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Repeated grading of weed abundance and multivariate methods to improve the efficacy of on-farm weed control trials

LIBÈRE NKURUNZIZA and PER MILBERG*

IFM Biology, Division of Ecology, Linköping University, Linköping, Sweden

We evaluated whether new information could be drawn from additional data collection and unconventional statistical analyses of an on-farm trial. First, we compared a conventional sampling method using a biomass estimate of weed abundance to repeated visual assessment of the percentage ground cover of weeds. The biomass was sampled once after the treatment, whereas the ground cover was repeatedly sampled once before weed control plus several occasions after weed control. Second, we contrasted the outcomes from analysis of variance (ANOVA), taking samples from a single point in time with repeated measures (rm)ANOVA and a multivariate method. As the outcomes and conclusions drawn were relatively similar, we conclude that the ground cover estimate of weed abundance was as reliable as the biomass estimate. The rmANOVA enabled us to follow the temporal trend in response to treatments in the most abundant species, including possible initial differences. Multivariate analysis went even further, by clearly displaying species-wise responses and treatment selectivity.

Key words: ground cover, multivariate methods, repeated measures analysis of variance, weed control.

INTRODUCTION

Weeds are humans' worst pest organisms, interfering with food production everywhere and reducing production, economic growth, and food security (Milberg & Hallgren 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, to evaluate the economic or other benefits of the new method compared with an established one. The end point of primary interest is then the crop yield. To be meaningful, such trials have to be located on farmers' fields, that is, the method has to be evaluated under realistic field conditions typical for producers in the region (Koenig *et al.* 2000). The second aim is often to evaluate the selectivity of the new method, that is, to what extent certain weed species will be more or less affected. For example, 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 the yields in treated plots and weed-free reference plots, can be $\leq 20\%$, even in the absence of weeds (Milberg & Hallgren 2004). This is an artefact related to spatial heterogeneity within a nearly weed-free crop stand. Weeds are even more patchily distributed than is the crop biomass or yield, contributing further to the heterogeneity. The current way to analyze these experiments, by pairing the data from the treated plots and the reference plots, means that a substantial part of the variation is created by the spatial heterogeneity of the weed population (Walter *et al.* 2002). Even where the researcher has artificially created the weed stand (e.g. Buhler 1997; Tamado *et al.* 2002), the initial number and composition of the weeds will not be identical in the plots. Therefore, a large number of similar experiments is needed to be able to evaluate selectivity with the currently prevailing sampling scheme (Rew & Cousens 2001; Milberg & Hallgren 2002).

There are large costs involved in establishing, maintaining, harvesting, processing, and analyzing this type of trial. Therefore, potentially much could be gained if better and more detailed information on weed responses could be collected and analyzed in these experiments. Repeated measures ANOVA (rmANOVA) or multivariate methods might be very useful for a more detailed assessment of the treatment effects in on-farm weed control trials. In various scientific disciplines, such as environmental assessment, medicine, econometrics, operations research, quality improvement, and ecology, rmANOVA is frequently used (Smith 2002; SAS 2005). Multivariate statistics are designed to summarize a complex data structure (Dieleman *et al.* 2000; Kenkel *et al.* 2002) and they are well-suited for community-level analysis when it comes to analyses of biological monitoring (Kedwards *et al.* 1999a, b). Apart from just summarizing complex data, multivariate analyses also can be used to test hypotheses (e.g. Hallgren *et al.* 1999) or evaluate designed experiments, like on-farm weed trials (Aguilar *et al.* 2003; Reberg-Horton *et al.* 2006). The possible advantages are that the issue of the differential control of individual species by different treatments is directly addressed, the signal in multivariate data might be stronger than in univariate data, and that multiple (species-wise) testing is avoided (Milberg & Hallgren 2002).

The aim of this study was to compare the sampling of above-ground biomass at the peak of the season, the data-collection method currently used in Sweden, with repeated assessments of percentage ground cover. The study also examined if

rmANOVA and multivariate statistics, on repeated recording, would add value to the information reached when using ANOVA on a single weed biomass assessment.

MATERIALS AND METHODS

Field trial

We carried out a conventional on-farm experiment at Vreta Kloster farm (58°27'N and 15°30'E), southern Sweden, managed by the Agricultural Society of Östergötland. The weed control experiment compared different mixtures of herbicides (Table 1) in spring-sown barley (*Hordeum vulgare* cv. “Astoria”; XXX, XXX). 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 fertilized with 89 kg nitrogen ha⁻¹ (330 kg ha⁻¹ Axan NS 27-3; Yara, Landskrona, Sweden). The experimental set-up consisted of a completely randomized block design with four blocks, 14 treatment plots, and one untreated control plot in each block. Each treatment plot was 39 m² (3 m × 13 m). The herbicide treatments consisted of early applications at the stage of 3–4 crop leaves (3 June 2005) and one late application ≈ 2 weeks later (20 June 2005) (Table 1). The herbicides were obtained from Agrilab AB, Uppsala, Sweden.

Table 1. Herbicides (doses/mixtures) used in the experiment and the date of application

Treatment	Herbicide applied (ha ⁻¹)
A	Not treated
B	5.0 g Express (tribenuron-methyl) and 0.1 L Lissapol Bio (alkyl etoxylat)†
C	5.0 g Express and 0.1 L Silwet Gold (trisiloxane ethoxylate)†
D	1.0 L Verigal (mecrop- <i>p</i> -ethyl)†
E	2.0 L Verigal†
F	1.0 L Verigal and 5.0 g Express†
G	1.0 L Verigal and 50 mL Primus (florosulan)†
H	2.0 L Ariane S (MCPA, fluroxypyr-1-methylheptylester, and clopyralid)†
I	75 mL Primus, 2.5 g Express, and 0.1 L Lissapol Bio†
J	40 g Ally Class (carfentrazone-ethyl and metsulfuron-methyl)†
K	50 g Hussar (idosulfuron-methyl) and 0.5 L Renol (oil adjuvant)†
L	75 g Hussar and 0.5 L Renol†
M	125 g Hussar and 0.5 L Renol†
N	150 g Checker (iodosulfon-methyl and amidosulfuron) and 0.5 L Renol†
O	7.5 g Express and 0.1 L Lissapol Bio‡

†Early treatment time (3 June 2005) at the stage of 3–4 crop leaves; ‡late treatment time (20 June 2005). The herbicides were obtained from Agrilab AB, Uppsala, Sweden.

Sampling

In each treatment plot, three permanent sampling points were randomly located on the first recording occasion. In order to locate them easily for the following sampling times, one tall 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 m². This methodology was appropriate in our case because late in the season, the crop might be a problem when using, for example, a frame that would be difficult to squeeze down bolted cereals. The sampling was undestructive in order to follow the weed species' dynamics during the whole cultural season.

We did the first recording before the early treatment to catch the initial flora, representing the baseline data (Lepš & Šmilauer 2003). A second recording was done before the application of the late treatment (2 weeks later). Afterwards, three other recordings were done with a 2 week interval from June to August. A visual estimate of the percentage ground cover for each present weed species was noted for each sampling unit.

In addition to the data collection described above, we used the data sampled by the Agricultural Society of Östergötland using the conventional method. In accordance with their field protocol, only three data-sets of the above-ground biomass were recorded: *Viola arvensis* Murray (the only species considered, according to the protocol, to be of sufficient abundance in the trial), “other annual weeds” (excluding *V. arvensis*), and “all annual weeds” taken together.

Statistical analysis

We used General Linear Models in STATISTICA 7.0 (StatSoft 2004) to run ANOVA and rMANOVA and CANOCO 4.5 (ter Braak & Šmilauer 1998) to conduct the partial redundancy analysis (pRDA). The latter is a method related to principal component analysis (PCA), but with the important difference that only the variation that can be mathematically accounted for by the linear combination of a number of explanatory variables is considered.

The untreated control was excluded from all analyses to be able to highlight the impacts of the different herbicides, but the data on the untreated control are occasionally given for reference. All data were square-root transformed before the analyses.

Analysis of variance and repeated measures analysis of variance

The conventionally used statistical analysis in Sweden, ANOVA, was applied to the data collected by the Agricultural Society of Östergötland and to the cover data-set sampled on the third occasion (both data-sets were collected in the same week). The full cover data-set from the five sampling occasions was submitted to rMANOVA (with “time” as the split plot factor and “treatment” as the main plot factor). For comparative purposes, we considered only the same three variables (*V. arvensis*, other annuals, and all annuals) that had been sampled using the conventional field protocol. In addition, as the latter only considered one value per treatment plot, the three sampling points per treatment plot were averaged to get one value per treatment plot, per species or group of species.

In case of significant treatment differences in the ANOVA results ($P < 0.05$), post-hoc Tukey HSD tests followed to group the treatments. For rMANOVA, graphs illustrated the treatment $\times$ time interaction term.

Partial redundancy analysis

Preliminary detrended correspondence analyses had been conducted to decide whether to use the linear or unimodal types 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 the linear method, pRDA.

The analyses were run at two levels: either with the data collected at one sampling time or the whole data-set from the repeated measures. The explanatory variables considered were the treatments (mixtures of herbicides) and time (one level only), while the block factor was taken as the covariable. All these variables and covariables were coded as a number of dummy variables.

Monte Carlo tests with 9999 permutations evaluated the significance of the pRDAs, testing between or within permutation blocks (treatment, time or interaction terms). The permutation tests took the experimental design into account, that is, repeated measures and blocks. Selected ordination graphs are presented to illustrate the results. These graphs illustrate the weed abundance with arrows and the treatment factors with centroids (black triangles). A PCA also was conducted, for illustration purposes only, and was used to display the time/treatment relationship.

RESULTS AND DISCUSSION

Among the 17 recorded weed species, there were two perennial species (*Cirsium arvense* (L.) Scop. and *Taraxacum* sp.) and the most abundant annual was *V. arvensis*.

Biomass compared to percentage ground cover

From the data recorded both for the biomass and the visual percentage ground cover estimates, we compared the treatment effects and found them relatively similar for both data types. The significance level of the differences among the treatments and the post-hoc tests according to treatment effectiveness were relatively identical (Table 2). These results are further supported by previous studies on the reliability of visual assessment of plant ground cover estimates (Sykes *et al.* 1983; Floyd & Anderson 1987; Kennedy & Addison 1987). The reliability, recognized here for weed ground cover as an estimate, suggests that it can be applied for repeated, undestructive data collection in weed control trials. It is desirable, however, to quantify the random and the systematic errors in such assessments. A major advantage with cover estimates in weed control trials, not exploited here, is that a larger part of the treatment plot can be assessed, compared with sampling for biomass.

Additional values from repeated assessments

With the data-set from the undestructive, repeated visual assessments of percentage cover, the rMANOVA (Table 2) and pRDA (Table 3) reached relatively similar conclusions to the ANOVA in relation to the ranking of the treatment effects (Table 2, Figs 1 and 2). However, some additional information was reached from the rMANOVA and pRDA.

Table 2. Comparisons of the outcomes (*P*-values) obtained with repeated measures analysis of variance on the biomass and cover estimates: summary of the quantitative conclusions

Species	ANOVA: biomass data	ANOVA: cover data	rmANOVA: cover data
<i>Viola arvensis</i>			
Block	0.1234	0.0316	0.0000
Treatment	0.0015	0.0000	0.0000
Time	–	–	0.0000
Block × time	–	–	0.0008
Treatment × time	–	–	0.0000
Tukey test	2 groups	3 groups	–
Best group	DEFGHIJKLMNO	DEFGHIJKLMN	–
Worst group	BCDFHIKLNO	CO	–
Other annuals			
Block	0.0140	0.1729	0.0752
Treatment	0.0167	0.0000	0.0000
Time	–	–	0.0000
Block × time	–	–	0.1933
Treatment × time	–	–	0.0003
Tukey test	2 groups	4 groups	–
Best group	B–N	D–N	–
Worst group	BCDEFHIJKLMNO	BCDFGHILMO	–
All annuals			
Block	0.0099	0.1097	0.0000
Treatment	0.0005	0.0000	0.0012
Time	–	–	0.0000
Block × time	–	–	0.0334
Treatment × time	–	–	0.0000
Tukey test	3 groups	4 groups	–
Best group	B–N	B, D–N	–
Worst group	BCDFHIKLNO	BCGO	–

Bold letters indicate overlap with the “best group”. ANOVA, analysis of variance; rmANOVA, repeated measures analysis of variance.

Table 3. Comparisons of the outcomes (*P*-values) reached with partial redundancy analysis

Variable	Sampling time	
	T ₃	T ₁ –T ₅
Block	0.0007	0.0001
Treatment	0.0001	0.0001
Time	–	0.0001
Treatment × time	–	0.0001

Repeated measures analysis of variance

Weed abundance varies in time according to the treatment and the degree of vulnerability of a weed species, and rmANOVA allows these dynamics to be followed. The baseline data in repeated measures, that is, before treatment, illustrated that weed abundance was not initially equally distributed. Furthermore, the baseline data helped us to illustrate that *V. arvensis* was generally difficult to control: its cover later in the season was large compared with other annuals, whose post-treatment cover rarely exceeded the baseline (Fig. 1).

Multivariate methods: Partial redundancy analysis and principal component analysis

Repeated assessments of weed cover used in the pRDA provided a similar grouping of treatments as for the above-mentioned statistical methods. Simultaneously, the most interesting feature was the ability to display, in one ordination graph, all weed species vis-à-vis each treatment (Fig. 2). This is advantageous because it potentially displays the selectivity of a treatment. For example, *V. arvensis* was not the only poorly controlled weed in the least effective treatments (O, B, C)

Fig. 1. Ground cover shown for the interaction between treatment and the first (T₁), fourth (T₄), and last (T₅) sampling times (with 95% confidence interval). (a) Square root of cover (%) for *Viola arvensis* and (b) square root of cover (%) for other annual weeds. (○), T₁; (□), T₄; (◇), T₅.

Fig.2. Weed species–treatment biplots, obtained with partial redundancy analysis, illustrating the effect of the treatment on the weed species ($P<0.001$). The letters represent a mixture of herbicides (see Table 1) (the untreated control was excluded from the analysis), whereas the arrows indicate the direction of increasing abundance of the species in question. All the sampling occasions (T_1 – T_5) were considered. Polyg., *Polygonum*.

according to ANOVA. *Polygonum convolvulus* L., *Galeopsis* spp., *C. arvensis*, and *Sinapis arvensis* L. also had a noteworthy level of abundance under these treatments.

The relationship between the three selected treatments and time was displayed in the PCA (Fig. 3). It is noticeable, first of all, that the initial weed abundance and composition were relatively similar given the position of centroids at the initial sampling (T_1) for the three treatments. From the second sampling occasion (T_2), there was a spread of centroids. The poor treatments, C and O, moved away from the efficient treatment, E, along the first principal component (PC1), which displays the major part of the variation in the data (Fig. 3). This graph also highlights the weed species turnover during the season (mainly the PC2; Fig. 3).

Although repeated assessments analyzed with rMANOVA and pRDA seem to provide interesting information, one must be aware of a few drawbacks. The sampling can be time-consuming given the project objectives, the earlier experience for observers, the time available for sampling, and the time frame within which sampling must be performed (Rew *et al.* 2000). In addition, due to a feature known as “observer drift” or “interobserver variability” (Ruxton & Colegrave 2003), it is worthwhile to bear in mind that the precision varies with the observers and over time (Sykes *et al.* 1983; Kirby *et al.* 1986; Carlsson *et al.* 2005). However, training, screening or calibration from a population of observers can improve the precision (Sykes *et al.* 1983), as might image analyses (Hutto *et al.* 2006).

Fig. 3. Principal component analysis (PCA) illustrating time and treatment effects. The PCA was conducted using 14 treatments (excluding the untreated control), but only three are visualized: the best treatment, E, and the two least effective treatments, C and O. (a) Trajectories over time in ordination space and (b) a representation of weed species abundance corresponding to these trajectories. Polyg. con., *Polygonum convolvulus*; Polyg. pers., *Polygonum persicaria*; T₁, the first sampling time; T₅, the last sampling time.

CONCLUSIONS

Repeated visual assessment of the percentage ground cover of weed species at permanent sampling points can be a surrogate for weed biomass estimates. Its outcomes were comparable to that of the weed biomass estimate and the agreement found between them was acceptable.

The rMANOVA was more informative than the ANOVA. Besides the ranking of treatment effects obtained with ANOVA at one sampling time, the currently prevailing method, one could see more details related to the time factor and the time × treatment interaction. Moreover, the multivariate method (pRDA) was found to complement the univariate methods because it clearly addressed the issue of selectivity among treatments. For a given treatment, one can detect the effect among several weed species forming a community in standing crops.

More studies in different agronomical and ecological conditions (i.e. other crops, fall-sown crops, and ecological zones) are required for better generalizations of these conclusions and a proper cost/benefit analysis would be welcome.

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