Final Report to Monsanto Canada, Inc.

Long-term Effects of Roundup-Ready Compared to Conventional Herbicide Systems in Ontario

Prepared by

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Executive Summary

The impact of Roundup-Ready corn and soybean cropping systems was compared to conventional herbicides in a corn-soybean and a corn-soybean-winter wheat crop rotation. Use of glyphosate consistently provided better weed control in corn and soybean compared to conventional herbicides. Mid-season weed densities in the two herbicide systems were influenced by crop and tillage system. Average mid-season weeds were smaller in corn and soybean when treated with glyphosate compared to conventional herbicides at the three conventional tillage (CT) locations. Despite differences in weed control, herbicide system influenced soybean yield at only three locations during 2003 to 2005 where soybean treated with glyphosate yielded more. Herbicide system had no impact on yield of corn and wheat. The effect of including winter wheat in the crop rotation improved soybean yield at two locations, but, in general, had little influence on weed control and yield in corn. Winter wheat yield was not affected by the herbicide system used in the previous corn and soybean crops, although over the 6 years, lower weed ground cover was observed in the winter wheat grown in the glyphosate-based herbicide system.

Weed communities were different among the locations and were strongly influenced by herbicide system and to a lesser degree crop rotation. Between corn and soybean, mid-season weed communities tended to be more similar when weeds were controlled with glyphosate in both crops than with conventional herbicides. The effect of the herbicide systems on the weed community were different in the two tillage systems. In general, more weed species were associated positively with the conventional herbicide system at the no-till (NT) locations, while in CT, more weed species were associated positively with the glyphosate-resistant herbicide system. Weed biodiversity indicators confirmed this observation. These results were likely caused by differences in weed emergence patterns in the two tillage systems in relation to the time of herbicide application. Few consistent treatment-species associations among all five locations were observed, indicating that local soil (edaphic) and/or climate factors also played a significant role in determining weed species by treatment associations.

1. Rationale

The short-term impacts of GR cropping systems have been well documented in the literature (Nurse et al. 2007, Nurse et al. 2006, Sikkema at al. 2005, Sikkema et al. 2004, Ivany 2004, Thomas et al. 2004, Johnson et al. 2000, Payne and Oliver 2000, Reddy and Whiting 2000) with comparatively little information available on the longer-term effects of intensive use of herbicide-resistant cropping systems (Heard et al. 2005, Heard et al. 2003a and 2003b). The objectives is research were to compare the effects of continuous use of glyphosate or conventional herbicides on weed control, weed biodiversity, crop yield and yield stability of glyphosate-resistant corn and soybean grown in rotation with or without winter wheat.

Materials and Methods

Experiment layout and establishment

To investigate the effects of glyphosate-resistant (GR) corn and soybean cropping systems on weed control and yield parameters, a long-term field study was established in 2000 at four locations in Ontario. Descriptions of the locations, soil type, and dates of planting and in-crop herbicide application are provided in Table 1. Two independent experiments were established at Woodstock with one under conventional tillage (CT) and one under No-tillage (NT) management next to each other, while the experiments at other locations were confined to one tillage system only. Individual plot size ranged from 60 to 104 m^2 and sites were managed using farm-scale equipment. Soil disturbance in conventional tillage experiments occurred with one pass of a chisel plow in the autumn and one secondary tillage pass with a disk or light cultivator before planting in the spring as is typical for the area. In the NT experiments, soil disturbance occurred only at the time of seeding.

The experiment was designed as a two-way factorial with herbicide system in corn and soybean as one main factor (conventional vs. Roundup Ready) and crop rotation as the second main factor (corn-soybean vs. corn-soybean-winter wheat). The herbicide regimes and application rates of conventional and Roundup Ready systems for all crops are provided in Table 2. Including winter wheat in the crop rotation provided a break from continuous in-crop glyphosate and allowed for the testing of residual effects of the herbicide systems used in corn and soybean. Each phase of each rotation was present each year resulting in 10 treatments per replicate with 4 replicates per experiment. The experiments were maintained for 6 years.

Fertilizer was applied according to soil test recommendations after soils were tested each spring. The bulk of fertilizer was broadcast before planting with the remainder side banded at the time of seeding. Crops were planted at recommended seeding rates which were about 75,000 plants ha^{-2} for corn, about 40 plants m^2 for soybean, and about 370 plants m^2 for winter wheat. Winter wheat was underseeded to red clover in the spring at a rate of about 10 kg ha^{-2}. All herbicides were applied at recommended rates (Table 2) and with the recommended water volumes. If deemed necessary by the site manager, a second in-crop application of glyphosate was applied 2 to 3 weeks after the first in-crop application of glyphosate (Table 2).

Data collection

Crop stand densities were determined in each plot shortly after the completion of emergence. Mid-season weeds were evaluated in early- to mid-August, about 7 weeks after the first in-crop application of glyphosate or 4 to 5 weeks after the second in-crop application of glyphosate where applied. Weed control determined as percent ground cover of grasses and broadleaf weeds were estimated visually on whole plots. Weed densities were determined by counting the number of weeds in at least five randomly placed 1 m^2 quadrats in the centre part of each plot or in the whole plot if densities were low.

Yields of corn, soybean, and winter wheat were determined at physiological maturity and grain moisture contents were adjusted to 15.5%, 13%, and 14.5%, respectively. Temperature and precipitation data were collected daily throughout the growing season and summarized as monthly means.

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Table 1. Herbicides, application timing, and rates in each crop of the conventional and glyphosate-resistant cropping systems.

Herbicide System	**Crop**	**Application**	**Herbicide**	**Rate**
				(kg ai ha^{-1})
Conventional	Soybean	PRE	flumetsulam / S-metolachlor	1.44
	Corn	PRE	S- metolachlor / benoxacor	1.37
		PRE	dicamba / atrazine	1.60
	Winter wheat	POST	bromoxynil / MCPA	0.56
		POST-harvest	glyphosate	1.80
Roundup Ready	Soybean	PRE	glyphosate	1.80
		POST	glyphosate (1 or 2 times)	0.90
	Corn	PRE	glyphosate	1.80
		POST	glyphosate (1 or 2 times)	0.90
	Winter wheat	POST	bromoxynil / MCPA	0.56
		POST-harvest	glyphosate	1.80

Table 2. Soil characteristics, seeding dates, and in-crop herbicide application dates (1st and 2nd where applicable) in soybean, corn, and winter wheat for each year at each location.

	Soil Characteristics				Seeding Dates			1st In-Crop Herbicide Application			2nd In-Crop Glyphosate			
											2-year		3-year	
Site	**Texture**	**OM (%)**	**pH**	**Year**	**Soybean**	**Corn**	**Winter Wheat**	**Soybean**	**Corn**	**Wheat**	**Soybean**	**Corn**	**Soybean**	**Corn**
Woodslee	clay loam	4.1	6.5	2003	18-Jun	18-Jun	Oct 10, '02	17-Jul	9-Jul	7-May	-	-	-	-
(42° 11' N, 82° 42' W)				2004	22-Jun	22-Jun	Oct 21, '03	16-Jul	16-Jul	13-May	-	-	-	-
				2005	13-May	26-Jun	Oct 28, '04	20-Jun	20-Jun	16-May	-	-	-	-
Huron Park	loam	4.3	7.7	2003	22-May	22-May	Oct 11, '02	21-Jun	16-Jun	14-May	3-Jul	3-Jul	3-Jul	3-Jul
(43° 21' N, 81° 29' W)				2004	1-Jun	1-Jun	Oct 23, '03	23-Jun	23-Jun	26-May	-	10-Jul	-	10-Jul
				2005	9-May	9-May	Oct 22, '04	9-Jun	4-Jun	26-May	-	-	-	-
Woodstock	loam	4.8	7.1	2003	2-Jun	20-May	Sep 30, '02	20-Jun	23-Jun	25-May	13-Jul	-	13-Jul	4-Jul
(42° 26' N, 81° 53' W)				2004	3-Jun	21-May	Oct 3, '03	28-Jun	15-Jun	11-May	-	-	-	-
				2005	27-May	19-May	Oct 13, '04	14-Jun	14-Jun	7-May	-	-	-	-
Ridgetown	sandy clay loam	5.3	6.8	2003	22-May	19-May	Oct 18, '02	24-Jun	18-Jun	21-May	-	8-Jul	-	8-Jul
(43° 8' N, 80° 45' W)				2004	1-Jun	29-May	Oct 18, '03	23-Jun	23-Jun	11-May	22-Jul	9-Jul	22-Jul	9-Jul
				2005	10-May	6-May	Oct 12 ,'04	9-Jun	9-Jun	5-May	-	20-Jun	-	20-Jun

2. Agronomic performance, yield stability, and weed control

Executive Summary

- The glyphosate weed control system consistently provided lower mid-season weed ground cover (i.e., improved mid-season weed control) in soybean and corn compared to weed control using conventional herbicides.

- When the same RR soybean genotype was grown in both herbicide systems during the second cycle of the rotation, yield was significantly greater when treated with glyphosate compared to conventional herbicides under No-till cultivation. A similar trend was observed in conventional tillage, however, this was not statistically significant.

- Despite some differences in mid-season weed ground cover in corn, herbicide system did not affect corn yield significantly in either tillage system or cycle.

- The glyphosate weed control system used in corn and soybean did result in lower mid-season weed ground cover in winter wheat at the No-till locations, however, this had no significant impact on the yield of winter wheat.

- Herbicide system did not affect yield stability significantly between site-years in corn and soybean.

Statistical analysis

Weed control and yield data from the five locations and two tillage systems were analyzed using the generalized mixed models and mixed models procedures, respectively, and summarized as described below. During the initial exploratory analysis, where tillage system was declared a random effect, the covariance parameter estimate indicated that tillage system was responsible for a substantial amount of variation. Therefore, tillage system was declared a fixed effect and the resulting 3-way interactions between tillage system and other factors precluded a combined analysis of weed control and yield data among tillage systems. Thus, the data were analyzed and summarized separately within tillage system (i.e. 3 conventional tillage sites and the 2 No-till sites). Due to the unbalanced replication of tillage system among sites, a direct, statistical comparison between tillage systems was not possible without violating the basic assumptions of statistical methods used. The effects of herbicide system and crop rotation on weed control and yield were also analysed within each location for comparison. These data are summarized in Appendix A (Tables A1 –A4).

Weed Control

From the August ratings that were consistent among all sites and years, total mid-season weed ground cover (= 100- total mid-season weed control) was determined by summing the percentage values for mid-season broadleaf and mid-season grassy weed ground cover. To eliminate zero values and thereby facilitate subsequent statistical analysis, 0.1 was added to each percentage value at this time. Mid-season ground cover data was not normally distributed and therefore was analysed using Proc GLIMMIX in SAS (version 9.1). This procedure fits mixed models to Gaussian as well as non-Gaussian error distributions and allows for the analysis of raw ground cover data without the need for transformation. Within tillage system, total mid-season weed, mid-season broadleaf, and mid-season grassy weed ground cover were analysed within each crop considering herbicide system, rotation, and cycle of the crop rotation as fixed effects, while location, year, and block within location were considered random effects. Model convergence was achieved using the lognormal error distribution with its default identity link function. Means from this analysis are presented as significant interactions dictated. The means presented are corrected for the lognormal distribution. In isolated instances, negative mean estimates have been replaced by zero values (i.e., mid-season grassy weed ground cover).

Yield

All yield data was anlaysed within crop. Yield data was tested for normality. To improve normality of the residuals and the raw data, outliers were removed (3 in soybean, 1 in corn) based on a studentized residual value greater than 3. The data were then subjected to ANOVA using the MIXED procedure of SAS. In the main analysis, herbicide system, rotation and cycle of the crop rotation were declared as fixed effects, while location, year and block within location were considered random. To determine the influence of location on crop yield, a second analysis was conducted where location was considered a fixed factor. To estimate the importance of mid-season total weed ground cover on yield, an ANCOVA analysis was conducted with weeds as the covariate. The data were summarized as significant interactions dictated.

Yield data with individual plot data outliers removed was used to determine whether differences in yield stability were attributable to herbicide system, rotation, and cycle of the rotation (where applicable) within each field (site-year) and between fields (among site years). For this analysis, standard errors of the yield means (SEMs) for each treatment were subjected to ANOVA using the MIXED procedure as a three-way factorial design with cycle of the rotation, herbicide system and crop rotation as the main factors. For the within field analysis, standard errors of the means for each treatment were determined from the four replicates for each site-year and subjected to ANOVA. These yield means were also used to determine the SEMs for the between field analysis. For this analysis, SEMs could be determined in two different ways, either by averaging the site-year means over years at each location (within locations) or by averaging the site-year means over locations for each year (within year). For the within location analysis, means were averaged and standard SEMs were calculated over years within each cycle of the rotation (n=3) or over all years of the study (n=6). For consistency with respect to the other analyses of yield stability, only the within cycle results are presented for the within location analysis.

Results and Discussion

Soybean

In general, broadleaf weeds contributed more to total mid-season weed ground cover (= 100 - weed control) than grassy weeds in these experiments. In soybean, herbicide system influenced total mid-season weed, mid-season broadleaf, and mid-season grassy weed ground cover similarly in both tillage systems and cycles of the crop rotation when mid-season weed populations were significant (Table 1). Mid-season total weed ground cover, mid-season broadleaf, and mid-season grassy weed ground cover were lower in the RR system (i.e. better mid-season weed control) compared to the conventional herbicide system and this was highly significant at all times. Crop rotation (*C-S* vs. *C-S-W*) did not affect mid-season weed ground cover in soybean (Table 2).

Soybean yield was affected by herbicide system and this effect differed between the two cycles of the crop rotation. In the second cycle of the rotation, when the same genotype was grown in both herbicide systems, a significant yield increase (12%) was observed at the No-till sites when soybean was treated with glyphosate compared to conventional herbicides (Table 3). A similar trend (5% yield increase) was observed at the conventional tillage locations, but this was not statistically significant. When tillage system was declared a random effect in the preliminary analysis, this yield increase was significant at all 5 locations and no interactions among location and herbicide system were observed, indicating that the yield increase caused by glyphosate was consistent among all locations (data not shown). Analysis of covariance indicated a difference in the influence of mid-season weed populations on soybean yield between the tillage systems. While mid-season total weed ground cover was highly significant in conventional tillage (p_{weeds} = 0.0007) and as a result appears to have influenced soybean yield, mid-season weed ground cover did not influence soybean yield at the No-till locations (p_{weeds} = 0.1081). This indicates that the soybean yield increase observed in No-till was not the result of lower mid-season weed pressure and suggests other factors were responsible for the observed reduction in yield. It is likely that the yield increase in soybean in the glyphosate system during the second cycle of the rotation were the result of reduced crop injury by glyphosate compared to conventional herbicides, thereby increasing yield potential. During the first cycle of the rotation where different soybean genotypes were grown in the different herbicide systems, this effect was not observed and may have been masked by differences in yield potential between the conventional and herbicide-resistant soybean genotypes.

Soybean yield was influenced by crop rotation under conventional tillage. In this tillage system, soybean yield was significantly greater (9%) in the 3-year rotation that included winter wheat compared to the 2-year corn-soybean rotation (Table 4). This yield increase was not observed in the No-till system. When tillage was declared a random effect during the preliminary analysis, the difference in soybean yield between crop rotation was observed among all locations with weak statistical significance (data not shown). Therefore, the combined analysis masked the clear interaction between tillage system and crop rotation on soybean yield. Data for weed control and yield in soybean at each location are provided in tables A1 and A4.

Table 1. The effect of herbicide system and cycle of rotation on mid-season total weed, broadleaf, and grassy weed ground cover for each crop within tillage system (CT = 18 site*years, NT = 12 site*years). Simple, untransformed means, S.E.M. (parentheses), and significance of main and or simple effects are inidcated.

Mid-season total weed ground cover

	Tillage system					
	CT			NT		
Years	**2000-2002**	**2003-2005**	Sign.$_{Cycle}$	**2000-2002**	**2003-2005**	Sign.$_{Cycle}$
	----------% ground cover----------			----------% ground cover----------		
Soybean						
RR	8.0 (0.9)	6.3 (0.6)	n.s.[a]	6.4 (0.5)	6.0 (0.7)	n.s.
Conv Herb	15.7 (1.5)	14.7 (1.3)		17.9 (1.7)	12.3 (1.2)	
Sign.$_{Herb}$	***			***		
Corn						
RR	8.7 (1.1)	10.0 (0.8)	n.s.	7.9 (1.3)	5.9 (0.6)	n.s.
Conv Herb	14.3 (2.0)	14.8 (1.3)	n.s.	24.8 (2.4)	11.6 (1.3)	n.s.
Sign.$_{Herb}$	***	n.s		***	**	
Wheat						
RR	13.6 (1.0)	8.2 (1.0)	*	14.4 (3.4)	6.0 (0.7)	n.s.
Conv Herb	15.4 (1.5)	9.8 (1.7)		14.6 (2.4)	7.9 (1.4)	
Sign.$_{Herb}$	n.s.			*		

Mid-season broadleaf ground cover

	Tillage system					
	CT			NT		
Years	**2000-2002**	**2003-2005**	Sign.$_{Cycle}$	**2000-2002**	**2003-2005**	Sign.$_{Cycle}$
	----------% ground cover----------			----------% ground cover----------		
Soybean						
RR	5.5 (0.8)	4.7 (0.6)	n.s.	3.2 (0.4)	2.1 (0.2)	n.s.
Conv Herb	11.1 (1.0)	12.1 (1.2)		8.7 (1.0)	6.2 (0.9)	
Sign.$_{Herb}$	***			***		
Corn						
RR	5.5 (0.8)	6.8 (0.4)	*	5.1 (1.2)	3.8 (0.5)	n.s.
Conv Herb	10.1 (1.5)	10.9 (1.0)	n.s.	11.8 (1.4)	6.8 (1.0)	n.s.
Sign.$_{Herb}$	***	n.s.		***	n.s.	
Wheat						
RR	9.8 (1.1)	4.8 (0.8)	n.s.	8.5 (2.1)	4.1 (0.7)	n.s.
Conv Herb	10.7 (1.9)	6.2 (1.4)	n.s.	8.2 (1.7)	6.0 (1.4)	n.s.
Sign.$_{Herb}$	n.s.	n.s.		n.s.	n.s.	

Mid-season grassy weed ground cover

	Tillage system					
	CT			NT		
Years	**2000-2002**	**2003-2005**	Sign.$_{Cycle}$	**2000-2002**	**2003-2005**	Sign.$_{Cycle}$
	----------% ground cover----------			----------% ground cover----------		
Soybean						
RR	2.3 (0.3)	1.4 (0.2)	n.s.	3.1 (0.3)	3.8 (0.6)	n.s.
Conv Herb	4.4 (0.9)	2.3 (0.3)	n.s.	9.0 (1.2)	5.9 (0.7)	**
Sign.$_{Herb}$	***			***	***	
Corn						
RR	3.0 (0.4)	3.0 (0.6)	n.s.	2.6 (0.6)	1.9 (0.3)	n.s.
Conv Herb	4.1 (0.9)	3.7 (0.8)		12.8 (1.4)	4.6 (0.6)	*
Sign.$_{Herb}$	n.s.			***	***	
Wheat						
RR	3.6 (0.6)	3.2 (0.6)	n.s.	5.8 (1.8)	1.7 (0.3)	n.s.
Conv Herb	4.5 (0.6)	3.5 (0.6)		6.3 (1.8)	1.8 (0.3)	n.s.
Sign.$_{Herb}$	n.s.			**	n.s.	

[a] *** $p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.05$, n.s. not significant

Table 2. The effect of crop rotation on mid-season total weed, broadleaf, and grassy weed ground cover in soybean, corn, and wheat within tillage system (CT = 18 site*years, NT = 12 site*years). Untransformed means, significance of main effect, and S.E.M. (parentheses) are indicated.

Mid-season total weed ground cover

	Tillage system					
	CT			NT		
Rotation	***C-S***	***C-S-W***	$\text{Sign.}_{\text{Rot}}$	***C-S***	***C-S-W***	$\text{Sign.}_{\text{Rot}}$
	----------% ground cover----------			----------% ground cover----------		
Soybean	11.8 (1.0)	10.4 (0.7)	n.s.[a]	11.4 (0.9)	9.9 (0.9)	n.s.
Corn	12.0 (0.9)	11.9 (1.0)	n.s.	14.6 (1.3)	10.5 (1.3)	**
Wheat	-[b]	11.5 (0.7)		-	10.4 (1.1)	

Mid-season broadleaf ground cover

	Tillage system					
	CT			NT		
Rotation	***C-S***	***C-S-W***	$\text{Sign.}_{\text{Rot}}$	***C-S***	***C-S-W***	$\text{Sign.}_{\text{Rot}}$
	----------% ground cover----------			----------% ground cover----------		
Soybean	8.6 (0.8)	8.1 (0.6)	n.s.	5.4 (0.6)	4.7 (0.5)	n.s.
Corn	8.5 (0.7)	8.2 (0.8)	n.s.	8.1 (0.9)	5.7 (0.7)	*
Wheat	-	7.7 (0.7)		-	6.5 (0.7)	

Mid-season grassy weed ground cover

	Tillage system					
	CT			NT		
Rotation	***C-S***	***C-S-W***	$\text{Sign.}_{\text{Rot}}$	***C-S***	***C-S-W***	$\text{Sign.}_{\text{Rot}}$
	----------% ground cover----------			----------% ground cover----------		
Soybean	3.0 (0.4)	2.2 (0.2)	n.s.	5.8 (0.6)	5.1 (0.6)	n.s.
Corn	3.4 (0.4)	3.6 (0.6)	n.s.	6.3 (0.8)	4.7 (0.7)	n.s.
Wheat	-	3.7 (0.3)		-	3.7 (0.3)	

[a] *** $p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.05$, n.s. not significant

[b] - not available

Table 3. The effect of herbicide system and cycle of the rotation on yield of soybean, corn, and winter wheat within tillage system (CT = 18 site*years, NT = 12 site*years). Means, S.E.M. (parentheses), and significance of main or simple effects are indicated.

	Tillage					
	CT			NT		
Years	**2000-2002**	**2003-2005**	Sign.$_{Cycle}$	**2000-2002**	**2003-2005**	Sign.$_{Cycle}$
	-------------------t ha^{-1}	-----------------		-------------------t ha^{-1}	-----------------	
Soybean						
RR	2.70 (0.08)	2.72 (0.08)	n.s.[a]	2.32 (0.12)	2.66 (0.10)	n.s.
Conv Herb	2.76 (0.09)	2.58 (0.07)		2.45 (0.11)	2.37 (0.11)	n.s.
Sign.$_{Herb}$	n.s.			n.s.	*	
Corn						
RR	7.17 (0.26)	8.43 (0.20)	n.s.	6.55 (0.35)	9.27 (0.19)	***
Conv Herb	6.90 (0.32)	8.28 (0.20)		6.05 (0.39)	9.14 (0.24)	
Sign.$_{Herb}$	n.s.			n.s.		
Wheat						
RR	5.45 (0.22)	5.26 (0.17)	n.s.	5.08 (0.29)	5.35 (0.26)	n.s.
Conv Herb	5.50 (0.18)	5.09 (0.17)		5.50 (0.32)	5.23 (0.22)	
Sign.$_{Herb}$	n.s.			n.s.		

[a] *** $p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.05$, n.s. not significant

Table 4. The effect of crop rotation on yield of soybean, corn, and wheat within tillage system (CT = 18 site*years, NT = 12 site*years). Means, significance of main effect, and S.E.M. (parentheses) are indicated.

	Tillage system					
	CT			NT		
Rotation	***C-S***	***C-S-W***	Sign.$_{Rot}$	***C-S***	***C-S-W***	Sign.$_{Rot}$
	-------------------t ha^{-1}	-----------------		-------------------t ha^{-1}	-----------------	
Soybean	2.57 (0.06)	2.81 (0.05)	*** [a]	2.45 (0.08)	2.44 (0.07)	n.s.
Corn	7.47 (0.18)	7.92 (0.19)	n.s.	7.72 (0.25)	7.78 (0.27)	n.s.
Wheat	-[b]	5.32 (0.09)		-	5.29 (0.13)	

[a] *** $p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.05$, n.s. not significant

[b] - not available

Corn

Similar to weed control in soybean, when statistically significantly different, mid-season weed ground cover in corn was lower (i.e. improved mid-season weed control) in the RR system than when weeds were controlled with conventional herbicides (Table 1). The effect of herbicide system on mid-season total weed ground cover in corn was different between the tillage systems during the second cycle of the rotation. Under No-till, weed control with glyphosate reduced mid-season total weed ground cover in corn by providing improved mid-season grassy weed control in this tillage system. This herbicide system effect was not observed under conventional tillage where mid-season total weed, mid-season broadleaf, and mid-season grassy weed ground cover were the same when controlled with glyphosate or conventional herbicides.

In contrast to weed ground cover in soybean, an interaction between crop rotation and tillage system was observed in corn. In No-till, mid-season total weed ground cover was greater in the 2 year rotation compared to the 3 year rotation, indicating a weed control benefit in corn when the rotation was expanded to include winter wheat (Table 2). Lower mid-season broadleaf weed ground cover in the 3 year rotation caused this difference between rotations. Mid-season grassy weed ground cover was not statistically significantly different between the rotations. Crop rotation did not affect mid-season total weed, mid-season broadleaf, and mid-season grassy weed ground cover under conventional tillage.

Despite improved weed control in the RR system in corn, herbicide system did not affect corn yield significantly throughout the duration of this study (Table 3). Interestingly, the RR corn varieties that were grown during the second cycle of the rotation, exclusively, resulted in a substantial yield increase compared to the RR and conventional genotypes grown during the first cycle of the rotation in the No-till system.

Under conventional tillage, the effect of crop rotation on corn yield tended to be similar to that observed in soybean (Table 4). Statistically, the crop rotation effect on corn yield was almost significant ($p_{rotation} = 0.0569$). As with soybean, crop rotation did not affect corn yield in the No-till system. Despite the lack of statistically significant effects of all tested factors on corn yield, analysis of covariance revealed that the effect of mid-season total weed ground cover on corn yield was highly significant in both tillage systems (ANCOVA conventional tillage $p_{weeds} = 0.0006$, No-till $p_{weeds} = 0.0001$). Data for weed control and yield in corn at each location are provided in tables A1 and A4.

Wheat

The herbicide system used in corn and soybean preceding the winter wheat crop did affect mid-season total weed and grassy weed ground cover differently in the two tillage systems. While no effect was observed under conventional tillage, mid-season total weed ground cover in both cycles of the rotation and mid-season grassy weed ground cover in the first cycle of the rotation were significantly lower (i.e. better weed control) in No-till winter wheat when weeds were controlled with glyphosate compared to conventional herbicides in the preceding corn and soybean crops (Table 2). However, this observed decrease in weed ground cover did not translate into significantly greater winter wheat yields, despite a significant effect of mid-season total weed ground cover as the covariate ($p_{weeds} = 0.0276$) (Table 4). Under conventional tillage, neither herbicide system, nor cycle of the rotation influenced winter wheat yield significantly and mid-season total weed ground cover also had no affect on winter wheat yield ($p_{weeds} = 0.5348$). Data for weed control and yield in wheat at each location are provided in tables A1 and A4.

Yield Stability in Corn and Soybean

To determine whether post-emergence weed control with glyphosate resulted in more stable yield production compared to conventional pre-applied soil residual herbicides, the standard errors (SEMs) associated with yield within each site-year and among site-years were examined. The probability values of this analysis are shown in Table 5 and many of the standard errors of the means can be gleamed from the yield data (Tables 3, 4 and A1). The within-field analysis which reflects spatial variability in yield within a parcel of land during the same cropping season, showed a significant interaction in soybean yield stability between herbicide system and crop rotation. Yield stability was greater (i.e., lower SEM) in the soybean treated with conventional herbicides in the 3-year rotation (SEM = 0.10 t ha^{-1}) compared to the 2-year rotation (SEM = 0.14 t ha^{-1}) (data not shown). Yield stability of soybean treated with glyphosate was intermediate (SEM = 0.11 and 0.13 t ha^{-1}). This suggests that treating a field of soybean with glyphosate may moderate the yield stability benefits observed by adding winter wheat in the rotation when weeds are controlled using conventional herbicides. In corn, the probability of a significant effect of herbicide system was 0.09 with SEMs of 0.42 and 0.50 t ha^{-1} for corn treated with glyphosate and conventional herbicides, respectively (data not shown). Although not considered statistically significant, this trend tends to support testimonials of more consistent corn yields, in this instance within a field, when treated with post-emergence glyphosate compared to conventional soil residual herbicides where efficacy is more dependent on soil moisture.

In the between-field analysis, significant differences were observed only in corn yield stability between the first and second cycles of the study. In both analyses, yield stability was lower in the first cycle (2000-2002) than in the second cycle (2003-2005) of the rotation. The reduced corn yield stability in the first cycle was the result of the large difference in corn yields observed at the NT compared to the CT locations (Table 3). Below normal precipitation in August in 4 of the 6 site-years at the NT locations likely contributed to this reduction in yield. Herbicide system did not affect yield stability in either corn or soybean between fields (site-years). These results are not surprising as the differences in weed control (Table 1) between the herbicide systems had a negligible effect on corn and soybean yield (Table 3). Moreover, the same genotype was grown in both herbicide systems during the second cycle of the rotation which eliminated the genotype component to yield variation between the herbicide treatments. Calculating SEMs for the between-field analysis over all site-years (n=6) rather than within each cycle of the rotation (n=3) did not influence the results (data not shown).

Table 5. ANOVA tables of yield variability (SEMs) within (at each location) and between fields (across locations) for conventional and glyphosate-resistant corn and soybean. Between field standard errors were determined by calculating the standard errors of the yield means within location or within year and subjecting to ANOVA.

		Variability in Yield							
		Within Field			**Between Fields**				
					Within Location			**Within Year**	
Effect	**DF_{Den}**	**Soybean**	**Corn**	**DF_{Den}**	**Soybean**	**Corn**	**DF_{Den}**	**Soybean**	**Corn**
		---------p value-------			---------p value-------			---------p value-------	
Cycle of rotation	104	0.6568	0.8899	28	0.6270	**0.0005**	12	0.8992	**0.0087**
Herb System	104	0.9522	0.0911	28	0.9070	0.5988	12	0.4321	0.3392
Cycle * System	104	0.9461	0.9326	28	0.6000	0.7983	12	0.7158	0.1728
Rotation	104	0.3707	0.2132	28	0.4501	0.9850	12	0.7949	0.5558
Cycle * Rotation	104	0.3249	0.7054	28	0.7956	0.3423	12	0.6134	0.2237
System * Rotation	104	**0.0191**	0.4649	28	0.8623	0.9302	12	0.8766	0.8878
Cycle * System * Rotation	104	0.9994	0.7409	28	0.8640	0.8050	12	0.7795	0.8157

Appendix A

Table A1. The effect of herbicide system and crop rotation on soybean and corn yield at each location. Significance of main effect and interactions are indicated, while the standard errors of the means are in parentheses.

	Soybean			Corn			Wheat		
	RR	**Conv. Herb.**	Sign.$_{Herb}$	**RR**	**Conv. Herb.**	Sign.$_{Herb}$	**RR**	**Conv. Herb.**	Sign.$_{Herb}$
	------------------t ha^{-1}----------------			------------------t ha^{-1}----------------			------------------t ha^{-1}----------------		
CT **Woodslee**									
C-S	2.81 (0.13)	2.93 (0.15)	[b]	7.28 (0.29)	7.00 (0.24)	n.s.			
C-S-W	2.83 (0.14)	2.94 (0.15)		7.73 (0.26)	7.52 (0.24)		5.41 (0.16)	5.44 (0.16)	n.s.
Sign.$_{Rot}$	n.s.[a]			**					
Huron Park									
C-S	2.43 (0.09)	2.38 (0.10)	*	7.26 (0.33)	7.32 (0.39)	n.s.			
C-S-W	2.96 (0.10)	2.70 (0.07)		7.83 (0.40)	7.88 (0.41)		5.48 (0.29)	5.15 (0.23)	n.s.
Sign.$_{Rot}$	***			*					
Woodstock									
C-S	2.42 (0.17)	2.45 (0.16)	n.s.	7.96 (0.55)	7.87 (0.68)	n.s.			
C-S-W	2.81 (0.17)	2.63 (0.14)		8.67 (0.56)	7.95 (0.70)		5.13 (0.23)	5.17 (0.27)	n.s.
Sign.$_{Rot}$	***			n.s.					
NT **Woodstock**									
C-S	2.52 (0.19)	2.29 (0.18)	**	8.32 (0.49)	7.11 (0.58)	***			
C-S-W	2.51 (0.17)	2.39 (0.18)		8.16 (0.60)	8.34 (0.69)	n.s.	5.12 (0.27)	5.32 (0.23)	n.s.
Sign.$_{Rot}$	n.s.			n.s.	***				
Ridgetown									
C-S	2.34 (0.12)	2.37 (0.11)	[b]	7.73 (0.45)	7.73 (0.46)	n.s.			
C-S-W	2.23 (0.11)	2.30 (0.09)		7.43 (0.40)	7.59 (0.45)		5.36 (0.29)	5.23 (0.28)	n.s.
Sign.$_{Rot}$	n.s.			n.s.					

[a] *** $p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.05$, n.s. not significant

[b] indicates a significant interaction with this effect and cycle of the rotation

Table A2. The effect of herbicide system and crop rotation on mid-season total weed, mid-season broadleaf, and mid-season grassy weed ground cover in soybean at each location. Significance of main effect and interactions are indicated, while the standard errors of the means are in in parentheses.

	Total weeds			**Broadleaf weeds**			**Grassy weeds**		
	RR	**Conv. Herb.**	Sign.$_{Herb}$	**RR**	**Conv. Herb.**	Sign.$_{Herb}$	**RR**	**Conv. Herb.**	Sign.$_{Herb}$
	---------------------% cover----------------			---------------------% cover----------------			---------------------% cover----------------		
CT									
Woodslee									
C-S	4.2 (1.5)	6.9 (1.9)	***^{ab}	3.9 (1.4)	6.1 (1.8)	***^{b}	0.3 (0.2)	0.8 (0.3)	***^{b}
C-S-W	4.8 (1.4)	9.2 (1.9)		4.5 (1.3)	6.6 (1.4)		0.3 (0.3)	2.6 (0.8)	
Sign.$_{Rot}$	**			*			n.s.		
Huron Park									
C-S	5.4 (1.1)	15.8 (2.7)	***^{b}	4.5 (0.9)	14.4 (2.6)	***^{b}	0.9 (0.2)	1.3 (0.2)	***
C-S-W	5.0 (1.3)	15.6 (1.9)		4.4 (1.2)	14.5 (1.8)		0.6 (0.1)	1.1 (0.3)	
Sign.$_{Rot}$	n.s.			n.s.			n.s.		
Woodstock									
C-S	8.3 (1.5)	21.7 (3.0)	***	5.3 (1.2)	13.6 (1.6)	***	3.0 (0.5)	8.1 (2.0)	**
C-S-W	7.4 (1.2)	12.8 (1.3)		4.7 (0.9)	10.2 (1.0)		2.7 (0.4)	2.6 (0.6)	
Sign.$_{Rot}$	*			n.s.			n.s.		
NT									
Woodstock									
C-S	7.4 (0.8)	17.8 (2.0)	***^{b}	1.8 (0.4)	5.6 (1.0)	***	5.6 (0.6)	12.3 (1.4)	***^{b}
C-S-W	7.6 (0.7)	15.3 (2.3)		2.3 (0.5)	4.8 (1.1)		5.3 (0.5)	10.6 (1.4)	
Sign.$_{Rot}$	n.s.			n.s.			n.s.		
Ridgetown									
C-S	2.8 (0.7)	12.8 (1.9)	***	2.3 (0.7)	10.0 (1.7)	***	0.5 (0.2)	2.9 (0.7)	***
C-S-W	2.5 (0.4)	9.9 (1.8)		2.1 (0.4)	7.5 (1.5)		0.3 (0.1)	2.3 (0.6)	
Sign.$_{Rot}$	n.s.			n.s.			n.s.		

[a] *** p < 0.001, ** 0.001 < p < 0.01, * 0.01 < p < 0.05, n.s. not significant

[b] indicates a significant interaction with this effect and cycle of the rotation

Table A3. The effect of herbicide system and crop rotation on mid-season total weed, mid-season broadleaf, and mid-season grassy weed ground cover in corn at each location. Significance of main effect and interactions are indicated, while the standard errors of the means are in in parentheses.

	Total weeds			**Broadleaf weeds**			**Grassy weeds**		
	RR	**Conv. Herb.**	Sign.$_{Herb}$	**RR**	**Conv. Herb.**	Sign.$_{Herb}$	**RR**	**Conv. Herb.**	Sign.$_{Herb}$
	---------------------% cover----------------			---------------------% cover----------------			---------------------% cover----------------		
CT									
Woodslee									
C-S	3.2 (0.6)	5.5 (1.7)	n.s.[ab]	3.0 (0.6)	5.2 (1.6)	n.s.	0.2 (0.1)	0.4 (0.3)	n.s.
C-S-W	4.8 (1.0)	7.3 (2.3)		4.3 (0.9)	6.5 (2.1)		0.5 (0.2)	0.8 (0.3)	
Sign.$_{Rot}$	n.s.[b]			n.s.			n.s.		
Huron Park									
C-S	6.7 (1.3)	10.9 (2.0)	n.s.[b]	5.1 (1.0)	9.0 (2.0)	n.s.[b]	1.6 (0.5)	1.9 (0.4)	n.s.[b]
C-S-W	9.1 (2.1)	9.9 (2.6)		5.3 (1.0)	5.9 (1.7)		3.8 (1.7)	4.0 (2.1)	
Sign.$_{Rot}$	n.s.			n.s.			n.s.		
Woodstock									
C-S	12.8 (1.2)	23.7 (2.8)	***	8.0 (1.1)	16.4 (1.4)	***	4.9 (0.7)	7.3 (1.9)	n.s.
C-S-W	11.3 (1.9)	20.7 (2.9)		8.0 (1.3)	15.6 (2.5)		3.3 (0.7)	5.1 (1.2)	
Sign.$_{Rot}$	n.s.			n.s.			n.s.		
NT									
Woodstock									
C-S	7.2 (1.5)	18.4 (2.8)	***[b]	3.1 (0.8)	6.3 (1.1)	***[b]	4.1 (0.9)	12.0 (1.9)	***[b]
C-S-W	3.3 (0.8)	16.4 (2.9)		1.1 (0.3)	7.5 (1.5)		2.2 (0.5)	8.8 (1.5)	
Sign.$_{Rot}$	**			n.s.			**		
Ridgetown									
C-S	7.8 (2.1)	20.1 (3.0)	***	7.6 (2.1)	13.3 (2.0)	***	0.2 (0.1)	6.9 (1.6)	***
C-S-W	4.4 (0.9)	13.3 (3.5)		3.9 (1.0)	8.1 (1.9)		0.5 (0.2)	5.2 (1.7)	
Sign.$_{Rot}$	**			***			n.s.		

[a] *** $p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.05$, n.s. not significant

[b] indicates a significant interaction with this effect and cycle of the rotation

Table A4. The effect of herbicide system and crop rotation on mid-season total weed, mid-season broadleaf, and mid-season grassy weed ground cover in wheat at each location. Significance of main effect and interactions are indicated, while the standard errors of the means are in in parentheses.

	Total weeds			**Broadleaf weeds**			**Grassy weeds**		
	RR	**Conv. Herb.**	Sign.$_{Herb}$	**RR**	**Conv. Herb.**	Sign.$_{Herb}$	**RR**	**Conv. Herb.**	Sign.$_{Herb}$
	---------------------% cover----------------			---------------------% cover----------------			---------------------% cover----------------		
Woodslee									
C-S-W	-[a]	-		-	-		-	-	
Huron Park									
C-S-W	10.8 (1.0)	14.5 (1.9)	*[b]	7.9 (1.1)	10.6 (1.9)	n.s.	2.8 (0.7)	3.9 (0.6)	*
Woodstock									
C-S-W	7.9 (1.3)	7.2 (0.8)	n.s.	5.0 (1.0)	4.3 (0.5)	n.s.	3.0 (0.6)	2.9 (0.6)	n.s.
NT									
Woodstock									
C-S-W	6.5 (1.3)	8.1 (1.5)	*	2.8 (0.5)	3.6 (0.5)	*	3.7 (1.0)	4.5 (1.2)	*[c]
Ridgetown									
C-S-W	10.4 (2.9)	11.3 (2.3)	n.s.	7.9 (1.8)	8.9 (1.8)	n.s.	2.5 (1.4)	2.3 (1.3)	n.s.

[a] - not available

[b] *** $p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.05$, n.s. not significant

[c] indicates a significant interaction with this effect and cycle of the rotation

2. Mid-Season Weed Species Composition (2003-2005)

Executive Summary

- There was little evidence for the selection for or enrichment of weed species considered to be naturally tolerant to or capable of avoiding in-crop glyphosate applications over the duration of this study.

- The occurrence and densities of volunteer corn and soybean were low (well below 1 plant m^{-2}) in both herbicide systems and neither volunteer crop caused significant problems.

- With the exception of weed diversity at both NT locations, there was little evidence of residual (long-term) effects on the weed community in winter wheat resulting from the herbicide system used in corn and soybean over the duration of this study.

- Weed communities in winter wheat tended to be more substantially different from those in corn and soybean (using either herbicide system). Therefore, the winter growth habit of the wheat crop was more significant in driving weed communities than other factors in this study.

- In corn and soybean, herbicide system (glyphosate vs. conventional) influenced the mid-season weed community to a greater extent than crop or rotation.

- At most locations, weed communities in corn and soybean treated with glyphosate were more similar than those in corn and soybean treated with conventional herbicides (i.e., more dense clustering of treatment means on biplots), indicating decreased variation in the mid-season weed community structure between corn and soybean in the glyphosate-resistant cropping system.

- In NT, more weed species were associated positively with the conventional herbicide system, while in CT, more weed species were associated positively with the glyphosate-resistant herbicide system. Weed biodiversity indicators confirmed this observation. The underlying cause for this remains unknown.

- Few consistent treatment-species associations were observed among the locations indicating that local soil (edaphic) and/or climate factors also played a significant role in determining weed species by treatment associations.

Statistical analysis

Multivariate analysis

Plant densities of individual species were converted to counts per square meter. For each location, crop * herbicide system minimum, median, and maximum values were plotted for each weed species. To investigate associations between herbicide system, crop, and rotation, multivariate analysis (MVA) was conducted. Substantially different weed spectra at each location precluded a combined MVA and therefore a separate MVA was conducted for each location. Prior to MVA, weed densities for each treatment were averaged over three years (2003-2005) within each replication. At each location, only species that were present in more than 15% of the replicate means were subjected to canonical discriminant analysis (CDA) to minimize the effects of joint absence bias of the multivariate analysis. This value was chosen to not exclude moderately rare species, but to exclude very rare species that contribute little to the overall community and may introduce the aforementioned biases in the MVA. To further improve the normality of the data and to eliminate zero values, weed densities were log transformed using (ln + 0.01). The CANDISC procedure in SAS was used for CDA of these data. Differences among treatment means were determined using Mahalanobis distances which refers to the most direct distance between two treatment means in multivariate space.

To display the results obtained by CDA, two-dimensional biplots were generated for the first two canonical variables for each analysis. Both axes were scaled the same to avoid skewing the relationships of ordinated values between the first and second canonical variables. Only weed species for which the total canonical structure coefficients were ≥ 0.3 for the first and second canonical variables were included in the biplots as only these were significant in defining the respective canonical axes (i.e. species that were of low density and/or present ubiquitously in all treatments). For each variable (weed species), the coordinates of the first and second canonical axis were plotted and joined to the origin with a vector to indicate direction (i.e. positive association) and magnitude. For each analysis, the end points of species vectors were scaled relative to the group means (treatments) to improve the ability to discern between variables and more clearly observe their associations with the group means.

Interpretation of Biplots

Vector length indicates the magnitude of association with either canonical axis. Sharp acute ($< 90^o$) angles between species vectors indicate a close positive association between species while vector angles approaching 180^o indicate a strong negative correlation between species. Angles approaching 90^o indicate no direct association between species. Increasing vector length signifies increased variance and a corresponding decrease in ubiquity of a variable (species) or a species with relatively greater densities among treatments, or both. Therefore, the species density plots facilitate the interpretation of the biplots. Shorter vectors indicate that a species is more rare or ubiquitous among treatments. The distance between treatment means is related to their similarity with increasing distance signifying greater dissimilarity. The perpendicular distance between species vector and a treatment mean indicates the strength of the association (positive or negative on the opposite side of the origin) between treatment-species pairs.

Weed density and estimated size

Total weed density and the size of individual mid-season weeds were estimated for each year at each location. For each plot, total weed densities were determined by summing the densities of all species counted. To estimate average size of each weed, total weed ground cover determined previously was divided by the number of weeds present in each plot. This provided an estimate of the proportion of ground covered by individual weeds and was taken as an estimate of relative mid-season weed size. To improve normality and meet the assumptions of analysis of variance, these data were log transformed before subjecting to ANOVA using the MIXED procedure in SAS. For each location, the data were analysed within crop due to the unbalanced nature of the experimental design. Herbicide system and rotation were considered fixed effects as a two-way factorial, while year and block were considered random. The data were presented as two-way interactions dictated.

Weed Biodiversity Indicators

Weed community data from each plot over the last three years of the study (2003-2005) was used to determine weed community indicators for each plot at each site in each year. Species richness (*J*) was determined by summing the number of species present in each plot. The Shannon-Wiener diversity index (H') (Shannon and Weaver 1949) was calculated using the following equation:

$$H' = -\sum_{i=1}^{s} \left(\frac{X_i}{X_0}\right)\left(\ln \frac{X_i}{X_0}\right) \qquad \text{(eq. 1),}$$

where X_i is the density of species *i* and X_0 is the density of all species in a plot. Greater values of H' indicate greater species diversity or less species dominance. The Shannon-Wiener index is a composite index whereby diversity increases with both increasing species richness and increasing species evenness. To assess the role of species richness relative to species evenness on the Shannon-Wiener index, species evenness (*E*) was calculated using $E = H' / \ln J$, for each plot. This provided a measure of the relative distribution among species in each plot. All indexes were subjected to analysis of variance using the MIXED procedure in SAS. Due to unbalanced nature of the experiment, these data were separated by crop and analysed within each location as a two-way factorial with herbicide system and crop rotation as factors.

Results and Discussion

Multivariate Treatment Separation and General Observations

Due to the substantially different weed communities with respect to species number (8 to 27) and identity among the five sites (Table 1), canonical discriminant analysis (CDA) was conducted within each location. Each phase of the rotation was present each year and therefore averaging over years 4 to 6 (2003-2005) moderated the effect of year on weed densities and minimized any spatial variability that may have been present within a replicate, thereby improving multivariate normality and reliability of these data. Species that were of sufficient frequency to be included in each analysis and those that were significant in defining the first two canonical variables are indicated in Table 1. The biplots on the first two canonical variables generated from these analyses are shown in Figure 1. Within location, CDA effectively separated the treatments (herbicide system, crop and rotation) and the first two canonical variables explained between 66.1 and 92.8% of the total variation observed in weed species among the combination of treatments. In many cases, these treatment differences were statistically significant (Table 2). At all locations, the mid-season densities of the weeds in each community clearly separated the conventional and glyphosate herbicide systems (Fig. 1). In most instances, the glyphosate treatments were ordinated in a more dense cluster than the treatments to which conventional herbicides were applied, indicating lower variation in the mid-season weed community between glyphosate-resistant corn and soybean crops. This was the likely the result of use of the same herbicide chemistry in glyphosate-resistant corn and soybean, whereas different herbicide chemistries, and therefore different selection pressures, were applied to each crop when treated with conventional herbicides. In winter wheat, however, the mid-season weed communities were distinctly different from those observed in either corn or soybean (Fig. 1) indicating that the selection pressures on the weed community of a winter annual crop are substantially different than those defining the weed spectrum in spring seeded corn and soybean crops treated with either herbicide system. At three of the four locations for which data was available (Table 2), the mid-season weed communities in winter wheat were not significantly different between the herbicide systems (i.e., the weed community in wheat following corn and soybean in which weeds had been treated with glyphosate compared to wheat following corn and soybean in which weeds had been treated with conventional herbicides), suggesting limited carry-over effect of the herbicides applied previously in corn and soybean in the weed community during years 4 to 6 of this study. Thus, the selection pressures defining the weed community in winter wheat appear to have been sufficiently strong to surpass any effects of previous herbicide applications for the 6 year duration of this study.

Some consistent trends in the behaviour of the mid-season weed communities were observed within the tillage systems with respect to weed species associations and the relative importance of crop vs. rotation in defining the weed communities within herbicide system. In NT, the majority of weed species were positively associated with the conventional herbicide system (Fig. 1). In CT, in contrast, more mid-season weed species were positively associated with the glyphosate herbicide system than the conventional herbicide program. Weed densities showed that this tillage system effect was primarily the result of differential densities of the weed species between the glyphosate and conventional herbicide systems used in corn and soybean, rather than from the presence or absence of a species between the herbicide systems which occurred in only a few instances (e.g., MATIN and LACSE at Ridgetown NT, POROL at Huron Park CT) (Fig. 2 and 3). Nevertheless, in CT, densities of these species tended to be low in most cases (< 1 plant m^{-2}) in both herbicide systems. This difference in weed species association with herbicide treatment between the tillage systems may be a reflection of different emergence times of these weeds relative to the time of herbicide application or may reflect increased herbicide efficacy of conventional soil-applied residual herbicides under CT compared to NT management.

Moreover, the ordination of treatment means on the first two canonical axes suggests contrasting relative importance of crop (corn vs. soybean) compared to rotation (2-yr vs. 3-yr) as defined by mid-season weed communities. In NT, the effect of crop species on mid-season weeds tended to be greater

Table 1. Bayer code, latin and common names, frequency of occurrence and significance of contribution to the first two canonical variables for each species at each location. Significance of contribnution is indicated as Y (yes) or N (no).

			Location									
			Huron Park CT		Woodslee CT		Woodstock CT		Woodstock NT		Ridgetown NT	
Bayer Code	Scientific name	Common name	Frequency	Biplot	Frequency	Biplot	Frequency	Biplot	Frequency	Biplot	Frequency	Biplot
annual												
ABUTH	*Abutilon theophrasti* Medic.	Velvetleaf			0.97	Y			0.23	Y	0.93	Y
AMARE	*Amaranthus retrofexus* L.	Pigweed, Redroot	0.90	Y	0.66	Y						
AMASS	*Amaranthus* species	Pigweed species					0.88	Y	0.95	Y	0.98	Y
AMBEL	*Ambrosia artemisiifolia* L.	Ragweed, Common	0.95	Y	0.56	Y	0.35	N	0.18	N	0.80	Y
ATXPA	*Atriplex patula* L.	Spreading atriplex									0.48	Y
CHEAL	*Chenpodium album L.*	Lambsquarters, Common	0.93	Y	0.97	Y	0.98	Y	0.83	Y	1.00	Y
DIGSA	*Digitaria sanguinalis* (L.) Scop.	Crabgrass, Large					0.85	Y	0.68	Y	0.68	N
ECHCG	*Echinchloa crus-galli (L.) Beauv.*	Bamyardgrass			0.34	N	0.73	Y	0.60	Y		
ERIAN	*Erigeron annuus* (L.) Pers	Annual fleabane					0.43	Y	0.10			
ERICA	*Conyza canadensis* (L.) Cronq.	Canada fleabane					0.08		0.05		0.50	Y
GLXMA	*Glycine max* (L.) Merr.	volunteer Soybean					0.15	N	0.05			
HIBTR	*Hibiscus trionum* L.	Mallow, Venice	0.78	Y								
PANCA	*Panicum capillare* L.	Witchgrass					0.68	Y	0.48	Y		
PANDI	*Panicum dichotomiflorum* Michx.	Panicum, Fall					0.13		0.03		0.40	Y
POLAV	*Polygonum aviculare* L.	KNtweed, Prostrate									0.23	Y
POLCO	*Polygonum convolvulus* L.	Buckwheat, Wild	0.88	Y			0.53	Y	0.05		0.70	N
POLLA	*Polygonum lapathifolium* L.	Smartweed, Pale	0.38	Y								
POLPE	*Polygonum persicaria* L.	Ladysthumb			0.69	Y	0.30	Y			0.50	Y
POROL	*Portulaca oleracea* L.	Purslane, Common	0.40	Y							0.58	N
SETFA	*Setaria faberi* Herrm.	Foxtail, Giant									0.18	Y
SETLU	*Setaria glauca* (L.) Beauv.	Foxtail, Yellow			0.22	N	0.30	N	0.05		0.60	Y
SETVI	*Setaria viridis* (L.) Beauv.	Foxtail, Green	1.00	Y			0.65	Y	0.98	Y	0.85	Y
SINAR	*Sinapis arvensis* L.	Mustard, Wild	0.43	Y								
SOLPT	*Solanum ptycanthum* Dun.	Nightshade, Eastern Black			0.41	Y	0.33	Y	0.03		0.35	N
SONOL	*Sonchus oleraceus* L.	Sowthistle, Annual					0.73	Y	0.55	N	0.73	N
STEME	*Stellaria media* (L.) Vill.	Chickweed, Common									0.35	N
ZEAMX	*Zea mays* L.	Volunteer Corn					0.20	N	0.18	Y	0.20	N
annual, biennial, perennial												
CERVU	*Cerastium fontanum* ssp. Vulgare (Hart.) Greuter & Burdet	Chickweed, Mouseear					0.20	Y	0.08			
DAUCA	*Daucus carota* L.	Carrot, Wild					0.05		0.08		0.60	Y
LACSE	*Lactuca serriola* L.	Prickly Lettuce									0.23	Y
MATIN	*Matricaria iNdora L.*	Scentless chamomile									0.20	Y
MELAL	*Silene latiflolia* Poir.	White Cockle	0.18	Y								
OXAST	*Oxalis stricta* L.	Woodsorrel, Yellow					0.40	N	0.15	Y		
TRFSS	*Trifolium species*	Clovers species									0.78	Y
perennial												
ASCSY	*Asclepias syriaca* L.	Milkweed, Common					0.18	Y	0.30	Y		
CIRAR	*Cirsium arvense* (L.) Scop.	Thistle, Canada					0.10					
CONAR	*Convolvulus arvensis* L.	Bindweed, Field	1.00	Y								
CYPES	*Cyperus esculentus* L.	Nutsedge, Yellow									0.43	Y
PLAMA	*Plantago major* L.	Broad-leaved Pantain	0.25	N			0.53	Y	0.30	Y	0.48	Y
POAPR	*Poa pratensis* L.	Kentucky bluegrass					0.43	Y	0.18	Y		
SONAR	*Sonchus arvensis* L.	Sowthistle, Perennial	0.85	Y								
TAROF	*Taraxacum officinale* Weber in Wiggers.	Dandelion					1.00	Y	1.00	Y	1.00	Y

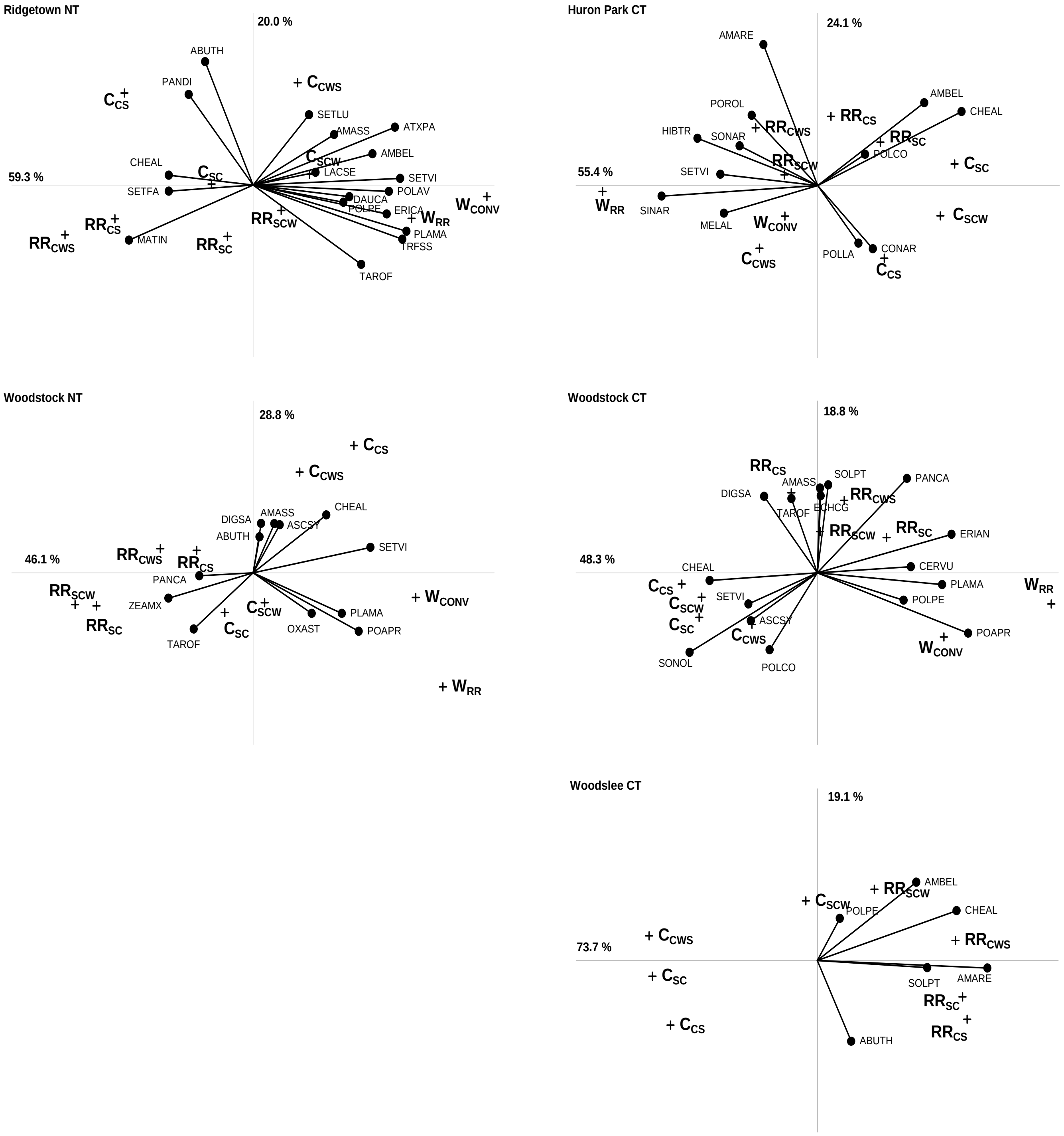


Fig 1. Biplots of the first two canonical variables of the species and treatment means at each location. For corn and soybean crops, main treatment codes indicate herbicide system (C = conventional herbicides, RR = glyphosate), while subscript codes indicate current crop and crop sequence (e.g. CWS = weed community in corn, preceded by wheat, preceded by soybean). In winter wheat treatments (main code = W), subscript code designates the herbicide system used in previous corn and soybean crops.

Table 2. Probabilities of pairwise comparisons based on separation using Mahalanobis distance (D2). Where applicable, the first location listed refers to the bottom left half of the table.

	Treatment									
	C_{CS}	C_{CWS}	RR_{CS}	RR_{CWS}	C_{SC}	C_{SCW}	RR_{SC}	RR_{SCW}	W_{CONV}	W_{RR}
Woodstock NT / Ridgetown NT										
C_{CS}	-	**	*	**	*	**	**	**	***	**
C_{CWS}	NS	-	**	**	**	*	**	*	**	**
RR_{CS}	**	*	-	NS	*	**	*	*	***	**
RR_{CWS}	**	*	NS	-	*	**	*	**	***	**
C_{SC}	***	**	**	**	-	NS	*	NS	**	**
C_{SCW}	***	**	*	**	*	-	*	NS	*	*
RR_{SC}	***	***	NS	NS	**	**	-	NS	**	**
RR_{SCW}	***	***	*	NS	**	***	NS	-	**	*
W_{CONV}	**	**	**	***	***	**	***	***	-	NS
W_{RR}	***	***	***	***	***	***	***	***	NS	-
Woodstock CT/ Huron Park CT										
C_{CS}	-	*	***	***	**	NS	**	***	**	***
C_{CWS}	NS	-	***	***	***	***	***	**	**	***
RR_{CS}	NS	*	-	NS	**	***	NS	**	***	***
RR_{CWS}	**	*	NS	-	***	***	**	**	**	***
C_{SC}	NS	NS	*	**	-	NS	NS	***	***	***
C_{SCW}	NS	NS	NS	*	NS	-	*	***	**	***
RR_{SC}	**	**	NS	**	**	**	-	*	**	***
RR_{SCW}	*	NS	NS	NS	*	NS	*	-	**	***
W_{CONV}	**	*	**	*	**	**	*	*	-	***
W_{RR}	***	**	**	**	***	***	**	**	NS	-
Woodslee CT										
C_{CS}										
C_{CWS}	*									
RR_{CS}	***	***								
RR_{CWS}	***	***	*							
C_{SC}	NS	NS	***	***						
C_{SCW}	***	**	***	**	***					
RR_{SC}	***	***	NS	NS	***	***				
RR_{SCW}	***	***	***	*	***	NS	**			
W_{CONV}	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A		
W_{RR}	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	

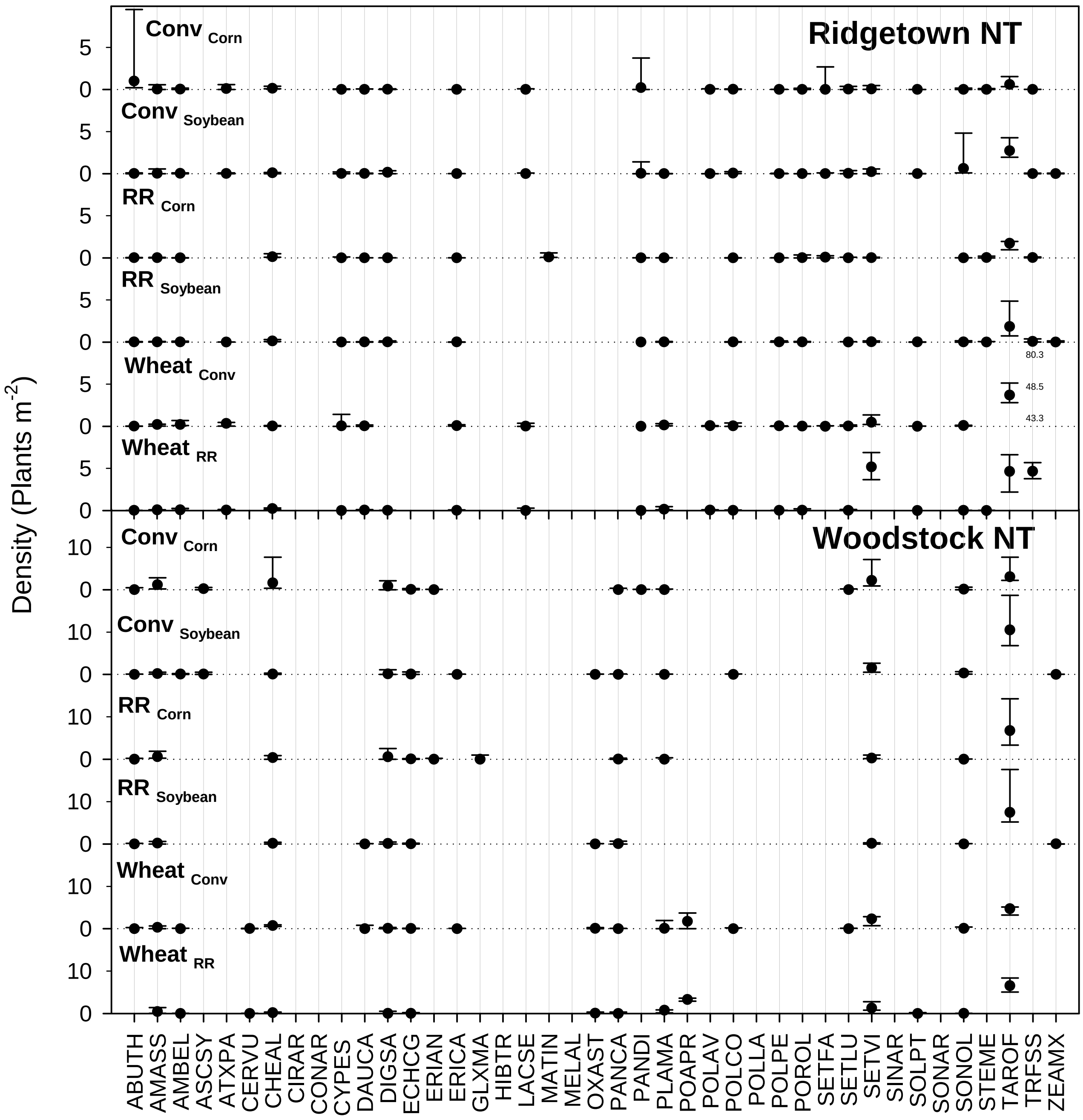


Fig. 2. Mid-season minimum, median, and maximum densities of each species in each crop by herbicide system treatment combination, where present, at the NT locations. For corn and soybean crops, main treatment codes indicate the herbicide system (Conv = conventional herbicides, RR = glyphosate) with subscript code indicating the crop. For these treatments, weed densities were combined for rotations. For winter wheat, the subscript code indicates the herbicide system used in the previous corn and soybean crops. Densities which exceeded the given scale are indicated as numbers.

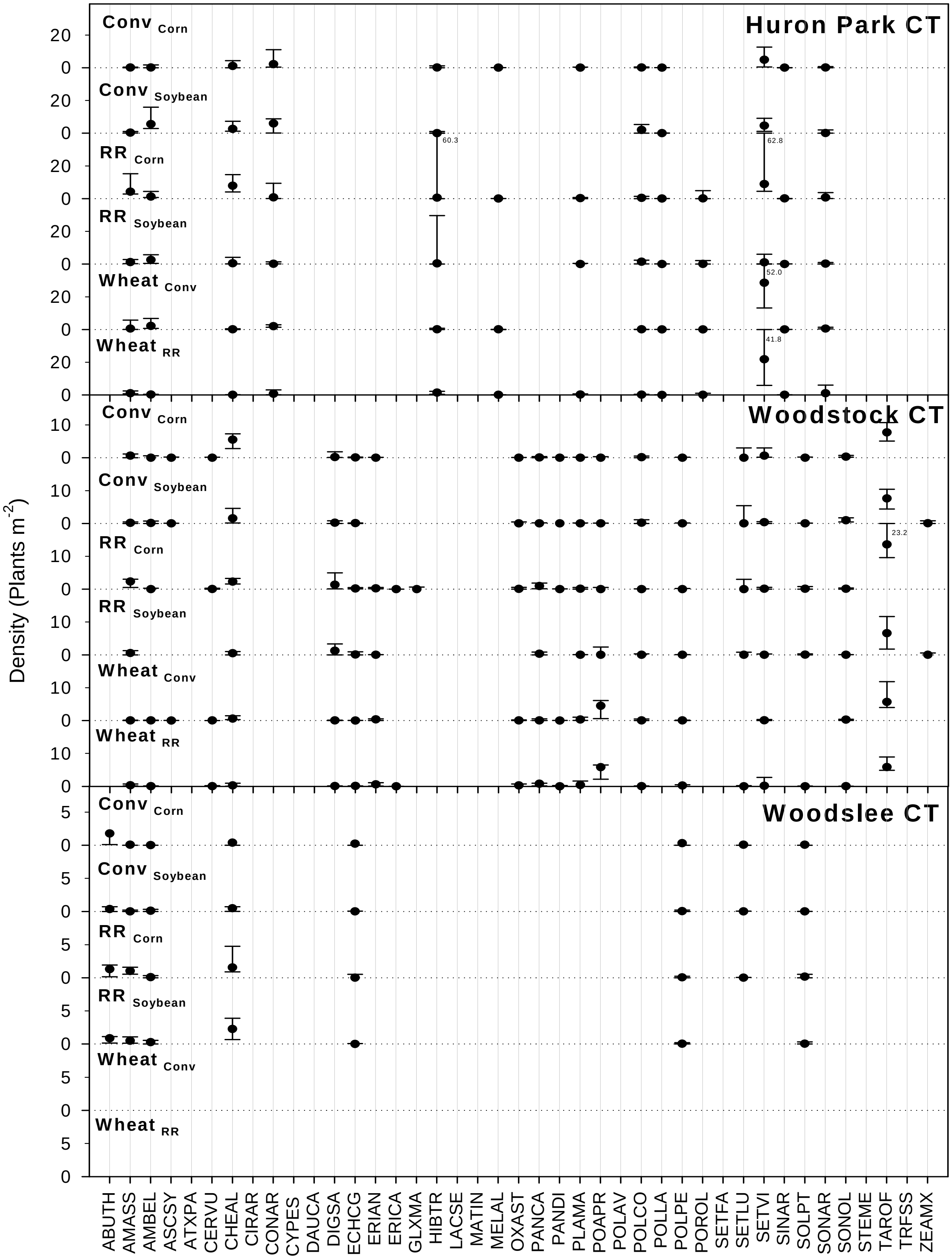


Fig. 3. Mid-season minimum, median, and maximum densities of each species in each crop by herbicide system treatment combination, where present, at the CT locations. For corn and soybean crops, main treatment codes indicate the herbicide system (Conv = conventional herbicides, RR = glyphosate) with subscript code indicating the crop. For these treatments, weed densities were combined for rotations. For winter wheat, the subscript code indicates the herbicide system used in the previous corn and soybean crops. Densities which exceeded the given scale are indicated as numbers.

than the effect of crop rotation, while the clustering of treatment means in CT suggests a greater influence of crop rotation relative to crop species. At all locations, mid-season densities of most species in the community were low (< 1 plant m^{-2}) when averaged over years (Figs. 2 and 3) and only few species were present at median densities greater than 5 plants m^{-2}.

Individual Locations

Ridgetown NT

At Ridgetown NT, of the 26 different mid-season weed species, 19 contributed significantly to the weed communities observed among the various treatments (Table 1). The first canonical variable (59.3%) distinguished between the wheat crop and the corn and soybean treatments while the second canonical variable (20.0%) primarily distinguished between the herbicide systems used in corn and soybean. Although all treatment means were considered statistically significantly different at this location (Table 2), crop rotation exhibited the smallest effect on the definition of treatment groups based on mid-season weed communities. With the exception of TAROF, median densities of all weed species were below 1 plant m^{-2} in treatments sprayed with glyphosate. In treatments to which conventional herbicides were applied, median densities of ABUTH, TAROF, and PANDI were greater than 1 plant m^{-2} in corn in the 2-year rotation treated with conventional herbicides in some years, but remained below 1 plant m^{-2} in all other treatments (Fig. 4) during the last 3 years of the study.

At Ridgetown, a number of mid-season weed species (e.g., PLAMA, ERICA, POLAV, DAUCA, POLPE) were directly associated with winter wheat in both herbicide systems. Inherent poor control of some of these species (e.g., PLAMA, ERICA, and DAUCA) may explain this association, however, reasons for the associations of POLPE and POLAV with this treatment are unclear. Species present at higher densities that defined the weed community in winter wheat included TRFSS and SETVI which are also species not controlled well by the herbicides used in winter wheat.

TAROF, another species with higher densities was closely associated with the RRscw treatment (= glyphosate treated soybean crop preceded by corn, preceded by wheat), but not with the remaining RR treatments. The RR corn treatments were mostly defined by MATIN which was present at relatively low densities (Fig. 2). The low growth habit of both species or late emergence TAROF seedlings may have allowed for avoidance of exposure to glyphosate.

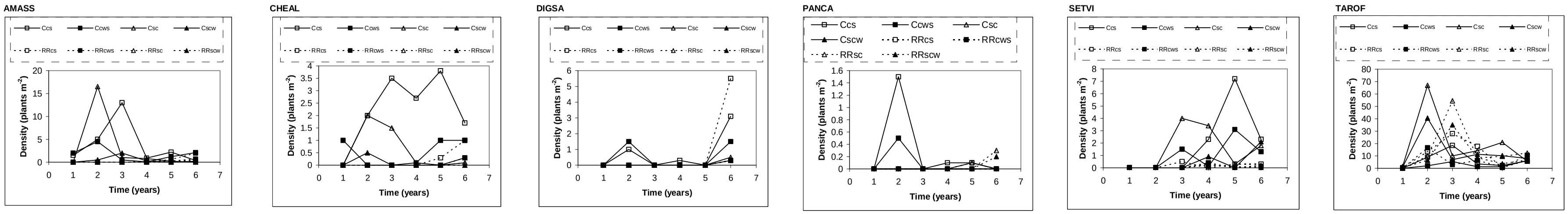
The soybean crop in the 2-yr rotation treated with conventional herbicides (Csc) was closely associated with CHEAL and SETFA, species with extended duration of or late emergence, respectively, while the soybean crop in the 3-year rotation treated with conventional herbicides (Cscw) was defined by AMBEL, LACSE, AXTPA, and AMASS. The inherent control of the conventional herbicide program used in soybean was reduced for AMBEL and AXTPA and poor for LACSE. Corn treated with conventional herbicides was weakly associated with PANDI, ABUTH, and SETLU, all late emerging species which may have avoided residual control by conventional herbicides. Many of the major weeds found in cropping systems in Ontario were primarily associated with the cropping systems using conventional herbicides (e.g., ABUTH, AMBEL, CHEAL, and *Setaria* spp.).

Woodstock NT

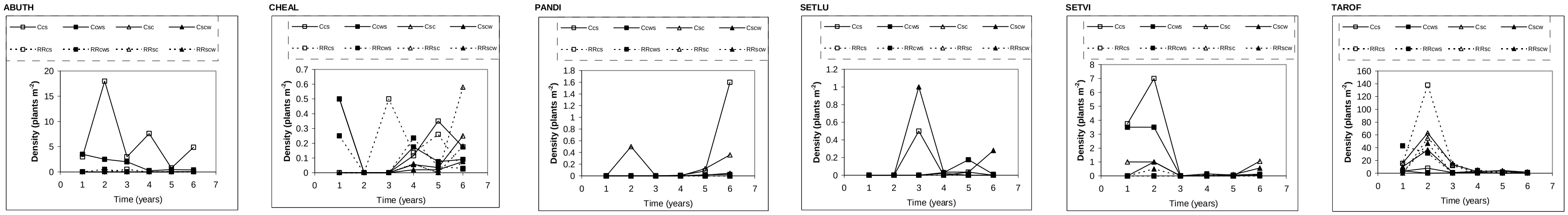
Similar to Ridgetown, the first canonical variable (46.1 %) differentiated primarily between the crops, while the second canonical variable (28.8%) improved the differentiation between the herbicide systems. At this location, crops and herbicide systems formed distinctly clustered pairs with no differences between crop rotation (Fig. 1, Table 2). At this location, eleven of thirteen weeds were significant in defining the weed communities. With the exception of TAROF, DIGSA, and AMASS (Fig. 4), median weed densities in plots treated with glyphosate were less than 1 plant m^{-2} for all species. In corn and soybean treated with conventional herbicides, median densities of SETVI, AMASS,

Fig. 4. Median weed denstities in corn and soybean treated with glyphosate or conventional herbicides of the most prominent species at Woodstock NT and Ridgetown NT over the duration of the study.

Woodstock NT



Ridgetown NT



CHEAL, TAROF, and DIGSA reached values greater than 1 plant m^{-2} in any one year during the last three years of the study (Fig. 4).

The RR treatments were most closely associated with PANCA and ZEAMX with ZEAMX only occurring in the soybean crop (RRsc and RRscw) (Fig. 1). Both species were present at low densities (< 1 plant m^{-2}). Similar to Ridgetown, PLAMA and SETVI showed associations with winter wheat. Weed species associated with soybean treated with conventional herbicides included TAROF and OXAST. TAROF was the most prominent mid-season weed at this location (median densities > 5 plants m^{-2}). The grass border strips surrounding the plots were heavily infested with TAROF and this likely contributed to the high incidence of this weed at this locations.

Corn treated with conventional herbicides was associated with AMASS, ASCSY, and CHEAL (Fig. 1). Association of CHEAL with the conventional herbicide system was likely due to the presence of triazine-resistant CHEAL at this location. Poor control of ASCSY by conventional herbicides contributed to this species-treatment association. Possible late emerging seedlings of AMASS likely contributed to the association of this species with conventional herbicide system.

Woodstock CT

The weed community at the Woodstock CT location included more weed species than that observed in the NT experiment located immediately next to the CT study. The first canonical variable differentiated between the crops and to some extent the herbicide systems, while the second canonical axis differentiated treatments based on herbicide system and crop species (Fig. 1). Most treatments were statistically significantly different from each other (Table 2) and clustered in distinct groups based on herbicide treatment (Fig. 1).

Based on median densities within each year, TAROF, CHEAL, and AMASS were the most prominent mid-season weeds at this location (5 to 30 plants m^{-2}) (Fig. 5). However, other species including DIGSA, PANCA, POLCO SETVI, and SONOL were found at median densities between 1 to 5 plants m^{-2} in isolated treatments (DIGSA and PANCA in RR treatments and PLOCO, SETVI, and SONOL under conventional herbicides) in some years (Fig. 5). Weed species that defined the RR cropping systems included ECHCG, DIGSA, SOLPT, AMASS, TAROF, PANCA, and ERIAN, however, with the exceptions of AMASS and TAROF, these species were present at low densities when averaged over the 3 years (Fig. 3). In all species, late emergence was the likely reason for these associations.

The conventional corn treatments were closely associated with CHEAL, SETVI, SONOL, ASCSY, and POLCO. CHEAL populations were resistant to conventional herbicides at this location. Reduced residual activity on SETVI and poor control of ASCSY and SONOL by conventional herbicides likely caused these species-treatment associations. The association of POLCO with corn in the three year rotation treated with conventional herbicides is unclear as two of the three herbicide programs (in corn and wheat) in this rotation exhibit excellent activity on this weed.

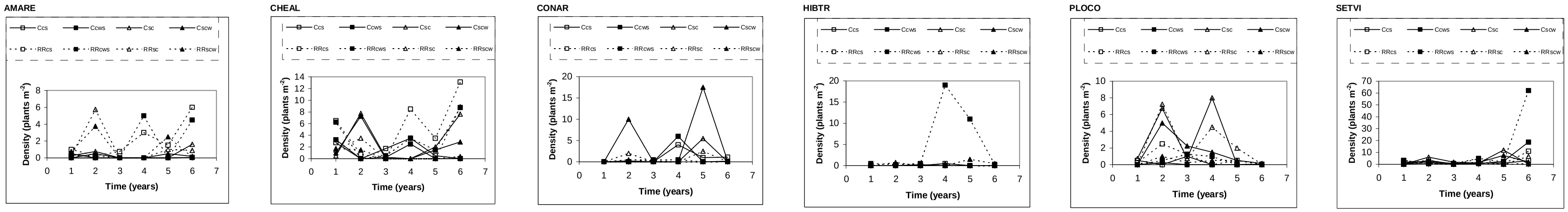
Similar to the NT locations, PLAMA was most closely associated with winter wheat grown in the RR cropping system. Other species associated with winter wheat at Woodstock CT were CERVU, POLPE, and POAPR. Lack of control of PAOPR and similarity in the life cycle coupled with herbicide avoidance due to a short stature in CERVU may explain these associations. Reasons for the association of PLOPE with winter wheat remain unclear.

Huron Park CT

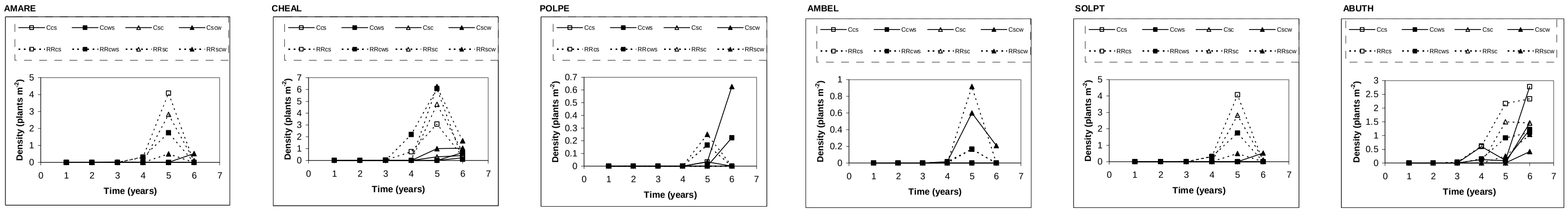
At this location, the first and second canonical axes was required to discern between crop, herbicide system and rotation (Fig. 1) as neither axis alone was able to discern treatments clearly between these main factors. Similar to other locations, the treatment means were primarily grouped according to herbicide system with a secondary grouping based on crop. At this location, mid-season weed densities were greater than at all other locations and reached median weed densities as high as 60

Fig. 5. Median weed denstities in corn and soybean treated with glyphosate or conventional herbicides of the most prominent species at Huoron Park CT, Woodslee CT, and Woodstock CT over the duration of the study.

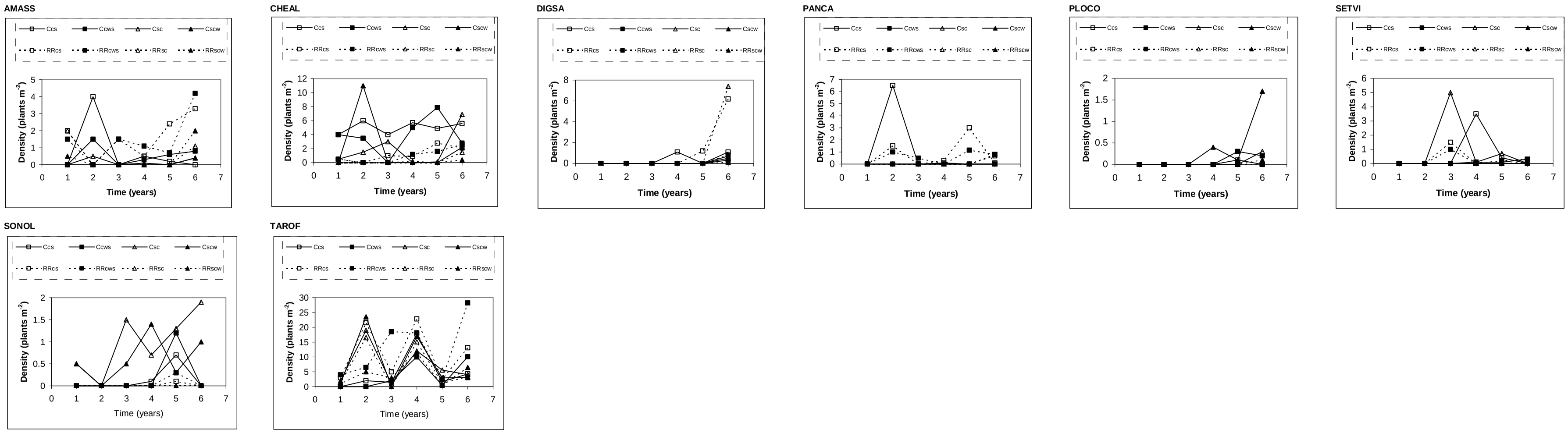
Huron Park CT



Woodslee CT



Woodstock CT



plants m^{-2} in SETVI in corn treated with glyphosate in the 3-year rotation in 2005 (Fig. 5). Many other mid-season weed species were also present at densities greater than 1 plant m^{-2} in at least one year during this study in both herbicide systems including AMARE (=AMASS), AMBEL, CONAR, CHEAL, and HIBTR.

In contrast to all other locations, a large difference was observed in the weed communities between the two winter wheat crops where W_{RR} (winter wheat following corn and soybean previously treated with glyphosate) ordinated distinctly separate from all other treatments. Reasons for this separation between winter wheat in the two herbicide systems are not clear as the weed species spectrum and median weed densities were similar between the winter wheat crops in the two herbicides systems (Fig. 3). W_{RR} was most closely associated with SINAR despite marginally greater median densities of this weed in Wconv when averaged over the 3 years (Fig. 3); however, median mid-season weed densities of other weed species were lower in W_{RR} which increased the relative dominance of this species in this treatment. MELAL was the other species directly associated with winter wheat. This may have been the result of inherent poor control of this species by herbicides used in winter wheat.

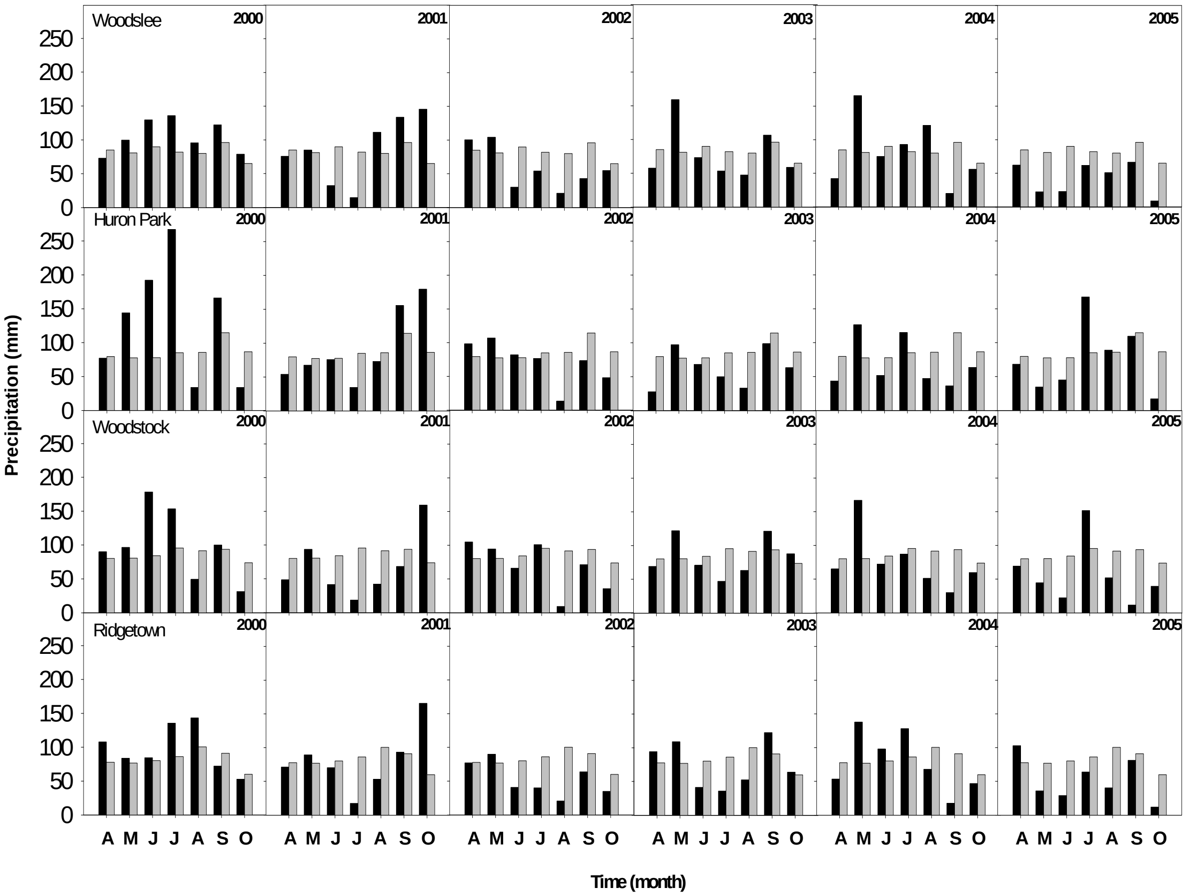
At this location, prominent weeds based on density that were associated with glyphosate were SETVI, AMBEL, and CHEAL in RR soybean and AMARE in RR corn. Herbicide avoidance through late (SETVI and AMARE) and extended (CHEAL) seedling recruitment likely resulted in these associations. Reasons for the association of AMBEL with the RR treatment are less clear as this is considered an early emerging weed with a medium emergence period and the conventional herbicide program used in soybean inherently provides reduced control of this weed. Species with lower densities that were associated with the RR treatments included PLOCO (RRsc), POROL (RRcws), SONAR and HIBTR (RRscw). These associations were likely due to inherently reduced efficacy of glyphosate on these species.

POLLA and CONAR were directly associated with Ccs, while no prominent direct species-treatment associations were found in the remaining conventional herbicide treatments (Ccws, Cscw, and Csc). The conventional herbicide program used in corn in theses experiments typically provides adequate control of CONAR, however, the inherent poor control of CONAR by conventional herbicides used in soybean likely resulted in the highest densities of this weed found in soybean treated with conventional herbicides (Figs. 3 and 5). Reduced residual activity of conventional herbicides on late emerging POLLA may have contributed to this species-treatment association as the activity of conventional herbicides used in both, corn and soybean, is considered excellent.

Woodslee CT

At this location, CDA separated herbicide treatment groups were primarily defined by the first canonical axis, while the second canonical axis defined the crop rotations (Fig. 1). Typical late seeding of crops and a soil type with heavier texture likely contributed weed species associating almost exclusively with corn and soybean treated with glyphosate. Only eight weed species were found at this location, six of which contributed significantly to the weed community (Table 1). Median weed densities tended to be greater in treatments to which glyphosate was applied with only POLPE and ABUTH densities surpassing those observed in the RR system when treated in conventional herbicides in isolated treatments during the last year of the study (Fig. 5). Species in which densities greater than 1 plant m^{-2} were observed in at least one of the last 3 years of the experiment included SOLPT, CHEAL, and AMARE (=AMASS). The lack of species associations with treatments to which conventional herbicides were applied, may have resulted from enhanced weed control due to prolonged residual activity of conventional herbicides applied to corn and soybean caused by below-normal precipitation from June through August in years 4 and 6 at this location (Fig. 6).

Fig. 6. Monthly growing season precipitation (black bars) during each yearat each location throughout this study. Grey bars indicate 30-year monthly mean precipitation at each location.



Weed species behaviour among locations

Total weed densities (Table 3) were not directly correlated with total weed ground cover (see report #1). While total weed ground cover was consistently lower in corn and soybean treated with glyphosate when significantly different, this consistent trend was not observed in weed densities between herbicide systems. For example, mid-season weed densities were greater in glyphosate treated soybean at Woodslee and glyphosate treated corn at Woodslee and Huron Park. The opposite was occurred at other locations, when significant differences were observed. However, despite these differences in mid-season weed densities between herbicide systems at various locations, the average proportion of ground covered by individual weeds, an estimate of weed size, was consistently lower in glyphosate treated corn and soybean than when treated with conventional herbicides when significant differences were observed (Table 4). In corn, this effect was observed at all CT locations, but was absent under NT management, while in soybean this effect was observed at all locations except at Woodstock NT. These results indicate smaller individual weed size in glyphosate treated corn and soybean and suggest that mid-season weeds observed in these treatments were primarily the result of seedlings that emerged after glyphosate application. In contrast, a greater proportion of mid-season weeds in corn and soybean treated with conventional herbicides likely established earlier in the season and possibly escaped herbicide control resulting in increased mean ground cover per individual. Thus, results obtained from mid-season weed densities alone can be somewhat deceiving. In winter wheat, weed densities and estimated mid-season weed size was not affected by the herbicide system used in previous corn and soybean crops.

The strength and the direction of the ordination of individual weed species varied among treatments and locations and showed little consistency between the tillage systems. For example, SETVI and CHEAL were correlated positively at Woodstock NT and CT, but negatively at Ridgetown NT and Huron Park CT. Presence of a herbicide-resistant population of CHEAL at Woodstock likely caused this discrepancy. In CT, no relationship between CHEAL and AMASS was observed with respect to herbicide treatment, while a positive correlation was observed in NT at Woodstock and a negative correlation was found in NT at Ridgetown between these two species. These findings indicate that edaphic (local soil) and/or climatic factors also contributed to the observations. Very few weeds showed consistent behaviour within tillage systems and with the exception of AMASS and PLAMA, these were all observed in NT. AMASS were consistently associated with conventional herbicides at the NT locations and with glyphosate treatments at the CT locations. PLAMA was present at 4 locations (Table 1). At the three sites (Ridgetown NT, Woodstock NT and Woodstock CT), where this species played a significant role in defining the first two canonical variables, PLAMA was most strongly associated with the winter wheat that followed glyphosate resistant crops. In NT, SETVI was associated with winter wheat in which the previous corn and soybean crops were treated with conventional herbicides and ABUTH consistently was associated with conventional herbicide systems. ABUTH was present at only one CT location (Woodslee), where it showed little direct association with either herbicide system. SETVI also showed no consistent trends between the two CT locations where it contributed significantly to the weed community. No distinct tendencies were observed for many other species. For example, POLCO showed a weak association with RR_{SC} in Ct at Huron Park, while showing a stronger association with conventional herbicide treatments in corn at Woodstock under the same tillage system (Fig. 1).

A number of weed species have been suggested to increase in significance in glyphosate-resistant cropping systems (e.g., ABUTH, CHEAL, CONAR, CYPES, DIGSA, and ERICA) by either elevated natural tolerance to or avoidance of application of this herbicide. Many of these species were present, but few showed preferential association with the glyphosate-resistant cropping systems during years 4 to 6 of this study. ERICA was present at three locations, but was only observed at sufficient frequencies for CDA at Ridgetown NT where this weed was strongly associated with winter wheat in the glyphosate-resistant herbicide system, but was present in all treatments (Table 1). At the Woodstock

Table 3. Avearge total mid-season weed densities in soybean and corn grown in a 2-yr (C-S) and a 3-yr (C-W-S) rotation treated with glyphosate (RR) or conventional herbicides at 5 locations. Mean mid-season weed densities in winter wheat treated with conventional herbicides are also indicated. Means and SEM (parentheses) are untransformed.

	Soybean			Corn			Wheat		
	RR	Conv Herb	Sign.$_{sys}$	RR	Conv Herb	Sign.$_{sys}$	RR	Conv Herb	Sign.$_{sys}$
	-------------plants m^{-2}-----------			-------------plants m^{-2}-----------			-------------plants m^{-2}-----------		
CT									
Woodslee									
C-S	4.6 (1.3)	1.0 (0.3)	***[a]	4.8 (1.1)	1.4 (0.5)	***	-[b]	-	
C-W-S	5.3 (1.3)	1.8 (0.4)		5.2 (1.4)	1.2 (0.4)		-	-	
Sign.$_{rot}$	**			n.s.					
Huron Park									
C-S	14.0 (2.5)	26.4 (2.9)	**	24.7 (3.5)	8.6 (1.6)	***	-	-	
C-W-S	13.1 (4.7)	23.0 (4.8)		76.8 (20.9)	15.1 (3.0)		30.6 (9.2)	39.0 (11.4)	n.s.
Sign.$_{rot}$	n.s.			**					
Woodstock									
C-S	10.5 (2.5)	13.0 (2.1)	***	23.1 (2.6)	18.4 (3.2)	**	-	-	
C-W-S	10.1 (3.3)	10.6 (2.0)		23.5 (4.0)	15.3 (1.5)		14.0 (1.9)	12.3 (2.1)	n.s.
Sign.$_{rot}$	n.s.			n.s.					
NT									
Ridgetown									
C-S	2.0 (0.4)	6.3 (1.2)	***	2.0 (0.5)	9.3 (3.0)	***	-	-	
C-W-S	3.6 (1.0)	4.5 (0.7)	n.s.	2.1 (0.5)	2.1 (0.3)		15.5 (1.3)	61.7 (23.9)	n.s.
Sign.$_{rot}$	*	n.s.			**				
Woodstock									
C-S	12.4 (2.2)	17.0 (2.5)	*	13.9 (3.1)	15.3 (2.1)	n.s.	-	-	
C-W-S	7.4 (1.0)	11.2 (1.4)		7.7 (1.7)	8.3 (1.5)		12.3 (0.8)	10.2 (1.6)	n.s.
Sign.$_{rot}$	*			n.s.					

[a] *** $p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.05$, n.s. not significant

[b] - not available

Table 4. Estimate of the size of mid-season weeds in soybean and corn grown in a 2-yr (C-S) and a 3-yr (C-W-S) rotation treated with glyphosate (RR) or conventional herbicides (Conv Herb) at 5 locations. Mean mid-season weed densities in winter wheat treated with conventional herbicides are also indicated Means and SEM (parentheses) are untransformed.

	Soybean			Corn			Wheat		
	RR	**Conv Herb**	**Sign.$_{sys}$**	**RR**	**Conv Herb**	**Sign.$_{sys}$**	**RR**	**Conv Herb**	**Sign.$_{sys}$**
	-----% ground cover plants^{-1}------			-----% ground cover plants^{-1}------			-----% ground cover plants^{-1}------		
CT									
Woodslee									
C-S	3.576 (1.37)	11.37 (1.90)	***	1.26 (0.19)	6.07 (0.69)	***	-[b]	-	
C-W-S	2.714 (0.90)	7.366 (1.34)		2.18 (0.52)	15.14 (4.69)		-	-	
Sign.$_{rot}$	n.s.			*					
Huron Park									
C-S	0.453 (0.12)	1.107 (0.26)	***	0.36 (0.05)	1.60 (0.31)	***	-	-	
C-W-S	0.874 (0.29)	2.342 (1.23)		0.20 (0.04)	1.00 (0.11)		0.575 (0.15)	0.535 (0.11)	n.s.
Sign.$_{rot}$	n.s.			***					
Woodstock									
C-S	0.80 (0.20)	1.12 (0.17)	**	0.55 (0.10)	1.22 (0.16)	***	-	-	
C-W-S	0.89 (0.37)	0.94 (0.10)		0.50 (0.14)	1.07 (0.12)		0.463 (0.14)	0.569 (0.15)	n.s.
Sign.$_{rot}$	n.s.			n.s.					
NT									
Ridgetown									
C-S	2.71 (1.20)	1.781 (0.29)	*	2.96 (0.83)	2.44 (0.57)	n.s.	-	-	
C-W-S	1.132 (0.38)	2.236 (0.56)		1.90 (0.45)	3.75 (0.70)		0.421 (0.10)	0.43 (0.10)	n.s.
Sign.$_{rot}$	n.s.			n.s.					
Woodstock									
C-S	0.934 (0.22)	0.913 (0.15)	n.s.	0.74 (0.12)	0.80 (0.18)	n.s.	-	-	
C-W-S	0.994 (0.13)	1.084 (0.22)		0.77 (0.12)	1.17 (0.26)	n.s.	0.43 (0.11)	0.62 (0.26)	n.s.
Sign.$_{rot}$	n.s.			n.s.	**				

[a] *** $p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.05$, n.s. not significant

[b] - not available

locations ERICA was found sporadically in corn and winter wheat in the glyphosate-resistant herbicide system only. At Woodstock, where a triazine-resistant population of CHEAL, a major weed in the region, was present, this species was associated with the conventional herbicide systems. This weed also was associated most closely with the conventional herbicide system in soybean at Ridgetown NT where the population was susceptible to conventional herbicides. At the remaining CT locations (Huron Park and Woodslee), however, CHEAL was associated with the RR herbicide system again indicating no clear trend among locations. DIGSA which was present at 3 sites showed an association with RR_{CS} at Huron Park CT, displayed an association with the conventional herbicide treatment C_{CWS} at Woodstock NT, and did not contribute significantly to differentiating the weed community at Ridgetown NT.

The occurrence of volunteer crops was infrequent, occurring at very low densities in only 5 and 2 of 30 site-years for volunteer ZEAMX and GLXMA, respectively (Tables 1 and 5). Herbicide systems did not appear to influence the occurrence of volunteer ZEAMX in subsequent soybean crops which tended to be present in soybean treated with conventional herbicides and glyphosate at similar, low densities. Volunteer GLXMA on the other hand, was only found in subsequent corn in the glyphosate-resistant cropping system, indicating consistent control of volunteer soybean in subsequent corn using the conventional herbicide program. Densities of volunteer GLXMA tended to be greater than those of volunteer ZEAMX (still <1 plant m^{-2}); however, the occurrence of this species was less frequent than volunteer ZEAMX. Neither species was detected at the Woodslee and Huron Park locations. At the densities and frequencies observed in this study, there is little evidence that volunteer crops were of greater concern in glyphosate-resistant cropping systems compared to systems using conventional herbicides in corn and soybean.

Weed Biodiversity Indicators

At each location, the effects of herbicide system and crop rotation on the mid-season species richness, the Shannon-Wiener diversity index, and species evenness were compared within each crop. Results from this analysis are shown in Table 6. As suggested in Table 1 and Fig. 1, species richness in individual plots was greatest at Ridgetown NT (up to 10 species per plot per year) and lowest at Woodslee CT (as few as 1 species per plot per year). Distinctly different trends in species richness as influenced by herbicide system were observed between the two tillage systems in corn and soybean. In NT, species richness was consistently lower in corn and soybean when treated with glyphosate compared to conventional herbicides indicating improved broad-spectrum weed control by glyphosate. At the Ct locations in corn, species richness was greater when treated with glyphosate, while in soybean no clear trend was observed among the three locations. The Shannon-Weiner diversity index analysis mirrored these trends, suggesting a direct relationship between these two biodiversity indicators. Moreover, species evenness was similar among herbicide systems and crop rotations in most cases, further supporting that the Shannon-Wiener index values were almost exclusively influenced by species richness. Lack of significant differences in species evenness also indicated that all species present tended to be distributed relatively uniformly within each plot, irrespective of herbicide system. These results confirm observations in the multivariate analysis regarding the number of weed species associated with each herbicide system at locations with the same tillage system. The observed differences in species richness and diversity between the tillage systems suggests either increased residual efficacy of conventional herbicides under CT, changes in weed seedling recruitment patterns, or a combination of these. Crop rotation exerted little influence on species richness, the Shannon-Wiener diversity index, and species evenness in corn and soybean at all locations and no clear trends in species evenness were observed between tillage systems (Table 6).

In wheat, no differences in species richness between the herbicide systems were observed at the four locations for which data were available (Table 6). These results indicate no carry-over effect of the herbicide system used in previous soybean and corn crops. At the two NT locations, however, Shannon-

Table 5. Mean densities of volunteer corn and soybean in the following soybean or corn crop, respectively , in conventional herbicide (Conv.) and glyphosate-resistant (RR) cropping systems. Standard errors of the means are provided in parentheses.

	Tillage system	CT						NT			
	Location	Woodslee		Huron Park		Woodstock		Woodstock		Ridgetown	
	Herbicide system	RR	Conv.	RR	Conv.	RR	Conv.	RR	Conv.	RR	Conv.
Species	**Year**	plants m^{-2}									
ZEAMX	**2000**	0	0	0	0	0	0	0	0	0	0
	2001	0	0	0	0	0	0	0	0	0	0
	2002	0	0	0	0	0	0	0	0	0	0
	2003	0	0	0	0	0.08 (0.07)	0.18 (0.10)	0.02 (0.004)	0.004 (0.004)	0.003 (0.003)	0.02 (0.02)
	2004	0	0	0	0	0	0	0	0	0.05 (0.04)	0.05 (0.03)
	2005	0	0	0	0	0	0	0	0	0.01 (0.01)	0
GLXMA	**2000**	0	0	0	0	0	0	0	0	0	0
	2001	0	0	0	0	0	0	0	0	0	0
	2002	0	0	0	0	0	0	0	0	0	0
	2003	0	0	0	0	0.25 (0.25)	0	0.15 (0.12)	0	0	0
	2004	0	0	0	0	0	0	0	0	0	0
	2005	0	0	0	0	0	0	0	0	0	0

Table 6. Weed community biodiverisity indicators including species richness, Shannon-Wiener index, and species eveness. Significance of main effects and interactions are shown where appropriate. Parenthetical values indicate the standard errors of the means.

		Species Richness			Shannon-Wiener Index			Species Evenness		
		RR	Conv	Sign.$_{sys}$	RR	Conv	Sign.$_{sys}$	RR	Conv	Sign.$_{sys}$
Soybean										
CT										
Woodslee										
	C-S	2.9 (0.7)	1.3 (0.3)	** [a]	0.85 (0.15)	0.32 (0.09)	***	0.76 (0.03)	0.66 (0.09)	*
	C-W-S	2.9 (0.6)	3.0 (0.5)	n.s.	0.68 (0.09)	0.97 (0.12)	n.s.	0.64 (0.05)	0.77 (0.09)	*
	Sign.$_{rot}$	n.s.	**		n.s.	***				
Huron Park										
	C-S	5.4 (0.8)	4.8 (0.5)	n.s.	1.04 (0.11)	1.01 (0.08)	n.s.	0.73 (0.05)	0.68 (0.09)	n.s.
	C-W-S	5.0 (1.0)	6.2 (0.8)	n.s.	0.88 (0.16)	1.20 (0.09)		0.57 (0.07)	0.54 (0.15)	
	Sign.$_{rot}$	n.s.	*		n.s.			n.s.		
Woodstock										
	C-S	3.8 (0.7)	5.3 (0.6)	n.s.	0.62 (0.18)	0.94 (0.15)	***	0.62 (0.06)	0.70 (0.03)	n.s.
	C-W-S	3.6 (0.9)	4.9 (0.8)		0.55 (0.16)	0.91 (0.17)		0.58 (0.07)	0.59 (0.04)	
	Sign.$_{rot}$	n.s.			n.s.			n.s.		
NT										
Ridgetown										
	C-S	5.8 (1.2)	8.8 (0.5)	**	0.68 (0.16)	0.91 (0.16)	*	0.55 (0.05)	0.54 (0.06)	n.s.
	C-W-S	10.0 (0.7)	10.7 (0.9)		1.05 (0.16)	1.18 (0.16)		0.51 (0.06)	0.67 (0.03)	
	Sign.$_{rot}$	n.s.			***			n.s.		
Woodstock										
	C-S	3.6 (0.5)	5.2 (0.4)	*	0.35 (0.09)	0.79 (0.09)	***	0.61 (0.09)	0.76 (0.04)	***
	C-W-S	2.9 (0.5)	4.6 (0.6)		0.39 (0.09)	0.79 (0.12)		0.52 (0.07)	0.83 (0.04)	
	Sign.$_{rot}$	***			n.s.			n.s.		
Corn										
CT										
Woodslee										
	C-S	3.9 (0.5)	2.0 (0.4)	***	0.98 (0.12)	0.34 (0.12)	***	0.78 (0.03)	0.69 (0.07)	n.s.
	C-W-S	4.3 (0.6)	2.3 (0.3)		0.84 (0.14)	0.60 (0.11)		0.61 (0.04)	0.78 (0.06)	
	Sign.$_{rot}$	n.s.			n.s.			n.s.		
Huron Park										
	C-S	6.6 (0.7)	4.5 (1.0)	***	1.29 (0.05)	0.78 (0.13)	***	0.73 (0.07)	0.69 (0.04)	n.s.
	C-W-S	7.3 (0.8)	4.2 (0.9)		1.04 (0.10)	0.55 (0.18)		0.67 (0.07)	0.72 (0.05)	
	Sign.$_{rot}$	n.s.			n.s.			n.s.		
Woodstock										
	C-S	7.2 (0.6)	5.7 (0.3)	**	1.24 (0.16)	1.21 (0.07)	n.s.	0.52 (0.09)	0.57 (0.07)	n.s.
	C-W-S	8.3 (0.8)	6.8 (0.8)		1.26 (0.20)	1.09 (0.13)		0.57 (0.09)	0.60 (0.07)	
	Sign.$_{rot}$	*			n.s.			n.s.		
NT										
Ridgetown										
	C-S	6.3 (0.8)	9.2 (1.1)	***	0.98 (0.10)	1.19 (0.17)	n.s.	0.43 (0.06)	0.41 (0.06)	n.s.
	C-W-S	5.3 (0.7)	9.3 (0.7)		0.83 (0.14)	1.49 (0.11)	***	0.51 (0.07)	0.50 (0.06)	
	Sign.$_{rot}$	n.s.			n.s.	n.s.		n.s.		
Woodstock										
	C-S	4.3 (0.6)	6.1 (0.4)	***	0.95 (0.17)	1.37 (0.09)	***	0.27 (0.04)	0.49 (0.06)	**
	C-W-S	3.8 (0.5)	5.0 (0.6)		0.62 (0.10)	1.25 (0.10)		0.32 (0.05)	0.49 (0.06)	
	Sign.$_{rot}$	n.s.			*			n.s.		
In following winter wheat										
CT										
Woodslee		- [b]	-		-	-		-	-	
Huron Park		6.4 (1.14)	5.0 (1.01)	n.s.	0.89 (0.12)	0.72 (0.12)	n.s.	0.55 (.07)	0.53 (0.07)	n.s.
Woodstock		5.4 (0.98)	5.7 (1.03)	n.s.	1.03 (0.13)	1.00 (0.12)	n.s.	0.67 (0.03)	0.64 (0.05)	n.s.
NT										
Ridgetown		7.8 (1.11)	9.5 (0.38)	n.s.	0.39 (0.06)	0.75 (0.20)	*	0.21 (0.04)	0.33 (0.09)	n.s.
Woodstock		4.1 (0.75)	4.7 (0.57)	n.s.	0.85 (0.15)	1.14 (0.09)	*	0.66 (0.07)	0.80 (0.03)	*

[a] *** $p < 0.001$, ** $0.001 < p < 0.01$, * $0.01 < p < 0.05$, n.s. not significant

[b] - not available

Wiener diversity was significantly lower in wheat when previous soybean and corn were treated with glyphosate compared to conventional herbicides. This finding indicates persistence of the diversity differences observed between the herbicide systems beyond the year of herbicide application. In contrast, no differences in weed diversity were detected in winter wheat under CT management.

Summary

In summary, although yield and agronomics showed few differences among the herbicide systems, multivariate analysis indicated that the mid-season weed communities in corn and soybean were strongly influenced by herbicide system and to a lesser extent crop rotation. Tillage system influenced the total number of species that ordinated with the herbicide system treatment means and also influenced biodiversity indexes (i.e., species richness and the Shannon-Wiener diversity index) between herbicide systems. Weed species diversity in winter wheat was affected by herbicide system at both NT locations, indicating a carry-over effect of weed management in previous corn and soybean. However, association of individual weed species with treatments tended to be variable among locations and also appeared to be affected by edaphic and/or climatic factors. With the exception of volunteer corn (ZEAMX) at Woodstock and ERICA in wheat grown after glyphosate-resistant corn and soybean at Ridgetown NT, there was little evidence suggesting selection for or enrichment of weed species that are naturally more tolerant to or avoid glyphosate application during the last 3 years of this 6 year study. Although differences in weed density observed between herbicide system were not always consistent, average estimated weed size was consistently smaller in glyphosate treatments when significantly different from those treated with conventional herbicides.