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SUMMARY

Introduction

In the Netherlands the area of maize has been increased substantially over the last decades. If large numbers of farmers in the future would switch to herbicide resistant varieties of maize the use of the complementary herbicides, would increase considerably. Since maize is grown all over the Netherlands, including in areas that are part of the National Ecological Network concern has been raised regarding the potential effects of this type of herbicides on natural off crop vegetation. Although these aspects are not yet included in the Dutch or EU approval procedure, and Liberty (glufosinate-ammonium) is approved for use in genetically modified glufosinate tolerant maize in the Netherlands since 2000, Bayer CropScience would like to anticipate on such aspects and asked the Institute of Environmental Sciences of Leiden University (CML) to conduct a field study on the potential effects of the use of Liberty (glufosinate-ammonium) in "Liberty Link Maize" (means: glufosinate tolerant maize) on off crop vegetation. Since there are only few examples of this kind of field studies, the present study also has a methodological aspect: investigate how these studies should be designed and conducted.

Aim

Therefore a field study has been carried out in the Netherlands for the period 2000-2002. The study had two aims:

- To acquire information on the effects of glufosinate-ammonium and recovery on adjacent vegetation at the community level in the field situation under "realistic" worst case conditions of use.
- To assemble knowledge about how to conduct field tests with vascular plants at the vegetation level regarding herbicides.

The proposed study design and interim results were presented and discussed in three workshops with representatives of chemical industry, authorities for the approval of pesticides and researchers from the Netherlands, Germany and the United Kingdom.

Set up

Since it was impossible to conduct a field study in a nature reserve or on natural vegetation, we therefore looked for habitats (ditch banks and road verges) in appropriate areas (potential exposure) with relatively natural vegetation. (no herbicides used on or adjacent to the vegetation in the last 10 years, relatively high number of species, diversity) representative for the Netherlands and for Mid-Europe. It was decided to study the effects of glufosinate-ammonium in a "realistic" worst case situation. Hereto the dosages were applied twice per season (highest rate was 64% of the registered rate with 2 x 800 g ai/ha per season in two consecutive years), as can be the (worst) case in practice and the vegetation is sprayed directly with the different dosages, thus simulating drift. The main question concerned the effects at the vegetation level. The central parameters are therefore vegetation surveys: records of species presence and abundance. The parameters were recorded (2000-2002) in May (before spraying) and in August (after spraying). The effects at the vegetation level were expressed in the two following research questions:

1) Are there any effects in August of the herbicide applications in May/June within the same year? (short / midterm effects)
2) Are there any effects in May before the herbicide applications due to the application in the year before ? (longer term effects)

Also some other parameters have been recorded:

- Exposure of the vegetation to the herbicide during application of the herbicide
- Phytotoxic symptoms of the vegetation shortly after spraying (short-term effects)
- Biomass: assessment of the effects of spraying on the above ground biomass (mid- and longer term effects).

Sites & pesticide treatments

The study was carried out at four sites, two sites were road verges on sandy soil and the other two are ditch banks, on clay and peat respectively. Plots measuring 25 m^2 (1 m x 25 m) were arranged in blocks to minimise within site variation. The study was carried out with 5 different treatment levels and a control (0%):

- Low dosages: 2% and 4% of the maximum registered field dosage of 2 x 800 g a.i. glufosinate-ammonium/ha per season. These low deposition levels (4%: 2 x 32 g a.i./ha), occur at approximately 2.5 m and 1 m resp. from sprayed fields
- Intermediate dosage: 16% (2 x 128 g a.i./ha).
- Higher dosages (positive controls): 64% (2 x 512 g a.i./ha) and 32% (2 x 256 g a.i./ha). At these dosages effects would be expected.

The number of replications was 5 per site per treatment. The herbicide was applied with an advanced handheld knapsack sprayer. The interval between the first and second spraying (May-June) was 15-20 days. Spray deposition was measured with water-sensitive paper at different heights above ground level and calculated with an image processing and analysis program.

Parameters

In order to quantify the short-term effects of spraying, the observable external damage to the plants was recorded 8-10 days after each spraying in each year by eye in intervals of 5% (*phytotox*). In 2001 the phytotox was also determined at about 33 and 62 days after the second application. The effects of the spraying on the *biomass* in 2000 at two sites and in 2001 and 2002 at all sites was studied. For this purpose three subsamples of 30 cm x 30 cm per plot (of 1m x 25m) were harvested, taking the entire above-ground biomass. Braun-Blanquet relevés were made in each plot in order to study the effects at the *vegetation level*. At all four sites data were gathered before spraying (May/June) and after spraying (August). From the relevé data the following parameters per plot were derived: total number of species, number of dicotyledonous species, number of pioneer species, total coverage, coverage of dicotyledon species, coverage of pioneer species, diversity (Shannon-Weaver index), evenness and plant species composition.

Statistical procedure

All analyses were conducted with the data packages per site and with the pooled data, but for each year separately. The statistical analysis is divided into two parts: univariate analysis of separate variables, e.g. phytotoxicity, biomass and number of species, on the one hand, and on the other hand the multivariate analysis of plant species composition (the Principle Response Curves: PRC). In the univariate analysis of the relevé, biomass and phytotox data comprised of three steps. First a standard analysis of variance was carried out. If an effect had been found with a significance value of $P<0.10$ in the first step, the second step followed with a Williams' test on trend (is there a monotonically increasing or decreasing relation between dosage and effect parameter). If there was a significant trend ($P<0.05$) in the second step, then in the third step the Williams' test was used again, to calculate the so called no observed effect dosages (NOED). The highest dosage that was not significantly different from the control was taken as the NOED. Since in most cases there was a significant autocorrelation between August and May relevé data of the same year and between May values of the different years, in the analysis of the effects the contrasts were used, instead of the data itself. The significance level α applied, is 0.05. If the statistical analysis resulted in a P-value of 0.05 or less than the dosage (etc.) had *a significant effect*. Because several variables were sampled or derived from the relevés, the significance level α for multiple comparisons was corrected by the improved Bonferroni procedure.

Three different multivariate tests were performed. First in the CANOCO computer program, Redundancy Analysis is accompanied by Monte Carlo permutation tests to assess the statistical significance of the effects of the explanatory variables on the species composition of the samples. The significance of the PRC diagram in terms of displayed treatment variance was tested by Monte Carlo permutation of the plots following the PRC analysis. Second it was tested if the treatment regime has a significant effect on the composition of the vegetation community on a particular sampling date, and third it was tested if a particular treatment had a significant effect on the composition of the vegetation community at a particular sampling date. Besides the overall significance of the treatment regime, we also wanted to know which treatment levels differed significantly from the controls. From the results at each site a $NOEC_{community}$ could be deduced for each sampling date, with the $NOEC_{community}$ being the highest treatment level for which no statistical significance of effects could be demonstrated.

Results

Pesticide deposition
Deposition on the off crop vegetation was measured during the first and second spraying. The results show that the dosages in the field were applied with great precision. During the first spraying in both years deposition significantly increased with increasing height. Differences in deposition during the first spraying are smaller in 2001 compared to 2000. During the second spraying deposition is dependent on height and dosage.

Short term phytotoxic effects
Significant short term phytotoxic effects (8-10 days after application) of glufosinate-ammonium were present in both years, after each treatment, at all sites and even at low dosages. At the low dosages 2% and 4% phytotox values ranged between 3 and 14% and phytotox values after the first application were higher then after the second application. NOED is in almost all cases lower than the lowest dosage of 2% used. In August of the same year of spraying, no significant effects on the vegetation could be detected anymore, even at the highest dosage (64%).

Mid- term ecological and community effects: within the years of application
Within the year of spraying at low concentrations (2% and 4% of the field dosage) there were *no significant effects* on the vegetation parameters studied. In the second year (2001) of the study no effect could be detected at low concentrations (or even high concentrations) on this type of parameters at all. At higher concentrations (32% or higher) in both years of the study *significant effects* were found on the vegetation community. In the community analysis the ditch bank locations (Lexmond and Zegveld) showed the most pronounced effects. In the first year of the study also significant effects on the vegetation biomass were found within the year of spraying (at 32%, all sites combined). In the second year at one site (Lexmond) a significant effect was found on the biomass (32%).

Longer term ecological and community effects: one or two years after application
In the experiment even at the highest dosages no significant effects have been found on the vegetation in May (2001 and 2002) of the herbicide applications of the years before (2000, 2001) for all relevant criteria / parameters. Thus, no longer term effects on the plant community were found one or two years after application.

Conclusions and discussion

The aims of the study were to acquire information on the effects of glufosinate-ammonium and recovery on adjacent vegetation at the community level in the field situation under "realistic" worst case conditions and to assemble knowledge about how to conduct field tests with vascular plants at the vegetation level regarding herbicides and non-target vegetation.

Concerning the effects of glufosinate-ammonium on off crop vegetation it can be concluded as follows:

- Short term effects: About 10 days after spraying in spring, *small, significant phytotoxic effects* could be detected on non target vegetation even at low concentrations of 2 and 4% of the field dosage. The effects are not visible any more later in the season (August).
- Mid term effects: Within the year of spraying at low concentrations (2% and 4% of the field dosage) there were *no significant effects* found on the vegetation parameters studied. At higher concentrations (32% or higher) in both years of the study *significant effects* were found on the vegetation community. At 32% also significant effects on the vegetation biomass were found within the year of spraying (in 2000 for all sites combined and in 2001 at one site).
- Longer term effects: In the pair wise comparison between years (2000/2001, 2001/2002 and 2000/2002) *no significant effects* of the sprayings could be detected in spring on all parameters.

Concerning the mid-term effects of this study the results should be handled with care in some instances there were nearly significant effects and clear negative trends between dosage and effect parameters.

From the study it is clear that large scale field studies can be conducted in practice. Moreover, sound and generally reproducible results could be generated. From the study some general methodological aspects could be derived regarding experimental set up (set up with drift simulation and not-adapted vegetation), field assessments (phytotox of plots incl. studying recovery, biomass and vegetation relevé's) and statistical procedures (multivariate and stepwise univariate approaches).

PREFACE

In this report the set up and results are presented of a field study of the effects of the herbicide glufosinate-ammonium on off crop vegetation, carried out in the Netherlands. The study was commissioned by Bayer CropScience GmbH (report CO45860) and carried out by the Institute of Environmental Sciences (CML) of Leiden University in the period 2000 - 2003.

Bayer report code: CO45860
Test item: Code: AE F039866 00 SL18 G2
Experimental start date: 31. May 2000
Experimental end date: 30. May 2002

Aim of the study is not only to acquire information concerning the effects of low dosages of glufosinate-ammonium on "natural" vegetation adjacent to the field but also to assemble knowledge about how to conduct higher tier tests (off crop) with vascular plants at vegetation level regarding herbicides.

Higher tier studies are normally not needed with regard to effects of herbicides on non target plants (NTP). Therefore, the set up of the study was communicated with experts in the field of the authorisation of pesticides. To this end a workshop was organised at CML (12-4-2000). The intermediate results were discussed at a second workshop (09-02-2001) at CML. The final results were presented and discussed at a third workshop (03-02-2003) at CML. See annex IV for participants of these three workshops. Moreover, during the study several field visits to the experimental sites were made by scientists, regulators and people from industry.

The authors would like to thank all people and organisations that made the research possible: Mr. F. Kool (farmer at Lexmond), Mr. K. van Houwelingen (researcher at the experimental farm Zegveld), Dr. G.-J. Blankema (City of Apeldoorn), Zuiveringsschap Hollandse Eilanden en Waarden and Hoogheemraadschap De Stichtse Rijnlanden.

During the research valuable comments were made both during the workshops, field trips and on the draft report by people from Bayer CropScience. We would like to thank specially, Jürgen Cremer, Peter Sowig, Klaus Stumpf, Rik Mekking and Maarten van Erp.

The research itself has been carried out by a large number of people of different backgrounds. Within the CML research team field surveys were made by Frank de Jong (now RIVM), Mike van der Linden (now DLV) and Rob van der Poll. Statistical support has been given by Nellie van der Hoeven (Ecostat) and Pim van Hooft (now University of Antwerp). The calculations were made - and reported - by Wil Tamis and Paul van der Brink (Alterra).

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1 INTRODUCTION

1.1 Background

If large numbers of farmers in the Netherlands in the future would switch to Liberty Link Maize varieties (genetically modified glufosinate tolerant maize), the use of Liberty (active ingredient = glufosinate-ammonium), the complementary herbicide, would increase considerably. Since maize is grown all over the country, including in areas that are part of the National Ecological Network (NEN) concern has been raised regarding the potential effects of this application on valuable off crop vegetation (Klepper, 1997, 1998). The NEN is composed of the major existing vulnerable and valuable nature areas and is to be extended with nature development areas and ecological corridors. Since the NEN often consists of rather narrow (10-25 m wide) corridors, the Network itself could be affected. Although these aspects are not yet included in the Dutch or EU approval procedure, and Liberty is approved for use in genetically modified glufosinate tolerant maize in the Netherlands since 2000, Bayer CropScience would like to anticipate on such aspects and asked the Institute of Environmental Sciences of Leiden University (CML) to conduct a field study on the potential effects of the use of Liberty on off crop vegetation. Since there are only few examples of this kind of field studies, the present study also has a methodological aspect: investigate how these studies should be designed and conducted.

On the basis of the knowledge available, a risk assessment of effects of drift on the vegetation of the NEN was made in 1997 by the Dutch National Institute for Public Health and the Environment (RIVM). At that time only laboratory data on the effects of high dosages of glufosinate-ammonium were available, for a number of weed species. Therefore a number of factors had to be extrapolated: high dosage effect to low dosage effect, available (annual) weed species to (perennial) species of concern for the National Ecological Network, short-term effects on individual species to longer-term effects on the vegetation as a whole. Based on the available data and the extrapolation, an unsprayed zone of 12 m (with 50% dose and 50% drift reduction) to 25 m (standard conditions) was suggested for glufosinate-ammonium (GA). If these distances are respected, 95% of the flora is protected, according to the model (Klepper, 1997).

Supported by Ganzelmeier drift data (Ganzelmeier *et al.*, 1995) Bayer CropScience concluded that considerably smaller buffer zones (1-4 m) would be sufficient. Both the conclusions of RIVM and Bayer CropScience are based on extrapolation of laboratory data and assumptions with regard to among others drift, wind direction and the species involved.

In a number of field studies (Shepperson & Sweet, 1997; Sweet *et al.*, 1999, Belyk, 1997), short term phytotoxic effects are found, followed by a quick recovery. In some of the studies of Shepperson & Sweet (1997) significant shifts in species composition in field margins could not be found for GA Also in the studies of Belyk (1997) no long term effects could be observed. However, in all cases vegetation composition was quite different from the vegetation of concern in the Netherlands.

1.2 Aims of the study

As regards the Netherlands, concern about the effects on off crop vegetation exists (Klepper, 1997, 1998). Therefore, a field study has been carried out in the Netherlands for the period 2000-2002. Further more guidelines for this type of studies are rare. The present study had two aims:

1) To acquire information on the effects of glufosinate-ammonium and recovery on adjacent vegetation at the community level in the field situation under "realistic" worst case conditions of use (two applications, respectively in May and June).
2) To assemble knowledge about how to conduct field tests with vascular plants at the vegetation level regarding herbicides and whether scientifically sound results can be derived.

1.3 General study design and the parameters

The proposed study design was presented and discussed in a workshop in 2000 with representatives of Bayer CropScience Germany and Netherlands, the Dutch authority for the approval of pesticides (CTB) and researchers from the Netherlands and the United Kingdom. The results of the workshop have been incorporated into the final design. The intermediate results were discussed at a second workshop in 2001 and the final results were presented and discussed at a third workshop in 2003 (see annex IV for participants of these workshops).

In the search for appropriate sites we looked for sites with natural vegetation, since the scope of the study is the National Ecological Network (NEN). It proved impossible to conduct a field study in a nature reserve or on natural vegetation. Spraying of herbicides in nature reserves is ethically questionable, and above that landowners in the reserves refused to participate in experiments, mainly for reasons of publicity. We therefore looked for habitats in appropriate areas with relatively natural vegetation. A number of criteria were applied:

- potential exposure to the compound (maize-growing region)
- no herbicides used on or adjacent to the vegetation in the last 10 years
- relatively high nature value (flowers, species, diversity)
- stable management over the years (i.e. mowing once or twice per year)
- representative for the Netherlands and for Mid-Europe.

It was decided to study the effects of glufosinate-ammonium in a *Realistic Worst Case* situation. Hereto the following design was chosen:

- dosages include a high dosage with a definite effect (positive control, 64% of the maximum registered dosage of two times 800 g active ingredient glufosinate-ammonium/ha) and an untreated control
- dosages are applied twice per season, as can be the (worst) case in practice
- the vegetation is sprayed directly with the different dosages, thus simulating drift
- relatively species rich vegetation types are chosen, that were likely not to be exposed to herbicide use in the past
- vegetation types are chosen, which could potentially be exposed to herbicides if Liberty-tolerant maize would be grown in an adjacent field.

In the Netherlands maize cropping traditionally takes place on sandy soils, but increasingly spreads all over the country to clay and peat soils. Non-target habitats in maize cropping regions are, for instance, ditch banks, hedgerows and road verges. For this study the trials are conducted on verges and ditch banks. Verges were selected for the extrapolation to Europe.

Ditch banks were selected because of the clay and peat soils. In all cases no fertiliser was applied and management included mowing and removal of the swot. Although the plots were part of the agricultural area and the ditch banks even part of a farm, this management ensured a relatively nutrient-poor situation, making for species-rich vegetation.
The main question concerned the effects at the vegetation level. The central parameters are therefore vegetation surveys: records of species presence and abundance. The parameters were recorded in May 2000, 2001 and 2002 (before spraying) and in August of 2000 and 2001 (after spraying), see Table 1.1.

Table 1.1 General lay-out of measurements (X) in 2000, 2001 and 2002; in 2000 and 2001 there were two herbicide applications in each year in May and June.

	2000			2001			2002
	May before appl	May-June after appl.	August	May before appl.	May-June after appl	August	May
	original reference	short term effects	mid term effects	long(er) term effects	short term effects	mid term effects	long(er) term effects
vegetation composition	X		X	X		X	X
pesticide deposition		XX			XX		
phytotox		XX			XXXX		
biomass			X	X		X	X

To explain the results, or in order to be able to trace other effects, a number of other parameters have also been recorded with respect to the carefulness of these putative implications:

- Exposure to the herbicide using water-sensitive paper; for tracing differences in exposure between sites (weather conditions); and for quantifying exposure high and low in the vegetation
- Phytotoxic symptoms at the time of maximum effect after both applications of the herbicide about 10 days after each herbicide application
- Biomass: assessment of the effects of spraying later in the growing season (August).

The main questions of this study concerning the effects at the vegetation level were expressed in the two following research questions:

- Effects within the year of applications (short term and mid term): *Are there any effects in August of the herbicide applications in June within the same year?*

- Effects one or two years after application (long(er) term): *Are there still detectable effects in May of the herbicide applications the years before?*

These two research questions were analysed for 2001 and 2002 separately.

1.4 Set up of the report

In Chapter 2 the lay-out of the field experiment, sampling and data analysis is described in detail. In Chapter 3 the results with respect to deposition of glufosinate-ammonium are being presented. In Chapter 4-9 the results of the study with respect to the effects of glufosinate-ammonium on the off crop vegetation are being presented. In Chapter 10 a synthesis is presented of the main results of the field experiment. At the workshop of 13-2-2003 also some more general results regarding improving the methodology on off crop vegetation issues were discussed, including the relevant information presented by the speakers. Conclusions and discussion regarding these methodological aspects are being incorporated as part of Chapter 11.

In the report on several pages there are Roman numbers which refer to end notes at the end of the report.

2 FIELD STUDY DESIGN

2.1 General design: sites & dosages

2.1.1 Selection of areas and sites

Maize cultivation in the Netherlands is concentrated in the higher regions on the sandy soils. These soils originally are nutrient poor. Fertiliser use in agriculture led to a nutrient rich status. Sites with a higher nature value however, among which many road verges, still are relatively poor in nutrients. Given the main aim of the study (vegetation in the National Ecological Network), the nutrient-poor verges were chosen for the trial. In the lower-lying parts of the Netherlands, ditch banks on clay and on peat soil were selected. The sites are shown in Fig. 2.1 and described below.

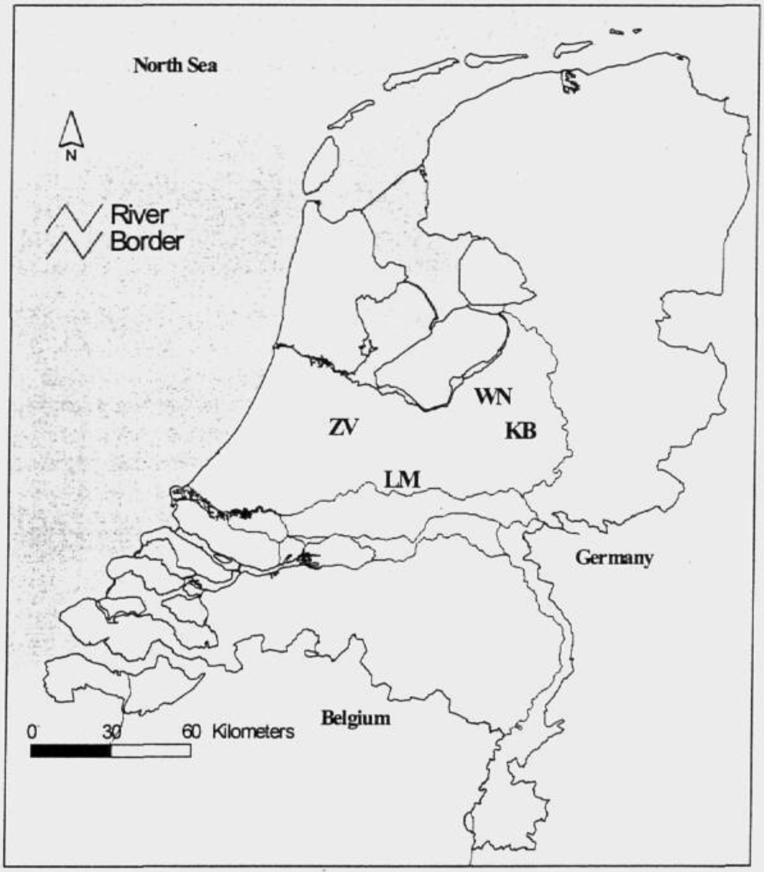


Fig. 2.1 Location of the four trial sites in the Netherlands; KB = Klarenbeek; WN = Wenum; LM = Lexmond; ZV = Zegveld.

2.1.2 Description of sites, blocks and plots

Sites Wenum (WN) and Klarenbeek (KB) are road verges on sandy soil near the city of Apeldoorn; sites Lexmond (LM) and Zegveld (ZV) are ditch banks, on clay and peat respectively. The Klarenbeek and Wenum sites are located in an agricultural area with cattle-breeding and maize-growing. Normally these verges are mowed twice a year. Because of the experiment, the first mowing was omitted so that the vegetation should produce leaves, thereby enabling it to be studied. These plots were mowed in September. The plots at Lexmond and Zegveld are situated on the banks of ditches belonging to cattle breeding farms. Normal management includes mowing and grazing. The plots were mowed at least 2 weeks before the applications of the herbicide. For our experiments, mowing and grazing were omitted during the trial, using fences to prevent grazing. These fences were removed after the last assessment, and the plots were mowed in September. In all four sites the plots were not adjacent to maize parcels, but to pastures.

Plots were arranged in blocks to minimise within site variation. In general a block contained five plots with different treatments, but in some cases this number was ten or even fifteen plots. Further detailed information on the blocks and plots can be found in Annexes II and III.

The exact site of the test areas was determined in such a way that they could easily be identified in the subsequent year. For this, an adjacent fixed reference point was used (in Lexmond and Zegveld) or the spot was marked either with wear-proof paint (if a road was present) or with zinc-coated pins hammered into ground level (in Klarenbeek and Wenum). Further detailed information on the sites can be found in Annexes I and II.

2.1.3 Selection and application of dosages

It is assumed that at 64% and 32% of the maximum registered field dosage of 2 x 800 g a.i. GA/ha (= 2 x 4 l Liberty/ha) per season, effects would be found, while 4% is a relevant low deposition level, occurring at approximately 1 m in the Dutch situation (according to Michielsen *et al.* 1999; Van der Zande *et al.*, 2000, see Fig. 2.2 and Table 2.1) from the area sprayed. In this study, 16% is taken as an intermediate dosage. We thus started with the dosages of: 0%, 4% (32 g a.i./ha), 16% (128 g a.i./ha), 32% (256 g a.i./ha) and 64% (512 g a.i./ha) of the maximum field dosage of 800 g a.i./ha (= 4 l Liberty/ha) applied twice per season.

Table 2.1 Distances at which the deposition levels used are expected, calculating with the model of Fig. 2.2, using drift reducing nozzles and a buffer zone of 50 cm.

dosage (%)	distance from last nozzle (m)	distance from field edge buffer zone 50 cm (m)
2	2.90	2.40
4	1.65	1.15
16	0.54	0.04
32	0.30	
64	0.17	

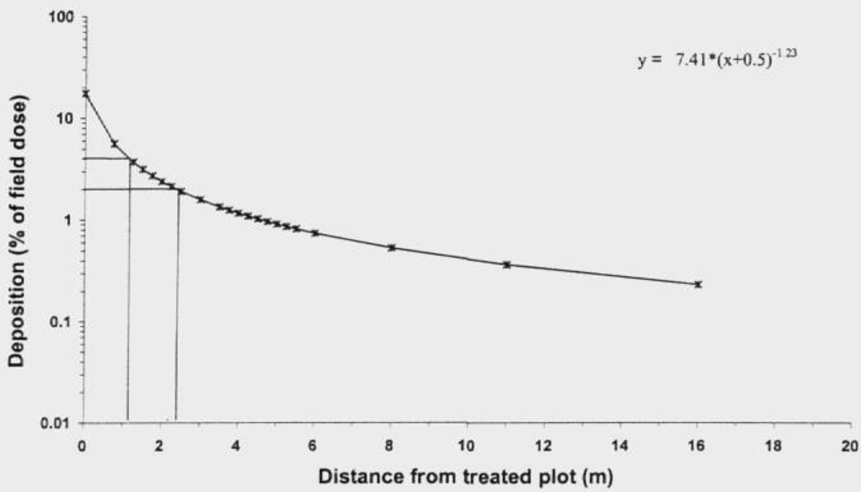


Fig. 2.2 Results of deposition measurements and additional modelling without a ditch; sources: Michielsen *et al.*, 1999; Van de Zande *et al.*, 2000; the formula describes the line, y = deposition and x = distance.

The first application (in Klarenbeek) showed clear phytotoxic symptoms at the simulated 4% drift level. For this reason the spraying scheme was changed: at the second site (Wenum) 2% (16 g a.i./ha) deposition was added and at the other two sites 2% was added and 32% was omitted. This resulted in the scheme shown in Table 2.2. Deposition of 2% can be expected on the vegetation types studied in 2-3 m distance from the treated fields (see Table 2.1). In total 105 plots have been examined.

Table 2.2 Distribution of the replicates over the sites and the application rates; full rate (100%) = 800 g active ingredient/ha.

	number of replications per dosage per site						
site	0%	2%	4%	16%	32%	64%	Total
Klarenbeek (KB)	5		5	5	5	5	25
Wenum (WN)	5	5	5	5	5	5	30
Lexmond (LM)	5	5	5	5		5	25
Zegveld (ZV)	5	5	5	5		5	25
total no. of replications	20	15	20	20	10	20	105

Table 2.3 shows the results of measurements of the dosages sprayed in practice during the trials. The actual dosages applied in practice were calculated by taking into account the remainder (r) of the total volume (V) of the spraying liquid (or vice versa the extra amount used): (V-r)/V*intended dosage. The data show that in 2000 cases the deviation from the intended dosage is less than 5% and in 2001 this is even less than 2%. So, it can be concluded that the applications have been carried out with high precision.

Table 2.3	Dosage-rate applied in practice during both applications on all sites; - = dosage not applied; ? = missing value.									
2000	dosage									
application	1	2	1	2	1	2	1	2	1	2
site	2%	2%	4%	4%	16%	16%	32%	32%	64%	64%
Klarenbeek (KB)	-	-	4.2	3.9	16.0	15.7	31.6	30.4	59.6	61.4
Wenum (WN)	2.0	2.0	3.8	3.8	15.4	15.5	30.2	32.1	61.2	63.4
Lexmond (LM)	2.0	2.0	3.8	3.7	15.4	15.1	-	-	62.5	60.3
Zegveld (ZV)	1.9	2.1	3.8	4.0	14.8	16.1	-	-	60.8	68.5
2001	dosage									
application	1	2	1	2	1	2	1	2	1	2
site	2%	2%	4%	4%	16%	16%	32%	32%	64%	64%
Klarenbeek (KB)	-	-	3.8	4.0	?	16.5	32.5	32.4	64.9	65.9
Wenum (WN)	1.9	2.0	3.9	3.9	15.4	16.1	32.0	32.1	64.5	64.7
Lexmond (LM)	2.0	1.9	4.0	4.0	16.1	15.8	-	-	65.0	?
Zegveld (ZV)	2.1	2.0	4.1	4.1	16.5	15.8	-	-	66.4	70.5

2.1.4 Number of replications per treatment

The number of replications per treatment over all four sites was 20 for most treatments (see Table 2.2) or 5 replications per site per treatment. With the number of 20 replicates differences between treated and untreated plots of 0.959 times the standard deviation can be detected, with a Power of 80% and α =0.05 (0.753 times the standard deviation, when a Power of 70% and a significance level α =0.10 is accepted). This means that the differences which can be traced depend on the variability of the parameter in question in the untreated control. Further information on the power analysis can be found in Annex V.

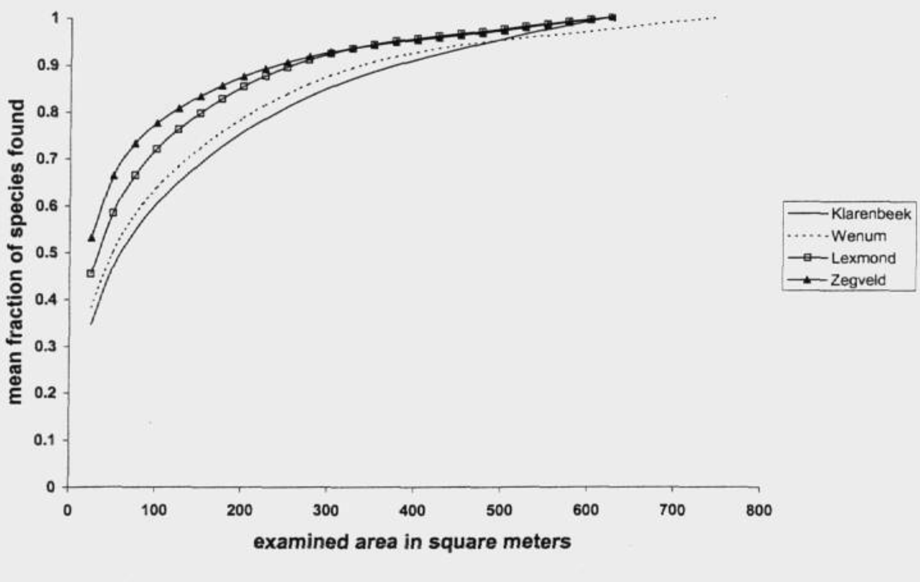


Fig. 2.3 Fraction of plant species found in relation to size of plot.

2.1.5 *Size and distribution of the plots*

All plots measure 25 m x 1 m = 25 m^2, a usual size for this kind of studies (Sýkora *et al.*, 1993). The results of Braun-Blanquêt relevés before the onset of the experiment showed that the percentage of species included in this way is higher on the sites on peat and clay than on sand (see Fig. 2.3 and Table 2.4). This probably is caused by the larger variation and species richness on the sand sites. As shown in Annex I and II the verges are situated along different roads and have for instance different expositions to sun and wind. From Fig. 2.3 it can be concluded that on the average, per treatment at least 65% of the species present will be taken into account

Table 2.4 Percentage of species in one and five plots (relative to the total number of species found on the site)

	% of total no. of species	
	25 m^2 (1 plot)	125 m^2 (5 plots)
Klarenbeek	35	65
Wenum	38	69
Lexmond	53	81
Zegveld	45	76

As an example of the distribution of the treatments over the plots the situation in Lexmond is presented. Since in Lexmond the plots were situated along two different ditches, there were two blocks, which were handled separately, among others to preserve the full scope of treatments in case of calamities. Fig. 2.4 shows the distribution of the number of dicotyledonous species over the plots. Since in general more effects were expected on the dicotyledonous species, the plots with different number of dicotyledonous species were distributed over the treatments as equally as possible (see Fig. 2.5).

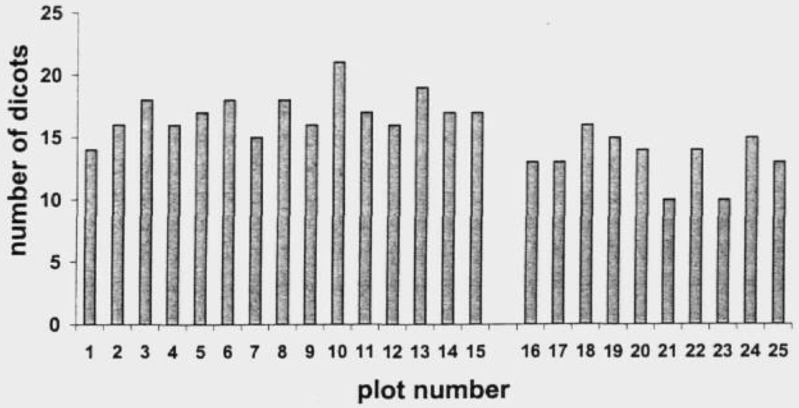


Fig. 2.4 Numbers of dicotyledonous species in the Lexmond plots before spraying.

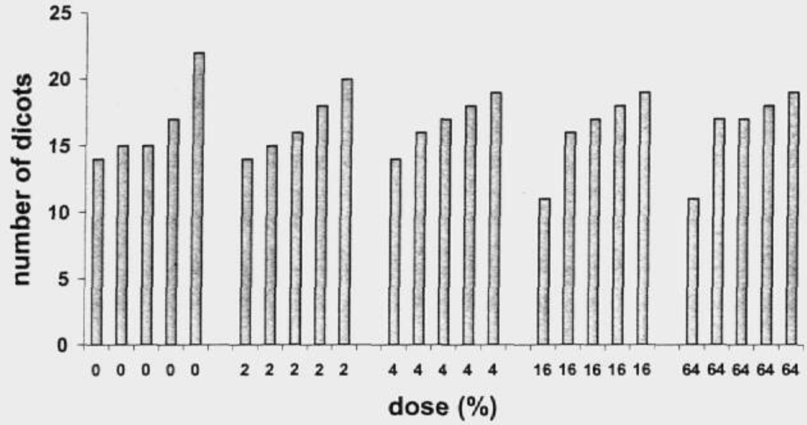


Fig. 2.5 Number of dicotyledonous species in the Lexmond plots before spraying, arranged per dosage.

The same procedure was followed at the other sites. In this way the mean number of dicotyledonous species before spraying was equal for the different treatments (15.6, 16.7, 16.1, 16.0 and 16.0 species for respectively 0%, 2%, 4%, 16% and 64% dosage, for all four sites).

2.2 Weather conditions

Table 2.5 shows the weather conditions during spraying in 2000 and 2001. In 2000 in general these weather conditions were quite similar for the different sites during first spraying but varied considerably in temperature and wind speed (range 2-6 m/s, median 4 m/s) for the second spraying. However, most sprayings were carried out with a low wind speed. In Wenum in 2000 it rained a little before and rather severely for 5 minutes between spraying of plots. In 2001 weather conditions were rather similar for the different sites and all sprayings were carried out at a low wind speed (range 2-5 m/s, median 4 m/s).

In Annex VI a summary is given of the weather surveys for the years 2000-2002 for the Netherlands. These three years can be characterised as very to exceptionally warm, wet and sunny.
The spring of 2000 can be characterised as exceptionally warm and wet with normal sunshine. The summer of 2000 can be characterised as normal. The autumn of 2000 can be characterised as very warm, wet and some less sunshine as normal. The winter of 2000/2001 is warm, wet and very sunny.
Spring and summer of 2001 are warm, wet and sunny. The autumn of 2001 can be characterised as very warm and very wet. The winter of 2001/2002 is very warm, wet and very sunny. Finally the spring of 2002 is very warm, dry and sunny.
The weather conditions in the research period are rather similar, with only the dry spring of 2002 different from the wet springs of 2000 and 2001, but since the winter of 2001/2002 was wet too, this probably was of little influence for the grassland vegetations investigated.

Table 2.5 Weather conditions during spraying in 2000 and 2001.

2000 site	date	temperature (Celsius)	air humidity (%)	wind speed (m/s)	wind direction	cloudiness (fraction)
Klarenbeek	31-05	18	60	2	NE	1/8
	20-06	33	40	4	S	1/8
Wenum	07-06	17	66	2-3	WWN	2/8
	22-06	22	60	2-3	SW	4/8
Lexmond	13-06	21	67	5-6	SW	8/8
	29-06	19	60	4	NW	1/8
Zegveld	16-06	20	55	3-4	NE	2/8
	03-07	26	55	4	SW	1/8

2001 Site	date	temperature (Celsius)	air humidity (%)	wind speed (m/s)	wind direction	cloudiness (fraction)
Klarenbeek	22-05	23	40	3	NE	0/8
	08-06	21	54	4	W	5/8
Wenum	30-05	27	46	3	NW	7/8
	14-06	24	35	2	NE	1/8
Lexmond	21-05	19	65	5	N	4/8
	07-06	17	54	4	SW	5/8
Zegveld	31-05	16	75	5	NW	6/8
	18-06	17	68	5	NW	7/8

2.3 Application of herbicides

2.3.1 Spraying conditions

The study focuses on the effects of herbicide drift. In practice it is almost impossible to study actual drift in a large scale experimental design under standardised conditions because weather conditions most likely will hamper an (experimentally postulated) optimised exposure to the herbicide. It was thus decided to apply the different dosages directly onto the vegetation, using for each dosage proper dilutions of the herbicide in the same amount of spray volume. In this way drift is simulated. Starting point was that the amount of active ingredient applied was equal to the amount calculated based on the drift model (see 2.1.3). For this aim the amount of spray volume was held constant, which is quite different from actual drift. Actual drift consists of smaller droplets with possibly higher concentrations of the compound - but less penetration power. Thus, this procedure simulates worst case conditions. The herbicide was applied by an experienced sprayer working for Bayer CropScience. He used an advanced handheld knapsack sprayer, with a specially constructed spray boom with two offside nozzles (Teejet, UB 8502). For practical reasons (plot size, volume of the knapsack sprayer, dilutions) the volume sprayed **was always equivalent to 240 l/ha**. Spray pressure was 2 bar. At all sites all replications per dosage were sprayed in succession, the remaining volume of the dilution being measured after five replications so that the dosage per treatment for all the replications together could be calculated. In all cases the order of spraying was from low to high dosage, to prevent contamination of the dilution with the compound.

Screens were put between the plots during spraying, downwind from the plot with a higher dosage than the adjacent plot. At Zegveld and Lexmond screens were placed between the treated area and the ditch to prevent contamination of the surface water. Measurements in the surface water after spraying adjacent to the plots which received the highest dosage, carried by the water board Rijnland (written communication), show that no traces of the compound were found (detection limit = 0.5 $\mu g/l$), showing that the screens were effective.

The dates of application are shown in Table 2.6. According to the recommendation in maize, the interval between the first and second spraying is between 15 and 20 days. Atmospheric humidity, temperature, wind speed and wind direction were measured before each application.

Table 2.6 Spraying dates in 2000 and 2001 and number of days between first and second application.

	first application		second application		days between applicat.	
site	2000	2001	2000	2001	2000	2001
Klarenbeek	31-05	22-5	20-06	08-06	20	17
Wenum	07-06	30-5	22-06	14-06	15	15
Lexmond	13-06	21-5	29-06	07-06	16	17
Zegveld	16-06	31-5	03-07	18-06	17	18

2.3.2 Deposition measurements

Spray deposition was measured in 2000 and 2001 with water-sensitive paper (2.5 x 7.5 cm) at 15, 30 and 60 cm above ground level. This was done in triplo during the first spraying with pure water in all 5 untreated plots to gain an impression of the exposure of different layers in the vegetation. During the second spraying deposition was measured at all four sites in one plot of each dosage. Subsequently the exposed papers were scanned and analysed with Scion Image, an image processing and analysis program, and deposition was calculated.

2.4 Effect variables

2.4.1 General

In the following paragraphs the effect variables studied and the way in which their sampling took place in the field experiment are described.

2.4.2 Phytotox

In order to quantify the short-term effects of spraying, the observable external damage to the plants was determined. This method, known as phytotox, is frequently used in efficacy studies. It involves a visual assessment of the percentage of plants affected by all kind of external visible damage such as discoloration and stunting (EPPO guideline PP1/117(2), 1998). Deviant from the EPPO guidelines the phytotox estimates were made at intervals of 5% (with 0%, 5%, 10% etc. as the class means[i]) because the variation in the plots at vegetation level made it hardly possible to estimate differences more accurately. At the site first sprayed (Klarenbeek) an estimate of the phytotox was made jointly with an Bayer CropScience representative well experienced with this method. 100% phytotox means that the plant is lethally damaged. The phytotox was always recorded 8 to 10 days after each spraying in each year. In 2001 the phytotox was also determined at 31-34 days and 59-62 after the second application.

The extent of phytotox is always related to the reference: the natural discoloration in the non-exposed vegetation or plant is, by definition, set at zero. Before assessing phytotox in the treated plots, the controls are examined. In this experiment the phytotox was estimated for the plot as a whole and for a number of abundant plant species, namely *Anthriscus sylvestris, Plantago lanceolata, Rumex acetosa, Cerastium fontanum* subsp. *vulgare, Ranunculus acris,* *R. repens, Heracleum sphondylium, Trifolium pratense* and *T. repens*. Per species an estimation of the phytotox for the plot was made. The phytotox estimations were always made by the same person, who did know the position of the untreated plots, but who did not know the dosages of the treated plots. As far as the monocotyledonous species are concerned, it turned out to be practically impossible to distinguish separate individuals in all species. Because of the dominancy of the monocotyledonous species in the vegetation it was assumed that the phytotox estimated for the entire plot also reflects the phytotox of the dominant monocotyledonous species.

2.4.3 Biomass

In order to get an indication of the effects of the spraying on the biomass in 2000 at two sites and in 2001 and 2002 at all sites the effect of spraying on the total biomass of the vegetation was studied. For this purpose three sub samples of 30 cm x 30 cm per plot were harvested cutting the vegetation with a garden-shears, taking the entire above-ground biomass. Sampling dates and distances of sub samples from the beginning of the plots are presented in Table 2.8. In August 2000 four plots per treatment in Wenum were collected and in five plots per treatment in all other site and year combinations. In 2000 sampling was carried out 63-64 days after second application. In 2001 first sampling was between 11 and 16 days before first application and second sampling was between 73 and 81 days after second application. In 2002 the sampling in May was 340 to 346 days after the second application in 2001. Before determining the dry weight, plant materials were also dried in a stove for 14 days at 80°C. Biomass results are expressed in g dry weight per dm^2.

Table 2.8 Sample dates of biomass; tree sub samples were taken per plot.

year	2000	2001		2002
site	August	May	August	May
sub sample distance from beginning plot	6, 12, 18 m	1, 3, 5 m		7, 9, 11 m
Klarenbeek	10-Aug	11-May	28-Aug	16-May
Wenum	17-Aug	14-May	29-Aug	23-May
Lexmond		10-May	27-Aug	13-May
Zegveld		16-May	30-Aug	30-May

2.4.4 Vegetation relevés

Braun-Blanquêt relevés

Relevés were made in each plot in order to study the effects at the vegetation level, 25 relevés at Klarenbeek, Lexmond and Zegveld and 30 relevés at Wenum (see Table 2.2). At all four sites data were gathered before spraying (May/June) and after spraying (August); see Table 2.9. During the second assessment (post-spraying) the untreated control plots were studied first, followed by the treated ones. The botanist did not know the dosages the treated plots received. In 2000 relevé sampling was carried out during three consecutive weeks, but this relatively large time span had not a large influence on the comparability of the data.

Table 2.9 Dates of vegetation relevé assessments in week numbers and days before herbicide application; number of days before assessment determined from last day of vegetation assessment, number of days after application determined from first day of vegetation assessments; - = no spraying or no assessment (in 2002).

site	first assessment		second assessment	
2000	week number	days before 1st spraying	week number	days after 2nd spraying
Klarenbeek	20	16	32	68
Wenum	21-23	2	32-33	65
Lexmond	19-20	29	34	69
Zegveld	23	7	34-35	73
site	first assessment		second assessment	
2001	week number	days before 1st spraying	week number	days after 2nd spraying
Klarenbeek	19	14	32	57
Wenum	20	16	32-33	54
Lexmond	18-19	19	31	53
Zegveld	20-21	13	33	56
site	first assessment		second assessment	
2002	week number	days before 1st spraying	week number	days after 2nd spraying
Klarenbeek	20	-	-	-
Wenum	20-21	-	-	-
Lexmond	19-20	-	-	-
Zegveld	21-22	-	-	-

Recording methodology

In the relevés, the abundance of the various plant species present in each plot were determined according to the method of Braun-Blanquêt, with the abundance scale as adjusted by Barkman (1964); see Table 2.10. The plant species are in accordance with *Heukel's Flora van Nederland* (Van der Meijden, 1996).

Table 2.10 The abundance scale used for vegetation relevés according to Braun-Blanquêt (adjusted by Barkman, 1964) with classes and class means.

code	abundance class		class mean
	%	number of plants per plot	%
1	<5	1-10 individuals per record	0.1
2	<5	10-24 individuals per record or 1-3 individuals per m²	0.2
3	<5	4-10 individuals per m²	1.0
4	<5	> 10 individuals per m²	3.0
5	5-12		8.5
6	13-25		18.5
7	26-50		37.5
8	51-75		62.5
9	76-100		87.5

Other vegetation parameters

For each relevé, besides abundance of each plant species present a number of other parameters was also determined in the field:

- total vegetation cover as a percentage of plot area
- cover of dicotyledonous species as a percentage of area covered by vegetation
- cover of monocotyledonous species as a percentage of area covered by vegetation
- cover of horsetail species (Equisetaceae) as a percentage of area covered by vegetation.

From the relevé data the following parameters per plot were derived:

- total number of species
- number of dicotyledonous species
- number of monocotyledonous species
- number of horsetail species
- number of pioneer species
- fraction of dicotyledonous species of total number of species
- fraction of monocotyledonous species of total number of species
- fraction of pioneer species
- diversity (explained below)
- evenness (explained below)

Information regarding the classification of species as dicotyledonous, monocotyledonuos or horsetails was taken from Van der Meijden (1996). Information on plant species to be regarded as pioneer species was retrieved from the BIOBASE database (CBS, 1997), see also Annex VII. The fractions of dicotyledonous and monocotyledonous species were calculated as the ratio between the respective numbers of these species and the total number of species and expressed as a percentage.

The parameter vegetation diversity can be derived from the number of species and the abundances of the species present. There are several different diversity indices available and in this study Shannon-Wiener's index H was used (Krebs, 1972):

$$H=-\sum_{i=1}^{i=S} p_i * \ln(p_i)$$

where S = total number of species and p_i = fraction calculated as the ratio between abundance of species i and sum of abundances of all plant species in the relevé. The diversity index combines information about number of species and (relative) abundance. An allied index is the evenness index E (Krebs, 1972), which is a measure of dominance. If a few plant species dominate the vegetation the index is low, and if most species have a similar abundance then it is high. Evenness is simply calculated as:

$$E = \frac{H}{H_{max} = \ln(S)}$$

During the project all these measured and derived relevé parameters were studied and some parameters selected for definitive analysis and presentation; see § 2.5

2.5 Statistical analysis

2.5.1 General

From the various parameters measured and derived from the field data (see § 2.4) a selection was made for definitive analysis and presentation, see Table 2.7. These parameters can be divided in two classes: univariate and multivariate variables. Statistical analysis in the study is itself divided into two parts: univariate analysis of eleven variables, e.g. phytotoxicity, biomass and number of species, on the one hand (see Table 2.7), and multivariate analysis of plant species composition on the other. The description of the multivariate statistical method, the Principle Response Curves (PCR) method, is incorporated in Chapter 9. The univariate statistical methods used are described in the following sections. This description is an abbreviated and adapted version of the statistical procedure formulated by Van der Hoeven (2002). The statistical analysis comprises of several consecutive steps. The first two steps, data transformation and autocorrelation, were applied to all univariate variables and are treated in § 2.5.2 and 2.5.3. In § 2.5.4 to 2.5.5 the following steps are described for deposition, phytotox and relevé-variables and biomass respectively.

Table 2.7 Overview of all effect variables in the field experiment presented in this report; NOED = No Observed Effect Dosage; ED = is effect dosage at which a chosen level of effect is found; chapter: the chapter in which the results are presented.

variable	univariate/multivariate	NOED	ED	chapter
variables deposition, phytotox and biomass				
deposition	univariate	-	-	3
phytotox	univariate	+	+	4
biomass	univariate	+	-	5
vegetation relevé derived variables				
coverage, total	univariate	+	-	6
coverage, dicotyledonous species	univariate	+	-	6
coverage, pioneer species	univariate	+	-	6
Shannon-Weaver index (H)	univariate	+	-	7
evenness index (E)	univariate	+	-	7
number of species	univariate	+	-	8
number of dicotyledonous species	univariate	+	-	8
number of pioneer species	univariate	+	-	8
vegetation community	multivariate	+	-	9

2.5.2 Data transformation and correction

One of the basic assumptions of standard statistical analysis is that there is homogeneity of variance. If variance is not homogeneous, then the problem can often be solved by data transformation. For all the variables studied the homogeneity of variance was investigated by plotting treatment means (site x date x dosage) versus plot residuals and by plotting standardised residuals in a normal quartile (Q-Q) plot (Sokal and Rolhf, 1995). Depending on the variable, the effects of several transformations (square root, logarithmic, logistic[ii]) were investigated. Table 2.11 presents the optimal transformations. The optimal transformation was defined as that transformation resulting in a distribution that was least deviant from the normal distribution. For the variables total coverage and phytotoxic effects ('phytotox'), logistic transformation was adapted by adding a small value of respectively 1% and 2.5%, to avoid calculation problems with zeros.

Phytotox data were corrected for natural discoloration by subtracting the phytotox value of the control plot (natural yellowing) from that of the treated plots (within each experimental block).

Table 2.11 Type of transformation applied for the univariate variables (x); - = no dimension.

variable	dimension	transformation
variables deposition, phytotox and biomass		
deposition	% of leaf area	logistic: ln(x/(100-x))
phytotox	% of vegetation	logistic: ln((x+2.5)/(102.5-x))
biomass	g/dm^2	logarithmic (base: 10): log(x)
relevé derived variables		
coverage, total	% of total area	logistic: ln((x+1)/(101-x))
coverage, dicotyledonous spp.	% of vegetation	logistic: ln(x/(100-x))
coverage, pioneer species	% of vegetation	logistic: ln(x/(100-x))
Shannon-Weaver index (H)	-	none
evenness index (E)	-	none
number of species	-	none
number of dicotyledonous spp.	-	none
number of pioneer species	-	none

2.5.3 Autocorrelation and calculations of contrasts

In case of repeated measurements, in time, of the same experimental units it is quite clear that these measurements are not independent. This is one of the basic assumptions of statistical analysis. A measure for dependence is the autocorrelation. If this autocorrelation is high, a statistical approach should be followed that take into account the dependence of the different measurements in time. There are several approaches, from which calculation of contrasts, further explained below, was the main approach used in this study.
Autocorrelation between August and May values of the same year and between May values from different years were calculated for all the variables, except for deposition. Autocorrelation analysis of phytotox data was only carried out for a series of three measurements after the second application of the herbicide in 2001. Calculation of the of autocorrelation coefficient, r_{ij}, was carried on the residuals of the (transformed) variables. Autocorrelation coefficients were tested by calculating W_{ij}. In the formula is k the number of observations minus the number of calculated means. The autocorrelation coefficient is significant if: $|W_{ij}| > t_{\alpha/2,k-1}$. In this formula is t the Student's t-value for the significance value $\alpha/2$ for k-1 d.f..

$$W_{ij} = r_{ij} * \sqrt{\frac{k-1}{1-r_{ij}^2}}$$

In most cases autocorrelations between August and May values of the same year and between May values of different years were significant, except for biomass and phytotox. Therefore the analysis of the effects within the year (in August) and after one or two years (in May) were analysed by only using the contrasts, instead of the data itself, except for biomass and phytotox.

The contrast (C) was defined as the difference between the (transformed) measurements (X) of the different dates, see formula.

$$C = X_{date2} - X_{date1}$$

2.5.4 General remarks on statistical analysis and presentation

In general, standard statistical techniques have been applied, using the statistical package SPSS 11.0. All analyses include block structure of the field experiment, with exception of deposition. In general dosage and site (and interaction) were used as explanatory variables, with exception of deposition where height (and interaction) was also used as explanatory variable.

The null hypothesis for the general analysis was that no differences in effect variables exist between treatments (dosage), site etc. or interactions. The alternative hypotheses was that there is at least one mean different from the other means.

All explanatory variables were treated as factors (nominal scale). Analyses were carried out for all sites per year combined (overall analysis: dosage + site +dosage x site) and in addition for each site per year individually (dosage). Data of the untreated (control) plots were always included in the analysis, except for the analysis for the phytotox data, because the data of the untreated (control) plots were already used to correct the data of the treated plots for natural yellowing.

The significance level α applied, is 0.05. If the statistical analysis resulted in a P-value of 0.05 or less than the dosage (etc.) had *a significant effect*. Significant P-values for dosage were marked in the tables with an asterisk *. For the variables derived from the relevés (see Table 2.11) a family-wise significance level 0.05/(n+1) is used; for further explanation see § 2.5.7.
If the statistical analysis resulted in a P-value for dosage between 0.05 and 0.10 (or between the family wise equivalents), these results were included in the tables, but not or only marginally discussed in the text. These results are marked in the tables with a plus sign +.

The mean values of the effect variables at the different dosages (or heights for deposition) are presented in separate diagrams for the different dates or contrasts. Mean values of transformed variables were first back-transformed before presentation.

2.5.5 Statistical analysis of deposition

In Table 2.12 the consecutive steps of the statistical analysis of the deposition measurements are summarised in a scheme. *Steps 1* and *2* have already been described in paragraphs 2.5.2 and 2.5.3 respectively.

There were two main analyses in *step 3*:
- effect of height of vegetation on deposition during first application in 2000 and 2001;

- effect of height of vegetation and effect of dosage from first application on deposition during second application in 2000 and 2001.

The last *step 10* (steps 4 to 9 are not relevant for deposition) has already been described in paragraph 2.5.4.

Table 2.12 Scheme of statistical analysis of deposition. Step numbering is discontinuous, because most steps in the scheme were not relevant for deposition (see Tab. 2.13, 14 for all steps discerned).

step	description	Remarks
1	data transformation	- logistic transformation
2	autocorrelation	- not relevant
3	general analysis effects height and dosage (GLM)	-for *2000* and *2001* during first application - three measurements in control, no block effect - all sites: site + height + site*height no multiple comparison because significant interaction - per site: height multiple comparison with LSD - for 2000 and 2001 during second application - one measurement per dosage, no block effect - all sites: S + H + D + S*H + S*D + H*D + S*H*D (S =- site, H = height, D = dosage) no multiple comparison because significant interaction - per site: height + dosage + site*dosage multiple comparison with LSD
10	presentation	- tables - diagrams - mean values per site per height and/or dosage - transformed means back transformed

2.5.6 Statistical analysis of phytotox

In Table 2.13 the consecutive steps of the statistical analysis of the phytotox measurements are summarised in a scheme. *Steps 1* and *2* have been described in paragraphs 2.5.2 and 2.5.3 respectively.

In *step 3* a general analysis was performed of the effect of dosage after each of the two applications of each year (2000 and 2001).

In *step 5* (step 4 was not relevant for phytotox) the analysis was continued with a test on the presence of a trend: is there a monotonically increasing relation between dosage and phytotox? For the analysis of monotonic trends in effects in relation to dosage, the parametric Williams' test (Williams, 1971, 1972) was used. The null hypothesis of this test is that the means are equal, against the alternative hypothesis of that the means are monotonically ordered. The monotonic trends were tested one-sidedly assuming a monotonically increasing phytotox with increasing dosage[iii]. Trend analyses were only carried out per site, since there were significant interactions between dosage and site, so effects of dosage differ per site and therefore cannot be combined.

Table 2. 13 Scheme of statistical analysis of phytotox. Step numbering is discontinuous, because one steps (4 in the scheme was not relevant for phytotox; see also Table 2.14.

step	description	Remarks
1	data transformation	- correction for natural discoloration: plot (treatment) – plot (control) - logistic transformation
2	autocorrelation	- between first application 2000 – second application 2000 - between first application 2001 – second application 2001
3	general analysis effects dosage (GLM)	- all sites: dosage + site + dosage*site + site/block - per site: dosage + block - for 2000 first application second application - for 2001 first application second application
5	trend analysis (Williams' test)	- if significance value in step 3 was lower than 0.10 then step 5 was carried out; this was always the case -no overall analysis, only per site since interaction dosage*site was always significant - for 2000 and 2001, first and second applications
6	NOED calculation (Williams' test)	- if step 5 was significant, then step 6 was carried out; this was always the case - see remarks step 5
7	effect dosages (logistic model of Van Ewijk & Hoekstra)	- ED5, ED10 and ED50 - see remarks step 6
8	correlation dosage and effect (Spearman correlation)	- carried out for each site for each assessment - see remarks step 5
9	disappearance general analysis effect dosage and time (GLM repeated measurements)	- three assessments after second application in 2001 - per site: dosage + time + dosage*time + block
10	Presentation	- tables (text) - diagrams - mean values per site per dosage - transformed means back transformed - also for 9 individual plant species - for 2000 and 2001, first and second application - minimum requirements number of measurements - information from sites combined

In *step 6*, the no observed effect dosage NOED, is determined per site also with the Williams' test. The highest dosage that was not significantly different from the control was taken as the NOED. However, in case of phytotox no control observations were available, because these data had already been used to correct the treatment values for natural yellowing effects. For the control the default dummy value 0 was used instead. The denominator of the test quantity was changed to $\sqrt{(MSE*(1/n+1))}$. In this formula is MSE the mean square error and n is the number of replicates per treatment.

In *step 7* the dosages which result in 5%, 10% and 50% phytotox had been calculated using a non-linear regression method in SPSS. To calculate the ED50, ED10 and ED5 of the phytotox at each site a three-parameter logistic model was applied (Van Ewijk & Hoekstra, 1993), see the formula for the ED50:

$$X = \frac{X_0}{1 + (\frac{dosage}{ED50})^{\eta}}$$

In this formula is X the variable; X_0 is the mean value for the control plots and η is a dosage-response parameter. Models were further adapted, depending on the type of transformation involved.

In addition to step 7, the Spearman correlation coefficient between dosage and effect variable were calculated in *step 8*.

In *step 9* the disappearance of the phytotoxic effects in time was studied after the second spraying in 2001. After the second spraying three measurements had been made. For each site it was investigated, with a GLM repeated measurements, if dosage and time had significant effects.

In addition to the general description of *step 10* in paragraph 2.5.4, the data of phytotoxic effects on certain dominant plant species were presented using a different approach. First means were calculated for each of these species per dosage per site in those cases where there were at least three observations per combination. Only those sites with at least four means, including control and highest dosage, were included in the further analysis. Next the means phytotox per dosage for each of these species over all selected sites were calculated.

2.5.7 Statistical analysis of biomass and relevé data

In Table 2.14 the consecutive steps of the statistical analysis of the biomass and the relevé data are summarised in a scheme. *Step 1* has already been described in paragraphs 2.5.2.

In *step 2* contrast have been calculated for all relevé parameters (see paragraph 2.5.3) between May and August data within the same year for the analysis of mid term effects and between the May months of different years. In case of biomass no contrasts were calculated and August data were used for the analysis of mid term effects and the May data were used for the long(er) term effects.

In *step 3* the general analysis of effect of dosages is carried out for all sites combined and also for each site separately (with exception of contrast May 2000-2002).

In *step 4* a correction is carried out for multiple comparisons. In total eight variables were sampled or derived from the relevés, e.g. total number of species, number of dicotyledonous species and number of pioneer species, see Table 2.11. By increasing the number of variables based on the same samples, the chance of finding a significant false-positive effect increases, which is an unwanted side-effect. There are several methods to correct the significance level α for multiple comparisons. The method used in this study is the improved Bonferroni procedure (Hacou & Meelis, 1992). Application of the improved Bonferroni procedure for these data indicates that significance values (P) over 0.025 are always to be interpreted as not significant and values below 0.006 as always significant. The significance of P-values between 0.006 and 0.025 depends on the rank number of the P-value (see Hacou & Meelis, 1992).

In *step 5* a test on the presence of a trend was performed (see former paragraph). This test was performed for all cases where the general analysis in step 3 resulted in a significance value P of less than 0.10. We hypothesised a monotonic increase between dosage on the one hand and number of pioneer plant species and coverage by pioneer plant species on the other. For all the other variables we hypothesised a monotonic decrease. Since interaction between site and dosage was never significant (in combination with an effect of dosage with a significance value of $P<0.10$) also an overall test was performed on the basis of overall mean effect values and mean square values (MSE) calculated without interaction.

In *step 6* the so-called no observed effect dosages NOED was calculated (see former paragraph). Since interaction between site and dosage was never significant (in combination with an effect of dosage with a significance value of $P<0.10$) also an overall NOED was calculated on the basis of overall mean effect values per dosage and mean square values (MSE) calculated without interaction. In case of significant trends the magnitude of the effects is calculated for the lowest dosage with a significant effect and for the highest dosage of 64%. This effect is expressed as the relative decrease in effect parameter relative to the values in the control situation. In case of effects on contrast values the control situation in the second period was used.
For *steps 8* and *10* (steps 7 and 9 were not relevant for these variables) see the remarks in Table 2.14.

Table 2. 14 Scheme of statistical analysis for relevé data and biomass. Step numbering is discontinuous, because two steps (7 and 9, see Table 2.14) in the scheme were not relevant.

step	description	remarks	relevé	Biomass
1	data transformation	general	+	+
2	autocorrelation and		+	+
	contrast calculation	- between May 2000 - August 2000	+	August 2000
	- effects within the year (mid term effects)	May 2000 - August 2001	+	August 2001
	- effects after one or two years (longer term)	- between May 2000 - May 2001	+	May 2001
		May 2001 – May 2002	+	May 2002
		May 2000 – May 2002	+	
3	general analysis effects dosage (GLM)	- all sites: dosage + site + dosage*site + site/block	+	+
		- per site: dosage + block (with exception May 2000- May 2002)	+	+
4	correction for multiple comparison (improved Bonferroni procedure)	- rank P-values from step 3 for 8 relevé variables from least (rank 1) to most (rank 8) significant	+	-
		- compare P-value with family-wise α 0.05/(rank+1)	+	-
		- if P-value: < family-wise α significant, otherwise not significant	+	-
		- correction has been applied to results step 2, 3, 8	+	-
5	trend analysis (Williams' test)	- P value step 3 <0.10 then step 5	+	+
		- all sites: if interaction dosage * site not significant	+	+
		- per site	+	+
6	NOED calculation (Williams" test)	- if trend analysis in step 5 significant	+	+
		- see remarks step 5	+	+
	calculation magnitude effect	- effect relative to control in (second) period	+	+
8	correlation dosage and effect (Spearman correlation)	- per site	+	+
		- irrespective of significance of dosage step 3	+	+

		- only for short term effects	+	+
10	presentation	- tables	+	+
		- diagrams	+	+
		- mean values per site per dosage	+	+
		- transformed means back transformed	+	+

3 DEPOSITION ON OFF CROP VEGETATION

3.1 General

Deposition of the herbicide glufosinate-ammonium was measured during the both applications in 2000 as well in 2001. This information is of general interest regarding the exposure of off crop vegetation to spraying under field conditions. During the first application deposition was only measured in the control plots, to study the deposition at different heights in the vegetation. The results are presented in § 3.2. During the second application deposition was measured at different heights in control and treatment plots, to study possible treatment effects on the vegetation structure. The results are presented in § 3.3. In the final paragraph, § 3.4 a synthesis of the results is presented.

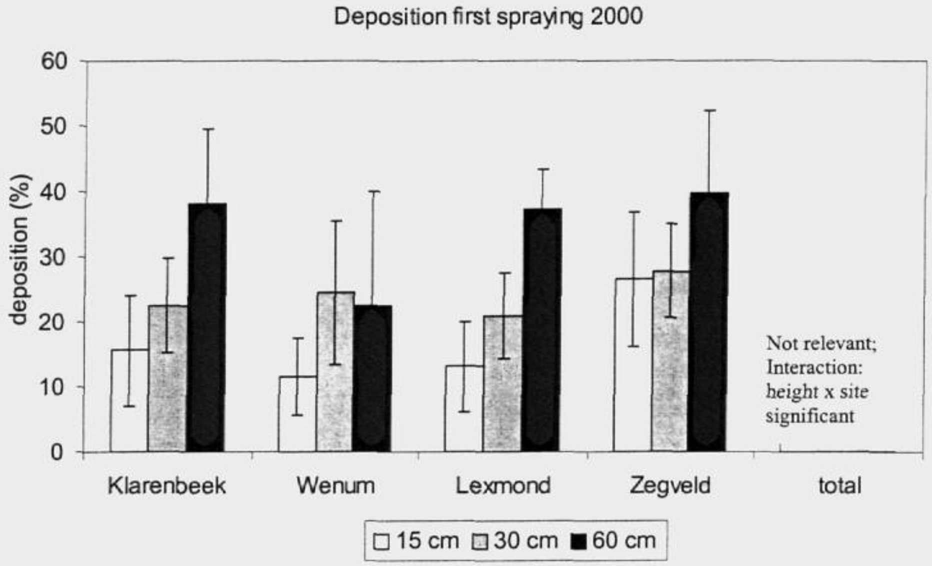
3.2 Deposition measurements during first spraying

During the first application, deposition is measured in the water-treated control at 15, 30 and 60 cm above ground level. As expected, deposition is highest at 60 cm (see Fig. 3.1 and Fig. 3.3). Differences for the mean deposition are statistically significant in 2000 and 2001 (Tab. 3.1). Plants in the higher vegetation layers receive in 2000 more than twice as much as plants in the lowest layers (Fig 3.1 and Fig. 3.3.) The smaller differences in 2001 may indicate that the plant coverage structure has changed. If we look at the individual sites, the same tendency is found. We have no explanation for the less pronounced situation in Wenum 2000. In all cases deposition at the 15 cm and 60 cm are statistically significant different. Not all differences between 30 cm and 15 or 60 cm are statistically significant at the individual site level (Tab. 3.1).

Table 3.1 Results statistical analysis deposition at three different heights (15, 30 and 60 cm) in control during first herbicide application in 2000 and 2001; * = P<0.05; h x s = interaction height x site; - = not relevant; see also below Table.

		effect			difference
year	sites	height	site	h x s	15 30 60 cm
2000	all sites	0.000*	>0.10	0.030*	-
	Klarenbeek	0.003*	-	-	a b b
	Wenum	0.013*	-	-	a a b
	Lexmond	>0.10	-	-	-
	Zegveld	0.029*	-	-	a a b
2001	all sites	0.000*	>0.10	>0.10	-
	Klarenbeek	>0.10	-	-	-
	Wenum	>0.10	-	-	-
	Lexmond	>0.10	-	-	-
	Zegveld	0.010*	-	-	a a b

Two different GLM-analyses were carried out each year: first all sites combined: height x site and then each site separate: height; difference = multiple comparisons with LSD; unequal letters indicate statistical difference (P<0.05) between individual heights.



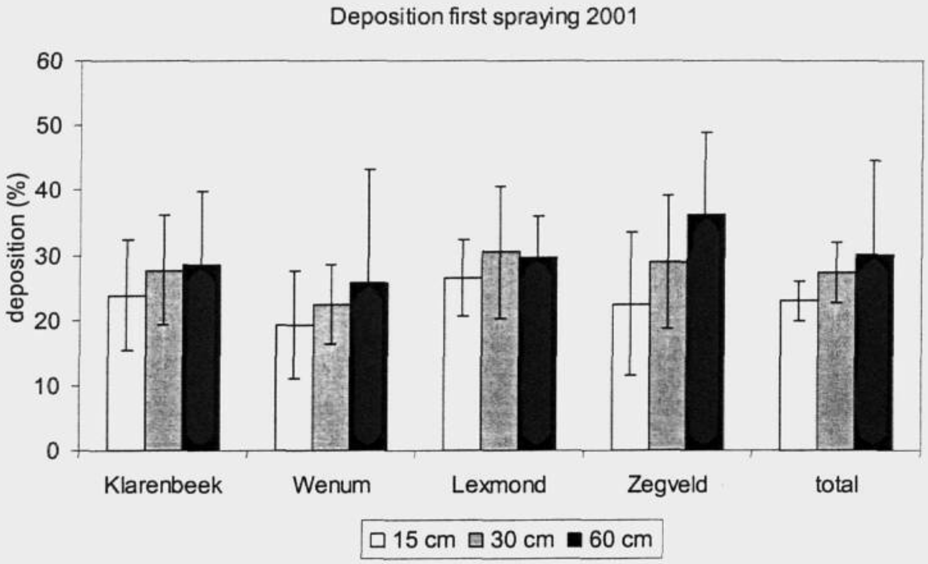


Fig. 3.1 Mean and standard deviation of the interception by water sensitive paper in the untreated control at different heights during the first application for separate sites in 2000 (above) and 2001 (below).

3.3 Deposition measurements during second spraying

During the second spraying the effects of the first spraying could influence the deposition, and deposition was measured in the different treatments. In Tab. 3.2 this can be seen as significant effects of dosages overall and for most sites individually. In Fig. 3.2 and 3.3 the deposition at three heights during the second application is shown for the separate sites (2000 and 2001 respectively). From these figures it is clear that the deposition in water-treated control is lowest in all heights. This can be explained by the fact that here the unaffected vegetation intercepts the deposition more effectively, even at 60 cm, than the vegetation in the treated plots.

Table 3.2 Results statistical analysis deposition at three different heights (15, 30 and 60 cm) at different dosages during second herbicide application in 2000 and 2001; * = P<0.05, + = 0.05<P<0.10; interaction are denoted by abbreviation x abbreviation; - = not relevant; see also under Table.

	effect							
	dosage d	height h	site s	d x h	d x s	h x s	d x h x s	difference 15 30 60 cm
2000								
all sites	0.000*	0.000*	0.000*	>0.10	0.000*	0.015*	>0.10	-
Klarenbeek	>0.10	>0.10	-	>0.10	-	-	-	-
Wenum	0.096+	0.000*	-	>0.10				a b c
Lexmond	0.001*	0.000*	-	>0.10	-	-	-	a b c
Zegveld	0.000*	>0.10	-	>0.10	-	-	-	-
2001								
all sites	0.004*	0.000*	0.000*	>0.10	0.000*	0.002*	0.075+	-
Klarenbeek	>0.10	0.009*	-	>0.10	-	-	-	a b b
Wenum	0.024*	0.000*	-	>0.10				a b c
Lexmond	0.004*	0.000*	-	>0.10	-	-	-	a b c
Zegveld	0.000*	0.000*	-	0.018*	-	-	-	-

Two different GLM-analyses were carried out each year: first all sites combined: dosage x height x site and then each site separate: dosage x height; difference = multiple comparisons with LSD; unequal letters indicate statistical difference (P<0.05) between individual heights.

In 2000 there are no clear differences in deposition related to dosage for Klarenbeek and Wenum (Tab. 3.2, Fig. 3.2) and there are no clear differences related to height for Klarenbeek and Zegveld (Tab. 3.2, Fig. 3.2).
In 2001 there is no clear difference in deposition related to dosage at Klarenbeek only. All other sites display the expected effect of height and dosage (Tab. 3.2, Fig. 3.3).

3.4 Synthesis

From the previous chapter it became clear that the dosages in the field were applied with great precision. Deposition on the off cop vegetation was measured during the first and second spraying. During the first spraying interception is about twice as high at 60 cm than at 15 and 30 cm in 2000. Differences in interception during the first spraying are much less in 2001, probably caused by a changed vegetation structure as a consequence of the herbicide applications. During the second spraying deposition is dependent on height and dosage. Interception increased with increasing height and dosage.

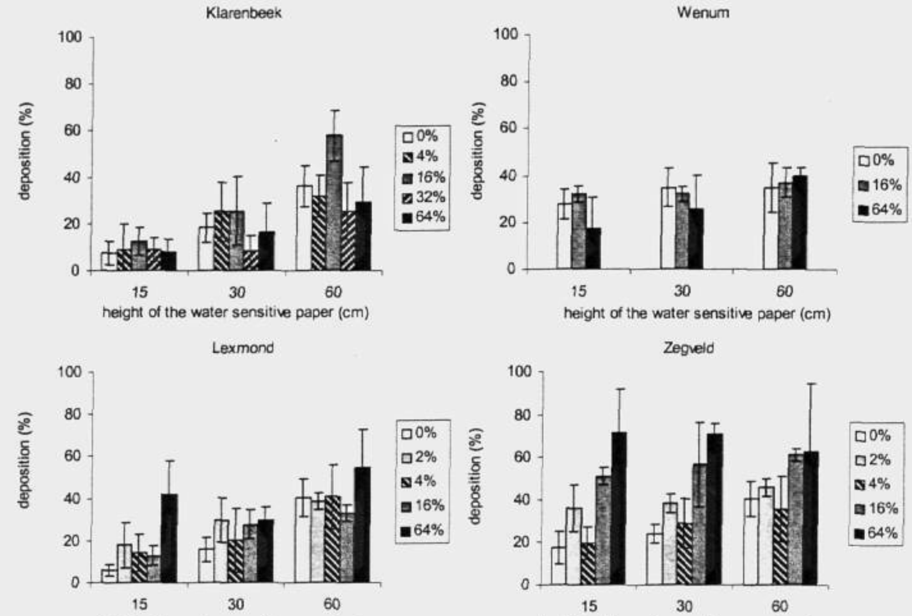


Fig. 3.3 Mean and standard deviation. of the interception at different heights in the vegetation in different treatments during the second application for all four sites in 2000.

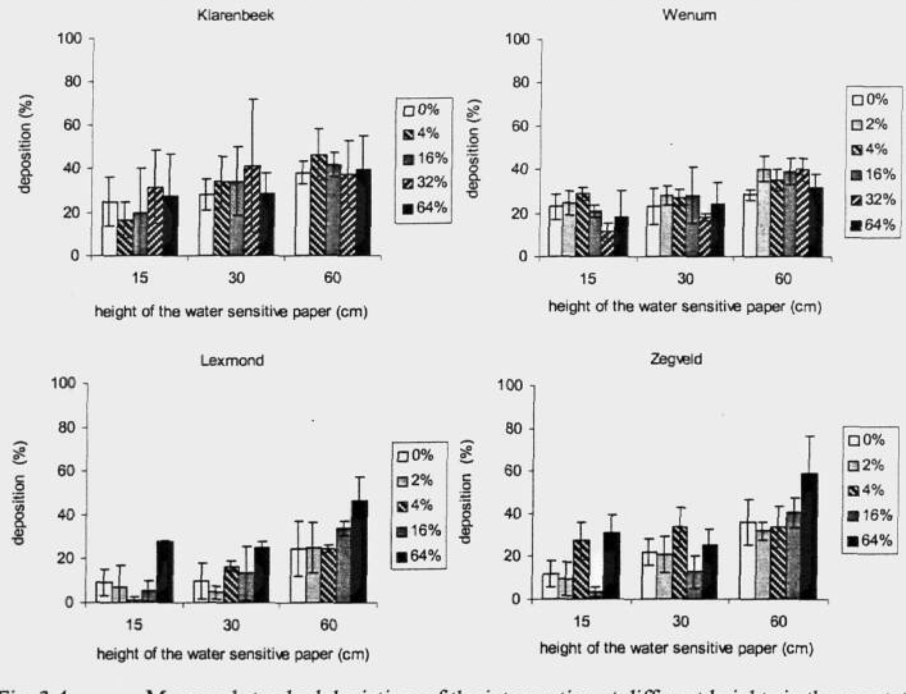


Fig. 3.4 Mean and standard deviation. of the interception at different heights in the vegetation in different treatments during the second application for all four sites in 2001.

4 SHORT TERM EFFECTS: PHYTOTOXIC EFFECTS OF GLUFOSINATE-AMMONIUM ON OFF CROP VEGETATION

4.1 General

In the period end May till end June of 2000 and 2001 there were two herbicide applications in each year. About a week after each application the short term phytotoxic effects of glufosinate-ammonium on the vegetation were assessed, by estimating the percentage of the vegetation with strong yellow discoloration. The results of these four phytotox assessments are presented in § 4.2 and 4.3
After the second herbicide application of 2001 three phytotox assessments were made at each site, in order to investigate the decline in time of the phytotoxic effects. The results of this analysis is presented in § 4.4. In § 4.5 phytotox effects on some dominant plant species are presented. In the final section § 4.6 a synthesis of the results is presented.

4.2 Phytotoxic effect after first and second application

Fig. 4.1 reviews the phytotoxic effects on off crop vegetation of the first and second herbicide application in 2000 and 2001 for all sites. The effects of site, dosage and interaction between dosage and site were analysed separately for each of these four assessments.
Dosages of glufosinate-ammonium had a significant effect in 2000 and 2001 after each application (GLM, $P<0.001$). The response to the treatment differed significantly between the sites after the first applications (GLM, $P<0.001$ for 2000 and $P=0.006$ for 2001) and after the second application for 2001 (GLM, $P=0.017$). Interactions between dosage and site were significant for all four assessments (GLM, $P<0.001$ for 2000 and first application 2001; $P=0.016$ for second application 2001). The effect of different dosages thus differed at different sites. The main cause is the small variation between plots.

After the first herbicide application in 2000, Zegveld showed lower phytotox values than the other sites. The phytotox values of Lexmond at low dosages were lower than for Klarenbeek and Wenum, but higher at high dosages. After the second application the phytotox values of the different sites were very similar. In comparison with the other two sites, Klarenbeek and Wenum had lower phytotox values at low dosages as well as at the highest dosage. After the first application in 2001 the phytotox value at Klarenbeek at dosage 16% appears to be a little higher and at Wenum at dosage 32% seems to be somewhat smaller compared with the other sites. After the second application in 2001 the Lexmond phytotox values were distinctly lower than at the other sites, with the exception for the highest dosage.

Table 4.1 Overall phytotoxic effect (years and sites combined), for all dosages 10 days after application on off crop vegetation; median and between brackets range.

dosage	after first spraying	after second spraying
2%	7% (4-14%)	3% (0-5%)
4%	14% (10-31%)	11% (5-14%)
16%	51% (43-57%)	48% (27-52%)
32%	65% (50-67%)	63% (54-70%)
64%	78% (69-85%)	80% (74-87%)

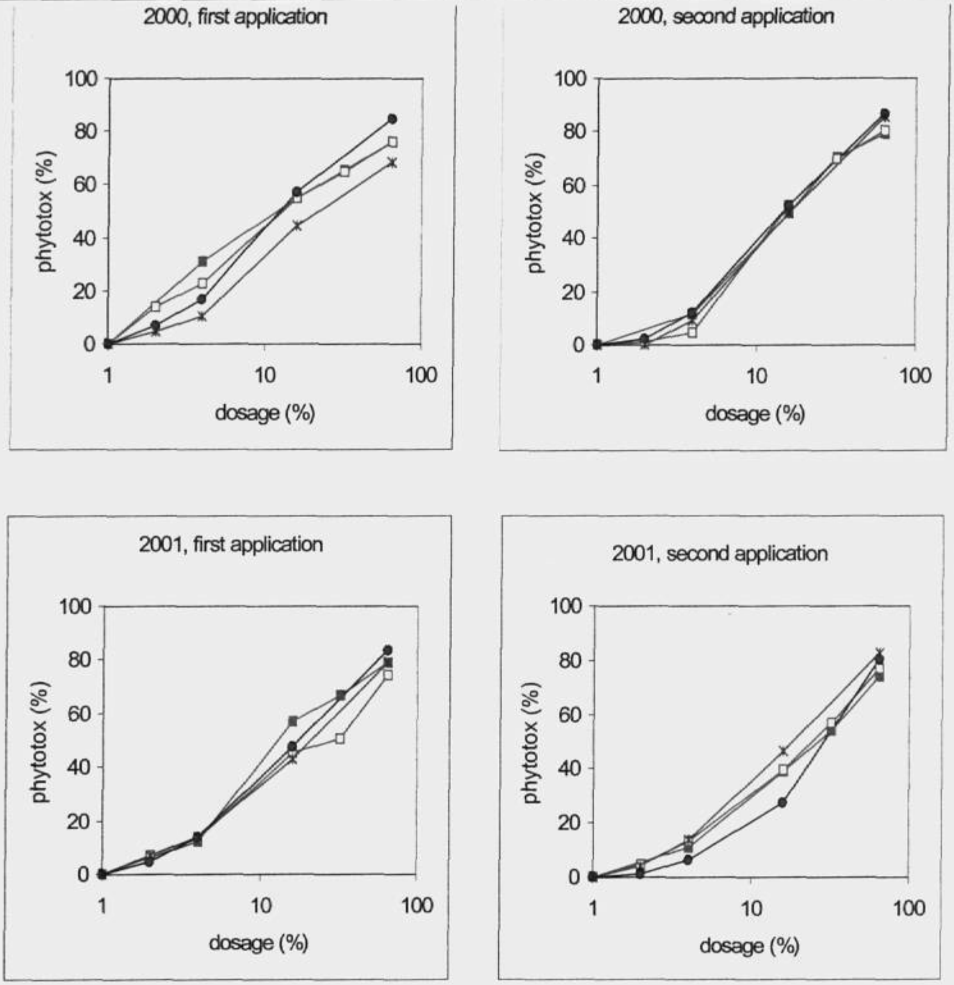


Fig. 4.1 Phytotoxic effect of several dosages of the herbicide glufosinate-ammonium on roadside grassland vegetations after first and second application (8-10 days after application) in 2000 and 2001 at four sites; filled square: Klarenbeek, open square: Wenum; filled circle: Lexmond and cross: Zegveld. N.B.) In the graphs the controls (0%) have been given the dummy dosage value 1%, so that control and treatment values can be plotted together with dosage on a logarithmic scale.

When the first and second applications in 2000 are compared, the phytotox values for the second are seen to be somewhat higher at the high dosages, but lower at the low dosages (see also Table 4.1). In 2001 the values at low dosages were lower after the second application only, but only somewhat. The phytotox values in 2001 were slightly lower than in 2000. The overall data (two years, all sites) on the effect of low dosages of glufosinate-ammonium on non-target vegetation are summarised in Table 4.1.
The effects of dosage were also analysed separately for each of the four sites and for each assessment after the first and second application for 2000 and 2001. At each site and after each spraying dosage was found to be significant (GLM, $P<0.001$).
Since there were two herbicide applications each year, with an interval of about 20 days, it was useful to examine whether there was any correlation between the effects of the first and second application. This was tested by calculating the residual autocorrelation between the

phytotox value of the first and second assessment in each year. In 2000 there was moderately high autocorrelation: 0.58 ($P<0.001$, 68 d.f.). Plots with relatively high phytotox values after the first application thus often have relative high values after the second, too. In 2001 autocorrelation was significant but low: 0.25 ($P=0.042$, 68 d.f.).

4.3 Trends, NOED, ED5, ED10 and ED50

There was a consistently significant increasing trend between dosage and effect (Williams' test, $P<0.001$, one-sided). In all cases the correlation between dosage and phytotoxic effect exceeded +0.95 (Spearman rank correlation, $P<0.001$).

Dosages resulting in a phytotoxic effect of 5%, 10% and 50%, the ED5, ED10 and ED50 respectively, and the no-observed effect dosage, the NOED was calculated for the assessments after the first and second application in 2000 and 2001 for each of the four sites (Table 4.2). In almost all cases the NOED was lower than the lowest dosage used, except for Wenum and Zegveld after the second application in 2000 and for Lexmond after the second application in 2001, where the NOED was equal to the lowest dosage of 2%. There were apparently no further differences in NOEDs between years and among sites.

Table 4.2 The no-observed effect dosage (NOED) and dosages resulting in 5% (ED5), 10% (ED10) and 50% (ED50) phytotoxic effects (8-10 days after application). N.B. full rate (100%) = 2x 800 g a.i./ha. NOED calculations based on Williams, 1972, ED calculations based on Hoekstra & Ewijk, 1992.

	Klaren-Beek (%)	Wenum (%)	Lexmond (%)	Zegveld (%)
NOED				
2000 - first application	<4	<2	<2	<2
2000 - second application	<4	2	<2	2
2001 - first application	<4	<2	<2	<2
2001 - second application	<4	<2	2	<2
ED5				
2000 - first application	0.2	0.4	1.1	1.3
2000 - second application	1.4	2.5	1.9	2.7
2001 - first application	1.3	1.0	1.5	1.1
2001 - second application	1.5	1.4	2.7	1.5
ED10				
2000 - first application	0.5	1.0	2.0	2.8
2000 - second application	2.6	4.2	3.2	4.3
2001 - first application	2.4	2.2	2.6	2.2
2001 - second application	3.0	2.7	4.6	2.8
ED50				
2000 - first application	10.4	13.3	12.5	23.4
2000 - second application	16.2	18.4	14.6	16.8
2001 - first application	15.6	21.2	15.2	17.2
2001 - second application	22.6	20.3	23.4	15.9

The ED5 ranges between 0.2% and 2.7%, with an overall median value of 1.5%, the ED10 between 0.5 and 4.6%, with median 2.7%, and the ED50 between 10% and 23%, with median 16.5%.

Inter-site differences in EDs were only small. In 2000 ED-values were higher after the second application than after the first. In 2001 this was also true, but to a lesser degree. The EDs were higher in 2000 than in 2001.

4.4 Disappearance of phytotoxic effect [iv]

After the second herbicide application of 2001 three phytotox assessments were made at each site, about 9, 33 and 61 days post-application (see also chapter Materials and Methods, see 2.4.2). The observed disappearance of phytotoxic effect at each site is shown in Fig. 4.2.

The effects of dosage and time (number of days after treatment) were analysed separately at each site. Dosage, time and interaction between dosage and site were to be significant at all sites (GLM repeated measurements; $P<0.001$). The significant interaction can be interpreted as signifying a different rate of disappearance for different concentrations.

The first phytotox assessment after the second application of 2001 showed effects persisting at all dosages. At the second assessment this held only at dosages of 16% or higher. At the final assessment effects were again found only at dosages of 16% or higher, especially at the Lexmond and Zegveld sites.

A complication during the phytotox assessments after the second herbicide application in 2001 (and 2000) was the yellowing of the vegetation later in the season in both the control and treated plots as a result of natural aging. The (median) percentages of vegetation showing 'natural yellowing' after the second application increased from 3% at the first assessment to 13% at the last assessment (not presented in Fig. 4.2).

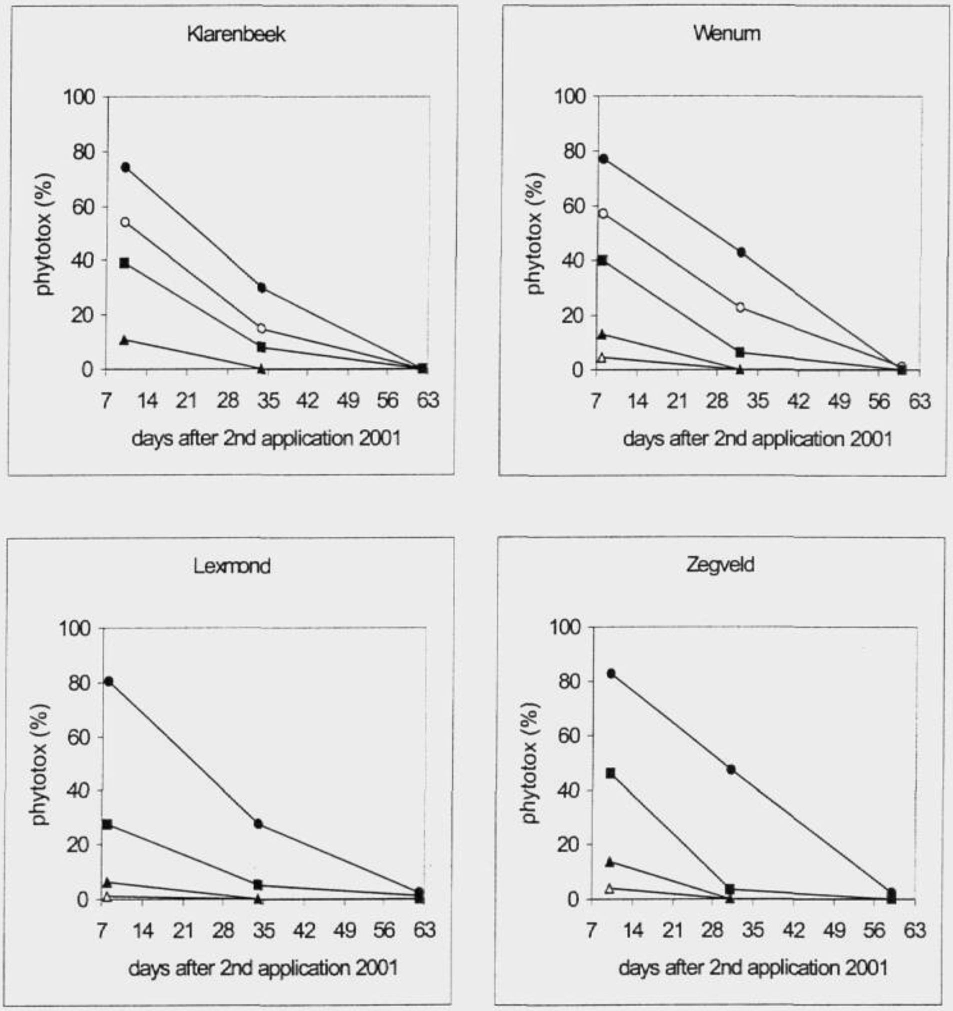
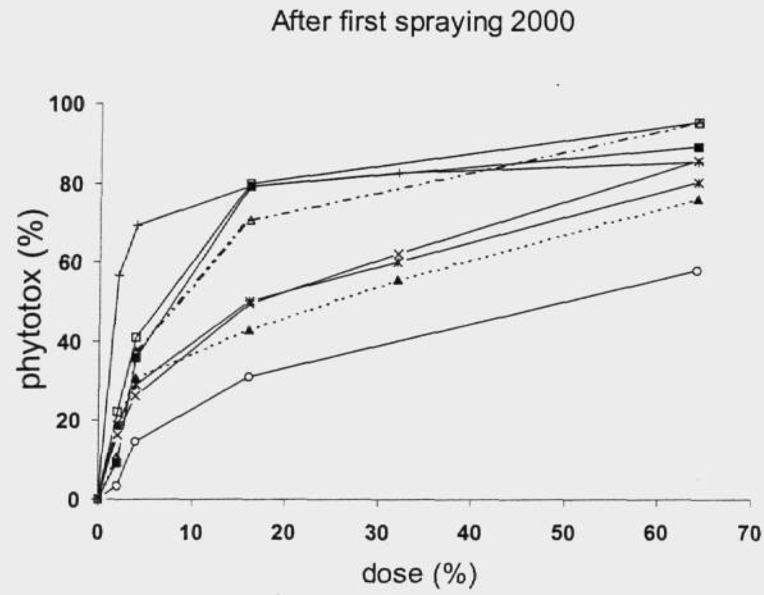


Fig. 4.2 Decline of phytotoxic effect of glufosinate-ammonium on roadside grassland vegetations after second application in 2001 at four sites; dosages: open triangle: 2%; filled triangle: 4%; filled square: 16%; open circle: 32% and filled circle: 64%.



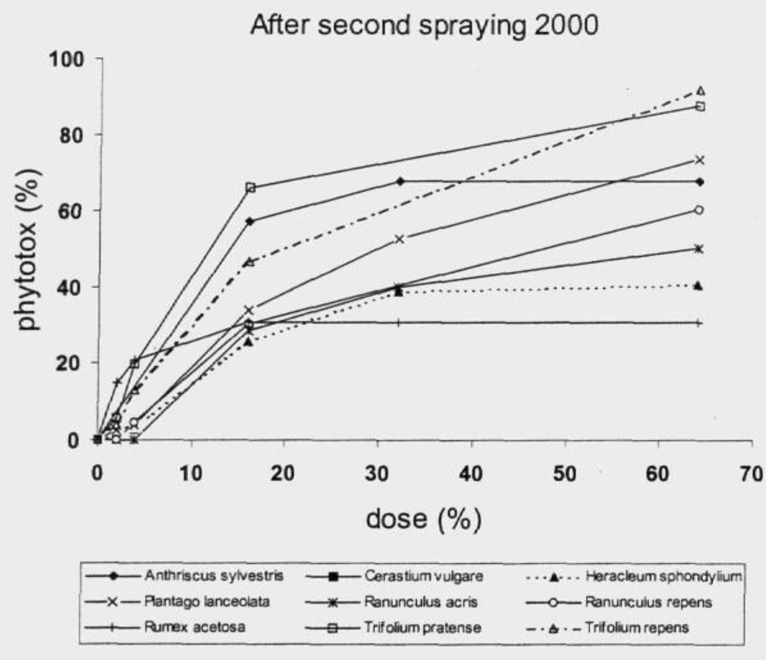


Fig. 4.3 Phytotoxic effects on some individual dicotyledonous plant species in 2000.

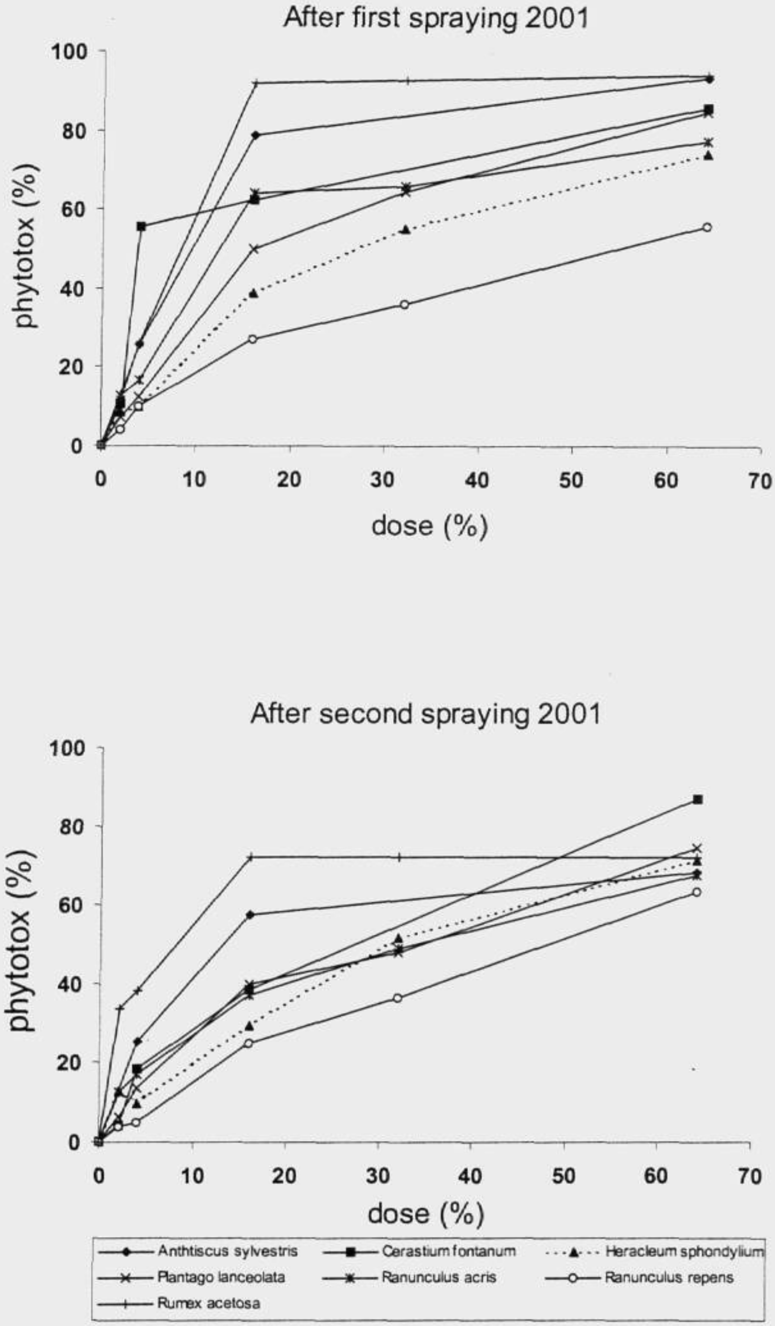


Fig. 4.4 Phytotoxic effects on some individual dicotyledonous plant species in 2001.

4.5 Phytotoxic effect on individual dicotyledonous plant species

In Fig. 4.3 and 4.4 the effects of glufosinate-ammonium on some common individual plant species are presented. In all figures it is clear that with an increasing deposition also the phytotoxic effect increases. In both years the phytotoxic effects are smaller after the second application compared to the first spraying. With respect to the species differences are large between species. For example, phytotoxic effects on *Rumex acetosa* seem to be strong, however, with the exception of the second spaying in 2000. For *Ranunculus repens* phytotoxic effects seems to be small in both years.

4.6 Synthesis

Short-term phytotoxic effects of glufosinate-ammonium were present in both years, after each treatment, at all sites and even at low dosages. At the low dosages phytotox values ranged between 3 and 14% and phytotox values after the first application were higher then after the second application. NOED is in almost all cases lower than the lowest dosage of 2% used. After the second application in 2001 the decline in time of the phytotoxic effects is assessed. At the species level large differences in the phytotoxic effects occur.

5 EFFECTS OF GLUFOSINATE-AMMONIUM ON BIOMASS OF OFF CROP VEGETATION

5.1 General

Vegetation biomass was measured at two sites in August 2000 after herbicide application, at all sites in 2001 before (May) and after (August) herbicide applications and at all sites in 2002 in May (see Materials and methods for details). In this chapter biomass is expressed as dry weight in gram per square decimetre. First the effects of the herbicide applications within the same year in August are presented (§ 5.2) and then in § (5.3) the effects of the herbicide applications after one or two years of herbicide applications in May. A synthesis of the results is presented in the last paragraph (§ 5.4). Only the significant results are treated in detail in the text; the remaining results are presented in the tables only.

5.2 Effects on biomass of herbicide applications within the same year

Biomass was measured after the second application of glufosinate-ammonium in August at two sites in 2000 and at four sites in 2001 (Fig. 5.1). The autocorrelation between the biomass values for spring 2001 and autumn 2001 was low: 0.17, and not significant, in contrast to spring autocorrelation (see next paragraph).

Table 5.1 Results of the statistical analysis of effects of dosages of glufosinate-ammonium within the same year of application, and of site on biomass of off crop vegetation in August in 2000 and 2001. See also explanatory text at the bottom of the Table.

		dosage			magnitude effect		dosage x site	site
Variable	site	P	trend	NOED	1stED	64%	P	P
August 2000								
Biomass	all sites	0.001*	↓	32%	-	-22%	>0.10	0.002
	Wenum	0.021*	↓	32%	-	-32%	-	-
	other sites	>0.10	-	-	-	-	-	-
August 2001								
Biomass	all sites	>0.10	-	-	-	-	>0.10	>0.10
	Lexmond	0.034*	↓	32%	-	-39%	-	-
	other sites	>0.10	-	-	-	-	-	-

Trend: Williams' test: ↓ = significant negative trend. NOED = No-Observed Effect Dosage. Magnitude effect 1stED and effect 64% = relative magnitude of effect at the 1st significant dosage and at the maximum significant dosage of 64%, *relative to the control*. - = not relevant.

The overall effects of site and dosage were first analysed per year. In 2000 dosage was significant (Tab. 5.1) and there was an overall significant, negative trend in dosage and the NOED was 32% (Tab. 5.1). The decrease in biomass at the highest dosage (64%) relative to the control was about 22% (Tab. 5.1). There was no significant interaction between dosage and site, indicating that effects of dosage is not dependent on site.

In 2001, however, dosage and the interaction, were not significant, though at some sites at the highest dosage (64%) biomass was lower than at the other dosages (Tab. 5.1, Fig. 5.1).

The effects of dosage were also analysed per site per year. In autumn 2000 there was a significant effect of dosage at Wenum only, although lower biomass values at dosages 32% and 64% were observed at Klarenbeek, too. At Wenum the negative trend between dosage and biomass was significant and the NOED was 32%. The decrease in biomass at the highest dosage (64%) relative to the control was about 32% (Tab. 5.1). In autumn 2001 there was a significant effect of dosage at Lexmond only. Here the negative trend was significant, and the NOED was 32%. The decrease in biomass at the highest dosage (64%) relative to the control was about 39% (Tab. 5.1).

Table 5.2 shows the correlations between dosage and biomass in August. All correlations are negative but low, signifying that overall biomass decreased with increasing dosage.

Table 5.2 Spearman rank correlation between dosage of the herbicide glufosinate-ammonium and biomass of road verge and ditch bank vegetation in August of 2000 and 2001 at four sites; *= P<0.05, + = 0.05<P<0.10; - = not relevant.

	site			
year	Klarenbeek	Wenum	Lexmond	Zegveld
2000				
biomass	-0.435+	-0.434+	-	-
2001				
biomass	-0.145	-0.011	-0.353+	-0.102

5.3 Effects on biomass of herbicide applications after one or two years

Biomass was determined in May of 2001 and 2002 before the herbicide applications in those years (Fig. 5.1). The general question is whether the effects of glufosinate-ammonium application in the one or two years before are still detectable in these spring data.

The autocorrelation between the biomass values in spring 2001 and 2002 is moderately high: 0.42 (P<0.001), indicating that in individual plots high values in the first spring are generally accompanied by high values in the second spring.

First the effects of site and dosage were analysed per year. No significant effects were found of dosage and of interaction dosage and site. [v] The effects of dosage were also analysed per site for May 2001 and 2002. At all four sites dosage is then not significant (Tab. 5.3).

Table 5.3 Results of the statistical analysis of effects of dosages of glufosinate-ammonium after one or two years of applications, and of site on biomass of off crop vegetation in May in 2001 and 2002. See also explanatory text at the bottom of the Table.

		dosage			magnitude effect		dosage x site	site
variable	site	P	trend	NOED	1stED	64%	P	P
May 2001								
biomass	all sites	>0.10	-	-	-	-	>0.10	0.063
	each site	>0.10	-	-	-	-	-	-
May 2002								
biomass	all sites	0.077+	↓	16%	-13%	-19%	>0.10	0.001
	each site	>0.10	-	-	-	-	-	-

Trend: Williams' test: ↓ = significant negative trend. NOED = No-Observed Effect Dosage. Magnitude Effect 1stED and 64% = relative magnitude of effect at the 1st significant dosage and at the maximum significant dosage of 64%, *relative to the control*. - = not relevant.

5.4 Synthesis

After the herbicide applications in 2000 there was a significant overall vegetation biomass decrease of 22% in August of the same year at the highest dosage. In August 2001, the effect of the herbicide application in the same year was significant at one site only at the highest dosage with a decrease of 39%. No effects were observed at the lowest dosages (2 and 4%). In May 2001 and 2002 there were no significant effects of glufosinate-ammonium applications from the previous years.

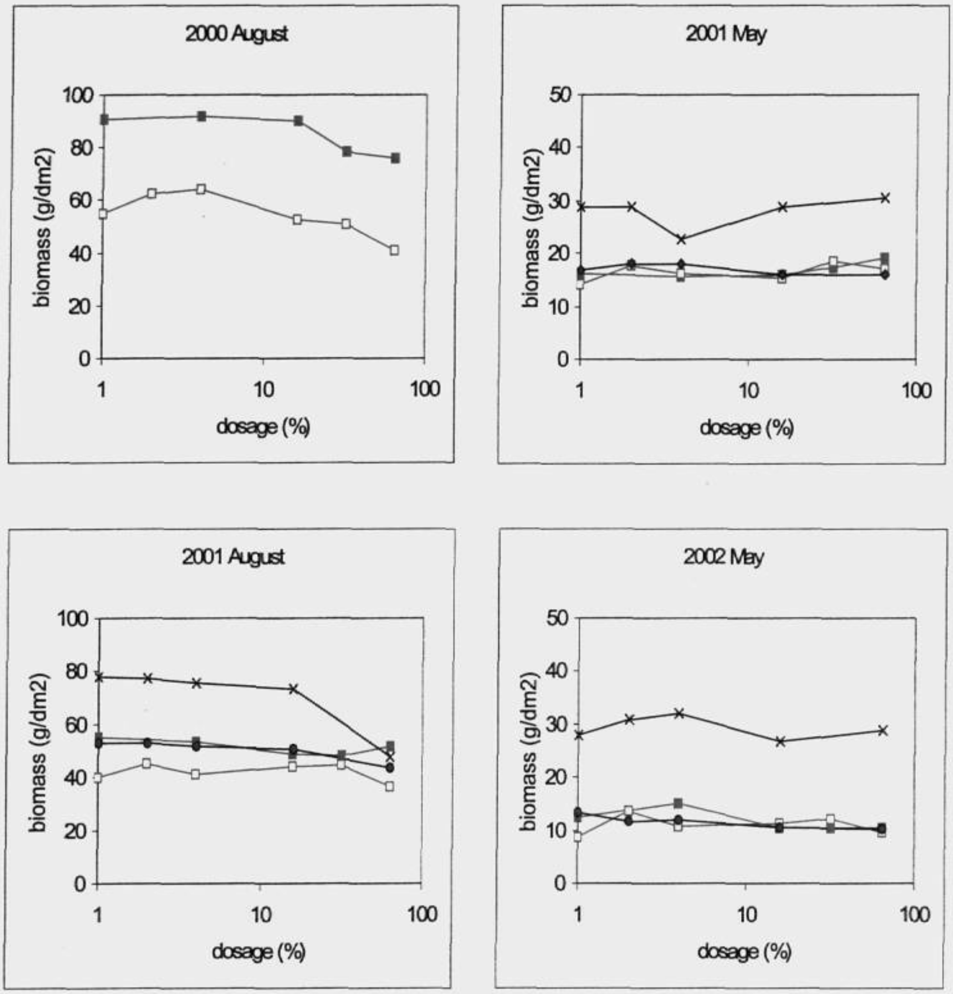


Fig. 5.1 Effect of several dosages of the herbicide glufosinate-ammonium on the biomass of road verges and ditch bank vegetation before (May) and after (August) applications in 2000, 2001 and 2002 at four sites; filled square: Klarenbeek, open square: Wenum; filled circle: Lexmond and cross: Zegveld. N.B. 1) In the graphs the controls (0%) have been given the dummy dosage value 1%, so that control and treatment values can be plotted together with dosage on a logarithmic scale. N.B. 2) Biomass scale for August is twice the scale for May.

6 EFFECTS OF GLUFOSINATE-AMMONIUM ON COVERAGE OF OFF CROP VEGETATION

6.1 General

Vegetation coverage was determined before the first herbicide applications in May 2000, 2001 and 2002 and after the second herbicide application in August 2000 and 2001. In this chapter data are presented on total coverage, coverage of dicotyledonous plant species and coverage of pioneer plant species. Total coverage is expressed as a percentage of total plot area, coverage of dicotyledonous and pioneer species as a percentage of total coverage. First the results on effects on coverage in August of the herbicide applications in the same year are presented (§ 6.2) and then the results on effects on coverage in May of the herbicide applications of one or two years before (§ 6.3). The last paragraph (§ 6.4) presents a synthesis of the results. Only the significant results are presented in detail in the text; the remaining results are presented in the tables only.

6.2 Effects on coverage of herbicide applications within the same year

The mean coverage in August after the second application of the herbicide is shown on the right-hand side of Fig. 6.1 (total coverage), 6.2 (dicotyledonous plant species) and 6.3 (pioneer plant species). The mean contrasts are presented in Fig. 6.4 for 2000 (left) and 2001 (right).

Autocorrelation between coverage values of May and August was determined (Table 6.1). The autocorrelation coefficients for coverage of dicotyledonous and pioneer species are much higher than for total coverage. All autocorrelations are significant, except for total coverage in 2000, indicating that a high coverage value in August is often accompanied by high coverage in May.

Table 6.1 Autocorrelations between August and May coverage data; * = $P<0.05$, + = $0.05<P<0.10$.

	May 2000-August 2000 correlation	May 2001-August 2001 correlation
coverage, total	0.267+	0.345*
coverage, dicotyledonous	0.717*	0.727*
coverage, pioneer	0.683*	0.572*

The overall effects of site and dosage were first analysed per year. In August 2000 and August 2001 there were no significant effects for dosage and site related to total, dicotyledonous or pioneer coverage. [vi]

Table 6.2 Results of the statistical analysis of effects of dosages of glufosinate-ammonium within the same year of application, and of site on coverage contrasts in 2000 and 2001. See also explanatory text at the bottom of the Table.

		dosage				magnitude effect		dosage x site	site
Variable	site	P	B-sign.	trend	NOED	1stED	64%	P	P
contrast August 2000 (versus May)									
total coverage	all sites	0.013	+	↓	32%	-	-1.1%	>0.10	>0.10
coverage dicots	all sites	0.053	ns	↓	2%	-31%	-33%	>0.10	>0.10
coverage pion.	all sites	>0.10	ns	-	-	-	-	>0.10	>0.10
total coverage	each site	>0.10	ns	-	-	-	-	-	-
coverage dicots	Wenum	0.041	ns	↓	<2%	-34%	-34%	-	-
	other sites	>0.10	ns	-	-	-	-	-	-
coverage pion.	each site	>0.10	ns	-	-	-	-	-	-
contrast August 2001 (versus May)									
total coverage	all sites	>0.10	ns	-	-	-	-	>0.10	>0.10
coverage dicots	all sites	>0.10	ns	-	-	-	-	0.042	>0.10
coverage pion.	all sites	>0.10	ns	-	-	-	-	>0.10	>0.10
total coverage	each site	>0.10	ns	-	-	-	-	-	-
coverage dicots	Klarenbeek	0.021	ns	↓	32%	-	-55%	-	-
	other sites	>0.10	ns	-	-	-	-	-	-
coverage pion.	each site	>0.10	ns	-	-	-	-	-	-

P = significance of GLM; B-sign.: significance after application of improved Bonferonni procedure: * = $P<0.05$, + = $0.05<P<0.10$, ns = not significant. Trend: Williams' test: ↓ = significant negative trend. NOED = No-Observed Effect Dosage. Magnitude effect 1stED and 64% = *relative* magnitude of effect at the 1st significant dosage and at the maximum significant dosage of 64%, *relative to the control in August*. - = not relevant.

The effect of dosage was also analysed per site per year. In 2000 and 2001 there were no significant effects of dosage for none of the sites (Tab. 6.2). [vii]

In general, in Fig. 6.4, coverage of dicotyledonous plant species showed throughout the year an overall decline (i.e. the contrast is negative), especially in 2001. This is due to a marked increase in monocotyledonous plant species, predominantly grasses. In contrast to the dicotyledonous plant species, pioneer plant species have higher coverage values later in the year (contrast is positive). The total coverage throughout the year showed an overall increase (i.e. the contrast is positive), especially in 2001.

In Table 6.3 the correlations between dosage and August contrast values for coverage are presented. In 2000 as well in 2001 correlation coefficients between dosage on the one hand and total coverage and coverage of dicots on the other are negative, but low, for all sites. So in general total biomass and biomass of dicots declined with increasing dosage. The opposite is the case for coverage of pioneers. Here, the correlation coefficients are positive, but low for all sites, so in general biomass increased with increasing dosage.

Table 6.3 Correlations (Spearman rank correlation) between August contrast values (difference between August and May data) of total coverage, coverage of dicotyledonous species and coverage of pioneer species on the one hand and dosages on the other; none of the correlations was significant.

	site			
	Klarenbeek	Wenum	Lexmond	Zegveld
2000				
coverage, total	-0.231	-0.353	-0.213	-0.320
coverage, dicot. spp	-0.174	-0.383	-0.206	-0.144
coverage, pioneer spp.	0.216	0.143	0.043	0.251
2001				
coverage, total	-0.060	-0.161	-0.205	-0.342
coverage, dicot. spp	-0.111	-0.248	-0.403	-0.362
coverage, pioneer spp.	0.055	0.330	0.075	0.012

6.3 Effects on coverage of herbicide applications after one or two years

Mean coverage in May is presented on the left-hand side of Figs. 6.1 (total coverage), 6.2 (dicotyledonous plant species) and 6.3 (pioneer plant species). Mean contrast values for May 2001 (after one year) and 2002 (after one year) are presented in Fig. 6.5 and in Fig. 6.6 for 2002 (after 2 years) respectively.

Table 6.4 Autocorrelations between coverage data in May 2000, 2001 and 2002; * = significant.

	May 2000- May 2001 correlation	May 2000- May 2002 correlation	May 2001-May 2002 correlation
coverage, total	0.307*	0.053	0.432*
coverage, dicotyledonous	0.642*	0.593*	0.840*
coverage, pioneer	0.592*	0.517*	0.582*

Table 6.4 shows the autocorrelations of the coverage data in May in 2000, 2001 and 2002. The autocorrelation coefficients for successive years (2000-2001, 2001-2002) are higher than for the years 2000-2002. Autocorrelation for the coverage of dicotyledonous and pioneer species is much higher than for total coverage.

The effects of the herbicide applications one of two years before were first analysed per year at all sites. In May 2001 (compared to May 2000) dosage and interaction dosage and site were not significant (Tab. 6.5). In May 2002 (compared to May 2001) there were no significant effects of dosage or of interactions between dosage and site. In May 2002 (compared to May 2000) there were no effects of dosage of the herbicide applications of the previous two years or of the interaction between dosage and site (Tab. 6.5).[viii]

Table 6.5 Results of the statistical analysis of effects of dosages of glufosinate-ammonium in May after one to two years of application, and of site or year on coverage in 2001 and 2002. See also explanatory text at the bottom of the Table.

		dosage				magnitude effect		dosage x site	site
variable	site	P	B-sign.	trend	NOED	1stED	64%	P	P
contrast May 2001 (versus May 2000)									
total coverage	all sites	>0.10	ns	–	-	-	-	>0.10	0.008
coverage dicots	all sites	>0.10	ns	–	-	-	-	>0.10	0.023
coverage pion.	all sites	>0.10	ns	–	-	-	-	>0.10	>0.10
total coverage	each site	>0.10	ns	–	-	-	-	-	-
coverage dicots	each site	>0.10	ns	–	-	-	-	-	-
coverage pion.	each site	>0.10	ns	–	-	-	-	-	-
contrast May 2002 (versus May 2001)									
total coverage	all sites	>0.10	ns	–	-	-	-	>0.10	0.011
coverage dicots	all sites	0.032	ns	↓	<2	-24%	-37%	>0.10	>0.10
coverage pion.	all sites	>0.10	ns	–	-	-	-	>0.10	>0.10
total coverage	each site	>0.10	ns	–	-	-	-	-	-
coverage dicots	each site	>0.10	ns	-	-	-	-	-	-
coverage pion.	each site	>0.10	ns	-	-	-	-	-	-
contrast May 2002 (versus May 2000)									
total coverage	all sites	>0.10	ns	-	-	-	-	0.027	0.003
coverage dicots	all sites	>0.10	ns	-	-	-	-	>0.10	0.032
coverage pion.	all sites	>0.10	ns	-	-	-	-	>0.10	>0.10

P = significance of GLM; B-sign.: significance after application of improved Bonferonni procedure: * = $P<0.05$, + = $0.05<P<0.10$, ns = not significant. Trend: Williams' test: ↓ = significant negative trend, n.s. = not significant. NOED = No-Observed Effect Dosage. $Effect_{1stED}$ and $effect_{64\%}$ = *relative* magnitude of effect (difference calculated at a coverage of 50%) at the 1st significant dosage and at the maximum significant dosage of 64%, *relative to the control*. - = not relevant.

The effects of the herbicide applications one or two years before were also analysed per year for each site individually. None of the sites showed an effect of dosage (Tab. 6.5).

6.4 Synthesis

No significant effects have been found on total coverage, coverage of dicotyledonous species and of pioneer species in August 2000 and 2001 of the herbicide applications in the same year. Also no significant effects were detected on coverages in May 2001 and 2002 of the herbicide applications one or two years before.

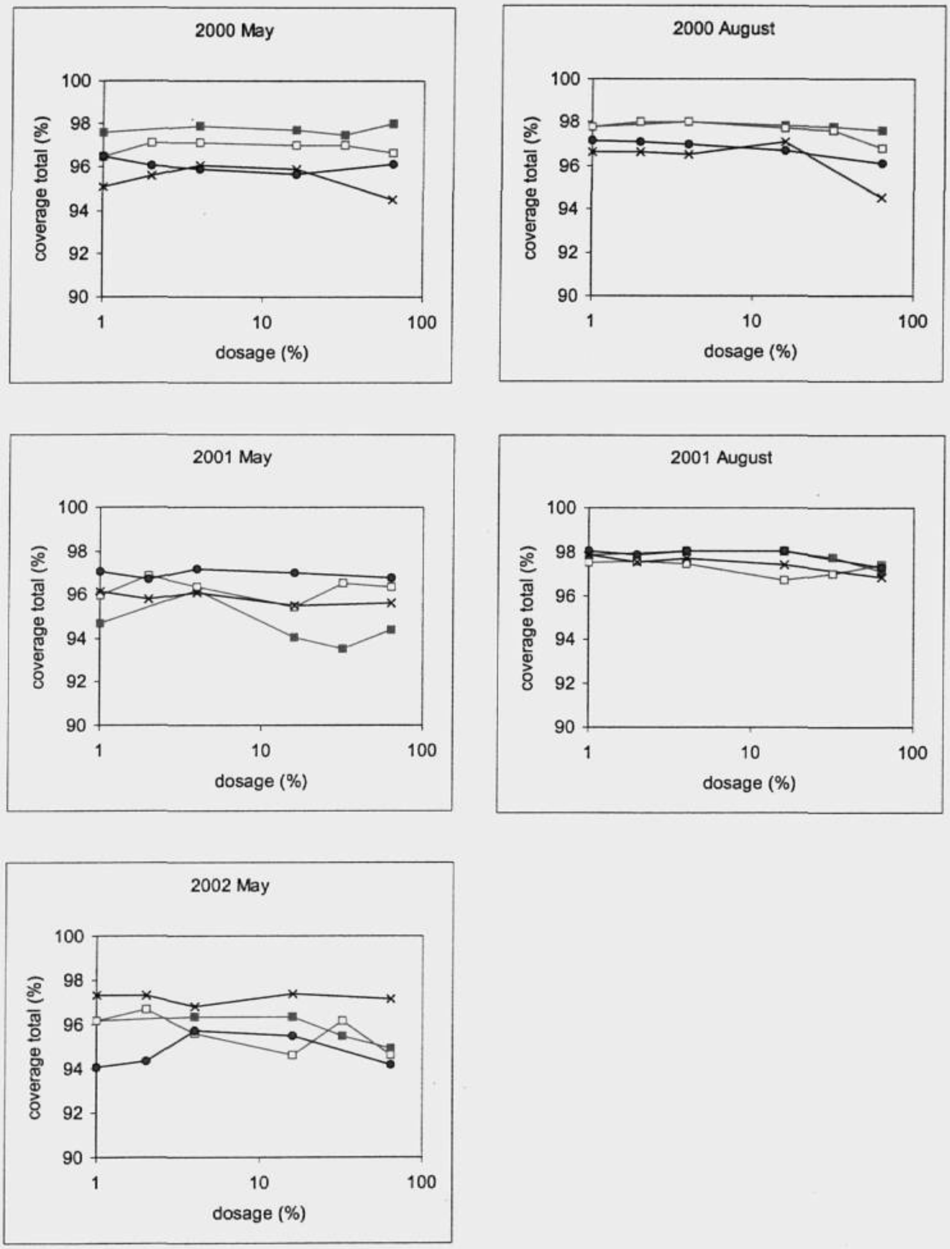


Fig. 6.1 Effect of several dosages of the herbicide glufosinate-ammonium on total coverage of plants in road verges and ditch bank vegetation before (May) and after (August) applications in 2000, 2001 and 2002 at four sites; filled square: Klarenbeek, open square: Wenum; filled circle: Lexmond and cross: Zegveld.; see N.B.1) in Fig. 5.1 (page 40), N.B. scale starts at 90%.

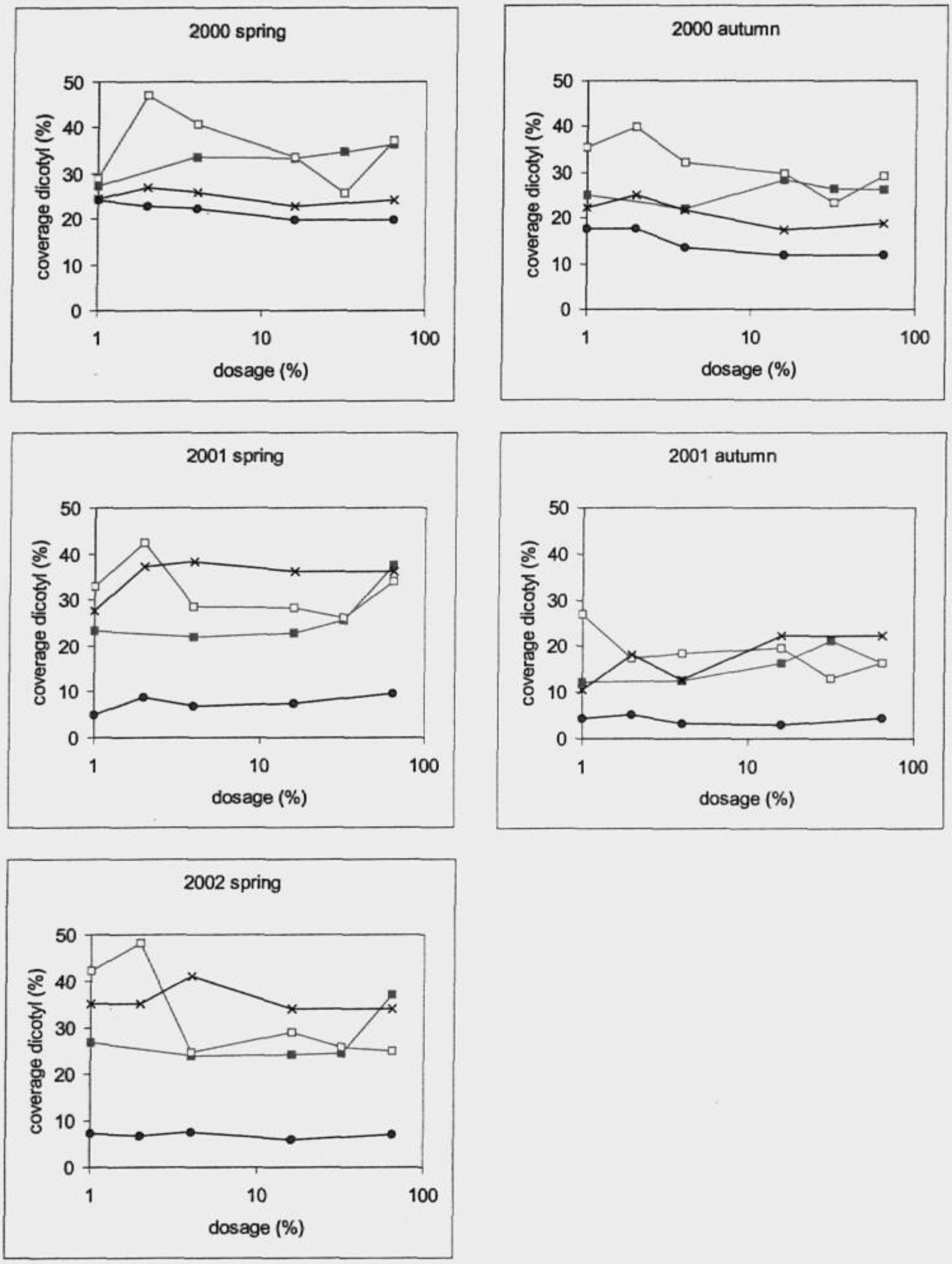


Fig. 6.2 Effect of several dosages of the herbicide glufosinate-ammonium on the coverage of dicotyledonous plant species in road verges and ditch bank vegetation before (May) and after (August) applications in 2000, 2001 and 2002 on four sites; filled square: Klarenbeek, open square: Wenum; filled circle: Lexmond and cross: Zegveld, see N.B.1) in Fig. 5.1 (page 40).

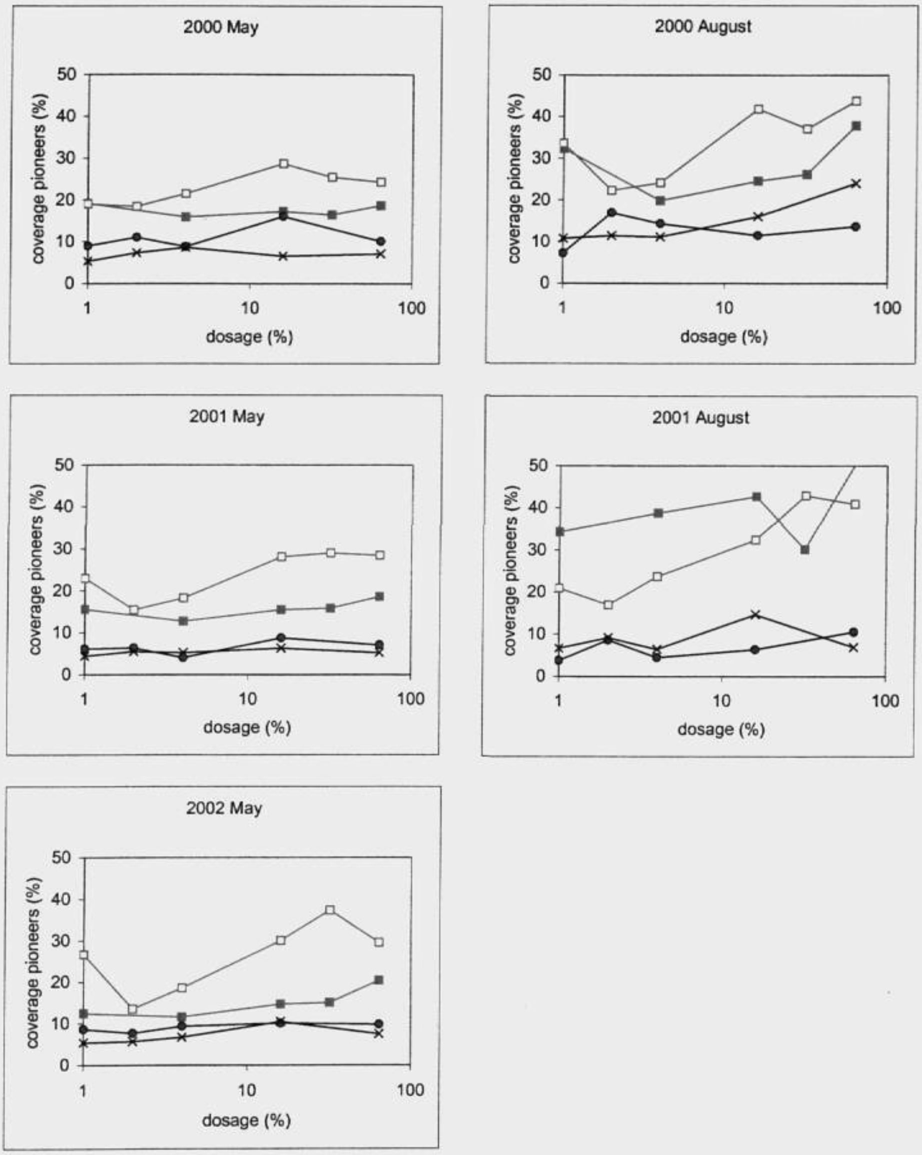


Fig. 6.3 Effect of several dosages of the herbicide glufosinate-ammonium on the coverage of pioneer plant species in road verges and ditch bank vegetation before (May) and after (August) applications in 2000, 2001 and 2002 at four sites; filled square: Klarenbeek, open square: Wenum; filled circle: Lexmond and cross: Zegveld, see N.B.1) in Fig. 5.1 (page 40).

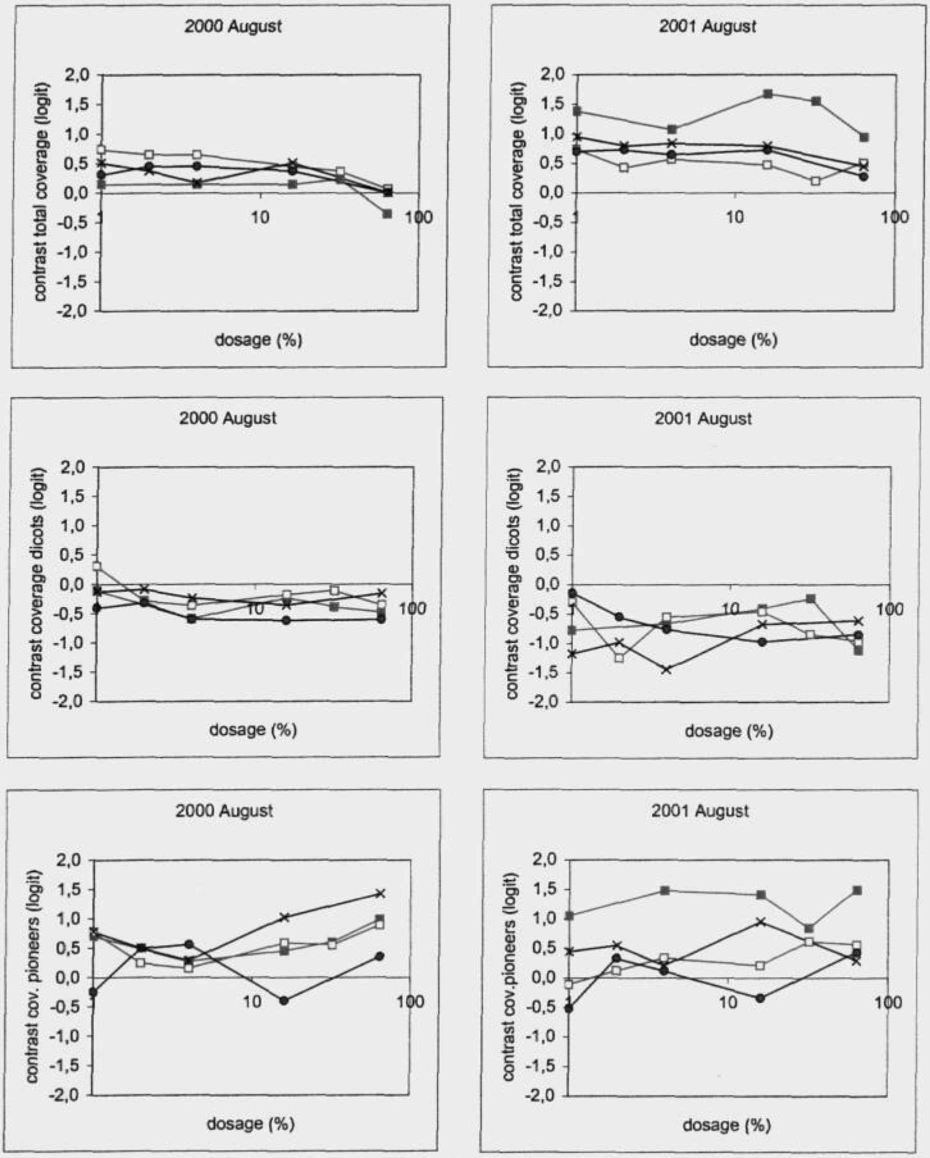


Fig. 6.4 Effect of several dosages of the herbicide glufosinate-ammonium on contrast (August corrected for May) of the coverage of total cover, dicotyledonous plant cover and pioneer plant cover in road verges and ditch bank vegetation after both herbicide applications in 2000 and 2001 at four sites; filled square: Klarenbeek, open square: Wenum; filled circle: Lexmond and cross: Zegveld., see N.B.1) in Fig. 5.1 (page 40).

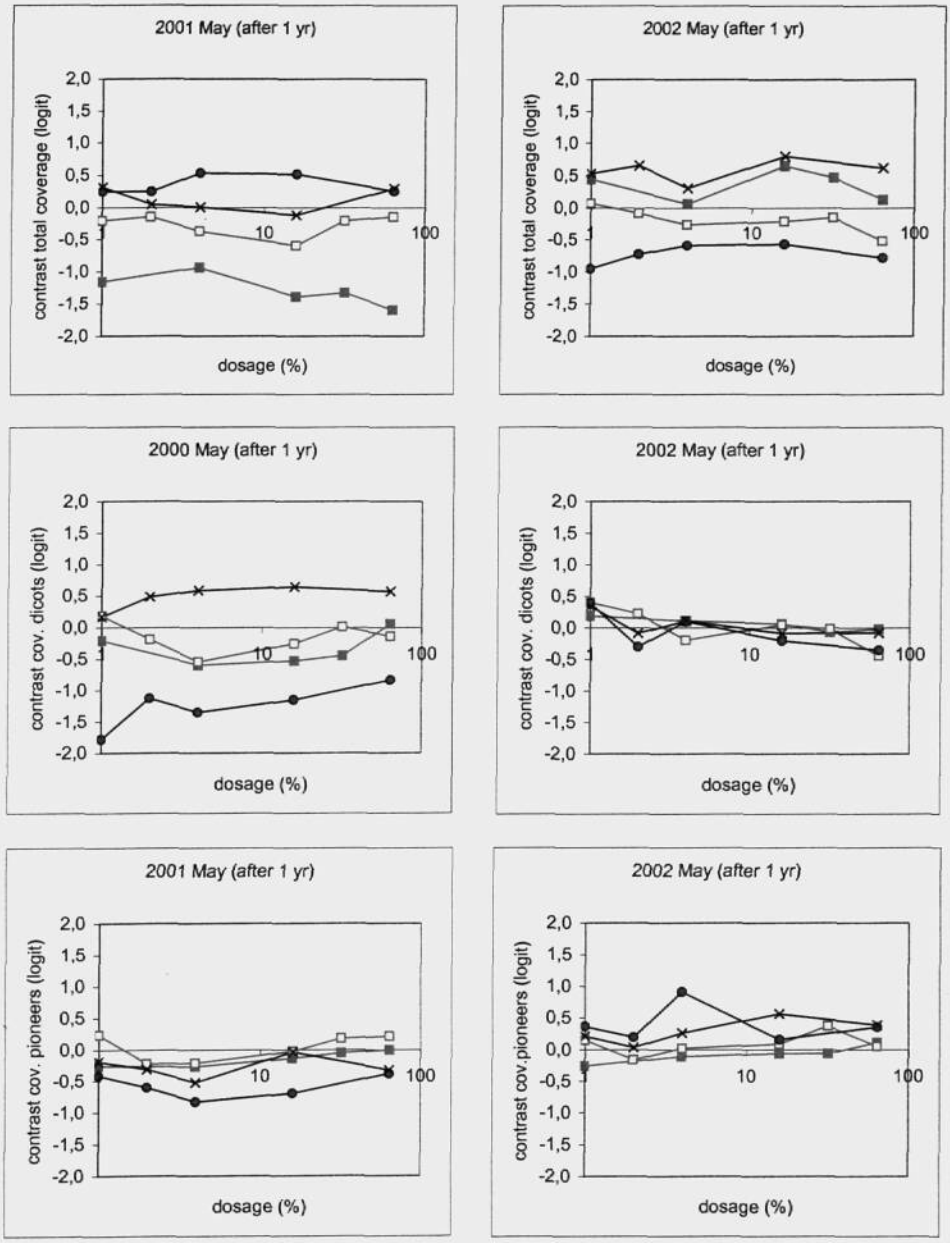


Fig. 6.5 Effect of several dosages of the herbicide glufosinate-ammonium on the contrast (May corrected for May data 1 yr ago) of total coverage, coverage of dicotyledonous and pioneer species in roadside grassland vegetations in May 2001 and 2002 at four sites; filled square: Klarenbeek, open square: Wenum; filled circle: Lexmond and cross: Zegveld, see N.B.1) in Fig. 5.1 (page 40).

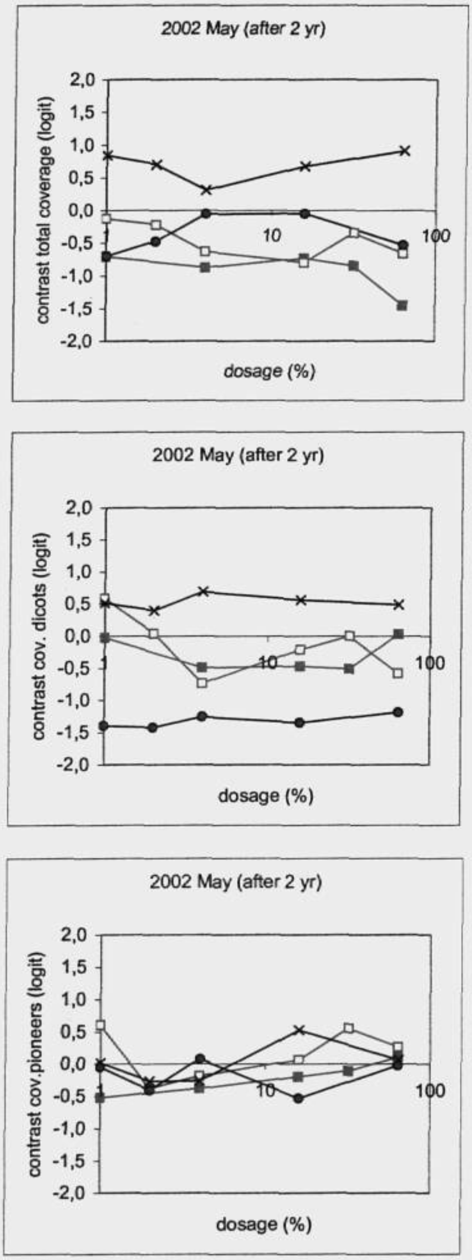


Fig. 6.6 Effect of several dosages of the herbicide glufosinate-ammonium on the contrast (May corrected for May data 2 yr ago) of total coverage, coverage of dicotyledonous and pioneer species in roadside grassland vegetations in May 2002 at four sites; filled square: Klarenbeek, open square: Wenum; filled circle: Lexmond and cross: Zegveld, see N.B.1) in Fig. 5.1 (page 40).

7 EFFECTS OF GLUFOSINATE-AMMONIUM ON DIVERSITY AND EVENNESS IN OFF CROP VEGETATION

7.1 General

Diversity was derived from the relevé data and was based on the number of species and their relative abundance. Evenness is a measure of dominance (low values mean few dominant species) and was calculated as the ratio between actual diversity and potential maximal diversity. Data on vegetation diversity are available for May 2000, 2001 and 2002 and after the second glufosinate-ammonium application in August 2000 and 2001. Data are presented for diversity (Shannon-Weaver H) and evenness (E). First the results of the effects of the herbicide applications on diversity and evenness in the same year of spraying in August are presented (§ 7.2) and then the results of the effects of the herbicide applications after one to two years in May (§ 7.3). In the last section (§ 7.4) a synthesis of the results is presented. Only the significant results are presented in detail in the text; the remaining results are presented in the tables only.

7.2 Effects on diversity and evenness of herbicide applications within the same year

Mean diversity and evenness in August after the second herbicide application in the same year are presented on the right-hand side of Figs. 7.1 and 7.2, respectively. The mean contrasts are presented in Fig. 7.3 for 2000 (left) and 2001 (right).

Autocorrelation between spring and autumn values of diversity and evenness was determined (Table 7.1). The autocorrelation coefficients for diversity are higher than for evenness and autocorrelations for diversity for both years are low but significant. The autocorrelation coefficients for evenness are even lower than for diversity.

Table 7.1 Autocorrelations between August and May diversity and evenness data in 2000 and 2001; * = $P<0.05$; + = $0.05<P<0.10$.

	August 2000–May 2000 correlation	August 2001-May 2001 correlation
diversity H	0.350*	0.280*
evenness E	0.213+	0.149

The overall effects of site and dosage were first analysed per year. There were no significant effects of dosage or of interaction dosage and site on diversity and evenness for August 2000 and 2001(Tab. 7.2).[ix] The effect of dosage was also analysed per site per year (Tab. 7.2). For none of the sites a significant effect was found of dosage in August 2000 and 2001.[x]

Table 7.2 Results of the statistical analysis of effects of dosages of glufosinate-ammonium within the same year of application, and of site on diversity and evenness in August in 2000 and 2001. See also explanatory text at the bottom of the Table.

		dosage				magnitude effect		dosage x site	site
Variable	site	P	B-sign.	trend	NOED	1stED	64%	P	P
contrast August 2000 (versus May)									
diversity H	all sites	0.023	ns	↓	4%	-10%	-10%	0.088	>0.10
evenness E	all sites	>0.10	ns	-	-	-	-	0.044	>0.10
diversity H	Klarenbeek	0.011	+	↓	32%	-	-17%	-	-
	Wenum	0.070	ns	↓	4%	-14%	-15%	-	-
	other sites	>0.10	ns	-	-	-	-	-	-
evenness E	Klarenbeek	0.043	ns	↓	32%	-	-17%	-	-
	other sites	>0.10	ns	-	-	-	-	-	-
contrast August 2001 (versus May)									
diversity H	all sites	>0.10	ns	-	-	-	-	>0.10	>0.10
evenness E	all sites	>0.10	ns	-	-	-	-	>0.10	>0.10
diversity H	Wenum	0.021	ns	↓	32%	-	-17%	-	-
	other sites	>0.10	ns	-	-	-	-	-	-
evenness E	Wenum	0.051	ns	↓	32%	-	-11%	-	-
	other sites	>0.10	ns	-	-	-	-	-	-

P = significance of GLM; B-sign.: significance after application of improved Bonferonni procedure: * = P<0.05, + = 0.05<P<0.10, ns = not significant. Trend: Williams' test: ↓ = significant negative trend. NOED = No-Observed Effect Dosage. Magnitude effect 1stED and 64% = relative magnitude of effect at the 1st significant dosage and at the maximum significant dosage of 64%, *relative to the control in August*. - = not relevant.

In Fig. 7.3 Klarenbeek, Wenum and to some extent also Lexmond show a clear decrease in diversity with increasing dosage in 2000. In 2001 the fluctuations are much larger than in 2000, but diversity still seems to be lower at higher dosages. The evenness sides of Fig. 7.3 show almost identical patterns, reflecting the fact that the contrast of evenness is mathematically almost identical to diversity multiplied with a constant, if diversity and evenness are relatively constant.

Table 7.3 presents the correlations between contrast values and dosages. In 2000 Klarenbeek, Wenum and Lexmond show negative correlations between diversity or evenness and dosage. In 2001 the correlations for Klarenbeek and Wenum are still negative, but much lower.

Table 7.3 Correlations (Spearman rank correlation) between contrast values (difference between autumn and spring data) of diversity and evenness on the one hand and dosages on the other; none of the correlations was significant.

	site			
	Klarenbeek	Wenum	Lexmond	Zegveld
2000				
diversity H	-0.329	-0.441	-0.212	0.012
evenness E	-0.322	-0.292	-0.231	0.157
2001				
diversity H	-0.114	-0.231	0.020	0.016
evenness E	-0.078	-0.245	0.071	-0.031

7.3 Effects on diversity and evenness of herbicide applications one or two years before

Mean diversity and evenness in May are presented on the left-hand side of Figs. 7.1 and 7.2, respectively. Mean contrast values (May values corrected for May values of the years before) are presented in Fig.7.4. Table 7.4 shows the autocorrelations of the May diversity and evenness data. Autocorrelation coefficients for diversity are somewhat higher than those for evenness. The coefficients are similar irrespective of the fact that the years are consecutive.

Table 7.4 Autocorrelations between May data for diversity and evenness; correlations and significance (P-values); * = P<0.05.

	May 2000-May 2001	May 2000- May 2002	May 2001- May 2002
diversity H	0.510*	0.466*	0.401*
evenness E	0.420*	0.410*	0.371*

The effects on diversity and evenness in May after herbicide applications one or two years before were first analysed per year at all sites. In May 2001 (relative to May 2000) dosage and interaction of dosage and site were not significant. (Tab. 7.5).

Table 7.5 Results of the statistical analysis of effects of dosages of glufosinate-ammonium after one or two year of applications, and of site on diversity and evenness in May in 2001 and 2002. See also explanatory text at the bottom of the Table.

		dosage				magnitude effect		dosage x site	site
variable	site	P	B-sign.	trend	NOED	1stED	64%	P	P
contrast May 2001 (versus May 2000)									
diversity H	all sites	>0.10	ns	-	-	-	-	0.014	0.001
evenness E	all sites	>0.10	ns	-	-	-	-	0.009	0.002
diversity H	Wenum	0.029	ns	↓	4%	-13%	-13%	-	-
	other sites	>0.10	ns	-	-	-	-	-	-
evenness E	Klarenbeek	0.056	ns	ns	-	-	-	-	-
	Wenum	0.064	ns	↓	2%	-10%	-12%	-	-
	other sites	>0.10	ns	-	-	-	-	-	-
contrast May 2002 (versus May 2001)									
diversity H	all sites	>0.10	ns	-	-	-	-	>0.10	>0.10
evenness E	all sites	>0.10	ns	-	-	-	-	>0.10	0.074
diversity H	each site	>0.10	ns	-	-	-	-	-	-
evenness E	each site	>0.10	ns	-	-	-	-	-	-
contrast May 2002 (versus May 2000)									
diversity H	all sites	>0.10	ns	-	-	-	-	>0.10	>0.10
evenness E	all sites	>0.10	ns	-	-	-	-	0.087	>0.098

P = significance of GLM; B-sign.: significance after application of improved Bonferonni procedure: * = P<0.05, + = 0.05<P<0.10, ns = not significant. Trend: Williams' test: ↓ = significant negative trend, ns = not significant. NOED = No-Observed Effect Dosage. Magnitude effect 1stED and 64% = magnitude of effect at the 1st significant dosage and at the maximum significant dosage of 64%, *relative to the control in 2001*. - = not relevant.

In May 2002 (relative to May 2001) no overall effects could be found of the applications the year before on diversity and evenness (Tab. 7.5). Also no overall effects could be found in May 2002 (relative to 2000) of the applications the two years before on diversity and evenness (Tab. 7.5).

Data of May were also analysed per site for each year. In May 2001 no significant effects of dosage on diversity and evenness were detected for any of the sites investigated..[xi] Also in May 2002 no effects of dosage were observed of the herbicide applications of the year before on diversity and evenness at the level of individual sites.

7.4 Synthesis

In August 2000 and 2001 there were no significant effects of dosage or interaction of dosage and site on diversity and evenness In May 2001 and 2002 no significant effects of the herbicide applications of the one or two years before were detected.

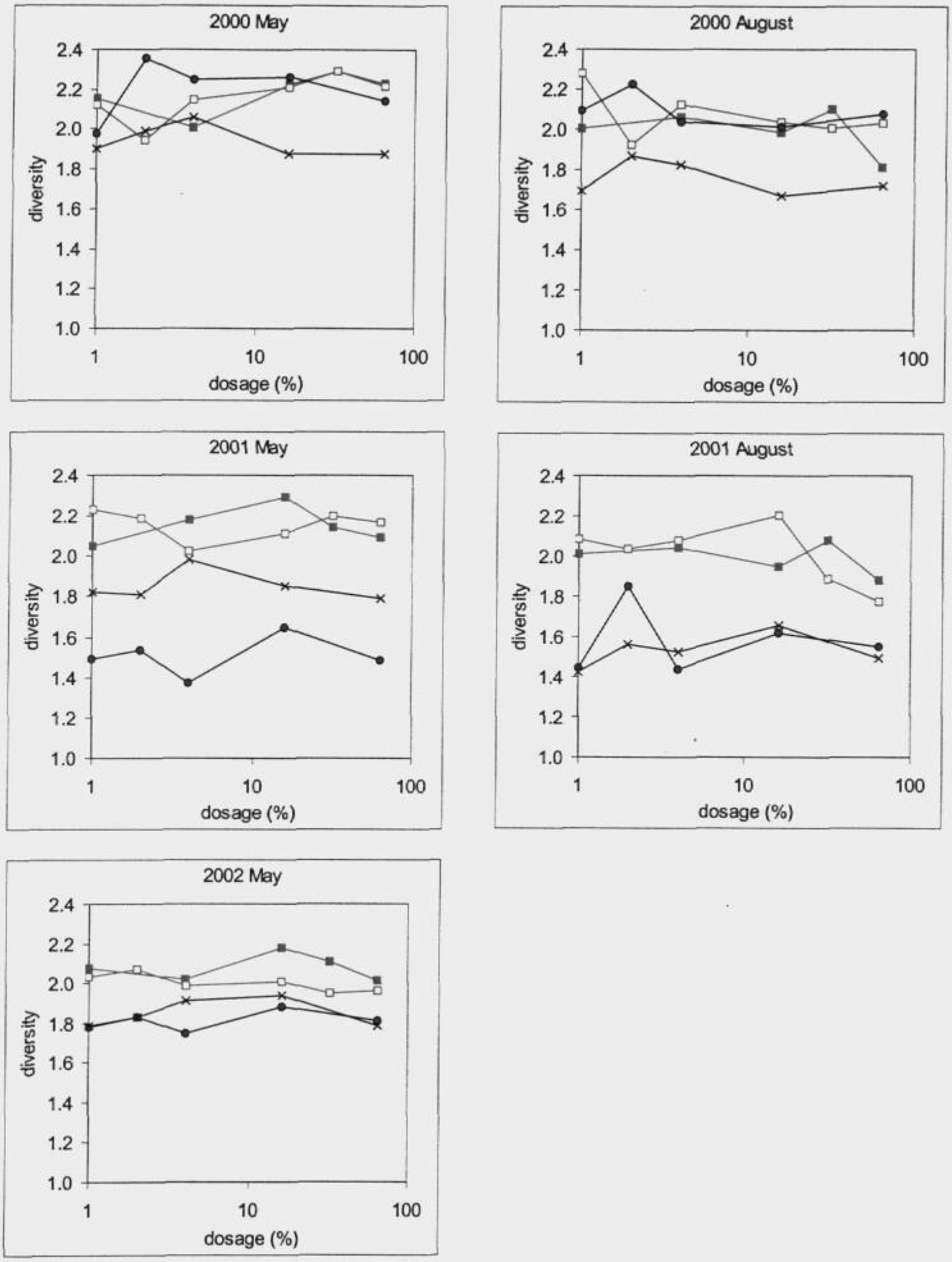


Fig. 7.1 Effect of several dosages of the herbicide glufosinate-ammonium on diversity of plant species in road verges and ditch bank vegetation before (May) and after (August) applications in 2000, 2001 and 2002 at four sites; filled square: Klarenbeek, open square: Wenum; filled circle: Lexmond and cross: Zegveld, see N.B.1) in Fig. 5.1 (page 40); N.B.: scale starts at 1.0.

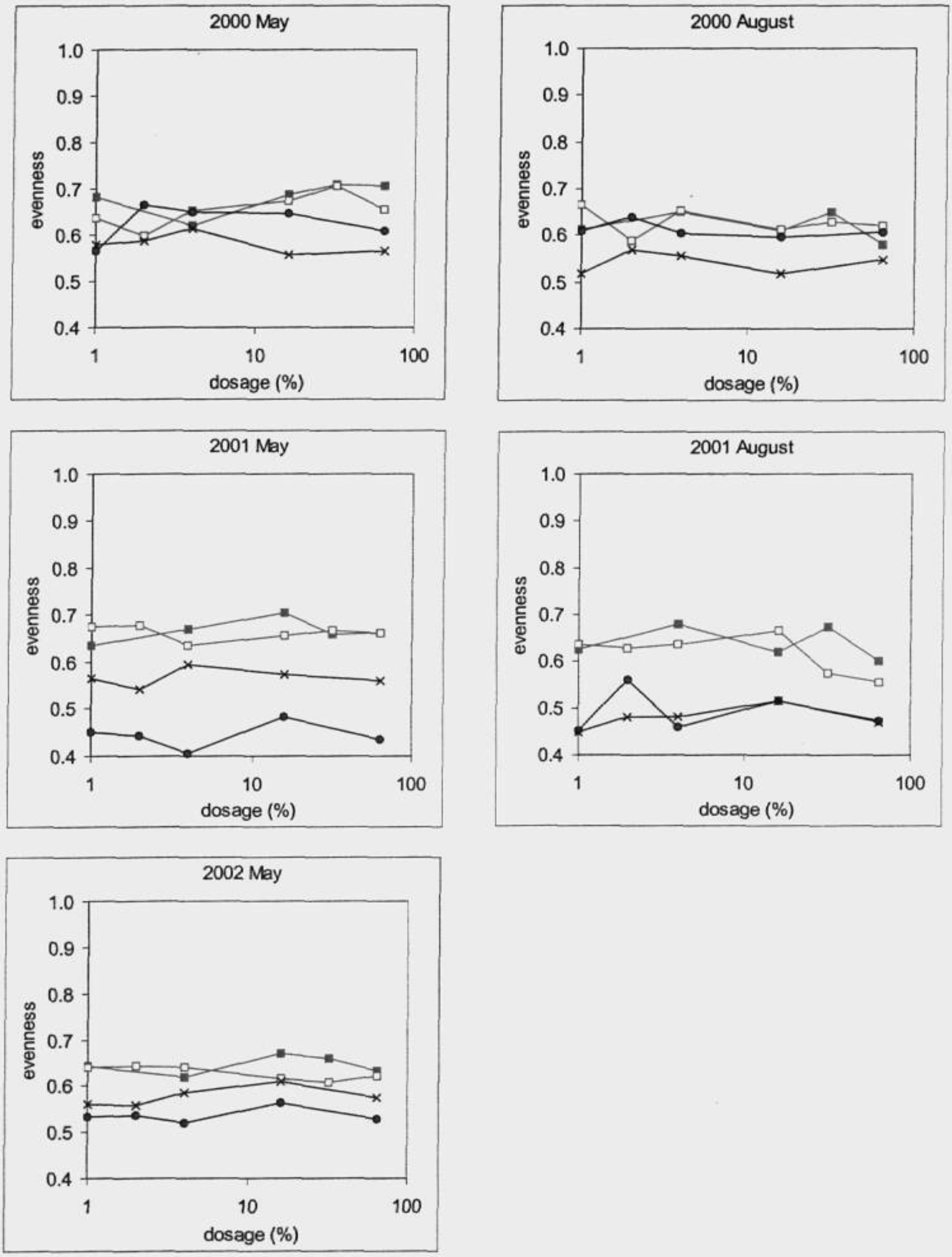


Fig. 7.2 Effect of several dosages of the herbicide glufosinate-ammonium on evenness in road verges and ditch bank vegetation before (May) and after (August) applications in 2000, 2001 and 2002 at four sites; filled square: Klarenbeek, open square: Wenum; filled circle: Lexmond and cross: Zegveld. see N.B.1) in Fig. 5.1 (page 40); N.B. scale starts at 0.4.

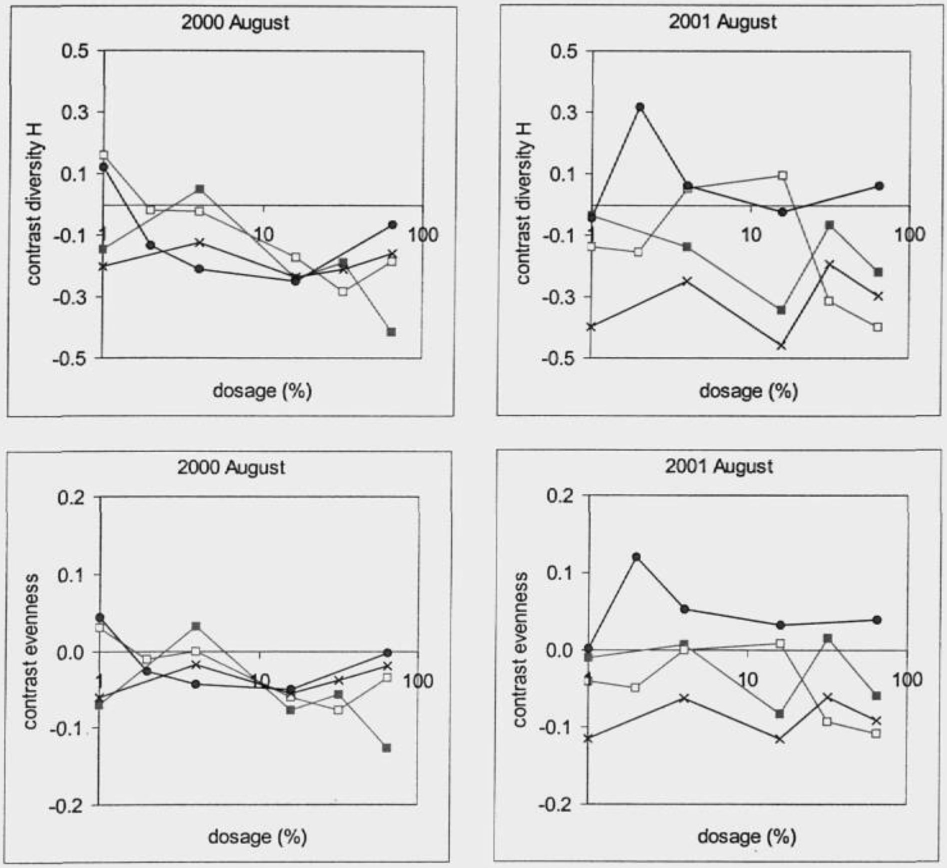


Fig. 7.3 Effect of several dosages of the herbicide glufosinate-ammonium on contrast (August corrected for May) of diversity and evenness of road verges and ditch bank vegetation and after both herbicide applications in 2000 and 2001 at four sites; filled square: Klarenbeek, open square: Wenum; filled circle: Lexmond and cross: Zegveld; see N.B.1) in Fig. 5.1 (page 40).

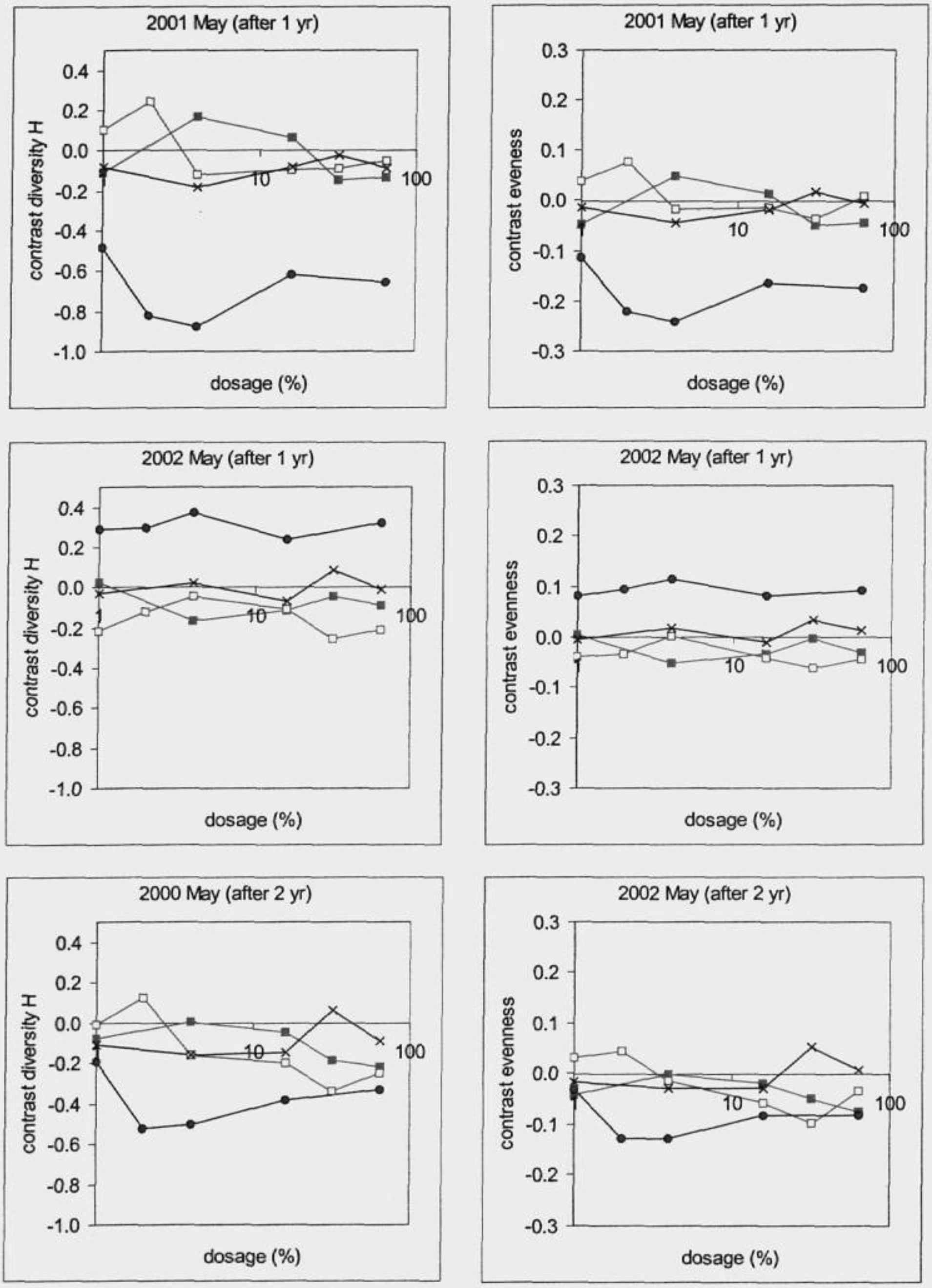


Fig. 7.4 Effect of several dosages of the herbicide glufosinate-ammonium on the contrast (May corrected for May data 1 or 2 yr ago) of diversity (left) and evenness (right) in roadside grassland vegetations in May *2001* and *2002* at four sites; filled square: Klarenbeek, open square: Wenum; filled circle: Lexmond and cross: Zegveld, see N.B.1) in Fig. 5.1 (page 40).

8 EFFECTS OF GLUFOSINATE-AMMONIUM ON NUMBER OF SPECIES IN OFF CROP VEGETATION

8.1 General

The number of species was derived from the relevé data, as were coverage, diversity and evenness. The number of plants species present was counted before herbicide application in May 2000, 2001 and 2002 and after the second herbicide application in August 2000 and 2001. Below, data are presented for total number of plants species, number of dicotyledonous plant species (broadleaved herbs) and number of pioneer species. First the results of August, the effects within the same year, are presented (§ 8.2) and then those of May, the effects after one to two years (§ 8.3). In the final section (§ 8.4) a synthesis of the results is presented. Only the significant results are treated in detail in the text; the remaining results are presented in the tables only.

8.2 Effects on number of species of herbicide applications in the same year

The mean number in August of species after herbicide applications are presented on the right-hand side of the Figs. 8.1 (all species), 8.2 (dicotyledonous species) and 8.3 (pioneer species). The mean contrast values (August values corrected for May values of the same year) for these variables are presented in Fig. 8.4 for 2000 (left) and 2001 (right).

The autocorrelation between values of May and August for number of species was calculated and is presented in Table 8.1. The autocorrelation coefficients for total number of species and for number of dicotyledonous species are high in 2000 and intermediate for 2001 and are all significant. The coefficients for pioneer plants are intermediate in 2000 and low in 2001 and significant in 2000 only. The lower autocorrelation values for pioneer species reflect the ephemeral nature of this group of species.

Table 8.1 Autocorrelations between August and May data on total number of plant species, number of dicotyledonous plant species and number of pioneer plant species; * = P<0.05t; + = 0.05<P<0.10.

	2000 May-2000 August correlation	2001 May-2001 August correlation
number, all species	0.804*	0.727*
number, dicotyledonous species	0.786*	0.710*
number, pioneer species	0.326*	0.275+

The overall effects of site and dosage were first analysed per year, so the information of all sites were evaluated per year as one data set. Dosage is not significant in August 2000 for total number of species, number of dicotyledonous and pioneer species (Tab. 8.2). Nevertheless the significance value for the total number of species, $P = 0.009$, is just above the significance level (in this specific case family-wise $\alpha = 0.006$).[xii] The interaction between dosage and site for total number of species, number of dicotyledonous and pioneer species in August 2000 is not significant. [xiii]

Table 8.2 Results of the statistical analysis of effects of dosages of glufosinate-ammonium within the same year of application, and of site on total number and number of dicotyledonous (dicot.) and pioneer of species in August in 2000 and 2001. See also explanatory text at the bottom of the Table.

		dosage				magnitude effect		dosage x site	site
Variable	site	P	B-sign.	Trend	NOED	1stED	64%	P	P
contrast August 2000 (versus May)									
total nr species	all sites	0.009	+	↓	<2%	-7%	-13%	>0.10	>0.10
nr dicot. Species	all sites	0.022	ns	↓	32%	-	-13%	>0.10	0.060
nr pioneer spec.	all sites	>0.10	ns	-	-	-	-	>0.10	0.046
total nr species	Wenum	0.090	ns	↓	32%	-	-14%	-	-
	other sites	>0.10	ns	-	-	-	-	-	-
nr dicot. Species	Wenum	0.066	ns	↓	32%	-	-20%	-	-
	other sites	>0.10	ns	-	-	-	-	-	-
nr pioneer spec.	each site	>0.10	ns	-	-	-	-	-	-
contrast August 2001 (versus May)									
total nr species	all sites	>0.10	ns	-	-	-	-	0.029	>0.10
nr dicot. Species	all sites	>0.10	ns	-	-	-	-	>0.10	>0.10
nr pioneer spec.	all sites	>0.10	ns	-	-	-	-	>0.10	>0.10
total nr species	each site	>0.10	ns	-	-	-	-	-	-
nr dicot. Species	each site	>0.10	ns	-	-	-	-	-	-
nr pioneer spec.	Klarenbeek	0.062	ns	ns	-	-	-	-	-
	other sites	>0.10	ns	-	-	-	-	-	-

P = significance of GLM; B-sign.: significance after application of improved Bonferonni procedure: * = P<0.05, + = 0.05<P<0.10, ns = not significant. Trend: Williams' test: ↓ = significant negative trend, ns = not significant. NOED = No-Observed Effect Dosage. Magnitude effect 1stED and 64% = relative magnitude of effect at the 1st significant dosage and at the maximum significant dosage of 64%, *relative to the control in August*. - = not relevant.

Dosage and interaction of dosage and site were not significant for total number of species, number of dicotyledonous and pioneer species in August 2001 (Tab. 8.2). The effect of dosage was also evaluated per site per year. For none of the sites a significant effect of dosage was detected (Tab. 8.2).[xiv]

Table 8.3 presents the correlations between contrast values and dosages. In 2000 all sites show a negative correlation between dosage and total number of species or number of dicotyledonous species, with one exception (Klarenbeek, no. of dicotyledonous spp.). Correlations between the number of pioneer species and dosage in 2000 are positive for Klarenbeek and Wenum, but negative for Lexmond and Zegveld. In 2001 all sites except Zegveld show negative correlations between dosage and total number of species or number of dicotyledonous species. All sites show positive correlations between number of pioneer species and dosage.

Table 8.3 Correlations (Spearman rank correlation) between contrast values (difference between August and May data) of total number of species, number of dicotyledonous species and number of pioneer species on the one hand and dosages on the other. Not one correlation was significant.

	site			
	Klarenbeek	Wenum	Lexmond	Zegveld
2000				
number, all species	-0.144	-0.389	-0.229	-0.265
number, dicotyledonous species	0.034	-0.351	-0.244	-0.316
number, pioneer species	0.040	0.021	-0.034	-0.124
2001				
number, all species	0.014	-0.097	-0.144	0.111
number, dicotyledonous species	0.014	-0.201	-0.079	0.184
number, pioneer species	0.002	0.190	-0.052	0.247

8.3 Effects on number of species of herbicide applications after one or two years

The mean number of species in May are presented on the left-hand side of Figs. 8.1, 8.2 and 8.3 for all species, dicotyledonous species and pioneer species, respectively. The mean contrast values (May values of 2001 corrected for May values of 2000 and May values of 2002 corrected for May values of 2001 or 2000) for these variables are presented in Fig. 8.5 for 2001 (left) and 2002 (right) and Fig. 8.6 respectively.

Table 8.4 shows the autocorrelations of the data of May on number of species. The autocorrelation coefficients for total number of species and number of dicotyledonous species are higher than for number of pioneer species. The autocorrelation between consecutive years (2000-2001 and 2001-2002) is higher than that between 2000 and 2002.

Table 8.4 Autocorrelations between data of May 2000, 2001 and 2002 on total number of plant species, number of dicotyledonous (dicot.) and pioneer plant species); * = $P<0.05$.

	May 2000-May 2001 correlation	May 2000-May2002 correlation	May 2001-May 2002 correlation
number, all species	0.818*	0.664*	0.798*
number, dicot. species	0.816*	0.607*	0.790*
number, pioneer species	0.406*	0.412*	0.518*

Effects after one or two years in May were first analysed per year at all sites. Dosage and interaction of dosage and site were not significant for total number of species, number of dicotyledonous and pioneer species in May 2001 (relative to May 2000) and May 2002 (relative to May 2001)

Table 8.5 Results of the statistical analysis of effects of dosages of glufosinate-ammonium in May after one to two years of application, and of site or year on total number, number of dicotyledonous (dicot.) and pioneer species in 2001 and 2002. See also explanatory text at the bottom of the Table.

		Dosage				magnitude effect		dosage x site	site
variable	site	P	B-sign.	trend	NOED	1stED	64%	P	P
contrast May 2001 (versus May 2000)									
total nr species	all sites	>0.10	ns	-	-	-	-	>0.10	0.064
nr dicot. species	all sites	0.055	ns	ns	-	-	-	>0.10	0.039
nr pioneer spec.	all sites	>0.10	ns	-	-	-	-	>0.10	0.093
total nr species	Wenum	0.087	ns	ns	-	-	-	-	-
	other sites	>0.10	ns						
nr dicot. species	Wenum	0.048	ns	ns	-	-	-	-	-
	other sites	>0.10	ns	-	-	-	-	-	-
nr pioneer spec.	each site	>0.10	ns	-	-	-	-	-	-
Contrast May 2002 (versus May 2001)									
total nr species	all sites	>0.10	ns	-	-	-	-	>0.10	>0.10
nr dicot. species	all sites	>0.10	ns	-	-	-	-	>0.10	>0.10
nr pioneer spec.	all sites	>0.10	ns	-	-	-	-	>0.10	>0.10
total nr species	each site	>0.10	ns	-	-	-	-	-	-
nr dicot. species	each site	>0.10	ns	-	-	-	-	-	-
nr pioneer spec.	each site	>0.10	ns	-	-	-	-	-	-
Contrast May 2002 (versus May 2000)									
total nr species	all sites	>0.10	ns	-	-	-	-	>0.10	>0.10
nr dicot. species	all sites	>0.10	ns	-	-	-	-	>0.10	>0.10
nr pioneer spec.	all sites	>0.10	ns	-	-	-	-	>0.10	0.099

P = significance of GLM-test; B-sign.: significance after application of improved Bonferonni procedure: * = $P<0.05$, + = $0.05<P<0.10$, ns = not significant. Trend: Williams' test: ↓ = significant negative trend. NOED = No-Observed Effect Dosage. Magnitude effect 1stED and 64% = magnitude of effect at the 1st significant dosage and at the maximum significant dosage of 64%, *relative to the control in August*. - = not relevant.

Dosage and interaction of dosage and site were not significant in May 2002 (relative to May 2001) after one year of herbicide application (Tab. 8.5). Dosage and interaction of dosage and site were also not significant in May 2002 (relative to 2001 and to 2000) after one or two years of herbicide applications (Tab. 8.5). The contrast values of May were also analysed per site for the years 2001 and 2002 after one year, individually (Tab. 8.5). No significant effects of dosage have been found for any of the sites investigated.

8.4 Synthesis

In August 2000 there were no significant overall effects of the herbicide applications of the same year on total number of species, number of dicotyledonous and pioneer species. Interaction of dosage and site in that year was not significant. There were no significant effects in August 2001 of dosage and of interaction dosage and site on number of dicotyledonous and pioneer species

In May 2001 and 2002 there were no significant effects of dosage and interaction dosage and site on total number of species, number of dicotyledonous and pioneer species of the herbicide applications one or two years before. Despite the almost significant effects of the herbicides applications on total number of species in August 2000, total number of species was recovered in the next spring (May 2001).

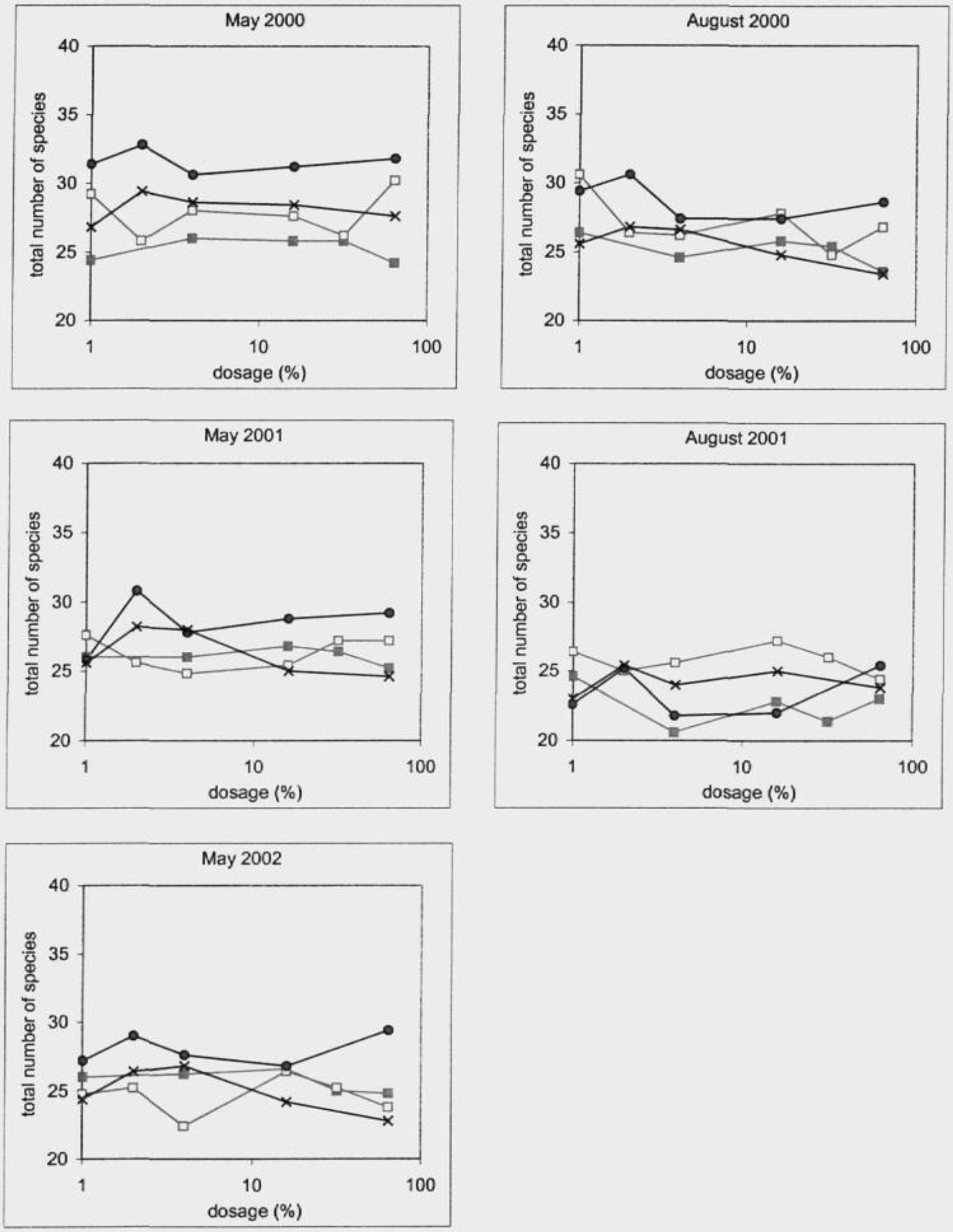


Fig. 8.1 Effect of several dosages of the herbicide glufosinate-ammonium on total number of plant species in roadside grassland vegetations before (May) and after (August) applications in 2000, 2001 and 2002 at four sites; filled square: Klarenbeek, open square: Wenum; filled circle: Lexmond and cross: Zegveld; see N.B.1) in Fig. 5.1 (page 40), N.B: scale starts at 20 species.

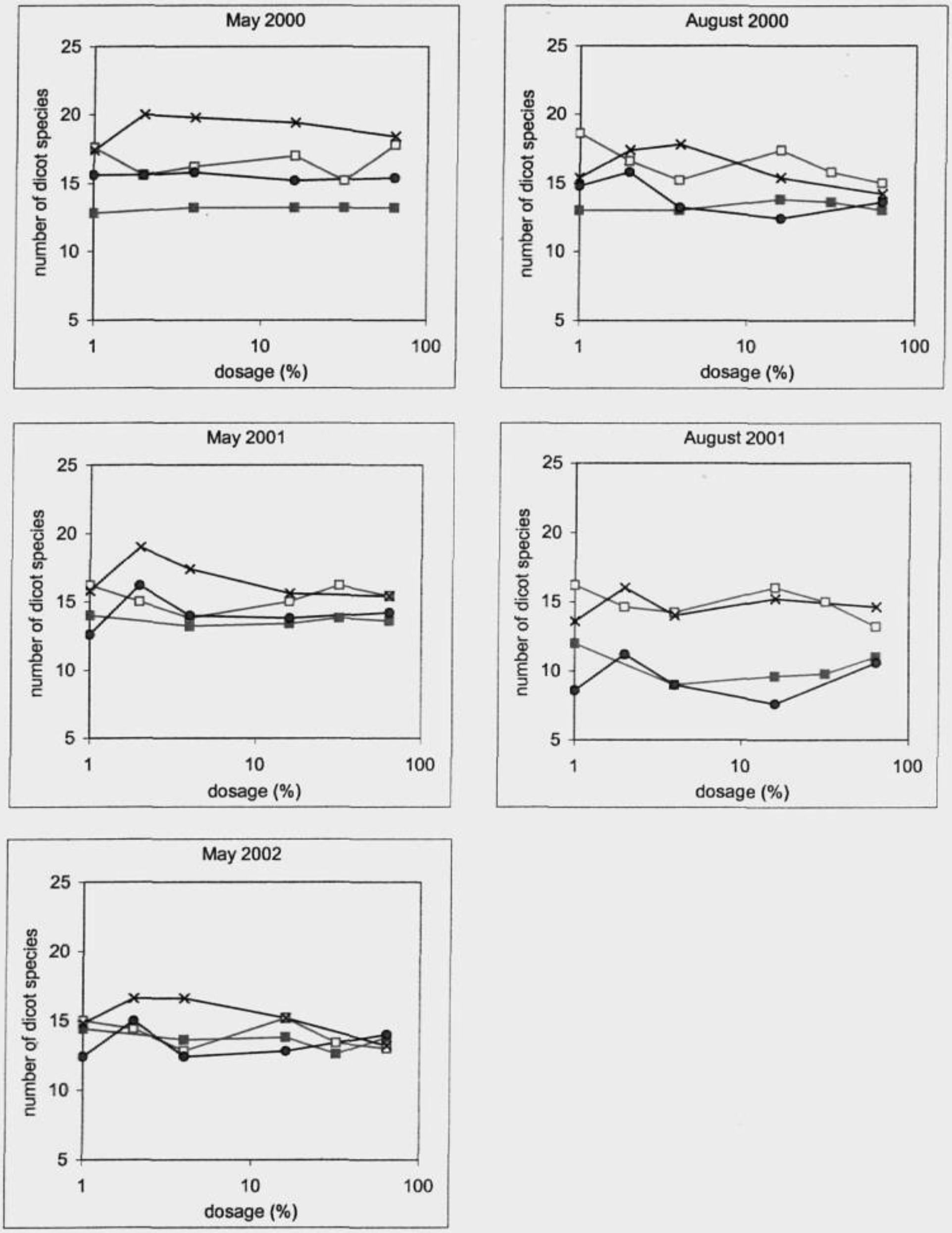


Fig. 8.2 Effect of several dosages of the herbicide glufosinate-ammonium on the number of dicotyledonous species in roadside grassland vegetations before (May) and after (August) applications in 2000, 2001 and 2002 at four sites; filled square: Klarenbeek, open square: Wenum; filled circle: Lexmond and cross: Zegveld; see N.B.1) in Fig. 5.1 (page 40), N.B.: scale starts at 5 dicotyledonous plant species.

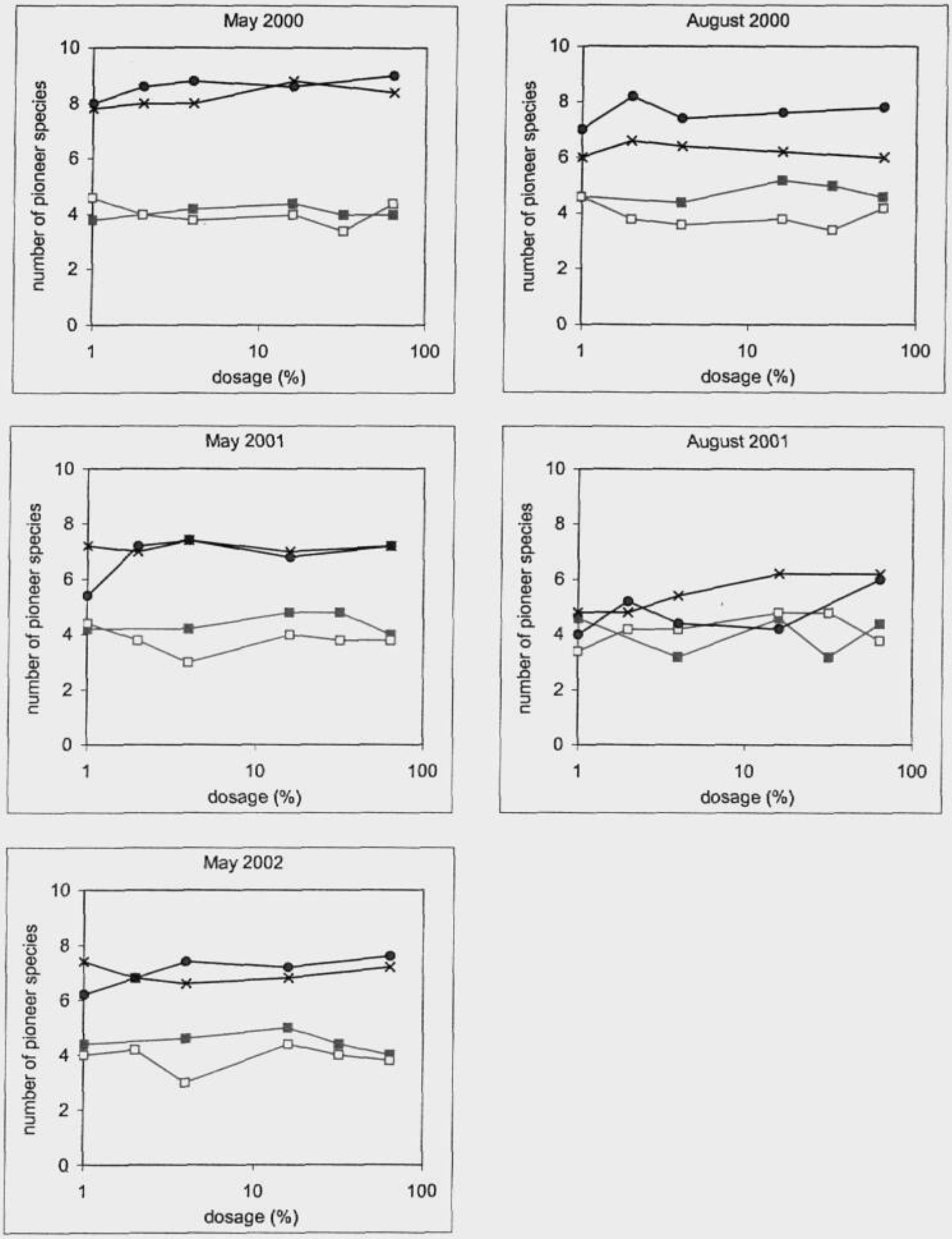


Fig. 8.3 Effect of several dosages of the herbicide glufosinate-ammonium on the number of pioneer plant species of roadside grassland vegetations before (May) and after (August) applications in 2000, 2001 and 2002 at four sites; filled square: Klarenbeek, open square: Wenum; filled circle: Lexmond and cross: Zegveld; see N.B.1) in Fig. 5.1 (page 40).

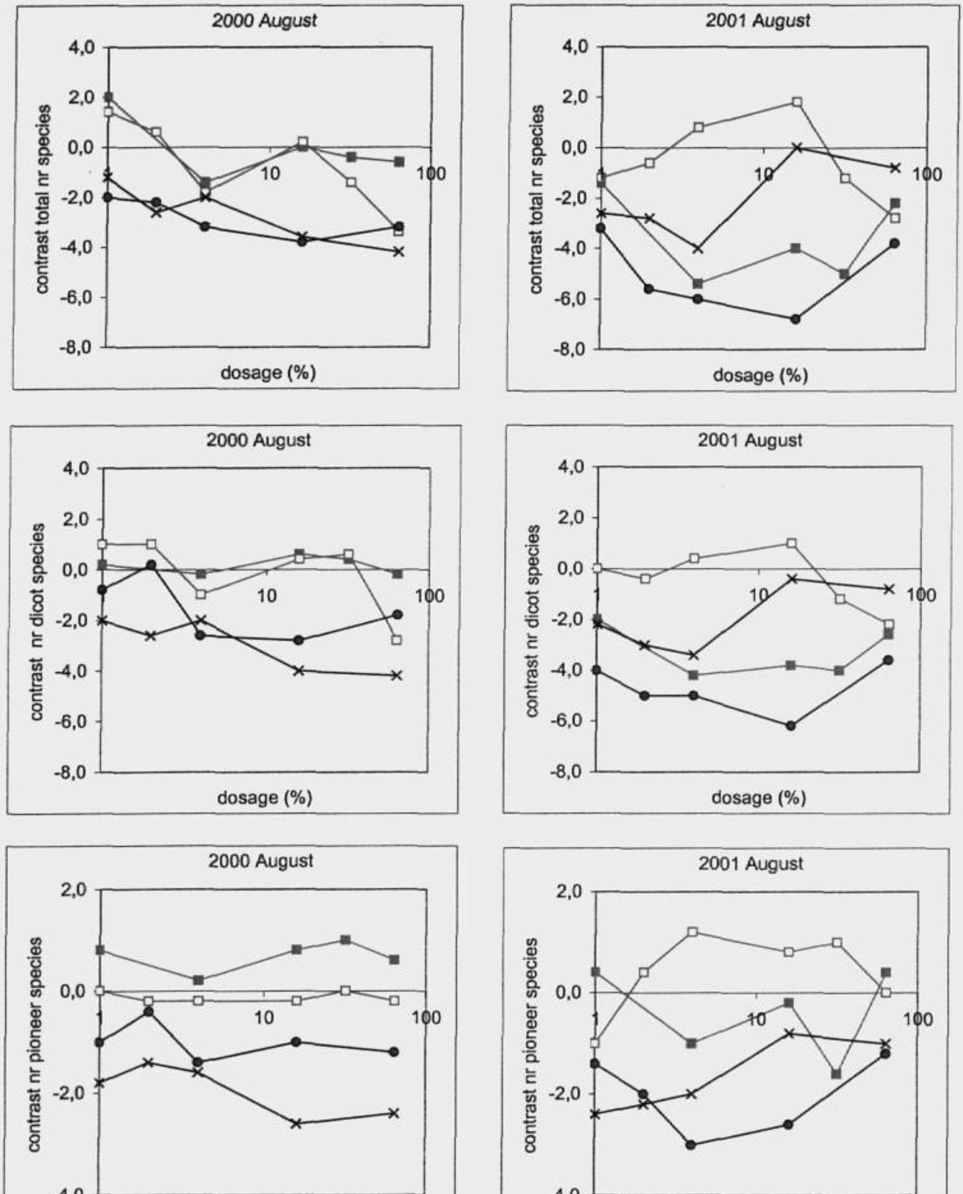


Fig. 8.4 Effect of several dosages of the herbicide glufosinate-ammonium on the contrast (August corrected for May) of total number of species, number of dicotyledonous and pioneer species in roadside grassland vegetations in August 2000 and 2001 at four sites; filled square: Klarenbeek, open square: Wenum; filled circle: Lexmond and cross: Zegveld, see N.B.1) in Fig. 5.1 (page 40).

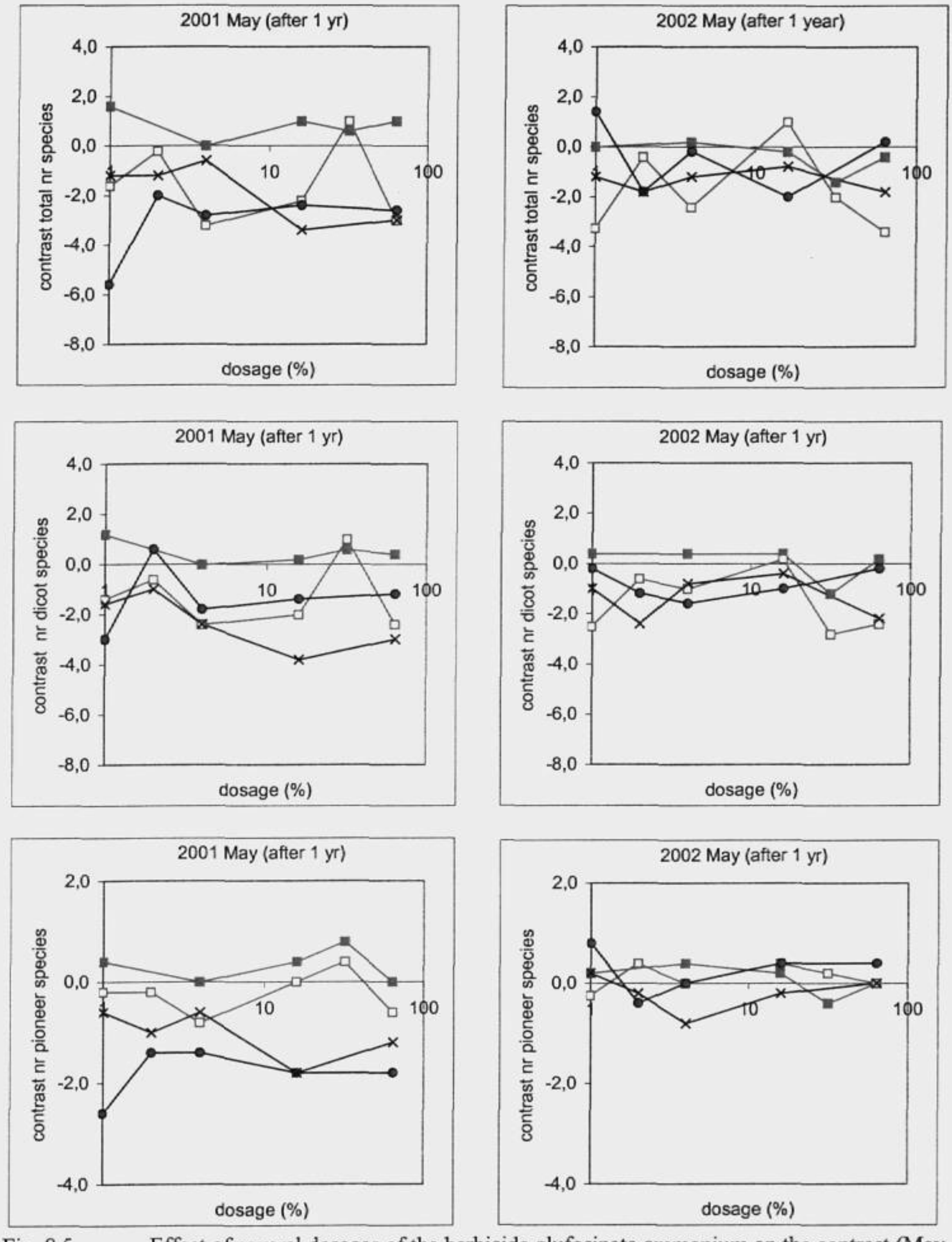


Fig. 8.5 Effect of several dosages of the herbicide glufosinate-ammonium on the contrast (May corrected for May data 1 yr ago) of total number of species, number of dicotyledonous and pioneer species in roadside grassland vegetations in May 2001 and 2002 at four sites; filled square: Klarenbeek, open square: Wenum; filled circle: Lexmond and cross: Zegveld, see N.B.1) in Fig. 5.1 (page 40).

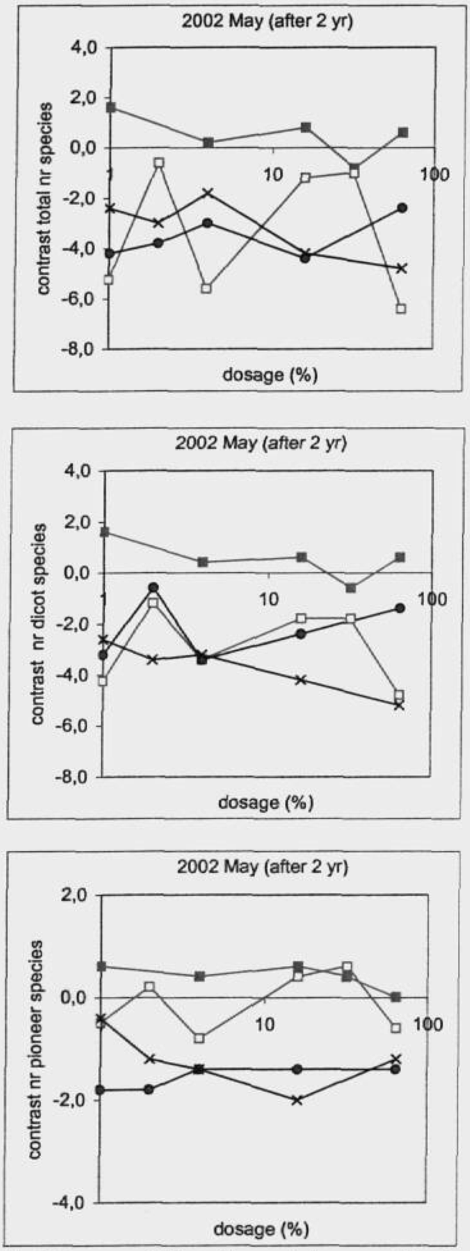


Fig. 8.6 Effect of several dosages of the herbicide glufosinate-ammonium on the contrast (May corrected for May data 2 yr ago) of total number of species, number of dicotyledonous and pioneer species in roadside grassland vegetations in May 2002 at four sites; filled square: Klarenbeek, open square: Wenum; filled circle: Lexmond and cross: Zegveld, see N.B.1) in Fig. 5.1 (page 40).

9 EFFECTS OF GLUFOSINATE-AMMONIUM ON OFF-CROP VEGETATION COMMUNITIES

9.1 Principal Response Curves

The effects of herbicide treatment at the vegetation community level were analysed by the Principal Response Curves method (PRC). PRC is based on the Redundancy Analysis ordination technique, the constrained form of Principal Component Analysis (Van den Brink and Ter Braak, 1999). The PRC method is a multivariate technique specially designed for the analysis of data from microcosm and mesocosm experiments. PRC results in a diagram showing the sampling dates on the x-axis and the first Principal Component of the treatment effects on the y-axis (see Fig. 9.1D for an example). This yields a diagram showing the deviations, in time, of treatments compared to controls. For instance, Fig. 1D indicates that for the sampling dates after the treatment in 2000 and 2001, the greatest deviations from the controls occurred at the highest treatment level. It also indicates smaller differences relative to the controls for the highest treatment level on the other sampling dates and very small differences for the other treatments. The species weights shown on the right-hand side of the diagram can be interpreted as the weight of each species for the response given in the diagram. Thus, *Ranunculus repens*, which has the greatest weight in the diagram, is shown to have decreased in abundance at the highest treatment level. The negative weight of *Stellaria media* in the diagram indicates that its numbers increased at the highest treatment level. In quantitative terms, multiplying the weight b_k of species k by the regression coefficient c_{dt} of a treatment d on a particular sampling date t yields the fitted difference on a nominal scale of this species between the treatment d and the controls (d=0). For instance, the mean nominal abundance (on the scale from 0 to 9, Braun-Blanquêt method) of *Ranunculus repens* in the plots receiving the highest treatment level on sampling date 2000_after is found to be (3.75*-0.49 =) -1.75 counts higher (equals 1.75 lower in Braun-Blanquêt scale) than the mean nominal abundance in the controls. The actual mean difference is 1.4. For *Stellaria media* a difference of (-4.55*-0.49 =) 2.23 is found between treatment and control (actual difference 3.20). For a complete description and discussion of the PRC method the reader is referred to Van den Brink and Ter Braak (1998, 1999). The PRC analyses were performed for each site separately using the CANOCO for Windows software package, version 4.5 (Ter Braak and Smilauer, 2002). In all PRC analysis the block structure was introduced into the covariables using nominal variables denoting blocks.

The results of the PRC analysis can also be evaluated in terms of the fractions of variance explained by sampling date, block structure, variation between replicates and treatment regime, and the fraction of the variance explained by the treatment regime shown in the PRC diagram.

9.2 Multivariate Statistical Tests

Three different multivariate tests were performed, as described below, headed by the question they answer.

1. Does the PRC diagram display a significant part of the variance in species composition explained by the treatment?

In the CANOCO computer program, Redundancy Analysis is accompanied by Monte Carlo permutation tests to assess the statistical significance of the effects of the explanatory variables on the species composition of the samples (Verdonschot and Ter Braak, 1994). The significance of the PRC diagram in terms of displayed treatment variance was tested by Monte Carlo permutation of the plots following the PRC analysis. This was done by permuting entire time series of plots in the partial redundancy analysis from which PRC is obtained, using an F-type test statistic based on the eigenvalue[xv] of the component (Van den Brink and Ter Braak, 1999).

2. Does the treatment regime have a significant effect on the composition of the vegetation community on a particular sampling date?

Monte Carlo permutation tests were also performed for each sampling date, using the ln-transformed dosage percentage as the explanatory variable (for rationale see Van den Brink et al., 1996) and block structure as covariable. This allows the significance of the treatment regime to be tested for each sampling date.

3. Does a particular treatment have a significant effect on the composition of the vegetation community at a particular sampling date?

Besides the overall significance of the treatment regime, we also wanted to know which treatment levels differed significantly from the controls. This was done by testing each treatment level against the controls using Monte Carlo permutation, introducing block structure as covariable. From the results at each site a $NOED_{community}$ could be deduced for each sampling date, with the $NOED_{community}$ being the highest treatment level for which no statistical significance of effects could be demonstrated.

9.3 Results

The Monte Carlo permutation tests following the PRC analyses of the 4 sites (test 1) indicated that the PRC diagrams of all individual sites did not display a significant amount of treatment variance. This can be a result of absence of strong herbicide effects and/or strong multidimensionality of effects. When all sites are combined into one analysis the PRC diagram does display a significant part of the treatment variance ($p < 0.05$). The PRC analyses of the individual sites showed that between 16 and 33% of the total variance can be explained by differences between sampling dates and between 14 and 29% can be recaptured by block structure, depending on the number of blocks. Between 44 and 49% of the total variance can be assigned to differences between replicates, the remaining 9 to 10% to the treatment (Table 9.1). Of this treatment variance, between 22 and 32% can be displayed in the PRC diagram. Fig. 9.1 shows the PRC diagrams of the sites. The PRC diagram of the Zegveld site (and to a lesser extent those of the Wenum and Lexmond sites) shows a clear deviation from the control for the highest treatment level, especially after the treatment took place in 2000 and 2001. Smaller differences are indicated for the other sampling dates, remarkably also for the pre-treatment sampling date.

When all sites are combined into one analysis the two highest treatment levels deviate from the controls, but this difference is also present in the pre-treatment period (Figure 9.2). The differences are slightly larger at the post-treatment sampling dates (2000_after and 2001_after).

Table 9.2 shows the results of statistical tests 2 through 4. For all sites treatment effects were demonstrated after the treatment in 2000 ($P<0.05$; test 2), effects of the 2001 treatment were demonstrated for the sites Wenum and Lexmond only. Occasionally, moderate overall treatment effects ($0.05<P<0.10$) were demonstrated for the 2001_after (Klarenbeek, Zegveld), 2002_before (Klarenbeek) and, remarkably the 2000_before (Zegveld) sampling dates. In the

overall analysis significant treatment effects are recorded for the post-treatment sampling dates (2000_after and 2001_after, Table 9.2).
When every treatment was tested separately against the control, p-values lower than 0.05 were found only at the highest treatment level (64%). When a more liberal threshold value of 0.10 was taken, effects were demonstrated at the 32 and 64% treatment levels. The derived $NOED_{community}$ (at 0.05 and 0.10 level) indicated 16% for three sites and 32% for one site. When all sites are combined in one analysis the $NOED_{community}$ was 32% (see Table 9.2).

Table 9.1 Variance all sites in PRC analysis and displayed treatment variance in PRC diagram.

	% of total variance explained by:					% of treatment
	time	block (no. of blocks)	differences between replicates	site	treatment	variance in PRC diagram
Klarenbeek	16	29(4)	45		10	32
Wenum	16	25(5)	49		10	23
Lexmond	33	14(2)	44		9	22
Zegveld	18	24(3)	48		10	29
All sites	*5*	*12 (5)*	*41*	*40*	*2*	*21*

Table 9.2 Results of multivariate statistical tests. The test number refers to the numbers given in the Materials and methods section; * = P<0.05; + = 0.05<P<0.10; - = not relevant.

	test number	2	3					
	result	P-value	2% vs control	4% vs control	16% vs control	32% vs control	64% vs control	$NOED_{community}$
Klarenbeek	2000_before	> 0.10	-	> 0.10	> 0.10	> 0.10	> 0.10	-
	2000_after	0.022*	-	> 0.10	> 0.10	0.085+	0.087+	16%
	2001_before	> 0.10	-	> 0.10	> 0.10	> 0.10	> 0.10	-
	2001_after	0.056+	-	> 0.10	> 0.10	> 0.10	> 0.10	-
	2002_before	0.059+	-	> 0.10	> 0.10	0.052+	> 0.10	32%[xvi]
Wenum	2000_before	> 0.10	> 0.10	> 0.10	> 0.10	> 0.10	> 0.10	-
	2000_after	0.020*	> 0.10	> 0.10	> 0.10	> 0.10	> 0.10	-
	2001_before	> 0.10	> 0.10	> 0.10	> 0.10	> 0.10	> 0.10	-
	2001_after	0.021*	> 0.10	> 0.10	> 0.10	> 0.10	0.088+	32%
	2002_before	> 0.10	> 0.10	> 0.10	> 0.10	> 0.10	> 0.10	-
Lexmond	2000_before	> 0.10	> 0.10	> 0.10	> 0.10	-	> 0.10	-
	2000_after	0.026*	> 0.10	> 0.10	> 0.10	-	0.034*	16%
	2001_before	> 0.10	> 0.10	> 0.10	> 0.10	-	> 0.10	-
	2001_after	0.029*	> 0.10	> 0.10	> 0.10	-	0.049*	16%
	2002_before	> 0.10	> 0.10	> 0.10	> 0.10	-	> 0.10	-
Zegveld	2000_before	0.060+	> 0.10	> 0.10	> 0.10	-	> 0.10	-
	2000_after	0.004*	> 0.10	> 0.10	> 0.10	-	0.021*	16%
	2001_before	> 0.10	> 0.10	> 0.10	> 0.10	-	> 0.10	-
	2001_after	0.054+	> 0.10	> 0.10	> 0.10	-	> 0.10	-
	2002_before	> 0.10	> 0.10	> 0.10	> 0.10	-	> 0.10	-
All sites	2000_before	> 0.10	> 0.10	> 0.10	> 0.10	> 0.10	> 0.10	-
	2000_after	0.002*	> 0.10	> 0.10	> 0.10	> 0.10	0.002*	32%
	2001_before	> 0.10	> 0.10	> 0.10	> 0.10	> 0.10	> 0.10	-
	2001_after	0.008*	> 0.10	> 0.10	> 0.10	> 0.10	0.015*	32%
	2002_before	> 0.10	> 0.10	> 0.10	> 0.10	> 0.10	> 0.10	-

9.4 Synthesis

Consistent effects on community structure were observed at the 64% treatment level, and intermediate effects at the 32% treatment level. At lower treatment levels no effects could be demonstrated. Effects were observed only on the sampling date following the application of the herbicide, so recovery was complete by the next year. Differences in observed effects between the four sites were demonstrated, but their NOEDs resembled each other.

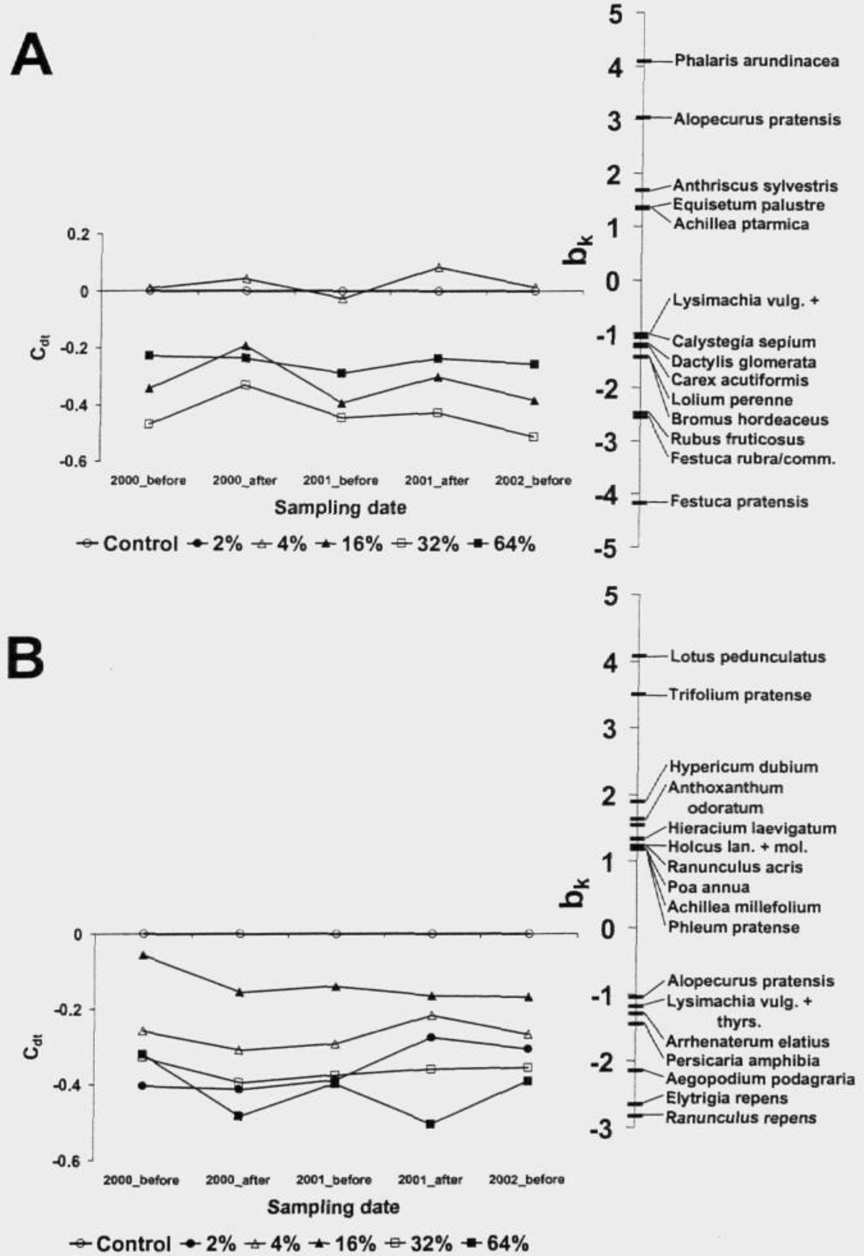


Fig. 9.1 PRC diagrams of the Klarenbeek (A) and Wenum (B) sites showing the effects of glufosinate-ammonium on the vegetation community. See Table 1 for variance allocation and displayed variance. See Table 2 for the results of Monte Carlo permutation tests on the significance of differences.

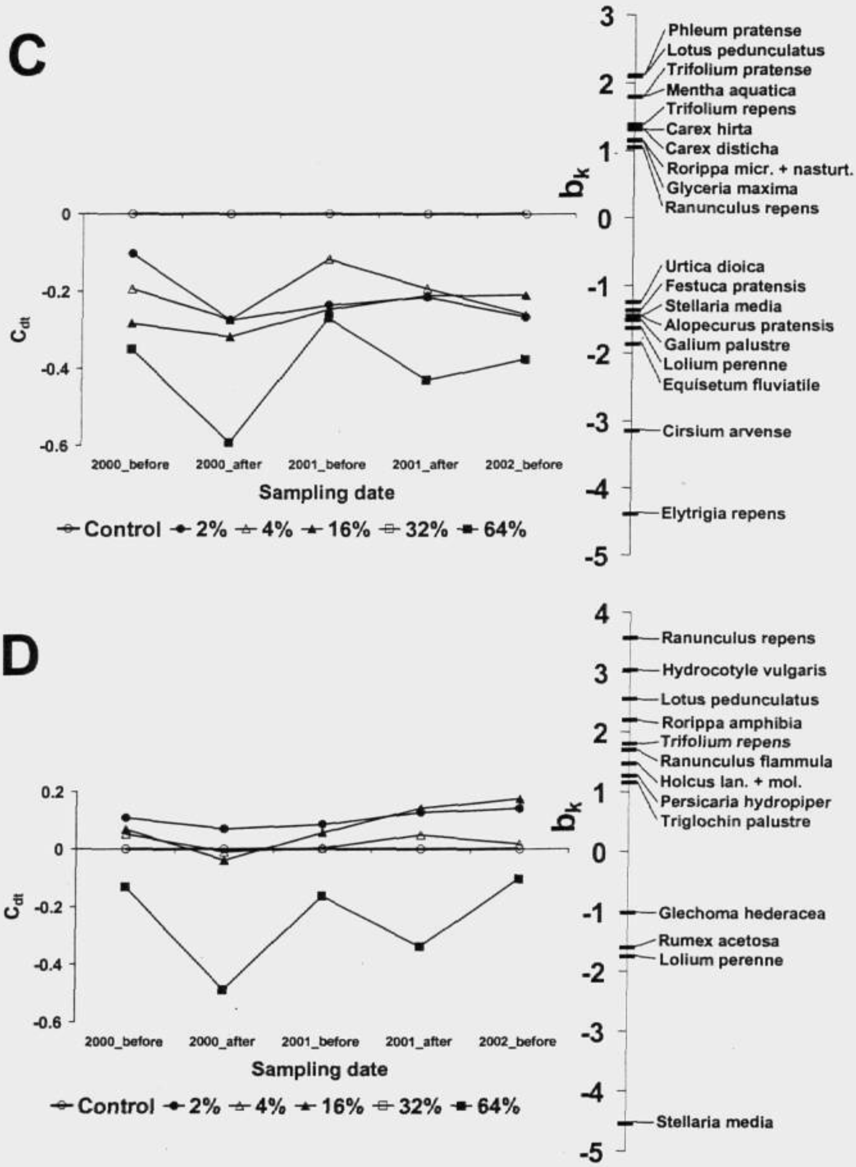


Fig. 9.1 (continued). PRC diagrams of the Lexmond (C) and Zegveld (D) sites showing the effects of glufosinate-ammonium on the vegetation community. See Table 1 for variance allocation and displayed variance. See Table 2 for the results of Monte Carlo permutation tests on the significance of differences.

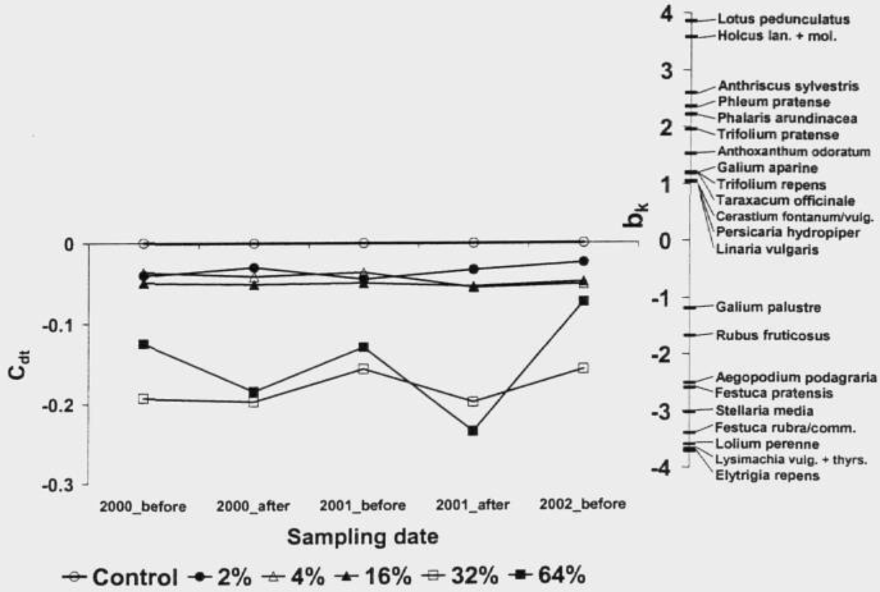


Figure 9.2. PRC diagram of the analysis including all sites showing the effects of glufosinate-ammonium on the vegetation community. See Table 9.1 for variance allocation and displayed variance. See Table 9.2 for results of Monte Carlo permutation tests on the significance of differences.

10 GENERAL SYNTHESIS

10.1 General

In the chapters 3-9 the results have been presented regarding the different parameters studied. In this chapter a short overview is given of the main out come of the study regarding these parameters. Therefore the main results are being summarised in some tables regarding the short and mid- and longer term effects of glufosinate-ammonium on off crop vegetation.

10.2 Short term phytotoxic effects and deposition

In Table 10.1 the short term phytotoxic effects are presented. Phytotoxic effects of glufosinate-ammonium were present in both years, after each treatment, at all sites and even at low dosages. At the low dosages 2% and 4% phytotox values ranged between 3 and 14% and phytotox values after the first application were higher then after the second application. NOED is in almost all cases lower than the lowest dosage of 2% used.
After the second application in 2001 the disappearance over time of the phytotoxic effects is assessed. The overall half-life or disappearance time (DT50), i.e. the time in days in which the phytotoxic effect declines with 50%, is for all sites and all dosages about 11 days. A problem with the half-life estimation is the presence of yellow discoloration in August and September as a consequence of natural senescence. At the species level large differences in the phytotoxic effects occur.

Table 10.1 Summary of the short term effects of dosage of glufosinate-ammonium for the phytotoxic data for 2000 and 2001 after first application (1st) and second application (2nd); * = significant, P<0.05; NOED are given for all significant cases; x = not calculated.

year	application	site Klaren-beek		Wenum		Lexmond		Zegveld		sites combined
2000	1st	*	<4	*	<2	*	<2	*	<2	x
	2nd	*	<4	*	2	*	<2	*	2	x
2001	1st	*	<4	*	<2	*	<2	*	<2	x
	2nd	*	<4	*	<2	*	2	*	<2	x

10.3 Mid-term ecological and community effects

10.3.1 Mid-term effects: effects of herbicide applications in the same year

In Table 10.2 the mid-term effects in August of the herbicide applications in that same year are summarised. In August 2000 there were no significant effects of dosage on number of species, diversity and coverage. Consistent significant effects on community structure and biomass were observed at the 32% treatment level. In all other cases in 2000 in Table 10.2, marked with a +, values decreased with increasing dosage.

Table 10.2 Summary of the mid-term effects of dosage of glufosinate-ammonium for the data for August 2000 and 2001 1) all ecological variables from the vegetation relevés: 2) from the community variable in the PRC-analysis and 3) from the biomass: -- = not significant (up to dosage of 64%); + = P>0.05/n and P<0.10; n = 1 for biomass and community and 2 or higher for the relevé data; * = significant effects (P<0.05/n); NOED are given for all cases where P<0.10; x = (could) not calculated or determined. Dicot.=dicotyledonous; sp., spec.=species.

variable	year	site: Klarenbeek		Wenum		Lexmond		Zegveld		sites combined	
RELEVÉ											
no. species total	2000	--		+	32%	--		--		+	<2
	2001	--		--		--		--		--	
no. dicot. species	2000	--		+	32%	--		--		+	32%
	2001	--		--		--		--		--	
no. pioneer species	2000	--		--		--		--		--	
	2001	+	x	--		--		--		--	
diversity H	2000	+	32%	+	4%	--		--		+	4%
	2001	--		+	32%	--		--		--	
evenness E	2000	+	32%	--		--		--		--	
	2001	--		+	32%	--		--		--	
coverage total	2000	--		--		--		--		+	32%
	2001	--		--		--		--		--	
coverage dicot. spec.	2000	--		+	<2%	--		--		+	2%
	2001	+	32%	--		--		--		--	
coverage pioneer sp.	2000	--		--		--		--		--	
	2001	--		--		--		--		--	
COMMUNITY											
	2000	*	16%	*	x	*	16%	*	16%	*	32%
	2001	+	x	*	32%	*	16%	+	x	*	32%
BIOMASS											
	2000	--		*	32%	x		x		*	32%
	2001	--		--		*	32%	--		--	

In August 2001 only an effect could be detected on the vegetation community at 32%. At one site (Lexmond) a significant effect was found on the biomass (32%). In all other cases in 2001 in Table 10.2, marked with a +, values decreased with increasing dosage.

After correction for multiple comparisons (Bonferroni) there were no significant effects on species numbers, diversity, evenness or plant coverage at any site or in any year.

From Table 10.2 it becomes clear that effects of dosage on ecological parameters derived from the relevés are found predominantly in 2000 and predominantly the effects were found in the same year of application of glufosinate-ammonium. Most pronounced effects were found at Klarenbeek and Wenum: the road verges. The effects on the ditch bank vegetation are much smaller (Zegveld and Lexmond). However in the community analysis Lexmond and Zegveld showed the most pronounced effects.

Table 10.3 Summary of the longer -term effects of dosage of glufosinate-ammonium for the May data for 2001 (for the relevé data relative to May 2000) and 2002 (for the relevé data relative to May 2001 and May 2000) See Table 10.2 for explanation.

variable	year	Klaren-beek (site)		Wenum (site)		Lexmond (site)	Zegveld (site)	sites combined	
RELEVÉ									
no. species total	2001 (1 yr)	--		+	x	--	--	--	
	2002 (1 yr)	--		-		--	--	--	
	2002 (2 yr)	x		x		x	x	--	
no. dicot. species	2001 (1 yr)	--		+	x	--	--	+	x
	2002 (1 yr)	--		--		--	--	--	
	2002 (2 yr)	x		x		x	x	--	
no. pioneer species	2001 (1 yr)	--		--		--	--	--	
	2002 (1 yr)	--		--		--	--	--	
	2002 (2 yr)	x		x		x	x	--	
diversity H	2001 (1 yr)	--		+	4%	--	--	--	
	2002 (1 yr)	--		--		--	--	--	
	2002 (2 yr)	x		x		x	x	--	
evenness E	2001 (1 yr)	+	x	+	2%	--	--	--	
	2002 (1 yr)	--		--		--	--	--	
	2002 (2 yr)	x		x		x	x	--	
coverage total	2001 (1 yr)	--		--		--	--	--	
	2002 (1 yr)	--		--		--	--	--	
	2002 (2 yr)	x		x		x	x	--	
coverage dicots. sp.	2001 (1 yr)	--		--		--	--	+	<2%
	2002 (1 yr)	--		--		--	--	--	
	2002 (2 yr)	x		x		x	x	--	
coverage pioneer sp.	2001 (1 yr)	--		--		--	--	--	
	2002 (1 yr)	--		--		--	--	--	
	2002 (2 yr)	x		x		x	x	--	
COMMUNITY									
	2000	--		--		--	--	--	
	2001	--		--		--	--	--	
	2002	+	32%	--		--	--	--	
BIOMASS									
	2000	x		x		x	x	x	
	2001	--		--		--	--	--	
	2002	--		--		--	--	+ 16%	

10.3.2 Longer term effects: effects of herbicide applications after one or two years

After correction for multiple comparisons (Bonferroni) no significant effects have been found of the herbicide applications of the year before. In all other cases in Table 10.3, marked with a +, values decreased with increasing dosage. So, despite the presence of effects within the same year of application in 2000, no effects are any longer visible in the following years, indicating that the vegetation had recovered.

11 CONCLUSIONS AND DISCUSSION

11.1 Conclusions regarding effects of glufosinate-ammonium

The aims of the study were to acquire information on the effects of glufosinate-ammonium and recovery on adjacent vegetation at the community level in the field situation under "realistic" worst case conditions and to assemble knowledge about how to conduct field tests with vascular plants at the vegetation level regarding herbicides and non target plants.

Therefore, a large field experiment has been conducted during 2000-2002 in which species rich vegetation on different soil types was sprayed directly, twice in the season with different concentrations of glufosinate-ammonium simulating drift under worst case conditions. Effects, including recovery, have been studied at vegetation level using a number of relevant variables. The data gathered from the field have been analysed in both a multivariate and a stepwise univariate approach (including a correction for multiple comparison). The general conclusions of the study, regarding the two aims, are formulated in this chapter:

Effects of Glufosinate-ammonium on non target plants

Concerning the effects of glufosinate-ammonium on off crop vegetation it can be concluded as follows:

- **Short term effects**: About 10 days after spraying in Spring, *small significant phytotoxic effects* could be detected on non target vegetation even at low concentrations of 2 and 4% of the field dosage corresponding to a distance less than 3 m under practical conditions. The effects will be not visible any more at the end of the season.
- **Mid term effects**: Within the year of spraying at low concentrations (2% and 4% of the field dosage) there were *no significant effects* found on the vegetation parameters studied. At higher concentrations (32% or higher) in both years of the study *significant effects* were found on the vegetation community. At 32% also significant effects on the vegetation biomass were found within the year of spraying (in 2000 for all sites combined and in 2001 at one site).
- **Longer term effects**: In the pair wise comparison between years (2000/2001, 2001/2002 and 2000/2002) *no significant effects* of the sprayings could be detected in spring on all parameters.

However, these results should be handled with care because in some instances there were nearly significant effects and clear negative trends between dosage and effect parameters, see also discussion (par.11.3).

Summarising, it can be concluded that using glufosinate-ammonium on agricultural fields can have a significant phytotoxic effects for a short period on the non target vegetation adjacent to sprayed fields. All effects on vegetation level in terms of impact on the species number, species cover, vegetation community, diversity, evenness and biomass will be limited to one season only or shorter. Even at the highest concentrations, no effects could be detected one year after spraying. Significant effects on vegetation level within the same year of spraying will be limited to a negative impact on the vegetation community and biomass for treatment levels of 32% or higher. Since this were the positive controls and deposition levels will be limited to the sprayed fields (incl. buffer zones) these types of effects on off crop flora will not be expected outside the treated fields.

11.2 Discussion about the results and interpretation of this field study

Due to the fact that in the first year of study, at the sandy soil areas, nearly significant effects and clear negative trends were detected at low treatment levels (2% and 4%) on the total number of species, species diversity and coverage of dicot species (but could not be detected in the second year of study) it is advisable to treat the results of the study concerning the effects on the vegetation within the year of spraying with care and a closer look to the ecological mechanism is necessary. These differences between the two years require additional attention. Some possible explanations are given as follows:

- *Exposure* did not differ between the years since deposition measurements and phytotox assessments in the second year were in the same order as in the first year of the trial.
- *Change of management* was necessary for methodological reasons especially at the ditch banks (one mowing in stead of two, preventing cattle grazing). This caused a decline of species in the control plots of the ditch banks, which was not observed on the road verges. Overruling effects cannot be denied but are unlikely to dominate over higher dose-rates.
- *Less common species in the relevés were not found after spraying in the first year*? As far as we look to the species level of the data, this seems to be not the case (Annex VIII).
- *Vegetation parameters not accurate within the year?* In most vegetation assessments a comparison is being made of the vegetation data gathered of the same period of the year, e.g. comparing Spring data between years. It might be that the accuracy of the parameters for the assessment within the year is too low, or too sensitive for species occurrence and non occurence.

11.3 General conclusions and discussion concerning field tests with off crop vegetation

From the study it can be concluded that large scale field studies can be conducted in practice. Moreover, sound and generally reproducible results could be generated. From the study some general methodological aspects could be derived regarding experimental set up, field assessments and statistical procedures:

1 Experimental set up: The study focuses on the effects of *herbicide drift off-crop*. In practice it is almost impossible to study actual drift in a large scale experimental design under standardised conditions because weather conditions most likely will hamper an (experimentally postulated) optimised exposure to the herbicide. It was thus decided to apply the different dosages directly onto the vegetation, in this way drift was simulated. For this aim the amount of spray volume was held constant, which is quite different from actual drift. Actual drift consists of smaller droplets with possibly higher concentrations of the compound - but less penetration power, being aware that this procedure reflects a worse case scenario compared to real drift. We did not compare our drift simulation approach with real drift but we think this is the only way of carrying out such a large trial. A second issue in the experimental set up is the fact that as far as we know this is one of the first large scale experimental field studies where spraying was carried out on potential exposed vegetation in agricultural areas which has not been exposed over a long period ('*Non-adapted vegetation*'). We did not carry out a comparison with 'herbicide adapted' vegetation, but we think that for studying the effects on non-target vegetation this is the best approach since it is sometimes reported that species could be affected adjacent to sprayed fields.

2 Field assessments: The assessment of the phytotox is frequently used in efficacy studies (short term, individual plants). Deviant from the EPPO guidelines in this study the phytotox estimates were used at vegetation level, assessments were made at intervals of 5% and also used for a longer time period. In principle we think this is also a possible methodology to investigate the short term effects on vegetation level in field studies. However, it is less suitable to study 'recovery' due to re-growth and the presence of yellow discoloration later in the year as a consequence of natural senescence. For the assessment of the effects on *biomass*, there should be more attention paid to standardisation of sampling, the number of replicates etc. in order to be useful for standard field tests. Finally the *vegetation releve's* according to the Braun-Blanquet methodology seems to be very useful for this type of studies.

3 Statistical procedures: In the study the data have been worked out by two different statistical approaches (uni- and multivariate). The outcome of the two approaches is sometimes different and for the interpretation of the outcome of field studies this should be worked out in more detail. The multivariate approach is not regularly used in non target plant tests at higher tier levels but seems to be promising. In the univariate analysis, there were three main steps in the statistical procedure. First a standard analysis of variance was carried out. If an effect had been found with a significance value of $P<0.10$ in the first step, the second step followed with a Williams' test on trend. If there was a significant effect ($P<0.05$) in the second step, then in the third step the Williams' test was used again to find the no observed effect dosage (NOED). We almost always found significant trends in the second step for near significant values ($0.05<P<0.10$) in the first step. The reason for this difference is obvious, since both analysis of variance and Williams' test have identical null hypotheses, no differences between means, but differ in their alternative hypotheses. The alternative hypothesis of the Williams' test is that means are monotonically ordered, resulting in a much more sensitive test procedure to detect effects in comparison with the standard analysis of variance. It can be argued that the first main step in the analysis is actually superfluous and that in future analyses the more powerful William's test (and improvements of this test, see Bretz, 1999) should be used directly to detect effects and trend in one analysis. If we had omitted the standard analysis of variance from the start, then more significant effects could have been expected.

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ANNEX I SITE DESCRIPTIONS AND MAPS

1. Klarenbeek

The plots are located 2 km west of Klarenbeek, in an area where the Netherlands' last primeval forest Beekbergerwoud once stood until its drainage and felling in 1871, in kilometre grid cells 199-464 (plots 1-5 and 12-25) and 199-465 (plots 6-11).
Average grade level is 9 m above NAP (*Normaal Amsterdams Peil)*, sloping locally from 10 m +NAP (one km to the west) to 7.5 m +NAP (Grote Wetering, 1.5 km to the east). The soil consists of fine sand overlying coarse sand at a depth of 40-120 cm (*gooreerdgronden*), with a shallow to moderately deep water table. In the near vicinity are podzolic soils (*veldpodzols, laarpodzols)*.
The actual plots are in the verges of the cited roads, viz. along either side of the metalled road and along one side of the unmetalled road; the latter plots border on a small dry ditch. Normal management consists of summer (June/July) and autumn (September) mowing, with removal of the swath. The whole area is predominantly agricultural: moderately heavily fertilised grassland in use as pasture (with recurrent ploughing and seeding), with some cropping of maize further away.

2. Wenum

The Wenum site is located in kilometre grid cell 195-474, one kilometre east of Wenum. Grade is about 10 m +NAP. The site is at the foot of the Veluwe ice-pushed ridge, at the transition to the IJssel valley. The metalled road traverses a small brook valley, at its deepest (about 1.5 m below grade) between plots 15 and 16.
The soil is a complex of loamy and fine sands overlying coarse sand at 40-120 cm depth (*beekeerdgronden*) and coarse-sandy podzols (*veldpodzols)* and sandy soils covered by 30-50 cm of agricultural topsoil. The water table varies from moderately deep to rather deep, to deep beneath the soils overlain by a topsoil.
Again these plots are all in road verges, only being bordered by a small dry ditch along the unmetalled road. They are mown twice a year (in June and September) and the swath removed. In this mainly agricultural district the land is not used intensively as grassland (mainly grazed) or pasture. In the wider area there is also maize cropping.

3. Lexmond

The Lexmond site lies between Lexmond and Ameide in kilometre grid cell 128-442. Grade level here is about 0.1m +NAP. The plots, inside the Lek river dyke, are on sandy clay loam overlying peat at 40-120 cm depth (*poldervaaggronden*). The water table is fairly shallow to moderately deep. Livestock trampling has flattened the banks into terraced slopes about 75 cm to one metre wide.
Because of the ditches behind the fields, the farmland has not been used very intensively in the (recent) past. Today the land is used as grassland with post-cut grazing; livestock density is 2.5 SLU/ha and nutrient input some 60 kg N/ha. There are 2 to 3 hay cuts a year. The plots themselves are on the ditch banks, which are not fertilised and only partly mown. Most of the neighbouring farmland is likewise used for hay-making, with post-cut grazing, although there is also some cultivation of maize and tree fruit.

4. Zegveld

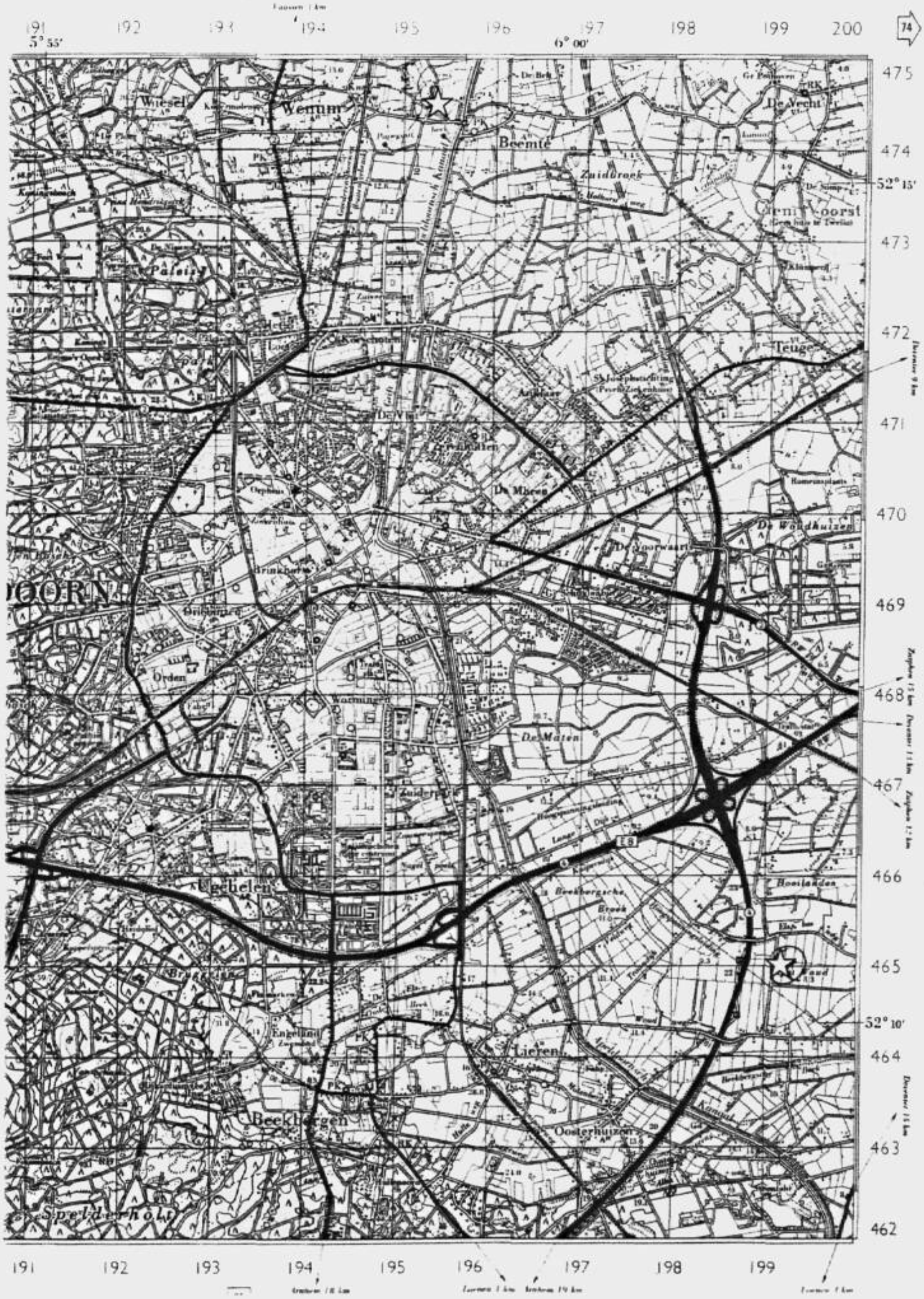
The Zegveld plots are in kilometre grid cell 116-461. Grade level is about 1.5-2 m below NAP. The soil consists of 7 metres of sedge and rush peat (*vlierveen*), surrounded by forest peat (*koopveengronden*). At its shallowest the average water table is less than 50 cm below grade. Livestock trampling has created terraced slopes over one-third to three-quarters of the entire plot width and grade is very uneven.
The fields are used for hay-making and as pastureland, under experimental conditions (for an agronomic study). Livestock density is about 1.5 SLU/ha and nutrient input about 200-260 kg N/ha, 40-80 kg P_2O_5/ha and 220-430 kg K_2O/ha. There is a twice-yearly cut. The ditch banks are not fertilised, but they are cut and grazed. All the farmland in the wide vicinity of the plots is also used for hay-making and as pasture. Somewhat further to the north and north-east lies an extensive, protected wetland (Nieuwkoopse Plassen).

At each of the sites the plots measure 25 x 1 metre. The precise location of each plot was carefully recorded to permit accurate subsequent relocation, using either a fixed and permanent reference point nearby (Lexmond, Zegveld) or on-site markings in permanent varnish or a galvanised stake sunk to grade level (Klarenbeek, Wenum).

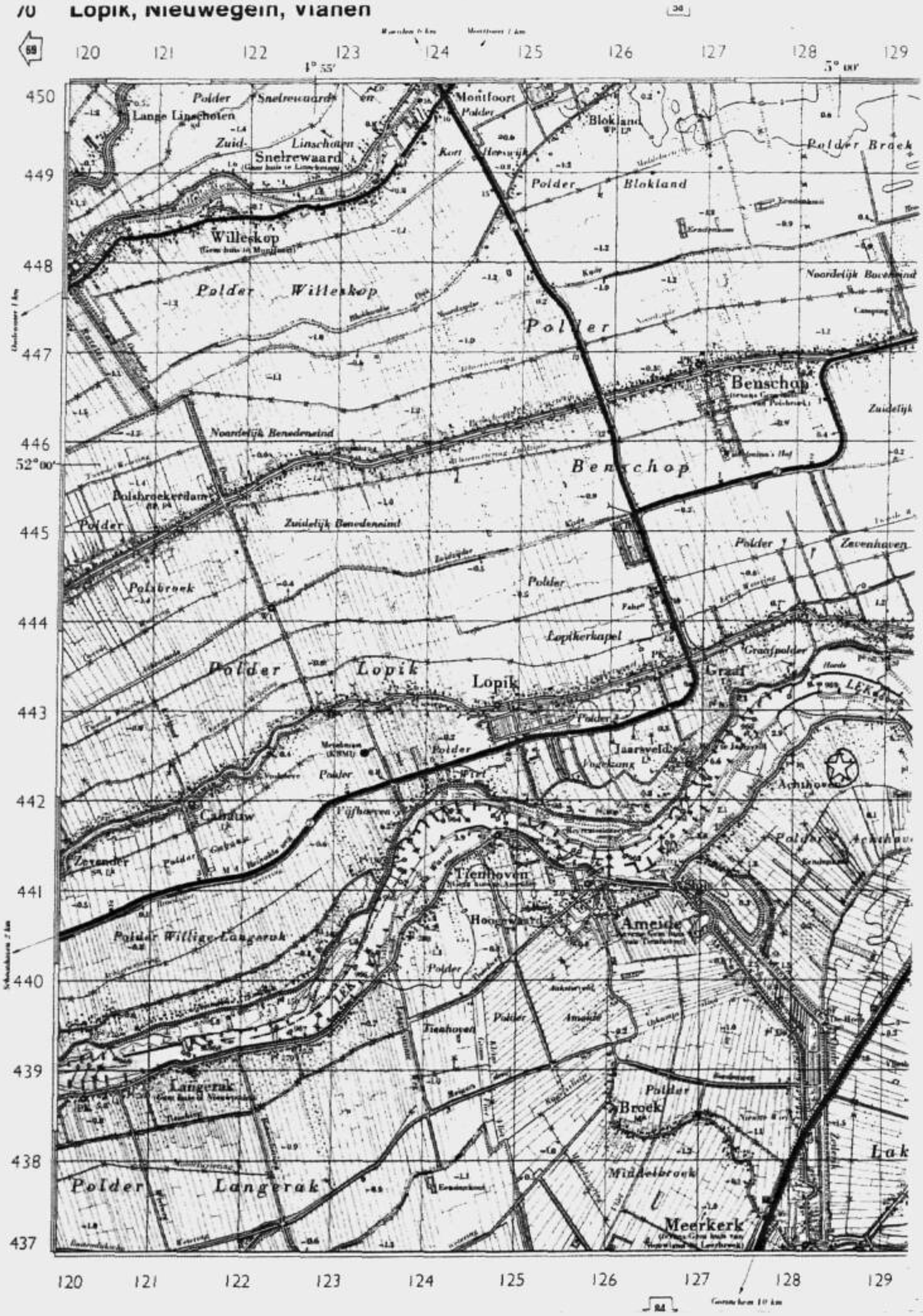
Geographical co-ordinates of the sites, see Table and Maps on the following pages.

Site	Plot nrs.	Coordinates		Map on page
		X	Y	
Klarenbeek	1 - 5 and 12 - 25	199	464	89
	6 - 11	199	465	89
Wenum	1 - 30	195	474	89
Lexmond	1 - 25	128	442	90
Zegveld	1 - 25	117	461	91

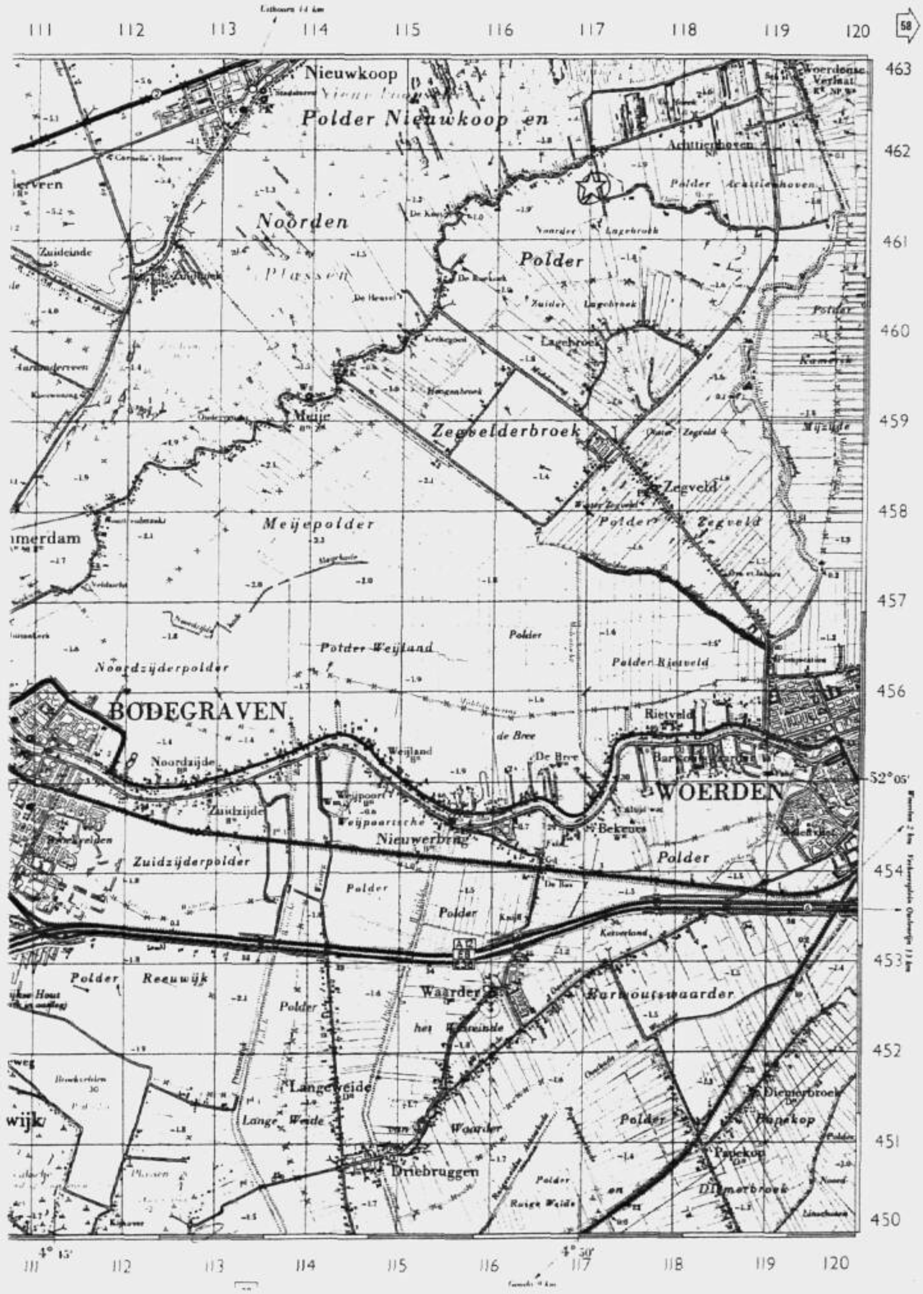
Map Klarenbeek (site = lower star) and Wenum (site = upper star)



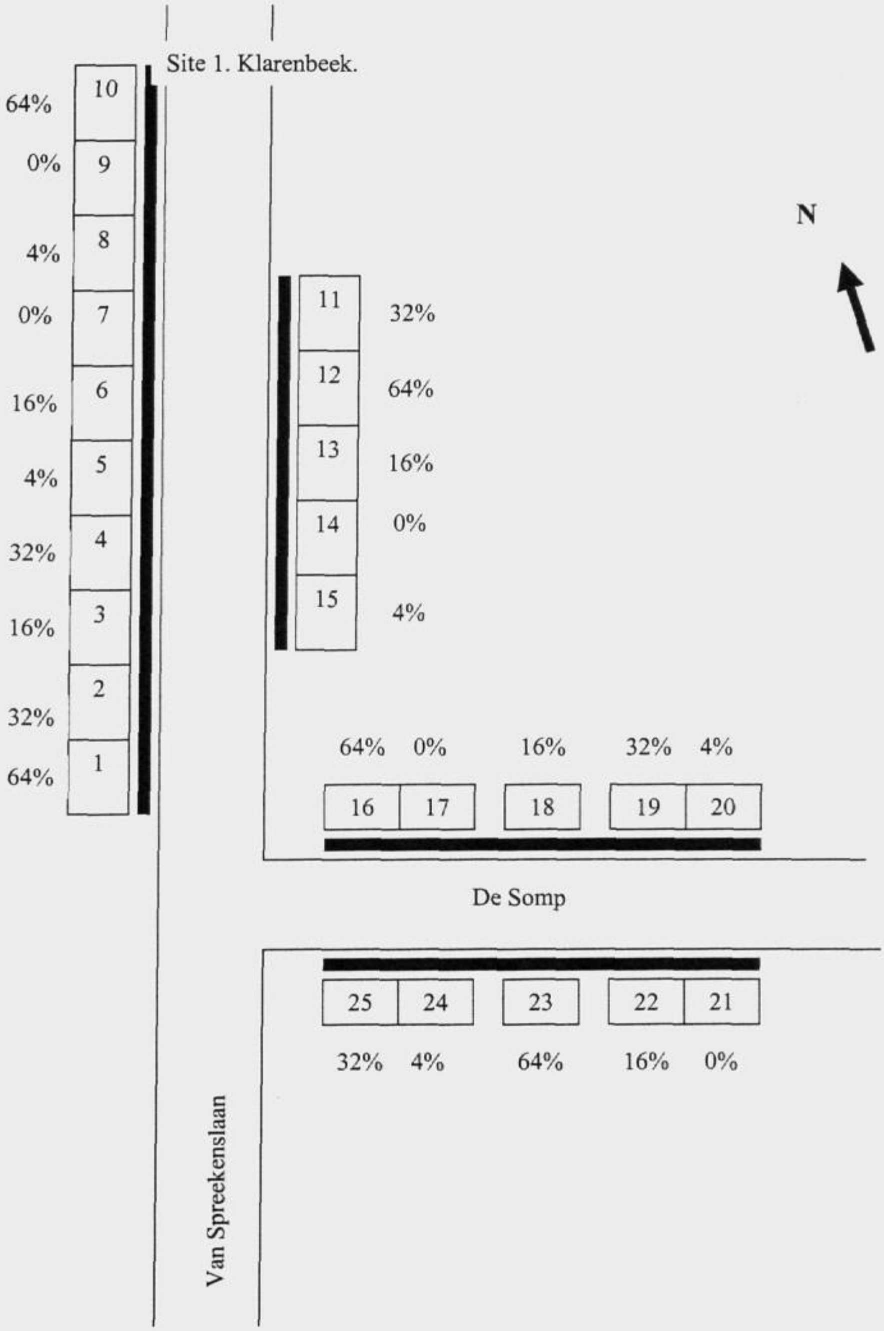
Map Lexmond (site = star)

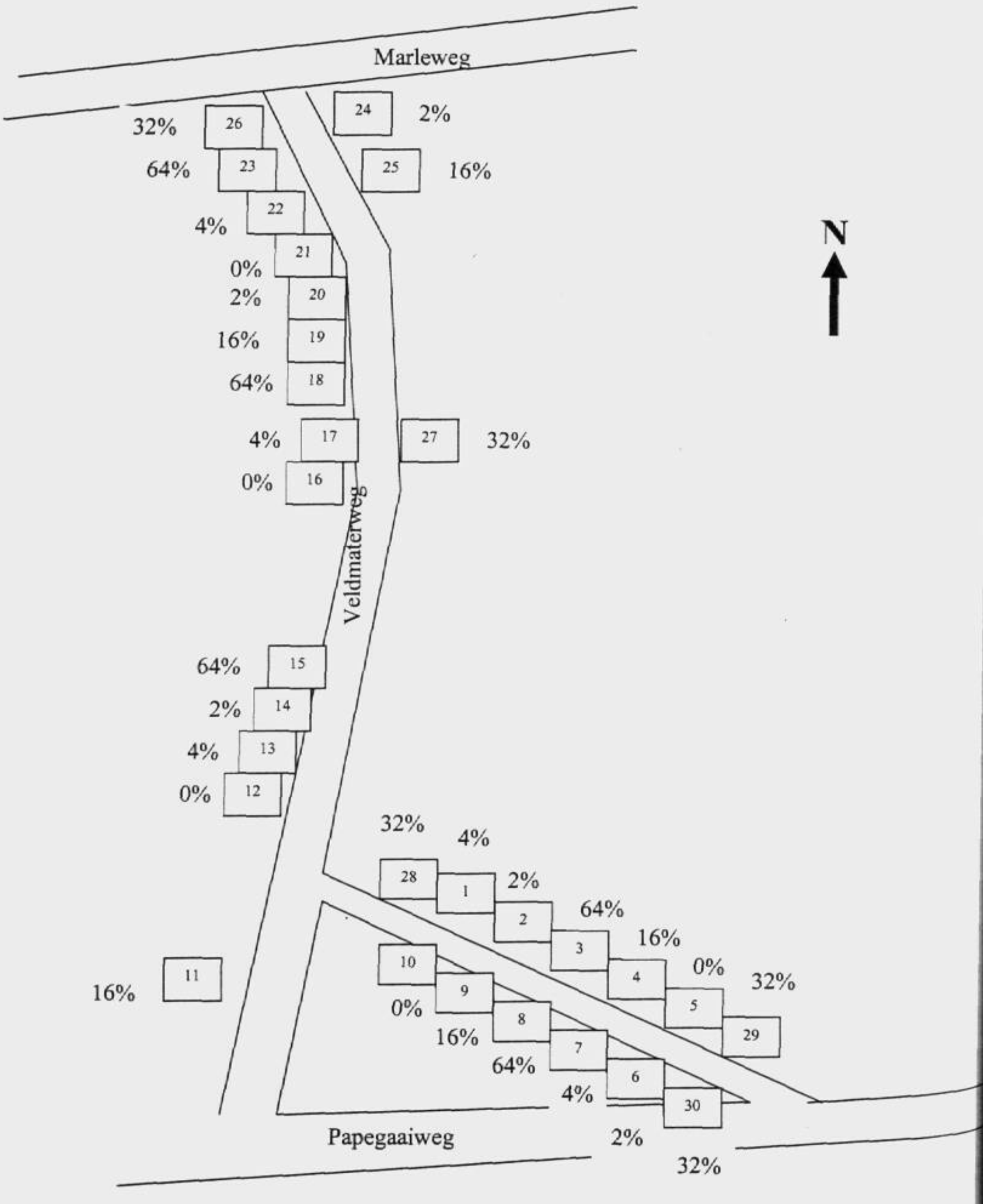


Map Zegveld (site = star)



ANNEX II DETAILED PLANS OF THE PLOTS OF EACH SITE




Marleweg
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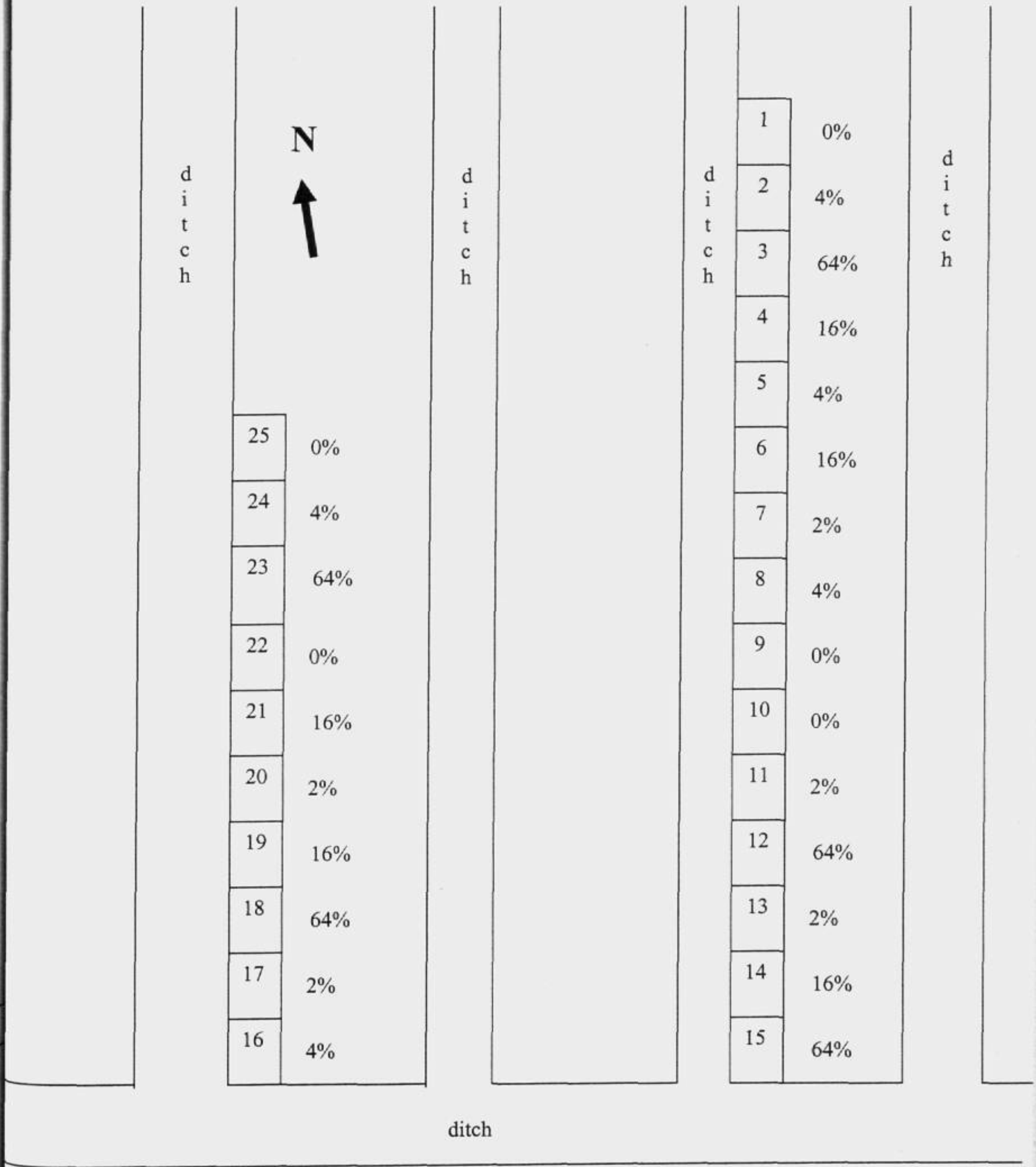
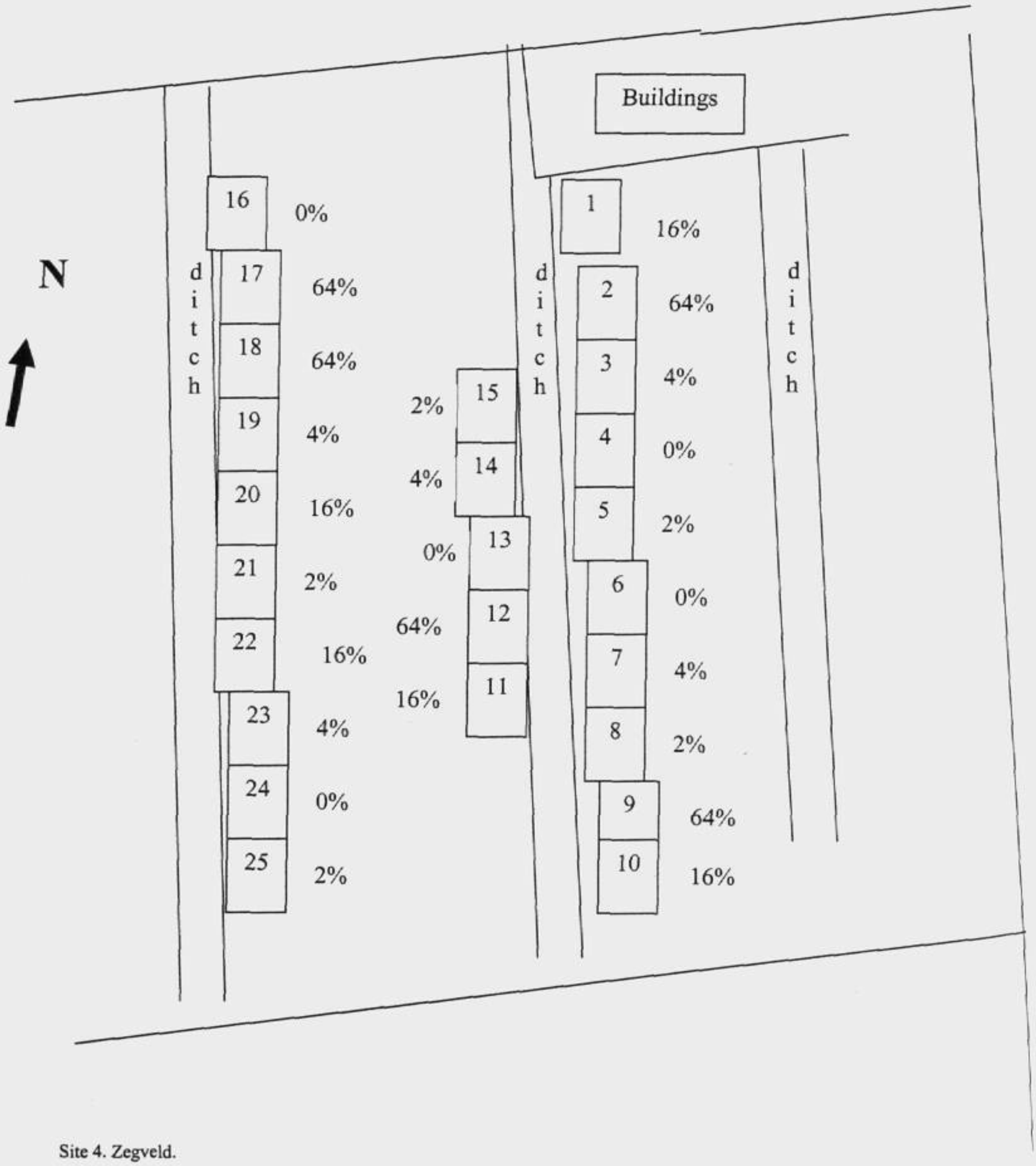
28
1
2%
2
64%
3
16%
4
0%
32%
5
29
16%
11
10
9
0%
8
16%
7
64%
6
4%
30
2%
32%

Site 2. Wenum.



Site 3. Lexmond.



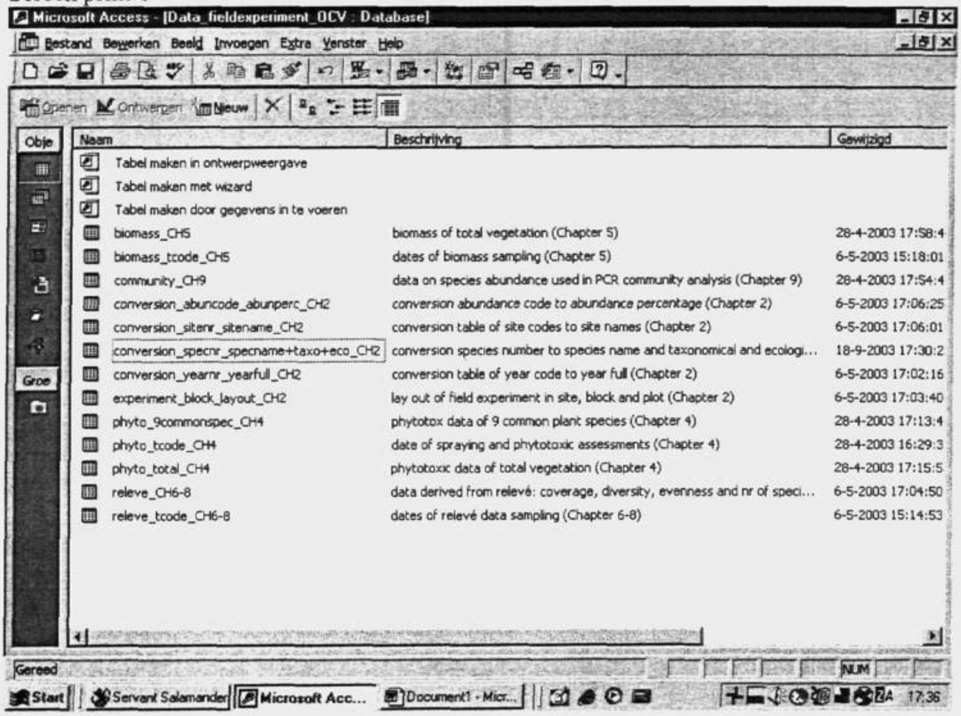
Site 4. Zegveld.

ANNEX III DESCRIPTION OF THE DATABASE WITH DATA ON RELEVÉS, BIOMASS AND PHYTOTOX AND SAMPLING AND SPRAYING DATES

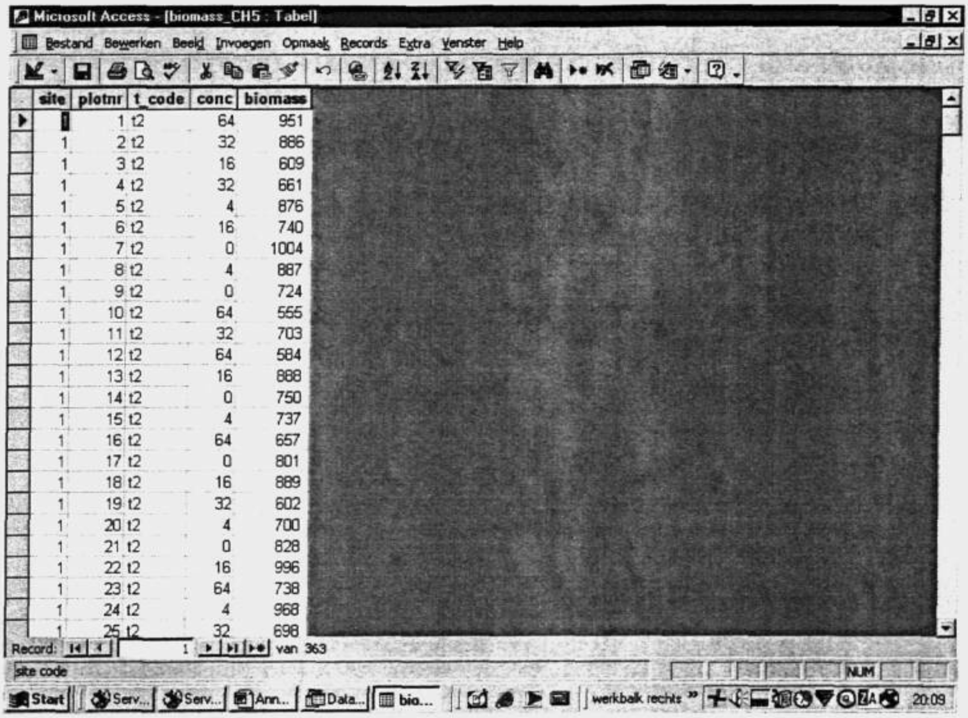
Name database: Data_fieldexperiment_OCV.mdb (Access 2000)

This database contains several tables (screen print 1) with the data for all the variables investigated, see e.g. biomass (screen print 2), and with the meaning for all the coded variables including the exact dates of all sampling, see e.g. the dates on which the biomass of the vegetation was harvested (screen print 4). All variables are described and explained in the properties section of the table (e.g. screen print 3 for table in screen print 2).

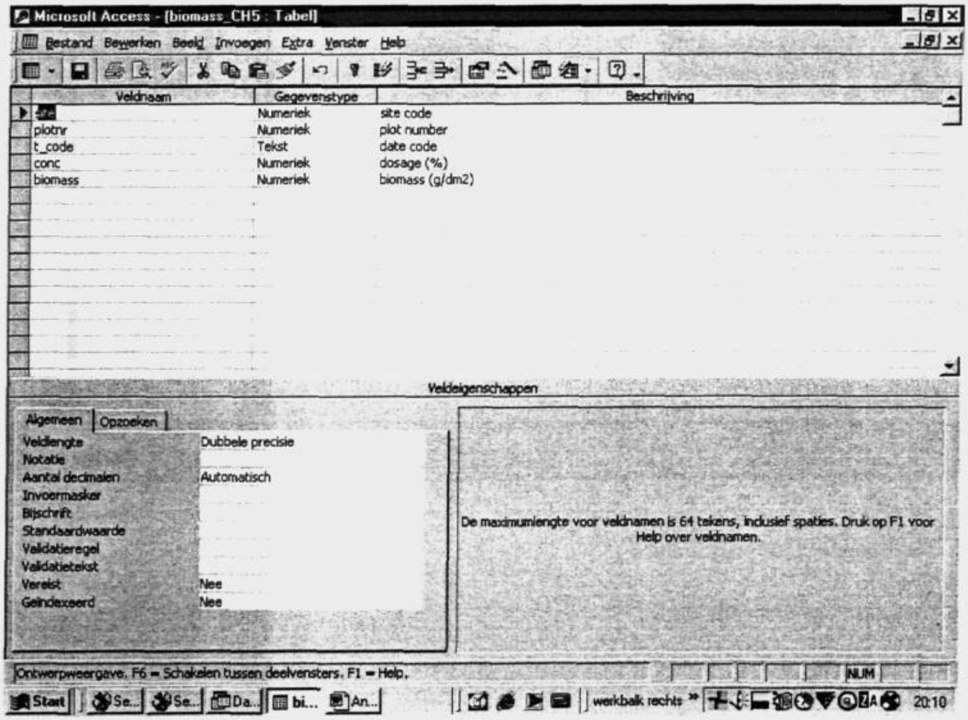
Screen print 1



Next page screen print 2 and 3

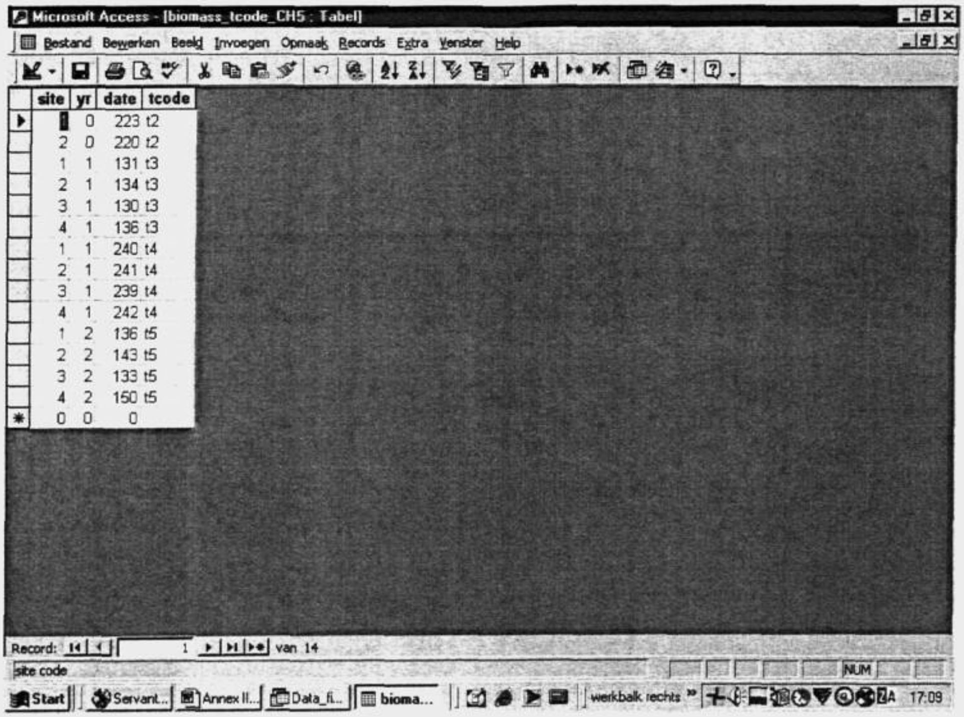


site	plotnr	t_code	conc	biomass
1	1	t2	64	951
1	2	t2	32	886
1	3	t2	16	609
1	4	t2	32	661
1	5	t2	4	876
1	6	t2	16	740
1	7	t2	0	1004
1	8	t2	4	887
1	9	t2	0	724
1	10	t2	64	555
1	11	t2	32	703
1	12	t2	64	584
1	13	t2	16	888
1	14	t2	0	750
1	15	t2	4	737
1	16	t2	64	657
1	17	t2	0	801
1	18	t2	16	889
1	19	t2	32	602
1	20	t2	4	700
1	21	t2	0	828
1	22	t2	16	996
1	23	t2	64	738
1	24	t2	4	968
1	25	t2	32	698



Veldnaam	Gegevenstype	Beschrijving
site	Numeriek	site code
plotnr	Numeriek	plot number
t_code	Tekst	date code
conc	Numeriek	dosage (%)
biomass	Numeriek	biomass (g/dm2)

screen print 4



site	yr	date	tcode
1	0	223	t2
2	0	220	t2
1	1	131	t3
2	1	134	t3
3	1	130	t3
4	1	136	t3
1	1	240	t4
2	1	241	t4
3	1	239	t4
4	1	242	t4
1	2	136	t5
2	2	143	t5
3	2	133	t5
4	2	150	t5
0	0	0	

ANNEX IV PARTICIPANTS OF WORKSHOPS

NL = the Netherlands, UK = United Kingdom

Workshop 12-04-2000 Leiden University, NL

Dr. J. Cremer	Bayer CropScience, Germany
Dr. P. Sowig	Bayer CropScience, Germany
Dr. E. Panman	Bayer CropScience, NL
Mr. R. Mekking	Bayer CropScience, NL
Dr. J. Sweet	NIAB, UK
Dr. C.E. Goewie	CTB, NL
Dr. ir. L. van der Eerde	TNO/UL, NL
Dr. R. Luttik	RIVM, NL
Dr. L. Bastiaans	Laboratory of Crop- and Weed Ecology, WUR, NL
Dr. J. Franzaring	Plant Research International, NL
Dr. C. Kempenaar	Plant Research International, NL
Drs. J.J.C. Karres	Ministerie v. Landbouw, NL
Dr. R. van der Meijden	Nationaal Herbarium Nederland, NL
Dr. J. van der Zande	IMAG, NL
Dr. P.A. Oomen	Plant Protection Service, NL
Dr. N. van der Hoeven	Ecostat, NL
Dr. F.M.W. de Jong	Institute of Environmental Sciences (CML), NL
Ing. R. van de Poll	Institute of Environmental Sciences (CML), NL
Drs. G. Barendse	Institute of Environmental Sciences (CML), NL
Prof. dr. G.R. Snoo	Institute of Environmental Sciences (CML), NL

Workshop 09-03-2001 Leiden University NL

Dr. J. Cremer	Bayer CropScience, Germany
Dr. P. Sowig	Bayer CropScience, Germany
Dr. K. Stumpf	Bayer CropScience, Germany
Mr. R. Mekking	Bayer CropScience, NL
Dr. N. de Schrijver	Bayer CropScience, NL
Ing. M. van Erp	Bayer CropScience, NL
Dr. J. Sweet	NIAB, UK
Drs. P. van Vliet	CTB, NL
Prof. dr. P. Zwerger	BBA, Germany
Drs. R. Luttik	RIVM, NL
Ing. T. Rotteveel	Plant Protection Service, NL
Prof. dr. H.A. Udo de Haes	Institute of Environmental Sciences (CML), NL
Drs. M. van der Linden	Institute of Environmental Sciences (CML), NL
Dr. F.M.W. de Jong	Institute of Environmental Sciences (CML), NL
Ing. R.J. van de Poll	Institute of Environmental Sciences (CML), NL
Prof. dr. G.R. Snoo	Institute of Environmental Sciences (CML), NL

Workshop 13-02-2003 Leiden University NL

Dr. J. Cremer	Bayer CropScience, Germany
Dr. P. Sowig	Bayer CropScience, Germany
Dr. K. Stumpf	Bayer CropScience, Germany
Mr. K. Pallet	Bayer CropScience, Germany
Dr. H.-U. Reuss	Bayer CropScience, Germany
Dr. W. Steinheuer	Bayer CropScience, Germany
Dr. A. Schueler	Bayer CropScience, Germany
Mr. R. Mekking	Bayer Crop Science, NL
Dr. N. de Schrijver	Bayer Crop Science Benelux, NL
Prof. dr. P. Zwerger	BBA, Germany
Dr. S. Martin	Umweltbundesamt, Germany
Dr. C. Kula	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit, Germany
Dr. H. Koch	Landsanstalt für Pflanzenbau und Pflanzenschutz, Germany
Drs. P.J.M. van Vliet	CTB, NL
Drs. R. Luttik	RIVM, NL
Dr. F.M.W. de Jong	RIVM, NL
Ing. T. Rotteveel	Plant Protection Service, NL
Dr. T. Dueck	Plant Research International, NL
Dr. G. Blom	Plant Research International, NL
Dr. P. van den Brink	Alterra, NL
Dr. N. van der Hoeven	Ecostat, NL
Dr. D. van der Schans	PPO-PAV, NL
Dr. J. de Rijk	Ministerie VROM, NL
Ir. J. van der Zande	IMAG, NL
Drs. R. Faasen	RIZA, NL
Dr. P. Soons	LNV, NL
Drs. A.J. Rossenaar	Foundation FLORON, NL
Prof. dr. H.A. Udo de Haes	Institute of Environmental Sciences (CML), NL
Ing. R. van der Poll	Institute of Environmental Sciences (CML), NL
Drs. Wil Tamis	Institute of Environmental Sciences (CML), NL
Prof. dr. Geert de Snoo	Institute of Environmental Sciences (CML), NL

ANNEX V INVESTIGATION INTO THE NUMBER OF REPLICAS NEEDED FOR THE DESIRED POWER IN A FIELD EXPERIMENT WITH 4 TREATMENT AND A CONTROL

Author: N. van der Hoeven.

1. How many replicas are needed to be able to observe a difference of size δ with probability P=1-β.

To analyse the power of the experiments, the one-sided Dunnett test is used as statistical test (Dunnett, 1955, 1964, 1985). This test is the appropriate multiple comparison method for comparing one control with several treatments if the data are normally distributed and the variance at all treatments is identical.

If we want to maximize the power of the experiment with a minimum amount of replicas, we should use more replicas in the control than in each of the concentrations. If we have k treatments and a control, the optimal experimental set-up is to use about $k^{1/2}$ times as many replicas in the control (n_c) as in each of the treatments (n_t). Let us take n_c as the smallest integer value $\geq k^{1/2} n_t$. To reach power P at a difference of δ

$$n_t \geq (1+\sqrt{1/k}\,)\bullet\left(\mathrm{U}_{\alpha,\nu,\mathrm{k}} + \Phi^{-1}(\mathrm{P})\right)^2 \bullet \left(\frac{\sigma}{\delta}\right)^2 \qquad [1]$$

where Φ^{-1} is the inverse of the cumulative standard normal distribution, σ the a priori available estimate of the standard deviation and $U_{\alpha,\nu,k}$ the appropriate one-sided critical value for a test with ν degrees of freedom and k comparisons between a treatment and the control at significance level α (see van der Hoeven, 1998).

However, if it is preferred to keep the number of replicas identical in the control and in each of the treatments ($n_c = n_t = n$), the necessary number of replicas to reach a power P at a difference of δ is

$$n \geq 2\bullet\left(\mathrm{U}_{\alpha,\nu,\mathrm{k}} + \Phi^{-1}(\mathrm{P})\right)^2 \bullet \left(\frac{\sigma}{\delta}\right)^2 \qquad [2]$$

In Table 1 the number of replicas needed to reach a power of 70% and 80% are given for the case that 4 treatments (k=4) are compared with the same control, and the number of replicas is identical in the control and each of the treatments (n).
In Table 1 the number of replicas needed to reach a power of 70% and 80% are also given for the case that 4 treatments (k=4) are compared with the same control, and the number of replicas in the control is twice the number of replicas in each of the treatments ($n_c = k^{1/2} n_t = 2n_t$).

Illustration of the use of Table 1.
Suppose that a treatment leading to an effect of 1.5 times the standard deviation has to be observed with a power of 80%, and that a significance level of 5% is used for the statistical test. Then, looking in Table 1, in the second column (power 80%, α=0.05, identical number of replicas in the control) the first value below 1.5 is 1.449 at n_t=9 or, looking in column 6 (power 80%, α=0.05, twice as many replicas in the control as in each treatment), the first value below 1.5 is 1.446 at n_t=7. The desired power can therefore be reached by either a control and 4 treatments, each with 9 replicas (sum: 45) or by a control with 14 replicas and 4 treatments with 7 replicas each (sum: 42).

It should be noted that it is assumed for this power analysis that we are interested in the power at only one treatment, the treatment with an effect of about size δ. It is assumed that the effect in the lower treatments (doses) is much smaller, and therefore the effects at lower treatment levels do not have to be observed with power P. Furthermore, it is assumed that the effect in the higher treatments (doses) is much larger, and will therefore almost always be observed (power very high). If it is assumed that the effect at each of the treatments can be in the order of δ, and they all together have to be detected with given power P, the necessary number of replicas will be higher. In Hsu (1996) an equation is given to calculate the necessary number of replicas for that case.

2. Increasing the power by blocking.

Sometimes, it will be possible to increase the power with the same number of replicas by blocking the data. If the experimental design is blocked, the residual variance is the variance of the data which cannot be explained by either the treatment or the block. This variance is σ^2_{rcb} 1 (rcb: randomized complete block design), and its estimate is indicated as $MSE = s^2_{rcb}$ 2. Using a completely randomized design, the residual variance, i.e. the variance of the data which cannot be explained by the treatment, would have been σ^2_{cr} 3 (cr: completely random design). If a randomized complete block design is used, i.e. each block contains each treatment and the control once ($n_c = n_t = n$) with (k+1) different treatments (including the control) and n blocks, σ^2_{cr} 4 can be estimated by

$$\frac{SSB + nk\ MSE}{n(k+1)-1} \qquad [3]$$

where SSB is the sum of squares of the deviation between the block means and the overall mean (Kuehl, 2000).

The efficiency of a randomised complete block design compared to a completely randomised design is RE, i.e. in the completely randomised design RE times as many replicas are needed to reach the same power as in the randomised complete block design. If both σ^2_{cr} 5 and σ^2_{rcb} 6 are known, $RE = \sigma^2_{cr} / \sigma^2_{rcb}$ 7. However, in analysing an experiment σ^2_{cr} 8 and σ^2_{rcb} 9 are estimated by s^2_{cr} 10 and s^2_{rcb} 11. Since the s^2 are only estimates, the information based on s^2 is less than the information based on σ^2. Therefore, the relative efficiency of blocking has to be corrected for the degrees of freedom in both estimates, s^2_{cr} 12 and s^2_{rcb} 13. The degrees of freedom are $f_{cr} = (n-1)(k+1)$ 14 and $f_{rcb} = (n-1)k$ 15, respectively. The relative efficiency based on the estimates s^2_{cr} 16 and s^2_{rcb} 17 then becomes

$$RE = \frac{s^2_{cr}}{s^2_{rcb}} \frac{(f_{rcb}+1)(f_{cr}+3)}{(f_{rcb}+3)(f_{cr}+1)} \qquad [4]$$

(Kuehl, 2000)

At the start of the experiment, all plots are monitored. It is recommended to monitor some more plots than needed in the experiment. This allows to remove from the experiments the plots deviating most from the mean plot characteristics. The data gathered in the initial monitoring can be used to block the plots. In blocking, the relative spatial position of the plots should also be considered.

3. Combining the experiments from different sites.

It is only possible to combine data gathered at different sites if the variance in the data at these sites is the same. The data from the two sites with hedgerows can probably be combined in the statistical evaluation of the experiments. The same holds for the two sites of nutrient poor verges. Whether the other sites can also be combined, can only be decided after the experiment. It is not a priori likely that the variance in the investigated floristic characteristics will be identical at all sites. Neither can it be ruled out that the effect of the test substance on the different vegetations will differ. If the *p*-values of all *r* sites are calculated for a given concentration at each site, the combination of these *p*-values can be evaluated by calculating

$$T_c = -2x \sum_{i=1}^{r} \ln\ p_i \qquad [5]$$

If the concentration has no effect, T_c is χ^2 distributed with $2 \times r$ degrees of freedom (Hedges & Olkin, 1985).

Table 1

An experiment is performed with a control and 4 treatments. The treatments are compared with the control using the Dunnett test. The significance level is either α=0.05 or α=0.10. If a given number of replicas is used in each treatment (n_t) a difference between control and one of the treatment is observed with power P=1-β=80% or 70% if the difference between that treatment and the control (δ) is larger than σ times the tabulated value. These values are tabulated both for the case that the number of replicas in the control is identical to the number of replicas in each treatment, and for the case that the number of replicas in the control is twice the number of replicas in each treatment.

	Number of replicas constant is twice number per treatment				Number of replicas in control			
	α=0.05		α=0.10		α=0.05		α=0.10	
n_t	power 80%	power 70%	power 80%	power 70%	power 80%	power 70%	power 80%	power 70%
2	3.694	3.377	3.089	2.772	3.062	2.787	2.628	2.353
3	2.702	2.443	2.341	2.082	2.324	2.100	2.040	1.816
4	2.262	2.038	1.981	1.757	1.963	1.768	1.735	1.541
5	1.991	1.790	1.752	1.551	1.733	1.559	1.538	1.365
6	1.800	1.617	1.589	1.405	1.570	1.411	1.397	1.238
7	1.656	1.486	1.464	1.295	1.446	1.299	1.288	1.141
8	1.542	1.383	1.365	1.207	1.347	1.210	1.202	1.064
9	1.449	1.299	1.284	1.135	1.267	1.137	1.131	1.001
10	1.371	1.229	1.216	1.074	1.199	1.076	1.071	0.948
11	1.304	1.169	1.158	1.023	1.141	1.024	1.020	0.903
12	1.247	1.117	1.107	0.978	1.091	0.979	0.976	0.863
13	1.196	1.072	1.063	0.938	1.047	0.939	0.937	0.829
14	1.151	1.031	1.023	0.903	1.008	0.904	0.902	0.798
15	1.111	0.995	0.988	0.872	0.973	0.873	0.871	0.770
16	1.075	0.963	0.956	0.844	0.941	0.844	0.843	0.745
17	1.042	0.933	0.927	0.818	0.913	0.818	0.817	0.723
18	1.012	0.906	0.900	0.795	0.886	0.795	0.794	0.702
19	0.984	0.881	0.876	0.773	0.862	0.773	0.772	0.683
20	0.959	0.859	0.853	0.753	0.840	0.753	0.752	0.666
21	0.935	0.837	0.833	0.735	0.819	0.735	0.734	0.649
22	0.913	0.818	0.813	0.718	0.800	0.717	0.717	0.634
24	0.874	0.782	0.778	0.687	0.766	0.686	0.686	0.607
26	0.839	0.751	0.747	0.659	0.735	0.659	0.659	0.583
28	0.808	0.723	0.720	0.635	0.708	0.635	0.635	0.561
30	0.780	0.698	0.695	0.613	0.684	0.613	0.613	0.542
32	0.755	0.676	0.673	0.594	0.662	0.593	0.593	0.525
34	0.732	0.655	0.653	0.576	0.642	0.575	0.576	0.509
36	0.712	0.637	0.634	0.559	0.624	0.559	0.559	0.495
38	0.692	0.620	0.617	0.544	0.607	0.544	0.544	0.481
40	0.675	0.604	0.601	0.530	0.591	0.530	0.530	0.469
45	0.636	0.569	0.567	0.500	0.529	0.474	0.500	0.442
50	0.603	0.539	0.538	0.474	0.504	0.452	0.474	0.419
55	0.575	0.514	0.512	0.452	0.482	0.432	0.452	0.400
60	0.550	0.492	0.491	0.433				
65	0.528	0.473	0.471	0.416				

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ANNEX VI WEATHER SURVEYS 2000-2002

Weather surveys 2000-2002 (Source: KNMI)

2000	general	temperature (Celsius)		precipitation (mm)		sunshine (hours)	
		measured	normal	measured	normal	measured	normal
year	exceptionally warm, wet, normal sunshine	10.9	9.4	897	794	1532	1487
SEASON							
winter	very soft, very wet, very sunny	5.0	2.6	283	193	216	158
spring	exceptionally soft, wet, normal sunshine	10.5	8.4	199	167	463	462
summer	normal summer	16.3	16.2	189	211	567	575
autumn	very soft, wet, a bit sulky	11.6	10.2	294	222	272	293

2001	general	temperature (Celsius)		precipitation (mm)		sunshine (hours)	
		measured	normal	measured	normal	measured	normal
year	very warm, very wet, sunny	10.4	9.8	956	797	1670	1550
SEASON							
winter	soft, wet, very sunny	4.1	2.6	219	192	227	155
spring	soft, wet, sunny	9.1	8.4	179	167	497	462
summer	warm, wet, sunny	17.4	16.2	229	211	646	575
autumn	very soft, very wet, normal sunshine	11.6	10.2	322	235	298	302

2002	general	temperature (Celsius)		precipitation (mm)		sunshine (hours)	
		measured	normal	measured	normal	measured	normal
year	very warm, wet, sunny	10.8	9.8	891	797	1671	1550
SEASON							
winter	very soft, very wet, very sunny	4.8	3.3	279	194	252	172
spring	very soft, dry, sunny	10.0	8.9	132	166	554	485
summer	warm, wet, a bit sulky	17.6	16.6	267	202	547	591
autumn	soft, dry, sunny	10.7	10.2	209	235	343	302

ANNEX VII LIST OF PIONEER SPECIES

nr: number Dutch botanical database
taxo: M = Monocotyledonidae (old, now: Liliidae) , D = Dicotyledonidae (old, now Magnoliidae); E = Equisetopsida
eco: P = pioneer -, G = grassland -, R = ruderal -, H = woodland - taxon

nr	scientific name	taxo	eco
40	Alopecurus geniculatus	M	P,G
101	Artemisia vulgaris	D	P,R
121	Atriplex prostrata	D	P
144	Bidens tripartita	D	P
200	Capsella bursa-pastoris	D	P
306	Chenopodium album	D	P
315	Chenopodium polyspermum	D	P
331	Cirsium arvense	D	P,R
446	Elytrigia repens	M	P,G,R,H
462	Equisetum arvense	E	P,R
520	Festuca rubra	M	P,G
543	Galeopsis tetrahit	D	P,R,H
570	Geranium dissectum	D	P
673	Juncus articulatus	M	P,G
706	Lamium purpureum	D	P
794	Matricaria recutita	D	P
1922	Myosotis laxa + Myosotis scorpioides	D	P,G,R
509	Odontites vernus subsp. serotinus	D	P,G
972	Persicaria hydropiper	D	P,H
977	Persicaria maculosa	D	P
976	Persicaria mitis	D	P
946	Plantago lanceolata	D	P,G
2320	Plantago major	D	P
952	Poa annua	M	P
968	Polygonum aviculare	D	P
1006	Potentilla anserina	D	P,G
1058	Ranunculus sceleratus	D	P
5201	Rorippa microphylla + Rorippa nasturtium-aquaticum	D	P,W
1076	Rorippa palustris	D	P
1094	Rumex acetosella	D	P
1098	Rumex crispus	D	P,G
1112	Sagina procumbens	D	P
1225	Sonchus oleraceus	D	P
1250	Stellaria media	D	P
1247	Stellaria uliginosa	D	P
1347	Veronica arvensis	D	P,G
1378	Viola arvensis	D	P

ANNEX VIII FURTHER EXPLORATION OF THE EFFECTS ON TOTAL NUMBER OF SPECIES IN 2000 AND 2001

It is remarkable that in 2000 near-significant effects and clear trends were found at very low dosages on total number of species and that in 2001 even at the highest dosage of 64% no effect could be found. In this appendix we give additional information on this finding.

We developed three hypotheses for explanation:
The first hypothesis is that less abundant species were affected (did not occur) in 2000 as a consequence of the herbicide applications and also, but somewhat later, the less abundant species in the control plots as a consequence of change in management during 2000 and 2001.
The second hypothesis starts with the observation that total number of species is a net result of occurring, (staying) and not occuring species. As a consequence it could be possible that for some unknown reason, in 2001 in contrast to 2000, the process of no(n) occurrence masked the process of occurence of species.
The third hypothesis is that variation in test variables in 2001 is larger than in 2000, so that the power to detect differences is less in 2001 than in 2000. We also checked whether the variation of contrast of the total number of species between May and August is larger than between May one year and May next year because of the large seasonal changes during the year (early and late species).

The first hypothesis is investigated in two steps. We checked first whether the species which did not occur, indeed are the less abundant species. This proved to be just the case. Since the change in number of species is a net result of a lower and a higher number of species throughout a year, we studied in further detail the species dynamics and checked in how much no(n) occurrences were masked occurrences. In table VIII.1 the total number of occurring and not occurring species in 2000 and 2001 are presented.

Table VIII.1 Sum of not occurring, occurring and turn-over (sum no(n) occurrence and occurence) over all plots per dosage in 2000 and 2001. - = not relevant.

	2000						2001					
site	0%	2%	4%	16%	32%	64%	0%	2%	4%	16%	32%	64%
no(n) occurrences												
Klarenbeek	16	-	22	19	22	26	24	-	34	35	31	24
Wenum	15	18	27	16	21	32	27	25	21	15	29	36
Lexmond	37	41	40	40	-	38	43	50	52	52	-	47
Zegveld	25	32	28	38	-	35	40	37	35	27	-	26
occurrences												
Klarenbeek	26	-	15	19	20	23	17	-	7	15	6	13
Wenum	22	21	18	17	14	15	21	22	25	24	23	22
Lexmond	27	30	24	21	-	22	27	22	22	18	-	28
Zegveld	19	19	18	20	-	14	27	23	15	27	-	22
turn-over												
Klarenbeek	42	-	37	38	42	49	41	-	41	50	37	37
Wenum	37	39	45	33	35	47	48	47	46	39	52	58
Lexmond	64	71	64	61	-	60	70	72	74	70	-	75
Zegveld	44	51	46	58	-	49	67	60	50	54	-	48

We do not see in 2001 from the table that the number of occurrences in relation to dosage is masked by the number of no(n) occurrences. The lower number of species is more obvious in the controls of the ditch sizes (Lexmond and Zegveld) compared to the road verges. Preventing cattle from the plots might have been a larger influence on species composition than postponement of mowing to September. So, the second hypothesis is not supported.

For hypothesis three we calculated the standard deviations for total number of species for May 2000, 2001 and 2002 and for August 2000 and 2001 and for the contrast of the total numbers within the year (May-August 2000 and May-August 2001) and between the years (May 2000-May 2001 and May 2001-May 2002). See for a summary of results Table VIII.2.
The variation in August for total number is consistently smaller than in May, but the variation of the contrast within in the year (May-August) is somewhat larger than between years (May-May). So, at least the seasonal aspect does not produce a excessive larger variation, and this means that total number of species (and contrasts) is not hampered by seasonal variation. We also see that variation in 2001 is consistently larger than in 2000. So, the detection power of the analysis in 2001 is smaller than in 2000.

Table VIII.2 Variances of total number of species in different periods and contrasts of total number of species between different periods. Values in the table are the medians over all sites and dosages.

standard deviation	period(s)					
	May 2000	August 2000	May 2001	August 2001	May 2002	overall
total number species	4.45	3.83	5.03	4.53	5.03	4.55
		May 2000-August 2000	May 2000-May 2001	May 2001 August 2001	May 2001-May 2002	overall
contrast total nr species		2.86	2.70	3.32	2.95	2.93

End notes

[i] The first class 0% has the approximate range of 0-1%; the second class has the approximate range of 1-7.5%, with a class mean of 5%, which is a slight overestimation, because the real class mean is 4.25%. The third class is 7.5-12.5% with class mean 10%, etc.

[ii] logistic transformation is the modern variant of the arcsincus transformation

[iii] For more information about and calculation examples of the Williams' test, see the original publications (Williams, 1971, 1972) and e.g
. http://www.csc.fi/cschelp/sovellukset/stat/sas/sasdoc/sashtml/lgref/z1016947.htm#z1079776

[iv] In Appendix IX an estimation procedure is presented for the estimation of the DT50.

[v] Nevertheless a significant trend, decreasing biomass with increasing dosage, was found for the data of the sites combined (Tab. 5.3). This contradicting result will be discussed in general in Chapter 11, Conclusions and discussion

[vi] Nevertheless, in two cases significant trends , decreasing coverage with increasing dosage, were found. These contradicting results will be discussed in general in Chapter 11, Conclusions and discussion.

[vii] Nevertheless, in two cases significant trends, decreasing coverage with increasing dosage, were found. These contradicting results will be discussed in general in Chapter 11, Conclusions and discussion.

[viii] Nevertheless a significant trend, decreasing coverage of dicotyledonous species with increasing dosage, was found for the data of the sites combined (Tab. 6.3). This contradicting result will be discussed in general in Chapter 11, Conclusions and discussion.

[ix] Nevertheless, a significant trend, decreasing diversity with increasing dosage, was found for the data combined in 2000 (Tab. 7.2).These contradicting results will be discussed in general in Chapter 11, Conclusions and discussion.

[x] Nevertheless, for two sites significant trends, decreasing diversity and evenness with increasing dosage, were found (Tab. 7.2) in August 2000 and 2001. These contradicting results will be discussed in general in Chapter 11, Conclusions and discussion.

[xi] Nevertheless, for Wenum significant trends, decreasing diversity and evenness with increasing dosage, were found (Tab. 7.5). These contradicting results will be discussed in general in Chapter 11, Conclusions and discussion.

[xii] In this case the test for the presence of a negative trend (decreasing total number of species with increasing dosage) gives significant results and the no observed effect dosage (NOED) is less than 2%. The effects ranges on average from about 8% less at 2% dosage to about 14% less at 64% dosage relative (Tab. 8.2, Fig. 8.4)

[xiii] Besides the nearly significant result for the total number of species, a significant trend, decreasing number of dicotyledonous species with increasing dosage, was found (Tab. 8.2). These conflicting results will be discussed in further detail in Chapter 11, Conclusions and discussion.

[xiv] Nevertheless, for two sites significant trends, decreasing total number of species and number of dicotyledonous species with increasing dosage, were found (Tab. 8.2). These conflicting results will be discussed in further detail in Chapter 11, Conclusions and discussion.

[xv] The eigenvalue is a measure of importance of the ordination axis in a multivariate analysis

[xvi] The NOED of 32% is estimated, based on the P valueof 0.052 at 32%, however no significant effect could be found at 64%.