Evaluation of 9,10 anthraquinone application to pre-seed set sunflowers for repelling blackbirds

Megan D. Niner,1 Department of Biological Sciences, North Dakota State University, Stevens Hall, Fargo, ND 58108, USA Megan.Niner@rockets.utoledo.edu

George M. Linz, USDA, APHIS, Wildlife Services' National Wildlife Research Center, Bismarck, ND 58501, USA

Mark E. Clark, Department of Biological Sciences, North Dakota State University, Stevens Hall, Fargo, ND 58108, USA

Abstract: Most nonlethal methods available for reducing blackbird (Icteridae) damage to sunflowers rely on fright responses (e.g., propane cannons, distress calls, pyrotechnics, raptor silhouettes) that birds quickly learn to ignore. Chemicals that cause taste or feeding aversions have potential to overcome the spatial and behavioral limitations of frightening methods. Anthraquinone (AQ) is an effective feeding repellent as a seed treatment to deter birds from eating freshly planted grains. In the United States, foliar application of AQ is not permitted on food crops except on small experimental plots. In August 2013, we applied 37.4 L/ha of an aqueous mix consisting of 15.1 L of a prototype AQ product (active ingredient = 25%) per 41.7 L water (5.6 kg AQ/ha). We applied the AQ product by high-clearance ground sprayer on 0.4-ha in a sunflower field in North Dakota. Sunflower development was at the R5.1 to R5.3 stages, or 10 to 14 days before usual onset of blackbird damage. We kept another 0.4-ha plot adjacent to the treated plot as an untreated reference. In early September, we placed 3 red-winged blackbirds (Agelaius phoeniceus) in 6 netted enclosures (2.4 m x 2.4 m x 2.4 m) in each plot. Supplemental rations of cracked corn and water were provided daily throughout the testing period that ended October 1, 2013. Treated enclosures had significantly greater damage (247.4 ± 5.8 cm$^2$) than reference enclosures (214.0 ± 8.6 cm$^2$). Statistical significance implied that AQ increased blackbird damage to sunflowers, contrary to the results of other studies. However, residue analysis of the backs of sunflower heads, bracts (our target areas for the spray), and achenes indicated that AQ residues may have been too low to produce a repellent effect. Our findings suggest that the effectiveness of AQ as a blackbird repellent is context-dependent when applied under commercial-grower conditions.

Key words: 9,10 anthraquinone, Avipel®, ground spraying, human–wildlife conflict, North Dakota, red-winged blackbird, sunflower damage

In 2013, U.S. agricultural producers planted >500,000 ha of oilseed sunflowers; 77% was planted in North Dakota and South Dakota (National Agricultural Statistics Service 2014). Most sunflower production in these states occurs within the Prairie Pothole Region (PPR), a fertile region pocked with numerous shallow wetlands (Ralston et al. 2007, Linz and Homan 2011). The PPR is the center of abundance for red-winged blackbirds (Agelaius phoeniceus), a major depredator of oilseed sunflowers (Peer et al. 2003). In North Dakota and South Dakota, resident populations of red-winged blackbirds number about 25 million in late August and early September, the peak period for blackbird damage (Klosterman et al. 2013; Figure 1). At the current price of oilseed sunflowers ($0.47/kg, [National Agricultural Statistics Service 2014]), red-winged blackbirds eat $5.1 million worth of the sunflower seeds produced annually in the PPR. Two other blackbird (Icteridae) species also cause damage to ripening sunflowers in the PPR, but red-winged blackbirds cause >50% of the damage (Peer et al. 2003, Linz et al. 2011). This amount of blackbird damage spread throughout the PPR would be inconsequential, but damage is localized in areas near wetland roosting sites (Linz and Homan 2011). Average damage levels may exceed 20% in these areas, far greater than the 5% damage threshold that is tolerable for most sunflower growers (Linz et al. 2012). Blackbird damage begins in late
August, when peripheral rows of achenes begin to develop following the dropping of ray petals. The birds access the peripheral achenes by removing bracts (i.e., petal-like structures that surround the sunflower heads). The damage season lasts until harvest in October, but about 75% of blackbird damage occurs in the 17 to 18 days after the ray petals drop (Cummings et al. 1989).

Blackbird damage not only causes economic losses to individual growers, but may have an impact on the sunflower industry as a whole. The effects of severe localized damage may permeate throughout the sunflower-growing area, leading to regional declines in plantings, because producers switch to less susceptible crops, such as corn and soybean (Kleingartner 2002, Klosterman et al. 2013).

Lethal methods to manage red-winged blackbirds are resource-intensive and, thus, lack cost effectiveness, because red-winged blackbirds are a short-lived, fecund species (Blackwell et al. 2003). Frightening devices, such as propane cannons, distress calls, raptor decoys, and pyrotechnics may be effective, in small fields, but an average sized sunflower field in the PPR is ≥65 ha. Most frightening devices have only short ranges of effectiveness, allowing birds in large fields to quickly habituate to the stimuli simply by moving out of a device’s range (Linz et al. 2011). Large fields would typically require many such devices, thereby greatly reducing efficacy by increasing total costs of defending the crop. Lure crops that are planted with the intention of attracting birds away from commercial sunflower fields have shown potential to mitigate blackbird damage to sunflowers; however, lack of cooperative funding has impeded use of this method (Linz et al. 2011). For over a decade, starting in the mid-1990s, thinning of cattail (Typha spp.) vegetation in roosting wetlands near sunflowers was a popular USDA program with sunflower producers, but funding for the program in the PPR has ended.

Inefficacy and unfavorable cost to benefit ratios of current methods used for reducing blackbird damage to sunflowers have led many resource managers to view chemical feeding repellents or taste repellents as the best options for protecting crops (Clapperton et al. 2012).

Effects of repellents range from immediate aversions upon contact with taste and smell receptors (i.e., taste repellents) to delayed gastrointestinal discomfort and vomiting following ingestion (feeding repellents). After suffering the negative consequences of a repellent, birds usually forage elsewhere. Four taste repellents are available for foliar use on sunflowers in the United States (Federal Insecticide, Fungicide, and Rodenticide Act, Section 3, registrations): Avian Control® (Avian Enterprises, LLC, Jupiter, Fla.); Bird Shield® (Bird Shield Repellent Corp., Pullman, Wash.); Avex® (Corvus Repellent Inc., Greeley, Colo.); and Flock Buster® (Skeet-R-Gone, West Fargo, ND). Active ingredients in these products are designated by the U.S. Food and Drug Administration as Generally Recognized as Safe. The first 3 products have methyl anthranilate as their active ingredient, whereas the last is a mixture of ingredients, including lemon grass oil, garlic oil, and clove oil. Research has shown that none are consistently effective at reducing feeding rates of blackbirds on sunflowers when applied at label-recommended concentrations (Linz et al. 2011).

The chemical compound, 9,10 anthraquinone, was identified as an avian feeding repellent in the 1940s (Avery 2003). It is a naturally occurring compound found in many plants and invertebrates for defense against herbivory and predation. Anthraquinone, once eaten, causes gastrointestinal discomfort or vomiting (Avery et al. 1997, 1998). Feeding repellents may be more effective than taste repellents.
for reducing bird damage (Werner and Clark 2003). Anthraquinone is an effective seed treatment (Avipel®, Arkion Life Sciences, New Castle, Del.) that deters granivorous birds from unearthing and eating freshly planted seeds and grains of crops, such as canola, rice, corn, and sunflowers. Use of AQ as a seed treatment is allowed under Federal Insecticide, Fungicide, and Rodenticide Act, Sections 18 or 24(c). AQ has reduced foraging of seeds and grains by ring-necked pheasant (Phasianus colchicus), sandhill cranes (Grus canadensis), common grackles (Quiscalus quiscula), and red-winged blackbirds (Blackwell et al. 2001; Werner et al. 2009, 2011, 2014). Enclosure studies have consistently shown that feeding rates of blackbirds are reduced by ≥80% with AQ treatments on seeds (Avery and Cummings 2003). Feeding rates of red-winged blackbirds were reduced ≥80% for birds in enclosures that were offered treated sunflower seeds at AQ concentrations of about 1,500 ppm (Werner et al. 2009).

Despite promising laboratory results, field studies have not provided evidence that AQ is effective. Except when AQ was applied by backpack sprayers (which is not a practical approach for treating large fields of sunflowers; see, Werner et al. 2011, 2014), results from field trials on AQ have been equivocal. For example, rice plots were protected for 7 days following aerial application, but similar tests on wild rice in California yielded no treatment effect (Avery and Cummings 2003). Results from preliminary field experiments in North Dakota indicated that AQ applied by either fixed-wing aircraft or ground-sprayer did not reduce blackbird damage to sunflowers (Linz and Homan 2012).

During August 2013 in North Dakota, we applied a foliar treatment of a repellent prototype that used AQ as its active ingredient (AV2022 [active ingredients = 25% 9,10 anthraquinone]; Arkion Life Sciences, LLC, New Castle, Del.). Sunflower development was at the R5.1 to R5.3 stages, which is about 10 to 14 days before onset of blackbird damage. The R5.1 to 5.3 (pre-achene set) stages occur when sunflower pollen is being released and is attracting insects. Spraying during this developmental period allowed us to evaluate the potential for applying AQ at the same time that insecticides are typically applied, thereby reducing application costs compared to a sole application of AQ. The effectiveness of applying Avipel to the back of sunflower heads prior to the petal drop stage (i.e., R-6) has yet to be tested. Food tolerance levels would not be needed for U.S. Environmental Protection Agency registration of this product for foliar use if AV2022 were effective at reducing damage when applied during the R5.1 to R5.3 stages and the AQ residues on achenes remained below detectable levels at harvest.

Our objectives were to (1) compare sunflower damage by male red-winged blackbird in treated and reference enclosures following AQ application by a method of foliar spraying commonly employed by agricultural producers in the PPR, and (2) measure AQ residues on the backs of sunflower heads, bracts, and achenes immediately following application and just before harvest. Our results will continue to build knowledge of AQ and its effectiveness when used in the field to protect ripening sunflowers.

**Study area**

Our study site was a 50-ha oilseed sunflower field located in east-central North Dakota in Barnes County (47° 2’ 10.16” N, 97° 40’ 40.22” W). Sunflowers were planted intermittently in this field as part of a crop rotation used by the producer. During prior rotations, the producer had experienced notable losses to foraging blackbirds. Flat agricultural fields of corn, wheat, and soybeans, characterized the surrounding landscape. The nearest cattail stand was located 1 km away along a stream. Average temperature and total precipitation over the study period between August 19, 2013 (first application of AV2022), and October 1, 2013 (removal of red-winged blackbirds from enclosures), was 18.4° C and 7.2 cm. These variables were 3.2° C above and 2.3 cm below 30-year averages. Weather data were collected 25 km southwest of the study site (National Oceanic and Atmospheric Administration 2014). Our study area was kept <4.05 ha to meet Environmental Protection Agency (EPA) regulations for testing unregistered pesticides. At the end of the study, all treated plants were destroyed.
Methods

Bird capture and care

In late May and early June 2013, we captured territorial male red-winged blackbirds in wetlands and waterways 40 to 50 km east of Bismarck, North Dakota (46.81° N, 100.78° W). We used a box trap specialized for capturing territorial male blackbirds (Bray et al. 1975). We transported captured birds in ventilated holding cages and held them at an aviary. Fresh water, cracked corn, sunflower achenes (seed and outer casing), millet, and meal worms were provided to the birds daily. To mimic the environment that the birds would face in netted field enclosures on the experimental plots, we proffered whole heads of sunflowers to the birds in early August. Additional red-winged blackbird males were captured by mist-netting in early August east of Bismarck to supplement the captured population. The capture, care, and use of birds in this study were approved by the Institutional Animal Care and Use Committees of North Dakota State University (Protocol #A13006) and National Wildlife Research Center (QA-2121, Study director, George M. Linz).

Plot preparation

We delineated a 128-m × 64-m plot (0.8-ha) in the study field in July. The plot was then halved (i.e., 128 m × 32 m). One plot was randomly selected for AQ treatment. The other plot served as an untreated reference. To allow unhindered growth of sunflowers in the plots, enclosures (2.4 m × 2.4 m × 2.4 m) were not placed until September 2, 2013. Enclosure panels were made of aluminum frames and plastic netting. Six enclosures were placed approximately 2.4 m apart in a straight line in the center of each plot. The width of the plots was about 1.5 m wider than the spray boom of the ground sprayer, allowing for a 3-m buffer between the treated and reference plot. Enclosures were located approximately 15 m from the plot buffer, roads, and field edges to reduce edge effects. The horizontal distance between the row of treated enclosures and reference enclosures was about 33 m, which helped ensure spray drift from contaminating reference enclosures. To prevent harassment, ingress, or predation from mammals, double-stranded electrified wire was placed around each line of enclosures. All vegetation <30 cm from the enclosures was cleared to prevent shorting of the electric fencing. A plastic, mesh covering, measuring 3.2 m × 3.6 m, was placed over the top of each enclosure to provide shading and prevent harassment by aerial predators. Each enclosure contained approximately 40 sunflower plants.

Anthraquinone application

We applied AQ on August 19, when plants had reached the R5.1 to R5.3 developmental stage. The AQ was applied to the treated plot using a high-vertical clearance ground sprayer (Ag Chem Rogator, Jackson, Minn.). The ground sprayer had a 30.5 m boom. The AQ was applied under 20.4 kg/ha using a size 1104 nozzle. The treated plot was sprayed in a single pass of the ground sprayer. More than 50% of the sunflowers were in the developmental stage of R5.1 (early flower development). This stage of development corresponds to the normal timing of insecticide application for the red seed weevil (S. micrnx fulvus), a common pest of sunflower crops. At the time of the first application, sunflowers were about 1.7 m in height. Sunflowers in the path of the sprayer were pushed from their vertically oriented positions but recovered their positioning in a few days. The first spray of AQ was aimed at a concentration of 37.4 L/ha, but a communication error occurred, and the plots were sprayed with only half the desired volume of water and AQ. On August 22, the plots were again sprayed with half of our desired spray to apply the full concentration. The sunflowers were in partial to full-bloom on the second application.

Enclosures

We placed 3 red-winged blackbirds in each of the 12 enclosures on September 9, 2013, when all sunflowers were at the R6 (petal drop) stage. On September 24, a fourth bird was added to each enclosure to increase the potential of sunflower damage. All birds were removed from the enclosures October 1, 2013. The enclosures remained standing and closed within the plots.

We calculated the amount of maintenance diet needed (100 g cracked corn) to support daily energy needs of adult male red-winged blackbird (Peer et al. 2003). The 100-g portions of cracked corn were dried overnight to <10%
moisture, labeled with enclosure identifiers, and placed in sealable plastic bags for distribution daily to the enclosures. We used metal feed pans to contain the cracked corn and prevent spillage. Fresh water was provided by a gravity-fed, 9.5 L container that was also placed in a feed pan. Deceased (reference enclosures = 12, treatment enclosures = 13) birds were removed and buried away from the enclosures. Prior to daily placement (about 1300 h CDT) of fresh 100-g portions of cracked corn, all unconsumed cracked corn from the previous day was collected, sealed in plastic containers, and labeled with the enclosure identifier. Unconsumed food from each enclosure was air dried overnight, then weighed on an electronic balance (±0.0001 g). We obtained the daily rate of consumption of cracked corn by subtracting the mass of the remaining food from 100 g. We discarded samples that were wet from rainfall.

Sunflower damage

We estimated sunflower damage in the enclosures on October 23, 2013, after the plants had been killed by frost. Experienced damage assessors alternated between treated and reference enclosures to reduce observer bias. The remaining area occupied by achenes was estimated by use of a 5-cm² template grid on all standing sunflowers inside each enclosure as described in Dolbeer (1975). Undamaged sunflowers were recorded as having 0 cm² damage. The diameter of all heads and the undeveloped center were recorded, regardless of damage. Percentage of damage estimates were then calculated by dividing the area of missing achenes by the total area of developed achenes and then multiplied by 100. Damage outside of the enclosures was not recorded.

Residue analysis

We collected a random sample of sunflower heads on 3 dates to provide bracts and achenes for analysis of AQ residues. Immediately following the August 19 application, 5 heads were randomly collected, and samples were

Figure 2. Food consumed per bird*day versus Julian day in treatment and reference plots shows a positive trend in relation to date and treatment ($F_{3,176} = 6.13, P = 0.001, r^2 = 0.10$). Reference observations are open circle, and the dashed line and treatment observations are represented by x’s and a solid line. Results did not change when the circled observation was excluded from the analysis. The gap in observations at days 269 to 271 represents a rainfall event where food samples were too wet to be used for analysis.
immediately sent to the USDA Wildlife Services’ National Wildlife Research Center chemical laboratory in Fort Collins, Colorado, where bracts and achenes were removed for residue analysis. Another 5 heads were collected following the August 22 application and sent out for residue analysis. Additional heads were collected from the field for bract and achene residue analysis on October 23.

Statistical analyses

A general linear model was used to examine the relationship between daily consumption per bird between the reference and treatment groups. Consumption was modeled as a function of treatment, day, and the interaction between treatment and day. If day did not have a significant effect on food consumption in the model, we excluded the term and reanalyzed food consumption using an Analysis of Variance (ANOVA) of consumption as a function of treatment. We also conducted a post-hoc analysis in which consumption prior to a rain event was modeled as a function of treatment, day and the interaction.

We used general linear models to examine damage per enclosure. We compared damage per enclosure between treatment and reference groups using an ANOVA. Damage per enclosure was also modeled as a function of the average consumption per bird per day, treatment, and the interaction of the 2 variables. We also tested for effects of observer, and used both area damaged and percentage of damage as the dependent variable in the analysis. For all statistical comparisons, significance was determined if \( P \leq 0.05 \), and we confirmed normality for all independent variables used in the analyses. Means are reported ± standard error.

Results

Maintenance diet consumption rates

Consumption of food per bird per day differed between treatment and reference enclosures (Figure 2). Although the full model explained <10% of the variation in daily consumption rates \( F_{3,176} = 6.13, P = 0.001, r^2 = 0.10 \), there were effects of both treatment \( F_{3,1} = 13.41, P \leq 0.001, r^2 = 0.07 \) and Julian day \( F_{3,1} = 3.99, P = 0.05, r^2 = 0.02 \). However, the interaction between treatment and Julian day was not significant \( F_{3,1} = 0.97, P = 0.33, r^2 = 0.01 \). After removing the time effect from the model and averaging daily consumption across the duration of the study, ANOVA did not show a difference between the amount of cracked corn consumed in treatment (1.5 g/bird/day ± 0.4) compared to reference enclosures (2.3 g/bird/day ± 0.4 \( F_{3,10} = 1.92, P = 0.196, r^2 = 0.16 \). A rain event (which resulted in the loss of 3 days of data) occurred prior to a noticeable increase in daily consumption rates (Figure 2), and, as such, we performed a post-hoc comparison in which data were grouped according to dates before the storm (pre-storm) and after the storm (post-storm). Julian day had an effect on post-storm consumption (Overall: \( F_{3,32} = 2.75, P = 0.06, r^2 = 0.21 \); Julian day: \( F_{3,1} = 6.22, P = 0.02, r^2 = 0.15 \), but not on pre-storm consumption (Overall: \( F_{3,140} = 5.29, P = 0.002, r^2 = 0.10 \); Julian day: \( F_{3,1} = 0.04, P = 0.84, r^2 \leq 0.01 \).

Sunflower damage

Sunflower damage was greater inside treatment enclosures compared to reference enclosures. Proportion of the developed area that was damaged in treatment enclosures (34.6 ± 1.8 %) was significantly greater than that in reference enclosures (24.9 ± 1.8 %; \( F_{1,10} = 13.86, P = 0.004, r^2 = 0.58; \) Figure 3). Absolute average sunflower damage inside each enclosure was also higher (Reference: 52.9 cm² ± 4.8; Treatment: 80.6 cm² ± 3.9) in the treatment enclosures \( F_{1,10} = 20.51, P \leq 0.001, r^2 = 0.67 \). Tests for observer bias found no difference between observers \( F_{1,10} = 0.03, P = 0.87, r^2 \leq 0.01 \) used to quantify damage.

We also found a difference between mean damage in the treatment and reference enclosures and the amount of cracked corn consumed. Treatment, average corn eaten per bird per day per enclosure, and the interaction of these 2 variables explained 76% of the variation in the proportion of damage observed in the enclosures \( F_{3,8} = 8.75, P = 0.007, r^2 = 0.77; \) Figure 4). However, only the treatment term had a significant effect \( F_{3,1} = 25.07, P = 0.001, r^2 = 0.73 \).

Residue analysis

The linear regression model indicated that the concentration of AQ on treated sunflower bracts decreased from August 19 to October 23 \( F_{1,23} = 33.23, P \leq 0.001, r^2 = 0.60, \) slope = -1.68).
Mean AQ concentration of achenes in treated enclosures was 166.9 μg AQ per gram of plant matter (SE = 66.6 μg/g) on August 19. Reference enclosures had an increase in AQ concentration on the achenes (Linear Regression: $F_{1,5} = 144,002.3$, $P \leq 0.001$, $r^2 = 1.00$, slope = 0.54). An initial sample of sunflower heads collected before the plot was sprayed produced a baseline of 2.5 μg/g AQ. Reference achenes revealed no detectable AQ levels, while treated achenes had concentrations ranging between 4.06 and 7.46 μg/g, with $\bar{x} = 4.95$ μg/g (SE = 0.34 μg/g).

**Discussion**

Our results indicate that AV2022, when sprayed on sunflowers prior to seed set, was not effective as an avian repellent. Birds in treated enclosures consumed less corn and caused more damage to sunflowers than birds in reference 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). We note, however, that none of these studies used a ground sprayer to apply the AQ, nor did any studies apply the repellant prior to seed set. The 2 prior studies using both enclosure 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 with backpack sprayers, allowing for more adequate coverage of the sunflower head than achieved by use of the high-clearance ground sprayer. Red-winged blackbirds readily forage in groups on small areas of agricultural fields (Yasukawa and Searcy 1995); hence, cage enclosures were utilized in this study to better examine the effectiveness of an AQ-based repellent when applied with a more commonly used mode of application for larger areas.

Our findings indicate that application of AQ under conditions approximately similar to those for commercial sunflower production did not reduce red-winged blackbird consumption of sunflower. In fact, we found significantly higher rates of sunflower depredation in enclosures of sunflowers treated with AQ. Hypotheses to explain the observed higher rates of consumption of plants with treated achenes include: (1) red-winged blackbirds prefer AQ; (2) concentrations of AQ on achenes were too low to elicit an adverse effect on birds that consumed treated product; or (3) other factors affected consumption rates of birds (e.g., reference cages were approximately 40 m closer to propane cannons directed at another crop). Given that no other study to date has found red-winged blackbirds to prefer AQ-treated seeds (Avery and Cummings 2003, Blackwell et al. 2003, Werner et al. 2009, Werner et al. 2011, Werner et al. 2014), our first hypothesis is unlikely. In the case of the second hypothesis, although the concentrations found on the plants immediately after application were below minimum recommended threshold concentrations, AQ residues were present on the plants and birds were exposed to these residues when feeding. We obtained residue levels of 167 μg/g, noticeably lower than the concentration of 380 μg/g obtained by Werner et al. (2014). Additionally, our application followed recommended sprayer application
methods and our conclusion that application at practical commercial levels does not reduce achene consumption by red-winged blackbirds remains valid. The third hypothesis, that AQ effectiveness at commercial application levels is context dependent, requires further study.

We demonstrated that spraying AQ on the bracts alone does not deter birds from pulling out the achenes in order to consume the sunflower seeds. Birds have the option of feeding around the bracts and can easily avoid coming into contact with AQ treated areas. This method will likely not be a very effective defense strategy against migratory birds, such as red-winged blackbirds, because naïve birds are less likely to encounter treated zones upon arrival, as earlier flocks will have already removed the sprayed bracts.

Logistically it is not possible to apply AQ on individual achenes of a sunflower head with commonly used, high-vertical clearance ground sprayers. Sunflower heads bend toward the ground as they mature, making it difficult to apply AQ directly to the achenes. This causes inadequate coverage by the sprayer. Better methodology for applying repellents to sunflower would increase spraying efficiency; Werner et al. (2014) suggested the use of upward facing nozzles.

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![Figure 4](image-url)  
**Figure 4.** Mean sunflower damage increased significantly with mean daily corn consumption in the treatment group (solid line and x’s) but not in the reference group (dashed line and circles; ANCOVA: $F_{3,8} = 8.76$, $P = 0.007$, $r^2 = 0.77$).
Department of Agriculture Wildlife Services’ National Wildlife Research Center, and North Dakota State University. Arkion Life Sciences LLC provided the 9,10 anthraquinone (AV2022) used in this study. This study was conducted under WS-NWRC study protocols QA-2121 (George M. Linz, Study Director) and in compliance with North Dakota State University IACUC protocol #A13006.

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Megan D. Niner graduated from North Dakota State University with her master’s degree in zoology in spring of 2014. She is currently a Ph.D. student in the Department of Environmental Sciences at the University of Toledo. Her current research is on the presence and evolution of the viral hemorrhagic septicemia virus in the Great Lakes at the Great Lakes Genetics-Genomics Lab at the Lake Erie center.

George M. Linz is a supervisory research wildlife biologist for the USDA, APHIS, Wildlife Services’ National Wildlife Research Center and leads the North Dakota Field Station in Bismarck, North Dakota. He earned B.S. and M.S. degrees in biology from Edinboro University of Pennsylvania and a Ph.D. degree in zoology from North Dakota State University. His research focuses primarily on developing methods of managing blackbird damage to sunflowers. During the last decade, however, he and his staff expanded the scope of their research to include investigating avian-borne diseases in dairies and feedlots across the United States and bird damage to fruit in Michigan.

Mark E. Clark is a faculty member in the Department of Biological Sciences at North Dakota State University. He is an ecologist studying population dynamics and quantitative ecology, focusing on seasonal variation in the reproductive dynamics of overwater nesting birds.