



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



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## ABSTRACT

Application strategies for avian repellents are needed to maintain efficacious repellent concentrations throughout the period of needed crop protection. We investigated the repellency of an ultraviolet (UV) feeding cue in the absence of postingestive consequences, the combination of the UV feeding cue and an UV-absorbent, postingestive repellent (i.e., a repellent that causes negative postingestive consequences), and a non-UV feeding cue combined with the UV-absorbent, postingestive repellent in red-winged blackbirds (*Agelaius phoeniceus*). In the absence of negative postingestive consequences, 0.2% of the UV feeding cue (wt/wt) was not aversive relative to untreated food (i.e., baseline preference test;  $P=0.1732$ ). Relative to the repellency of food treated only with the anthraquinone-based repellent, synergistic repellency (i.e., 45–115% increase) was observed when 0.2% of the UV feeding cue was combined with 0.02% or 0.035% anthraquinone (wt/wt). In contrast, <10% repellency was observed for 0.2% of a non-UV feeding cue (red #40 aluminum lake dispersion) paired with 0.02% anthraquinone. Aversion performance was therefore not attributed to characteristics of either conditioned or unconditioned stimuli but their combinations, and enhanced repellency of anthraquinone plus the UV-absorbent cue was attributed to UV wavelengths. Thus, the addition of an UV feeding cue can enhance avian repellency at repellent concentrations realized from previous field applications on agricultural crops (e.g.,  $\leq 1000$  ppm anthraquinone).

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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.

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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<sup>-1</sup> (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 postingestive consequences (i.e.,

postingestive 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 postingestive 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, postingestive 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 postingestive 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, postingestive 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 postingestive consequences, (2) the combination of the UV feeding cue and an UV-absorbent, postingestive repellent, and (3) a non-UV feeding cue combined with the UV-absorbent, postingestive 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, FD&C 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

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