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Application strategy for an anthraquinone-based repellent and the protection of soybeans from Canada goose depredation

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Abstract: Agricultural crops can sustain extensive damage caused by Canada geese (*Branta canadensis*) when these crops are planted near wetlands or brood-rearing sites. From 2000 to 2015, South Dakota Game, Fish and Parks spent >\$5.6 million to manage damages caused by Canada geese to agricultural crops (primarily soybeans) in South Dakota, USA. For the purpose of developing a repellent application strategy for nonlethal goose damage management, we comparatively evaluated the width of anthraquinone applications (i.e., 9.4 L Flight Control® Plus goose repellent/ha [active ingredient: 50% 9,10-anthraquinone] at 0–36 m versus 0–73 m perpendicular to the edge of wetlands in 2014), the timing of the first repellent application (i.e., 9.4 L Flight Control Plus goose repellent/ha at 50% versus 75% seedling emergence in 2015), the yield of soybeans (*Glycine max*) within repellent-treated and untreated subplots, and anthraquinone chemical residues in Day County, South Dakota. Soybean yield was greater in subplots 73 m from the water's edge than that in the 36-m subplots ($P < 0.02$). Among subplots first sprayed at 50% seedling emergence, soybean yield was greater at 73 m and 82 m than that at 36 m ($P < 0.005$). In contrast, we observed no difference in yield at 36 m, 73 m, or 82 m in the subplots first sprayed at 72% seedling emergence ($P > 0.09$). We therefore conclude that goose damages were effectively managed in subplots first sprayed at 72% seedling emergence. Anthraquinone residues averaged 674 and 629 ppm anthraquinone upon the first application of the repellent (June to July), 22 and 35 ppm anthraquinone in the mid-season hay (August to September), and 36 and 28 ppb anthraquinone in the harvested seed (October to November) in 2014 and 2015, respectively. Our results suggest that a 73-m bandwidth of anthraquinone-based repellents first applied at approximately 72% or 65–85% seedling emergence can protect soybeans from Canada goose depredation.

Key words: 9,10-anthraquinone, biopesticide, *Branta canadensis*, Canada geese, chemical repellent, nonlethal, wildlife damage management

CANADA GEESE (*Branta canadensis*) historically nested throughout the Great Plains of the United States, but they were nearly extirpated in portions of this range in the early twentieth century (Vaa et al. 2010, South Dakota Game, Fish and Parks [SDGFP] 2016). Subsequent reintroduction efforts were successful, and Canada goose populations began to increase throughout much of the United States in the late 1960s (Dieter and Anderson 2009). The overall Canada goose population in North America increased 4.5-fold from 1.26 million in 1970 to 5.69 million in 2012 (Dolbeer et al.

2014). The increased abundance and localized overabundance of geese have caused pest management concerns in North America and Eurasia, including agricultural depredation, strikes with aircraft, disease transmission and ecosystem disservices (Dolbeer et al. 2014, Simonsen et al. 2016, Buij et al. 2017).

Emerging corn (*Zea mays*), winter wheat (*Triticum aestivum*), and soybeans (*Glycine max*) are common agricultural crops in North America that are consumed by Canada geese, and these crops can sustain extensive goose damage when planted near wetlands or brood-

rearing sites (Whitford 2008). Most goose-caused crop damage in South Dakota, USA occurs during their flightless period (i.e., 25 days in late June to mid-July; Anderson 2006) when adult geese are molting and young geese are still flightless (Radtke 2008, Radtke and Dieter 2010). From 2000 to 2015, SDGFP spent >\$5.6 million to manage damages caused by Canada geese to South Dakota's agricultural crops (primarily soybeans; SDGFP 2016). In 2012, SDGFP spent \$720,000 on goose damage management, primarily electric fence barriers constructed between the commercial soybean fields and the wetlands used by Canada geese during their brood-rearing and molting periods (SDGFP 2016). Although relatively expensive, the SDGFP electric fence program can effectively deter flightless geese from depredating South Dakota soybeans (Radtke 2008, Radtke and Dieter 2011).

The SDGFP electric fences have been previously used to establish field plots directly adjacent to wetlands occupied by flightless geese to determine the efficacy of foliar applications of chemical repellents and the protection of emergent soybeans (Warner 2013, Dieter et al. 2014). Chemical repellents could provide an effective, nonlethal strategy for goose damage management and/or an important component of an integrated pest management program for the protection of soybean production (Dieter et al. 2014). Methyl anthranilate-based products, including Rejex-It Migrate Turfguard® (Ceannard Inc., Gastonia, North Carolina, USA), Bird Shield® (Bird Shield Repellent Corp., Pullman, Washington, USA) and Avian Control® (Avian Enterprises LLC, Jupiter, Florida, USA), were ineffective at reducing soybean damages caused by Canada geese; soybean damage was 100% on all plots treated with 1 of these 3 products (Dieter et al. 2014). Goose occupancy of field plots was similar between plots treated with Avian Control and reference plots ($P = 0.99$). Moreover, goose occupancy of field plots treated with Rejex-It Migrate Turfguard or Bird Shield increased between the pre- and post-treatment periods of the study ($P < 0.02$). In contrast, geese occupied plots treated with an anthraquinone-based repellent (Avipel®; Arkion Life Sciences, New Castle, Delaware, USA) less than reference plots ($P < 0.01$), and soybean damage was less on Avipel-treated plots than reference plots

($P < 0.01$). Additional research was therefore suggested to assess the rates and timing of foliar applications of anthraquinone-based repellents for the reduction of damage to soybeans (Dieter et al. 2014).

Anthraquinone is a naturally occurring compound that was identified as a promising avian repellent in the early 1940s (Heckmanns and Meisenheimer 1944). As nonlethal biopesticides, anthraquinone-based repellents have been used to effectively protect rice seeds and emergent rice seedlings from blackbirds (Icteridae), turf from Canada geese, whole-kernel and ripening corn from sandhill cranes (*Grus canadensis*) and blackbirds, and sunflowers (*Helianthus annuus*) from blackbirds (DeLiberto and Werner 2016). A threshold concentration of 1,450 ppm anthraquinone was needed to reduce Canada goose consumption of whole corn by 80% in captivity (Werner et al. 2009). In addition to these laboratory efficacy data, field efficacy and chemical residue data are needed for the U.S. registration of anthraquinone-based biopesticides for agricultural applications and goose damage management.

For the purpose of developing a nonlethal strategy for goose damage management, we used the SDGFP electric fences to establish experimental 0.25–0.28-ha plots for our evaluation of an anthraquinone-based repellent applied to emergent soybean fields in Day County, South Dakota. Our primary research objectives were to comparatively evaluate (1) the width of repellent applications in 2014 (i.e., 36-m vs. 73-m bandwidth of soybeans perpendicular to the edge of wetlands); (2) the timing of the first repellent application in 2015 (i.e., upon 50% vs. 75% seedling emergence); (3) the yield of soybeans within repellent-treated and untreated subplots and (4) anthraquinone chemical residues upon the first application of the repellent (June to July), on the mid-season hay (August to September), and on harvested seed (October to November) at the conclusion of the 2014 and 2015 soybean growing seasons. This field study was conducted in accordance with the U.S. National Research Council's Guide for the Care and Use of Laboratory Animals - Field Investigations, and the Quality Assurance standards of the U.S. Department of Agriculture's (USDA) National Wildlife Research Center (NWRC; QA-2149; S. Werner, study director).

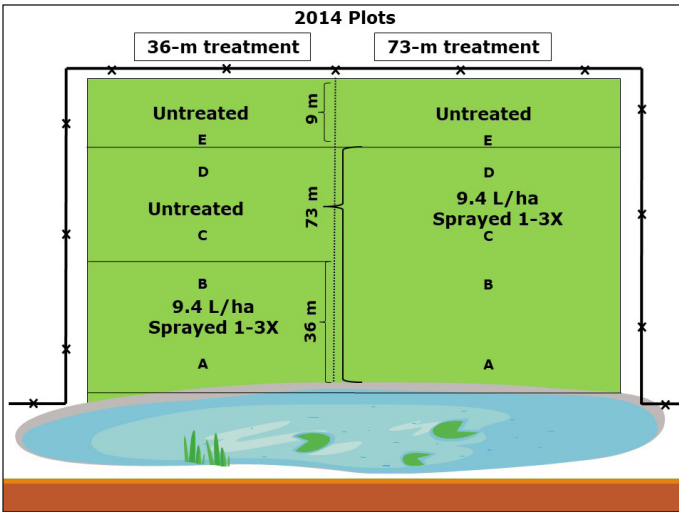


Figure 1. Schematic of soybean field plots used to develop an application strategy for an anthraquinone-based goose repellent (Arkion Life Sciences, New Castle, Delaware, USA) when applied at 0–36 m or 0–72 m from the edge of wetlands in Day County, South Dakota, USA.

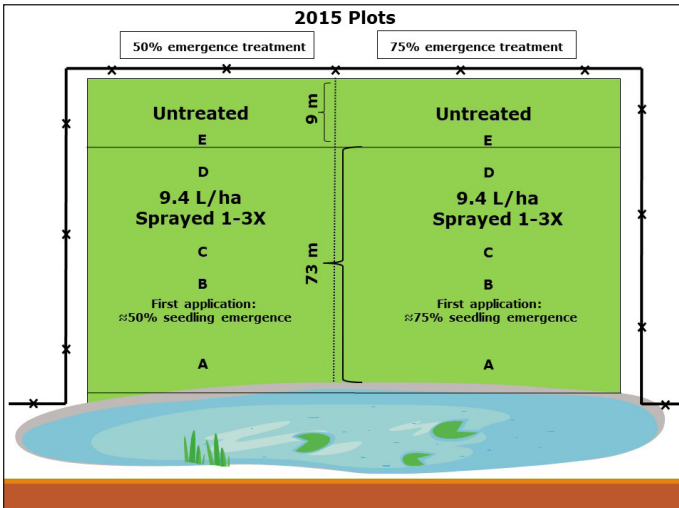


Figure 2. Schematic of soybean field plots used to develop an application strategy for an anthraquinone-based goose repellent (Arkion Life Sciences, New Castle, Delaware, USA) when first applied at 50% or 72% soybean seedling emergence in Day County, South Dakota, USA.

Study area

We selected potential field sites early in April to May of 2014 and 2015 in northeastern South Dakota (Day County). Day County is characterized by rolling hills, plant and animal agriculture (i.e., spring wheat, soybeans, cattle), and numerous lakes and wetlands used by abundant wildlife including breeding and migratory waterfowl. Field sites were selected based upon their proximity to a wetland and

minimal visual obstruction (Flann 1999, Radtke and Dieter 2010). Ideal sites had exposed shorelines, goose loafing areas (e.g., islands within wetlands) and a single soybean field planted to the wetland edge. Some sites that initially appeared to be ideal were later excluded from the study because adult geese moved large groups of goslings to other water bodies (Dieter and Anderson 2009).

We established field plots by constructing electric fences with materials provided by the SDGFP or by modifying electric fences already constructed by SDGFP field staff. Fences consisted of a solar fencer connected to a single strand of wire (Gallagher Animal Management Systems®, Riverside, Missouri, USA) adjacent to the portion of the field where geese were likely to damage soybeans. Solar fencers were powered by a 6-volt, 7 AH rechargeable battery that could store up to 0.17 Joules. The wire was constructed of 9 metal strands that provided 209 ohms/km. Fences were grounded by placing a 0.5-m metal post into the ground and secured in place by 1.2-m plastic posts (Dare Products Incorporated®, Battle Creek, Michigan, USA). Wires were clipped to posts approximately 0.5 m above ground level. Fences surrounding field plots were reinforced with a second strand of wire.

In 2014, we established 12 plots within emergent soybean fields in Day County. Field plots were each approximately 0.25 ha and measured 30 m parallel to the water’s edge (width) and 82 m perpendicular to the water’s edge (depth). For the purpose of determining the sufficient repellent-application bandwidth for goose repellency within soybean fields in 2014, we divided each field plot into 2 subplots that were treated with the repellent at 0–36 m or 0–73 m from the water’s edge (Figure 1). Control

portions of these subplots were left untreated at 36–82 m or 73–82 m from the water's edge for subsequent comparisons of soybean yield.

In 2015, we established 14 plots (0.25 ha each) within emergent soybean fields in Day County. For the purpose of comparatively evaluating the timing of the first repellent application in 2015, we divided each field plot into 2 subplots that were each treated with the repellent at 0–73 m from the water's edge (Figure 2). We visually estimated seedling emergence during our daily plot visits. The first application of the repellent was completed when approximately 50% of soybean seedlings had emerged within 1 subplot. Within the adjacent subplot, the first repellent application was completed when approximately 75% of soybean seedlings had emerged. Control portions of these subplots were left untreated at 73–82 m from the water's edge for subsequent comparisons of soybean yield.

Methods

Repellent applications

We applied 9.4 L Flight Control® Plus goose repellent/ha (active ingredient: 50% 9,10-anthraquinone; Arkion Life Sciences) during each of 1–3 applications in 2014 and 2015. Subsequent to the first repellent application on each field plot, we monitored all plots twice during the first week and thereafter every 2 days to identify new goose damage. If new damage was found within a field plot during our site visits, we applied a subsequent repellent treatment within both treated subplots, not to exceed 3 applications per plot in each of 2014 and 2015. All soybeans within treated subplots were destroyed after harvest and were thereby removed from food and feed uses at the end of each study year.

Chemical residue sampling and analysis

For the purpose of quantifying anthraquinone concentrations on treated soybeans throughout the 2014 and 2015 growing seasons, we sampled soybean phytomass and harvested soybean seeds exposed to foliar applications of the repellent. We collected 1–3 200-g samples of treated soybean leaves and a 200-g sample of untreated soybean leaves within each of 3 randomly selected field plots at 2–3 hours after the first repellent application from June to July.

In 2014, these 3 random plots included 1 plot that was sprayed once, 1 plot that was sprayed twice, and 1 plot that was sprayed 3 times. In 2015, these random plots included 2 plots that were sprayed twice and 1 plot that was sprayed 3 times. For the mid-season hay sampling from August to September, we collected 1 treated and 1 untreated whole-plant sample at the R3–R4 growth stage (i.e., pods 50% developed) within the 3 field plots randomly selected for first-application residue analyses. For the harvested seed sampling from October to November, we collected 3 1-kg samples of treated soybean seed and a 1-kg sample of untreated soybean seed within each of the 3 field plots randomly selected for first-application residue analyses. We immediately froze all residue samples within labeled plastic bags and maintained them at <0°C until shipped for residue analysis.

In each of 2014 and 2015, we shipped all frozen residue samples overnight to the Analytical Chemistry Unit of the NWRC in Fort Collins, Colorado, USA. Anthraquinone concentrations were analyzed in triplicate foliar subsamples per sample (i.e., first-application and mid-season hay) using high performance liquid chromatography (\pm 1 ppm anthraquinone). Anthraquinone concentrations were analyzed in 3–5 seed subsamples per sample (\pm 1 ppb anthraquinone) using gas chromatography/mass spectrometry (GC/MS/MS) and baseline corrected to quality control recoveries.

Soybean yield estimates

All field plots were harvested using a Massey Ferguson small-plot combine (AGCO Corporation, Duluth, Georgia, USA) in each of 2014 and 2015. Two soybean samples were collected from each of the untreated control and treated portions of each subplot. Each sample consisted of all soybeans harvested within the combine swath that was approximately 1.7 m wide and 9 m long. The combine harvester provided mass and moisture content data for each sample. We estimated soybean yield per subplot treatment (\pm 1 kg/ha; dry mass) by integrating the combine data with the associated area harvested within each subplot.

Statistical analysis

The response variable was comparative soybean yield associated with repellent-

Table 1. Soybean yield (mean \pm SE; kg/ha) among soybean field plots treated with an anthraquinone-based goose repellent (Flight Control[®] Plus; Arkion Life Sciences, New Castle, Delaware, USA).

Year	Treatment	Subplot	Subplot distance from water's edge (m)	Yield (kg/ha)
2014	0–36 m	Treated	0–36	1,057.9 \pm 234.0
		Untreated	36–73	2,039.1 \pm 232.7
	0–73 m	Treated	0–36	1,065.9 \pm 204.4
		Treated	36–73	2,282.5 \pm 218.6
		Untreated	73–82	2,202.5 \pm 191.7
	2015	50% emergence	Treated	0–36
Treated			36–73	2,967.1 \pm 193.7
Untreated			73–82	3,054.5 \pm 77.3
72% emergence		Treated	0–36	2,265.7 \pm 297.3
		Treated	36–73	2,777.5 \pm 230.0
		Untreated	73–82	3,179.0 \pm 183.6

application treatments in each of 2014 and 2015. We analyzed the subplot-by-distance-by-treatment interaction associated with repellent application bandwidth (2014) and the timing of the first repellent application (2015) using a mixed model ANOVA (SAS v9.4). The random variable of our models was field site, or plot. The independent variables were subplot, distance (i.e., 0–36 m, 36–73 m, and 73–82 m from water's edge), treatment (repellent, untreated control), and replicate sample. In 2014, paired subplots contained the 0–36-m and 0–73-m repellent treatments (Figure 1). In 2015, paired subplots were first sprayed with the repellent upon 50% or 75% seedling emergence (Figure 2). We used Tukey's tests to separate the means of ANOVA interactions ($\alpha = 0.05$). We used descriptive statistics (average \pm SE, min, max) to comparatively summarize soybean yield and anthraquinone residues within field subplots.

Results

Repellent-application bandwidth (36-m vs. 73-m banding)

We applied the anthraquinone-based repellent on 12 field plots in 2014. Of these 12 plots, geese occupied the wetlands but never accessed 3 of the adjacent field plots; these 3 plots were therefore omitted from the study. An additional site was censored because the harvesting personnel

were unable to access the study site. The first repellent application on sites 1–8 was completed from June 13 to July 9. The second application occurred on sites 1–7 from June 30 to July 18, and a third repellent application was completed only on site 1 on July 9. Among our site visits, minimum estimates of goose abundance at these 8 sites were 40, 50, 60, 60, 100, 125, and 150 geese (average = 81 geese per site) in 2014.

We observed an interaction of soybean subplots-by-distances-by-repellent treatments for soybean yield in 2014 ($F_{4,75} = 8.15$, $P < 0.0001$). Among the subplots sprayed with the repellent at 0–36 m from the water's edge, soybean yield was greater in the untreated portion of the subplot at 36–73 m from the water's edge than in the repellent-treated portion of the subplot ($P = 0.0139$; Table 1).

Among the subplots sprayed with the repellent at 0–73 m from the water's edge, soybean yield was greater in the treated portion of the subplot at 36–73 m from the water's edge ($P = 0.0015$) and in the untreated portion of the subplot at 73–82 m from the water's edge ($P = 0.0035$) than in the treated portion of the subplot at 0–36 m from the water's edge (Table 1). We observed no difference in soybean yield between the treated portion of the subplot at 36–73 m and the untreated portion of the subplot at 73–82 m from the water's edge ($P = 0.9990$). Chemical

Table 2. Anthraquinone residues among soybean field plots treated with an anthraquinone-based goose repellent (Flight Control® Plus; Arkion Life Sciences, New Castle, Delaware, USA).

Year	Residue sampling	Subsamples				Anthraquinone (ppm)			Anthraquinone (ppb)		
		n	Mean	Min, max	MLOQ ^a	Mean	Min, max	MLOQ	Mean	Min, max	MLOQ
2014	First application	Treated	27	674	374, 979	5.5					
		Untreated	9	<MLOQ	<MLOQ						
	Mid-season hay	Treated	9	22	<MLOQ, 45	7.1					
		Untreated	9	<MLOQ	<MLOQ						
2015	Harvested seed	Treated	45					36	<MLOQ, 320		8.7
		Untreated	15					<MLOQ	<MLOQ, 41		
	First application	Treated	9	629	370, 908	7.1					
		Untreated	9	<MLOQ	<MLOQ						
Mid-season hay	Treated	9	35	<MLOQ, 104	11.7						
	Untreated	9	<MLOQ	<MLOQ							
Harvested seed	Treated	27					28	<MLOQ, 147		8.7	
	Untreated	12					<MLOQ	<MLOQ, 16			

^a The method limit of quantitation (MLOQ) was estimated from the mean chromatographic response of anthraquinone-treated vs. untreated soybean samples.

residues among treated field plots averaged 674 ppm anthraquinone upon the first application of the repellent, 22 ppm anthraquinone in the mid-season hay, and 36 ppb anthraquinone in the harvested seed in 2014 (Table 2).

Timing of first repellent application (50% vs. 75% seedling emergence)

We applied the repellent on 14 field plots in 2015. Of these 14 plots, 6 plots were destroyed by

a severe thunderstorm with high wind speeds and hail on June 21–22, and geese occupied the wetlands but never accessed an additional 4 field plots. These 10 plots were therefore omitted from the study. One additional plot was omitted from the study because all soybean plants had emerged prior to our first repellent application. The first repellent application on sites 1–3 was completed from June 4–18. Upon the first repellent application,

soybean emergence averaged 50% (41–55%) in the subplots selected for the 50% seedling emergence treatment and 72% (65–86%) in the subplots selected for the 75% emergence treatments. The second application occurred on sites 1–3 from June 15–25, and a third repellent application was completed only on site 1 on July 21. Among our site visits, minimum estimates of goose abundance at these 3 sites were 40, 70, and 80 geese (average = 63 geese per site) in 2015.

Although we observed no difference in soybean yield among subplots first treated at 50% versus 72% seedling emergence at 0–36 m ($P = 0.3601$) or 36–73 m from the water's edge ($P = 0.9924$), we observed an interaction of soybean subplots-by-distances-by-repellent treatments for soybean yield in 2015 ($F_{5,30} = 6.52$, $P = 0.0003$). Among the subplots first treated at 50% seedling emergence, soybean yield was greater at 36–73 m ($P = 0.0035$) and 73–82 m ($P = 0.0017$) than that within 36 m of the water's edge. Among the subplots first treated at 72% seedling emergence, we observed no differences in soybean yield between 0–36 m and 36–73 m ($P = 0.6482$; Table 1), 0–36 m and 73–82 m ($P = 0.0985$), or 36–73 m and 73–82 m from the water's edge ($P = 0.8334$). Chemical residues among treated field plots averaged 629 ppm anthraquinone upon the first application of the repellent, 35 ppm anthraquinone in the mid-season hay, and 28 ppb anthraquinone in the harvested seed in 2015 (Table 2).

Discussion

We generally observed greater soybean yield in the 73-m treatments than the 36-m treatments during the 2014 growing season. Similarly, we observed greater yield at 36–73 m and 73–82 m than within 36 m of the water's edge in subplots first sprayed at 50% seedling emergence in 2015. In contrast, we observed no difference in soybean yield at 36 m, 73 m, or 82 m from the water's edge in subplots first sprayed at 72% seedling emergence, and yield was generally greater in 72% emergence subplots (Table 1). We therefore conclude that goose damages were effectively managed in subplots first sprayed at 72% seedling emergence. Although applying deterrents early in the growing season is extremely important for reducing goose damage (Radtke 2008, Radtke and Dieter

2011), chemical repellents can be applied too early in the growing season. First repellent applications at <50% seedling emergence may result in ineffective and wasteful applications of the repellent on bare soil. Moreover, first repellent applications that occur too late in the growing season (e.g., after goose damage is observed) will likely succeed most annual goose damage (Radtke 2008). Our results suggest that a 73-m bandwidth of anthraquinone-based repellents first applied at approximately 72% or 65–85% seedling emergence can be effective in protecting soybeans from Canada goose depredation.

Anthraquinone residues among treated field plots averaged 674 and 629 ppm anthraquinone upon the first application of the repellent, 22 and 35 ppm anthraquinone in the mid-season hay, and 36 and 28 ppb anthraquinone in the harvested seed in 2014 and 2015, respectively. These field residues are less than the threshold concentration of anthraquinone estimated for Canada geese in captivity (i.e., 1,450 ppm anthraquinone; Werner et al. 2009). We applied 9.4 L Flight Control Plus goose repellent (active ingredient: 50% 9,10-anthraquinone) per ha, per application. At 1–3 applications per plot, we provided 9.4–28.2 L Flight Control Plus/ha on our field plots in each of 2014 and 2015. For comparison, Warner (2013) sprayed 132.5 L tank mixture/ha each 7 days throughout July and August when light winds (< 15 km/hour), no precipitation, and goose activity enabled access to field plots for repellent applications (Warner 2013, Dieter et al. 2014). The repellent tank mixture was prepared by adding 1 part Avipel (active ingredient: 50% 9,10-anthraquinone) to 6 parts water (Dieter et al. 2014), thus providing approximately 18.9 L Avipel per ha, per application. Assuming 8–9 applications from July to August, that would have provided 151.4–170.4 L Avipel/ha on field plots during the 2012 soybean growing season.

With further regard to goose damage management, Flann (2009) suggested that the visual obstruction of vegetation may influence where geese penetrate field barriers more than the width of the barrier. Agricultural damage can be greatest in the area where geese entered crop fields (Radtke 2008). Distance from water to crops and visual obstruction are therefore important factors of where geese access crop

fields and damage management planning (Flann 1999, Radtke 2008, Radtke and Dieter 2010). Previous research suggested that the greatest distance traveled by geese to access soybeans was 36 m and crops planted within 36 m of water are potentially susceptible to damage by flightless geese (Radtke 2008, Radtke and Dieter 2010). Indeed, we observed a lack of repellent efficacy in our 36-m treatments first sprayed at 50% seedling emergence. The importance of distance between crops and standing water may have implications for the design of buffer strips used to deter geese; buffer strips need to be at least 36 m wide to sufficiently deter geese (Radtke 2008, Radtke and Dieter 2010). However, the sufficient width of a buffer strip may be related to the distance that geese need to travel to access soybeans on alternative, locally available field sites (Radtke 2008, Radtke and Dieter 2010). We therefore suggest subsequent evaluations of combined visual obstruction in the area where geese enter soybean fields and chemical repellents for the protection of soybeans from Canada geese.

The registration of anthraquinone-based biopesticides is presently needed for the protection of agricultural crops from goose depredation. Flight Control Plus is currently registered by the U.S. Environmental Protection Agency as a goose repellent for turf throughout the United States (except California). Use sites for Flight Control Plus include terrestrial areas at or near airports; grassy areas at commercial sites, industrial office sites, municipal sites, or in developed urban areas; golf courses; turf areas, including sports fields, park grounds, home lawns, and cemeteries; and landfills and dumpsites (Arkion Life Sciences).

These field and chemical residue data will help the development of nonlethal repellents for the protection of soybeans from Canada goose depredation. Future pest management research should evaluate the efficacy of foliar applications of these and other nonlethal chemical repellents for the protection of ripening agricultural crops under field conditions (i.e., >100 ha with applicable experimental use permitting). We suggest the need for replicated field plots and untreated controls (e.g., ≥10 ha per plot), reliable estimates of crop damage and crop yield, representative observations of the feeding behavior and movements of

target pests, and comparative measurements of chemical residues throughout the period of needed crop protection in subsequent field efficacy studies of wildlife repellents.

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