Department of Physics, Chemistry and Biology

Final Thesis

Repeated Grading of weed Abundance and Multivariate Methods to Improve Efficacy in On-farm Weed Control Trials

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1 Abstract

Thousands of field trials are conducted annually to evaluate the usefulness of various techniques in weed control. Conventional data collection and statistical methods lead to relatively scant information from trials because of the spatial heterogeneity and temporal changes in weed abundance. To evaluate whether additional information could be drawn from new methods in on-farm trials, two experiments were carried out to compare different data collection and statistical methods. First, we compared conventional sampling method using biomass estimate of weed abundance to repeated visual assessment of the percentage ground cover. Biomass was sampled once after the treatment whereas ground cover was repeatedly sampled once before plus several occasions after the weed control. Secondly, we contrasted outcomes from ANOVA taking samples from a single point in time with repeated measures ANOVA and a multivariate method (pRDA). It was concluded that ground cover estimate of weed abundance was as reliable as biomass estimate because the outcomes and conclusions drawn were relatively similar. The repeated measures ANOVA enabled to follow the temporal dimensional trend and the initial flora differences. Multivariate analysis went even further by displaying species-wise the impact of each control tool in the frame of the environmental gradients.

Key words: ANOVA, Ground cover, Multivariate analysis, Repeated measures, Weed control

2 Introduction

Weeds are Man's worst pest organisms, interfering with food production everywhere and reducing production, economic growth and food security (Pimentel *et al.* 1999, Milberg & Hallgren 2004, Sinden *et al.* 2004, Jones *et al.* 2005). Therefore, worldwide thousands of field trials are conducted annually to evaluate the usefulness of various techniques for weed control. There are normally two aims combined in these trials. First, it is to evaluate economical or other benefits of the new method compared with an established one. The end point of primary interest is then crop yield. To be meaningful, such trials have to be located on farmers' fields, i.e. the method has to be evaluated under realistic field conditions typical for producers in the region (Koenig *et al*. 2000, Petheram 2000). The second aim is to evaluate the selectivity of the new method, i.e. to what extent certain weed species will be more or less affected (e.g. is a problematic weed species better controlled by the new method compared with the conventional one?).

Unfortunately, these trials produce data with very large uncontrolled variation. For example, the parameter "yield loss due to weeds", which is calculated from yields in treated plots and weed-free reference plots, can be up to 20% even in the absence of weeds (Milberg & Hallgren 2004). This is an artefact due to spatial heterogeneity within a weed-free crop stand. Weeds, however, are even more patchily distributed than the crop biomass is. The current way to analyse these experiments, by pairing data from treated plots and reference plots, means that a substantial part of the variation is created by the spatial heterogeneity of the weed population (Walter *et al*. 2002). Or, the plots do not have the same initial weed flora. Even where the researcher has artificially created the weed stand (e.g. Buhler 1997, Tamado *et al*. 2002), initial number and composition of weeds will not be identical in plots. Therefore, large number of similar experiments is needed to be able to evaluate selectivity (Rew & Cousens 2000, Milberg & Hallgren 2002).

There are large costs involved in establishing, maintaining, harvesting, processing and analysing this type of trials. The spatial variability in weed composition, however, makes them ill suited to analyse treatment selectivity among weed species. Therefore, much could potentially be gained if better and more detailed information on weed responses could be collected and analysed in these experiments.

To circumvent the spatial variability that may affect the outcomes and to include the time dimension in the experiment, repeated measures and some specific statistical methods might be very useful for a more detailed assessment of treatment effects in on-farm weed control trials. In fact, such a repeated sampling method together with repeated measures analysis of variance (rmANOVA) are frequently used in various scientific disciplines such as environmental assessment, medicine, econometrics, operations research, quality improvement, ecology, etc. (Smith 2002, Hopkin 2003, SAS 2005).

In addition, multivariate statistics (ordination methods) is designed to summarise a complex data structure in a low-dimensional space, while retaining as much of the underlying trended variation as possible (Dieleman 2000, Kenkel 2002, Hallgren *et al.* 1999). This method is favoured and proposed for community level analysis, rather than repeated measures ANOVA, when it comes to analyse biological monitoring studies at the community level (Kedwards 1999a, 1999b). Therefore, it might be worthwhile to investigate this statistical method in repeatedly sampled weed abundance.

The aim of this study was to compare the current sampling method, the above ground biomass at the peak of the season, to repeated visual assessments of percentage ground cover. In addition, a correlation analysis was run to examine the reliability of the percentage ground cover vis-à-vis to the biomass estimate. We also examined if rmANOVA and multivariate statistics on repeated measures would add value to the information reached when using ANOVA.

3 Material and methods

3.1 Field trials

Our study targeted two on-farm weed control trials (Klostergården and Tegneby) managed by the Agricultural Society in Östergötland (ASÖ), southern Sweden, during the period of April to August 2005.

3.1.1 Klostergården

The experiment, located 15 km north of Linköping city (Klostergården 58^{o} 27' N and 15^{o} 30' E), compared different herbicide weed control methods in spring sown barley (*Hordeum vulgare* L.). A quantity of 175 kg ha^{-1} of seeds was sown on 22 April 2005 on a clay soil, rich in humus with a pH of 6.7. The field was fertilised with 330 kg ha^{-1} NPK (89,0,0).

The experimental set-up consisted of a completely randomised block design with four blocks and 15 treatments. Apart from one untreated control, 14 treatments consisted of herbicides that were mixtures of chemicals at different doses. Each treatment plot was 39 m^{2} (3 m x 13 m). An early herbicide application concerned 13 treatments at the stage when the crop had 3 to 4 leaves whereas a late treatment occurred at the Zadoks stage 37 (Anderson *et al*. 2002) two weeks later (Table 1). The herbicides applied were obtained from Agrilab AB of Uppsala in Sweden.

3.1.2 Tegneby

Tegneby (58^{o}28' N, 15^{o}41' E) is located 30 km north-west of Linköping. This experiment compared three different mechanical implements to control of *Cirsium arvense* in oats (*Avena sativa*) ecological farming. Implements were equipped with a "straight cutting edge" (A), "oblique cutting edge" (B) or a "goose foot-like cultivator" (C). In the present study we were interested in the possible differences in all weed species with respect to these implements.

The experiment was a split-plot design with unreplicated main plots representing treatment time and sub-plots that represented the three implements A, B and C with two replications. The first whole-plot (A1, B1

and C1) was treated on 3rd June whereas the second one (A2, B2 and C2) was treated on the 20th the same month.

Table 1: Description of the used herbicides (doses/mixtures) and the date of application at Klostergården

Plot name	*Herbicide*
A	*Not treated*
B	*1.0 tabl (5.0 g) Express + 0.1 l Lissapol Bio **
C	*1.0 tabl (5.0 g) Express + 0.1 l Silwet Gold **
D	*1.0 l Verigal **
E	*2.0 l Verigal **
F	*1.0 l Verigal + 1.0 tabl (5.0 g) Express **
G	*1.0 l Verigal + 50 ml Primus **
H	*2.0 l Ariane S **
I	*75 ml Primus + 0.5 tabl (2.5 g) Express + 0.1 l Lissapol Bio **
J	*40 g Ally Class **
K	*50 g Hussar + 0.5 l Renal **
L	*75 g Hussar + 0.5 l Renal **
M	*125 g Hussar + 0.5 l Renal **
N	*150 g Checker + 0.5 l Renol **
O	*1.5 tabl (7.5 g) Express + 0.1 l Lissapol Bio l ***

**: The first treatment times (3rd June 2005)*

***: The second treatment occasion (20th of June 2005)*

3.2 Sampling methods

Repeated assessments of percentage ground cover for each weed species were done for both experiments. For each treatment, three permanent sampling points were randomly located on the first recording. In order to locate them easily for the following sampling times, one stick was fixed on the chosen point. At each occasion, we considered a circular sampling unit by using a string of 28.3 cm that was rotated around the fixed stick. Thus the area of the sampling unit was equal to 0.25 m^2. This methodology was appropriate because late in the season the crop might be a problem when using other kind of frame such as grid (e.g. squeezing a frame down bolted cereals). The sampling was non-destructive in order to follow weed species dynamic during the whole cultural season.

We did the first recording before treatment to catch the initial flora or baseline data (Lepš *et al.* 2003). Before the second treatment (two weeks later), a second recording was done. Afterwards, three and four other recordings were taken at Klostergården and Tegneby respectively with a two weeks interval from June to August. A visual estimate of the ground cover (%) for each weed species present was noted for each sampling unit.

Fogelfors (1977) and Korsmo *et al.* (1981) facilitated the identification of weed species.

We also sampled weed biomass before harvest of the crop (late August) at Tegneby and used the biomass data sampled in early July by the ASÖ at Klostergården. Weeds were cut at soil surface, sorted according to species and dried at 85^{o} C for four days and weighted (Blumenthal 2003, Hyvönen 2004).

3.3 Statistical methods

First of all, ANOVA and rmANOVA from STATISTICA 7.0 software (StatSoft Inc. 2004) using General Linear Models were run. In the same software, for Tegneby where the biomass and percentage ground cover estimates emerged from the same sampling units, an analysis of correlation between them was established. Second, partial Redundancy Analysis (pRDA) from multivariate ordination in CANOCO 4.5 (ter Braak & Šmilauer 1998) was applied to our data sets.

For the data set from Klostergården, the untreated control was excluded from the analyses because we were interested in the comparison of the impacts of herbicides among themselves rather than individual herbicide impact.

3.3.1 ANOVA and Repeated Measures ANOVA

ANOVA, the conventionally used statistical analysis in the region (Arvidsson & Andersson 2003), was applied to the data set sampled on the third occasion at Klostergården and the last sampling time at Tegneby. And, rmANOVA included the analyses of treatment and time factor effects but also their interaction effect through the season.

The response variables in ANOVA consisted of either a single species or a group of species. The three sampling points per treatment plot were averaged to get one value per plot/treatment, per species or group of species. At Klostergården, V*iola* spp., "other annuals" and "all annual weeds" variables were analysed because *Viola* spp. was the most abundant weed and we wanted to group species as the ASÖ did. At Tegneby, *Cirsium arvense* was taken alone because it was of interest for the ASÖ. *Sinapis arvensis* was identified to be a best reference to compare biomass and ground cover estimates due to its visible morphological changes of cover along the seasonal development.

In case of significant differences ($P< 0.05$), we did post-hoc Tukey HSD tests to group the treatments for ANOVA whereas for the rmANOVA graphs illustrated the variations due to considered factors.

3.3.2 Partial Redundancy Analysis

The main environmental variables taken into account were the treatments (herbicides or implements) and time while block factor was taken as covariable. All these variables and covariables were coded as a number of dummy variables.

Preliminary analysis using Detrended Correspondence Analysis (DCA) was conducted to decide whether to use the linear or unimodal type of ordination method. As the beta diversity in the community composition was relatively low, we followed the advice of Lepš and Šmilauer (2003) and used linear method: partial Redundancy Analysis (pRDA). Monte Carlo permutation test with 9999 permutations allowed significance test between or within permutations blocks (treatment, time or interaction terms). Analyses were run at two levels: either data collect at one sampling time or the whole data set from the repeated measures. The permutation tests at the one-time sampled data sets level concerned the explainable variation in species composition between treatments and blocks whereas for the repeated measures pRDA, the experimental design was taken into account (i.e. repeated measures and blocks). Principal Component Analysis (PCA) was used for illustration purposes in some cases.

In cases where the P-value was <0.05, ordination graphs were presented. Graphs illustrated the weed abundance with arrows whereas environmental factors were illustrated with centroids (black triangles).

4 Results

4.1 Klostergården

In this experiment, 17 weed species were recorded (Appendix). Among them, 15 were annual species with very few perennial species (*Cirsium arvense* and *Taraxacum officinale)*.

4.1.1 *Viola* spp.: ANOVA

Even though ground cover and biomass estimates did not emerge from the same sampling units, there were, in both cases, significant differences among treatment, time and interactions terms (Table 2). But the block effect was non-significant for ground cover.

Post-hoc Tukey HSD tests classified the best treatments as E, J, D and M that best controlled *Viola* spp. for both ground cover and biomass. The less effective treatments were B, C and O whereas F, G, H, I, K, L and N were moderately effective to control *Viola* spp. (Table 3).

In both cases, the untreated plots showed higher abundance compared to treated plots but the extent to which this shown differs. The biomass for the control was more than three fold the abundance of the less effective herbicide C (17.5 versus 5.5 g m^{-2}) whereas for ground cover, however, they did not differ considerably (4.1 % for the control A and 3.8 % ground cover for treatment O).

Table 2: ANOVA (for the third sampling time) and rmANOVA for Viola spp at Klostergården. (: Significant and NS: Non significant)*

Variable	Effect	Df	MS	F	P
Biomass (g m^{-2})	Treatment	13	6,46	19,42	0,0000 *
	Block	3	1,45	4,36	0,0096 *
	Error	39	0,33		
Ground cover (%)	Treatment	13	14,11	3,76	0,0006 *
	Block	3	5,86	1,56	0,2145 NS
	Error	39	3,75		
Repeated measures Ground cover (%)	Treatment	13	3.88	17.35	0,0000*
	Block	3	4.77	21.36	0,0000*
	Error	39	0.22		
	Time	4	21.53	119.64	0,0000*
	Time * Block	12	0.53	2.99	0,0008*
	Time * treatment	52	0.46	2.60	0,0000*
	Error	156	0.18		

Table 3: Summary of Tukey HSD tests after ANOVA for ground cover and biomass vis-à-vis the treatment factor: Viola spp. at the third sampling time at Klostergården

Ground cover (%)			Biomass (g m^{-2})		
Treat	Mean	Group	Treat	Mean	Group
Control	4.08		Control	17.50	
D	0.00	a	E	0.25	a
J	0.00	a	J	0.25	a
M	0.00	a	G	0.50	ab
E	0.08	a	M	0.50	ab
L	0.12	a	D	0.75	abc
F	0.12	a	H	1.50	abc
N	0.21	a	F	1.75	abc
H	0.46	a	I	2.25	abc
G	0.71	a	N	2.25	abc
K	0.75	a	K	2.50	abc
I	1.00	ab	L	2.50	abc
B	2.33	bc	B	5.25	bc
C	3.21	cd	O	5.25	bc
O	3.83	d	C	5.50	c

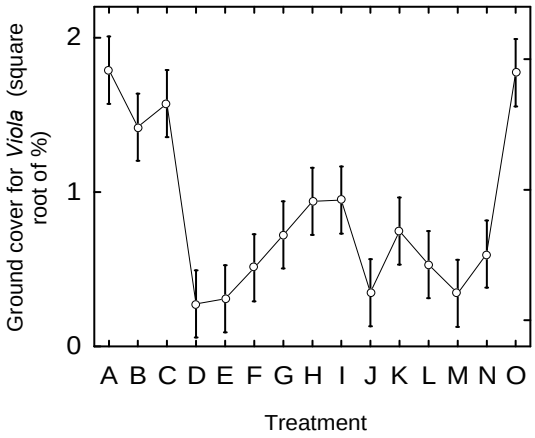


Figure 1. Square root of mean percentage ground cover of Viola iand treatments at Klostergården (with 95 % confidence interval)

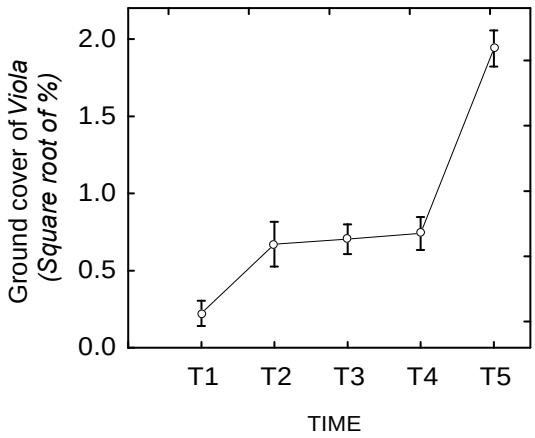


Figure 2. Square root of mean percentage ground cover of Viola over time at Klostergården (with 95 % confidence interval)

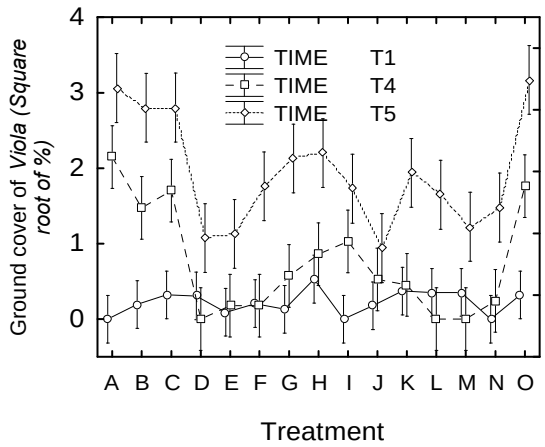


Figure 3. Square root of mean percentage ground cover of Viola (with 95% confidence interval) with respect to interaction between treatment and the first (T_1), fourth (T_4) and last (T_5) sampling times at Klostergården

The rmANOVA illustrated the same hierarchy among herbicides as for the ANOVA (Figures 1-3). Furthermore, they illustrated how weed abundance was time dependent. The first sampling time was characterised by sparse cover of V*iola* spp. and it increased with time except for four treatments (D, E, L and M) for which weed abundance was lower than the initial weed flora. Looking at sampling times two (T_2), three (T_3) and four (T_4), there were no apparent differences in ground cover (Figure 2). For clarity, only one of them was used illustrating the interactions between treatment and time factors (Figure 3).

4.1.2 Other and all annual weeds: ANOVA

For other annual weeds, the biomass estimate did not show differences among treatments whereas the ground cover showed high significance for the third sampling time (Table 4).

Table 4: ANOVA (third sampling time) and repeated measures ANOVA for other and all annual weeds (: Significant and NS: Non significant)*

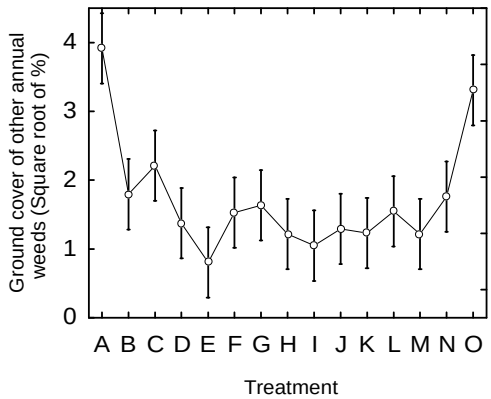
Variable	Estimate	Effect	Df	MS	F	P
Other annual weeds	Biomass (g m^{-2})	Treatment	13	126.87	1.68	0.1038 NS
		Block	3	165.35	2.19	0.1041 NS
		Error	39	75.35		
	Ground cover (%)	Treatment	13	67.14	13.25	0.0000 *
		Block	3	8.71	1.72	0.1784 NS
		Error	39	5.06		
	Repeated measures Ground cover (%)	Treatment	13	7.46	6.02	0.0000 *
		Block	3	3.07	2.48	0.0752 NS
		Error	39	1.23		
		Time	4	22.46	43.99	0.0000 *
		Time * Block	12	0.69	1.35	0.1933 NS
		Time * treatment	52	1.05	2.06	0.0003 *
		Error	156	0.51		
All annual weeds	Biomass (g m^{-2})	Treatment	13	211.06	2.59	0.01073 *
		Block	3	203.20	2.49	0.07370 NS
		Error	39	81.31		
	Ground cover (%)	Treatment	13	105.36	18.98	0.0000 *
		Block	3	12.11	2.18	0.1053 NS
		Error	39	5.54		
	Repeated measures Ground cover (%)	Treatment	13	10.57	9.99	0.0012 *
		Block	3	6.74	6.37	0.0000 *
		Error	39	1.05		
		Time	4	25.64	55.13	0.0000 *
		Time * Block	12	0.90	1.94	0.0334 *
		Time * treatment	52	1.126	2.41	0.0000 *
		Error	156	0.46		

Treatments grouped by post-hoc Tukey test showed minor differences between the outcomes from biomass and cover(Table 5 and Figure 4a).

Table 5: Summary of Tukey HSD tests after ANOVA for ground cover and biomass vis-à-vis treatment factor: Other and all annual weeds (NS effect judged to appear for comparison)

All annual weed species						Other annual weed species					
Ground cover (%)			Biomass (g m^{-2})			Ground cover (%)			Biomass (g m^{-2}) *		
Treat	Mean	Group	Treat	Mean	Group	Treat	Mean	Group	Treat	Mean	Group
A	19.58		A	163.00		A	15.50		A	145.00	
E	0.16	a	G	0.50	a	K	0.00	a	G	0.00	a
J	0.29	a	E	1.75	a	E	0.08	a	I	0.50	a
K	0.75	ab	J	1.75	a	J	0.29	a	J	1.50	a
M	1.00	ab	M	2.50	ab	I	0.29	a	E	1.50	a
N	1.00	ab	I	2.75	ab	H	0.62	a	M	2.00	a
H	1.08	ab	D	3.50	ab	N	0.79	a	N	2.00	a
F	1.20	ab	F	4.00	ab	M	1.00	a	F	2.25	a
I	1.29	ab	N	4.00	ab	F	1.08	a	D	2.75	a
D	1.41	ab	H	5.25	ab	L	1.37	a	H	3.75	a
L	1.50	ab	L	7.00	ab	D	1.41	a	L	4.50	a
G	3.45	ab	K	8.50	ab	B	2.16	a	K	6.00	a
B	4.50	ab	B	13.00	ab	G	2.70	a	C	7.50	a
C	6.54	b	C	19.50	ab	C	3.33	a	B	14.25	a
O	19.87	c	O	25.00	b	O	16.04	b	O	25.00	a

a)



b)

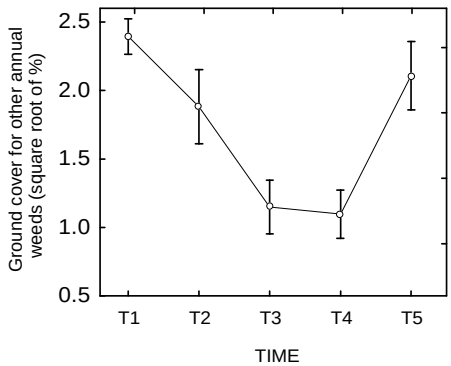


Figure 4*. Percentage ground cover of other annual weeds at Klostergården (with 95% confidence interval). a) Treatment factor, b) Time factor*

The best treatments were E, J and M for both cover and biomass. Treatment K was found to be among best for percentage cover whereas G was classified best when biomass was considered. Treatments B, C and O remained less effective treatments. Weeds abundance decreased T_2 to T_4 (lowest abundance) and increased again at the T_5 during weed recruitment (Figures 4b and 5).

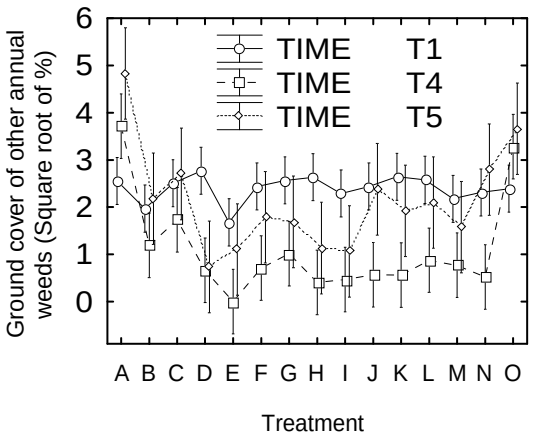


Figure 5. *Ground cover of other annual weeds at Klostergården (with 95% confidence interval) showing interaction between treatment and the first (T_1), fourth (T_4) and last (T_5) sampling times*

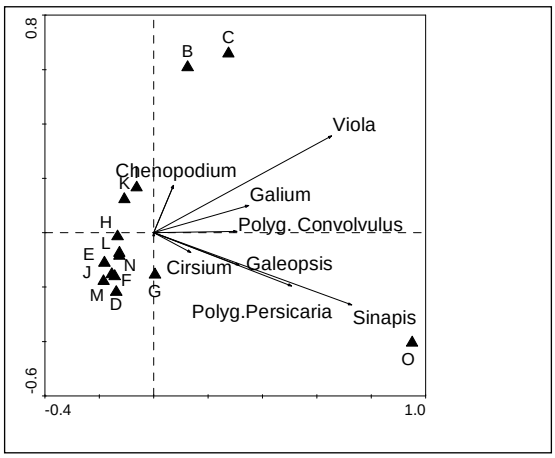
4.1.3 Partial RDA

All the environmental variables tested (treatment, blocks, time and interaction between time and treatment) showed significant effects for the abundance records at the third sampling time and the repeated assessments (Table 6). It was deducted from the ordination graphs (Figure 6) that the treatment O, B and C were the least effective because they had the largest abundance of all weed species. All other treatments were located to the opposite direction of the positive increase of environmental gradient of most weed species. In these least effective treatments, it was not only V*iola* spp. but also *Polygonum convolvulus*, *Galeopsis* spp., *Cirsium arvense*, *Sinapis arvensis* etc. had noteworthy abundances. The outcomes from ground cover sampled at the third time compared to the whole data set obtained from all sampling times were similar (Figure 6).

Table 6: Summary of Monte Carlo permutation test in pRDA (with 9999 permutations) resulting from different analyses of variance for each one of the following factors when all others were taken as covariables. The sampling unit within each plot was taken as covariable in all tests. Permutation blocks took into account the experimental design (repeated measures and blocks). The control plots were excluded from the tests.

Variable & time	Factors	Trace	F-ratio	P-Value
Ground cover (%) at T3 for all present species	Treatment	0.35	6.56	0.0001 *
	Block	0.03	2.33	0.0007 *
Biomass (g m^{-2}) at T3 only for a few weed species	Treatment	0.42	2.72	0.0002 *
	Block	0.12	3.26	0.0056 *
Ground cover (%) for all sampling times and all present weed species	Treatment	0.11	10.90	0.0001 *
	Time	0.20	64.14	0.0001 *
	Block	0.02	8.85	0.0001 *
	Time * treat	0.04	4.77	0.0001 *

a)



b)

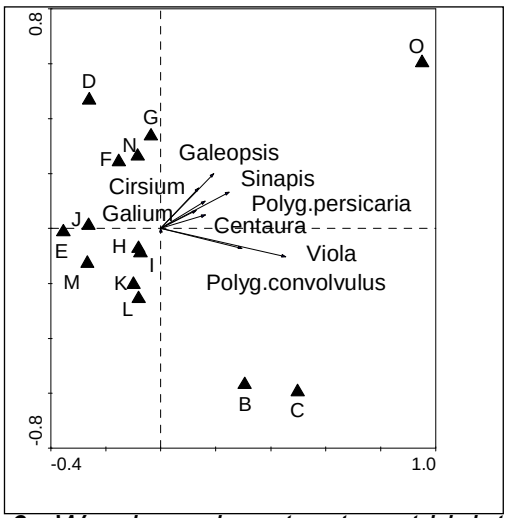
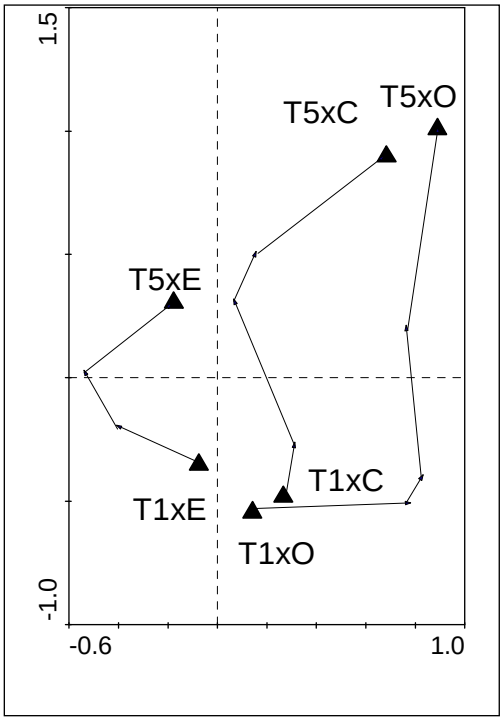


Figure 6. Weed species - treatment biplots obtained with pRDA at Klostergården illustrating the effect of the treatment on the weed species. Letters represent treatments corresponding to herbicides in Table 1 (untreated control excluded) whereas the arrows indicate the direction of increasing abundance of the species in question. Graph a) shows the third sampling time and graph b) represents the repeated measures obtained from T_1-T_6.

a)



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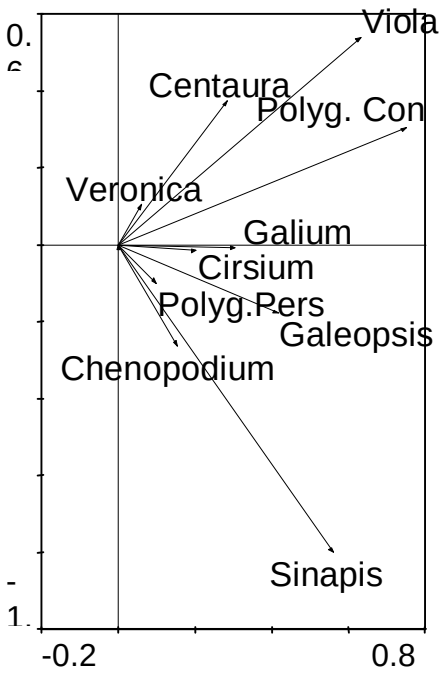


Figure 7. Representation of interaction between time and treatment factors at Klostergården using PCA. The PCA was conducted using all 14 treatments, but three only were selected, one best and the two least effective. a) trajectories over time in ordination space; and b) representation of weed species abundance corresponding to these environmental factors.

The dynamics of weed abundance (cover) over time for the two least effective treatments (C and O) and the most effective treatments (E) as illustrated using PCA (Figure 7). At T_1, the initial weed abundance was almost the same for the three treatments. From the second sampling occasion (T_2) there was a spread of centroids. Treatments C and O were spread towards the environmental gradients favouring weed species presence. Adversely, treatment E evolved towards the opposite direction. There was a shift in weed species over the season as noted for O and C from *Sinapis arvensis* early in the season towards *Viola* spp. later in the season. Treatment O was the least effective during the whole season. PC1 displayed the differences between treatments whereas PC2 highlighted the time factor.

4.2 Tegneby

Eighteen weed species were recorded with 13 annual weed species and 5 perennials (Appendix).

4.2.1 *Sinapis arvensis*, other annual weeds and *Cirsium arvense:* ANOVA

ANOVAs on percentage ground cover and biomass sampled at the sixth occasion reached very similar outcomes. Implement and block factors did not affect biomass or cover of *Sinapis arvensis, Cirsium arvense* or other annuals. Repeated measures ANOVA highlighted significant effects in abundance among sampling times and treatment times in some cases. Even with this kind of analysis, there were no apparent effects of implement and blocks. For *Sinapis arvensis* and other annuals, differences were found between plots treated earlier in the season compared to those treated later. Time factor did not affect species abundance for *Sinapis arvensis* and other annuals. Time-treatment interaction effects were not significant (Table 7).

Treatment time (early versus late treatments) shed light on differences. Independently to the variable considered, the plots treated later were characterised by higher weed abundance than those treated early. "Other annual weeds" increased from T_1 to T_5 but before harvest (T_6) there was no substantial increase. Adversely, when looking at *Sinapis arvensis* a progressive decrease of cover from T_3 to the end of the experiment was displayed (Figure 8).

Moreover, the outcomes after comparison between the cover for other annual weeds and *Sinapis arvensis* at the first recording and before harvest behaved differently. Other annual weeds displayed a high ground cover at the end of the experiment whereas the cover of *Sinapis arvensis* diminished (Figure 8-9).

*Table 7: ANOVA and rmANOVA for Sinapis arvensis, other annual and Cirsium arvense. ANOVAs concern ground cover and biomass at the sixth sampling time. RmANOVAs concern all sampling times except the time two when some of the plots were not yet treated. (NS: Non significant differences; *: Significant differences)*

			Sinapis arvensis				Other annual weeds				*Cirsium arvense*			
Time	Variables	Effect	Df	MS	F	P	Df	MS	F	P	Df	MS	F	P
T 6	Ground cover (%)	Implement	2	0.51	0.93	0.43 NS	2	0.29	1.36	0.31 NS	2	4.71	2.27	0.17 NS
		Treatment time	1	1.47	2.72	0.14 NS	1	0.35	1.62	0.24 NS	1	5.49	2.65	0.14 NS
		Block	1	0.001	0.002	0.97 NS	1	1.06	4.86	0.06 NS	1	0.27	0.13	0.72
		Error	7	0.54			7	0.21			7	2.07		
	Biomass ($g\ m^{-2}$)	Implement	2	72.79	0.94	0.43 NS	2	95.51	1.36	0.17 NS	2	46.60	0.76	0.50 NS
		Treatment time	1	77.74	1.00	0.34 NS	1	114.43	2.73	0.14 NS	1	177.1	2.91	0.13 NS
		Block	1	18.70	0.24	0.63 NS	1	6.18	0.14	0.71 NS	1	6.32	0.10	0.75 NS
		Error	7	77.36			7	41.82			7	60.84		
T1,T3, T4,T5, and T6	Ground cover (%)	Implement	2	5.28	6.88	0.02 *	2	215.42	4.29	0.06 NS	2	134.4	1.48	0.28 NS
		Block	1	2.31	3.01	0.12 NS	1	236.15	4.70	0.06 NS	1	14.67	0.16	0.69 NS
		Treatment time	1	25.09	32.69	0.00 *	1	603.15	12.0	0.01 *	1	177.8	1.96	0.20 NS
		Error	7	0.76			7	50.20			7	90.35		
		Time	4	7.57	15.07	0.00 *	4	1841.27	53.9	0.00 *	5	26.99	0.87	0.50 NS
		Time * Implement	8	0.37	0.73	0.65 NS	8	50.70	1.48	0.20 NS	10	33.33	1.08	0.40 NS
		Time * Block	4	0.48	0.96	0.44 NS	4	41.15	1.20	0.33 NS	5	3.74	0.12	0.98 NS
		Time * Treatment time	4	1.32	2.64	0.05 NS	4	62.92	1.20	0.14 NS	5	40.96	1.33	0.27 NS
		Error	28	0.50			28	34.10			35	30.74		

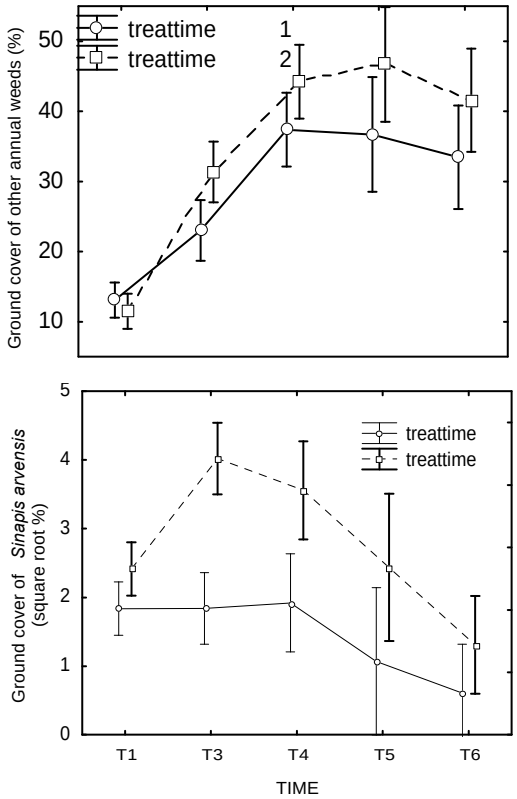


Figure 8. Dynamics of cover according to the early versus the late treatment time for "other annual weeds" and Sinapis arvensis.

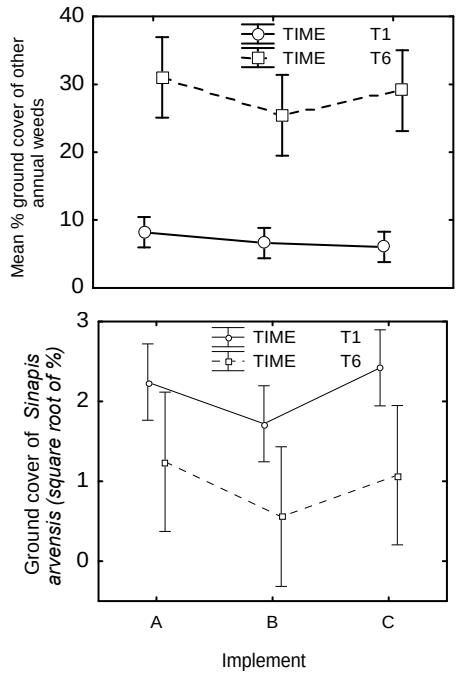


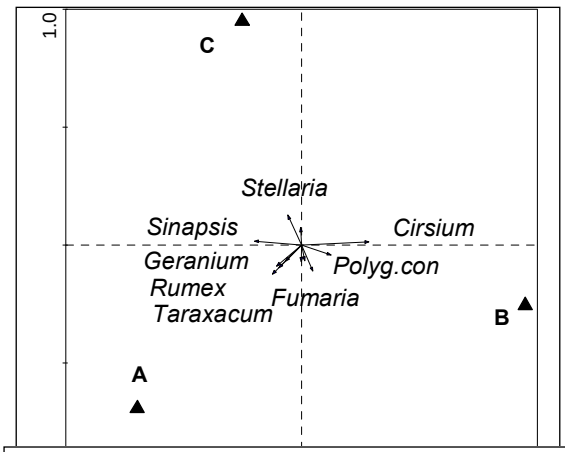
Figure 9. Comparison between ground cover from the first and the last (before harvest) recordings of other annual weeds and Sinapis arvensis

4.2.2 Partial RDA

Table 8: Summary of Monte Carlo permutation test in pRDA (with 9999 permutations) resulting from different analyses of variance for each one of the following factors when all others were taken as covariables. The sampling unit within each plot was taken as covariable in all tests. The control plots were made supplementary.

Data analysed	Factor	Trace	F	P
All sampling times (% Ground cover)	Implement	0.03	4.97	0.0001 *
	Time	0.22	13.98	0.0001 *
	Treatment time	0.05	16.68	0.0001 *
	Time * Implement	0.25	5.16	0.0001 *
Sixth sampling time (% ground cover	Implement	0.08	1.62	0.3430 NS
Sixth sampling time (g m^{-2})	Implement	0.05	0.63	0.5075 NS

a)



b)

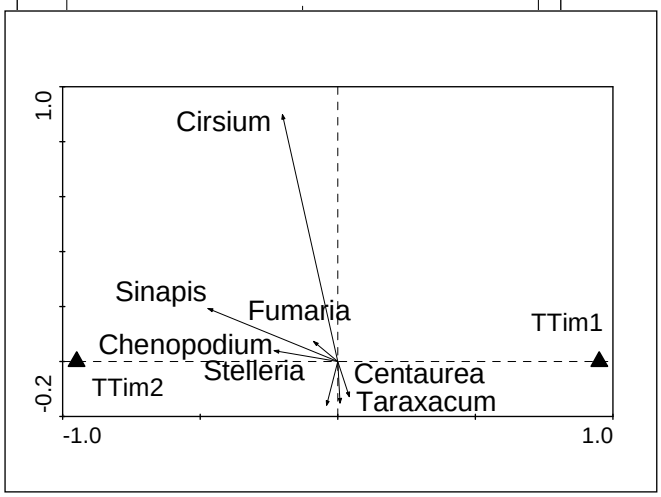


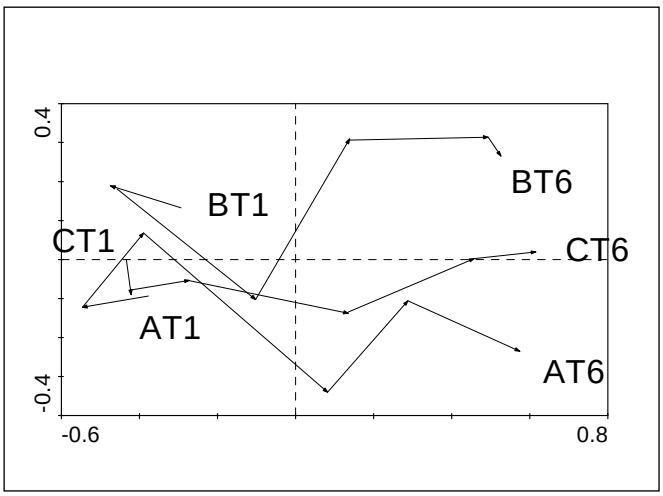
Figure 10: Ordination graphs showing differences of factors with respect to percentage ground cover. a) Implements. b) Treatment time

With the Monte Carlo test, ground cover recorded in all occasions showed significant effects for implement and time factors and interactions terms. However, the implement effect was not detected in case of one sampling time. This was true for both biomass and cover estimate (Table 8). The three tested implements were highly different ($P<0.001$) and

implement B was shown to be less effective against *Cirsium arvense* than implement A and C. Treating weed infestation at the late treatment time was found to be of low impact (Figure 10).

The interaction of time and implements highlighted the differentiation of implements and weeds over time. *Sinapsis arvensis* was most abundant during the earlier samplings while the weed species composition was almost the same for all implements. Later in the season, other species such as *Stellaria media* became the most abundant and the implement effect showed that B implement was the least effective against *Cirsium arvense* (Figure 11).

a)



b)

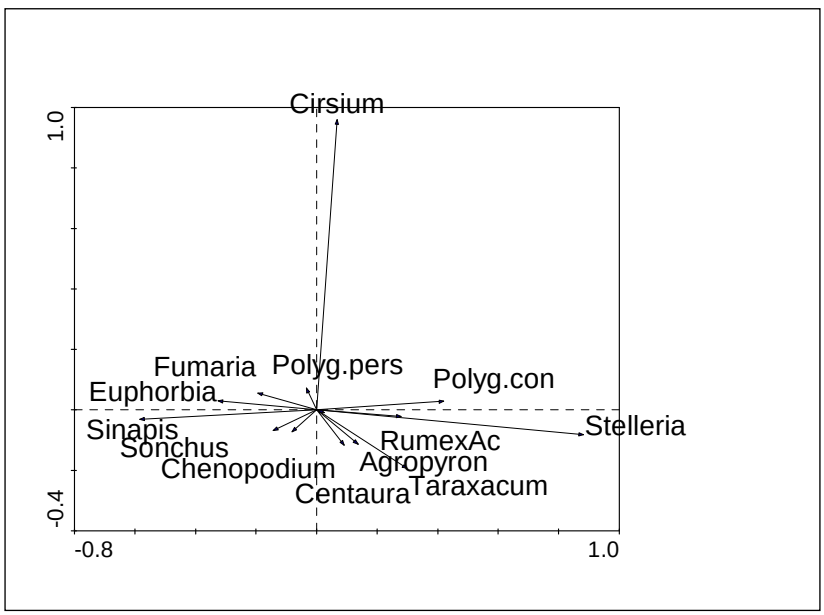


Figure 11: Representation of time x implement interactions obtained using PCA from the data set of Tegneby. a) Trajectories over time of the implement centroids (AT_1 - AT_6, BT_1 - BT_6 and CT_1 - CT_6) in ordination space b) Arrows are representing species.

4.2.3 Relationship between ground cover and biomass

The coefficient of correlation in the relationship between percentage ground cover and square root of biomass was shown higher for the averages of three sampling units (r = 0.84) than the direct comparison between each sampling unit (r = 0.71) (Figure 12).

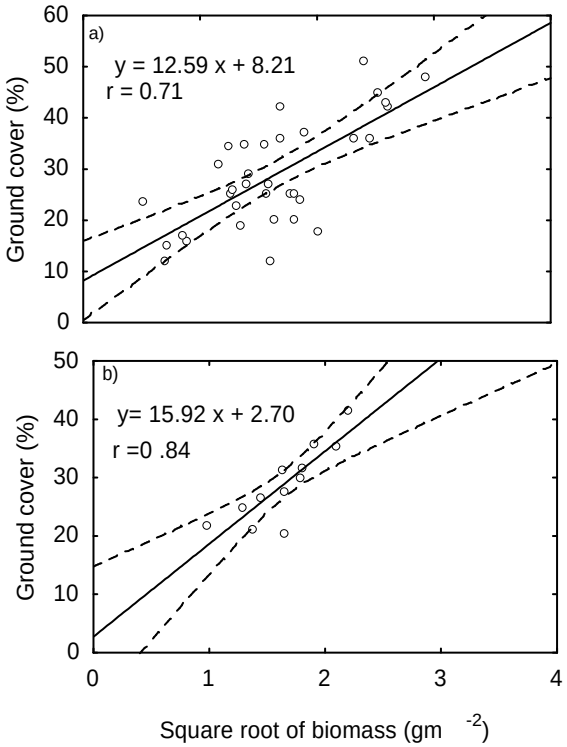


Figure 12: The relationship between biomass and ground cover of "other annual weed species" at the sixth sampling time at Tegneby (wit the confidence interval of 95 %). a) Comparison based on sampling unit per sampling unit (n= 36). b) Comparison of mean of biomass and ground cover per plot (i.e. the mean of three sampling units per plot: n= 12). P < 0.05

5 Discussion

5.1 Percentage ground cover versus biomass estimates

The results suggested that the outcomes reached from percentage ground cover and biomass were relatively similar (Table 9). At Tegneby, no effects for different factors were found for the data sets considered. However, at Klostergården, some differences were noticed after the post-hoc Tukey test. These differences might be partly attributed to the spatial heterogeneity and the size of sampled units because, for this experiment, the sampling of biomass was done by the ASÖ field staff. Their data were used because, for the trial at Klostergården, they followed the conventional sampling method. In contrast, at Tegneby the sampling was unconventional since their interest was principally focused only on *Cirsium arvense*.

Table 9: Comparisons of the outcomes obtained with ANOVA on biomass and ground cover estimates: summary of the quantitative conclusions (S: Significant differences. NS: Non Significant differences after ANOVA)

Compared results	Similarities	Differences
Klostergår-den (Tables 2, 3, 4 and 5)	***Viola:*** 1. Treatment factor: **S** 2. Tukey HSD test showed four best (D, J, M and E) versus three least effective treatments (B, C and O). **Other annuals:** 1. Block factor: **NS** 2. Treatments I, J and E were the best ranked for both estimates and B, C and C were at the bottom of the list **All annuals:** 1. Treatment factor: **S** Block factor: **NS** 2. Treatments I, J and M were best ones versus B, C and O that were least effective.	***Viola:*** 1. Block factor (biomass: **S** but cover: **NS**) 2. For biomass, the means abundance after Tukey HSD test were grouped into three groups where group a, b and c overlapped whereas the cover estimate showed four groups with a and b overlapping and b overlapping with c and d. **Other annuals:** 1. Biomass for treatment factor: **NS** 2. The late treatment O was different from other treatments for the cover estimate 3. K and G showed different behaviours respectively to biomass and cover estimates. K was found among best treatments for the cover whereas G came among best for the biomass **All annuals:** 1. Two overlapping groups were obtained for biomass estimate versus three overlapping groups for ground cover. 2. Treatments G and K remained ambiguous due their ranking
Tegneby (Table 7)	For all environmental factor and responses variables: **NS**	

These results supported several previous studies dedicated to the determination of the reliability of the visual assessment of the plant ground cover estimate. Such studies have quantified the random and systematic error in the assessments (Sykes *et al.* 1983, Floyd & Anderson 1987, Kennedy & Addison 1987). Although percentage ground cover has been recognised as a god estimate in plant ecology (Margurran 2004), and even used in recent studies in weed science (Paruelo *et al.* 2000, Major *et al.* 2005), some precautions need to be considered. For the present study, the reached similarities of outcomes from biomass and cover comparison might be partly attributable to time series sampling method. As concluded by Kennedy and Addison (1987), the precision of visual cover in biological monitoring can be improved by time series sampling. Previous sampling occasions might have contributed to reduce the visual estimation errors by increasing familiarity with species identification, following the species-morphology, the species occurrence and density. Also, an average of several estimates will give more reliable estimate than a one-time assessment.

On the other hand, the relationship between the sampled biomass and ground cover (Figure 12) showed that there was a positive correlation. The correlation was stronger and positive between sampled biomass and cover records when averages per plot were compared (r=0.84, n=12) than the direct comparison sampling unit per sampling unit (r=0.71, n=36). Alternative explanations to the reached patterns can be suggested. Either, more than one sampling point per plot improves the reliability than just a direct comparison of sampling units from plots especially in this case of spatial heterogeneity. Or, merely, the enhanced coefficient of correlation was attributable to the fact that the more data points (n) in correlation analysis, the more unexplained variation can occur.

With the starting point that biomass is obtained from an instrument that can be calibrated and therefore can be closer to the standard measurement, we compared the outcomes from the two data sets. It is, however, worth mentioning that comparisons made do not mean that biomass, so far considered the "gold standard" in weed field trials, is exempt of errors. There are various sources of errors occurring in all measurement methods and these need to be identified and minimised. Beside the weed patchiness, which is the actual problem in field trials, errors can take place during different processing such as harvest, drying and weighting.

5.2 Statistical methods

The present study revealed some specific features to rmANOVA and pRDA analyses that added more information to the results and conclusions reached with the conventional tests run with ANOVA (Table 10 and 11).

At Klostergården, the overall information drawn was that the highlights of the ranking issued from the different statistical methods on the effects of the studied factors did not display notable dissimilarities in the trends. However, the most important and indubitable value of rmANOVA was the ability to show to what extent the time factor affected weed species abundance (Figures 2-5).

Viola spp. increased in percentage ground cover in contrast to "other or all species" from time one to four. However, from time four to the harvest, cover increased for *Viola* and "other annual" weeds as well. Probably, herbicides were no longer effective at that stage of growth or negligible rainfall during that period would have prevented new recruitment or vegetative growth of weed species (Figures 2 & 4b). By including the baseline flora in the time trend analysis, it was illustrated to what extent the treatments were effective or not towards a given individual weed species or a group of weeds (Figures 3 and 5). "Other annual weed

species", as shown by the Figure 6, were affected by the treatments given the decrease of their weed abundance. In contrast, for *Viola* spp. at Klostergården, the initial flora was the lowest of all sampling times. Weed abundance increased after application of herbicides despite the treatment (Figure 3).

Table 10: Comparisons of the outcomes reached with different statistical analyses at Klostergården.

Compared results	Similarities	Differences
ANOVA versus rmANOVA (Tables 2 and 3; Figures 1, 2 and 3)	***Viola:*** 1. Treatment and block effects were significant 2. Similar outcomes after Tukey HSD test for twelve of fourteen treatments	***Viola:*** 1. K and H became exchangeable respectively to eleventh and ninth places after ranking of the treatment effect. 2. RmANOVA showed differences among weed abundance at different sampling times. 3. Despite the treatment, there was an increase of weed abundance from sampling time one to two, a stationary weed abundance between sampling times two and four followed by a highly increase weed abundance. 4. The before treatment assessment (Time 1) showed weed abundance variability but the after sampling times were even more illustrating the variability of weed abundance: spatial heterogeneity + treatment effect.
ANOVA versus rmANOVA (Table 4, Figures 4 and 5)	**Other annuals:** 1. Differences were found between treatments but not between blocks.	**Other annuals:** 1. The ranking are different: i) the K treatment, which is ranked first with one time sampled data set, came fifth for repeated samples; ii) the B treatment was ranked as being best than G treatment with one sampling time. 2. From the sampling time one to four, weed abundance decreased and increased again shortly before the harvest. The same variability in weed abundance at the before sampling time noticed for viola was seen even with other annuals but due to treatment effect, the weed abundance later on was lower.
ANOVA versus RDA (at T3) (Figure 6)	***Viola:*** 1. All the tested factors (treatments, block) were found significantly different. 2. The ranking was exactly similar	***Viola:*** ANOVA can handle one species or a group of species at once whereas RDA shows all the present species when raking the factor effects.
rmANOVA versus RDA (repeated measures) (Tables 2 and 6; Figures 2, 3, 6 and 7)	***Viola:*** 1. All the tested factors (treatments, block, time and interaction between time and treatments) were found significantly different. 2. There was a highlight of the spatial and temporal heterogeneity in weed abundance.	***Viola:*** 1. ANOVA can handle one species or a group of species at once whereas RDA shows all the present species with the possibility to rank the treatment or temporal effect for each of them.

RDA distinguished the treatment, time effects and interaction terms vis-à-vis each individual weed species abundance. Within the two dimensional ordination space plotted, treatments followed the first principal component whereas time followed the second one (Figures 6, 7, 8, 9 and 11). With pRDA, it was easier to consider each of the weed species for each treatment and time. Furthermore, the turnover of weed species over time was highlighted with PCA (Figures 7 and 11). This was in accordance with previous study of multivariate methods in general (Guisan *et al*. 1999, Kedwards *et al.* 1999, van den Brink& ter Braak 1999). They are more readily implemented for many species at the community level rather than individual or groups of species.

At Tegneby, ANOVA did not distinguish implements or blocks neither for biomass nor ground cover. However, rmANOVA could at least shed light on differences between the treatment time effect, the time effect (Table 10) for *Sinapis arvensis* and "other annual weed species" but not for *Cirsium arvense*. At the same time, Figure 9 showed, as stated by Sykes *et al.* (1983), how the species morphology can be a source of error in both measurements and conclusions drawn. "Other annuals" had a higher ground cover at the end of the season, in contrast to *Sinapis arvensis* for which the cover diminished, compared to the initial flora (Figures 8a and 9a) . The progressive loss of leaves later in the season, morphological changes of *Sinapsis arvensis,* meant a decrease in percentage ground cover in repeated measures. This can possibly lead, contrary to biomass estimate, to wrong conclusion (i.e. stating wrongly a control effect) for some specific weed species that have broad leaves only at the beginning of the season.

RDA enabled to differentiate the effect of all factors considered in the repeated assessment data set ($P<0.001$ with Monte Carlo test). The B implement was the least effective against the weed community, followed by C and the A implement that was controlled weeds best (Figure 10a). The earlier treatment time was more appropriate to control weed species than the later one. At the sixth sampling time, no differences were found between implements because it was too late in the season after treatments to detect the implement effects. This suggests that sampling time during the crop-weeds development must be considered to detect management effects on weeds.

Table 11: Comparisons of the outcomes reached from different statistical analyses at Tegneby

Compared results	Similarities	Differences
ANOVA versus rmANOVA (Table 7; Figues 8 and 9)	No differences between blocks	1. With rmANOVA, differences between implements, time x implement and treatment time were shown for *Sinapis arvensis*. However, only time and time treatment could show differences for "other annual weeds" whereas any of those factors showed effect on the weed abundance for *Cirsium arvense*. 2. RmANOVA allowed to follow the dynamics of weed abundance according to the treatment time but also the effect of the morphological characteristics of weeds towards the determination of the treatment effect.
ANOVA and rmANOVA versus RDA (Tables 7 and 8; Figures 10 and 11)	1. No significant differences between implements for sampling time six. 2. Treatment time and time factors were shown different after both rmANOVA and RDA considered *Sinapis arvensis* and other annual weeds.	1. Only with RDA, significant differences were found between implements, time, treatment time, and interaction between time and implement. 2. The ordination space allowed to find out which implement was effective to what species. (e.g implement B is effective against *Cirsium arvense* than A and C). At the same time, the earlier treatment controlled relatively best the present weed species. 3. RDA could as well highlight the turn over of different weed species. *Stellaria* was more abundant later in the season.

So far, for both experiments, we have demonstrated the additional value of rmANOVA and pRDA after the highlights of the sampling method using time series, more sampling units per plots that contribute to the reliability/precision of the visual assessment of the percentage ground cover estimate. Moreover, visual assessment can allow observations on much larger areas than harvest plots and this might reduce errors due to spatial variability. Nevertheless, one must be cautious since time series sampling can be time consuming and therefore costly given the project objectives, earlier experience for observers, time available for sampling and timeframe within which sampling must be performed as suggested by Rew *et al*. (2000). Even though visual assessment of weed abundance seems to be quicker than harvesting, drying and weighting the above ground biomass, in case of large field trials with limited personnel to collect the data, it would be difficult to cover the whole field in due time. At this stage then, a rising question would be to know if samples are to be made by individual species or per categories.

To paraphrase Rew and Cousens (2001), the determination of the sampling methods are subsequent to the analytical methods to be used and the end-use of the results of the study. With ANOVA, it would be fine to sample the most infectious weeds in separate groups and to consider the rest as another group. In contrast, to take advantage of multivariate

methods, each species considered separately will require more time for fieldwork. On the other hand, one must bear in mind that precision varies with observers (Sykes *et al*. 1983, Kirby *et al*. 1986), a feature known as observer drift or inter-observer variability (Ruxton and Colegrave 2003). According to Sykes *et al.* (1983), training, screening or calibration from a population of observers can improve precision.

6 Conclusion

Visual estimate of percentage ground cover can be a surrogate of the biomass weed estimate. Outcomes from the two estimates, analysed with ANOVA, were comparable and relationship found between their records seemed to be acceptable.

The repeated assessments, rmANOVA and multivariate statistics (partial RDA) were shown to be more informative than one sampling time analysed with ANOVA. In repeated sampling, a pre-treatment sampling can be relevant in case of remarkable weed abundance values. Several sampling occasions post-treatment might be required to level out gross errors that might be caused by the observer's imprecision in visual estimation of ground cover. Besides the ranking of treatment effects obtained with ANOVA rmANOVA and pRDA give more details on time factor and treatment-time interactions. Even, multivariate methods illuminate an eventual solution to the herbicide selectivity problem.

As for other disciplines such as ecotoxicology (Kedwards *et al.* 1999) and other environmental impact assessments, rmANOVA and multivariate methods were found well suited to follow the changes of biological response of weed species to managerial decision or other environmental perturbations.

More studies in different ecological conditions (i.e. other crops, autumn sown crops and ecological zones) are required for more light and generalisations of these conclusions whilst comparisons in terms of involved costs would be established.

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Appendix : List of present weed species and the percentage occurrence in all plots per experiment

Scientific name (Family)	Life cycle	Occurrence (%)	
		Klostergården	Tegneby
Sinapis arvensis (Cruciferae)	A	30.1	64.4
Galeopsis spp. (Labiatae)	A	18.4	90.8
Chenopodium spp. (Chenopodiaceae)	A	5.2	51.4
Cirsium arvense (Compositae)	P	3.11	46.8
Polygonum convolvulus (Polygonaceae)	A	33.1	73.7
Stellaria media (Caryophyllaceae)	A	0.1	57.5
Viola spp. (Violaceae)	A	54.1	-
Fumaria officinalis (Papaveraceae)	A	0.3	3.2
Taraxacum officinale (Compositae)	P	0.1	8.7
Polygonum persicaria (Polygonaceae)	A	2.4	1.3
Euphorbia peplus (Euphorbiaceae)	A	-	13.8
Sonchus oleraceus (Compositae)	A	0.3	0.9
Agropyron repens (Poaceae)	P	-	3.2
Lamium spp. (Labiatae)	A	1.2	-
Veronica spp. (Scrophulariaceae)	A	1.0	-
Thlaspi arvense (Cruciferae)	A	0.5	-
Centaurea cyanus (Compositae)	A	7.7	2.6
Myosotis arvensis (Boraginaceae)	A	0.1	0.9
Spergula arvensis (Caryophyllaceae)	A	-	0.9
Galium aparine (Rubiaceae)	A	12.2	-
Rumex acetosella (Polygonaceae)	P	-	6.9
Geranium spp. (Geraniaceae)	P	-	0.4
Matricaria inodora (Compositae)	A	-	0.9

A: Annual weed; P: Perennial weed;

The calculation of the occurrence in percentage of each species was based on the number of time that a given species was recorded. An occurrence was counted from all the sampling units, plots and all time samplings (i.e. how much time the species was found in the total of 900 samples at Klostergården versus 216 samples at Tegneby).