

Agricultural Weeds in Glyphosate-Resistant Cropping Systems in the United States

Bryan G. Young, David J. Gibson, Karla L. Gage, Joseph L. Matthews, David L. Jordan, Micheal D. K. Owen, David R. Shaw, Stephen C. Weller, and Robert G. Wilson*

A segment of the debate surrounding the commercialization of genetically engineered (GE) crops, such as glyphosate-resistant (GR) crops, focuses on the theory that implementation of these traits is an extension of the intensification of agriculture that will further erode the biodiversity of agricultural landscapes. A large field-scale study was conducted in 2006 in the United States on 156 different field sites with a minimum 3-yr history of GR corn, cotton, or soybean in the cropping system. The impact of cropping system, crop rotation, frequency of using the GR crop trait, and several categorical variables on emerged weed density and diversity was analyzed. Species richness, evenness, Shannon's H' , proportion of forbs, erect growth habit, and C_3 species diversity were all greater in agricultural sites that lacked crop rotation or were in a continuous GR crop system. Rotating between two GR crops (e.g., corn and soybean) or rotating to a non-GR crop resulted in less weed diversity than a continuous GR crop. The composition of the weed flora was more strongly related to location (geography) than any other parameter. The diversity of weed flora in agricultural sites with a history of GR crop production can be influenced by several factors relating to the specific method in which the GR trait is integrated (cropping system, crop rotation, GR trait rotation), the specific weed species, and the geographical location. The finding that fields with continuous GR crops demonstrated greater weed diversity is contrary to arguments opposing the use of GE crops. These results justify further research to clarify the complexities of crops grown with herbicide-resistance traits, or more broadly, GE crops, to provide a more complete characterization of their culture and local adaptation.

Nomenclature: Glyphosate; corn, *Zea mays* L. ZEAMX; cotton, *Gossypium hirsutum* L. GOSHI; soybean, *Glycine max* (L.) Merr. GLXMA.

Key words: Agroecology, Benchmark Study, corn, cotton, crops, genetically engineered crops, genetically modified crops, herbicide-resistant soybean.

Management of farmland in the United States and Europe since 1960 escalated as a consequence of economic incentives and technical advancements in agricultural mechanization, crop genetics, synthetic fertilizers, pesticides, and production practices (Donald et al. 2006; Matson et al. 1997). The development and subsequent widespread adoption of transgenic crops in the United States, as well as other countries, has been viewed as a relatively recent contributor in the progressive intensification of farmland management (Johnson and Hope 2000; Perry et al. 2003). Moreover, the improvement of crop genetics, including biotechnology and transgenes, has been cited as the single most important factor impacting crop management and ecology over the past 50 yr (Crookston 2006). Transgenic corn, soybean, and cotton has been planted on 85, 91, and 88% of the production area for each crop in the United States, respectively, which corresponds to over 60 M ha of transgenic crop production (U.S. Department of Agriculture [USDA] 2009). Resistance to specific herbicides have been the most commonly adopted transgenic crop trait comprising 68, 91, and 71% of corn, soybean, and cotton plantings, respectively, in the United States in 2009 (USDA 2009). The continued improvement of

weed management and the benefits imparted by the herbicide-resistance traits, especially resistance to the herbicide glyphosate, have been well documented in numerous cropping systems (Gianessi 2005).

The benefits of the Green Revolution have long been contrasted against the inherent detriment from some practices placed on the environment. In particular, the intensification of crop production since 1960 has been implicated in a steady decline in biodiversity in agricultural fields (Benton et al. 2003; McLaughlin and Mineau 1995; Potts et al. 2010). While the societal, agricultural, and ecosystem costs and benefits of the Green Revolution are debated (Tilman 1998), management strategies to increase the sustainability of agricultural production are being sought (Matson et al. 1997; Pretty 2007).

The Farm Scale Evaluations (FSEs) conducted in the United Kingdom from 2000 to 2002 were the first large-scale research studies implemented to contrast conventional crops with GE crops, specifically those with transgenic traits for herbicide resistance, and their associated crop management techniques on flora and fauna biodiversity and population densities (Champion et al. 2003). Differences in weed population density between conventional and GE beet and oilseed rape were dependent on the sampling period during the cropping season (Heard et al. 2003b). Initial weed population densities shortly after planting were greater in the GE beet and oilseed rape, while late-season weed population densities were greater in the conventional crop. In contrast, weed population density was higher during the entire growing season of GE corn compared with conventional corn (Heard et al. 2003b). A similar trend was observed on individual weed species; a reduction in the survival of individual weed species was observed in GE beet and oilseed rape, but an increase was noted in GE corn (Heard et al. 2003a). Even though this research demonstrated an association of weed diversity and the

DOI: 10.1614/WS-D-12-00001.1

*First and fourth authors: Professor and Assistant Scientist, respectively, Department of Plant, Soil, and Agricultural Systems, Southern Illinois University, Carbondale, IL 62901; second and third authors: Professor and Graduate Assistant, respectively, Department of Plant Biology, Center for Ecology, Southern Illinois University, Carbondale, IL 62901-6509; fifth author: Professor, Department of Crop Science, North Carolina State University, Raleigh, NC 27695; sixth author: Professor, Agronomy Department, Iowa State University, Ames, IA 50011; seventh author: Professor, Department of Plant and Soil Sciences, Mississippi State University, Mississippi State, MS 39762; eighth author: Professor, Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN 47907; ninth author: Professor, Department of Agronomy and Horticulture, University of Nebraska, Scottsbluff, NE 69361. Corresponding author's E-mail: bgyoung@siu.edu

culture of GE crops, the authors acknowledged that the specific implementation and management of the GE crop may interact and further complex the impact on biodiversity (Squire et al. 2003). Thus, the specific cropping system and the management practiced by individual growers within a region on an agricultural site may be more critical than the adoption of a specific GE crop trait. A recent study in Canada surmises that weed communities and species richness was linked more to glyphosate use and tillage than the adoption of a GR crop (Gulden et al. 2009, 2010).

The FSE study provided mixed conclusions on the biodiversity resulting from the adoption of GE crops and were not able to draw conclusions on the influence of different cropping systems and management implemented by growers over a large geography (Ammann 2005). The uniqueness of crop production practices and the resident flora and fauna within diverse geographical regions must be considered when determining the effect of agriculture in agroecosystems (McLaughlin and Mineau 1995). A field-scale study similar to the FSE study has never been conducted in cropping systems found within the United States where the use of transgenic traits in the major agronomic crops of corn, soybean, and cotton has become the norm. This adoption has also impacted other elements of the cropping system, such as greater use of conservation tillage practices (Cerqueira and Duke 2006) in which soil disturbance can be minimal. The impact of GE crops on weed diversity, in particular, must be characterized within the context of the specific cropping systems and management tactics to further describe how the adoption of these crops shapes the composition of the weed flora in agricultural sites, which will have an indirect influence on the surrounding ecosystem.

The objectives of this study (Benchmark Study) were to quantify the weed flora across broad regions of the United States in agricultural fields in response to the rotation of the glyphosate-resistance trait and crops. In particular, we tested the following hypothesis: The impact of utilizing the GE crop trait for resistance to glyphosate on the weed flora in agricultural fields is dependent on crop rotation, frequency using the GR crop trait, and geography.

Materials and Methods

Study Sites. A research protocol was outlined that included 156 commercial field sites across six U.S. states (Figure 1) in corn, soybean, and cotton production with a minimum 3-yr field history of the following cropping systems: (1) a single continuous GR crop, (2) a rotation of two GR crops, and (3) a GR crop rotated with a non-GR crop. The inclusion of commercial field sites without a history of GR crops was not possible due to their low frequency in the landscape and the nonrandom approach that would have been required to identify these sites. A complete list of agronomic crop producers who previously used a GR crop trait in six states, Illinois, Indiana, Iowa, Mississippi, Nebraska, and North Carolina in the United States was compiled in fall 2005. These states represent the major crop growing regions in the United States, have a high adoption of the GR crops, and represent a diversity of environments and cropping systems for production of corn, soybean, and cotton. Further detail on the procedures and criteria used for field site selection are reported in Shaw et al. (2011).



Figure 1. Location of 156 field-scale research sites in Illinois, Indiana, Iowa, Mississippi, Nebraska, and North Carolina in the United States.

In spring 2006, each field was divided into two (approximately) equal sections to establish a treatment that would be managed by the grower and a treatment managed by a university specialist who implemented best management practices (BMPs) to manage weeds and deter the evolution of GR weed species. The focus of this paper was to characterize the effect of grower adoption of GR cropping systems on agricultural weed flora. Thus, research data from half of each field research site utilizing BMPs was not used for the analysis presented here and was published by Wilson et al. (2011).

Data Collection. The grower managed side of the field was sampled using a W-pattern (Thomas 1985) with one sample point per 0.4 ha for a total of 20 0.5 by 1 m quadrats in most fields. The sampling locations were referenced by global positioning system at the first sample time in order to relocate the sample sites for subsequent data collection. The fields were sampled for emerged weeds, enumerated by species at four time periods during the cropping season: (1) prior to crop planting, (2) just prior to any POST herbicide applications, (3) 2 wk following the POST herbicide application, and (4) at crop harvest.

Univariate Analyses. Data analyzed were population densities of individual species per 0.5 m² sample plot per site, population density by functional group, and number of species in terms of richness, Shannon's index H' and evenness and by functional group. Species were grouped into the following functional groups: summer annuals, winter annuals, forbs, prostrate herbs, erect herbs, perennials, climbing species, grasses, C₃ plants, C₄ plants, *Ipomea* spp. and *Amaranthus* spp., and species in which biotypes have been selected previously for resistance to glyphosate.

Fourteen weed species were considered to occur frequently enough (present in > 20% of the sites at any one sample time) for analyses as individual species. Many species were absent or were infrequent at one sample time or in one or more regions necessitating the elimination of some sample time periods or the merging of regions (e.g., creating a north vs. south comparison), or both. Four species were only present frequently enough for analysis in only one of the sample times, i.e., redroot pigweed (*Amaranthus retroflexus* L.), yellow nutsedge (*Cyperus esculentus* L.), annual bluegrass (*Poa annua* L.), and mouseear chickweed [*Cerastium fontanum* ssp. *vulgare* (Hartman) Greuter and Burdet]. These species were analyzed

using the same procedures outlined below but for just the sample time during which they occurred in > 20% of the sites.

A mixed model analysis was conducted in SAS 9.1 (SAS Institute, Cary, NC) using PROC GLIMMIX and PROC MIXED following Littell et al. (2006). Cropping system (1, 2, 3), crop rotation (0 = no rotation, 1 = rotation), GR trait rotation (0 = no rotation, 1 = rotation), and crop in 2006 (1 = corn, 2 = cotton, 3 = soy) were considered as categorical fixed effects. There were too few sites to allow the development of a complete model, thus four analyses were conducted representing the main effects and their interactions of (a) cropping system, region, and time, (b) crop rotation, region, and time, (c) GR trait rotation, region, and time, and (d) crop in 2006 (1 = corn or 3 = soy), cropping system, and time in USDA hardiness zone region 5 only (U.S. National Arboretum 2003). Region (USDA hardiness zones 4, 5, 6, 7, or 8; U.S. National Arboretum 2003) was considered as a random effect (PROC GLIMMIX) or fixed effect (PROC MIXED) in the first three analyses (i.e., the analysis was conducted both ways). The USDA hardiness zone in which a site was located was included in the analysis rather than the specific state to avoid the use of political boundaries in the analysis (although results were qualitatively similar when state was included). Time (i.e., four sample times) was treated as a repeated measure with site as the subject. The response variable was log transformed prior to analysis (Shaw et al. 2011). Untransformed variables were used for means calculations. Using PROC GLIMMIX, the response variable was assumed to have an overdispersed Poisson distribution because weeds are distributed in patches, thus the variance is many times the mean (Quinn and Keough 2002). The covariance structure in the analysis was unstructured (type = un) although runs with a first order auto-regression (type = ar[1]) or constant variance and covariance (type = cs) produced identical results. Using PROC MIXED, the covariance structure used was that which returned the lowest Akaike information criterion fit statistic using either type = un, cs, or ar[1] covariance structure. Means separation of significant interactions and, if appropriate, main effects, were based on least squares means tests.

Multivariate Analyses. Weed species population density were analyzed using Nonmetric Dimensional Scaling (NMDS), a nonparametric ordination procedure in DECODA (Database for Ecological Communities, ANUTECH Pty. Ltd., Technology Marketing Division, Canberra, Australia) that we used similarly in a previous experiment on the weeds invading soybean fields (Gibson et al. 2008). Data consisted of the mean weed density of each species in each field from the four separate sample times during the cropping season. Mean total weed population density of all species, species richness, Shannon's H' , and evenness were calculated in DECODA as independent continuous variables.

Separate ordinations were undertaken for each of the four sample times, along with a separate ordination over all sample times based upon the maximum density each species attained over the four sample times. Weed density data were double standardized to adjust species and sites to unit maxima prior to analysis based on the Bray-Curtis dissimilarity coefficient. Twenty random starting configurations were initiated running up to 200 iterations to obtain one to four dimensional

solutions. The minimum number of dimensions necessary to obtain a useful interpretation of the data was retained after inspection of stress plots, and minimum stress with R values. In all cases, a three-dimensional solution was retained.

The relationship between the ordination solution and independent continuous variables (as listed above, as well as site longitude and latitude) were investigated by fitting vectors of maximum correlation. Vector significance was assessed following permutation tests to generate correlation values. Vectors significantly correlated with the ordination were retained for plotting in ordination space relative to the ordination centroid.

The importance of categorical variables (six states, 2006 crop [corn, cotton, soybean], crop rotation [yes, no], cropping system [three categories], and USDA hardiness zone [numbers 4, 5, 6, 7, and 8]) were quantified by testing for differences among *a priori* groups (e.g., Illinois sites vs. Iowa sites) using analysis of similarity (ANOSIM) in DECODA. ANOSIM compares within- vs. among-group similarity based upon the Bray-Curtis dissimilarity coefficient (itself based upon the density of each weed species per site) using random permutations of group membership to calculate an R -value that is then tested for significance as the proportion of permuted R -values for sites within a group compared to members of another group.

The relationship of individual weed species to the ordination was assessed by calculating species scores for each species in the NMDS space. The species scores were calculated as the weighted average of the density scores of the samples in which the species occurred in each dimension. These weighted averages were used to plot species as points in the NMDS ordination and are referred to as species centroids because they show the center of the species' distribution with respect to the ordination axes. The relationship between weed species density and the independent variables retained as significant vectors (as outlined above) was assessed by examining two-way ordered tables. These tables show the density of species in sites arranged along vectors of particular interest (e.g., latitude).

Results and Discussion

Of 156 field sites included in the study, two were omitted because no weeds were present throughout the four sample times. Within the 154 remaining fields, 187 species were found across all sample times. Forty-four angiosperm plant families were represented with the largest number of species in the Poaceae (36 species), Asteraceae (23 species), and Brassicaceae (20 species) families, respectively. The 10 species with the highest density over all sample times were giant foxtail (*Setaria faberi* Herrm.; mean of 9.0 plants per m^2), henbit (*Lamium amplexicaule* L.; mean of 5.9), common waterhemp (*Amaranthus rudis* Sauer; mean of 5.1), common chickweed [*Stellaria media* (L.) Vill.; mean of 3.2], annual bluegrass (mean of 3.1), redroot pigweed (mean of 3.0), large crabgrass [*Digitaria sanguinalis* (L.) Scop.; mean of 3.0], prickly sida (*Sida spinosa* L.; mean of 2.7), fall panicum (*Panicum dichotomiflorum* Michx.; mean of 2.3), and mouseear chickweed (mean of 2.0). The 10 most frequent species over all sample times were giant foxtail (occurred in 36.8% of fields), velvetleaf (*Abutilon theophrasti* Medik.; 29.7%), common lambsquarters (*Chenopodium album* L.; 27.7%), common waterhemp (25.2%), prickly sida (25.2%),

henbit (23.9%), horseweed [*Conyza canadensis* (L.) Cronq.; 23.1%], annual bluegrass (23.1%), redroot pigweed (21.3%), and large crabgrass (20.6%).

Univariate Analyses. Individual Weed Species. The density of seven weed species (redroot pigweed, mouseear chickweed, horseweed, ivyleaf morningglory [*Ipomoea hederacea* Jacq.], annual bluegrass, prickly sida, and common chickweed) was related to cropping system, crop rotation, or GR trait rotation (Table 1; Figure 2). Of these seven weeds, all except ivyleaf morningglory and prickly sida had higher density in cropping system 1 compared with cropping systems 2 or 3, or both. Five of the seven (i.e., not *C. canadensis* and *S. spinosa*) had highest density in fields managed without crop rotation, although this pattern was only evident in regions 6 and 7 for *S. media* (Figure 2g) and in region 6 for ivyleaf morningglory (Figure 2h). Redroot pigweed, prickly sida (in region 4 only), and common chickweed (prior to POST herbicide) had highest density in fields managed with continuous compared with discontinuous GR trait rotation (Figures 2b, 2c, and 2f).

Functional Groups. The density of 7 of 13 functional groups (winter annuals, forbs, perennials, climbing weeds, C₃ weeds, and *Ipomoea* spp.) was highest in cropping system 1 compared with cropping systems 2 and 3 (which were equal) (Table 1), although this relationship was restricted to two time periods for climbing weeds and *Ipomoea* spp. (prior to POST herbicide and prior to crop harvest) (Figure 3). By contrast, C₄ weeds had the lowest density in cropping systems 1 and 2 compared with cropping system 3 prior to crop planting, but had a higher density in cropping system 1 compared with cropping system 2 after POST herbicide. Eight of 13 functional groups (summer annuals, winter annuals, forbs, prostrate weeds, perennials, climbing weeds, C₃ weeds and *Ipomoea* spp.) had the highest density in the absence of crop rotation at one or more sample times, again with C₄ weeds showing an opposite pattern. Six of the functional groups (forbs, prostrate weeds, perennials, climbing weeds, C₃ weeds, *Ipomoea* spp., and *Amaranthus* spp.) had the highest density in fields managed with GR traits at one or more sample times, while the density of summer annuals and grasses had the highest density in fields managed with crops rotating the GR trait. Total weed population density was higher in cropping system 1 compared with cropping system 3 prior to harvest, and higher in fields managed with crop rotation than without during sample times 1 and 4.

Diversity. Whether measured as H' or richness, diversity was highest in cropping system 1 compared with cropping systems 2 and 3, which were equal, and highest in the absence of crop or GR trait rotation (Table 1; Figure 4a). Evenness showed the same pattern for crop and GR trait rotation but for cropping system, evenness was highest and equal for cropping systems 1 and 2 compared with cropping system 3. The number of GR species was also highest under cropping system 1, but only in region 6 (Figure 4b). The number of winter annuals was similarly highest under cropping system 1, and also in the absence of crop rotation (Figure 4c).

Multivariate Analyses. The composition of weed flora was most strongly related to location whether expressed as

longitude, latitude, state, or USDA hardiness zone. This relationship held for each sample time (Table 2). Changes in weed species density and diversity have also been observed across a north-south transect in the United States (Scursoni et al. 2006). However, there was a significant signal in the data indicating a relationship to the crop and cropping system, particularly the former; the weed communities under corn were consistently different to those under cotton and soybean. At sample time 2 (prior-to-post), the weed communities were different under all three crops. Cropping system effects were weak, albeit significant. In particular, the weed community under cropping system 1 was different to the weed community under cropping systems 2 and 3 pooled over all sample times and at each sample time when they were considered separately (Table 2). However, low R values from the ANOSIM test indicate that this was a weak relationship (Figure 5). This pattern was related to crop rotation at sample times 2 and 3 (prior-to-post and post-post), and glyphosate trait rotation for the sample time prior to crop harvest. These compositional differences in the weed communities were related to weed diversity, and this relationship became stronger as the season progressed (Table 2). Prior to harvest, vector correlations with the NMDS ordination for evenness, Shannon's H', and total density indicated that the composition of the weed community was related to both these components of diversity and weed density (Figure 5).

Weed flora in related research has been influenced variably by the alteration of different components of cropping systems such as crop species, crop rotation, and tillage (Andersson and Milberg 1998; Légère et al. 2005; Osten et al. 2007; Thomas and Frick 1993; Tuesca et al. 2001). Tuesca et al. (2001) reported weed populations were more consistently impacted by tillage in a summer annual crop compared with a winter annual crop. Légère et al. (2005) found that tillage had a greater influence on the composition of weed populations than on weed diversity. Research in southern Sweden documented that weed species composition and density was mostly determined by experimental location and crop species, whereas crop rotation was only a minor determinant in weed flora (Andersson and Milberg 1998). Thus, the complexity of cropping systems in terms of tillage, herbicide use, crop life cycle, and crop rotation compounded with geographical differences arguably produce the variability observed in weed flora response to these parameters when investigated individually.

The affinity of individual species to the cropping system-weed community patterns is unclear because of the multivariate nature of the relationship; however, some general patterns can be seen (Figure 2). Prior to crop harvest, there was a higher evenness of species population densities in cropping system 1 reflecting more evenness than in cropping systems 2 and 3 (Table 2; Figure 5). Prickly sida, henbit, and horseweed were more abundant in cropping system 1 than cropping systems 2 and 3 (Table 1), and, of these species, the centroid for prickly sida (Figure 5, bottom panel) was closely associated in multivariate space with cropping system 1 sites (Figure 5, top panel).

Species Density and Frequency. The 10 most abundant or frequent species in this study encompass mostly annual grass and broadleaf species, some of which have prolific seed production (Sellers et al. 2003; Walker and Oliver 2008), which may result in high density in infested field sites. Most of these species have been cited as common and problematic

Table 1. Summary of mixed model univariate analyses on the number and density of weed species recorded in agricultural fields according to cropping system, crop rotation, and glyphosate trait rotation. All differences shown are significant at $P < 0.05$.

Parameter	Number of species ^a			Density ^b		
	Cropping system ^c	Crop rotation ^d	GR trait rotation ^e	Cropping system	Crop rotation	GR trait rotation
H'	1 > 2 = 3 r, f	0 > 1 r, f	0 > 1 regions 4 & 6, f	n/a	n/a	n/a
Evenness	1 = 2 > 3 (2 = 3), r	0 > 1 r	0 > 1 f	n/a	n/a	n/a
Richness	1 > 2 = 3 r, f	0 > 1 r, f	0 > 1 f	n/a	n/a	n/a
Summer annuals	ns	ns	ns	1 > 2 = 3 r	0 > 1 r	0 < 1 t ₃ , t ₄ , f
Winter annuals	1 > 2 = 3 r	0 > 1 r	ns	1 > 2 = 3 r	0 > 1 r, f	ns
Forbs	1 > 2 = 3 r, f	0 > 1 r, f	0 > 1 r, f	1 > 2 = 3 r	0 > 1 t ₁ , t ₄ , r	0 > 1 f
Prostrate	1 > 2 = 3 r, f	0 < 1 t ₂ , t ₄ , r	0 > 1 f	1 = 3 > 2 r	0 > 1 t ₁ , t ₄ , r	0 > 1 f
Erect	1 = 2 > 3 (2 = 3), r, f	0 > 1 r	0 > 1 f	1 = 2 < 3 t ₁ , t ₂ , f	0 > 1 t ₄ , r, 0 > 1 regions 4, 5, 6, & 8 at different times	0 > 1 t ₄ , r, 0 < 1 t ₂ region 8, 0 > 1 t ₃ region 4 f
Perennial	1 > 2 = 3 r, f	0 > 1 t ₄ , f	0 > 1 f	1 > 2 = 3 f	0 > 1 t ₁ , t ₂ , t ₄ , r, f	0 > 1 t ₁ , t ₄ , r, f
Climbing	1 > 2 (1, 2 = 3) r, f	Cro*region*t	Gly*region*t	1 > 2 = 3 t ₂ , t ₄ , f	0 > 1 t ₂ , t ₃ , t ₄ , r, f	0 > 1 t ₂ , t ₄ , f
Grasses	1 < 2 = 3 region 5, t ₃ , t ₄	ns	ns	1 = 3 < 2 f	ns	0 < 1 t ₂ , t ₄ , f
Glyphosate resistant	1 > 2 = 3 region 6, f	0 > 1 f	ns	1 > 2 = 3 r	ns	ns
C ₃	1 > 2 = 3 r, f	0 > 1 r, f	0 > 1 f	1 > 2 < 3 t ₁ , r	0 > 1 t ₁ , t ₄ r	0 > 1 f
C ₄	1 < 2 = 3 t ₁ , f	ns	ns	1 > 2 = 3 t ₃ , r	0 < 1 t ₁ , r	ns
<i>Ipomea</i> spp.	n/a	n/a	n/a	1 > 2 = 3 t ₂ , t ₄ , f	0 > 1 t ₂ , f	0 > 1 t ₂ , f
<i>Amaranthus</i> spp.	n/a	n/a	n/a	ns	ns	0 > 1 r
Total density	n/a	n/a	n/a	1 > 3 = 2 t ₄ f	0 > 1 t ₁ , t ₄	0 > 1 r, f
Redroot pigweed	n/a	n/a	n/a	1 > 2 (both = 3) r	0 > 1 f	0 > 1 r
Mouseear chickweed	n/a	n/a	n/a	1 > 2 > 3 in soy region 5	0 > 1 r	ns
Horseweed	n/a	n/a	n/a	1 > 2 (both = 3) f	ns	ns
Ivyleaf morningglory	n/a	n/a	n/a	ns	0 > 1 region 6 f	ns
Annual bluegrass	n/a	n/a	n/a	1 > 2 = 3 r	0 > 1 r	ns
Prickly sida	n/a	n/a	n/a	ns	ns	ns
Common chickweed	n/a	n/a	n/a	1 > 3 (both = 2) r, f	0 > 1 f, regions 6 & 7 f	0 > 1 t ₄ , r, f

^a Statistical notations: ns, nonsignificant treatment effect; n/a, test not applied; r, random effect; f, fixed effect.

^b Time notations: t, time; t₁, prior to crop plantings; t₂, prior to POST herbicide; t₃, after POST herbicide; t₄, prior to crop harvest.

^c Cropping systems: 1, continuous glyphosate-resistant (GR) crop, same crop; 2, rotation of GR crops; 3, rotation of GR/non-GR crops.

^d Crop rotation: 0, continuous croppings; 1, crop rotation.

^e GR trait rotation: 0, continuous GR trait crops; 1, rotation of GR trait.

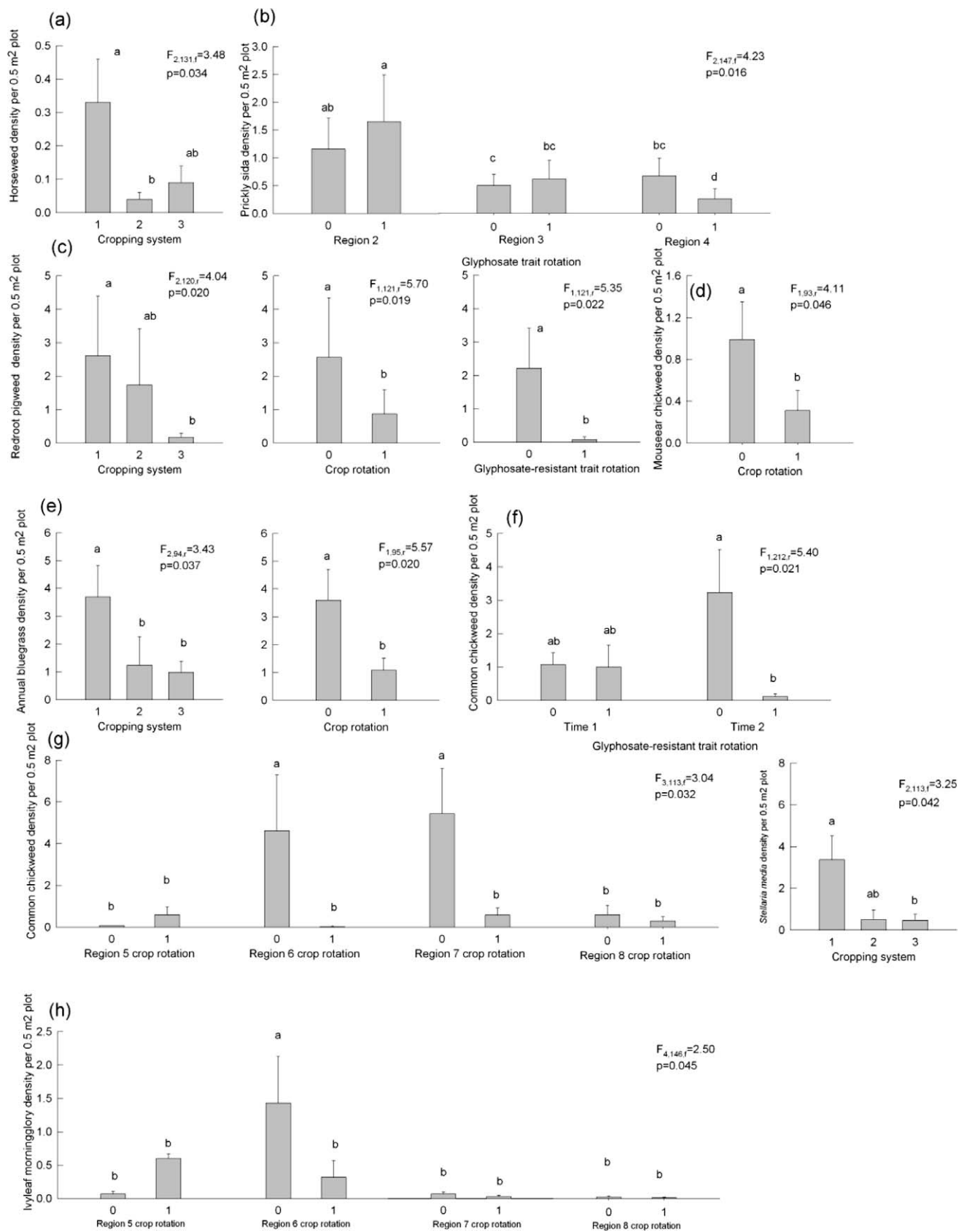


Figure 2. Density of individual weed species (pooled over time period or at individual time periods) in response to cropping system, crop rotation, and glyphosate-resistant (GR) trait rotation; (a) horseweed, (b) prickly sida, (c) redroot pigweed (common chickweed shows same patterns), (d) mouseear chickweed, (e) annual bluegrass, (f) and (g) common chickweed, and (h) ivyleaf morningglory. An “r” or “f” subscript following the degrees of freedom for the F values denotes whether Region was considered as random or fixed effect, respectively, in the mixed model analysis. Cropping systems: (1) = continuous GR crop, same crop; (2) = rotation of GR crops; (3) = rotation of GR/non-GR crops. Time notations: (t1) = prior to crop planting; (t2) = prior to POST herbicide; (t3) = after POST herbicide; (t4) = prior to crop harvest. Crop rotation: (0) = continuous cropping; (1) = crop rotation. GR trait rotation: (0) = continuous GR trait crops; (1) = rotation of GR trait. Regions denote the U.S. Department of Agriculture hardiness zones. Mean values represented by bars sharing the same letter in a panel were not significantly different ($P < 0.05$, least squares means test).

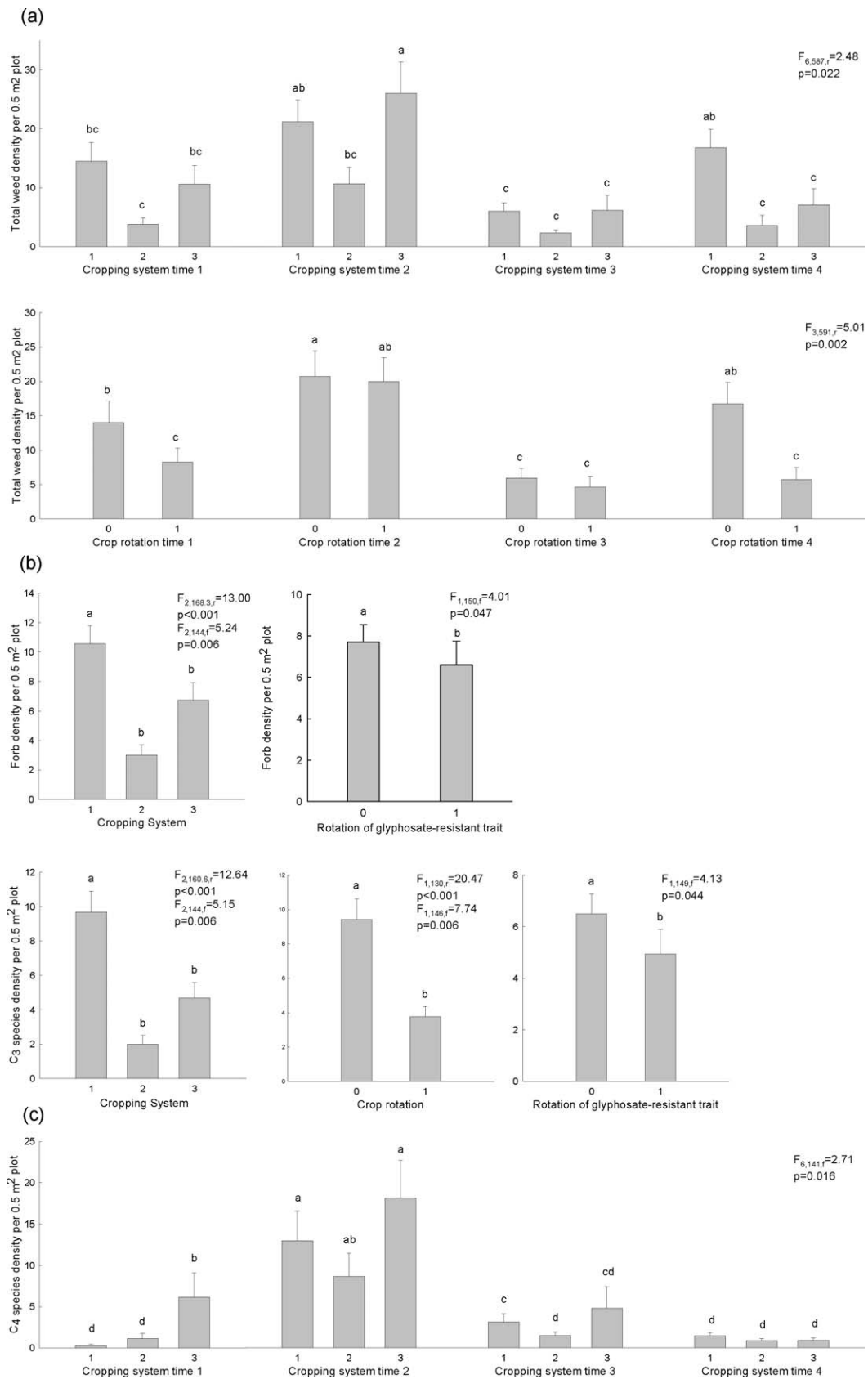


Figure 3. Density of weeds by functional group in response to cropping system, crop rotation, and glyphosate-resistant (GR) trait rotation; (a) total weed density, (b) forbs (same pattern for *Ipomoea* spp.), (c) C₃ (same pattern for perennials and climbing species), and (d) C₄ species. An “r” or “F” subscript following the degrees of freedom for the F values denotes whether Region was considered as random or fixed effect, respectively, in the mixed model analysis. Cropping systems: (1) = continuous GR crop, same crop; (2) = rotation of GR crops; (3) = rotation of GR/non-GR crops. Time notations: (t1) = prior to crop planting; (t2) = prior to POST herbicide; (t3) = after POST herbicide; (t4) = prior to crop harvest. Crop rotation: (0) = continuous cropping; (1) = crop rotation. GR trait rotation: (0) = continuous GR trait crops; (1) = rotation of GR trait. Mean values represented by bars sharing the same letter in a panel were not significantly different ($P < 0.05$, least squares means test).

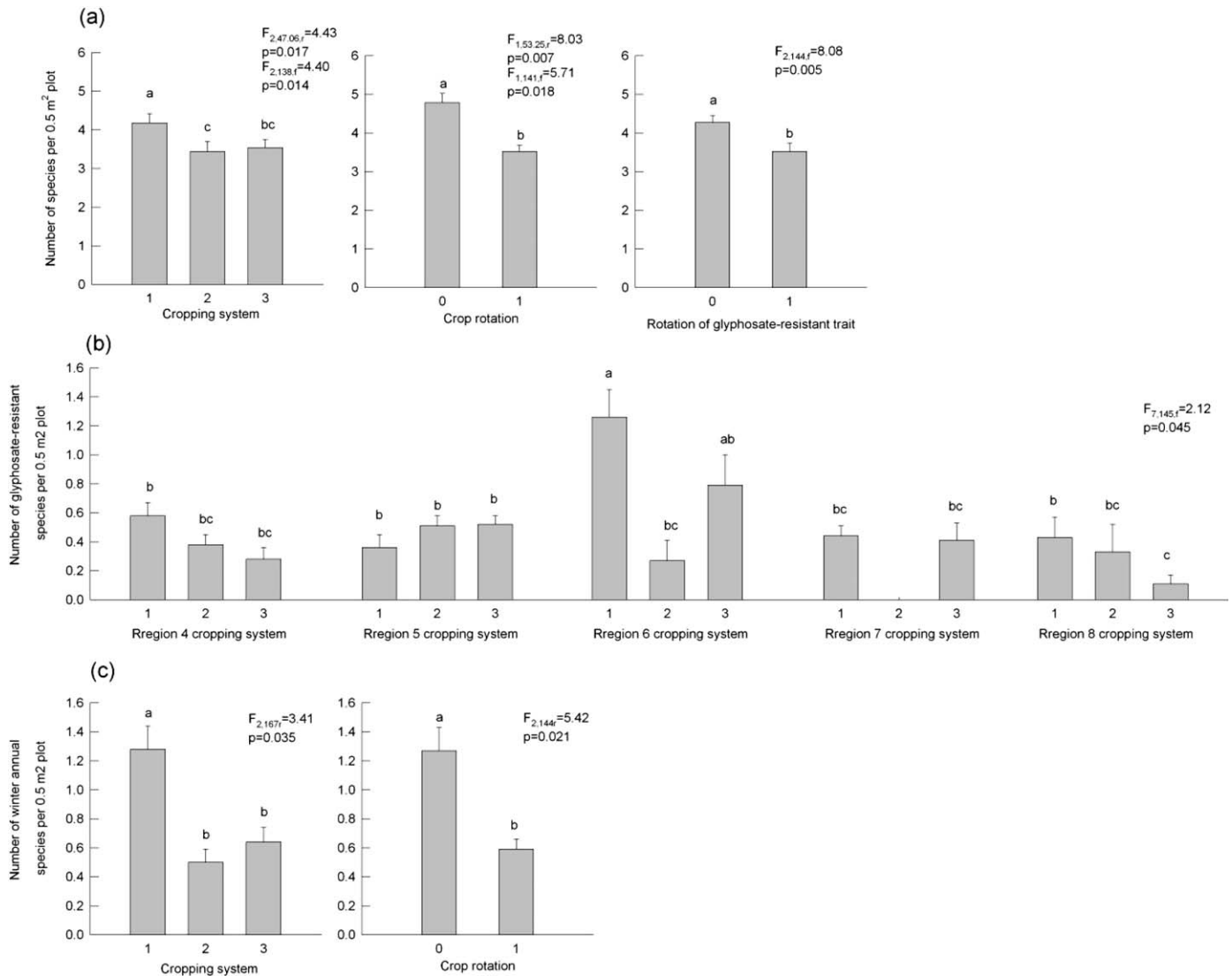


Figure 4. Number of weed species in response to cropping system, crop rotation, and glyphosate-resistant (GR) trait rotation; (a) number of species per site, (b) number of GR species, and (c) number of winter annuals (same pattern for the number of forbs, prostrate, erect, perennial, climbing, and C₃ species). An “r” or “f” subscript following the degrees of freedom for the F values denotes whether Region was considered as random or fixed effect, respectively, in the mixed model analysis. Cropping systems: (1) = continuous GR crop, same crop; (2) = rotation of GR crops; (3) = rotation of GR/non-GR crops. Crop rotation: (0) = continuous cropping; (1) = crop rotation. GR trait rotation: (0) = continuous GR trait crops; (1) = rotation of GR trait. Regions denote the U.S. Department of Agriculture hardiness zones. Mean values represented by bars sharing the same letter in a panel were not significantly different ($P < 0.05$, least squares means test).

in agronomic field sites (Gibson et al. 2005; Johnson et al. 2009; Kruger et al. 2009; Webster and Coble 1997) and have evolved resistance to multiple herbicide modes of action (Heap 2011). Common waterhemp, Palmer amaranth (*Amaranthus palmeri* S. Wats.), and horseweed are the primary species in our field research, which have evolved resistance to glyphosate in these regions of the United States (Heap 2011).

Species Diversity and Population Density. The intensification of agriculture and the relatively recent integration of GE crops, more specifically GR crops, have been broadly implicated in the reduction of farmland biodiversity and related deleterious effect on agroecosystems (Altieri 2005; Benton et al. 2003; Johnson and Hope 2000; Tilman 1998). However, the impact of GR crops or any other agricultural input has been argued to reside in the specific crop management systems of herbicide use and tillage and the

local adaptation in a geographical region (McLaughlin and Mineau 1995; Menalled et al. 2001; Squire et al. 2003), which would not directly implicate the GR crop trait. The weed flora at our sites was influenced by one or a combination of the specific cropping system, crop rotation, or rotation of the GR trait. The parameters of species richness, evenness, Shannon’s H' , numbers of species of forbs, erect growth habit, possessing a perennial life cycle (at crop harvest time only), and C₃ species were all greater in fields that lacked crop rotation or were in a continuous GR crop system. There was not just a larger number of weed species under continuous crops compared with under crop rotation, but more evenness suggesting enhanced niche complementarity and resource sharing (Cerabolini et al. 2009). By contrast, Ulber et al. (2009) investigated the weed species richness and composition in wheat fields as influenced by previous crop rotation intensity concluding that the preservation of weed species richness requires a diversified crop rotation.

Table 2. Summary of multivariate analyses on weed densities recorded in agricultural fields managed for glyphosate-resistant crop production.

Parameter ^a	Sample time			
	Pooled over all sample times (maximum density per weed species)	Prior to crop planting	After the POST herbicide	Before crop harvest
Number of samples	154	85	150	122
Number of species	190	97	135	110
Mean no. spp. (no. 0.5 m ²)	10.5 ± 0.4	4.9 ± 0.4	6.1 ± 0.3	4.7 ± 0.3
Mean density (no. 0.5 m ²)	37.1 ± 3.6	17.3 ± 2.7	20.9 ± 2.6	12.6 ± 2.1
Continuous variables fitted as vectors (maximum R, P value)				
Longitude	0.68, < 0.0001	0.77, < 0.0001	0.54, < 0.0001	0.58, < 0.0001
Latitude	0.89, < 0.0001	0.84, < 0.0001	0.86, < 0.0001	0.70, < 0.0001
Total density	0.27, < 0.0003	ns ^b	ns	0.51, < 0.0001
No. spp.	0.57, < 0.0001	ns	ns	0.25, 0.05
Evenness	0.33, < 0.002	ns	0.23, 0.05	0.31, 0.013
	ns	ns	ns	0.36, < 0.0001
Categorical variables tested using ANOSIM ^c (R, P value)				
State	0.52, < 0.0001, All states different from each other	0.47, < 0.0001, IA vs. IL, MS vs. NC are ns, all others different	0.43, < 0.0001, All states different from each other	0.36, < 0.0001, All states different from each other
USDA hardiness zone	0.29, < 0.0001, 4 vs. 5, 5 vs. 6 – ns, all other pairs different < 0.0001	0.26, < 0.0001, 4 vs. 5, 4 vs. 6, 5 vs. 6, 7 vs. 8 – ns, all other pairs < 0.0001	0.25, < 0.0001, 4 vs. 5, 5 vs. 6 – ns, all other pairs different	0.16, < 0.0001, 4 vs. 5, 5 vs. 6, 5 vs. 7, 7 vs. 8 – ns, all other pairs different
Crop in 2006	0.14, < 0.0001, Corn different to both cotton and soy	0.08, 0.028, Corn different to both cotton and soy	0.19, < 0.0001, Each crop different to the others	0.10, < 0.0001, Corn different to both cotton and soy
Crop rotation	0.06, 0.001	ns	0.05, < 0.0001	ns
GR trait rotation	ns	ns	ns	0.04, 0.05
Cropping system	0.05, 0.001, 1 vs. 2, 1 vs. 3 different, 2 vs. 3 ns	0.13, < 0.0001, 1 vs. 2 and 1 vs. 3 different, 2 vs. 3 ns	0.04, 0.007, 1 vs. 2 and 1 vs. 3 different, 2 vs. 3 ns	0.05, 0.004, cropping system 1 vs. 2 and 1 vs. 3 different

^a The following additional variables were fitted as significant vectors to the overall multivariate analysis: number of summer annuals, perennials, grasses, forbs, prostrate, climbing, glyphosate resistant, and *Ipomoea* and *Amaranthus* species.

^b Statistical notation: ns, nonsignificant treatment effect.

^c Abbreviations: ANOSIM, analysis of similarity; IA, Iowa; IL, Illinois; MS, Mississippi; NC, North Carolina; USDA, U.S. Department of Agriculture.

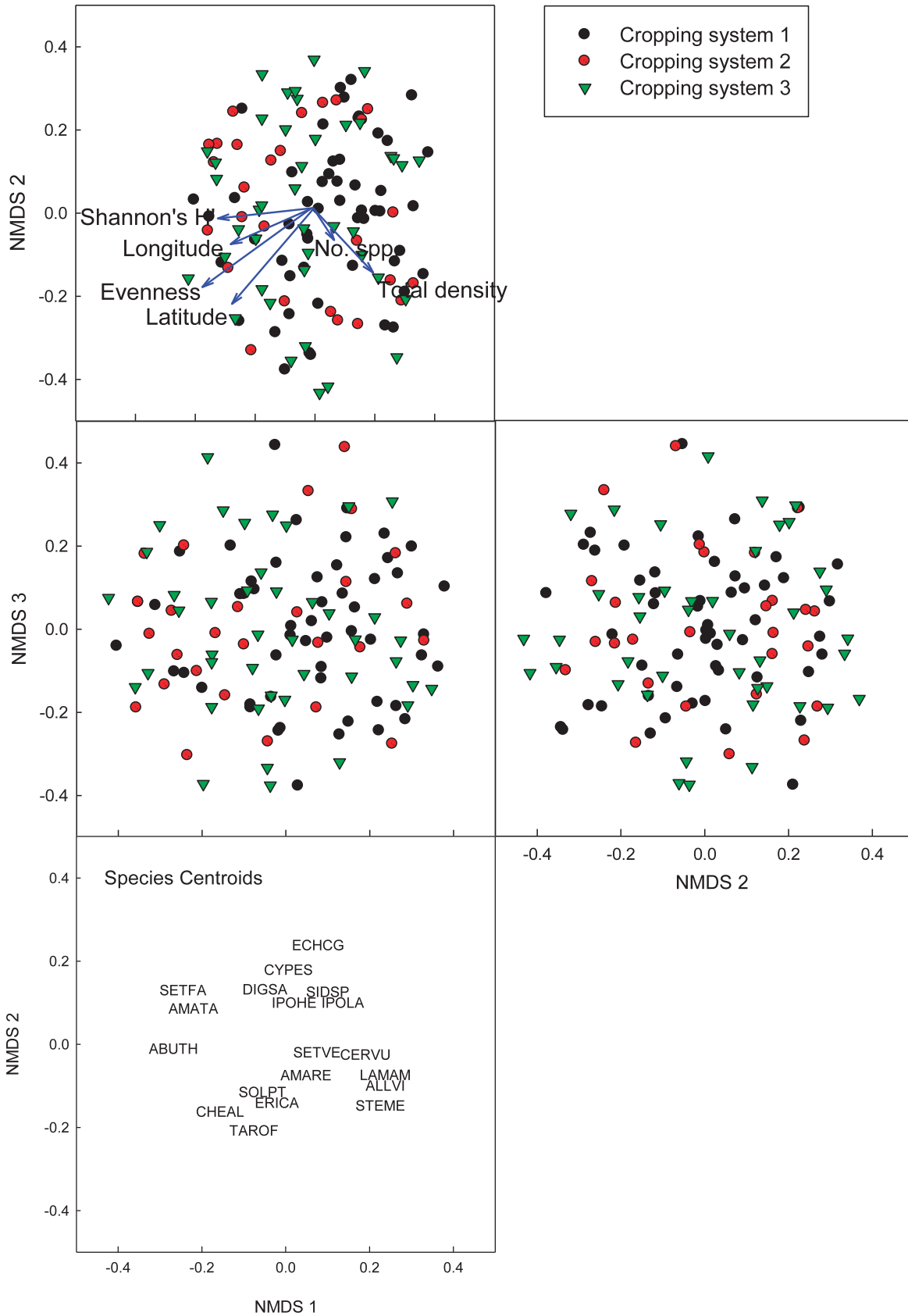


Figure 5. Plots of 3D Nonmetric Dimensional Scaling (NMDS) solution of weed flora prior to crop harvest. The vectors are presented on just the top panel to avoid repetition in the lower two panels. Plots are labeled with symbols representing the cropping system (1 = continuous glyphosate-resistant [GR] crop, same crop; 2 = rotation of GR crops; 3 = rotation of GR/non-GR crops). The distribution of cropping system 1 is significantly different to the distribution of both cropping systems 2 and 3, but the distribution of cropping systems 2 and 3 are not significantly different to each other (analysis of similarity [ANOSIM] $R = 0.05$, $P = 0.04$). Significant ($P < 0.05$) vectors of maximum correlation of continuous variables are shown in blue. The bottom panel shows a plot of species centroids of taxa occurring in $> 10\%$ of the fields prior to crop harvest. Species labeled using Bayer codes (<http://www.wssa.net/Weeds/ID/WeedNames/namesearch.php>).

The specific cropping systems described in our field-scale research can be further characterized by changes in crop rotation and the frequency of using the GR trait. Rotation out of the GR trait reduced Shannon's H' in some geographical regions of the Midwest (Table 1), whereas there was no difference in Shannon's H' for other regions (i.e., the southern region). This greater biodiversity of weed flora evident under continuous use of the GR trait is likely due to the specific weed management tactics implemented by growers. Growers utilizing GR soybean, and to a lesser extent GR corn, have typically applied glyphosate at one or two periods during crop growth, which allows for multiple weed emergence events and weed growth between glyphosate applications from crop planting to harvest (Givens et al. 2009; Young 2006). This result is in contrast to the view that crop rotation reduces the density of dominant weed species allowing for a more diverse weed flora (Liebman and Dyck 1993). In general, the management of GR cotton in the southern region of the United States would commonly include some residual herbicides (Givens et al. 2009), which maintain weed populations at reduced levels compared with managing weeds solely with multiple applications of glyphosate. At the grower field sites included in this article, residual herbicides were used on 83% of the GR cotton fields over 2006 and 2007 compared with 41 to 68% and 21 to 30% in GR corn and soybean, respectively (Wilson et al. 2011). These crop differences in weed management surrounding the GR trait provide the rationale for at least partially explaining the difference in Shannon's H' as influenced by geography. Another contributing factor may be the specific weed species found in the southern region vs. the northern region of the United States. McLaughlin and Mineau (1995) surmised that the impact of agricultural practices is realized not only by geography, but also by the particular species responding to the input in that geography.

Fields with a continuous crop monoculture (cropping system 1) had a greater number of species classified as winter annuals and GR biotypes compared with cropping systems that included crop rotation (cropping systems 2 and 3). However, the number of species found within these two categories of weed species was not found to be impacted by the rotation of the GR trait. From a weed management standpoint, this may suggest that rotating out of the GR trait is not as important as crop rotation for long-term management of these two weed categories. This finding is of particular importance in efforts to mitigate the evolution of GR weeds since moving away from the GR trait may not alter the composition of weed species classified as GR in fields. Rather, increasing crop diversity may have a greater impact on deterring the evolution of GR weeds than avoiding the use of the GR crop trait. Crop rotation and the concomitant rotation of herbicide modes of action have been cited as an important component of BMPs to deter the evolution of weed resistance to herbicides (Beckie 2006; Johnson et al. 2009).

Weed Community Composition. Parameters associated with location (longitude, latitude, state, USDA hardiness zone) were strongly related to the composition of weed flora over all four sample times during the growing season (Table 2). These findings provide further support for the univariate analyses of individual weed species and life forms, and diversity in that geographical location was an underlying factor that can alter

the influence of crop rotation and GR traits on weed diversity. The differences observed in weed communities in corn compared with cotton and soybean may be related to the inherent differences in the cultivation and growth habit of corn, a monocot species vs. two dicot crop species. Certainly, the frequent use of atrazine (Givens et al. 2009; Young 2006), which provides residual and foliar control of dicot weeds in corn, may have a marked effect on weed composition differences since atrazine cannot be used in cotton and soybean. Factors such as these that are common to the production of a specific crop are difficult to separate from the effect of crop rotation on weed dynamics (Doucet et al. 1999). Interestingly, the collective weed abundance and density as well as the impact on individual weed species in corn were considerably different than in beet and rape in the FSE study (Hawes et al. 2003; Heard et al. 2003a,b).

Elucidating the impact of a specific crop input or technology such as GE crops is exceptionally complicated given the interconnected management practices that may be implemented along with the GE crop in local environments. The FSE study pioneered large-scale research on GE crops and provided evidence that weed biodiversity may be slightly influenced transiently throughout the crop growing season in a positive or negative manner depending on the specific GE crop (Heard et al. 2003a,b). Furthermore, the FSE study authors recognized the potential interaction with the specific crop management system (Squire et al. 2003), which may be a much greater contributor to the alteration of weed species composition than the adoption of an individual GE crop trait for herbicide resistance. Our research provides evidence of the multiple underlying factors (cropping system, crop rotation, GR trait rotation, geographical location) that may contribute to the impact of GR crops on weed diversity and the related implications on the overall agroecosystem and the desire to conserve heterogeneity. Undoubtedly, the specific weed management tactics implemented by individual growers will have a significant influence on weed diversity (Ulber et al. 2009). Ironically, the continuous production of a single GR crop that demonstrated greater weed diversity in our research is the cropping system, particularly in cotton and soybean, which has most frequently been associated with the evolution of GR weeds in commercial field sites (Culpepper et al. 2006; Legleiter and Bradley 2008; Pollard et al. 2004; VanGessel 2001). Thus, weed management that promotes the greatest diversity in field populations of weeds may not be robust as an isolated strategy to mitigate the evolution of GR weeds. Rather, the selection pressure exerted on weed populations from any single herbicide or herbicide mode of action would be a more meaningful focal point in developing BMPs for herbicide resistance (Beckie 2006).

Some research infers the intensification of agriculture as a result of GE crop culture will potentially lead to a reduction in biodiversity (Altieri 2005). The evidence in the FSE study and our "Benchmark Study" would not support such broad conclusions in terms of diversity within weed communities, but rather points to the cropping system being implemented and the specific crop rotation, frequency of the use for the GR crop trait, the geographical region, and weed species under management that contribute to weed diversity in agroecosystems. Thus, weed community diversity in U.S. cotton, corn, and soybean production, at least two-thirds containing GR crop traits, will not be determined solely by the GR trait. Rather, the method in which a grower integrates the GR

technology into a cropping system and manages weeds with other tactics will determine the diversity of agricultural weeds. In a much broader context, weed management, whether it includes GE traits or not, will be determined by biology and to a much greater extent the socioeconomic considerations for crop producers (Tilman et al. 2002).

Acknowledgments

We thank the growers who hosted the research on their field sites, the numerous research staff and students at each university who aided in the conduct of this research, Philip Dixon and Laura Kirwan for statistical advice, and Monsanto Company for funding to support this research.

Literature Cited

Altieri, M. A. 2005. The myth of coexistence: why transgenic crops are not compatible with agroecologically based systems of production. *Bull. Sci. Tech. Soc.* 25:361–371.

Ammann, K. 2005. Effects of biotechnology on biodiversity: herbicide-tolerant and insect-resistant GM crops. *Trends Biotechnol.* 23:388–394.

Andersson, T. N. and P. Milberg. 1998. Weed flora and the relative importance of site, crop, crop rotation, and nitrogen. *Weed Sci.* 46:30–38.

Beckie, H. J. 2006. Herbicide-resistant weeds: management tactics and practices. *Weed Technol.* 20:793–814.

Benton, T. G., J. A. Vickery, and J. D. Wilson. 2003. Farmland biodiversity: is habitat heterogeneity the key? *Trends Ecol. Evol.* 18:182–188.

Cerabolini, B., S. Pierce, A. Luzzaro, and A. Ossola. 2009. Species evenness affects ecosystem processes in situ via diversity in the adaptive strategies of dominant species. *Plant Ecol.* 207:333–345.

Cerdeira, A. L. and S. O. Duke. 2006. The current status and environmental impacts of glyphosate-resistant crops: a review. *J. Environ. Qual.* 35:1633–1658.

Champion, G. T., M. J. May, S. Bennett, D. R. Brooks, S. J. Clark, R. E. Daniels, L. G. Firbank, A. J. Haughton, C. Hawes, M. S. Heard, J. N. Perry, Z. Randle, M. J. Rossall, P. Rothery, M. P. Skellern, R. J. Scott, G. R. Squire, and M. R. Thomas. 2003. Crop management and agronomic context of the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Philos. Trans. R. Soc. Lond. B* 358:1801–1818.

Crookston, R. K. 2006. A top 10 list of developments and issues impacting crop management and ecology during the past 50 years. *Crop Sci.* 46:2253–2262.

Culpepper, A. S., T. L. Grey, W. K. Vencill, J. M. Kichler, T. M. Webster, S. M. Brown, A. C. York, J. W. Davis, and W. W. Hanna. 2006. Glyphosate-resistant Palmer amaranth (*Amaranthus palmeri*) confirmed in Georgia. *Weed Sci.* 54:620–626.

Donald, P. F., F. J. Sanderson, I. J. Burfield, and F.P.J. van Bommel. 2006. Further evidence of continent-wide impacts of agricultural intensification on European farmland birds, 1990–2000. *Agric. Ecosyst. Environ.* 116:189–196.

Doucet, C., S. E. Weaver, A. S. Hamill, and J. Zhang. 1999. Separating the effects of crop rotation from weed management on weed density and diversity. *Weed Sci.* 47:729–735.

Gianessi, L. P. 2005. Economic and herbicide use impacts of glyphosate-resistant crops. *Pest Manage. Sci.* 61:241–245.

Gibson, D. J., K. Millar, M. Delong, J. Connolly, L. Kirwan, A. J. Wood, and B. G. Young. 2008. The weed community affects yield and quality of soybean [*Glycine max* (L.) Merr.]. *J. Sci. Food Agric.* 88:371–381.

Gibson, K. D., W. G. Johnson, and D. E. Hillger. 2005. Farmer perceptions of problematic corn and soybean weeds in Indiana. *Weed Technol.* 19:1065–1070.

Givens, W. A., D. R. Shaw, W. G. Johnson, S. C. Weller, B. G. Young, R. G. Wilson, M.D.K. Owen, and D. Jordan. 2009. A grower survey of herbicide use patterns in glyphosate-resistant cropping systems. *Weed Technol.* 23:156–161.

Gulden, R. H., P. H. Sikkema, A. S. Hamill, F. J. Tardif, and C. J. Swanton. 2009. Conventional vs. glyphosate-resistant cropping systems in Ontario: weed control, diversity, and yield. *Weed Sci.* 57:665–672.

Gulden, R. H., P. H. Sikkema, A. S. Hamill, F. J. Tardif, and C. J. Swanton. 2010. Glyphosate-resistant cropping systems in Ontario: multivariate and nominal trait-based weed community structure. *Weed Sci.* 58:278–288.

Hawes, C., A. J. Haughton, J. L. Osborne, D. B. Roy, S. J. Clark, J. N. Perry, P. Rothery, D. A. Bohan, D. R. Brooks, G. T. Champion, A. M. Dewar, M. S. Heard, I. P. Woiwod, R. E. Daniels, M. W. Young, A. M. Parish, R. J. Scott, L. G. Firbank, and G. R. Squire. 2003. Responses of plants and invertebrate

trophic groups to contrasting herbicide regimes in the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Philos. Trans. R. Soc. Lond. B* 358:1899–1913.

Heap, I. 2011. International Survey of Herbicide Resistant Weeds. <http://www.weedsience.org/In.asp>. Accessed: December 30, 2011.

Heard, M. S., C. Hawes, G. T. Champion, S. J. Clark, L. G. Firbank, A. J. Haughton, A. M. Parish, J. N. Perry, P. Rothery, D. B. Roy, R. J. Scott, M. P. Skellern, G. R. Squire, and M. O. Hill. 2003a. Weeds in fields with contrasting conventional and genetically modified herbicide-tolerant crops: II. Effects on individual species. *Philos. Trans. R. Soc. Lond. B* 358:1833–1846.

Heard, M. S., C. Hawes, G. T. Champion, S. J. Clark, L. G. Firbank, A. J. Haughton, A. M. Parish, J. N. Perry, P. Rothery, R. J. Scott, M. P. Skellern, G. R. Squire, and M. O. Hill. 2003b. Weeds in fields with contrasting conventional and genetically modified herbicide-tolerant crops: I. Effects on abundance and diversity. *Philos. Trans. R. Soc. Lond. B* 358:1819–1832.

Johnson, B. and A. Hope. 2000. GM crops and equivocal environmental benefits. *Nature Biotechnol.* 18:242.

Johnson, W. G., V. M. Davis, G. R. Kruger, and S. C. Weller. 2009. Influence of glyphosate-resistant cropping systems on weed species shifts and glyphosate-resistant weed populations. *Eur. J. of Agron.* 31:162–172.

Kruger, G. R., W. G. Johnson, S. C. Weller, M.D.K. Owen, D. R. Shaw, J. W. Wilcut, D. L. Jordan, R. G. Wilson, M. L. Bernards, and B. G. Young. 2009. U.S. grower views on problematic weeds and changes in weed pressure in glyphosate-resistant corn, cotton, and soybean cropping systems. *Weed Technol.* 23:162–166.

Légère, A., F. C. Stevenson, and D. L. Benoit. 2005. Diversity and assembly of weed communities: contrasting responses across cropping systems. *Weed Res.* 45:303–315.

Legleiter, T. R. and K. W. Bradley. 2008. Glyphosate and multiple resistance in common waterhemp (*Amaranthus rudis*) populations from Missouri. *Weed Sci.* 56:582–587.

Liebman, M. and E. Dyck. 1993. Crop rotation and intercropping strategies for weed management. *Ecol. Appl.* 3:92–122.

Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. SAS for Mixed Models. 2nd ed. Cary, NC: SAS Institute.

Matson, P. A., W. J. Parton, A. G. Power, and M. J. Swift. 1997. Agricultural intensification and ecosystem properties. *Science* 277:504–509.

McLaughlin, A. and P. Mineau. 1995. The impact of agricultural practices on biodiversity. *Agric. Ecosyst. Environ.* 55:201–212.

Menalled, F. D., K. L. Gross, and M. Hammond. 2001. Weed aboveground and seedbank community responses to agricultural management systems. *Ecol. Appl.* 11:1586–1601.

Osten, V. A., S. R. Walker, A. Storrie, M. Widderick, P. Moylan, G. R. Robinson, and K. Galea. 2007. Survey of weed flora and management relative to cropping practices in the north-eastern grain region of Australia. *Aust. J. Exp. Agric.* 47:57–70.

Perry, J. N., P. Rothery, S. J. Clark, M. S. Heard, and C. Hawes. 2003. Design, analysis and statistical power of the Farm-Scale Evaluations of genetically modified herbicide-tolerant crops. *J. Appl. Ecol.* 40:17–31.

Pollard, J. M., B. A. Sellers, and R. J. Smeda. 2004. Differential response of common ragweed to glyphosate. R. G. Hartzler, ed. Page 27 *in* Proc. North Central Weed Sci. Soc. Champaign, IL: North Central Weed Science Society.

Potts, G. E., J. A. Ewald, and N. J. Aebischer. 2010. Long-term changes in the flora of the cereal ecosystem on the Sussex Downs, England, focusing on the years 1968–2005. *J. Appl. Ecol.* 47:215–226.

Pretty, J. 2007. Agricultural sustainability: concepts, principles and evidence. *Phil. Trans. R. Soc. Lond. B* 363:447–465.

Quinn, G. P. and M. J. Keough. 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge, UK: Cambridge University Press. 520 p.

Scursoni, J., F. Forcella, J. Gunsolus, M. Owen, R. Oliver, R. Smeda, and R. Virdine. 2006. Weed diversity and soybean yield with glyphosate management along a north-south transect in the United States. *Weed Sci.* 54:713–719.

Sellers, B. A., R. J. Smeda, W. G. Johnson, J. A. Kendig, and M. R. Ellersieck. 2003. Comparative growth for six *Amaranthus* species in Missouri. *Weed Sci.* 51:329–333.

Shaw, D. R., M. D. Owen, P. M. Dixon, S. C. Weller, B. G. Young, R. G. Wilson, and D. L. Jordan. 2011. Benchmark study on glyphosate-resistant cropping systems in the United States. Part 1: introduction to 2006–2008. *Pest Manage. Sci.* 67:741–746.

Squire, G. R., D. R. Brooks, D. A. Bohan, G. T. Champion, R. E. Daniels, A. J. Haughton, C. Hawes, M. S. Heard, M. O. Hill, M. J. May, J. L. Osborne, J. N. Perry, D. B. Roy, I. P. Woiwod, and L. G. Firbank. 2003. On the rationale and interpretation of the Farm Scale Evaluations of genetically modified herbicide-tolerant crops. *Philos. Trans. R. Soc. Lond. B* 358:1779–1799.

Thomas, A. G. 1985. Weed survey system used in Saskatchewan for cereal and oilseed crops. *Weed Sci.* 33:34–43.

- Thomas, A. G. and B. L. Frick. 1993. Influence of tillage systems on weed abundance in southwestern Ontario. *Weed Technol.* 7:699–705.
- Tilman, D. 1998. The greening of the green revolution. *Nature* 396:211–212.
- Tilman, D., K. G. Cassman, P. A. Matson, R. Naylor, and S. Polasky. 2002. Agricultural sustainability and intensive production practices. *Nature* 418:671–677.
- Tuesca, D., E. Puricelli, and J. C. Papa. 2001. A long-term study of weed flora shifts in different tillage systems. *Weed Res.* 41:369–382.
- Ulber, L., H. H. Steinmann, S. Klimek, and J. Isselstein. 2009. An on-farm approach to investigate the impact of diversified crop rotations on weed species richness and composition in winter wheat. *Weed Res.* 49:534–543.
- [USDA] U.S. Department of Agriculture. 2009. Acreage Report. <http://usda.mannlib.cornell.edu/usda/current/Acre/Acre-06-30-2009.pdf>. Accessed: August 23, 2009.
- U.S. National Arboretum. 2003. USDA Plant Hardiness Zone Map. USDA Miscellaneous Publication No. 1475. <http://www.usna.usda.gov/Hardzone/ushzmap.html>. Accessed: December 30, 2011.
- VanGessel, M. J. 2001. Glyphosate-resistant horseweed from Delaware. *Weed Sci.* 49:703–705.
- Walker, E. R. and L. R. Oliver. 2008. Weed seed production as influenced by glyphosate applications at flowering across a weed complex. *Weed Technol.* 22:318–325.
- Webster, T. M. and H. D. Coble. 1997. Changes in the weed species composition of the southern United States: 1974 to 1995. *Weed Technol.* 11:308–317.
- Werth, J. A., C. Preston, I. N. Taylor, G. W. Charles, G. N. Roberts, and J. Baker. 2008. Managing the risk of glyphosate resistance in Australian glyphosate-resistant cotton production systems. *Pest Manage. Sci.* 64:417–421.
- Wilson, R. G., B. G. Young, J. L. Matthews, S. C. Weller, W. G. Johnson, D. L. Jordan, M. D. Owen, P. M. Dixon, and D. R. Shaw. 2011. Benchmark study on glyphosate-resistant cropping systems in the United States. Part 4: weed management practices and effects on weed populations and soil seedbanks. *Pest Manage. Sci.* 67:771–780.
- Young, B. G. 2006. Changes in herbicide use patterns and production practices resulting from glyphosate-resistant crops. *Weed Technol.* 20:301–307.

Received January 1, 2012, and approved June 12, 2012.