Registered Pesticides and Citrus Terpenes as Blackbird Repellents for Rice

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ABSTRACT Nonlethal management alternatives are needed to minimize bird depredation of agricultural crops. We conducted 8 caged feeding tests and 2 field studies to evaluate 2 registered fungicides (GWN-4770, Gowan Company, Yuma, AZ; Quadris®, Syngenta Crop Protection, Greensboro, NC), a neem oil insecticide (Aza-Direct®, Gowan Company), and a novel terpene formulation (Gander Gone, Natural Earth Products, Winter Springs, FL) as avian repellents. For all candidate repellents, red-winged blackbirds (*Agelaius phoeniceus*) discriminated between untreated and treated rice during preference-testing in captivity. We observed a positive concentration–response relationship among birds offered rice treated with 2,500 ppm, 5,000 ppm, 7,500 ppm, 11,000 ppm, or 22,000 ppm GWN-4770. Relative to pretreatment, blackbirds consumed 34% and 77% less rice treated with 11,000 ppm and 22,000 ppm GWN-4770, respectively, during the concentration–response test. Maximum repellency among other tested compounds was <40% during the concentration–response test. Blackbirds consumed 28% of rice seeds treated with 20,000 ppm GWN-4770 and 68% of untreated seeds broadcast within rice fields in southwestern Louisiana, USA. We observed 50% fewer unprotected seedlings than those treated with 10,000 ppm GWN-4770 within a drill-seeded rice field in southeastern Missouri, USA. The manufacturer subsequently applied for a United States patent for the active ingredient of GWN-4770 as an avian repellent. Although additional registration criteria and formulation optimization must be satisfied to enable the commercial availability of GWN-4770 as an avian repellent, additional efficacy studies of GWN-4770 and other promising repellents under extended field conditions are warranted for protection of newly planted and ripening rice. (JOURNAL OF WILDLIFE MANAGEMENT 72(8):1863–1868; 2008)

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Red-winged blackbirds (*Agelaius phoeniceus*), common grackles (*Quiscalus quiscula*), and brown-headed cowbirds (*Molothrus ater*) cause extensive damage to newly planted and ripening rice in the mid-South of the United States (Cummings et al. 2002, 2005). Cummings et al. (2005) estimated that the economic loss to the rice industry in Louisiana, Arkansas, Texas, California, and Missouri from blackbirds in 2001 due to direct damage, prevention, and lost price support was US$21.5 million. These losses have motivated rice producers to use various bird-damage management practices, including chemical repellents.

Development of chemical repellents is constrained by environmental regulations and registration costs (Avery et al. 2005, Linz et al. 2006, Werner et al. 2008). Thus, few avian repellents are currently registered by the United States Environmental Protection Agency for agricultural applications. Although anthraquinone effectively protects rice seed from blackbirds under captive and field conditions (Avery et al. 1997, Cummings et al. 2002), this compound is not registered as a blackbird repellent in the United States. Avery et al. (2005) suggested that caffeine may be an effective, economical, and environmentally safe chemical repellent for reducing bird damage to newly seeded rice. Formulation improvements are needed, however, for the development of caffeine as an avian repellent (Werner et al. 2007). Werner et al. (2005) concluded that Bird Shield™ (a registered formulation of methyl anthranilate; Bird Shield Repellent Corporation, Spokane, WA) was not effective for repelling blackbirds from ripening rice and sunflower fields. Although we previously observed repellency of 3 registered pesticides (Dividend Extreme®, Karate®, Tilt®; Syngenta Crop Protection, Greensboro, NC) for blackbirds in caged feeding trials, the label application of Tilt fungicide did not reduce blackbird consumption within a maturing rice field (Werner et al. 2008). Our purpose was to further evaluate the efficacy of candidate blackbird repellents for reducing rice damage.

We conducted caged feeding tests and 2 field studies to evaluate 2 natural compounds and 2 registered fungicides as avian repellents. We tested natural compounds and registered pesticides because of the aforementioned regulatory and economic constraints on development of chemical repellents. We used preference and concentration–response tests to evaluate Aza-Direct® (Gowan Company, Yuma, AZ), Gander Gone (Natural Earth Products, Winter Springs, FL), GWN-4770 (Gowan

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Company), and Quadris® (Syngenta Crop Protection). Aza-Direct, GWN-4770, and Quadris are currently registered for rice production in the United States. The active ingredient of Aza-Direct (i.e., 1.2% azadirachtin; triterpenoid insecticide) is extracted from neem seeds and effectively repels insects and birds from particular foods (Xie et al. 1995, Mason and Matthew 1996). Gander Gone contains citrus terpenes, or plant-derived hydrocarbons that repel arthropod (Snyder et al. 1993) and mammalian (Villalba et al. 2002) herbivores (see also Watkins et al. 1999 for structure–activity relationships of terpenoids as avian repellents). Active ingredients of the tested fungicides were flutolanil (benzanilide fungicide; GWN-4770) and 22.9% azoxystrobin (strobilurin fungicide; Quadris). The mode of action of these fungicides as avian repellents is unknown. We conducted 2 subsequent field studies to evaluate repellency of GWN-4770 within fields where rice had been broadcast or drill-seeded.

STUDY AREA

We conducted preference and concentration–response tests with red-winged blackbirds at the United States Department of Agriculture (USDA), National Wildlife Research Center's (NWRC) outdoor animal research facility in Fort Collins, Colorado, USA. We maintained all birds in 4.9 × 2.4 × 2.4-m cages (25–40 birds/cage) within an open-sided building for $\geq$2 weeks prior to testing. We provided free access to water, grit, and maintenance food (2 millet:1 milo:1 safflower:1 sunflower) to all birds during quarantine and holding. We conducted feeding tests within individual cages (0.9 × 1.8 × 0.9 m) in an open-sided building.

In February 2004, we evaluated repellency of treated seed-rice broadcast on fallow rice fields in Vermilion and Cameron Parish, Louisiana. All fields were drained and leveled prior to our study. We completed a supplemental field study in April 2004 at the Southeast Missouri State University Rice Research Farm (Malden, MO) to evaluate repellency within a drill-seeded rice field. Study plots were drained, rolled, and leveled prior to the study.

METHODS

Preference-Testing

We conducted a preference-test for each candidate repellent. We captured 87 experimentally naïve red-winged blackbirds in 2003–2004 near Fort Collins, Colorado, and transported them to NWRC. Because gender affects rice consumption among blackbirds (Avery et al. 2005), we used adult males for all feeding tests. We randomly assigned 18–23 blackbirds to each of 4 tests conducted from June 2003 to June 2004. We transferred birds to individual cages following group quarantine and holding, and offered birds untreated seed rice (ad libitum) in each of 2 food bowls (30 cm wide × 9 cm high) for 3–5 days of acclimation. We provided water ad libitum to all birds throughout preference-testing (i.e., acclimation, pretreatment, test). The NWRC Institutional Animal Care and Use Committee approved capture, care, and use of birds associated with feeding tests (NWRC Study Protocol QA973).

We offered 30 g of untreated rice in each of 2 bowls to all birds during each day of the 4-day pretreatment. We collected unconsumed rice (remaining in food bowls) and spilt rice (remaining in trays beneath each bowl) at 0800–0930 hours daily and determined their mass ($\pm$0.1 g). We measured pretreatment and test consumption independently for food bowls located on the north and south sides of each cage. We accounted for changes in the mass of rice independent of rice consumption (e.g., desiccation) by weighing rice offered within a vacant cage. We offered untreated rice (ad libitum) in 2 bowls for the 3 days between pretreatment and test periods.

We offered one bowl of untreated rice and one bowl of rice treated with one of the candidate repellents (30 g rice in each bowl) to all birds during each day of the 4-day test. We formulated our repellent treatments based upon manufacturer recommendations for agricultural applications. Treatments included 28.0 mL Aza-Direct per kg rice (28,000 ppm), 17.0 mL/kg Gander Gone (17,000 ppm), 11.0 g/kg GWN-4770 (11,000 ppm), and 10.3 mL/kg Quadris (10,300 ppm). Whereas pesticide applications for rice production often include anti-transpirants and stickers, we added 6 mL Transfilm® (PBI/Gordon, Kansas City, MO) to each formulation (per manufacturer label). We applied treatment solutions (60 mL/kg) to 10 kg certified seed rice (Louisiana State University Rice Research Station, Crowley, LA) using a rotating mixer and household spray equipment. We randomized the north–south positioning of treatments within individual cages on the first day and alternated positioning on subsequent days of the test.

The dependent measure for preference-testing was average (daily) rice consumption during pretreatment and test periods. We used the general linear model procedure (SAS Institute, Inc., Cary, NC) to conduct an analysis of variance (ANOVA) associated with preference-testing for each candidate repellent. Repellent treatments were fixed among bird subjects. The independent variables (and associated error terms) of these analyses were testing periods and the period–treatment interaction (subject–period–treatment), and the period–day interaction (residual error). We offered one bowl of untreated rice on each of the north and south sides of all cages throughout pretreatment. Thus, we regarded pretreatment consumption of treated rice as that which was offered on the north (days 1 and 3) and south side (days 2 and 4) for statistical analyses. We used Tukey's tests to separate means of ANOVA effects ($\alpha = 0.05$) and descriptive statistics ($\bar{x} \pm$ SE) to summarize test consumption of treated and untreated rice.

Concentration–Response Testing

We conducted a concentration–response test for each candidate repellent. We captured 160 experimentally naïve red-winged blackbirds (ad M) in 2003–2004 near Fort Collins, Colorado, and transported them to NWRC. We randomly assigned 40 blackbirds to each of 4 tests conducted from June 2003 to June 2004. We repeated all quarantine, holding, acclimation, and pretreatment procedures (using one food bowl; 30 cm wide $\times$ 9 cm high) used during preference-testing.

For each test, we ranked blackbirds based upon average pretreatment consumption and assigned them to 1 of 5 groups (n = 6–9 birds/group) such that each group was similarly populated with birds that exhibited high–low daily consumption. We randomly assigned treatments among groups. We used treatment groups to evaluate decreased rice consumption (i.e., repellency) associated with each of 5 concentrations for all candidate repellents. We selected repellent concentrations that represented between 10–20% and 100–200% manufacturer-recommended application rates. Our repellent treatments included 5.0 mL, 10.0 mL, 15.0 mL, 20.0 mL, and 28.0 mL Aza-Direct/kg rice; 4.0 mL/kg, 8.0 mL/kg, 12.5 mL/kg, 17.0 mL/kg, and 33.0 mL/kg Gander Gone; 2.5 g/kg, 5.0 g/kg, 7.5 g/kg, 11.0 g/kg, and 22.0 g/kg GWN-4770; and 1.3 mL/kg, 2.6 mL/kg, 5.1 mL/kg, 10.3 mL/kg, and 20.6 mL/kg Quadris. We applied treatment solutions (60 mL/kg), including 6-mL Transfilm, to 10 kg certified seed rice. We offered one bowl of treated rice (30 g) to all birds and measured consumption during the 1-day test.

We hypothesized that repellency would be directly related to repellent concentration. We predicted that test consumption associated with efficacious treatments would be <25% (i.e., ≥75% repellency; Schneider 1982) of pretreatment consumption. The dependent measure for concentration–response testing was percent repellency (i.e., test day 1 relative to average pretreatment consumption) as a function of repellent concentration. We used regression procedures (SAS Institute, Inc.) to analyze repellency exhibited during concentration–response testing.

Field Evaluation of Broadcast Seed

We conducted a field evaluation to investigate the spatial extent of inferences from our preference-testing results. We established 5 field sites (0.6–0.8 ha/site) near traditional blackbird roosting areas or under predominant flight lines emanating from those roosts in southwestern Louisiana. Sites were separated by 1–23 km. We prebaited each site with untreated seed rice for 3–5 days to establish blackbird feeding activity. We broadcast rice at a rate of 10 kg/lane on each of 4 lanes (10 m wide, 50–70-m long) at each site. Lanes were separated by 25 m. We observed blackbirds foraging at each site between 0700 and 1100 hours daily for 3 days. All sites had intermediate blackbird activity (approx. 150–300 birds/site; 75–85% red-winged blackbirds, 10–20% brown-headed cowbirds, 5–10% common grackles) throughout the study. The NWRC Institutional Animal Care and Use Committee approved the use of birds associated with our field evaluations (NWRC Study Protocol QA1127).

We treated 18 kg of seed rice with 360 g GWN-4770 (20,000 ppm), 20 mL Transfilm, and 620 mL water for each site. We applied the treatment solution for 4 minutes using household spray equipment within a rotating cement mixer. We stored all treated rice for <24 hours before uniformly broadcasting it on 2 randomly selected lanes using an all-terrain vehicle and electric spreader on each site. We baited the 2 remaining lanes with untreated seed rice (9 kg/lane).

We established 10 permanent sampling plots (0.3 $\times$ 0.3 m) along the center line of each lane at all sites to estimate daily consumption of seed rice by birds. We systematically placed plots at 9-m intervals along the lane beginning with a random starting point between 1 m and 9 m. We manipulated each plot daily to contain 25 rice seeds that visually matched the surrounding density of broadcast rice seed at the beginning of the 3-day evaluation. We assessed plots daily until all seed rice was consumed, blackbirds abandoned the test site, or 3 days had elapsed. We observed 23 mm (average = 3 mm/day; range = 0–22 mm/day) of rain during the field study.

The dependent measure was the average number of consumed rice seeds within each plot throughout the 3-day evaluation. Repellent treatments were fixed among field sites. The independent variables (and associated error terms) were the treatment effect (site-treatment) and the day–treatment interaction (residual error). We used descriptive statistics ($\bar{x} \pm$ SE) to summarize blackbird consumption of treated and untreated rice seeds.

Field Evaluation of Drilled Seed

We established 12 test plots (3 $\times$ 5 m) within an experimental rice field in southeastern Missouri to further evaluate efficacy of GWN-4770 as an avian repellent under field conditions. We randomly assigned plots to 1 of 2 treatments. We drill-seeded 6 plots with rice treated with 10,000 ppm GWN-4770 and 1,000 ppm Transfilm and 6 plots with untreated seed rice. We observed continuous blackbird activity (approx. 100 M red-winged blackbirds) within the experimental field (i.e., among all plots) and 103 mm (average = 4 mm/day; range = 0–38 mm/day) of rain during the study.

We randomly established 8 subplots (0.5 $\times$ 0.5 m) within each plot. We excluded birds from 4 reference subplots in each plot using woven wire. We used the remaining subplots (birds not excluded) to estimate bird damage to treated and control seedlings. We counted the number of emergent seedlings within each subplot 27 days following planting.

The dependent measure was the average number of rice seedlings within each subplot at the conclusion of the evaluation. We randomized repellent treatments among test plots. The independent variables (and associated error terms) were the treatment effect (plot[treatment]), exclosure (i.e., birds present or absent), and the treatment–exclosure interaction (exclosure $\times$ plot[treatment]). We used Tukey's tests to separate means of ANOVA effects (SAS Institute, Inc.; $\alpha = 0.05$).

RESULTS

Preference-testing

We observed less consumption of rice treated with 28,000 ppm Aza-Direct (Fig. 1A) than that of untreated rice during preference-testing (i.e., period–treatment interaction; $F_{2,66}$ = 8.93, $P < 0.001$). On average, blackbirds consumed 4.1

($\pm$0.34; SE) g/bird/day of untreated rice and 1.9 ($\pm$0.31) g/bird/day of rice treated with Aza-Direct ($P < 0.05$). We observed no difference in overall consumption during the pretreatment and test (i.e., period effect; $F_{1,66} = 0.00$, $P = 0.963$) or among days of these testing periods (period–day interaction; $F_{6,194} = 1.03$, $P = 0.405$).

Blackbirds consumed less rice treated with 17,000 ppm Gander Gone (Fig. 1B) than untreated rice during the test ($F_{2,88} = 63.53$, $P < 0.001$). Average consumption was 8.3 ($\pm$0.36) g/bird/day of untreated rice and 1.8 ($\pm$0.34) g/bird/day of treated rice ($P < 0.05$). We observed no period effect ($F_{1,88} = 2.59$, $P = 0.111$) or period–day interaction ($F_{6,270} = 0.53$, $P = 0.788$) during the Gander Gone preference-test.

We observed less consumption of rice treated with 11,000 ppm GWN-4770 (Fig. 1C) than that of untreated rice during the test ($F_{2,88} = 418.35$, $P < 0.001$). On average, blackbirds consumed 9.1 ($\pm$0.23) g/bird/day of untreated rice and 0 ($\pm$0.09) g/bird/day of treated rice ($P < 0.05$). We observed no period effect ($F_{1,88} = 2.18$, $P = 0.143$) or period–day interaction ($F_{6,270} = 0.39$, $P = 0.886$) during the GWN-4770 preference-test.

Blackbirds consumed less rice treated with 10,300 ppm Quadris (Fig. 1D) than untreated rice during the test ($F_{2,80} = 97.37$, $P < 0.001$). Average consumption was 5.9 ($\pm$0.27) g/bird/day of untreated rice and 0.4 ($\pm$0.14) g/bird/day of treated rice ($P < 0.05$). We observed no period effect ($F_{1,80} = 0.53$, $P = 0.470$) or period–day interaction ($F_{6,234} = 0.65$, $P = 0.687$) during the Quadris preference-test.

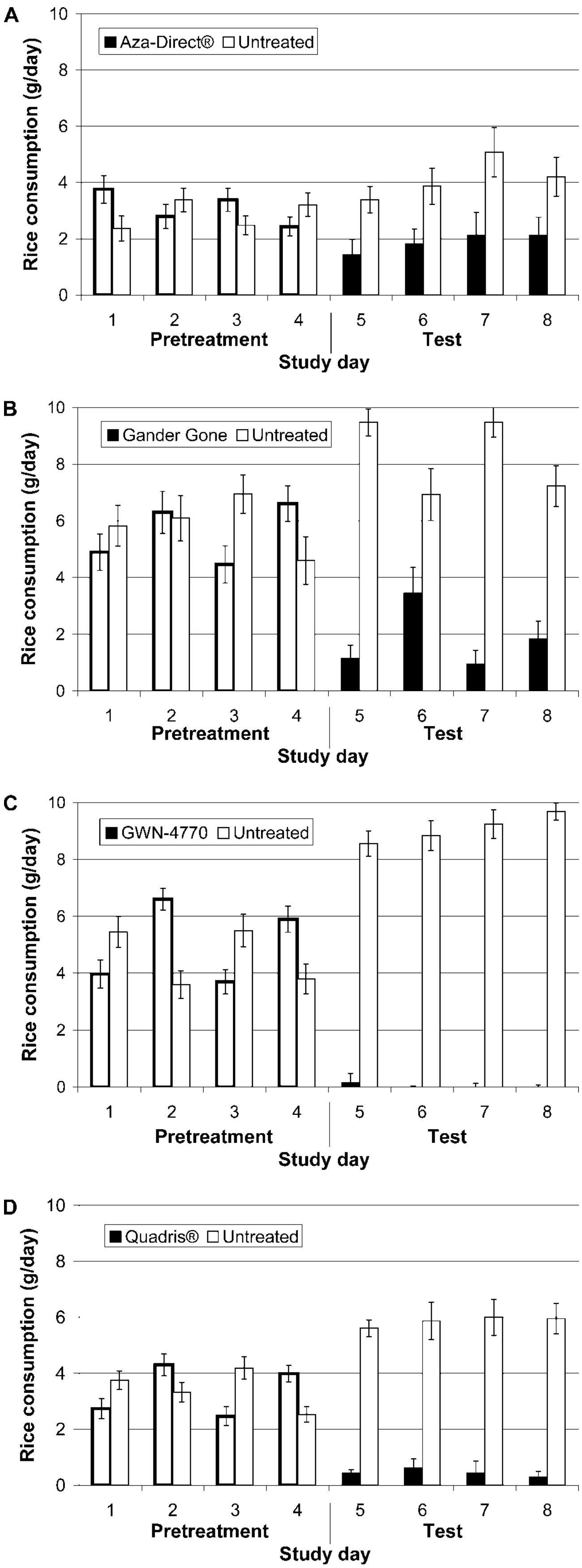

Figure 1. Rice consumption ($\bar{x} \pm$ SE) among red-winged blackbirds offered untreated rice and rice treated with (A) 28,000 ppm Aza-Direct® insecticide (Gowan Company, Yuma, AZ), (B) 17,000 ppm Gander Gone

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terpene formulation (Natural Earth Products, Winter Springs, FL), (C) 11,000 ppm GWN-4770 fungicide (Gowan Company), or (D) 10,300 ppm Quadris® fungicide (Syngenta Crop Protection, Greensboro, NC) at the National Wildlife Research Center in Fort Collins, Colorado, USA, June 2003–June 2004. We offered all birds 2 bowls of untreated rice during the pretreatment.

Concentration–Response Testing

We observed no concentration–response relationship for Aza-Direct or Gander Gone. Repellency (i.e., test relative to pretreatment consumption) of rice treated with Aza-Direct was unrelated to tested concentrations ($r^2 = 0.007$, $P = 0.646$). Maximum repellency (31%) of Aza-Direct was associated with the 10,000 ppm (second-lowest) concentration. Repellency was also unrelated to tested concentrations of Gander Gone ($r^2 = 0.004$, $P = 0.691$). Maximum repellency (25%) was associated with 12,500 ppm Gander Gone (i.e., the middle concentration tested).

Relative to pretreatment, blackbirds consumed 34% and 77% less rice treated with 11,000 and 22,000 ppm GWN-4770, respectively, during the test (Fig. 2). We observed a direct concentration–response relationship among tested concentrations of GWN-4770 ($r^2 = 0.600$, $P < 0.001$). Thus, rice consumption decreased with increasing concentrations of this registered fungicide.

Repellency was also related to tested concentrations of Quadris fungicide ($r^2 = 0.154$, $P = 0.012$). Maximum repellency, however, was only 37% for 20,600 ppm Quadris

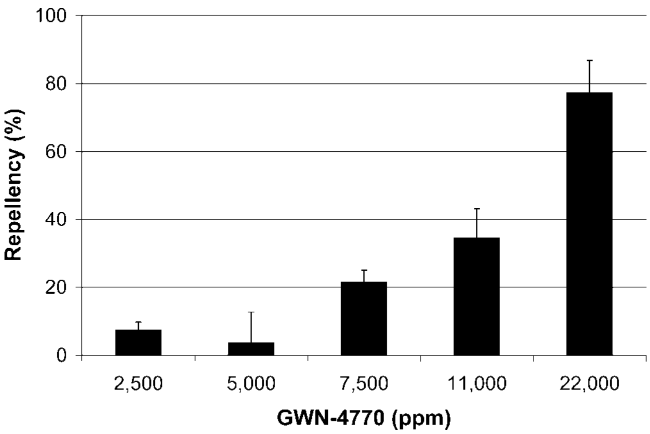

Figure 2. Avian repellency ($\bar{x} \pm$ SE) associated with 5 concentrations of GWN-4770 fungicide (Gowan Company, Yuma, AZ; $N = 8$ caged birds/group) at the National Wildlife Research Center in Fort Collins, Colorado, USA, August 2003. Repellency represents test (day 1) consumption relative to average pretreatment rice consumption.

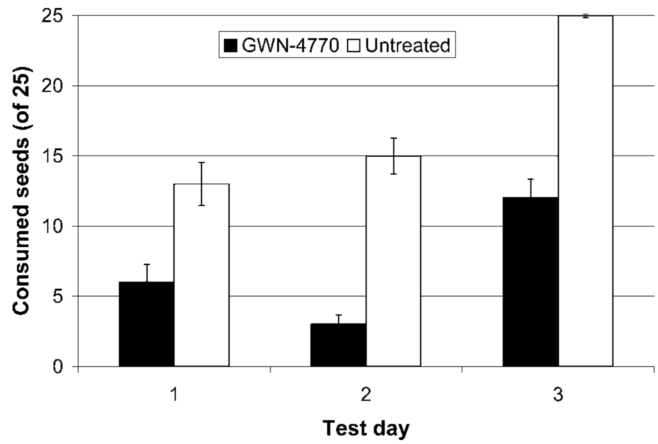

Figure 3. Rice seeds ($\bar{x} \pm$ SE) consumed by blackbirds on lanes baited with treated (20,000 ppm GWN-4770; Gowan Company, Yuma, AZ) and untreated rice for 3 days in southwestern Louisiana ($N = 5$ study sites), USA, February 2004.

(i.e., the highest concentration tested). Thus, we proceeded with field evaluations of GWN-4770.

Field Evaluation of Broadcast Seed

Birds consumed more untreated rice than rice treated with 20,000 ppm GWN-4770 ($F_{1,8} = 5.72$, $P = 0.044$) within fields where seed had been broadcast above ground. Of 25 seeds placed on permanent plots within treated and control lanes, birds consumed an average of 7 ($\pm$0.7) treated seeds and 17 ($\pm$0.8) untreated seeds throughout the 3-day test (Fig. 3). Thus, birds consumed 28% of rice seeds treated with GWN-4770 and 68% of untreated seeds broadcast within fallow rice fields in southwestern Louisiana.

Field Evaluation of Drilled Seed

We observed a treatment–exclosure interaction ($F_{1,10} = 6.25$, $P = 0.032$). We counted more treated seedlings than untreated seedlings within nonexclosed (i.e., birds present) subplots ($P < 0.05$; Fig. 4). We observed no difference in number of emergent seedlings between treated and untreated plots (i.e., among all subplots; $F_{1,10} = 2.13$, $P = 0.175$). We also observed no difference in seedling numbers within exclosed and nonexclosed subplots (i.e., among all plots; $F_{1,10} = 2.95$, $P = 0.116$). Thus, we observed 50% fewer unprotected seedlings than those treated with 10,000 ppm GWN-4770 within a drill-seeded rice field in southeastern Missouri.

DISCUSSION

Among tested compounds, GWN-4770 was most effective for reducing blackbird consumption of rice. Blackbirds preferred untreated seed rice to rice treated with any of the candidate repellents throughout preference-testing. We observed a positive concentration–response relationship among tested concentrations of GWN-4770 and Quadris. We also observed a targeted reduction (i.e., $\geq$75% repellency; Schneider 1982) in consumption of seed rice treated with GWN-4770. Blackbirds also exhibited preference for untreated rice during our field evaluations of 10,000 ppm (drilled seed within an experimental rice field) and 20,000 ppm (broadcast seed) GWN-4770.

Most rice producers in the mid-South of the United States sustain bird depredation during late winter and early spring as rice is planted, and during late summer and early autumn prior to harvest. Much rice in the southern extent of the mid-South growing region is planted via water seeding, or planting of presoaked seeds within flooded fields. Other plantings utilize grain drills within drained fields. We evaluated efficacy of GWN-4770 when treated seeds were broadcast and drill-seeded within drained fields. Additional efficacy studies are needed to evaluate GWN-4770 and other promising repellents for protection of newly planted and ripening rice under extended field conditions.

MANAGEMENT IMPLICATIONS

Subsequent to our study, the manufacturer applied for a United States patent for the active ingredient of GWN-4770 as an avian repellent. In addition to cage- and field-

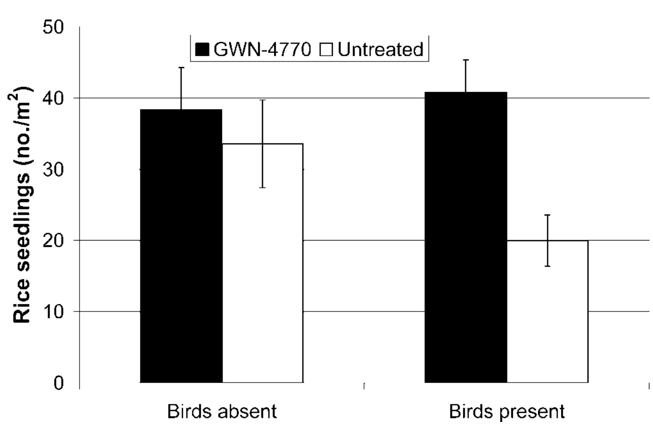

Figure 4. Rice seedlings ($\bar{x} \pm$ SE) on treated (10,000 ppm GWN-4770; Gowan Company, Yuma, AZ) and untreated plots within a drill-seeded rice field in southeastern Missouri ($N = 6$ plots), USA, April 2004. We excluded blackbirds from birds-absent subplots and enabled them to freely forage within birds-present subplots for 27 days after planting.

efficacy data, several registration requirements (e.g., product and residue chemistry, toxicology, environmental fate) are necessary to develop avian repellents for agricultural applications. Although GWN-4770 is currently registered by the United States Environmental Protection Agency as a systemic fungicide for rice, additional registration criteria and formulation optimization must be satisfied to enable the commercial availability of this product as an avian repellent. The cost associated with the label application rate of GWN-4770 for rice is approximately $54/ha (United States currency; G & H Seed Company, Inc., Crowley, LA). The existing label for GWN-4770 allows this product to be applied within rice fields up to 30 days prior to harvest. Thus, this product is formulated to maintain residues throughout the 3–4 weeks of ripening phenology associated with most blackbird depredation. In addition to potential rice applications, the development of GWN-4770 as an avian repellent is dependent upon its market justification for protecting sunflowers (Linz et al. 2006), corn (Stone et al. 1972), blueberries (Nelms et al. 1990), cherries (Tobin et al. 1991), lettuce (Cummings et al. 1998), and other agricultural crops affected by bird depredation.

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