instances where birds exited the video frame to handle achenes. We excluded sunflowers from the analyses where birds did not remove 10 achenes.

After an achene was removed from the sunflower head, we recorded handling behaviors that may influence ingestion and repellent exposure if the repellent reached the achenes. We tested whether achenes were crushed with the beak as opposed to pried open or dropped (Table

3.1). Crushing occurred when achenes were fully or partially within the beak and often resulted in the internal seed being visually apparent. We evaluated the position of the achene relative to the beak (i.e., perpendicular, parallel, or both) when crushing occurred, and the part of the achene in contact with the beak (i.e., wide-end, narrow-end, or side; Table 3.1). We recorded how blackbirds pry into the achene hull by recording the location where achenes were pried open. We measured the presence of beak wiping after each achene was handled, given this behavior may result from contact with repellent residue or increase exposure to the repellent on the vegetative parts of the sunflower. We recorded the proximity of the depredated achene in relation to pre-existing damage to evaluate if the removal of achenes influenced subsequent depredation. That is, achene removal may become easier with missing achenes in a tightly-packed sunflower head causing birds to focus on one sunflower. We measured achene proximity as the count of achenes depredated that were adjacent to a previously depredated achene. Achene proximity did not include the first achene removed due to lack of pre-existing damage.

We evaluated the proportion of the 10 achenes that were consumed to inform the amount seed wasted in the process of foraging. However, seeds are not always completely consumed and the amount of seed consumed was difficult to visually quantify. Therefore, we recorded seed depredation as either uneaten or eaten, where any part of the seed was consumed to any degree. The amount of wasted seed is valuable when using bioenergetics models to evaluate damage estimates as this type of seed loss is not often accounted for in these models (Peer et al. 2003).

We conducted one-way ANOVAs to evaluate if handling rate, number of achenes crushed, presence of beak wiping, and proximity to damage on treated sunflowers differed as a function of treatment (n = 26); Trts 1-7, see Table 2.1). The treatments (Trt 1-7) were not significantly different in handling behavior on the treated sunflower, thus we combined birds (n

= 18) from every treatment to evaluate behavior on treated and untreated sunflowers. We used a paired-sample t-test to assess whether handling rate, number of achenes crushed, presence of beak wiping, and achene proximity differed with treated and untreated sunflowers within the same cage. Avian behavior on treated and untreated sunflowers were not significantly different when combining birds from all treatments, thus in evaluating moisture content we used every bird (n = 37) regardless of treatment or the sunflower it foraged on (i.e., treated or untreated). We calculated linear regressions to evaluate the relationship between handling rate, number of achenes crushed, beak wiping, and achene proximity with percent moisture. If no significant effect of moisture was found, we performed linear regressions to evaluate the relationships of handling rate, number of achenes crushed, presence of beak wiping, and proximity to damage to increasing anthraquinone residues on treated sunflowers (Trt 1-5).

We preformed all statistical analyses as detailed above using R (version 3.5.2; R Core Team 2019). We tested all data concerning assumptions of normality using a Shapiro-Wilk *W* statistic and homogeneity using Bartlett's test. A log-transformation was used to normalize handling rates when evaluating moisture content. All other dependent variables followed a normal distribution.

Results

Blackbirds did not show early preference toward untreated sunflowers regarding the sunflower head that was first perched upon ($\chi^2_{(df = 1, n = 38)} = 0.0$, p = 1.0), pecked at ($\chi^2_{(df = 1, n = 38)} = 0.4$, P = 0.5), or depredated ($\chi^2_{(df = 1, n = 38)} = 0.1$, p = 0.7). Additionally, there was no significant differences in the proportion of birds which depredated an achene from untreated sunflowers first between any of the seven treatments (Fisher's Exact Test, p = 0.69). Although not significant,

>80% of sunflowers treated with Avipel™ (Trt 6; lacking visual inert) were depredated prior to untreated sunflowers, which was 2x higher than other treatments (Figure 3.1).

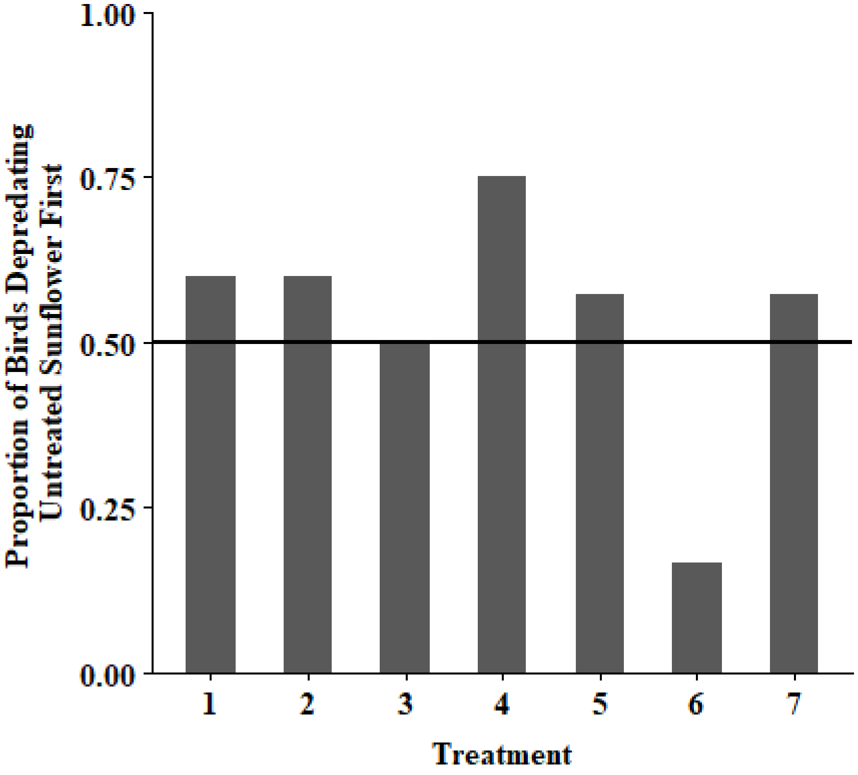

Figure 3.1. Blackbird preference for untreated sunflowers at initial depredation. Bars represent proportion of blackbirds which initially depredated the untreated sunflower for each treatment (Trt 1-7; see Chapter 2, Table 2.1). Black line represents expected proportion if no effect is present.

During early observations, blackbirds spent 79% of the time on one of the two sunflowers. Time spent on a treated sunflowers was not statistically different between treatments ($F_{5,27}$ = 1.4, P = 0.25; Figure 3.2A). Time spent on treated sunflowers did not differ with increasing anthraquinone residues on achenes (R^2 = 0.03, p = 0.19). Within the same cage, blackbirds did not spend more time on untreated sunflowers (minutes ± SE; 7.2 ± 0.6) than treated sunflowers (7.0 ± 0.6; t_{30} = 0.18, P = 0.86). When on a sunflower for the first 17 minutes, blackbirds spent an average of 41% of their time gaping vegetative disk flowers. Within the same cage, blackbirds did not spend more time gaping on untreated sunflowers (minutes ± SE; 2.8 ± 0.3) than treated sunflowers (2.8 ± 0.3; $t_{30} = 0.009$, $p = 0.99$). The amount of time spent gaping disk flowers decreased with increasing anthraquinone residue on disk flowers (adjusted R^2 of 0.23, $p = 0.003$; Figure 3.2B).

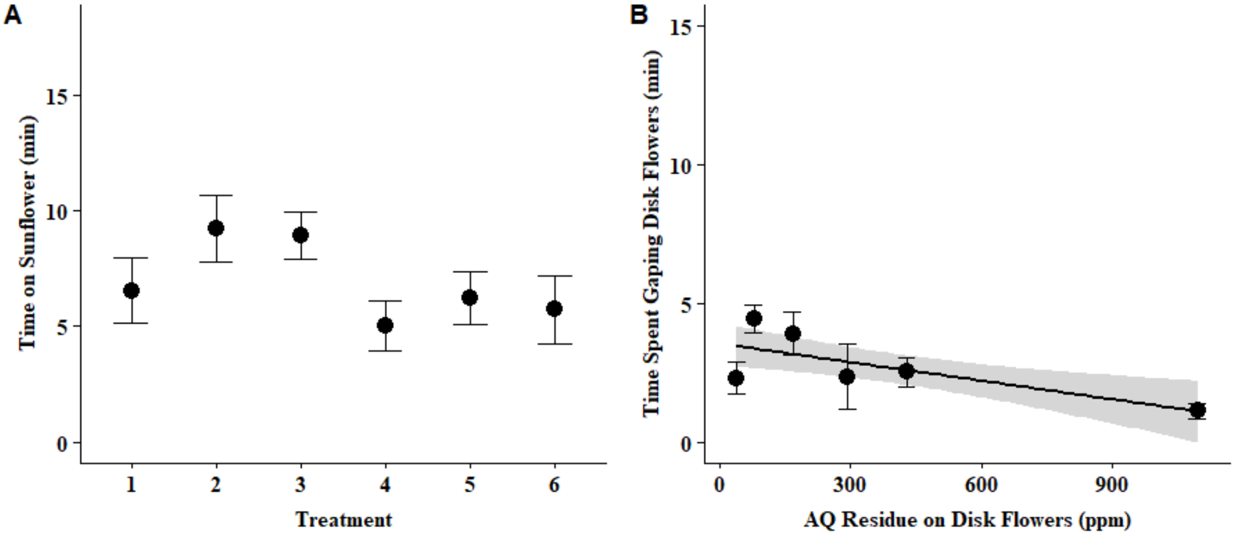

Figure 3.2. A) Amount of time blackbirds spent on treated sunflowers in the first 17 minutes of exposure on the test day (Day 4). B) Relationship between anthraquinone (AQ) residue on disk flowers (range = 40-1,095 ppm) and the time spent gaping disk flowers (mean ± SE; adjusted $R^2 = 0.23$; $p = 0.003$). For every 100 ppm increase in disk flower residue, there is a 0.002 min decrease in time spent gaping ($y = -0.002(x) + 3.5$; shaded area indicates 95% confidence interval).

We did not find significant differences in handling rates (s/achene) between treatments (Trts 1-7, [see Table 2.1]; $F_{6,19} = 0.9$, $p = 0.5$; Figure 3.3A). Handling rates did not differ with increasing anthraquinone residue on achenes ($R^2 = -0.05$, $p = 0.87$). Blackbirds did not differ in the time spent (sec/achene) handling untreated (time ± SE; 12.1 ± 1.2 s) and treated sunflowers (11.2 ± 1.0 s; $t_{17} = 1.04$, $P = 0.31$) in the same cage. The log-transformed handling rates did not differ with moisture content ($R^2 = -0.02$, $p = 0.51$; Figure 3.3B).

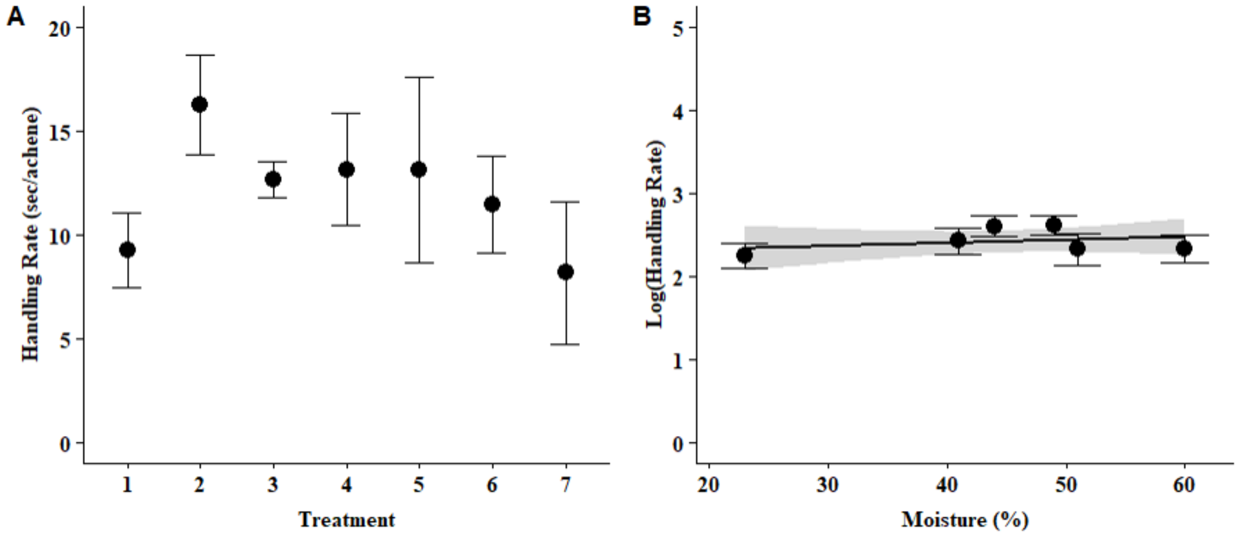

Figure 3.3. A) Blackbird handling rates by treatments. B) Relationship between blackbird handling rates ($\log_{10}$ scale mean ± SE) moisture content of achenes (adjusted $R^2 = -0.02$; p = 0.51; shaded area indicating 95% confidence interval).

We did not find a significant effect of treatment on the number of achenes crushed ($F_{6,19} = 1.18$, p = 0.36; Figure 3.4A). We found no significant difference in the number of achenes crushed in untreated (mean ± SE; 7.6 ± 0.6) and treated sunflowers with the same cage (6.2 ± 0.7; $t_{17} = 1.69$, P = 0.11). The number of achenes crushed did increase with increasing percent moisture (adjusted $R^2 = 0.09$, p = 0.04; Figure 3.4B). However, percent moisture of 41% may have heavily influenced the relationship. Therefore, a regression was calculated excluding this group but was still significant (adjusted $R^2 = 0.11$; p = 0.04). We did not evaluate the relationship between the number of achenes crushed and increasing anthraquinone residues on achenes because moisture content influenced the number of achenes crushed.

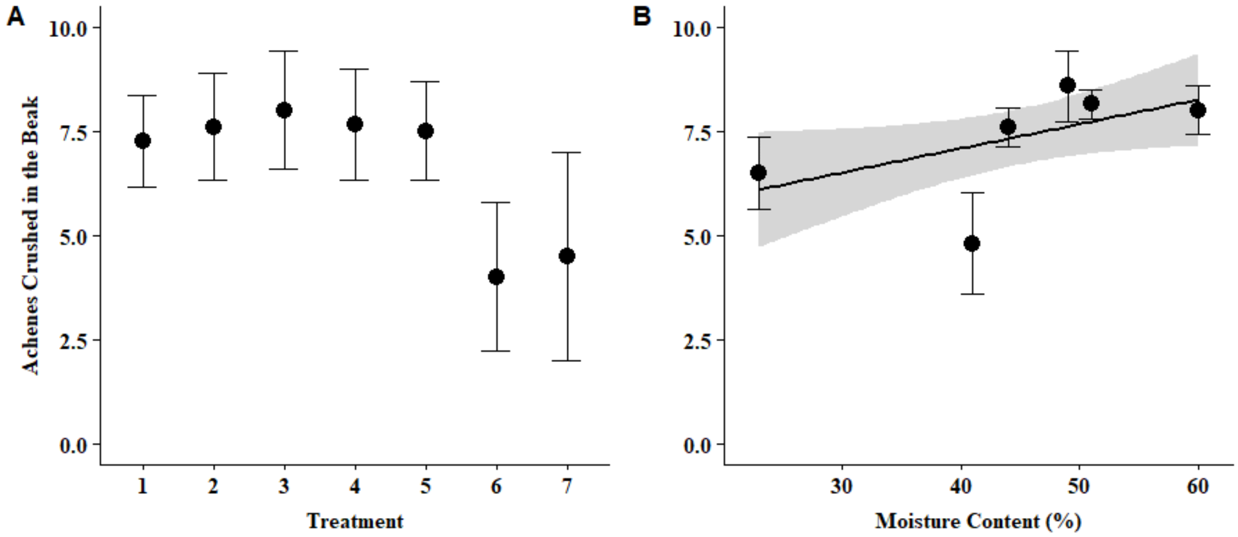

Figure 3.4. A) Average number of achenes (out of a possible 10 achenes; mean ± SE) crushed in the beak by treatment. B) Relationship between moisture content of achenes and the number of achenes crushed in the beak (mean ± SE; adjusted $R^2 = 0.09$; $p = 0.04$). For every 10% increase in moisture content, there is a 0.6 increase in achenes crushed ($y = 0.06(x) + 4.7$; shaded area indicates 95% confidence interval).

A majority of achenes crushed in the beak were positioned perpendicular to the beak (64%), with less occurrences of crushed achenes positioned parallel to the beak (26%). The lowest occurring crush position was the use of both perpendicular and parallel positions (10%). Crushing achenes in a perpendicular position primarily resulted in the wide end of achenes within the beak (96%) with narrow ends rarely being contained in the beak (4%). Crushed achenes in a parallel position resulted in the whole achene occurring in the beak (66%) with the side edge of achenes within the beak less often (34%). Crushing achenes in both perpendicular and parallel positions resulted in the entire achene within the beak at some point (100%). Prying achenes open was observed much less frequently than crushing achenes (19%). Blackbirds pried open achenes from the wide end (44%), side (39%), and narrow end of achenes (17%).

We did not find a significant effect of treatment on the presence of beak wiping after handling an achene ($F_{6,19} = 1.52$, $p = 0.224$; Figure 3.5A). The presence of beak wiping after handling an achene did not differ with increasing anthraquinone residues on achenes ($R^2 = 0.01$, $p = 0.28$). There was no significant difference in beak wiping between untreated (mean ± SE; 6.2 ± 0.5) and treated sunflowers (6.2 ± 0.5; $t_{17} = 0.09$, $p = 0.9$). The presence of beak wiping after handling an achene did not differ with moisture content ($R^2 = -0.01$, $p = 0.43$; Figure 3.5B).

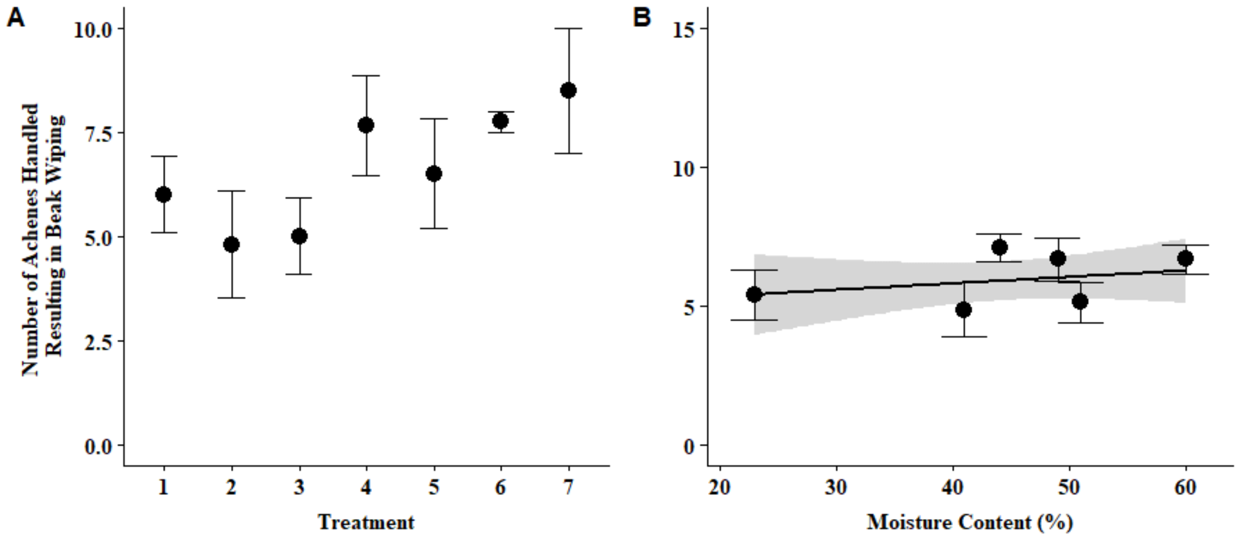

Figure 3.5. A) Number of achenes (out of a possible 10 achenes; mean ± SE) which resulted in beak wiping by treatment. B) Relationship between moisture content of achenes and the number of achenes resulting in beak wiping (mean ± SE; $R^2 = -0.01$; $p = 0.43$; shaded area indicates 95% confidence interval).

We did not find a significant effect of treatment on the location of achenes predated ($F_{6,19} = 0.88$, $p = 0.53$; Figure 3.6A). The location of achenes predated did not differ with increasing anthraquinone residues on achenes ($R^2 = -0.05$, $p = 0.81$). There was not a significant difference in the number of depredated achenes bordering pre-existing damage for untreated (mean ± SE; 6.1 ± 0.5) and treated sunflowers (5.8 ± 0.5; $t_{17} = 0.54$, $P = 0.6$). Achene depredation adjacent to pre-existing damage did not differ with moisture content ($R^2 = 0.04$, $p = 0.12$; Figure 3.6B).

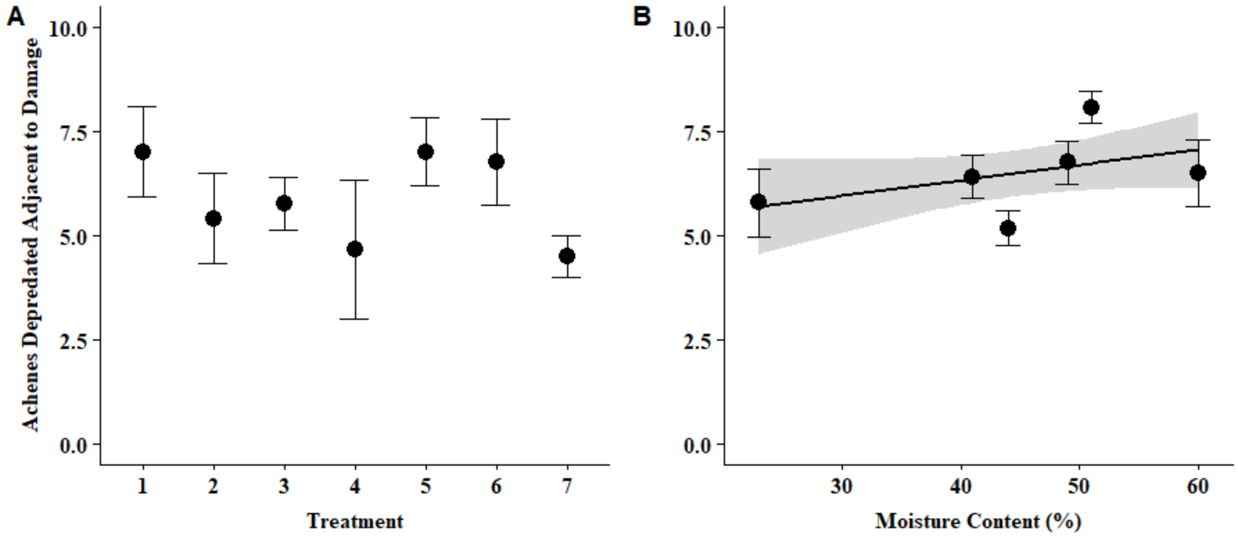

Figure 3.6. A) Number of achenes (out of a possible nine achenes; mean ± SE) depredated adjacent to pre-existing damage by treatment. B) Relationship between moisture content and the number of achenes depredated adjacent to pre-existing damage (mean ± SE; adjusted $R^2 = 0.04$; $p = 0.12$; shaded area indicating 95% confidence interval).

Overall, we observed 550 depredated achenes from 37 blackbirds, all of which were removed from the head. However, only 65% of depredated achenes were successfully consumed. When blackbirds crushed achenes with their beak, handling resulted in successful consumption 82% of the time. When blackbirds pried open achenes, handling resulted in successful consumption 56% of the time. However, when both crushing and prying the achene open were implemented, the success rate was 85%.

Discussion

In the context of our study, blackbirds did not show early preference towards untreated sunflower. This is not surprising, given the repellent requires ingestion to be effective (Avery et al. 1997b; 1998). However, it should be noted that blackbirds did show an increased preference to depredate sunflowers treated with Avipel™ (Treatment 6). This is of interest as Avipel™ lacks a visual inert which has been found to increase the efficacy of anthraquinone-based repellents when included in repellent formulations (Werner et al. 2009). In Treatments 1-6, disk flowers experienced higher residues than achenes and would therefore be the matrix on which the visual inert is acting upon. The presence of repellent may influence how the blackbirds perceive the sunflower, specifically at different phenological stages. As sunflowers progress from maturity to harvest, the green features of the plant turn yellow and then brown (Schneiter & Miller 1981). If blackbirds use the visual coloration of sunflowers as a feeding cue, the presence of a repellent may change how the sunflower is perceived, especially in the ultra-violet spectrum. Anthraquinone absorbs near-UV wavelengths (Du et al. 1998), which are visible to most birds (Hart & Hunt 2007) and can be used to detect food quality such as ripeness (Cuthill et al. 2000; Werner et al. 2012). Although, our laboratory study does not capture how free-ranging birds would perceive a repellent-treated crop when visually selecting fields to depredate while flying above.

Until now, interactions between blackbirds and disk flowers have been of little interest, as repellent application primarily focused on above canopy strategies where repellent fails to reach disk flowers of downward facing sunflower heads (Linz & Homan 2012; Niner et al. 2015). Disk flowers are present across the face of ripening sunflower and must be removed to gain access to achenes below. Blackbirds appeared to be very efficient at removing disk flowers while gaping, which made up 41% of early activity while on a sunflower. Similar vegetative interaction has been observed with red-winged blackbirds manipulating the corn husk (Bernhardt et al. 1987). Although the presence of intact disk flowers was ephemeral, the initial gaping disk flowers by blackbirds may constitute prolonged bouts of repellent contact with the beak. And while minimal floral matter was consumed while gaping disk flowers, some repellent ingestion likely occurred. The presence of repellent did not affect time spent on the sunflower, but increasing anthraquinone residues on disk flowers reduced the amount of time blackbirds spent gaping disk flowers.

Our results suggest repellent on the disk flowers may play an important role in early blackbird interaction with sunflowers. Potentially the relationship between repellent residue on disk flowers and gaping activity is a result of other sensory systems such as the visual perception or tactile feel of the repellent. Alternatively, the repellent may decrease the abundance of invertebrates in the sunflower head to effectively reduce gaping, a foraging technique often used to access invertebrate prey (Orians & Angell 1985). Regardless of why the repellent treatment reduced gaping, the reduction in gaping did not result in reduced consumption of treated sunflowers (see Chapter 2). Disk flowers covered with repellent may temporarily influence exploratory behaviors such as gaping, but may be limited to the short term as disk flowers can easily be removed.

Blackbird achene handling rates were not influenced by repellent or moisture content. Blackbirds have shown differential feeding behavior when presented clay-coated rice seeds compared to uncoated seeds, (Daneke & Decker 1988) although a physically obstructive clay-coating is likely to have a different influence on handling than achenes partially coated with repellent. Avery et al. (1997a) suggests that increased handling rates of treated food items would result in longer exposure to repellent. Conversely, achenes treated while remaining in the head are subject to repellent only on the wide end of achenes, resulting in minimal treated surface area. If enough repellent could be administered, handling rates could potentially be increased if birds were forced to avoid the repellent (Greig-Smith & Crocker 1986). This scenario would only be possible if 1) enough repellent was ingested to influence handling techniques or 2) the repellent on achenes influences the appearance or texture of the treated achene in a way perceptible to the blackbird. Additionally, handling rates may be influenced by the position of the sunflower head. Sunflowers in preference experiments were positioned at a 90^o angles, which may have restricted blackbirds to interact with the sunflower towards the top. As sunflower crops phenologically progress, the heads face downwards, allowing the entire back of the head to serve as a stable platform. Head position may directly influence handling times as birds may require dexterity to handle achenes without dropping them prematurely. Previous studies focusing on morphological bird-resistant traits of sunflower have shown that downward facing heads are more difficult to damage (Mah et al. 1990).

Achenes depredated from sunflower heads were only successfully consumed 65% of the time. Success of achene consumption may be a result of head position. Previous studies have shown higher success rates in achene consumption (77-85%) when the sunflower heads were more inverted (i.e., downward facing) (Mah & Nuechterlein 1991). Overall blackbird damage to crops is the main focus for damage mitigation, thus this data is of interest for bioenergetics models aimed to predict damage based on dietary needs of individuals and population densities (Peer et al. 2003). Our data on successful consumption of seeds from depredated achenes illustrates that damage likely includes a large proportion of wasted achenes (uneaten). Bioenergetics models predict potential impacts of blackbird damage by considering metabolic rates, energy value, and percentage of sunflower in diet but likely underestimates the economic cost of blackbirds by not including wasted achenes (Peer et al. 2003).

Blackbirds overwhelmingly handled achenes by crushing the achene in their beak, whereas prying achenes open or using both crushing and prying were much less common. Pried achenes were set down and held stationary with the foot. The decision to set down achenes to hull them may be influenced by morphologic characteristics (e.g., sunflower head position or hull characteristics) of specific sunflower varieties (Mah & Nuechterlein 1991). Although repellent treatment did not affect prevalence of achene crushing, depredated achenes were increasingly crushed in the beak as the percent moisture increased. Percent moisture, which can be used as a physiological proxy for sunflower maturity, decreases across the growing season until achenes are dry enough to be harvested (Anderson 1975). Achenes with higher moisture content may be easier to squeeze open due to a softer hull, resulting in more crushing to remove the internal seed. Albeit, we did not have moisture values <23%, so crushing may also be employed with drier, harder achenes found at harvest (10% moisture).

Our data suggests crushing achenes may be used by blackbirds more during early stages of sunflower maturity. This is of interest given a majority of damage to sunflower crops occurs during the first the first two to three weeks after reaching R_6 (Cummings et al. 1989). Furthermore, crushing was observed to occur most prominently with the achene perpendicular to the beak and the wide-end of the achene inside the beak. Repellent applied to the face of the sunflower is limited to the surface area of achenes not embedded in the head (i.e., wide-end). Although, contact with the repellent-treated portion of achenes inside of the beak does not necessarily mean ingestion is occurring, increasing repellent on this area may increase the probability of ingestion. Achieving higher repellent residue on the achenes or continual ingestion of lower residues may be an avenue for repellent to be ingested.

Prying achenes occurred less frequently than crushing achenes in the beak and resulted in a lower success rate of seed consumption. Prying achenes open occurred when a blackbird stabbed an achene and opened the hull with their beak. Achene handling in this manner would likely warrant no ingestion of repellent as prying occurred on all portions of the achene and did not focus on the wide-end where repellent would be located. Additionally, prying did not result in achene hulls entering the beak, reducing the potential of repellent ingestion. It is important to note that as moisture and the prevalence of crushing decreased, prying open achenes increased. Blackbirds may change their strategy for handling achenes at different phenological stages. If this is the case, repellents would could become less effective as sunflowers progress towards a harvestable percent moisture.

Management Implications

Our study provides insight on the process and strategies male red-winged blackbirds use while foraging on mature sunflowers. Our results indicate that anthraquinone residue applied to the sunflower face failed to reduce consumption by male red-winged blackbirds (see Chapter 2) and were not shown to greatly impact foraging behavior. However, lack of significance may be limited by small sample sizes in the foraging study. Anthraquinone residue on the achenes were limited to the exposed, wide-end of the achene. After removing an achene from the head, much of the surface area of the achene is untreated. If post-ingestive repellent must be ingested to be effective, blackbirds must handle achenes in a way which promotes repellent ingestion. Low anthraquinone residue on achenes did not impact handling rates of achenes nor overall handling strategies. Our data also indicated the moisture content of achenes is an important factor to consider when evaluating blackbird foraging behavior. The majority of damage to ripening sunflower occurs within the first 18 days after anthesis (Cummings et al. 1989). During this period, achenes are at a higher moisture content, which seems to motivate a higher rate of crushing achenes. Pairing knowledge of blackbird feeding behavior on sunflower plants at different moisture contents with sunflower application limitations may further dictate the potential efficacy of anthraquinone as a foliar applied avian repellent. Repellent application in the field can reach achenes but is limited to the wide-end of the achene. Although blackbirds mainly interact with the wide-end, this behavior is subject to sunflower phenology and thus repellent effectiveness may decrease as sunflower progress towards harvest. However, current application strategies result in limited residue on achenes that did not reduce consumption (see Chapter 2) or influence foraging behavior.

References

Anderson A, Lindell C, Moxcey KM, Siemer W, Linz GM, Curtis P, Carroll J, Burrows C, Boulanger JR, Steensma K. 2013. Bird damage to select fruit crops: the cost of damage and the benefits of control in five states. *Crop Protection* **52**:103-109.

Anderson W. 1975. Maturation of sunflower. *Australian Journal of Experimental Agriculture* **15**:833-838.

Avery ML, Cummings JL. 2003. Chemical repellents for reducing crop damage by blackbirds (eds G.M. Linz). Pages 41-48. In Management of North American Blackbirds, Proceedings of a special symposium of The Wildlife Society, 9th Annual Conference, USDA-APHIS-Wildlife Services, National Wildlife Research Center, Bismarck, ND.

Avery ML, Fischer DL, Primus TM. 1997a. Assessing the hazard to granivorous birds feeding on chemically treated seeds.

Avery ML, Humphrey JS, Decker DG. 1997b. Feeding deterrence of anthraquinone, anthracene, and anthrone to rice-eating birds. *Journal of Wildlife Management* **61**:1359-1365.

Avery ML, Humphrey JS, Primus TM, Decker DG, McGrane AP. 1998. Anthraquinone protects rice seed from birds. *Crop Protection* **17**:225-230.

Avery ML, Mason JR. 1997. Feeding responses of red-winged blackbirds to multisensory repellents. *Crop Protection* **16**:159-164.

Avery ML, Primus TM, Defrancesco J, Cummings JL, Decker DG, Humphrey JS, Davis JE, Deacon R. 1996. Field evaluation of methyl anthranilate for deterring birds eating blueberries. *The Journal of Wildlife Management*:929-934.

Barzen J, Gossens A, Lacy A. 2018. Sandhill crane foraging behavior and damage estimates in cornfields during spring (eds J. Austin and R.P. Urbanek). Pages 67-80. In Proceedings of the 14th North American Crane Workshop, Chattanooga, Tenn., North American Crane Working Group, Baraboo, Wisconsin, USA.

Bernhardt GE, Van Allsburg L, Dolbeer RA. 1987. Blackbird and Startling Feeding Behavior on Ripening Corn Ears.

Bollinger EK, Caslick JW. 1985. Factors influencing blackbird damage to field corn. *The Journal of Wildlife Management*:1109-1115.

Boudreau GW. 1972. Factors related to bird depredations in vineyards. *American Journal of Enology and Viticulture* **23**:50-53.

Carlson JC, Tupper SK, Werner SJ, Pettit SE, Santer MM, Linz GM. 2013. Laboratory efficacy of an anthraquinone-based repellent for reducing bird damage to ripening corn. *Applied Animal Behaviour Science* **145**:26-31.

Conover MR, Chasko GG. 1985. Nuisance Canada goose problems in the eastern United States. *Wildlife Society Bulletin (1973-2006)* **13**:228-233.

Cummings J, Shwiff S, Tupper S. 2005. Economic impacts of blackbird damage to the rice industry (eds D.L. Nolte, K.A. Fagersone). Pages 317-322. In Proceedings of the 11th Wildlife Damage Management Conference.

Cummings JL, Guarino JL, Knittle CE. 1989. Chronology of blackbird damage to sunflowers. *Wildlife Society Bulletin (1973-2006)* **17**:50-52.

Cummings JL, Pochop PA, Engeman RM, Davis Jr JE, Primus TM. 2002. Evaluation of Flight ControlR to reduce blackbird damage to newly planted rice in Louisiana. *International Biodeterioration & Biodegradation* **49**:169-173.

Cuthill IC, Partridge JC, Bennett AT, Church SC, Hart NS, Hunt S. 2000. Ultraviolet vision in birds. Pages 159-214. *Advances in the Study of Behavior*. Elsevier.

Dambach C, Leedy D. 1948. Ohio studies with repellent materials with notes on damage to corn by pheasants and other wildlife. *Journal of Wildlife Management* **12**:392-398.

Daneke D, Decker DG. 1988. Prolonged seed handling time deters red-winged blackbirds feeding on rice seed (eds A.C. Crabb, R.E. Marsh). Pages 287-292. In Proceedings of the 13th Vertebrate Pest Conference, University of California, Davis USA.

DeHaven R, Hothem R. 1981. Estimating bird damage from damage incidence in wine grape vineyards. *American Journal of Enology and Viticulture* **32**:1-4.

Dolbeer RA, Seamans TW, Blackwell BF, Belant JL. 1998. Anthraquinone formulation (Flight Control™) shows promise as avian feeding repellent. *Journal of Wildlife Management*:1558-1564.

Du H, Fuh RCA, Li J, Corkan LA, Lindsey JS. 1998. PhotochemCAD‡: a computer-aided design and research tool in photochemistry. *Photochemistry and Photobiology* **68**:141-142.

Elser J, Anderson A, Lindell C, Dalsted N, Bernasek A, Shwiff S. 2016. Economic impacts of bird damage and management in US sweet cherry production. *Crop Protection* **83**:9-14.

Emlen J. 1937. Bird damage to almonds in California. *Condor* **39**:192-197.

Greig-Smith P, Crocker D. 1986. Mechanisms of food size selection by bullfinches (Pyrrhula pyrrhula L.) feeding on sunflower seeds. *Animal Behaviour* **34**:843-859.

Hart NS, Hunt DM. 2007. Avian visual pigments: characteristics, spectral tuning, and evolution. *American Naturalist* **169**:S7-S26.

Klug P. 2017. The future of blackbird management research. Pages 217-234. *Ecology and Management of Blackbirds (Icteridae) in North America.* (eds G.M. Linz, M.L. Avery & R.A. Dolbeer) CRC Press/Taylor & Francis, Boca Raton, Florida, USA.

Levey DJ. 1987. Seed size and fruit-handling techniques of avian frugivores. *American Naturalist* **129**:471-485.

Lindell CA, Eaton RA, Lizotte EM, Rothwell NL. 2012. Bird consumption of sweet and tart cherries. *Human-Wildlife Interactions* **6**:283-290.

Linz GM, Homan HJ. 2012. Preliminary evaluation of 9, 10 anthraquinone bird repellent for managing blackbird damage to ripening sunflower. In Proceedings of the 34th National Sunflower Association Research Forum.

Linz GM, Homan HJ, Werner SJ, Hagy HM, Bleier WJ. 2011. Assessment of Bird-management Strategies to Protect Sunflowers. *Bioscience* **61**:960-970.

Mah J, Linz GM, Hanzel JJ. 1990. Relative Effectiveness of Individual Sunflower Traits for Reducing Red-Winged Blackbird Depredation. *Crop Protection* **9**:359-362.

Mah J, Nuechterlein GL. 1991. Feeding-Behavior of Red-Winged Blackbirds on Bird-Resistant Sunflowers. *Wildlife Society Bulletin* **19**:39-46.

Niner MD, Linz GM, Clark ME. 2015. Evaluation of 9, 10 anthraquinone application to pre-seed set sunflowers for repelling blackbirds. *Human-Wildlife Interactions* **9**:4-13.

Orians G, Angell T. 1985. Blackbirds of the Americas.

Peer BD, Homan HJ, Linz GM, Bleier WJ. 2003. Impact of blackbird damage to sunflower: Bioenergetic and economic models. *Ecological Applications* **13**:248-256.

Salmon T, Crabb A, Marsh R. 1986. Bird damage to pistachios. *California agriculture* **40**:5-8.

Sayre RW, Clark L. 2001. Effect of primary and secondary repellents on European starlings: an initial assessment.

Schneiter AA, Miller JF. 1981. Description of Sunflower Growth-Stages. *Crop Science* **21**:901-903.

Team RC. 2019. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. *URL:(https://www. R-project. org)*.

Tobin ME, Dolbeer RA, Webster CM, Seamans TW. 1991. Cultivar differences in bird damage to cherries. *Wildlife Society Bulletin (1973-2006)* **19**:190-194.

Tracey JB, Bornford M, Hart Q, Saunders G, Sander R 2006. Managing bird damage to fruit and other horticultural crops. Commonwealth Government-Department of Agriculture, Fisheries and Forestry ….

Tupper SK, Andelt WF, Cummings JL, Weisner C, Harness RE. 2010. Polyurea elastomer protects utility pole crossarms from damage by pileated woodpeckers. *Journal of Wildlife Management* **74**:605-608.

Vogt PF. 1997. Control of Nuisance Birds by Fogging with ReJeX-iT® TP-40. Pages 63-66. In Proceedings of the Great Plains Wildlife Damage Control Workshop.

Werner SJ, Carlson JC, Tupper SK, Santer MM, Linz GM. 2009. Threshold concentrations of an anthraquinone-based repellent for Canada geese, red-winged blackbirds, and ring-necked pheasants. *Applied Animal Behaviour Science* **121**:190-196.

Werner SJ, Clark L. 2003. Understanding blackbird sensory systems and how repellent applications work (ed G.M. Linz). Pages 31-40 in Linz GE, editor. In Management of North American Blackbirds, Proceedings of a special symposium of The Wildlife

Society, 9th Annual Conference, USDA-APHIS-Wildlife Services National Wildlife Research Center, Bismarck, North Dakota, USA.

Werner SJ, Homan HJ, Avery ML, Linz GM, Tillman EA, Slowik AA, Byrd RW, Primus TM, Goodall MJ. 2005. Evaluation of Bird Shield (TM) as a blackbird repellent in ripening rice and sunflower fields. *Wildlife Society Bulletin* **33**:251-257.

Werner SJ, Tupper SK, Carlson JC, Pettit SE, Ellis JW, Linz GM. 2012. The role of a generalized ultraviolet cue for blackbird food selection. *Physiology & Behavior* **106**:597-601.

Werner SJ, Tupper SK, Pettit SE, Ellis JW, Carlson JC, Goldade DA, Hofmann NM, Homan HJ, Linz GM. 2014. Application strategies for an anthraquinone-based repellent to protect oilseed sunflower crops from pest blackbirds. *Crop Protection* **59**:63-70.

CHAPTER 4: CONCLUSIONS AND FUTURE DIRECTIONS

Based on our data we conclude that secondary repellents, such as anthraquinone, are not currently a suitable tool for protecting ripening sunflower from blackbird damage due to plant structure. One obstacle for foliar application is the paradox of requiring high enough residues to reducing feeding, while simultaneously conforming to the United States Environmental Protection Agency (US EPA) food tolerance regulations (Eisemann et al. 2011). If research on anthraquinone-based repellents for ripening crops is to continue, three objectives must be addressed: 1) repellents will need to be optimized for the individual crops including phenological changes within the damage period; especially if visual cues will be incorporated as a method to reduce anthraquinone residues at harvest. 2) Required concentrations of these optimized repellents will need to be established for each crop and each pest species to effectively reduce feeding in a diversity of conflict situations. 3) A cost-effective method to apply repellent at the field scale will need to be developed for individual crops to deliver the necessary residues to reduce avian feeding. Research to address these objectives may be slow moving and costly, especially for unregistered repellents (Werner & Avery 2017).

Although blackbirds interact with components of the plant inoculated with repellent, effective residues have not been achieved in a manner practical for commercial application. Increased concentrations of repellent using drop-nozzle equipped ground rigs improve application, but has not been achieved to the point where the repellent is effective. Alternatively, primary and secondary repellents used in combination have been shown to effectively reduce free-ranging house sparrow (*Passer domesticus*) feeding on wheat (Day et al. 2012). However, repellent applied in high quantities or a combination of products would likely not be cost-effective. Thus, increased residues are not a likely avenue for success, an alternative suggestion is to focus on optimizing the repellent for each crop of interest (e.g., sunflower).

The optimization of anthraquinone-based repellents have focused on exploiting other sensory cues (i.e., visual) to reduce the residue of the active ingredient (anthraquinone) while maintaining efficacy (Werner & Clark 2003; Werner et al. 2014). However, visual cues are likely perceived differently on loose, dry achenes than on the sunflower plant. The use of visual exploitation may still hold promise if optimized on the sunflower plant or other crop of interest. The use of visual cues can also be used without the negative stimulus of a repellent. For instance, cryptic seeds matching the coloration of soil have reduced predation by visually-cued avian predators (Porter 2013). Hiding commercial fields is not practical, but visual cues may change how birds perceive the crop at different phenological stages, thus “camouflaging” the crop to look less ripe. Modifying the ripe appearance of crops may dissuade transient flocks from establishing the crop as a food source. Additional research would benefit from understanding the cues birds use to select fields or portions of fields for foraging.

A precursor for blackbird damage to crops could be insect abundance before the crop reaches maturity. Blackbird diet is primarily comprised of insects in the summer, leading into the fall season when ripening crops become available and their diet switches to seeds (Linz et al. 1984). Blackbirds may first be drawn to fields due to insect abundance. In this scenario, controlling insect pests may indirectly reduce crop susceptibility to bird damage. Previous studies have found that controlling insects with pesticides indirectly reduced bird damage to corn (Woronecki & Dolbeer 1980). However, reducing insect abundance may come at the cost of lower yields if control effects also impact pollinator abundance (Bommarco et al. 2012; Park et al. 2015). The presence of birds in crop fields may benefit producers by consuming herbivorous or non-beneficial insects (Whelan et al. 2015), but may lead to birds staying in fields to then directly damage ripening crops. Ultimately, producers should weigh the costs and benefits of ecosystem services provided by birds (Kross et al. 2016).

Although the use of repellents sprayed directly on crops may not be suitable for commercial sunflower, primary repellents may be an alternative strategy if paired with an efficient delivery method. Additional work should focus on the use of unmanned aircraft systems (UAS) as a form of precision agriculture (Ampatzidis et al. 2015). Pairing UAS with a payload containing a primary repellent, such as Rejex-it® TP-40 (methyl anthranilate), may allow for direct control of flocks as opposed to broad scale spraying of crops. Precision use of primary repellents as a fog has been shown to be effective at removing pest birds from runways at airfields (Engeman et al. 2002). The use of aerial hazing with a primary repellent allows direct application to the birds actually preforming the damage and the aerosolized nature of the methods may create a negative stimulus to a greater proportion of the flock to better encourage departure from the field (Klug 2017; Egan 2018).

References

Ampatzidis Y, Ward J, Samara O. 2015. Autonomous system for pest bird control in specialty crops using unmanned aerial vehicles. ASABE Annual International Meeting. American Society of Agricultural and Biological Engineers. p. 6.

Bommarco R, Marini L, Vaissière BE. 2012. Insect pollination enhances seed yield, quality, and market value in oilseed rape. *Oecologia* **169**:1025-1032.

Day TD, Clapperton BK, Porter RE, Waas JR, Matthews LR. 2012. Responses of free-ranging house sparrows to feed containing primary and secondary repellents. *New Zealand Journal of Crop and Horticultural Science* **40**:127-138.

Egan CC. 2018. Evaluating the Potential Utility of Drones to Deter Birds from Areas of Human-Wildlife Conflict. MS Biological Sciences. North Dakota State University, Fargo, ND, USA. p. 93.

Eisemann JD, Werner SJ, O'hare JR. 2011. Registration considerations for chemical bird repellents in fruit crops. *Outlooks on Pest Management* **22**:87-91.

Engeman RM, Peterla J, Constantin B. 2002. Methyl anthranilate aerosol for dispersing birds from the flight lines at Homestead Air Reserve Station. *International Biodeterioration & Biodegradation* **49**:175-178.

Klug P. 2017. The future of blackbird management research. Pages 217-234. *Ecology and Management of Blackbirds (Icteridae) in North America.* (eds G.M. Linz, M.L. Avery & R.A. Dolbeer) CRC Press/Taylor & Francis, Boca Raton, Florida, USA.

Kross SM, Kelsey TR, McColl CJ, Townsend JM. 2016. Field-scale habitat complexity enhances avian conservation and avian-mediated pest-control services in an intensive agricultural crop. *Agriculture, Ecosystems & Environment* **225**:140-149.

Linz GM, Vakoch DL, Cassel JF, Carlson RB. 1984. Food of Red-Winged Blackbirds, *Agelaius-phoeniceus*, in Sunflower Fields and Corn Fields. *Canadian Field-Naturalist* **98**:38-44.

Park MG, Blitzer E, Gibbs J, Losey JE, Danforth BN. 2015. Negative effects of pesticides on wild bee communities can be buffered by landscape context. *Proceedings of the Royal Society B: Biological Sciences* **282**:20150299.

Porter SS. 2013. Adaptive divergence in seed color camouflage in contrasting soil environments. *New Phytologist* **197**:1311-1320.

Werner SJ, Avery ML. 2017. Chemical Repellents. Pages 135-158. *Ecology and Management of Blackbirds (Icteridae) in North America*. (eds G.M. Linz, M.L. Avery & R.A. Dolbeer) CRC Press/Taylor & Francis, Boca Raton, Florida, USA.

Werner SJ, Clark L. 2003. Understanding blackbird sensory systems and how repellent applications work (ed G.M. Linz). Pages 31-40 in Linz GE, editor. In Management of North American Blackbirds, Proceedings of a special symposium of The Wildlife Society, 9th Annual Conference, USDA-APHIS-Wildlife Services National Wildlife Research Center, Bismarck, North Dakota, USA.

Werner SJ, DeLiberto ST, Pettit SE, Mangan AM. 2014. Synergistic effect of an ultraviolet feeding cue for an avian repellent and protection of agricultural crops. *Applied Animal Behaviour Science* **159**:107-113.

Whelan CJ, Şekercioğlu ÇH, Wenny DG. 2015. Why birds matter: from economic ornithology to ecosystem services. *Journal of Ornithology* **156**:227-238.

Woronecki PP, Dolbeer RA. 1980. The influence of insects in bird damage control (eds J.P. Clark, R.E. Marsh). Pages 53-59. In Proceedings of the 9th Vertebrate Pest Conference, Universtiy of California, Davis, California, USA.