1 EFFECTS OF HERBICIDE DRIFT ON HIGHER PLANTS

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In this section of the report the effects of herbicide drift on plants and plant communities are assessed under field conditions. The aim was to develop no-spray buffer zones to protect sensitive sites such as SSSIs. Throughout this study and in our previous contract, a bioassay approach has been adopted, where test plants have been placed downwind of a tractor-mounted hydraulic sprayer. The effects of herbicide drift from this sprayer was then assessed by measuring damage and yield reduction of the test plants.

A series of experiments were reported under the previous contract, starting with single species bioassays using established perennial plants. This work suggested that damage was more or less confined near to the sprayer and that buffer zones in the order of 6-10 m were adequate Marrs et al. (1989). In the second experiment Marrs et al. (1991a) showed that damage in the most sensitive area (i.e. 0-4 m zone downwind of the sprayer) differed according to herbicide, species, plant age and the structure of surrounding vegetation.

These preliminary studies took no account of community dynamics, either in established plant communities or in regeneration. Both are likely to be important. At the established community level herbicide deposition on individual plants may be affected by the structure of its surrounding neighbours (Marrs et al, 1991a), and any deleterious effect on one species may promote the growth of another, which may intensify inter-specific competition. Any deleterious impact is likely to be small because of the sub-lethal doses encountered (Elliott & Wilson, 1983; Williams et al., 1987). Thus, it may take several exposures for these 'subtle' effects to develop, and hence long-term experiments are required. At the regeneration level impacts on establishing seedlings could be a critical phase in the long-term persistence of a given community, and young plants can be more susceptible than older ones (Marrs et al, 1991a).

This report attempts to address both these areas through standardized bioassay experiments where,

(1) artificially created communities in microcosms (Section 1.1), and

(2) a range of establishing seedlings (Section 1.2).

were exposed downwind of a tractor mounted hydraulic sprayer. The aims were to test whether the original buffer zone distances suggested in the earlier trials were adequate, or whether they needed to be expanded in the light of more sensitive and detailed studies of community processes.

1.1 EFFECTS OF HERBICIDE DRIFT ON PLANT COMMUNITIES: THE USE OF MICROCOSMS

There are 3 main approaches that could be used to investigate the effects of low doses of herbicides affecting the balance of species in semi-natural communities:

(1) Experimental treatment of existing communities under field conditions (Yemm & Willis, 1962; Parr & Way, 1984; Marrs, 1985; Marshall, 1988; Willis, 1988). This is the most realistic option, but there are major drawbacks. For example, it is often difficult to obtain experimental plots with the same species complements for detailed comparisons. Moreover, there are usually background fluctuations in species abundance which will complicate interpretation (Watt, 1960; Van den Berg, 1979; Marrs, Bravington & Rawes, 1988, Marrs, 1990); and there may be unknown processes (e.g. herbivore activity) which change as a result of herbicide use and may also affect species composition (Marrs, 1984, 1985). Separation of background noise from signal is especially difficult when the signal itself is small, for example where change is brought about by sub-lethal damage from herbicides.

(2) Classic competition experiments (De Wit, 1960; Jolliffe, Minjas & Runeckles, 1984). These experiments are a useful approach in studying the interactions between species under experimental conditions (Breeze, Thomas & West, 1990), but are difficult to use in multi-species investigations.

(3) Microcosms. Here artificial communities are created and experimentally manipulated (Grime et al., 1987). This approach is essentially a hybrid one between the field and laboratory. It has the advantage over field experiments that vegetation heterogeneity and background fluctuations can be minimized by standardization, but the effects are still assessed under realistic field spray application conditions. On the negative side it does not allow direct measurements of competitive interactions. The effects of the experimental treatments on the balance of species can, however, be assessed relative to unsprayed controls.

Here the use of microcosms was preferred, because the communities could be accurately standardized at the start. Mixtures of perennial dicotyledonous species (with and without the perennial grass *Lolium perenne*) were exposed to drift of glyphosate, MCPA and mecoprop. The 3 herbicides were chosen to reflect ones widely used in Britain (Williams et al., 19887). Mecoprop and MCPA are used to control broad-leaved weeds, since grasses are generally resistant (Roberts, 1982). Glyphosate, on the other hand is a broad-spectrum herbicide and both dicotyledons and grasses are affected (Roberts, 1982).

Recovery from the first dose and subsequent effects of a repeat dose were then assessed. Assessment of yield was done annually, but various non-destructive measures of performance were also made in 1989 ('88 microcosms only) and in 1990 (flowering performance, yield and seed viability).

1.1.1 **Methods**

A summary timetable of events for these experiments is shown in Table 1.1.

(a) Experimental procedure

One study started in 1988 and 3 started in 1989; these are referred to as '1988' and '1989' studies respectively. In each study there were 60 artificially created microcosms. Each microcosm consisted of a container (27 cm diameter x 12 cm deep) filled with SAI GP compost in which 8 individuals of the dicotyledon species were planted. The species used were: Digitalis purpurea, Filipendula ulmaria, Galium mollugo, Hypericum hirsutum, Lychnis flos-cuculi, Primula veris, Ranunculus acris and Stachys sylvatica in the '1988' experiment, and Campanula rotundifolia, Centaurea nigra, Digitalis purpurea, Filipendula ulmaria, Geum urbanum, Lotus corniculatus, Primula veris and Silene dioica in '1989' (nomenclature follows Clapham, Tutin & Moore, 1987). The plants were produced from seed sown in the autumn of the previous year and matched plants transferred into the microcosms when they had 4 expanded leaves. A standard arrangement was used (Figure 1.1) with the structure designed so that the taller species (when mature) could be positioned on the downwind side. Half of the microcosms in each experiment were sown with the grass Lolium perenne Majestic at a rate equivalent to 20 kg seed ha^{-1} in the April before their first spray to create an additional treatment comparison (grass versus no grass). At the time of first spraying the grass had reached a height of c. 6 cm. After the microcosms were produced they were assigned randomly to treatment position, i.e. downwind of the sprayer or unsprayed control.

The microcosms were exposed to drift annually; to date the '1988' microcosms have received 4, and the '1989' microcosms 3, applications. The same method was used in each year. Five replicate microcosms with Lolium perenne and five without grass were placed at 0 m (i.e. receiving the full application rate from the outer 50 cm of the spray jet) and at 1 m, 2 m, 4 m and 8 m downwind from the sprayer. Four sequential upwind swathes from the 0 m position were sprayed in each experiment. Application rates were: glyphosate = 2.2 kg ai ha^{-1};MCPA = 1.5 kg ai ha^{-1};mecoprop = 2.4 kg ai ha^{-1}. Five microcosms with Lolium perenne and 5 without the grass remained unsprayed to act as controls. After exposure to the spray drift the microcosms were placed in a glasshouse for 24 h and watered carefully to prevent herbicide being washed off, before transfer to an open-air plunge bed. Thereafter, plants were maintained using standard horticultural

Table 1.1 A summary timetable of events in the '1988' and '1989' microcosm experiments.

Propagation and husbandry

Study	Dicotyledonous species sown	Species transplanted to microcosm	*L. perenne* sown	Fertilized
'1988'	Sept '87	Oct-Nov '87	Apr '88	Apr '88, '89, '90, '91
'1989'	Sept '88	Oct-Nov '88	Apr '89	Apr '89, '90, '91

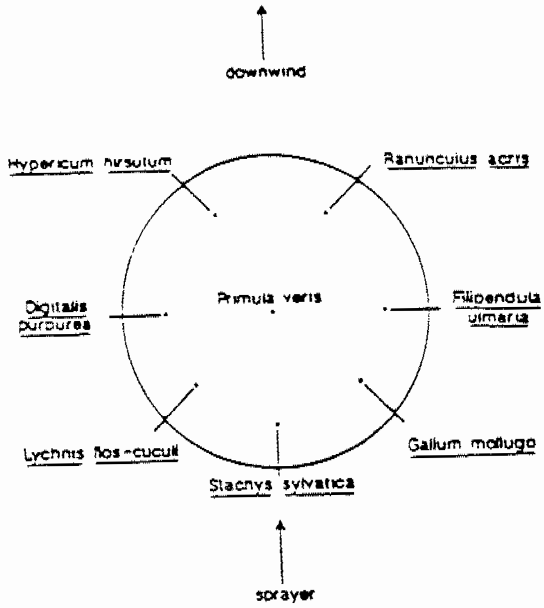
Herbicide treatment

Study		Spray Treatment (Done in June) 1st	2nd	3rd	4th
'1988'	mecoprop	'87	'88	'90	'91
'1989'	glyphosate) MCPA) Mecoprop)	'89	'91	'91	-

Assessment

Study	End of season yield (Done in Sept) 1	2	3	4	Performance: Various non-destructive performance measures	Flowering	Seed production
'1988'	'88	'89	'90	'91	'89	May-Aug '90	May-Aug '90
'1989'	'89	'90	'91	-	-	May-Aug '90	May-Aug '90

'1988'



'1989'

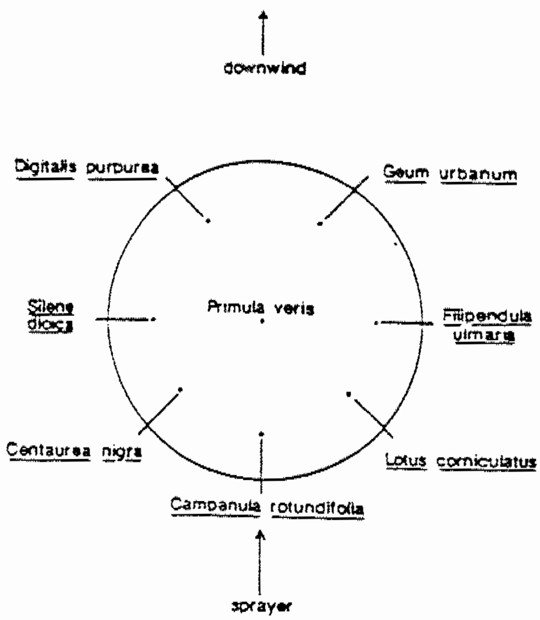


Figure 1.1

Planting arrangement in the '1988' and '1989' microcosms; the arrangement was designed so that taller species at maturity would be on the downwind side of the sprayer as shown above.

procedures. After the first year of the experiment all microcosms were given 10 g of fertilizer (Nitrophoska $N:P_2O_5:K_2O:MgO$ 12:12:17:2) each Spring.

Spraying was done in June of each year with a tractor-mounted Team sprayer to reproduce field conditions. The sprayer had a 6 m boom, which was fitted with 12 Lurmark Red 03-F80 (BCPC code F110/1.20/3) fan nozzles, and held 80 cm above the vegetation surface. A tank pressure of 2 bar and a tractor speed of 6 km h^{-1}, which distributed c. 200 litres solution ha^{-1}, was used throughout. Wind speed and direction were monitored with a R500 recording anemometer. In both years the wind speed during spraying was between 2-3 m s^{-1} at a height of 2 m.

(b) Recording

Recording was done in 4 ways:

(1) In September of each year all individual dicotyledonous plants plus the entire herbage of <u>Lolium perenne</u> were harvested at ground level, dried at 80°C and weighed.

(2) In June 1989 the recovery of plants from the first application of mecoprop in the '1988' study was assessed using a series of non-destructive measures of performance. The measures used for each species are outlined in Table 1.2.

(3) During the summer of 1990 the numbers of flowers produced by each dicotyledonous species were counted weekly.

(4) During the summer of 1990 seed was collected from each dicotyledonous flower head weekly as it was produced. The seeds were extracted through a combination of gentle crushing and sieving and then weighed. The viability of the seeds was then assessed using standardized germination tests.

(c) Data analysis

The following assumptions were made; (1) that the data for each species in the unsprayed 'control' microcosms was the norm, and (2) that any deviation from the data for the unsprayed microcosms was an effect brought about directly or indirectly by exposure to herbicide drift in the zone downwind of the sprayer.

As the harvesting was spread over a 3-4 week period each set of 30 microcosms (ie grass sown or unsown sets) were harvested separately in a sequential sequence. To prevent any errors associated with time of sampling interfering with the interpretation, data for each experiment are analyzed individually. The yield data for each year were treated as

Table 1.2 The range of non-destructive measures used to assess the effects of plant recovery in microcosms after spraying with mecoprop the previous year.

Species	Counts	Measurements
Digitalis purpurea	leaves	longest leaf length mean leaf length flowering stem height
Filipendula ulmaria	leaves	mean leaf length
Galium mollugo	stems	mean stem length
Hypericum hirsutum	stems	mean stem length
Lychnis flos-cuculi	stems flowers	mean flower stem height
Primula veris	leaves flowering stems flowers	leaf length mean flower stem height
Ranunculus acris	leaves stems flowers	mean flower stem height
Stachys sylvatica	stems	mean stem height

separate datasets, and for all datasets (apart from seed viability) 3 analyses were done:

(1) A contrast between the means at 8 m and the untreated control. This was to assess whether there was any significant effect at the greatest test distance.

(2) A contrast between means (expressed as a of untreated values) at 0 m and the pooled mean of 1, 2, 4 and 8 m. This was to test whether plants given the full dose was reduced in performance relative to those in the downwind zone.

(3) Regressions between the performance measure (expressed as a percentage of untreated values) and distance downwind; both linear and quadratic equations were fitted (n=25). Only significant equations are presented here, and only the one which accounted for the greatest variation is discussed.

Only significant results from these analyses are presented. To aid interpretation and clarity they statistical information is presented in Appendix I, with summary Tables and Figures in the main report.

For seed viability, because only a limited range of data was available, Kendall rank correlation coefficients were calculated between viability and distance downwind.

1.1.2 Results

Effects on end of season yield

<u>Seasonal variations in yield</u>
The combined yield of all species growing in the unsprayed microcosms varied between years (Table 1.3). In all cases the greatest yield occurred in the first year where the measured response was a function of both the initial size of the transplant and the growth during the first summer when soil resources in the microcosms were at a maximum. After the first year yields were lower reflecting regrowth from cut plants.

In the '1988' study there was initially a greater yield where <u>L. perenne</u> was sown, but the difference between the two grass treatments disappeared by the third year. In the '1989' study there was no difference between microcosms sown with <u>L. perenne</u> and those without, but there were differences between the unsprayed microcosms allocated to the different herbicide experiments (Table 1.3). This result is mainly because of the different sampling times for each study.

Analysis 1: Contrast between 8 m and untreated

Relatively few species showed a significant difference in either end-of-season yield or flowering performance in the 8 m

Table 1.3 Total yield (g) of vegetation per microcosm in each year of the '1988' and '1989' studies; mean values ± standard errors (n=5) are presented.

Study	Herbicide	Year	-*L. perenne*	+*L. perenne*
'1988'	mecoprop	'88	48.2±1.1	65.1±3.6
		'89	11.8±0.9	16.5±0.7
		'90	9.4±0.4	8.3±0.3
		'91	18.7±2.7	16.9±1.9
'1989'	glyphosate	'89	86.4±2.3	81.2±0.9
		'90	34.9±2.2	35.3±1.6
		'91	52.6±10.8	52.1±5.7
	MCPA	'89	77.9±2.1	64.2±1.4
		'90	42.7±2.0	30.0±1.4
		'91	15.5±7.7	13.4±3.8
	mecoprop	'89	80.5±1.0	64.5±2.0
		'90	29.3±1.1	39.2±2.6
		'91	25.4±6.4	44.4±1.4

microcosms compared to untreated controls (Table 1.4). The number of potential significant effects was 132 and 201 for the '1988' and '1989' studies respectively, yet only 7 were found in each study. Moreover, within these significant results there was both stimulation and suppression at 8 m. Presence of grass appeared to have no effect.

In seed production out of 64 potential contrasts only one was significant, with suppression of L. corniculatus in the '1989' no grass microcosms sprayed with Mecoprop. This species was unaffected in the microcosm sowed with grass and where other herbicides were applied.

Table 1.4 A summary of the significant contrasts between species grown in the microcosms at 8 m with those untreated; + = grass and - = no grass treatments (Full data in Appendix I-I).

Study	Herbicide	Suppression 8 < Untreated	Stimulation 8m > untreated
Yield and Performance			
'88	Mecoprop	D. purpurea (-) H. hirsutum (-) R. acris (+) S. sylvatica (-)	F. ulmaria (-) L. perenne (+) P. veris (-)
'89	Glyphosate		C. rotundifolia (-) S. dioica (+)
	MCPA	G. urbanum (-) P. veris (+)	
	Mecoprop	L. corniculatus (-)	C. nigra (+) S. dioica (-)
Seed yield '90			
'89	Mecoprop	L. corniculatus (-)	

Analysis 2: Contrast between 0 m and the pooled mean of 1, 2, 4 8 m

As in the previous analysis relatively few species showed a significant difference in either end-of-season yield or flowering performance in the 0 m microcosms compared to those downwind (Table 1.5). The number of potential significant effects was 148 and 201 for the '1988' and '1989' studies respectively, yet only 33 were found in each study. Surprisingly, some species showed an increased yield or performance directly underneath the sprayer.

Table 1.5 A summary of the significant contrasts between species grown in the microcosms at 0 m with those in the downwind zone; + = grass and - = no grass treatment (Full data in Appendices I-II and I-III).

Study	Herbicide	Stimulation 0 m > pooled mean 1,2,4 8 m	Suppression 0 m < pooled mean 1,2,4 8 m
Yield and Performance			
'88	Mecoprop	F. ulmaria (-) H. hirsutum (-) L.perenne (+) P.veris (-)	F. ulmaria (+) G. mollugo (±) L. flos-cuculi (±) P. veris (+) S. sylvatica (-)
'89	Glyphosate	C. rotundifolia (+)	C. nigra (±) D. purpurea (±) F. ulmaria (-) G. urbanum (+) L. corniculatus (±) S. dioica (+)
	MCPA	C. rotundifolia (+) L. perenne (+)	L. corniculatus (±) S. dioica (±) P. veris (-)
	Mecoprop	C. rotundifolia (-) L. corniculatus (+) L. perenne (+)	S. dioica (-)
Seed yield '90			
'89	MCPA	G. urbanum (-)	

The presence of grass appeared to affect some species. In 1991 F. ulmaria was stimulated in the no grass treatment and suppressed where L. perenne was sown in the '1988' microcosms. P. veris showed different responses in the grass treatments but in different years in the '1988' microcosms and L. corniculatus showed different responses with grass treatment when exposed to different herbicides.

The seed production of only one species was affected, and here seed yield of G. urbanum was increased in the no grass microcosm exposed but in no other treatment.

Analysis 3: Regression analysis between measured responses of plants in microcosms and distance downwind of the sprayer

Many species showed significant relationships between yield or performance and distance downwind of the sprayer. They were broadly classified into those species which were stimulated near the sprayer with a decline through the downwind zone and those that were reduced near the sprayer and gradually increased with distance downwind. The 2 types of response were described by both linear and quadratic equations and typical examples are shown in Figure 1.2.

The majority of species in the '1988' microcosms were reduced near the sprayer, but 2 species L. perenne and S. sylvatica were stimulated under the sprayer (Table 1.6). In the '1989' microcosms different species were stimulated and suppressed by the different herbicides (Table 1.6), but some common themes emerged:

(1) C. nigra and S. dioica were consistently suppressed by all herbicides and C. rotundifolia was consistently stimulated near the sprayer.

(2) L. perenne was stimulated near the sprayer where MCPA and Mecoprop were sprayed but not Glyphosate.

3) The effect of grass treatment had differential effects on some species and not others, and there was no apparent consistent pattern.

It should be noted that many of the regressions show that within the 0 - 8 m downwind zone there is stimulation of growth to greater than the levels found in untreated microcosms.

Analysis 4: Relationship between seed viability in 1990 with exposure downwind of the sprayer

Sufficient data were available for analysis for L. flos-cuculi and R. acris in the '1988' microcosms and for C. nigra, D. purpurea, G. urbanum, L. corniculatus, P. veris and S. dioica in the '1989' microcosms. Only 2 significant correlations ($P < 0.05$) were found, both for G. urbanum downwind of MCPA applications.

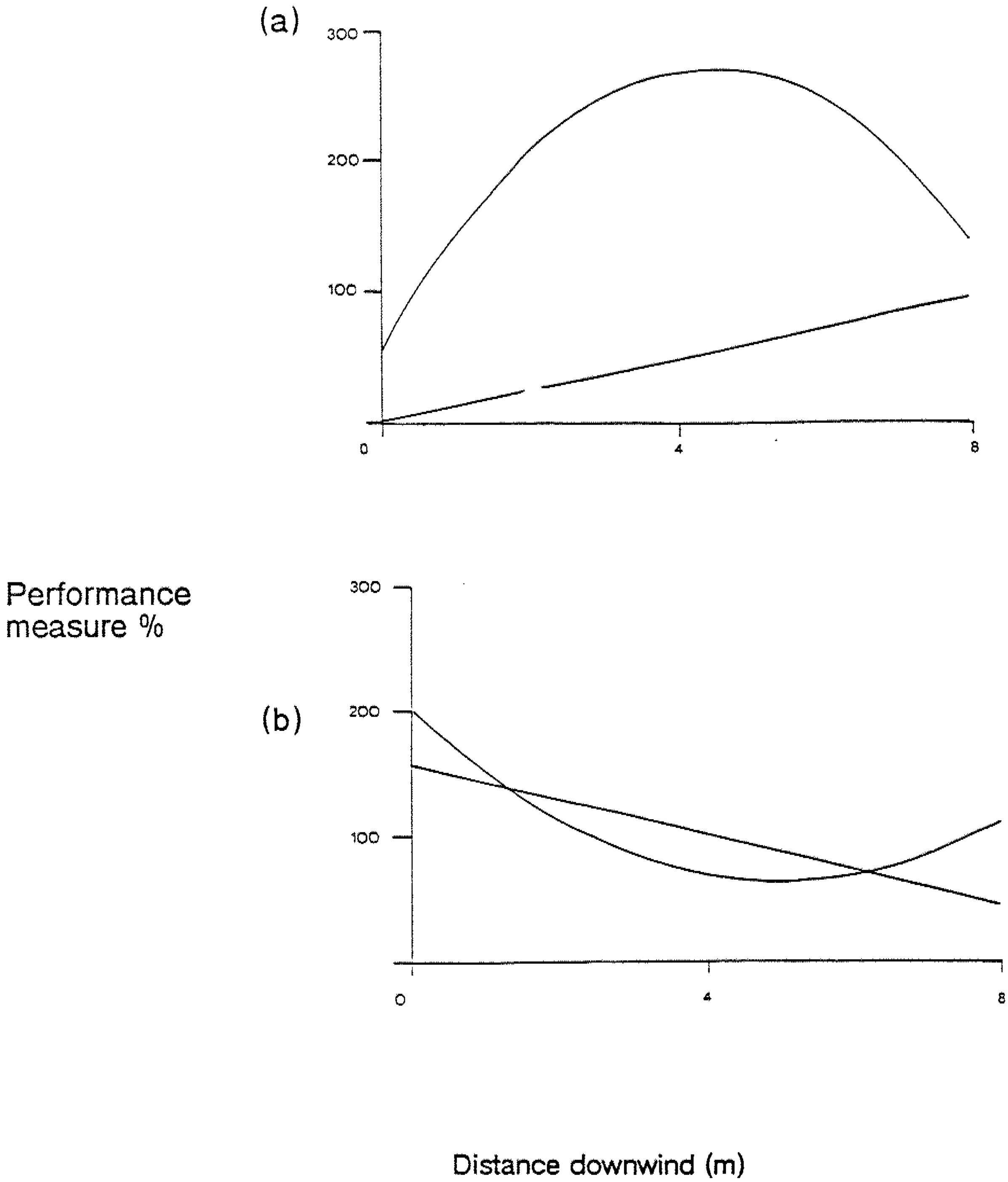


Figure 1.2

Typical relationships found between species performance in microcosms and distance downwind;

(a) reduction under the sprayer, and
(b) stimulation under the sprayer.

In the grass treatment positive correlation (r_k = 0.59, $P < 0.02$) was found, but in the no grass treatment the correlation was negative (r_k = -0.49, $P < 0.04$).

Table 1.6 Summary of the regression analyses between measured response and distance downwind from the sprayer; + = grass and - = no grass treatments (Full data in Appendices I-IV and I-V).

Study	Measure	Herbicide	Response in 0-8 m zone Positive	Negative
'88	Yield	Meco	F. ulmaria (+) G. mollugo (±) L. flos-cuculi (±) P. veris (+) R. acris (+)	L. perenne (+) S. sylvatica (+)
	Perf '89		D. purpurea (+) G. mollugo (±) L. flos-cuculi (±) P. veris (±) R. acris (+)	H. hirsutum (-) S. sylvatica (+)
'89	Yield	Glyp	C. nigra (±) D. purpurea (+) G. urbanum (+) L. corniculatus (-) S. dioica (±)	C. rotundifolia (+)
		MCPA	C. nigra (+) F. ulmaria (-) G. urbanum (+) L. corniculatus (±) P. veris (+) S. dioica (+)	C. rotundifolia (±) L. perenne (+) P. veris (-)
		Meco	C. nigra (±) D. purpurea (+) F. ulmaria (-) S. dioica (±)	C. rotundifolia (-) F. ulmaria (+) G. urbanum (-) L. perenne (+)
	Perf '90	Glyp	C. nigra (+)	
		MCPA		L. corniculatus (+)
		Meco	S. dioica (-)	
Seed Yield '90		Glyp	C. nigra (+)	
		MCPA	G. urbanum (-)	

1.1.3 Discussion

The aims of this part of the project were to investigate the cumulative effects of drift of 3 herbicides on mixtures of species typical of semi-natural communities. An experimental approach with standardized microcosms was used, and although we accept that this is an artificial approach, microcosms appear to offer some promise as a useful investigative tool for the study of 'subtle' inter-actions between species. Part of the artificiality of the approach centres on the cutting treatment given here purely for the purposes of determining plant yield rather than as a experimental treatment. Cutting, therefore, may have interacted with herbicide effects by helping to promote the species which can withstand cutting. The inclusion of this cutting treatment means that the microcosms were treated in a similar way to roadside vegetation bounding sprayed agricultural land, when cut at a time suitable for the maintenance of floristically-rich vegetation (Wells & Bayfield, 1990). The cutting treatment was, however, more severe than that given to roadside vegetation. In spite of this artificiality, the results presented here show clear effects on the balance of species between microcosms exposed to herbicide drift compared to those unsprayed in the second year of exposure.

The results that few species were affected at 8 m downwind from the sprayer compared to untreated 'controls', even when grown in mixture and when cumulative exposures are given, support the original studies of Marrs et al. (1989) that buffer zones of this order of magnitude are reasonable. Moreover, effects on flowering and seed production and seed viability also appear to be unaffected. However, these ecological significance for the stimulation or suppression of the few species that were affected at this distance requires further detailed study. Stimulated growth downwind of the sprayer has been noted in other studies (Marrs et al. 1991a,b), and indeed was extensively found in this study between 0-8 m. What is perhaps more surprising was that so few species were adversely affected at the 0 m position, where they would have received a full dose of herbicide. Clearly, many established perennial plants are able to withstand herbicide damage and can recover rapidly.

Effects on species interactions

Herbicide spray drift affected the species in different ways. It is, however, important to realize that the data reported here are not clear cut. We have, where possible, attempted to draw together data to highlight the important trends, but in many of the analyses that we did there were no significant differences detected. It is difficult, therefore, to extrapolate from these experiments to the wider countryside. We are a very long way from be able to predict the outcome of spray events on multi-species mixtures. Nevertheless, the following four generalizations were found:

First, different species showed different responses (reduction, stimulation or no effect) when exposed to a full dose of herbicide directly under the sprayer. This result supports that of Marrs et al. (1991a) and confirms that some species may be able to survive at the expense of others in land adjacent to heavily sprayed crops. Species which are likely to survive include C. rotundifolia, H. hirsutum, L. perenne, S. sylvatica, and possibly R.acris.

Second, the regressions between species yield and distance downwind from the sprayer separate species into two groups; (a) those that are promoted near the sprayer, either because of reduced herbicide interception or reduced competition - C. rotundifolia, H. hirsutum, L. perenne, S. sylvatica, and (b) those that are reduced near the sprayer probably because of a direct effect of herbicide - C. nigra, D. purpurea, F. ulmaria, G. mollugo, G.urbanum, L. flos-cuculi, L. corniculatus, P.veris, R.acris, and S.dioica.

Third, there are some unknown differences in the response of dicotyledons in microcosms sown with grass and those left unsown. This effect may be caused by differential interception of the herbicide (Marrs et al., 1991a), but increased competition from grass may also be important.

Finally, there were differences between herbicides, with some species showing a response to one herbicide but not another. Perhaps the most important example was the different responses of Lolium perenne, with maximum growth under the sprayer and a reduction with distance downwind for MCPA and mecoprop, but no significant relationship with glyphosate. This may be because the first 2 herbicides affect mainly broad-leaved species and the latter is a non-specific herbicide which also affects grasses. Where mecoprop and MCPA were used Lolium perenne was unaffected and managed to exploit the reduced competition from other species, yet with glyphosate Lolium perenne was also susceptible and did not increase near the sprayer. Continuous exposure to drift of MCPA or mecoprop, may, therefore, lead to a dominance of this perennial grass in the longer term. Increased dominance of grasses has been reported in several experiments where herbicides/growth retardants have been applied at recommended rates to semi-natural grasslands (Marshall, 1988; Parr & Way, 1984).

An important result from these studies is that exposing plants in mixtures to spray drift gives different results depending on the species present, whether a grass was present or not, and the herbicide used. This means that it is extremely difficult to extrapolate results derived from either laboratory toxicological tests or single species trials to predict the outcome in the field. This statement is borne out by comparing laboratory responses of G. mollugo and L. perenne to topical applications of mecoprop in the laboratory with our '1988' microcosm data. The laboratory data were similar for both species (ED_{10} = 170 - 200 μg per plant and ED_{50} = > 900 μg per plant - Breeze et al., 1989), yet G. mollugo was reduced and L. perenne increased near the sprayer in the microcosm study.

1.2 EFFECTS OF HERBICIDE SPRAY DRIFT ON SEEDLING ESTABLISHMENT

In our previous work on the effects of spray drift on higher plants we have concentrated on mature plants (Marrs et al., 1989, 1991a,b; Section 1.1), although we have always pointed out that effects could also occur at the germination and establishment stages. For example, we found that younger plants of some species were more susceptible to herbicide drift under some situations than older ones. Here we consider the effects of glyphosate drift on establishment seedlings. Two studies were done; first a series of experiments using L. flos-cuculi to determine both buffer zone distance and the effects of different surrounding grass structure on seedling mortality, and second a comparative study to determine the buffer zone distance for a range of 15 species.

1.2.1 **Methods**

(a) L. flos-cuculi studies

Lychnis flos-cuculi seeds were sown thinly in 48 seed trays (220 mm x 175 mm x 55 mm) with SAI GP compost. Approximately two weeks after sowing the numbers of establishing seedlings in each tray ranged from 140 - 250 seedlings per tray. Trays were then placed at varying distances downwind of the sprayer (with appropriate untreated controls) in three separate experiments:

Experiment 1: This was done in September 1990 with 4 replicate trays placed at 0 m (i.e. receiving the full application rate from the outer 50 cm of the spray jet) and at 0.5 m, 1 m, 1.5 m, 2 m, 3 m, 4 m, 5 m, 6 m, 8 m and 10 m downwind from the sprayer.

Experiment 2: This was done in September 1991 with 5 replicate trays at 0 m, 5 m, 10 m, 12 m, 14 m, 16 m, 18 m, 20 m, 25 m, 30 m, 35 m and 40 downwind from the sprayer.

Experiment 3: This was done in September 1991 with 4 replicate trays at 0 m, 5 m, 10 m, 15 m and 20 m downwind of the sprayer in each of two contrasting grassland structure - short (c. 5 cm) and tall (c. 80 cm).

(b) Multi-species comparison

Seedlings of 15 species (Betonica officinalis, Digitalis purpurea, Galium verum, Geum urbanum, Hypericum perforatum, Lotus corniculatus, Lycopus europaeus, Pimpinella saxifraga, Plantago media, Primula elatior, P. vulgaris, Ranunculus acris, Silene alba, Teucrium scorodonia and Verbascum thapsus) were established in small plant containers. Between 20 - 120 individual seedlings, depending on species, were placed at 0 m, 5 m, 10 m, 12 m, 14 m, 16 m, 18 m, 20 m, 25 m, 30 m, 35 m and 40 m downwind of the sprayer.

In all experiments glyphosate was applied at 2.2 kg ai ha^{-1} in four sequential upwind swathes from the 0 m position. After exposure to the spray drift the trays were maintained in a glasshouse and watered carefully to prevent herbicide being washed off. Spraying was done using the same methods outlined in

Section 1.1 in a wind speed of between 2-3 m s^{-1} at a height of 2 m.

Three to four weeks after exposure to the herbicide drift each seedling was examined and classified visually as either healthy or dead (in experiment 1 with L. flos-cuculi seedlings a severely damaged category was also included). Mortality was then calculated as a percentage of control plants.

1.2.2 Results

(a) L. flos-cuculi studies

Experiment 1: Large numbers of seedlings were either killed or damaged over the entire 10 m distance downwind of the sprayer (Figure 1.3a). All seedlings were either killed under the sprayer and at 0.5 m downwind. Thereafter there was a steady decline in numbers killed until 30 % mortality was found at 10 m. In contrast the numbers of seedlings damaged increased with increasing distance downwind (Figure 1.3b). Very few individuals were healthy even at 10 m compared to > 80 % in the untreated controls (Figure 1.3c).

Experiment 2: In this experiment mortality declined rapidly to 10 % over the first 10 m (Figure 1.4), thereafter it remained at 10 % up to 18 m and after 20 m declined to the levels found in the untreated controls (1%).

Experiment 3: In this experiment similar results were obtained to experiment 2 with a mortality of 10 % found at 10 m (Table 1.7). The only point at which the grassland structure appeared to have any effect on mortality was at 5 m downwind of the sprayer, where mortality was less in the tall grass (Table 1.7).

Table 1.7 Mortality of L. flos-cuculi seedlings after exposure downwind from a glyphosate application in both short and tall grassland; mean values ± standard errors are presented.

Distance downwind of the sprayer (m)	Short grassland	Tall grassland
0	91±1	98±1
5	35±5	15±3
10	9±2	10±2
15	3±0.5	1±0.3
20	2±0.6	1±0.2

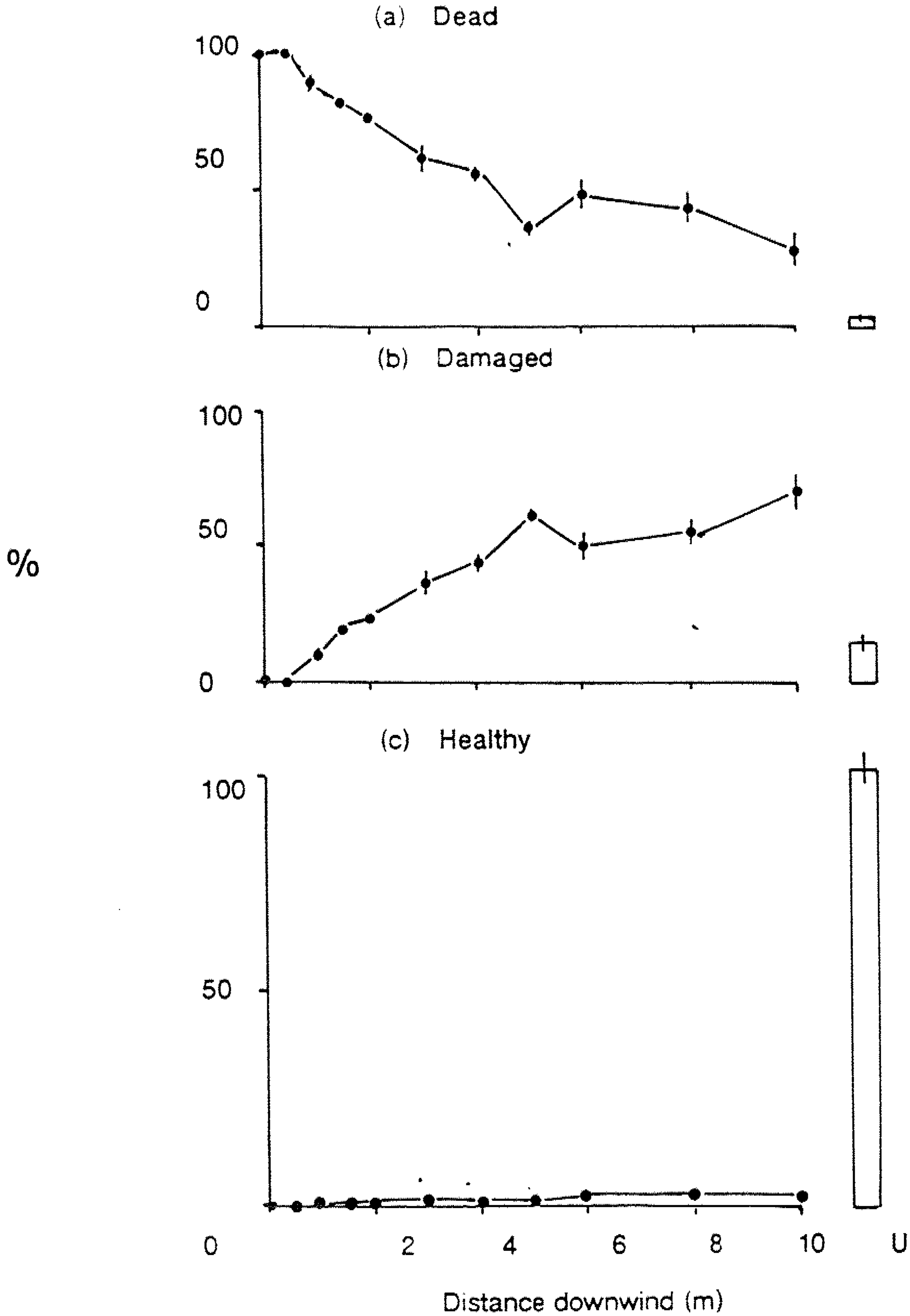


Figure 1.3.

Effects of glyphosate on L. flos-cuculi seedlings downwind of a sprayer in 1990: mean values ± standard errors (n=4) are presented, U = unsprayed.

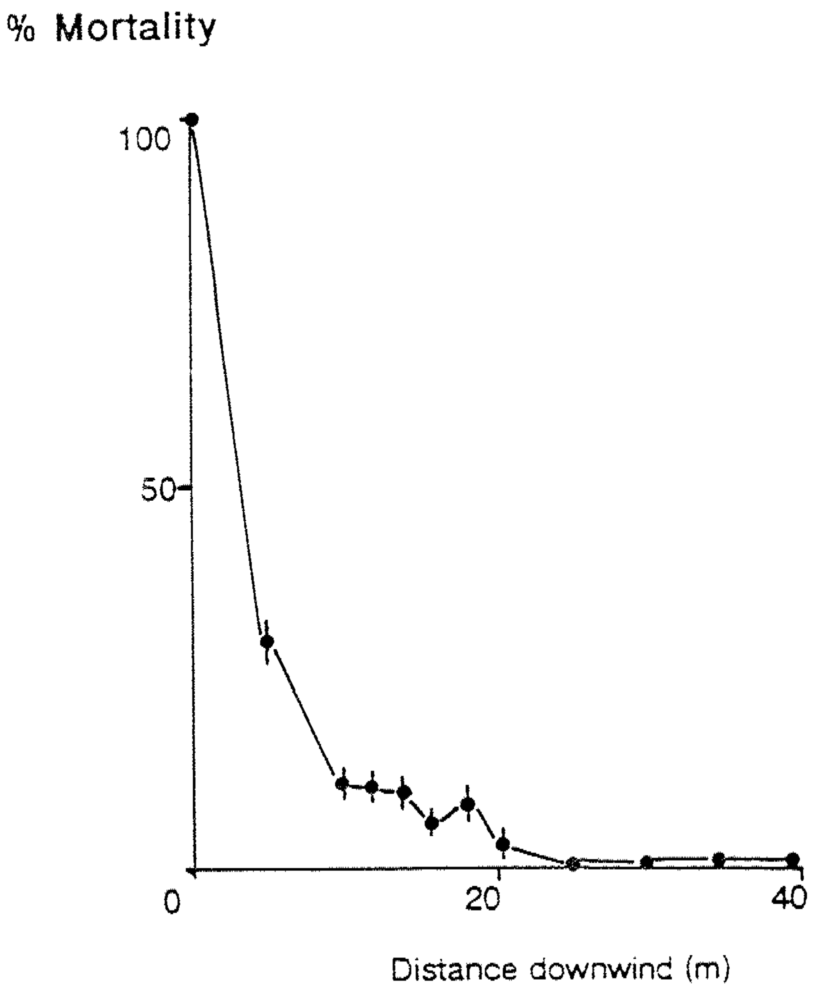


Figure 1.4

Effects of glyphosate on L. flos-cuculi seedlings downwind of a sprayer in 1991; mean values ± standard errors (n=5) are presented.

b) Multi-species comparison
In this experiment logistic equations were fitted to the data (Table 1.8) with extremely good fits for most of the species. There was a wide range of response ranging from 5 species with an LD_{50} mortality between 0 - 5 m to the other extreme with H. perforatum at 15 - 20 m (Table 1.8). Data from two examples (P. media and H. perforatum) are shown on Figure 1.5, and even the most sensitive species shows little damage at distances > 20 m downwind of the sprayer.

1.2.3 Discussion

The most obvious difference in this study was that seedlings were apparently more sensitive to herbicide drift downwind of glyphosate spray than established perennial plants. There was a wide variation in susceptibility in L. flos-cuculi within 3 experiments. In the first the seedlings were dead or severely damaged up to the maximum distance tested (10 m), indicating that no effect buffer zones needed to be greater than 10 m. In the second experiment effects were not as severe at 10 m, but were still present between 10 - 18 m. In the third experiment little effect was found after 10 m and there was very little significant interaction with grassland structure. The multi-species study also highlighted a wide range in sensitivity with the most sensitive H. perforatum having an LD_{50} of between 15 - 20. There was, however, little effect after 20 m.

The conclusions from these studies were, therefore, that buffer zones may need to greater in situations, where seedling establishment is an important feature of community regeneration. A first approximation of the size of buffer zones in situations like this is 20 m.

1.3 GENERAL CONCLUSIONS

The results from the microcosm studies confirm the recommendations of Marrs et al. (1989) that buffer zones of between 6-10 m were adequate to protect established vegetation downwind of a tractor-mounted sprayer. The earlier conclusions were based on a single year's study of individual plants, and the authors warned that the data were preliminary because no account was taken of species interactions or repeat applications. The data presented here extend the earlier work to include interactions between species and repeat applications, and the results do not suggest that wider zones are needed. However, the impact of drift within the buffer zone may be greater than suggested previously.

However, where seedling establishment is an important feature of the regeneration of the downwind community buffer zones need to be greater and should be at least 20 m.

These conclusions have been derived from bioassay experiments and need to be tested in detailed long-term field experiments.

Table 1.8 Mortality of seedlings in the comparative species experiment: data for logistic equations relating % mortality after exposure downwind from a glyphosate application and LD_{50} ranges are presented. The logistic equation fitted was; MORTALITY (%) =A+C/(1+EXP(-B(DISTANCE(m)-M)))

LD_{50} range (m)	Species	A	C	B	M	Variation accounted by regression
0-5	*Geum urbanum*	0	70.0	4.10	-2.00	99
	Pimpinella saxifraga	-3.74	3656	-15.65	-0.23	62
	Plantago media	0.40	99.6	4.02	-1.97	99
	P. vulgaris	8.70	78.3	4.63	-1.99	99
	Silene alba	-0.41	101.9	8.24	-0.54	98
7-10	*Digitalis purpurea*	1.74	112.8	5.82	-0.32	99
	Lycopus europaeus	2.01	103.7	5.75	-0.49	99
	Primula elatior	-1.88	123.7	6.93	-0.31	94
10-15	*Betonica officinalis*	2.79	115.4	8.75	-0.23	98
	Galium verum	4.01	89.7	10.44	-1.08	99
	Lotus corniculatus	-0.28	103.6	11.10	-0.74	98
	Ranunculus acris	1.79	98.2	11.01	-1.84	99
	Teucrium scorodonia	2.42	97.8	12.59	-1.88	99
	Verbascum thapsus	15.48	74.0	12.13	-0.45	94
15-20	*Hypericum perforatum*	2.16	98.3	16.6	-0.74	99

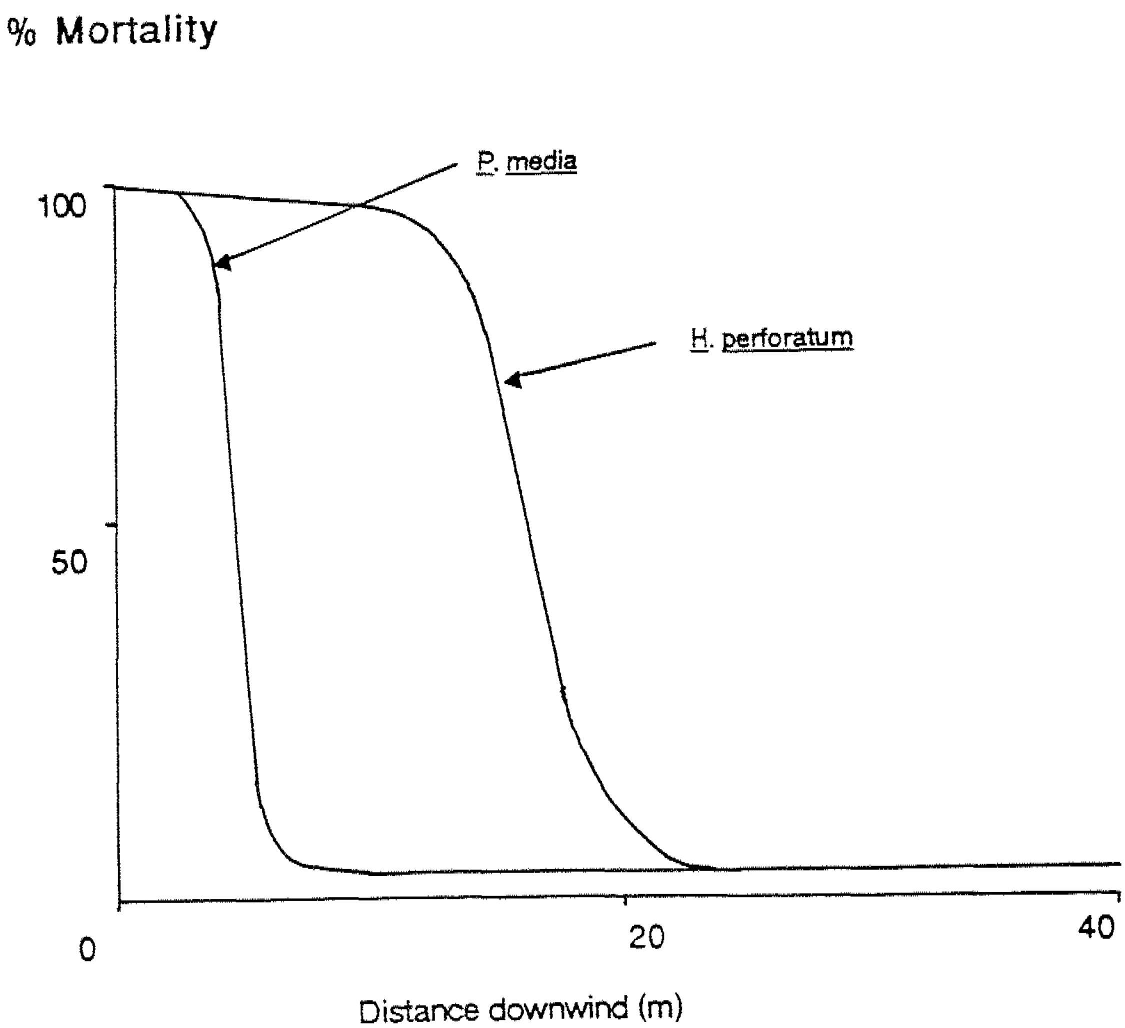


Figure 1.5.

Effects of glyphosate on two species with contrasting responses in the multi-species trial, where seedlings were exposed downwind of a sprayer; fitted logistic equations are presented.

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