Synergistic effect of an ultraviolet feeding cue for an avian repellent and protection of agricultural crops

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1. Introduction

The gregarious feeding behavior of some wild birds causes economic losses annually to world-wide agricultural production. For example, red-winged blackbirds (Agelaius phoeniceus; Werner et al., 2008b, 2009), common grackles (Quiscalus quiscula), yellow-headed blackbirds (Xanthocephalus xanthocephalus) and brown-headed cowbirds (Molothrus ater) negatively impact rice (Avery et al., 1997, 1998, 2005; Cummings et al., 2002a,b, 2011; Werner et al., 2008a, 2010), corn (Carlson et al., 2013) and sunflower (Linz et al., 2011; Werner et al., 2010, 2011) production each year in the United States of America. Cummings et al. (2005) estimated that blackbirds caused approximately $13.4 million of damage to USA rice production in 2001. Similarly, blackbird damage to sunflower was estimated to be $5.4 million annually in the prime sunflower growing area of North America (i.e., North Dakota, South Dakota, Minnesota; Peer et al., 2003) and $3.5 million in North Dakota (Klosterman et al., 2013). These losses have motivated the use of several blackbird damage management techniques, including non-lethal behavioral approaches such as chemical repellents.
The effectiveness and commercial development of blackbird repellents are dependent upon the repellent’s efficacy under field conditions, cost relative to expected damages of unmanaged crops, environmental impacts, and food and feed safety (Werner et al., 2008a, 2009). Optimized repellent formulations and application strategies are needed for agricultural crop protection in context of these economic, environmental, and safety thresholds. Thus, much research on repellents for agricultural applications has been focused to investigate the repellency of fungicides and insecticides already registered by the United States Environmental Protection Agency for agricultural applications (Linz et al., 2006; Werner et al., 2008a,b, 2010), and naturally-occurring compounds such as 9,10-anthraquinone (Carlson et al., 2013; Cummings et al., 2011; Werner et al., 2009, 2011).

Although anthraquinone is a naturally-occurring substance that was identified as a promising avian repellent in the early 1940s (Heckmanns and Meisenheimer, 1944), no anthraquinone-based repellents are currently registered for agricultural applications in the United States of America. Thus, data regarding efficacy, chemical residues, and application strategies are presently needed for the development of anthraquinone-based repellents and the protection of agricultural crops. Anthraquinone has been used to effectively protect rice seeds and emergent rice seedlings from blackbirds under captive and 2–ha field conditions (Avery et al., 1997, 1998; Cummings et al., 2002a,b, 2011; Neff and Meanley, 1957), turf from Canada goose (Branta canadensis) grazing in captivity (Blackwell et al., 1999; Dolbeer et al., 1998), whole-kernel corn and ripening corn from captive sandhill cranes (Grus canadensis) and blackbirds (Blackwell et al., 2001; Carlson et al., 2013), and sunflower achenes from blackbirds under captive and <0.2–ha field conditions (Werner et al., 2009, 2011). Blackbird repellency was not observed within 2–5–ha rice fields aerially sprayed with 9.3 or 18.6 L Flight Control®/ha (active ingredient [a.i.] 50% 9,10-anthraquinone, Arkion® Life Sciences, New Castle, DE, USA; Avery et al., 2000a) or within 0.33–0.4 ha rice fields aerially sprayed with 18.3 or 54.9 L Flight Control® ha⁻¹ (Avery et al., 2000b). Anthraquinone residues among these treated rice plots ranged from approximately 175–475 ppm anthraquinone (Avery et al., 2000a) and 275–1000 ppm anthraquinone (Avery et al., 2000b) on the day subsequent to the repellent application. Thus, blackbird repellency under field applications was limited by repellent concentrations realized from previous field applications on agricultural crops (i.e., >1000 ppm anthraquinone).

Because field applications of anthraquinone-based repellents have provided ≤1000 ppm anthraquinone and the threshold repellent concentration was estimated as 1475 ppm anthraquinone for red-winged blackbirds (Werner et al., 2009), our purpose was to develop an efficacious application strategy for a blackbird repellent at repellent concentrations realized from previous field applications on agricultural crops (i.e., ≤1000 ppm anthraquinone; Avery et al., 2000a,b). Anthraquinone is a cathartic purgative and its action is principally on the large intestine (Merck, 1991); thus, anthraquinone-based repellents cause negative postigestive consequences (i.e., postigestive repellent). Interestingly, anthraquinone also absorbs near-UV wavelengths (Du et al., 1998) that are visible to most birds (i.e., 300–400 nm; Hart and Hunt, 2007). Based upon these biochemical and physical characteristics (i.e., inextricable sensory cue plus postigestive consequence), anthraquinone is a quintessential avoidance conditioning agent for wild birds (Werner et al., 2009) and an effective chemical repellent for the protection of agricultural crops. Indeed, blackbirds conditioned with a UV-absorbent, postigestive repellent (a.i. 50% 9,10-anthraquinone) subsequently avoided food treated only with an UV-absorbent or UV-reflective feeding cue (Werner et al., 2012).

If aversion performance is not determined primarily by the nature of either conditioned or unconditioned stimuli but their combinations (i.e., cue-consequence specificity; Domjan, 1985), and if avian repellency can be optimized by independently varying the concentrations of the UV visual cue and the postigestive consequence, then we predicted that the addition of an UV-absorbent feeding cue can enhance the concentration-response relationship, or efficacy of an UV-absorbent, postigestive repellent for wild birds associated with agricultural depredation. Our objectives were to comparatively investigate the blackbird repellency of (1) an UV feeding cue in the absence of postigestive consequences, (2) the combination of the UV feeding cue and an UV-absorbent, postigestive repellent, and (3) a non-UV feeding cue combined with the UV-absorbent, postigestive repellent.

2. General methods

All feeding experiments were conducted in October 2012–February 2013 at the National Wildlife Research Center’s (NWRC) outdoor animal research facility in Fort Collins, Colorado (USA). We live-captured 121 male red-winged blackbirds for the experiments. The capture, care, and use of all birds associated with our feeding experiments were approved by the NWRC Animal Care and Use Committee (NWRC Study Protocol QA-1968; S.J. Werner-Study Director).

Blackbirds were maintained in 4.9 × 2.4 × 2.4-m cages (35–45 birds/cage; Werner et al., 2009) within a wire mesh-sided building for at least 2 weeks prior to the experiments (i.e., quarantine, holding). Free access to grit and a maintenance diet was provided to all birds during quarantine and holding. The maintenance diet included two parts millet: one cracked corn: one milo: one safflower. Blackbird feeding experiments were conducted in visually-isolated, individual cages (0.9 × 1.8 × 0.9 m) within a wire mesh-sided building. Water was provided ad libitum to all birds throughout the experiments.

An anthraquinone-based repellent (Avipel®; Arkion® Life Sciences, New Castle, DE, USA), and titanium dioxide (Evonik Goldschmidt Corporation, Hopewell, VA, USA) and red feeding cues (red #40, F&D aluminum lake dispersion; Roha USA, St. Louis, MO, USA) were used for the feeding experiments. A Genesys® 2, 336002 spectrophotometer (Thermo Spectronic US, Rochester, NY, USA) was previously used to determine that both the Avipel repellent and the titanium dioxide feeding cue absorb near UV
wavelengths (Werner et al., 2012) that are visible to red-winged blackbirds (Chen et al., 1984; Chen and Goldsmith, 1986). Seed treatments for all experiments were formulated by applying aqueous suspensions (60 mL/kg) to whole oilseed sunflower (Ranch-Way Feed Mills, Fort Collins, CO, USA) using a rotating mixer and household spray equipment.

3. Experiment 1: Baseline preference test of UV feeding cue

A baseline preference (i.e., choice) feeding experiment was conducted to (1) evaluate blackbird consumption of untreated sunflower seeds versus those treated with the UV feeding cue and (2) identify a numerically preferred concentration of the UV feeding cue for our subsequent feeding experiments.

3.1. Materials and methods

Daily sunflower consumption was measured throughout the preference experiment (test days 1–4). Unconsumed sunflower seeds (remaining in each food bowl) and spillage were collected (at 08:00 h, daily) and weighed (±0.1 g). Weight change (e.g., desiccation) of sunflower seeds was measured daily by weighing seeds offered within a vacant cage throughout the preference experiment.

Eleven red-winged blackbirds (experimentally naïve) were randomly assigned to the baseline preference experiment. All blackbirds were offered untreated sunflower seed ad libitum in two food bowls for 5 days of acclimation in individual cages. Each blackbird was subsequently offered one bowl of untreated sunflower and one bowl of sunflower treated with 0.2% of the titanium dioxide feeding cue (targeted concentration, wt/wt) at 08:00 h, daily throughout the 4-day test. The north–south placement of food bowls was randomized on the first day and alternated on subsequent days of the experiment.

The dependent measure for the baseline preference experiment was average (i.e., daily) test consumption of treated and untreated sunflower seeds. After successfully conducting Levene’s test for equal variances (α = 0.05) and affirmatively inspecting the normality of residuals, consumption data were subjected to a repeated measures analysis of variance (ANOVA). The random effect of our model was bird subjects, the between-subjects effect was treatment (treated vs. untreated seed), and the within-subject effect was test day. The treatment effect was analyzed using a mixed model (SAS v9.1). Descriptive statistics (X ± S.E.M.) were used to summarize consumption of treated and untreated seeds throughout the preference experiment.

3.2. Results

Relative to average consumption of untreated sunflower, blackbirds non-significantly preferred sunflower treated with 0.2% of the UV feeding cue (F1,10 = 2.15, P = 0.1732). Blackbirds consumed an average of 3.3 ± 0.4 g of treated sunflower and 2.5 ± 0.3 g of untreated sunflower during the 4-day experiment (Fig. 1). Thus, the UV feeding cue was not itself aversive (i.e., in the absence of negative postigestive consequences).

4. Experiment 2: Concentration-response of repellent plus added UV feeding cue

This experiment was designed to establish a concentration-response relationship of anthraquinone plus 0.2% titanium dioxide-treated sunflower seeds for blackbirds in captivity. We predicted that (1) the threshold concentration of anthraquinone necessary for blackbird feeding repellency could be reduced by varying the concentration of the anthraquinone-based repellent when combined with the titanium dioxide feeding cue and (2) this optimized anthraquinone concentration would be less than the threshold anthraquinone concentration previously established for red-winged blackbirds offered treated oilseed sunflower seeds (i.e., 1475 ppm anthraquinone; Werner et al., 2009). Daily sunflower consumption was measured throughout the pretreatment and test phases of the experiment as previously described for Experiment 1 (Section 3.1).

We hypothesized that repellency would be directly related to repellent concentration during the repellent concentration-response feeding experiments. We operationally defined laboratory efficacy as ≥80% repellency during captive feeding experiments (Werner et al., 2009, 2011). Thus, we predicted that consumption of efficacious treatments would be ≤20% of pretreatment consumption during the concentration-response experiments.

4.1. Materials and methods

Fifty-five red-winged blackbirds (experimentally naïve) were offered untreated sunflower seed ad libitum in one food bowl for 5 days of acclimation in individual cages. Each blackbird was subsequently offered 30 g of untreated sunflower seeds in one bowl during each of study days 1, 2 and 3. Blackbirds were ranked based upon average
pretreatment consumption and assigned to one of six treatment groups \(n = \text{nine to 10 birds per group}\) such that each group was similarly populated with birds that exhibited high–low daily consumption (i.e., observed range of pre-treatment food consumption was represented in each test group).

Subsequent to the pretreatment, treatments were randomly assigned among groups \((0.02\%, 0.035\%, 0.05\%, 0.1\%, 0.25\%, \text{and 0.5}\% \text{anthraquinone; targeted concentrations, wt/wt; Werner et al., 2009})\). Each of these seed treatments also included 0.2% of the titanium dioxide feeding cue (Werner et al., 2012). We offered 30 g of treated sunflower seeds in one bowl to all birds on study day 4, and determined the combined mass \((\pm 0.1 \text{ g})\) of uneaten seeds and seed spillage at 08:00 h on study day 5. A 200 g sample of each seed treatment was collected for subsequent analysis of actual anthraquinone concentrations \((\pm 10 \text{ ppm anthraquinone})\) via high performance liquid chromatography (HPLC; Werner et al., 2009, 2011).

The dependent measure for the concentration-response experiments was calculated as test consumption relative to average pretreatment consumption (percent repellency). Non-linear regression procedures (SAS v9.1) were used to analyze repellency as a function of anthraquinone concentration (ppm). Descriptive statistics \((\bar{x} \pm \text{S.E.M., mg anthraquinone/kg body mass})\) were used to summarize consumption of treated seeds during the concentration-response feeding experiments.

4.2. Results

We observed a positive concentration-response relationship during the experiment with varying concentrations of the anthraquinone-based repellent and 0.2% of the UV feeding cue. Blackbirds exhibited 100% repellency for sunflower treated with 2270 ppm anthraquinone \((27.4 \pm 13.2 \text{ mg anthraquinone/kg body mass})\) and 0.2% of the UV feeding cue (Fig. 2). Moreover, to the repellency of sunflower treated only with 0.02% or 0.035% anthraquinone (i.e., 222 and 556 ppm anthraquinone; lower curve, Fig. 2), the addition of the UV feeding cue in Experiment 2 (upper curve, Fig. 2) caused an 45–115% increase in blackbird repellency (i.e., 22.8% to 49.1% repellency at 166 ppm anthraquinone, and 34.0% to 49.4% repellency at 272 ppm anthraquinone). Thus, we observed a synergistic effect of the combined UV feeding cue and anthraquinone-based repellent. With the addition of 0.2% of the UV feeding cue, blackbird repellency \((y)\) was a function of anthraquinone concentration \((x)\): \(y = 19.22 \ln(x) - 57.23\) \((r^2 = 0.88, P = 0.006)\). We therefore predicted a threshold concentration of 1300 ppm anthraquinone (i.e., 80% repellency) for blackbirds offered sunflower treated with the anthraquinone-based repellent and the UV feeding cue (upper curve, Fig. 2).

5. Experiment 3: Concentration-response of UV feeding cue

This experiment was designed to establish a concentration-response relationship of titanium dioxide plus 0.02% anthraquinone-treated sunflower seeds for blackbirds in captivity. We predicted that the threshold concentration of anthraquinone necessary for blackbird feeding repellency could be minimized by varying the concentration of the titanium dioxide feeding cue. We also predicted that the optimized anthraquinone concentration would be less than the threshold anthraquinone concentration previously established for red-winged blackbirds (Werner et al., 2009).

5.1. Materials and methods

The acclimation, pretreatment and test phases of Experiment 2 (Section 4.1) were replicated with 44 experimentally naïve red-winged blackbirds. Treatment groups 1–4 \((n = \text{11 birds/group})\) received 0.04%, 0.08%, 0.12% and 0.16% (respectively) of the titanium dioxide feeding cue (targeted concentrations, wt/wt). Each of these seed treatments also included 0.02% anthraquinone (targeted concentration, wt/wt). A 200 g sample of each seed treatment was collected for subsequent analysis of actual anthraquinone concentrations \((\pm 1 \text{ ppm})\) via HPLC. The statistical analyses of Experiment 2 (Section 4.1) were repeated for this concentration-response experiment for the UV feeding cue.

5.2. Results

We did not observe a positive concentration-response relationship during the experiment with varying concentrations of the UV feeding cue and 0.02% of the anthraquinone-based repellent \((r^2 = 0.18, P = 0.571)\). Blackbirds exhibited <10% repellency for sunflower treated with 0.04–0.16% of the UV feeding cue and 164–205 ppm anthraquinone (Fig. 3). Thus, <0.2% of the UV feeding cue
did not enhance the repellency of anthraquinone-treated sunflower in red-winged blackbirds.

6. Experiment 4: No-choice test of non-UV feeding cue

This experiment was designed to evaluate the repellency associated with the combination of the anthraquinone-based repellent and a non-UV feeding cue. This experiment was therefore a crucial test of our hypothesis regarding the cue-consequence specificity (Domjan, 1985) of the UV-absorbent feeding cue and the UV-absorbent, postigestive repellent paired in the previous concentration-response experiments. If enhanced repellency of anthraquinone (i.e., UV absorbent, postigestive repellent) plus the UV-absorbent feeding cue can be attributed to UV wavelengths, then less repellency should be observed for anthraquinone plus visual cues >400 nm (e.g., red-treated food).

6.1. Materials and methods

The acclimation, pretreatment and test phases of Experiment 2 (Section 4.1) were replicated with 11 experimentally naïve red-winged blackbirds. The test treatment (n = 11) included 0.02% anthraquinone and 0.2% of red #40 (Roha USA, St. Louis, MO, USA; Werner et al., 2008c). A 200 g sample of each seed treatment was collected for subsequent analysis of actual anthraquinone concentrations (±1 ppm) via HPLC.

We again predicted that consumption of efficacious treatments would be <20% of pretreatment consumption during the no-choice feeding experiment. Linear regression procedures (SAS v9.1) were used to analyze percent repellency as a function of 0.04–0.16% of the UV feeding cue (arc sine transformed). Descriptive statistics (\( \bar{x} \pm S.E.M. \)) were again used to summarize consumption of treated seeds during the no-choice feeding experiment.

6.2. Results

Blackbirds exhibited 4.5% repellency for sunflower treated with 150 ppm anthraquinone and 0.2% of red #40 (i.e., non-UV feeding cue). Compared to the previous concentration-response experiment with varying concentrations of the anthraquinone-based repellent and 0.2% of the UV feeding cue (49% repellency at 166 ppm anthraquinone; Fig. 2), we observed less repellency of the anthraquinone-based repellent when paired with a non-UV feeding cue. Thus, 0.2% of the non-UV feeding cue did not enhance repellency of anthraquinone-treated sunflower in red-winged blackbirds.

7. Discussion

In the absence of negative postigestive consequences, the UV feeding cue was not aversive during our baseline preference experiment (Experiment 1, Fig. 1). Red-winged blackbirds use affective processes (i.e., flavor–feedback relationships; Provenza, 1995; Provenza and Villalba, 2006) to shift preference for both novel and familiar flavors, and cognitive associations (i.e., visual cue-postigestive feedback) to avoid food, subsequent to toxin exposure (Werner et al., 2008c). Ultraviolet cues alone, however, are unlikely to function as aposematic signals in wild birds (Lyytinen et al., 2001). Indeed, feeding repellents based merely on sensory cues (e.g., flavor, visual cues) are not likely to be effective in the absence of aversive postigestive effects (Provenza, 1997).

Because the addition of 0.2% of the UV-absorbent cue enhanced the repellency of 166–272 ppm anthraquinone (Experiment 2, Fig. 2), we observed cue-consequence specificity (Domjan, 1985) for visual cues and a postigestive repellent in red-winged blackbirds. Blackbirds cognitively associate pre- and postigestive consequences with visual cues, and reliably integrate visual and gustatory experience with postigestive consequences to procure nutrients and avoid toxins (Werner and Provenza, 2011). These visual cues include UV-absorbent and UV-reflective cues for blackbird feeding behavior (Werner et al., 2012).

Whereas 0.04–0.16% of the UV-absorbent cue resulted in <10% repellency when paired with 164–205 ppm anthraquinone (Experiment 3, Fig. 3), we suggest that 0.2% is a threshold concentration of titanium dioxide to reliably cue the repellent consequences of the anthraquinone-based repellent (Fig. 2). Unlike 0.04–0.16% titanium dioxide, we suggest that 0.2% is a salient cue for anthraquinone repellency, including sub-threshold concentrations of anthraquinone for red-winged blackbirds (i.e., <1475 ppm anthraquinone; Werner et al., 2009). To further test our hypotheses regarding the relationship of the UV feeding cue and the postigestive repellent, we conducted a crucial experiment including a non-UV feeding cue (Experiment 4).

Because the non-UV cue did not enhance the repellency of anthraquinone (i.e., UV-absorbent, postigestive repellent) during the no-choice experiment (Experiment 4), we observed cue-consequence specificity (Domjan, 1985) of a UV feeding cue and a postigestive repellent in red-winged blackbirds (Fig. 2). Moreover, aversion...
performance among our experiments was not determined primarily by the nature of either conditioned or unconditioned stimuli, but their combinations (Domjan, 1985). In addition to the cue-consequence specificity of taste-illness and exteroceptive-peripheral combinations (Domjan, 1985), we suggest that birds reliably associate visual cues with postigestive consequences to avoid food subsequent to toxin exposure (Werner et al., 2008c) and these visual cues include UV feeding cues (Werner et al., 2012). These results have implications for subsequent field applications of chemical repellents and the management of avian depredation.

Our purpose was to develop an efficacious strategy for field applications of an avian repellent and the protection of agricultural crops. In addition to providing sufficient repellent concentrations under field conditions, field efficacy of chemical repellents is constrained by the local overabundance of blackbirds and the assumed daily immigration of repellent-naïve birds within damaged agricultural fields. Interestingly, repellent-naïve birds benefit from repellent-experienced flockmates by learning where to forage for untreated food (Avery, 1994) rather than a socially-facilitated aversion for repellent-treated food. Thus, daily immigration of repellent-naïve blackbirds may not prohibit repellency under field conditions if sufficient repellent concentrations are offered throughout the period of needed protection to some portion of the depredating flock.

The synergistic repellency observed in this study will not instantly maximize the proportion of repellent-experienced flockmates. Rather, the addition of an UV feeding cue can enhance blackbird repellency at repellent concentrations (e.g., <1000 ppm anthraquinone; upper curve, Fig. 2) realized from previous field applications on agricultural crops (Avery et al., 2000a,b). Several chemical repellents and cues exhibit similar UV spectra, and might therefore be used in a field application strategy including an initial application of a repellent and subsequent applications of a visual cue with spectral characteristics sufficiently similar to the repellent (Werner, 2009).

8. Conclusion

Whereas the UV feeding cue was not itself aversive (Experiment 1) and the addition of 0.2% of the UV feeding cue in Experiment 2 increased repellency relative to that observed for anthraquinone alone (Werner et al., 2009), we observed synergistic repellency of the combined UV feeding cue and anthraquinone-based repellent in red-winged blackbirds. Because <10% repellency was observed when a non-UV feeding cue was paired with the anthraquinone-based repellent in Experiment 4, enhanced repellency of anthraquinone plus the UV-absorbing cue can be attributed to UV wavelengths. The addition of an UV feeding cue can enhance avian repellency at concentrations realized from previous field applications on agricultural crops (e.g., <1000 ppm anthraquinone).

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References


