



- CHAPTER 10 -

ENRICHMENT OF AGRICULTURALLY IMPROVED GRASSLAND USING SEED

It has been shown that a good deal may be done in the fight against weeds by the application of manure and artificial fertilisers. All kinds of nitrogenous manures stimulate the growth of grasses; under such favourable conditions these develop so vigorously that Buttercups, Hawkweeds, and many other weeds will be choked.

Bastin S. Leonard (1915) The warfare against weeds. *Journal of the Bath & West & Southern Counties Society* **10**: 59-70.

SUMMARY

1. The sowing of wildflower seed has been the most widespread practice in grassland restoration. For grasslands subjected to intensive agricultural improvement, it is often recommended to create a completely new seedbed through turf stripping in order to remove both the eutrophied soil layer, and the *weed* dominated soil seed bank, and then resow with wildflower seed mixtures. However, oversowing with wildflower seed is also becoming a standard procedure for the enrichment of improved swards. Therefore it was necessary to compare the efficacy of seed inoculation with the main transplant inoculation treatments.
2. This eight species seed inoculation experiment was accommodated within the transplant field set-up, and carried out using similar inoculation treatments. Preparation started in July 1996. Six rows were delineated, comprised of 16 sowing positions so that each row received all the species, but in a randomised order. As for the transplant experiments, 30-cm, 15-cm and 0-cm *competition-free* gaps were created using herbicide. Inoculum sets were prepared by counting out 12 doses of 50 seed, of each species, for each subplot, and then temporarily storing them in paper sachets. In early November 1996, the seed was sown into the prepared gaps. In addition to the field experiment, pot trials were set up in order to investigate the viability of the seed under conditions of zero competition. The primary census procedure was the count of seedlings found at each gap position. From 1998 onwards, the seedlings had mostly developed into adult plants, and performance parameters were also recorded.
3. Large seedling losses took place within the early establishment phase, but those individuals that managed to entrench generally survived up to the final census a year later. The species assembly in terms of ranking also changed significantly over the first year, though remained similar for the subsequent two years. There were no significant differences between plots for the germination and establishment counts over the whole research period. The subplot counts also did not follow the gradient of improvement that is apparent in the other experiments. Overall, seed germination and subsequent seedling establishment did not correlate with any of the established productivity variables. *Primula veris* germination and conversion to adult plants was far higher in all plots than the other species. In contrast, the few *Scabiosa columbaria* seedlings that emerged were extinct by summer 1998.
4. Except *Leontodon hispidus*, the performance parameters showed little further size increments between 1998-9, suggesting that by summer 1998 maximum adult size had largely been attained. Height performances for *Malva moschata*, *Leucanthemum vulgare* and *Primula* were clearly higher in Plot 1 and parallels the transplant results. Flower production across all plots for both yearly censuses was very low. *Filipendula vulgaris* stands out as the only species not to produce a single inflorescence.
5. Unlike for the transplants, herbicide spray gaps did seem to have a positive impact on seed enrichment. For all censuses, in terms of *total* seed conversion/survival, 30-cm diameter gaps encouraged highest germination and seedling survival. However, *Primula* seed inoculation seemed to be highly effectual with or without herbicide gaps. Management treatment regime did not seem to produce demonstrable differentials in seed germination and seedling survival.
6. Using seed appears to be far cheaper than using either pot or plug transplants for the same relative unit of enrichment. However, seed-derived seedlings have the negative potential of remaining indefinitely as juveniles or vegetative adults under productive conditions. A key finding is that whilst transplants were unassisted by the use of artificial gaps, enrichment using seed seems to be comparatively heavily dependent. In fact, gap creation in productive swards seems to be an essential treatment for successful seed inoculation.

Keywords: wildflower seed, oversowing, restoration, agriculturally improved, competition-free gap, inoculum, inoculation, seedling, germination, establishment, conversion, performance, productivity.

INTRODUCTION

In the context of grassland creation and restoration, the sowing of wildflower seed has so far been the most widespread practice (Wathern & Gilbert 1978; Wells *et al* 1981; Wells *et al* 1989; Wells 1990; Ireland 1992; Frame *et al* 1994; Hopkins *et al* 1997; Pywell *et al* 1997; Jones & Hayes 1999; Smith *et al* 2000). The results of many studies show that perennial forb recruitment from seed into natural populations is infrequent (Cavers & Harper, 1967; Silvertown & Dickie 1980; Rusch & Fernández-Palacios 1995; Jakobsson & Erikson 2000), compounded by the dispersal barriers of contemporary agricultural landscapes (Tilman 1997; Strykstra *et al* 1998). However, the limitations of natural seed dispersal, colonisation, and recruitment can be circumvented through the direct sowing of naturally harvested or commercially grown propagules (Turnbull *et al* 2000). Broadcasting species-rich mixtures onto newly prepared seedbeds is the easiest method to produce “instant grasslands” (Gilbert & Anderson 1998), containing all the requisite species, though usually without the α - and β -diversity of unimproved old grasslands (Kendle 1993; Gordon & Duncan 1994). Certainly for grasslands that have been subjected to intensive species-depauperisation through agricultural or amenity *improvement*, i.e. ploughing, re-sowing, and artificial fertiliser applications; it is usually most expeditious to create a completely new seedbed through turf stripping in order to remove both the eutrophied soil layer, and the *weed* dominated soil seed bank. After this field preparation, wildflower seed mixtures can be sown onto a surface re-optimised for the germination and establishment of a species-rich sward (Pywell *et al* 1997; Gilbert & Anderson 1998).

Attempting to enrich depauperated swards with seed, rather than starting with *ground-zero* conditions, is a different proposition. The massive over-application of artificial fertilisers in the post WWII period has meant that even with fertiliser cessation, grassland productivity takes a long time to revert to conditions receptive to wildflowers (Marrs 1993; Hopkins & Pinto 1998). For this reason starting from scratch i.e. turf/soil removal and resowing, has many attractions for the restorationist, especially for pure floristic creation. However turf and soil removal also eliminates the majority of the valuable extant invertebrate and soil ecology, and, on an economic level, continuity of farming income (Kirby 1992; Cullen & Wheeler 1993; Wells *et al* 1989; Smith *et al* 2000).

Oversowing unimproved grasslands with desirable agricultural species has a relatively long history in grassland management (Davies & Davies 1997), and has lately become a standard procedure for the enrichment of improved swards with desirable wildflower species (Gilbert & Anderson 1998; Hopkins *et al* 1999). The application of oversowing is certainly far less expensive in practice than the implantation of container-grown transplants, however the comparative efficacy of both methods is at question (Davies *et al* 1999; Coulson *et al* 2001; Westbury 2001). Seed inoculation into extant swards has received more research attention than transplant use, largely due to the technology cross-over from conventional agriculture (Davies 1997; Hopkins *et al* 1999), and Well's 1983 concern that: "It is generally thought that throwing herb seed onto old grassland is wasteful, but there has been little research on this aspect of establishment", still remains largely true. However, there is adequate research information for Gilbert and Anderson (1998) to consider oversowing to be worthwhile only if natural establishment gaps are abundant (low soil nutrient and sparse sward), or when a consistent provision of gaps can be maintained through management (Crofts & Grayson 1999). Evidently, these receptivity factors are least prevalent in agriculturally improved grasslands (Boyce 1994; Davies *et al* 1999).

A growing body of research suggests that at least one species, *Rhinanthus minor*, can be successfully introduced into productive swards by oversowing, though this is largely due to its hemi-parasitic physiology which allows it to facilitate its own establishment and spread (Davies *et al* 1997; Smith *et al* 2000, Westbury & Dunnett 2000; Westbury 2001). For most other forb species, research suggests that when sown into closed swards, germination is low, seedlings suffer high mortality, and there is an unacceptably low rate of conversion to adulthood. Within these constraints, it is easy to anticipate that species with complex germination requirements are best established as transplants rather than seed, but it is also possible that some species may establish as well - or even better - from seed than transplanted stock (Dixie & Bisgrove 1996; Davies *et al* 1999; Westbury 2001).

Techniques have been established in order to give seed a greater chance of germination and seedling establishment. Most of these techniques can be understood on a superficial reading of the concepts 'safe site' and 'regeneration niche' theories set down by Harper *et al* (1961) and Grubb, (1977) respectively. In practical terms, this means creating gaps in the sward into

which seed is sown, which may promote germination, and effectively foster seedling survival and establishment. Typical grassland management such as mowing, and especially grazing, are means of creating regeneration gaps for seed inoculation (Crofts & Grayson 1999; Smith *et al* 2000, Coulson *et al* 2001). Artificial methods, which have had widespread currency, include creating gaps by removing turf divots, or by spraying out areas with chemical herbicides (Luscombe & Scott 1994; Hopkins *et al* 1999). Wells *et al* (1989), for example, used agricultural slot-seeding technologies to inoculate turf.

While transplant inoculation was the central focus of the restoration research, it was a necessary comparative addition to attempt the enrichment of existing swards using seed. In doing so, the results would provide a direct comparison with the plug and pot transplant experiments, thus raising theoretical and practical questions concerning plant recruitment, establishment and survival. This follows from the assertions of Coulson *et al* (2001) that: “there is a wide variation in the success of sown species, in terms of establishment and spread, and this is often addressed by sowing large quantities of seed. The problem is that little is known about the processes controlling colonisation of sown species or how management may affect this.”

METHODS

Inoculation species

The eight species chosen for this seed inoculation experiment were the same phytometers (Cavers & Harper 1967) used in **Chapter 8** used to assess seasonality in transplant survivorship. The only alteration to the species assemblage was the addition of *Filipendula vulgaris*. *Filipendula* was meant to be included in the seasonal transplant experiment but not enough stock could be grown in time for implantation. **Table 10.01** summarises the main germinule characteristics of the selected species. For simplicity, germinules are generically referred to as *seeds* throughout the text unless more detailed specific description is required.

Table 10.01 List of species used in the experiment. Also included are main ecological seed characteristics for comparison.

Species	Germinule weight (mg)	Germinule size Dimensions (mm)	Dispersal timing (inclusive of hay	Germination requirements	Soil seed bank persistency
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			making agency)		
<i>Centaurea nigra</i>	2.55	3.1 x 1.7	July >	Drying	I
<i>Filipendula vulgaris</i>	1.00ap	4.0 x 1.9ap	July>	Chill	I
<i>Leontodon hispidus</i>	0.85	6.5 x 1.0	July >	-	II
<i>Leucanthemum vulgare</i>	0.33	2.4 x 0.5	July>	-	III
<i>Lotus corniculatus</i>	1.67	1.9 x 1.5	July>	Scarify	III
<i>Malva moschata</i>	2.00ap	1.5 x 1.5ap	July>	Scarify	III
<i>Primula veris</i>	0.69	1.7 x 1.4	July>	Chill	I
<i>Scabiosa columbaria</i>	1.32	2.6 x 1.5	July>	-	II

Data from Emorsgate (2001); Grime *et al* 1988; Hodgson *et al* 1995; ap = approximated from categories presented in Hodgson *et al* 1995

While clearly each species has a *natural* seed dispersal phenology (Smith & Rushton 1994), **Table 10.01** presents the observation that all these species have usually produced mature seed by the end of July. As hay making is usually finished in August, the mechanised process of hay making can act as a significant vector in liberating and dispersing seed earlier than the natural-term (Coulson *et al* 2001).

Inoculation methodology

So as to provide a direct comparison with the transplants, this seed sowing experiment was accommodated within the same field set-up, and carried out using similar inoculation treatments. In the initial experimental configuration (see **Chapter 3**), allowance had been made for further enrichment experiments by providing expansion border areas in the subplots. However, in order to include a comparable number of replicates, and to be able to include both the autumn transplant, and seed experiments, it was necessary to reduce the span between sowing positions to 60-cm instead of the metre distances between transplants used in the spring 1994 experiment. However, this was still a far greater nearest-neighbour distance than used by Wells *et al* (1989).

Field preparation for seed sowing was started after hay cut and removal in late July 1996. Six rows (three either side of the central 1994 transplanted area) were delineated, with 16 sowing positions in each row. To locate the sowing positions, an electric fencing wire was graduated at 60-cm intervals and stretched between permanent markers at each end of the row. The sowing scheme was organised in a stratified-random sequence, with each row containing all the species, but in a randomised order. As for the spring planting experiment, 30-cm, 15-cm and 0-cm *competition-free* gaps were created using glyphosate herbicide sprayed over stencils with the appropriate diameters. Spraying was completed by late August 1996. In November 1996, the herbicide had effectively removed the vegetation and exposed sufficient (80%) mineral soil for sowing to take place. Inoculum sets were prepared by counting out 12 doses of

50 seed, of each species, for each subplot, and then temporarily storing them in paper sachets. According to the randomised sowing plan, two doses of seed for each species were sown into gaps in each row. The seed was sown into the central 10-cm diameter area irrespective of gap size (Morgan 1997). In all, therefore, 600 seeds of each species were sown into each subplot. The quantity of seed per dose was derived from commercial sowing rates g/m^2 (Emorsgate 2001), attenuated to 10% of prescribed, in accordance with natural viable seed production (Hodgson *et al* 1995) and cost considerations.

Germination tests

In addition to the field experiments, pot trials were also set up in order to investigate the viability of the seed under conditions of zero competition. Rather than an optimum laboratory controlled environment – which was not anyway available - these trials were undertaken using similar moisture inputs as in the field. However, with hindsight, it is clear that an attempt should have been made to assess viability more formally utilising standard procedures such as sowing onto moist filter paper under controlled conditions (Thompson 1993). Such tests would have provided a baseline of viability with which to compare the germination of both the field experiments and the pot sown seed.

For each species, ten, 1 litre pots were filled with multipurpose peat-free compost, and 50 seeds sown into each pot. The seed was sown directly on to the medium surface without further incorporation, as was initially experienced by the field sown seed. Therefore, each seed-species had ten replicates, with 50 seeds in each pot. The pots were then set outside on horticultural matting and kept moist if precipitation was not absolutely sufficient (i.e. droughting). Placing the pots outside of greenhouse conditions meant that the seed would be acted upon by the same weather conditions as the seed sown in the field, thus making the germination results relatively comparable except for the sward competition factor.

Censusing methods

The census methodology was similar to that used for the transplants i.e. the use of basic plant counts. The graduated wire used for locating the sowing positions was also used to relocate each sown position for surveillance purposes. The primary census procedure was the count of seedlings found at each gap position. Any circumstantial evidence of

damage, such as from molluscs, was also recorded. For 1997, the censuses were carried out in March, May and September. The summer census (May) was earlier than for subsequent censuses (June) in order to more closely assess germination patterns before peak standing crop. From 1998 onwards, the seedlings had mostly developed into adult plants, and performance parameters were recorded for comparison with the transplants. Because of time limitations the performance parameters of plant height and rosette width were only recorded for the largest individual recruit in each sowing position. However, counts were made for all flowers, from all recruits in each position. As with the transplant experiments, height and flower records were taken in the June census, and rosette diameter in September.

Data analysis

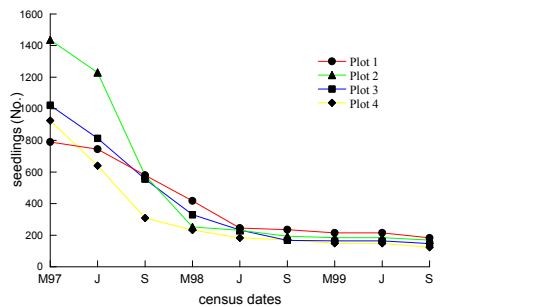
Data were analysed at both subplot and plot scales, depending on which tier seem to produce the best explanatory power. In general, though, as with the results of **Chapter 7**, the plot scale was the default. The primary analyses were calculated using transformed data, in line with the transplant analyses, as most of the datasets were composed of simple seedling-plant counts and thus considered as not *normally* distributed (Fowler & Cohen 1990). It was therefore necessary to normalize the data using logarithmic (\log_{10}) transformation, as the variances of the samples were larger than the means (Norušis 1998). This enabled parametric tests to be used within acceptable limits (Fowler & Cohen 1990). In order to integrate and transform zero counts, 1 was added to each data point ($x + 1$). For convenience and analytical power, One-way Analysis of Variance was used throughout. Least-squares linear regression, in combination with the Pearson correlation coefficient (r) (two-tailed) were used to examine the relationships between key environmental variables and seedling survival responses. Due to high seedling mortality across all subplots, few seed recruits survived until the final 1999 census. Because of sparse populations and concomitant small, uneven datasets, even with log-transformation, performance, in some instances, comparisons could only be undertaken using the non-parametric Kruskal-Wallis test (Fowler & Cohen 1990). All statistical analyses were conducted using SPSS ver 9.0 (1998).

RESULTS

Comparisons of seed germination and seedling survival

Figure 10.01 shows how the initial germination differentials between plots, as established by the first census in March 1997, gradually converge over the three years. Whilst Plot 2 had a markedly higher seedling count in 1997, by 1999 the seedling levels were very similar to the other plots. This finding may indicate that the factors influencing germination may have been discrete from those influencing seedling survival and establishment. However, the results of one-way ANOVA comparisons for each census showed no significant differences between plots over the whole research interval.

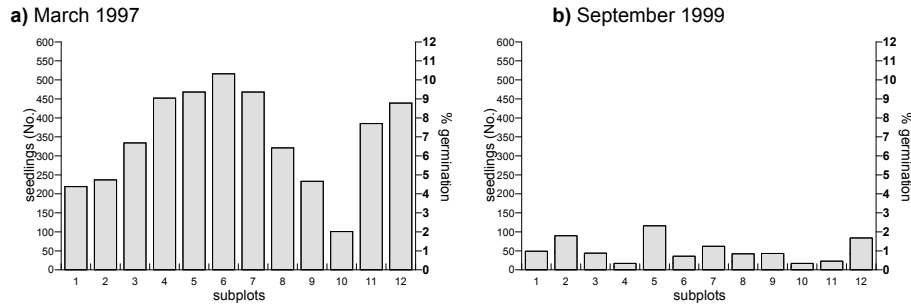
Figure 10.01 Survival curves for total seedling counts from the four plots.



However, it must be noted that these seedling counts are amalgamated into one artificial ‘cohort’ i.e. a count of seedlings at each census date without respect to germination flushes. The low frequency of censusing imposed by resource constraints meant that it was not in practicality possible to track the episodic germination patterns associated with these wild species (Waite 1984; Fenner 1985; Kelly 1989). Whilst for plant ecology research *sensu stricto*, tracking each cohort is essential, it was deemed unnecessary for the present restoration research purposes. Thus, within this agenda, the results presented in **Figure 10.02** reinforce the germination/survival disparity. **Figures 10.02a&b** show the 1997 and 1999 seedling census data in terms of both total subplot counts and as a percentage of seed sown. The datasets do not follow the established subplot *level of improvement* gradient that is apparent in the experiments presented in the other chapters. In fact neither dataset showed significant correlations with the subplot scheme (1997 $r = 0.053$; $P = 0.871$; 1999 $r = -0.193$ $P = 0.547$). In addition, the two census results do not significantly correlate ($r = 0.244$ $P = 0.445$), indicating that germination and subsequent survival do not seem to be positively related. Furthermore,

comparing the seedling counts across the subplot gradient using one-way ANOVA showed no significant differences for either dataset, indicating that germination (1997), and survival (1999) were relatively homogenous across the subplot swards.

Figure 10.02 Subplot census data displayed in terms of both seedlings/plant counts and as a percentage of total seed sown.



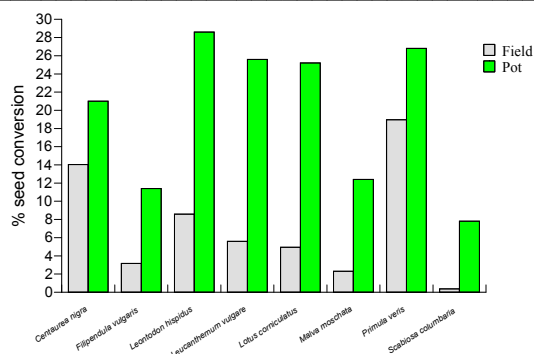
Finally, the 1999 seedling count was correlated with the results of the transplant inoculation experiments (**Chapters 7 & 8**), in order to evaluate how the conversion of sown seed to adult plants compared to transplant survival. This once again produced non-significant relationships, with spring cohort survival ($r = -0.105$), autumn cohort survival ($r = 0.124$), and even soil seed bank size ($r = 0.075$).

Comparisons of seedling-species survival and seed conversion

The germination tests used on the seed used in the field trials were set-up to reproduce elements of the environmental stressors encountered in the field, specifically droughting, at the same time excluding competition from neighbouring sward components. **Figure 10.03** is a comparison of species pot-seedling counts with those of field counts for the same species. The pot counts are lower than that expected from comparable straight viability tests (Westbury 2001), with only four species reaching above 25% seed germination. However, there is a very significant positive linear relationship between the datasets ($r = 0.864$; $P = 0.006$), indicating that both pot and field seed conversions partly relate to initial seed viability. In contrast, however, the one-way ANOVA results presented in **Figure 10.03** indicate that pot conditions, i.e. competition-free, as would be generally be expected, and had significantly higher seed conversion to seedling than under field conditions.

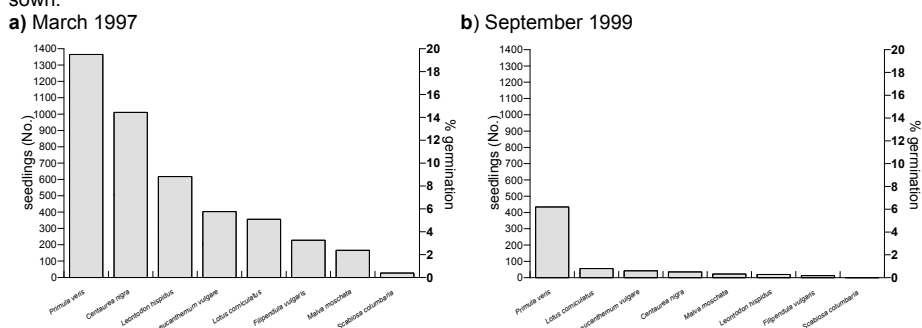
Figure 10.03 Comparison of field seed germination (conversion) with seed sown at the same time in pots – April 1997. Analysis using one-way ANOVA.

$$F_{1,14} = 8.79; P = 0.010$$



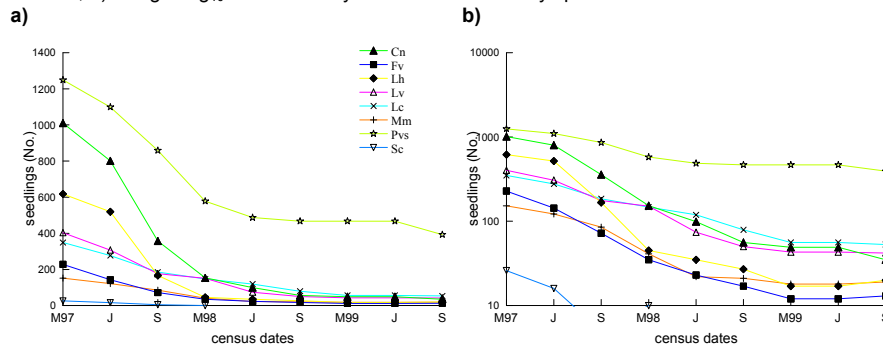
Utilising total subplot counts for the March 1997 census, one-way ANOVA reveals a very highly significant difference in germination between species ($F_{7,88} = 30.41$; $P = 0.000$), as presented graphically in **Figure 10.04a**. Further evaluation with Tukey HSD shows that, while most species are significantly differentiated on the basis of seedling number, *Scabiosa columbaria* stands out because of its very poor germination and *Primula veris* for having the highest seedling abundance. Even with the removal of both *Scabiosa* and *Primula* datasets from the analysis, there is still a highly significant variation between the species ($F_{5,66} = 14.62$; $P = 0.000$).

Figure 10.04 Species census data displayed in terms of both seedlings/plant counts and as a percentage of total seed sown.



By the September 1999 census, *Scabiosa* was extinct, and most of the other species severely reduced. The massive depletion in seed recruits is demonstrated by statistically comparing the first census species totals with the last census three years later using one-way ANOVA ($F_{1,22} = 85.6$; $P = 0.000$). Comparison of species plant counts within the last 1999 census also shows a highly significant variation ($F_{6,77} = 16.59$; $P = 0.000$), with Tukey HSD again indicating stand-out *Primula* survival as the dominating variable. Removing *Primula* from the analysis produces a non-significant result ($F_{5,66} = 1.16$; $P = 0.33$), suggesting that, as evinced by **Figure 10.05a** continued seedling mortality has led to overall convergence.

Figure 10.05 Sown species depletion curves plotted from data collected over the three-year monitoring period. The curves are presented: **a)** using a linear y-axis axis in order to show the extreme convergence of all species except *Primula*; **b)** using a \log_{10} -transformed y-axis in order to clarify species trends.

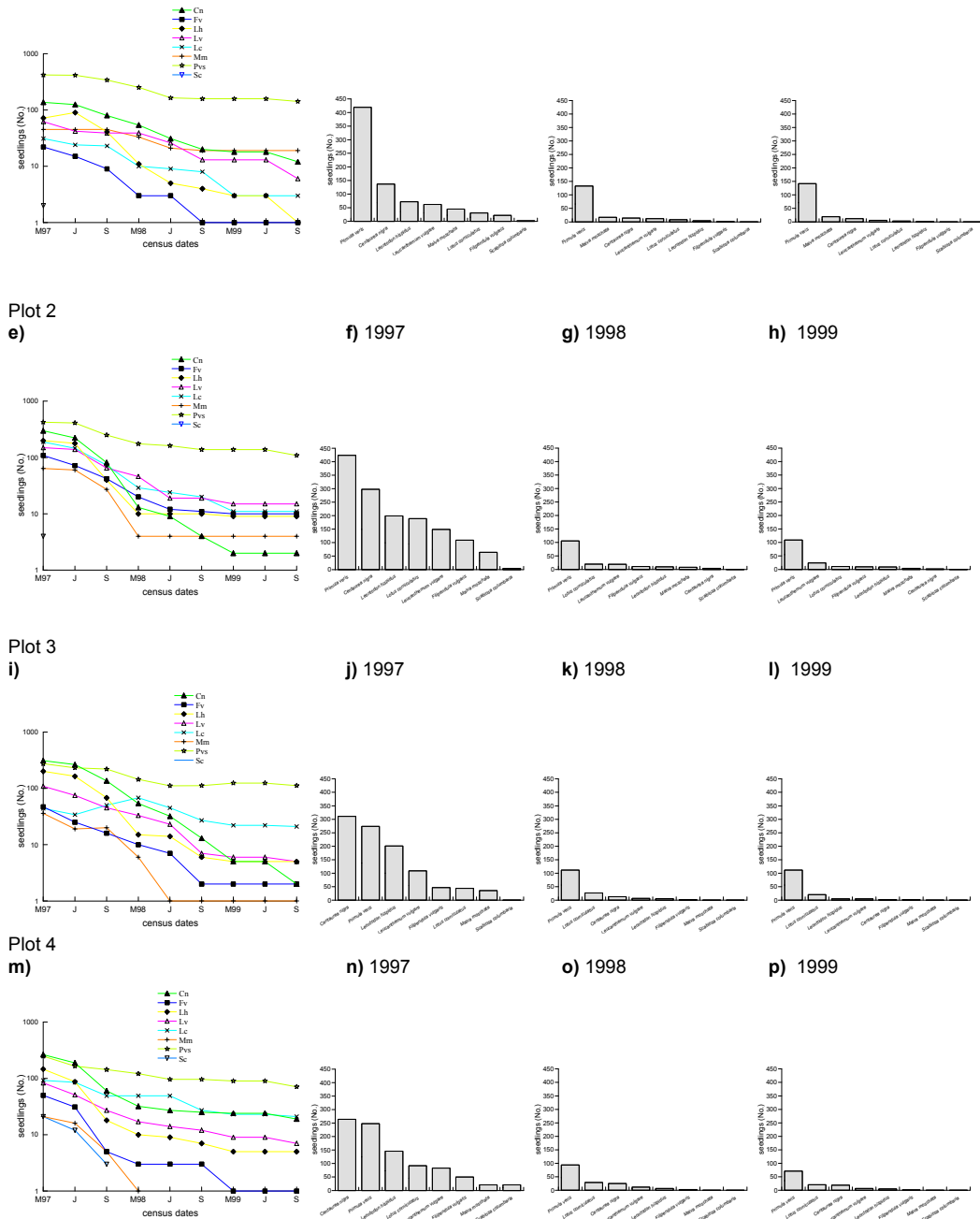


A number of researchers have found a positive correlation between seed weight and ability to germinate and establish under productive sward conditions. To ascertain if a similar association was occurring, seed weights of the eight species (Grime *et al* 1988) were correlated with the final counts of established adult plants (September 1999). The relationship was not only not significant ($r = -0.33$) but surprisingly also negative. This result was probably because *Primula* has a low seed weight (0.69mg) and had high establishment, and *Scabiosa* a relatively high seed weight (1.32mg) and zero establishment.

Figure 10.06 below compares the species data at the plot level, both in terms of depletion curves (line charts), and (column charts) germination (1997); survival/establishment (1998); and final adult plant counts (1999). As with the total (combined plot) count, what is most readily apparent from all the charts is the disparity between *Primula* seedling counts and the other seven species. Both *Primula* germination success and conversion to adult plants was far higher in all plots than the other species. One-way ANOVA for the March 1997 census using total subplot count data shows a highly significant difference in germination ($F_{7,88} = 30.41$; $P = 0.000$).

Figure 10.06 Seedling-species depletion curves plotted on a \log_{10} transformed y-axis: charts a), e), i) & m). Included are column charts ranked by total seedlings per species: data from 3/97; 6/98; 9/99 censuses.

Plot 1
a) **b) 1997** **c) 1998** **d) 1999**



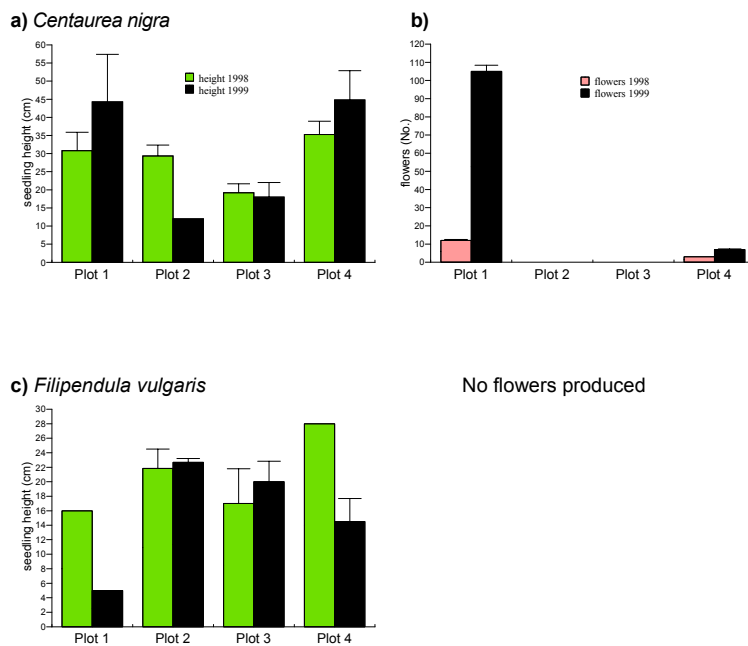
Both Plots 1 & 2 counts (**Figures 10.06a-h**) exemplify the quantitative gulf between *Primula* conversion and the other seven species. The census sequence of the column charts also displays two other interesting patterns. Firstly, the 1998 and 1999 trends are very similar in terms of number of seedlings. Large losses took place between the 1997 and 1998 censuses, and those individuals that managed to establish generally survived up to the final census a year later. Secondly, the species assembly in terms of ranking also changed significantly over the first year, though remained almost the same for all

four plots for the subsequent two years. Thus, the selection processes affecting the germination phase seem to be markedly different from the establishment phase.

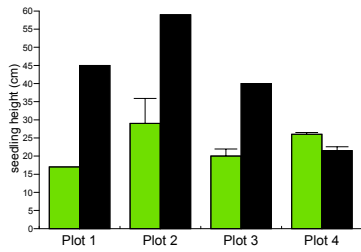
Species' performance

Along with survival counts, data were collected for seed-derived plant performance over the first two years, in essence charting age-state developments from seedling to adult. The parameters measured were the same as for the transplants, though because of methodological constraints, only the largest individual recruit from each sown-position was measured, though all flowers from each sown patch were counted. Summarised accounts of the results are presented in **Figure 10.07** in terms of plot-tier data.

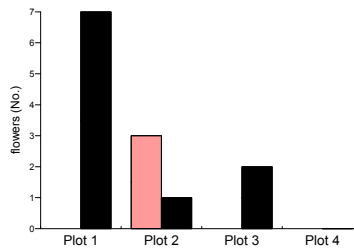
Figure 10.07 Plot comparisons of performance of sown seed recruits after two (1998), and three (1999) years. Plant heights were calculated from the tallest individual per sowing position, averaged across the plot. Flower counts are totals from each sowing position i.e. from all plants averaged across the plot. Bars = Standard Error.



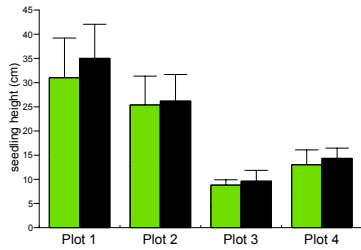
d) *Leontodon hispidus*



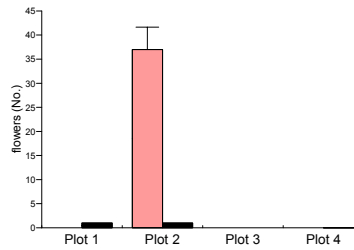
e)



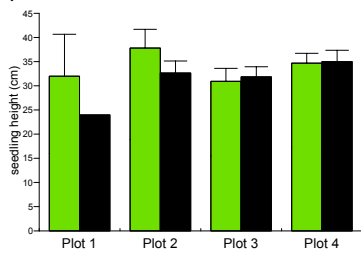
f) *Leucanthemum*



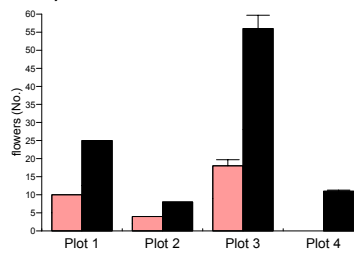
g)



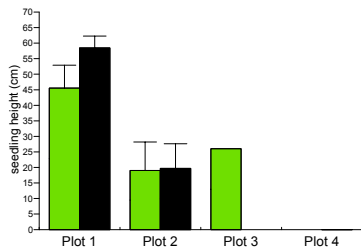
h) *Lotus corniculatus*



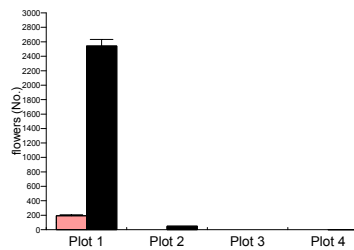
i)



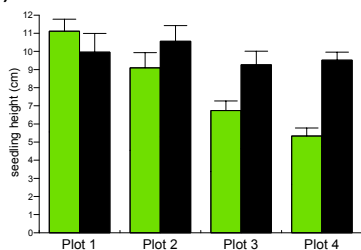
j) *Malva moschata*



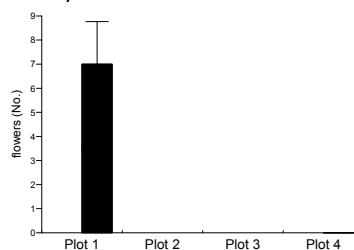
k)



l) *Primula veris*



m)



The only species not presented is *Scabiosa columbaria* because the few seedlings that did convert from seed were extinct by summer 1998. Except *Leontodon*, comparisons between the two censuses seem to show little further size increments between 1998-9, suggesting that, at least for the largest patch individual, by summer 1998 maximum

adult size had largely been attained within the plot-specific productivity constraints. In fact for *Filipendula* average plant size was higher for 1998 than 1999 suggesting retrenchment to an earlier age-state perhaps due to increased competition. Height performance for *Malva*, *Leucanthemum* and *Primula* was clearly higher for Plot 1 - the most productive grassland – than for the other plots, which also parallels the transplant results (see **Chapter 7**). In contrast, for *Filipendula* height performance was notably lower for Plot 1, and effectively equal for the other grasslands averaged over the two census years.

Flower production across all plots for both yearly censuses was very low, in line with the very low seedling survival statistics. Even in terms of total (combined plot) flowering performance, production was not significantly different between the two censuses ($P = 0.334$), or across plots ($P = 0.366$). *Malva* was the only species to show a significant differential between the two years, with summer 1999 flower counts higher than 1998 (Kruskal-Wallis test: $\chi^2=7.73$; $P<0.05$). *Filipendula* stands out again as the only species not to produce a single inflorescence in any of the plots the over two-and-a-half years since the seed was sown. *Primula* flower attainment was also very limited, but probably largely due to the management treatments than underlying productivity-competition factors. Otherwise, only *Leontodon* and *Lotus* seemed to produce even limited parity of flower production across the plots.

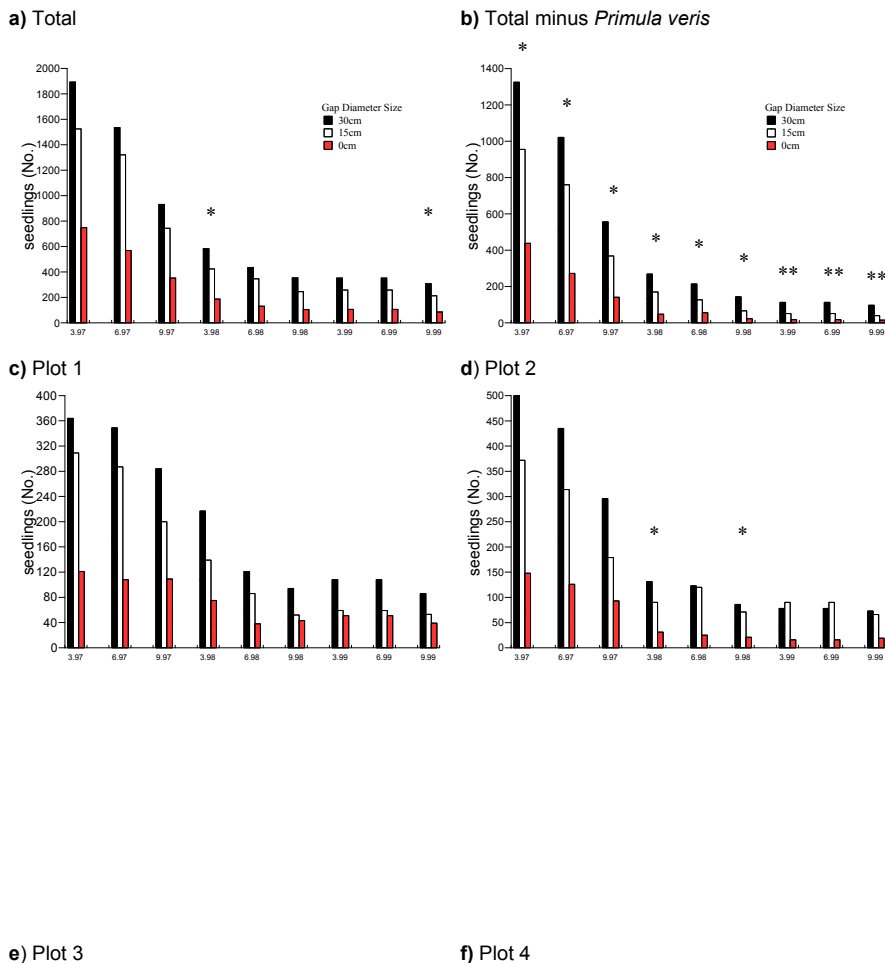
Artificial gap size: seedling germination and survival

What is apparent from the previous experiments using transplants is that the creation of artificial competition-free gaps using herbicide has limited efficacy in promoting transplant survival and establishment. Nevertheless, for seed inoculation, herbicide spray gaps do seem to have a much greater impact on enrichment. **Figure 10.08a** shows that for all censuses in terms of *total* seed conversion/survival, 30-cm diameter gaps encouraged highest germination and seedling survival. In terms of statistical importance, gap effects were significant in March 1998 ($F_{2,18} = 3.97$; $P = 0.037$), and the final census of the research period September 1999 ($F_{2,18} = 3.66$; $P = 0.05$). What is clear from **Figure 10.06** is that *Primula* seed germination and establishment were significantly higher than for all the other species put together. In addition, *Primula* seed inoculation seems to be highly effectual with or without an herbicide gap. Because the

relative dominance of *Primula* inoculation success skews the overall results, **Figure 10.08b** demonstrates seed/gap influence with the removal of *Primula* from the estimations. Subtraction of the *Primula* data presents the zero gap with significantly lower seedling generation than for gap throughout the three years of monitoring, and 30-cm diameter gaps as the most effective means of promoting germination, seedling establishment, and development to adulthood.

In terms of plot data, gap creation led to higher seedling generation than no-gap, though only Plots 2 (March 1998, $F_{2,18} = 3.83$; $P = 0.04$; September 1998 $F_{2,18} = 4.28$; $P = 0.03$) & 3 (March 1998, $F_{2,18} = 3.71$; $P = 0.04$) recorded significant differentials. For Plot 2 (**Figure 10.08d**) the actual gap size, whether 30- or 15-cm diameter, was apparently largely unimportant, with 15-cm gaps attaining equal seedling counts.

Figure 10.08 Column charts showing seedling totals per gap size, and b) total seedling counts minus *Primula veris*. * = one-way ANOVA significant at $P < 0.05$; ** < 0.001 ; Charts g) and h) are plot comparisons for seedling germination and survival – also given are results of the χ^2 test for homogenous frequencies, ** $P < 0.01$



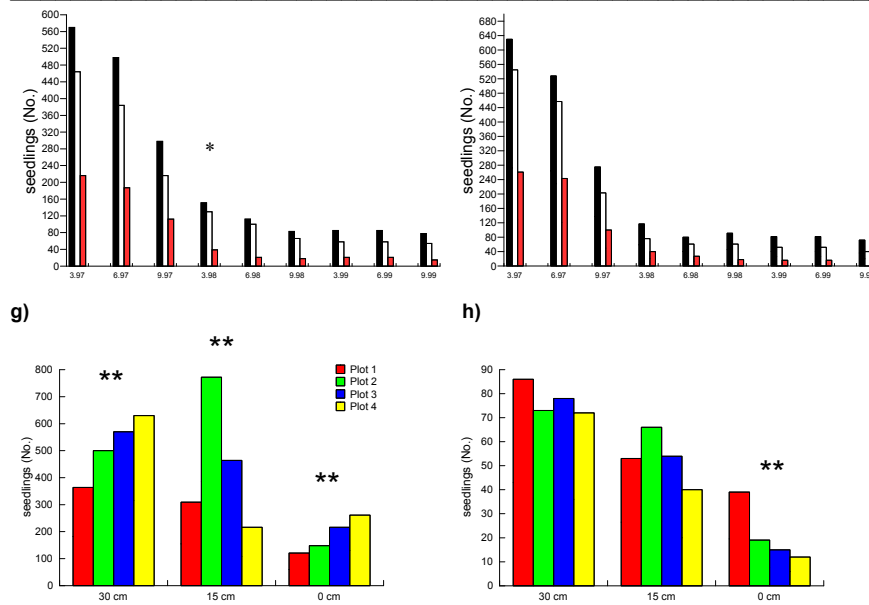


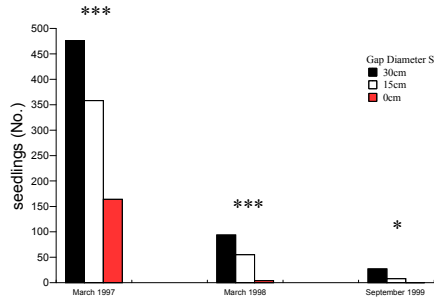
Figure 10.08g&h compare the germination (March 1997) and survival (September 1999) data for the three gap sizes across the plots. In terms of germination there was a significant departure from homogeneity between the four plots, inferring Plot 1 as recording the lowest germination for both 30-cm and 0-cm plots, and Plot 4 the highest. The clear anomaly is Plot 2 for the 15-cm gap size, which had significantly higher germination than the other three plots. **Figure 10.08h** shows that after three years the differentials across the plots for gap size had reduced due to the high mortality risk, with only the 0-cm gap showing a significant departure from homogeneity. Rather surprisingly, the rank order recorded for germination was reversed by 1999, with Plot 4 recording the lowest counts and Plot 1 the highest.

Primula establishment was so superior to the other species; its dominance obscures other significant relationships in the gap dataset. By looking at the impact of gaps on individual species, **Figure 10.09** reveals the fact that each species presents different patterns in establishment/survival over the three years of evaluation. *Centaurea*, and *Primula* exhibit highly significant differences between gap and non-gap over the three years, and therefore stand out as the two species that gained the most by gap creation. While for all species 30-cm diameter gaps produced the highest survival, the differential between the 30- and 15-cm gaps was smallest for *Primula*, and 15-cm gap survival was actually higher for the first ‘germination’ census.

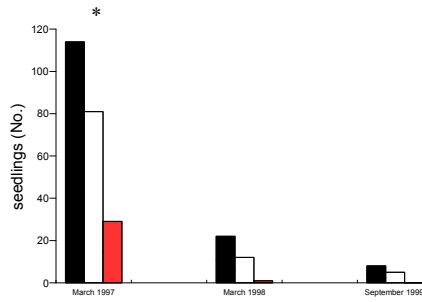
Figure 10.09 Column charts showing effect of gap size on individual sown species. * = One-way ANOVA significant at $P < 0.05$; ** < 0.001; *** < 0.000

a) *Centaurea nigra*

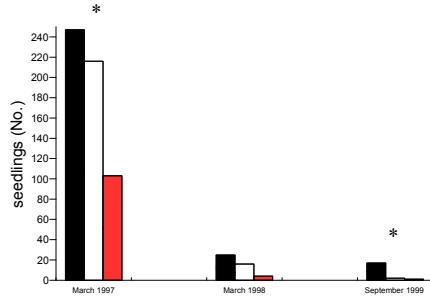
b) *Filipendula vulgaris*



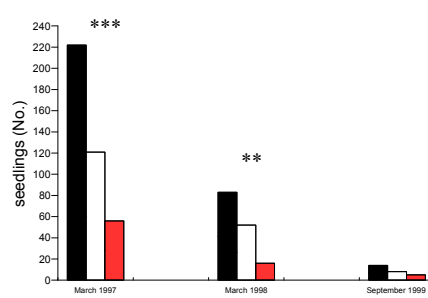
c) *Leontodon hispidus*



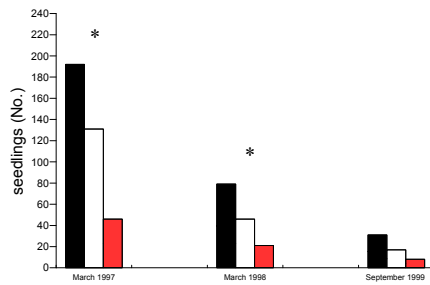
d) *Leucanthemum vulgare*



e) *Lotus corniculatus*



f) *Malva moschata*



g) *Primula veris*

The only other species to display a significant final count differential i.e. gap creation survival higher than no gap, was *Leontodon*. Otherwise, of the remaining four species, *Leucanthemum*, *Lotus* and *Malva* all evinced significantly higher seedling survival in gaps than no gap for the first two censuses, but by the final census the differential was negated by continuing overall depletion. Only *Filipendula* showed no significant results, though clearly again 30-cm gap survival was dominant. Because of the

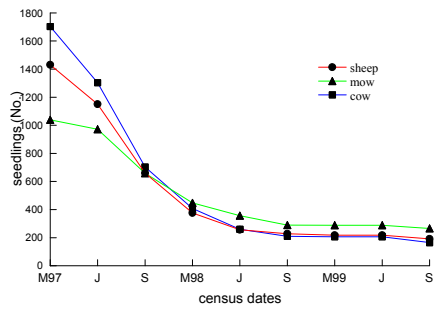
negligible germination counts of *Scabiosa columbaria* assessment of gap impact was essentially irrelevant.

Management treatments: seedling germination and survival

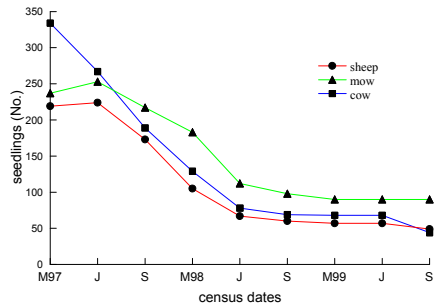
Management treatment does not seem to have created consistent trends in seed germination and seedling survival over time. **Figure 10.10** shows that for combined plot totals of seedling survival; there is virtually no difference between the treatments, indicating that other factors override that of the method by which the grasslands are defoliated. The curves of **Figures 10.10b-e** show a largely consistent rank order. Once the germination differential had been formed six months after sowing, this differential was maintained throughout the experiment. Thus, for Plots 1 & 2, the mown subplots had the highest seedling numbers right up until the final census; whilst the sheep grazed subplots had the lowest. Conversely, in Plots 3 & 4, sheep grazing promoted the highest germination and seedling survival, and mowing the lowest. Despite these obvious trends, only Plot 4 showed statistically significant management treatment effects. One-way ANOVA shows that for the first year after sowing, germination and subsequent seedling survival were significantly lower for the mown grassland than the grazed subplots (March 1997 ($F_{2,21} = 4.05$; $P = 0.03$); June 1997 ($F_{2,21} = 6.33$; $P = 0.007$); September 1997 ($F_{2,21} = 3.59$; $P = 0.04$). Apart from the September 1998 census ($F_{2,21} = 3.58$; $P = 0.04$), after this first year, the levels of seed-derived plants converged between management treatments, the differential becoming non-significant, though the rank order remained the same. Of note, however, is the fact that the number of seed recruits in the cow grazed subplot crashed during the first year, rapidly aligning with the very low counts found in the mown grassland (**Figure 10.10e**). Sheep grazing seemed to more effectively ameliorate sward conditions for continued seedling survival, though of course other environmental factors may have been in play.

Figure 10.10 Management treatment effects on seed germination and seedling survival (no sigs)

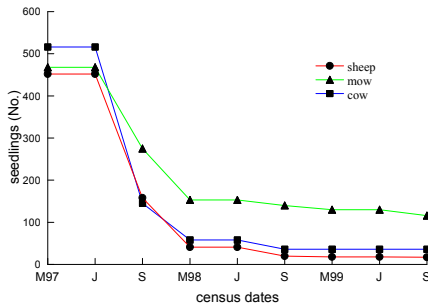
a) Combined data



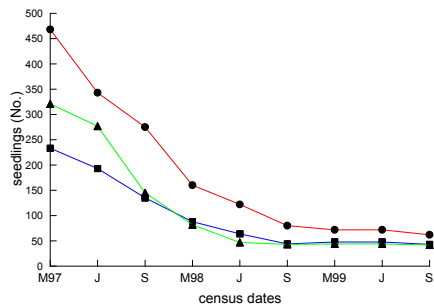
b) Plot 1



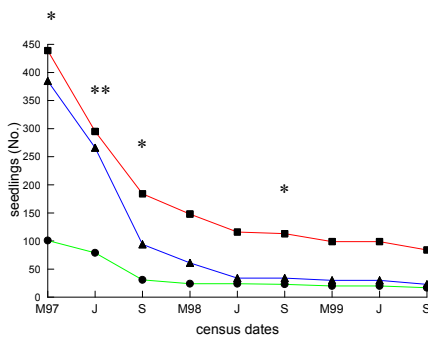
c) Plot 2



d) Plot 3



e) Plot 4



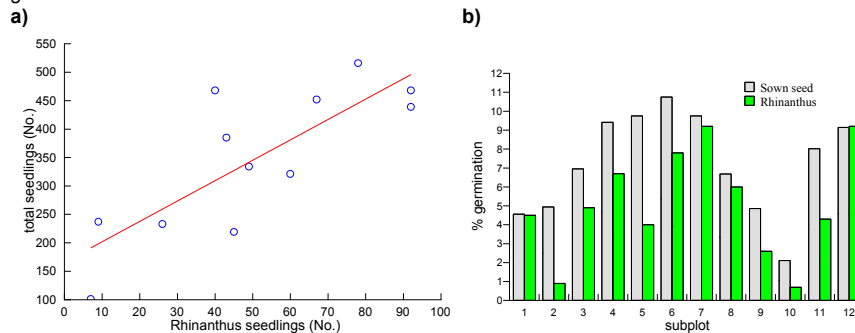
Grassland productivity factors: seedling germination and survival

The previous chapters have identified soil nitrogen, phosphorus and potassium as key factors in the survival of transplants. Also, average sward height, phytomass and field layer light (lux) levels have also demonstrated significant correlations with sward diversity and transplant survival performance. It was therefore deemed appropriate that this sown-seed data should be analysed in the same way to identify significant trends. As with the above analyses, the March 1997 seedling counts were accepted as indicative of germination success. However none of the 1995-1997 productivity factors mentioned above correlated significantly with the results of this first seedling census i.e. none attained $P < 0.05$, two-tailed correlation coefficient (r). Equally, the September 1999 census, equating to seedling survival over this specific three-year research period, also did not correlate with any of the productivity factors collated from 1996-7, or 1999. The only significant (negative) correlation was between seedling count and the

March 1998 quantification of soil potassium ($r = -0.595$; $P = 0.043$). Overall then, with one exception, seed germination and subsequent seedling establishment did not correlate with any of the established productivity variables. In addition, neither final spring or autumn transplant survival significantly correlated with seed germination and establishment.

Whilst this lack of correlations with the productivity factors seemed curious in comparison to the responses shown for transplants, the results were supported by a separate experiment not presented in this thesis. In mid February 1997 *Rhinanthus minor* was also sown into the subplot swards using similar artificial gap treatments. The germination and subsequent seedling counts were recorded until the plants had produced seed and senesced. Correlating the *Rhinanthus* seedling counts with the above March 1997 seedling counts produced a highly significant positive relationship (**Figure 10.11**: $r = 0.797$; $P = 0.002$). This indicates that the same factors involved in *Rhinanthus* germination were similar to those affecting the suite of seed-species. However, it is still difficult to ascertain without further experimentation what the main variables affecting germination are.

Figure 10.11 Charts showing the significant positive relationship between sown seed and *Rhinanthus minor* germination.



In a final assessment as to whether there were any significant responses between seed inoculation and the above productivity factors, analysis was applied at the species-level. **Table 10.02** presents only the statistically significant ($P < 0.05$; two-tailed) results. What is immediately clear is that there are far more *species-germination* correlations. Phosphorus is negatively correlated only with *Scabiosa*, though *Filipendula*, *Leucanthemum* and *Lotus* are all negatively correlated with potassium. Both *Malva* and *Primula* are positively correlated with phosphorus. *Malva* also positively correlated with average sward height, and *Centaurea* with soil nitrogen.

Table 10.02 Pearson (*r*) correlation coefficients comparisons with: a) 1997 seed *germination* census and average sward production/ 1998 soil nutrient levels; b) 1999 seed *establishment* and average sward production/ 2000 soil nutrient levels.

a) <i>Germination</i> March 1997 Census	<i>Centaurea nigra</i>	<i>Filipendula vulgaris</i>	<i>Leontodon hispidus</i>	<i>Leucantheu m vulgare</i>	<i>Lotus corniculatus</i>	<i>Malva moschata</i>	<i>Primula veris</i>	<i>Scabiosa columbaria</i>
Av Sward height						0.603*		
AV Biomass	0.724**							
N 1998						0.708*	0.663*	- 0.612*
P 1998								
K 1998		- 0.620*		- 0.682*	- 0.685*			
b) <i>Establishment</i> Sept 1999 Census								
AV sward height								
AV Biomass								
N 1998						- 0.790**		
P 2000						0.578*		
K 2000						0.585*		

Of the *seed-establishment* census, the only species to evince any significant correlations was *Malva*. These results for *Malva* were very similar to the autumn transplant performance results (see **Chapter 8**), that is, survival-establishment was positively related to soil phosphorus and potassium, and very negatively associated with soil nitrogen.

DISCUSSION

The results of this three-year study have shown that: i) Neither germination and seedling survival, nor development to adult plants, showed significant adherence to the gradient of grassland improvement/productivity: ii) The number of seedlings which emerged at the beginning of the study bore no significant relationship to the final count of adult plants; iii) There were no significant differences between seedling counts between the plots, for any census over the three years; iv) The final count of adult plants generated from the seed sown in each subplot did not correlate with either transplant survival, or soil seed bank size: v) Two species stand out - *Scabiosa columbaria* and *Primula veris* - for respectively opposite reasons; *Scabiosa* because of very poor germination, and total failure in survival; and *Primula* for its high relative germination and high seedling survivorship: vi) The use of artificial competition-free gaps, unlike for transplants, did significantly enhance total seed germination and seedling survival and establishment: vii) Management treatment may have had a significant impact on seed germination and seedling survival, though this was only statistically significant for Plot 4.

Overall trends in germination, seedling survival and development

Unlike many seed enrichment studies (Wells *et al* 1989; Smith *et al* 2000; Westbury 2001), instead of an estimate, this research used a known quantity of seed, sown in doses into each subplot. Whilst this vastly reduced the volume of sown propagules – Smith *et al* used a rate of 1.5kg ha^{-1} commercial seed, compared to 0.11kg ha^{-1} applied in this study – it did enable exact quantification of seed derived recruitment. Therefore, the ratio of seeds sown to the number plants counted at each census can be calculated with some certainty, and even the performance of individual plants followed throughout the study. Because of this more detailed monitoring level, it is convenient to discuss the results in terms of three basic age-state intervals: germination; seedling establishment/survival; and development to adulthood.

Germination

The results have demonstrated a wide range of seed inoculation efficacies between the phytometer species - from the stand out conversion of *Primula*, to the complete failure of *Scabiosa*. As suggested by **Figure 10.03**, one reason for this variation may be due to the inherent viability of the seed stock used. The conditions of the pot trials promoted significantly higher germination. This result was to be largely expected, as the pot conditions were competition-free, and predation-free, unlike field conditions. It must be stated though, with hindsight, that to provide a more strict assessment of seed viability, germination tests should have been exercised under laboratory conditions (K. Thompson pers. comm.). However, with this caveat highlighted, the results do show that both pot and field germination results were significantly positively related, indicating that seed viability was influential irrespective of receptor site conditions. The species, which most notably demonstrated this relationship, are *Scabiosa*, which had the lowest germination for both pot and field trials, and *Primula*, which had high germination for both trials. Against this trend, *Leucanthemum*, *Lotus*, and especially *Leontodon*, demonstrated relatively high pot germination, and low field germination. It is therefore perhaps fair to assume that for these species, in particular, the differentials between inoculation trials were largely due to the respective autecologies (Grime *et al* 1988; Handa & Jefferies 2000) of each species interacting with the properties of the recipient grasslands (Bobbink & Willems 1993; Davies *et al* 1999; Smith *et al* 2000).

Although the germination behaviour of wild flower seed is often drawn-out and/or episodic (Wells *et al* 1981; Fenner 1985), the majority of the species used in this experiment persist relatively briefly as viable seed in the soil, and none are recognised as long-term seed bank formers (see **Table 10.01**: Hodgson *et al* 1995; Hutchings & Booth 1996; Thompson *et al* 1997). The March 1997 seedling count - some five months after sowing – was undertaken after winter vernalisation and spring diurnal temperature and light fluctuations had acted on the seed, and therefore it should be acceptable as an estimate of peak germination (Bakker *et al* 1980; Francis & Morton 2001). The fact that the highest seedling counts were recorded from this census may bear out the assertion. However, because of the low frequency of censuses, further germination and seedling deaths may have taken place in the between-census intervals without being recorded (Hutchings 1986). Also, some seed of species such as *Lotus* and *Malva* may have been incorporated into the persistent soil seed bank, with germination meted out over a long time frame than was observable within the monitoring scheme (Bakker *et al* 1996; Davies & Waite 1998).

The fact that neither the subplot nor plot germination datasets showed significant correlations with the agricultural improvement gradient suggests a major departure from the evaluations made in the other chapters using transplants. In addition, the productivity parameters affecting transplant inoculations, soil seed bank sizes, and even gap occlusions, were not significantly implicated in seed germination. Thus, seed inoculation did not correlate with any of the established productivity variables (Bakelaar & Odum 1978; Howe & Snaydon 1986; Mountford *et al* 1993). This is perhaps a surprising result considering that much research suggests that seed would be the most sensitive indicators of agricultural improvement levels (Hopkins *et al* 1997; Hopkins *et al* 1999). That is to say, trying to infiltrate species into improved grasslands using seed would in theory seem to test the productivity-related resistance to invasion most pertinently. Transplant use is designed to circumvent the prohibitions impacting on seed regeneration (Kendle 1992; Davies *et al* 1999), yet transplant survival broadly followed the improvement gradient, and seed inoculation did not. If none of the recorded productivity factors correlate with the germination counts, it is germane to speculate as to what factors actually influenced the trend presented in **Figure 10.02a**. The robustness of the trend is corroborated by the fact that *Rhinanthus* germination also followed the same

pattern (**Figure 10.11b**). The low germinations in subplots 8 & 9, and particularly 10, seem to go conspicuously against the transplant inoculations results. The fact that both subplots 9 and 10 are mown suggests that management may play a part in retarding germination, though for no obvious reasons, particularly as the spray-gaps produced adequate regeneration opportunities. These subplots may have higher rates of seed predation (Hulme 1994; Edwards & Crawley 1999), pathogens (Hitchmough *et al* 1996), low levels of soil nitrate (Karssen & Hilhorst 1992), or even allelopathic substances produced by certain vegetation components (Fenner 1978). In contrast to the low germination of Plot 3 and subplot 10, the high relatively high germination results for Plot 2 were a surprise. The inhibitory factors operating in Plot 3 are perhaps lacking in Plot 2, alternatively higher levels of promotional factors may occur in Plot 2 e.g. soil nitrate (Karssen & Hilhorst 1992).

What is immediately clear (**Table 10.02**), however, is that the results do show productivity correlations with individual species. *Filipendula*, *Leucanthemum* and *Lotus* are all negatively correlated with soil potassium, and *Scabiosa* with phosphorus, and this result is paralleled by the negative correlations for transplant survival described in the previous chapters. *Filipendula*, *Lotus*, and *Scabiosa* in particular, are generally categorised as stress-tolerators (Grime 1979; Hodgson *et al* 1995), and the fact that these species established relatively poorly in these productive swards would be the general expectation. In contrast, however, both *Malva* and *Primula* positively correlated with phosphorus. *Malva* also positively correlated with average sward height, and *Centaurea* with soil nitrogen. In terms of transplant survivorship, none of these species showed positive relationships with the productivity variables (**Chapter 8; Table 8.06**), so clearly for these species, the seed inoculation dynamics were notably different. Only nitrogen (nitrate) is known as a major germination stimulant (Karssen & Hilhorst 1992), so perhaps the seed-derived seedlings of the above species were able to respond positively to the respective soil macronutrients in their early stages of growth. Fenner & Lee (1989) propose that most seedlings benefit from immediate external sources of N and K, and it is known also that P can be limiting for newly germinated seedlings (Fenner & Lee 1989). The results suggest that species generally considered to be stress tolerant require only low soil P, higher levels of which will confer vigour to dominant species exacerbating competitive exclusion pressures (Grime & Curtis 1976; Kitajima & Tilman 1996; Jones &

Hayes 1999). However *Malva* and *Primula* accord with a more competitive established strategy (Hodgson *et al* 1995), as higher levels of soil phosphorus seemingly promoted germination and early seedling growth.

Seedling establishment/survival

Fenner (1985) states, “a seedling is considered fully established when it has become effectively independent of its seed reserves”. Following this definition, certainly by the September 1998 census the germination phase was essentially over, and the surviving juveniles were endeavouring to entrenchment. Unlike a number of studies, seed size did not correlate with seedling establishment success (Fenner 1985; Leishman & Westoby 1994; Erikson & Jakobson 1998), though any significant correlations were perhaps confounded by the use of artificial gaps. The early stages of seedling growth typically have high mortality rates, with causes of death such as desiccation, burial, predation, disease and competition (Fenner 1985; Kitajima & Tilman 1996). The results presented in **Figure 10.06** show that the sown populations also demonstrate high mortality. Large losses took place between germination in 1997, and the September 1998 census. Secondly, the species assembly in terms of ranking also changed significantly over the first year, though remained almost the same for all four plots for the subsequent two years. Thus, the selection processes affecting the germination phase seem to be markedly different from the establishment phase. This accords with by Silvertown & Dickie (1980) who assert that heavy mortality is often observed in the seedling and juvenile phases and that this may be a major component of the species selection. Turnbull *et al* (2000) reinforce this contention: “for many species, conditions for establishment are more stringent than conditions for germination.” Unfortunately, no clear associations between biotic and abiotic factors, germination, and subsequent seedling establishment were apparent from the analyses. What caused the differential between seedling germination and seedling survival could not be satisfactorily explained. Fenner (1985) states that competition from neighbouring vegetation is probably the greatest single hazard faced by colonising seedlings. This is shown by the fact that greatest mortality tends to occur during periods favourable to growth. Also predation, especially by molluscan herbivores, is known as an important cause of mortality in seedlings (Cavers & Harper 1967; Hanley 1995; Jones & Hayes 1999). The selective pressure of predation therefore may have altered between germination and

establishment, thus not only reducing overall seedling numbers, but also changing relative species abundances.

As with germination, none of the survival censuses (up until 1999), presented significant correlations with the gradient of agricultural improvement. Fundamentally, the connotations are that the trends in sown-seed populations established during the germination stage were too influential to be altered by the dynamics of later seedling development, and/or seedling establishment continued to be effected by the same parameters which influenced germination, which were not clearly linked to any of the measured productivity factors. Paradoxically, the seedling-plugs used in the transplant experiments *were* significantly affected by grassland productivity factors, even though intuitively they should be less susceptible than seed inoculation to productivity factors (Dixie & Bisgrove 1996; Davies *et al* 1999). In terms of individual species, the only species to evince any significant correlations with the productivity variables was *Malva moschata*. The *Malva* sown-seed results were very similar to the autumn transplant performance results (see **Chapter 8**), that is, seedling survival and establishment were positively related to soil phosphorus and potassium, and negatively associated with soil nitrogen. In terms of high P and K soil concentrations, *Malva* seems to react competitively (Burke & Grime 1996). Though *Malva* seemed to perform conspicuously badly with increased levels of soil nitrogen.

Adulthood

Turnbull *et al* (2000) note that many seed sowing experiments do not follow the seed through germination to adulthood. The present study however did monitor the recruitment during all phases, and the final census in September 1999 can be taken as the evaluation of how many of the initial seed doses converted to adult plants. Overall, from the 57,000 seed sown across the plots, 623 adult plants resulted. **Figure 10.06** suggests that those individuals that managed to establish by 1998, were successfully entrenched, generally surviving up to the final census a year later. The early massive depletion in seed recruits is demonstrated by the statistically significant differential between the first census species totals (1997) with the last evaluation, three years after sowing (1999). However, the differential between the 1998 census and final 1999 census was slight, indicating that after the initial period of high mortality, seedling

mortality risk stabilised. In these agriculturally eutrophied grasslands the immediate post germination period seems to be the most selective phase, particularly inhibiting stress-tolerant species, excluding *Scabiosa* completely, and promoting the more competitive species. However, one species – *Primula veris* - eclipsed the other species not only in terms of germination success, but also in conversion to adult plants. Because *Primula* germination was so relatively high, even with large reductions in survival counts, *Primula* conversion to adult plants was far higher in all plots than all the other species put together, with *Primula* comprising 70% of total plants counted in the final census.

As with the previous developmental stages, the final count of adult plants showed little correlation with the productivity gradient. The only significant (negative) correlation was between the final total count of plants and soil potassium. This negative relationship with K was paralleled in the transplant studies, yet seed-plant counts did not show a significant relationship with spring transplant survival, autumn transplant survival, or even soil seed bank size. In other words, seed inoculation produced relatively homogenous recruitment irrespective of soil nutrient capital or grassland physiognomy, and this pattern differed demonstrably from the gradients demonstrated not only through transplant use, but soil seed bank size, and gap occlusion. Of all the species, only *Malva* continued to show significant correlations with productivity factors (**Table 10.02**), positively for both P and K, though the strongest correlation was negative with soil N. Thus, the broad interpretation is that *Malva* germination and development is actually promoted by high soil K and P. Yet, with increasing soil N, perhaps accumulated through succession, *Malva* performs less well. In Plot 1, this species showed particular high recruitment, as soil P was very high, and soil N was very low due to regular ploughing preventing organic matter build-up (see **Chapter 4**). Thus, *Malva* seemed to respond most positively to the grassland conditions created by cessation of fertiliser applications to the *Lolium perenne* (MG 7) ley (Rodwell 1992). Nevertheless, the overall interpretation of the results is that, beyond germination and initial development, seedling numbers showed no conditioning at all by the variables found so explanatory in the transplant work.

Looking at individual species performance provided a more detailed review of seed inoculation. After three years since sowing, all of the remaining plants could be said to have reached an *adult* age-state (Gatsuk *et al* 1980). Except *Leontodon*, comparisons between the two censuses seem to show little further size increments between 1998-9, suggesting that, at least for the largest patch individual, by summer 1998 maximum adult size had largely been attained within the plot-specific productivity constraints. However, though adult, this did not mean necessarily that they had reached generative potential. Clearly vegetative expansion had taken place for all individuals since germination, and in terms of height performance, *Malva*, *Leucanthemum* and *Primula* were promoted in Plot 1, taking advantage of the high soil nutrient capital, which also parallels the transplant performance results (see **Chapter 7**). In contrast, *Filipendula* height performance was notably lower for Plot 1, and effectively equal for the other grasslands. Yet, for flower and seed production, more diverse responses were recorded. For instance, although the remaining *Filipendula* plants attained foliage heights equivalent to the remaining transplants, none flowered. In comparison, 7% of the surviving transplants flowered in the same year (1999). For the other species, except *Leucanthemum* (**Figure 10.07f&g**), overall inflorescence production increased from 1998 to 1999, demonstrating complete generational development, from seed, to seed bearing adults. *Malva* was the only species to show a significant differential between the two years, with summer 1999 flower counts higher than 1998. However, in line with its more ruderal strategy (Grime *et al* 1988), *Leucanthemum* inflorescence production peaked in 1998 and dropped dramatically back a year later. *Primula* inflorescence attainment was very limited compared to its seed-to-adult conversion, but this was probably largely due to the management treatments than underlying productivity-competition factors. In order to more effectively tease out the interactions of productivity, species' regeneration ecologies, and management, further detailed experimentation is required (Bazzazz 1986; Handa & Jefferies 2000; Turnbull *et al* 2000). This viewpoint is echoed by Burke & Grime (1996) who state that: "No serious attempt to assess the risk of biological invasions can be made without a reasonable understanding of the structure and regenerative characteristics of the 'target' community, matched with some knowledge of the functional characteristics of the species likely to invade."

Influence of artificial gaps on seed inoculation

In the transplant studies, artificial gap creation only significantly enhanced recruitment under very specific circumstances (see **Chapter 7**). The results of this experiment demonstrate an important departure from the transplant studies in that spray gaps had a broad, significant impact on sown seed recruitment. Gap creation was shown to promote total seedling survival in both the March 1998, and final census (September 1999) of the research period. For all censuses, gap creation aided sown-seed recruitment compared to no herbicide gap. Except for Plot 2, all the charts of **Figures 10.08** and **10.09** show that the 30-cm gaps enhanced both seed germination and seedling survival compared to the 15-cm gap or no gap at all. This means that, in effect, the 30-cm gaps promoted greatest recruitment as predicted by the work of Goldberg & Werner (1983), though in straight statistical terms, the differential between 30- and 15-cm gaps was not significant. Because of the relative dominance of *Primula*, the removal of this species from the estimations amplified the result (**Figure 10.08b**), showing significant positive effects of initial gap creation continuing long after gap occlusion. These results reinforce the value of the common agricultural and amenity practice of applying herbicide before sowing or slot seeding (Wells 1983; Wells *et al* 1989; Anderson 1995; Dixie & Bisgrove 1996). In addition, the lack of significant impacts of artificial gap creation on transplantation suggests that in comparison to seed inoculation, the use of transplants *does* mitigate the exclusionary pressures such as competition for light, mineral nutrients, water etc, that seem to impact more directly on sown-seed (Fenner 1985).

With regard to the plot gradient of agricultural improvement, once again seed inoculation confounded expectations derived from the transplant work. One interpretation of **Figures 10.08g&h** is that they show artificial-gaps to be most effective in promoting germination in the grasslands with the lowest productivity (Plots 3 & 4), including in terms of survival to adulthood. Thus, what would be considered the most amenable sward for seedling survival proved to be the least. It is possible, however, that rather than impacts of the competitive environments, the germination results could more accurately reflect patterns in seed predation and disease mortality (Hillier 1990). Perhaps the zero gap treatment best highlights the differentials between plots, as there was no amelioration of adverse competition factors. Consequently, **Figures 10.08g&h**

show a clear linear ranking, with germination success increasing *along* the plot gradient. In contrast, this trend is reversed for the final count of adult plants, suggesting that the environment for germination was most deleterious in the productive plots (Plots 1 & 2), though proved to lower relative to the other plots as the surviving seedlings established. Whereas, for the less productive plots, the relatively open swards were seemed to be less hostile to germination, against expectation, mortality risk increased relative to the productive plots during the establishment phase.

The impact of gaps on seedling recruitment also showed significant effects at the species-level (see **Figure 10.09**). *Centaurea* and *Primula* seem to be the two species that gained the most from gap creation, demonstrating highly significant differences between gap and non-gap recruitment over the three years. In the case of *Centaurea*, gap creation proved to be the crucial factor in establishing populations, as it was extinct in the zero gaps after three years of depletion. This pattern was equally true of *Filipendula* and *Leontodon*, whereas, of course, *Scabiosa*, seemingly the most sensitive phytometer, failed completely. Otherwise, of the remaining four species, *Leucanthemum*, *Lotus* and *Malva*, all evinced highest recruitment in the 30-cm diameter gaps, though not significantly so by the final census.

Grassland management through grazing and/or cutting is the traditional means by which regeneration gaps can be created (Bakker *et al* 1980; Hopkins 1989; Smith *et al* 1996). As with the transplant studies, management did not seem to have had a statistically discernable impact on seed recruitment. In fact **Figure 10.10** shows that for the combined plot totals, there was virtually no difference between the treatments. It is notable, however, that for the two most productive grasslands, the mowed subplots had the highest recruitment, and for the two least productive, cow grazing seemed to be more advantageous. Certainly, overall, mowing produced the shortest swards (see **Chapter 4**), and for the most productive grassland plots this may have had an impact in facilitating sown-seed recruitment. Only Plot 4 showed statistically significant management treatment effects, largely due to very poor germination and establishment in the mown subplot (10). It is not possible to distinguish whether the low seedling counts were related to the management regime, or to other less obvious factors.

Practical implications for grassland enrichment

In theory, sowing seed directly into established swards is one of the cheapest ways of enriching swards (Buckley 1989; Wells *et al* 1989; Luscombe & Scott 1994; Smith *et al* 1997). Whilst this may have particular efficacy in unproductive grasslands (Hillier 1990), under productive conditions the resistance to recruitment from oversowing seed seems to be much greater (Cavers & Harper 1967; Fenner 1978; Ehrlén & van Groenendael 1998). The benefit of the present research is that direct comparisons can be made between enrichment techniques such as sowing seed and using transplants. In pure initial cost terms, using seed was far cheaper than plugs, and particularly pot transplants. For cross-treatment comparisons, the inoculation units used in this project were 50 seeds = 1 plug = 1 pot. Using an averaged quantity of seed per gram (888), at an average cost of £1.00 per gram (Emorsgate Seeds 2001), a dose of 50 seeds calculates at £0.06, and only £5.76 for all doses per subplot, £69 total project cost. For plugs the cost is substantially greater i.e. 12 plugs x £0.25 x 8 species (Dixie & Bisgrove 1994), £24.00 per subplot; £288 total project cost: and for pots transplants: 12 pots x £0.80 x 8 species, £76.80 per subplot, £921 total project cost, substantially more so (Watson & Hack 2000). Therefore plug usage is 31% of pot material cost, and seed only 7.5% of pot cost. These large disparities do not even take into account the labour costs to sow the different media (Handa & Jeffries 2000). As noted in **Chapter 3**, plugs were far easier to plant than pot transplants, and seed even more so, though for the purposes of this experiment, it must be mentioned that it was time consuming counting out the individual 50 seed doses, whilst for non research application a scoop with known seed capacity would speed up efficiency considerably. In order to equate the various techniques, **Table 10.03** displays a quantification of the relative costs of each inoculation medium i.e. one unit equals (*at least*, vis-à-vis seed inoculation) one surviving plant.

Table 10.03 Inoculation cost efficiency: comparative unit costs of pot, plug and seed inoculation. *Filipendula* and *Leontodon* were omitted, because though they were sown in the seed trials, they were not planted in the autumn transplant project due to lack of stock. Pot and plug final counts were doubled in order to approximate to the seed doses (144 per species).

Species	Pot (x 2)	Plug (x 2)	Seed
<i>Centaurea nigra</i>	84	50	15

<i>Leucanthemum vulgare</i>	18	24	15
<i>Lotus corniculatus</i>	58	14	20
<i>Malva moschata</i>	72	24	12
<i>Primula veris</i>	96	78	87
<i>Scabiosa columbaria</i>	0	0	0
Total Units (total cost of units)	328 (£262)	190 (£47)	149 (£9)
Unit Price	£2.10 per unit	£1.14 per unit	£0.34 per unit

The clear finding from this evaluation is that after three years, using seed appears to be far cheaper than using either pot or plug transplants for the same relative unit of enrichment. Sowing seed would also normally be far less expensive than pot or plug implantation in terms of manpower (Wells *et al* 1989; Westbury 2001). In the case of the six species that could be compared, the results seem unequivocal in support for utilising seed in enrichment of depauperate swards. In addition, where conversion is high, using seed, especially in the case of *Primula* and *Centaurea*, can create patches of recruits rather than the limited foci of individual transplants. It is possible that a single-species patch or *phalanx* (Begon *et al* 1990; Gibson 2000) may offer competitive advantages in productive grasslands.

Obviously, the above economic analysis does not do is take into account the relative performance efficacies of the inoculation types, especially in relation to the aim of producing generative adult plants. That is, seed inoculation may be most effective in establishing juvenile plants, whilst transplants which establish are probably more capable of attaining both sexual and vegetative reproduction. Even though *Primula* was very successful as sown seed, surprisingly, *Malva* performed relatively poorly, even though it is one of the few species in the project that has actually managed to naturally recruit new individuals through seed dispersal. In the final 1999 census, the number of flowering sown-seed *Malva* recruits was only 20% of the autumn transplants, with *Leontodon* (18%) and *Centaurea* (12%) even less. Thus, without factoring in cost, the transplants seem to offer more rapid, if not greater restoration efficiency. At least the implantation of adult plants has the generative potential to build up species populations. This potential may be particularly important for the most scarce, stress-tolerant species for which large volumes of seed are expensive and/or difficult to obtain (Tallowin & Smith 2001). Also, seed-derived seedlings have the negative potential of remaining indefinitely as juveniles or vegetative adults, thus remaining relatively neutral in terms of species enrichment. Overall, *Scabiosa* was the

most extreme case, as it failed completely as sown seed, whilst the transplants were at least able to reach flowering and seed dispersal before the populations became extinct (see **Chapter 9**). Unfortunately, no further recruitment was recorded as derived from this copious seed production.

Perhaps the most useful evaluation to emerge from this study concerns the restorative value of artificial competition-free gaps. In plain terms, the implication is that whilst transplants are relatively speaking unassisted by the use of artificial gaps, enrichment using seed seems to be comparatively heavily dependent. In fact, gap creation in productive swards seems to be an essential treatment for successful seed inoculation. Spraying out areas before sowing seems to mitigate the invasion resistance of productive grasslands, and the positive carry-over effect continues long after the gaps have occluded. The largest gap used in this experiment, 30-cm diameter, was also the most successful in terms of recruitment, indicating that in terms of sown-seed at least, size does matter (Bakker *et al* 1980). As mentioned in **Chapter 3**, herbicide treatment was used in preference to other gap creation techniques such as scarification (Westbury 2001) and turf removal (Luscombe & Scott 1994) because of the speed and ease of application. However, in terms of effect, it is possible that in particular, sowing into scaled patches may have an even more significant positive effect on recruitment as nutrient enriched soil is removed, and the speed of gap occlusion likely to be reduced. Pywell *et al* (1997) certainly found that seed sowing in combination with turf stripping was the most effective way to diversify species-depauperate swards. In contrast, the poor recruitment in the zero gaps suggests that under productive conditions, for most species, management treatments, in the short term cannot be relied on to produce sufficiently frequent and large gaps for effective seed infiltration. With reducing productivity, it is hoped and expected that continued traditional management, particularly cow grazing in conjunction with the summer hay cut, would gradually facilitate natural recruitment and self-sustaining populations (Bullock *et al* 1995; Crofts & Grayson 1999; Turnbull *et al* 2000; Coulson *et al* 2001).

ACKNOWLEDGEMENTS

The Usual Suspects: Ian and Edel Davies managed to facilitate the inoculations with undue difficulty from a recalcitrant patient. Ian and Owen Davies continued the surveillance with legitimate quibbles. Lottie supplied the illegitimate quibbles...

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