Crop Protection 59 (2014) 63–70

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Application strategies for an anthraquinone-based repellent to protect oilseed sunflower crops from pest blackbirds

Scott J. Werner [a,*], Shelagh K. Tupper [a], Susan E. Pettit [a], Jeremy W. Ellis [a], James C. Carlson [a], David A. Goldade [a], Nicholas M. Hofmann [a], H. Jeffrey Homan [b], George M. Linz [b]

[a] United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, 4101 LaPorte Avenue, Fort Collins, CO 80521-2154, USA

[b] United States Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, National Wildlife Research Center, North Dakota Field Station, 2110 Miriam Circle, Bismarck, ND 58501-2502, USA

ARTICLE INFO

Article history:
Received 3 September 2013
Received in revised form
27 January 2014
Accepted 31 January 2014

Keywords:
Agelaius phoeniceus
Anthraquinone
Chemical repellent
Esfenvalerate
Helianthus annuus
Pyraclostrobin

ABSTRACT

Non-lethal alternatives are needed to manage the damage caused by wild birds to oilseed sunflower crops (*Helianthus annuus* Linnaeus). We evaluated field residues and experimental applications of an anthraquinone-based repellent (active ingredient 50% 9,10-anthraquinone) to minimize red-winged blackbird (*Agelaius phoeniceus* Linnaeus) depredation of oilseed sunflower. Chemical residues from experimental applications of the anthraquinone-based repellent (4.7 l/ha and 9.4 l/ha; low, high) in a ripening oilseed sunflower field were 481 ppm and 978 ppm anthraquinone at the beginning of blackbird damage, and 385 ppm and 952 ppm anthraquinone at the end of blackbird damage, respectively. Prior to harvest, we observed 402 ppm and 462 ppm anthraquinone in the oil, and 27 ppm and 165 ppm anthraquinone in the pomace from crushed sunflower achenes previously sprayed with the low and high applications, respectively. For the purpose of developing application strategies useful for avian repellents, we subsequently investigated blackbird feeding response to oilseed sunflower treated with the anthraquinone-based repellent and either a registered insecticide or a registered fungicide popularly used for ripening sunflower. We observed a positive concentration–response relationship among blackbirds exposed to anthraquinone and the insecticide (a.i. 8.4% esfenvalerate), or anthraquinone and the fungicide (a.i. 23.6% pyraclostrobin). Blackbirds reliably discriminated between untreated sunflower and that treated with 1810 ppm anthraquinone and 0.1% of the insecticide or 1700 ppm anthraquinone and 0.14% of the fungicide during our preference experiments. Given that ripening achenes are inverted from conventional pesticide applications throughout much of the period associated with blackbird depredation, we also evaluated blackbird repellency of the anthraquinone-based repellent applied to involucral bracts (i.e., the back of sunflower heads) of oilseed sunflower. Blackbirds did not discriminate between untreated involucral bracts and those treated with foliar applications comparable to 4.7 l/ha or 9.4 l/ha; blackbirds consumed more achenes from untreated sunflower heads than from those treated with 18.7 l/ha of the anthraquinone-based repellent. Supplemental repellent efficacy studies should investigate blackbird response to anthraquinone-based repellents (e.g., ≥4.7 l/ha) within 10–100 ha sunflower fields and include independent field replicates with predicted bird damage, repellent application strategies developed for protection of ripening crops, pre- and at-harvest repellent residues, and bird damage and crop yield measurements.

Published by Elsevier Ltd.

1. Introduction

The feeding behavior of red-winged blackbirds (*Agelaius phoeniceus* Linnaeus), common grackles (*Quiscalus quiscula* Linnaeus) and yellow-headed blackbirds (*Xanthocephalus xanthocephalus* Bonaparte) negatively impacts production of confectionery and oilseed sunflower (*Helianthus annuus* Linnaeus) each year in the

* Corresponding author. Tel.: +1 970 266 6136; fax: +1 970 266 6138.
E-mail address: Scott.J.Werner@aphis.usda.gov (S.J. Werner).

http://dx.doi.org/10.1016/j.cropro.2014.01.016
0261-2194/Published by Elsevier Ltd.

United States of America (Linz and Hanzel, 1997; Linz et al., 2011; Werner et al., 2005, 2009, 2011). Blackbird damage to sunflower was estimated to be $5.4 million annually in the prime sunflower growing area of North America (Peer et al., 2003). The effectiveness of lethal damage management is typically associated with localized populations closed to immigration; thus, non-lethal alternatives are needed to manage damage caused by non-localized, or mobile blackbirds to sunflower crops (Linz et al., 2011).

Chemical repellents provide a non-lethal alternative to managing wildlife damages to agricultural production, including blackbird depredation of sunflower crops (recently reviewed by Linz et al., 2011). Anthraquinone was identified as a promising avian repellent in the early 1940's (Heckmanns and Meisenheimer, 1944). Anthraquinone-based repellents have been used to effectively protect rice seed from blackbirds under captive and field conditions (Avery et al., 1997, 1998; Cummings et al., 2002a, 2002b; Neff and Meanley, 1957), turf from Canada goose grazing in captivity (Blackwell et al., 1999; Dolbeer et al., 1998), and whole-kernel corn and ripening corn from captive sandhill cranes and blackbirds, respectively (Blackwell et al., 2001; Carlson et al., 2013). Anthraquinone is a naturally-occurring, cathartic purgative; its action is principally on the large intestine, and it is not effective if transit through the small intestine is delayed (Fraser and Bergeron, 1991). No anthraquinone-based repellents are registered currently for agricultural applications in the United States of America.

We previously conducted laboratory efficacy experiments to estimate the threshold concentration of an anthraquinone-based repellent (active ingredient 50% 9,10-anthraquinone; Arkion Life Sciences, New Castle, DE, USA) for red-winged blackbirds (1475 ppm anthraquinone; Werner et al., 2009) and common grackles (9200 ppm anthraquinone; Werner et al., 2011). We conducted laboratory residue testing with confectionery sunflower heads treated with applications of the anthraquinone-based repellent comparable to 9.4 l/ha, 18.7 l/ha and 37.4 l/ha; we observed 3489 ppm, 6001 ppm and 16,638 ppm anthraquinone among sunflower seeds (i.e., achenes) sampled from these treated sunflower heads, respectively (Werner et al., 2011). Based upon these laboratory results, we predicted that our CO_2 backpack application of 18.7 l/ha to all sunflower heads in treated enclosures (i.e., experimental application strategy with expected maximized residues) would effectively repel common grackles within a confectionery sunflower field (Werner et al., 2011). We observed 18% sunflower damage among anthraquinone-treated enclosures and 64% damage among untreated enclosures populated with common grackles (Werner et al., 2011).

Although laboratory and field efficacy studies have been conducted for several chemical repellents on rice and confectionery sunflower (Avery et al., 2005; Cummings et al., 2002a, 2002b, 2011; Linz et al., 2006; Werner et al., 2007, 2008a, 2008b, 2010), additional research was needed to (1) evaluate field residues of blackbird repellents on oilseed sunflower and (2) develop repellent application strategies for protection of ripening agricultural crops (e.g., ground-based or aerial applications useful for field applications avian repellents). Although field studies of experimental pesticides are limited to <4.05 ha by the United States Environmental Protection Agency, these field residue and laboratory efficacy data are necessary for the commercial development and registration of agricultural pesticides in the United States of America. Thus, our purpose was to investigate experimental applications of an anthraquinone-based repellent for non-lethal protection of oilseed sunflower crops. Cost-effective field applications of agricultural pesticides often include a single application of combined chemicals (e.g., insecticides, fungicides, avian repellents). Thus, our objectives were to evaluate (1) the chemical residues from field applications of the anthraquinone-based repellent on ripening oilseed sunflower, and blackbird feeding responses to the anthraquinone-based repellent when (2) combined with either a registered insecticide or a registered fungicide and (3) applied to the back of sunflower heads (i.e., surface available for foliar repellent applications on ripening crops). The capture, care and use of all birds associated with our studies were approved by the Animal Care and Use Committee of the United States Department of Agriculture's (USDA) National Wildlife Research Center (NWRC Study Protocols QA-1739, QA-1793; S.J. Werner- Study Director).

2. Methods

Field residue studies and laboratory efficacy experiments are necessary for the development, registration, and commercialization of experimental pesticides in the United States of America, including blackbird repellents for sunflower crop protection (Werner et al., 2009, 2011). We conducted a field residue study to evaluate an anthraquinone-based repellent (Arkion Life Sciences) as a red-winged blackbird repellent within a ripening oilseed sunflower field. We conducted five subsequent laboratory efficacy experiments to evaluate blackbird feeding responses to experimental applications of the anthraquinone-based repellent on oilseed sunflower.

2.1. Field residue study in ripening oilseed sunflower

We established 24 enclosures, or netted plots (each 3.7 m long × 4.0 m wide × 1.8–3.1 m tall; 1.5-cm mesh, polypropylene netting) within the maturing oilseed sunflower field on near Steele, North Dakota on July 20–21, 2010. All nets were suspended with plots constructed of 5-cm diameter, galvanized poles. The bottom of all nets was secured within the field using 45-cm rebar stakes driven vertically through 3.7-m wooden boards (extended horizontally to complete enclosures). A zipper was installed in one end of each enclosure to enable daily care of test subjects throughout the field residue study.

For ripening sunflower, >75% of annual blackbird damage occurs within the first 18 days after anthesis (i.e., flowering period; Cummings et al., 1989). The end of anthesis for sunflower is marked by the emergence of the last anther, which coincides with the beginning of yellow ray flower drop (Siddiqui, 1975). We planned our field residue study with red-winged blackbirds based upon our previous laboratory and field efficacy results (Werner et al., 2009, 2011). Thus, a backpack CO_2 sprayer was used to apply 0 l/ha, 4.7 l/ha, or 9.4 l/ha of the anthraquinone-based repellent to the achenes on all sunflower heads within treated field enclosures (n = eight enclosures per treatment, including untreated control) on August 24, when >50% of sunflower within our enclosures was at the R-6 growth stage (i.e., anthesis complete, ray flowers wilting or falling, heads drooping toward ground). A 100 ml sample of each repellent tank formulation was collected and all liquid samples were frozen in a labeled amber jar for anthraquinone residue analyses. Ultraviolet/visible (UV/VIS) spectrophotometry was used to analyze anthraquinone concentrations (±1 ppm anthraquinone) among tank mixtures associated with our field residue study.

On the day subsequent to the repellent application (August 25), we populated each of the 24 enclosures with ten experimentally-naïve red-winged blackbirds. Ten blackbirds per enclosure were maintained throughout the 14-day study. Maturing sunflower between enclosures (1.5–2 m tall) provided visual isolation among enclosures throughout the study. Blackbirds fed freely within field enclosures throughout the study. We offered 300 g of a maintenance diet (milo) in all treated and untreated enclosures, daily throughout the field study (Werner et al., 2011). Consumption of the maintenance diet (±1 g) was measured on odd days throughout the study (beginning day three). Blackbirds were removed from all enclosures on test day 15 (September 8). Sunflower achenes were sampled among enclosures on August 25, September 8, and October 20 (i.e., prior to field harvest) for subsequent analytical chemistry.

Reversed-phase, high performance liquid chromatography (HPLC) with ultraviolet detection was used to quantify anthraquinone concentrations among pomace and oil samples from treated sunflower heads (±1 ppm anthraquinone). Crushed sunflower achenes and sunflower oil were weighed (0.25 g) and extracted with chloroform. Acetonitrile was added to the extracts (one:one ratio) and the samples were filtered. An aliquot was passed through an aminopropyl solid phase extraction (SPE) column followed by evaporation of the solvent. The residue was reconstituted in methanol, filtered and transferred into an HPLC vial, capped, and analyzed (Table 1). A six-level, linear calibration curve was used to calculate the concentration of anthraquinone at 254 nm. Peaks at the 325 nm wavelength were used only to confirm the presence of anthraquinone. The method limit of detection (MLOD) was calculated to be 1.7 ppm anthraquinone in pomace from crushed sunflower achenes and 4.2 ppm anthraquinone in sunflower oil using a one-tailed critical *t*-test ($\alpha = 0.005$) and control matrices fortified at the 4.0 μg/g level. Descriptive statistics ($\bar{x} \pm$ SE) were used to summarize anthraquinone residues on sunflower achenes sampled within repellent-treated and untreated enclosures.

Because field studies of experimental pesticides are limited to <4.05 ha by the United States Environmental Protection Agency and local abundance of migratory birds can change daily (i.e., independent of experimental treatments), we previously used these experimental enclosures to successfully investigate field efficacy of an anthraquinone-based repellent for newly-planted rice and ripening confectionery sunflower (Cummings et al., 2011; Werner et al., 2011). Thus, repellent efficacy was evaluated for oilseed sunflower based upon comparative sunflower damage and harvested achene mass (i.e., sunflower yield) between repellent-treated and untreated enclosures. All sunflower heads were manually harvested within treated and untreated enclosures on September 9–10. Upon manual harvest, we visually estimated damage (i.e., achene removal; ±10% surface area) of each head in all enclosures using graduated-transparency templates (10 cm, 15 cm, 20 cm, 25 cm and 30 cm diameter; Werner et al., 2011). A stationary thresher (USDA Agricultural Research Service, Akron, CO, USA) was used to remove sunflower achenes from harvested heads. All harvested achenes were dried and weighed to determine sunflower yield for each enclosure (±1.0 kg). All treated sunflower was destroyed upon the completion of the study per existing pesticide regulations.

After considering independence, inspecting data for normality and successfully conducting Levene's test for equal variances ($\alpha = 0.05$), we used an ANOVA to analyze percent damage to sunflower heads and comparative sunflower yield associated with our field residue study (Proc GLM, SAS v9.2). Tukey's tests were used to separate means of ANOVA effects ($\alpha = 0.05$) and descriptive statistics (95% confidence intervals and $\bar{x} \pm$ SE) were used to summarize maintenance diet consumption, and sunflower damage and yield between repellent-treated and untreated enclosures.

Table 1
Operating conditions of a high performance liquid chromatograph for determination of anthraquinone residue concentrations on crushed sunflower achenes and sunflower oil from a field residue study, and sunflower achenes from laboratory efficacy experiments.

Parameter	Operating conditions	
	Field residue study	Laboratory efficacy experiments
Mobile phase	De-ionized water (42% at 0 min and 0.25 min; and 5% at 1.25 min), and	De-ionized water with 0.1% trifluoroacetic acid (75% at 0 min, 2 min, 16.01 min, and 17.5 min; and 0% at 16.00 min), and
	Acetonitrile (58% at 0 min and 0.25 min; and 95% at 1.25 min)	Acetonitrile with 0.1% trifluoroacetic acid (25% at 0 min, 2 min, 16.01 min, and 17.5 min; and 100% at 16.00 min)
Injection volume	5.0 μL	5.0 μL
Analytical column	Restek Pinnacle DB Biphenyl 3 μm, 100 × 2.1 mm	Phenomenex Gemini 3 μm C18 110A, 150 × 3.0 mm
Temperature	50.0 °C	60.0 °C
Run time	7 min	20 min
Flow rate	0.300 mL/min at 0 min, 0.25 min, and 1.25 min	0.500 mL/min at 0 min, 2 min, and 17.5 min; and 0.800 mL/min at 16.0 min
Detector	Ultraviolet @ 254 nm and 325 nm	Ultraviolet @ 254 nm and 325 nm

2.2. Concentration–response experiments

Two concentration–response (no-choice) feeding experiments were conducted in January–March 2011 to establish a concentration–response relationship for red-winged blackbirds offered oilseed sunflower achenes treated with the anthraquinone-based repellent and Asana® XL insecticide (a.i. 8.4% esfenvalerate; DuPont, Wilmington, DE, USA; experiment one) or the anthraquinone-based repellent and Headline® fungicide (a.i. 23.6% pyraclostrobin; BASF Corp., Research Triangle Park, NC, USA; experiment two). Asana® XL insecticide and Headline® fungicide were selected for these experiments because they are the most commonly used pesticides for late-season applications concurrent with blackbird depredation in the United States of America (L. Kleingartner, National Sunflower Association; pers. commun.). Our laboratory efficacy experiments were conducted at the NWRC outdoor animal research facility in Fort Collins, Colorado, USA.

We maintained 110 male red-winged blackbirds in 3.1 m long × 6.2 m wide × 3.1 m tall cages (20–40 birds/cage) within an outdoor aviary for at least two weeks prior to our concentration-response experiments. The outdoor aviary consists of a wire mesh-sided building designed to hold blackbirds and starlings in an outdoor environment. Blackbirds were visually isolated among all cages; only the top 30 cm of all cages were visible between each of four rows of cages. We provided free access to a maintenance diet for all blackbirds during quarantine and holding, and water *ad libitum* throughout our laboratory efficacy experiments. The maintenance diet for our laboratory efficacy experiments included two parts millet:one cracked corn:one milo:one safflower.

Concentration–response experiments were conducted in individual cages (0.9 m long × 1.8 m wide × 0.9 m tall) within the small-bird testing facility. The small-bird testing facility consists of wire mesh-sided building that contains 66 cages designed for choice and no-choice feeding experiments with individual birds in an outdoor environment. Seed treatments for our laboratory efficacy experiments were formulated by applying aqueous solutions (60 ml/kg solution achenes) to oilseed sunflower using a rotating mixer and household spray equipment.

We used previously-described HPLC to quantify anthraquinone concentrations among our experimental seed treatments (±10 ppm anthraquinone). We did this by collecting a 200 g sample of each seed treatment shortly after formulation for anthraquinone residue analyses. Samples of sunflower achenes were ground and 0.25 g replicates were extracted with chloroform and filtered. An aliquot was passed through an aminopropyl solid phase extraction (SPE) column followed by evaporation of the solvent. The residue was reconstituted in methanol, filtered and transferred into an HPLC vial, capped, and analyzed (Table 1). A five-level, linear calibration curve was used to calculate the concentration of anthraquinone at 254 nm. Peaks at the 325 nm wavelength were used only to confirm the presence of anthraquinone. The MLOD was calculated to be 3.9 ppm anthraquinone using a one-tailed critical *t*-test ($\alpha = 0.005$) and control matrices fortified at the 40.0 μg/g level.

For each of the two experiments, we offered 55 experimentally-naïve red-winged blackbirds untreated oilseed sunflower achenes *ad libitum* in one food bowl for five days of pre-experiment acclimation in individual cages. We subsequently offered each blackbird 30 g of untreated oilseed sunflower achenes in one bowl at 08:00 h, daily on study days one to three. Blackbirds were ranked based upon average pretreatment consumption and assigned them to one of six treatment groups (n = nine to ten blackbirds/group) such that each group was populated with birds that exhibited similar daily consumption. Treatments were randomly assigned among groups (0.02%, 0.035%, 0.05%, 0.1%, 0.25% and 0.5% anthraquinone; targeted concentrations, wt/wt; Werner et al., 2009, 2011). For all groups, seed treatments also included either 0.1% Asana® XL insecticide (i.e., 1 ml Asana®/kg; experiment one) or 0.14% Headline® fungicide (i.e., 1.4 ml Headline®/kg; experiment two). Treatment concentrations of Asana® XL and Headline® were based upon manufacturer-recommended label rates. Chemical compatibility of the anthraquinone-based repellent was observed when combined with Asana® XL insecticide or Headline® fungicide (i.e., stable dispersion and no obvious settling 1-h subsequent to initial inversion).

For the purpose of establishing a concentration–response relationship of the anthraquinone-based repellent in blackbirds, we offered 30 g of treated sunflower achenes in one bowl to all birds on study day four and determined the mass (±0.1 g) of uneaten achenes and spillage at 08:00 h on study day five (Werner et al., 2009, 2011). Daily achene consumption was measured throughout the experiments (study days two to five). Unconsumed achenes (remaining in food bowls) and spillage were collected (at 08:00 h, daily) and weighed (±0.1 g). Weight change of achenes (e.g., desiccation) was measured daily by weighing achenes offered within a vacant cage throughout our experiments.

We hypothesized that (1) repellency would be directly related to repellent concentration during our concentration–response experiments and (2) repellency would not be negatively affected by formulations including a registered pesticide previously developed for its insecticidal or fungicidal activity. We previously defined ≥80% repellency as efficacious during our laboratory feeding experiments (Werner et al., 2009, 2010, 2011). Thus, we predicted that consumption of efficacious treatments (i.e., threshold repellency) would be <20% of pretreatment consumption during the blackbird concentration-response experiments. The dependent measure of our concentration-response experiments was calculated as test consumption of treated achenes relative to average pretreatment consumption of untreated achenes (i.e., percent repellency = [one − (test consumption/average pretreatment consumption)] * 100). Non-linear regression procedures (Proc Reg, SAS v9.2) were used to analyze repellency as a function of anthraquinone concentration (Werner et al., 2009, 2011).

2.3. Preference experiments

Both concentration-response testing and preference testing are recommended to reliably investigate the behavioral response of test subjects to chemical repellents (Werner et al., 2010). Thus, two preference (choice) feeding experiments were conducted in January–March 2011 to evaluate red-winged blackbird consumption of untreated oilseed sunflower achenes versus those treated with the anthraquinone-based repellent and Asana® XL insecticide (experiment one) or the anthraquinone-based repellent and Headline® fungicide (experiment two).

We maintained 22 male red-winged blackbirds in a 3.1 m long × 6.2 m wide × 3.1 m tall cage within an outdoor aviary for at least two weeks prior to our preference experiments (i.e., quarantine, holding). In supplement to our concentration-response experiments, preference experiments were conducted in individual cages (0.9 m long × 1.8 m wide × 0.9 m tall) within the small-bird testing facility. Blackbirds were visually isolated among all cages. Daily achene consumption was measured throughout the experiments (study days two to five). Unconsumed achenes (remaining in food bowls) and spillage were collected (at 08:00 h, daily) and weighed (±0.1 g). Consumption was measured independently for the food bowls offered on the north and south sides of each cage during the preference experiments. Weight change of achenes (e.g., desiccation) was measured daily by weighing achenes offered within a vacant cage throughout our experiments.

Eleven experimentally-naïve red-winged blackbirds were randomly to each of the two experiments. All blackbirds were offered untreated oilseed sunflower achenes *ad libitum* in two food bowls for five days of pre-experiment acclimation in individual cages. Each blackbird was subsequently offered 30 g of untreated sunflower in one bowl, and 30 g of sunflower treated with 0.25% anthraquinone (targeted concentration, wt/wt; Werner et al., 2009, 2011) and 0.1% Asana® XL insecticide (experiment one) or 0.25% anthraquinone and 0.14% Headline® fungicide (experiment two) in the second bowl at 08:00 h, daily on study days one to four. The north–south placement of food bowls was randomized on the first day and alternated on subsequent days of the preference experiments. A 200 g sample of each seed treatment was collected shortly after formulation for subsequent quantification of anthraquinone concentrations via previously-described HPLC (Table 1).

The dependent measure for our preference experiments was average (daily) test consumption of treated and untreated achenes. Consumption data were subjected to a repeated measures ANOVA (Proc Mixed, SAS v9.2). The random effect of our model was bird subjects, the between-subjects effect was treatment (treated vs. untreated achenes) and the within-subject effect was test day. We analyzed the treatment effect and the treatment by day interaction. Descriptive statistics ($\bar{x} \pm$ SE) were used to summarize consumption of treated and untreated achenes during the preference experiments.

2.4. Anthraquinone-based repellent applied to back of sunflower heads

Ripening sunflower achenes are not effectively treated with aerial repellent applications because ripening heads face downward six to eight weeks prior to harvest (i.e., vertically opposed to downward spray). Thus, one additional preference experiment was conducted in March 2011 to evaluate red-winged blackbird response to our treatment of involucral bracts (i.e., the back of sunflower heads) with the anthraquinone-based repellent.

We maintained 44 male red-winged blackbirds in 3.1 m long × 6.2 m wide × 3.1 m tall cages (20–40 birds/cage) within an outdoor aviary for at least two weeks prior to our laboratory efficacy experiment (i.e., quarantine, holding). Our preference experiment was conducted with experimentally-naïve birds in individual cages (0.9 m long × 1.8 m wide × 0.9 m tall) within the small-bird testing facility. Blackbirds were visually isolated among all cages;

only the top 30 cm of all cages were visible between each of two paired rows of cages. Involucral bracts were treated by applying aqueous solutions to the backs of oilseed sunflower heads using a CO_2 backpack sprayer. A 200 g sample of sunflower achenes was collected from treated heads shortly after formulation for subsequent quantification of anthraquinone concentrations via previously-described HPLC (Table 1). All blackbirds were offered 50 g of untreated oilseed sunflower in each of two food bowls for five days of pre-experiment acclimation in individual cages. Blackbirds were ranked based upon average acclimation consumption and assigned them to one of four treatment groups ($n = 11$ blackbirds/group) such that each group was populated with birds that exhibited similar daily consumption. Treatments were randomly among groups (untreated control; and treatments comparable to 4.7 l/ha, 9.4 l/ha and 18.7 l/ha of the anthraquinone-based repellent).

For each group, one sunflower head was offered on each of the north and south sides of each cage. All sunflower heads were extended vertically within cages using 40-cm stalks attached to wooden stands (i.e., sunflower heads were naturally attached to their stalks). Because diameter varied among sunflower heads (10–18 cm), we balanced the diameter of offered heads for each bird (i.e., paired, treated–untreated heads had similar diameters; ± 2.3 cm) and among treatment groups (±0.76 cm). For the control group, both sunflower heads were untreated. For the remaining groups, one sunflower head was treated with the anthraquinone-based repellent and the other head was untreated. The north-south placement of sunflower heads was randomized on the first day and alternated on the subsequent day of the two-day preference experiment. The mass of all sunflower heads was measured prior and subsequent to our two-day experiment. Achene spillage was collected and weighed (±0.1 g) at 08:00 h, daily throughout the experiment. Consumption was measured independently for treated and untreated sunflower heads.

The dependent measure for our preference experiment was the difference in the average mass of treated and untreated sunflower heads prior and subsequent to our experiment. For each treatment (0 l/ha, 4.7 l/ha, 9.4 l/ha and 18.7 l/ha of the anthraquinone-based repellent), we used Wilcoxon two-sample tests (Proc npar1way Wilcoxon, SAS v9.2) to analyze and 95% confidence intervals to summarize differences in mass of sunflower heads subsequent to the preference experiment.

3. Results

3.1. Field residue study in ripening oilseed sunflower

The concentration of anthraquinone within tank mixtures was 27,432 ppm and 47,347 ppm anthraquinone for the 4.7 l/ha and 9.4 l/ha of the anthraquinone-based repellent, respectively. From test day one to day 15, anthraquinone concentrations on achenes treated with 4.7 l/ha and 9.4 l/ha decreased from 481 ppm to 385 ppm anthraquinone, and 978 ppm–952 ppm anthraquinone, respectively (Table 2). Anthraquinone concentrations observed prior to field harvest also varied in relation to the treatments (Table 2). Anthraquinone concentrations in oil from crushed achenes was similar from both treatments; relative to the 4.7 l/ha treatment, more anthraquinone was observed in pomace from crushed achenes treated with 9.4 l/ha of the anthraquinone-based repellent (Table 2). We detected <10 ppm and <1.7 ppm anthraquinone residues in untreated oil and untreated pomace samples, respectively (Table 2). A total of 8.5 cm of rain was recorded in Steele, North Dakota during our 15-day field study.

Blackbirds within treated enclosures caused less (Tukey's $P < 0.05$) sunflower damage (33–34%) than those within untreated enclosures (44%; $F_{2,23} = 8.02$, $P = 0.003$; Fig. 1). Sunflower yield within treated enclosures (2.6 kg, dry weight) was higher (Tukey's $P < 0.05$) than that within untreated enclosures (2.1 kg; $F_{2,23} = 5.81$, $P = 0.010$; Fig. 1). As predicted, blackbirds within treated enclosures consumed more maintenance diet (58.5–81.2 g milo/day for 4.7 l/ha treatment, 64.3–80.3 g/day for 9.4 l/ha; 95% CI) than those within untreated enclosures (26.0–43.1 g/day) throughout the 15-day field study.

Table 2
Anthraquinone residues (AQ; $\bar{x} \pm$ SE) detected within a ripening oilseed sunflower field treated with an anthraquinone-based repellent (a.i. 50% 9,10-anthraquinone). The repellent was applied on August 24, 2010 to all sunflower heads within treated blackbird enclosures using a CO_2 backpack sprayer at the R-6 growth stage (anthesis complete, ray flowers wilting/falling). The method limit of detection (MLOD) for the residue analysis of pomace from crushed sunflower was 1.7 ppm anthraquinone.

Sample	Sample date	Treatment	AQ (ppm)
Sunflower achenes from treated heads			
Enclosures populated with birds	August 25, 2010	4.7 l/ha	481 ± 78
		9.4 l/ha	978 ± 197
Enclosures depopulated with birds	September 8, 2010	4.7 l/ha	385 ± 40
		9.4 l/ha	952 ± 112
Prior to field harvest	October 20, 2010	4.7 l/ha	304 ± 53
		9.4 l/ha	789 ± 27
Oil from crushed achenes	October 20, 2010	0 l/ha	6 ± 1
		4.7 l/ha	402 ± 22
		9.4 l/ha	462 ± 9
Pomace from crushed achenes	October 20, 2010	0 l/ha	<MLOD
		4.7 l/ha	27 ± 2
		9.4 l/ha	165 ± 16

3.2. Concentration–response experiments

We observed a positive concentration–response relationship among tested concentrations of anthraquinone on oilseed sunflower achenes also treated with Asana® XL insecticide (Fig. 2) or Headline® fungicide (Fig. 3). Blackbirds exhibited >80% repellency for sunflower treated with (1) 1810 ppm anthraquinone and 0.1% Asana® XL insecticide (Fig. 2), and (2) 1700 ppm anthraquinone and

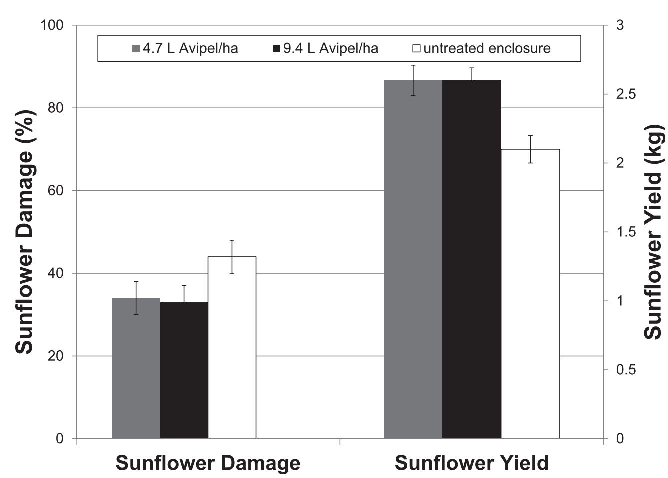

Fig. 1. Mean sunflower damage and sunflower yield (±SE) among repellent-treated and untreated enclosures in a ripening oilseed sunflower field. Red-winged blackbirds (*Agelaius phoeniceus* Linnaeus) were maintained in experimental enclosures ($n =$ eight enclosures per treatment, ten blackbirds/enclosure) for 14 days subsequent to the repellent application. The anthraquinone-based repellent (4.7 and 9.4 l/ha; a.i. 50% 9,10-anthraquinone) was applied to all sunflower heads within treated enclosures using a CO_2 backpack sprayer at the R-6 growth stage (anthesis complete, ray flowers wilting/falling).

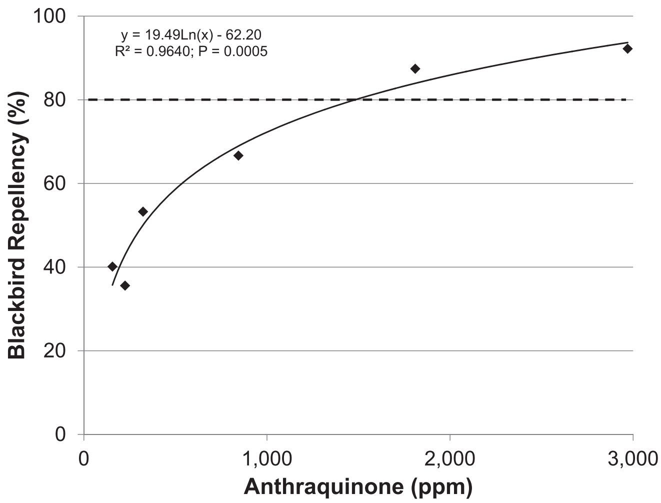

Fig. 2. Mean feeding repellency associated with varying concentrations of the anthraquinone-based repellent (a.i. 50% 9,10-anthraquinone) plus Asana® XL insecticide (a.i. 8.4% esfenvalerate) offered to red-winged blackbirds (*Agelaius phoeniceus* Linnaeus). Repellency represents test consumption relative to average, pretreatment consumption of oilseed sunflower (n = nine to ten blackbirds/concentration).

0.14% Headline® fungicide (Fig. 3). Based upon statistical functions relating blackbird repellency (y) and anthraquinone concentration (x), a threshold concentration of 1475 ppm anthraquinone was predicted to elicit 80% blackbird repellency for oilseed sunflower achenes also treated with Asana insecticide (Fig. 2) or Headline fungicide (Fig. 3).

3.3. Preference experiments

Red-winged blackbirds reliably discriminated between untreated oilseed sunflower achenes and those treated with 1810 ppm anthraquinone and 0.1% Asana® XL insecticide ($F_{1,10} = 168.57$, $P < 0.001$). On average, blackbirds consumed less than 1 g (±0.4 g) of treated sunflower and 7.3 ± 0.4 g of untreated sunflower during the four-day experiment (Fig. 4a). No treatment by day interaction was observed during the first preference experiment ($F_{6,60} = 1.25$, $P = 0.2961$).

Blackbirds also reliably discriminated between untreated oilseed sunflower achenes and those treated with 1700 ppm anthraquinone and 0.14% Headline® fungicide ($F_{1,10} = 1379.33$, $P < 0.001$). On average, blackbirds consumed less than one gram (±0.1 g) of treated sunflower and 6.4 ± 0.2 g of untreated sunflower during the four-day experiment (Fig. 4b). A treatment by day interaction was observed during the second preference experiment ($F_{6,60} = 2.68$, $P = 0.0228$); blackbirds consumed more untreated sunflower than sunflower treated with anthraquinone and Headline® fungicide on test days 1–4 (Tukey's $P < 0.05$).

3.4. Anthraquinone-based repellent applied to back of sunflower heads

We observed no difference in the mass of untreated sunflower heads offered to blackbirds in the control group subsequent to the two-day preference experiment ($Z = -0.7880$, $P = 0.4307$). Blackbirds exposed to sunflower heads treated with an application rate comparable to 4.7 l/ha of the anthraquinone-based repellent consumed 0.6–9.0 g from treated heads (95% CI) and 2.0–8.2 g from untreated heads ($Z = -0.1642$, $P = 0.8696$). Blackbirds in the 9.4 l/ha treatment group consumed <1 g–7.7 g from treated heads and 2.6–16.0 g from untreated heads ($Z = -1.9048$, $P = 0.0568$).

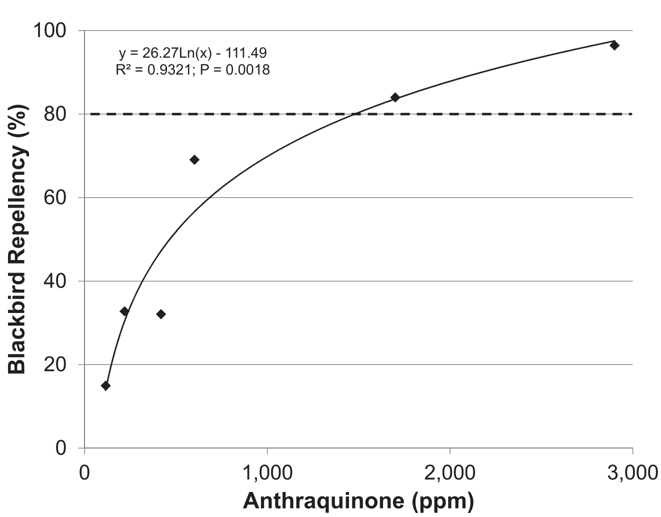

Fig. 3. Mean feeding repellency associated with varying concentrations of the anthraquinone-based repellent (a.i. 50% 9,10-anthraquinone) plus Headline® fungicide (a.i. 23.6% pyraclostrobin) offered to red-winged blackbirds (*Agelaius phoeniceus* Linnaeus). Repellency represents test consumption relative to average, pretreatment consumption of oilseed sunflower (n = nine to ten blackbirds/concentration).

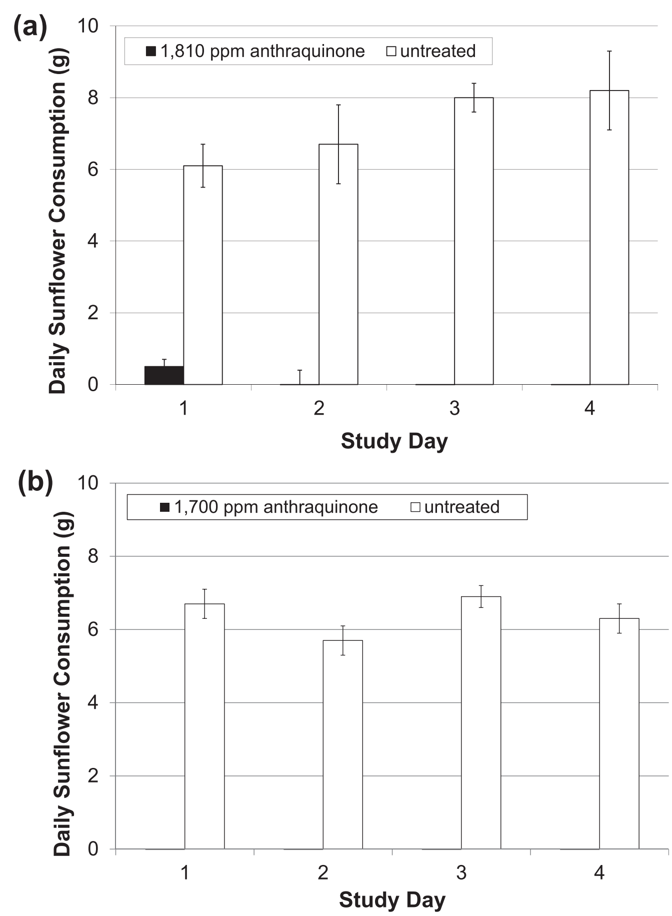

Fig. 4. Mean consumption (±SE) of oilseed sunflower achenes offered to red-winged blackbirds (*Agelaius phoeniceus* Linnaeus). Blackbirds were offered (a) untreated achenes and those treated with the anthraquinone-based repellent (a.i. 50% 9,10-anthraquinone) plus Asana® XL insecticide (a.i. 8.4% esfenvalerate), or (b) untreated achenes and those treated with the anthraquinone-based repellent plus Headline® fungicide (a.i. 23.6% pyraclostrobin).

Blackbirds in the 18.7 l/ha treatment group consumed <1 g–5.0 g from treated heads and 7.4–11.4 g from untreated heads ($Z = -2.8580$, $P = 0.0043$). Thus, non-overlapping confidence intervals were observed only among blackbirds exposed to sunflower heads treated with an application rate comparable to 18.7 l/ha. The 4.7 l/ha, 9.4 l/ha and 18.7 l/ha treatments yielded 45 ppm, 141 ppm and 320 ppm anthraquinone on sunflower achenes, respectively.

4. Discussion

We observed field efficacy of a CO_2 backpack application of the anthraquinone-based repellent that provided 380–480 ppm (4.7 l/ha) and 950–980 ppm anthraquinone (9.4 l/ha) throughout our field residue study, respectively. Field efficacy was therefore observed at anthraquinone concentrations less than the threshold anthraquinone concentration previously estimated for red-winged blackbirds (i.e., 1475 ppm anthraquinone; Werner et al., 2009). Thus, laboratory and field efficacy testing are both necessary for developing reliable recommendations of effective field application rates, species-specific repellency, and individual and group feeding responses.

Although anthraquinone concentrations remained relatively stable throughout our field residue study, we also detected >400 ppm anthraquinone in oil from crushed sunflower achenes, and 27 ppm and 165 ppm anthraquinone in pomace from crushed sunflower achenes within enclosures treated with CO_2 backpack applications of 4.7 l/ha and 9.4 l/ha, respectively. Replicated field residue studies are necessary to quantify at-harvest repellent concentrations associated with aerial or ground-based applications on ripening crops. We also detected 6 ppm anthraquinone in oil from untreated sunflower achenes. Interestingly, four analogs of 9,10-anthraquinone were isolated from the culture of an endophytic fungus (*Phoma sorghina* Sacc.) associated with Mexican sunflower (*Tithonia diversifolia* Hemsl.; de Souza Borges and Tallarico Pupo, 2006).

We observed 10% less damage and greater yield within sunflower enclosures treated with 4.7 l/ha or 9.4 l/ha of the anthraquinone-based repellent than in untreated enclosures. During the 2012 growing season, sunflower yield was 1696 kg/ha and 745,042 ha were harvested in the United States of America (National Sunflower Association; http://www.sunflowernsa.com/). At $0.64/kg sunflower achenes (United States dollars) received by growers during the 2011–2012 marketing year (http://www.sunflowernsa.com/), a 10% decrease in sunflower damage would therefore represent $108.54/ha. Thus, the anthraquinone-based repellent effectively protected ripening oilseed sunflower from monetary damages caused by red-winged blackbirds during our field study.

Our field application was made using a CO_2 backpack sprayer within small enclosures (3.7 m long × 4.0 m wide). Development of commercial application strategies is presently needed for chemical repellents and protection of ripening agricultural crops. For example, ground-based spray equipment with drop nozzles and upward-oriented spray tips might be used to effectively treat the underside of ripening sunflower heads (i.e., sunflower receptacle including ripening achenes) with foliar pesticides, including avian repellents (Linz et al., 2011). Replicated field residue studies should be conducted to evaluate such ground-based spray equipment for avian repellent applications. Although we recommend supplemental field efficacy testing of anthraquinone-based repellents and other chemical repellents using larger plots, pesticide regulations limit agricultural applications of unregistered products to <4.05 ha annually in the United States of America.

The addition of Asana® XL insecticide or Headline® fungicide to the anthraquinone-based repellent formulations did not affect blackbird repellency. We predicted a threshold concentration of 1475 ppm anthraquinone for red-winged blackbirds based upon our concentration-response experiments with the anthraquinone-based repellent plus 0.1% Asana® XL insecticide or 0.14% Headline® fungicide. The comparable threshold concentration for red-winged blackbirds offered sunflower achenes treated only with anthraquinone was also 1475 ppm anthraquinone (Werner et al., 2009). Thus, anthraquinone can be effectively added to tank mixtures including these commonly used, late-season pesticides. Additional testing is warranted to investigate the potential effects of anthraquinone-based repellents to insecticide and fungicide efficacy.

Sunflower achenes treated with the anthraquinone-based repellent plus Asana® XL insecticide or Headline® fungicide effectively repelled red-winged blackbirds in captivity. Red-winged blackbirds previously consumed an average of 5.1 g/bird (±0.4 SE) of oilseed sunflower treated with 0.046% Asana® XL insecticide and 6.3 ± 0.4 g/bird of untreated oilseed sunflower (Linz et al., 2006). In contrast, blackbirds consumed less than one gram of sunflower treated with 1810 ppm anthraquinone and 0.1% Asana® XL insecticide or 1700 ppm anthraquinone and 0.14% Headline® fungicide, and 6–7 g of untreated achenes during our preference experiments. Similarly, blackbirds consumed less than one gram (±0.2 SE) of sunflower achenes treated only with 1778 ppm anthraquinone and 6.5 ± 0.4 g of untreated achenes during our previous preference experiment (Werner et al., 2009). Contingent upon its registration for agricultural applications, an anthraquinone-based repellent could therefore be included in late-season pesticide applications for combined protection from insect, fungus and blackbird damages. Such combined applications (i.e., single versus multiple applications) would minimize fixed-cost expenditures associated with agricultural pesticide applications (e.g., fuel, maintenance, labor).

Blackbirds were not effectively repelled by applications comparable to <18.7 l/ha of the anthraquinone-based repellent to involucral bracts (i.e., the back of sunflower heads). Thus, an aerial application of this repellent (e.g., ≤9.4 l/ha) is unlikely to influence sufficient blackbird repellency. At our application rate comparable to 18.7 l/ha, blackbirds were repelled by exposure to anthraquinone-treated involucral bracts and/or the 320 ppm anthraquinone on inverted sunflower achenes. Because this concentration is much less than our predicted threshold concentration for red-winged blackbirds offered anthraquinone-treated oilseed sunflower (1475 ppm anthraquinone; Werner et al., 2009), additional field testing (e.g., 14-day behavioral response under field conditions) is needed to evaluate the spatial extent of inferences from these captive preference data.

Our field residue and repellent efficacy results provide a reliable basis for pesticide registrations, and planning for future field applications of anthraquinone-based repellents and the protection of ripening crops from pest blackbirds. Cost-effective applications of blackbird repellents might include a single application of combined pesticides (e.g., insecticides, fungicides, avian repellents) and/or back-of-the-heads applications within ripening sunflower fields. Future repellent efficacy studies should investigate the behavioral response of pest blackbirds to anthraquinone-based repellents (e.g., ≥4.7 l/ha) within 10–100 ha sunflower fields and include independent field replicates with predicted blackbird damage, repellent application strategies developed for protection of ripening crops, pre- and at-harvest repellent residues, and bird damage and crop yield measurements.

Acknowledgments

This research was supported by the National Sunflower Association (Mandan, ND, USA). Our feeding experiments were conducted with the anthraquinone-based repellent (Arkion® Life Sciences, New Castle, DE, USA), Asana®XL insecticide (DuPont, Wilmington, DE, USA) and Headline® fungicide (BASF Corp., Research Triangle Park, NC, USA). Corporate collaborations do not imply endorsement by the United States Department of Agriculture. We thank W. Bleier (North Dakota State University [NDSU], Fargo, ND, USA), T. DeKrey, M. Klosterman, B. Larson, L. Penry, J. Schanandore, M. Strassburg and D. Stonefish for their dedicated assistance with our 2010 sunflower field study. D. Weiskopf (USDA Agricultural Research Service [ARS], Fargo, ND, USA) shelled sunflower head samples and D.P. Wiesenborn (NDSU, Fargo) provided assistance and equipment for preparation of oil and pomace samples from our field study. M.F. Vigil and D.G. Koch (USDA ARS, Arkron, CO, USA) provided assistance and equipment for stationary threshing of achene samples and M.M. Santer (Arkion® Life Sciences) conducted analytical chemistry for our field study. We appreciate the National Wildlife Research Center animal care staff that provided daily care of all birds throughout quarantine and holding for our repellent efficacy studies. We also thank L. Addy Orduna, S.B. Canavelli, J.D. Eisemann, B.A. Kimball, A.M. Mangan, E.N. Rodriguez and M.E. Tobin for constructive feedback from their review of our manuscript.

References

Avery, M.L., Humphrey, J.S., Decker, D.G., 1997. Feeding deterrence of anthraquinone, anthracene, and anthrone to rice-eating birds. J. Wildl. Manag. 61, 1359–1365.
Avery, M.L., Humphrey, J.S., Primus, T.M., Decker, D.G., McGrane, A.P., 1998. Anthraquinone protects rice seed from birds. Crop Prot. 17, 225–230.
Avery, M.L., Werner, S.J., Cummings, J.L., Humphrey, J.S., Milleson, M.P., Carlson, J.C., Primus, T.M., Goodall, M.J., 2005. Caffeine for reducing bird damage to newly seeded rice. Crop Prot. 24, 651–657.
Blackwell, B.F., Seamans, T.W., Dolbeer, R.A., 1999. Plant growth regulator (Stronghold™) enhances repellency of anthraquinone formulation (Flight Control™) to Canada geese. J. Wildl. Manag. 63, 1336–1343.
Blackwell, B.F., Helon, D.A., Dolbeer, R.A., 2001. Repelling sandhill cranes from corn: whole-kernel experiments with captive birds. Crop Prot. 20, 65–68.
Carlson, J.C., Tupper, S.K., Werner, S.J., Pettit, S.E., Santer, M.M., Linz, G.M., 2013. Laboratory efficacy of an anthraquinone-based repellent for reducing bird damage to ripening corn. Appl. Animal Behav. Sci. 145, 26–31.
Cummings, J.L., Guarino, J.L., Knittle, C.E., 1989. Chronology of blackbird damage to sunflowers. Wildl. Soc. Bull. 17, 50–52.
Cummings, J.L., Avery, M.L., Mathre, O., Wilson, E.A., York, D.L., Engeman, R.M., Pochop, P.A., Davis Jr., J.E., 2002a. Field evaluation of Flight Control™ to reduce blackbird damage to newly planted rice. Wildl. Soc. Bull. 30, 816–820.
Cummings, J.L., Pochop, P.A., Engeman, R.M., Davis Jr., J.E., Primus, T.M., 2002b. Evaluation of Flight Control® to reduce blackbird damage to newly planted rice in Louisiana. Int. Biodeterior. Biodegr. 49, 169–173.
Cummings, J.L., Byrd, R.W., Eddleman, W.R., Engeman, R.M., Tupper, S.K., 2011. Effectiveness of AV-1011® to reduce damage to drill-planted rice from blackbirds. J. Wildl. Manag. 75, 353–356.
de Souza Borges, W., Tallarico Pupo, M., 2006. Novel anthraquinone derivatives produced by *Phoma sorghina*, an endophyte found in association with the medicinal plant *Tithonia diversifolia* (Asteraceae). J. Braz. Chem. Soc. 17, 929–934.
Dolbeer, R.A., Seamans, T.W., Blackwell, B.F., Belant, J.L., 1998. Anthraquinone formulation (Flight Control™) shows promise as avian feeding repellent. J. Wildl. Manag. 62, 1558–1564.
Fraser, C.M., Bergeron, J.A., 1991. In: Merck Veterinary Manual, seventh ed. Merck & Co., Inc., USA, p. 1383.
Heckmanns, F., Meisenheimer, M., 1944. Protection of seeds against birds. Patent 2,339,335. U.S. Patent Office, Washington, D.C.
Linz, G.M., Hanzel, J.J., 1997. Birds and sunflower. sunflower technology and production. Agron. Monogr. 35, 881–894.
Linz, G.M., Homan, H.J., Slowik, A.A., Penry, L.B., 2006. Evaluation of registered pesticides as repellents for reducing blackbird (Icteridae) damage to sunflower. Crop Prot. 25, 842–847.
Linz, G.M., Homan, H.J., Werner, S.J., Hagy, H.M., Bleier, W.J., 2011. Assessment of bird-management strategies to protect sunflowers. BioScience 61, 960–970.
Neff, J.A., Meanley, B., 1957. Research on Bird Repellents: Bird Repellent Studies in the Eastern Arkansas Rice Fields. Wildl. Res. Lab., Denver, CO, p. 21.
Peer, B.D., Homan, H.J., Linz, G.M., Bleier, W.J., 2003. Impact of blackbird damage to sunflower: bioenergetic and economic models. Ecol. Appl. 13, 248–256.
Siddiqui, M.Q., 1975. Growth stages of sunflowers and intensity indices for white blister and rust. Plant Dis. Rep. 59, 7–11.
Werner, S.J., Homan, H.J., Avery, M.L., Linz, G.M., Tillman, E.A., Slowik, A.A., Byrd, R.W., Primus, T.M., Goodall, M.J., 2005. Evaluation of Bird Shield™ as a blackbird repellent in ripening rice and sunflower fields. Wildl. Soc. Bull. 33, 251–257.
Werner, S.J., Cummings, J.L., Tupper, S.K., Hurley, J.C., Stahl, R.S., Primus, T.M., 2007. Caffeine formulation for avian repellency. J. Wildl. Manag. 71, 1676–1681.
Werner, S.J., Cummings, J.L., Pipas, P.A., Tupper, S.K., Byrd, R.,W., 2008a. Registered pesticides and citrus terpenes as blackbird repellents for rice. J. Wildl. Manag. 72, 1863–1868.
Werner, S.J., Cummings, J.L., Tupper, S.K., Goldade, D.A., Beighley, D., 2008b. Blackbird repellency of selected registered pesticides. J. Wildl. Manag. 72, 1007–1011.
Werner, S.J., Carlson, J.C., Tupper, S.K., Santer, M.M., Linz, G.M., 2009. Threshold concentrations of an anthraquinone-based repellent for Canada geese, red-winged blackbirds, and ring-necked pheasants. Appl. Animal Behav. Sci. 121, 190–196.
Werner, S.J., Linz, G.M., Tupper, S.K., Carlson, J.C., 2010. Laboratory efficacy of chemical repellents for reducing blackbird damage in rice and sunflower crops. J. Wildl. Manag. 74, 1400–1404.
Werner, S.J., Linz, G.M., Carlson, J.C., Pettit, S.E., Tupper, S.K., Santer, M.M., 2011. Anthraquinone-based bird repellent for sunflower crops. Appl. Animal Behav. Sci. 129, 162–169.