2021

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Research Article

Received: 2 August 2020 Revised: 13 October 2020 Accepted article published: 4 November 2020 Published online in Wiley Online Library: 26 December 2020

(wileyonlinelibrary.com) DOI 10.1002/ps.6171

Inefficiency of anthraquinone-based avian repellents when applied to sunflower: the importance of crop vegetative and floral characteristics in field applications

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Abstract

BACKGROUND: Blackbirds (Icteridae) cause significant damage to sunflower (*Helianthus annuus* L.) prompting the need for effective management tools. Anthraquinone-based repellents can reduce feeding by > 80% in laboratory settings, but require birds to learn the negative association through repellent ingestion. We evaluated an anthraquinone-based repellent applied directly to mature sunflower plants for its ability to reduce bird damage. We used captive male red-winged blackbirds (*Agelaius phoeniceus*) to evaluate efficacy of two anthraquinone-based formulations in varying concentrations and applied in a manner attainable by sunflower producers. We also assessed field application methods for repellent coverage and anthraquinone residues when using ground-rigs equipped with drop-nozzles situated below the crop canopy.

RESULTS: The repellents failed to reduce feeding and birds did not exhibit a preference between untreated and treated sunflowers at concentrations 2.7× the suggested application rate (i.e. 9.35 L ha^{-1} of repellent). In the absence of disk flowers, which obstruct repellent from reaching the achenes, the repellents failed to reduce consumption. Anthraquinone concentrations in field applications were considerably less than those in the laboratory experiments and did not reduce bird damage.

CONCLUSION: Efficacy is difficult to achieve in the field due to application issues where growth patterns and floral components of sunflower limit residues on achenes, thus contact with foraging birds. Although field residues could be improved by increasing anthraquinone concentrations in tank mixtures and decreasing droplet size, repellents optimized for loose achenes are inefficient in reducing avian consumption of sunflower when applied to intact plants in a manner representative of commercial agriculture.

Keywords: foliar treatment; human–wildlife conflict; oilseeds; row crops; vertebrate pest; wildlife damage management

1 INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an important crop grown worldwide.[1] In North America ripening sunflower is prone to blackbird (*Icteridae*) damage.[2] During the fall, birds feed on readily-available, highly-nutritious crops (e.g. corn and sunflower), as they molt and form flocks in preparation for migration.[3,4] These mixed flocks can number > 100 000 individuals and primarily contain red-winged blackbirds (*Agelaius phoeniceus*), but also include yellow-headed blackbirds (*Xanthocephalus xanthocephalus*), common grackles (*Quiscalus quiscula*), and European starlings (*Sturnus vulgaris*).[1] Although regional blackbird damage to sunflower is ~2%, localized crop damage often exceeds levels where it becomes non-economical to harvest.[5,6] Repeated annual bird damage and a long damage window (6–8 weeks) is financially taxing to agricultural operations,[1,2,7] thus producers require cost-effective management strategies to combat bird damage.[8]

Non-lethal chemical repellents hold the potential to be a cost-effective management tool for broad-scale agriculture, provided application difficulties can be overcome.[1,9] Anthraquinone

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(9,10-anthraquinone) (The E-Pesticide Manual, Version 3.02003), a post-ingestive secondary repellent, has been identified as a candidate for reducing blackbird depredation of corn, rice, sunflower, and other crops.[10–12] Anthraquinone acts on the digestive system and must be ingested for the negative consequence and learned aversion to take effect.[11] Anthraquinone is registered by the United States Environmental Protection Agency (US EPA) to repel vertebrate pests from turf and as a seed treatment at planting.[13] In these scenarios, the repellent conforms to US EPA food tolerance or maximum residue levels for entry into the human or animal food stream. A major hurdle for expanding the registration to include foliar application is developing methods that maintain repellency but reduce residues at harvest.[14] Substantial work has focused on incorporating inert ingredients (e.g. visual components) that act in synergy with anthraquinone to increase efficacy at lower residue levels.[15] These repellent formulations are optimized in laboratory settings using loose, dry sunflower achenes. Thus, efficacy when applied to the vegetative and floral components of sunflower is unknown, but important given that the repellent is applied to intact plants and not loose achenes in the field.

Although a > 80% reduction in consumption has been achieved in laboratory trials where sunflower achenes are fully coated with the repellent, field trials have not been able to replicate this efficacy, potentially due to the complex vegetative growth patterns of sunflower and limitations in application strategies.[10,16,17] Repellent deployed above the crop canopy (e.g. aerial crop dusters and high-clearance sprayers) results in the product landing on the back of the downward-facing sunflower head, which fails to reduce blackbird feeding as insufficient repellent reaches parts of the plant manipulated by the bird.[16,18–20] Repellent applied directly to the sunflower face has been shown to reduce blackbird damage when applied using a carbon dioxide (CO_2) backpack sprayer resulting in extremely high residues on achenes.[16] However, this intense and direct application is not feasible at the scale of commercial sunflower production.

Innovative application strategies, such as using upward-oriented spray nozzles situated below the leaf canopy, may improve the delivery of a repellent to the sunflower face and increase contact between foraging birds and the repellent.[1] Although, if the repellent reaches the sunflower face, achene residues may still be limited due to obstruction by disk flowers. This may be an obstacle by which secondary repellents will be deemed ineffective in sunflower, or depending on how blackbirds interact with the disk flowers, an avenue to limit anthraquinone residues on harvested achenes while simultaneously being an effective repellent. For example, corn husks fully coated in anthraquinone reduce blackbird consumption of sweet corn while simultaneously reducing the residue on the edible parts of the crop.[12]

To determine the benefit of anthraquinone-based repellents to sunflower producers, we evaluated if (i) a repellent optimized using harvested achenes is effective when applied to intact sunflower plants, especially in the presence of disk flowers; and (ii) field application strategies deposit sufficient repellent onto the sunflower face to effectively reduce blackbird consumption. We conducted laboratory-based experiments to evaluate the efficacy of repellents for reducing blackbird consumption on intact sunflower when applied under cost-effective tank mixtures and simulated commercial spraying operations. We conducted a field study to assess the ability of drop-nozzles to increase repellent coverage and residue on the sunflower face to effectively reduce bird damage.

2 MATERIALS AND METHODS

2.1 Laboratory feeding experiments

2.1.1 Animal care

We captured 130 male red-winged blackbirds (hereafter 'blackbirds') in Colorado, USA in February 2017 and 43 blackbirds in North Dakota, USA from May to July 2018. We used male blackbirds because annual sunflower consumption by males is greater than females when considering beak morphology, field metabolic rates, and percentage of sunflower in diets.[7] We housed blackbirds at the Red River Zoo, North Dakota State University (NDSU) Conservation Sciences Aviary in Fargo, North Dakota, USA in a 4.8 m × 4.8 m × 2.4 m cage (< 60 birds per cage) or smaller 2.4 m × 2.4 m × 2.4 m cages (< 20 birds per cage) under a natural light–dark cycle. Birds had free access to equal parts millet, milo, sunflower, safflower, and corn with grit and water *ad libitum*.

2.1.2 Sunflower

We planted oilseed sunflower (Daytona, Nuseed®) at the NDSU Agriculture Research Experimental Station (Prosper, ND, USA) and NDSU Casselton Agronomy Seed Farm (Casselton, ND, USA). We established four plots (3 m × 30.5 m; rows = 6; row spacing = 61 cm; stand count = 100 plants) and staggered plantings to provide consistency in sunflower maturity across weeks. We cut sunflower stalks for feeding trials ~15 cm below the head and placed them inside a 40-cm tube, securing the heads so that each face was perpendicular to the cage floor.[21] We selected sunflowers at R6 maturity (i.e. anthesis or petal drop) given this is when majority of blackbird damage occurs.[7,22] Additionally, we chose sunflowers based on lack of disease, disk flower retention, and the size, flatness, and symmetry of the head. Disk flowers are tiny tubular florets that grow over the top of the embedded achenes and are retained by the plant until maturity when they desiccate and fall off. Thus, disk flowers are a potential barrier to a repellent when targeting the achene. We used achene moisture content to gauge maturity given that capitula color can be subjective.[23] We measured achene moisture content within the plots every 2 days throughout the trials. We collected two achene wedges (5–8 g each) from two to three heads and weighed achene samples before and after placement in a convection oven (110 °C for 24 h) to determine percent moisture.[24] We accounted for differences in weekly achene moisture by standardizing achene moisture at 10%. Percent moisture ranged from 23 to 64% ($\bar{x}$ = 51.3 ± 4.1%). We measured weekly achene oil content in 2017 at the R6 growth stage via extraction using *n*-hexane in an accelerated solvent extraction (NDSU Agricultural and Biosystems Engineering Department, Fargo, ND, USA). Achene oil content ranged from 6 to 23% ($\bar{x}$ = 12.5 ± 2.6%).

2.1.3 Repellent application

We used anthraquinone-based repellent formulations (9,10-anthraquinone; Arkion® Life Sciences, LLC, New Castle, DE, USA) at 13% [AV-5055] and 50% [Avipel™] anthraquinone mixed with water and R-11® Nonionic Surfactant Spreader Activator (Wilbur-Ellis Company, Fresno, CA, USA) to produce tank mixtures that could be achieved with a commercial sprayer. The treatments varied in the percent anthraquinone and inclusion of inert ingredients (i.e. sensory cue or visual inert) in the formulation and thus the amount of formulation and anthraquinone in the tank mixture (Table 1). We applied tank mixtures at 126.3 L ha^{-1} to the sunflower face using an automated spraying machine (Control Assemblies Co., Fargo, ND, USA) equipped with one flat-fan nozzle

Table 1. Summary of laboratory feeding experiments evaluating the efficacy of repellents in reducing red-winged blackbird (*Agelaius phoeniceus*) damage on treated sunflower heads conducted in 2017–2018 in Fargo, ND, USA

	Year[d]	Repellent formulation[e]	AQ in formulation[f] (%)	Trt[g]	Sample size	Application rate of formulation (L ha^{-1})	Formulation in tank mix (%)	AQ in tank mix (%)	AQ residues on achenes (mg kg^{-1} ± SE)	AQ residues on disk flowers (mg kg^{-1} ± SE)
Concentration response experiments[a]	2017	AV-5055	13	1	13	3.18	2.5	0.60	0.36 ± 0.08	39.97 ± 2.20
				2	12	6.36	5	0.95	0.77 ± 0.10	78.71 ± 2.31
				3	12	12.63	10	1.64	1.80 ± 0.36	167.71 ± 14.10
				4	13	25.26	20	4.29	2.81 ± 0.38	294.14 ± 9.01
Preference test experiments[b]	2017	AV-5055	13	1	10	3.18	2.5	0.60	0.36 ± 0.08	39.97 ± 2.20
				2	9	6.36	5	0.95	0.77 ± 0.10	78.71 ± 2.31
				3	9	12.63	10	1.64	1.80 ± 0.36	167.71 ± 14.10
				4	10	25.26	20	4.29	2.81 ± 0.38	294.14 ± 9.01
	2018	AV-5055	13	5	9	25.26	20	4.14	4.33 ± 3.08	429.50 ± 50.50
	2018	Avipel	50	6	9	25.26	20	10.21	5.99 ± 2.38	1095.00 ± 95.00
	2018	AV-5055	13	7	8[c]	25.26	20	4.24	49.35 ± 17.75	N/A

[a] Evaluated repellency when blackbirds were provided a single sunflower in a no-choice scenario. Consumption of treated sunflowers during test days were compared to consumption of untreated sunflowers on pretest days to determine repellency (%).
[b] Evaluated preference and reduction in feeding when birds were provided both a treated and untreated sunflower in a two-choice scenario. Consumption of treated sunflowers was compared to untreated sunflowers during test days to determine preference. Total consumption on test days was compared to pretest days to determine a reduction in feeding.
[c] Evaluated preference and reduction in feeding when disk flowers were removed and tank mix was sprayed directly on achenes embedded in the sunflower face.
[d] Feeding trials occurred over 7 weeks in 2017 (August–October) and 3 weeks in 2018 (August–September). Feeding trials in 2018 employed control cages ($n = 13$) to evaluate if the reduction in feeding found in 2017 was due to a cage effect or the avian repellent.
[e] AV-5055 (Arkion® Life Sciences, LLC, New Castle, DE, USA) contains a visual inert found to have a synergistic effect with anthraquinone (AQ) to increase efficacy at lower residues.[15] Avipel™ (Arkion® Life Sciences, LLC) does not contain a visual inert and thus has a higher AQ%.
[f] Remainder of both avian repellents consisted of proprietary ingredients (Arkion® Life Sciences, LLC).
[g] In 2017 we tested four concentration levels (Trts 1–4) for both concentration response and preference experiments. In 2018, we repeated the high concentration from 2017 (Trt 5) and added a treatment using Avipel™ to create a tank mix with higher AQ% (Trt 6) and a treatment with disk flowers removed (Trt 7). Trt 4 and 5 are identical except conducted in different years.

Table 2. Summary of treatments (F1–F5) used to evaluate the effectiveness of high clearance sprayers with drop-nozzles to apply an avian repellent to the face of sunflower plants in a repellent application field study at the NDSU Carrington Research Extension Center in Carrington, ND, USA. On September 7, 2018, four plots received no repellent (untreated control) with each of the five treatments having four replicates

Trt	Tractor speed ($m\ s^{-1}$)	Tank pressure (PSI)	Tank mixture application rate ($L\ ha^{-1}$)	Repellent application rate[a] ($L\ ha^{-1}$)	Nozzles[b]	Spray action	Residue on achenes at application ($mg\ kg^{-1} \pm SE$)	Residue on disk flowers at application ($mg\ kg^{-1} \pm SE$)	Residue on achenes at harvest ($mg\ kg^{-1} \pm SE$)
F1	1.07	50	187	9.35	VK3 XR11001	continuous	7.4 ± 1.2	258.6 ± 14.8	6.1 ± 0.8
F2	1.07	50	187	18.71	VK3 XR11001	continuous	27.9 ± 7.4	1021.9 ± 274.8	12.3 ± 3.7
F3	0.54	50	187	9.35	VK3 XR11001	50% pulse	6.0 ± 0.6	342.42 ± 21.4	5.6 ± 1.4
F4	1.07	70	221	11.03	VK3 XR11001	continuous	13.3 ± 0.8	404.6 ± 48.3	12.3 ± 1.7
F5	2.20	50	187	9.35	VK3 AIXR11002	air induction	4.1 ± 0.5	165.5 ± 12.0	4.9 ± 0.6

[a] Tank mixtures had 5% Avipel™ mixed with water, except treatment F2 which had 10% Avipel™.
[b] VK3 angled backport (hollow cone; $n = 1$); XR11001 side ports (extended range flat fan; $n = 2$); AIXR11002 side ports (air induction flat fan; $n = 2$).

(8001EVS; TeeJet Technologies, Wheaton, IL, USA). We treated sunflowers the day before use in the feeding trials. Using different sunflowers, we quantified percent coverage of the repellent using Syngenta Water Sensitive Paper (76.2 mm × 25.4 mm; Spraying Systems Inc., Wheaton, IL, USA) pinned to the sunflower face. We calculated percent coverage using 'DepositScan'[25] and conducted a Kruskal–Wallis (KW) test in R (version 3.5.2; www.r-project.org) to compare coverage between treatments. We also collected weekly samples of achenes (20 g) and disk flowers (9–15 g) from two additional plants per treatment to analyze anthraquinone residues ($\bar{x}$ ± standard error) on repellent-treated sunflower heads (USDA-APHIS-WS-NWRC, Fort Collins, CO, USA; see Kaiser[26]). We used a linear regression to evaluate the relationship between disk flower and achene residues in R (version 3.5.2).

2.1.4 Concentration response experiment

We conducted a concentration response experiment to evaluate the relationship between repellent applied directly to the sunflower face and the reduction in blackbird consumption under four application scenarios feasible for large-scale commercial agriculture (Table 1). We placed blackbirds, naïve to anthraquinone, in individual cages (1.2 m × 0.6 m × 0.8 m) for 4 days, including 1 day of acclimation (Day 1), two pretest days (Days 2–3), and one test day (Day 4). On Day 1 we provided 30 g of maintenance diet and a sunflower head to acclimate birds to the cage and the test diet. Following acclimation, birds were offered one untreated sunflower head during each pretest day (Days 2–3) and one sunflower head treated with repellent on the test day (Day 4). We used one test day in the concentration response experiment because previous studies have shown blackbird repellency after 1 day of exposure to anthraquinone on achenes[10,15] and a need to minimize the potential for starvation if the repellent successfully reduced feeding. We ranked blackbirds based on average pretest consumption and assigned birds so each treatment was populated with birds exhibiting high-low daily consumption.[10,16,27] We offered access to the sunflowers for a 10-h period (08:00–18:00), when blackbirds were active.[28] Outside of this period, birds were offered 30 g of the maintenance diet. We weighed sunflowers before and after each day and collected sunflower waste from the spill tray below. We measured response variables including bird damage to the sunflower (Δ sunflower mass) and consumption (damage − spillage) at the end of each 10-h day (Days 2–4).

Percent repellency was calculated by comparing test Day 4 consumption to the average pretest consumption on Days 2–3 (repellency = [1 − (test consumption/average pretest consumption)] × 100).[16] We used an analysis of variance (ANOVA) to compare blackbird repellency among the four tank mixtures (Table 1). We assessed differences in consumption using a mixed ANOVA via the 'ez' package[29] in R (version 3.5.2; www.r-project.org) with bird as a random effect, four tank mixture treatments as a between-subject variable, and day of the experiment as a within-subjects repeated-measures variable. The dependent measure for mixed ANOVA met assumptions of normality (Shapiro–Wilk W statistic), equality in variance (Bartlett's test of homogeneity of variance), and sphericity (Mauchly's tests). We used pairwise t-tests for multiple comparisons using a Bonferroni correction ($P < 0.05$).

2.1.5 Preference experiments

We conducted preference experiments to compare blackbird consumption of treated and untreated sunflowers. In 2017 we evaluated four treatments with varying amounts of anthraquinone

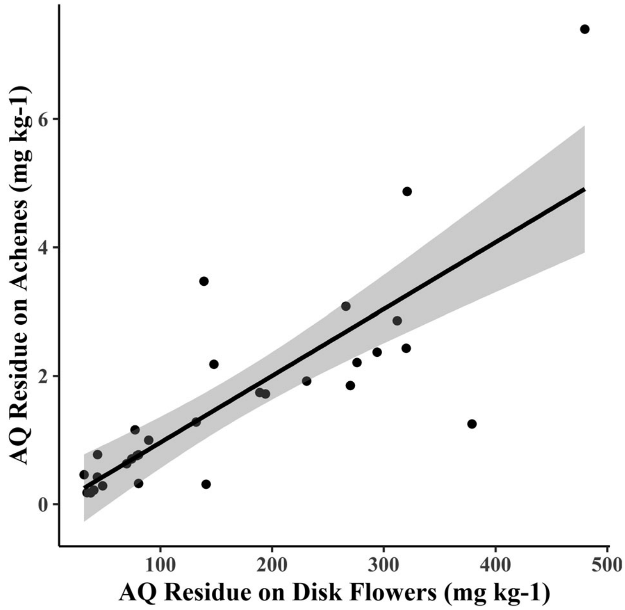

Figure 1. Relationship between anthraquinone (AQ) residues observed on sunflower disk flowers and achenes ($P < 0.0001$, adjusted $R^2 = 0.64$; $y = 0.01(x) - 0.08$) when applied with an automated sprayer used in laboratory feeding experiments.

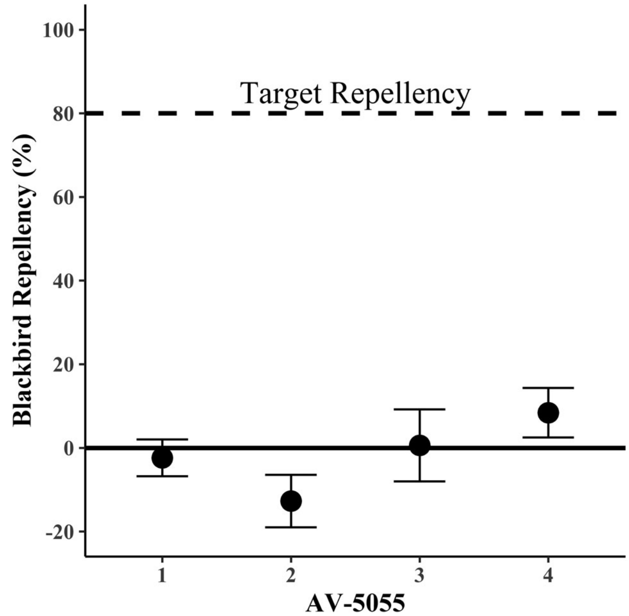

Figure 2. Red-winged blackbird (*Agelaius phoeniceus*) repellency calculated as consumption (mean ± SE) of treated sunflower (Day 4) compared to average consumption of untreated sunflower (Days 2–3) at four concentration levels of avian repellent (AV-5055) in a concentration-response experiment.

(Table 1). We placed blackbirds naïve to anthraquinone in individual cages for 5 days including 1 day of acclimation (Day 1), two pretest days (Days 2–3), and two test days (Day 4–5). We followed Werner *et al.*[15] for the preference study design and used multiple test days to evaluate repellent efficacy over time; birds also had untreated sunflower available for forage if the repellent successfully reduced consumption. We offered two untreated sunflower heads during the pretest, and one untreated and one treated sunflower head on test days. We alternated the side on which the treated sunflower was placed to overcome potential side bias independent of the effect of the repellent treatment. Consumption represented daily consumption on treated or untreated sunflowers separately, whereas total consumption was daily consumption of both sunflowers combined.

In 2017, we saw a decline in total consumption on the final test day (Day 5) of the preference experiment. Therefore, in 2018 we conducted an additional preference experiment to evaluate if the reduction in total consumption was due to cumulative ingestion of the repellent (i.e. added test days) or cage effects (i.e. added control cages with no treated sunflower). In 2018, we evaluated three repellent formulations varying in anthraquinone concentration including: (1) AV-5055, (2) Avipel™, and (3) AV-5055 applied after disk flowers were removed in an attempt to increase repellent residue on sunflower and determine a threshold for repellent effectiveness (Table 1). We offered repellent treatments to 8–9 blackbirds naïve to anthraquinone along with 4–5 blackbirds in control cages each week. The feeding experiments included 1 day of acclimation (Day 1), two pretest days (Days 2–3), and four test days (Day 4–7). We offered two untreated sunflowers heads to birds in control cages, daily.

We used a two-way mixed ANOVA to evaluate consumption of untreated and treated sunflowers and a one-way mixed ANOVA to evaluate total consumption using R (version 3.5.2). Consumption was calculated for both untreated and treated sunflowers by averaging consumption on test days (2017: Days 4–5 and 2018: Days 4–7). We used bird as a random effect, treatment (repellent concentrations; see Table 1) and sunflower (untreated and treated) as between-subject effects, and test day as within-subjects repeated measure effect. Total consumption (both sunflowers heads combined) was calculated by averaging consumption on test days (2017: Days 4–5 and 2018: Days 4–7). We used bird as a random effect, treatment (treated and control cages) as between-subjects effect, and test day as within-subjects repeated-measure effect. Dependent measures for each mixed ANOVA met assumptions of normality (Shapiro–Wilk W statistic), equality in variance (Bartlett's test of homogeneity of variance), and sphericity (Mauchly's tests). We performed pairwise t-tests for multiple comparisons with Bonferroni corrections ($P < 0.05$) to determine which values differed significantly.

2.2 Repellent application field study

We evaluated foliar application of an anthraquinone-based repellent in a field experiment at NDSU Carrington Research Extension Center (Carrington, ND, USA). We planted oilseed sunflower (Pioneer P64ME0 hybrid) on June 7, 2018 to establish four plots (1.5 m × 9 m; rows = 3; row spacing = 76 cm; stand count = 103 ± 2.9 plants) for each of the five treatments and a control (Table 2). The application treatments (F1–F5) varied in tractor speed (in m s^{-1}), tank pressure (in pound-force per square inch, PSI), spray action, nozzle type, and both tank mixture and repellent application rates (in L ha^{-1}; Table 2). On September 7, 2018, we used 360 Undercover® drop nozzle sprayers (360 Yield Center, Morton, IL, USA) attached to pulse-width ground sprayer with boom applicator to apply the repellent (Avipel™ [50% anthraquinone]) when 50% of the sunflowers had completed anthesis (growth stage R6).[30] We used Avipel™ because it contained the highest concentration of anthraquinone for establishing highest possible residues on sunflower heads. We pinned Syngenta Water Sensitive Paper (76.2 mm × 25.4 mm) to the faces of five

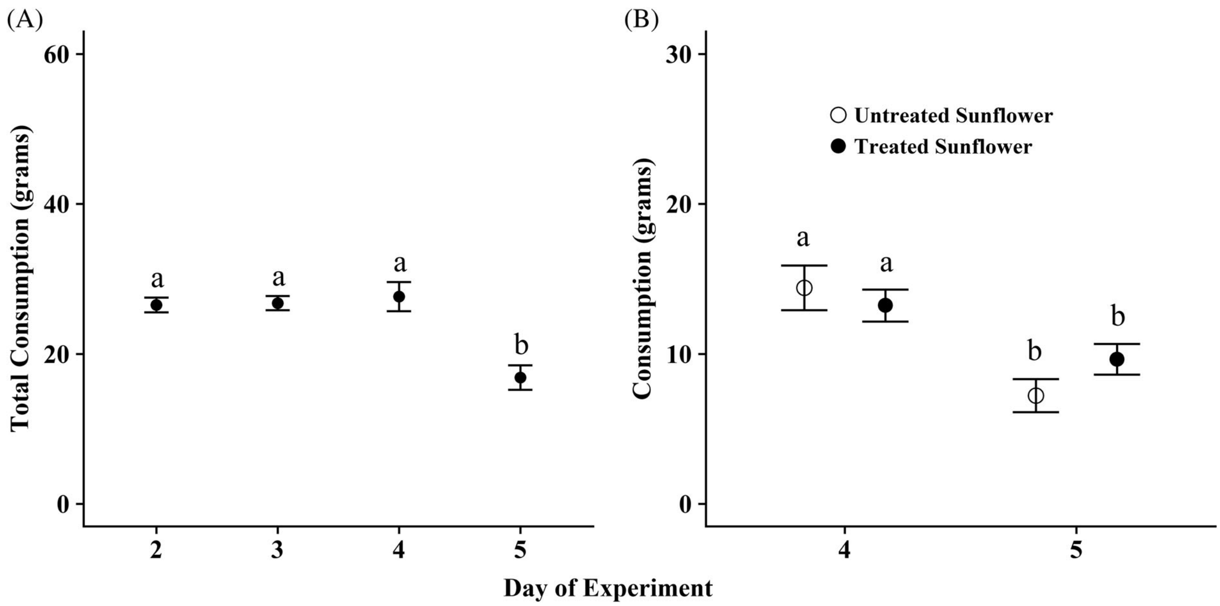

Figure 3. In 2017, (A) total consumption (mean ± SE) by red-winged blackbirds (*Agelaius phoeniceus*) including treated and untreated sunflowers (combined) in the same cage, and (B) consumption of untreated (open circles) and treated (closed circles) sunflowers in the same cage in a preference experiment. Lowercase letters signify significantly different means.

sunflower heads within each plot to assess repellent coverage. We collected sunflower samples for residue analyses at application (September 7, 2018) and at harvest (October 31, 2018). At application, we collected 5 g of achenes from four sunflowers (i.e. 20 g plot^{-1}). Whereas, at harvest, we collected 20 g plot^{-1} of achenes from the harvested sample. We collected disk flowers from four sunflowers (i.e. 20 g plot^{-1}) at application, given that most disk flowers were lost prior to harvest. We used KW tests to evaluate differences in repellent coverage and anthraquinone residues (Table 2). We confirmed a lack of bird damage within each plot prior to repellent application and estimated final damage prior to harvest. Percent damage on each head in the middle row was obtained by dividing the total area of damage by the total area available minus the area of the undeveloped center and multiplying by 100.[5,31] Sunflower was harvested with a small plot harvester and yield (in kg ha^{-1}) was corrected to a standardized 10% moisture. Repellent efficacy was based upon comparative bird damage and sunflower yield between treated and untreated plots using a KW test (Table 2). The estimate of bird damage was from free-ranging birds of unknown species or abundance, but likely included finches, sparrows, and blackbirds.

3 RESULTS

3.1 Laboratory feeding experiments

3.1.1 Repellent application

When using the automated spray machine, coverage ranged from 39 to 62% with no significant differences among the six tank mixtures applied to sunflower with intact disk flowers (Trt 1–6; KW, $\chi^2_5 = 8.95$, $P = 0.11$). We found anthraquinone residues to be 100× greater on disk flowers than achenes (Fig. 1).

3.1.2 Concentration response experiment

We observed no significant differences in repellency between tank mixtures of AV-5055 with increasing anthraquinone. All anthraquinone concentrations failed to meet the target of 80% repellency (Fig. 2). We found no significant differences in consumption between tank mixtures ($F_{3,46} = 0.37$, $P = 0.78$, $\eta_G^2 = 0.02$), but experiment day had a significant effect ($F_{2,92} = 4.92$, $P = 0.009$, $\eta_G^2 = 0.02$). Consumption on pretest Day 2 ($\bar{x} = 12.6 \pm 0.7$ g) was less than pretest Day 3 ($\bar{x} = 14.4 \pm 0.7$ g), with test Day 4 averaging 13.7 g (± 0.8).

3.1.3 Preference experiments

In 2017, we found no significant differences in blackbird consumption among tank mixtures ($F_{3,68} = 0.56$, $P = 0.65$, $\eta_G^2 = 0.017$), but there was a significant effect of experiment day ($F_{1,68} = 33.78$, $P < 0.0001$, $\eta_G^2 = 0.128$) and a sunflower (untreated *versus* treated) by day interaction ($F_{1,68} = 4.12$, $P = 0.046$, $\eta_G^2 = 0.018$). Blackbirds did not exhibit a preference between untreated ($\bar{x} = 10.8 \pm 1.0$ g) and treated sunflower ($\bar{x} = 11.4 \pm 0.8$ g) over test Days 4–5. However, total consumption (both sunflowers combined) on the final test day (Day 5) was significantly lower than that on previous test days (Days 2–4), decreasing between 36 and 39% (Fig. 3).

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

Over test Days 4–7, we observed no significant difference in total consumption between blackbirds in control cages ($\bar{x} = 18.0 \pm 1.0$ g) and in cages treated with Avipel™, the highest concentration of anthraquinone (Trt 6 [see Table 1]; $\bar{x} = 17.8 \pm 1.1$ g; Fig. 4(B)). However, we observed a significant effect of experiment day ($F_{3,33} = 7.6$, $P < 0.001$, $\eta_G^2 = 0.22$). Blackbirds ate more on test Day 7 (21.9 ± 0.9 g) than Day 5 (13.4 ± 1.1 g). We observed no

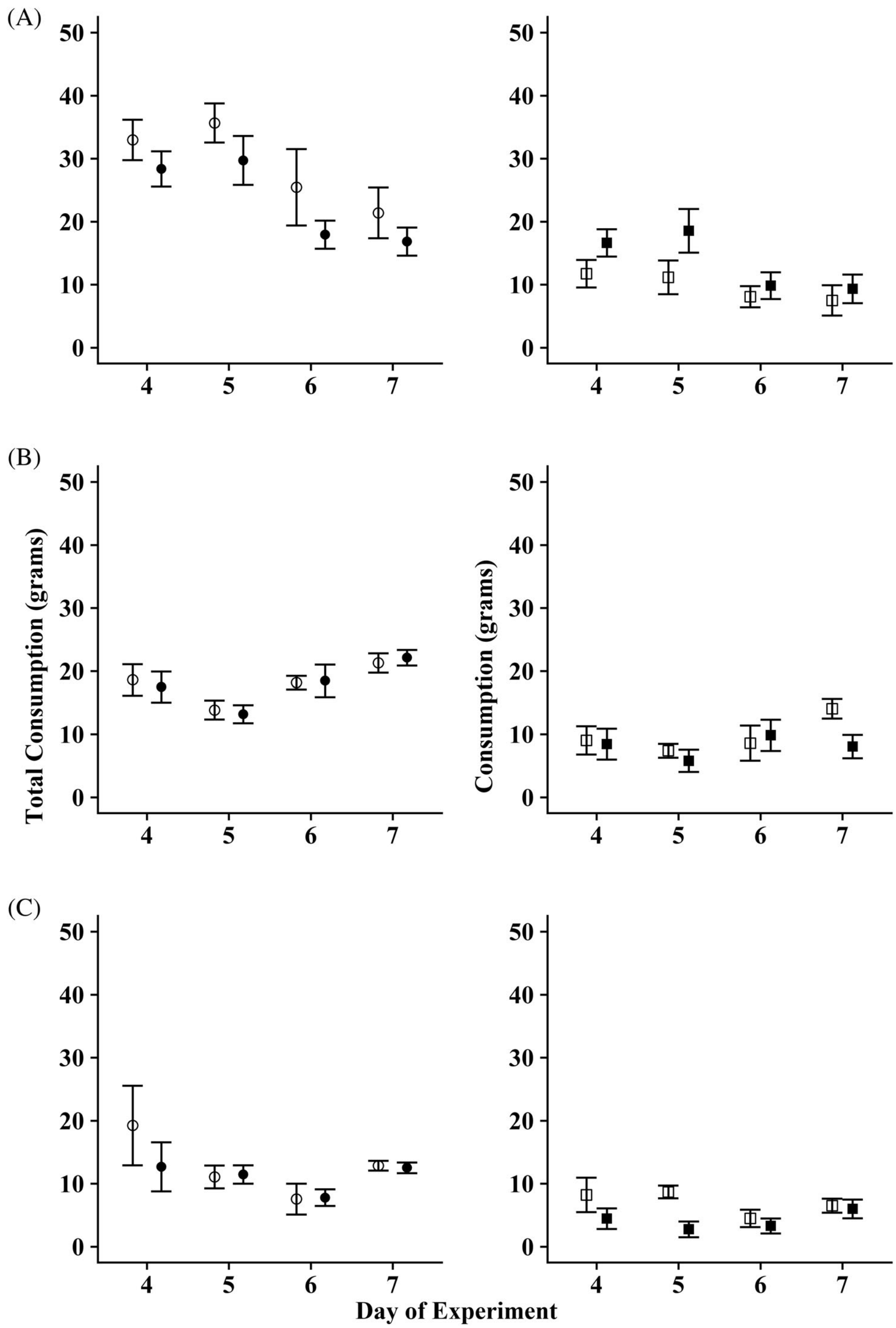

Figure 4. On the left, total consumption (mean ± SE) by red-winged blackbirds (*Agelaius phoeniceus*) including both sunflowers in control cages (open circles) and cages with treated sunflowers (closed circles) in a preference experiment. On the right, consumption of untreated (open squares) and treated (closed squares) sunflower within the treatment cages. The sunflowers were treated with (A) AV-5055 (Trt 5), (B) Avipel™ (Trt 6), and (C) AV-5055 after disk flower removal (Trt 7; see Table 1).

significant difference between consumption of untreated sunflowers ($\bar{x}$ = 9.8 ± 1.1 g) and sunflowers treated with Avipel™ (Trt 6; $\bar{x}$ = 8.0 ± 1.1 g; Fig. 4(B)).

Over test Days 4–7, total consumption did not differ between control cages ($\bar{x}$ = 12.7 ± 1.9 g) and cages where sunflowers were treated with AV-5055 after disk flowers were removed (Trt 7 [see Table 1]; $\bar{x}$ = 11.1 ± 1.1 g; Fig. 4(C)). We observed a significant effect of experiment day ($F_{3,33} = 4.2$, $P = 0.01$, $\eta_G^2 = 0.17$) with blackbirds consuming more on test Day 4 (15.2 ± 3.4 g) and Day 7 (12.6 ± 0.6 g) than Day 6 (7.7 ± 1.2 g). We observed no significant difference between consumption of untreated ($\bar{x}$ = 7.0 ± 0.9 g) and treated sunflowers with disk flowers removed ($\bar{x}$ = 4.1 ± 0.7 g; Fig. 4(C)).

3.2 Repellent application field study

Repellent coverage ranged from 0 to 76% ($\bar{x}$ = 19 ± 2%) and did not significantly differ among treatments (KW, χ^2_4 = 3.1,

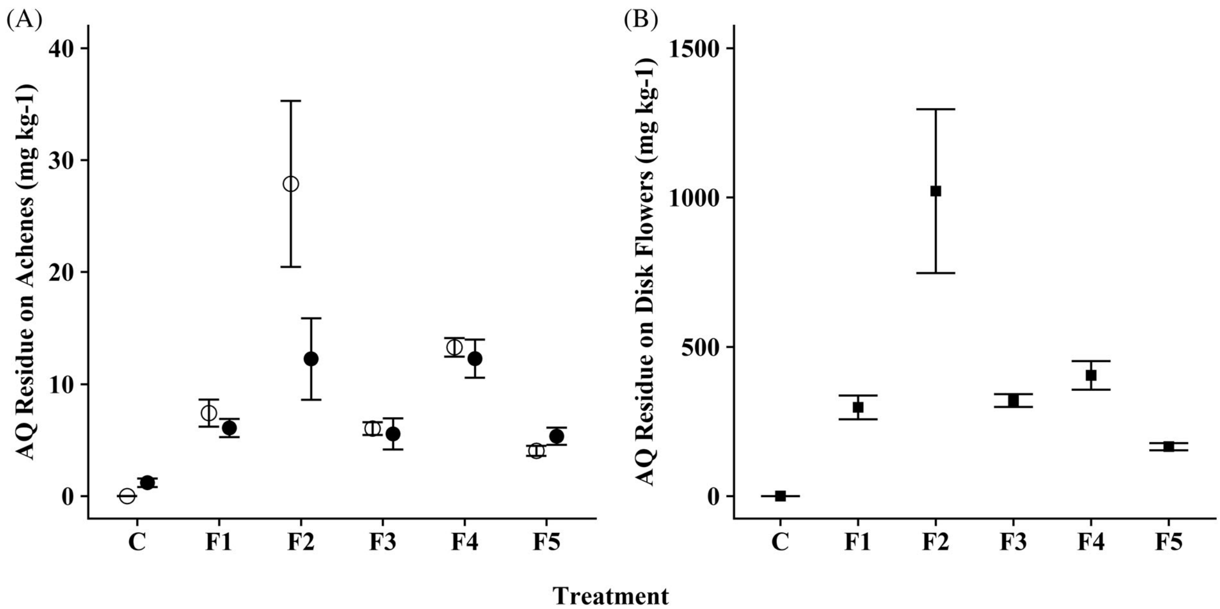

Figure 5. Anthraquinone (AQ) residue (mean ± SE) on sunflower (A) achenes at application (open circles) and after harvest (closed circles); and on (B) disk flowers at application for a control and treatments in a repellent application field study. The application treatments (F1–F5) varied in the tractor speed (m s^{-1}), tank pressure (PSI), spray action, nozzle type, and both tank mixture and repellent application rates (L ha^{-1}) (Table 2).

$P = 0.54$). We found a significant difference in residues among repellent treatments on achenes (KW, $\chi^2_5 = 20.7$, $P < 0.001$; Fig. 5(A)) and disk flowers (KW, $\chi^2_5 = 18.1$, $P < 0.003$; Fig. 5(B)). On treated plots, anthraquinone residue on achenes at application was less than that on disk flowers (Table 2). Bird damage did not differ statistically (KW, $\chi^2_5 = 2.9$, $P = 0.70$) and was relatively low in both treated ($\bar{x} = 5.0 \pm 0.4\%$) and control plots ($\bar{x} = 3.9 \pm 0.7\%$), due to overall low bird pressure. Average agronomic yield was not statistically different (KW, $\chi^2_5 = 5.4$, $P = 0.37$) between treated ($\bar{x} = 1883 \pm 55.3$ kg ha^{-1}) and control plots ($\bar{x} = 1976 \pm 67.6$ kg ha^{-1}). The average area of developed sunflower was similar (KW, $\chi^2_5 = 6.9$, $P = 0.23$) in treated (194.3 ± 10.2 cm^2) and control plots (196.2 ± 16.1 cm^2). Oil content was similar (KW, $\chi^2_5 = 4.5$, $P = 0.48$) in treated ($38.8 \pm 0.2\%$) and control plots ($38.4 \pm 0.3\%$).

4 DISCUSSION

Our results highlight the difficulty of translating the efficacy found for avian repellents optimized for loose, dry achenes in laboratory-settings to the efficacy of those repellents when applied to intact sunflower plants. The significant effect of day on daily consumption could be due to (i) changes in daily ambient temperature and humidity influencing metabolism or rate of digestion, (ii) the amount of food consumed on the previous day and status of energy reserves,[28] (iii) neophilia with presentation of the fresh sunflower that was not part of their maintenance diet,[32] (iv) a cage effect where lack of alternative enrichment resulted in increased feeding,[33] (v) differences in the caloric content of the sunflower heads,[7] or any combination of these factors. We were unable to tease apart the reason for the day effect with our experimental design. Regardless, blackbirds in the control and treatment cages closely tracked each other for total consumption with the average difference between control and treatment cages ranging from 0.22–7.51 g ($\bar{x} = 2.75 \pm 0.82$) in a given day and the difference in total consumption between days ranging from 0.26 to 13.57 g ($\bar{x} = 5.6 \pm 0.89$). For a repellent to be deemed effective, any significant influence on reducing consumption would have to be greater than the small differences and variation in consumption between days, which we did not observe.

In applying repellents to intact plants, it must be considered that only a fraction of each achene is exposed to repellent deposition due to the achene being embedded in the sunflower head and protected by disk flowers. This differs from previous laboratory studies where hulls of dry, loose achenes were entirely coated with repellent.[10,15] Thus, to obtain the residue required to reduce avian feeding on an intact plant you would need to achieve the same anthraquinone residue on achenes (~1000 mg kg^{-1}) but on a fraction of the surface.[15] Although achene residues increased as repellent in the tank mixtures increased, anthraquinone (0–49 mg kg^{-1}) was still well below amounts shown to reduce feeding in studies performed on loose achenes (> 385 mg kg^{-1}).[16] Werner *et al.*[16,34] found a 33–34% reduction in blackbird feeding on intact sunflower, but this was due to repellent application via a backpack sprayer directly targeting the sunflower face, which is unattainable for broad-scale commercial application. Additionally, tank mixtures, including > 20% of the repellent active ingredient, applied at > 126 L ha^{-1}, are not economically nor logistically feasible for producers.[35]

Our results are the first to quantitatively measure the role of disk flowers as a barrier for repellent reaching the embedded sunflower achenes. Disk flowers have been overlooked in previous blackbird repellents research. Previous field efficacy studies involved the application of repellents from above the canopy, assuming the repellent would reach the sunflower face.[19,20] Ideally, residues on disk flowers would reduce feeding while keeping the residue on achenes low enough to conform to US EPA food tolerance levels.[14] In our concentration-response and preference experiments, anthraquinone residue on disk flowers (40–1095 mg kg^{-1}) was 183× higher than residue on achenes protected by disk flowers (0–6 mg kg^{-1}). For comparison, a threshold concentration of 1475 mg kg^{-1} anthraquinone was predicted for blackbirds offered repellent-treated loose achenes.[10,15] However, disk flowers with higher residues failed to reduce blackbird consumption, suggesting that treated disk flowers play an insignificant role. Even though disk flowers were removed by blackbirds in order to reach the underlying achenes, the presence of treated disk flowers did not enhance repellent efficacy. Whereas laboratory-based tests using whole achenes showed repellency, even though the birds crack open the achenes to ingest the seeds and do not consume the residue-laden shells. When we removed disk flowers prior to repellent application, achene residues (49 ± 18 mg kg^{-1}) were still low due to achenes being embedded in the sunflower head and thus failed to reduce consumption. However, consumption of sunflowers treated with the repellent after removing disk flowers ($\bar{x}$ = 4.1 ± 0.7 g) was almost half of that of untreated sunflowers ($\bar{x}$ = 7.0 ± 0.9 g), suggesting residues may have been approaching the necessary concentration to reduce blackbird feeding.

The incorporation of sensory cues in chemical repellents has shown potential to increase cost-effectiveness by reducing the residue required to effectively decrease blackbird feeding.[15] However, repellents with sensory cues were optimized in the laboratory on loose, dry achenes offered in a bowl,[15] and it is unknown how blackbirds visually perceive the repellent on the vegetative and floral parts of mature crops.[36] Blackbirds did not exhibit a preference between untreated and treated sunflowers in the same cage. We hypothesize that the structural complexity of a mature sunflower head (e.g. achenes imbedded in the head and covered by disk flowers) may inhibit the sensory component from providing the same deterrence found on loose achenes because the blackbird visual system may perceive the color or contrast of the repellent against vegetative or floral components differently than when applied directly to black achenes.[36] For example, sunflowers change color from a deep green to pale yellow to a yellowish-brown over the damage period.[30] Blackbirds may use visual cues to evaluate crop maturity and select fields or plants for foraging. Thus, the addition of a repellent with or without added visual cues, may interfere in crop selection (i.e. a repellent may change the way blackbirds perceive the plant and potentially make it appear more mature). Niner *et al.*[19] observed more damage to sunflower treated with an anthraquinone-based repellent compared to control plots when the repellent was applied above the canopy. Although not statistically significant, we saw increased consumption of sunflower treated with AV-5055, which included a sensory cue (Fig. 4(A)-Consumption). Further work should consider how repellents may alter the visual properties of the crop and how this may influence foraging cues in blackbirds.

Our field applications via drop-nozzles positioned beneath the crop canopy allowed the repellent to reach the sunflower face, but was subject to high variation due to variation in sunflower head position in relation to the spray nozzles. Insufficient repellent residue was deposited on the sunflower face, and we did not document any differences in percent bird damage or agronomic yield among treatments (i.e. the 5% difference in yield was likely due to fine scale differences in soil properties or other unmeasured variables). Our results from testing field application strategies highlight the difficulties in scaling-up from individual plants to the field, given repellent coverage was relatively low and highly variable (0–76%; $\bar{x}$ = 19 ± 2%). Increased repellent concentration in the tank mixture resulted in 3.7× the residue on achenes (despite a 33% decrease in coverage), suggesting higher concentrations are needed for repellent efficacy. Reducing tractor speed should result in increased coverage with similar output, but we saw a decrease when speed was reduced by 50% and spraying action was at a 50% pulse. As suggested by Knoche[37] and Nuyttens *et al.*,[38] droplet size may be more important for increasing coverage. However, when pressure was increased to 70 PSI at an application rate of 221 L ha^{-1}, we attained similar coverage and only slightly higher residues. The importance of droplet size is not well understood for anthraquinone-based repellents but finer droplets may be required to infiltrate disk flowers. We suggest that increased repellent concentrations and manipulated droplet size should be explored further to enhance repellent efficacy in sunflower fields.

5 CONCLUSIONS

At this time, application strategies capable of depositing sufficient repellent to the sunflower face are not available and future research is needed to overcome issues of plant structure. As harvest approaches, disk flowers fall off, which would reduce the floral barrier and allow more repellent to reach the achenes. Thus, applying a bird repellent after disk flower loss may increase achene residues, but drawbacks include crop vulnerability to lodging with use of in-field tractors at this stage and no protection during earlier periods of heavy damage (i.e. 18 days after petal drop).[22] Multiple applications across the damage season may allow for increased protection but would decrease cost-effectiveness. Thus, repellent efficacy would have to compensate for additional cost as well as additional loss in yield from lodging. Even under an ideal spraying environment, we were unable to effectively reduce avian consumption using currently recommended application rates. Thus, we did not find anthraquinone-based repellents to be a suitable option for the protection of sunflower from blackbird damage. Sufficient residue levels on achenes were not achieved due to complications with plant structure and application strategies representative of commercial agriculture. A limitation likely present in other crops where repellent is ineffective at the field scale (e.g. methyl anthranilate on blueberries[39,40]).

ACKNOWLEDGEMENTS

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

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