Managing the evolution of herbicide resistance

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Abstract

BACKGROUND: Understanding and managing the evolutionary responses of pests and pathogens to control efforts is essential to human health and survival. Herbicide-resistant (HR) weeds undermine agricultural sustainability, productivity and profitability, yet the epidemiology of resistance evolution – particularly at landscape scales – is poorly understood. We studied glyphosate resistance in a major agricultural weed, *Amaranthus tuberculatus* (common waterhemp), using landscape, weed and management data from 105 central Illinois grain farms, including over 500 site-years of herbicide application records.

RESULTS: Glyphosate-resistant (GR) *A. tuberculatus* occurrence was greatest in fields with frequent glyphosate applications, high annual rates of herbicide mechanism of action (MOA) turnover and few MOAs field−1 year−1. Combining herbicide MOAs at the time of application by herbicide mixing reduced the likelihood of GR *A. tuberculatus*.

CONCLUSIONS: These findings illustrate the importance of examining large-scale evolutionary processes at relevant spatial scales. Although measures such as herbicide mixing may delay GR or other HR weed traits, they are unlikely to prevent them. Long-term weed management will require truly diversified management practices that minimize selection for herbicide resistance traits.

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Keywords: resistance evolution; glyphosate resistance; *Amaranthus tuberculatus*; common waterhemp; modes of action; herbicide rotation; herbicide mixing

1 INTRODUCTION

Widespread, rapid evolution of herbicide-resistant (HR) weeds is destabilizing weed management in commercial agriculture. This problem has reached epidemic levels, driving up crop production costs, decreasing farm profitability, 1, 2 and forcing farmers out of business in some cases (Norsworthy JK, private communication, 2014). Current efforts to impede the evolution and spread of HR weeds focus on diversifying the herbicide mechanisms of action (MOAs) (i.e. herbicidal active chemistries that affect and disrupt specific target sites or biochemical processes) 3 to which weed populations are exposed. 4, 5 Herbicide rotations and sequences (also called cycling) reduce selective pressure on individual target sites by employing herbicides with different MOAs in successive growing seasons or within the same season, respectively, whereas herbicide mixing exposes weeds to multiple MOAs simultaneously. 4 Drug mixture and rotation strategies used to combat drug-resistant infectious agents are based on the same principles; the evolution of drug and herbicide resistance are parallel evolutionary processes. 6

Although herbicide rotation and mixing are commonly recommended for preventing HR weeds, almost no quantitative data exist on the effectiveness of either strategy under conditions relevant to production farming 7 (see the supporting information). Current understanding is derived from simulation models, laboratory studies and small-scale-plot studies focused on proximate instead of ultimate causes of resistance. There is a large gap between the urgency and scale of the problem and the scope of relevant knowledge. Given the epidemic proportions of the problem, we believe it is time to take an epidemiological approach to understanding it.

Our goal was to identify risk factors associated with resistance to the broad-spectrum herbicide glyphosate in *Amaranthus tuberculatus* (Moq.) Sauer var. *rudis* (Sauer) Costea & Tardif (common waterhemp) in field crops (see the supporting information). In recent decades, *A. tuberculatus* has expanded from its native range in Midwest US riparian areas to become a highly competitive weed in arable systems of this region and beyond. It also has become emblematic of HR weeds in general; 8 numerous populations of *A. tuberculatus* possess multiple herbicide resistance mechanisms that, in the most serious cases, severely limit the number of remaining effective herbicide options. 9

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Herbicide resistance evolves within a complex of environmental and management factors acting within agroecosystems. To detect risk factors for herbicide resistance evolution that apply broadly to real-world production systems, as opposed to narrowly defined experimental systems, we took an epidemiological approach. We compiled A. tuberculatus glyphosate resistance frequency, soil, landscape, farm management and weed data, and almost 500 site-years of field-level herbicide application records from 105 central Illinois grain farms managed by a single custom retail applicator from 2004 to 2010 (Fig. 1). In addition to deciphering environmental and management signals linked to herbicide resistance evolution, our analysis examined putative causal relationships between past herbicide use and contemporary glyphosate resistance frequencies. Because producers have questioned whether they will be able to manage resistance effectively if their neighbors do not, we addressed spatial contagion in a separate analysis.

2 MATERIALS AND METHODS
2.1 Data collection and processing

Fields selected for inclusion in the study were identified by a commercial custom applicator that provides services for several hundred fields in central Illinois. The applicator released field-specific management histories for 2004–2011, and provided access for our team to collect seeds, soil samples and other site data. The fields in the study were distributed over an area of approximately 300 miles² (777 km²). While small on a continental scale, this is an area of high strategic importance and provided the opportunity to study glyphosate resistance evolution in waterhemp as it was unfolding.

We quantified the glyphosate resistance frequencies of A. tuberculatus at each field in the greenhouse as the proportion of plants grown from field-collected seeds that survived glyphosate exposure. Two people each sampled seeds from ten plants per field (N = 20 plants field⁻¹) in September 2010. The total seed produced by each sample plant was added to one bulk seed sample per field. Thus, the sample was representative of the frequency of glyphosate resistance traits contributed to the soil seedbank in 2010 rather than the frequency of those traits in the sampled parent plants. We sampled plants along transects according to the following level of priority: infield (highest), head-row (intermediate), field border (lowest). The majority of plants were sampled from within the fields. All plants sampled, including the minority that came from field borders, were exposed to any pollen-bearing plants within the field.16 Because we sampled seeds at the end of the growing season, the sample weed populations were potentially enriched for resistance traits against the MOAs that had already been used that year (i.e. plants resistant to the herbicides applied were most likely to survive and produce seed for collection). This is a potential source of bias, but this method allowed us to capture a representative sample of the seeds contributing to the next year’s weed population. Seeds were threshed, pooled from within each field and stratified at 4 °C for at least 6 weeks to break dormancy. Seeds were germinated on moistened filter paper in petri dishes incubated in a growth chamber set to 12 h days at 35 °C/15 °C day/night temperatures. After 48 h, germinated seedlings were transplanted to a growth medium of 3:1:1:1 commercial potting mix:soil:peat:sand in 3.8 cm × 21 cm cone-tainers. Seedlings were grown in a greenhouse with 16 h days at 28–30 °C/24–26 °C day/night temperatures, and with supplemental lighting to maintain a minimum of 800 μmol m⁻² s⁻¹. Twenty-one uniform plants 4–5 cm tall were sprayed with a commercial formulation of glyphosate at 1260 g ae ha⁻¹ using a moving-nozzle spray chamber.9 This glyphosate rate was chosen on the basis of preliminary experiments to distinguish effectively known glyphosate-resistant (GR) and sensitive populations in a high-throughput greenhouse screen. Plants were visually evaluated for glyphosate resistance 14 days after treatment and rated as sensitive (dead) or resistant (alive). A. tuberculatus populations previously determined to be sensitive or resistant to glyphosate were included in all herbicide application runs as controls, and most field populations were evaluated in at least two separate runs (yielding a sample of 42 plants field⁻¹ for most fields). Glyphosate resistance frequencies in each field were estimated using a logistic regression with site as a fixed effect and the number of resistant versus sensitive plants per field as the binomial response.

Data used as covariates in the analyses were compiled from several sources. Farm management records were obtained from the custom retail applicator. Landscape attributes, including field area, field perimeter length, distance to nearest road, field margin composition, presence of and proximity to waterways, etc., were obtained from analyses of aerial imagery in Google Earth. Weed community composition was assessed and soil samples collected in April 2010. Soil samples were analyzed for chemical and physical properties by Great Lakes Laboratories.

To quantify within-year herbicide diversity, we calculated the total unique herbicide MOAs year⁻¹, as well as the mean and maximum MOAs application⁻¹ year⁻¹, each averaged across years. The distinctions among these are important. The mean MOAs year⁻¹ measures average herbicide diversity per growing season. In contrast, the per-application metrics quantify the diversity of herbicides applied simultaneously through herbicide mixing. We calculated the proportion of consecutive 2 year sequences in which glyphosate was applied in both years (i.e. a ‘run’ of glyphosate years), the overall proportion of years that glyphosate was applied and the mean number of glyphosate applications per year. Herbicide MOA temporal diversity was quantified with Whittaker’s 𝛽w,11 Harrison’s 𝛽w,12 and our own index of herbicide turnover across
of resistance probability (glyphosate resistance presence/absence field⁻¹) and the proportion of GR plants site⁻¹ separately for eight measures of herbicide diversity. The GLMMs had binomial errors and random slopes and intercepts within spatial clusters of sites to control for spatial correlations. We then ranked the models using Akaike weights (\(w_i\)) and AICc\(^{18}\) to identify the predictors and random effects (or lack of random effects) best supported by the data for each response variable (proportion of resistant plants or probability of resistance 4–6 years later). Random slopes and intercepts were retained in the proportional resistance models, but no random effects were supported in the binary (probability of resistance) models, which were thus reduced to generalized linear models (logistic regressions). In these models we used data from 40 sites with complete management records for the period 2004–2006, within 5 km of the nearest neighboring site, and identified using hierarchical clustering as belonging to a spatial cluster of three or more sites.

To verify that these results were independent of proximity effects, we calculated pairwise differences in glyphosate resistance (\(\Delta GR\)), maximum MOAs application⁻¹ (2004–2006 data) (\(\Delta MOA\)) and intersite distance for all site pairs. We then used semi-partial correlations\(^{19}\) to quantify the variance in \(\Delta GR\) uniquely explained by (a) \(\Delta MOA\) after controlling for distance effects and (b) distance after controlling for \(\Delta MOA\). See the supporting information for further details on model fitting and semi-partial correlations. The data reported in this paper and the analytical code necessary to reproduce the analyses are archived in the supporting information.

### 3 RESULTS AND DISCUSSION

Management practices were most predictive of glyphosate resistance among 66 environmental, soil, landscape, weed and management variables analyzed (supporting information Table S2). Frequency of glyphosate resistance among seeds produced within a given field was highest (supporting information Table S2; Fig. 2) in fields with at least 57% turnover in herbicide MOAs year⁻¹ (i.e. rotation) and at least 0.93 glyphosate applications year⁻¹ (mean GR = 18% of plants field⁻¹). Likewise, the presence of GR A. tuberculatus in fields was associated with high annual turnover in herbicide MOAs, using fewer herbicide MOAs year⁻¹ and using glyphosate in over 75% of years (supporting information Table S2; Fig. 3). Contrary to prevailing wisdom, both binary and proportional models indicated that herbicide turnover increased the frequency of resistance. Logistic regression models supported this result, with positive or non-significant effects of several metrics of herbicide MOA turnover and temporal \(\beta\) diversity on proportion of GR plants (Fig. 4).

Herbicide mixing was strongly linked with reduced selection for glyphosate resistance. A field with a mean herbicide complexity of 2.5 MOAs application⁻¹ in 2004–2006 was 83 times less likely to produce GR A. tuberculatus seeds 4–6 years later than one with a mean complexity of 1.5 MOAs application⁻¹ (Fig. 5A) (odds ratio = 0.012; \(\Delta AICc = 4.2; w = 0.83\)). Similarly, GR seed relative abundance decreased as the maximum MOAs application⁻¹ year⁻¹ increased after controlling for random spatial effects (Fig. 5B) (odds ratio = 0.0195; \(\Delta AICc = 16.0; w > 0.99\)). A. tuberculatus seeds in fields with maximum annual application mixtures of three MOAs were 51 times less likely to be glyphosate resistant than those from fields with two MOAs per application. Model rankings are shown in the supporting information Tables S3 and S4.

After partialling out distance effects, \(\Delta MOA\) explained 19.4% of the variance in \(\Delta GR\) (\(r = -0.440, P < 0.0001, N = 780\), while
Figure 2. Final regression tree of proportional herbicide resistance. $R^2 = 0.27$. HR is the mean proportion of herbicide-resistant plants per field at each node. The percentage of fields at each node is shown below the node. The bold text indicates the splitting criteria at each node. Fields where the criterion is true are moved down the tree to the right to the next node or terminal leaf. For example, the first split indicates that if the herbicide turnover index is at least 0.57, move to the right. If it is less than 0.57, move to the left. Observations were weighted by the number of years of management data available. $N = 76$ fields.

Figure 3. Final classification tree of the presence/absence of the resistance trait within the field. $R^2 = 0.53$, accuracy = 0.60. At each node, HR is the mean probability of the glyphosate resistance trait occurring within a field, followed by the percentage of fields represented at the node or leaf. For example, the first node includes 100% of the farms in the dataset, of which 49% have glyphosate resistance. The first split is based on manure use. The label indicates that if manure is applied, go to the left. The leaf to the left includes 18% of the fields in the dataset that together have a mean resistance probability of 0.15. Note: for all other splits, if the splitting criterion is true, observations are included in the branch or leaf to the right of the split. Observations were weighted by the number of years of management data available. $N = 103$ fields.

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Figure 4. Logistic regressions of the per capita probability of glyphosate resistance (proportion of plants resistant) versus three indices of herbicide MOA diversity. Each metric was calculated for the 3 year period 2004–2006 (n = 55 sites; left column) and for all available years for sites with at least 3 years of data (n = 76 sites; right column). The three metrics are Harrison’s $\beta_1$ ($\beta_{H1}$), Whittaker’s $\beta$ ($\beta_W$) and our own herbicide turnover index (see methods).

analysis does not specifically address application rates, we suggest caution when considering application rate reductions for herbicide mixtures.

Herbicide mixes are not a permanent solution to the problem of target-site resistance; herbicidal mixtures may delay evolution of resistance, but they do not prevent it. HR weed evolution is inevitable, enabled by the current, nearly exclusive reliance on herbicides and the greatly reduced discovery and commercialization of new herbicide chemistries over the last two decades. Long-term, cost-effective, environmentally sound weed management will require truly diversified management practices that minimize selection for herbicide resistance traits. Combining chemical, cultural, physical and biological tactics can provide cost-effective weed management while reducing reliance on herbicides.

The striking parallels between pesticide-resistant weeds and insects and drug-resistant pathogens stem from their identical underlying evolutionary causes, which explains the similarities in their responses to management. This is why, for example, antibiotic cycling in hospitals is unlikely to suppress evolution and spread of antibiotic-resistant microbes, while mixtures or ‘cocktails’ of multiple drugs administered simultaneously can suppress HIV and drug-resistant bacterial infections. These measures work because the probability of target-site resistance to multiple drug MOAs is the product of the individual resistance probabilities. By killing all bacterial or viral particles in the host, the evolution of resistance traits is halted. Likewise, MOA rotation strategies can
fail by allowing resistance to evolve if the cost of resistance is low relative to the rotation interval.\textsuperscript{27} If the fitness cost of a resistance trait is high, depletion will occur rapidly in the absence of the selective agent. If it is small, depletion will take a long time. In a wind-pollinated weed such as \textit{A. tuberculatus}, low-cost resistance traits can persist in individuals growing outside crop fields (e.g. in field margins) with minimal depletion and thus can survive a multi-year herbicide MOA rotation schedule. Even if the other agents in the rotation are 100\% effective, the resistance trait can reinoculate the field.

We have a high level of confidence in our conclusions, given (a) the broad inference domain of our study, (b) the consistent management signals we detected through noisy, real-world data and (c) that in an analogous problem from the field of health care the same strategy of administering multiple MOA drug mixtures has repeatedly proven to be effective at managing drug-resistant infections.\textsuperscript{8} We will encounter resistance evolution repeated in natural systems managed for human benefit. Sustainable stewardship of these systems will depend on recognizing that we are always applying selective pressures, and that management responses need to grow from our understanding of applied evolution.

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Supporting Information

Supporting information may be found in the online version of this article.

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