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Repellent application strategy for wild rodents and cottontail rabbits

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ABSTRACT

Effective chemical repellents and repellent application strategies are needed to manage damages caused by wild rodents and rabbits to agricultural resources. For the purpose of comparatively investigating the behavioral response of wild rodents and rabbits to a chemical repellent, we experimentally evaluated the concentration-response relationship of an anthraquinone-based repellent in California voles (*Microtus californicus* Peale), Richardson's ground squirrels (*Urocitellus richardsonii* Sabine), deer mice (*Peromyscus maniculatus* Wagner) and cottontail rabbits (*Sylvilagus audubonii* Baird) in captivity. We observed 52–56% feeding repellency for whole oats treated with 10,800 ppm anthraquinone or 18,500 ppm anthraquinone in mice and squirrels, and 84–85% repellency for oats treated with 18,300 ppm anthraquinone or 19,600 ppm anthraquinone in voles and rabbits, respectively. In addition to providing the negative postingestive consequences necessary for conditioned food avoidance, the anthraquinone-based repellent also absorbs ultraviolet (UV) wavelengths that are visible to most wild birds. For the purpose of developing a repellent application strategy to modify the behavior of vertebrate pests, we therefore conducted a conditioned avoidance experiment by offering repellent- and UV-treated food to California voles in a subsequent behavioral assay. Relative to unconditioned test subjects ($P = 0.3161$), voles conditioned with the UV, postingestive repellent subsequently avoided whole oats treated only with an UV cue ($P = 0.0109$). These behavioral responses to anthraquinone-based repellents and UV feeding cues can be exploited as a repellent application strategy for wild mammals. We discuss potential applications of preplant seed treatments and surface treatments that include postingestive repellents and related visual cues for the protection of agricultural resources associated with mammalian depredation.

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1. Introduction

The opportunistic feeding behavior and fecundity of some wild rodents and rabbits cause economic losses annually to world-wide agricultural production (Gebhardt et al., 2011; Jacob and Tkadlec, 2010; Johnson and Timm, 1987; Pelz, 2003; Salmon, 2008; Witmer and Singleton, 2010). For example, voles (*Microtus* spp. Schrank and *Arvicola* spp. La Cépède) are known to cause damage in the United States of America and Europe to agricultural crops such as alfalfa, peas and wheat, and reforestation efforts (Baldwin et al., 2014; Giusti, 2004; Jacob and Tkadlec, 2010; Sullivan and Sullivan, 2008; Witmer et al., 2007). Ground squirrels (*Spermophilus* spp. Cuvier) cause millions of dollars of damage to alfalfa production in the western United States and Canada (Johnson-Nistler et al., 2005; Proulx, 2010). Ground squirrels caused $17.9–23.9 million in crop losses and $11.9–17.9 million (dollars projected for 2016 valuation) in physical damages to materials such as structures, levees and earthen dams as well as damages to nut crops, tree fruits and rangeland forage (Baldwin et al., 2013; Marsh, 1998). Deer mice (*Peromyscus* spp. Gloger) cause damage to corn, almonds, avocados, citrus, pomegranate and sugar beet crops (Pearson et al., 2000; Witmer and Moulton, 2012). Cottontail rabbits (*Sylvilagus floridanus* Allen) damage tree seedlings, shrubs, hay, soybean and rangeland forage (Dugger et al., 2004; Johnson and Timm, 1987).

Agricultural depredation caused by wild rodents and rabbits is a persistent problem with few cost-effective solutions. Methods to alleviate damage caused by wild rodents and rabbits include behavioral applications (e.g. physical exclusion, chemical repellents) and lethal removal. The need for effective solutions to mammal depredation remains despite prior evaluations of numerous chemical repellents (Agnello et al., 2014; Baldwin et al., 2014; Gurney et al., 1996; Nolte and Barnett, 2000; Nolte et al., 1993; Sutherland, 2003; Williams and Short, 2014). The effectiveness and commercial development of wildlife repellents are dependent upon the repellent's

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efficacy under field conditions, cost relative to expected damages of unprotected resources, environmental impacts, and food and feed safety (Werner et al., 2009). Thus, data regarding efficacy, chemical residues and application strategies are presently needed for the development of non-lethal repellents and the protection of agricultural resources from wild rodents and rabbits.

Although anthraquinone is a naturally-occurring compound that was identified as a promising avian repellent in the early 1940s (Heckmanns and Meisenheimer, 1944), an anthraquinone-based seed treatment (AV-1011; Arkion® Life Sciences, New Castle, DE, USA) was first registered by the United States Environmental Protection Agency for the protection of newly-planted rice in January 2016. Anthraquinone has been used to effectively repel blackbirds (Avery et al., 1997, 1998; Carlson et al., 2013; Cummings et al., 2002a,b, 2011; Neff and Meanley, 1957; Werner et al., 2009, 2011a, 2014b,c), Canada geese (*Branta canadensis* Linnaeus; Blackwell et al., 1999; Dolbeer et al., 1998; Werner et al., 2009), sandhill cranes (*Grus canadensis* Linnaeus; Blackwell et al., 2001), ring-necked pheasants (*Phasianus colchicus* Linnaeus; Werner et al., 2009), European starlings (*Sturnus vulgaris* Linnaeus; Tupper et al., 2014), wild turkeys (*Meleagris gallopavo* Linnaeus; Werner et al., 2014a), horned larks (*Eremophila alpestris* Linnaeus), great-tailed grackles (*Quiscalus mexicanus* Gmelin) and American crows (*Corvus brachyrhynchos* Brehm; Werner et al., 2015).

Relatively few studies, however, have evaluated anthraquinone as a mammalian repellent. Santilli et al. (2005) discovered that wild boar (*Sus scrofa* Linnaeus) consumed 86.5% less corn treated with 0.64% anthraquinone than untreated corn. Werner et al. (2011b) observed 24–37% repellency in black-tailed prairie dogs (*Cynomys ludovicianus* Ord) offered corn seeds treated with 0.5–4.0% anthraquinone. Cowan et al. (2015) observed an aversion to anthraquinone-treated baits in black rats (*Rattus rattus* Linnaeus; 0.1% and 0.25% anthraquinone) and possums (*Trichosurus vulpecula* Kerr; 0.25% anthraquinone). Relative to the consumption of control baits (0.01–0.03% cinnamon, green carrots), the consumption of anthraquinone-treated baits was less in brown rats (*R. norvegicus* Berkenhout; 0.04% and 0.08% anthraquinone) and no different in possums (*T. vulpecula,* 0.08% anthraquinone; Clapperton et al., 2015). Although Hansen et al. (2015) observed that female common voles (*M. arvalis* Pallas) consumed 47% less wheat treated with 5% anthraquinone and chloroform than wheat treated only with chloroform, Hansen et al. (2016a) found no difference in consumption of wheat treated with 15% anthraquinone and chloroform in male common voles and greater consumption of wheat treated with 15% anthraquinone and chloroform in male house mice (*Mus musculus* Linnaeus) relative to wheat treated only with chloroform.

The purposes of this study were to comparatively investigate the behavioral response of wild rodents and rabbits to a chemical repellent, and develop an effective application strategy for the protection of agricultural resources commonly damaged by these wild mammals. Our objectives were to (1) experimentally evaluate the concentration-response relationship of an anthraquinone-based repellent for California voles (*M. californicus* Peale), Richardson's ground squirrels (*Urocitellus richardsonii* Sabine), deer mice (*P. maniculatus* Wagner) and cottontail rabbits (*S. audubonii* Baird), and (2) develop a repellent application strategy by exploiting the behavioral responses of wild rodents and rabbits to anthraquinone-based repellents and associated visual cues.

Most placental mammals (e.g. wild rodents, rabbits) are dichromatic, having two classes of cone photopigment (i.e. long- and short-wave sensitive visual pigments; David-Gray et al., 2002). The short-wave sensitive (SWS) visual pigments of vertebrate cone photoreceptors are divided into two molecular classes, SWS1 and SWS2. Only the SWS1 class is present in mammals. The SWS1 class has been subdivided into violet-sensitive (VS; peak maximum absorbance, or λ_{max} = 400–430 nm) and ultraviolet-sensitive visual pigments (UVS, λ_{max} < 380 nm; Cowing et al., 2002). Although ultraviolet (UV) sensitivity is widespread among animals, UVS visual pigments are considered rare in mammals (Douglas and Jeffery, 2014). Animals without UVS visual pigments, however, will be sensitive to UV wavelengths if they have ocular media that transmit UV wavelengths, as all visual pigments absorb significant amounts of UV if the energy level is sufficient (Douglas and Jeffery, 2014). For the purpose of developing an effective repellent application strategy, we were therefore interested to investigate the conditioned avoidance of UV visual cues subsequent to exposure to an UV, postingestive repellent in California voles.

2. Concentration-response feeding experiments

Four concentration-response feeding experiments were conducted at the headquarters of the National Wildlife Research Center (NWRC) in Fort Collins, Colorado (USA). We live-captured 38 California voles adjacent to commercial artichoke fields in California USA, 28 Richardson's ground squirrels within alfalfa fields in Montana, and 34 deer mice and 30 cottontail rabbits adjacent to NWRC-Fort Collins using appropriate Scientific Collection Permits. We used 8–10 test subjects per treatment group (Werner et al., 2009, 2011b) and thus 3–4 concentrations for each of the four tested species based upon the availability of test subjects subsequent to live-captures. The capture, care and use of all test subjects associated with each experiment were approved by the NWRC Animal Care and Use Committee (NWRC Study Protocols QA-2104, QA-2243, QA-2333; S.J. Werner- Study Director).

All test subjects were offered a maintenance diet for at least one week prior to each of the feeding experiments (i.e. quarantine, holding). For the purpose of comparatively investigating the intra- and interspecific efficacy of a chemical repellent, all test subjects were maintained within individual cages throughout the experiments (quarantine, holding, acclimation, pre-test, test). California voles, Richardson's ground squirrels and cottontail rabbits were maintained within visually-isolated, individual cages (23 × 41 × 18-cm cages for voles, 62 × 50 × 42-cm for ground squirrels, 62 × 50 × 42-cm for rabbits) in an NWRC indoor animal research building. Deer mice were maintained within individual cages (46 × 24 × 19-cm) in the NWRC outdoor animal research facility throughout the experiment to reduce the potential exposure of researchers to hantavirus. Free access to water and environmental enrichment were provided to all test subjects throughout the feeding experiments.

An anthraquinone-based repellent (Avipel® Shield, active ingredient: synthetic 9,10-anthraquinone; Arkion® Life Sciences, New Castle, DE, USA) was used for each of the experiments (Werner et al., 2009, 2010, 2011a,b). Seed treatments for all concentration-response experiments were formulated by applying aqueous suspensions (100 ml/kg) to the test diet using a rotating mixer and household spray equipment (Werner et al., 2014a). The test diet for each of the concentration-response feeding experiments was whole oats.

We hypothesized that repellency would be directly related to repellent concentration during our concentration-response experiments. We operationally defined ≥80% repellency as efficacious during our previous laboratory feeding experiments (Werner et al., 2009, 2011a, 2014a,b,c). Thus, we predicted that consumption of efficacious treatments (i.e. threshold repellency) would be ≤20% of average, pre-test consumption during the concentration-response experiments.

For each test group, the dependent measure of our concentration-response experiments was calculated as average test consumption of treated test diet relative to average, pre-test consumption of untreated test diet (i.e. percent repellency). The NWRC Analytical Chemistry Unit used high performance liquid

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