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TESTING THREE CHEMICALS FOR DETERRING CROP DAMAGE BY CRANES

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Abstract: Damage to planted corn seed by cranes has the potential to cause great economic loss in areas where both intersect. In 2000 the International Crane Foundation (ICF) tested limonene (LIM), methyl anthranilate (MA), and 9,10-anthraquinone (AQ) as possible replacements for the insecticides lindane and diazinon that had been used as deterrents to cranes damaging corn seed and seedlings. LIM, MA, and AQ lowered germination rates (down to 85, 90, and 92%, respectively) as compared to a germination rate of 96% in untreated corn. A 1.0% solution of AQ was effective as a crane deterrent, while LIM and MA were not. Both LIM and MA metabolized in the soil too quickly to be effective during the entire period when corn seedlings were vulnerable to crane herbivory. In 2001, a 0.5% concentration of AQ in 2 different soils (sand and organic) was tested in 2 different time periods (trial 1, 15 May to 14 June; trial 2, 26 June to 7 July 2). The concentration of AQ did not degrade to below effective levels in either soil type or in either time period. In all trials, AQ concentration of 0.5% prevented crane herbivory. Crane response to AQ-treated corn was to continue foraging in fields without damaging the planted crop. We believe AQ is an effective chemical deterrent and will prove useful for preventing crane damage to planted corn.

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Greater sandhill cranes (*Grus canadensis tabida*) use agricultural fields in Wisconsin for food (Bennett 1978, Melvin 1978, Barzen et al. 2018). While cranes foraging in fields can be beneficial by removing waste grain and invertebrate pests, they can also feed on the kernels of newly geminated corn plants (Bennett 1978), damaging significant corn acreage each year (Barzen and Ballinger 2018). Often, more traditional methods of deterrence, such as lure crops and scare methods, do not resolve the problem. These methods move birds (and thus the problem) from 1 field to another but they do not stop the damage from occurring (O'Connor and Shrubb 1986, Knittle and Porter 1988). In some cases, lure crops can even attract more birds to a specific field or area, exacerbating the existing problem. Crop damage compensation programs require and maintain an antagonistic relationship between wildlife and landowner in that the compensation growers receive rarely reimburses the growers at the rate they believe they have lost, and they do not address the actual problem, that of preventing loss in the first place (Barzen and Ballinger 2017).

Scare tactics and lure crops move cranes among habitats (Austin and Sundar 2018). As a result, cranes (or other bird species causing damage) often readily habituate to various scare tactics if these strategies (e.g., propane cannons) attempt to prevent cranes from using the whole field (that cranes highly prefer). Rather than removing the whole field from availability to cranes, chemical taste deterrents used on an individual food item remove 1 type of food among many items (Johnson 1980) and protect the crop while still allowing cranes to remain in preferred fields (Barzen et al. 2018). To date, 119 chemical repellents have been tested for avian deterrence (Werner and Avery 2017).

The insecticides lindane (gamma isomer of hexachlorocyclohexane, g-HCH; Blus et al. 1984) or diazinon (Schafer et al. 1983) can prevent damage to corn seedlings by many bird species. Both chemicals likely work as taste deterrents after gut irritation provides a conditioned response (Werner and Clark 2003), causing cranes to avoid foraging on treated seed but allowing the birds to continue foraging on other food items in the field. However, lindane is no longer available for use on corn seed (U.S. Environmental Protection Agency 2006), because it persists in the environment (Cheah et al. 1998), is resistant to photolysis and hydrolysis (except at high pH), and degrades slowly by microbial actions (Walker et al. 1999); diazinon has very limited use in the U.S. and has been removed as a seed treatment (U.S. Environmental Protection Agency 2007). An alternative deterrent could provide farmers with an

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environmentally appropriate, but economically viable, substitute to prevent crane damage to germinating corn and allow landowners to use new deterrents while not requiring costly, ineffective compensation programs (Barzen and Ballinger 2017).

During the 2000 growing season, we tested 3 possible alternatives to lindane and diazinon: 9, 10-anthraquinone (AQ), methyl anthranilate (MA), and limonene (LIM). A naturally occurring quinone compound, AQ is found in plants (Izhaki 2002), has long been recognized as a feeding deterrent, and is currently registered as a seed treatment to prevent bird damage for corn and other crops (DeLiberto and Werner 2016). AQ can be detected through taste, sight, or smell (Blackwell et al. 2001), but the mechanism for deterrence is post-ingestion irritation (Avery et al. 1997) followed by conditioned avoidance (Werner and Provenza 2011, Werner et al. 2014). AQ has been tested on a variety of bird species (DeLiberto and Werner 2016), including captive sandhill cranes (*Grus canadensis*; Blackwell et al. 2001, Barzen and Ballinger 2018).

As a plant-derived, food grade additive (Werner and Avery 2017), MA works as a taste deterrent by irritating the trigeminal nerve and prevents animals from eating grain seed (Mason and Clark 1995, Aronov and Clark 1996). Deterrence of crop herbivory by MA has been tested on many birds, including sandhill cranes (Blackwell et al. 2001), red-winged blackbirds (*Agelaius phoeniceus*; Avery et al. 1995), fish crows (*Corvus ossifragus*; Avery and Decker 1994), European starlings (*Sturnus vulgaris*; Mason et al. 1989), and greater snow geese (*Chen caerulescens*; Mason and Clark 1995).

Limonene is a plant-derived monoterpene (Abrahim et al. 2000) that may act as a contact repellant to European starlings (Clark 1997). Other uses of LIM as a repellent are unknown (Werner and Avery 2017).

The objectives of our 2000 experiment were to: 1) test the effectiveness of these 3 chemicals as a deterrent to sandhill crane damage in an agricultural context; 2) examine the degree of below-ground breakdown for each of the 3 chemicals used to treat corn seed; and 3) test the effect on germination and compatibility of each of the chemicals for ease of use within the agricultural system. Based on test results from 2000, we redefined our objectives in 2001 to: 1) test the effectiveness of a lower concentration of AQ in the field and 2) examine the degree of chemical degradation below ground at a lower concentration during a longer testing period and in different soil types.

STUDY AREA

The study area was located on a commercial farm near Briggsville, Wisconsin (43°39′N, 89°35′W), during 2000 and 2001. The area immediately surrounding the study fields was approximately 40% agriculture and 20% wetland, including an important crane roosting wetland located less than 1 km south of the test fields (Fig. 1). The remaining 40% of surrounding land cover consisted of forested and grassy non-agricultural areas.

METHODS

In 2000, our experiment occurred from 25 June to 21 July and was performed under an experimental use permit from the U.S. Environmental Protection Agency (EPA) that stipulated the crop would be destroyed at the end of the trial period. Prior to planting, standard warm germination tests (27°C/7 days; Martin et al. 1988) were performed on treated seed by the Tryon Group (Madison, WI).

Under the experimental use permit from the EPA, we were allowed to plant up to 10 acres (4.05 ha) of each type of treated seed. Seed treated with AQ was planted on 4 ha next to 9.31 ha planted with non-treated seed; 4 ha were planted with MA-treated seed next to 4.86 ha planted with non-treated seed. Finally, LIM-treated seed was planted on 4 ha next to the MA field so the control (non-treated) field was adjacent to both LIM and MA fields (Fig. 1). A liquid 1% solution (10 g chemical/1 kg seed) of each chemical was applied to seed before planting.

In 2001, a total of 24.28 ha was used to test AQ in 2 separate trials with 12.14 ha in each trial (Fig. 1). The first trial occurred from 15 May to 14 June, coinciding with peak corn planting by other landowners in the area. The second trial occurred between 26 June and 7 July to correspond to the time period used in the 2000 study; this would also replicate a situation in which a farmer was forced to replant a damaged field. Each trial in 2001 included a treated and untreated portion of the field of 6.07 ha each. A concentration of 0.5 % AQ (5 g chemical/1 kg seed) was used based on experiments with captive cranes at Patuxent Wildlife Research Center, Laurel, Maryland (Blackwell et al. 2001).

Chemical Tests

In 2000, chemical residues were tested from 6 corn kernels taken from each of 5 fields (AQ, MA, LIM, and 2 control fields) every other day. The kernels

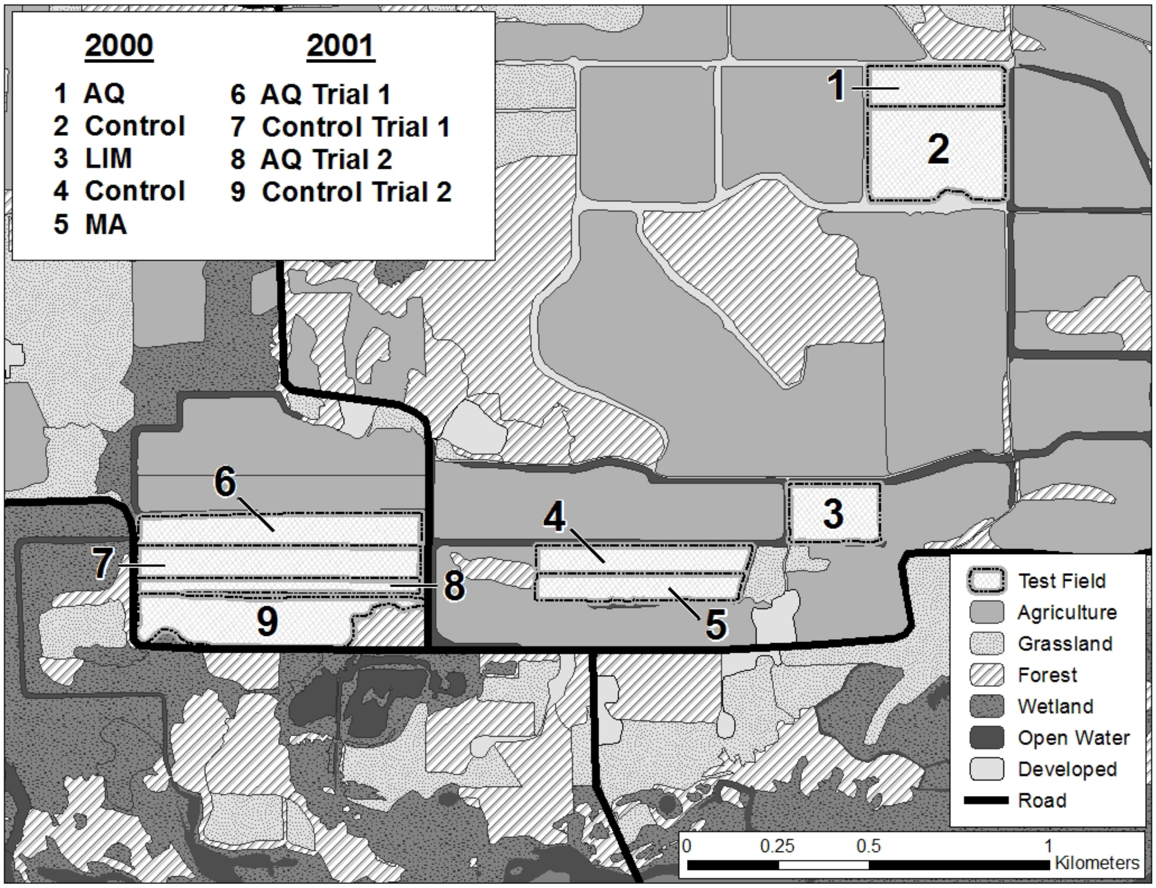

Figure 1. Study area showing experimental fields used to test anthraquinone (AQ), methyl anthranilate (MA), and limonene (LIM) in Briggsville, Wisconsin, in 2000 and 2001.

were collected using a stratified random approach so the beginning, middle, and end of planted rows were all sampled. In 2001, chemical residues were tested from 6 corn kernels collected each day of the experiment from 2 soil types (organic and sand). Corn seedlings were collected until the endosperm had been fully metabolized and there was no seed remaining underground, which usually occurred by 17 days post-germination (Barzen et al. 2018). All kernels, packaged as blind samples, were sent to Arkion Life Sciences LLC (Wilmington, DE) for laboratory analysis. After being taken from frozen storage, the kernels were separated from plant and roots by a sharp blade. The kernels were then extracted using a measured amount of acetonitrile and filtered according to standard high-pressure liquid chromatography. Chromatograms were then analyzed for the presence of MA, LIM, and AQ. The minimum detectable level (MDL) for AQ was 0.043 µg/ml; LIM and MA were too volatile to set a MDL (K. Ballinger, Arkion Life Sciences LLC, personal communication). Chemical longevity was analyzed with linear regression in R (R Core Team 2013).

Corn Seedling Density

We delineated areas within each field by regions with low and high crane use. For each experimental field, corn seedling density was measured in 1 area of the field that had been used by cranes and another area of the field that had no observed crane use. Each sampling plot was 40 m long and 15 corn rows wide. The number of seedlings/m was determined for each row and averaged for each plot. The number of seeds planted (5.65 seeds/m) was also compared to each germination rate to determine the expected seedling density for each treatment. Results for damage estimates were shown via box plot and *t*-tests were used to compare crane use between treated and non-treated fields by using R (R Core Team 2013).

Crane Use

Crane use of experimental and control fields was determined by surveying each field a minimum of 2 times per day from the time the corn was planted to the time corn seeds were no longer vulnerable (Barzen et al. 2018). Observations occurred once in the morning and once in the evening during peak foraging times (Barzen et al. 2018). For each survey, crane number, location, and behavior were recorded on aerial photos. The average number of crane observations per day was calculated for each experimental field; *t*-tests were used to compare use between treated and non-treated fields by using R (R Core Team 2013). Research with sandhill cranes was implemented through a permit (#007, amended on 5 Mar 2000) from the Animal Care and Use Committee at the International Crane Foundation.

RESULTS

Chemical Tests

Germination rates varied with each of the experimental chemicals under standard warm germination conditions tested immediately after treatment. As compared to 96% germination for non-treated seeds, germination rates for treated seeds were 85% (LIM), 90% (MA), and 92% (AQ) (Table 1).

In 2000, the concentration of AQ on planted seeds treated at 1.0% changed over the 2-week testing interval (Table 2), but the concentration increased rather than decreased ($y = 5.2506x - 192429$). The average concentration (317.33 µg AQ/kernel) remained above levels that were necessary to deter crane herbivory. The concentration of MA declined rapidly after planting to undetectable levels within days of planting (Fig. 2) while LIM on treated seeds was never detectable. Verification of seed treatment for LIM was assured, however, because the characteristic lemon smell of LIM was strong in the seed bags at planting.

In 2001, the amount of AQ on seeds treated at 0.5% planted in sandy soils did not decrease during trial 1 or trial 2 (Table 2), but the average residual concentration of the chemical on the seeds planted in organic soil (285.72 µg AQ per kernel in trial 1 after 30 days) was less than the concentration of AQ in organic soil from trial 2 (396.53 µg AQ per kernel in trial 2 after 13 days; Table 2).

Chemical Effectiveness and Crane Use

To assess the effectiveness of different chemicals on crane deterrence, we subtracted the mean seedling density in crane non-use areas from seedling density in crane use areas. If no damage occurred, seedling

Table 1. Expected seedling densities of test field corn treated with 3 experimental deterrents (anthraquinone [AQ], methyl anthranilate [MA], and limonene [LIM]) at Briggsville, Wisconsin, 2000-2001. Planting rate of 5.65 seeds/m (from grower interviews) was multiplied by germination rate to determine expected seedling density. Except for percent reduction and germination rate, all measurements are seedlings/m.

Year	Treatment	Germination rate	Expected seedling density	Crane unused area	Crane used area	Percent reduction[b]
2000	AQ control	0.96	5.42	4.69	4.24	9.59
	AQ	0.92	5.20	4.63	4.75	−2.59
	MA control	0.96	5.42	4.38[a]	0.71	83.79
	MA	0.90	5.08	4.38[a]	3.59	18.04
	LIM	0.85	4.80	3.81	2.43	36.22
2001	AQ trial 1	0.92	5.20	3.60	4.03	−11.94
	AQ control 1	0.96	5.42	3.85	0.72	81.30
	AQ trial 2	0.92	5.20	3.99	4.52	−13.28
	AQ control 2	0.96	5.42	4.64	2.17	53.23

[a] Estimates of seedlings/m for crane unused areas in MA and MA control fields were not available because all areas of the field were used by foraging cranes; thus we used the average of unused seedling densities measured in AQ, AQ control, and LIM unused areas to estimate corn seedling densities in MA control and MA fields.

[b] Percent reduction is calculated as [(crane unused seedling density − crane use seedling density)/crane unused] × 100. A negative number indicates areas that cranes used had higher seedling densities than did areas that cranes did not use. Negative values are likely a result of sampling error or variation in germination in response to in-field variations in soil characteristics.

Table 2. Results of linear regressions of the concentrations of methyl anthranilate (MA) and anthraquinone (AQ) on planted seeds at Briggsville, Wisconsin, 2000-2001. In 2001 only planted seeds with an AQ concentration of 0.05% were used in 2 different soil types (sand and organic).

Year	Treatment	F (df)	P	R^2
2000	AQ	155.892 (1,11)	0.000[a]	0.934
	MA	3.94 (1,11)	0.079	0.283
2001	AQ Trial 1 organic	1.055 (1,30)	0.313	0.034
	AQ Trial 1 sand	0.071 (1,27)	0.793	0.003
	AQ Trial 2 organic	1.510 (1,10)	0.248	0.131
	AQ Trial 2 sand	0.028 (1,10)	0.609	0.027

[a] P values reported as 0.000 indicate $P < 0.001$.

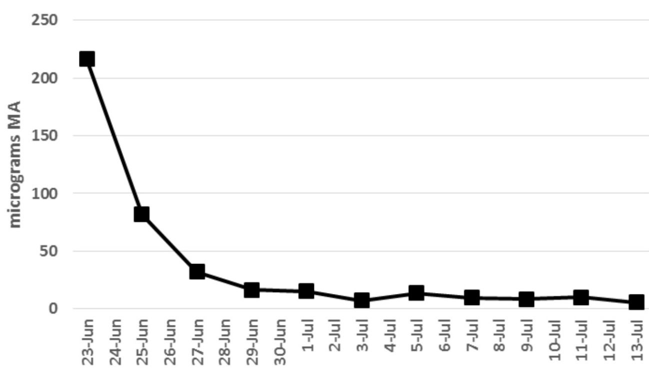

Figure 2. Concentration of methyl anthranilate (MA) found on treated seeds post-planting over the course of the experimental trial in Briggsville, Wisconsin, 25 June to 21 July 2000.

densities in the 2 areas would be the same so their difference would be zero. Due to extensive use of MA and MA control fields by foraging cranes, it was not possible to obtain a sample of seedling density in an area not used by cranes. Instead, we used the average seedlings/m of the AQ, AQ control, and LIM fields as a proxy for the unused areas of the MA and MA control fields. In 2000, seedling density was substantially reduced in the LIM (36%) and the MA (18%) treated field, as well as in the 2 control fields (10% and 84% respectively; Table 1, Fig. 3). Only the AQ-treated field had a seedling difference that did not differ from zero (Table 3, Fig. 3), meaning that seedling densities in used and unused areas did not differ. In 2001, AQ control fields in both trials suffered significant damage (Fig. 4) with 81% and 53% of seedling density lost in crane foraging areas during trial 1 and 2, respectively (Table 1). In contrast, both of the fields treated with AQ were undamaged, having seedling densities in areas

Table 3. Comparison of seedlings/m from crane unused – crane used areas of each treatment (anthraquinone [AQ], methyl anthranilate [MA], and limonene [LIM]) to zero, from Briggsville, Wisconsin, 2000-2001. Positive values for the 95% confidence interval (CI) indicate damage (i.e., lower seedling densities in areas that cranes used vs. areas that cranes did not use).

Year	Treatment	t	P	±95% CI
2000	AQ control	2.737	0.010	0.112, 0.780
	AQ	−1.154	0.258	−0.328, 0.091
	MA control	13.526	0.000[a]	0.90, 1.230
	MA	−8.869	0.000	−1.924, −1.203
	LIM	9.410	0.000	1.080, 1.680
2001	AQ trial 1 control	33.529	0.000	2.767, 3.126
	AQ trial 1	−5.731	0.000	−0.837, −0.397
	AQ trial 2 control	15.262	0.008	2.21, 2.894
	AQ trial 2	−2.877	0.000	−0.880, −0.146

[a] P values reported as 0.000 indicate $P < 0.001$.

that cranes foraged higher than areas where they did not forage, which resulted in negative differences (Fig. 4). A negative difference would indicate that there were more seedlings/m in the area where cranes were observed foraging; these differences could be attributable to inconsistent germination rates, due to soil and moisture variation on a microscale, making differences in AQ-treated fields difficult to interpret.

We found no difference in the mean number of cranes/day observed in the experimental fields in 2000; an average 10.30 cranes/day used treated fields and 13.48 cranes/day used non-treated fields. However, in the first trial of 2001, 2.85 cranes/day used the treated field while 9.21 cranes/day used the non-treated field, a difference approaching significance ($t = -1.98$, 38 df, $P = 0.06$). During trial 2, crane use of treated (4.30 cranes/day) and non-treated (2.74 cranes/day) fields did not differ.

DISCUSSION

Even though MA, LIM, and AQ have provided effective deterrence in feeding trials with captive birds (e.g., Aronov and Clark 1996, Abrahim et al. 2000, Blackwell et al. 2001), considerable variation occurred among the substances in our study when tested under field conditions. MA and LIM did not effectively deter crane herbivory in our study whereas AQ did. Since tested germination rates were relatively high for MA and AQ, the low seedling densities in MA fields

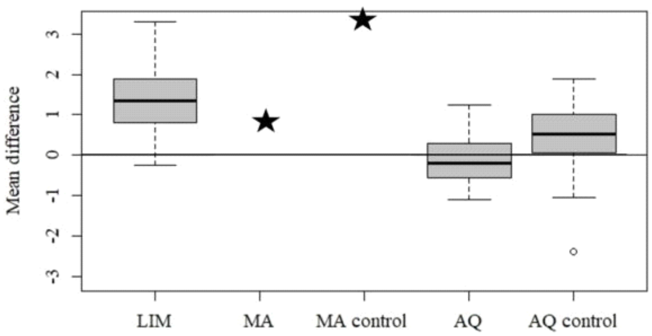

Figure 3. Box plots showing the mean difference in seedlings/m (seedlings/m in crane unused areas – seedlings/m in crane used areas) from anthraquinone (AQ), methyl anthranilate (MA), and limonene (LIM)-treated experimental fields, Briggsville, Wisconsin, 25 June to 21 July 2000. On each box, the dark midline indicates median, with lower and upper quartile; open circle is an outlier. Due to extensive damage in MA and MA control fields, the mean seedlings/m of the used areas was subtracted from mean seedlings/m from crane unused areas of AQ, AQ control, and LIM fields (4.38, see Table 1) and thus represented by 1 number (stars).

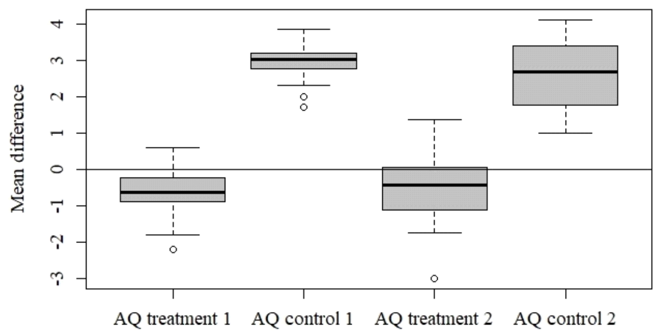

Figure 4. Box plots showing the mean difference in seedlings/m (seedling/m in crane unused areas – seedling/m in crane used areas) from anthraquinone (AQ)-treated and control fields, Briggsville, Wisconsin. On each box, the dark midline indicates median, with lower and upper quartile; open circles are outliers. Trial 1 was conducted 15 May to 14 June 2001, coinciding with peak corn planting by other landowners in the area. Trial 2 occurred between 26 June and 7 July 2001, coinciding with the timing of replanted fields.

were likely due to extensive crane herbivory. Despite documented crane use of both AQ and MA fields, only MA experienced heavy damage by cranes. Abrahim et al. (2000) found that monoterpenes such as LIM could interfere with germination; we found that germination rates for corn treated with LIM were lower than kernels treated with both AQ and MA. The fact that LIM may be more difficult to use in a planter because of its sticky texture is an additional practical limitation to its use as a crane deterrent (Roderick Gumz, co-owner, Gumz Farms LLC, personal communication). Overall, LIM appeared to be unsuitable for this agricultural application.

Previous studies have established that MA does not persist on treated seeds; Aronov and Clark (1996) found that MA is quickly metabolized by aerobic bacteria in dark conditions, such as is found in the soil when corn is planted. The rapid decline of MA concentrations on planted seed to undetectable levels may have left seed unprotected for most of its lifespan in the soil and resulted in crane herbivory of treated seeds that differed little from herbivory of untreated seeds. The reported threshold value to deter birds (80 µg/kg MA; Werner and Avery 2017) was greater than what was found on the seeds after only 3-4 days in the soil (Fig. 2).

At both the 1% and 0.5% treatment levels, AQ was the only deterrent that exhibited the characteristics desired in a chemical repellent for protecting planted corn seeds from crane damage. Though organic soils degraded AQ faster than sandy soils, the concentration of 0.5% was adequate to persist throughout the period that corn kernels are vulnerable to crane herbivory in either soil type. A variety of soil temperatures also did not reduce the effectiveness of AQ. DeLiberto and Werner (2016) estimated that the AQ threshold concentration for cranes was 0.25% (250 µg AQ/kg corn), well below the minimum concentrations that we measured on seed at the end of the study.

In 2001, fields that were planted concurrently with other agricultural fields in the area (trial 1) received regular use by cranes during the experiment. Although the rate of crane use between the treated and non-treated field was nearly equal, corn seedling densities were markedly lower in the non-treated versus the treated fields. Other factors beyond our control (number of cranes in the area, farmer disturbance, weather, etc.) may have influenced crane use of experimental fields. Cranes did not avoid the AQ-treated fields during trial 1 even though there were other cornfields in the area at the same stage of germination and even though they did not consume planted corn in treated fields. Other foods or other reasons for using the field likely existed for cranes that continued to use AQ-treated fields. Crane use of a field was not an accurate gauge of potential damage.

Trial 2 in 2001 occurred when most other cornfields located within the study area were no longer vulnerable to crane damage, thereby putting this germinating field at higher risk to damage by crane herbivory (Barzen et al. 2018). Crane use of the AQ-treated field in trial 2 did not differ from crane use of the untreated field, yet no damage occurred in the treated field even though both fields experienced heavy foraging pressure.

Overall, the use of AQ was effective at preventing damage caused by foraging cranes on newly germinating corn observed in our study. In contrast, MA and LIM dissipated too quickly in the soil to provide effective deterrent, and LIM both inhibited germination and was difficult to plant due to its sticky nature. Neither MA nor LIM was useful when deployed in agricultural fields. AQ-treated seeds had adequate germination rates and were easy to incorporate into the planting regime. AQ-treated corn also provided adequate deterrence without displacing birds to other fields and potentially causing more damage elsewhere. As such, AQ successfully mitigated damage problems caused by cranes and was able to be integrated successfully into the agriculture system, demonstrating that it could be effective at solving crop damage on a broad scale (Barzen and Ballinger 2018).

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