THE EFFECTIVENESS OF 9, 10 ANTHRAQUINONE AS A REPELLENT TO PROTECT OILSEED SUNFLOWER FROM BLACKBIRD DEPREDATION

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MASTER OF SCIENCE

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ABSTRACT

Across North America, blackbirds (Icteridae) cause heavy damage to sunflower crops annually, but effective methods limit blackbird damage to sunflower crops are lacking. I tested two repellents (active ingredient 9, 10 anthraquinone) under application conditions appropriate for large-scale, sunflower farming. In 2012, I conducted point counts and weekly crop damage surveys to assess blackbird use of plots of sunflowers, half of which were sprayed with Avipel. In 2013, six enclosures (three red-winged blackbirds, *Agelaius phoeniceus*, each) in a plot of sunflower treated with AV2022, and another six enclosures in an adjacent plot of sunflower left untreated. Results from 2012 indicate blackbird use of sunflower plots did not vary with Avipel treatment. Results from 2013 showed AV2022 treated sunflower plants had more seed loss than the untreated plot. I conclude that 9, 10 anthraquinone does not reduce blackbird damage to sunflower crops under typical methods for pesticides in large-scale, commercial agriculture.

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LIST OF ABBREVIATIONS

AQ…………………Anthraquinone

COGR …………….Common grackle

IACUC……………Institutional Animal Care and Use Committee

NDSU……………..North Dakota State University

PPR……………….Prairie Pothole Region

RWBL ……………..Red-winged blackbird

YHBL …………..…Yellow-headed blackbird

UV………………....Ultra violet

US EPA……………. United States Environmental Protection Agency

USDA………………United States Department of Agriculture

GENERAL INTRODUCTION

Sunflower is an important crop for the Prairie Pothole Region (PPR). North Dakota and South Dakota's total production comprises over 70% of the United States' total sunflower seed yield (Blackwell *et al.,* 2003). The sunflower plant (*Helianthus annuus*) originated in North America and has been selectively cultured to produce flowers with large, seed-filled heads (Burke *et al.,* 2002). An increasing demand for oilseed sunflower comes from its growing use for confectionary oil and biodiesel.

In addition to providing ideal conditions for growing sunflower, the PPR also provides habitat for several blackbird species. Large populations of three native blackbird species, red-winged blackbird (*Agelaius phoeniceus),* yellow-headed blackbird (*Xanthocephalus xanthocephalus*), and common grackle (*Quiscalus quiscula*), use the PPR for nesting and migration (Yasukawa and Searcy, 1995; Twedt and Crawford, 1995; Peer and Bollinger, 1997). High densities of cattail-dominated wetlands in the area not only provide nesting habitat for resident blackbirds, but later in the season also provide roosting habitat for resident breeders, their offspring and migratory blackbirds. Individuals from all the age groups are building energy reserves for migration at this time, and sunflower plants provide readily available energy-rich seeds in a convenient locale. Most of the damage done to sunflower crops takes place 18 days after the last petals drop from the sunflower heads (Cummings *et al.,* 1989). Since the mid-1970s, blackbird depredation has become an increasingly common problem for farmers in the Prairie Pothole Region of the United States and Canada (Blackwell et al., 2003). Damage from the blackbirds costs the growers of sunflower alone around $5 to $10 million each year in crop damage (Peer *et al.,* 2003; Klosterman *et al.* 2010).

Multiple methods of protecting crops exist, but none have proved to be cost-effective. Lethal control methods are not target-specific, and are unpopular with the general public, scaring devices have variable effectiveness requiring frequent location to be efficient, and cattail management can only be used with landowner consent (Linz *et al.*, 2011). Thus, a non-lethal, chemical-based repellent would be a promising alternative (Avery and Cummings, 2003).

The compound 9, 10 anthraquinone (AQ) is known to repel blackbirds. Repellency was first demonstrated in the 1940s, but today AQ is only approved for use in the United States in Flight Control, a chemical formula used to prevent damage to lawns or other high-value sites by Canada geese, *Branta canadensis* (Avery, 2002). Currently, the patent holder Arkion (Arkion® Life Sciences LLC., 551 Mews Drive Suite J., New Castle, Delaware, 19720, USA) is applying for registration with the FDA for use of AQ on crops (EPA FIFRA Section 3). AQ is a secondary repellent because it has no immediate effect on birds that consume it. However, it affects digestion, causing birds to experience discomfort and sometimes regurgitation (Avery *et al.*, 1997, Avery *et al.*, 1998).

A new AQ-based repellent, Avipel®, has recently shown effectiveness as a repellent in laboratory studies. Wild caught blackbirds were shown to reliably discriminate between AQ-treated and untreated rice (Werner *et al.*, 2009). Seeds treated with 12,220 ppm AQ boasted a repellency rate of more than 80% against common grackles. In a field cage study performed by Werner *et al.* (2009) examined the effectiveness of Avipel® after application to oilseed sunflower plots when >50% of the flowers were at the end of the flowering stage (R6). The results showed captive grackles preferred untreated sunflower seeds (Werner *et al.*, 2011).

The objectives of this study were to determine if blackbirds would avoid of fields sprayed with AQ, if rates of damage to sunflower were affected by AQ, and to quantify change in AQ residues on sunflower heads in fields sprayed with AQ over time. We hypothesized that plots treated with AQ would sustain less damage than untreated plots from blackbirds.

This thesis is divided into three chapters. The first chapter is an in-depth review of blackbird depredation on sunflower crops in North America. Chapter two details the 2012 field study on the effectiveness of late-season application of AQ for reducing blackbird damage to sunflower. The third and final chapter details the 2013 field experiment in which red-winged blackbirds were placed in enclosures with sunflower treated with AQ (applied earlier in the season at the approximate time when general insecticides would be applied) to determine the effectiveness of mid-season AQ application on reducing blackbird damage to sunflower. Chapter three also contains the effects of the spray on bee pollination and the results from a cage enclosure study.

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CLARIFICATION OF DUAL-AUTHORED CHAPTERS

Two of the following three chapters of this thesis are coauthored. I, Megan Niner had primary responsibility for collecting samples in the field and for interpreting the data collected as well as developing all the conclusions. Each chapter was also drafted and revised by myself. Mark Clark, George Linz, and Jeff Homan served as proofreaders and checked the math in the statistical analysis conducted by Megan Niner.

CHAPTER 1. THE IMPORTANCE OF SUNFLOWER IN AGRICULTURE ACROSS THE NORTHERN PLAINS OF NORTH AMERICA

Literature Review

On the Origin of Sunflower in Agriculture

Around 8,000 BP, humans adopted agriculture in the southern regions of North America. First to be domesticated were different species of gourds, such as bottle gourd, gourd squash, and egg-gourd (Ford, 1981). Four thousand years would pass before the sunflower, *Helianthus annuus*, would be domesticated as well (Lentz *et al*, 2001). While scientists agree that the sunflower was domesticated in North America sometime around 4000 BP, a heated debate still remains over the actual area of origin for the domesticated variety. One group argues that humans domesticated the sunflower in the present-day Eastern United States based on morphological and genetic similarities to the wild populations across the country (Harter *et al.*, 2004). The other group claims their more recent research shows the domestic sunflower originated in Mexico. This claim is based on archeological evidence of carbonized sunflower remains found at numerous archeological sites that are too large in size to have come from the wild population. Sunflower was also commonly used in cultural practices passed down by the numerous indigenous peoples of the region (Lentz *et al.*, 2008). Regardless of origin, both groups can agree that the major trait selected for seed in today's cultivar is the characteristic large head, jam-packed with seeds that stay in place until the head dries out (Burke *et al.*, 2002).

H. annuus belongs to the Asteraceae family which developed sometime during the Cretaceous period, around 50 million years ago. The species itself did not arise until 0.5 to 1 million years ago (Lentz *et al.*, 2008). Wild *H. annuus* grows across the continental United States and Northern Mexico resembling the domestic version with its namesake sun-shaped, yellow flowers. This is where most similarities end, however. Heads of wild sunflower are much smaller and multiple flowers grow on the branched stalks of a single plant. As the plant reaches maturity, the seeds in the head 'shatter' or are released, long before desiccation. Domesticated *H. annuus* grows on a larger, taller, single stalk and maintains its seeds even past desiccation. These simple changes are the result of hundreds of years of artificial selection resulting in a plant that reliably produces a large seed load that is retained until it is fully ripened for human consumption (Burke *et al.*, 2002).

As a C4 plant, the sunflower is able to fix carbon at a higher rate than other crops under conditions of high temperatures and intense light, also making it rather drought resistant to a certain extent (Gimenez *et al.*, 1991). When oxygen levels in the soil are low, either from excess water or low mineral content, germinating sunflower will alleviate this problem by enlarging the cells just above the root to continue the movement of oxygen and other gas products through the plant that would otherwise build up due to these conditions. While the plant will survive under these less than optimal conditions, the overall biomass of the plant is greatly reduced (Kriedemann and Sands, 1984).

A standardized method for describing the different growth stages of the sunflower was developed in the 1980s as the crop began to become more commonly grown and the need for a simple and accurate way to describe where the plant was in terms of development was necessary. There are two main stages in this system; the V, or vegetative stage, and the R, or reproductive stage. The V stage is further broken down into two main parts: emergence and true leaf development. Depending on the variety of sunflower grown, the true leaf development phase can have multiple steps as well based on how many leaves with a length greater than four centimeters are put out before the flower begins to develop. The R stage follows and is divided into nine phases based on the flower development. R1 begins with the appearance of the inflorescence and R9 ends the development stage when the bracts of the sunflower have turned brown and the seeds are ready for harvest (Schneiter and Miller, 1981).

Commercially, sunflower is grown in two varieties; oil seed and confectionary. Oil seed sunflowers are characterized by smaller seeds with high oil content. Sunflower oil is highly valued for its lack of odor, clear color, and high levels of polyunsaturated linoleic acid (Harris *et al.*, 1978). The average seed size of oilseed sunflower is 9.52 mm by 5.12 mm by 3.27 mm in length, width and thickness, respectively. The inner kernel is slightly smaller and where the majority of the oil is located (Gupta and Das, 1996). During the seed development, the oil contents change from palmitic and oleic acid to linoleic acid, reaching the maximum oil content shortly before the plant is ready for harvest. Studies have looked to show a link between temperature and oil content, but have thus far been unsuccessful. However, it appears that diseases such as rust, head rot and stem rot do not affect the overall fatty acid content of the seeds (Harris *et al.*, 1978). Overall, the amount of water provided to the crop during anthesis, or the development of the flower, has a direct effect on the number of seeds per head (Anderson *et al.*, 1997; Goyne *et al.*, 1978).

Current research is ongoing to determine the relationships between sunflower genes and certain adaptations such as drought-tolerance and germination time (Kane and Rieseberg, 2007; Blackman *et al.*, 2011). This matter will become increasingly important as global climate change alters many areas and rainfall becomes less predictable. Future growers of sunflower may eventually see a switch from the well-established annual variety to a perennial crop. As perennial crops offer many advantages over annuals, such as more carbon storage, better soil and water usage, and more conservative use of nutrients, the push for a perennial variety is present. The

Land Institute is conducting current research and development of a perennial sunflower, Maximilian sunflower, in order to establish a nonshattering variety (Cox *et al.*, 2006). Until an economical yield of seed can be obtained from the perennial variety, possible use as a decoy crop could be implemented.

Sunflower is grown around the world for both its oil and confectionary purposes. Each year, an estimated twenty-four million hectares are planted globally in places such as Australia, China, Europe, India, North America, Pakistan, Russia, South America, and Ukraine (Linz *et al.* 2011). In North America, sunflower is grown in the central region with the vast majority in the PPR of the United States and Canada, with the majority in North and South Dakota. Within the United States alone, the Dakotas produce more than seventy percent of the total sunflower crop (Blackwell *et al.*, 2003). Receding glaciers left behind a flat landscape abundant in wetlands around 10,000 BP during the Wisconsin Glacial Age (Peer *et al.*, 2003). Here, the moist soil and cool climate conditions at the beginning of the growing season allow for yields in excess of 1,800 kilograms per hectare. As field sizes in the region are expansive, usually the smallest are at least 65 ha; a large yield of sunflower can be harvested each year (Linz *et al.*, 2011).

Diseases such as *Sclerotinia,* Verticillium wilt, and Rhizopus head rot are the most common across the sunflower fields in the United States and can have debilitating effects in localized outbreaks, but are not a major cause of concern on the whole. Dramatic weather, namely droughts and floods are of more concern, but less can be done to control these unexpected events (Gulya, 2002). Depredation from migratory avian species is substantial as the PPR is also prime habitat for a few native species of blackbirds with populations well into the millions. Each year, an estimated US $70 million of damage to sunflower crops occurs by birds in this geographic location alone and is the leading cause of the decline of farmers attempting to grow the crop (Linz *et al.,* 2011). This problem is not a new one; in the late 1960s, shortly after the sunflower was recognized by farmers for its economic value, a sharp increase in bird depredation was witnessed in direct correlation with the increase in area used for growing the crop (Peer *et al.,* 2003).

Sunflower seeds are an important crop for multiple reasons. While confectionary seeds are a common snack food and a major component of birdseed for feeders, oil seed is used for producing sunflower oil and ethanol. Ethanol is commonly used in the production of biodiesel, an environmentally friendlier fuel than fossil fuels. Benefits of crop-derived biodiesel include decrease in acid rain, decreased greenhouse gas emissions and a biodegradable fuel-source (Anotlin *et al*., 2001). With a growing demand for cleaner energy and more food production, it is important that an effort be made to help relieve some of the problems growers are faced with each year.

Blackbird Life History

All across the continent of North America, people are often familiar with their resident blackbird species. To some, they represent the coming or going of the seasons, while to others these birds can be seen as harbingers of devastation to their ripening crops. I focus this section of my literature review on the three most common species seen in North Dakota of the *Icteridae* family: red-winged blackbird, common grackle, and yellow-headed blackbird. These three species, RWBL, COGR, and YHBL respectively, share many similarities in their life history and are often seen flocking and foraging together during fall migration. All three are found in their highest densities across the PPH region of the United States and Canada. The state of North Dakota has the highest concentration of RWBL, YHBL, and COGR of any state or province in the USA or Canada (Peer *et al*., 2003). All of these species are granivorous passerines and share a basic morphology of the beak that allows for the rapid and effective consumption of a large quantity of seed in a short amount of time. Birds can store more food than can immediately fit into their stomach because they have a crop that aids in food storage. After loading up, the birds can fly to a safe area and digest their meal rather than make repeated trips or stops while on migration (Klasing, 1999).

Red-winged Blackbird

Perhaps the most common bird in North America, the red-winged blackbird, *Agelaius phoeniceus* is easily identified by its distinct plumage coloration in the males. A common sight in roadside ditches abundant with cattails, the RWBL, is a jet-black bird with shoulder patches of bright red and yellow on its wings. A very vocal species, male is often heard singing its distinct ‘o-ka-lee’ song while perched at the top of the cattail stands or some other area where he will be visible to all. Females, along with the first-year males are more cryptically colored and are a mottled brown color with a distinct white eye-band (Yasukawa and Searcy, 1995).

The RWBL has a vast distribution that spans the North American continent from Mexico to just below the Arctic Circle and from the Atlantic to Pacific coastlines. Habitat selection is diverse and the species is known to dwell in various substrates mostly in wetland, upland, or agricultural areas. Though they have a preference for cattails, RWBL will nest in bulrush, sedge, reed, golden rod, alder, button brush, wheat, barley, alfalfa, and rice or other vertically growing shoots (Yasukawa and Searcy, 1995).

Spring migration usually begins in most of the region mid-March and carries on through mid-May. Males arrive first to the breeding grounds and establish territories in which several female reside. Female selection of a male strongly correlates with habitat quality; therefore it is critical for a male bird to defend his territory against other males. Successful territorial male birds will have correspondingly large harems; the number of female birds in a single male's territory has been recorded as high as fifteen. However, RWBL are a highly promiscuous species, and a male with a high number of female birds does not necessarily have greater reproductive success. While a male may have a dozen females within his individual territory, females will seek out extra pair copulations with males from neighboring territories to help ensure their own reproductive success (Searcy and Yasukawa, 1983).

Red-winged blackbird nests are open cups made of reeds and other plant matter, usually lined with mud and built solely by the female bird. While she builds the nest, the male continues to vigorously guard his territory against other males until the female lays her eggs. After this point, the male guards the territory, but is less aggressive as he has ensured the passing of his own genes. Eggs are laid in clutches of two to four eggs and incubation is carried out for eleven to thirteen days by the female bird. Both the male and female spend time feeding the chicks and cleaning the nest. The more effort the male puts into feeding the chicks, the more likely the offspring are to survive (Searcy and Yasukawa, 1983, Yasukawa and Searcy, 1995). At fledging, juvenile males are over 30% larger in mass than their female siblings, and all juveniles retain their plumage until the following spring (Fiala and Congdon, 1983).

As with most birds, the diet of the RWBL varies seasonally to meet the energy requirements of the birds' life stage. During the breeding season, invertebrates make up the majority of their diet in order to supply the breeding birds, as well as the newly-hatched young, with adequate protein (Yasukawa and Searcy, 1995). In late summer, the diet composition changes to a seed-based diet. Corn, sunflower, and rice are just a few among the agricultural crops the red-winged blackbird choose to consume. As a long migration from Canada to Mexico is in front of some of these birds, they can be found commonly foraging for food sources near their roosting sites. With large scale farming common across most of the species' range, much conflict arises between growers and birds during the non-breeding season (Homan *et al.,* 1994).

Common Grackle

Perhaps more familiar to urban dwelling humans, is the common grackle, *Quiscalus quiscula*. At first glance, the both sexes appear rather drab with a dark blackish-brown overall body color and bright yellow irises. Yet, if given more than a passing glance, the iridescence of this bird quickly becomes noticeable. Geographical range appears to have some correlation on the slight color differences of this species. They are in fact different enough that three distinct subspecies can be distinguished based on geographical locations alone. Overall the trend in color is Eastern populations have a purple body and green tail, while more Western populations of COGR have a bronze body with a purple tail (Peer and Bollinger, 1997).

A primarily eastern bird, the COGR, inhabits a large area of continental United States and southeastern Canada. With the planting of shelterbelts along fields in the prairie region, the COGR has expanded its range westward to the Rocky Mountains. Like RWBL, COGR migrates Northward beginning in February and March for their spring breeding grounds. Populations along the Gulf Coast tend to be non-migratory thanks to suitable year-round weather conditions. Fall migration starts in August and September in the Northern range of the bird's territory. During this migration COGR can be seen in large mixed-species flocks, usually joining RWBL, European starlings, and brown-headed cowbirds (Peer and Bollinger, 1997).

Once on the mating grounds, COGR males and females form mating pairs while the female bird searches for an appropriate nest site. A strong preference for building a nest in conifer trees has been noted across the entire species range, but the COGR will also use deciduous trees and shrubs should they find them more suitable. Once the nest site is selected, the female bird will build a cup-shaped nest. The female lays and incubates four to five light blue to gray eggs with dark scrawls per clutch. When the eggs hatch the male usually deserts the female. The young are then raised by the female, who feeds them until they are adept fliers (Peer and Bollinger, 1997).

The massive success of this species is due to its extremely opportunistic behavior in regards to both habitat and diet. Commonly found in urban areas, the COGR consumes human trash. In more rural areas, the bird eats what it can find (Peer and Bollinger, 1997). In North Dakota, the primary diet item during the late summer is almost exclusively sunflower seed (Homan *et al.,* 1994).

Yellow-headed Blackbird

Preferring habitat with deeper waters and very sparse in trees, yellow-headed blackbirds, *Xanthocephalus xanthocephalus*, are the least common of the group in focus. Named for its striking appearance the YHBL is characterized by a bright yellow-colored head, throat and breast in the males with a dark black body and white wing bars visible while the bird is in flight. Females and juveniles also have the characteristic yellow on their breasts and necks along with a yellow eyebrow, but the intensity of color is more muted than adults. The remainder of their bodies is a dull black to brown color. Unlike the RWBL, songs of the YHBL are less varied and can be described as buzzy sounding screech.

This species is more selective of its habitat and has a far more condensed range than either the COGR or RWBL. Ranging from the Southern reaches of Mexico northwards to central Canada, the YHBL is found mostly in the central region of the continent. During the winter months, it spends the non-breeding season in the extreme south-central United States and its

Mexican range. During the breeding season it migrates northward when the temperatures are not as harsh, usually late-March through May (Twedt and Crawford, 1995).

Males arrive on the breeding grounds first, and like the RWBL, establish territories and defend them against other males. Both these species of blackbird have the same preference for cattail stands for nesting grounds resulting in species competition. It has been observed that in the cases where the two overlap, YHBL is dominant to RWBL. Harem sizes are slightly smaller in this species ranging from zero to eight females per male, yet the species is still highly promiscuous. The average clutch size is three to four eggs, which are gray to greenish white in color, marked with even blotches and spots. After hatching, the female feeds and takes care of the hatchlings until they are able fliers (Twedt and Crawford, 1995).

The overall dietary choices of the YHBL are similar to those of the COGR and the RWBL. Food items with extra protein content are selected more in the breeding season, most of this comes in the form of aquatic invertebrates near nesting sites. Later in the season, YHBL forage in agricultural fields, targeting small grains such as wheat, barley, and oats, along with corn and sunflower (Twedt and Crawford, 1995).

Avian Management

Human and avian interactions play an important role in how local species are viewed. During the eighteenth and nineteenth centuries in the United States, the massive spread of agriculture spurred by westward expansion, saw the clearing of areas for farming as well as planting shelterbelts to protect large fields from wind erosion. By the end of the 1940s, studies of experimental ways to repel birds and other animal pests began to make their way into the scientific community as a growing problem was soon recognized (Damback and Leady, 1948). As humans expanded their range into the formerly undeveloped areas, they removed the natural food sources for birds and provided a ready and concentrated alternative in the form of crops. In return, hungry birds will seek out food and where better to eat than in a field full of readily available crops? From the birds' perspective, an agricultural field provides them with more energy than they need and less energy is required to obtain their food (Avery, 2002b).

North America is not alone in their struggle between farmers and birds; globally, avian depredation is a major problem for many of the same crops. Yet in each corner of the world, a different species poses the problem, creating the need for a variety of techniques to be used to address each case individually (Linz *et al.*, 2011). The following description will focus on North American solutions aimed at managing primarily avian depredation.

In North America, crops such as corn, rice, and sunflower are among the most damaged by avian species. At the top of the list for worst offenders are the red-winged blackbird and other *Icteridae* family members. Rice growers in Louisiana have seen the complete depredation of their fields by a flock of around five hundred blackbirds. With a foraging range up to fifty-six km in radius from their roosting site, damage done by RWBL is proportional to the amount of roosting blackbirds in the area (Cummings and Avery, 2003). Despite an overall declining trend in blackbird populations nation-wide, the sunflower growing regions of the United States have reported an increase in blackbird population. Nationally, the RWBL remains the most abundant bird across the lower forty-eight (Werner *et al.*, 2010).

The conflict between growers and birds is exacerbated by the coincidence of the growing seasons with the annual migration of the blackbirds. As the crops ripen in northern regions of the continent at the end of summer, millions of newly fledged first-year blackbirds, along with the surviving members of the older generations, must bulk up for their fall migration. Sunflower is grown in large quantities across the central United States, right along a major portion of the blackbird migration corridor. More than seventy-five percent of all damage done to sunflower crops occurs within the first eighteen days following petal drop (Cummings *et al.,* 1989). Their migration south is coordinated with the ripening of further crops until they reach their wintering grounds. Overwintering in states such as LA and AR causes another crop timing problem. These states are two of the largest producers of rice in the United State, and in November, the rice crops are still being harvested. With the arrival of millions of migratory birds at the end of their trip, the blackbirds often descend into the rice fields and spend the winter months feeding on the crop (Cummings and Avery, 2003).

Birds chose to eat food items from agricultural fields for more reasons than beyond mere convenience. Should a wild habitat with the birds' natural food sources be nearby, they still might choose to feed in the farmers' fields for a variety of reasons. First of all, the crop field usually provides the birds with a higher quality food source, usually in terms of caloric content. Humans have altered the crops grown for larger seeds and bigger yields to provide more nutritional rewards, however, these same principals of nutrition apply to birds as well. Secondly, the crop may be easier for the birds to handle and consume. With less handling time, more food can be consumed in the same amount of time as a food that requires more effort. As less energy is required to manipulate the food before consumption and less time is wasted, the overall result is a higher caloric gain. Thirdly, the reduced diversity of animals foraging in agricultural fields means less competition with other species for the same resources. Less competition can be a playing factor in the birds' decision when the need for amassing extra body weight is high. Finally, the birds may feel that the predation risk is lower in the agricultural setting than in their natural areas. Safety and security reduce stress and conserve energy, another bonus for the birds.

These factors, whether alone or combined, can make a world of difference in how much damage is sustained by a field (Avery, 2002a).

An estimated 277g of sunflower seed are consumed annually by each male RWBL foraging in a given year. Females, being smaller in size and appetite, consume about 168g of sunflower annually. In 2003, the cost of damages to growers was estimated at $0.09 per male and $0.05 per female RWBL in their fields (Peer *et al.*, 2003). As more than eighty percent of the birds foraging in sunflower fields are RWBL, a significant portion of the economic loss may be attributed to this species alone (Cummings *et al.*, 1989). Profit margins for farmers are considered negligible when damage exceeds five percent of the field and total loss occurs when more than seventy percent of the field has been lost (Blackwell *et al.*, 2003; Linz and Homan, 2011). With the massive numbers of birds in a flock, damage of these quantities can be reached rapidly.

In order to accurately measure the amount of damage inflicted on a field, different techniques have been developed to gather estimates quickly and efficiently. Two methods are commonly used to obtain an accurate measure of damage on sunflower heads. One option is to use the ‘template method’ in which a clear piece of plastic is cut into a semi-circle. The template is divided into even sections of 2 cm. When held up to a sunflower head, the damage in each section is recorded as well as the diameter of the un-developed center. By summing up the damages in each section, a total damage estimate can be obtained. A second option to estimate the damage on a sunflower head is to use a wire cross, labeled at 2 cm intervals to visually estimate the percent damage done in each quadrant when it is held up to the center of the flower. This second method is quicker, but less precise than the template (Dolbeer, 1975).

Local and federal organizations, such as the United States Department of Agriculture, work with farmers to help manage these widespread problems and alleviate some of the damage. The main goals of such collaborations are to determine the status of the species causing the damage, accurately determining how much damage is done to the total production, developing and evaluating possible repellents and management techniques, improving current techniques, and to develop other strategies to deter bird depredation (Cummings and Avery, 2003).

Ethics are major concern to take into account when looking at avian depredation. To the farmer whose entire livelihood is gobbled up by a flock of hungry birds, local and regional laws start to become less important and may be ignored. From their view either they have two choices; allow the birds to eat their fields and suffer the economic consequences or get rid of the birds and be able to put food on their own table. In some cases growers will take matters into their own hands, not realizing their impact on the environment or the bird's overall population. By reducing the damage caused by birds, the deaths of birds caused by illegal killings are also reduced (Avery *et al.,* 2001).

There are a few major hurdles that must be overcome when developing new devices and chemicals for controlling avian damage. First, and perhaps foremost, is the amount of funding or monetary incentive for private companies to consider damage mitigation methods. The market for avian deterrence from agricultural fields is rather low as the number of farms is small compared with the overall area of land in agricultural use. Secondly, for the existent repellent techniques known to be effective, the cost often outweighs the profit from growing the crops. Third, the problem-causing species number in the millions, flocks in large groups, and travel long distances every year. Finally, the problem species are also native birds and lethal control methods are highly unpopular with the general public (Avery, 2002b).

The overall effectiveness of a repellent is dependent on four major factors. 1) The repellent must be effective under field conditions, 2) the cost relative to the expected damages must be equivalent or less, 3) the repellent must be safe for food and feed in order to be applied directly, and 4) there must be no negative environmental impacts. In order for a repellent to become registered it must display each of the above characteristics (Werner *et al.,* 2010).

Lethal Control

For many growers, the first instinctive solution to a pest problem is to eliminate the pest. Multiple attempts have been made with different compounds to control blackbird populations with lethal baiting. A problem with this approach is encountered when non-target species also consume the bait. DRC-1339, commonly used around nesting sites of blackbirds in the south has the unintentional effect of being consumed by species such as savannah sparrows (*Passerculus sandwichensis*), and other tree sparrows, resulting in their deaths as well (Cummings and Avery, 2003). Another chemical, PA-14, was used from 1974 to 1992 in attempts to reduce the overall breeding population of RWBL, COGR, and other pest bird species. When sprayed directly to the feathers of a bird, the chemical allows water to penetrate and saturate the birds. With a good amount of additional water, usually via rainfall, the birds would eventually succumb to hypothermia and die (Heisterberg *et al*., 1987). Even with an average of two million bird deaths per year, the chemical alone was ineffective for reducing the overall populations as they quickly rebounded the following breeding season (Dolbeer *et al.,* 1995). Although lethal measures make intuitive sense, they are not very effective on a large scale as the populations of the targeted species are extremely large and mobile. Public dislike of these control techniques also further deters farmers from using such drastic measures. As such, other control measures, such as scare devices, repellents, habitat management and decoy crops are more commonly considered and used (Linz *et al.*, 2011).

Physical Barriers and Handling Time

The second easiest solution would appear be to physically prevent the birds from reaching the crops. A common effective method used to create this barrier is to use netting to cover the targeted crop. This works well and is very economic on a small-scale level, but remains highly impractical in the context of large fields of sunflower and other such crops (Avery, 2002b).

In addition to accessibility, handling time is a major factor in how much damage is done to a crop. If a bird can quickly consume a large quantity of food items from a field without much trouble, it will be hard to dissuade the birds from feeding. The rate of food consumed per unit of time is a very important factor for a migrating bird's choice on where to feed as they strive to bulk up as fast as possible for their migration. While physical barriers can increase handling time, if not completely exclude the bird from feeding, they are not cost-effective over large areas. Instead, protecting just the portion of the plant being damaged can sometimes be an option. In the case of planting seeds, coating with non-toxic clay can greatly impede the progress of a bird's feeding rate. In order to consume the seed, a bird will need to remove the clay from the seed and later themselves. The cleaning process greatly impedes the bird's consumption rate, often causing them to search elsewhere for more ready-to-eat food items. This simple treatment has been shown to be effective when planting rice in high-blackbird areas (Avery and Cummings, 2003).

Decoy Crops

Decoy crops are another possible alternative and provide other benefits to the environment as well. By planting a more appetizing crop closer to a known roosting site, farmers can reduce damage done to their own fields by providing an alternative energy source to the birds. This practice works well when protecting a high-value crop, such as confectionary sunflower. Plants chosen to serve as decoy crops should ideally cost less to plant and come into maturity earlier than the crop to be protected. By planting a variety with different maturation times, the decoy plot can prove effective for a longer length of time (Cummings *et al.*, 1987). Additionally, perennial varieties would reduce time, money, and energy spent sowing new decoys each year. The major drawbacks of this method are the cost to plant decoy fields and the loss of space that could be used to plant other crops. Currently, this method is not very popular, but with the development of perennial crops, there could be an increase seen sometime in the future (Linz *et al.*, 2011).

Cattail Management

Both species of blackbird commonly roost in cattail stands and grackles often forage around them as well. As these features are common across the vast wetlands of the PPR, they are often located in close proximity to sunflower fields as they also provide the growers with an excellent source of irrigation. Since the late 1800s, an introduced species of cattail from Europe began to spread and hybridized with the native species. The result of this cross was the extremely generalist species known simply as ‘hybrid cattail’. Their progeny now occupies most of the wetlands across the continent rapidly covering any open, shallow water areas. By removing these large stands of cattails, blackbirds would be forced to search elsewhere for roosting and nest-building sites. Glyphosate, commercially available in multiple forms, is the most commonly sprayed herbicide for cattail removal (Linz and Homan, 2011). With a single aerial application, a stand of cattails can be controlled for about four years or longer in areas with deeper water (Solberg and Higgins, 1993). The cost of such a spray is offset by the benefits of blackbird density reduction if around 238 blackbirds are present per day on a single hectare of cattail. The reduction of cattail stands offers another benefit; increased diversity in both plant and animal species that require open shallows for suitable habitat (Linz and Homan, 2011). Yet, cattails are quick to colonize open wetlands that are shallow enough for them to grow, making colonization an issue those using this method will have to contend with (Peer *et al*., 2003). Additionally, a decline in invertebrates in areas treated with glyphosate has been documented, but the long-term effects of this decline are currently unknown (Solberg and Higgins, 1993).

Scare Tactics

Scare tactics are also effective means for reducing bird damage. Research has shown devices that make loud, unexpected noises are effective at scaring birds out of fields. The disturbance startles the birds and causes stress. If enough stress is caused to outweigh the benefits of an easy dinner, the disturbance will be effective (Avery, 2002b). Propane cannons are among the most common of scare tactics used to combat bird depredation. However, the use of such devices is limited due to cost per unit and the amount needed to effectively cover a large field characteristic of north-central North America. In 1999, it was estimated by York *et al*. (2000) that the average grower in California spent around $120 per hectare when utilizing scaring by shooting techniques, even though many of them find the effectiveness questionable. An estimated two to three hectares can be protected by one cannon and as most fields in the region are at least sixty-five hectares, the cost of the cannons would negate most of the profit from the crop. To remedy this, frequently moving a few cannons around the fields as well as changing the direction and timing of the blasts is suggested. The required time and energy for the farmer to relocate each cannon makes this option rather unappealing (Linz *et al.*, 2011).

Chemical Repellents

Chemical repellents are often turned to for reducing bird depredation for many reasons. These chemical repellents are characterized as non-lethal, easy to apply, and generally low-cost, and are an ideal solution to the bird damage. This topic will be covered in-depth in the next sections.

Chemical Avian Repellents

Repellents for use in agriculture have been developed since the beginning of the twentieth century as means of both protecting crops from bird depredation and protecting birds from harmful insecticides and other harmful materials (Avery, 2002a). A repellent is any chemical that produces a behavioral response of avoidance in an animal. They are non-lethal and can be made from a multitude of natural and synthetic components (Avery *et al.*, 2000b). In order to be licensed in the United States for use on consumable crops, the developer must comply with strict guidelines from the Food and Drug Administration. Other countries do not have the same regulations and may use compounds that have been considered toxic in the United States. The overall goal of a repellent is not to completely eliminate damage being done, but rather to disperse it more evenly to lessen the economic burden that comes with loss (Peer *et al.*, 2003).

Repellent properties are sometimes found in other chemicals already in use for other agricultural purposes. Some, such as Thiram, a fungicide, are found to be effective for repelling birds often by accident (Kennedy and Connery, 2008). It is less expensive to register an existing chemical for an additional use than to simply develop a new repellent from scratch, it is easier for fungicides and the like to be used as a repellent should positive results be found during trial studies (Werner *et al.,* 2008c).

There are two major types of repellents that all repellents fall under: primary and secondary. Primary repellents have immediate effects on the organisms that consume them. Usually, these work by causing bad taste, immediate irritation, or other fast-acting symptoms (Avery, 2002a). The most commonly used repellent in this category is methyl anthranilate, abbreviated as MA. This chemical compound is considered safe for human consumption by the US Food and Drug Administration and used widely in the artificial flavoring of food items such as beverages to obtain a grape or fruity flavor. Although humans experience no discomfort when consuming MA, blackbirds have been observed to suffer pain as the compound stimulates the bird's trigeminal nerve when it comes into contact with the eyes, nostrils or mouth of the bird (Avery and Cummings, 2003). Blackbirds are not alone in their aversion to the compound; all birds that MA has been tested on find the taste repulsive to some extent (Avery *et al.,* 1996). Despite the positive repellent effects, MA, when used as a repellent, has very limited short-term effects lasting under a week and inconsistent repellency (Blackwell *et al.,* 1999). On sunflower and rice, the MA containing Bird Shield^{TM} failed to make any difference between damages from blackbirds (Werner *et al.,* 2005). Additionally, plants treated with MA often suffer yellowing on their leaves, indicating phytotoxicity from the chemical (Avery *et al.,* 1996). While primary repellents require no learning to be effective, they are often mot as effective at creating a long-term avoidance pattern (Day *et al.,* 2003).

The other type of repellent, secondary repellents, are based on the concept of conditional learning because they are not immediately aversive. Most repellents in this category induce illness or discomfort after ingestion of treated matter. The overall effectiveness of a secondary repellent is based upon how unpleasant the reaction is to the items consumption as well as the length of the time interval between onset of symptoms and illness (Lee, 1999; Day *et al.*, 2003). The association made between consuming the contaminated item and the resulting illness is critical for a secondary repellent to function. This learned aversion has been demonstrated throughout the vertebrate class and has been seen in many invertebrates as well (Lee, 1999). Major repellents of this sort include 9, 10 anthraquinone and methiocarb. Both can induce indigestion and vomiting at least ten minutes after consumption. Methiocarb was originally created as an insecticide, but was quickly seen to be an effective bird repellent as well. A slightly toxic compound, birds that consumed treated food items experience vomiting, retching, and temporary paralysis with the severity linked to the dosage. All negative effects wear off in afflicted birds after an hour post-consumption (Avery and Cummings, 2003). However, this effective repellent is not licensed for use on crops for human consumption after the manufacture declined to provide further information to meet new standards set by the United States Environmental Protection Agency (Blackwell *et al.*, 1999; Avery *et al.*, 1996). Today, methiocarb is still used to protect ornamental crops and the eggs of endangered birds from avian depredation (Avery and Cummings, 2003). A need for a new repellent has been created by the absence of this somewhat effective repellent for the consumable crop industry.

Regardless of primary or secondary effects, repellents work best in tandem groups with other stimuli such as taste, color, or patterns. Nature’s own strategies for repellency often are a combination of several signals. For example, the brightly colored wings of the monarch butterfly herald its bad taste; thus birds instinctively know to leave it alone after a single sample. Plants too use coloration as a natural defense; many fruits change in color to indicate ripeness. The color change is often accompanied by the reduction of naturally occurring repellent chemicals

(Avery *et al.,* 1997). Not many studies currently exist that have examined this concept for repellents in the field, but many have shown positive enhancement of repellents attempted in the lab. When color is added to a chemical repellent, it adds to the repulsive effects where birds are concerned as they rely on their vision most for food selection (Avery, 1984; Avery, 1995).

Repellents can be applied in a variety of ways, depending on the crop and stage of growth to which it will be applied. To protect seeds, a coating of a repellent can be applied to the outer shell of the seed, so long as it will still be able to germinate. Not all seeds need to be treated if the repellent is adverse enough that just one ‘bad’ seed can dissuade a hungry bird to look elsewhere for food. Where depredation of the ripening product is of greatest concern, fields of ripening crop, such as sunflower, can be sprayed directly. Common methods of application include aerial application by plane, ground spraying with a large rig, or hand spraying with a backpack sprayer for smaller fields. In some cases it is possible to spray only certain sections of fields, provided the response induced by the repellent is enough to cause avoidance (Avery, 2002a).

Application is often complicated by the issue of uniformly covering the targeted area. In rice, the outer covering is discarded by the bird before the inner seed is consumed, thus, less of the repellent is actually consumed than was applied (Avery *et al.,* 2000a). Fruits, such as blueberries, also have an issue of complete coverage. It has been suggested that use of a surfactant or sticker compound could help uniformly apply the repellents used to the targeted fruit (Avery *et al.*, 1996). A similar problem exits with sunflowers as the heads droop with the increasing weight of the seeds overtime. Optimal application time of a repellent would be just before the onset of bird depredation, but it is at this point in development, R6, that the sunflowers are often facing downward. When this happens, conventional spray methods are unable to apply repellent to the targeted area. Therefore, a more advantageous spraying time for sunflowers is while the heads are still upright in the earlier portions of R5, or at the beginning of flower development (Kandel *et al.*, 2009).

Damage to sunflowers typically occurs immediately following the flowering stage known as anthesis, or R6. As the yellow petals fall, the birds begin to come into the fields in massive numbers. Around 75% of the annual damage from avian depredation occurs within the first eighteen days following the end of anthesis. This poses the biggest problem for growers, as they must wait for the heads to dry out before they harvest. While anthesis (petal drop) occurs in mid to late August, growers in the Prairie Pothole Region wait for their crops to dry out and harvest early to mid October, two months after the time the bird problem reaches its climax (Werner *et al.*, 2010).

No matter how good a repellent might appear to be, when crops are under continual and heavy depredation by birds, no current repellent exists that can provide effective relief (Kennedy and Connery, 2008). The cost in energy or stress must be high in order to effectively outweigh the many benefits birds gain from foraging in the fields (Avery, 2002a). As laboratory and field enclosure settings cannot replicate the many complexities of the field, repellents that show lots of promise in early studies ultimately fail to show repellency in the field (Werner *et al.*, 2008b).

Avian Sensory Implications

Taste and visual cues play significant roles in how well a repellent can work. Though not as well understood as the mammalian system, what we do know about how avian sensory works could potentially aid the efforts to make an effective repellent. As more knowledge becomes available in this area, the potential for better designing species-specific repellents will grow.

Some primary repellents are suggested to work by providing a bad taste for the bird consuming the treated item. With taste receptors on both the upper and lower jaw in addition to the tongue, birds interpret this sense rather differently than do mammals. While some species of birds have up to 375 taste buds, this number is dramatically lower than the around 9000 taste buds found in humans (Klasing, 1999). When coupled with the lack of mastication during consumption, along with the low quantity of salvia secreted, it is generally accepted that birds have a far lesser taste acuity than that of humans (Roura *et al*., 2013).

The sense of taste evolved in order to aid organisms in selecting foods with the nutritional requirements they needed and to avoid potentially toxic items. Despite the smaller number of taste buds, genomic evidence from the domestic chicken, turkey, and zebra finch, all similar in dietary preferences to blackbirds, have taste genes for umami, sour, salt, bitter, calcium, and lipids. Of the five major tastes that mammals generally have, birds only are missing the sweet sensory (Roura *et al.*, 2013). Researching and utilizing this knowledge could greatly help in the design of a new repellent scheme. Repellents that function using bad taste alone inevitably fail after a given period of time as the novelty concept wears off; eventually the animals realize that nothing bad will happen. As such, pairing a repellent that causes gastrointestinal distress with a bad flavor is far more likely to have a longer lasting effect (Werner and Provenza, 2011).

Granivorous birds have ridges in the tomia of their beaks that enable them to easily cut into the tough exterior shells of seeds. With the aid of a dexterous tongue able to position and reposition the seed in these grooves until the inner seed is free of its casing, passerines can make quick work of even some of the toughest shelled seeds (Klasing, 1999). This being said, it has been observed that many birds are tolerant of different acidic and alkaline solutions, normally off-putting to mammals, which may allow them to eat unripe crops long before humans could tolerate the bitter tastes (Beason, 2003). A study comparing RWBL to European starling taste preferences showed that a higher tolerance of tannins in the former than the latter. The researchers attribute this to the higher presence of tannins in many of the natural food choices of the RWBL. However, as the concentration of tannins increased, both species consumed less of the substances offered (Espaillat and Mason, 1990). Further research into how blackbirds interpret taste could open new doors to more repellents targeted at the species in specific.

It is a vastly accepted fact that the majority of avian species have excellent eyesight. Like mammals, they have both rods and cones, but where humans only have three classes of cones, birds have four to five, depending on species. These additional classes of cones allow birds to perceive many more colors and even ultra-violet colors that are not visible to humans. The ability to see in the ultra violet range is utilized in the plumage of various species during mating season and has also been suggested to play a role in the selection of food items as well (Werner *et al.*, 2012). Additionally, the beak of most birds lies within their field of vision, allowing them to assist their feeding with their vision (Beason, 2003).

Drawing on the above information, combining a repellent with a visual cue, such as an ultra violet color, the effectiveness of a repellent can be enhanced. An experiment performed by Avery that showed house finches were more apt to avoid treated seed when the containing bowl was also marked with red tape than just treated or marked alone (Avery, 1984). Individual birds, like other organisms, have unique seed preferences per individual, which can also play a role in selection of food, though perhaps a more minor one (Cummings and Avery, 2003). Furthermore, RWBL have been observed to avoid UV-absorbent food items for up to eighteen days after being conditioned with food labeled by a UV signal and treated with a post-ingestive repellent (Werner *et al.,* 2012). Researchers in Australia have observed that birds will attack ripening grape crops based on color, sugar, and aroma, thus suggesting that nature itself uses a combination of signals that the birds follow when selecting their food items (Saxton *et al.,* 2009). We see the same trend when looking at aposematic insects that are brightly colored and have a chemical defense. Birds learn quickly which insects are safe to eat and which ones are not (Skelhorn and Rowe, 2005; Skelhorn *et al.,* 2008). It is reasonable to conclude that a stimulus of color alone will be unlikely to provide any protection from foraging birds, as the novelty of the stimulus will wear off without any repercussions from consumption (Werner *et al.*, 2008a). It is important to realize that in order for a combination of color and chemical stimuli to work that the entire area needing protection is colored or the birds will simply have been provided with an easier way to discriminate between treated and untreated (Avery and Mason, 1997). Additionally, conditioning with LiCl to cause illness, only worked in tandem with flavors to create avoidance in RWBLs (Werner *et al.*, 2008a). Therefore, color cues may not be all too practical on a large scale.

By combining multiple cues, such as taste and color, to a repellent, the effect will be learned quicker and repellency thus boosted of local birds (Gustavson *et al.*, 1982). A recent study by Werner and Provenza displayed that RWBL would reliably avoid foods flavored a certain way after experiencing gastrointestinal illness during conditioning (Werner and Provenza, 2011). These studies suggest that there is the potential for a combination to be effective if the bird can learn to associate stimuli with consequences. Again, the difficulty here is found when trying to apply these techniques to the field. During migration, many naïve birds show up in large flocks and the learning must begin again for each group (Werner *et al.,* 2008b).

Social cues are another avenue that, with some additional research, could also improve our tactics when deterring avian species from feeding on ripening crops. RWBL have been demonstrated in a laboratory setting to learn from watching others members of their own species who have been pre-conditioned to avoid food in certain marked dish. Additionally, the RWBLs also learned the same avoidance from a COGR trained the in the same manner and vice versa (Mason *et al.,* 1984). Perhaps this could imply that if a few birds in a flock quickly learn to avoid the treated food, the effectiveness of a repellent could act faster.

9,10 Anthraquinone

Anthraquinone is a naturally derived chemical repellent found in both plants and animals and is believed to be the chemical responsible for antiherbivory functions and predator defense in the various organisms in which is found. The chemical absorbs near ultra-violet light, which is visible to birds (Werner *et al.*, 2011). While the actual mechanism that AQ uses to act as a repellent is unknown, birds that consume the compound occasionally vomit, suggesting that it is a gastrointestinal irritant (Avery *et al.,* 1997). In addition, there is speculation that consumption of AQ-treated food items may cause malabsorption of nutrients and possibly dehydration as well (Werner *et al.,* 2009). As a secondary repellent, birds that consume treated items are not immediately repelled, but begin to avoid treated areas and food when they make the association between the discomfort and consumption of the contaminated food (Blackwell *et al.*, 1999). Repellents containing AQ as the active ingredient have been found to be non-toxic to most organisms when applied at the suggested rate (Barbee *et al.*, 2010). The low toxicity levels and quick decomposition rate make this compound a good candidate for use on crops for human consumption in addition to seed treatments (Cummings *et al.*, 2006).

Different repellents incorporating AQ as the active ingredient have been implemented for various bird problems around the world. In Ireland, the use of AQ formulated as Morkit showed moderate repellency of crows from treated wheat seed (Kennedy and Connery, 2008). In Europe, Flight Control^{TM} is used as a seed treatment while in Venezuela it is being tested for protecting the ripened rice crop (Avery *et al.,* 2001). In New Zealand, the use of Avex on poison baits is used to repel the native bird species from eating a lethal amount (Day *et al.,* 2003).

In 1944, anthraquinone was patented as a bird repellent in the United States, but was never registered for actual use. Despite this, the chemical's use in repellents has not abated and continues today (Cummings *et al.,* 2002). Early use of AQ was utilized in hopes of preventing ring-necked pheasants from eating corn seedlings in the Midwest region of the United States, but no clear evidence of its effectiveness was observed (Dambach and Leedy, 1948). There are a vast amount of applications for the repellents that AQ is considered for use as; seed treatments, ripening crop protection, grass defense, and even as a deterrent to stop birds from receiving a lethal dose of rodenticide baits (Werner *et al.,* 2011).

The afore mentioned Flight Control has been tested widely in hopes of its use as an effective repellent to protect various crops. A known repellent of blackbirds, the chemical mix contains 50% AQ as its only active ingredient. The rest of the chemical is composed of 2% surfactants and 48% latex-based filler material (Blackwell *et al.,* 2001). RWBL have been frequently seen vomiting in laboratory settings shortly after ingesting treated seeds (Avery *et al.,* 2000b). With a low toxicity level and a pH in the range of 7.5 to 8.5, the US EPA has deemed Flight Control non-toxic for both birds and mammals (Blackwell *et al.,* 2001; Cummings *et al.,* 2006). Flight Control has been proven effective as a repellent when sprayed on turf that Canada geese graze on and is commonly used at airports and golf courses for such matters (Blackwell *et al.,* 1999; Ayers *et al.,* 2010). At the current moment, turf-application is the only currently registered use of AQ registered by the EPA and for seed treatment in 20 states under EPA Special Local Use agreements (Blackwell *et al.,* 2001).

While successful in many a laboratory trial, Flight Control studies in the field encounter a plethora of problems. A field trial in California saw no difference between the crop yield of treated and untreated rice paddies. However, the fields not only provided a food source for the birds, but a roosting site as well, the pressure for the birds to stay far outweighed the effects of the repellent, even when sprayed twice at rates of 18.6 and 55.8L/ha. Cage studies were also done by the same group of researchers and while the bird preferred the untreated rice to the treated, treated rice was still consumed at a higher than expected rate (Avery *et al*., 2000a). Another study, this time dealing with rice seedlings, showed that residues from spraying were not consistent and left many areas insufficiently protected, suggesting the need for a new way of application or a higher spray rate (Avery *et al.*, 2000b). Positive results were shown concerning Flight Control treated corn and sandhill crane deterrence in cage studies where the birds had the choice of treated and untreated corn (Blackwell *et al.*, 2001).

Mixed results were even seen with Flight Control while treating in caged enclosures. A 1999 study using Flight Control to protect lettuce seedling showed only a 20% reduction in damage while another study in 2006 displayed around a 90% reduction. It should be noted that both of these studies were cage enclosures and may not be indicative of the actual field conditions (Cummings *et al*., 2006; York *et al.,* 2000).

Avipel® is the latest of the anthraquinone-containing chemicals to be developed as an avian repellent. Like Flight Control, Avipel is comprised of 50% AQ and 50% inert ingredients. The liquid chemical is miscible in water, a light tan in color with a weak aromatic odor, and has a pH on the slightly basic side between 7.5 and 8.5. The lethal oral dose for rats is greater than 5,000 mg/kg and the aquatic toxicity is 5,300 mg/L making this chemical non-toxic at label-suggested levels of application according to US EPA guidelines (Arkion® Life Sciences, 2008).

Already proven to be an effective seed treatment, the patent holding company, Arkion® Life Sciences (551 Mew Drive Suite 5, New Castle, DE, 19720, USA) is in the process of registering the compound on a national level for protecting seed corn (Linz and Homan, 2012). No detrimental effects have been observed on treated sunflower germination, growth, or seed yield after treatment (Kandel *et al.,* 2009; Werner *et al.,* 2011).

Laboratory trials have quickly established that this new formulation could be very effective for repelling a few common avian pest species. Canada geese were repelled from corn treated with Avipel at more than 80% when the concentration was 1764ppm AQ. Ring-necked pheasants avoided treated rice more than 80% of the time when it was treated with 9000ppm AQ. Most importantly, RWBLs were repelled at a rate of 80% when sunflower seeds were treated with 1994ppm AQ. Interestingly, the body mass of the bird is not directly proportional to the amount of repellent needed to cause avoidance. As the chemical also absorbs light at near ultra violet levels, which is visible to birds, the authors of this study also suggest that a visual stimulus may aid in the avoidance of treated seed. Overall, a suggested 1475ppm AQ is considered the threshold concentration for effective blackbird repellency (Werner *et al*., 2009).

Trials with Avipel® on sunflower have been inconclusive in terms of bird repellency in the field. Thus far, problems with determining adequate concentrations and the timing of the spray relative to the sunflower growth stages have caused less than stellar results when using conventional methods such as planes and ground sprayers. Spray too early and the AQ residues wear off before the birds arrive, but spraying later when the sunflower has developed results in very little residue on the seeds due to the weight of the seeds causing the head to face downwards (Kandel *et al*., 2009). Utilizing a backpack sprayer to apply Avipel to the heads of ripening sunflowers, one study showed a reduction in damage done by COGRs kept in field enclosures for fifteen days. Avipel was applied at a rate of 18.7L/ha and resulted in 18% damage to treated enclosures versus 64% damage in untreated enclosures (Werner *et al.*, 2011). A follow-up study using an airplane for application and a rate of 9.31 L/ha proved ineffective when repelling wild birds (Linz and Homan, 2012).

Conclusion

With only a small amount of area allowed to be sprayed with test chemicals by the U.S. EPA, it can be difficult to quantify the effectiveness of a repellent on a large scale, such as the average sunflower field of the PPR of North America (Werner *et al.*, 2006). With the added frustration of having to destroy any crop treated with a new compound, growers must be provided with compensation from an external source in order to make the test even worth their time. Eventually testing repellents can become a very costly operation in both money and effort (Avery *et al.*, 1996). These factors limit the ability to hold a trial that effectively tests the repellent. Future testing of repellents should focus on a variety of factors such as application strategies for different crops, using large-scale fields, targeting the application rate for the species of concern, residue analysis at after the each spray and just before harvest, detailed bird damage measurements, and crop yield comparisons between treated and untreated areas (Werner *et al.*, 2011).

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CHAPTER 2. THE EFFECTIVENESS OF AVIAN REPELLENT 9,10 ANTHRAQUINONE WHEN APPLIED TO SUNFLOWERS AT THE R6 STAGE OF DEVELOPMENT

To be submitted to *Crop Protection* as: Megan D. Niner[a], George M. Linz[b], Mark E. Clark[a], and Jeffery Homan[b]. The effectiveness of avian repellent 9,10 anthraquinone when applied to sunflowers at the R6 stage of development.

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Abstract

Blackbird populations in the Prairie Pothole Region (PPR) of the United States and Canada are increasing in size and no effective method to protect sunflowers from blackbird damage has created a demand for chemical repellents. We evaluated the effectiveness of Avipel®, a new blackbird repellent, in an open field test on oilseed sunflowers. In late August of 2012, we used a highboy ground sprayer to apply Avipel to half of the total area of three sunflower fields. We used daily point counts to quantify bird use of sprayed and unsprayed (i.e., control) plots. We also conducted weekly damage assessments and vegetation measurements to further quantify variation in damage and crop quality among plots. Over the two-month study period, we found no differences in plant growth, bird activity, or damage sustained between treatment and control plots. Non-blackbird species accounted for approximately 80% of the species observed in point counts, indicating that our findings may not be conclusive for blackbirds per se.

Introduction

Since the latter half of the 1900s, blackbird depredation has become an increasingly common problem for farmers in the PPR of the United States and Canada (Blackwell *et al.*, 2003). The problem is expected to persist with the population of red-winged blackbirds (*Agelaius phoeniceus*) on the rise across the region (Werner *et al.,* 2010). Blackbird damages to sunflower crops cost growers across the United States an estimated US $5.4 million in 2003. With the rising value of sunflower over the past decade and the increase in blackbird population in the PPR, farmers can expect to see even more monetary loss in the future if an effective form of protection is not found (Peer *et al.,* 2003).

Due to the coincidence of the fall migration, fledging of millions of juvenile blackbirds, and the ripening of the sunflower seeds, blackbird damage typically occurs in a narrow time frame (Avery and Cummings, 2003). More than 75% of the total blackbird damage occurs within eighteen days following anthesis (i.e., when the last of the petals fall from the sunflower head) (Cummings *et al.,* 1989). Thus, there is a defined window in which protection of sunflowers is most critical.

Various methods for protecting sunflower crops have been developed, with varying levels of effectiveness. Mechanical decoys can be successful for small areas, but they require frequent repositioning or the birds will become acclimated to the disturbance. Decoy plots provide an alternative, preferable food source, but are not cost-effective and require large areas to be effective. Lethal baiting and cattail destruction can be effective, but both can have negative impacts on non-target species as well (Linz *et al.,* 2011). To date, none of these methods have provided growers with an effective deterrent for birds. New research has shifted its focus to determine the effectiveness of different chemical-based repellents (Avery and Cummings, 2003).

FDA registered repellents for sunflowers, Birdshield®, Flockbuster®, and Avex are not effective at repelling blackbirds (Linz et al., 2011). Growers use these repellents regardless of results as they feel that doing something is better than nothing. In the end, the use of these repellents only adds to their overall monetary loss should bird damage occur.

The chemical compound, 9, 10 anthraquinone (AQ), is the active ingredient in some newly-developed repellents (Arkion® Life Sciences LLC., 551 Mews Drive Suite J., New Castle, Delaware, 19720, USA). AQ has no immediate effects on the birds that consume it. During digestion, birds experience discomfort and occasional regurgitation (Avery *et al.*, 1997; Avery *et al.*, 1998). Secondary repellents become effect after the bird makes the connection between the treated food item and the discomfort caused (Werner and Clark, 2003).

AQ has been licensed for use in Europe and other countries worldwide. However, AQ is not as widely used in the United States. Flight Control, used to keep Canada geese (*Branta canadensis*) from grazing on grass, is the only licensed AQ product on the market (Avery 2003).

Avipel® is a newly formulated, AQ-based repellent that has shown some effectiveness as a repellent for birds in laboratory studies. Avipel® has been shown to be effective in promoting avoidance of treated seeds and rice in Canada geese (*Branta canadensis*), red-winged blackbirds, and ring-necked pheasants (*Phasianus colchicus*). Wild caught blackbirds were shown to reliably discriminate between treated and untreated rice and sunflower seeds after a few days (Werner *et al*., 2009). Additionally, Avipel® has been shown to be effective in protecting treated oilseed sunflowers in early germination from ring-necked pheasants. The same study also found that seeds treated with 12,220 ppm AQ had a repellency rate of more than 80% against common grackles (*Quiscalus quiscula*) (Werner *et al*., 2011).

Avipel has also been evaluated in field-based studies. Werner *et al*. (2009) examined the effectiveness of Avipel® for protecting ripening confectionary sunflower from common grackles by placing 10 adult male birds into field enclosures on either treated or non treated fields. AQ was applied to the plots when over 50% of the flowers were at the end of the flowering stage (R6). The results found significantly less damage in the cages where 18.7L Avipel® per ha had been used (Werner *et al*., 2011).

However, large-scale field studies of AQ-based repellents using application methods commonly used in commercial agriculture have not been conducted. Field studies are a logical extension of laboratory-based studies because they provide assessments under conditions more similar to those encountered in commercial agriculture (Kandel *et al.,* 2009; Linz and Homan, 2012).

In a preliminary study in 2011, AQ was applied aerially to a field of oilseed sunflower. The application rate was 1.53L/ha, but the residue analysis of the bracts and seeds did not show any AQ. The backs of sunflower heads were not analyzed for residue. This preliminary study indicated that aerial application of AQ is insufficient (Linz and Homan, 2012), but ground-based applications have not been evaluated.

The objectives of this study were to quantify: 1) the use of AQ-treated and untreated sunflower plots by blackbirds, 2) the rates of damage to AQ-treated and untreated sunflower plots, and 3) the change in AQ residues on sunflower heads in fields treated with AQ by ground-sprayer over time. We hypothesized that fewer blackbirds would be counted in plots treated with AQ compared to control plots, that rates of damage would be lower in plots treated with AQ compared to control plots, and that AQ residues on sunflower heads would decrease over time.

Study Site

Our study site was located in McLean County, North Dakota, on land currently used for commercial sunflower production. In early 2012, we secured permission to establish experimental plots in three fields from a commercial sunflower grower in McLean County, North

Dakota. Oilseed sunflower was planted in the three fields, each of which had experienced significant blackbird damage in prior years. All three fields are located in Township 146 N, Section 29, Range 81 W. The surrounding landscape is characterized by hilly prairie and expansive agricultural plots (Figure 1). The closest urban areas are more than 45 kilometers from the site. An altered drainage through the site provides an abundance of nearby cattail (Figure 1).

Figure 1. Aerial view of field locations at the McLean County, North Dakota study site. (Bing Maps 2014).

Methods

Field Setup

Experimental plots were established in three fields of sunflower. Fields varied in size (Field One 2.23 ha, Field Two 1.12 ha, Field Three 2.23 ha) and adjacent field characteristics. Each field was separated into 0.56ha (61 x 91.4 m) plots and all plots were then randomly assigned as treatment or control. Treatment plots were sprayed with AQ while control plots were left unsprayed. A 15.2 m buffer was placed between each plot inside the fields to prevent any overlap from the sprayer. Layout of the plots within each field is illustrated in Figure 2.

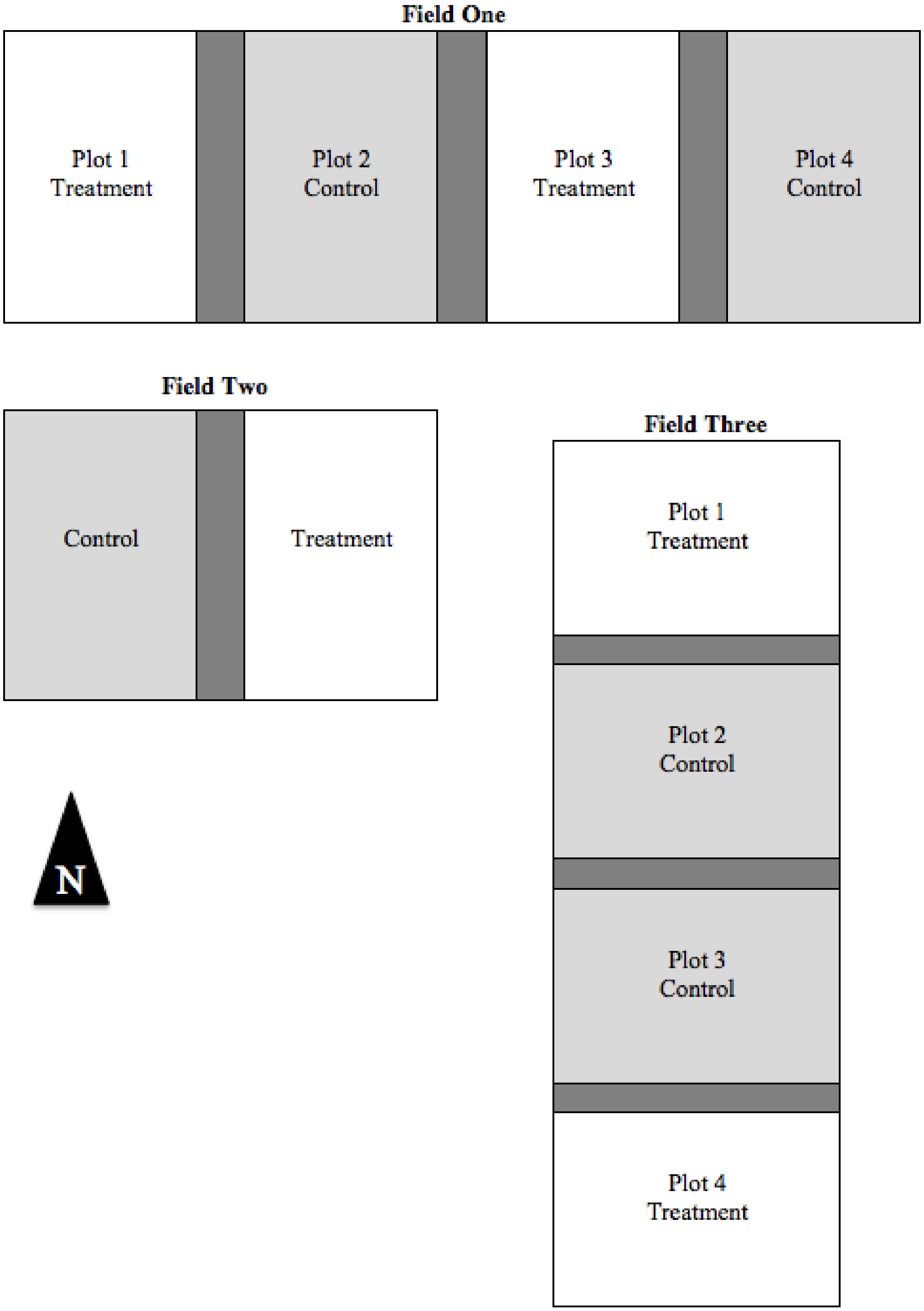

Figure 2. Field design for divisions of treatment and control plots for each field in 2012. Each plot is approximately 61.0 m x 91.4 m. Dark areas represent buffer areas; Field One and Field Two had 15.24 m buffers whereas Field Three had 6.10 m buffers.

Application of Anthraquinone

Application of AQ to treatment plots occurred on two occasions. A ground sprayer outfitted with electronic pulse control and GPS system was used to ensure consistent concentrations throughout the treatment plots. First application was on 17 August, 2012 (when we estimated 50% of the plants were approximately at the R5.8 stage) and the second application was 31 August (when plants were approximately at the R6 stage). Some plants in treatment plots in Field One received damage during spraying because the sprayer boom made contact with the tops of the plants. Furthermore, a control plot in Field Three received an AQ treatment spray on the second application, while a treatment plot in this field failed to receive the second spray application.

Point Counts

We conducted point counts using half-circles (radius of 25 m) from each side of the plots. Point counts began each morning at a half an hour after sunrise and again three hours before sunset. One pair of plots was surveyed each day from all fields via random selection. Two biologists watched each area for five minutes while standing on two-meter stepladders and recorded all birds seen flying into and out of the selected area. A wait time of three minutes was used before each count to allow birds to settle. On days that it rained or winds were in excess of 24 km/hour, we did not conduct point counts to prevent complications from altered bird behavior. Birds flushing from the field upon the arrival of the biologists were recorded separate from the point count data. We recorded counts according to five categories 1) total birds observed in the field, 2) blackbirds observed in the fields, 3) blackbirds flushed from the field at arrival, 4) non-blackbirds observed in the field, and 5) non-blackbirds flushed from the field at arrival.

Vegetation Sampling

Following the first spray, we sampled the vegetation in each plot on a weekly basis. Two, one-m^2 quadrats were randomly selected (and location recorded using hand-held GPS units) from each plot for vegetation sampling. Each week we recorded the number of standing sunflower plants, the height of the tallest plant, and the number of non-sunflower plants in each quadrat. We estimated canopy cover using a spherical crown densiometer placed in the middle of each quadrat. We then averaged the number of sunflower plants, heights of the tallest plants, number and diversity of non-sunflower plants and the canopy cover between the two quadrats to quantify the vegetation characteristics in each plot during each week.

Damage Assessment

Once a week, damage to sunflowers was estimated from ten sunflower heads in each plot was recorded. To make sure the same sunflowers were sampled each week, a white spot was applied to the back of the head away from the bracts. We used the template method (Dolbeer, 1975), in which seeds visible in a semicircular template placed on the heads of selected sunflower plants are counted, to estimate the amount of damage to sunflower heads in each plot. We randomly selected ten plants in each plot, recorded head diameter and seed counts to estimate total damage in the plot.

Residue Analysis

We collected vegetation samples following each application of AQ and at the end of the study to determine the change in AQ concentrations on the plants over the course of the study. We collected five sunflower heads from each plot the day after the first AQ application, the day after the second AQ application and on 9 October (the end of the study). Sunflower heads were placed in plastic bags and frozen (-2ºC) within two hours of collection. Frozen heads were sent to the USDA APHIS Lab at Fort Collins, CO for analysis of AQ via liquid chromatography. We also collected 100 ml samples (frozen at -2ºC in amber jars within two hours of collection) from each sprayer tank after each application, which were also sent to the lab for analysis to determine actual AQ concentrations applied in the study.

Statistical Methods

We used Analysis of Covariance (ANCOVA) to examine the effects of AQ treatment on bird use of sunflower plots. In the ANCOVA, we modeled count (by category) as a function of plot, time, and the interaction between plot and time (in weeks). If time did not have a significant effect on count in the ANCOVA, we excluded the term and reanalyzed the count using a simple Analysis of Variance (ANOVA) of count as a function of plot to increase our sample size. Because plot was technically nested in field, we ran ANOVAs to examine potential field effects if no variations were seen between treatment and control.

ANCOVAs were used to examine any temporal effects of crop density, canopy cover, non-crop density, and maximum sunflower height. In this ANCOVA, each crop characteristic was modeled individually as a function of plots, time (in weeks), and the interaction between plot and time. After determining if a trend was present or not, individual ANOVAs were run to examine differences in crop characteristics between the three fields. Because plot was technically nested in field, we ran ANOVAs to examine potential field effects if no variations were seen between treatment and control.

We also used ANCOVA to determine if damage estimates were affected by treatment. Again we modeled damage estimate as a function of plot, time (in weeks) and the interaction between plot and time. We also repeated the analysis for damage as a function of plot when time did not have a significant effect in the ANCOVA. Because plot was technically nested in field, we ran ANOVAs to examine potential field effects if no variations were seen between treatment and control. Simple linear regressions were used to examine any relationships between blackbird counts and flushes based on vegetation characteristics.

For the residue analysis, we used linear regression to determine if the concentration of AQ changed significantly with time on bracts and achenes, separately.

Results

Point Counts

We observed a total of 100 birds (20 of which were blackbird species) in treatment plots and 112 birds (44 of which were blackbird species) in control plots over the course of the study. Non-blackbird species using the fields included American goldfinch (*Carduelis tristis*), sparrows (family Emberizidae), American robin (*Turdus migratorius*), and mourning dove (*Zenaida macroura*). These numbers do not include birds outside of 25 m radius or the birds that flushed from the field upon arrival.

The number of birds observed using fields did not differ between treatment and control plots. In the ANCOVA of total birds counted per treatment, less than 16% of the variation in the counts were explained by treatment, time and the interaction between treatment and time ($F_{6,35} = 1.109$, $p = 0.498$, $r^2 = 0.159$). We measured time as weeks from the start of the study and considered it to be a continuous variable. Similarly, less than 16% of the variation in the number of blackbirds counted was explained by treatment, time and the interaction between treatment and time ($F_{6,35} = 0.915$, $P = 0.498$, $r^2 = 0.159$), and less than 15% of the variation in non-blackbird species counted was explained by treatment, time and the interaction between treatment and time ($F_{6,35} = 0.798$, $P = 0.579$, $r^2 = 0.1417$).

When we repeated the analysis without time (i.e., as an ANOVA), we still did not detect significant differences in bird counts between the control and treatment plots. Less than 1% of the variation in total birds counted was explained by treatment ($F_{1,34} = 0.022$, $P = 0.884$, $r^2 = 0.001$), less than 2% of the variation in blackbirds counted was explained by treatment ($F_{1,34} = 0.437$, $P = 0.513$, $r^2 = 0.013$), and less than 8% of the variation in non-blackbirds counted was explained by treatment ($F_{1,34} = 2.615$, $P = 0.115$, $r^2 = 0.071$).

We observed 5,276 blackbirds flushing from treatment plots compared to 6,068 blackbirds from the control plots over the course of the study. All birds were observed flushing from treatment and control plots in Field Two. No blackbirds were observed flushing from either treatment or control plots in Fields One and Three at the start of any counts. Non-blackbirds flushing from the field numbered 54 from the treatment plots and 82 from the control plots.

Similar to the point counts, flush counts were not related to treatment. Approximately 23% of the variation in the total number of birds flushed per treatment and control plots was explained by treatment, time and the interaction between treatment and time ($F_{6,35} = 1.428$, $P = 0.238$, $r^2 = 0.228$). Less than 16% of the variation in the number of blackbirds flushed from treatment ($F_{6,35} = 0.915$, $P = 0.498$, $r^2 = 0.159$) and less than 15% of the variation in non-blackbird species flushed from treatment ($F_{6,35} = 0.798$, $P = 0.579$, $r^2 = 0.142$) was explained by treatment, time and the interaction of treatment and time.

Again, when we excluded time from the model, variation in the number of birds flushed did not differ between treatment and control plots. Less than 1% of the variation in all birds flushed ($F_{1,34} = 0.016$, $P = 0.899$, $r^2 = <0.001$), less than 1% of the variation in the number of blackbirds flushed ($F_{1,34} = 0.0151$, $P = 0.9031$, $r^2 = <0.001$) and less than 3% of the variation in non-blackbirds flushed ($F_{1,34} = 0.920$, $P = 0.344$, $r^2 = 0.026$) was explained by treatment.

Vegetation Sampling

Weekly vegetation characteristics (Table 1) displayed a relationship with week and field. Our ANCOVA explained 76% of the variation for crop density, 91% for percent canopy cover, 86% for non-crop density, and 86% for max height (Table 2). Significant trends were observed for all categories of vegetation sampling between fields (Figure 3), but no significant trends were observed for treatment in any vegetation category.

Despite these differences, no significance was found in post-hoc analysis of blackbirds seen in the field when run against individual vegetation characteristics. Linear regression found no influence of vegetation characteristics on blackbird flushes from crop density ($F_{1,34} = 3.298$, $P = 0.078$, $r^2 = 0.088$), percent canopy cover ($F_{1,34} = 0.134$, $P = 0.717$, $r^2 = 0.004$), non-crop density ($F_{1,34} = 2.032$, $P = 0.163$, $r^2 = 0.056$), and max sunflower height ($F_{1,34} = 0.734$, $P = 0.398$, $r^2 = 0.021$). Linear regression found no influence of vegetation characteristics on blackbirds counted in the fields from crop density ($F_{1,34} = 2.376$, $P = 0.133$, $r^2 = 0.065$), percent canopy cover ($F_{1,34} = 1.108$, $P = 0.300$, $r^2 = 0.032$), non-crop density ($F_{1,34} = 0.342$, $P = 0.563$, $r^2 = 0.001$), and max sunflower height ($F_{1,34} = 0.031$, $P = 0.861$, $r^2 = 0.001$).

Table 1. Weekly averages of plant characteristics per field treatment. T represents treated and C represents control plots. Max height given in cm.

Date	Plot	Crop Density	% Canopy Cover	Non Crop Density	Max Height
23-Aug	1T	5.5	74	0.5	74.55
	1C	7	89.08	1	81.6
	2T	12	77.12	0	56.7
	2C	10	72.96	0	60
	3T	8.5	45.14	5.5	54.6
	3C	7	50.09	3.25	55.8
30-Aug	1T	4.8	70.1	0.25	72.88
	1C	6	78.94	1	80
	2T	7	49.04	0	54.75
	2C	9	67.24	0.5	61.75
	3T	5.5	37.08	3.75	50.63
	3C	4.5	44.36	4.75	52.13
7-Sep	1T	4.75	67.5	0.25	75.13
	1C	6	76.08	0.5	81.5
	2T	7	49.04	0	51.25
	2C	9	49.04	0	57.75
	3T	6	27.72	6	50.88
	3C	5.75	37.34	2.25	51.75
14-Sep	1T	4.5	52.94	0.25	71.63
	1C	6	71.14	0.25	80.5
	2T	7	26.68	0	53.75
	2C	8.5	28.24	0	58
	3T	5.75	24.6	3.25	50.75
	3C	5.75	35.75	2.25	50.88
20-Sep	1T	4.25	35.78	0.25	71.31
	1C	6	42.8	0	80.5
	2T	7	12.12	0	54.38
	2C	8.5	24.08	0	58.5
	3T	5.75	15.24	2.5	49.63
	3C	5.75	17.32	1.75	51.44
27-Sep	1T	4.25	19.14	0	68.75
	1C	6	30.32	0	76.63
	2T	6.5	15.24	0	53.75
	2C	8.5	17.84	0	56.75
	3T	5.75	13.42	2.5	49.19
	3C	5.75	17.06	1.5	50.88

Table 2. Test statistics from vegetation characteristic ANCOVAs.

	Full Model	**Field**	**Time (Week)**	**Treatment[Field]**
Crop Density	$F_{6,35}$ = 15.459 P <0.0001 r^2 = 0.762	$F_{6,2}$ = 33.634 P <0.0001 r^2 = 0.552	$F_{6,1}$ = 12.129 P = 0.002 r^2 =0.100	$F_{6,3}$ = 11.389 P = 0.011 r^2 = 0.110
Percent Canopy Cover	$F_{6,35}$ = 49.633 P <0.0001 r^2 = 0.911	$F_{6,2}$ = 45.180 P <0.0001 r^2 = 0.277	$F_{6,1}$ = 196.702 P <0.0001 r^2 = 0.602	$F_{6,3}$ =3.5776 P = 0.0258 r^2 = 0.0.034
Non-Crop Density	$F_{6,35}$ = 31.081 P = <0.0001 r^2 = 0.865	$F_{6,2}$ = 81.031 P <0.0001 r^2 = 0.062	$F_{6,1}$ = 13.443 P = 0.001 r^2 = 0.051	$F_{6,3}$ = 3.6589 P = 0.0237 r^2 = 0.752
Max Height	$F_{6,34}$ = 413.775 P <0.0001 r^2 = 0.865	$F_{6,2}$ = 1154.119 P <0.0001 r^2 = 0.013	$F_{6,1}$ = 33.584 P < 0.0001 r^2 = 0.056	$F_{6,3}$ = 46.943 P <0.0001 r^2 = 0.919

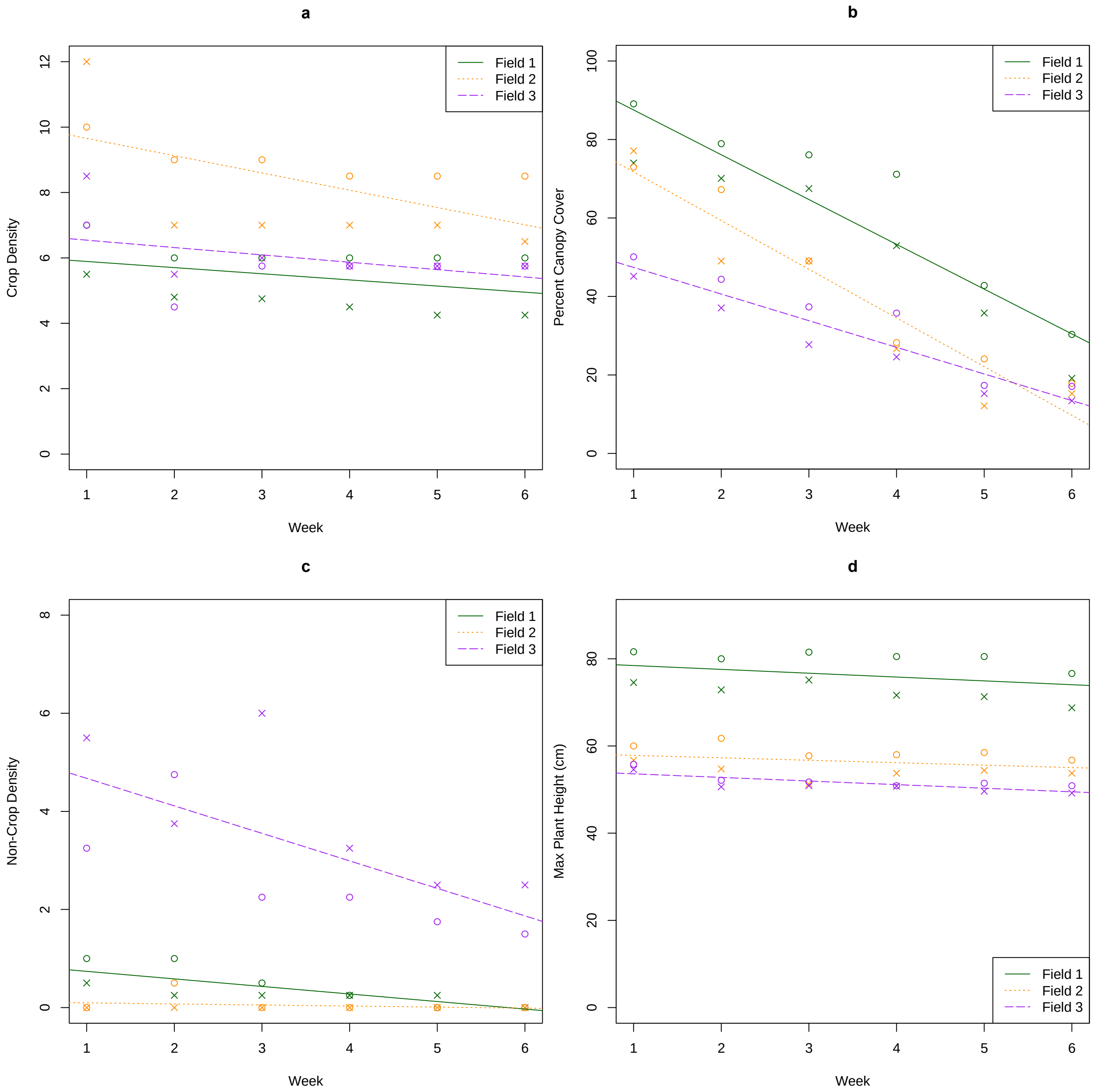

Figure 3. Time and field effects on vegetation sampling. Control plots are represented by circles and treatment plots are represented by 'x's. Field effects were seen in each of the characteristics sampled between the fields indicating a disparity in crop quality. All ANCOVAs revealed significant field effects as well as time effects (Overall models: **a**: $F_{5,35} = 12.499$, $P < 0.0001$, $r^2 = 0.676$; **b**: $F_{5,35} = 63.035$, $P < 0.0001$, $r^2 = 0.913$; **c**: $F_{5,35} = 39.843$, $P < 0.0001$, $r^2 = 0.869$; **d**: $F_{5,35} = 83.344$, $P < 0.0001$, $r^2 = 0.933$).

Damage Estimates

From our weekly collection of damage estimates, we saw that damage to sunflower plants increased over time, but did not differ between treatment and control plots. Approximately 8% of the variation in damage was explained by treatment, time and the interaction between treatment and time ($F_{3,56} = 1.721$, $P = 0.173$, $r^2 = 0.084$). However, effects of treatment ($F_{3,1} = 0.055$, $P = 0.816$, $r^2 = 0.001$) and the treatment and time interaction ($F_{3,1} < 0.001$, $P = 0.9838$, $r^2 < 0.001$) were not significant, whereas the effect of time was significant ($F_{3,1} = 5.1069$, $P = 0.028$, $r^2 = 0.001$), with damage increasing over time (Figure 4).We suspected a field effect could be present, so we ran another ANCOVA using field instead of treatment and found 94% of the data explained by this model ($F_{5,54} = 156.948$, $P < 0.0001$, $r^2 = 0.936$). Effects of field ($F_{5,2} = 260.511$, $P < 0.0001$, $r^2 = 0.621$), time ($F_{5,1} = 152.2105$, $P < 0.0001$, $r^2 = 0.181$), and field and time interaction ($F_{5,2} = 96.842$, $P < 0.0001$, $r^2 = 0.231$) were all statistically significant (Figure 5).

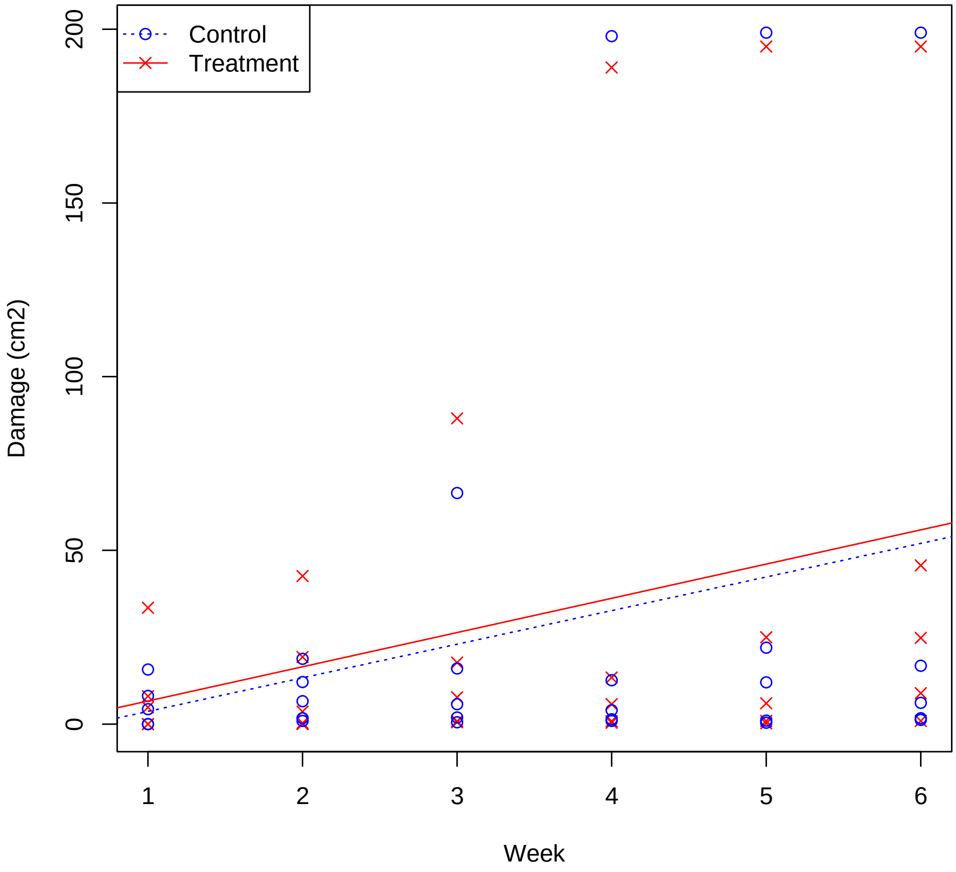

Figure 4. Damage to sunflowers over time. Circles represent control plots and ‘x’s represent treatment plots. This graph represents the damage accumulated in each treatment over the six weeks of the study. An ANCOVA revealed a significant time effect (Overall: $F_{6,56} = 1.721$, $P = 0.173$, $r^2 = 0.084$).

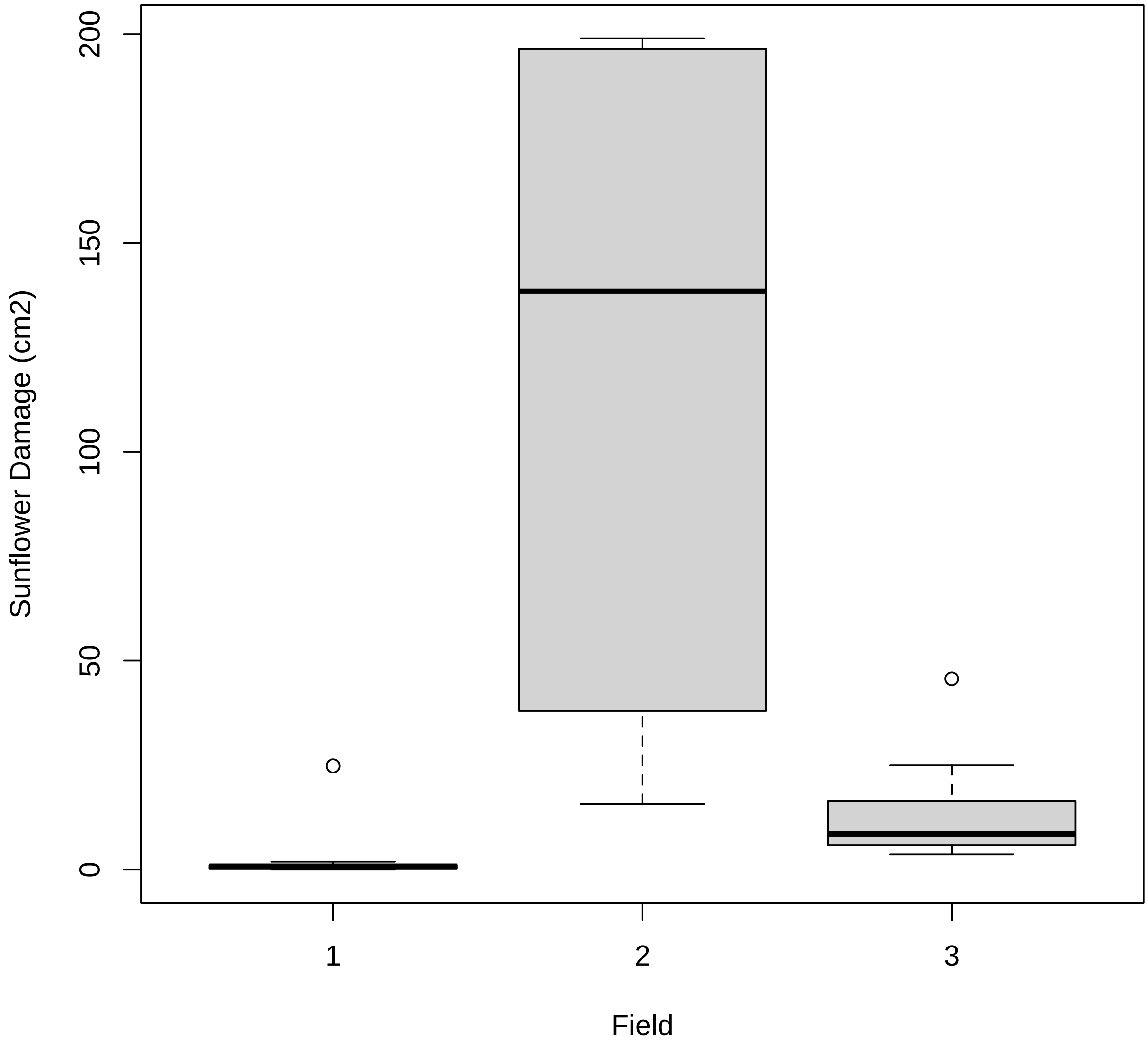

Figure 5. Box and whisker plot displaying sunflower damage by field. The ends of the whiskers indicate the 95% and 25% percentiles, the mean is represented by the bold bar, and open circles represent outliers. A clear field effect was found in the ANCOVA when field replaced the treatment term ($F_{5,54} = 156.948$, $P < 0.0001$, $r^2 = 0.936$).

Residue Analysis

Concentrations of AQ on sunflower bracts increase over time, but did not change over time for achenes. The linear regression of AQ concentration on bracts indicated over 66% of the variation in concentration was explained by time ($F_{1,5} = 24.269$, $P = 0.002$, $R^2 = 0.669$, slope = 4.436) (Figure 6). However AQ concentrations on the achenes of the treated plots did not vary significantly over time ($F_{1,5} = 0.277$, $P = 0.621$, $R^2 = 0.053$, slope = -0.042).

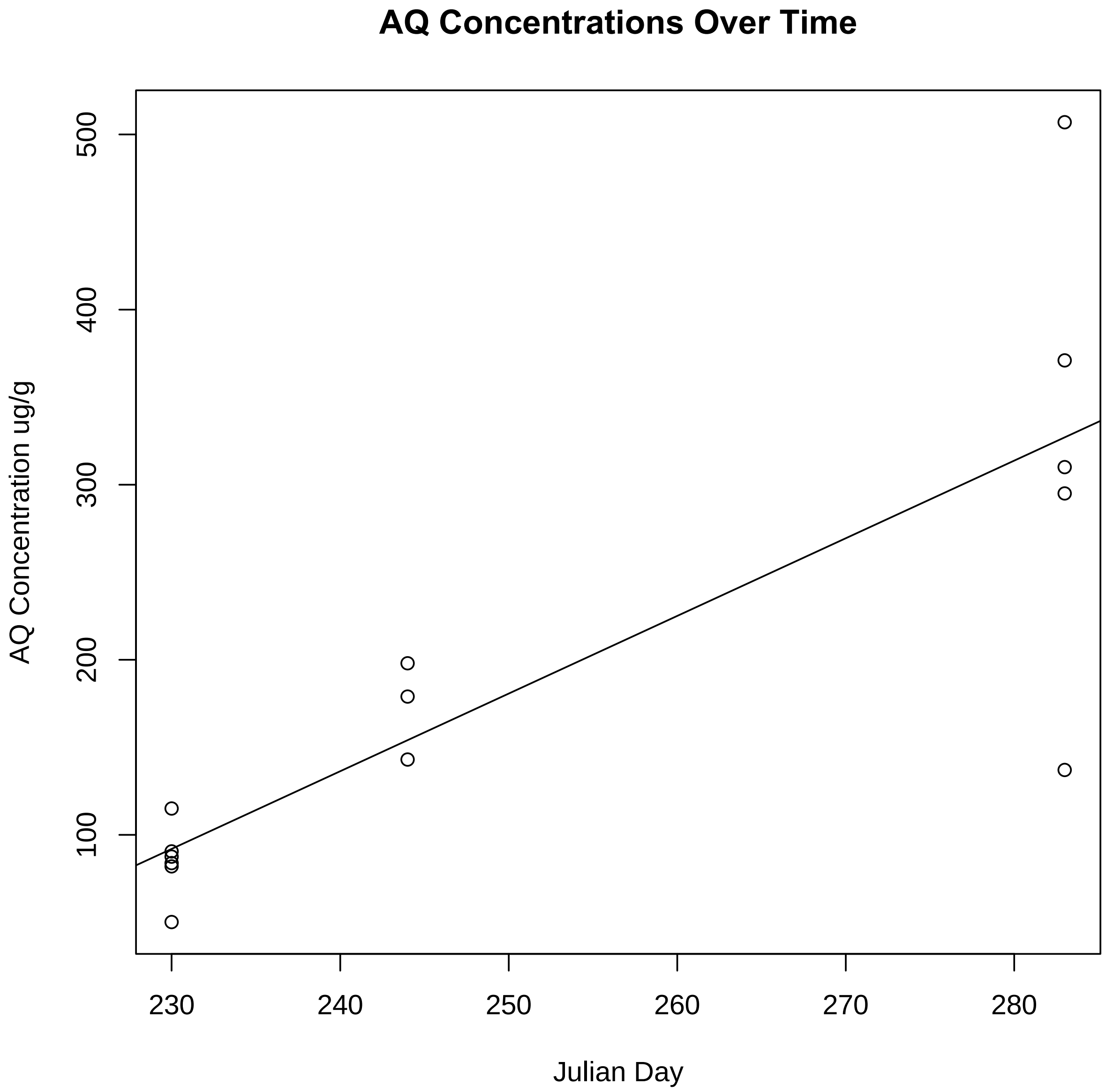

Figure 6. AQ concentrations on bracts after each spray event and harvest. A baseline measurement of 2.8 ug/g was obtained from untreated samples. Drying out of the plant caused heads to shrink towards the end of the study and it may have been possible that the increase is the result of a larger percentage of the plant being sampled than compared to prior samples. (Linear Regression: $F_{1,5} = 24.269$, $P = 0.002$, $r^2 = 0.669$, slope = 4.436)

Discussion

Our results suggest that Avipel, when applied foliarly at the R6 (petal drop) stage of sunflower growth, is not an effective repellent of blackbirds. Data collected indicates that blackbirds used treated and non-treated plots equally and the rates of damage to sunflower by birds also do not differ. This occurred even as AQ concentrations on the treated sunflowers increased over time. Point counts and flush counts both revealed no statistical differences between treatment and control plots. Damage estimates displayed an increase in loss of sunflower seed over time (presumably to birds) (Figure 4), but the rate of loss was not different between AQ-treated and untreated plots. Instead, significant differences in damage were observed among fields, suggesting factors other than AQ treatment had more of an impact on bird selection of foraging sites in sunflower fields.

These findings are the first of their kind, as no large-scale open field test had been conducted. However, the lack of repellency shown by our data contrasts with findings of other studies of the repellent Avipel. Laboratory trials have demonstrated repellency of Canada geese, ring-necked pheasants, and red-winged blackbirds with various AQ-treated food items including sunflower (Werner *et al.* 2009). In a cage enclosure study with common grackles, Werner *et al*. (2011) found that treated enclosures sustained only 18% damage to sunflowers whereas untreated sunflowers took 64% damage. However, Werner used a backpack sprayer and applied Avipel directly to the heads of each sunflower in the treated cages. This amount of coverage is something large-scale farming equipment cannot replicate.

We frequently observed feeding birds flushing upon the approach of the observers. These birds subsequently landed in the back of the fields beyond the 25-m count radius and thus were excluded by our sampling technique. Therefore, bird behavior associated with avoiding humans may exceed other behaviors associated with foraging (e.g. avoidance of AQ-treated plants). The may have caused limitations in detecting differences in feeding behaviors of blackbirds.

Additional human disturbance could have added to the behavior of foraging birds based on where the fields were located. Significant differences in field level use by blackbirds were detected in this study. Field use may be the response to larger landscape-level effects (e.g., proximity to roost sites, better food sources, human disturbances, etc.). However, other studies found that the use of sunflower fields is more related to the crop quality than outside field land use (Hagy *et al*. 2010). Our data from the vegetation characteristics (Figure 3) seems to be in agreement with this ideal because significant field effects were found in crop density, percent canopy cover, non-crop density, and maximum crop height. However, we did not find any significant relationships between bird presence and any of the characteristics we sampled. Bird presence may have been in response to other environmental factors we did not sample for.

Avipel was not applied directly to the seeds of the sunflower due to challenges in application to this part of the plant. As sunflowers mature the head becomes too heavy for the stalk to support, and it faces the ground by the time the petals are falling off. The current design for commercial sprayers is only able to apply the repellent to the back of the heads when this happens. Residue analysis indicates that the achenes (seeds) did not acquire as much of the spray as compared to bracts (heads) following each spray. This was expected as the achenes were in early development when the plants were sprayed. The bracts were also more readily exposed to the sprayer because of the orientation of the maturing sunflower heads. Consistent with this, there was no change in the AQ concentration on the achenes found at the beginning and end of the study. However, the AQ concentrations on the bracts increased from initial spray to the second spray and to harvest. We hypothesize that increasing concentrations over time reflect loss of water in the plant tissues as the sunflower plants mature and the heads dry out (Robinson 1983). Nevertheless, our data show that detectable AQ concentrations remain (indeed increase significantly) for an extended period of time, and that birds foraging in treated plots over time were continually exposed to the repellent, yet did not avoid those areas.

The 2012 season revealed several logistical problems associated with the field experiments. Drought, differential crop growth rates, and unexpected bird behaviors were the largest of the problems we experienced. For the two months of August and September (our study ran from 13 August to 9 October, 2012), Turtle Lake, ND reported 4.293cm of total rainfall while the historical normal amount is 8.331cm (North Dakota Agricultural Weather Network 2014). While this lack of rain may have affected the overall crop quality, our goal was to observe if the AQ-treated sunflowers would take less damage, which our data suggests did not happen.

However, we have used our findings as well as knowledge of potential problems to assist in the study design for the future. We recommend protecting fields from blackbirds during the critical first three weeks after the petals drop when the most damage normally occurs by the local populations to ensure high seed densities are present during the study. We also will apply AQ concurrent with insecticide applications at the R5.1 stage when pollen is released. In order to avoid landscape-level factors that might affect bird field preference, we will use enclosures to observe the rate and total damage of by blackbirds in treated and untreated plots.

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Center, and North Dakota State University. Arkion Life Sciences LLC provided the 9,10 anthraquinone (Avipel®) used in this study. This study was conducted under WS-NWRC study protocols QA-2038 and 2121 (George M. Linz, Study Director) and in compliance with NDSU IACUC protocol #A13006.

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CHAPTER 3. EFFECTS OF APPLYING 9,10 ANTHRAQUINONE TO PRE-SEED SET SUNFLOWERS ON HONEYBEES AND BLACKBIRDS

To be submitted to *Crop Protection* as: Megan D. Niner[a], George M. Linz[b], H. Jeffery Homan[b], & Mark E. Clark[a]. Effects of applying 9,10 anthraquinone to pre-seed set sunflowers on bees and blackbirds.

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Abstract

Blackbird (Icteridae) damage to sunflowers has been gaining attention as a major problem for sunflower growers in the Prairie Pothole Region of North America. With no effective method to protect sunflower crops from flocks of birds numbering in the thousands, there is a need for an effective bird repellent. AV2022 is a new repellent containing 25% 9, 10 anthraquinone (AQ) as the active ingredient and was tested in field enclosures on oilseed sunflowers. Our objectives were to determine if the AV2022 would be an effective repellent when sprayed at the same time as typical insecticide applications, examine any avoidance by honeybees and to quantify its effectiveness at preventing blackbird foraging on sunflower. AQ was sprayed over half of a 0.819 ha field when the sunflowers were at growth stage R5.1-R5.3 (early bloom and when most insecticides are typically applied), and an identically sized plot of sunflower next to the sprayed plot was left untreated as a control. We erected cages after the plants reached the R6 (petal drop) stage, and three to four blackbirds were maintained inside each cage (and provided access to cracked corn and fresh water) for three weeks. Results showed that damage to sunflowers in the treated cages was significantly higher than damage in control cages ($F_{1,10} = 20.508$, $P < 0.001$, $r^2 = 0.672$). We conclude that AV2022 is not an effective repellent when applied to sunflowers at the R5.1 stage.

Introduction

Sunflower has been an important crop for the continent of North America since its domestication around 4,000 BP (Lentz *et al.*, 2001). Noting its useful properties, early European explorers took the crop to their home countries and spread it across the continent and into Asia. Commercial desire for the crop would not develop until the 18th century in Russia, where farmers began to produce oil on a large scale (National Sunflower Association, 2012). Today, the United States alone plants around 800,000 ha of sunflower seeds for commercial production per year (National Sunflower Association, 2012).

In the Prairie Pothole Region (PPR) of North America, large fields of sunflower are grown annually. Three native birds species, red-winged blackbirds (*Agelaius phoeniceus*), common grackles (*Quiscalus quiscula*), and yellow-headed blackbirds (*Xanthocephalus xanthocephalus*) are the most common species that attribute damage to sunflower fields. With plentiful food in a concentrated area, migrating birds descend on the fields in desperate need of refueling for their flight of several hundred miles (Avery, 2003). Flocks of single or mixed species can easily number in the thousands during migration season. Rife with shallow standing water and dense cattail stands, the PPR is also prime habitat for these species (Kleingartner, 2003). Estimates of monetary damages totaled around US$5.4 million annually at the turn of the century (Peer *et al*., 2003). In the decade since these estimates, the prices of sunflower crops have increased by at least threefold (Klosterman *et al.*, 2011).

Conflicts between birds and sunflower growers began to gain attention at the start of the 1960s when commercial production of the crop greatly expanded across much of the PPR (Blackwell *et al.*, 2003). Each year, millions of fledging blackbirds begin to prepare for their first migration as they join the adult population. This massive influx of birds into the foraging population matches in time with the ripening of many sunflower fields across the PPR. The coincidence of these two events makes for large local losses to growers in the country (Avery and Cummings, 2003). More than 75% of the total blackbird damage occurs within eighteen days post anthesis, or following the drop of the petals from the sunflower head (Cummings *et al.*, 1989). It is during this time that sunflower protection is most needed.

Many methods exist for protecting crops from bird damage, but none are effective. Propane cannons, pop-up scarecrows, and other mechanical devices are known to have some success, but they are only meant for small areas. Birds can become acclimated to these disturbances once they learn that no negative effects will follow. Planting more appealing crops as decoys can work to a limited degree, but this method is often costly in time, space, and money (Linz *et al*., 2011). Thinning cattail stands near sunflower fields using herbicide is another effective control method. While this method remains effective for many years, it has the side effect of harming non-target species (Linz and Homan, 2011).

Mathematical modeling of lethal methods indicates these methods are economically ineffective as a means of preventing avian damage to crops. Public opinion also prevents the employment of such lethal control methods (Blackwell *et al*., 2003). While there is measureable public sympathy for farmers experiencing crop damage, there is also measurable public concern for the protection of wildlife (Conover and McCoy, 2002). Baiting poses numerous problems, including the mortality of non-target species because blackbirds are not the only birds that consume sunflower seeds (Custer *et al.*, 2003). To avoid non-target mortalities and other concerns, repellents are currently seen by resource managers as the best option to protect agricultural fields from depredation (Clapperton *et al*., 2012).

Chemical repellents are considered effective if they reduce the damage to the crop to a defined level based on the costs and time for the plant to grow. Instead of preventing damage completely, the aim of a repellent is to distribute damage evenly over a larger area. Ideally, the chemical repellent should cause the cost in time and energy of eating the seed to exceed the energy gain from consumption. Costs associated with consuming treated food items vary from bad taste to severe gastrointestinal discomfort. Birds should then look elsewhere for a more profitable energy source (Avery and Cummings, 2003). In the United States, Birdshield®, Flockbuster®, and AVEX™ are the only repellents that have passed the FDA's approval for use on sunflowers, but none have proven to be effective after multiple studies have been conducted by agents with no financial gains at stake (Linz *et al.,* 2011).

9, 10 anthraquinone (AQ) has been known to be a repellent of blackbirds since the 1940s and is currently used in a few bird repellents worldwide (Avery, 2003). This naturally occurring compound can be found in various plants and invertebrates as a chemical defense against predation (Werner *et al.,* 2011). Flight Control™ is the only AQ containing repellent registered in the United State and is used for controlling Canada geese (*Branta canadensis*) browsing on grass (Avery, 2003). The patent holder of the compound, Arkion® Life Sciences, is in the application process to obtain FDA registration to use various formulations of AQ for use on crops such as corn, rice and sunflower.

Anthraquinone does not immediately cause adverse reactions in the birds that consume it. During digestion, birds have displayed mild discomfort to vomiting (Avery *et al.,* 1997; Avery *et al.,* 1998). Prior studies have shown that repellents that cause gastrointestinal illness over other forms of discomfort are more effective in reducing bird depredation over the long-term than primary repellents that cause immediate reactions (Werner and Clark, 2003).

Research in recent years has looked at the effectiveness of Avipel®, an AQ containing repellent. In the laboratory, Avipel displayed promising results (Werner *et al.*, 2011). Canada geese, red-winged blackbirds, and ring-necked pheasants were all shown to avoid Avipel treated food items in preference tests under laboratory settings (Werner *et al*., 2009). Common grackles and red-winged blackbirds were shown to have an avoidance of Avipel treated seeds of more than 80% when the concentration of AQ was at 12,220ppm (Werner *et al*., 2011, Werner *et al*., 2014).

Significant repellency was obtained in one field enclosure study conducted by Werner *et al.*, (2011). The group of researchers examined the effectiveness of Avipel® in protecting ripening confectionary sunflower from common grackles when 18.7 L per ha of AQ was applied to the plots after 50% of the flowers were at the R6 (petal drop) stage (Werner *et al*., 2011).

Despite promising results in the laboratory, large-scale field studies have not been conducted as frequently, nor have they provided any overwhelming evidence that Avipel is effective. Uncontrollable variables in the field can create scenarios that cannot be replicated in the laboratory. Taking into account the problems of prior field studies, we tested a concentration of 50% AQ on three fields of sunflower in 2012 using a highboy commercial ground sprayer. A first spray of 4.68L/ha was followed a week later with a second spray with a higher concentration of 14.03L/ha was applied since not all of the fields had been at the R6 (petal drop) stage when the first spray was applied. Due to a drought reducing crop quality and birds only visiting one of the three fields, our results were highly inconclusive.

For the growing season of 2013 we used the repellent AV2022 (i.a., 25% AQ) concentration from Avipel® (i.a., 50%) and focused our efforts to protecting fields from blackbird damage during the first three weeks after petal drop when the most damage normally occurs. Spraying earlier allowed us to examine the potential for AQ to be mixed in with insecticides, around the R5.1-5.3 (pre-seed set) stage when pollen is beginning to be released and insecticides are typically applied. Cage studies were used to better quantify consumption of the treated and non-treated sunflower seeds, as bird movement was a major challenge in the 2012 study.

At present, no one has tested the effectiveness of applying Avipel to the back of sunflower heads prior to the R6 (petal drop) stage. Should AV2022 prove effective for pre-seed set spraying and the residues are below the detectable levels at harvest, a food tolerance would not need to be established prior to registration. Our study area was limited to 4.05 ha to meet EPA regulations for testing unregistered pesticides. At the end of the study, all treated plants were destroyed.

Inspired in part by the growing concern of Colony Collapse Disorder in western honeybees (*Apis mellifera*), we also estimated the change in abundance of honeybees over time in the treated and control plots. Colony Collapse Disorder is an unexplained phenomenon that has been devastating western honeybee populations kept for use in pollinating large-scale agricultural fields in the winter of 2006/2007. Afflicted colonies experience a loss of the majority of the adult forager bees over the winter, and currently there is no known cause (vanEngelsdorp *et al.*, 2009). With this in mind, there is an added concern for any negative effects caused by repellents on bees. We were concerned that by spraying AV2022 during the earlier development stages of the sunflower would potentially repel the honeybees and other important pollinator species. This possible loss of pollination would negatively impact the seed-set and quality of the crop.

AQ is known to be visible in the ultraviolet spectrum, suggesting that spraying could potentially affect bee pollination (Werner *et al.* 2011; Jones and Buchmann, 1974). Prior to the study, it was hypothesized that no difference would be seen between bee abundance in the treated and control plots. Birds are also able to the shorter wavelengths of UV and are known to use this range when selecting a mate. The added characteristic of coloration may help with the birds' observational learning of which areas are treated and could result in a better overall repellency rate.

The major objectives for the 2013 season were to: (1) quantify consumption of sunflower by male blackbirds maintained in enclosures with AQ-treated versus untreated sunflower, (2) analyze the AQ residues on the backs, bracts, and seeds of the sunflower heads from the treated plots and (3) estimate honeybee abundance over time in treated and control plots. Based on the results of a similar study by Werner *et al.,* (2011), we hypothesized that sunflowers treated with AQ would sustain less damage than those left untreated. Furthermore, we hypothesize that concentrations of AQ on treated sunflower plants will decline over time, and that honeybee abundance will not differ between AQ-treated plots and control plots. The results obtained from this study will continue to build on the knowledge of AQ and its effectiveness when used to protect sunflower fields. As no registered repellent has been found to be effective, our study will provide new information regarding the effectiveness of a potential new repellent.

The capture, care, and use of all birds in this study was approved by both the Intuitional Animal Care and Use Committee of North Dakota State University (Protocol #A13006) and by the Animal Care and Use Committee of the United States Department of Agriculture's National Wildlife Research Center.

Study Site

Our study site was located in Barnes County, North Dakota (Township 141N, Section 14, and Range 56W), on land currently used for commercial sunflower production. In the summer of 2013, we secured permission to establish an experimental plot on the corner of one field outside of Oriska, ND. Oilseed sunflower was planted in this field and the grower had experienced significant losses from blackbirds in prior years. The surrounding landscape is characterized by flat agricultural fields, of corn, wheat, and soybeans (Figure 7). The 2013 field site has less topographic variation, fewer trees and larger agricultural fields in the surrounding landscape compared to the 2012 field site.

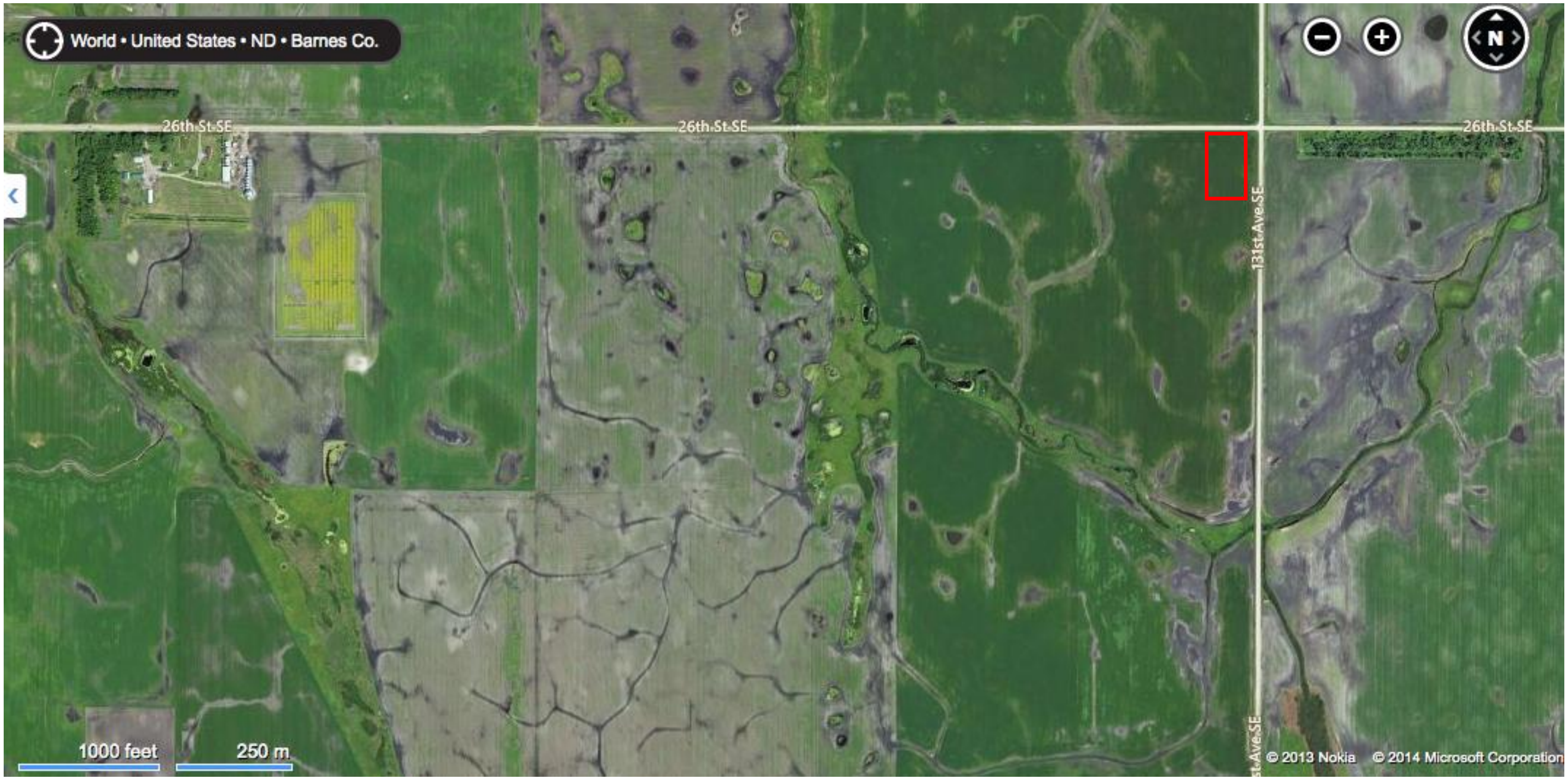

Figure 7. Location of 2013 field in Barnes County, North Dakota. The area is relatively flat and dominated by agricultural fields, mainly of corn, wheat, and soy. Field dimensions 64 m x 128 m (Bing 2014).

Methods

Bird Capture and Care

We used a box trap to capture adult second-year (after hatch) red-winged blackbirds. The birds were captured in late May and early June of 2013 (Bray *et al.*, 1975). Up to twenty birds were captured in a single visit to an area and were transported back in cages plentiful in water and food. Trapping was only carried out in the early morning or late evening when conditions were not hot or raining.

Birds were transported to the holding facility of the USDA-APHIS located in Bismarck, North Dakota. Trapped birds were placed into large enclosures with areas for perching as well as access to overhead shelter. Fresh food and water was provided daily, along with whole sunflower heads as they became available.

Field Setup

In late summer, we established six cages in the AQ-treated and six cages in the control plots to house blackbirds in sunflower field near Oriska, North Dakota used in the study. An area of 128 m x 64 m was staked out on the corner of the field at the intersection of two dirt roads to mark the treatment and control plots. The field was marked at each corner, with a small buffer of approximately 3 meters from the edge. At each corner, a single metal stake was pounded in and a 3.66 m pole with blue flagging was placed on top to clearly mark the boundaries of the field and the respective treatments. We randomly selected half of the field to be sprayed with AQ when plants reached the R5.1-R5.3 stage. We maintained a 3 m buffer between the treatment and control plots to prevent any residual spray from drifting into the control plot. A post for each cage was used to mark sites of approximately 2.44 m x 2.44 m area and each site was separated by 7.62 m. Sites were placed in the middle of the treatments to minimize potential edge effects.

All vegetation was cleared up to 30.5 cm away from the sites where the cages were to be located to allow for the placement of an electric fence around each line of cages (Figure 8). Double strand wire was placed around the perimeter of the cages to deter predators from attacking the caged birds.

Anthraquinone Application

On 19 August, 2013, a spray rig from the local farmer's co-op was hired to apply AV2022 to the treatment plot using an Ag Chem Ragator rig with a 30.48 m boom, an application pressure of 20.43 kg and an 1104 nozzle size. The rig's sprayer spanned a width of 30.5 meters across, covering the width of the entire treatment plot in a single pass. More than 50% of the sunflowers were at the reproductive stage of R5.1 (early flower development) corresponding to the normal timing of pesticide application for the red seed weevil, a common pest of sunflower crop. At the time of the spray, sunflowers stood around 167.6 cm tall. Those in the path of the sprayer's body were knocked backwards, but recovered after a few days. The fields were sprayed again on 22 August as the first application was only at half of the desired concentration of 37.4 L/ha mixed with 56.8 L of water. The sunflowers were still in partial to full-bloom at this time.

Honeybee Counts

Honeybee counts were carried out daily prior to the set up of the caged enclosures. From 23 August to 30 August, 2013, surveys were conducted between the hours of 13 and 14, when the day had warmed up and the flowers were open to obtain data from the height of bee activity. The technique of line-transects, developed for observing birds, was adapted to estimate bee population in the two plots. The treatment we started with was switched every day. A recorder followed a single observer for all observations as they traveled straight line transect of 365.8 m down both the treated and control plots. Eight equidistant stops were made on each transect at which we would stop and count the bees seen on the sunflowers facing forward for one meter on either side of the observer and three rows back. Bees seen to the left and right were recorded as well as an estimate of distance from the line transect. Other insects seen were not recorded. We did not count during any form of precipitation.

Field Enclosure Setup

Six 244 cm x 244 cm field enclosures made of aluminum frames and plastic netting were erected in both the treatment and control plots. Enclosures located within the respective treatments are referred to as treatment enclosures or control enclosures, respectively. Side panel dimensions were 239 cm x 244 cm and the top panels measured 244 cm x 249 cm. Enclosures were placed a distance of approximately 2.44 m apart in a straight line along each half of the field. Random distribution was not practical for the set up of an electric fence to protect the birds from ground-based predators. Cages in each treatment were located approximately 15.24 m from the buffer, roads, and the edges of the field to reduce edge effects.

Enclosures were set up on 2 September, 2013. Sunflowers immediately surrounding the cages were knocked down for easier accessibility. Inside the enclosures, two large metal feed pans were placed randomly. One dish served as a feeding dish for the maintenance diet while the other held a gravity fed 9.5 L water container. An additional plastic mesh covering, measuring 325cm x 365cm, was placed over the top of each enclosure to prevent harassment by aerial predators.

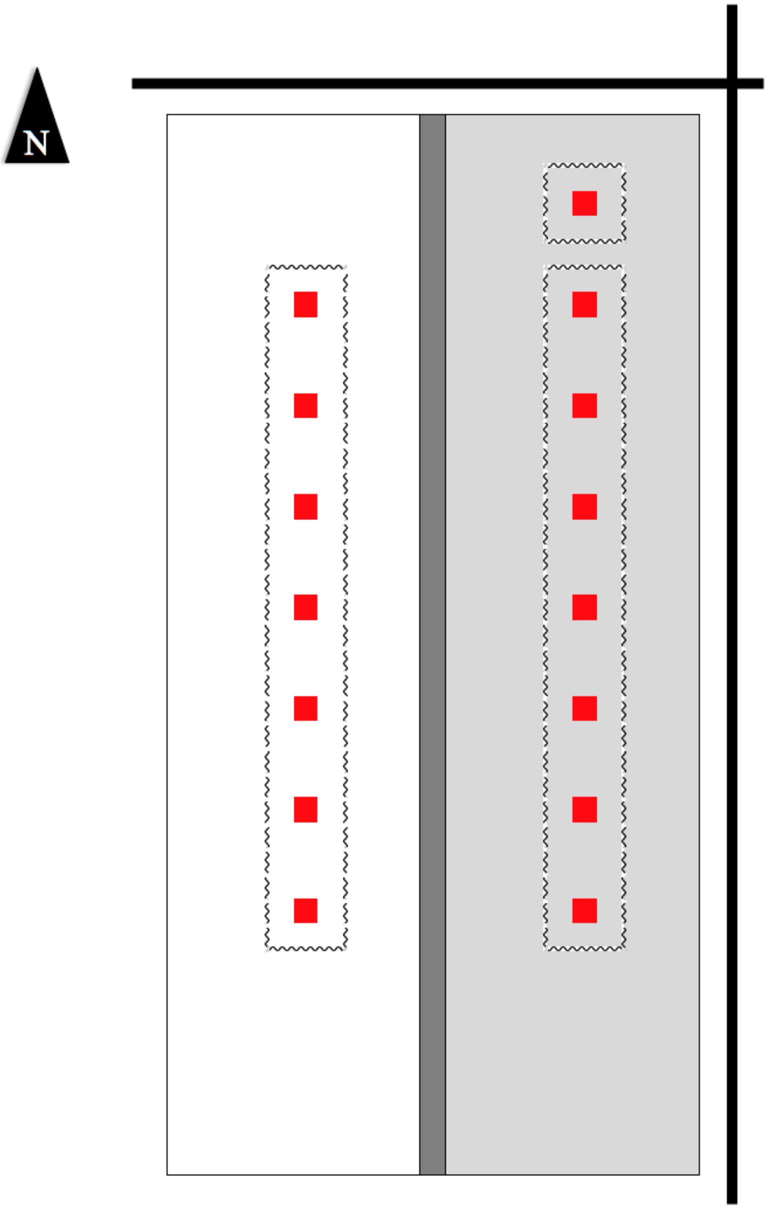

Figure 8. Field setup for 2013 cage enclosures. Field dimensions were 128 m x 64 m. The shaded area represents the control plot and the white area represents the treated area. The dark middle line represents a 3m buffer. Enclosures are represented by red squares. Wavy lines represent the electric fence. Thick black lines to the North and East of the field are dirt roads. The lone cage in the control side of the field represents the holding cage for extra birds.

Blackbird Observations

Prior to the study, the amount of maintenance diet was determined by estimating the daily energy needs of adult male red-winged blackbirds. We estimated individual daily metabolic rate (y, kJ/d) as $y = 10.4x^{0.68}$ where x represents body mass (g) (Peer *et al.*, 2003). Using the average weight of an adult male red-winged blackbird of 70.5 g (Yasukawa and Searcy, 1995), the daily energy needs of each bird was estimated to be 187.85 kJ/day.

We supplied a diet of cracked corn to the birds inside enclosures. The energy content of ground cornmeal is roughly 13.9 kJ/g (Lardy, 2013), meaning that the average RWBL needs around 13.51 g/day. In order to assure that each bird was provided with ample food, an amount of at least 20 g/bird was placed in each dish on a daily basis.

Dry samples were weighed out, recorded, and placed in gallon-sized freezer zip-lock bags for distribution to cages in the treatments each day. Uneaten food was collected every day from each enclosure and place into clean plastic square containers with watertight lids. Upon returning to the lab, samples were air dried overnight and weighed the next morning on an electronic balance (± 0.0001 g). The difference between the initial amount of food and the remainder obtained the next day was used to determine the rate of consumption of maintenance diet. Uneaten food samples were collected daily at approximately 1300 to allow sufficient time for drying prior to collection. We discarded wet samples.

We placed three birds were placed in each enclosure on 9 September 2013, when all sunflowers were at the R6 (petal drop) stage and maintained until 1 October, 2013. On 24 September 2013, a fourth bird was added to each enclosure to increase damage.

All birds in this study were cared for by the guidelines set by the USDA and NDSU IACUC protocols. Approval of these methods was obtained prior to the start of the study.

Sunflower Damage

We waited until 23 October, 2013 before collecting damage estimates, giving the sunflowers time to further dry out and for a frost to stop any further growth of the plants. Field enclosures were left standing with closed doors to prevent any other birds from entering and feeding on the remaining sunflowers. Two teams of observers alternated between treatment and control cages in order to reduce observer bias while obtaining damage estimates. Remaining seeds were counted on all standing sunflowers inside each cage were measured.

Damage estimates were recorded by placing a plastic, half circle cutout with grid openings of 5 cm^2, the amount of missing achenes in each quadrant was estimated, recorded, and summed up for a total approximation of bird damage. The diameter of the head and that of the undeveloped center were also measured to the nearest centimeter to provide a better approximation of the amount of sunflower seeds consumed by the birds.

Residue Analysis

Immediately following the 19 August spray, heads were collected from the field by a biologist wearing protective gear to minimize exposure to the newly applied chemical. Samples were immediately sent to the USDA Wildlife Services National Wildlife Research Center chemical laboratory in Fort Collins, Colorado for residue analysis. Heads were again collected from the field for residue analysis when damage estimates were conducted.

Statistical Methods

An Analysis of Covariance (ANCOVA) was used to examine the effects of AQ treatment of honeybee use of sunflower treatments. In the ANCOVA, bee count was modeled as a function of treatment, time, and the interaction between treatment and time. Plot was treated as a random sample in our analysis.

ANCOVA was also used to examine the relationship between daily consumption per bird between the control and treatment groups. Consumption was modeled as a function of treatment, time, and the interaction between treatment and time. If time did not have a significant effect on count in the ANCOVA, we excluded the term and reanalyzed the count using an Analysis of Variance (ANOVA) of consumption as a function of treatment.

We also used ANCOVA to examine damage per cage. Damage per cage was modeled against consumption per bird, per day, treatment, and the interaction of the two variables. Consumption per bird, per day served as the covariate. Further post hoc tests were used to check for underlying factors. For all the above tests, statistical significance was only accepted if $\alpha \leq 0.05$.

Results

Honeybee Counts

No statistically significant differences in honeybee abundance were observed between treatment and control plots. We saw an average of 94 bees per day in the treatment plot, while an average of 91 bees per day was seen in the control plot. Daily temperatures ranged from 23.9°C to 30°C, with a mean of 28.7°C for the dates when counts took place. Point counts were carried out until a clear decline was observed in both treatment and control plots as the sunflowers matured and stopped producing pollen. An ANCOVA, explaining 83% of the data, revealed no differences between honeybees counted in the control and treatment plots (Figure 9), but did show a significant effect of the Julian day (Overall: $F_{3,44} = 71.69$, $P < 0.0001$, $r^2 = 0.830$; Treatment: $F_{3,1} = 0.335$, $P = 0.566$, $r^2 = 0.001$; Julian day: $F_{3,1} = 214.736$, $P < 0.0001$, $r^2 = 0.829$; Treatment x Julian day: $F_{3,1} = 0.002$, $P = 0.964$, $r^2 < 0.001$).

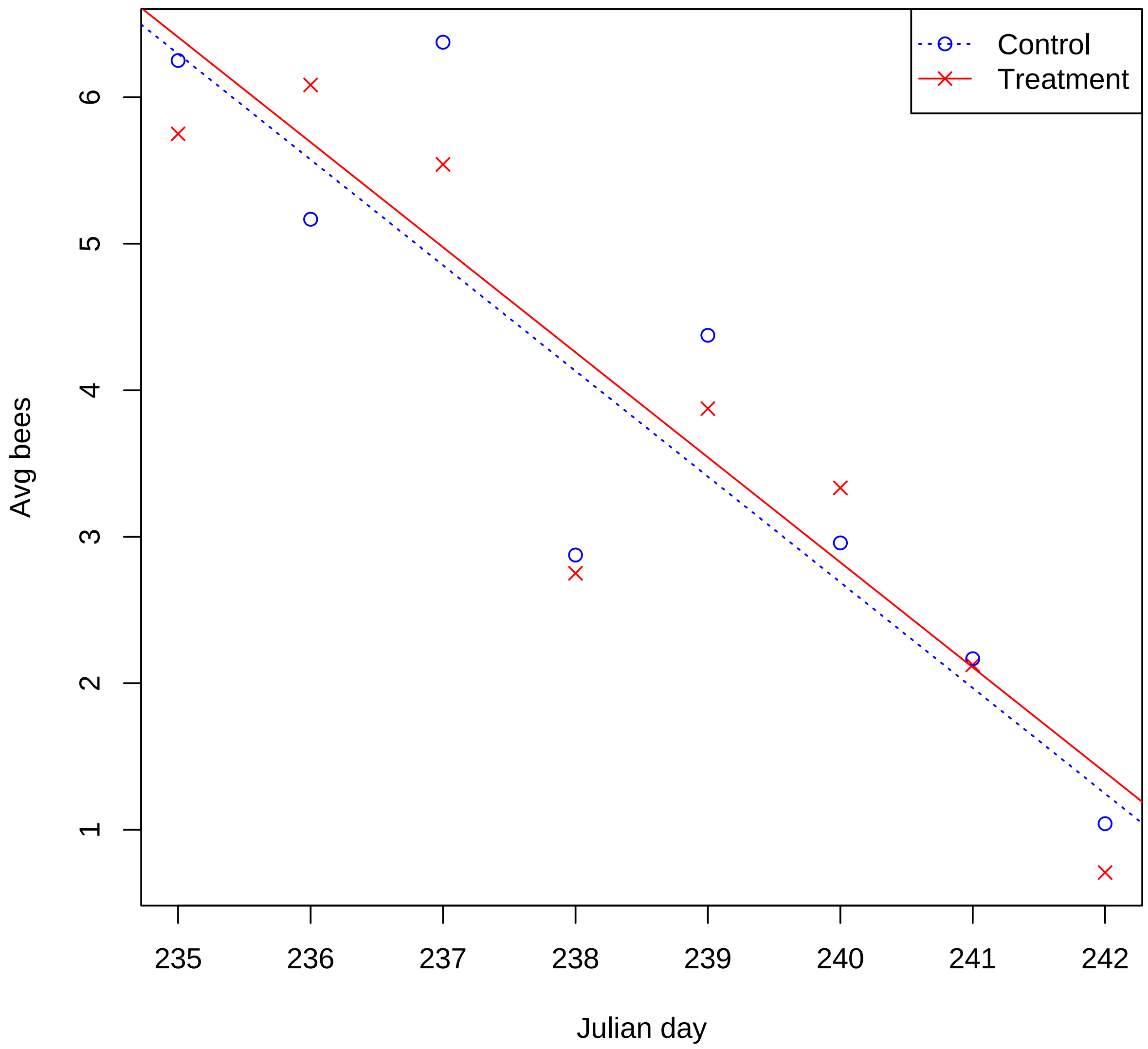

Figure 9. Average number of bees per Julian day in treatment versus control plots (ANCOVA: $F_{3,44} = 71.691$, $P < 0.0001$, $r^2 = 0.830$). Bees were counted daily along linear transects with eight counts in both control and treatment halves of the field. No statistically significant difference was observed between treatments. An overall trend of decline was observed in relationship to Julian day, as there were fewer flowers for the bees to pollinate in the field as a whole. (ANCOVA: Julian day: $F_{3,1} = 214.736$, $P < 0.0001$, $r^2 = 0.829$).

Maintenance Diet Consumption Rates

Consumption of food per bird day was differed significantly between treatment and control enclosures as indicated by our ANCOVA (Figure 10). Although the full model explained less than 10% of the variation in daily consumption rates ($F_{3,176} = 6.125$, $P = 0.001$, $r^2 = 0.095$), there were significant effects of both treatment ($F_{3,1} = 13.413$, $P < 0.001$, $r^2 = 0.069$) and Julian day ($F_{3,1} = 3.993$, $P = 0.047$, $r^2 = 0.021$), but the interaction between treatment and Julian day was not significant ($F_{3,1} = 0.968$, $P = 0.327$, $r^2 = 0.005$). Removing the time effect, we ran an ANOVA (Figure 11) and again found a significant difference between the amount of cracked corn consumed in treatment and control enclosures (Control: 2.316 g/bird*day ± 0.168, Treatment: 1.477 g/bird*day ± 0.158, Mean ± Standard Error; ANOVA: $F_{1,176} = 13.193$, $P < 0.001$, $r^2 = 0.069$). A rainstorm (which resulted in the loss of three days of data) occurred prior to a noticeable increase in daily consumption rates (Figure 10), and as such we performed post-hoc ANCOVAs in which data were restricted to dates before the storm (pre-storm) and after the storm (post-storm). Julian day had a significant effect post-storm (Overall: $F_{3,32} = 2.753$, $P = 0.059$, $r^2 = 0.205$; Treatment: $F_{3,1} = 2.037$, $P = 0.163$, $r^2 = 0.051$; Julian day: $F_{3,1} = 6.217$, $P = 0.018$, $r^2 = 0.154$; Julian day x Treatment: $F_{3,1} = 0.004$, $P = 0.953$, $r^2 < 0.001$), but not pre-storm (Overall: $F_{3,140} = 5.290$, $P = 0.002$, $r^2 = 0.102$; Treatment: $F_{3,1} = 11.883$, $P = 0.001$, $r^2 = 0.076$; Julian day: $F_{3,1} = 0.043$, $P = 0.837$, $r^2 < 0.001$; Julian day x Treatment: $F_{3,1} = 3.944$, $P = 0.049$, $r^2 = 0.025$).

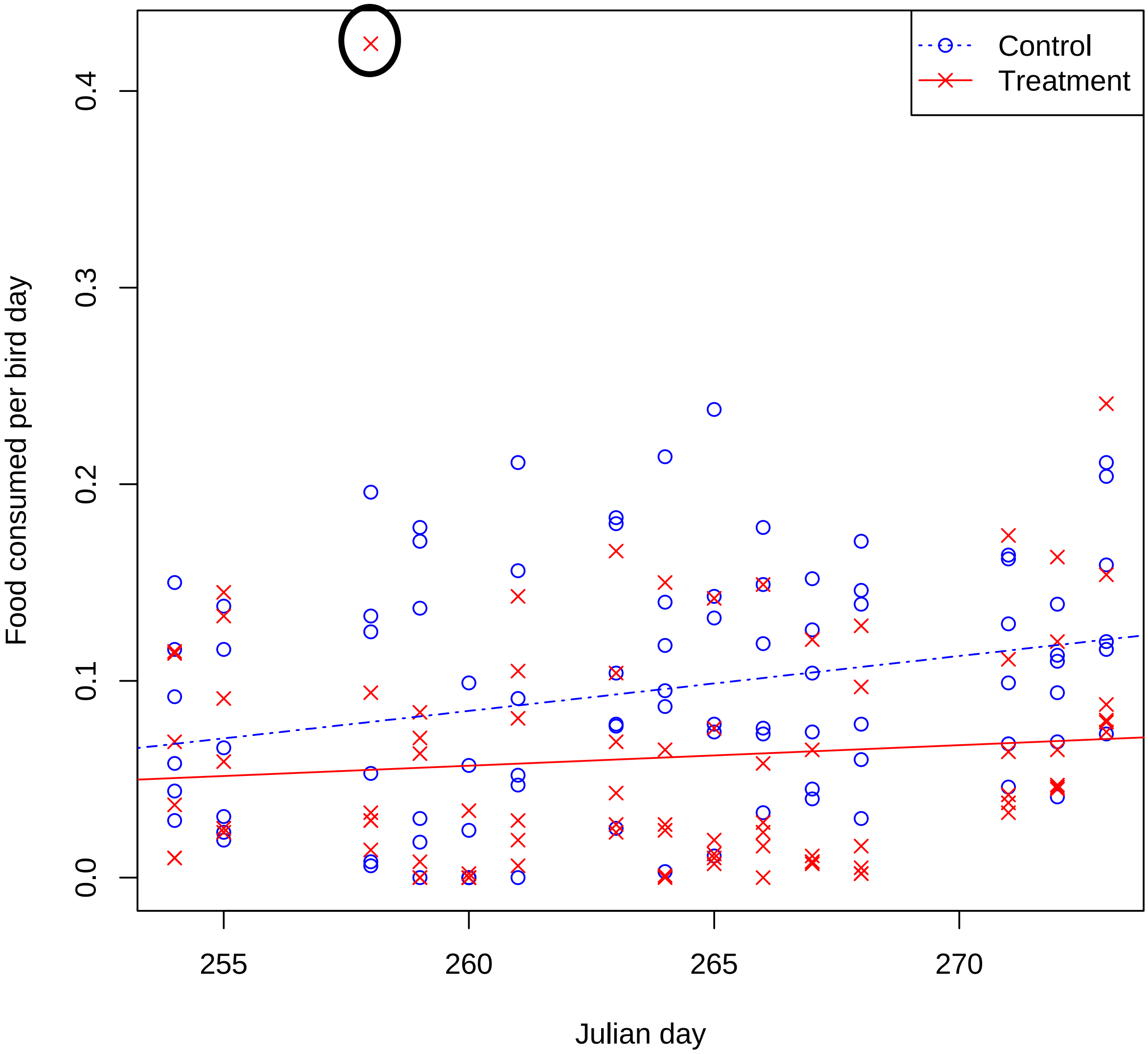

Figure 10. Food consumed per bird*day per Julian day in treatment and control plots. The circled outlier could not be disproven as a recorder error; however, no significant changes were observed when it was excluded from analysis. Large gaps in data represent periods of rain where food samples were too wet to be salvaged for reliable analysis. ANCOVA revealed significant trend in relation to date and treatment ($F_{3,176} = 6.125$, $P = 0.001$, $r^2 = 0.095$).

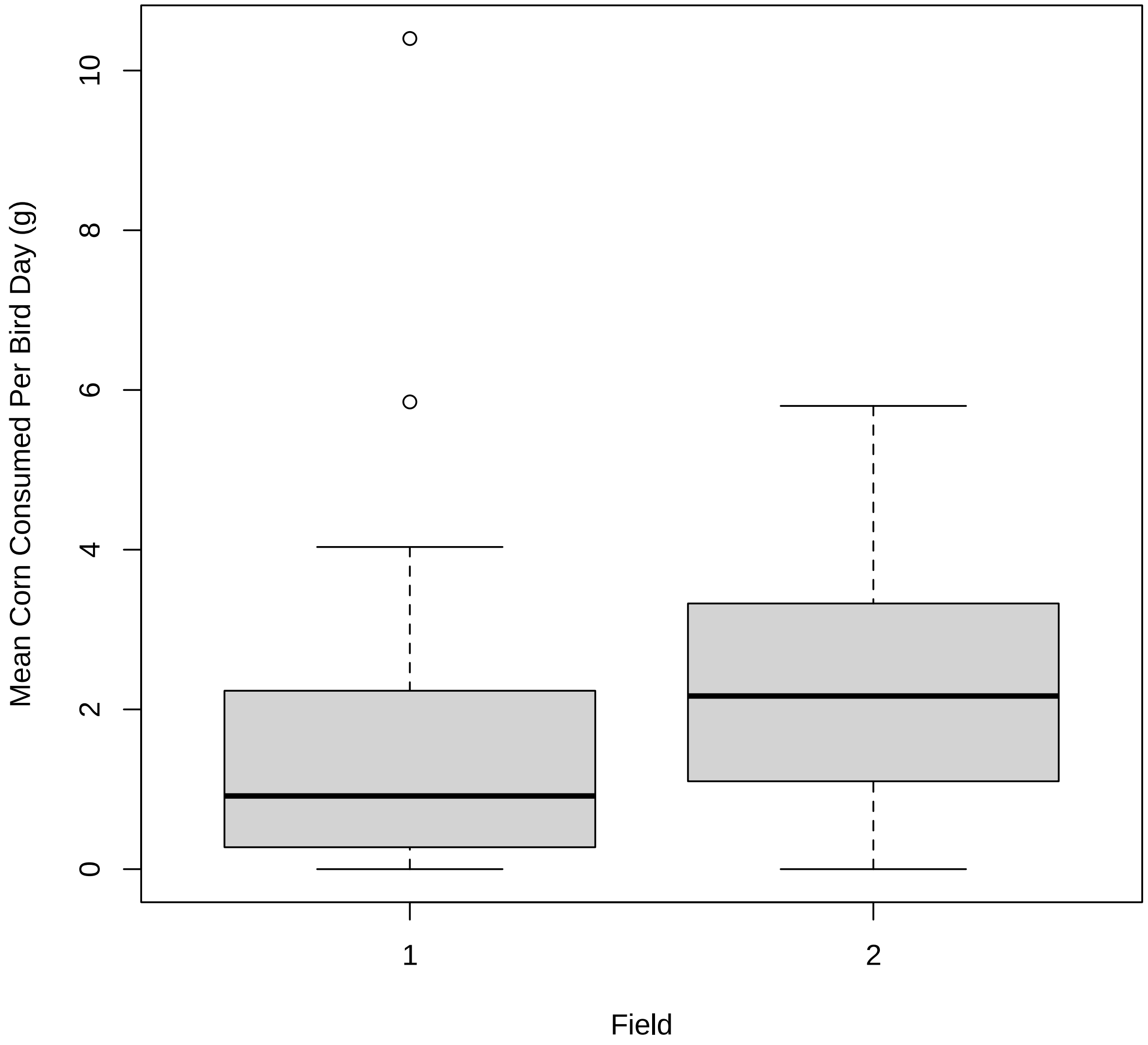

Figure 11. Differences in average food consumption between treatment and control plots. Field 1 is treatment and field 2 is control. Bold lines represent the means, ends of whiskers represent the 95% and 25% percentiles, and open circles are outliers. ANOVA revealed a significant difference (Control: 2.316 g/bird*day ± 0.168, Treatment: 1.477 g/bird*day ± 0.158; $F_{1,176}$ = 13.193, $P < 0.001$, $r^2 = 0.069$).

Sunflower Damage

ANOVA indicated average sunflower damage inside each cage was significantly higher (Control: 52.875 cm^2 ± 4.751; Treatment: 80.601 cm^2 ± 3.861) in the treatment enclosures ($F_{1,10} = 20.508$, $P < 0.001$, $r^2 = 0.672$). Sunflowers in the cages treated with AV2022 had taken substantially more damage (Figure 12). Post hoc tests were conducted to check for observer bias, but no significance was found ($F_{1,10} = 0.029$, $P = 0.865$, $r^2 = 7.828e^{-5}$). Additionally, the size of sunflower heads in treatment and control cages (Control: 221.598 cm^2 ± 5.842, Treatment: 247.415 cm^2 ± 7.565) were also found insignificant by an ANOVA. Developed sunflower head area was slightly larger in the treatment cages than in the control, but this difference was not significant ($F_{1,10} = 3.892$, $P = 0.077$, $r^2 = 0.280$). Despite the lack of significance, we looked at the damage as a fraction of the developed area on the sunflower (Control: 213.969 cm^2 ± 8.615, Treatment: 234.435 cm^2 ± 5.780; ANOVA: $F_{1,10} = 13.864$, $P = 0.004$, $r^2 = 0.581$) and again following a logit transformation to unbound the data (ANOVA: $F_{1,10} = 13.065$, $P = 0.005$, $r^2 = 0.566$). Both of these ANOVAs showed a significant difference was present in the damage the control and treated flowers accumulated.

A significant difference between average damage in the enclosures and food consumed was revealed by our ANCOVA ($F_{3,8} = 8.754$, $P = 0.007$, $r^2 = 0.766$, Intercept: $t = 9.00$, $P < 0.0001$) (Figure 13). Treatment, corn eaten per bird day, and an interaction of the two variables were run against the average damage for each cage. Seventy-six percent of the data was explained by this model and it suggests a significant difference between the slope and intercept of the treatments for both average damage and food consumed per bird day. Of the variables tested, only the treatment had a significant effect ($F_{3,1} = 25.067$, $P = 0.001$, $r^2 = 0.731$) suggesting a difference between both damage and maintenance diet consumed between treatment and control.

A post-hoc analysis on consumption was run on both sides of the rainstorm around Julian day 270. Statistical significance was found in both before ($F_{3,11} = 9.167$, $P = 0.006$, $r^2 = 0.775$) and after ($F_{3,8} = 6.707$, $P = 0.014$, $r^2 = 0.716$). Of the variables run in the model, only treatment had an effect in both before ($F_{3,1} = 25.972$, $P <0.001$, $r^2 = 0.732$) and after ($F_{3,1} = 19.693$, $P = 0.002$, $r^2 = 0.700$).

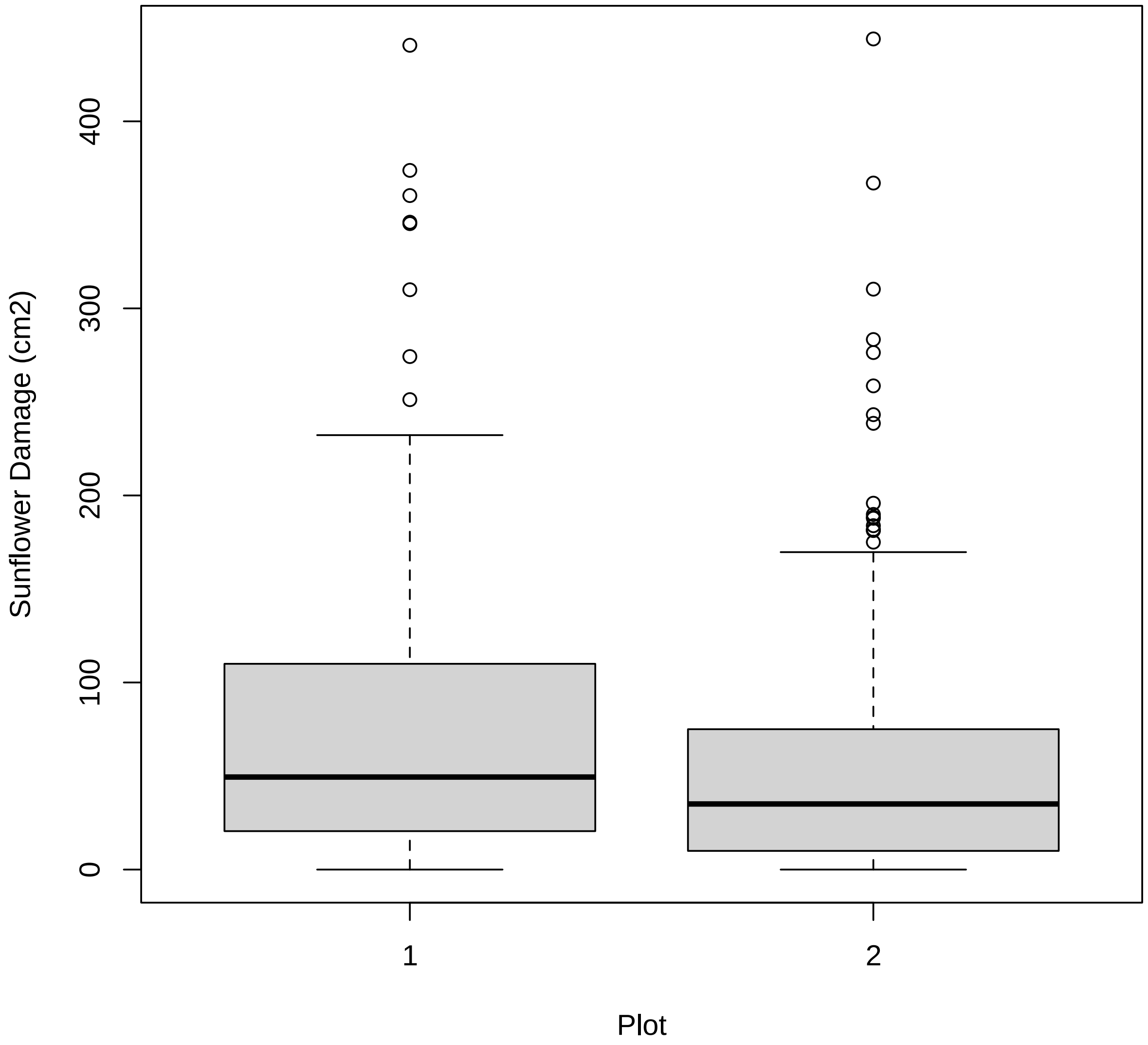

Figure 12. Box and whisker plot of sunflower damage inside control and treatment cages. Plot 1 represents treatment and plot 2 represents control. Bold lines are the mean, extended lines represent the 95% and 25% percentiles, and open circles are outliers (Control: 52.875 cm^2 ± 4.751; Treatment: 80.601 cm^2 ± 3.861). Significant difference found with ANOVA ($F_{1,10}$ = 20.5082, P <0.001, r^2 = 0.672).

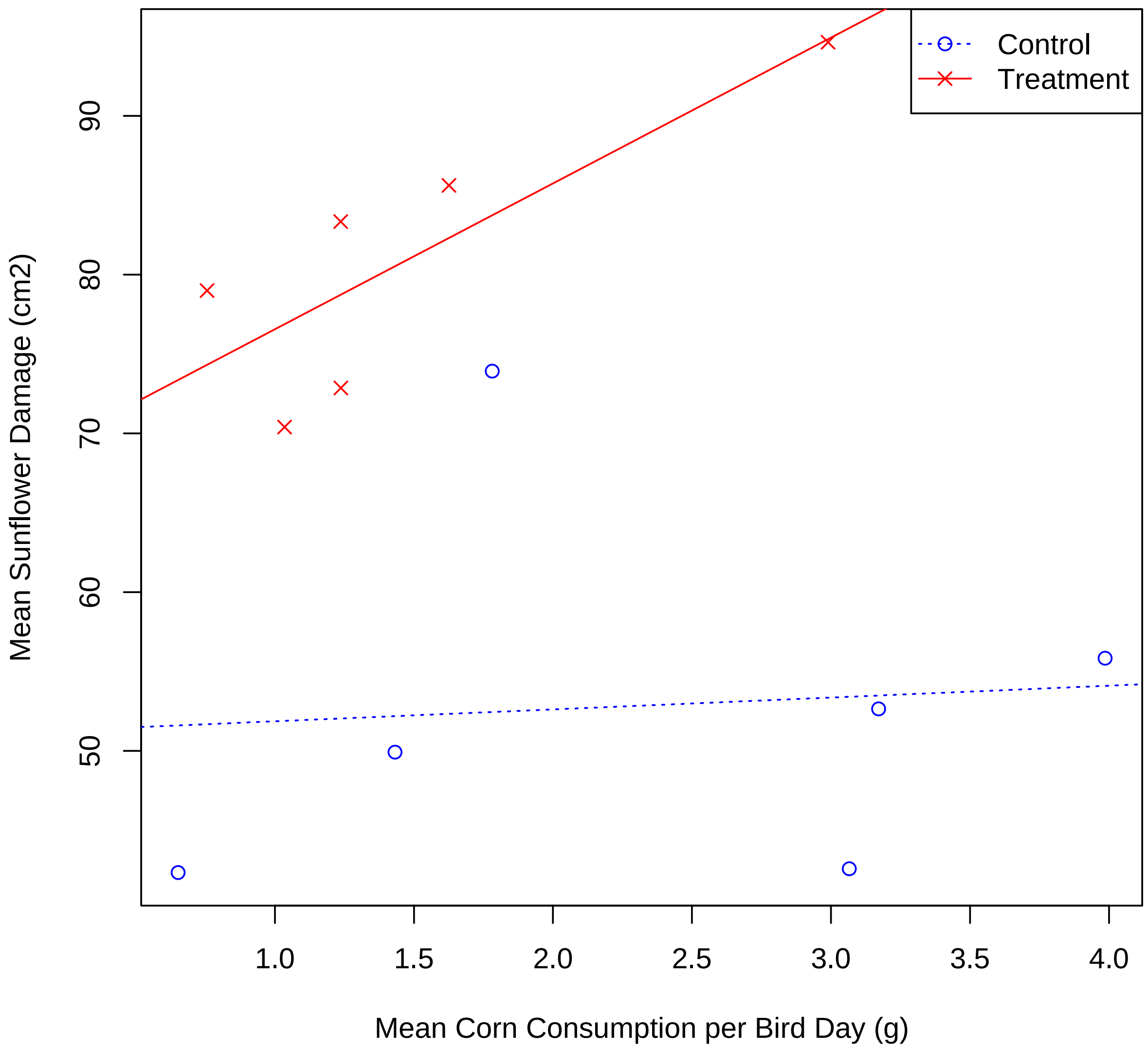

Figure 13. Relationship between the covariate mean corn consumption and mean sunflower damage. Cages where less corn was consumed had significantly higher rates of damage to the sunflowers suggesting that the birds were supplementing their diet with sunflower when not eating the maintenance diet. (ANCOVA: $F_{3,8} = 8.754$, $P = 0.007$, $r^2 = 0.766$).

Residue Analysis

Data from the linear regression model showed a significant decrease in the concentration of AQ on treated sunflower bracts (heads) over time ($F_{1,23}$ = 33.231, P <0.0001, r^2 = 0.602, slope = -1.684). Control sunflowers showed an increase in AQ concentration (Linear Regression: $F_{1,5}$ = 144002.3, P <0.0001, r^2 = 1.000, slope = 0.540). Initial control samples were collected before the field was sprayed and produced a baseline reading of 2.5 ug/g.

Control achenes (seeds) revealed no detectable AQ while treated achenes had concentrations ranging between 4.06 ug/g and 7.46 ug/g with a mean of 4.95 ug/g.

Discussion

Potential Risks to Pollinators

AV2022 is a chemical aimed at protecting sunflower crops from blackbird depredation via use of the secondary repellent, 9, 10 anthraquinone. Known to have a UV component, the potential for accidental repellency of the major pollinator the western honeybee, was of interest to this study. With colony collapse a pressing issue for crop-growers nation-wide, it was critical to make sure AQ did not pose any adverse effects on the local honeybee activity. The data collected in August during the development of the sunflowers showed no statistically significant differences between the number of honeybees foraging in either treatment or control plots. This suggests that the UV component of the AQ, when applied foliarly to R5 to R6 stage oilseed sunflowers has no effect on honeybee preference. The overall decline of bee foraging seen in our data was the result of the decline of flowers available for pollination.

Should AQ be desired for use on any bee-pollinated crop, studies to check the concentration of residues in the wax inside the hive and any effects a potential build up may have on overall hive health. Future studies should look for effects ideally over multiple fields for higher-power statistics.

Implications for Use as an Avian Repellent

While not completely indicative of actual field conditions, the netted cage enclosures used in this study were essential to testing if blackbirds would be repelled by AV2022 when applied via ground sprayer. During a prior year of related research, we used point counts, but there was much disparity between bird activity observed in the different fields. Based on this source of confusion, we decided to use the enclosures as a way to force birds to forage in specific areas we selected. Using field enclosures also provided us with the chance to observe any learning-response to the repellent in the treatment cages as the birds were constantly exposed to AQ.

Our results indicate that AV2022, when sprayed on sunflower prior to seed set, is not effective as an avian repellent. Despite our initial predictions that more damage would be seen on the untreated sunflower heads, our study found the opposite effect. Birds in treatment enclosures consumed less corn than birds in control enclosures, and treatment enclosures exhibited more sunflower damage than control enclosures. This is contrary to multiple prior studies conducted in the lab and field that have all seen repellency with AQ containing compounds (Avery *et al.,* 1998; Blackwell *et al.,* 2001; Werner *et al.,* 2011, Werner *et al.,* 2014). It should be noted though, that none of these studies used a ground sprayer to apply the AQ, nor did any studies apply the repellent prior to seed set. The two cage-enclosure studies with AQ-based repellents showed repellency for common grackles (Werner *et al.*, 2011) and red-winged blackbirds (Werner *et al.* 2014), when applying compounds with 50% AQ (twice the active ingredient of

AV2022 used in this study) with backpack sprayers, allowing for more adequate coverage of the sunflower head at a higher concentration than what the ground sprayer allowed.

Results from the residue analysis indicate that AQ concentrations declined over time. Rain may have contributed to the declining concentrations of AQ in treated sunflowers. From the second spray on 22 August to 23 October of 2013, the area received a total of 8.82 inches of rain (North Dakota Agricultural Weather Network, 2014).

Our data shows that treatment group birds ate less of the cracked corn and had higher damage rates in the cages compared to the birds in the control group. This brings us to consider what might have caused this trend to appear. Birds inside the treated enclosures may have mistaken the aversive effects as coming from the corn diet instead of the sunflowers. When they were first introduced to the cage, the birds may have sampled both sunflower and cracked corn. As they pealed back the bracts on the sunflower, contact with AQ would have been made. From this exposure, the birds may have falsely assumed the cause of any discomfort was the cracked corn rather than the sunflower bracts. Additionally, the birds may have figured out that less residue was on the achenes (seeds) and simply eaten around the treated areas. As we do not have any video of birds feeding, we cannot prove or disprove these hypotheses. Adequate time for learning should not have been an issue in this study as all birds had plenty of time to experience any discomfort from consuming AQ.

Another possible explanation for the greater sunflower damage in treatment enclosures was the placement of our cages. For instance, propane cannons were placed in another area by the landowner, which happened to be closer to the treatment plot (as this half of our field bordered a far large portion of the larger field) than the control plot (which ran alongside the road). Because the treatment plot was closer to the scare devices, birds in treatment enclosures may have been more stressed than birds in control cages. Stress may have caused birds in treatment enclosures to expend more energy each day, and therefore consume more seeds in order to maintain mass. We am not convinced this was the sole factor as the control birds would have only been about 40 m further away and the sounds could still be clearly heard in each treatment. This was an issue of logistics in setup as we only had two electric fences available. Because our cages are not true replicates (pseudo replicates), we inflated our sample size. However, the results still found no evidence that treatment of sunflower heads with AQ prior to seed set was effective as a blackbird repellent.

Finally, there is the possibility that the blackbirds preferred the sunflower treated with AQ. Our results not only suggest that the repellent does not work to repel blackbirds, but possibly the blackbirds increased their consumption of sunflower when it was treated with the repellent.

As shown in this experiment, spraying AQ on the bracts alone does not deter birds from eating the sunflower. Birds have the option of feeding around the bracts and can easily avoid coming into contact with AQ treated areas. This poses a problem with migratory birds, as naïve populations are less likely to encounter treated zones over time as flocks that arrive earlier will have already eaten the treated seed.

Application problems can be brought into question; coating individual seeds on a sunflower head is not possible with common spray rigs. As the flower head bends over with the added weight it accumulates as it matures, the less area can be coated with any repellent. This causes inadequate coverage by the sprayer. Better methodology for applying repellents to sunflower would greatly aid repellent application, such as upward facing nozzles as suggested by

Werner *et al.* (2014). However, the real test would be the overall effectiveness of the repellent, which is currently in doubt.

Although AQ application at the R5.1 stage did not affect pollinators, AV2022 does not appear to be an effective avian repellent for sunflower crops either. Future efforts should be made to determine whether or not this is the result of bird preference, application timing or coverage. By assessing the residues on sunflowers sprayed at different growth stages, the optimal timing for the most spray residue could be determined.

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