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Examination of commercially available bird feed for weed seed contaminants

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Abstract

In 2016 and 2017, 98 separate commercially available bird feed mixes were examined for the presence of weed seed. All weed seed contaminants were counted and identified by species. *Amaranthus* species were present in 94 of the 98 bags of bird feed. *Amaranthus* species present in bird feed mixes included waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer], redroot pigweed (*Amaranthus retroflexus* L.), Palmer amaranth (*Amaranthus palmeri* S. Watson), smooth pigweed (*Amaranthus hybridus* L.), and tumble pigweed (*Amaranthus albus* L.). *Amaranthus palmeri* was present in 27 of the 98 mixes. Seed of common ragweed (*Ambrosia artemisiifolia* L.), kochia [*Bassia scoparia* (L.) A.J. Scott], grain sorghum [*Sorghum bicolor* (L.) Moench], wild buckwheat (*Fallopia convolvulus* L., syn: *Polygonum convolvulus*), common lambsquarters (*Chenopodium album* L.), large crabgrass [*Digitaria sanguinalis* (L.) Scop.], and *Setaria* species were also present in bird feed mixes. A greenhouse assay to determine *Amaranthus* species seed germinability and resistance to glyphosate revealed that approximately 19% of *Amaranthus* seed in bird feed mixes are readily germinable, and five mixes contained *A. tuberculatus and A. palmeri* seed that were resistant to glyphosate. Results from linear regression and *t*-test analysis indicate that when proso millet (*Panicum miliaceum* L.), grain sorghum, and corn (*Zea mays* L.) were present in feed mixes, *Amaranthus* seed contamination was increased. The presence of proso millet and grain sorghum also increased contamination of grass weed species, while sunflower (*Helianthus annuus* L.) increased *A. artemisiifolia* contamination and safflower (*Carthamus tinctorius* L.) increased contamination of *Bassia scoparia.*

Introduction

A survey conducted by the U.S. Fish and Wildlife Service (USFWS) in 2016 reported that 56.8 million homeowners own a bird feeder as an attractant for avian wildlife (U.S. Department of the Interior et al. 2016). Henke et al. (2001) estimated that 289 million kg of bird feed were distributed across the United States in 1999. However, this number is likely even higher today, as the number of people that feed birds around their homes has increased by 8.8 million in the most recent USFWS survey (U.S. Department of the Interior et al. 2016). While homeowners may have good intentions with bird feeding, this popular hobby may have unintended consequences. For decades, bird feed has been examined as a source for weed seed introduction into new areas (Chauvel et al. 2004; Frick et al. 2011; Hanson and Mason 1985; Vitalos and Karrer 2008). Hanson and Mason (1985) surveyed bird feed mixes in Britain and reported on 438 weed species that they believed to have been introduced through the bird feed industry. Bird feed was also identified as a vector in the introduction of common ragweed (*Ambrosia artemisiifolia* L.) into England, where it was reported at levels as high as 531 seeds kg^{-1} mix (Vitalos and Karrer 2008). Brandes and Nitzsche (2006) also reported that bird feed was the main source for the establishment of *A. artemisiifolia* in Germany. Additionally, a Swiss study reported that 22% to 57% of samples screened from 2005 through 2009 were contaminated with *A. artemisiifolia* and that contamination reached as high as 303 seeds kg^{-1} of bird feed mix (Frick et al. 2011). Chauvel et al. (2004) identified a correlation between the presence of *A. artemisiifolia* in bird feed mixes when sunflower (*Helianthus annuus* L.) was used as an ingredient. Similarly, Wilson et al. (2016) reported that proso millet (*Panicum miliaceum* L.), sunflower, and grain sorghum [*Sorghum bicolor* (L.) Moench ssp. *Arundinaceum* (Desv.) de Wet & Harlan] used in bird feed is unlikely to undergo any processing to remove weed seed or alter its ability to germinate. The amount of weed seed that is present in grain after harvest can depend on a multitude of factors, including end of season weed control and combine sieve and fan adjustments (Clay et al. 2009; Davis 2008). Wilson et al. (2016) indicated that separating weeds from crops would likely be more difficult in small cereal grains, flax (*Linum usitatissimum* L.), and proso millet than in larger-seeded crops such as corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.].

In Australia, the Queensland Agricultural Merchants have developed standards for bird feed that require all bird feed to be examined for weed seed contamination; they established that any

Management Implications

Seeds of noxious and other invasive plant species can spread to new regions through a variety of avenues. Identifying methods of dispersal is a critical step in preventing the spread of these species into new geographies where they did not previously occur. This study examined the commercial bird feed trade as a pathway for weed seeds to travel long distances into new regions. A total of 98 bird feed mixes were purchased from various retail locations in the eastern portion of the United States. Each feed mix was screened for weed seed contaminants. Seeds were identified and *Amaranthus* spp. were planted to determine their germinability. A total of 29 species of weed seeds were identified in the feed mixes. *Amaranthus* spp. were identified in 96% of bird feed mixes at an average of 384 seeds kg^{-1} of feed mix, but were also determined to reach levels as high as 6,525 seeds kg^{-1} of feed mix. Of these mixes, 71% contained *Amaranthus* spp. seed capable of germination. The *Amaranthus* spp. identified included *Amaranthus palmeri* (Palmer amaranth), *Amaranthus tuberculatus* (common waterhemp), *Amaranthus hybridus* (smooth pigweed), *Amaranthus retroflexus* (redroot pigweed), and *Amaranthus albus* (prostrate pigweed). Additional analyses identified proso millet as an ingredient most likely to contribute *Amaranthus* spp. seed to feed mixes. Mixes containing processed ingredients such as dried fruits and hulled nuts contained the lowest amount of weed seeds. Bird feed mixes are not subject to any seed laws, as they are not intended for planting. It is difficult to estimate the role bird feed plays in the spread of weed species to new regions; however, bird feed is typically placed outdoors where conditions are favorable for germination. It is also possible that endozoochory, the dispersal of weed seed by animals, could play a role in weed seed dispersal from the bird feeder. Managing weed infestations in crop fields designated for bird feed would be a practical approach for mitigating weed seed contamination in commercial bird feed. Implementing weed seed limits in feed mixes has proven effective in reducing contamination in countries outside of the United States. Additional sieving by packaging companies would likely be effective in reducing contamination of many weed species as well.

presence of noxious seeds or weed seeds in amounts that exceed the tolerance levels are rejected (QAM 1998). In Switzerland, screening of bird feed mixes also resulted in regulations against *A. artemisiifolia* contamination, with a 10 seeds kg^{-1} threshold being established for bird feed. Additional members of the European Union (Germany, Denmark, and Slovenia) followed suit in the implementation of this threshold, and noticeable reduction of contaminated seed mixtures occurred in just 2 yr (Frick et al. 2011). In the United States, however, the Federal Seed Act enforced by the Department of Agriculture regulates agricultural seeds, which are defined as grass, forage, and field crop seeds that the Secretary of Agriculture finds useful for seeding purposes (USDA 1940). By definition, bird feed is not covered in the Federal Seed Act, and bird feed manufacturers are not required to reveal seed composition percentages such as the percentage of weed seed contamination, including noxious species. The Federal Drug Administration Center for Veterinary Medicine is the primary regulator of animal feed in the United States, but imposes regulations that are primarily directed toward contamination by pesticides or harmful foreign material such as metal shavings. Therefore, these regulations do not address weed seed contamination of bird feed. Additional guidelines were set forth by the Wild Bird Feed Industry in the Wild Bird Feed Industry Standards, which were adopted in 2004 and set guidelines for bird feed to be of a consistent quality (WBFI 2004). However, these standards also make no mention of weed seed contamination thresholds for bird feed distributed within the United States and Canada.

Palmer amaranth (*Amaranthus palmeri* S. Watson) has been documented as the most troublesome weed species present in agroecosystems today (VanWychen 2016). *Amaranthus palmeri* was historically a weed of the southwestern United States and Mexico; however, over time it has moved into more northern and eastern geographies (Heap 2019; Webster and Nichols 2012). The successful establishment of *A. palmeri* in new areas can be attributed to many factors, including its prolific seed production, highly competitive nature, and distinct ability to evolve resistance to herbicides (Legleiter and Johnson 2013). Human-mediated activities during the 20th and 21st century, such as movement of machinery contaminated with seed, animal feed, and manure, as well as contaminated pollinator planting seed mixes are largely to blame for the spread of this troublesome weed species across the country (Chahal et al. 2015). However, Farmer et al. (2017) reported that waterfowl were capable of spreading *A. palmeri* long distances, and other studies have identified *Amaranth* species seeds in water runoff, so natural dissemination is possible as well (Wilson 1980). Human-mediated dispersal will often result in a more rapid dispersal of a new species into a geography than natural introduction, due to multiple introductions occurring across large areas simultaneously (Taylor et al. 2012). No previous research has examined the potential for weed seed contamination of bird feed mixes that are commercially available in the United States, or more specifically focused on the possibility of *A. palmeri* contamination in these mixes. Therefore, the objectives of this research were to: (1) identify contamination levels of weed species in commercially available bird feed in the United States, (2) determine the viability and glyphosate-resistance status of any *Amaranthus* seed present in commercial bird feed mixes, and (3) determine the effects of ingredient composition and location of purchase on the presence and abundance of weed species in bird feed mixes.

Materials and Methods

Bird Feed Collection

Results were compiled from bird feed mixes purchased from a variety of common retail locations in Missouri, Kentucky, Tennessee, Arkansas, Illinois, Virginia, and North Carolina in 2016 and 2017 that represented 22 different brands (Table 1). Mixes were selected at random from these retail locations similar to Henke et al. (2001). The majority of bird feed mixes were purchased in Columbia, MO ($n = 62$). Mixes ranged in size from 1 to 9 kg and ranged from single-ingredient mixes to combinations of multiple-ingredient feed mixtures (Table 1).

Bird Feed Screening

In all cases, the entire bag was examined to be certain all weed seed contaminants were extracted from the mix. All bird feed mixes were poured through a series of sieves to separate seeds by size for a more accurate assessment of contaminants. Large-seeded ingredients like sunflower and safflower (*Carthamus tinctorius* L.) were initially separated with a 10-mm^2 sieve followed by the separation of medium-sized seeds like grain sorghum, proso millet,

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Table 1. Ingredient composition and purchase location of feed mixes used in the experiment.

Brand	Variety	Ingredients	State purchased[a]
3D	Nut & Berry	Processed, safflower, sunflower	MO
	Premium Songbird	Processed, safflower, sunflower	IL
	Premium Woodpecker	Processed, safflower, sunflower	IL, MO*
Ace	Safflower Seed	Safflower	MO
Audubon Park	Cardinal Supreme	Proso millet, processed, safflower, sunflower	MO
	Colorful Bird Blend	Proso millet, nyjer, processed, safflower, sunflower	NC, VA
	Patio and Garden Blend	Corn, proso millet, processed	MO
	Premium Nut and Fruit	Proso millet, nyjer, sunflower	MO
	Signature Harvest	Processed, grain sorghum	MO
	Songbird Selections	Proso millet, safflower, grain sorghum, sunflower	VA
	Wild Bird Food	Corn, proso millet, grain sorghum, sunflower	NC, VA
Cole’s	Blue Ribbon Blend	Corn, proso millet, processed, sunflower	MO
	Critter Munchies	Corn, sunflower	MO
	White Millet	Proso millet	MO
Enchanted Garden	Midwest Blend	Proso millet, nyjer, safflower, sunflower	MO
	No Waste	Corn, proso millet, processed, safflower, sorghum	MO
Feathered Friend	Birdsnack	Canarygrass, corn, proso millet, grain sorghum, sunflower, wheat	MO
	Economy Bird Feed	Corn, proso millet, grain sorghum, sunflower, wheat	MO
	Finch Delight	Canarygrass, nyjer, processed	MO
Garden Treasures	Cardinal Blend	Corn, proso millet, safflower, sunflower	KY, MO
	Finch Blend	Canarygrass, proso millet, nyjer	KY, MO
	Songbird Blend	Corn, proso millet, safflower, grain sorghum, sunflower	KY
	Wild Bird Food	Corn, proso millet, grain sorghum, sunflower	KY, TN
Harvest Seed	No Waste	Canarygrass, proso millet, processed	TN
Kaytee	Birder’s Blend	Corn, proso millet, safflower, grain sorghum, sunflower, wheat	MO
	Southern Blend	Proso millet, processed, safflower, grain sorghum, sunflower	MO
	Waste Free	Canarygrass, corn, proso millet, processed	MO
	Wild Finch	Canarygrass, proso millet, nyjer, processed	MO
Kroger	Wild Bird Seed	Proso millet, grain sorghum, sunflower	MO
Morning Song	Deluxe Bird	Proso millet, grain sorghum, safflower, sunflower	MO*
	Dove and Ground	Proso millet, grain sorghum, sunflower	MO
	Wild Finch	Canarygrass, proso millet, nyjer, processed	MO*
	Birdwatcher’s Blend	Proso millet, processed, sunflower, safflower	MO
National Audubon Society	Cardinal Mix	Safflower, sunflower	MO
	Deluxe Blend	Processed, safflower, sunflower	TN
	Finch Blend	Proso millet, nyjer, processed	TN
	Wild Bird Food	Corn, proso millet, grain sorghum, sunflower	KY
Nature’s Own	Cracked Corn	Corn	MO
	Finch Food	Proso millet, nyjer, sunflower	VA
	Fruit and Nut	Corn, proso millet, processed, sunflower	MO
	Safflower Seed	Safflower	MO
Nature’s Song	Cardinal Blend	Processed, safflower, grain sorghum, sunflower	MO
	Safflower Bird Seed	Safflower	MO
	Thistle Seed	Nyjer	MO
	Wild Bird Seed	Proso millet, grain sorghum, sunflower	MO
	Wild Finch	Proso millet, nyjer	MO
Orschlen’s	Bulk Bird Seed	Corn, proso millet, safflower, grain sorghum, sunflower	MO
Pennington	Birder’s Blend	Proso millet, safflower, grain sorghum, sunflower, wheat	MO*
	Black Oil Sunflower	Sunflower	IL
	Classic Wild Bird Feed	Proso millet, grain sorghum, sunflower, wheat	IL
	Harvest Deluxe	Processed, safflower, sunflower	TN
	Premium Select Blend	Proso millet, safflower, grain sorghum, sunflower, wheat	NC
	Safflower	Safflower	MO
	Songbird Blend	Processed, safflower, sunflower	MO
	Supreme Wild Finch	Canarygrass, nyjer, proso millet	MO
	Ultra Fruit and Nut	Corn, processed, safflower, sunflower	KY, MO*
	Ultra Waste Free	Canarygrass, corn, processed	MO, TN
Petco	All Purpose Seed Mix	Corn, proso millet, grain sorghum, sunflower	MO
Royal Wing	Cardinal Mix	Canarygrass, safflower, sunflower	AR
	Nut and Fruit Blend	processed, safflower, sunflower	AR
	Splendid Blend	Grain sorghum, sunflower, wheat	AR, MO
	Wild Finch Blend	Canarygrass, proso millet, nyjer	AR

(*Continued*)

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Table 1. (*Continued*)

Brand	Variety	Ingredients	State purchased[a]
Shafer	White Millet	Proso millet	VA
Stokes Select	Premium Cardinal	Safflower, sunflower	IL
	Supreme Blend	Safflower, sunflower	IL
Valley Splendor	Premium Blend	Proso millet, safflower, grain sorghum, sunflower	MO
	Wild Bird Food	Corn, proso millet, grain sorghum, sunflower	MO
Wagner's	Sunflower Seed	Sunflower	NC
	Cardinal Blend	Safflower, sunflower	MO*
	Cracked Corn	Corn	MO
	Deluxe Blend	Canarygrass, proso millet, nyjer, processed	MO, VA
	Finches Deluxe	Nyjer, processed	IL
	Finches Supreme	Canarygrass, proso millet, nyjer, processed	NC
	Greatest Variety	Canarygrass, corn, proso millet, nyjer, processed, grain sorghum, safflower, sunflower	IL, MO*
	Wild Bird Food	Corn, proso millet, grain sorghum, sunflower	MO
Wild Delight	Buffet for Birds	Proso millet, grain sorghum, sunflower	MO*
	Nut and Berry	Processed, safflower, sunflower	MO
	Songbird Food	Processed, safflower, sunflower	VA
	Special Finch	Processed, nyjer	MO

[a]Asterisk (*) indicates mix was purchased at two separate locations in the same state.

cracked corn, wheat (*Triticum araraticum* L.), and nyjer thistle [*Guizotia abyssinica* (L. f.) Cass.] with a 5-mm^2 sieve. All remaining ingredients were passed through a 1-mm^2 sieve, which allowed primarily for the passage of smaller-sized weed seeds like the *Amaranthus* species. Finally, remaining seeds and residue were placed in a 0.5-mm^2 sieve, which allowed for the removal of dust and powder residues from larger seeds that could interfere with *Amaranthus* seed detection. Each stage of seed separation was examined for weed seed contaminants, which were removed for further identification. For this experiment, all seeds that were not listed as ingredients of a mix were considered weed seed. Bird feed ingredients commonly used in bird feed that were included in the analysis include sunflower, proso millet, grain sorghum, safflower, wheat, nyjer thistle, and annual canarygrass (*Phalaris canariensis* L.). Certain mixes contained dried fruits and hulled nuts, which were categorized as processed ingredients due to a significantly higher level of handling before incorporation into the final commercial bird feed mix.

Amaranthus Identification and Resistance Screening

For all weed species except the *Amaranthus* species, identification was possible without the necessity of seed germination. All *Amaranthus* species seeds collected from bird feed mixes were broadcast in 54 by 27 by 6 cm greenhouse flats (Hummert International, Earth City, MO) containing a commercial potting medium (Pro-Mix BX, Premier Tech Horticulture, Quakertown, PA) and were maintained in a greenhouse at 30 C. Natural light was supplemented with metal-halide lamps (600 μmol photon m^{-2} s^{-1}) providing a 14-h photoperiod, and flats were watered as needed. Approximately 14 d after planting, a germination percentage was recorded to ensure any plants that did not survive until identification were accounted for. When *Amaranthus* species reached 5 cm in height, they were identified by species and transplanted into individual 10 by 10 cm diameter pots with a 1:1 ratio of the same commercial potting medium and field topsoil. Once plants reached 10 cm in height, identification was confirmed, and a discriminating dose of 3.3 kg ha^{-1} of glyphosate (Roundup PowerMax®, 540 g ai L^{-1}, Monsanto, St Louis, MO) was applied to all plants using a CO_2-pressurized backpack sprayer applying 140 L ha^{-1} water volume at 144 kPa with a XR 8002 flat-fan nozzle (TeeJet®, Spraying Systems, Wheaton, IL). Visual injury was estimated at 21 d after application on a 0% to 100% scale, with 0% indicating no phytotoxic effects present and 100% indicating complete plant death. If any mix contained plants that survived this application of glyphosate, that mix was marked as containing glyphosate-resistant seed. Survival was determined visually in a subjective evaluation of each plant's ability to survive and reproduce following the application of glyphosate.

Statistical Analysis

A linear regression model (PROC REG, SAS® 9.4, SAS Institute, Cary, NC) was generated to determine what bird feed ingredients best predicted weed seed contamination. Feed mix ingredients were predictor variables and quantities of seed per weed species were response variables. Models were developed for each weed species detected, and weed species with significant regression models were analyzed further. Of the 29 weed species extracted from bird feed mixes, a significant model was developed for *Amaranthus* species, grass weed species, *A. artemisiifolia*, and kochia [*Bassia scoparia* (L.) A.J. Scott]. For each model, an equation could be developed to predict the abundance of each of these four weed species based on the ingredients present in the feed mix. An example equation for each weed species is represented by: $[y = x(\text{ingredient}) + \text{Intercept}]$, wherein y is the weed species, x is the parameter estimate for seed abundance, and (ingredient) is 1 if present and 0 if absent from the feed mix. When ingredient parameter estimates were significant, the prediction of the increase or decrease in overall weed seed abundance from that ingredient is considered significantly different from zero. However, to predict contamination levels, all factors must be included in the equation regardless of significance. The *B. scoparia* and *A. artemisiifolia* data sets included a large number of zeros, as they were less common ingredients present in feed mixes than *Amaranthus* and grass weed species. The data sets could not be normalized through the use of data transformations, so to support linear regression, an additional binomial logistic regression was conducted for each weed species.

This binomial logistic regression predicts only the probability of weed seed presence or absence. The binomial logistic regression also does not require the assumption that data are normally distributed, so significance is not impacted by the large number of bird feed mixes without contamination (Cox 1958).

Certain bird feed ingredients are more common in feed mixes than others. For example, proso millet was used in 60 mixes, and grain sorghum was used in 38 mixes. However, grain sorghum was only present in three feed mixes in which proso millet was not. Because of this, it could be possible for a more common ingredient such as proso millet to conceal the true weed seed contribution of an ingredient like grain sorghum when all ingredients are included in the model. Therefore, an additional analysis was performed using independent-sample *t*-tests to evaluate how individual ingredients are associated with weed seed quantity present in bird feed mixes (PROC TTEST, SAS®). Variance equality was assessed using the folded *F* method, and for instances when variances were unequal, the Satterthwaite method was used to calculate *t*-values (Satterthwaite 1946). The Satterthwaite method is appropriate when variances of two groups are unequal.

Finally, differences in *Amaranthus* species abundance from feed mixes purchased from different states were tested through a linear mixed-effects model using the PROC GLIMMIX procedure (SAS® 9.4). The states were treated as a random factor in this analysis to determine whether the origins of the bird feed mixes significantly affected the weed seed contamination.

Table 2. Descriptive analysis of weed species detected in bird feed mixes.

Weed species	No. of mixes present	Mean	Maximum	SD
Grass species		——Seeds kg^{-1} of bird feed mix——		
Cenchrus longispinus	9	0.1	4	0.6
Digitaria sanguinalis	31	19	1,078	110
Echinochloa crus-galli	11	2.5	113	13
Setaria spp.	39	69	2,768	300
Sorghum bicolor	37	13	200	29
Sorghum halepense	10	0.3	13	1.4
Broadleaf species				
Abutilon theophrasti	15	1.1	22	3.9
Amaranthus spp.	94	384	6,525	897
Ambrosia artemisiifolia	36	16	296	40
Ambrosia trifida	7	0.1	4	0.6
Bassia scoparia	13	0.7	15	2.5
Brassica spp.	15	1.2	41	5.2
Chenopodium album	8	1.1	52	5.6
Cirsium spp.	9	0.6	15	2.2
Convolvulus arvensis	6	0.4	18	2.1
Fagopyrum esculentum	33	2.8	56	7.3
Ipomoea spp.	17	0.7	14	2.1
Portulaca oleracea	4	0.1	6	0.7
Rumex crispus	7	0.2	5	0.7
Salsola tragus	7	0.2	6	0.8
Tribulus terrestris	6	0.1	6	0.7
Xanthium strumarium	2	0.1	2	0.2

Results and Discussion

Bird Feed Screening

There was not a significant effect of the state from which bird feed mixes were purchased ($P = 0.98$); therefore, mixes from all locations were combined for analysis. From the 98 bird feed mixes evaluated in this research, 29 different species of weeds were identified (Table 2). The most frequently identified weed species were *Amaranthus* species, which were found in 96% of mixes and averaged 384 seeds kg^{-1} across all mixes. Certain mixes contained very high levels of *Amaranthus* seed, such as one sample that contained 6,525 seeds kg^{-1}. Collectively, grass weed species were the second most abundant weed seeds present in the bird feed mixes, and these consisted of the foxtail species giant foxtail (*Setaria faberi* Herrm.), yellow foxtail [*Setaria pumila* (Poir.) Roem. & Schult.], and green foxtail [*Setaria viridis* (L.) P. Beauv.], large crabgrass [*Digitaria sanguinalis* (L.) Scop], barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.], shattercane [*Sorghum bicolor* (L.) Moench ssp. *verticilliflorum* (Steud.) de Wet ex Wiersema & J. Dahlb.], johnsongrass [*Sorghum halepense* (L.) Pers.], and longspine sandbur [*Cenchrus longispinus* (Hack.) Fernald]. Grass weed species were identified in 76% of the bird feed mixes at levels ranging from 0 to 3,896 seeds kg^{-1} of feed mix. The third most frequently identified species present in bird feed mixes was *A. artemisiifolia*. Seed of this species was present in 43% of feed mixes and was measured at levels as high as 296 seeds kg^{-1} of feed mix. In a similar study, Vitalos and Karrer (2008) reported *A. artemisiifolia* seeds in 37% of bird feed mixes screened at levels as high as 531 seeds kg^{-1}. The next most common and abundant weed screened in our study was wild buckwheat (*Fallopia convolvulus* L.; syn: *Polygonum convolvulus*). *Fallopia convolvulus* was present in 30% of mixes and reached levels of 56 seeds kg^{-1} of feed mix. Additional weeds that were identified in bird feed mixes that have relevance as troublesome species (VanWychen 2016) include *B. scoparia*, morningglory species (*Ipomoea* spp.), common lambsquarters (*Chenopodium album* L.), and velvetleaf (*Abutilon theophrasti* Medik.), which were identified in 13%, 17%, 10%, and 13% of mixes, respectively. Hanson and Mason (1985) also reported each of these weed species in a bird feed screening conducted in Great Britain; however, they did not report on the quantities present in each mix.

Amaranthus Species Identification and Resistance Screening

Amaranthus species germination ranged from 0% to 78%, while 19% of all *Amaranthus* seeds planted were readily germinable. Five different *Amaranthus* species were identified. These included redroot pigweed (*Amaranthus retroflexus* L.), common waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer], smooth pigweed (*Amaranthus hybridus* L.), *A. palmeri*, and tumble pigweed (*Amaranthus albus* L.). We were unable to identify *Amaranthus* seed at the species level when seed present in a mix was not viable. Of the 94 seed mixes that contained *Amaranthus* seed, 71% contained seed that was readily germinable. *Amaranthus retroflexus* was the *Amaranthus* species that was most common in seed mixes (50%); however, only 16 mixes contained *Amaranthus* seed of only one species. Bird feed mixes are most often composed of seed collected from more than one field and often from multiple crop species, so weed seed contamination was shown to vary greatly even within the same mix. *Amaranthus albus* was the second most common *Amaranthus* species identified (34%), followed by *A. palmeri* (28%) and *A. tuberculatus* (23%). The least common *Amaranthus* species identified was *A. hybridus*, which was present in only 4% of mixes screened. These results are consistent with previous research that reported *A. retroflexus* as the most common *Amaranthus* species present in a Canadian grain sampling program that took place from 2007 through 2015 (Wilson et al. 2016). Although *A. retroflexus* and *A. albus* were the two most

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common *Amaranthus* species identified in these experiments, neither of these species exhibited resistance to glyphosate in any of the bird feed mixes tested. All *A. hybridus* plants were also controlled by the discriminating dose of glyphosate and were not deemed resistant. To date, there are no known cases of glyphosate resistance in *A. retroflexus* or *A. albus*, and only three known cases of glyphosate resistance occur in *A. hybridus* in Argentina; therefore, these results seem consistent with the status of glyphosate resistance in these species in the United States (Heap 2019). However, of the 26 bird feed mixes that contained readily germinable *A. palmeri* seed, four contained glyphosate-resistant *A. palmeri* plants. Similarly, of the 23 bird feed mixes that contained readily germinable *A. tuberculatus* seed, three contained glyphosate-resistant plants. An additional two mixes contained both *A. palmeri* and *A. tuberculatus* seeds that were resistant to glyphosate. It is important to note that in two of the three mixes that contained glyphosate-resistant *A. tuberculatus* and in all four mixes that contained glyphosate-resistant *A. palmeri*, all plants screened were determined to be resistant. This segregation in resistance suggests that in some cases, the *Amaranthus* species that are present in a bird feed mix could be originating from one source. To date, glyphosate-resistant *A. palmeri* has been documented in 26 states in the United States, as well as Argentina and Brazil, while glyphosate-resistant *A. tuberculatus* occurs in 18 states in the United States and also in Canada (Heap 2019). These results not only demonstrate another possible avenue for the spread of *Amaranthus* species, but also another route for the spread of glyphosate-resistant *Amaranthus* species throughout the United States.

Prediction of Amaranthus Seed Contamination

The equation [*Amaranthus* seed = 66.1(proso millet) + 2.9(grain sorghum) + 1.4(corn) + 2.3(sunflower) − 1.5(safflower) + 1.1(wheat) − 2.0(nyjer thistle) − 4.8(processed) + 4.0(canarygrass) + 977.2] (Table 3) best predicted the likelihood of *Amaranthus* contamination ($P \leq 0.0001$). Additionally, from the *t*-test analysis (Figure 1), it was determined that when proso millet, grain sorghum, and corn were present in seed mixes, there was an overall increase in *Amaranthus* seed presence. While the results from the *t*-test analysis suggests several ingredients could potentially increase *Amaranthus* seed contamination, proso millet is the only ingredient that demonstrated a positive effect in both analyses. *Amaranthus* seed size varies from 0.32 to 0.63 mm^2 (Farmer et al. 2017), and because proso millet is a small-seeded crop, mechanical separation will be especially difficult (Duary 2014; Wilson et al. 2016). Additionally, proso millet that is used for bird feed is unlikely to undergo any additional processing or cleaning to reduce weed seed contamination (Wilson et al. 2016). Corn and grain sorghum also increased the contamination of *Amaranth* seeds in the *t*-test analysis (Figure 1). These results are in agreement with previous research in which grain sorghum was determined to be a major source of weed seed contamination, including contamination of *Amaranthus* species, in Japanese feed imports (Kurokawa 2001). Another study reported *Amaranthus* species were one of six species that were consistently present at harvest time in Illinois cornfields (Davis 2008). In contrast, when processed ingredients were present in the feed mix, there was a decrease in *Amaranthus* seed contamination (Figure 1). The decrease in contamination as a result of the presence of processed ingredients is likely explained by the reduction in weed seed that will inevitably occur when grain products are subject to processing practices such as milling, shelling, or seed cleaning (Hoseney 1994). Additionally, it is expected that processed ingredients such as raisins and nuts would be free of *Amaranthus* seed contaminants due to the differences in harvesting methods and processing elements in comparison with raw agronomic grain. These results indicate that contamination of *Amaranthus* species in bird feed mixes could be originating from proso millet, grain sorghum, and corn and that further processing of these feed ingredients to remove these seeds may have the potential to reduce *Amaranthus* seed contamination.

Table 3. Prediction of *Amaranthus* species seed contamination in commercially available bird feed mixes based on linear regression analysis.

Ingredient	Parameter estimate	SE	*t*-value	P-value[a]
Intercept	977.23	2.95	6.34	<0.0001*[b]
Proso millet	66.06	2.75	4.11	<0.0001*
Grain sorghum	2.88	2.88	1.00	0.3215
Corn	1.40	0.36	0.72	0.4347
Sunflower	2.34	3.23	0.72	0.4759
Safflower	−1.47	2.57	−0.41	0.6808
Wheat	1.13	4.57	0.08	0.9344
Nyjer thistle	−2.03	3.52	−0.57	0.5732
Processed	−4.81	2.62	−1.63	0.1074
Canarygrass	3.95	3.71	1.05	0.2967

[a]Model is significant at $P \leq 0.0001$. When P-value from individual ingredient is significant, the parameter estimate from that ingredient is different from zero.
[b]Asterisks indicate ingredient is significant at $P<0.05$.

Prediction of Grass Weed Species Seed Contamination

The equation [grass weed species seed = 4.57(proso millet) + 1.27 (grain sorghum) − 1.62(corn) − 2.29(sunflower) − 1.37(safflower) + 2,802(wheat) + 7.76(nyjer thistle) − 10.15(processed) + 1.04 (canarygrass) + 358.3] ($P \leq 0.0001$) best predicted contamination of grass weed species (Table 4). The *t*-test analysis demonstrated an increase in grass weed seeds when wheat, grain sorghum, and proso millet were present in the mix (Figure 1). Shimono and Konuma (2008) reported similar results, with Poaceae species appearing the most often in wheat grain samples. Historically, the control of grass weeds in monocotyledonous crops like these has proven difficult due to the limited availability of selective herbicides used for grass control and lack of herbicide-resistant cultivars. Shimono and Konuma (2008) determined that in-field abundance of weeds and weed height were two factors that correlated to the number of weed seeds that contaminated wheat. Processed ingredients decreased weed seed in the model ($P = 0.0470$) as well as in the *t*-test analysis ($P = 0.0027$), similar to results with the *Amaranthus* species. Grass weed seed was also lower when safflower was in the mix. The relatively large seed size of safflower would allow for more effective mechanical separation of the desired crop and weed seed by harvesting equipment. Additionally, previous research has shown that the cyclohexanedione and aryloxyphenoxypropionate (WSSA Group 1) herbicides are highly effective in controlling grass weed species in safflower production (Blackshaw et al. 1990). These results suggest that the monocotyledonous crop species commonly used in bird feed mixes like wheat, grain sorghum, and proso millet are primary contributors to grass weed seed contamination in feed mixes. Therefore, bird feeders placed directly in homeowner yards could be responsible for the introduction of weeds such as *D. sanguinalis* and *S. viridis*. It is also worth noting that the amount of glyphosate- and multiple-resistant grass weed species continues to increase (Heap 2019). To reduce the amount of grass weed seed

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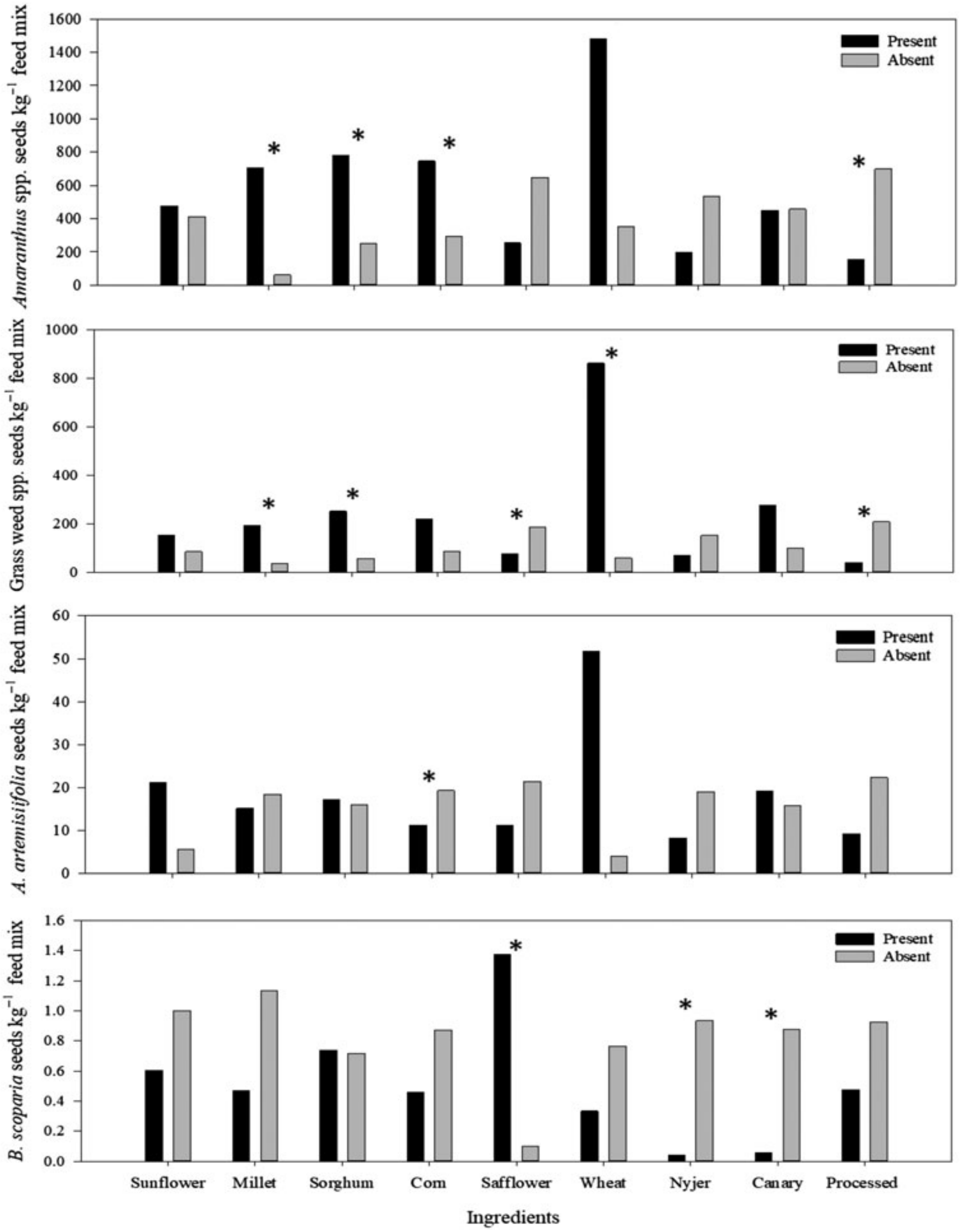

Figure 1. Weed seed contamination based on the presence and absence of common bird feed ingredients. Dark bars illustrate weed seed contamination when a given ingredient is present; lighter bars illustrate weed seed contamination when that ingredient is absent from bird feed mixes. Canary, canarygrass. Asterisks indicates significant difference between paired bars based on *t*-test analysis. Values are averages.

transported in bird feed, mixes that incorporate processed ingredients or safflower should be promoted.

Prediction of Ambrosia artemisiifolia Contamination

The model [*A. artemisiifolia* = −5.88(proso millet) − 6.72(grain sorghum) − 20.89(corn) + 89.12(sunflower) − 15.48(safflower) + 1.58(wheat) + 1.43(nyjer thistle) − 4.09(processed) + 3.33(canarygrass) + 37.15] (P = 0.0017) best predicted contamination of *A. artemisiifolia*. The logistic regression supported these results for all species except safflower (Table 5). Safflower was also not determined to decrease *A. artemisiifolia* in the *t*-test analysis, suggesting that the linear regression may have overestimated its contribution to *A. artemisiifolia* contamination due to large variance in the data set for this species. Corn decreased *A. artemisiifolia* levels in all analyses. This can likely be explained by the variety of corn herbicides that are effective in controlling *A. artemisiifolia* as well as the ability of most harvesting machines to mechanically separate corn grain from *A. artemisiifolia* seeds (Heap 2019; Wilson et al. 2016). Sunflower increased contamination in both regression analyses but was not a factor in the *t*-test analysis (P = 0.0769). Many other studies have determined sunflower to be an important factor in *A. artemisiifolia* contamination. Vitalos and Karrer (2008) reported that all samples that were contaminated with *A. artemisiifolia* seed contained sunflower. They also reported the highest levels of *A. artemisiifolia* seed (531 seeds kg^{-1}) in bird feed mixes that contained only sunflower as an ingredient. Bohren et al. (2006) also reported that *A. artemisiifolia* was commonly identified in imported sunflower and deemed it nearly

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Table 4. Prediction of grass weed species seed contamination in commercially available bird feed mixes based on linear regression analysis.

Ingredient	Parameter estimate	SE	*t*-value	P-value[a]
Intercept	358.9	3.63	4.55	<0.0001*[b]
Proso millet	4.57	3.31	1.26	0.2126
Grain sorghum	1.27	3.54	0.19	0.8507
Corn	1.62	3.02	0.44	0.6585
Sunflower	−2.29	3.09	−0.59	0.5592
Safflower	−1.37	3.14	−0.28	0.7838
Wheat	2802	6.21	4.35	<0.0001*
Nyjer thistle	7.76	4.46	1.37	0.1735
Processed	−10.15	3.15	−2.01	0.0470*
Canarygrass	1.04	4.67	0.03	0.9780

[a]Model is significant at $P \leq 0.0001$. When P-value from individual ingredient is significant, the parameter estimate from that ingredient is different from zero.
[b]Asterisks indicate ingredient is significant at $P<0.05$.

Table 5. Prediction of *Ambrosia artemisiifolia* seed contamination in commercially available bird feed mixes based on linear regression analysis.

Ingredient	Parameter estimate	SE	*t*-value	P-value[a]
Intercept	37.15	3.09	3.19	0.0019*[b]
Proso millet	−5.88	2.89	−1.69	0.0953
Grain sorghum	−6.72	3.03	−1.72	0.0891
Corn	−20.89	2.67	−3.09	0.0027*
Sunflower	89.12	3.44	3.64	0.0005*
Safflower	−15.48	2.72	−2.75	0.0073*
Wheat	1.58	4.94	0.29	0.7727
Nyjer thistle	1.43	3.71	0.27	0.7843
Processed	−4.09	2.73	−1.40	0.1647
Canarygrass	3.33	3.92	0.88	0.3836

[a]Model is significant at $P \leq 0.0017$. When P-value from individual ingredient is significant, the parameter estimate from that ingredient is different from zero.
[b]Asterisks indicate ingredient is significant at $P<0.05$.

Table 6. Prediction of *Bassia scoparia* seed contamination in commercially available bird feed mixes based on linear regression analysis.

Ingredient	Parameter estimate	SE	*t*-value	P-value[a]
Intercept	1.23	1.62	−0.45	0.6510
Proso millet	−1.04	1.54	−0.12	0.9049
Grain sorghum	2.18	1.61	1.64	0.1043
Corn	−1.59	1.51	−1.10	0.2740
Sunflower	−4.79	1.69	−2.95	0.0041*[b]
Safflower	3.80	1.53	3.11	0.0025*
Wheat	−1.05	1.98	−0.09	0.9317
Nyjer thistle	−2.23	1.74	−1.46	0.1490
Processed	−1.36	1.51	−0.72	0.4756
Canarygrass	−1.34	1.79	−0.54	0.5922

[a]Model is significant at $P \leq 0.0203$. When P-value from individual ingredient is significant, the parameter estimate from that ingredient is different from zero.
[b]Asterisks indicate ingredient is significant at $P<0.05$.

Table 7. Logistic regression analysis of bird feed ingredient effects on *Ambrosia artemisiifolia* and *Bassia scoparia* seed contamination.

Bird feed ingredient	*A. artemisiifolia*	*B. scoparia*
	—— Pr > *F* ——	
Proso millet	0.0619	0.5779
Grain sorghum	0.6000	0.0926
Corn	0.0331	0.1360
Sunflower	0.0279	0.0182
Safflower	0.1064	0.0592
Wheat	0.5849	0.6973
Nyjer thistle	0.4166	0.1020
Processed	0.4331	0.9102
Canarygrass	0.8512	0.9981

impossible to separate *A. artemisiifolia* from the desired crop. Brandes and Nitzsche (2006) also proposed that sunflower should routinely be checked for *A. artemisiifolia* contamination and also proposed a certified *Ambrosia*-free bird feed classification. *Ambrosia artemisiifolia* has been determined resistant to four classes of herbicides in the United States (WSSA Groups 2, 5, 9, and 14) (Heap 2019), and the presence of this seed in feed mixes could provide a route for herbicide-resistant *A. artemisiifolia* seed to spread into new geographies.

Prediction of Bassia scoparia Contamination

Bassia scoparia was identified in bird feed mixes at quantities much lower than any of the other weed species discussed, but is also an economically important weed on WSSA's list of top 10 most troublesome weeds in the United States (VanWychen 2016). The model [*B. scoparia* = −1.04(proso millet) + 2.18(grain sorghum) − 1.59(corn) − 4.79(sunflower) + 3.80(safflower) − 1.05(wheat) − 2.23(nyjer thistle) − 1.36(processed) − 1.34(canarygrass) + 1.23] ($P = 0.0203$) best predicted *B. scoparia* contamination in feed mixes (Table 6). Factors in the model of the linear regression align with results from the logistic regression, suggesting the linear regression was not affected by the nonnormalized data set for this species (Table 7). The *t*-test analysis determined that canarygrass and nyjer thistle reduced *B. scoparia* contamination. The control of *B. scoparia* in canarygrass production would likely be achievable with WSSA Group 4 herbicides, reducing any seeds present at harvest. The reduction observed with nyjer thistle is likely due to the fact that this species is often harvested by hand and not by mechanical means, which could allow for manual separation of *B. scoparia* from nyjer thistle plants (Duke 1983). Safflower increased contamination in both analyses. Several previous studies have noted the problematic nature of *B. scoparia* in safflower production (Anderson 1987; Berglund et al. 2007; Blackshaw et al. 1990). These results indicate that *B. scoparia* contamination in bird feed mixes originates primarily from safflower. In fact, *B. scoparia* was the only weed species analyzed that did not result in a significant intercept in the model, which indicates that *B. scoparia* is not expected to be present in the mix unless safflower is used as an ingredient.

The results of this research draw attention to what may be an overlooked and underestimated pathway of seed spread of troublesome weed species. Many weed seeds are being transported in bird feed mixes, including *Amaranthus* species, which are some of the most troublesome weeds in the United States. Our screening has also proven that glyphosate-resistant *Amaranthus* seed is being transported in bird feed mixes. In an earlier similar study, Watts and Watts (1979) suggested that the series of chance events that would make it possible for a component of a bird feed mix to escape and ultimately settle in an area conducive for its germination may happen more than expected. Others doubt bird feed plays much more than possibly a minor role in the introduction of weed species into new territories (Vitalos and Karrer 2008). Regardless, endozoochory may be involved, as several studies have reported weed seeds to remain viable after alimentary excretion in cattle, waterfowl, and other avian species (Dowsett-Lemaire 1988; Farmer et al.

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2017; Lhotska and Holub 1989; Powers et al. 1978). When this issue was exposed in Europe, European governmental agencies imposed regulations for bird feed contamination, which subsequent data suggest led to decreased overall contamination levels. Perhaps similar regulations could lessen contamination levels in North American bird feed mixes. Across our entire screening, we measured an average of 363 *Amaranthus* seeds kg^{-1} of bird feed. Using the results from our study in conjunction with data from the USFWS survey, it could be possible that 105 million *Amaranthus* seeds are transported in bird feed mixes each year.

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