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Plant life span and persistence of soil seedbanks predict the emergence of herbicide resistance in noxious weeds

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Abstract

Plant control methods have been developed to reduce weed species that are often problematic in agricultural systems. However, these methods can create new challenges, such as herbicide resistance. Determining which plant traits are associated with herbicide resistance can assist managers in identifying species with the potential to develop herbicide resistance and to better understand factors contributing to the evolution of herbicide resistance. We used random forest models to model herbicide resistance of noxious weeds as a function of 10 biological and ecological plant characteristics. Three noxious weed characteristics—plant life span, seedbank persistence, and occurrence in riparian or wetland microsites—predicted herbicide resistance with 87% accuracy. Species with persistent seedbanks and with short life spans (i.e., annuals) that occurred outside riparian or wetland areas were most likely to develop herbicide resistance. Short life spans indicate short generation times enabling faster evolution for herbicide resistance. Persistent seedbanks may increase the survival of resistant genotypes within a population or may be co-selected as an alternate form of escape from control methods. Species occurring in riparian or wetland microsites may be a case of "avoidance" rather than resistance, as managers typically avoid applying herbicide in these areas. Currently, 47 of the noxious weed species analyzed in this study are herbicide resistant, and our models identified an additional 63 species with traits that are highly associated with herbicide resistance, potentially indicating species that are at risk of developing resistance under conducive conditions. Further data-driven analyses with more plant traits and species from around the world could help refine current risk assessment of herbicide-resistance development.

Introduction

The development of synthetic herbicides in the 1940s provided a breakthrough in the control of weed populations. The widespread adoption of herbicides by landowners and land managers has allowed for increased economic yields in agricultural systems (Oerke 2006) and control of noxious invasive weeds (Weidlich et al. 2020), and assisted in the restoration of invaded native plant communities (Kettenring and Adams 2011). Yet the efficacy of synthetic herbicides to control weed populations is increasingly threatened by the emergence of herbicide-resistant (HR) genotypes that can survive a lethal dose of herbicide (dePrado et al. 1997; Mortensen et al. 2012). Since herbicide resistance was first documented in 1957 (Hilton 1957), the number of HR weed species has risen rapidly. At this writing, herbicide resistance has been reported in populations of 263 species across 71 countries and continues to rise, with almost 20% of all recorded instances of herbicide resistance occurring in the past decade alone (Heap 2021). Weed populations have evolved resistance to 23 of the 26 herbicide mechanisms of action (MOAs), which are the various methods by which a herbicide can disrupt biological or enzymatic processes, thereby preventing normal plant development and growth in a given weed species.

Given the economic and ecological threats posed by HR weeds, a critical challenge in weed science is predicting which species are most likely to evolve herbicide resistance and under what conditions. A considerable amount of research in recent decades has focused on identifying the molecular mechanisms responsible for conferring resistance in individual species (Délye 2013; Powles and Yu 2010) and the evolutionary mechanisms and patterns of herbicide usage most likely driving herbicide resistance in a given species (e.g., Diggle et al. 2003; Gressel 2009; Maxwell et al. 1990; Neve et al. 2011). These studies have revealed that the probability and rate at which herbicide resistance evolves in a weed population should be a function of herbicide use patterns, herbicide MOA, and the intrinsic biological and population characteristics of the species (Délye et al. 2013).

A key finding of these studies has been that the probability of herbicide resistance emerging in a population and the rate at which it evolves depend on the population size and rate of recombination (Kreiner et al. 2018; Maxwell et al. 1990; Neve et al. 2009). Gene variants conferring resistance are more likely to be preexisting or to arise through spontaneous mutation in large, genetically diverse populations (Kersten et al. 2021; Lanfear et al. 2014), and the rate at which herbicide resistance evolves is suggested to depend on the life span and the longevity of seeds in the soil seedbank (a reservoir of dormant or nondormant seeds; Baskin and Baskin 2014). Therefore, annuals and species with short-lived seedbanks are predicted to evolve resistance faster than perennials and species with long-lived seedbanks.

While the results from these studies have been critical in the development of management recommendations (Norsworthy et al. 2012) and risk assessments (Moss et al. 2019) regarding the types of species and patterns of herbicide use likely to result in herbicide resistance, there have been relatively few data-driven analyses on the ecological, morphological, and life-history characteristics associated with herbicide resistance (but see Darmency et al. 2017; Holt et al 2013; Kreiner et al. 2018). To date, data-driven analyses have compared the characteristics of HR and non-HR weeds and confirmed that annual weed species (Holt et al. 2013) and outcrossing species (Kreiner et al. 2018) have evolved resistance much more often and more quickly than biennial or perennial weed species or self-pollinating species. However, any search for traits associated with herbicide resistance is complicated by the fact that currently non-HR species may have the intrinsic biological traits conducive to evolving herbicide resistance but have not yet been exposed to the right conditions to do so.

To examine plant characteristics that potentially contribute to herbicide resistance, we analyzed a set of 283 noxious weed species in the United States along with their herbicide-resistance status, morphological, ecological, and life-history characteristics. A noxious weed is a legal designation passed by the U.S. Congress to control the entry and spread of harmful weeds (7 U.S.C. ch. 104, §§7110 et seq.). A species is formally listed as a noxious weed when it has been determined to cause damage to crops, livestock, poultry, or other resources of the United States (Plant Protection 2012). Using this list of noxious weed species, we specifically asked:

1. Which morphological, ecological, and life-history characteristics are most associated with developing herbicide resistance?
2. Can these traits be used to identify which current noxious weeds are most "at risk" of becoming HR?

We hypothesized that species with short life spans and short-lived seedbanks would be most likely to be HR. We used model results to identify noxious weed species that are currently non-HR, but that have characteristics associated with the evolution of herbicide resistance.

Materials and Methods

Overview

In this study we used random forest models, a machine learning algorithm, to model the herbicide-resistance status (yes/no) of noxious weeds as a function of 10 biological and ecological characteristics postulated to influence the evolution of herbicide resistance, including life-history traits, traits that affect the uptake of herbicides into plant tissue, and information on long-distance gene flow. We assessed which set of covariates resulted in the most accurate identification of the herbicide-resistance status of species and used model results to create a ranked assessment of the "risk" that current non-HR species may become HR in the future under appropriate conditions.

Data Description

We obtained data on plant characteristics from a U.S. noxious weed database created by the Western Wildlands Threat Assessment Center (WWETAC). Data were categorical, descriptive, and continuous, and described the ecology, management, and functional traits of nonnative, naturalized grass, forb, and vine species classified as noxious weeds in the United States (https://plants.usda.gov/java/noxiousDriver). These data included species' life history, environmental tolerances, leaf traits, known dispersal vectors, and whether species were known to be HR or not (NEG, unpublished data). We selected 10 variables from this data set that may be conducive to the evolution of herbicide resistance. These included: life-history traits (plant life span, plant life-form, and persistence of seeds in the seedbank); leaf traits linked to the efficacy of herbicide uptake (mean leaf area and leaf surface properties); whether seeds were known to be dispersed long distances (dispersed by wind; or transported by vehicles; or in shipments of soil, seeds, or hay); and ecological characteristics likely to expose a species to herbicide use (found in cultivated habitats vs. riparian or wetland habitats). We verified the herbicide-resistance status of each species in August 2021 against the International Herbicide-Resistant Weed Database (www.weedscience.org; Heap 2021).

Life-form data were categorical with three classes: graminoids, herbaceous forbs, and vines. Species life span was categorical with four classes: annual, biennial, perennial (average life span ≤5 yr), or long-lived perennial (average life span >5 yr). Persistence of seeds in a soil seedbank was categorical with five classes: none (species not known to produce a seedbank), short (seeds survive 1–4 yr on average), moderate (seeds survive 5–10 yr on average), long (seeds survive 11–20 yr on average), and very long (>20 yr). We estimated mean leaf area as the natural log of mean leaf length (cm) multiplied by mean leaf width (cm), using mean length and width values for each species as provided by regional flora. We categorized whether species had leaf traits (yes/no) that may affect the efficacy of herbicide delivery into plant tissues (e.g., presence of trichomes, succulence, or waxy or leathery leaves; Hess and Falk 1990; Wang and Liu 2007). We categorized whether species' seeds had the capacity for long-distance seed dispersal by wind (yes/no) or transport (e.g., in soil, seed, or hay shipments) (yes/no). As an assessment of whether a species was likely to be exposed to herbicide use, we categorized whether the species was a known weed of cultivated habitats (yes/no) or riparian and wetland habitats (yes/no).

We used the function *hclustvar* in the package CLUSTOFVAR (Chavent et al. 2012) in R v. 3.4.3 (R Core Team 2020) to test for redundancy among covariates. CLUSTOFVAR uses clustering algorithms to find clusters of variables that are strongly related to each other within data sets that contain a mixture of quantitative and qualitative variables (Chavent et al. 2012; see Supplementary Material for details).

Analyses

Random forest models were implemented using the package RANDOMFOREST (Liaw and Wiener, 2002) in R (R Core Team 2020) to model herbicide-resistance status as a function of selected species' traits. Random forest models are ensemble models in which numerous classification or regression tree models are built using recursive binary partitioning to split predictor covariates (Cutler et al. 2007; Olden et al. 2008). During the growth of each "decision tree", a random subset of predictor covariates is used to split the data at each classification node, and each decision tree is trained on a bootstrap sample of the original data set (the "training" data, approximately two-thirds of the data set). The predictive accuracy of each decision tree is then tested on the remaining one-third of the data set (the "testing" data). Final results are aggregated over all decision trees to produce the out-of-bag (OOB) error rate, the percentage of incorrectly classed observations. (Note that the terms "random forest" and "decision trees" deal with the form of analysis rather than actual plants that were studied in the analysis.)

Because our data were unbalanced (far fewer HR species than non-HR species; Table 1), we adjusted the random forest bootstrapping technique to sample equally from each class (Chen and Breiman 2004). For all analyses, we set the number of decision trees grown per forest at 1,000 and used $\sqrt{n}$ for the number of covariates considered at each classification node, where n is the number of predictor covariates in the model.

We assessed the overall predictive accuracy of the model using the OOB error rate. We assessed the importance of each covariate to the predictive accuracy of the model by examining permutation importance values, which quantify the difference in the predictive accuracy of a model when the value of a particular covariate is randomly permuted compared to when it is not (Breiman 2001). The higher the score, the greater the contribution of that covariate to model accuracy. To optimize model structure, we sequentially removed covariates with negative permutation importance scores until only covariates with positive contributions to model accuracy remained.

We assessed the strength and direction (positive or negative) of associations between covariates and species herbicide-resistance status using partial dependence values. Partial dependence values denote the univariate effect of each covariate on the probability that a species is or is not classified as HR, controlling for the influence of all other covariates (Friedman 2001), as:

$$\bar{f}(x) = \frac{1}{n} \sum_{i=1}^{n} f(x, x_{iC}) \qquad [1]$$

where n is the number of covariates, x is the covariate for which partial dependence is sought, and x_{iC} represents all other covariates in the data. The summand is the predicted logit function for the response variable:

$$f(x) = \log p_k(x) - \frac{1}{K} \sum_{j=1}^{K} \log p_j(x) \qquad [2]$$

where $K = 2$, the number of classes of the response variable (HR or non-HR), k is the class of interest for the response variable (HR), and p_j is the proportion of votes for class j.

Results and Discussion

The model with the best predictive power consisted of only three covariates: species life span, seedbank persistence, and whether species occurred in riparian/wetland habitats. Using these three

Table 1. The number of herbicide-resistant (HR) and non-HR species correctly classified by the model and the proportion of HR and non-HR species that were incorrectly classified (classification error).[a]

Herbicide-resistance status	Total no. of species	No. classified as HR by model	No. classified as non-HR by model	Classification error
HR	47	41	6	0.128
Non-HR	236	62	174	0.263

[a]The out-of-bag error rate of the overall model was 24.03%.

Table 2. Permutation importance values for each trait in the model with the highest classification accuracy of the herbicide-resistance status of noxious weeds in the United States.

Covariate	Importance	Mean decrease in Gini index[a]
Species life span	18.59	7.85
Seedbank persistence	17.32	8.89
Occurs in riparian habitats	5.10	2.16

[a]The Gini index measures the average gain in homogeneity when the data are split by a given variable.

traits, the model was able to accurately classify 87% of the HR species in our database (41 out of 47; Table 1). Overall, the model classified the herbicide-resistance status of noxious weeds with 76% accuracy (i.e., an OOB error rate of 24%). The lower overall accuracy was due to the occurrence of false positives, with the model classifying 62 of the 236 current non-HR species as HR.

Species life span and seedbank persistence had the greatest effect on model accuracy (Table 2). In support of our hypothesis, HR weeds were more likely to be annuals; but contrary to our hypothesis, HR weeds had moderate (5–10 yr), long (11–20 yr), or very long lived (>20 yr) seedbanks. Partial dependence values of each covariate indicated that the herbicide-resistance status of a species was approximately inversely proportional to its life span, with annuals most likely, and perennials and long-lived perennials least likely to have evolved herbicide resistance (Figure 1A). In contrast, seedbank persistence was directly proportional to herbicide resistance (Figure 1B). Species with either no or short-lived seedbanks were less likely to have evolved herbicide resistance, while species with moderate, long, or very long-lived seedbanks were more likely to have evolved herbicide resistance. Occurrence in riparian habitats had a small effect on overall model accuracy, but decreased the number of false positives classified by the model from 73 to 62. Species found in riparian and wetland habitats were less likely to have evolved herbicide resistance than species that did not occur in riparian habitats (Figure 1C).

Our finding that the occurrence of herbicide resistance in species is inversely proportional to species life span is consistent with evolutionary theory (Kreiner et al. 2018; Neve et al. 2009) and previous data-driven analyses (Holt et al. 2013). The short generation time of annual species and their greater reliance on sexual reproduction compared to vegetative reproduction typically result in higher levels of genetic variation and more rapid rates of evolutionary change than found in long-lived species (Kreiner et al. 2018; Neve et al. 2009). In contrast, persistent soil seedbanks, in which seeds may survive for long periods in the soil, have been postulated to slow the evolution of herbicide resistance in a population (Gressell and Segel 1978). Herbicides act as a strong selection force on plant populations, eliminating susceptible genotypes from a population and leaving individuals that possess genes conferring
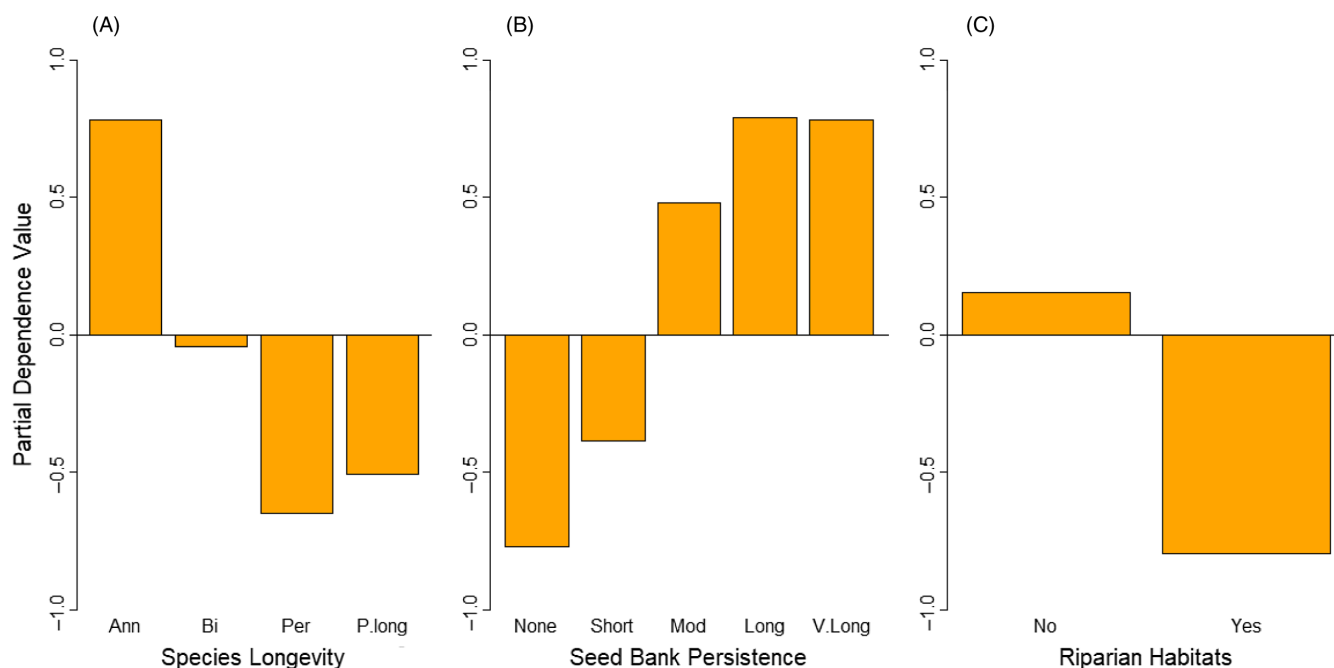
Figure 1. Partial dependence values denoting the relative logit contribution of a species' life span (A), soil seedbank persistence (B), and recorded presence in riparian or wetland habitats (C) to the probability that the species is herbicide resistant (HR). Positive/negative values (*y* axis) indicate that a species with this characteristic was more likely/less likely to be HR; zero indicates the trait had no effect on herbicide-resistance status. Trait value abbreviations for species life span: Ann, annual; Bi, biennial; Per, perennial with ≤5-yr life span; P.long, perennial with >5-yr life span.

partial or complete resistance. After multiple herbicide applications, only individuals with resistant genotypes are likely to remain. However, in species with persistent seedbanks, "old" susceptible genotypes can persist in a population, emerging and recombining with resistant genotypes, breaking apart adapted gene complexes, and diluting the proportion of resistant individuals.

A number of factors could explain why species with long-lived seedbanks are more likely to have evolved herbicide resistance. First, persistent seedbanks increase effective population size by increasing the number of individuals in a population (Lundemo et al. 2009; Nunney 2002), and large effective population size has been linked to the emergence of herbicide resistance. Modeling the population genetics of plant species with persistent seedbanks, Koopmann et al. (2017) found that while more persistent seedbanks did slow down the time for advantageous allele fixation, they also decreased the probability that advantageous alleles were lost due to random drift, thus potentially allowing for the persistence and survival of resistant genotypes. Species with long-lived, persistent seedbanks are also likely to be troublesome agricultural weeds and thus are likely to be exposed to persistent herbicide use, thus increasing the probability of emergence of resistance.

It is also possible that long-lived seedbanks and herbicide resistance are two traits that have emerged in some species, independently or together, as a response to intensive weed control practices. Delayed seed germination has been shown to evolve as a mechanism to escape early-season weed management practices, independent of herbicide usage (Sbatella and Wilson 2010). It has also been suggested that selection for herbicide resistance may have a pleiotropic effect on dormancy rates, and thus seedbank persistence (Owen et al. 2015). Reviewing published studies on HR and non-HR populations of 55 species that had evolved herbicide resistance, Darmency et al. (2017) found 22 species in which seeds from HR populations also had delayed germination and/or higher dormancy rates than conspecific non-HR populations, 19 species in which HR populations had accelerated germination or lowered dormancy, and 14 species with no difference. In this study, we used data on species' mean seedbank persistence, as opposed to data from specific HR or non-HR populations, and thus the association between seedbank persistence and herbicide resistance across species may be more indicative of either selection for traits that provide an escape from intensive weed control efforts or a preexisting advantage for the emergence of herbicide resistance in species with long-lived seedbanks.

The association between long-lived seedbanks and herbicide resistance is noteworthy for the management of noxious weeds. For many years, recommendations to prevent the evolution of herbicide resistance included rotating applications of herbicides with different MOAs (Beckie 2006; Gressel and Segel 1990; Norsworthy et al. 2012). In theory, rotating MOAs increases temporal heterogeneity of exposure to any one class of herbicide, thereby reducing selection pressure and slowing the evolution of resistance to any one class of herbicide (Diggle et al. 2003). Yet this recommendation is less effective for species with long-lived seedbanks, as only the photosynthesizing plants rather than seeds of the weed population will be killed by each alternating MOA (Darmency et al. 2017).

Our model suggests that riparian and wetland species were less likely to evolve herbicide resistance. Previous studies have noted that wetland and aquatic species are underrepresented in HR species (Heap and LeBaron 2001; Holt et al. 2013). This may reflect patterns of herbicide usage, which is typically restricted in these habitats (Holt et al. 2013; Radosevich et al. 2007). Herbicides that have aquatic labels enabling them to be used in riparian areas often have very short residence times in the system (e.g., Hall et al. 2014). Herbicides with shorter residence times would have reduced selection pressure to develop herbicide resistance than herbicides with longer residence times. Herbicide effectiveness is dependent on

Table 3. List of non–herbicide resistant (non-HR) noxious weed species that the model identified as HR.

Species name	Family	Proportion classified as HR[a]	Life span	Seedbank persistence[b]	Riparian
Cucumis melo L.	Cucurbitaceae	0.99	Annual	Long	N
Cucumis myriocarpus E. Mey. ex Naud.	Cucurbitaceae	0.98	Annual	Long	N
Phyllanthus urinaria L.	Euphorbiaceae	0.98	Annual	Unknown	N
Hibiscus trionum L.	Malvaceae	0.98	Annual	Very long	N
Tribulus terrestris L.	Zygophyllaceae	0.98	Annual	Long	N
Phyllanthus tenellus Roxb.	Euphorbiaceae	0.98	Annual	Unknown	N
Polygonum cespitosum Blume	Polygonaceae	0.98	Annual	Long	N
Carthamus creticus L.	Asteraceae	0.98	Annual	Moderate	N
Microstegium vimineum (Trin.) A. Camus	Poaceae	0.98	Annual	Moderate	Y
Carthamus oxyacanthus M. Bieb.	Asteraceae	0.98	Annual	Moderate	N
Carthamus leucocaulos Sm.	Asteraceae	0.98	Annual	Moderate	N
Emex spinosa (L.) Campd.	Polygonaceae	0.98	Annual	Moderate	Y
Anchusa arvensis (L.) M. Bieb.	Boraginaceae	0.98	Annual	Moderate	N
Hyptis suaveolens (L.) Poit.	Lamiaceae	0.98	Annual	Moderate	N
Carthamus lanatus L.	Asteraceae	0.98	Annual	Moderate	N
Emex australis Steinh.	Polygonaceae	0.97	Annual	Moderate	N
Lepidium coronopus (L.) Al-Shehbaz	Brassicaceae	0.97	Annual	Moderate	N
Carduus tenuiflorus W. Curtis	Asteraceae	0.97	Annual	Moderate	N
Euphorbia oblongata Griseb.	Euphorbiaceae	0.97	Annual	Moderate	N
Erodium cicutarium (L.) L'Hér. ex Aiton	Geraniaceae	0.97	Annual	Moderate	N
Ipomoea turbinata Lag.	Convulvulaceae	0.97	Annual	Moderate	N
Halogeton glomeratus (M. Bieb.) C.A. Mey.	Chenopodiaceae	0.97	Annual	Moderate	N
Aegilops cylindrica Host	Poaceae	0.97	Annual	Moderate	N
Taeniatherum caput-medusae (L.) Nevski	Poaceae	0.97	Annual	Moderate	N
Onopordum tauricum Willd.	Asteraceae	0.90	Biennial	Long	N
Onopordum illyricum L.	Asteraceae	0.88	Biennial	Long	N
Medicago polymorpha L.	Fabaceae	0.82	Annual	Short	N
Salsola paulsenii Litv.	Chenopodiaceae	0.82	Annual	Short	N
Thymelaea passerina (L.) Coss. & Germ.	Thymelaeaceae	0.82	Annual	Short	N
Aegilops triuncialis L.	Poaceae	0.81	Annual	Short	N
Striga asiatica (L.) Kuntze	Scrophulariaceae	0.81	Annual	Unknown	N
Salsola collina Pall.	Chenopodiaceae	0.81	Annual	Short	N
Fatoua villosa (Thunb.) Nakai	Moraceae	0.81	Annual	Unknown	Y
Crupina vulgaris Cass.	Asteraceae	0.81	Annual	Unknown	N
Pennisetum pedicellatum Trin.	Poaceae	0.81	Annual	Short	N
Cenchrus echinatus L.	Poaceae	0.81	Annual	Short	N
Chorispora tenella (Pall.) DC.	Brassicaceae	0.81	Annual	Unknown	N
Lolium temulentum L.	Poaceae	0.81	Annual	Short	N
Centaurea sulphurea Willd.	Asteraceae	0.81	Annual	Unknown	N
Chaenorhinum minus (L.) Lange	Scrophulariaceae	0.81	Annual	Unknown	N
Milium vernale M. Bieb.	Poaceae	0.80	Annual	Short	N
Aegilops geniculata Roth	Poaceae	0.80	Annual	Short	N
Centaurea melitensis L.	Asteraceae	0.80	Annual	Unknown	N
Torilis arvensis (Huds.) Link	Apiaceae	0.80	Annual	Short	N
Secale cereale L.	Poaceae	0.80	Annual	Short	N
Verbascum blattaria L.	Scrophulariaceae	0.79	Biennial	Very long	N
Verbascum thapsus L.	Scrophulariaceae	0.77	Biennial	Very long	N
Onopordum acanthium L.	Asteraceae	0.77	Biennial	Very long	N
Silybum marianum (L.) Gaertn.	Asteraceae	0.77	Biennial	Moderate	N
Glaucium flavum Crantz	Papaveraceae	0.76	Biennial	Moderate	N
Pastinaca sativa L.	Apiaceae	0.75	Biennial	Moderate	N
Phalaris arundinacea L.	Poaceae	0.67	Perennial long	Very long	Y
Ranunculus repens L.	Ranunculaceae	0.65	Perennial	Long	N
Panicum antidotale Retz.	Poaceae	0.64	Perennial	Long	N
Solanum elaeagnifolium Cav.	Solanaceae	0.64	Perennial	Long	N
Bryonia alba L.	Cucurbitaceae	0.61	Perennial	Long	Y
Polygonum ×*bohemicum* (J. Chrtek & Chrtková) Zika & Jacobson [*cuspidatum* × *sachalinense*]	Polygonaceae	0.60	Perennial long	Long	Y
Hypericum perforatum L.	Clusiaceae	0.59	Perennial long	Very long	N
Calystegia sepium (L.) R. Br.	Convulvulaceae	0.59	Perennial long	Very long	N
Polygonum polystachyum Wall. ex Meisn.	Polygonaceae	0.59	Perennial long	Long	Y

[a] Proportion of model decision trees (out of 1,000) in which species were classified as HR.
[b] Seedbank persistence: Short, seeds known to survive in seedbank for 1–4 yr; Moderate, 5–10 yr; Long, 11–20 yr; Very long, >20 yr; Unknown, seedbank persistence is unknown.

Table 4. Herbicide-resistant (HR) species that the model identified as non-HR.

Species name	Family	MOA[a]	Life span	Seedbank persistence[b]	Riparian
Arctotheca calendula (L.) Levyns	Asteraceae	D, O	Perennial	Short	N
Centaurea stoebe L. ssp. *micranthos* (Gugler) Hayek	Asteraceae	O	Perennial	Moderate	N
Convolvulus arvensis L.	Convulvulaceae	D	Perennial long	Very long	N
Cyperus esculentus L.	Cyperaceae	B	Perennial	Very long	Y
Daucus carota L.	Apiaceae	O	Biennial	Moderate	N
Ranunculus acris L.	Ranunculaceae	B, O	Perennial	Long	Y

[a]MOA, mechanism of action of herbicide to which species has evolved resistance. MOAs: B, inhibition of acetohydroxyacid synthase (AHAS, ALS); D, diversion of electrons transferred by photosystem I ferredoxin (Fd); O, stimulation of transport inhibitor response protein 1 (TIR1).
[b]Seedbank persistence: Short, seeds known to survive in seedbank for 1–4 yr; Moderate, 5–10 yr; Long, 11–20 yr; Very long, >20 yr.

precise timing of application to have the maximum effect on plant mortality. Herbicides with shorter residence times would have shorter windows of opportunity to effectively kill plant populations, thus reducing herbicide resistance selection pressures.

While our model was successful in predicting "true positives" (i.e., correctly classifying HR species as HR) based on species characteristics, it also had a relatively high false-positive rate, classifying approximately 26% of currently non-HR species as HR (Table 1). The false-positive species (Table 3) have a combination of traits associated with herbicide resistance: short life span and/or a long-lived seedbank and/or not found in riparian habitats. One reason for the relatively higher false-positive rate may be that our analysis is based on observational data, as opposed to experimental data, and not all of the noxious weeds included in our analysis have been exposed to the same levels of herbicide use. These species may have traits conducive to the evolution of herbicide resistance, but may not have experienced the conditions to evolve herbicide resistance. For example, our data set includes species that do not occur in cultivated agricultural systems as well as species that form expansive populations, the entirety of which is not feasible or practical to control with herbicides. Both field and simulation studies have shown that the evolution of herbicide resistance can be slowed by increased spatial heterogeneity in herbicide application (Beckie et al. 2004; Dauer et al. 2009; Roux et al. 2008). Expansive populations with gene flow from reservoirs of susceptible individuals into the portions of the population controlled by herbicides could hinder the evolution of resistance, which may occur in widespread species, such as common mullein (*Verbascum thapsus* L.), or species with long-distance seed dispersal, such as *Salsola* spp.

Inclusion of additional plant characteristics that have been shown to influence the evolution of herbicide resistance, such as mating system and reproductive output (Kreiner et al. 2018), could improve the predictive accuracy of our model. Self-pollinating species have relatively low rates of recombination, typically resulting in smaller effective population sizes, factors that limit the speed of herbicide resistance evolution and the spread of resistant genotypes (Maxwell and Mortimer 1994). Seed production potential can also influence the emergence of herbicide resistance. Species producing few seeds produce fewer genetic combinations and thus a lower chance of developing a resistant individual (Jasieniuk et al. 1996). Including these characteristics could increase the overall accuracy of future models.

Six species known to have evolved herbicide resistance were classified as non-HR by our model (i.e., false negatives; Table 4). These six species all have life spans ranging from 2 yr to more than 20 yr and seedbanks that can persist in the soil from 2 yr to more than 20 yr. Detailed analyses of the specific populations of these species that evolved herbicide resistance and the conditions under which herbicide resistance

evolved may increase our understanding of the evolutionary forces at play and the accuracy of current risk assessments (Moss et al. 2019).

Data-driven analyses such as this one can help in the identification of species most at risk of becoming HR and provide clues to the relative importance of the evolutionary forces at work. In this study, we examined a relatively small set of species' characteristics and focused only on noxious weed species found in the United States. Further data-driven analyses including more species and species traits from around the globe could help refine risk of the evolution of herbicide resistance. In particular, expanding the number of HR and susceptible species used in analyses would permit evaluating whether traits associated with herbicide resistance differ by MOA. Ideally, such a study would compare HR and non-HR species with a variety of trait values that had been exposed to herbicides at the same levels, under the same conditions, and for the same length of time to determine the plant traits associated with an increased risk of evolving herbicide resistance.

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