

**THE DEVELOPMENT OF MULTI-LAYER HERBACEOUS  
PLANT COMMUNITIES FOR USE IN URBAN PARKS  
AND GREEN SPACES**

BY:

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## ABSTRACT

The aims of this study were to develop practices to develop robust multi-layer plant communities for use in urban parks and green spaces. Such communities can provide a strong impact on the lay public through flowers which bloom in spring, summer and autumn. Potentially, these kinds of communities display a spring flowering, a late spring/early summer, and an autumn component. Such herbaceous plant communities have many attributes such as being potentially easier to manage, more resistant to weed invasion, providing greater diversity and providing a dramatic visual impact over a longer period. Plant community development is affected by many factors but competition with other species for light, water and nutrients are particularly important. A series of experiments have been undertaken to work out how to develop multiple layer herbaceous plant communities in urban parks and green spaces. These commenced with seed germination studies in response to different chilling treatments, seedling survival at different sowing ratios and species mixtures, and productivity across soil gradients. A microcosm experiment was conducted to explore the actual competitive relationships between different canopy layers sown in a wide range of ratios. The survivorship of different canopy layer species in 2006 and/or 2007 as a percentage of the number of seedlings in the 2005 data was significantly different ( $P=0.006$ , Kruskal-Wallis test) within the canopy layers, with the medium canopy layer showing the highest survivorship (81.35%), followed by tall (76.65%) and low (62.73%) canopy layers. The growth of different canopy layer plants in 2006 and/or 2007 as a percentage of those present in the 2005 data was significantly different ( $P=0.001$ , Kruskal-Wallis test) between the canopy layers with the tall canopy layers showing the highest percentage increase in biomass than the medium and low canopy layer. To assess the practicality of creating multi-layers by field sowing, a large scale field experiment was conducted. Seed ratios and species mixture for creating multi-layer communities at two different productivities was studied. Many species showed a greater emergence on sand mulch than on clay subsoil mulch. At the end of the first growing season, many *Helenium*, *Phlox*, *Rudbeckia*, *Silphium* plus some individuals of *Aster*, *Echinacea*, *Eupatorium*, *Helianthus* and *Silene* were flowering in the treatment mixes. In terms of their survival and growth performance during the first two years of growth, most of the multi-layer plant communities showed more positive results on sand mulch when compared to clay subsoil mulch treatment.

## DECLARATION

No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or institute of learning.

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# CONTENTS

	<b>Page</b>
Abstract	ii
Table of contents	v
List of figures	xii
List of tables	xxviii
Appendices for figures	xxxi
Appendices for tables	xxxii

## **CHAPTER 1:INTRODUCTION**

<b>1.1 Background</b>	<b>1</b>
<b>1.2 Overall aim of the research</b>	<b>3</b>
<b>1.3 Research questions</b>	<b>4</b>
<b>1.4 Research objectives</b>	<b>4</b>
<b>1.5 Research activities</b>	<b>4</b>

## **CHAPTER 2: REVIEW OF LITERATURE AND PRACTICE: THE BASIS IN CREATION OF MULTI-LAYER HERBACEOUS VEGETATION**

<b>2.1 Application of ecological ideas to urban landscape vegetation</b>	<b>6</b>
<b>2.2 Semi-natural woodland</b>	<b>7</b>
2.2.1 Herbaceous woodland understorey	10
<b>2.3 Prairie vegetation</b>	<b>11</b>
2.3.1 Prairie vegetation in urban landscapes and its ecological strategies	13
<b>2.4 Plant community establishment by sowing seed <i>in situ</i></b>	<b>16</b>
2.4.1 Species diversity	17
<b>2.5 Species competition</b>	<b>18</b>
2.5.1 Type of substrate	20
<b>2.6 C-S-R plant strategies concept</b>	<b>20</b>
<b>2.7 Mechanisms of competition</b>	<b>23</b>
2.7.1 Competition for water	24
2.7.2 Competition for nutrients	24
2.7.3 Competition for light	25
<b>2.8 The effect of competition on community composition</b>	<b>26</b>

## CHAPTER 3: SEED GERMINATION AND CHILLING REQUIREMENTS OF SELECTED HERBACEOUS SPECIES

3.1	<b>Introduction</b>	28
3.1.1	Species selection	29
3.1.1.1	List of the species	29
3.1.2	Objectives	30
3.2	<b>Materials and methods</b>	31
3.2.1	Data collection	32
3.2.1.1	Squash test	32
3.2.2	Statistical analysis	32
3.3	<b>Results</b>	32
3.3.1	<b>Low canopy (understorey species)</b>	32
3.3.1.1	<i>Ajuga reptans</i>	36
3.3.1.2	<i>Anemone nemorosa</i>	36
3.3.1.3	<i>Anemone ranunculoides</i>	36
3.3.1.4	<i>Anemone sylvestris</i> ('Madonna')	36
3.3.1.5	<i>Cordalis solida</i>	36
3.3.1.6	<i>Dodecatheon meadia</i>	39
3.3.1.7	<i>Doronicum orientale</i> 'Magnificum'	39
3.3.1.8	<i>Galium odoratum</i> ( <i>Asperula odorata</i> )	39
3.3.1.9	<i>Glechoma hederacea</i>	39
3.3.1.10	<i>Lathyrus vernus</i>	44
3.3.1.11	<i>Montia sibirica</i>	44
3.3.1.12	<i>Phlox divaricata</i>	44
3.3.1.13	<i>Polemonium reptans</i>	48
3.3.1.14	<i>Primula elatior</i>	48
3.3.1.15	<i>Primula veris</i>	48
3.3.1.16	<i>Primula vulgaris</i>	48
3.3.1.17	<i>Viola labradorica</i>	53
3.3.1.18	<i>Viola odorata</i> 'Konigin Charlotte'	53
3.3.1.19	<i>Viola pedatifida</i>	53
3.3.1.1.1	Squash test	57

3.3.2	<b>Medium canopy</b>	57
3.3.2.1	<i>Aster azureus</i>	59
3.3.2.2	<i>Phlox maculata</i>	59
3.3.2.3	<i>Phlox pilosa</i>	59
3.3.2.4	<i>Solidago speciosa</i>	63
3.3.2.5	<i>Tradescantia ohioensis</i>	63
3.3.2.6	<i>Zizia aptera</i>	63
3.3.2.1.1	Squash test	67
3.3.3	<b>Tall canopy</b>	67
3.3.3.1	<i>Aster laevis</i>	69
3.3.3.2	<i>Aster novae-angliae</i> ‘Septemberrubin’	69
3.3.3.3	<i>Eupatorium maculatum</i>	72
3.3.3.4	<i>Helianthus mollis</i>	72
3.3.3.5	<i>Silphium integrifolium</i>	72
3.3.3.6	<i>Veronicastrum virginicum</i>	76
3.3.3.1.1	Squash test	76
3.4	<b>Discussion</b>	78
3.4.1	Effect of chilling on seed germination	78
3.4.1.1	Germination in understorey species	78
3.4.1.2	Germination in medium canopy species	79
3.4.1.3	Germination in tall canopy species	79
3.4.2	Germination in the fridge during chilling incubation	81
3.4.3	Seed viability	81
3.5	<b>Conclusion</b>	82
<b>CHAPTER 4:MICROCOSM STUDIES INTO SEEDLING SURVIVAL IN DIFFERENT SOWING RATIOS AND SPECIES MIXTURES</b>		
4.1	<b>Introduction</b>	84
4.1.1	Species selection	85
4.1.2	Objectives	86
4.2	<b>Materials and methods</b>	87
4.2.1	Data collection	91
4.2.2	Statistical analysis	92

4.3	<b>Results</b>	93
4.3.1	Field emergence in the microcosm	93
4.3.2	<i>Effect of sowing ratio, density and plant layer in the first year of the growing season (2005)</i>	95
4.3.2.1	Survival and recruitment of forb species from May-September 2005	95
4.3.2.1.1	Low canopy, understorey forbs	95
4.3.2.1.2	Medium canopy forbs	96
4.3.2.1.3	Tall canopy forbs	98
4.3.2.2	Growth (above ground dry weight) of species in September 2005	99
4.3.2.2.1	Low canopy, understorey forbs	99
4.3.2.2.2	Medium canopy forbs	100
4.3.2.2.3	Tall canopy forbs	101
4.3.2.3	Growth (above ground dry weight) of individual plants in September 2005	101
4.3.2.3.1	Low canopy, understorey forbs	101
4.3.2.3.2	Medium canopy forbs	103
4.3.2.3.3	Tall canopy forbs	104
4.3.2.4	Cover value in 2005	105
4.3.3	<i>Effect of sowing ratio, density and plant layer in the second year of the growing season (2006)</i>	106
4.3.3.1	Growth (above ground dry weight) of species in September 2006	106
4.3.3.1.1	Low canopy, understorey forbs	106
4.3.3.1.2	Medium canopy forbs	107
4.3.3.1.3	Tall canopy forbs	107
4.3.3.2	Growth (above ground dry weight) of individual plants in September 2006	108
4.3.3.2.1	Low canopy, understorey forbs	108
4.3.3.2.2	Medium canopy forbs	108
4.3.3.2.3	Tall canopy forbs	110
4.3.3.3	Cover value in year 2006	111
4.3.3.4	Phenology of the species by 2006	115



4.3.4	<i>Effect of sowing ratio, density and plant layer in the third year of the growing season (2007)</i>	117
4.3.4.1	Growth (above ground dry weight) of species in May 2007	117
4.3.4.1.1	Low canopy, understorey forbs	117
4.3.4.1.2	Medium and tall canopy forbs	118
4.3.4.2	Growth (above ground dry weight) of individual plants in May 2007	118
4.3.4.2.1	Low canopy, understorey forbs	118
4.3.4.2.2	Medium and tall canopy forbs	119
4.3.5	<i>Effect of sowing ratio and density for the multi-layer community as a whole (2005-2007)</i>	120
4.3.5.1	Survival of sown forbs as a percentage of those present in 2005	120
4.3.5.1.1	Low canopy, understorey forbs	120
4.3.5.1.2	Medium canopy forbs	121
4.3.5.1.3	Tall canopy forbs	123
4.3.5.2	Density of seedlings between 2005 and 2007	124
4.3.5.3	Growth (above ground dry weight) of species at final harvest as a percentage of harvest weight in 2005	127
4.3.5.3.1	Low canopy, understorey forbs	127
4.3.5.3.2	Medium canopy forbs	128
4.3.5.3.3	Tall canopy forbs	129
4.4	<b>Discussion</b>	131
4.4.1	Seedling emergence in the microcosm	131
4.4.2	The multi-layer herbaceous plant communities as a whole	132
4.4.2.1	Effect of sowing ratio	132
4.4.2.2	Effect of sowing density	133
4.4.3	Growth and survival of species in relation to 2005	134
4.4.4	Aesthetic aspects of the study	137
4.5	<b>Conclusion</b>	137

## CHAPTER 5: FIELD EXPERIMENT INTO MULTI-LAYER COMMUNITY DEVELOPMENT

5.1	<b>Introduction</b>	139
5.1.1	Species selection	141
5.1.2	Objectives	143
5.2	<b>Materials and methods</b>	143
5.2.1	Data collection	148
5.2.2	Statistical analysis	149
5.3	<b>Results</b>	149
5.3.1	Effect of mulch and underlying soil type on seedling emergence	149
5.3.2	Effect of mulch and underlying soil type on seedling emergence of individual species	150
5.3.3	Comparison of field emergence with the previous microcosm experiment	152
5.3.4	Number of seedlings after adjustment	152
5.3.5	Growth in the first year (2006)	158
5.3.5.1	Impact of flowering in 2006	159
5.3.5.2	Cover value in year 2006	160
5.3.6	Effect of the second year growth (2007) on the development of multi-layer community	162
5.3.6.1	Survival of sown forbs in April 2007 as a percentage those present in September 2006	164
5.3.6.1.1	Low canopy, understorey forbs	164
5.3.6.1.2	Medium canopy forbs	165
5.3.6.1.3	Tall canopy forbs	167
5.3.6.2	Survival of individual sown forbs in April 2007 as a percentage of those present in September 2006	169
5.3.6.2.1	Low canopy, understorey forbs	169
5.3.6.2.2	Medium canopy forbs	171
5.3.6.2.3	Tall canopy forbs	173
5.3.6.3	Growth of understorey forb assessed by number of plants in flower in April 2007	174
5.3.6.4	Cover values in the second year (2007)	176
5.3.6.5	Effect of slug grazing by June 2007	180

<b>5.4</b>	<b>Discussion</b>	182
5.4.1	Effect of mulch and topsoil v subsoil on overall percentage emergence in the first year growth (2006)	182
5.4.2	Effect of mulch and topsoil v subsoil on emergence of individual species	183
5.4.3	Effect of mulch and topsoil v subsoil on growth of multi-layer community in the first year growth (2006)	184
5.4.4	Effect of mulch and topsoil/subsoil on plant survival in the second year (2007)	185
5.4.5	Effect of mulch and topsoil/subsoil on growth and flowering of multi-layer community in the second year growing season (2007)	186
5.4.6	Effect of slug predation in 2007	187
5.4.7	Effect of sowing ratios and density on seedling survival and growth (2006-2007)	188
<b>5.5</b>	<b>Conclusion</b>	188

## **CHAPTER 6: OVERALL DISCUSSION AND CONCLUSION**

<b>6.1</b>	<b>Discussion</b>	190
6.1.1	Is it possible to create multi-layer herbaceous vegetation by sowing?	190
6.1.2	Do fast growing species typically eliminate low, slow growing species?	192
6.1.2.1	Effect of sowing ratios	194
6.1.2.2	Effect of density	195
6.1.3	Are multi-layer plant communities visually attractive, low maintenance and sustainable over a longer period?	196
<b>6.2</b>	<b>Conclusions</b>	197
<b>6.3</b>	<b>Recommendation for future research</b>	198

<b>REFERENCES</b>	199
-------------------	-----

<b>APPENDICES</b>	210
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## LIST OF FIGURES

### Chapter 1

- Figure 1.1 Conceptual development of multi-layer herbaceous vegetation in spring, summer and autumn shown in plan and section. Spring species are typically short, autumn species tall and summer species intermediate. ....3
- Figure 1.2 Key phases in the development of multi-layer herbaceous plant communities studies. ....5

### Chapter 2

- Figure 2.1 Profile of semi-natural woodland (adapted from Anonymous (2002))..... 8
- Figure 2.2 Semi-natural woodland understorey dominated by bluebells (*Hyacinthoides non-scripta*) ..... 10
- Figure 2.3 Structure of prairie vegetation (adapted from Steiger, 1930) showing community response to moisture and aspect. Plants are shortest on the southern exposure (on the left of the image)..... 13

### Chapter 3

- Figure 3.1 Germination of *Ajuga reptans* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM. ....37
- Figure 3.2 Number of new emerged seedlings per count of *Ajuga reptans* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. ....37
- Figure 3.3 Mean maximum percentage germination of *Ajuga reptans* seeds in response to chilling treatments. Error bars represent 1 SEM. ....37
- Figure 3.4 Germination of *Anemone sylvestris* ('Madonna') seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM. ....38
- Figure 3.5 Number of new emerged seedlings per count of *Anemone sylvestris* ('Madonna') in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. ....38
- Figure 3.6 Mean maximum percentage germination of *Anemone sylvestris* ('Madonna') seeds in response to chilling treatments. Error bars represent 1 SEM. ....38

- Figure 3.7 Germination of *Dodecatheon meadia* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM. ....40
- Figure 3.8 Number of new emerged seedlings per count of *Dodecatheon meadia* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. ....40
- Figure 3.9 Mean maximum percentage germination of *Dodecatheon meadia* seeds in response to chilling treatments. Error bars represent 1 SEM. ....40
- Figure 3.10 Germination of *Doronicum orientale* ‘Magnificum’ seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM. ....41
- Figure 3.11 Number of new emerged seedlings per count of *Doronicum orientale* ‘Magnificum’ in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. ....41
- Figure 3.12 Mean maximum percentage germination of *Doronicum orientale* ‘Magnificum’ seeds in response to chilling treatments. Error bars represent 1 SEM. ....41
- Figure 3.13 Germination of *Galium odoratum* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM. ....42
- Figure 3.14 Number of new emerged seedlings per count of *Galium odoratum* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. ....42
- Figure 3.15 Mean maximum percentage germination of *Galium odoratum* seeds in response to chilling treatments. Error bars represent 1 SEM. ....42
- Figure 3.16 Germination of *Glechoma hederacea* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM. ....43
- Figure 3.17 Number of new emerged seedlings per count of *Glechoma hederacea* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. ....43
- Figure 3.18 Mean maximum percentage germination of *Glechoma hederacea* seeds in response to chilling treatments. Error bars represent 1 SEM. ....43

- Figure 3.19 Germination of *Lathyrus vernus* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM. ....45
- Figure 3.20 Number of new emerged seedlings per count of *Lathyrus vernus* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. ....45
- Figure 3.21 Mean maximum percentage germination of *Lathyrus vernus* seeds in response to chilling treatments. Error bars represent 1 SEM. ....45
- Figure 3.22 Germination of *Montia sibirica* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM. ....46
- Figure 3.23 Number of new emerged seedlings per count of *Montia sibirica* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. ....46
- Figure 3.24 Mean maximum percentage germination of *Montia sibirica* seeds in response to chilling treatments. Error bars represent 1 SEM. ....46
- Figure 3.25 Germination of *Phlox divaricata* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM. ....47
- Figure 3.26 Number of new emerged seedlings per count of *Phlox divaricata* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. ....47
- Figure 3.27 Mean maximum percentage germination of *Phlox divaricata* seeds in response to chilling treatments. Error bars represent 1 SEM. ....47
- Figure 3.28 Germination of *Polemonium reptans* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM. ....49
- Figure 3.29 Number of new emerged seedlings per count of *Polemonium reptans* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. ....49
- Figure 3.30 Mean maximum percentage germination of *Polemonium reptans* seeds in response to chilling treatments. Error bars represent 1 SEM. ....49

Figure 3.31 Germination of <i>Primula elatior</i> seeds in the growth cabinet at 20/10°C after chilling treatments. Error bars represent 1 SEM. ....	50
Figure 3.32 Number of new emerged seedlings per count of <i>Primula elatior</i> in the growth cabinet after chilling treatments. ....	50
Figure 3.33 Mean maximum percentage germination of <i>Primula elatior</i> seeds in response to chilling treatments. Error bars represent 1 SEM. ....	50
Figure 3.34 Germination of <i>Primula veris</i> seeds in the growth cabinet at 20/10°C after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM. ....	51
Figure 3.35 Number of new emerged seedlings per count of <i>Primula veris</i> in the growth cabinet after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. ....	51
Figure 3.36 Mean maximum percentage germination of <i>Primula veris</i> seeds in response to chilling treatments. Error bars represent 1 SEM. ....	51
Figure 3.37 Germination of <i>Primula vulgaris</i> seeds in the growth cabinet at 20/10°C after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM. ....	52
Figure 3.38 Number of new emerged seedlings per count of <i>Primula vulgaris</i> in the growth cabinet after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. ....	52
Figure 3.39 Mean maximum percentage germination of <i>Primula vulgaris</i> seeds in response to chilling treatments. Error bars represent 1 SEM. ....	52
Figure 3.40 Germination of <i>Viola labradorica</i> in the growth cabinet at 20/10°C after chilling treatments. Error bars represent 1 SEM. ....	54
Figure 3.41 Number of new emerged seedlings per count of <i>Viola labradorica</i> in the growth cabinet after chilling treatments. ....	54
Figure 3.42 Mean maximum percentage germination of <i>Viola labradorica</i> seeds in response to chilling treatments. Error bars represent 1 SEM. ....	54
Figure 3.43 Germination of <i>Viola odorata</i> 'Konigin Charlotte' in the growth cabinet at 20/10°C after chilling treatments. Error bars represent 1 SEM. ....	55
Figure 3.44 Number of new emerged seedlings per count of <i>Viola odorata</i> 'Konigin Charlotte' in the growth cabinet after chilling treatments. ....	55
Figure 3.45 Mean maximum percentage germination of <i>Viola odorata</i> 'Konigin Charlotte' seeds in response to chilling treatments. Error bars represent 1 SEM. ....	55

Figure 3.46 Germination of <i>Viola pedatifida</i> in the growth cabinet at 20/10°C after chilling treatments. Error bars represent 1 SEM. ....	56
Figure 3.47 Number of new emerged seedlings per count of <i>Viola pedatifida</i> in the growth cabinet after chilling treatments. ....	56
Figure 3.48 Mean maximum percentage germination of <i>Viola pedatifida</i> seeds in response to chilling treatments. Error bars represent 1 SEM. ....	56
Figure 3.49 Germination of <i>Aster azureus</i> seeds in the growth cabinet at 20/10°C after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM. ....	60
Figure 3.50 Number of new emerged seedlings per count of <i>Aster azureus</i> in the growth cabinet after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. ....	60
Figure 3.51 Mean maximum percentage germination of <i>Aster azureus</i> seeds in response to chilling treatments. Error bars represent 1 SEM. ....	60
Figure 3.52 Germination of <i>Phlox maculata</i> seeds in the growth cabinet at 20/10°C after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM. ....	61
Figure 3.53 Number of new emerged seedlings per count of <i>Phlox maculata</i> in the growth cabinet after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. ....	61
Figure 3.54 Mean maximum percentage germination of <i>Phlox maculata</i> seeds in response to chilling treatments. Error bars represent 1 SEM. ....	61
Figure 3.55 Germination of <i>Phlox pilosa</i> seeds in the growth cabinet at 20/10°C after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM. ....	62
Figure 3.56 Number of new emerged seedlings per count of <i>Phlox pilosa</i> in the growth cabinet after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. ....	62
Figure 3.57 Mean maximum percentage germination of <i>Phlox pilosa</i> seeds in response to chilling treatments. Error bars represent 1 SEM. ....	62
Figure 3.58 Germination of <i>Solidago speciosa</i> seeds in the growth cabinet at 20/10°C after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM. ....	64



Figure 3.59	Number of new emerged seedlings per count of <i>Solidago speciosa</i> in the growth cabinet after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. ....	64
Figure 3.60	Mean maximum percentage germination of <i>Solidago speciosa</i> seeds in response to chilling treatments. Error bars represent 1 SEM. ....	64
Figure 3.61	Germination of <i>Tradescantia ohioensis</i> seeds in the growth cabinet at 20/10°C after chilling treatments. Error bars represent 1 SEM. ....	65
Figure 3.62	Number of new emerged seedlings per count of <i>Tradescantia ohioensis</i> in the growth cabinet after chilling treatments. ....	65
Figure 3.63	Mean maximum percentage germination of <i>Tradescantia ohioensis</i> seeds in response to chilling treatments. Error bars represent 1 SEM. ....	65
Figure 3.64	Germination of <i>Zizia aptera</i> seeds in the growth cabinet at 20/10°C after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM. ....	66
Figure 3.65	Number of new emerged seedlings per count of <i>Zizia aptera</i> in the growth cabinet after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. ....	66
Figure 3.66	Mean maximum percentage germination of <i>Zizia aptera</i> seeds in response to chilling treatments. Error bars represent 1 SEM. ....	66
Figure 3.67	Germination of <i>Aster laevis</i> seeds in the growth cabinet at 20/10°C after chilling treatments. Error bars represent 1 SEM. ....	70
Figure 3.68	Number of new emerged seedlings per count of <i>Aster laevis</i> in the growth cabinet after chilling treatments. ....	70
Figure 3.69	Mean maximum percentage germination of <i>Aster laevis</i> seeds in response to chilling treatments. Error bars represent 1 SEM. ....	70
Figure 3.70	Germination of <i>Aster novae-angliae</i> 'Septemberrubin' seeds in the growth cabinet at 20/10°C after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM. ....	71
Figure 3.71	Number of new emerged seedlings per count of <i>Aster novae-angliae</i> 'Septemberrubin' in the growth cabinet after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. ....	71
Figure 3.72	Mean maximum percentage germination of <i>Aster novae-angliae</i> 'Septemberrubin' seeds in response to chilling treatments. Error bars represent 1 SEM. ....	71

Figure 3.73 Germination of <i>Eupatorium maculatum</i> seeds in the growth cabinet at 20/10°C after chilling treatments. Error bars represent 1 SEM. ....	73
Figure 3.74 Number of new emerged seedlings per count of <i>Eupatorium maculatum</i> in the growth cabinet after chilling treatments. ....	73
Figure 3.75 Mean maximum percentage germination of <i>Eupatorium maculatum</i> seeds in response to chilling treatments. Error bars represent 1 SEM. ....	73
Figure 3.76 Germination of <i>Helianthus mollis</i> seeds in the growth cabinet at 20/10°C after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM. ....	74
Figure 3.77 Number of new emerged seedlings per count of <i>Helianthus mollis</i> in the growth cabinet after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. ....	74
Figure 3.78 Mean maximum percentage germination of <i>Helianthus mollis</i> seeds in response to chilling treatments. Error bars represent 1 SEM. ....	74
Figure 3.79 Germination of <i>Silphium integrifolium</i> seeds in the growth cabinet at 20/10°C after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM. ....	75
Figure 3.80 Number of new emerged seedlings per count of <i>Silphium integrifolium</i> in the growth cabinet after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. ....	75
Figure 3.81 Mean maximum percentage germination of <i>Silphium integrifolium</i> seeds in response to chilling treatments. Error bars represent 1 SEM. ....	75
Figure 3.82 Germination of <i>Veronicastrum virginicum</i> seeds in the growth cabinet at 20/10°C after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM. ....	77
Figure 3.83 Number of new emerged seedlings per count of <i>Veronicastrum virginicum</i> in the growth cabinet after chilling treatments. (*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. ....	77
Figure 3.84 Mean maximum percentage germination of <i>Veronicastrum virginicum</i> seeds in response to chilling treatments. Error bars represent 1 SEM. ....	77
Figure 3.85 Chilling regimes associated with maximum germination in the study. ....	78

## Chapter 4

- Figure 4.1 Experimental sites at the Tapton Experimental Gardens, University of Sheffield, United Kingdom. .... 89
- Figure 4.2 Arrangement of the treatment plot in the competition experiment. .... 91
- Figure 4.3 Emergence of species in the microcosm as a percentage of their maximum germination in the growth cabinet in the laboratory. .... 93
- Figure 4.4 Effect of multi-layer treatment and density on percentage of survival in low canopy species between May and September 2005. (a) Survival of species in different density treatments. Significant differences (Mann-Whitney *U*-test) between low and high densities are indicated by: ns, not significant. (b) Mean survival of species as pooled across density treatments. Bars labeled with different letters are significantly different at  $P=0.001$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-tests). Error bars represent 1 S.E.M. .... 96
- Figure 4.5 Survival of individual low canopy species in response to different multi-layer treatments. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between treatments are indicated by: \*  $P=0.05$ ; ns, not significant. *Primula elatior* and *Dodecatheon meadia* were excluded from this analysis due to insufficient data. .... 96
- Figure 4.6 Effect of multi-layer treatment and density on percentage of survival in medium canopy species between May and September 2005. (a) Survival of species in different density treatments. Significant differences (Mann-Whitney *U*-test) between low and high densities are indicated by; \*\*\*  $P=0.001$ ; ns, not significant. (b) Mean survival of species as pooled across density treatments. Bars labeled with different letters are significantly different at  $P=0.001$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-test). Error bars represent 1 S.E.M. .... 97
- Figure 4.7 Survival of individual medium canopy species in response to different multi-layer treatments. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between treatments are indicated by: ns, not significant. .... 98
- Figure 4.8 Effect of multi-layer treatment and density on percentage of survival in tall canopy species between May and September 2005. (a) Survival of species in different density treatments. Significant differences (Mann-Whitney *U*-test) between low and high densities is indicated by; ns, not significant. (b) Mean survival of species as pooled across density treatments. Bars labeled with the same letters are not significantly different at  $P=0.394$  (Kruskal-Wallis test). Error bars represent 1 S.E.M. .... 98
- Figure 4.9 Survival of individual tall canopy species in response to different multi-layer treatments. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between treatments are indicated by: \*  $P=0.05$ ; ns, not significant. .... 99

Figure 4.10 Effect of multi-layer treatment and density on biomass of low canopy species per quadrat in September 2005. (a) Total biomass of species in different densities. Significant differences (Mann-Whitney *U*-test) between low and high densities are indicated by: ns, not significant. (b) Total biomass of species as pooled across density treatments. Bars labeled with different letters are significantly different at  $P=0.001$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-tests). Error bars represent 1 S.E.M. .... 100

Figure 4.11 Effect of multi-layer treatment and density on biomass of medium canopy species per quadrat in September 2005. (a) Total biomass of species in different densities. Significant differences (Mann-Whitney *U*-test) between low and high densities are indicated by; \*  $P=0.05$ ; ns, not significant. (b) Total biomass of species as pooled across density treatments. Bars labeled with different letters are significantly different at  $P=0.001$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-tests). Error bars represent 1 S.E.M. .... 100

Figure 4.12 Effect of multi-layer treatment and density on biomass of tall canopy species per quadrat in September 2005. (a) Total biomass of species in different densities. Significant differences (Mann-Whitney *U*-test) between low and high densities are indicated by; \*  $P=0.05$ ; ns, not significant. (b) Total biomass of species as pooled across density treatments. Bars labeled with different letters are significantly different at  $P=0.001$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-tests). Error bars represent 1 S.E.M. .... 101

Figure 4.13 Effect of multi-layer treatment and density on mean biomass of individual plants in low canopy species in September 2005. (a) Mean biomass in response to different multi-layer treatments at different densities. Significant differences (Mann-Whitney *U*-test) between low and high densities are indicated by: ns, not significant. (b) Mean biomass in response to different multi-layer treatments as pooled across density treatments. Bars labeled with the same letters are not statistically different at  $P=0.259$  (Kruskal-Wallis test). Error bars represent 1 S.E.M. .... 102

Figure 4.14 Mean biomass of individual species (g/plant) in response to composition of sowing mix. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between multi-layer treatments are indicated by: ns, not significant. *Primula elatior* and *Dodecatheon meadia* were excluded from this analysis due to insufficient data. .... 102

Figure 4.15 Effect of multi-layer treatment and density on mean biomass of individual plants in medium canopy species in September 2005. (a) Mean biomass in response to different multi-layer treatments at different densities. Significant differences (Mann-Whitney *U*-test) between low and high densities are indicated by; \*  $P=0.05$ ; ns, not significant. (b) Mean biomass in response to different multi-layer treatments as pooled across density treatments. Bars labeled with the same letters are not statistically different at  $P=0.146$  (Kruskal-Wallis test). Error bars represent 1 S.E.M. .... 103

- Figure 4.16 Mean biomass of individual species (g/plant) in response to composition of sowing mix. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between multi-layer treatments are indicated by: \*  $P=0.05$ ; \*\* $P=0.01$ ; \*\*\* $P=0.001$ ; ns, not significant. .... 103
- Figure 4.17 Effect of multi-layer treatment and density on mean biomass of individual plants in tall canopy species in September 2005. (a) Mean biomass in response to different multi-layer treatments at different densities. Significant differences (Mann-Whitney *U*-test) between low and high densities are indicated by; ns, not significant. (b) Mean biomass in response to different multi-layer treatments as pooled across density treatments. Bars labeled with different letters are significantly different at  $P=0.001$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-tests). Error bars represent 1 S.E.M. .... 104
- Figure 4.18 Mean biomass of individual species (g/plant) in response to composition of sowing mix. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between multi-layer treatments are indicated by: \*  $P=0.05$ ; \*\* $P=0.01$ ; \*\*\* $P=0.001$ ; ns, not significant. .... 105
- Figure 4.19 Effect of seed ratios and sowing density on cover values (a) low v high density (b) mean values for both density in September 2005. Bars labeled with different letters are significantly different at  $P=0.001$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-test). Error bars represent 1 S.E.M. .... 105
- Figure 4.20 Effect of multi-layer treatment and density on biomass of low canopy species per quadrat in September 2006. (a) Total biomass of species in different densities. Significant differences (Mann-Whitney *U*-test) between low and high densities are indicated by: ns, not significant. (b) Total biomass of species as pooled across density treatments. Bars labeled with different letters are significantly different at  $P=0.001$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-tests). Error bars represent 1 S.E.M. .... 106
- Figure 4.21 Effect of multi-layer treatment and density on biomass of medium canopy species per quadrat in September 2006. (a) Total biomass of species in different densities. Significant differences (Mann-Whitney *U*-test) between low and high densities are indicated by: ns, not significant. (b) Total biomass of species as pooled across density treatments. Bars labeled with different letters are significantly different at  $P=0.01$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-tests). Error bars represent 1 S.E.M. .... 107
- Figure 4.22 Effect of multi-layer treatment and density on biomass of tall canopy species per quadrat in September 2006. (a) Total biomass of species in different densities. Significant differences (Mann-Whitney *U*-test) between low and high densities are indicated by ns, not significant. (b) Total biomass of species as pooled across density treatments. Bars labeled with different letters are significantly different at  $P=0.01$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-tests). Error bars represent 1 S.E.M. .... 108
- Figure 4.23 Effect of multi-layer treatment and density on mean biomass of individual plants in medium canopy species in September 2006. (a) Mean biomass in response to different multi-layer treatments at different densities. Significant differences (Mann-Whitney *U*-test) between low and high densities are

indicated by; ns, not significant. (b) Mean biomass in response to different multi-layer treatments as pooled across density treatments. Bars labeled with different letters are not significantly different at  $P=0.05$  (Kruskal-Wallis test, pairwise Mann-Whitney  $U$ -tests). Error bars represent 1 S.E.M. .... 109

Figure 4.24 Mean biomass of individual species (g/plant) in response to composition of sowing mix. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between multi-layer treatments are indicated by: \*  $P=0.05$ ; \*\* $P=0.01$ ; \*\*\* $P=0.001$ ; ns, not significant. .... 109

Figure 4.25 Effect of multi-layer treatment and density on mean biomass of individual plants in tall canopy species in September 2006. (a) Mean biomass in response to different multi-layer treatments at different densities. Significant differences (Mann-Whitney  $U$ -test) between low and high densities are indicated by; ns, not significant. (b) Mean biomass in response to different multi-layer treatments as pooled across density treatments. Bars labeled with the same letters are not significantly different at  $P=0.05$  (Kruskal-Wallis test). Error bars represent 1 S.E.M. .... 110

Figure 4.26 Mean biomass of individual species (g/plant) in response to composition of sowing mix. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between multi-layer treatments are indicated by: \*  $P=0.05$ ; \*\* $P=0.01$ ; \*\*\* $P=0.001$ ; ns, not significant. .... 111

Figure 4.27 Effect of weeks and multi-layer herbaceous plant communities treatments on cover in March-June 2006. Error bars represent 1 S.E.M. .... 112

Figure 4.28 (a) Cover values (>70%) representing T1 (Understorey), T4 (Mix, understorey dominant), T5 (Mix, tall canopy dominant) and T6 (Mix equivalent understorey, medium, tall) in this study (18th May 2006). (b) Cover values (<60%) representing T2 (Medium canopy) and T3 (Tall canopy) in this study (18th May 2006). This coverage may allow weed seedlings to invade. .... 114

Figure 4.29 Effect of seed ratios and sowing density on cover values (a) low v high density (b) mean values for both density in May 2005. Bars labeled with the same letters are significantly different at  $P=0.001$  (Kruskal-Wallis test). Error bars represent 1 S.E.M. .... 114

Figure 4.30 Effect of seed ratios and sowing density on cover values (a) low v high density (b) mean values for both density in September 2006. Bars labeled with the same letters are not significantly different ( $P=0.493$ , Kruskal-Wallis test). Error bars represent 1 S.E.M. .... 115

Figure 4.31 Effect of multi-layer treatment and density on biomass of low canopy species per quadrat in May 2007. (a) Total biomass of species in different densities. Significant differences (Mann-Whitney  $U$ -test) between low and high densities are indicated by: ns, not significant. (b) Total biomass of species as pooled across density treatments. Bars labeled with different letters are significantly different ( $P<0.01$ ; Kruskal-Wallis test, pairwise Mann-Whitney  $U$ -tests). Error bars represent 1 S.E.M. .... 117

- Figure 4.32 Effect of multi-layer treatment and density on mean biomass of individual plants in low canopy species in May 2007. (a) Mean biomass in response to different multi-layer treatments at different densities. Significant differences (Mann-Whitney *U*-test) between low and high densities are indicated by; ns, not significant. (b) Mean biomass in response to different multi-layer treatments as pooled across density treatments. Bars labeled with different letters are not significantly different at  $P=0.05$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-tests). Error bars represent 1 S.E.M..... 118
- Figure 4.33 Mean biomass of individual species (g/plant) in response to composition of sowing mix. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between multi-layer treatments are indicated by: \*  $P=0.05$ ; \*\* $P=0.01$ ; \*\*\* $P=0.001$ ; ns, not significant. .... 119
- Figure 4.34 (a) Survival of different canopy layer plants in 2006 as a percentage of number of seedlings in 2005. Error bars represent 1 S.E.M. Low layer plant was excluded from this analysis due to insufficient data. Bars labeled with the same letters are not significantly different ( $P=0.243$  ns, Mann-Whitney *U*-test). (b) Survival of low canopy layer plants in 2007 as a percentage of number of seedlings in 2005. Medium and tall canopy layer plants were excluded from this analysis due to insufficient data. Error bars represent 1 S.E.M..... 120
- Figure 4.35 Effect of multi-layer treatments on survival of low species in 2007 as a percentage of number of seedlings in 2005. Error bars represent 1 S.E.M. ( $P=0.795$  ns, Kruskal-Wallis test). .... 121
- Figure 4.36 Survival of individual low canopy species in response to different multi-layer treatments. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between treatments are indicated by: ns, not significant. *Dodecatheon meadia* was excluded from this analysis due to insufficient data. .... 121
- Figure 4.37 Effect of multi-layer treatments on survival of medium species in 2006 as a percentage of number of seedlings in 2005. Error bars represent 1 S.E.M. ( $P=0.151$  ns, Kruskal-Wallis test). *Tradescantia ohioensis* was excluded from this analysis due to insufficient data..... 122
- Figure 4.38 Survival of individual medium canopy species in response to different multi-layer treatments. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between treatments are indicated by: \*  $P=0.05$ ; ns, not significant. *Tradescantia ohioensis* was excluded from this analysis due to insufficient data. .... 122
- Figure 4.39 Effect of multi-layer treatments on survival of tall species in 2006 as a percentage of 2005. Bars labeled with different letters are significantly different at  $P=0.008$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-tests). Error bars represent 1 S.E.M..... 123
- Figure 4.40 Survival of individual tall canopy species in response to different multi-layer treatments. Significant differences (Kruskal-Wallis test) between

treatments are indicated by: \* P=0.05; ns, not significant. Error bars represent 1 S.E.M. .... 123

Figure 4.41 (a) Dry weight of different canopy layer plants in 2006 as a percentage of their survival in 2005. \* Dry weight of low canopy layer was excluded from statistical analysis as it does not produce a realistic assessment of canopy mass due of loss foliage in these species by harvest time. Error bars represent 1 S.E.M. Bars labeled with different letters are significantly different at P=0.001 (Kruskal-Wallis test, pairwise Mann-Whitney *U*-test). (b) Dry weight of low canopy layer plants in 2007 as a percentage of their survival in 2005. \* Dry weight of medium and tall canopy layers was excluded from statistical analysis as it does not produce a realistic assessment of canopy mass in these species by harvest time..... 127

Figure 4.42 (a) Effect of multi-layer treatments on dry weight per total individual of low canopy species in 2006 as a percentage of 2005. Error bars represent 1 S.E.M. (P=0.715 ns; Kruskal-Wallis test). (b) Effect of multi-layer treatments on dry weight per individual of low canopy species in 2007 as a percentage of 2005. Error bars represent 1 S.E.M. (P=0.789 ns; Kruskal-Wallis test)..... 128

Figure 4.43 Dry weight of individual low canopy species in response to different multi-layer treatments. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between treatments are indicated by ns, not significant. .... 128

Figure 4.44 Effect of multi-layer treatments on dry weight per individual of medium species in 2006 as a percentage of 2005. Error bars represent 1 S.E.M. (P=0.826 ns; Kruskal-Wallis test). .... 129

Figure 4.45 Dry weight of individual medium canopy species in response to different multi-layer treatments. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between treatments are indicated by ns, not significant. .... 129

Figure 4.46 Effect of multi-layer treatments on dry weight per individual of tall species in 2006 as a percentage of 2005. Error bars represent 1 S.E.M. (P=0.161 ns; Kruskal-Wallis test)..... 130

Figure 4.47 Dry weight of individual tall canopy species in response to different multi-layer treatments. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between treatments are indicated by: \* P=0.05; ns, not significant. .... 130

## Chapter 5

Figure 5.1 Arrangement of the treatment plot in the productivity experiment..... 144

Figure 5.2 Cut and fill process to obtained subsoil (unproductive treatment) in the study. .... 145



Figure 5.3	Experimental site at the Lower Walkley, Sheffield, United Kingdom; (a) Plywood frames was used to prevent ‘seed blow’ during sowing, (b) Overall views of treatment plots. ....	146
Figure 5.4	Summary of seedling emergence (mean of all species) in response to mulch type and soil underneath. Error bars represent 1 S.E.M.....	149
Figure 5.5	Percentage emergence of individual sowing mix species in response to mulching type. Error bars represent 1 S.E.M. Significant differences (Mann-Whitney <i>U</i> -test) between sand and subsoil mulch are indicated by; * <i>P</i> =0.05; ** <i>P</i> =0.01; *** <i>P</i> =0.001; ns, not significant. ....	150
Figure 5.6	Field emergence of species as a percentage of their maximum emergence in the microcosm experiment. ....	152
Figure 5.7	Creating multi-layer communities on different productivity and mulches during the first year of growing season; (A) the plot was treated with sand mulch and subsoil mulch; showing seedling start to growth and (B,C) the same site in September 2006, showing most of prairie species start to producing flowers.....	158
Figure 5.8	The same experiment in the first year of growing season, showing how medium-tall shade intolerant plant compete with each other to develop attractive multi-layer communities.....	158
Figure 5.9	<i>Andropogon gerardii</i> reached up to 150 cm in height (October 2006).....	158
Figure 5.10	Some of the prairie forbs were producing flowers in year one; (a) <i>Aster azureus</i> , (b) <i>Aster novae-angliae</i> , (c) <i>Echinacea purpurea</i> , (d) <i>Eupatorium maculatum</i> , (e, f) <i>Helenium autumnale</i> , (g) <i>Helianthus mollis</i> , (h) <i>Phlox amplifolia</i> , (i) <i>Silene regia</i> , (j, k) <i>Rudbeckia</i> spp., (l) <i>Silphium integrifolium</i> . All images were taken in September 2006.....	159
Figure 5.11	Effect of seed ratios and sowing density on cover values for both densities in October 2006. Bars labeled with the same letters are not significantly different at <i>P</i> =0.05 (Kruskal-Wallis test). Error bars represent 1 S.E.M. ...	160
Figure 5.12	Effect of soil type and mulch treatments on percentage cover values of multi-layer plant communities in October 2006. Error bars represent 1 S.E.M.....	161
Figure 5.13	Effect of multi-layer treatments and mulch on topsoil/ subsoil on multi-layer cover in October 2006. Error bars represent 1 S.E.M.....	162
Figure 5.14	The experimental plot in the second growing season; (A) most of the forbs are dormant in winter, (B,C) the same site showing some of the understorey forbs start to grow and flower in April 2007 and (D,E) most of the prairie forbs grow vigorously from May to June 2007.....	163
Figure 5.15	Effect of seed ratios and sowing density on low canopy plant survival in April 2007 (mean values for both densities). Bars labeled with the same	

- letters are not significantly different at  $P=0.05$  (Kruskal-Wallis test). Error bars represent 1 S.E.M. .... 164
- Figure 5.16 Effect of substrate on percentage survival of the understorey plant community in April 2007. Bars labeled with different letters are significantly different at  $P=0.05$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-test). Error bars represent 1 S.E.M. .... 165
- Figure 5.17 Effect of mulching type on percentage survival of the understorey plant community in April 2007. Bars labeled with the same letters are not significantly different at  $P=0.05$  (Mann-Whitney *U*-test). Error bars represent 1 S.E.M. .... 165
- Figure 5.18 Effect of seed ratios and sowing density on medium canopy plant survival in April 2007 (mean values for both densities). Bars labeled with the same letters are not significantly different at  $P=0.05$  (Kruskal-Wallis test). Error bars represent 1 S.E.M. .... 166
- Figure 5.19 Effect of substrate on percentage survival of medium canopy plant communities in April 2007. Bars labeled with different letters are significantly different at  $P=0.01$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-test). Error bars represent 1 S.E.M. .... 167
- Figure 5.20 Effect of mulching type on percentage survival of medium canopy plant communities in April 2007. Bars labeled with different letters are significantly different at  $P=0.001$  (Mann-Whitney *U*-test). Error bars represent 1 S.E.M. .... 167
- Figure 5.21 Effect of seed ratios and sowing density on tall canopy plant survival in April 2007 (mean values for both densities). Bars labeled with the same letters are not significantly different at  $P=0.05$  (Kruskal-Wallis test). Error bars represent 1 S.E.M. .... 168
- Figure 5.22 Effect of substrate on percentage survival of tall canopy plant communities in April 2007. Bars labeled with different letters are significantly different at  $P=0.01$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-test). Error bars represent 1 S.E.M. .... 169
- Figure 5.23 Effect of mulching type on percentage survival of tall canopy plant communities in April 2007. Bars labeled with different letters are significantly different at  $P=0.001$  (Mann-Whitney *U*-test). Error bars represent 1 S.E.M. .... 169
- Figure 5.24 Effect of substrate/mulch type on number of plant of individual low species in April 2007 as a percentage of those present in August 2006. Significant differences (Kruskal-Wallis test) between productivity gradient are indicated by: \* $P=0.05$ ; \*\* $P=0.01$ ; ns, not significant. Error bars represent 1 S.E.M. .... 170
- Figure 5.25 Effect of mulching type on number of plant of individual low species in April 2007 as a percentage of those present in August 2006. Significant differences (Kruskal-Wallis test) between sand and subsoil mulched are

indicated by: \*P=0.05; \*\*P=0.01; \*\*\*P=0.001; ns, not significant. Error bars represent 1 S.E.M. .... 171

Figure 5.26 Effect of substrate type on number of plant of individual medium species in April 2007 as a percentage of those present in August 2006. Significant differences (Kruskal-Wallis test) between productivity gradient are indicated by: \*P=0.05; \*\*P=0.01; ns, not significant. Error bars represent 1 S.E.M..... 172

Figure 5.27 Effect of mulching type on number of plant of individual medium species in April 2007 as a percentage of those present in August 2006. Significant differences (Kruskal-Wallis test) between sand and subsoil mulched are indicated by: \*P=0.05; \*\*P=0.01; \*\*\*P=0.001; ns, not significant. Error bars represent 1 S.E.M. .... 172

Figure 5.28 Effect of substrate type on number of plant of individual tall species in April 2007 as a percentage of those present in August 2006. Significant differences (Kruskal-Wallis test) between productivity treatments are indicated by: \*P=0.05; \*\*P=0.01; ns, not significant. Error bars represent 1 S.E.M..... 173

Figure 5.29 Effect of mulch type on number of plant of individual tall species in April 2007 as a percentage of those present in August 2006. Significant differences (Kruskal-Wallis test) between sand and subsoil mulches are indicated by: \*P=0.05; \*\*P=0.01; \*\*\*P=0.001; ns, not significant. Error bars represent 1 S.E.M. .... 174

Figure 5.30 Effect of weeks and treatment mixes on plant coverage in April-May 2007. Error bars represent 1 S.E.M..... 177

Figure 5.31 Effect of seed ratios and sowing density on cover values for both densities in May 2007. Bars labeled with the same letters are not significantly different at P=0.05 (Kruskal-Wallis test). Error bars represent 1 S.E.M. ... 178

Figure 5.32 Effect of soil type and mulch treatments on percentage cover values of multi-layer plant communities in May 2006. Error bars represent 1 S.E.M. .... 179

Figure 5.33 Effect of multi-layer treatments and mulch on topsoil/ subsoil on multi-layer cover in May 2007. Error bars represent 1 S.E.M..... 180

Figure 5.34 The effect of predation during the establishment of plants in the second year growing (May 2007); plants growth is much more abundant on sand mulch (left view) than on subsoil mulch (right view) in part due to slug grazing. .... 182

## LIST OF TABLES

### Chapter 2

Table 2.1	Structural types of semi-natural woodland and their characteristics from a landscape architects perspective (Gustavsson, 2004)	9
Table 2.2	Commonly cultivated prairie species and their preferred growing conditions (Curtis, 1959; Hitchmough, 2004)	12
Table 2.3	Relative palatability of some adult prairie species to slug and snails when emerging in spring (from Hitchmough (2004)).	14
Table 2.4	Combination of productivity and disturbance resulting in three basic plant strategies (source from Grime (2001)).	21
Table 2.5	Some of the plant growth characteristics based on the primary CSR strategy.	22

### Chapter 3

Table 3.1	Plant species chosen for the study based on its flowering. Native species marked with an asterisk (*).	30
Table 3.2	Effect of duration of chilling on germination of understorey species at 20/10°C in a growth cabinet. Maximum germination is indicated by bold type.	33
Table 3.3	Effect of duration of chilling on germination of understorey species in the fridge during chilling at 4°C. Maximum germination is indicated in bold.	34
Table 3.4	Germination of understorey species in a growth cabinet after adequate chilling treatments, and during chilling incubation in a fridge.	35
Table 3.5	Percentage viability of seed in understorey species according to squash test after 90 days chilling. Viability was assessed after 30 days observation post placement in the growth cabinet.	57
Table 3.6	Effect of duration of chilling on germination of medium forbs at 20/10°C in a growth cabinet. Maximum germination is indicated by bold type.	57
Table 3.7	Effect of duration of chilling on germination of medium forbs in the fridge during chilling at 4°C. Maximum germination is indicated in bold.	58
Table 3.8	Germination of medium canopy species in a growth cabinet after adequate chilling treatments, and during chilling incubation in a fridge.	59
Table 3.9	Percentage viability of seed in medium canopy species according to squash test after 90 days chilling. Viability was assessed after 30 days observation post placement in the growth cabinet.	67

Table 3.10	Effect of duration of chilling on germination of tall forbs at 20/10°C in a growth cabinet. Maximum germination is indicated in bold. ....	67
Table 3.11	Effect of duration of chilling on germination of tall forbs in the fridge during chilling at 4°C. Maximum germination is indicated in bold. ....	68
Table 3.12	Germination of tall canopy species in a growth cabinet after adequate chilling treatments, and during chilling incubation in a fridge. ....	69
Table 3.13	Percentage viability of seed in tall canopy species according to squash test after 90 days chilling. Viability was assessed after 30 days observation post placement in the growth cabinet. ....	76

## Chapter 4

Table 4.1	Understorey and prairie forbs species used in the study .....	86
Table 4.2	Ratios in terms of seed sown for each plant functional group tested in this study. ....	87
Table 4.3	Actual seed sown for each species per treatment community in each microcosm tray (0.24m <sup>2</sup> ). ....	88
Table 4.4	Actual number of seedling per quadrat (500 x 300 mm) for each species in each replicate after adjustment. ....	90
Table 4.5	Comparison of field emergence in the microcosm and germination in the laboratory. ....	94
Table 4.6	Flowering phenology of each species under multi-layer communities in the second growing year of this study. ....	116
Table 4.7	Density of low species at different counted years; 2005, 2006 and 2007. <i>P</i> -values refer to the difference in seedling numbers between July 2005 and the final census date (May 2007). ....	124
Table 4.8	Density of medium species at different counted year; 2005, 2006 and 2007. <i>P</i> -values refer to the difference in seedling numbers between July 2005 and the final census date (May 2007). ....	125
Table 4.9	Density of tall species at different counted year; 2005, 2006 and 2007. <i>P</i> -values refer to the difference in seedling numbers between July 2005 and the final census date (May 2007). ....	126
Table 4.10	Total biomass (g) for each plant layer groups per quadrat, based on data shown in Figures 4.10, 4.11,4.12, 4.20,4.21,4.22 and 4.31. Dry weight at the peak of standing biomass is indicated by bold type. ....	135

## Chapter 5

Table 5.1	Understorey and prairie forbs, and prairie grass species used in the study	142
Table 5.2	Physical and chemical properties of the soil types/mulch used in the experiment (source from Hitchmough et. al. (2004)).	143
Table 5.3	Different sowing ratios for each plant functional group tested in this study.	146
Table 5.4	Estimation of seed numbers for each species with different combination treatments and densities per subplot (0.6m <sup>2</sup> ).	147
Table 5.5	Percentage emergence of individual species in response to soil type underneath when sown in sand and subsoil mulch.	151
Table 5.6	Comparison of field emergence with microcosm experiment.	153
Table 5.7	Comparison of target number of plants per replicate and those actually present (in parentheses) in August 2006.	154
Table 5.8	Mean percentage of plants in flower (pooled across density treatments) of understorey forb in response to different productivity gradient.	175
Table 5.9	Numbers of slug on the experiment plot on a wet and a dry day in June 2007.	181
Table 5.10	Categorisation of forbs in terms of treatment that best aided survival between 2006 and 2007, based on statistical significance shown in Figures 5.13, 5.16 and 5.19.	186
Table 5.11	Categorisation of forbs in terms of treatment that best aided establishment, based on plant flower in Table 5.9, plus statistical significance shown in Figures 5.13, 5.16 and 5.19.	187

## APPENDICES FOR FIGURES

### Chapter 4

- Figure A4.1 The illustrated of understorey forb seedlings at early establishment in the microcosm experiment. ....217
- Figure A4.2 The illustrated of medium canopy seedlings at early establishment in the microcosm experiment. ....218
- Figure A4.3 The illustrated of tall canopy seedlings at early establishment in the microcosm experiment. ....219
- Figure A4.4 Some of the emerged seedlings of high density treatment (4000 seed/m<sup>2</sup>) at early establishment in the microcosm experiment. Site photographs on the 9th May 2005. ....220

### Chapter 5

- Figure A5.1 Effect of seed ratios and sowing density on cover values; low v high density in October 2006. Bars labeled with the same letters are not significantly different (Mann Whitney *U*-test,  $P > 0.05$ ). Error bars represent 1 S.E.M. .221
- Figure A5.2 Effect of seed ratios and sowing density on low canopy plant survival in April 2007 (low v high density). Bars labeled with the same letters are not significantly different (Mann Whitney *U*-test,  $P > 0.05$ ). Error bars represent 1 S.E.M. ....222
- Figure A5.3 Effect of seed ratios and sowing density on medium canopy plant survival in April 2007 (low v high density). Bars labeled with the same letters are not significantly different (Mann Whitney *U*-test,  $P > 0.05$ ). Error bars represent 1 S.E.M. ....223
- Figure A5.4 Effect of seed ratios and sowing density on tall canopy plant survival in April 2007 (low v high density). Bars labeled with the same letters are not significantly different (Mann Whitney *U*-test,  $P > 0.05$ ). Error bars represent 1 S.E.M. ....224
- Figure A5.5 Effect of substrate type on number of plant of individual low species in April 2007 as a percentage of those present in August 2006. Significant differences (Kruskal-Wallis test) between treatment mixes are indicated by: \* $P = 0.05$ ; ns, not significant. Error bars represent 1 S.E.M. ....225
- Figure A5.6 Effect of substrate type on number of plant of individual medium species in April 2007 as a percentage of those present in August 2006. Significant differences (Kruskal-Wallis test) between treatment mixes are indicated by: \* $P = 0.05$ ; ns, not significant. Error bars represent 1 S.E.M. ....226

Figure A5.7 Effect of substrate type on number of plant of individual tall species in April 2007 as a percentage of those present in August 2006. Significant differences (Kruskal-Wallis test) between treatment mixes are indicated by: \*P=0.05; ns, not significant. Error bars represent 1 S.E.M.....227

Figure A5.8 Effect of seed ratios and sowing density on cover values; low v high density in May 2007. Bars labeled with the same letters are not significantly different (Mann Whitney U-test, P>0.05). Error bars represent 1 S.E.M. .228

## APPENDICES FOR TABLES

### Chapter 4/5

Table A4.1 Ecological characteristics of the species used in the experiment (Curtis, 1959; Lang, 1985; Hitchmough et al., 2001; Hendy, 2005; Nearctica, 2005; Prairiemoon, 2005; USDA, 2006), plus the observations of the author. Ecological strategy assessment derived from Grime (2001).....211

Table A5.1 Mean percentage of number of plant flower across all treatment mixes of understory forb in response to different productivity gradient at different densities.....216



## CHAPTER 1: INTRODUCTION

---

### 1.1 Background

Vegetation plays an important role in creating the urban landscape, representing nature and responding to aesthetic, ecological and recreational needs in the urban environment. There is currently much interest in perennial herbaceous plants (forbs) as components of urban plantings. People appreciate the beauty of ornamental herbaceous plants, with often showy flowers in various colours, sometimes fragrant and sustained for long periods. Perennial plant communities that provide good impact through flowers in spring, summer and autumn are most likely to be liked by the public. At present, significant resources are required to develop and manage cultivated plantings of this type in urban areas such as public parks, streets, children's playground and other places. Peoples are often interested in planting that changes over time in terms of plant structure and floristic characteristics. These changes are needed to avoid users or visitors to the area sensing monotony or feeling unexcited.

Allocations of funds and resources for the maintenance of landscape plantings remain static or have decreased (CABE Space, 2004). As such, action needs to be taken so that the resources needed for future plantings can be minimised whilst still allowing the provision of attractive vegetation. This might involve plant communities that do not require any fertilizer, are resistant to pests, diseases, and weed invasion and not requiring any watering beyond the establishment period.

One approach to addressing the above issues involves implementing an ecological approach to landscape planting. This approach is explained by Dunnett and Hitchmough (2004) in the '*The Dynamic Landscape: Design, Ecology and Management of Naturalistic Urban Planting*'. However, in practice, herbaceous plant communities which are naturalistic, dynamic, long flowering and sustainable are still not fully explored. In landscape practice, most urban vegetation is planted as a series of monocultural "patches". Whilst these are often relatively easy to maintain, they are sometimes monotonous and prone to loss if a disease epidemic or severe climatic conditions are experienced. The main weakness of this type of planting is however that it is difficult to maintain a lengthy floral display from one or a few species. Thus, the

application of ecological principles to create vegetation communities based on many species may prove to be more sustainable. These designed herbaceous plant communities resemble semi-natural plant communities in structure and general appearance.

Several research studies on the characteristics of “naturalistic” herbaceous plant communities used in urban landscape have been published (Hitchmough and Woudstra, 1999; Hitchmough, 2000). However, these are largely concerned with plant communities that are designed to consist of one layer only, which limits the range of species present and hence the possible duration of the flowering season.

Many spring flowering herbaceous plants are low growing woodland species, for example, *Primula vulgaris* and *Primula elatior* occur in open and shaded habitats (Whale, 1984). Species flowering in late spring and early summer are often associated with under-canopy layers, for example *Dodecatheon meadia* and *Tradescantia ohioensis*. In temperate regions late summer flowering is often associated with tall North American prairie species such as *Helianthus mollis*, *Silphium integrifolium* and *Veronicastrum virginicum* while many *Aster* and *Solidago* species flower in autumn (Curtis, 1959; Hitchmough et al., 2004).

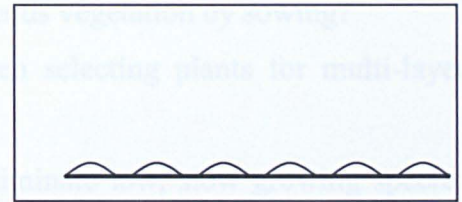
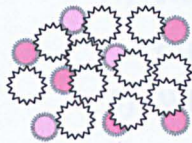
There is a strong case for developing plant communities with multiple layers. These kinds of communities potentially have a spring flowering layer (normally low growing species) a later spring-early summer component, and an autumn component. These ideas are shown graphically in Figure 1.1. In this context, multi-layer herbaceous plant communities potentially have many attributes such as being potentially easier to manage, providing more vegetation diversity per unit area, and giving dramatic visual impact over a longer period.

A layer of foliage at ground level, then layers of foliage above this, are likely to make it much more difficult for weeds to invade, as more of the potential gaps are occupied. Hence vegetation with this kind of structure is likely to be easier to manage and more likely to persist under low maintenance. However, to date there are no published accounts of attempts to create plantings with these structural characteristics.

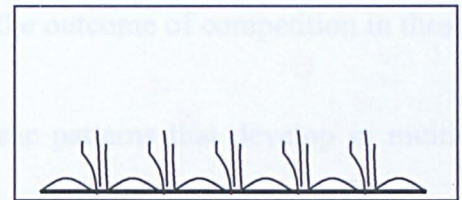
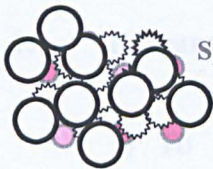
## 1.3 Research questions

**SPRING LAYER**

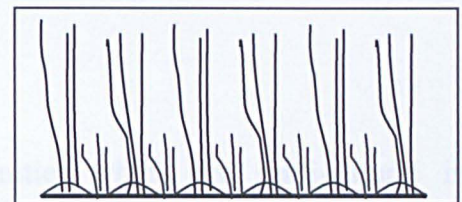
Spring layer in full sun

**SUMMER LAYER**

Spring layer in semi shade

**AUTUMN LAYER**

Spring and summer layers in shade



**Figure 1.1** Conceptual development of multi-layer herbaceous vegetation in spring, summer and autumn shown in plan and section. Spring species are typically short, autumn species tall and summer species intermediate.

By mixing selected species drawn from understorey vegetation subject to heavy shade during the summer, with species from tall grass prairie vegetation, it should theoretically be possible to create a multiple layered plant community which flower at different growing seasons and which is sustainable for long periods. These kinds of community mirror the existing structure of woodland and prairie ecosystems.

## 1.5 Research activities

**1.2 Overall aim of the research**

To provide information to assist in the development of robust multi-layer herbaceous plant communities for use in urban parks and green spaces.

## 1.5 Research activities

Three main activities were carried out in the laboratory (Chapter 3). Secondly, microcosm studies into seedling survival at different sowing ratios and species mixtures (Chapter 4), and thirdly, field experiment into multi-layer community development across productivity gradients have been conducted (Chapter 5).

### 1.3 Research questions

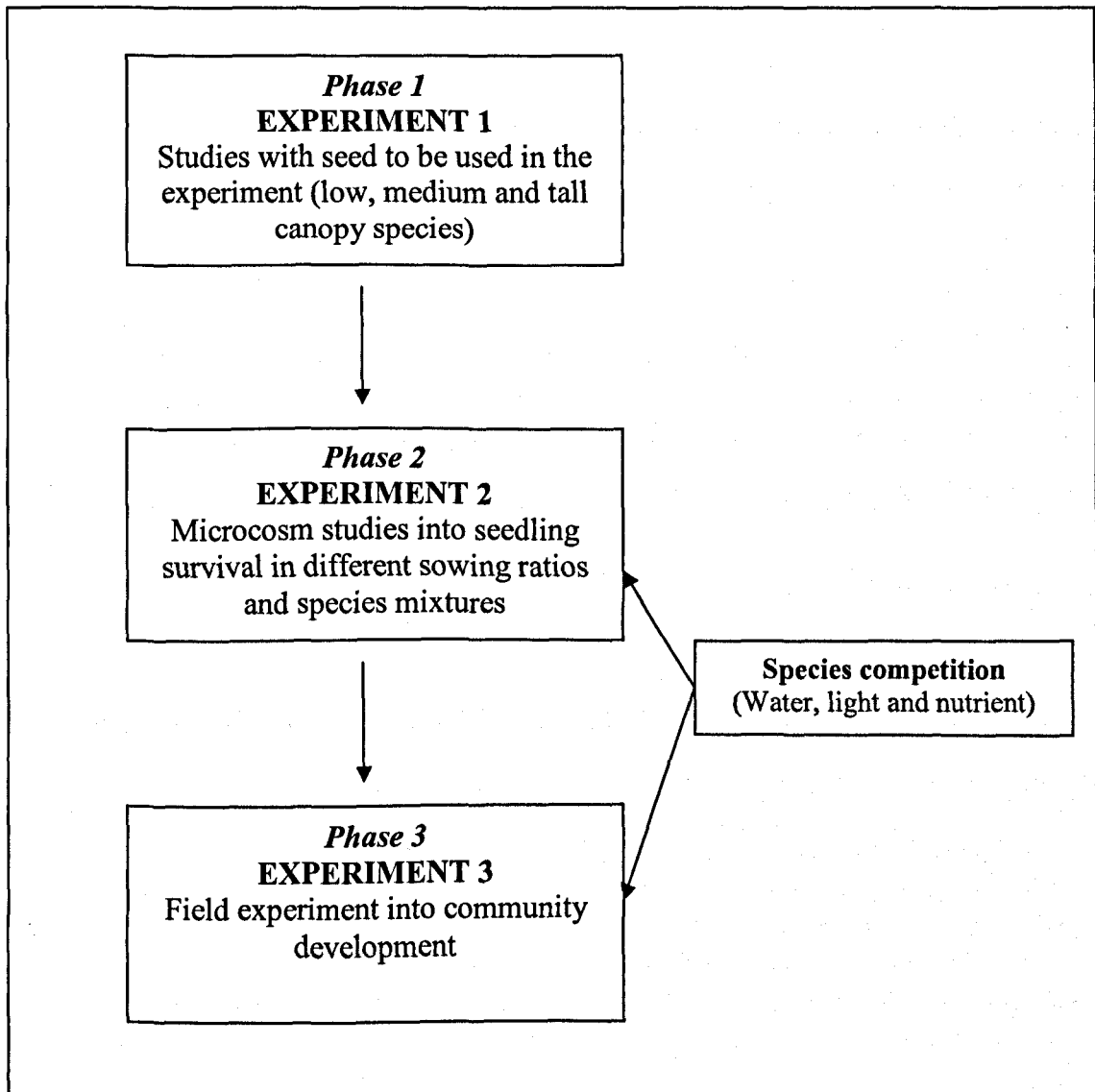
- 1) Is it possible to create multi-layer herbaceous vegetation by sowing?
- 2) What criteria should be considered when selecting plants for multi-layer communities?
- 3) Do tall fast growing species typically eliminate low, slow growing species and how is this affected by the ratio of species present?
- 4) How does substrate and predation affect the outcome of competition in these experiment communities?
- 5) What are the structural forms and floristic patterns that develop in multi-layer communities?

### 1.4 Research objectives

- 1) To develop multi-layer plant communities which are semi-natural in appearance, visually attractive, low maintenance and sustainable over a longer period.
- 2) To determine a suitable ratios and species mixtures for creating multi-layer plant communities.
- 3) To monitor and characterize growth and flowering habits of multi-layer herbaceous plant communities.
- 4) To study performance and species competition in multi-layer plant communities on different substrates.

### 1.5 Research activities

A series of experiments were undertaken to assess how to construct multi-layer herbaceous plant communities in urban parks and green spaces. Three major experiments were conducted to achieve the aim and objective of this study (see Figure 1.2). Firstly, a study on seed germination in response to different chilling treatments was carried out in the laboratory (**Chapter 3**). Secondly, microcosm studies into seedling survival at different sowing ratios and species mixtures (**Chapter 4**), and thirdly, field experiment into multi-layer community development across productivity gradients have been conducted (**Chapter 5**).



**Figure 1.2** Key phases in the development of multi-layer herbaceous plant communities studies.

## **CHAPTER 2: REVIEW OF LITERATURE AND PRACTICE: THE BASIS IN CREATION OF MULTI-LAYER HERBACEOUS VEGETATION**

---

### **2.1 Application of ecological ideas to urban landscape vegetation**

Currently, interest in applying ideas about sustainability in traditional landscape practice and the ecological approach in urban landscape practice both have a high profile. The application of ecological theories to urban landscape vegetation covers a wide range of ideas. This includes the development of plant communities with the intention of maintaining species richness and complexity whilst allowing regeneration with minimum input which can be sustained over time (Hitchmough, 2004). Ecological ideas about planting design are often based on the premise that 'nature knows best' (Hull and Robertson, 2000; Anonymous, 2004), and most of these approaches have long been implemented over time mainly with wildflowers and other native species.

To realise this concept in practice, information on the ecological characteristics of each ornamental species (such as phenology, plant canopy, habitat, distribution and other aspects) is required. Hitchmough et al., (2004) and Kircher (2004) have explored plant selection for urban landscapes. Seedling establishment has also been explored by Hitchmough et al., (2001; 2004) and plant habitat requirements by Whale (1984) and Grime et al. (1988). Dunnett et al. (1998; 2004), Hitchmough and de La Fleur (2006) has focused on the dynamic nature of plant communities whilst Hunningher (2001) investigated the ecology of garden plants.

In landscape practice, understanding of a wide range of plant species suitable for the development of a structurally sound plant community and a more sustainable and environmental friendly landscape design is still lacking. Most of the literature is aimed at plant ecologists rather than designers or managers and gives only basic information about plants: for example about shade tolerance/intolerance (Whale, 1984; Grime, 2001), distribution and habitat (Halliday and Elkington, 1981; Pigott, 1981; Sjors, 1981; Archibold, 1995). The mechanisms of how plants interact with each other, in particular in different layered communities of herbaceous and prairie species, are poorly understood. The same is true of competition between species in shade/sun with limited resources. Therefore, determining the 'rules' of competition between species during

establishment is needed to assist in developing effective, long-term herbaceous planting in the future.

## 2.2 Semi-natural woodland

Lang (1985) stated that '*woodlands seldom consist of a single species of trees, but a mixture of many, with an understorey of lesser shrubs, and a ground flora*'. Natural woodland communities, consists of species that have evolved over thousands of years in a particular region, and often contains a wide diversity of native species (Pigott, 1981; Lang, 1985; Archibold, 1995; Lauver et al., 1999). In Britain woodlands often contain long naturalised species that are now considered as natives, for example *Galanthus nivalis* (Pigott, 1981). Kendle and Rose (2000) reported that (in the context of Britain): '*a native plant is one that has arrived before neolithic times, or has arrived since without human agency*'.

The flora of many European woodland is profoundly influenced by human management practices and have been for 1000's years. Generally, woodland vegetation can be classified by structural (height and spacing) and floristic (species present) characteristics. This classification has been commonly used to described natural woodland vegetation structure and components present (Pigott, 1981; Lauver et al., 1999; Gustavsson, 2004; McElhinny et al., 2005). This classification covers the wide range of woodland types in Europe and North America temperate vegetation.

In woodland, trees are obviously the dominant plants, but a shrub layer and a ground layer of grasses, sedges, ferns, forbs and geophytes are also present (see Figure 2.1). Normally, herbaceous plants that grow in the ground layer are shade-tolerant species. They can survive in the lower light levels beneath closed canopy trees. The natural distribution of these species is closely associated with soil and climatic conditions (Struik and Curtis, 1962; Brattons, 1976; Thompson, 1980; Peterson and Rolfe, 1982; Archibold, 1995). In Britain for example, *Primula veris* is common in drier sites on clay soil, chalk and limestone (Lang, 1985), *Primula vulgaris* is common in damper clay soil (Lang, 1985; Archibold, 1995), *Anemone nemorosa* is often found in moist and wet soils (Shirreffs, 1985) and *Hyacinthoides non-scripta* is common in drier sites (Archibold, 1995). Similarly, in North American woodland ecosystems, a forb rich

perennial herbaceous flora is present in the understorey (Levin, 1967; Turner and Quarterman, 1968; Gilliam et al., 1995). *Dodecatheon meadia* for example, is often found in open woods, moist meadows and prairies (Turner and Quarterman, 1968), and *Phlox divaricata* is common in rich soils of moist woodlands, but also exists in meadows or on rocky slopes (Levin, 1967).

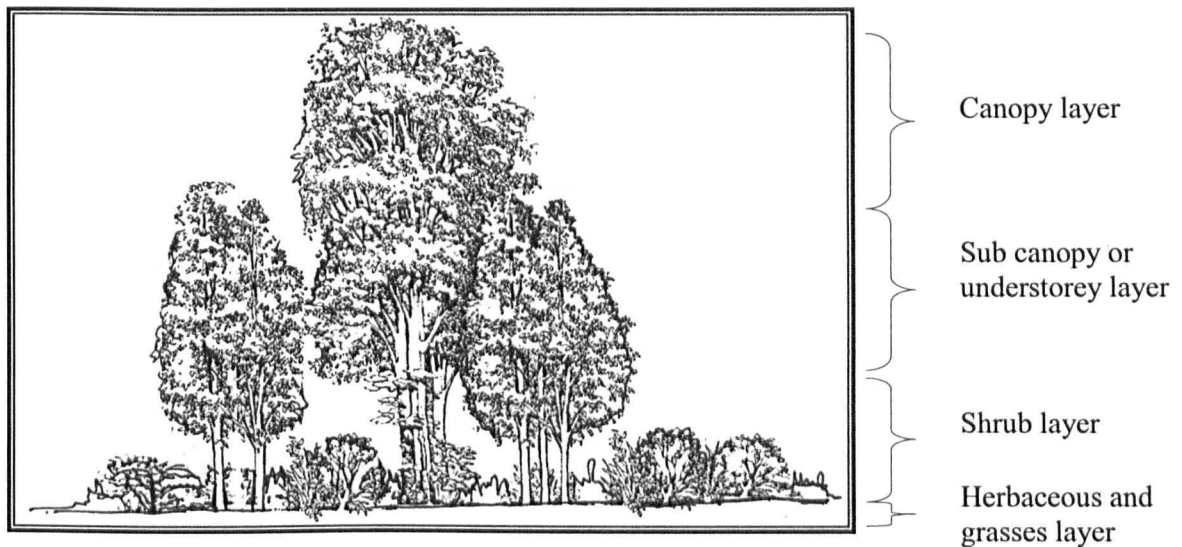


Figure 2.1 Profile of semi-natural woodland (adapted from Anonymous (2002)).

Original woodland in Britain was dominated by lime 5000 years ago (Peterken, 1996). Nowadays, semi-natural woodland may be dominated by oak, ash, beech and hornbeam (Pigott, 1981; Peterken, 1996). Hornbeam-oak woods for example, grow mainly on moist to wet soil (Peterken, 1996). In North America, temperate forest ecosystems are varied and may be dominated by oak-hickory, oak-chestnut, oak-pine, beech-maple, maple-basswood, mixed mesophytic, western mesophytic, southeastern evergreen and hemlock-white pine-northern hardwood (Archibold, 1995). From a landscape-ecological perspective, semi-natural woodland can be developed, and divided into different structural types and characteristics as shown in Table 2.1.

In a woodland system, many species compete for resources such as light, water and nutrients. Tall canopy layer species with deep root systems obtain nutrient resources from deeper in the soil or from soil outside the canopy (Scholes, 1990; Archibold, 1995). In deciduous woodland, in autumn most leaves are shed and decompose to make available nutrient resources for other members of the plant community (Archibold, 1995). Plants with shallow root systems obtain nutrients from the soil surface. Nutrient recycling in this way makes woodland system sustainable in the longer term.



**Table 2.1 Structural types of semi-natural woodland and their characteristics from a landscape architects perspective (Gustavsson, 2004).**

Woodland structure	Basic characteristics	Key species
Dark high woodlands; one storey high stand	Homogeneity, dominated by one tree species. Tall canopy layer.	Beech, maple, lime, elm, hornbeam and horse chestnut.
Light high woodland	Homogeneity, one species or a mixing of two extra species. i.e. birch and oak, or birch and wild cherry. Sub canopy layer; small trees/shrubs.	Poplar, birch, ash, pine, oak and cherry.
Many layered woodland	Species-rich with multiple-layered components.	Upper tree layer: ash, oak and aspen. Low tree layer: lime, rowan, whitebeam, hornbeam, beech, wild cherry, bird cherry, maple and hazel.
Low woodlands (low stands)	Low woodland with multi-stemmed trees and high shrubs.	Hazel, lime, <i>Salix</i> spp., hawthorn, hornbeam, oak, beech, rowan, ash, maple, elm, birch and bird cherry. Exotic species; <i>Pterocarya</i> , <i>Hamamelis</i> , etc.
Shrub based system	Combination planting of high and low shrubs.	Light demanding shrub species; blackthorn and roses. Shade-tolerant species: <i>Ribes alpinum</i> , <i>Sambucus</i> , <i>Virbunum opulus</i> , etc.
Half-open land and small-scale mosaics	Interactive system planting. Open grown. Informal/formal patterns. Evenly or unevenly spread trees/shrubs over a grassland/meadow area.	Tree characters; light-giving, small and narrow-crowned with attractive flowers. Oaks, beeches, hornbeams, lime trees and maples, and exotics; horse chestnut and sweet chestnut.
Edge types	Edge types are varied. Three-staged edge with an outdrawn profile to a one-staged edge.	Specific plant communities of the edge zones; suitable in different climates and soil types.

In temperate ecosystems, the seasonal changes in the woodland trees influence understorey layer species. As the tree canopy leafs out in spring and becomes fully expanded in summer the amount of the light transmission to the ground layer, which is reflected in seed germination, and the survival and vegetative growth of understorey species, declines dramatically. Incident radiation (light transmission) at the woodland floor decreases to <10% when the canopy closes (Archibold, 1995). The light intensities interferences under the canopy layer much depends on the types and angles of the leaves

(Koike, 1989), and the angle of the sun (Anderson et al., 1969; Mitchell and Woodward, 1987).

### 2.2.1 Herbaceous woodland understorey

In semi-natural woodlands there is generally a ground layer in the woodland structure which consists of various forbs and grasses that mostly bloom in spring. Some are evergreen and some are deciduous in summer. The phenology of these woodland understorey species are associated with the seasonality of changes in the tree canopy. Many forbs in the ground layer are dormant in winter but start growing and blooming in the early spring before the tall canopy layer trees develop (Archibold, 1995). Grasses and forbs compete for space within this layer sometimes leading to multiple-layered communities (Garcia-Albarado, 2005). These communities can become very species rich and sometimes visually exciting, especially in spring (Kingsbury, 2004). As a result of the dense shade and moisture stress generated by the canopy, the ground layer is often seasonal, with many species entering dormancy in summer to be replaced by bare soil or leaf litter. This is the case with geophytes such as bluebells (*H. non-scripta*) (Figure 2.2) and wood anemone (*A. nemorosa*).



**Figure 2.2** Semi-natural woodland understorey dominated by bluebells (*Hyacinthoides non-scripta*).

Artificial manipulation of shade takes place in coppiced woodlands, but generally the understorey forbs persist because the low light levels and competition for soil moisture

in summer prevent them being excluded by taller more productive herbaceous plants (Archibold, 1995; Gilliam et al., 1995; Grime, 2001).

Plants associated with very low light levels, under a closed canopy layer are able to reduce their growth rates (Grime, 2001). Evergreen species like *P. vulgaris* often grow very slowly at very low light levels in summer and tolerate severe drought by reducing their foliage to a small rosette (Whale, 1984; Valverde and Silvertown, 1995). North American shade-tolerant herbaceous species (*Trillium grandiflorum* and *Solidago flexicaulis* for example) also demonstrate lower photosynthetic rates under shaded conditions.

Some vernal species (defined as species that flower at approximately the same period that the deciduous tree layers develop into leaf) are however are extremely shade sensitive and drought intolerant. By growing in winter, and blooming in spring and then going dormant in summer these species avoid both shade and drought. This is the case with *H. non-scripta* (Pigott, 1990).

The various types of understorey plant strategies for survival have been investigated. *Anemone nemorosa* (Shirreffs, 1985) and *Primula elatior* (Lang, 1985) for example, like moist soils during their spring growing period but tolerate the soil drying out in summer when they are dormant. *Primula veris* is evergreen and hence has to tolerate drought in the summer whilst in leaf, i.e. it's a drought tolerator rather than a drought avoider as in the first two. *Primula vulgaris* loses most of its leaves in summer in dry situations (Whale, 1984), but keeps them in moist conditions. This is an intermediate strategy with aspects of both drought tolerance and avoidance.

### 2.3 Prairie vegetation

The word 'prairie' originates from the French word meaning a meadow. This is a North American plant community dominated by tall grasses and forbs. In a contemporary urban design sense, it has come to refer in Britain to any designed plant community of medium to tall herbaceous plants, irrespective of geographical origin. Prairie was historically found from the Rocky Mountains to the Appalachians and from South Canada to Texas. It is associated with open habitat, on wet, mesic and dry soils (Curtis,

1959) and has developed over the past 10,000 years in response to regular aboriginal burning (Pauly, 1997). Prairie consists of perennial forbs and grasses, referred to as C4 grasses which are more drought tolerant than C3 grasses (Steiger, 1930; Hitchmough, 2004). The distribution and composition of prairie is determined by the soil moisture, although some species commonly persist across a wide range of moisture conditions, for example *Aster laevis* and *Monarda fistulosa* (Hitchmough, 2004). Based on soil moisture, prairie can be divided into different types; dry prairie, moist prairie (mesic) and wet prairie (hydric) (Curtis, 1959). Examples of the prairie species commonly occurring under different moisture regimes are given in Table 2.2.

**Table 2.2** Commonly cultivated prairie species and their preferred growing conditions (Curtis, 1959; Hitchmough, 2004).

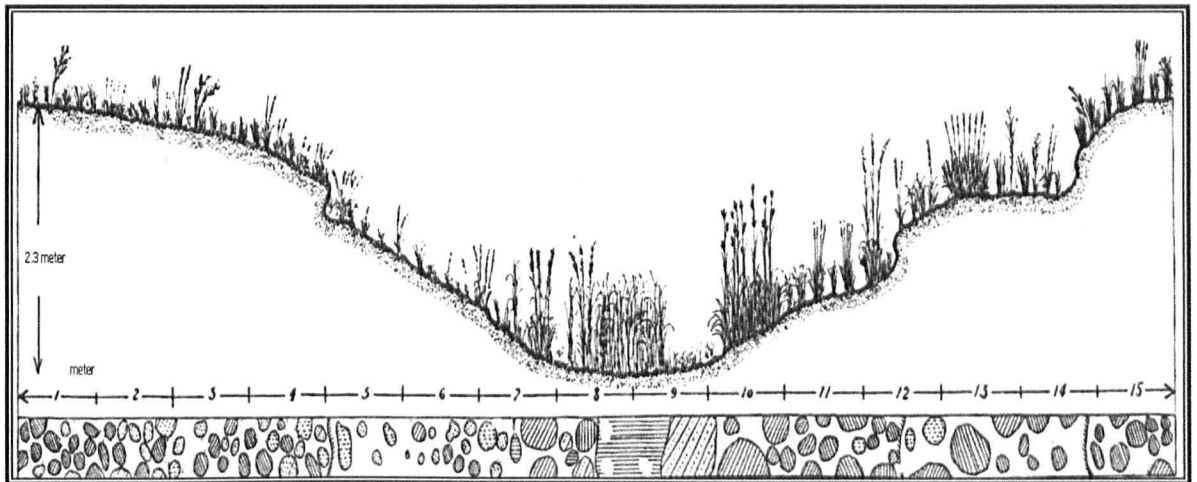
Dry prairie	Moist prairie	Wet prairie
<i>Amorpha canescens</i>	<i>Andropogon gerardii</i> *	<i>Aster laevis</i>
<i>Anemone cylindrica</i>	<i>Aster laevis</i>	<i>Aster nova-angliae</i>
<i>Aster laevis</i>	<i>Baptisia australis</i>	<i>Dodecatheon meadia</i>
<i>Coreopsis palmata</i>	<i>Dodecatheon meadia</i>	<i>Eupatorium maculatum</i>
<i>Euphorbia corollata</i>	<i>Echinacea pallida</i>	<i>Helenium autumnale</i>
<i>Helianthus laetiflorus</i>	<i>Echinacea purpurea</i>	<i>Monarda fistulosa</i>
<i>Phlox pilosa</i>	<i>Monarda fistulosa</i>	<i>Rudbeckia subtomentosa</i>
<i>Monarda fistulosa</i>	<i>Silphium integrifolium</i>	<i>Solidago ohioensis</i>
<i>Solidago rigida</i>	<i>Solidago speciosa</i>	<i>Spartina pectinata</i> *
<i>Sporobolus heterolepis</i> *	<i>Sporobolus heterolepis</i> *	<i>Veronicastrum virginicum</i>

\* Grasses

Individual prairies differ in height and peak flowering, depending on species and growing seasons. Flowering on dry and mesic prairies commences in spring (Curtis, 1959) with the shooting star (*D. meadia*), downy plox (*Phlox pilosa*), spiderwort (*Tradescantia ohioensis*) and prairie violet (*Viola pedatifida*). Species such as *T. ohioensis* will die down by midsummer. Coneflower (*Echinacea pallida*) and compass plant (*Silphium* spp.) flower in midsummer and are followed in autumn by aster (*Aster* spp.), goldenrods (*Solidago* spp.) and sunflowers (*Helianthus* spp.) (Curtis, 1959). As a general rule, the later a species flowers in the year, the taller it grows.

The structure of prairie vegetation consists of different plant architecture as shown in Figure 2.3. This structure depends on edaphic (especially soil moisture) and aerial factors (light and evaporation rate) in the environment (Steiger, 1930). The tallest

species are associated with the moisture soils, as soils become drier and less fertile prairie species become much less tall and the community more open.



**Figure 2.3** Structure of prairie vegetation (adapted from Steiger, 1930) showing community response to moisture and aspect. Plants are shortest on the southern exposure (on the left of the image).

### 2.3.1 Prairie vegetation in urban landscapes and its ecological strategies

In Britain many species that are naturally found in prairie vegetation in North America have been widely used in urban landscape development and as plants in the garden since the C19th (Thomas, 1976). These species play an important role because they provide flowers in summer and autumn when most European species do not. These are a mainstream of contemporary European gardening (Oudolf and Kingsbury, 1999; Kingsbury, 2004). The appreciation and awareness of nature like planting determined by designers has caused this type of urban planting to become increasingly popular in Britain. For example, prairie species from the genera *Aster*, *Echinacea*, *Euphorbia*, *Helianthus*, *Liatris*, *Petalosporum*, *Ratibida*, *Rudbeckia*, *Silphium* and *Solidago* have been successfully cultivated in the development of semi-naturalistic herbaceous plantings for the Eden Project, Cornwall (Hitchmough, 2004).

Prairie species ‘well fitted’ to the British climate with attractive flowers include *A. laevis*, *Eupatorium maculatum*, *Helianthus mollis*, *Rudbeckia fulgida*, *Rudbeckia subtomentosa*, *Silphium integrifolium* and *Veronicastrum virginicum* (Hitchmough, 2004). These species are tolerant of competition, robust but non-invasive. Generally, they flower in summer and autumn. This period of flowering is useful in the UK, because a lot of public activities are conducted in urban parks and green spaces at this time of year.

The disadvantage of prairie type plantings is that to persist they require husbandry practices (when implemented on an extensive scale in public space) such as burning or spraying with a defoliant herbicide in spring. The burning technique is practiced between March and April to remove leaf debris and eliminate molluscs and annual weeds which compete with the forbs (Hitchmough, 2004). However, this unfamiliar practice is not suitable in all urban landscapes.

Recently studies have been conducted into the establishment of prairie species in this country as a plant community rather than as individual species. This has dealt with seedling establishment and long-term community development (Hitchmough et al., 2004; Hitchmough and de La Fleur, 2006). The main findings of this research are as follows:

- The North American prairie vegetation can be established by field sowing.
- Slugs and snails must be controlled during the emergence period to establish prairie vegetation community in urban parks.
- Sand mulching reduces slug grazing in spring and minimises weed germination in the first growing season, hence facilitating prairie seedling dominance.

An important factor in the long-term persistence of prairie plants in Britain is palatability to slugs and snails (Table 2.3). Many species disappear after a few years due to repeated grazing of their foliage in spring.

**Table 2.3** Relative palatability of some adult prairie species to slug and snails when emerging in spring (from Hitchmough (2004)).

Highly unpalatable	Intermediate palatable	Highly palatable
Grasses (most species)	<i>Aster laevis</i>	<i>Echinacea purpurea</i>
<i>Helianthus cvs</i>	<i>Aster novae-angliae</i>	<i>Liatris aspera</i>
<i>Helianthus mollis</i>	<i>Baptisia australis</i>	<i>Ratibida pinnata</i>
<i>Rudbeckia fulgida</i> var. <i>deamii</i>	<i>Echinacea pallida</i>	
<i>Silphium integrifolium</i>	<i>Eupatorium maculatum</i>	
<i>Veronicastrum</i>	<i>Rubecckia</i>	
	<i>Solidago</i>	

Hitchmough (2004) noted that as a general rule ‘as seedlings age they become increasingly less palatable, due to increases in the concentrations of various chemical substances and, in some cases, morphological features, such as surface hairs’. For example, *H. mollis* (Hitchmough, 2004) and *Trollius europaeus* (Hitchmough, 2003) are highly palatable as young seedlings, but are unpalatable to and/or tolerant at molluscs as adult plants. However, some species (*Echinacea purpurea* for example) remain palatable as adults (Hitchmough and de La Fleur, 2006) and hence disappear unless specific management activities that disadvantage molluscs are undertaken. During the early emergence stage the density of these species can probably be increased and persist in the longer term.

Persistence of cultivated prairie communities (consisting of C3 forbs and C4 grasses) is also affected by individual plant interactions and changes due to seasonality. Tall warm-season prairie grasses (C4), are dormant in winter. They enter dormancy in September to October and emerge in April (Fowler, 1981). They have slow growth in spring but are vigorous from mid summer till autumn. As a result, during mild winters in the British climate, the planting surface in prairies is bare, with dead grass and this facilitates weed emergence, usually C3 grasses (cool-season). This happens because of the cool-season; plants normally break dormancy in September to October and continue to grow until May to June (Fowler, 1981). Therefore, when prairie forbs are used in the landscape, a winter evergreen plant which can cover bare soil surface during the winter season may be valuable. In order to achieve this, Hitchmough (2004) has suggested a non competitive and winter evergreen, shade tolerant species like *P. vulgaris* as an understorey species to restrict weed invasion.

Tall prairie species like *Coreopsis tripteris* and *V. virginicum* can create structural communities up to 200 cm (Hitchmough et al., 2004) which provides shelter and shading to the lower species. The space beneath tall prairie species is therefore similar to the space beneath trees and shrubs in a woodland so it may be possible to mix tall grass prairie (tall canopy) and woodland species (low canopy) to create multiple-layer communities and a new “synthetic” type of urban designed vegetation.

By combining two groups of plants with different phenology such as low growing winter green and taller winter dormant plants it may be possible to create a desired

ecological community in which weed invasion is reduced, a longer season of interest for people is generated, more opportunities are provided for wildlife and the whole is more sustainable and manageable. However, in landscape practice this approach has not yet been explored. Very simple versions of this sort of planting has been studied as 'horticultural inter-cropping systems' in relation to specific objectives such as biomass production (Hallam et al., 2001), suppressing weeds (Weiner et al., 2001), species dynamics in mixtures (Park et al., 2001; Gathumbi et al., 2004) and exploring dynamic and sustainable systems (Jolliffe, 1997). Information on indicators and experimental approaches in 'inter-cropping' research are discussed by Connolly (2001).

#### 2.4 Plant community establishment by sowing seed *in situ*

The establishment of herbaceous plant communities by sowing seeds *in situ* (planting seed directly in the soil) is one method for creating semi-natural vegetation in urban spaces. It has been demonstrated for herbaceous perennials by Hitchmough (2004) and Kircher (2004). Hitchmough et al., (2004) demonstrated that North American prairie vegetation could be established by field sowing on an urban site in Northern England.

There are several advantages to sowing herbaceous vegetation by seed *in situ*; it is much cheaper, produces a fluid, naturalistic effect and moreover it is sustainable. However, the disadvantages are that it requires specialized skills to weigh out small quantities of seed, treatment to overcome seed dormancy, calibrated sowing equipment and good control during the germination stage (Hitchmough, 2004).

The seeds of some herbaceous perennials are dormant and need chilling to germinate. They also require appropriate environmental factors such as moist soil and suitable temperatures for germination. On productive soil some species, especially slower germinating seeds may not survive due to competition with weed seedlings (Hitchmough, 2004).

The most effective technique to overcome seed dormancy is winter chilling *in situ*. Seeds are sown from autumn through to early winter and allowed to chill in the soil. Following germination sown seeds compete with each other and with weeds. Without control of fast growing weeds, the slower growing and shade intolerant species will be



eliminated. Hitchmough and de La Fleur (2004) provide evidence that by using coarse sand as a surface mulch, reduce weed invasion and greatly increased the survival of seedlings in the first growing season.

In landscape practice, the creation of naturalistic herbaceous plant communities by sowing *in situ* is potentially more problematic than conventional planting, due to the need to control seedling weeds (Hitchmough, 2004). Usually weeds are aggressive, have faster growth rates than the sown species, and compete more effectively for light, water and nutrients. Therefore, sowing density may play an important role in determining the plant community structure.

Plant communities with a high density of sown seedlings are potentially more resistant to weed competition. There is evidence that the advantage of size in competition increases with density, so weeds will be more suppressed at higher sown densities than at lower ones (Weiner et al., 2001). As sown communities mature and the seedlings become adult, density will potentially reduce weed colonization and multi-layered structures might aid this process. Low light intensity under a multi-layered canopy may suppress weed colonization due to deteriorating light for photosynthesis.

#### 2.4.1 Species diversity

Species diversity can be defined as '*the number of species present in a community*' (Menge and Sutherland, 1976). Many researchers (Barbault, 1995; Rookwood, 1995; Grime, 2001) have discussed the impact of species diversity on the behavior of plant communities. Tilman (1999) proposes for example that a multi-species are commonly more productive than monocultures. At higher plant diversity, the complete use of limiting resources is achieved. In the end, this 'mechanism' leads to a greater number of species present. Brown and Bugg (2001) and Tilman et al. (1997) suggests however that as additional species are added the contribution to productivity and other aspects of ecosystem functioning diminishes.

Dunnett (2004) and Barbault (1995) suggest that a high diversity of species in vegetation is more able to maintain species richness and to enhanced exploitation of plant resources (such as water, light and nutrients) than single species. Mixing diverse

species may offer the widest selection of plants able to adapt to all possible ecological conditions; such as droughts, frost, fires, floods, no snow or heavy grazing (Grime, 2001). It also maintains ecosystem functioning, such as nutrient cycling, water relations and genetic diversity. In addition, by growing multi-species together, particularly unpalatable species (Hitchmough, 2004), invertebrate (in particular slugs and snails) predation may be less problematic as increased spatial complexity within the vegetation may limit the capacity of predators to locate the most palatable species.

High plant species diversity also produces attractive patterns due to changes in space and time (Dunnett, 2004). This leads to more “products” on offer, exhibiting a long season of flowering and possibly attracting more fauna. To achieve this, species diversity is determined by the intensity of species competition, stress and/or disturbance (Grime, 2001). The relationship between species richness and productivity is typically ‘hump-shaped’ or unimodal, particularly in plant communities. In landscape practice, competition between species can cause low species diversity in designed communities of plants.

Plant diversity and species richness are often used to mean the same thing in the long-term, habitat conditions with minimal environmental stress and disturbance should be avoided due to this favouring aggressive competitor species. In general, intermediate environmental stress and disturbance are favourable conditions to maintaining species diversity. These two environmental factors; stress and disturbance are discussed in section 2.7.

## **2.5 Species competition**

In general, competition is an interaction between two individual plants that reduces the fitness of one or both of them. Fitness is usually measured in terms of growth rate or most importantly, reproductive output, for example by mean yield of a population (Mead, 1968). Competition happens when individual species are negatively affected by competing for limited resources (water, light and nutrients).

Species competition falls into two basic categories; interspecific and intraspecific. Interspecific competition means interactions between individuals of different species.

Intraspecific competition means interactions between individuals of the same species. Competition theory is usually applied to individuals, but the consequences scale-up to the level of community and ecosystem. Competition can be defined as '*the tendency of neighbouring plants to utilize the same quantum of light, ion of mineral nutrient, molecule of water, or volume of space*' (Grime, 1979). Competition theory shows how it is possible for any number of competing species to exist in a given area, depending upon the level and kind of interspecific trade between them.

Competition may involve either or both of above ground (shoot competition) (Brenda and Robert, 1997; Haugland and Tawfiq, 2001) and below ground (root) competition (Brenda and Robert, 1997). In newly sown vegetation, competition is initially concentrated in the root zone for water and nutrients. As the cultivated plant grows, shoot competition for light and space become more significant. The capacity of individual community plants to compete successfully for given resources depends on the plant characteristics such as growth rate, height, spread, canopy architecture and phenology. These characteristics play an important role in classifying C-S-R strategies by Grime (2001). Plant species are categorised based on their primary (competitors (C), stress (S) and ruderals (R)) and secondary strategies. Competition between species produces attractive structural and floristic vegetation in landscape, which appear and change over time (Dunnett et al., 1998).

Competition is greatest in productive sites. In certain cases, competitive elimination of a species by its neighbour occurs where competition is asymmetrical (Schwinning and Weiner, 1998). This is due to differences in seedling size and growth rate between different species in communities. Fast growing species produce dense foliage shading the slow growing species and eliminating small seedlings. *Veronicastrum virginicum*, for example, was eliminated in a plant community soon after establishment (Hitchmough et al., 2004).

Competitive elimination through shading is most likely to occur with slow growing species that are adapted to grow in full sun. By cultivating species that are highly tolerant of both shade and drought with sun species the opportunities for coexistence should be increased. Forbs such as *P. veris* and *P. vulgaris* associated with woodland edges/tall grassland and woodland respectively show tolerance of shade and drought

(Whale, 1984). These kind of strategies demonstrates that these species are 'competition tolerant' and could survive and establish themselves if planted in a multiple-layer community.

### 2.5.1 Type of substrate

Species competition in created plant communities may be affected by type of substrate, commencing after sowing the seed mix on the soil surface. During emergence, particularly when seedling roots are restricted to the soil surface, they are competing for the resources to survive (Hitchmough et al., 2004). This mechanism may affect the pattern of species density and richness in communities in a long-term. On productive sites, on top soil for example, species competition is most intense (Buckland and Grime, 2000). Vigorous seedlings may eliminate small and slow growing seedlings, hence reduce the number of species present (species density) in a created community. Stevens and Carson (1999) proposed that the decline in species density across productivity gradient may be due to increasing size in certain species. It means that fast growing, vigorous and dominant species potentially inhibit slow growing species. As supported by previous studied, under productive soils, plant biomass production increased with declining in plant species density and species richness (Wilson and Tilman, 1993; Buckland and Grime, 2000; Gough et al., 2000).

Grime (2001) has noted that unproductive soil is more likely to give an advantage for the creation of herbaceous plant communities of stress-tolerating forbs. By using unproductive soil the capacity of more productive species such as prairie forbs to out compete less productive forbs such as woodland species, may be reduced. Several researchers have noted that on unproductive soils, species competition and productivity decreased, but the species diversity and individual plant numbers increased (Buckland and Grime, 2000; Hitchmough and de La Fleur, 2006)

## 2.6 C-S-R plant strategies concept

'Stress' and 'disturbance' are two environmental factors which commonly affect plant growth and survival. All plants are subject to different levels and combinations of these two factors. Stress is referred to as the phenomena which limits sources (light, water

and nutrients) that affect plant growth or photosynthetic production (Grime, 2001). Other stress factors includes heavy shade, low or high temperature and drought (Dunnett, 2004). Disturbance results directly from the destruction of the plant biomass and may arise from the activities of herbivores, man and from phenomena such as hurricanes, soil erosion, drought, fire and frosting (Grime, 2001).

As shown in Table 2.4, there are three basic responses or 'strategies' for plant survival in sites of different productivity, when subjected to various combinations of low to high stress and disturbance. High disturbance and productive habitats are exploited by ruderals (R) and stress-tolerators (S) respectively. In habitats where the effects of stress (high productive) and disturbance are minimal, competitor (C) species exist. It is shown that the combination of minimal environmental stress and disturbance is an important factor on a productive site. In highly disturbed habitats the effect of continuous and severe stress (low productivity) inhibits the establishment of vegetation (Grime, 2001).

**Table 2.4** Combination of productivity and disturbance resulting in three basic plant strategies (source from Grime (2001)).

Intensity of disturbance	Productivity	
	Low	High
Low	Stress-tolerators ( S )	Competitors ( C )
High	No viable strategy	Ruderals ( R )

Intermediate strategies were also identified by Grime as competitive ruderals (CR), stress-tolerant competitors (SC), stress-tolerant ruderals (SR) and C-S-R strategies correlated with medium habitat conditions. In general, based on the C-S-R strategies, there are several growth characteristics associated with the different types of plant strategy as shown in Table 2.5.

**Table 2.5** Some of the plant growth characteristics based on the primary CSR strategy.

Strategy	Characteristics
Competitors	Vigorous vegetative growth High dense canopy / leaves Large growing; robust leaf form Extensive lateral spread (above and below ground)
Stress-tolerator	Slow vegetative growth Wide range of growth forms Often small growing; small leaf forms Occurs in habitat with limited resources
Ruderals	Vigorous growth, short lived - often annuals Limited lateral spread Various growing; various leaf forms Persistence depends on successful seed production and germination

Currently, in landscape research, many non-native species have shown intermediate strategies between these extremes. For example, moist-wet prairie plants like *Aster azureus* tend to be associated with stress factors (soil type) and disturbance (management practices) in long-term plant community development (Hitchmough and de La Fleur, 2006). These species showed high persistence on sand mulch compared to subsoil and topsoil. This happens because sand mulching reduced molluscs predation in spring, hence facilitated higher regeneration from seed, and reduced competition from weeds (Hitchmough et al., 2004; Hitchmough and de La Fleur, 2006).

The prairie forbs used in urban landscape plantings vary considerably in terms of plant strategy. Based on the habitat preferences and growth characteristics, several prairie species were estimated for their ecological strategy (Hitchmough et al., 2004). Species such as *A. laevis*, *R. subtomentosa* and *V. virginicum* demonstrate the strategy of a stress-tolerant competitor (SC). It was also observed that *A. azureus* and *Solidago speciosa* are demonstrably stress tolerator-CSR. In this study, the estimated type of plant strategy for the woodland and prairie species selected is shown in Appendix Table A4.1. As a whole, the CSR model can be advantageous to landscape planting design especially for matching species to site conditions and their management in the longer term (Dunnett, 2004).

## 2.7 Mechanisms of competition

In semi-natural herbaceous communities, many diverse species of plant grow together, with the same species occurring randomly throughout the planting. Any other plant growing amongst a cultivated community is a weed, which competes for resources. In general, all species require an appropriate amount of water, nutrients and light from the soil that surrounds its roots (below ground) and the air that surrounds its shoots and leaves (above ground) in order to establish and survive. Species do however vary considerably in the level of each resource they require.

Root and shoot competition plays an important role in influencing the success or failure of a plant in the community. Competition is dependent on the availability of growth factors; on unproductive sites (limited nutrients and water for example) root competition increases and shoot competition decreases (Haugland and Froud-Williams, 1999). Root competition significantly reduces the plant biomass and increases the root: shoot ratio.

Shoot competition was found to have a greater effect than root competition when the root competition was reduced either by watering or fertilising (Wilson and Tilman, 1993). Haugland and Tawfiq (2001) demonstrates that shoot competition increases (plant dry weight increases) with time when new seedlings of *Trifolium pratense* were planted into established grassland. It was also found that in the first year's field experiments, root competition in newly sown seedlings had a greater effect on seedling biomass than shoot competition. However, full competition (both root and shoot) compared with no competition showed the same decreasing trend with time.

As each year passes, competition between root and shoot can cause problems within species in landscape communities. Slow growing species may be eliminated by vigorous species making it difficult to maintain species richness, leading to low diversity naturalistic plant communities in urban landscapes. By choosing the right species and understanding their interactions in the community it may possible to resolve this problem.

### 2.7.1 Competition for water

Plants compete for water to increase their size and rate of survival. This is associated with the below ground competition and the availability of water (Friedman and Orshan, 1974; Inouye et al., 1980). Much work has been undertaken on the effects of herbaceous vegetation on establishing trees and shrubs; competition for water is critical in this situation (Belsky, 1994; Breshears et al., 1997).

In urban landscape practice, the creation of semi-natural plant communities by direct sowing requires adequate rainfall or irrigation during seedling emergence after winter sowing (Hitchmough et al., 2004). During emergence, seedlings compete for water to establish. Enough water determines the success of seedling establishment especially in the summer and during dry conditions. As a sown or planted community establishes they require less water, and ultimately no irrigation is undertaken (Hitchmough and de La Fleur, 2006), because established prairie forbs are able to absorb available groundwater from the soil, particularly in summer, where temperatures and plant transpiration are at a high. Prairie forbs (*Artemisia frigida*, *Chrysopsis villosa* and *Lygodesmia juncea* for example) and grasses (*Andropogon gerrardi*, *Panicum virgatum* and *Spartina pectinata* for example), which consist of deep root systems of up to 200 cm, absorb water efficiently from the soil (Archibold, 1995), and tolerate long droughts (Weaver and Albertson, 1943; Curtis, 1959).

### 2.7.2 Competition for nutrients

Nutrient availability plays an important role in plant growth. There is a relationship within herbaceous plant communities between primary productivity and species richness which is often consistently detected when comparisons are made across community structures. Species richness typically increases as nutrient supply decreases (Al-Mufti et al., 1977; Buckland and Grime, 2000). In landscape ecological approaches, low nutrition, using minimal fertilising for example, helps to maintain species richness in the field. Buckland and Grime (2000) found that under low soil fertility treatments, the rate of competition and shoot biomass production decreased in herbaceous plant communities. However, species richness and number of individual plants increased.



When there is a higher availability of nutrients, Peace and Grubb (1982) reported that shade tolerant plants, *Impatiens parviflora* for example, increased biomass production under low light levels. However, in herbaceous communities the rate of plant growth was rapid and dominated by fast growing species when soil fertility increased (Buckland and Grime, 2000). Gough et al., (2000) demonstrated that aboveground biomass increased but the plant species density decreased when nutrient levels were increased in herbaceous plant communities. Rapid plant growth tends to lead to an elimination of small seedlings, of slow growing and shade intolerant species during competition for resources. In some cases, it has been shown that the nutrient supply only had a significant detrimental effect on the understorey forbs with high light availability. Nutrient addition is not detrimental to plant biomass production and distribution at low light levels (Elemans, 2004). Also, initial species composition is an important factor in response to nutrient addition in a plant community. Individual species that respond immediately to nutrient supply will be expected to dominate the area and eliminate other species (Tilman, 1993).

### 2.7.3 Competition for light

Most plants require sufficient light to establish themselves and hence seedlings and established plants in communities compete for this resource. Competition for light is more significant when seedlings start to grow and plant communities develop. This competition often leads to differing plant architecture at different height (Grime, 2001), biomass distribution and production (Elemans, 2004). These differences are associated with light rotation and quality of radiation (Grime, 2001). Competition for light between species can be expressed by stem growth and shoot elongation as this depends on stored and newly produced photosynthates. Although light is required for plant photosynthesis, too high light intensities (light stress) can be injurious to landscape plants (Griffin and Ranney, 2001). As a result, plant leaves will show reduced green pigmentation (chlorophyll destruction), necrotic and abscission. Also, prolonged light stress can cause plant death.

In woodland understorey forbs, most individual plants require partial or filtered sunlight most of the day. For example, *P. veris* and *P. vulgaris* required partial sunlight for greatest growth (Whale, 1984). In its natural habitat, these species (shade tolerant) are

understorey, associated with low light intensity under canopy trees and shrubs. As demonstrated by Koike (1989), the foliage of a tree canopy intercepts light and decreases the light intensity. This happens because of active foliage multiplication on the canopy area and light interception by matured leaves. In landscape design, the possibility of using natural shade medium-tall prairies in planting design has never been evaluated. This planting design seems to create semi-natural conditions with low light intensity under a medium-tall prairie canopy, especially during the summer period.

Conversely, the majority of exotic herbaceous prairie species (shade intolerant) have been observed and successfully grown in site conditions with full sun (Hitchmough and Woudstra, 1999). Site conditions with insufficient sunlight may influence the growth performance and shoot elongation of these species. According to Grime (2001), competition for light is most strongly demonstrated by herbaceous species with tall leafy shoots.

## **2.8 The effect of competition on community composition**

'Sustainable' and 'manageable' outcomes within plant community competition are the final aim in the development of semi-naturalistic landscape planting. It is desirable to maintain species richness, diversity, functional and aesthetic values. Studying the relationship between competition and environmental factors may give a better understanding of the effect of competition on community composition.

The effect of plant competition on survival, reproduction of individual plants and distribution of a species has been discussed (Fowler, 1986; Goldberg, 1987; Hitchmough and de La Fleur, 2006). The growth of transplants has been explored by Goldberg (1987), Peltzer and Kochy (2001). Some of the species in these studies responded positively to the removal of competitors. Fowler (1981), showed how the impact of the numbers of individuals present on community composition depended on the availability of growth resources and species interaction.

In urban landscapes, the effects of competition have been examined in only a limited community system, for example, community development in native meadows (Hitchmough et al., 2001), prairie forbs (Hitchmough and de La Fleur, 2006) and mixed

native-exotic forbs (Hitchmough, 2000). The effect of competition is greatest on productive soil. However, the effect of competition did not differ significantly in terms of above ground biomass between native meadows and prairie forbs in unproductive conditions (Hitchmough et al., 2003). Hitchmough and de La Fleur (2006) demonstrated that sand mulch (50 mm deep) had a significant effect on the persistence of the prairie community. It is effective in reducing weed invasion and mortality from slug predation in spring.

Currently, the effects of competition upon the abundance of species present in semi-naturalistic landscape planting are little understood. The following chapters explore key aspects of these relationships and attempt to identify the 'rules' that determine the outcome of initial competition on community composition particularly on multi-layer herbaceous communities.

## CHAPTER 3: SEED GERMINATION AND CHILLING REQUIREMENTS OF SELECTED HERBACEOUS SPECIES

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### 3.1 Introduction

Native and exotic species of herbaceous vegetation are an important element in the urban landscape. These species can be established by planting or by the sowing of seeds where they are to grow in urban spaces to create semi-natural plant communities. The success of vegetation established by sowing *in situ* is largely attributable to its seed germination and emergence characteristics. Freshly harvested seed of herbaceous species is sometimes non-dormant and is easy to germinate under favourable conditions (Grime et al., 1981; Baskin and Baskin, 1988; Meyer and Kitchen, 1992). As seeds are subjected to dry storages post harvesting seeds of some temperate forbs develop some type of physical or physiological dormancy (Baskin and Baskin, 1988; Derek, 1997; Baskin and Baskin, 2001) and this further complicates using laboratory tests to estimate field emergence. It is believed that environmental factors such as temperature, light and darkness contribute to the induction of seed dormancy (Slade and Causton, 1978; Baskin and Baskin, 1988). Derek (1997) defined dormancy as '*the failure of an intact viable seed to complete germination under favourable conditions*'. Dormant seeds may occur due to the embryo being constrained by its surrounding structures (seed coat dormancy), or the embryos themselves being dormant (embryo dormancy). Generally, the dormant states benefits the species in synchronising their life cycles according to the changes of the seasons.

Dormancy in seeds of many temperate forbs can be broken by chilling under artificial conditions in the laboratory. Using this technique, imbibed seeds are exposed to the low temperatures (Slade and Causton, 1978; Hitchmough et al., 2000) for certain periods before they can germinate. Baskin and Baskin (1988) found that temperature plays an important environmental role that influences seed dormancy and determines the success or failure of germination. Many temperate forb species require imbibed seed to experience temperatures between 2-10°C before germination can proceed (Grime et al., 1981; Grime, 2001). In general, chilling temperatures used in the laboratory are commonly between 4°C and 6°C (Willemsen, 1975; Slade and Causton, 1978; Hoffman, 1985; Shimono and Washitani, 2004) with chilling incubation periods up to 105 days

(Willemsen, 1975). Chilling periods to break dormancy may increase due to prolonged dry-storage (Qaderi et al., 2005). Hence, the length of the required chilling period varies from several days to months (Grime, 2001).

Most of the research into the germination of herbaceous plants has been concerned with the techniques used to terminate seed dormancy on common herbaceous vegetation (Slade and Causton, 1978; Parmenter et al., 1996; Baskin and Baskin, 2001). Overall however, there is a shortage of data concerning the germination of herbaceous species used in the creation of designed plant communities, particularly the breaking of seed dormancy through chilling treatments after a long storage. In this experiment, seed from more than 30 herbaceous species was tested. The purpose of this experiment was to establish the minimum chilling requirements to allow establishment in subsequent experiments.

### **3.1.1 Species selection**

Several native and exotic forbs species have been identified for this study. The criteria for plants selection are as follows:

- Commercial availability of seed.
- Low, medium and tall canopy herbaceous vegetation species that are extremely attractive in flower in either spring, summer or autumn.
- Diverse yet complementary ecological strategies and life forms.
- Species that are generally unattractive to slugs and snails as adults.
- Species that can be adapted to likely management regimes.

#### **3.1.1.1 List of the species**

The understorey and prairie species chosen for this study are shown in Table 3.1. All seeds were obtained from commercial seed suppliers, Prairie Nursery, Westfield, WI, USA, Prairie Moon Nursery, Winona, Minnesota, USA and Jellito Seeds, Schwarmstedt, Germany.

**Table 3.1** Plant species chosen for the study based on its flowering. Native species marked with an asterisk (\*).

Plant Types (Height)	Spring	Early Summer	Summer	Autumn
<b>Low canopy (understorey species) (&lt; 300 mm)</b>	<i>Ajuga reptans*</i>	<i>Dodecatheon meadia</i>		
	<i>Anemone nemorosa*</i>	<i>Glechoma hederacea</i>		
	<i>Anemone ranunculoides</i>	<i>Phlox divaricata</i>		
	<i>Anemone sylvestris</i> (‘Madonna’)	<i>Polemonium reptans</i>		
	<i>Cordalis solida</i>			
	<i>Doronicum orientale</i> (‘Magnificum’)			
	<i>Galium odoratum*</i> ( <i>Asperula odorata</i> )			
	<i>Lathyrus vernus</i>			
	<i>Montia sibirica</i>			
	<i>Primula elatior*</i>			
	<i>Primula veris*</i>			
	<i>Primula vulgaris*</i>			
	<i>Viola labradorica*</i>			
<i>Viola odorata</i> (‘Konigin Charlotte’)				
<i>Viola pedatifida</i>				
<b>Medium canopy (300-600 mm)</b>		<i>Phlox maculata</i>	<i>Aster azureus</i>	<i>Solidago speciosa</i>
		<i>Phlox pilosa</i>		
		<i>Tradescantia ohioensis</i>		
		<i>Zizia aptera</i>		
<b>Tall canopy (&gt; 900 mm)</b>			<i>Eupatorium maculatum</i>	<i>Aster laevis</i>
			<i>Helianthus mollis</i>	<i>Aster novae-angliae</i> (‘Septemberrubin’)
			<i>Silphium integrifolium</i>	
			<i>Veronicastrum virginicum</i>	

### 3.1.2 Objectives

The specific objectives of this study were:

- 1) To generate data on the duration of chilling required to break dormancy in the chosen species in the laboratory.

- 2) To assess the capacity of the laboratory germination tests to estimate likely emergence in the field environment.

### 3.2 Materials and methods

The germination experiment was conducted under laboratory conditions at the Annex Block, University of Sheffield for a period of 6 months. The seed germination experiment consisted of six treatments are as follows:

- 0 day chilling + growth cabinet at 20/10°C
- 30 days chilling + growth cabinet at 20/10°C
- 60 days chilling + growth cabinet at 20/10°C
- 90 days chilling + growth cabinet at 20/10°C
- 120 days chilling + growth cabinet at 20/10°C
- 150 days chilling + growth cabinet at 20/10°C

The experimental design for this study was a randomized complete block design (RCBD) with 3 replications. It involved 31 species x 6 treatments x 3 replications. A total of 558 petri dishes consisting of 20 seeds each were used. The procedures for the seed germination study were as follows:

- Three layers of filter paper (Whatman No. 1 900 mm) were placed in a petri dish.
- In each petri dish, the filter paper was moistened with approximately 4.5 ml of de-ionised water.
- Twenty (20) seeds from each species were placed onto filter paper in a petri dish and sealed with Parafilm to maintain moisture content.
- All petri dishes were placed in the fridge at 4°C for chilling treatment. They were rotated and re-randomised once a week.
- After chilling treatments, petri dishes were placed in a growth cabinet at 20/10°C, lit for a 16 hour day by fluorescent lamps. They were rotated randomly once a week as above.

### 3.2.1 Data collection

Every 3 days until day 30, the numbers of germinated seeds in the growth cabinet were counted. Seed germination was defined as the protrusion of the radicle tip through the seed coat (Shimono and Washitani, 2004). Germinated seeds were then removed from the petri dish after being recorded. During chilling incubation, all seeds in petri dishes were observed once a week, and counted if any of them germinated.

#### 3.2.1.1 Squash test

A squash test (Gunn, 2001) was undertaken to assess the viability of the seed. This test was conducted only on seeds which did not germinate after 90 days chilling. At this stage, the individual seed which did not germinate in the growth cabinet after 30 days incubation was gently squeezed using a pair of tweezers. The results were visually inspected (using a small hand lens if necessary) and the numbers of viable seeds (firm and creamy-white in colour) were counted.

### 3.2.2 Statistical analysis

Statistical analysis was undertaken using *SPSS version 12* for Windows. Descriptive statistics was used to obtain the mean and standard error for individual species.

## 3.3 Results

### 3.3.1 Low canopy (understorey species)

Seed germination of the understorey was significantly greater after chilling treatments (Table 3.2). It can be seen in Table 3.2 that the greatest percentage of mean germination in the growth cabinet was in *Dodecatheon meadia* (97%) and *Primula vulgaris* (82%), both after 60 days chilling, and the smallest percentage of germination was in *Galium odoratum* (2%) after 30 days chilling and *Primula elatior* (2%) after 150 days chilling. However, there was a maximum percentage of mean germination in *Montia sibirica* (97%) and *Polemonium reptans* (55%) without chilling treatment.



Rate of germination (in terms of radicle protrusion) for most understorey species in the growth cabinet is considerably increased with 60 days exposure chilling rather than other chilling durations. Rate of germination (in terms of emerged seedlings per count) of the understorey is erratic in all species tested. There was no specific trend or pattern in the number of new seedlings germinated per count after chilling (see graph under section results in each species tested).

Some of the species tested showed substantial germination in the fridge during chilling incubation (Table 3.3) particularly when chilled for >60 days. This contributed to declining germination of some understorey species in the growth cabinet following long chilling. Over 80% of *Phlox divaricata* seed germinated in the fridge when chilled for 120 and 150 days. Understorey species naturally associated with shady habitats were most likely to demonstrate high levels of germination during chilling.

**Table 3.2** Effect of duration of chilling on germination of understorey species at 20/10°C in a growth cabinet. Maximum germination is indicated by bold type.

Species	Days of chilling in the fridge at 4°C					
	0	30	60	90	120	150
<b>Shade tolerant understorey forbs</b>						
<i>Ajuga reptans</i>	5.00	<b>20.00</b>	16.67	<b>20.00</b>	16.67	13.33
<i>Anemone nemorosa</i>	0.00	0.00	0.00	0.00	0.00	0.00
<i>Anemone ranunculoides</i>	0.00	0.00	0.00	0.00	0.00	0.00
<i>Anemone sylvestris</i> ('Madonna')	83.33	85.00	<b>86.67</b>	61.67	33.33	21.67
<i>Cordalis solida</i>	0.00	0.00	0.00	0.00	0.00	0.00
<i>Dodecatheon meadia</i>	0.00	93.33	<b>96.67</b>	81.67	76.67	16.67
<i>Doronicum orientale</i> 'Magnificum'	76.67	<b>80.00</b>	28.33	5.00	1.67	0.00
<i>Galium odoratum</i> ( <i>Asperula odorata</i> )	0.00	<b>1.67</b>	0.00	0.00	0.00	0.00
<i>Glechoma hederacea</i>	5.00	16.67	16.67	<b>21.67</b>	13.33	15.00
<i>Lathyrus vernus</i>	58.33	<b>61.67</b>	1.67	1.67	5.00	5.00
<i>Montia sibirica</i>	<b>96.67</b>	41.67	11.67	8.33	5.00	3.33
<i>Phlox divaricata</i>	0.00	10.00	35.00	<b>56.67</b>	8.33	0.00
<i>Polemonium reptans</i>	<b>55.00</b>	16.67	18.33	11.67	10.00	3.33
<i>Primula elatior</i>	0.00	0.00	0.00	0.00	0.00	<b>1.67</b>
<i>Primula veris</i>	1.67	48.33	<b>61.67</b>	33.33	18.33	13.33
<i>Primula vulgaris</i>	33.33	70.00	<b>81.67</b>	25.00	1.67	5.00
<i>Viola labradorica</i>	1.67	6.67	<b>11.67</b>	8.33	0.00	0.00
<i>Viola odorata</i> 'Konigin Charlotte'	0.00	0.00	<b>16.67</b>	<b>16.67</b>	1.67	0.00
<i>Viola pedatifida</i>	3.33	3.33	<b>11.67</b>	10.00	6.67	5.00

**Table 3.3** Effect of duration of chilling on germination of understorey species in the fridge during chilling at 4°C. Maximum germination is indicated in bold.

Species	Days of chilling in the fridge at 4°C				Mean for post 60 days chilling
	60	90	120	150	
<b>Shade tolerant, understorey forbs</b>					
<i>Ajuga reptans</i>	0.00	<b>1.67</b>	<b>1.67</b>	0.00	1.11
<i>Anemone nemorosa</i>	0.00	0.00	0.00	0.00	0.00
<i>Anemone ranunculoides</i>	0.00	0.00	0.00	0.00	0.00
<i>Anemone sylvestris</i> ('Madonna')	0.00	28.33	46.67	<b>63.33</b>	46.11
<i>Cordalis solida</i>	0.00	0.00	0.00	0.00	0.00
<i>Dodecatheon meadia</i>	1.67	18.33	20.00	<b>78.33</b>	38.88
<i>Doronicum orientale</i> 'Magnificum'	38.33	63.33	<b>80.00</b>	53.33	65.55
<i>Galium odoratum</i> ( <i>Asperula odorata</i> )	0.00	1.67	6.67	<b>11.67</b>	6.67
<i>Glechoma hederacea</i>	0.00	0.00	0.00	0.00	0.00
<i>Lathyrus vernus</i>	<b>63.33</b>	51.67	55.00	60.00	55.56
<i>Montia sibirica</i>	55.00	<b>70.00</b>	56.67	53.33	60.00
<i>Phlox divaricata</i>	0.00	15.00	<b>83.33</b>	<b>83.33</b>	60.55
<i>Polemonium reptans</i>	1.67	10.00	13.33	<b>43.33</b>	22.22
<i>Primula elatior</i>	0.00	0.00	0.00	0.00	0.00
<i>Primula veris</i>	3.33	48.33	50.00	<b>75.00</b>	57.78
<i>Primula vulgaris</i>	1.67	43.33	66.67	<b>76.67</b>	62.22
<i>Viola labradorica</i>	0.00	15.00	6.67	<b>21.67</b>	14.45
<i>Viola odorata</i> 'Konigin Charlotte'	0.00	<b>45.00</b>	36.67	36.67	39.45
<i>Viola pedatifida</i>	0.00	0.00	0.00	0.00	0.00

As a whole, seed germination of understorey species can be divided into three groups (Table 3.4), with low, medium and high percentage after adequate chilling treatments (0-150 days).

Table 3.4 Germination of understorey species in a growth cabinet after adequate chilling treatments, and during chilling incubation in a fridge.

Germination	Low germination percentage (< 40%)	Medium germination percentage (40%-70%)*	High germination percentage (> 70%)
In a growth cabinet	<i>Ajuga reptans</i>	<i>Lathyrus vernus</i>	<i>Anemone sylvestris</i> ('Madonna')
	<i>Anemone nemorosa</i>	<i>Phlox divaricata</i>	<i>Dodecatheon meadia</i>
	<i>Anemone ranunculoides</i>	<i>Polemonium reptans</i> *	<i>Doronicum orientale</i> 'Magnificum'
	<i>Cordalis solida</i>	<i>Primula veris</i>	<i>Montia sibirica</i> *
	<i>Galium odoratum</i> ( <i>Asperula odorata</i> )		<i>Primula vulgaris</i>
	<i>Glechoma hederacea</i>		
	<i>Primula elatior</i>		
	<i>Viola labradorica</i>		
	<i>Viola odorata</i> 'Konigin Charlotte'		
	<i>Viola pedatifida</i>		
In a fridge	<i>Ajuga reptans</i>	<i>Anemone sylvestris</i> ('Madonna')	<i>Dodecatheon meadia</i>
	<i>Anemone nemorosa</i>	<i>Lathyrus vernus</i>	<i>Doronicum orientale</i> 'Magnificum'
	<i>Anemone ranunculoides</i>	<i>Polemonium reptans</i>	<i>Montia sibirica</i>
	<i>Cordalis solida</i>	<i>Viola odorata</i> 'Konigin Charlotte'	<i>Phlox divaricata</i>
	<i>Galium odoratum</i> ( <i>Asperula odorata</i> )		<i>Primula veris</i>
	<i>Glechoma hederacea</i>		<i>Primula vulgaris</i>
	<i>Primula elatior</i>		
	<i>Viola labradorica</i>		
	<i>Viola pedatifida</i>		

\* Without chilling treatment.

The results of each species after and throughout chilling treatments are as follows:

### **3.3.1.1 *Ajuga reptans***

The germination of *A. reptans* was very low. It can be seen from Figure 3.1 that the best germination (approximately 20%) of this species in the growth cabinet was achieved in the seed treated with 30 days chilling. The number of new seedlings germinated after 30 days chilling was very low (<2 seedlings) from day 9 to 27 (Figure 3.2). Low seed germination (approximately of 2%) also occurred in the fridge during incubation between 90 and 120 days chilling (Figure 3.3).

### **3.3.1.2 *Anemone nemorosa***

Chilling of any length had no effect on the seed germination of *A. nemorosa*.

### **3.3.1.3 *Anemone ranunculoides***

Chilling of any length had no effect on the seed germination of *A. ranunculoides*.

### **3.3.1.4 *Anemone sylvestris* ('Madonna')**

As shown in Figure 3.4, greatest mean germination (approximately 87%) for *A. sylvestris* in the growth cabinet was achieved after 60 days chilling. There was no specific trend in the number of new seedlings germinated per count after chilling (Figure 3.5). Seed germination also occurred in the fridge during chilling. As shown in Figure 3.6, the highest germination (approximately 63%) was obtained around a period of 150 days chilling.

### **3.3.1.5 *Cordalis solida***

This species failed to germinate irrespective of chilling regime.

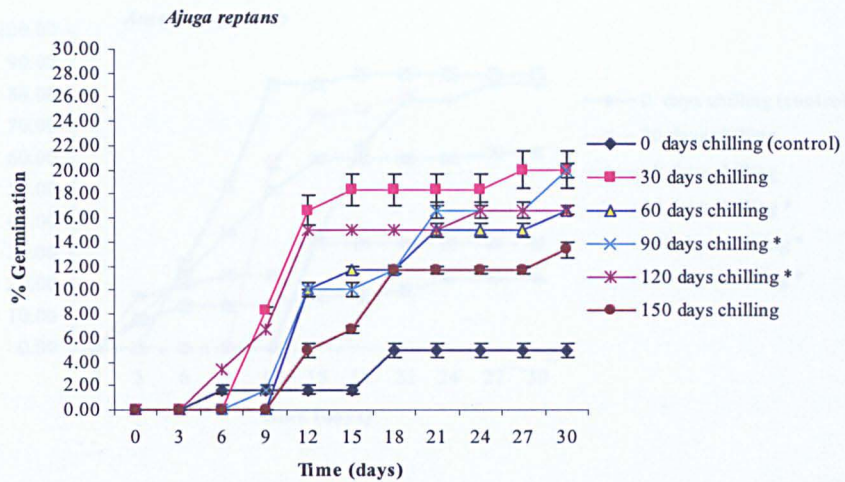


Figure 3.1 Germination of *Ajuga reptans* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM.

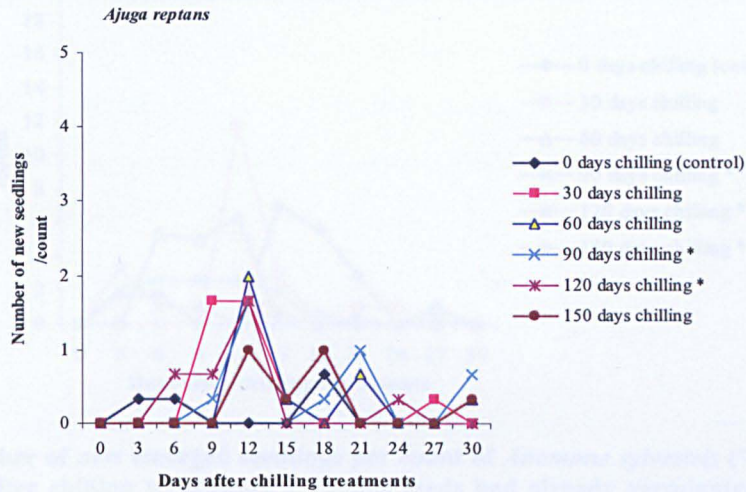


Figure 3.2 Number of new emerged seedlings per count of *Ajuga reptans* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included.

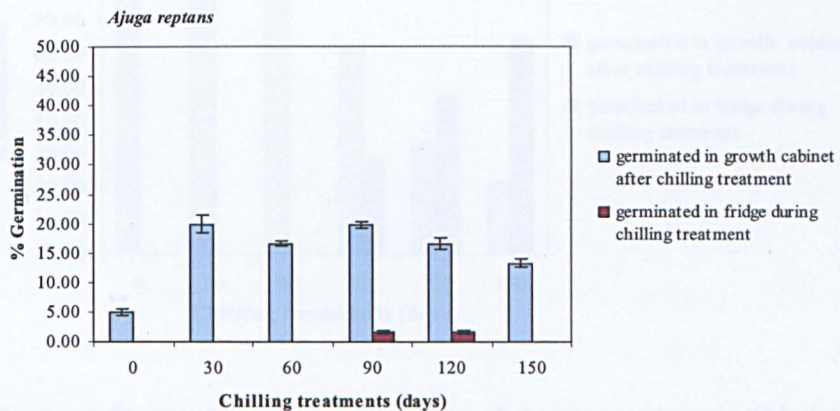


Figure 3.3 Mean maximum percentage germination of *Ajuga reptans* seeds in response to chilling treatments. Error bars represent 1 SEM.

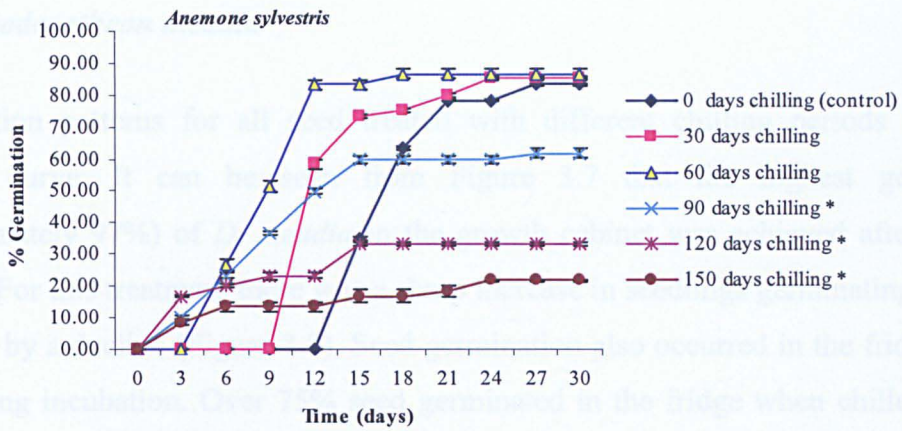


Figure 3.4 Germination of *Anemone sylvestris* ('Madonna') seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM.

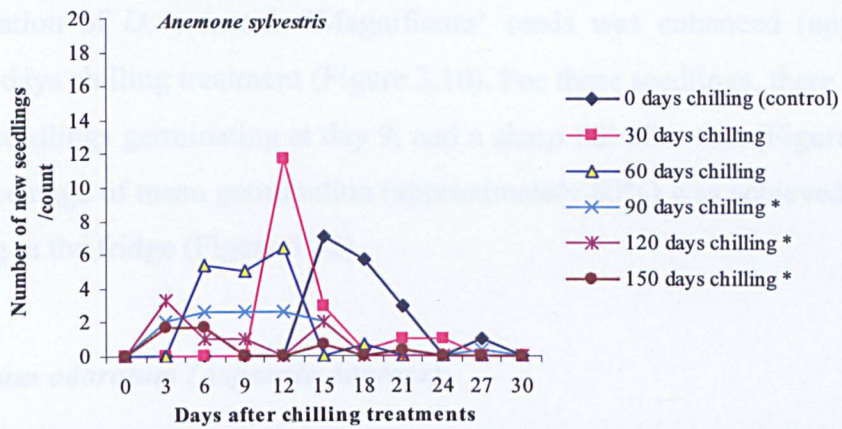


Figure 3.5 Number of new emerged seedlings per count of *Anemone sylvestris* ('Madonna') in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included.

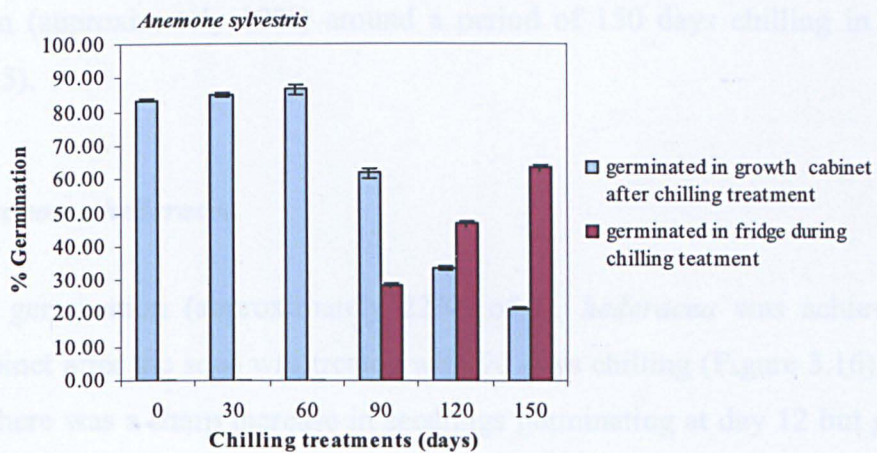


Figure 3.6 Mean maximum percentage germination of *Anemone sylvestris* ('Madonna') seeds in response to chilling treatments. Error bars represent 1 SEM.

### 3.3.1.6 *Dodecatheon meadia*

Germination patterns for all seed treated with different chilling periods showed a sigmoid curve. It can be seen from Figure 3.7 that the highest germination (approximately 97%) of *D. meadia* in the growth cabinet was achieved after 60 days chilling. For this treatment there was a sharp increase in seedlings germinating at day 6, followed by a decline (Figure 3.8). Seed germination also occurred in the fridge during the chilling incubation. Over 75% seed germinated in the fridge when chilled for 150 days chilling (Figure 3.9).

### 3.3.1.7 *Doronicum orientale* 'Magnificum'

The germination of *D. orientale* 'Magnificum' seeds was enhanced (approximately 80%) by 30 days chilling treatment (Figure 3.10). For these seedlings, there was a sharp increase in seedlings germinating at day 9, and a sharp fall after that (Figure 3.11). The greatest percentage of mean germination (approximately 80%) was achieved during 120 days chilling in the fridge (Figure 3.12).

### 3.3.1.8 *Galium odoratum* (*Asperula odorata*)

Chilling of any length had no effect on the seed germination of *G. odoratum* as seen in Figures 3.13 and 3.14. The highest percentage of mean germination was just under 2% after 30 days chilling. However, the chilled seeds gave a better percentage of mean germination (approximately 12%) around a period of 150 days chilling in the fridge (Figure 3.15).

### 3.3.1.9 *Glechoma hederacea*

Maximum germination (approximately 22%) of *G. hederacea* was achieved in the growth cabinet after the seed was treated with 90 days chilling (Figure 3.16). For these seedlings there was a sharp increase in seedlings germinating at day 12 but germinants went down after that (Figure 3.17). The percentage of mean germination in the growth cabinet increased steadily from approximately 5% to 22% between 0 and 90 days chilling followed by a decline (Figure 3.18). There was no germination in the fridge until 150 days chilling.

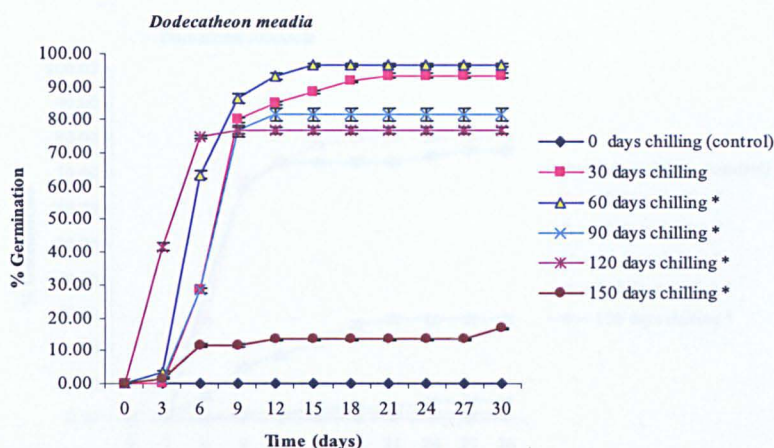


Figure 3.7 Germination of *Dodecatheon meadia* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM.

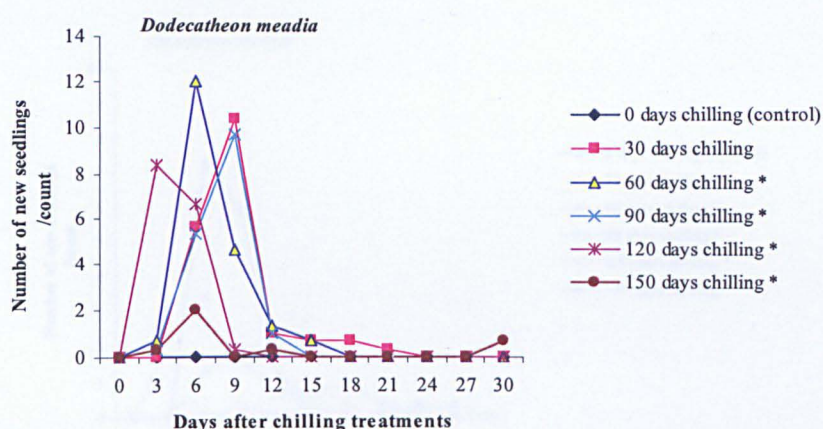


Figure 3.8 Number of new emerged seedlings per count of *Dodecatheon meadia* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included.

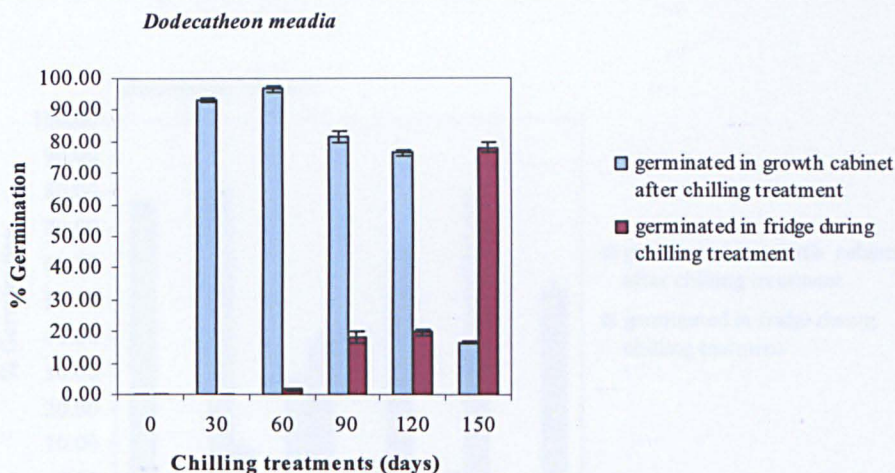


Figure 3.9 Mean maximum percentage germination of *Dodecatheon meadia* seeds in response to chilling treatments. Error bars represent 1 SEM.



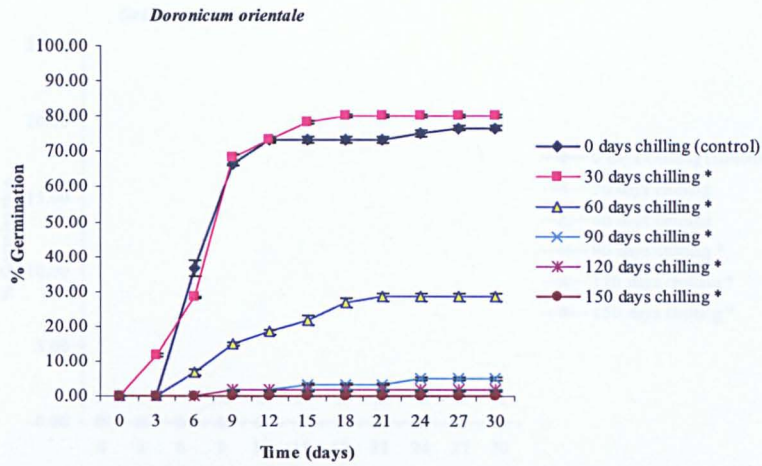


Figure 3.10 Germination of *Doronicum orientale* ‘Magnificum’ seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM.

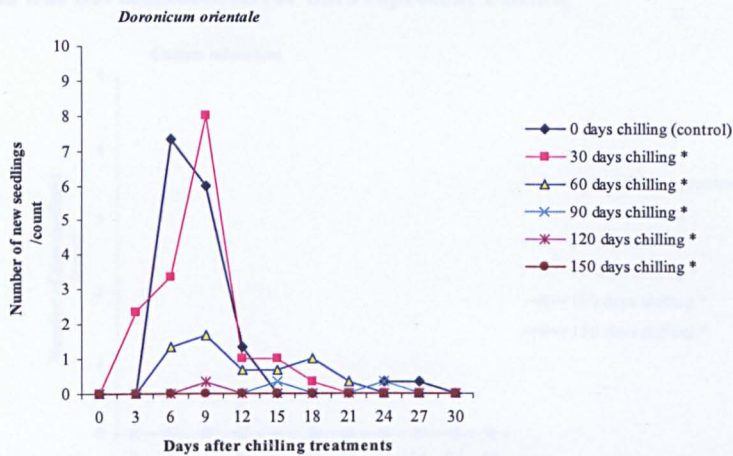


Figure 3.11 Number of new emerged seedlings per count of *Doronicum orientale* ‘Magnificum’ in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included.

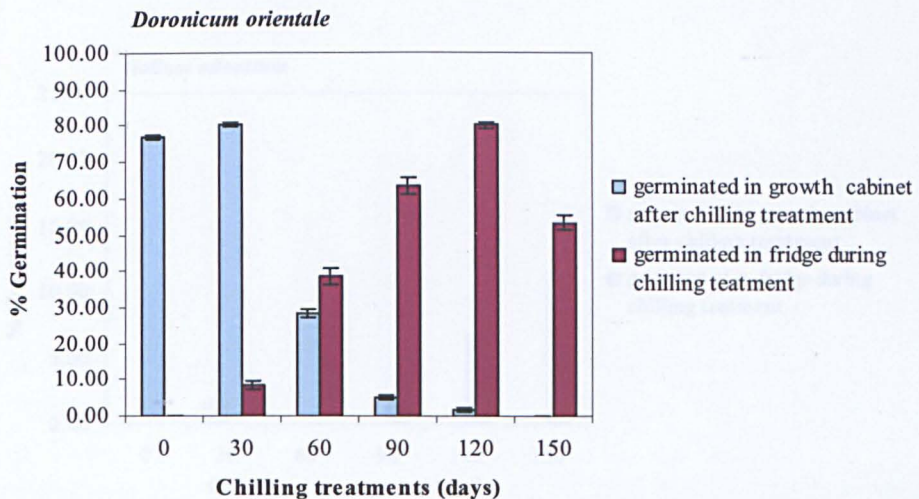


Figure 3.12 Mean maximum percentage germination of *Doronicum orientale* ‘Magnificum’ seeds in response to chilling treatments. Error bars represent 1 SEM.

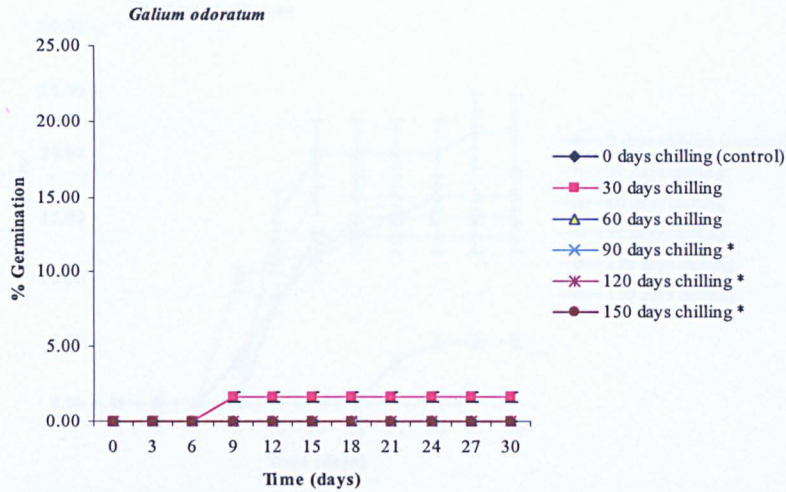


Figure 3.13 Germination of *Galium odoratum* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM.

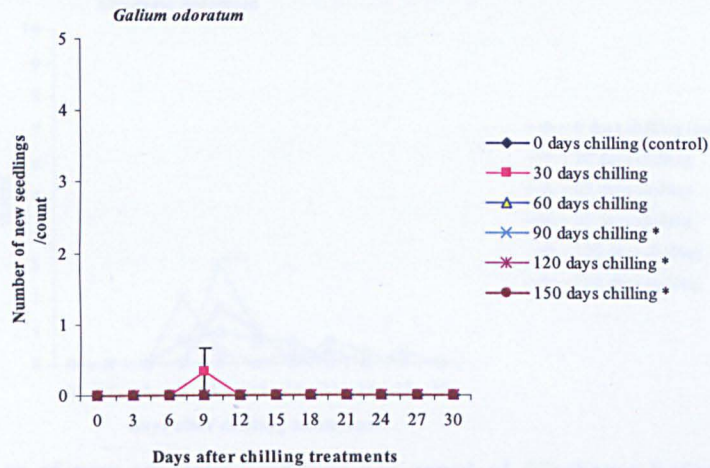


Figure 3.14 Number of new emerged seedlings per count of *Galium odoratum* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included.

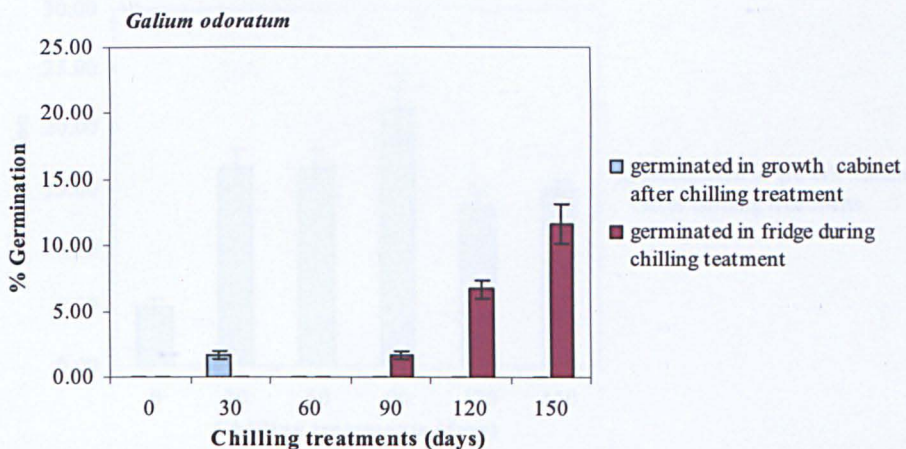


Figure 3.15 Mean maximum percentage germination of *Galium odoratum* seeds in response to chilling treatments. Error bars represent 1 SEM.

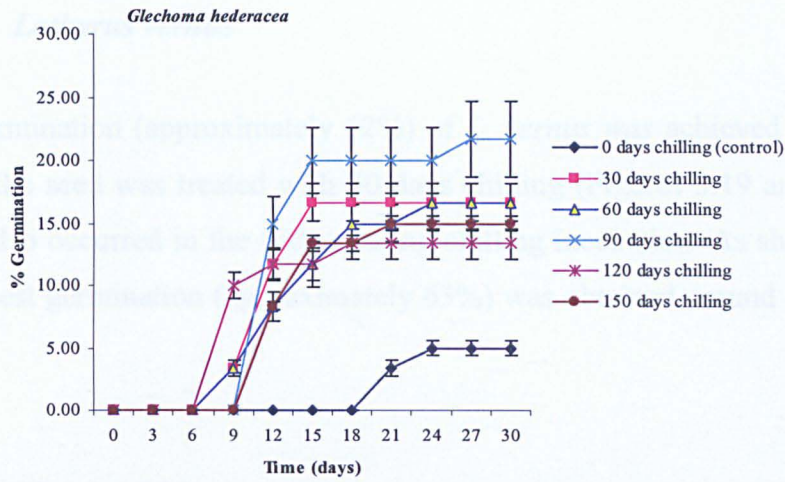


Figure 3.16 Germination of *Glechoma hederacea* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM.

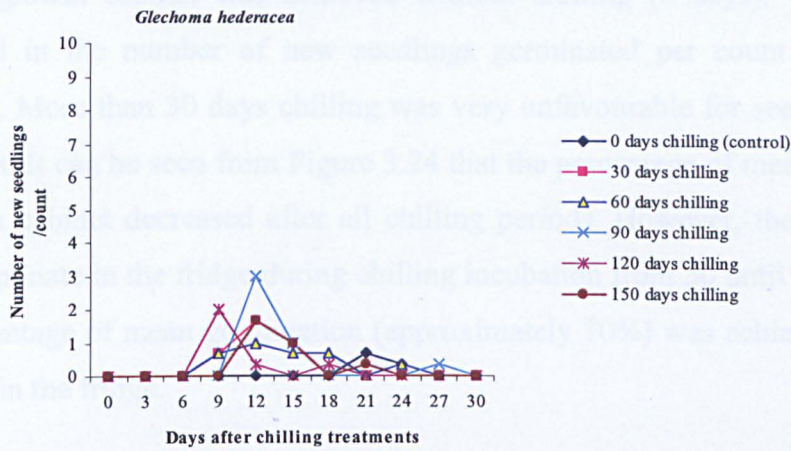


Figure 3.17 Number of new emerged seedlings per count of *Glechoma hederacea* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included.

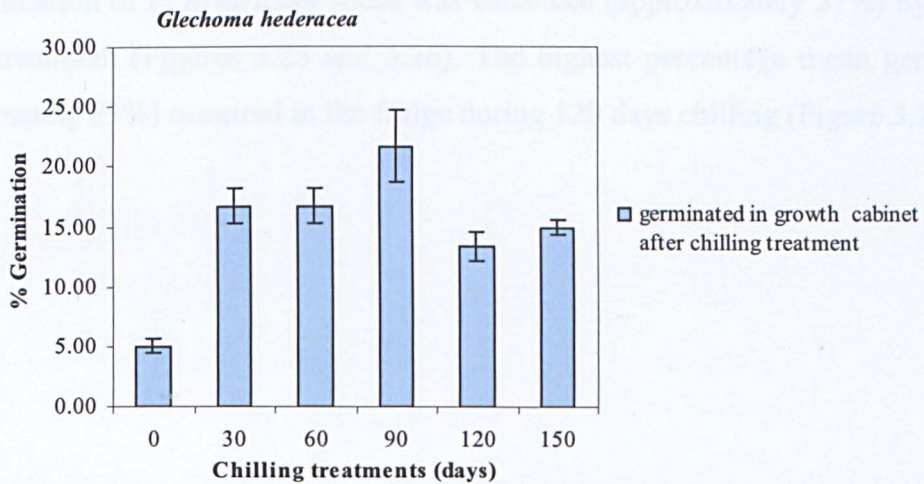


Figure 3.18 Mean maximum percentage germination of *Glechoma hederacea* seeds in response to chilling treatments. Error bars represent 1 SEM.

### 3.3.1.10 *Lathyrus vernus*

Maximum germination (approximately 62%) of *L. vernus* was achieved in the growth cabinet after the seed was treated with 30 days chilling (Figures 3.19 and 3.20). Seed germination also occurred in the fridge during chilling incubation. As shown in Figure 3.21, the highest germination (approximately 63%) was obtained around a period of 60 days chilling.

### 3.3.1.11 *Montia sibirica*

As shown in Figure 3.22, the highest germination (approximately 97%) of *M. sibirica* seed in the growth cabinet was achieved without chilling (0 days). There was no specific trend in the number of new seedlings germinated per count after chilling (Figure 3.23). More than 30 days chilling was very unfavourable for seed germination of this species. It can be seen from Figure 3.24 that the percentage of mean germination in the growth cabinet decreased after all chilling periods. However, the chilled seeds started to germinate in the fridge during chilling incubation from 30 until 150 days. The greatest percentage of mean germination (approximately 70%) was achieved during 90 days chilling in the fridge.

### 3.3.1.12 *Phlox divaricata*

The germination of *P. divaricata* seeds was enhanced (approximately 57%) by 90 days chilling treatment (Figures 3.25 and 3.26). The highest percentage mean germination (approximately 83%) occurred in the fridge during 120 days chilling (Figure 3.27).

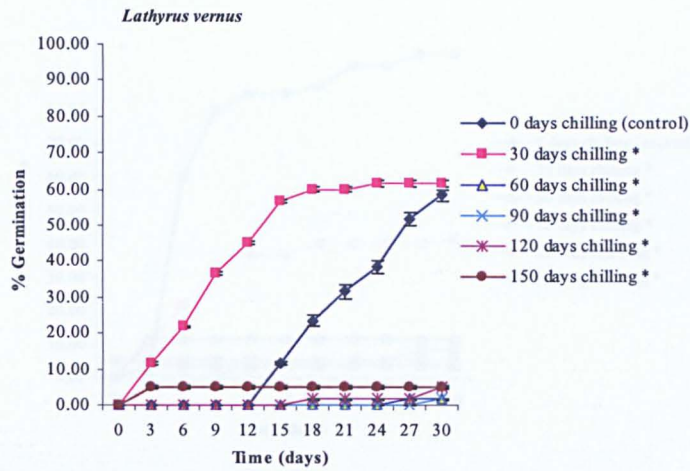


Figure 3.19 Germination of *Lathyrus vernus* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM.

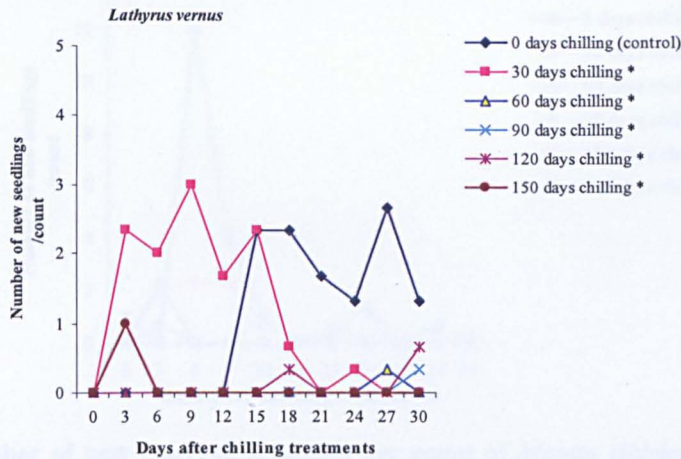


Figure 3.20 Number of new emerged seedlings per count of *Lathyrus vernus* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included.

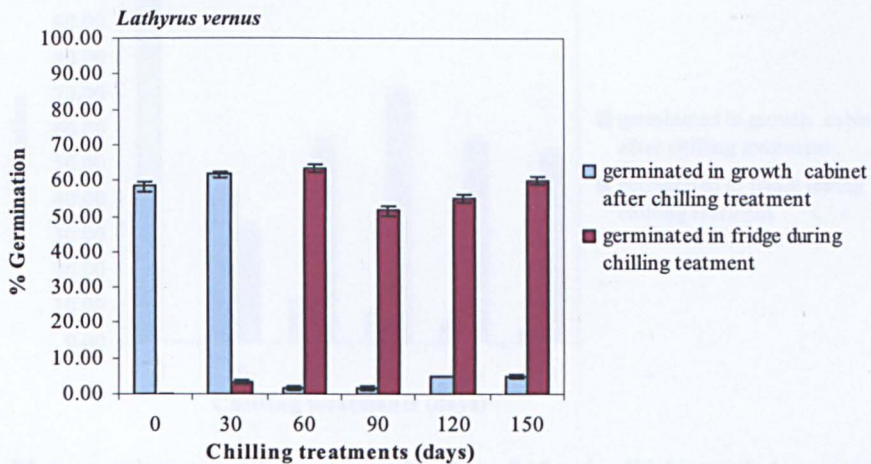


Figure 3.21 Mean maximum percentage germination of *Lathyrus vernus* seeds in response to chilling treatments. Error bars represent 1 SEM.

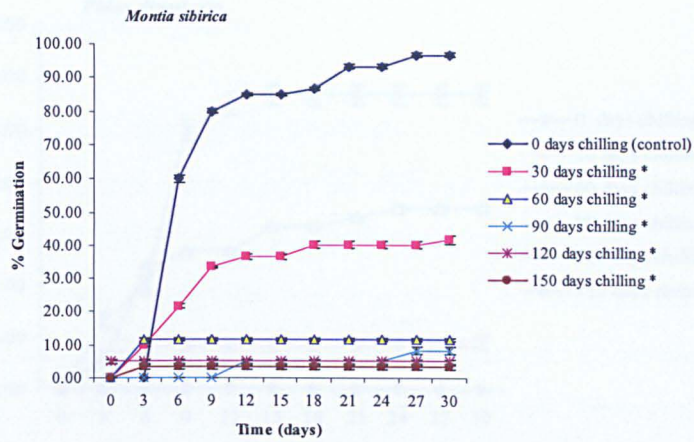


Figure 3.22 Germination of *Montia sibirica* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM.

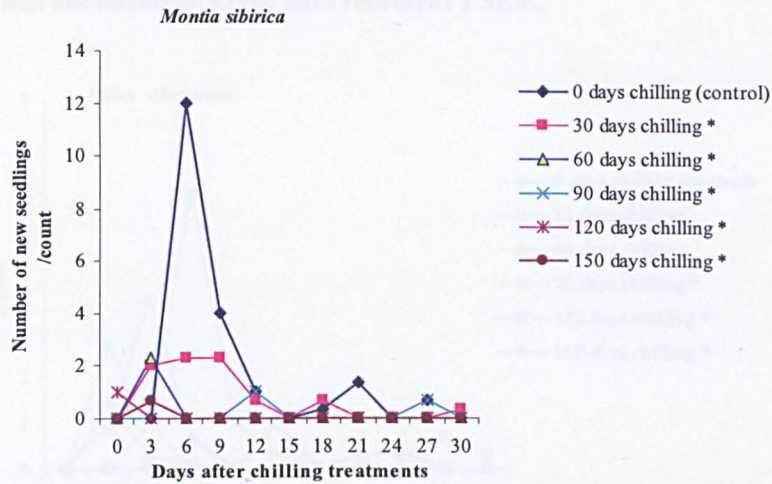


Figure 3.23 Number of new emerged seedlings per count of *Montia sibirica* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included.

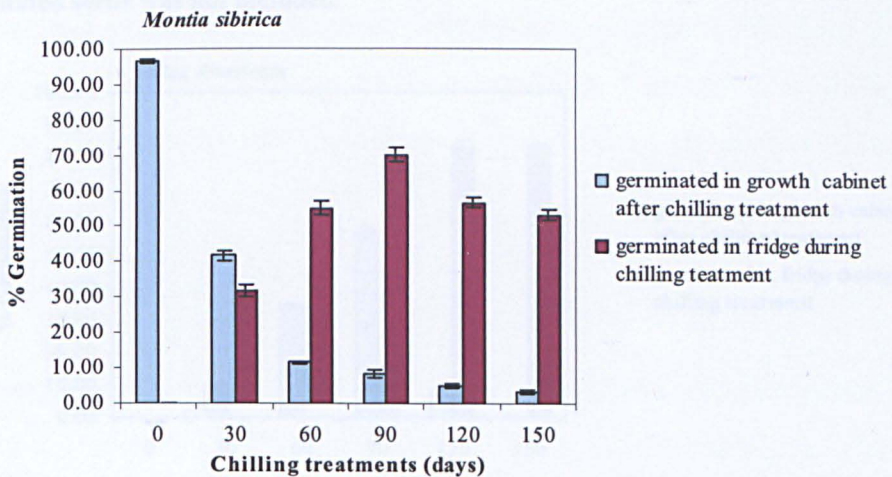


Figure 3.24 Mean maximum percentage germination of *Montia sibirica* seeds in response to chilling treatments. Error bars represent 1 SEM.

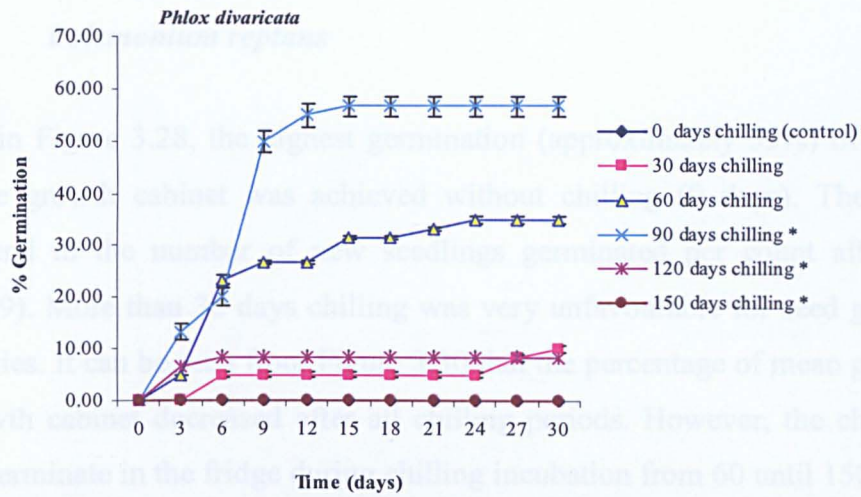


Figure 3.25 Germination of *Phlox divaricata* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM.

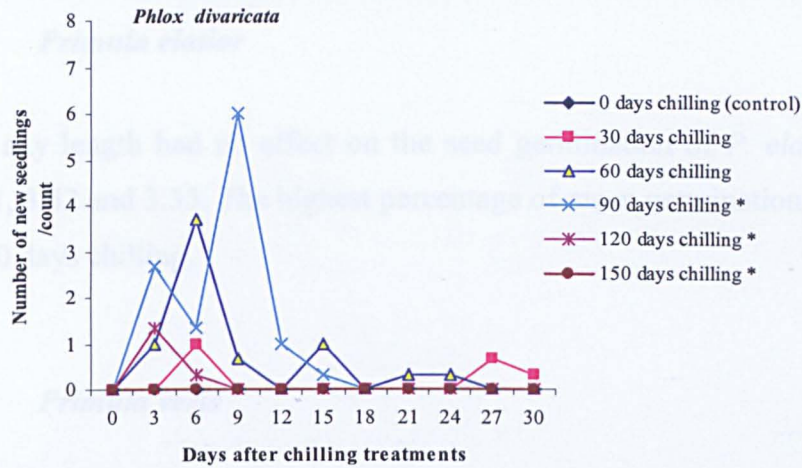


Figure 3.26 Number of new emerged seedlings per count of *Phlox divaricata* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included.

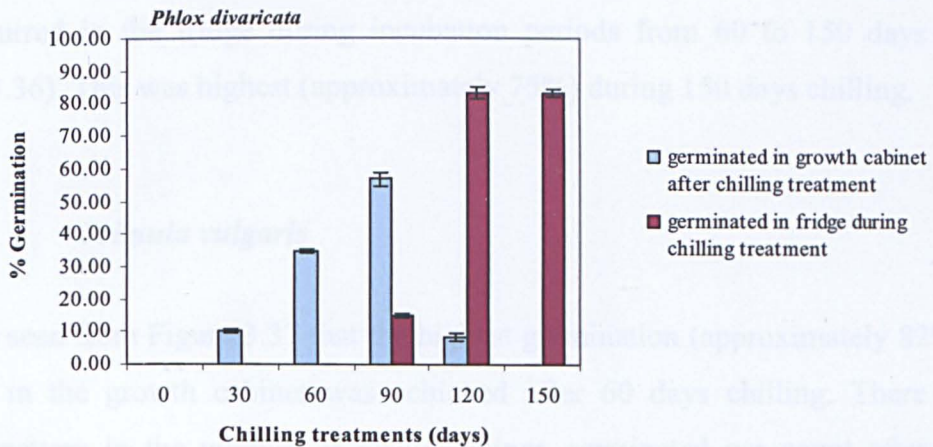


Figure 3.27 Mean maximum percentage germination of *Phlox divaricata* seeds in response to chilling treatments. Error bars represent 1 SEM.

### 3.3.1.13 *Polemonium reptans*

As shown in Figure 3.28, the highest germination (approximately 55%) of *P. reptans* seed in the growth cabinet was achieved without chilling (0 days). There was no specific trend in the number of new seedlings germinated per count after chilling (Figure 3.29). More than 30 days chilling was very unfavourable for seed germination of this species. It can be seen from Figure 3.30 that the percentage of mean germination in the growth cabinet decreased after all chilling periods. However, the chilled seeds started to germinate in the fridge during chilling incubation from 60 until 150 days. The percentage of mean germination during chilling increased steadily from approximately 2% in 60 days to 43% in 150.

### 3.3.1.14 *Primula elatior*

Chilling of any length had no effect on the seed germination of *P. elatior* as seen in Figures 3.31, 3.32 and 3.33. The highest percentage of mean germination was just under 2% after 150 days chilling.

### 3.3.1.15 *Primula veris*

It can be seen from Figure 3.34 that the maximum germination (approximately 62%) of *P. veris* was achieved after 60 days chilling. There was no specific trend in the number of new seedlings germinated per count after chilling (Figure 3.35). Seed germination also occurred in the fridge during incubation periods from 60 to 150 days chilling (Figure 3.36). This was highest (approximately 75%) during 150 days chilling.

### 3.3.1.16 *Primula vulgaris*

It can be seen from Figure 3.37 that the highest germination (approximately 82%) of *P. vulgaris* in the growth cabinet was achieved after 60 days chilling. There was no specific pattern in the number of new seedlings germinated per count after chilling (Figure 3.38). Seed germination also occurred in the fridge during chilling incubation with approximately 77% after 150 days chilling (Figure 3.39).



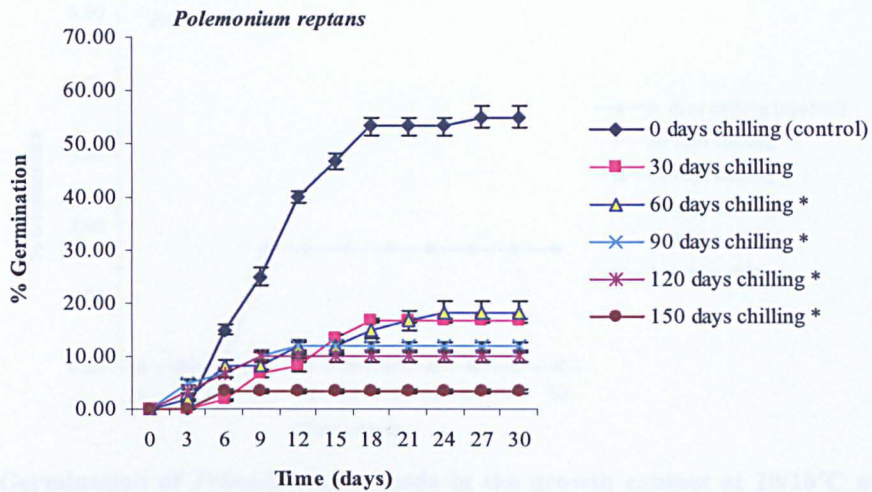


Figure 3.28 Germination of *Polemonium reptans* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM.

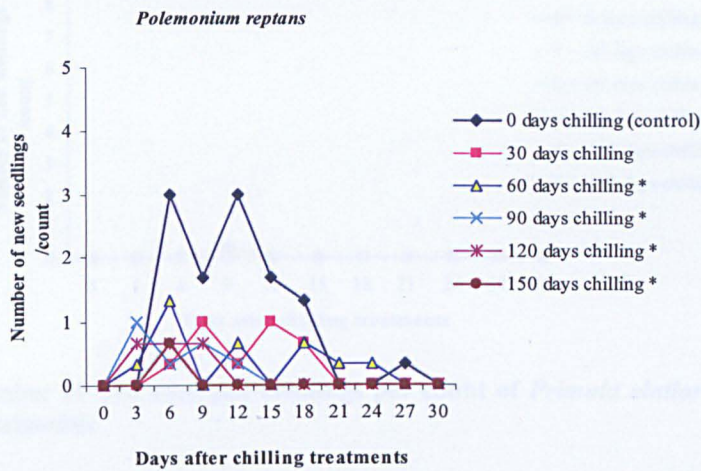


Figure 3.29 Number of new emerged seedlings per count of *Polemonium reptans* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included.

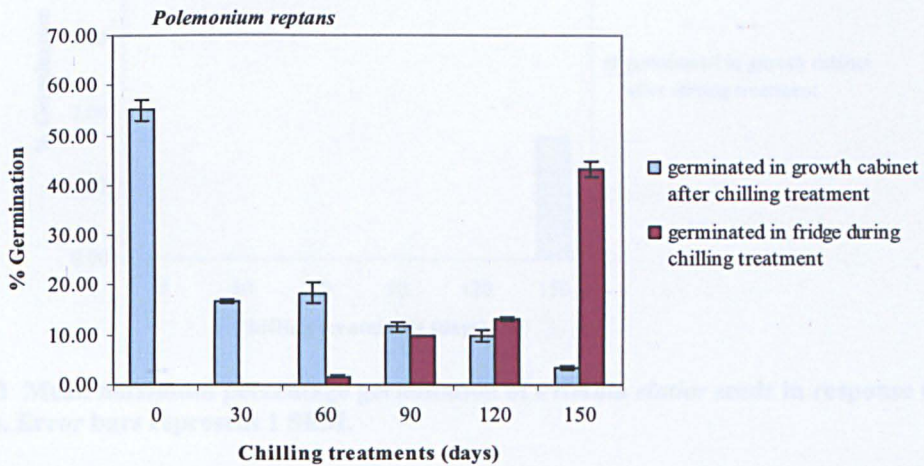


Figure 3.30 Mean maximum percentage germination of *Polemonium reptans* seeds in response to chilling treatments. Error bars represent 1 SEM.

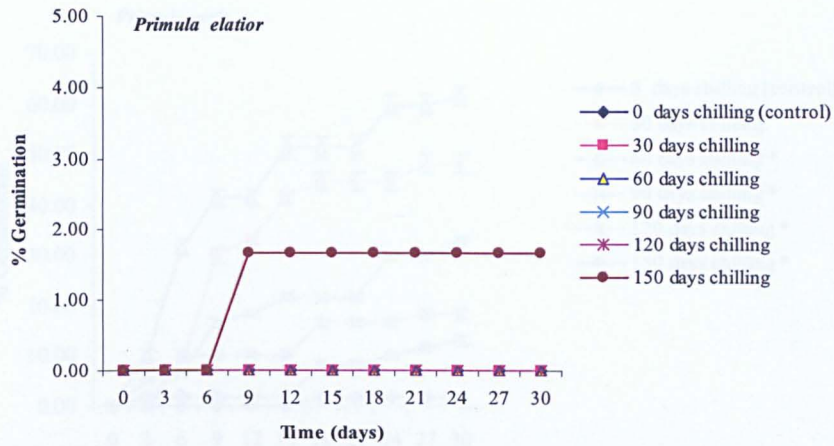


Figure 3.31 Germination of *Primula elatior* seeds in the growth cabinet at 20/10°C after chilling treatments. Error bars represent 1 SEM.

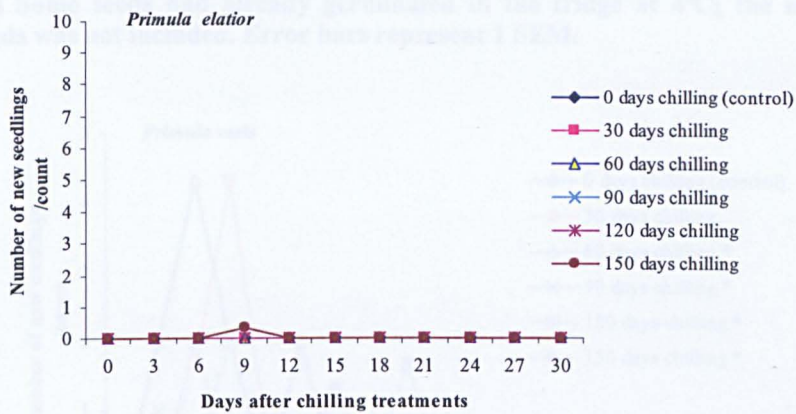


Figure 3.32 Number of new emerged seedlings per count of *Primula elatior* in the growth cabinet after chilling treatments.

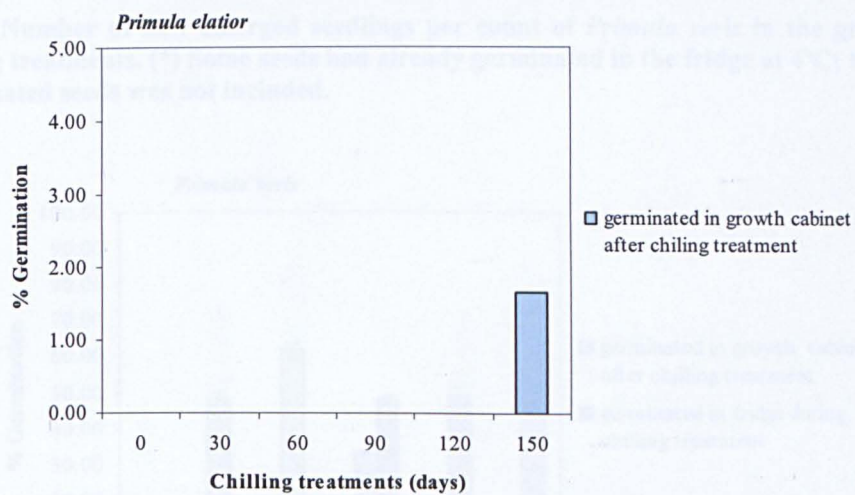


Figure 3.33 Mean maximum percentage germination of *Primula elatior* seeds in response to chilling treatments. Error bars represent 1 SEM.

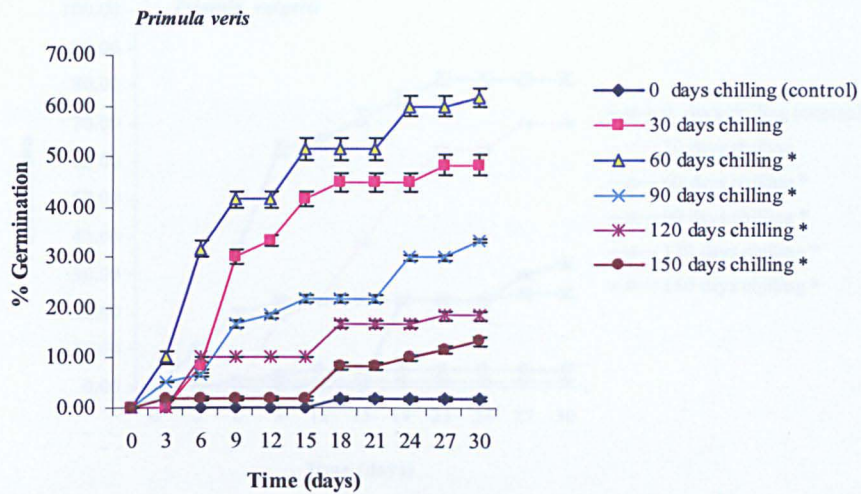


Figure 3.34 Germination of *Primula veris* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM.

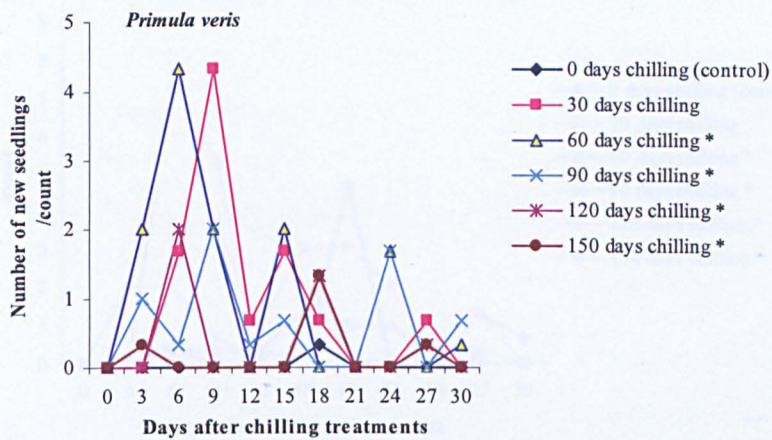


Figure 3.35 Number of new emerged seedlings per count of *Primula veris* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included.

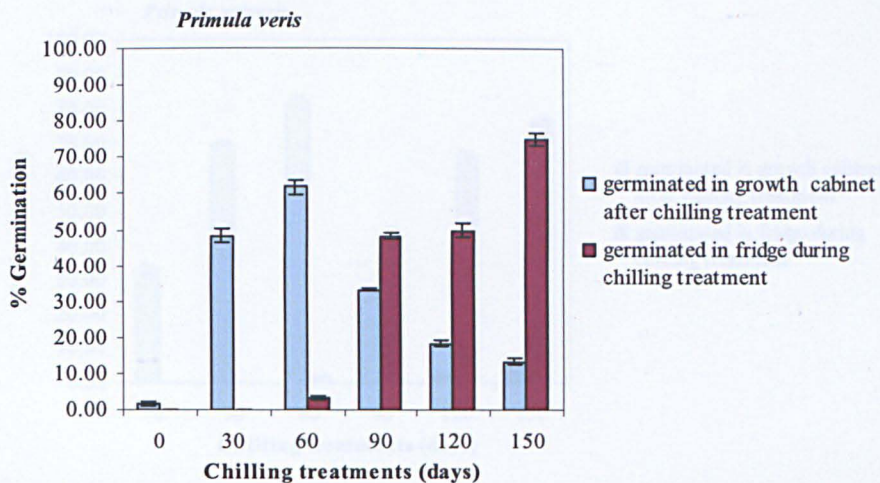


Figure 3.36 Mean maximum percentage germination of *Primula veris* seeds in response to chilling treatments. Error bars represent 1 SEM.

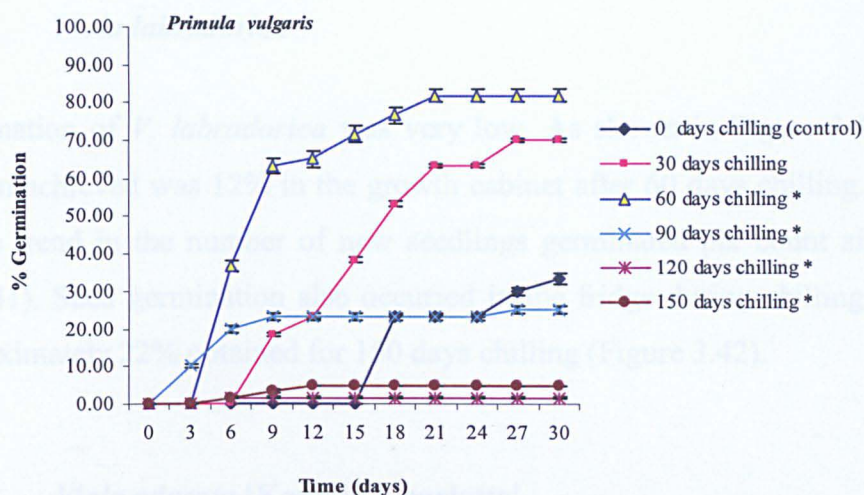


Figure 3.37 Germination of *Primula vulgaris* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM.

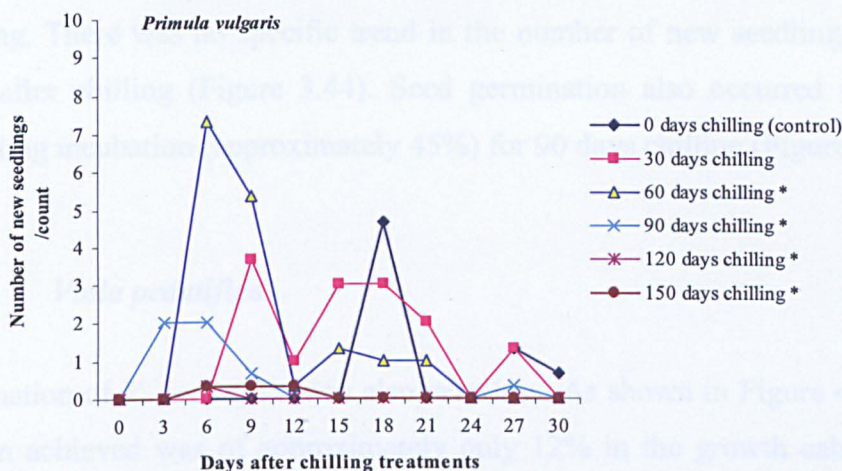


Figure 3.38 Number of new emerged seedlings per count of *Primula vulgaris* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included.

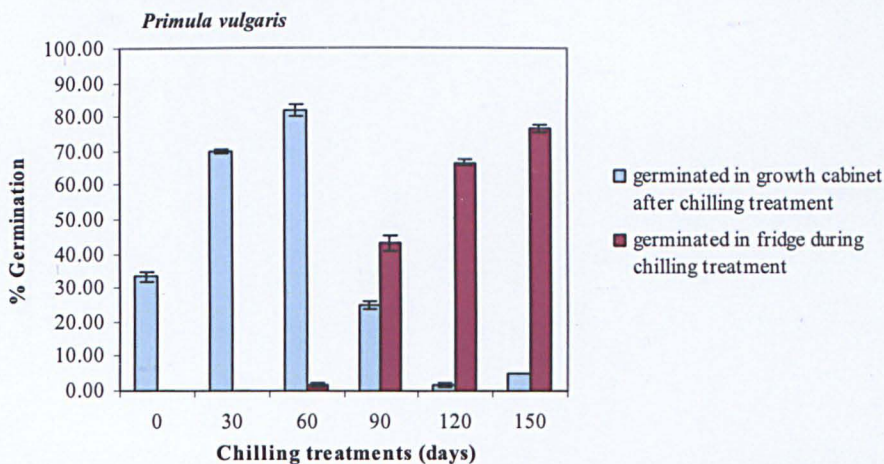


Figure 3.39 Mean maximum percentage germination of *Primula vulgaris* seeds in response to chilling treatments. Error bars represent 1 SEM.

**3.3.1.17 *Viola labradorica***

The germination of *V. labradorica* was very low. As shown in Figure 3.40, the best germination achieved was 12% in the growth cabinet after 60 days chilling. There was no specific trend in the number of new seedlings germinated per count after chilling (Figure 3.41). Seed germination also occurred in the fridge during chilling incubation with approximately 22% obtained for 150 days chilling (Figure 3.42).

**3.3.1.18 *Viola odorata* ‘Konigin Charlotte’**

The germination of *V. odorata* was very low. As shown in Figure 3.43, the best germination achieved was of approximately only 17% in the growth cabinet after 60 days chilling. There was no specific trend in the number of new seedlings germinated per count after chilling (Figure 3.44). Seed germination also occurred in the fridge during chilling incubation (approximately 45%) for 90 days chilling (Figure 3.45).

**3.3.1.19 *Viola pedatifida***

The germination of *V. pedatifida* was also very low. As shown in Figure 4.46, the best germination achieved was of approximately only 12% in the growth cabinet after 60 days chilling. There was no specific trend in the number of new seedlings germinated per count after chilling (Figure 3.47). No germination occurred in the fridge until 150 days chilling (Figure 4.48).

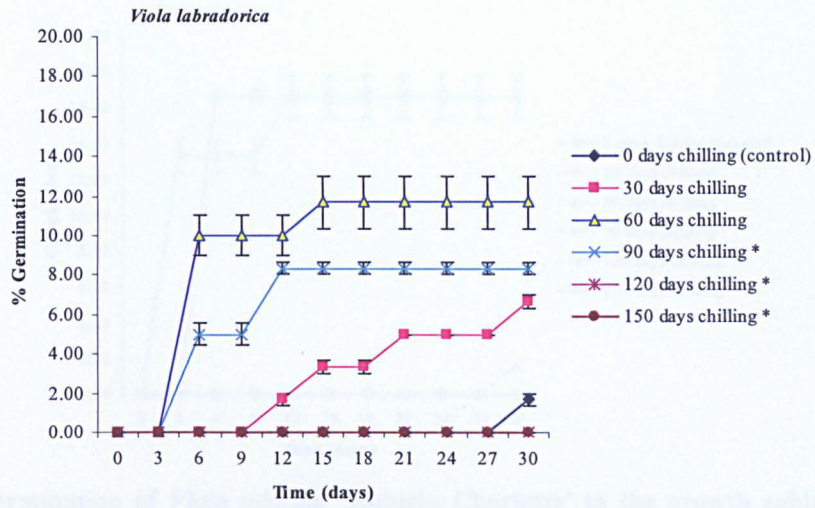


Figure 3.40 Germination of *Viola labradorica* in the growth cabinet at 20/10°C after chilling treatments. Error bars represent 1 SEM.

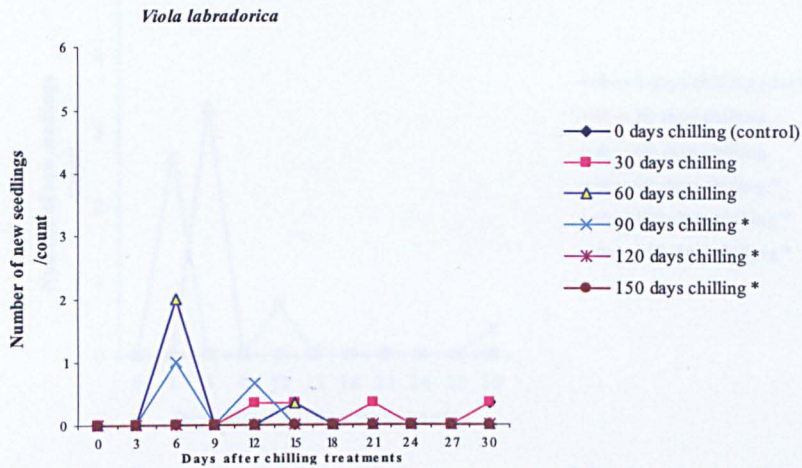


Figure 3.41 Number of new emerged seedlings per count of *Viola labradorica* in the growth cabinet after chilling treatments.

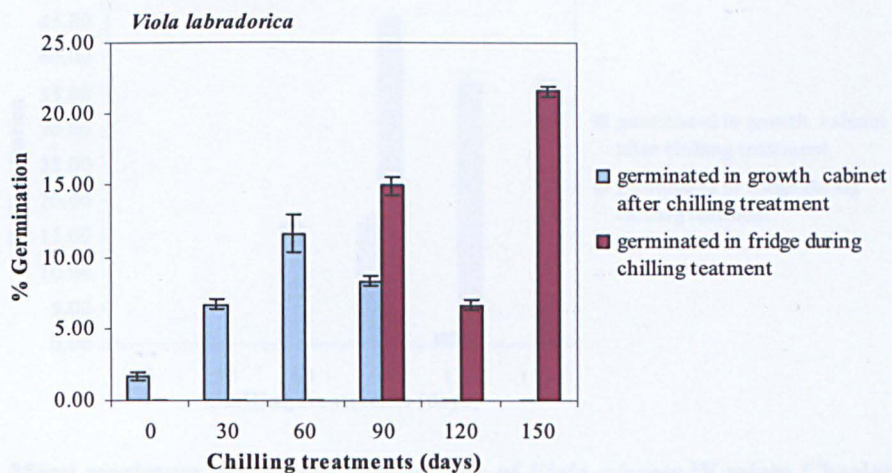


Figure 3.42 Mean maximum percentage germination of *Viola labradorica* seeds in response to chilling treatments. Error bars represent 1 SEM.

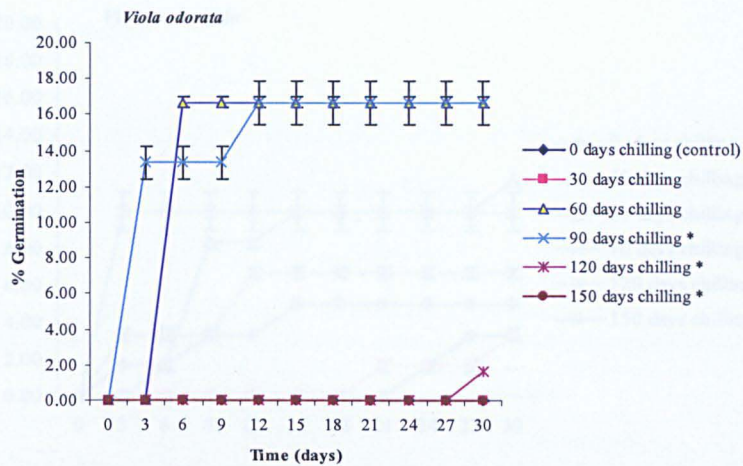


Figure 3.43 Germination of *Viola odorata* 'Konigin Charlotte' in the growth cabinet at 20/10°C after chilling treatments. Error bars represent 1 SEM.

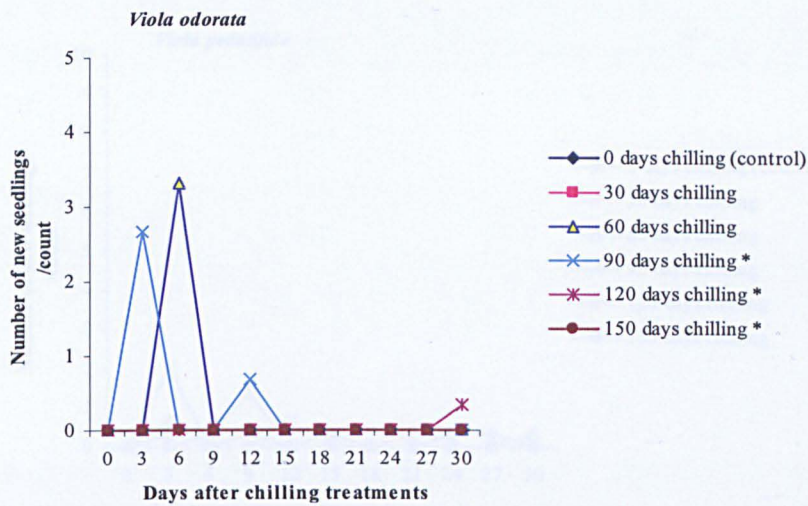


Figure 3.44 Number of new emerged seedlings per count of *Viola odorata* 'Konigin Charlotte' in the growth cabinet after chilling treatments.

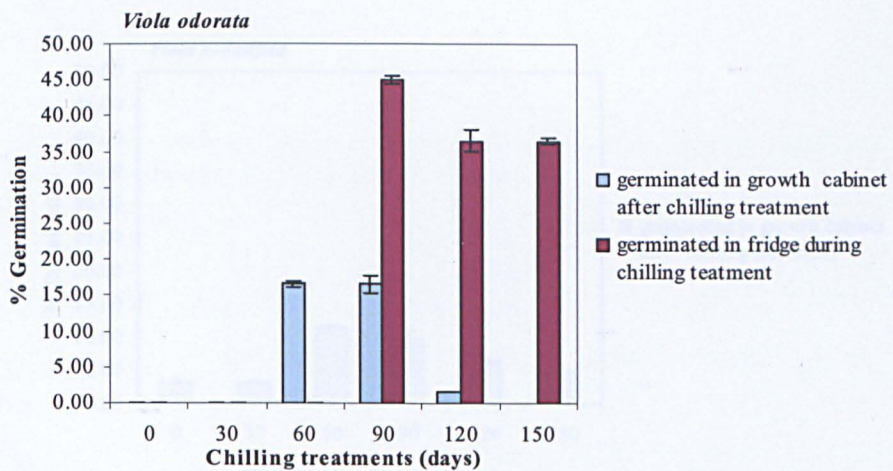


Figure 3.45 Mean maximum percentage germination of *Viola odorata* 'Konigin Charlotte' seeds in response to chilling treatments. Error bars represent 1 SEM.

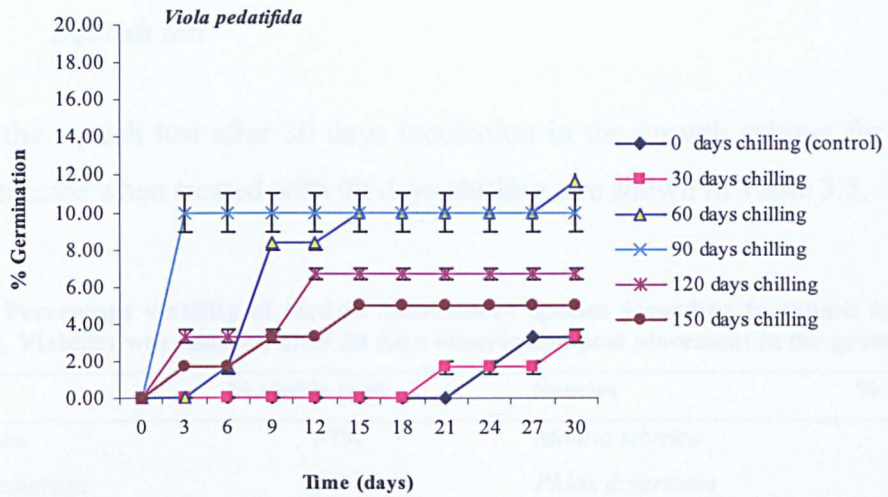


Figure 3.46 Germination of *Viola pedatifida* in the growth cabinet at 20/10°C after chilling treatments. Error bars represent 1 SEM.

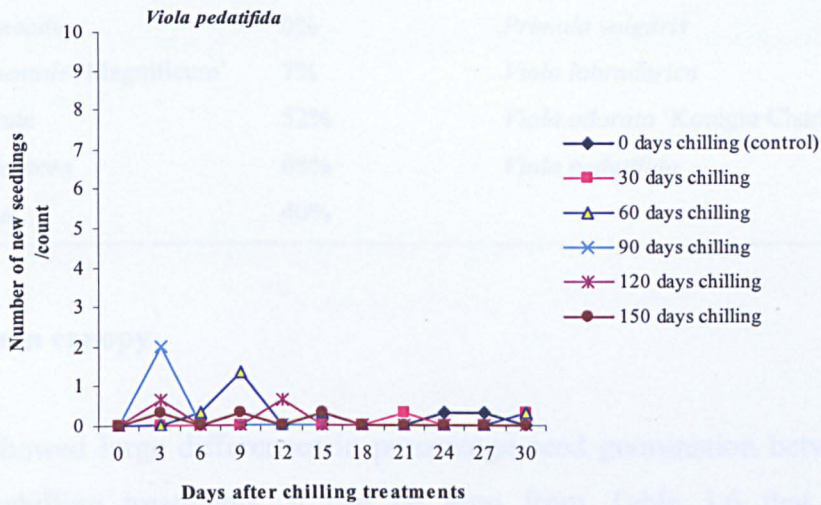


Figure 3.47 Number of new emerged seedlings per count of *Viola pedatifida* in the growth cabinet after chilling treatments.

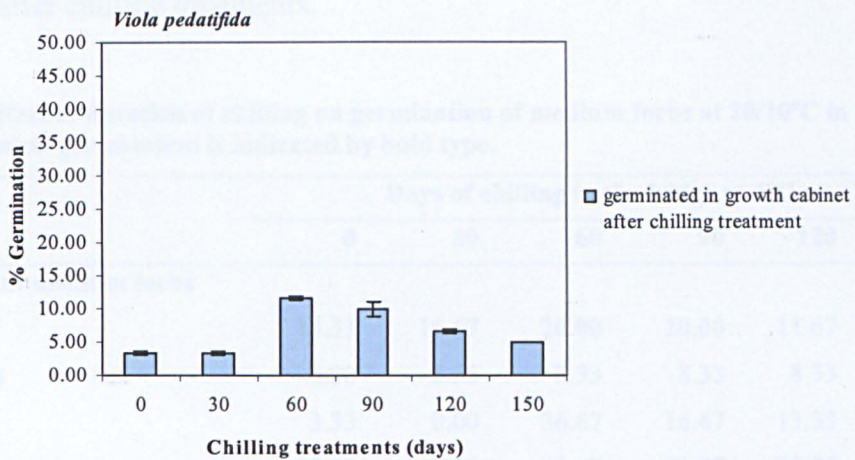


Figure 3.48 Mean maximum percentage germination of *Viola pedatifida* seeds in response to chilling treatments. Error bars represent 1 SEM.



### 3.3.1.1.1 Squash test

Results of the squash test after 30 days incubation in the growth cabinet for seed that did not germinate when treated with 90 days chilling, are shown in Table 3.5.

**Table 3.5** Percentage viability of seed in understorey species according to squash test after 90 days chilling. Viability was assessed after 30 days observation post placement in the growth cabinet.

Species	% viable seed	Species	% viable seed
<i>Ajuga reptans</i>	37%	<i>Montia sibirica</i>	18%
<i>Anemone nemorosa</i>	38%	<i>Phlox divaricata</i>	15%
<i>Anemone ranunculoides</i>	27%	<i>Polemonium reptans</i>	58%
<i>Anemone sylvestris</i> ('Madonna')	8%	<i>Primula elatior</i>	5%
<i>Cordalis solida</i>	82%	<i>Primula veris</i>	7%
<i>Dodecatheon meadia</i>	0%	<i>Primula vulgaris</i>	2%
<i>Doronicum orientale</i> 'Magnificum'	7%	<i>Viola labradorica</i>	17%
<i>Galium odoratum</i>	52%	<i>Viola odorata</i> 'Konigin Charlotte'	25%
<i>Glechoma hederacea</i>	68%	<i>Viola pedatifida</i>	2%
<i>Lathyrus vernus</i>	40%		

### 3.3.2 Medium canopy

The results showed large differences in percentage seed germination between species tested after chilling treatments. It can be seen from Table 3.6 that the highest germination was in *Tradescantia ohioensis* (73%) and the lowest was in *Phlox maculata* (10%), both after 150 days chilling. Overall, medium canopy seeds increase their germination after chilling treatments.

**Table 3.6** Effect of duration of chilling on germination of medium forbs at 20/10°C in a growth cabinet. Maximum germination is indicated by bold type.

Species	Days of chilling in the fridge at 4°C					
	0	30	60	90	120	150
<b>Shade intolerant medium forbs</b>						
<i>Aster azureus</i>	18.33	16.67	<b>20.00</b>	<b>20.00</b>	11.67	5.00
<i>Phlox maculata</i>	0.00	8.33	8.33	8.33	8.33	<b>10.00</b>
<i>Phlox pilosa</i>	3.33	0.00	<b>36.67</b>	16.67	13.33	6.67
<i>Solidago speciosa</i>	38.33	<b>48.33</b>	41.67	41.67	10.00	6.67
<i>Tradescantia ohioensis</i>	10.00	50.00	71.67	71.67	60.00	<b>73.33</b>
<i>Zizia aptera</i>	5.00	28.33	30.00	<b>41.67</b>	11.67	6.67

Generally, rate of germination (in terms of radicle protrusion) for most medium canopy species in the growth cabinet increased after between 60 and 150 days exposure to the low temperature. Rate of germination (in terms of emerged seedlings per count) of the medium canopy is erratic in all species tested. There was no specific trend or pattern in the number of new seedlings germinated per count after chilling (see graph under section results in each species tested).

Some of the species tested showed diverse germination in the fridge during the chilling periods (Table 3.7) particularly when chilled >60 days. Over 40% of *Phlox pilosa* seeds germinated in the fridge when chilled for 150 days. However, *T. ohioensis* seeds did not germinate at all in the fridge. These medium canopy species naturally associated with tall canopy plants were more likely to demonstrate intermediate levels of germination during chilling periods.

**Table 3.7** Effect of duration of chilling on germination of medium forbs in the fridge during chilling at 4°C. Maximum germination is indicated in bold.

Species	Days of chilling in the fridge at 4°C				
	60	90	120	150	Mean for post 60 days chilling
<b>Shade intolerant medium forbs</b>					
<i>Aster azureus</i>	0.00	5.00	<b>13.33</b>	10.00	9.44
<i>Phlox maculata</i>	0.00	0.00	0.00	<b>1.67</b>	0.56
<i>Phlox pilosa</i>	0.00	15.00	28.33	<b>43.33</b>	28.89
<i>Solidago speciosa</i>	0.00	1.67	20.00	<b>28.33</b>	16.67
<i>Tradescantia ohioensis</i>	0.00	0.00	0.00	0.00	0.00
<i>Zizia aptera</i>	0.00	3.33	16.67	<b>31.67</b>	17.22

As a whole, seed germination of medium canopy species can be divided into three groups (Table 3.8), with low, medium and high percentage after adequate chilling treatments (0-150 days).

**Table 3.8** Germination of medium canopy species in a growth cabinet after adequate chilling treatments, and during chilling incubation in a fridge.

Germination	Low germination percentage (< 40%)	Medium germination percentage (40%-70%)	High germination percentage (> 70%)
In a growth cabinet	<i>Aster azureus</i>	<i>Solidago speciosa</i>	<i>Tradescantia ohioensis</i>
	<i>Phlox maculata</i>	<i>Zizia aptera</i>	
	<i>Phlox pilosa</i>		
In a fridge	<i>Aster azureus</i>	<i>Phlox pilosa</i>	
	<i>Phlox maculata</i>		
	<i>Solidago speciosa</i>		
	<i>Zizia aptera</i>		

The results of each species after and throughout chilling treatments are as follows:

### 3.3.2.1 *Aster azureus*

The germination of *A. azureus* was very low. It can be seen from the Figure 3.49 that the best germination (approximately 20%) of this species in the growth cabinet was achieved after 60 days chilling. There was no specific pattern in the number of new seedlings germinated per count after chilling (Figure 3.50). Low seed germination also occurred in the fridge during chilling incubation. As shown in Figure 3.51, the highest germination in the fridge (approximately 13%) was obtained after 120 days chilling.

### 3.3.2.2 *Phlox maculata*

The germination of *P. maculata* was also very low (approximately 10%) after 150 days chilling (Figures 3.52 and 3.53). Maximum germination within the fridge was 2% and occurred during 150 days incubation (Figure 3.54).

### 3.3.2.3 *Phlox pilosa*

The highest germination (approximately 37%) of *P. pilosa* in the growth cabinet was achieved after 60 days chilling (Figures 3.55 and 3.56). Seed germination also occurred in the fridge during chilling incubation. As shown in Figure 3.57, the highest germination (approximately 43%) was obtained during 150 days chilling.

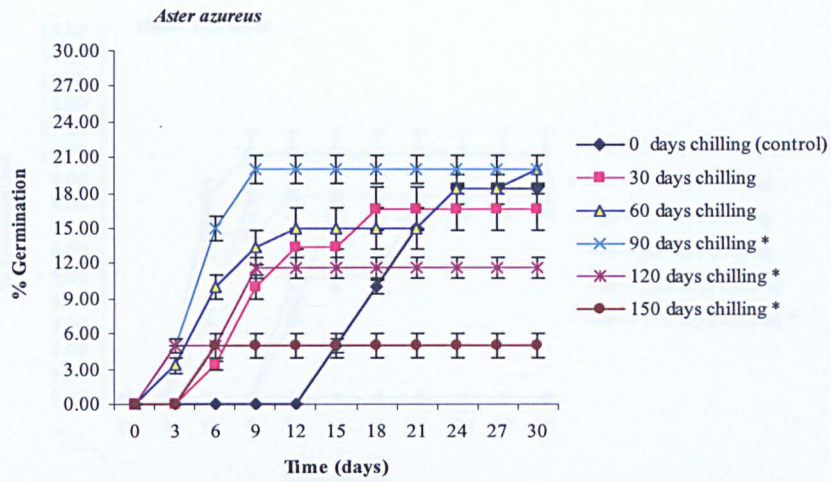


Figure 3.49 Germination of *Aster azureus* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM.

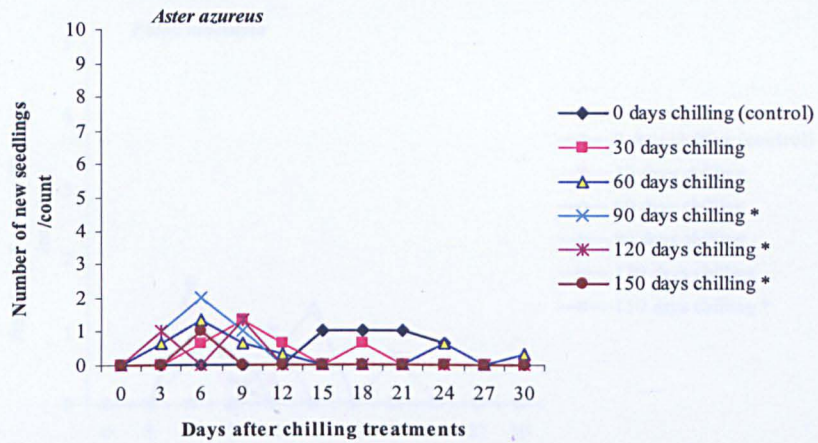


Figure 3.50 Number of new emerged seedlings per count of *Aster azureus* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included.

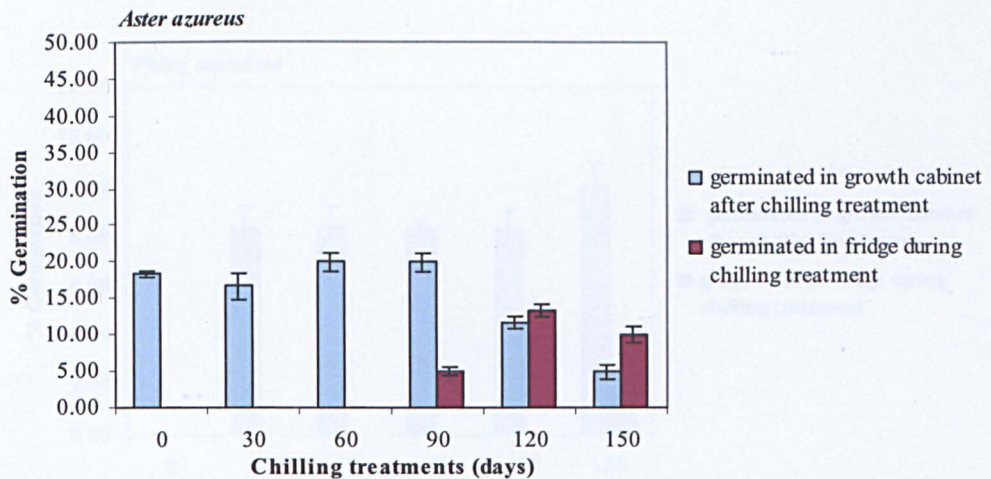


Figure 3.51 Mean maximum percentage germination of *Aster azureus* seeds in response to chilling treatments. Error bars represent 1 SEM.

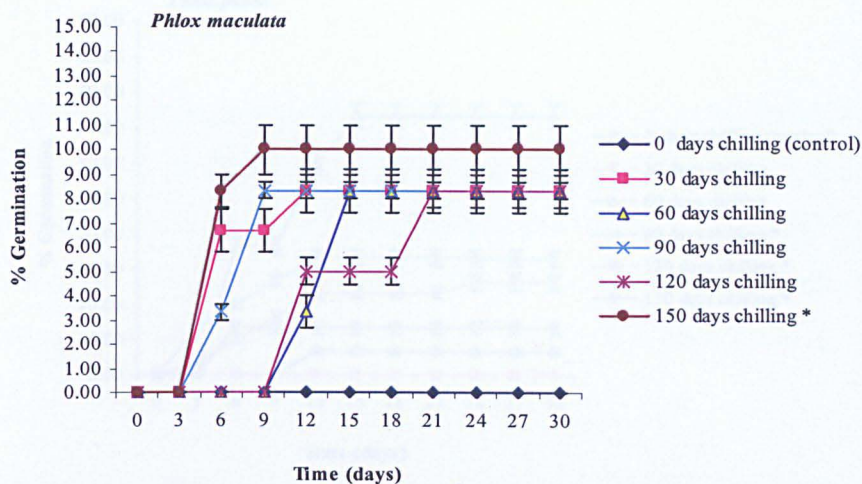


Figure 3.52 Germination of *Phlox maculata* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM.

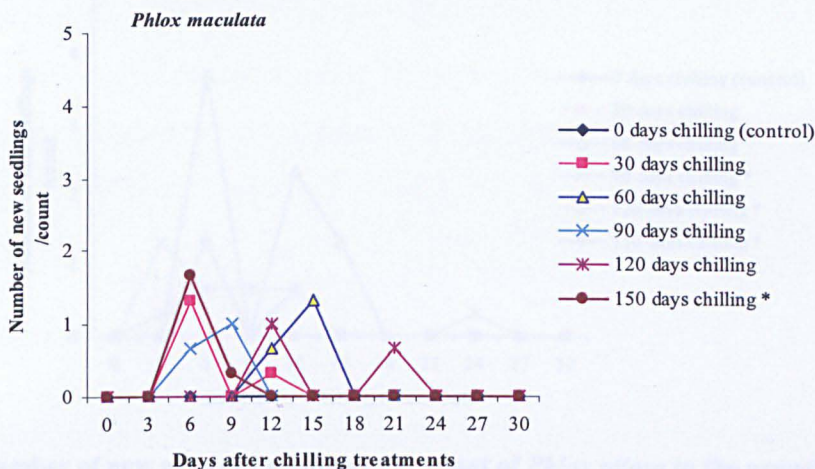


Figure 3.53 Number of new emerged seedlings per count of *Phlox maculata* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included.

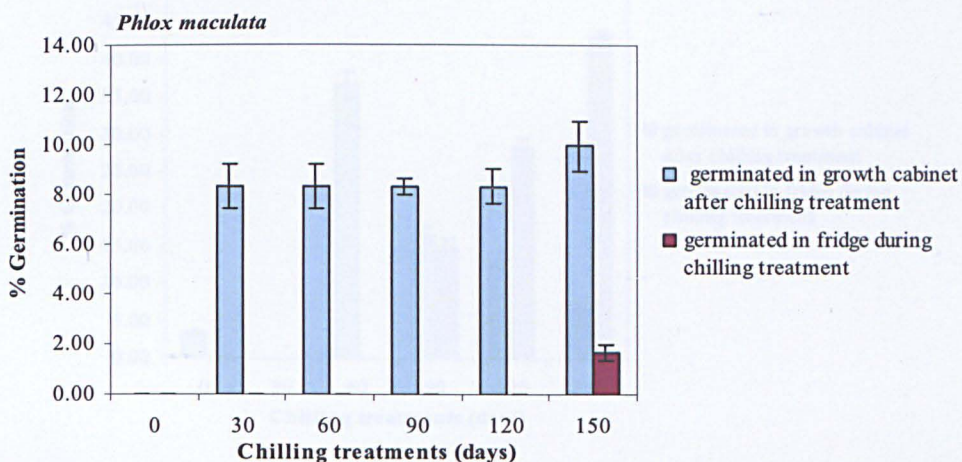


Figure 3.54 Mean maximum percentage germination of *Phlox maculata* seeds in response to chilling treatments. Error bars represent 1 SEM.

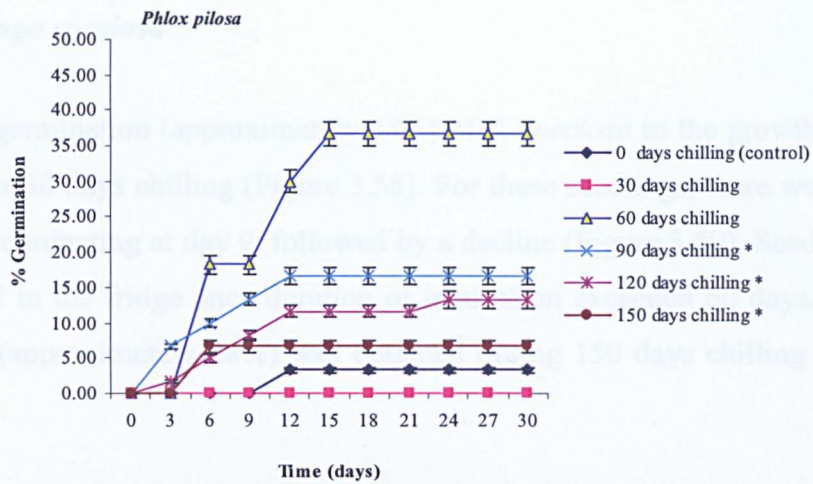


Figure 3.55 Germination of *Phlox pilosa* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM.

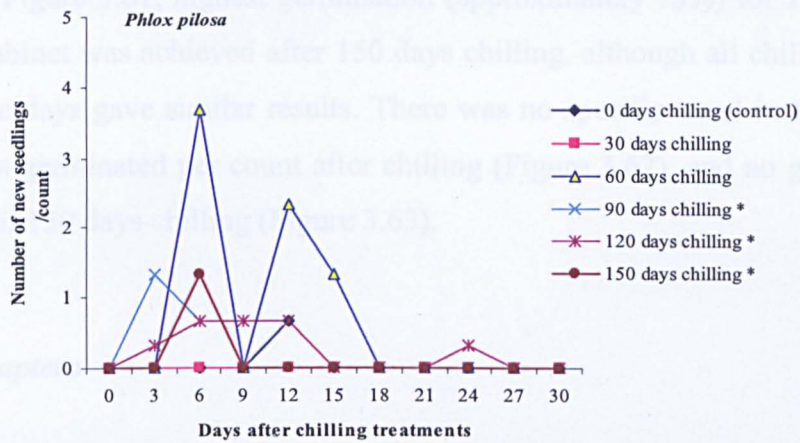


Figure 3.56 Number of new emerged seedlings per count of *Phlox pilosa* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included.

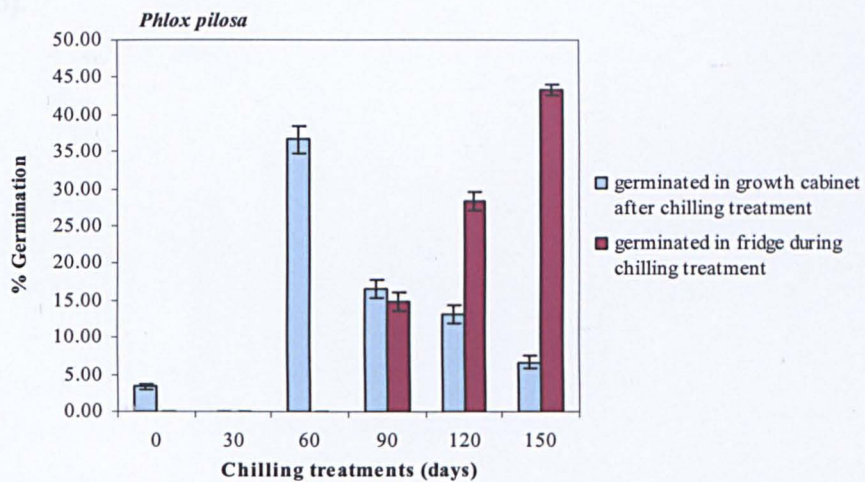


Figure 3.57 Mean maximum percentage germination of *Phlox pilosa* seeds in response to chilling treatments. Error bars represent 1 SEM.

#### **3.3.2.4 *Solidago speciosa***

The highest germination (approximately 48%) of *S. speciosa* in the growth cabinet was achieved after 30 days chilling (Figure 3.58). For these seedlings, there were just under 6 seedlings germinating at day 9, followed by a decline (Figure 3.59). Seed germination also occurred in the fridge once duration of incubation exceeded 60 days. The highest germination (approximately 28%) was obtained during 150 days chilling in the fridge (Figure 3.60).

#### **3.3.2.5 *Tradescantia ohioensis***

As shown in Figure 3.61, highest germination (approximately 73%) for *T. ohioensis* in the growth cabinet was achieved after 150 days chilling, although all chilling durations of 60 or more days gave similar results. There was no specific trend in the number of new seedlings germinated per count after chilling (Figure 3.62), and no germination in the fridge until 150 days chilling (Figure 3.63).

#### **3.3.2.6 *Zizia aptera***

The highest germination (approximately 42%) of *Z. aptera* in the growth cabinet was achieved after 90 days chilling (Figures 3.64 and 3.65). Seed germination (approximately 32%) also occurred in the fridge during 150 days chilling incubation (Figure 3.66).

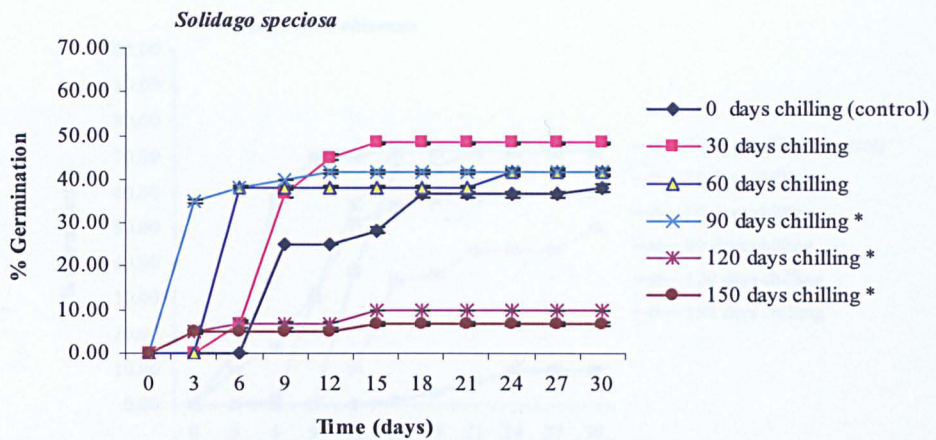


Figure 3.58 Germination of *Solidago speciosa* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM.

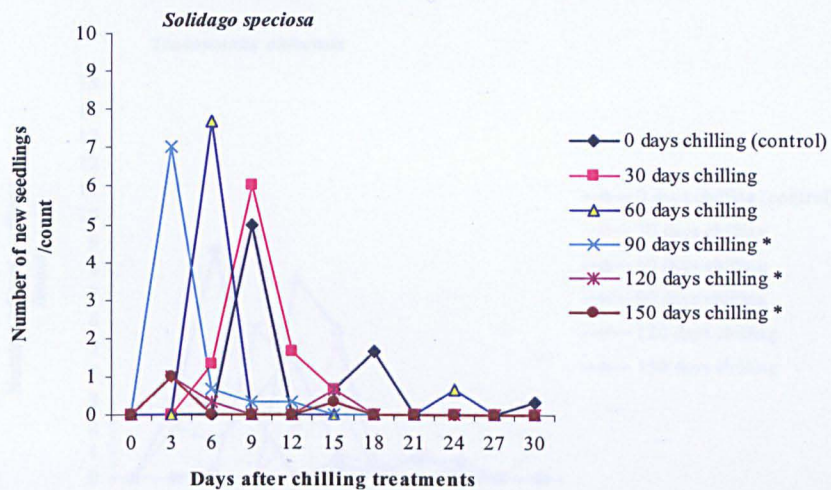


Figure 3.59 Number of new emerged seedlings per count of *Solidago speciosa* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included.

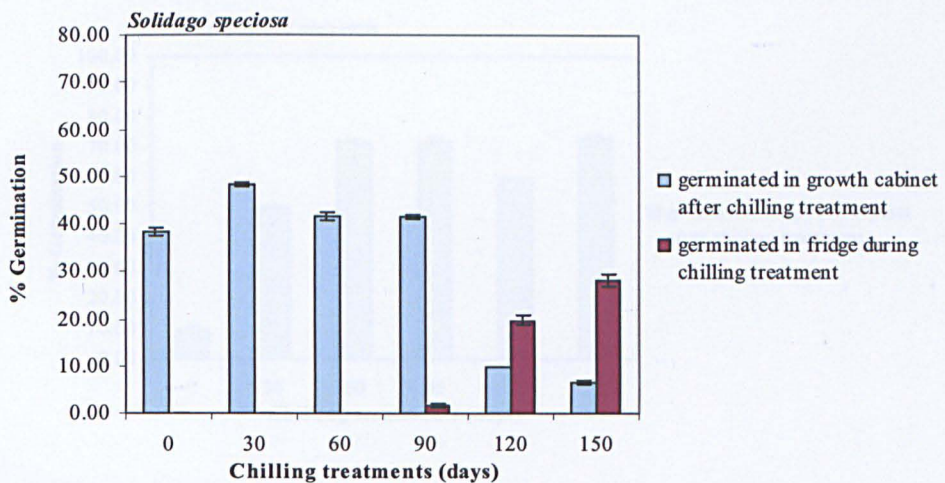


Figure 3.60 Mean maximum percentage germination of *Solidago speciosa* seeds in response to chilling treatments. Error bars represent 1 SEM.



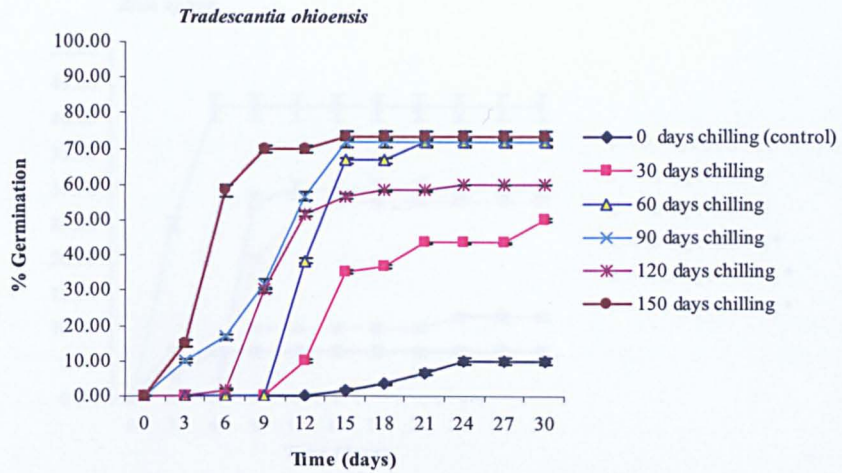


Figure 3.61 Germination of *Tradescantia ohioensis* seeds in the growth cabinet at 20/10°C after chilling treatments. Error bars represent 1 SEM.

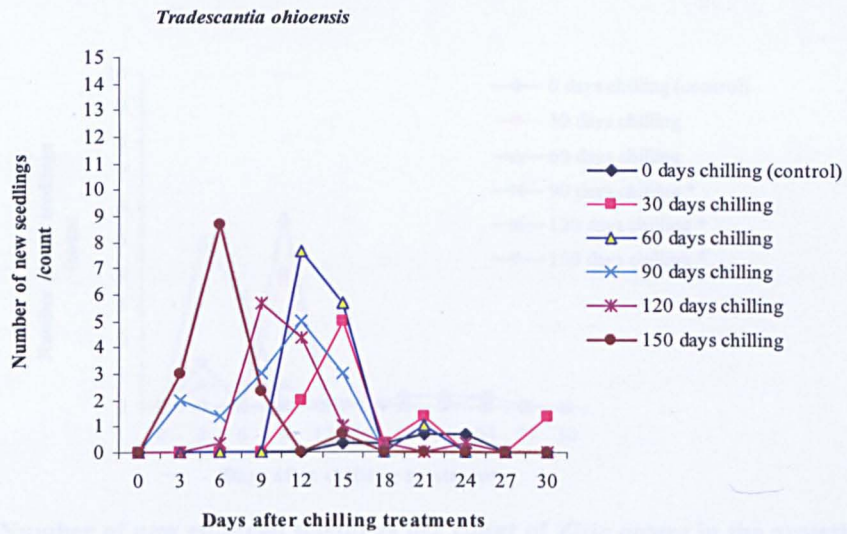


Figure 3.62 Number of new emerged seedlings per count of *Tradescantia ohioensis* in the growth cabinet after chilling treatments.

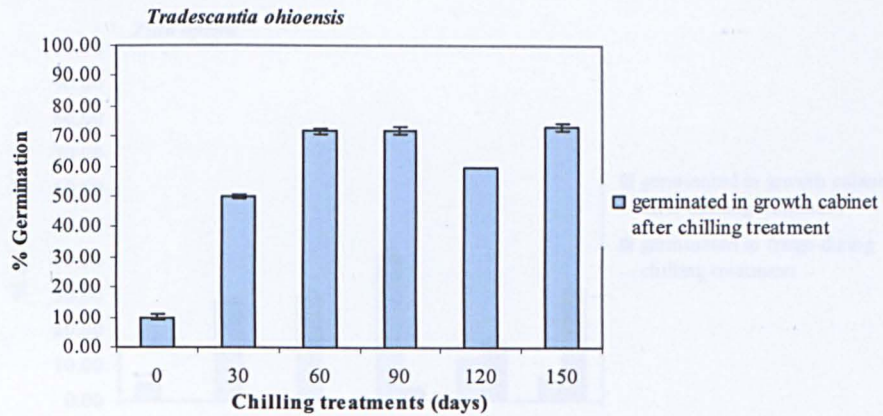


Figure 3.63 Mean maximum percentage germination of *Tradescantia ohioensis* seeds in response to chilling treatments. Error bars represent 1 SEM.

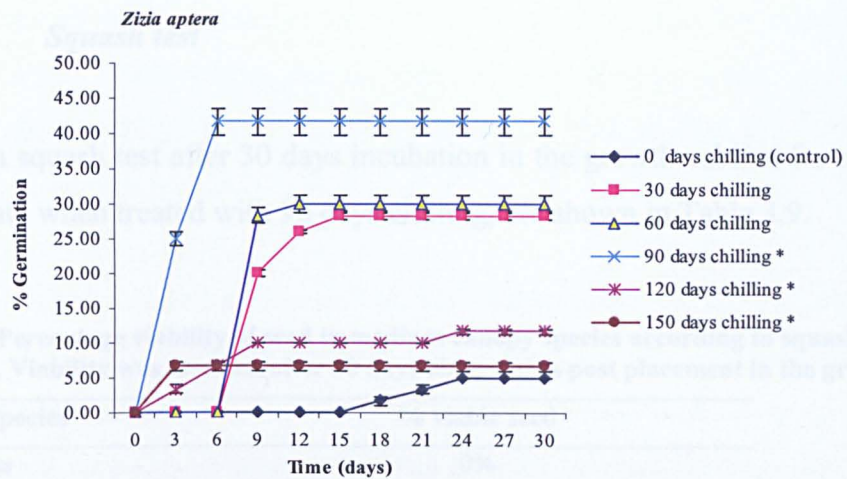


Figure 3.64 Germination of *Zizia aptera* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM.

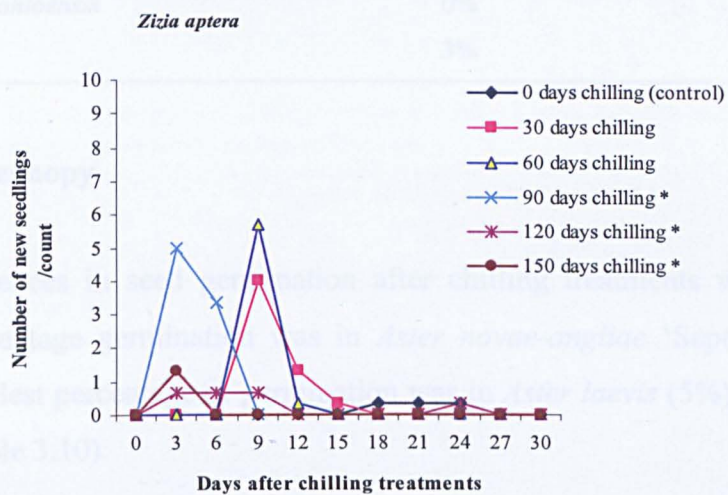


Figure 3.65 Number of new emerged seedlings per count of *Zizia aptera* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included.

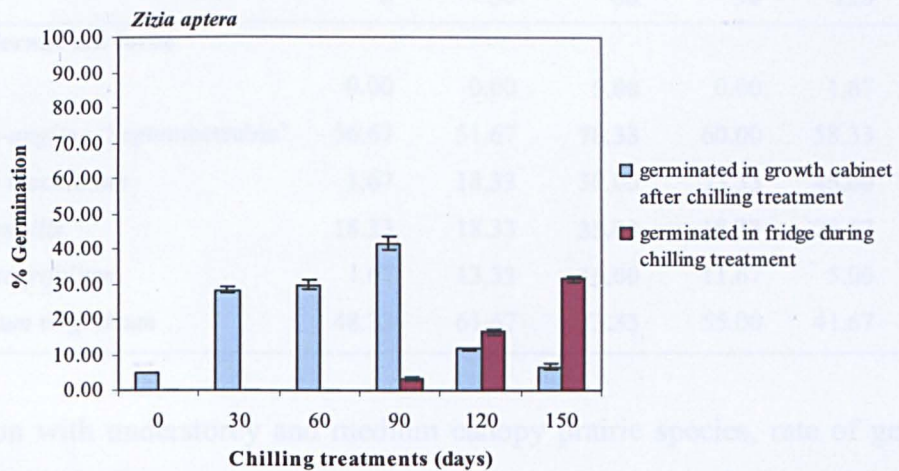


Figure 3.66 Mean maximum percentage germination of *Zizia aptera* seeds in response to chilling treatments. Error bars represent 1 SEM.

### 3.3.2.1.1 *Squash test*

Results of a squash test after 30 days incubation in the growth cabinet for seed that did not germinate when treated with 90 days chilling, are shown in Table 3.9.

**Table 3.9** Percentage viability of seed in medium canopy species according to squash test after 90 days chilling. Viability was assessed after 30 days observation post placement in the growth cabinet.

Species	% viable seed
<i>Aster azureus</i>	0%
<i>Phlox maculata</i>	8%
<i>Phlox pilosa</i>	17%
<i>Solidago speciosa</i>	0%
<i>Tradescantia ohioensis</i>	0%
<i>Zizia aptera</i>	3%

### 3.3.3 Tall canopy

Large differences in seed germination after chilling treatments were observed. The highest percentage germination was in *Aster novae-angliae* 'Septemberrubin' (78%) and the smallest percentage of germination was in *Aster laevis* (5%), both after 60 days chilling (Table 3.10).

**Table 3.10** Effect of duration of chilling on germination of tall forbs at 20/10°C in a growth cabinet. Maximum germination is indicated in bold.

Species	Days of chilling in the fridge at 4°C					
	0	30	60	90	120	150
<b>Shade intolerant tall forbs</b>						
<i>Aster laevis</i>	0.00	0.00	<b>5.00</b>	0.00	1.67	0.00
<i>Aster novae-angliae</i> 'Septemberrubin'	56.67	51.67	<b>78.33</b>	60.00	58.33	43.33
<i>Eupatorium maculatum</i>	1.67	18.33	30.00	33.33	<b>45.00</b>	43.33
<i>Helianthus mollis</i>	18.33	18.33	<b>35.00</b>	18.33	26.67	26.67
<i>Silphium integrifolium</i>	1.67	13.33	<b>15.00</b>	11.67	5.00	10.00
<i>Veronicastrum virginicum</i>	48.33	61.67	<b>73.33</b>	55.00	41.67	30.00

In common with understorey and medium canopy prairie species, rate of germination (in terms of radicle protrusion) for most tall canopy species in the growth cabinet was considerably increased, effectively with 60 days exposure chilling than other chilling

duration. Rate of germination (in terms of emerged seedlings per count) of the tall canopy is erratic in all species tested. There was no specific trend or pattern in the number of new seedlings germinated per count after chilling (see graph under section results in each species tested).

Some of the species tested germinated in the fridge during the chilling periods (Table 3.11) particularly when chilled for >60 days. These species, however, germinated at very low percentages ranging from approximately 1% (*Silphium integrifolium*) to 26% (*Veronicastrum virginicum*) based on means for post 60 days chilling. Species such as *A. laevis* and *Eupatorium maculatum* did not germinate at all in the fridge. These, shade intolerant species naturally associated with the open habitats were more likely to demonstrate low levels of germination during the chilling periods.

**Table 3.11** Effect of duration of chilling on germination of tall forbs in the fridge during chilling at 4°C. Maximum germination is indicated in bold.

Species	Days of chilling in the fridge at 4°C				
	60	90	120	150	Mean for post 60 days chilling
<b>Shade intolerant tall forbs</b>					
<i>Aster laevis</i>	0.00	0.00	0.00	0.00	0.00
<i>Aster novae-angliae</i> 'Septemberrubin'	0.00	6.67	<b>3.33</b>	10.00	10.00
<i>Eupatorium maculatum</i>	0.00	0.00	0.00	0.00	0.00
<i>Helianthus mollis</i>	0.00	0.00	<b>10.00</b>	1.67	3.89
<i>Silphium integrifolium</i>	0.00	0.00	0.00	<b>1.67</b>	0.57
<i>Veronicastrum virginicum</i>	0.00	3.33	21.67	<b>53.33</b>	26.11

As a whole, seed germination of tall canopy species can be divided into three groups (Table 3.12), with low, medium and high percentages after adequate chilling treatments (0-150 days).

**Table 3.12 Germination of tall canopy species in a growth cabinet after adequate chilling treatments, and during chilling incubation in a fridge.**

Germination	Low germination percentage (< 40%)	Medium germination percentage (40%-70%)	High germination percentage (> 70%)
	<i>Aster laevis</i>	<i>Eupatorium maculatum</i>	<i>Aster novae-angliae</i> 'Septemberrubin'
<b>In a growth cabinet</b>	<i>Helianthus mollis</i> <i>Silphium integrifolium</i>		<i>Veronicastrum virginicum</i>
	<i>Aster novae-angliae</i> 'Septemberrubin'	<i>Veronicastrum virginicum</i>	
<b>In a fridge</b>	<i>Helianthus mollis</i> <i>Silphium integrifolium</i>		

The results of each species after and throughout chilling treatments are as follows:

### 3.3.3.1 *Aster laevis*

The germination of *A. laevis* was very low. As can be seen from the Figure 3.67 the best germination (approximately 5%) was observed in the growth cabinet after 60 days chilling. For these seedlings, there was no specific trend in the number of new emerged seedlings per count after chilling (Figure 3.68). There was no seed germination in the fridge until 150 days chilling (Figure 3.69).

### 3.3.3.2 *Aster novae-angliae* 'Septemberrubin'

The germination patterns for all seed treated by chilling showed a likeness to a sigmoid curve. As shown in Figure 3.70, the highest germination (approximately 78%) of *A. novae-angliae* 'Septemberrubin' in the growth cabinet was achieved after 60 days chilling. There was no specific trend in the number of new seedlings germinated per count after chilling (Figure 3.71). Seed germination also occurred in the fridge during incubation of between 90 and 150 days chilling (7% to 10% respectively (Figure 3.72)).

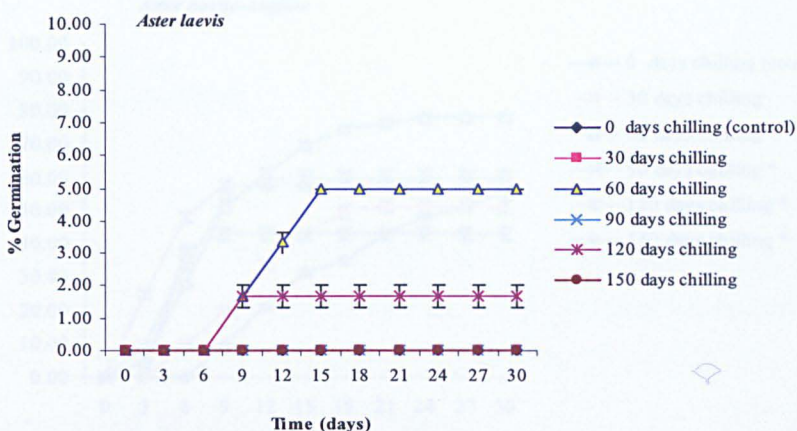


Figure 3.67 Germination of *Aster laevis* seeds in the growth cabinet at 20/10°C after chilling treatments. Error bars represent 1 SEM.

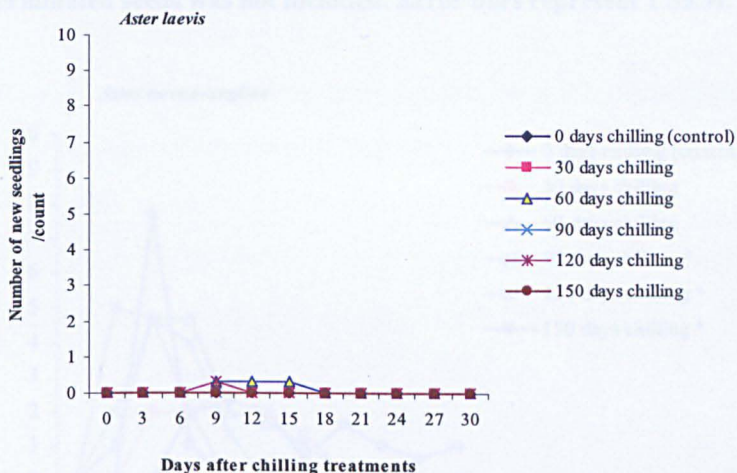


Figure 3.68 Number of new emerged seedlings per count of *Aster laevis* in the growth cabinet after chilling treatments.

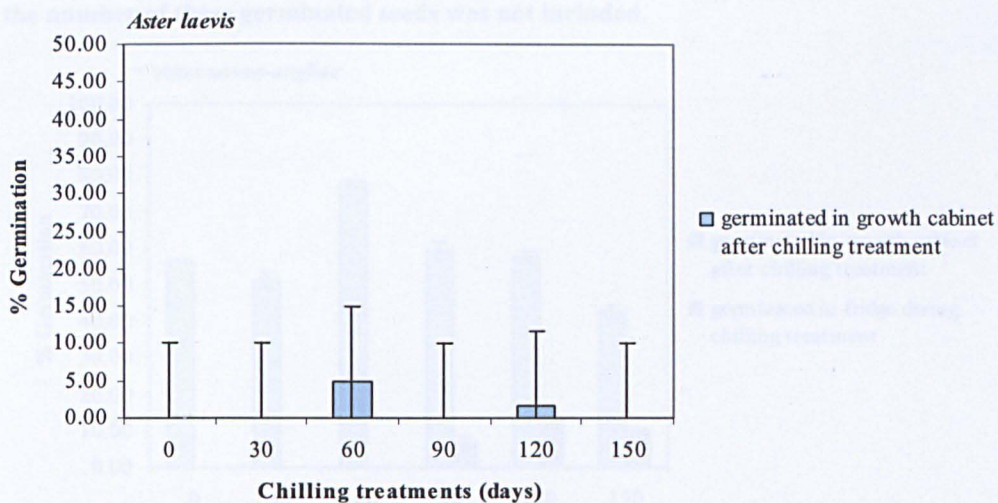


Figure 3.69 Mean maximum percentage germination of *Aster laevis* seeds in response to chilling treatments. Error bars represent 1 SEM.

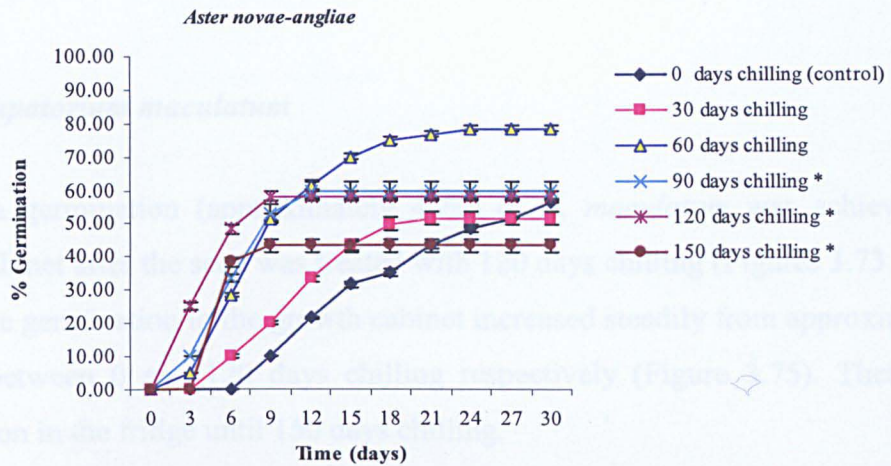


Figure 3.70 Germination of *Aster novae-angliae* 'Septemberrubin' seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM.

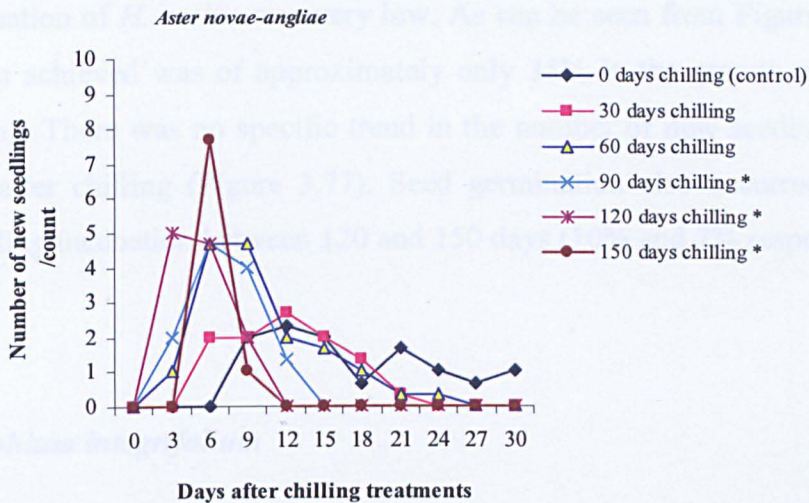


Figure 3.71 Number of new emerged seedlings per count of *Aster novae-angliae* 'Septemberrubin' in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included.

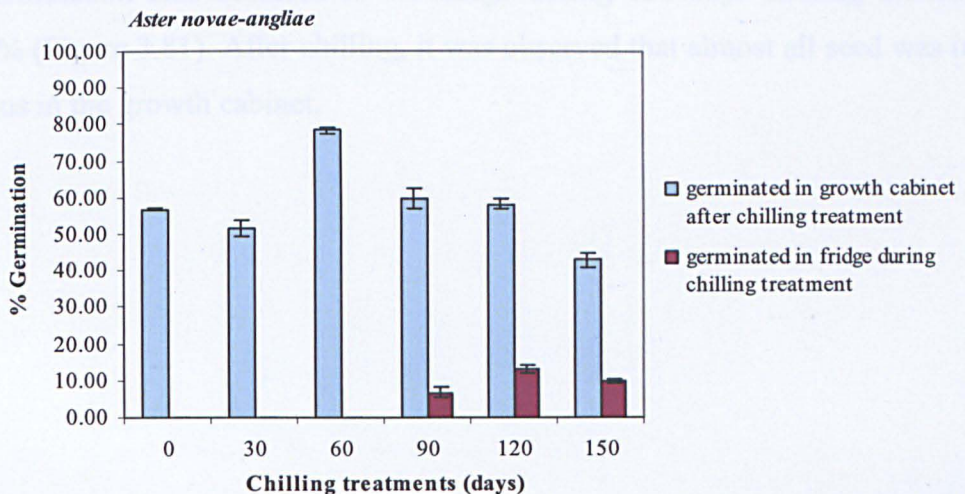


Figure 3.72 Mean maximum percentage germination of *Aster novae-angliae* 'Septemberrubin' seeds in response to chilling treatments. Error bars represent 1 SEM.

### 3.3.3.3 *Eupatorium maculatum*

Maximum germination (approximately 45%) of *E. maculatum* was achieved in the growth cabinet after the seed was treated with 120 days chilling (Figures 3.73 and 3.74). Percentage germination in the growth cabinet increased steadily from approximately 2% to 45% between 0 and 120 days chilling respectively (Figure 3.75). There was no germination in the fridge until 150 days chilling.

### 3.3.3.4 *Helianthus mollis*

The germination of *H. mollis* was very low. As can be seen from Figure 3.76, the best germination achieved was of approximately only 35% in the growth cabinet after 60 days chilling. There was no specific trend in the number of new seedlings germinated per count after chilling (Figure 3.77). Seed germination also occurred in the fridge during chilling incubation between 120 and 150 days (10% and 2% respectively (Figure 3.78)).

### 3.3.3.5 *Silphium integrifolium*

Maximum germination (approximately 15%) of *S. integrifolium* was achieved in the growth cabinet after the seed was treated with 60 days chilling (Figures 3.79 and 3.80). Seed germination also occurred in the fridge during 150 days chilling incubation of about 2% (Figure 3.81). After chilling, it was observed that almost all seed was infected by fungus in the growth cabinet.



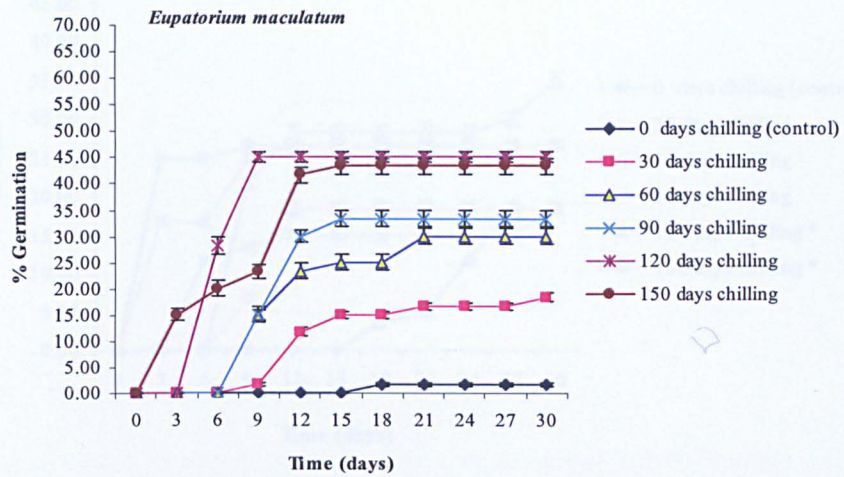


Figure 3.73 Germination of *Eupatorium maculatum* seeds in the growth cabinet at 20/10°C after chilling treatments. Error bars represent 1 SEM.

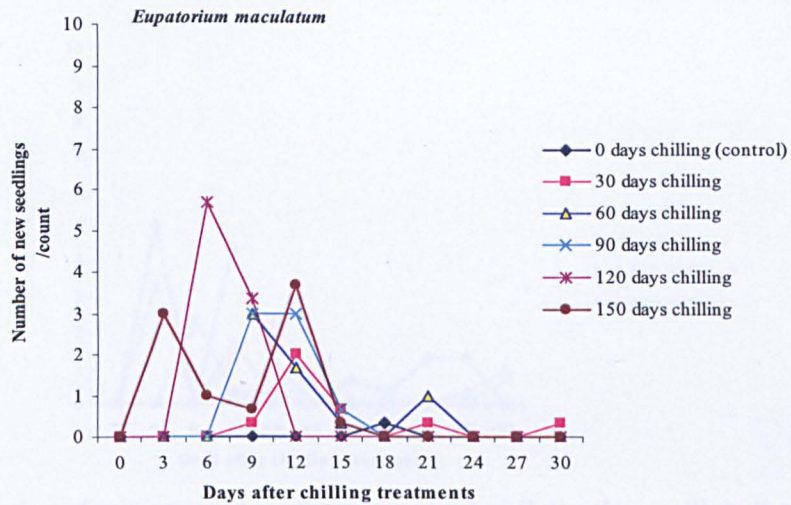


Figure 3.74 Number of new emerged seedlings per count of *Eupatorium maculatum* in the growth cabinet after chilling treatments.

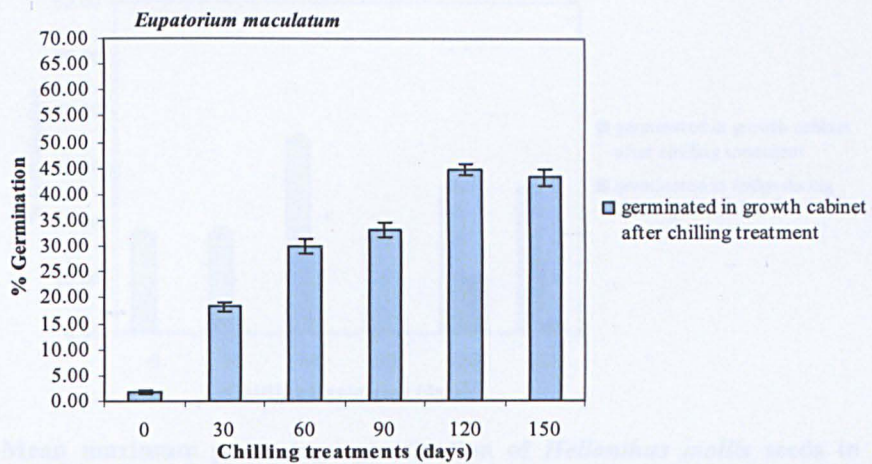


Figure 3.75 Mean maximum percentage germination of *Eupatorium maculatum* seeds in response to chilling treatments. Error bars represent 1 SEM.

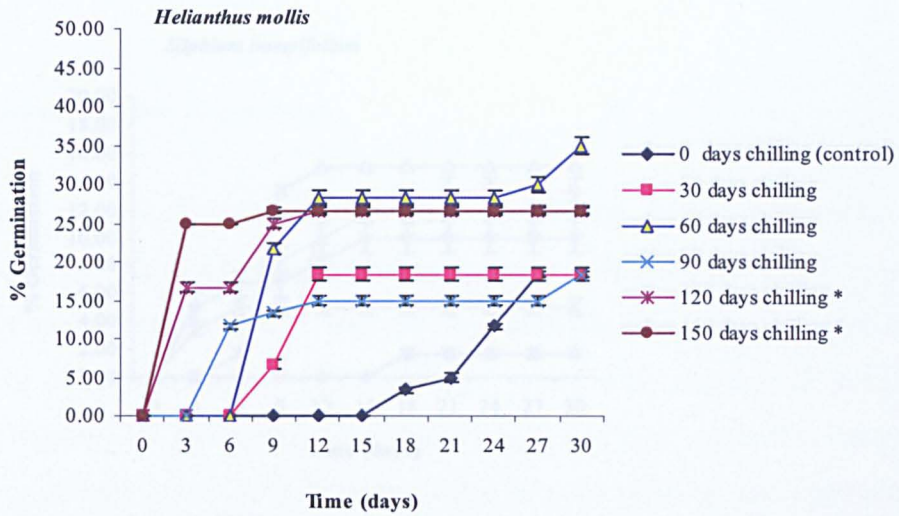


Figure 3.76 Germination of *Helianthus mollis* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM.

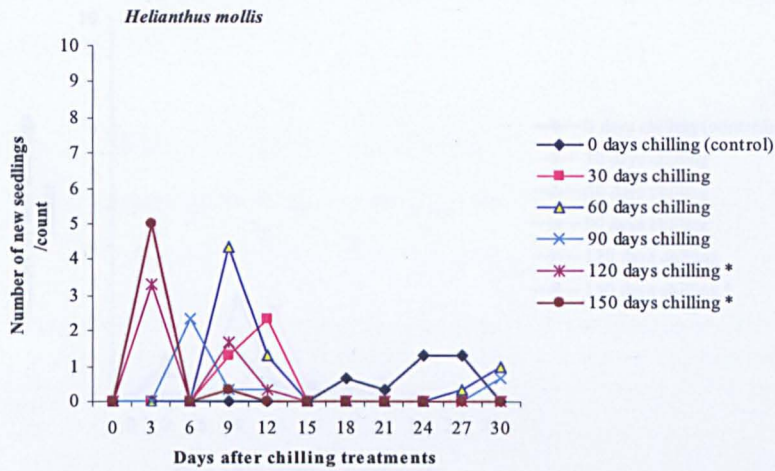


Figure 3.77 Number of new emerged seedlings per count of *Helianthus mollis* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included.

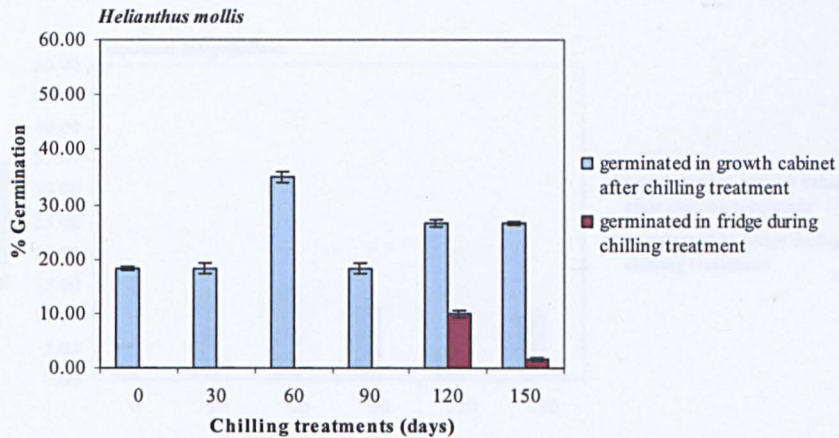


Figure 3.78 Mean maximum percentage germination of *Helianthus mollis* seeds in response to chilling treatments. Error bars represent 1 SEM.

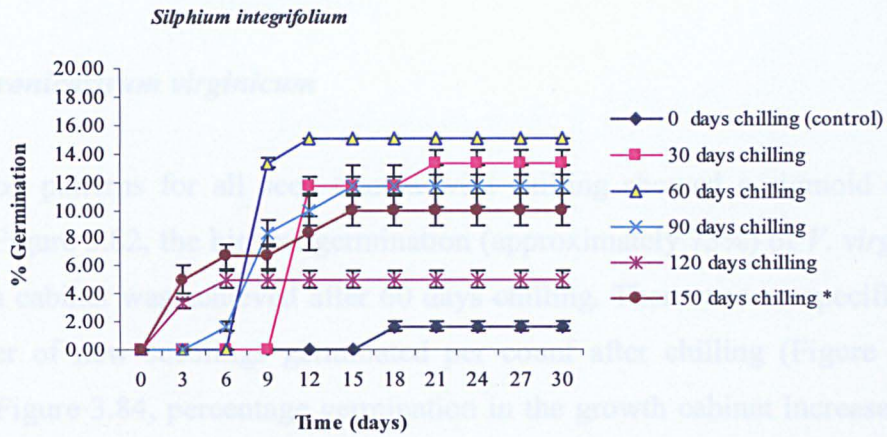


Figure 3.79 Germination of *Silphium integrifolium* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM.

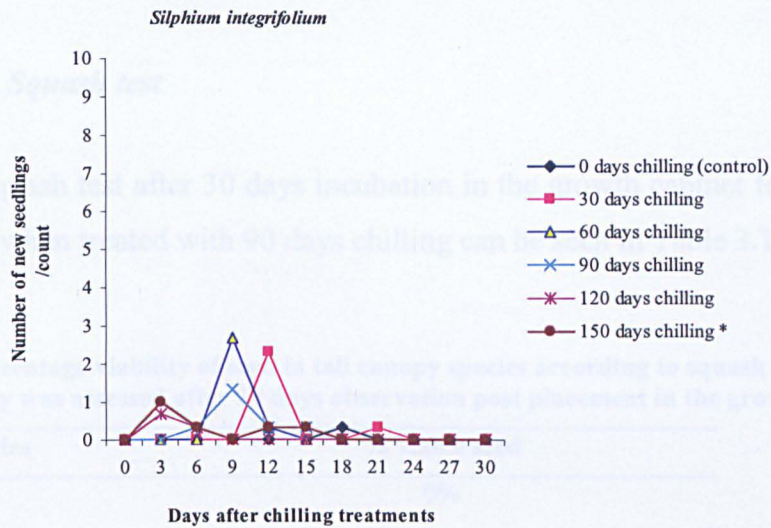


Figure 3.80 Number of new emerged seedlings per count of *Silphium integrifolium* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included.

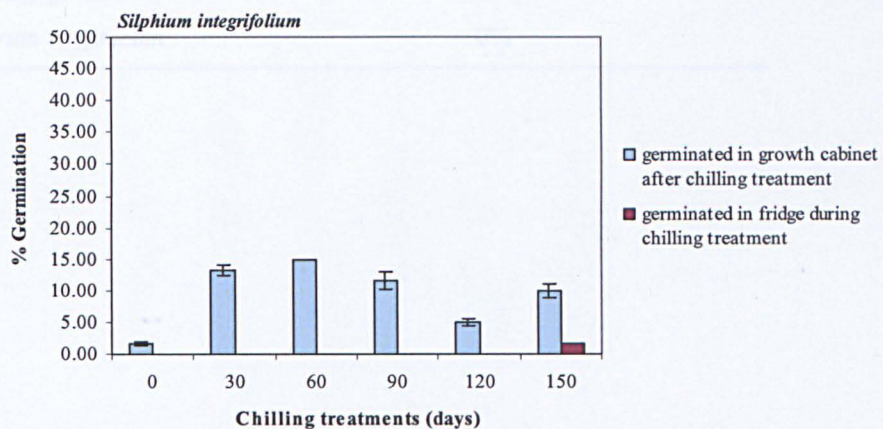


Figure 3.81 Mean maximum percentage germination of *Silphium integrifolium* seeds in response to chilling treatments. Error bars represent 1 SEM.

### 3.3.3.6 *Veronicastrum virginicum*

Germination patterns for all seed treated with chilling showed a sigmoid curve. As shown in Figure 3.82, the highest germination (approximately 73%) of *V. virginicum* in the growth cabinet was achieved after 60 days chilling. There was no specific trend in the number of new seedlings germinated per count after chilling (Figure 3.83). As shown in Figure 3.84, percentage germination in the growth cabinet increased steadily from approximately 2% to 73% between 0 and 60 days chilling, and decreased steadily to 30% after 150 days chilling. Seed germination also occurred in the fridge during chilling incubation (approximately 53%) during 150 days chilling.

#### 3.3.3.1.1 *Squash test*

Results of a squash test after 30 days incubation in the growth cabinet for seed that did not germinate when treated with 90 days chilling can be seen in Table 3.13.

**Table 3.13** Percentage viability of seed in tall canopy species according to squash test after 90 days chilling. Viability was assessed after 30 days observation post placement in the growth cabinet.

Species	% viable seed
<i>Aster laevis</i>	0%
<i>Aster novae-angliae</i> 'Septemberrubin'	0%
<i>Eupatorium maculatum</i>	5%
<i>Helianthus mollis</i>	0%
<i>Silphium integrifolium</i>	0%
<i>Veronicastrum virginicum</i>	0%

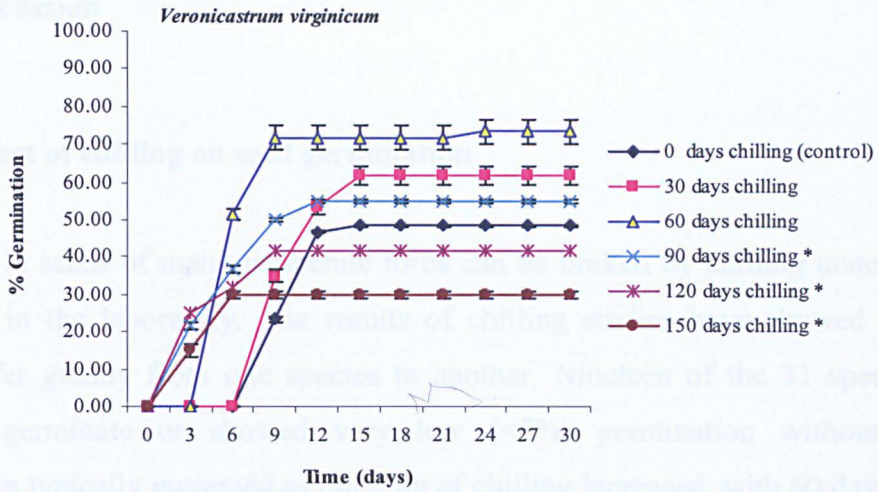


Figure 3.82 Germination of *Veronicastrum virginicum* seeds in the growth cabinet at 20/10°C after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included. Error bars represent 1 SEM.

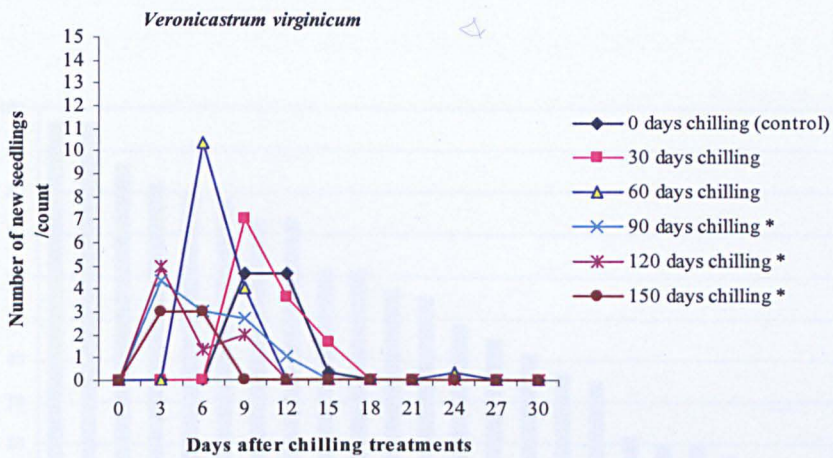


Figure 3.83 Number of new emerged seedlings per count of *Veronicastrum virginicum* in the growth cabinet after chilling treatments. (\*) Some seeds had already germinated in the fridge at 4°C; the number of these germinated seeds was not included.

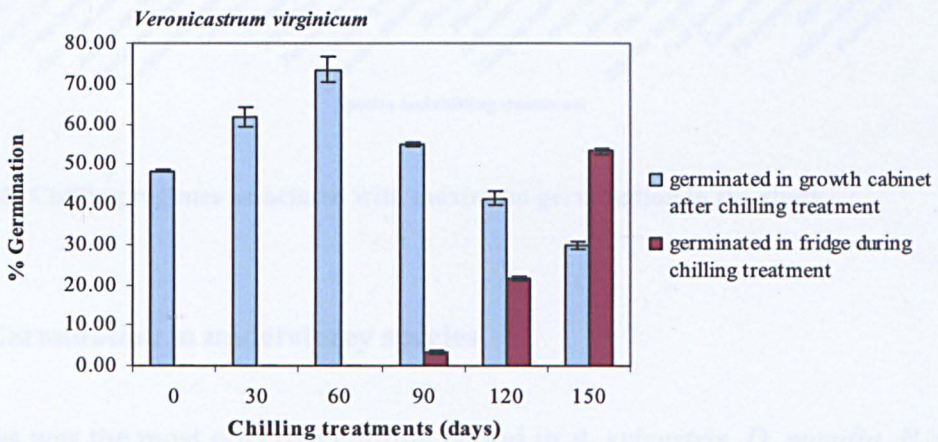


Figure 3.84 Mean maximum percentage germination of *Veronicastrum virginicum* seeds in response to chilling treatments. Error bars represent 1 SEM.

## 3.4 Discussion

### 3.4.1 Effect of chilling on seed germination

Dormancy in seeds of many temperate forbs can be broken by chilling under artificial conditions in the laboratory. The results of chilling studies have showed that these effects differ greatly from one species to another. Nineteen of the 31 species tested failed to germinate or showed very low (<5%) germination without chilling. Germination typically increased as duration of chilling increased, with 60 days the most effective chilling period in many woodland and medium-tall canopy species. Figure 3.85 summarises the maximum germination of each species in the growth cabinet in this study.

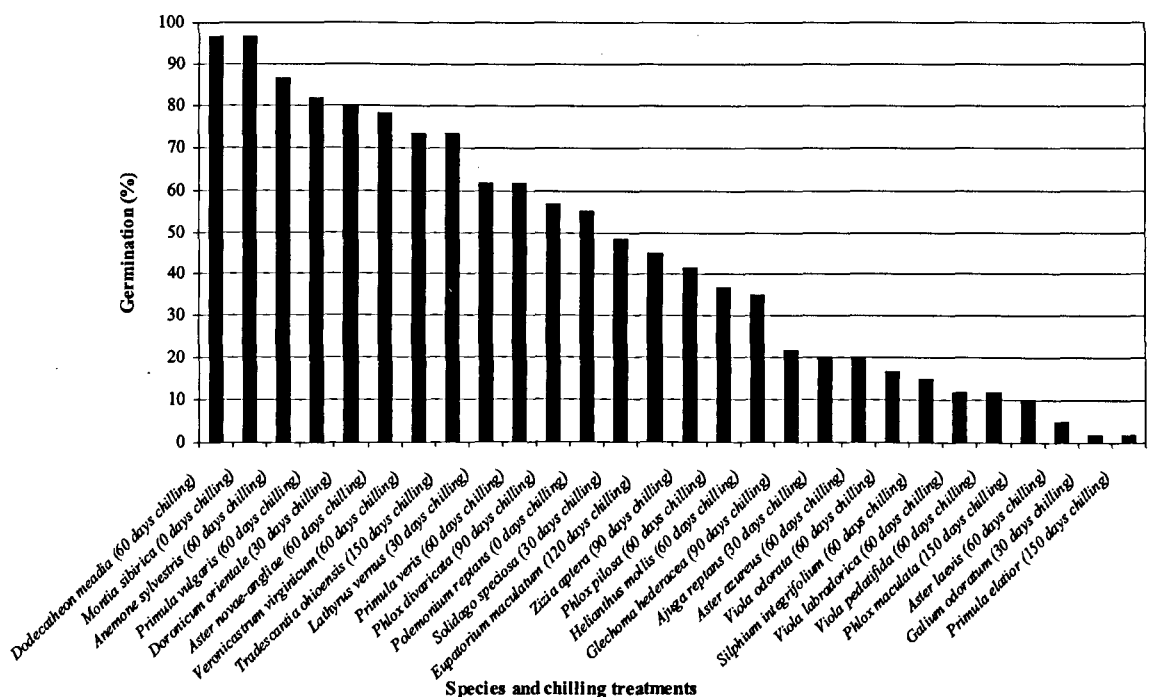


Figure 3.85 Chilling regimes associated with maximum germination in the study.

#### 3.4.1.1 Germination in understory species

Sixty days was the most effective chilling period in *A. sylvestris*, *D. meadia*, *P. veris*, *P. vulgaris*, *V. labradorica*, *V. odorata* and *V. pedatifida*. These results are consistent with

the work of Willemsen (1975) who found 60 days chilling at temperature 4°C is more effective in breaking the dormancy of ragweed seeds. Also, Meyer (2000) has demonstrated that 56 days chilling at low temperatures (2°C) is effective in breaking the dormancy of herbaceous seed (*Eriogonum racemosum*).

Seed of *M. sibirica* and *P. reptans* is non-dormant, and the best germination occurs in the growth cabinet without chilling. Chilling treatment did not increase the germination percentage of these species. It suggests that these species may germinate in its natural habitat in late summer soon after seed dispersal. Similar patterns were also obtained in germination of herbaceous seeds such as *Bromus ciliatus*, *Elymus glaucus*, *Agastache urticifolia*, *Erigeron elatior*, *Senecio serra* and *Solidago spathulata* (Hoffman, 1985). Our study also demonstrated that *M. sibirica* and *P. reptans* can germinate in the fridge at 4°C during chilling incubation. In the context of landscape practice, it shows that there is a higher likelihood of these species being established when sown in winter with the other dormant seed. Such seed mixes will germinate in spring when the temperature increases after chilling in the winter month.

#### 3.4.1.2 Germination in medium canopy species

Two (*A. azureus* and *P. pilosa*) of the 6 species medium canopy showed the greatest germination after 60 days chilling. Other species (with the exception of *S. speciosa*) require longer chilling periods to obtain a maximum germination. These were *P. maculata* (150 days chilling), *T. ohioensis* (150 days chilling) and *Z. aptera* (90 days chilling). This suggests these more deeply dormant species need to be sown in landscape sites during autumn to ensure they received sufficient natural chilling, if high levels of emergence are to be achieved. Similar pattern was also demonstrated by Hoffman (1985) who found 60 to 120 days chilling to be effective in the germination of *Delphinium barbeyi*, approximately 4% after 60 days chilling and up to 24% and 80% in light and darkness, respectively, after 120 days chilling.

#### 3.4.1.3 Germination in tall canopy species

The study showed that 60 days chilling incubation was the most effective period in *A. laevis*, *A. novae-angliae* 'Septemberrubin', *H. mollis*, *S. integrifolium* and *V.*

*virginicum*. These species are slightly dormant and associated with intermediate chilling period in order to achieve a higher germination. However, high germination (>50%) was recorded in *A. novae-angliae* 'Septemberrubin' without chilling treatment. It suggests that this species was essentially non-dormant and may be capable of germination in its natural habitat in late summer soon after seed dispersal. Hitchmough (2004) and Baskin and Baskin (2001) report that most *Aster* species are non-dormant.

As a whole, many deeply dormant seeds (medium forbs for example) possessed a marked chilling requirement for germination (Baskin and Baskin, 2001) possibly as a strategy to delay emergence until late spring by which time the soil temperature increases (Bokhari et al., 1975). There are however exceptions to this trend, for example the medium prairie forb *Solidago* requires low chilling requirements, suggesting it may germinate in its habitat in early spring soon after exposure to low temperature. This emergence behaviour has also been demonstrated by tall prairie forb from genera *Aster* (Baskin et al., 1992). Similar emergence behaviour has also been demonstrated by understory forbs from genera such as *Polemonium*, *Montia* (see Table 3.2 in this study), *Milium* and *Silene* (Slade and Causton, 1978). Clearly there was no evidence that medium sized prairie forbs per se have increased levels of dormancy.

Chilling requirements were not significantly correlated with seed weight nor plant family, with for example, the two genera in the Polemoniaceae, *Phlox* (medium canopy forb) and *Polemonium* (understorey forb) showing strongly contrasting behaviour. This was also true within genera in the Asteraceae (tall canopy forb). Overall, the best predictor of likely chilling requirements was the genus to which a species belonged. Genera that typically demonstrate low chilling requirements include *Aster*, *Polemonium*, *Solidago* and *Veronicastrum* (Hoffman, 1985; Baskin and Baskin, (2001). By contrast *Eupatorium* and *Phlox* species have high chilling requirements (Nichols, 1934; Greene and Curtis, 1950; Baskin and Baskin, 2001) with the rest of the genera intermediate, with chilling for up to 60 days improving germination percentage.



### 3.4.2 Germination in the fridge during chilling incubation

In general, almost all of the understorey species germinated at 4°C in the fridge after 60 days chilling. After 90 days chilling a further increase in germination occurred in these species. Some of the understorey species such as *G. odoratum* (12%), *L. vernus* (63%), *P. divaricata* (83%), *P. veris* (75%), *V. labradorica* (22%) and *V. odorata* (45%) demonstrated their maximum germination during chilling in the fridge. This shows that these species are highly dormant and require a long chilling period in order to achieve a higher germination, and are able to do this at low temperatures. One explanation for this is that in understorey habitats that are heavily shaded by taller plants from early summer on, species have evolved to germinate early in the year to increase their capacity to photosynthesise and establish successfully before they are heavily shaded. This strategy is common in woody plant seedlings (Jones et al. 1997) and has been shown to occur in wild populations of *Primula sieboldii* which germinate at temperatures >5°C following winter chilling (Washitani and Kabaya, 1988). This species typically occurs as vernal element in tall wet meadow vegetation.

Three of the twelve shade intolerant species (*A. laevis*, *E. maculatum* and *T. ohioensis*) did not germinate in the fridge, and other species demonstrated low germination (<30%, mean for post 60 days chilling). Shade intolerant species naturally associated with open habitats were more likely to demonstrate greater germination in spring at higher soil temperatures. This strategy is common for North American prairie plant seedlings (Nichols, 1934) and has been demonstrated for prairie sown seed mixes which germinate in March-April at temperatures >10°C following winter chilling (Hitchmough et al., 2004).

### 3.4.3 Seed viability

After 90 days chilling, species such as *A. laevis*, *P. elatior*, *P. maculata*, *S. integrifolium* and *V. pedatifida* still showed very low germination. This can probably be attributed to issues of seed quality. “Squash tests” (Gunn, 2001) used on non-germinating samples confirmed this. Tetrazolium tests, a standard seed viability test that stains living seed tissue red when treated with solution of triphenyl tetrazolium chloride (TZ), were not used because of the difficulties of interpretation, especially when dealing with non-crop

species with small seed. Using this test, a sample of seeds is cut in half lengthwise, treated with TZ solution, and the seed examined with a hand lens.

Low viability and germination of *A. azureus*, *S. speciosa*, *Z. aptera* (see Table 3.9), *A. laevis*, *H. mollis* and *S. integrifolium* (see table 3.13) after 90 days chilling might be due to bacterial or fungal infection during fridge chilling. It was shown in this experiment that most of these seed exhibit heavy levels of seed coat infection in petri dishes that caused low germination (less than 50%), both in the growth cabinet and in the fridge. Similar results were observed in *A. laevis* which was obtained from the American prairie seed industry, and commonly showed high levels of seed infection in the laboratory (Hitchmough, unpublished data) and this was the case in this study. It is clear that the quality of seed offered, especially by seed producers whose main market is the wildflower/prairie restoration industry, is often extremely low, and this is a significant problem for practice.

Some of the species didn't germinate well after long chilling treatments (up to 150 days) even though their seeds viability was high (>50%), such as *C. solida*, *G. hederacea* and *P. reptans*. These species remained dormant despite the chilling periods. This may be due to insufficient duration of chilling, or insufficient fluctuation during chilling of temperature, as well as the phenomenon of waxy covering to the seed coat (Voigt, 1977) and deep embryo dormancy (Derek, 1997). Additional treatments such as scarification, pre-soaking in GA3 and leaching may give better germination.

### 3.5 Conclusion

This research shows that high germination (>70%) after chilling was obtained from understorey species (*A. sylvestris*, *D. meadia*, *D. orientale*, *M. sibirica* and *P. vulgaris*), medium canopy (*T. ohioensis*) and tall canopy (*A. novae-angliae* 'Septemberrubin' and *V. virginicum*). It shows that chilling enhanced the percentage and increased germination rate for many species in all plant types or habitat groups of herbaceous vegetation. Generally, rates of germination (in terms of radicle protrusion) in the growth cabinet is considerably increased with 60 days chilling.

This germination study provides the first published data on germination patterns and percentages of a range of herbaceous species under laboratory conditions. Whilst germination following natural chilling in the field over winter may be more effective than laboratory chilling at constant temperature in the absence of leaching and other stimuli, these data are valuable in shedding light on likely minimum chilling requirements and also in supporting the choice of species to be used in subsequent experiments in this study. From a practice perspective, perhaps the most valuable aspect of these data is that they reveal how chilling seed mixes of many species with different chilling requirements in moist sand in a fridge for more than 60 days is likely to lead to premature emergence within the fridge and subsequent death of such emergents.

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## CHAPTER 4: MICROCOSM STUDIES INTO SEEDLING SURVIVAL IN DIFFERENT SOWING RATIOS AND SPECIES MIXTURES

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### 4.1 Introduction

Seedling establishment of herbaceous plants in naturalistic plant communities has been explored by Hitchmough (2004) and the dynamics of these plant communities in the longer term, by Hitchmough and de La Fleur (2006). This 'naturalistic design' is potentially of considerable interest to urban planners, and increasingly popular in the urban parks and green spaces in towns and cities (Dunnett and Hitchmough, 2004; Hitchmough et al., 2004). However, the idea of creating a multi-layer herbaceous community in urban parks and green spaces by sowing *in situ* has largely remained unexplored. To realise this idea in practise, predictable outcomes in terms of establishment and survival of sown seedlings of each species derived from different plant types or habitat groups must be made available by the study of sowing ratios and species mixes. This requires the ability to predict how much seed of each species and plant type (i.e. low, medium and tall canopy forbs) must be sown per m<sup>2</sup> to achieve a target population in a multi-layer community.

The establishment of multi-layer communities created from two contrasting plant types/habitat groups of understorey and medium-tall canopies is likely to be affected by factors such as seedling density (Hitchmough et al., 2004; Martin et al., 2004), the ratio of seed sown of each species (Fischbach et al., 2006), the range of species in mixtures (Fone, 1989; Peltzer and Kochy, 2001) and competition for resources (Fone, 1989; Hitchmough and de La Fleur, 2006). From previous studies, Martin et al. (2004) demonstrated in prairie restoration that the survival rate of species fell with increasing seedling density. However, there was no significant effect on diversity by increasing initial densities (Zamfir and Goldberg, 2000; Martin et al., 2004). Other factors (the ratio of seed sown of each species, the range of species in mixtures and competition for resources) stated above, had a direct influence on species establishment, survival and growth. Tieborger and Kadmon (2000) found the growth of understorey species was greater under canopies than in an open habitat. This also proved to be true in woodland *Primula* (Whale, 1984). Conversely, recent research (Hitchmough and de La Fleur, 2006) has shown that some relatively low growing prairie species have the lowest

survival rate when subjected to heavy shading by the foliage of taller neighbours in the plant community. These data suggest there is a significant interaction between tall canopies and understorey species in communities. However, information on the relationships and interactions amongst these species (understorey and medium-tall canopy) particularly from two contrasting plant types/habitat groups grown together in composition are lacking.

The manipulation of seed mixes provides a means by which to examine factors that affect establishment and survival of species in multi-layer communities in urban parks and green spaces. The rate (seeds/m<sup>2</sup>) at which low and tall species are sown, determines the likelihood of a neighbour being tall, fast and therefore highly competitive or small, slow and highly uncompetitive. Identifying which species tend to be dominant or even aggressive and therefore likely to eliminate other species is commonly used to formulate herbaceous sowing mixes in the field. In a previous study, Hitchmough and Woudstra (1999) reported that fast growing prairie species such as *Solidago* and *Aster* tended to eliminate initially slow growing (but ultimately long lived) species such as *Veronicastrum virginicum*, in a sown community in the first year (Hitchmough et al., 2004). This suggests that fast growing, competitive species play an important role in determining the survival of slow and less competitive species.

The purpose of this study was to determine threshold seed ratios and densities for the establishment, survival and development of species in multi-layer plant communities in experimental microcosms. These experiments also provided an opportunity to compare and contrast emergence under surrogate field conditions with that of laboratory germination environments. The hypotheses of this study were as follows: tall forbs will demonstrate a higher survival and growth than medium forbs; medium forbs will demonstrate a higher survival and growth than low species; and seed ratios and density will have a significant impact upon survival and growth.

#### 4.1.1 Species selection

Based on the review in **Chapter 2** and studies conducted in **Chapter 3**, more than 30 forb species with low, medium and tall foliage canopies were identified. Of these, for this study, a total of 18 native and exotic forbs species were selected (Table 4.1) based on the criteria described in section 3.1.1 in **Chapter 3**. Seed was obtained in October

and November 2004 from Jelitto Seeds, Schwarmstedt, Germany for European species and Prairie Moon Nursery, Winona, MN, USA for North American species. Seed was dry stored at approximately 4°C in the fridge prior to sowing. Detailed characteristics of the species selected in the experiment are shown in Appendix Table A4.1.

**Table 4.1 Understorey and prairie forbs species used in the study.**

Species	Plant type/ habitat	Canopy type*
<i>Aster azureus</i>	prairie forb	medium
<i>Aster laevis</i>	prairie forb	tall
<i>Aster novae-angliae</i> 'Septemberrubin'	prairie forb	tall
<i>Dodecatheon meadia</i>	understorey forb	low
<i>Eupatorium maculatum</i>	prairie forb	tall
<i>Helianthus mollis</i>	prairie forb	tall
<i>Phlox divaricata</i>	understorey forb	low
<i>Phlox maculata</i>	prairie forb	medium
<i>Phlox pilosa</i>	prairie forb	low-medium
<i>Polemonium reptans</i>	understorey forb	low
<i>Primula elatior</i>	understorey forb	low
<i>Primula veris</i>	understorey forb	low
<i>Primula vulgaris</i>	understorey forb	low
<i>Silphium integrifolium</i>	prairie forb	tall
<i>Solidago speciosa</i>	prairie forb	medium
<i>Tradescantia ohioensis</i>	prairie forb	medium
<i>Zizia aptera</i>	prairie forb	low-medium
<i>Veronicastrum virginicum</i>	prairie forb	tall

\*Under typical garden condition; low = < 450 mm, medium = 450-900 mm and tall = >900 mm in height

#### 4.1.2 Objectives

The specific objectives of this study were:

- 1) To determine the survival of individual species in the microcosm over a 3 year period in response to initial sowing ratios and density.
- 2) To determine the aboveground dry weight of individual species in the microcosm over a 3 year period in response to initial sowing ratios and density.
- 3) To record cover values for the different sowing ratios x density communities across the 3 year period.
- 4) To record the phenology of the different species across the experiment.

## 4.2 Materials and methods

The experiment was initially conducted at Tapton Experimental Gardens, University of Sheffield, United Kingdom in December 2004, as a fully randomized design. As shown in Table 4.2 the seed mixes in this study consisted of a total of 18 species of understorey and prairie forbs, sown at low (2000 seed/m<sup>2</sup>) and high density (4000 seed/m<sup>2</sup>) in six different ratios of low, medium and tall canopy species.

**Table 4.2 Ratios in terms of seed sown for each plant functional group tested in this study.**

Sowing mixes (Treatments)	Herbaceous species with different canopy height (%)		
	Low species	Medium species	Tall species
T1	100	0	0
T2	0	100	0
T3	0	0	100
T4	70	20	10
T5	10	20	70
T6	33	33	33

The amount of seed sown was increased (see Table 4.3) in certain species to ensure that enough numbers of seedling were established per ‘tray’ achieved in each treatment. This was done with the intention of removing excess seedlings as required. When making up the various sowing mixes for the experiment seed was weighed rather than counted, with three replicates to establish mean seed weights for each species. Seed mixes were sown in 600 x 400 x 150 mm deep plastic “trays” (microcosm). Thus, a total of 36 microcosms (6 species ratios x 2 planting densities x 3 replicates) were used for each sowing density. A soil based sowing substrate was used, John Innes No 2 (85%) plus coarse sand (15%). This substrate was formulated to create appropriate conditions for seedling growth across a 2-3 year period.

Table 4.3 Actual seed sown for each species per treatment community in each microcosm tray (0.24m<sup>2</sup>).

Species	Seed weight <sup>a</sup> (mg/seed)		Approximate no of seed sown/ 0.24m <sup>2</sup>											
			T1 (100%L)		T2 (100%M)		T3 (100%T)		T4 (70%L: 20%M:10%T)		T5 10%L: 20%M:70%T)		T6 (33%L: 33%M:33%T)	
			LD	HD	LD	HD	LD	HD	LD	HD	LD	HD	LD	HD
<b>Low canopy (L)</b>														
<i>Dodecatheon meadia</i> **	0.236	0.37	80	160					56	112	8 (48)	16 (96)	26 (156)	52
<i>Phlox divaricata</i> **	2.380	1.62	80	160					56	112	8 (48)	16 (96)	26 (156)	52
<i>Polemonium reptans</i> ***	1.269	1.51	80	160					56	112	8 (32)	16 (64)	26 (104)	52
<i>Primula elatior</i> ***	0.903	1.23	80	160					56	112	8 (32)	16 (64)	26 (104)	52
<i>Primula veris</i> ***	1.125	0.46	80	160					56	112	8 (32)	16 (64)	26 (104)	52
<i>Primula vulgaris</i> ***	0.936	0.69	80	160					56	112	8 (32)	16 (64)	26 (104)	52
<b>Medium canopy (M)</b>														
<i>Aster azureus</i> ***	0.318	0.43			80	160			16 (64)	32	16 (64)	32	26 (104)	52
<i>Phlox maculata</i> *	2.478	1.36			80 (160)	160 (320)			16 (128)	32 (64)	16 (128)	32 (64)	26 (208)	52 (104)
<i>Phlox pilosa</i> **	1.388	3.25			80	160			16 (96)	32	16 (96)	32	26 (156)	52
<i>Solidago speciosa</i> ***	0.244	0.50			80	160			16 (64)	32	16 (64)	32	26 (104)	52
<i>Tradescantia ohioensis</i> **	3.037	1.64			80	160			16 (96)	32	16 (96)	32	26 (156)	52
<i>Zizia aptera</i> **	1.538	3.10			80	160			16 (96)	32	16 (96)	32	26 (156)	52
<b>Tall canopy (T)</b>														
<i>Aster laevis</i> *	0.387	0.32					80 (160)	160 (320)	8 (64)	16 (128)	56 (112)	112 (224)	26 (208)	52 (104)
<i>Aster novae-angliae</i> *** 'Septemberrubin'	0.434	0.21					80	160	8 (32)	16 (64)	56	112	26 (104)	52
<i>Eupatorium maculatum</i> **	0.314	0.62					80	160	8 (48)	16 (96)	56	112	26 (156)	52
<i>Helianthus mollis</i> ***	3.094	3.40					80	160	8 (32)	16 (64)	56	112	26 (104)	52
<i>Silphium integrifolium</i> ***	14.646	57.04					80	160	8 (32)	16 (64)	56	112	26 (104)	52
<i>Veronicastrum virginicum</i> *	0.031	0.12					80 (160)	160 (320)	8 (64)	16 (128)	56 (112)	112 (224)	26 (208)	52 (104)
<b>TOTAL</b>			480	960	480	960	480	960	480	960	480	960	480	960
					(640)	(1280)	(800)	(1600)	(1296)	(1568)	(1472)	(1920)	(2976)	(1272)

<sup>a</sup> Mean of three replicates; LD= Low density (2000 seed/ m<sup>2</sup>); HD= High density (4000 seed/ m<sup>2</sup>)

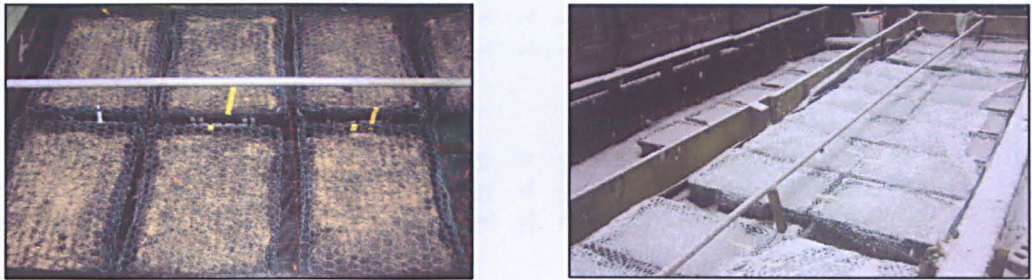
Germination was predicted as: \*Low germination rate (>10%); Number of seed sown was marked-up 8 or 2 times in selected treatments, as shown in brackets.

\*\*Medium germination rate (10-20%); Number of seed sown was marked-up 6 times during sowing, as shown in brackets.

\*\*\*High germination rate (>20%); Number of seed sown was marked-up 4 times during sowing, as shown in brackets.



Seed mixes for each tray were mixed with sawdust to aid distribution and carefully broadcast in two directions at right angles to one another. Plots were raked to incorporate seed and lightly compressed. Sowing was completed on 20th December 2004 to allow between 90-120 days of natural chilling before seeds germinated in spring. The microcosms were placed outdoors in an open area (Figure 4.1). To reduce the impact of slug predation on seedling emergence in spring, plots were baited post-sowing with metaldehyde containing pellets at approximately 40 pellets/m<sup>2</sup>. Slug pellets were re-applied at approximately 2 weekly intervals until the end of May 2005.



**Figure 4.1** Experimental sites at the Tapton Experimental Gardens, University of Sheffield, United Kingdom.

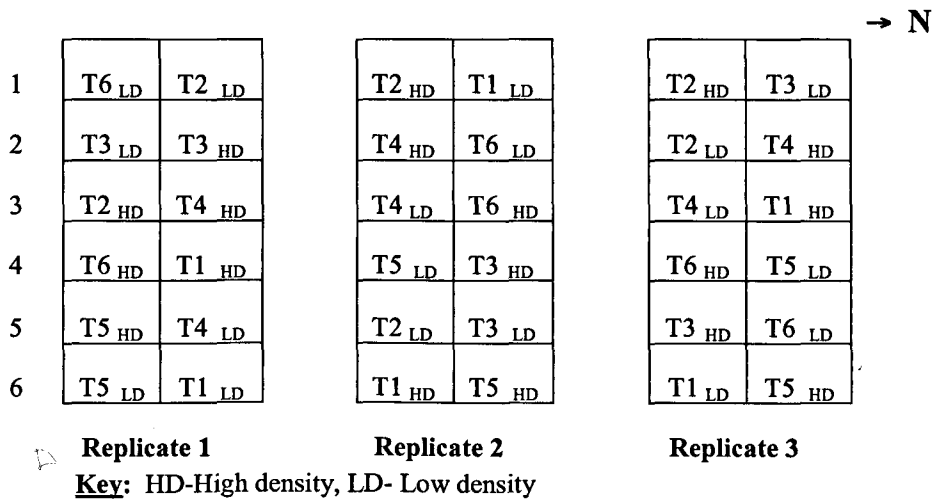
From 26th May to 2nd June 2005, emerged seedlings (see Appendix Figures A4.1-A4.4) were identified and counted and the data was compared with germination in the petri dished experiment. To achieve the target seed ratios (see Table 4.2), the numbers of each species were corrected by removal (thinning) of existing seedlings, or the additional (transplanting) of new seedlings depending on how many of each species emerged per microcosm. As there was an excess of germinated seedlings, thinning of the most abundant species was undertaken. Moreover, plants which were grown in clumps or aggregations, and were very large in relation to other species were also removed thereby allowing for even spatial distribution, equal numbers of each species and target seedling ratios based on the plants functional group.

The thinning and transplanting of new seedlings was completed on 8th July 2005, with approximate rates of 900 seedlings/m<sup>2</sup> ( $\approx$ 40 seedlings/quadrat) for low density and 1500 seedlings/m<sup>2</sup> ( $\approx$ 220 seedlings/quadrat) for high density. The amended seedling numbers per species for each treatment are shown in Table 4.4. In summer (5th August 2005), these microcosms were moved to a permanent experimental site which was open and sunny at Lower Walkley, a suburb of Sheffield in Northern England. The experimental design is illustrated graphically in Figure 4.2.

Table 4.4 Actual number of seedling per quadrat (500 x 300 mm) for each species in each replicate after adjustment.

Species	Approximate no of seedling after adjustment											
	T1 (100%L) (R1, R2, R3)		T2 (100%M) (R1, R2, R3)		T3 (100%T) (R1, R2, R3)		T4 (70%L:20%M:10%T) (R1, R2, R3)		T5 (10%L:20%M:70%T) (R1, R2, R3)		T6 (33%L:33%M:33%T) (R1, R2, R3)	
	LD	HD	LD	HD	LD	HD	LD	HD	LD	HD	LD	HD
<b>Low canopy (L)</b>												
<i>Dodecatheon meadia</i>	65, 56, 59	111, 89, 104					36, 37, 51	54, 60, 53	3, 3, 3	4, 4, 4	4, 10, 18	18, 13, 14
<i>Phlox divaricata</i>	16, 21, 28	30, 27, 34					16, 10, 6	22, 20, 15	7, 3, 3	4, 3, 4	9, 8, 8	11, 7, 7
<i>Polemonium reptans</i>	15, 15, 17	34, 29, 27					19, 15, 5	27, 20, 21	2, 3, 3	4, 5, 4	6, 9, 17	16, 12, 16
<i>Primula elatior</i>	0, 0, 0	0, 0, 0					0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0	0, 0, 0
<i>Primula veris</i>	44, 51, 51	69, 87, 96					34, 32, 34	50, 47, 55	6, 3, 3	4, 4, 2	10, 27, 6	20, 22, 17
<i>Primula vulgaris</i>	<u>40, 37, 20</u> <u>180, 180, 175</u>	<u>82, 70, 38</u> <u>326, 302, 299</u>					<u>21, 32, 23</u> <u>126, 126, 119</u>	<u>43, 42, 38</u> <u>196, 189, 182</u>	<u>2, 3, 3</u> <u>20, 15, 15</u>	<u>4, 4, 4</u> <u>20, 20, 18</u>	<u>31, 6, 11</u> <u>60, 60, 60</u>	<u>15, 26, 26</u> <u>80, 80, 80</u>
<b>Medium canopy (M)</b>												
<i>Aster azureus</i>			13, 18, 18	22, 25, 22			6, 8, 8	6, 10, 5	4, 3, 2	4, 11, 8	9, 3, 33	14, 9, 16
<i>Phlox maculata</i>			32, 23, 10	45, 32, 32			6, 6, 3	10, 5, 13	5, 5, 4	8, 6, 6	12, 8, 8	13, 13, 13
<i>Phlox pilosa</i>			24, 19, 6	28, 19, 21			4, 4, 5	8, 12, 13	5, 5, 6	5, 4, 4	7, 8, 15	14, 16, 12
<i>Solidago speciosa</i>			9, 14, 10	15, 15, 11			8, 6, 5	10, 6, 6	5, 3, 3	10, 10, 3	19, 11, 8	16, 17, 14
<i>Tradescantia ohioensis</i>			22, 27, 13	36, 35, 28			8, 9, 5	9, 10, 10	4, 5, 4	7, 5, 8	11, 24, 10	10, 16, 11
<i>Zizia aptera</i>			<u>21, 17, 20</u> <u>121, 118, 77</u>	<u>24, 32, 26</u> <u>170, 158, 140</u>			<u>4, 3, 8</u> <u>36, 36, 34</u>	<u>13, 11, 5</u> <u>56, 54, 52</u>	<u>3, 5, 5</u> <u>26, 26, 24</u>	<u>4, 4, 7</u> <u>38, 40, 36</u>	<u>2, 6, 14</u> <u>60, 60, 88</u>	<u>13, 9, 14</u> <u>80, 80, 80</u>
<b>Tall canopy (T)</b>												
<i>Aster laevis</i>					4, 9, 2	7, 4, 4	1, 4, 1	4, 2, 1	4, 2, 2	4, 5, 3	3, 3, 3	2, 1, 1
<i>Aster novae-angliae</i> 'Septemberrubin'					25, 35, 27	46, 58, 40	9, 5, 7	7, 6, 4	52, 48, 62	73, 78, 59	17, 25, 17	24, 31, 24
<i>Eupatorium maculatum</i>					8, 19, 6	17, 16, 20	3, 2, 1	6, 8, 12	4, 13, 5	6, 8, 9	1, 4, 8	13, 5, 25
<i>Helianthus mollis</i>					5, 5, 5	3, 4, 7	1, 1, 0	0, 1, 3	3, 5, 1	1, 6, 4	0, 4, 5	8, 3, 4
<i>Silphium integrifolium</i>					1, 0, 2	1, 3, 0	0, 2, 0	2, 0, 1	3, 0, 1	1, 0, 0	1, 0, 0	2, 1, 0
<i>Veronicastrum virginicum</i>					<u>37, 27, 24</u> <u>80, 95, 66</u>	<u>54, 54, 69</u> <u>128, 139, 140</u>	<u>4, 4, 8</u> <u>18, 18, 17</u>	<u>9, 10, 5</u> <u>29, 27, 26</u>	<u>25, 23, 13</u> <u>91, 91, 84</u>	<u>28, 43, 51</u> <u>113, 140, 126</u>	<u>38, 24, 27</u> <u>60, 60, 61</u>	<u>31, 39, 26</u> <u>80, 80, 80</u>
Approx. ~Seedling ratios per replicate	1L : 0M : 0T		0L : 1M : 0T		0L : 0M : 1T		7L : 2M : 1T		1L : 2M : 7T		1L : 1M : 1T	

Key: LD=Low density; HD=High density; R=Replicate



**Figure 4.2** Arrangement of the treatment plot in the competition experiment.

During summer 2005, the microcosms were watered as necessary to prevent severe stress. On average watering took place at three day intervals between May and September at which microcosms were returned to field capacity. To improve seedling growth and ensure adequate competition (as nutrients were leached from the compost), microcosms were fertilized with water soluble fertilizer, Miracle-Gro (NPK 15-10-15) at approximately 5g/l per microcosm in August 2005. Fertilizers were re-applied 2 times at approximately 2 weekly intervals until the first week of September 2005. The microcosms sat on an area of 'weed mat', and the roots of the plants soon grew out of bottom of the trays and through this mat into the underlying soil. This allowed the microcosms to be largely independent of watering in years 2 and 3.

#### 4.2.1 Data collection

Permanent quadrats (500 x 300 mm) were established for data collection. Within each quadrat, plant numbers, cover value and biomass data were collected. The number of seedlings of each species within each quadrat was recorded in May and September 2005 and 2006. At the end of the first full growing season September 2005, all plants of each species were harvested from each microcosm to provide dry weight and count data. Both understorey and prairie forbs were cut at ground level with scissors, and the above ground biomass of each plant carefully placed into individual coded envelopes. Samples were initially dried at an ambient temperature and then transferred to a laboratory oven at 80°C and dried to constant weight. The total biomass for each plant layer per quadrat was used to generate a mean value per treatment. The individual samples in each treatment mix was used to generate plant numbers per species. Canopy cover was also

estimated in September 2005 at the peak of standing biomass. Canopy values were estimated visually using a Sykes (1983) method, with fixed quadrats. Photographs of microcosms were undertaken at intervals throughout the experiment.

In the second growing season (2006), the development of the multi-layer plant community was continuously monitored. The cover values were estimated between March and April 2006 and also at the peak of standing biomass (September 2006). Reproductive and flowering phenology was recorded for all species. The methodology of Dunne (2003) that involves having at least five plant blooming per species was used. In September 2006, all plants of the medium and tall species were harvested via the same method as in the first year growing season. Photographs of the experiment were undertaken throughout the year.

In the third growing season (spring 2007), all plants of the understorey species were harvested as previously described. The medium-tall prairie forbs were only counted to provide plant numbers. This split final harvesting was employed to gain an estimation of the summer growing prairie species and the spring growing but partly summer dormant understorey species.

#### 4.2.2 Statistical analysis

As Kolmogorov-Smirnov tests indicated that counted data was non-normal and could not be adequately improved by transformation, analysis was undertaken using a non-parametric tests (Dytham, 2003). Statistical analysis was undertaken using *SPSS version 12* for Windows. The Mann-Whitney *U*-test was used in lieu of *t*-tests for paired comparisons. This test was used to compare the significant differences between low and high density sowing treatments. The Kruskal-Wallis test was used to compare the significant differences amongst the treatment mixes. Where a Kruskal-Wallis test gave a significant result ( $P < 0.05$ ), a Mann-Whitney *U*-test was undertaken to allow comparison and ranking of means. Mean in figures and tables that are statistically significantly different ( $P < 0.05$ ) are indicated by the use of suffix subscript letters.

To investigate the effect of different plant canopy layers on survival and dry weight, data were sorted into the three plant layer groups; low, medium and tall canopy species. Of the original 18 species 3 were excluded from the analysis. *Primula elatior* did not

germinate and *Dodecatheon meadia* and *Tradescantia ohioensis* had entered dormancy and disappeared by the September harvest date.

### 4.3 Results

#### 4.3.1 Field emergence in the microcosm

Emergence of shade tolerant understory forbs commenced in the third week of March 2005 once the average soil temperature (100 mm depth) exceeded 5°C (approximately 3 months post sowing). Field emergence of shade intolerant medium-tall prairie forbs commenced 2 weeks later.

Emergence of species in the field microcosm was compared with that of the same species in the laboratory experiment (see Chapter 3). Emergence in microcosm was considerably lower for most species than the maximum germination values recorded in the laboratory (Figure 4.3). The same was also true (with the exception of *Aster laevis*) of the comparison between emergence in the microcosm and the laboratory germination after broadly equivalent periods of chilling (120 days), (Table 4.5).

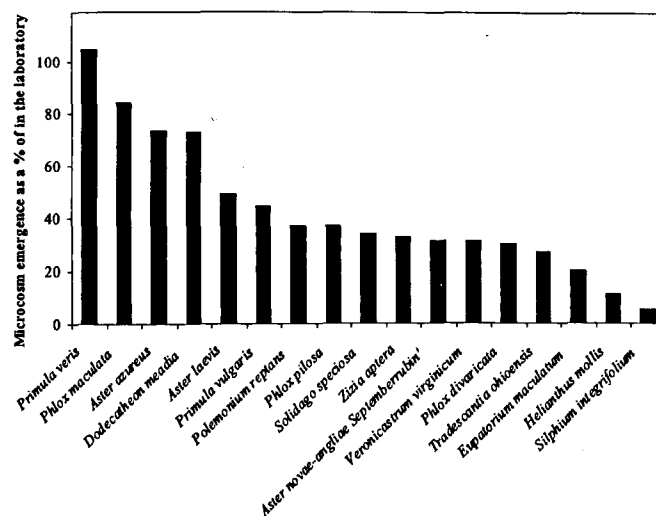


Figure 4.3 Emergence of species in the microcosm as a percentage of their maximum germination in the growth cabinet in the laboratory.

The species that were most similar in terms of germination/emergence between the maximum recorded in the laboratory and microcosm emergence, were; *A. azureus*, *A. laevis*, *D. meadia*, *P. maculata*, *P. elatior* and *P. veris*. Mean percentage field emergence of shade tolerant understory species (excluding *P. elatior*) was 33.68% as opposed to 12.16% in shade-intolerant, medium-tall species ( $P=0.055$ , Mann-Whitney *U*-test).

Table 4.5 Comparison of field emergence in the microcosm and germination in the laboratory.

Species	Laboratory				Microcosm		P-value	P-value
	Percentage germination at 120 days chilling (in fridge + growth cabinet)		(Maximum percentage germination in growth cabinet)		(Percentage emergence in sowing mix)		Growth cabinet maximum vs. field	Growth cabinet 120 days chilling vs. field
	Mean	SE	Mean	SE	Mean	SE		
<b>Shade tolerant understory forbs</b>								
<i>Dodecatheon meadia</i>	96.67	3.33	96.67	3.33	70.33	7.54	0.07 ns	0.070 ns
<i>Phlox divaricata</i>	91.66	4.41	56.67	10.14	17.14	2.75	0.004 **	0.004 **
<i>Phlox pilosa</i>	41.66	6.01	36.67	8.82	13.53	2.38	0.018 *	0.004 **
<i>Polemonium reptans</i>	23.33	7.26	55.00	10.00	20.39	2.20	0.004 **	0.536 ns
<i>Primula elatior</i>	0.00	0.00	1.67	1.67	0.00	0.00	0.448 ns	1.000 ns
<i>Primula veris</i>	68.33	12.02	61.67	8.82	64.48	5.22	1.00 ns	0.734 ns
<i>Primula vulgaris</i>	68.34	1.67	81.67	8.33	36.21	3.90	0.004 **	0.004 **
<i>Zizia aptera</i>	28.34	3.33	41.67	9.28	13.69	2.48	0.004 **	0.031 *
<b>Shade intolerant medium-tall forbs</b>								
<i>Aster azureus</i>	25.00	2.89	20.00	5.77	14.67	1.74	0.448 ns	0.031 *
<i>Aster laevis</i>	1.67	1.67	5.00	0.00	2.44	0.51	0.101 ns	0.295 ns
<i>Aster novae-angliae</i> 'Septemberrubin'	71.33	4.41	78.33	4.41	24.25	2.86	0.004 **	0.004 ns
<i>Eupatorium maculatum</i>	45	5.00	45.00	5.00	8.99	2.52	0.004 **	0.004 **
<i>Helianthus mollis</i>	36.67	4.41	35.00	5.00	3.88	2.95	0.004 **	0.004 **
<i>Phlox maculata</i>	8.33	3.33	10.00	5.00	8.40	1.31	0.734 ns	0.734 ns
<i>Silphium integrifolium</i>	5.00	2.89	15.00	0.00	0.76	0.47	0.004 **	0.180 ns
<i>Solidago speciosa</i>	30.00	5.77	48.33	1.67	16.29	1.23	0.004 **	0.031 *
<i>Tradescantia ohioensis</i>	60.00	0.00	71.67	4.41	19.29	2.44	0.004 **	0.004 **
<i>Veronicastrum virginicum</i>	63.34	6.67	73.33	16.67	22.65	5.36	0.018 *	0.031 ns

Significant differences (Mann-Whitney *U*-test) between *in vitro* and field germination are indicated by asterisks; ns: not significant: \* P=0.05; \*\*P=0.01

### 4.3.2 *Effect of sowing ratio, density and plant layer in the first year of the growing season (2005)*

#### 4.3.2.1 Survival and recruitment of forb species from May-September 2005

##### 4.3.2.1.1 Low canopy, understorey forbs

The Mann-Whitney *U*-test revealed that survival data were not statistically different between low and high density treatments (Figure 4.4a) so these were pooled for analysis. The Kruskal-Wallis test was used for comparisons between different treatment mixes. As shown in Figure 4.4b, there were significant differences ( $P=0.001$ ) in survival of understorey forbs planted in combination with medium-tall layers (T4 (Mix, understorey dominant), T5 (Mix, tall canopy dominant) and T6 (Mix equivalent understorey, medium, tall) to those planted in a single understorey layer T1 (Understorey). Recruitment from ungerminated seed between May-September 2005 was particularly marked in understorey species in T5 (Mix, tall canopy dominant) and T6 (Mix equivalent understorey, medium, tall). This leads to survival values exceeding 100%.

Survival was highest for all low canopy species (excluding *Dodecatheon meadia*) tested in T5 (Mix, tall canopy dominant), and significantly so ( $P<0.05$ ) for *Phlox divaricata* and *Primula veris* (Figure 4.5). Survival was generally lowest in T1 (Understorey) and T4 (Mix, understorey dominant) for all understorey species. The species that showed increased survival (>100%) were *P. divaricata*, *Polemonium reptans*, *P. veris* and *Primula vulgaris* in treatment T5 (Mix, tall canopy dominant) and T6 (Mix equivalent understorey, medium, tall). This increase may be due to active recruitment from sown seed or regrowth from the roots of seedlings that had been removed as part of the seedling number re-adjustment in June-July. With the exception of *D. meadia* (which entered dormancy and hence were not observable at the September count) the survival of understorey forbs was highest (>80%) when they were mixed with taller species.

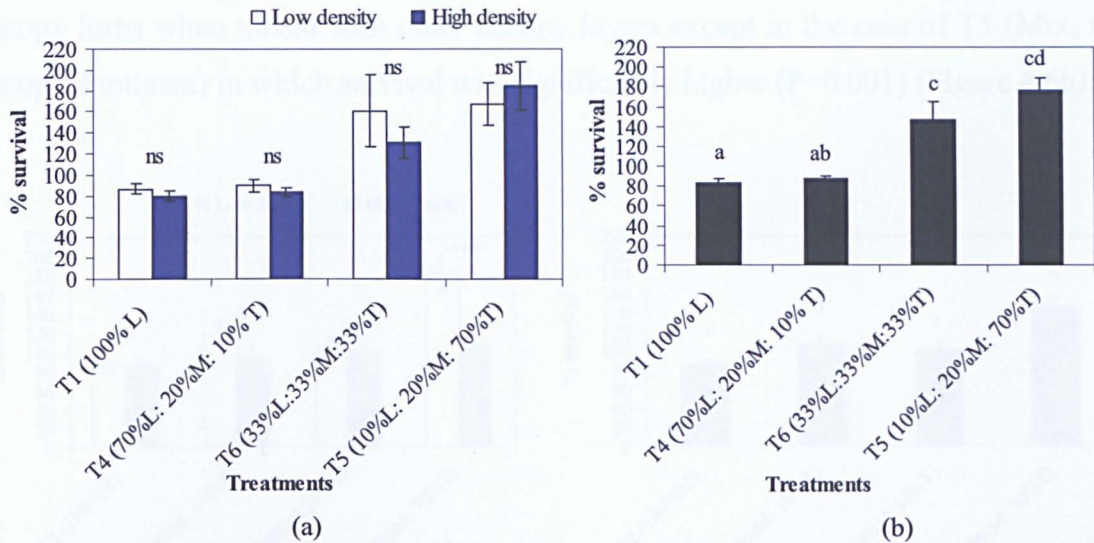


Figure 4.4 Effect of multi-layer treatment and density on percentage of survival in low canopy species between May and September 2005. (a) Survival of species in different density treatments. Significant differences (Mann-Whitney  $U$ -test) between low and high densities are indicated by: ns, not significant. (b) Mean survival of species as pooled across density treatments. Bars labeled with different letters are significantly different at  $P=0.001$  (Kruskal-Wallis test, pairwise Mann-Whitney  $U$ -tests). Error bars represent 1 S.E.M.

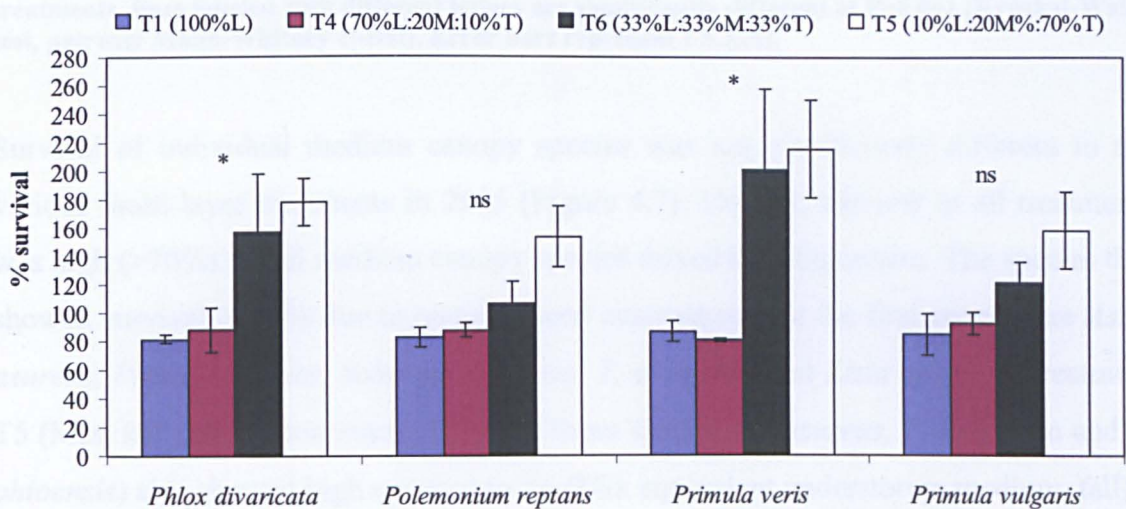


Figure 4.5 Survival of individual low canopy species in response to different multi-layer treatments. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between treatments are indicated by: \*  $P=0.05$ ; ns, not significant. *Primula elatior* and *Dodecatheon meadia* were excluded from this analysis due to insufficient data.

#### 4.3.2.1.2 Medium canopy forbs

The Mann-Whitney  $U$ -test found that survival was not statistically different between low and high density treatments with the exception of T5 (Mix, tall canopy dominant) (Figure 4.6a). Subsequent analysis was conducted on pooled density data. A Kruskal-Wallis test indicated that there were no significant differences in the survival of medium



canopy forbs when mixed with other canopy layers except in the case of T5 (Mix, tall canopy dominant) in which survival was significantly higher ( $P=0.001$ ) (Figure 4.6b).

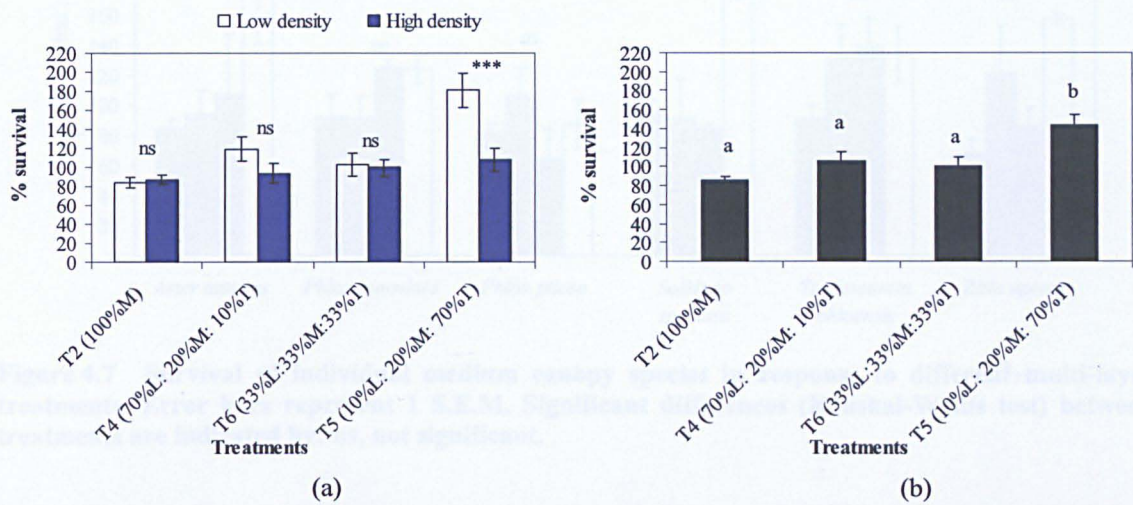


Figure 4.6 Effect of multi-layer treatment and density on percentage of survival in medium canopy species between May and September 2005. (a) Survival of species in different density treatments. Significant differences (Mann-Whitney  $U$ -test) between low and high densities are indicated by; \*\*\*  $P=0.001$ ; ns, not significant. (b) Mean survival of species as pooled across density treatments. Bars labeled with different letters are significantly different at  $P=0.001$  (Kruskal-Wallis test, pairwise Mann-Whitney  $U$ -test). Error bars represent 1 S.E.M.

Survival of individual medium canopy species was not significantly different in the various multi-layer treatments in 2005 (Figure 4.7). Overall, survival in all treatments was high ( $>70\%$ ) for all medium canopy species mixed in composition. The species that showed survival  $>100\%$  due to ongoing seed emergence post the first count were *Aster azureus*, *Phlox maculata*, *Solidago speciosa*, *T. ohioensis* and *Zizia aptera* in treatment T5 (Mix, tall canopy dominant). Some of these species (*A. azureus*, *P. maculata* and *T. ohioensis*) also showed high survival in T6 (Mix equivalent understorey, medium, tall).

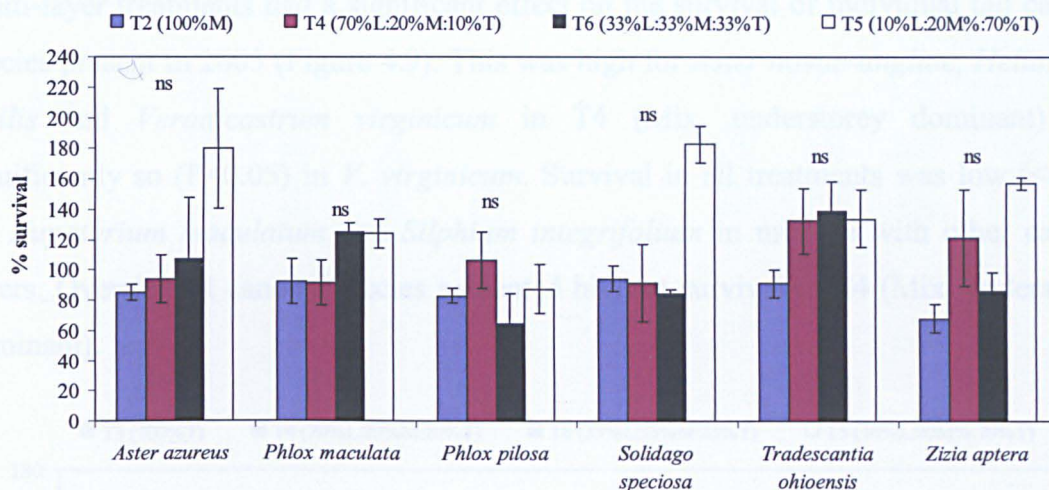


Figure 4.7 Survival of individual medium canopy species in response to different multi-layer treatments. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between treatments are indicated by: ns, not significant.

#### 4.3.2.1.3 Tall canopy forbs

As survival data were not statistically different between low and high density treatments (Figure 4.8a), data were pooled across density treatments. As shown in Figure 4.8b, there were no significant differences in the survival ( $P=0.394$ , Kruskal-Wallis test) of tall canopy layer forbs when combined with other canopy layers as opposed to when grown alone. Unlike medium forbs, survival values in tall forbs did not exceed 100%.

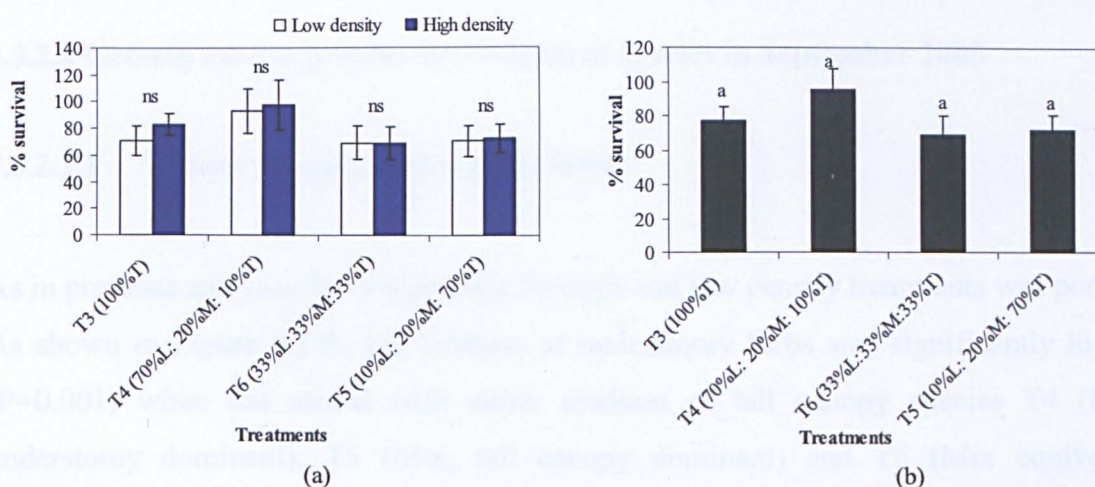


Figure 4.8 Effect of multi-layer treatment and density on percentage of survival in tall canopy species between May and September 2005. (a) Survival of species in different density treatments. Significant differences (Mann-Whitney  $U$ -test) between low and high densities is indicated by: ns, not significant. (b) Mean survival of species as pooled across density treatments. Bars labeled with the same letters are not significantly different at  $P=0.394$  (Kruskal-Wallis test). Error bars represent 1 S.E.M.

Multi-layer treatments had a significant effect on the survival of individual tall canopy species present in 2005 (Figure 4.9). This was high for *Aster novae-angliae*, *Helianthus mollis* and *Veronicastrum virginicum* in T4 (Mix, understorey dominant) and significantly so ( $P < 0.05$ ) in *V. virginicum*. Survival in all treatments was low ( $< 65\%$ ) for *Eupatorium maculatum* and *Silphium integrifolium* in mixture with other canopy layers. Overall, tall canopy species presented highest survival in T4 (Mix, understorey dominant).

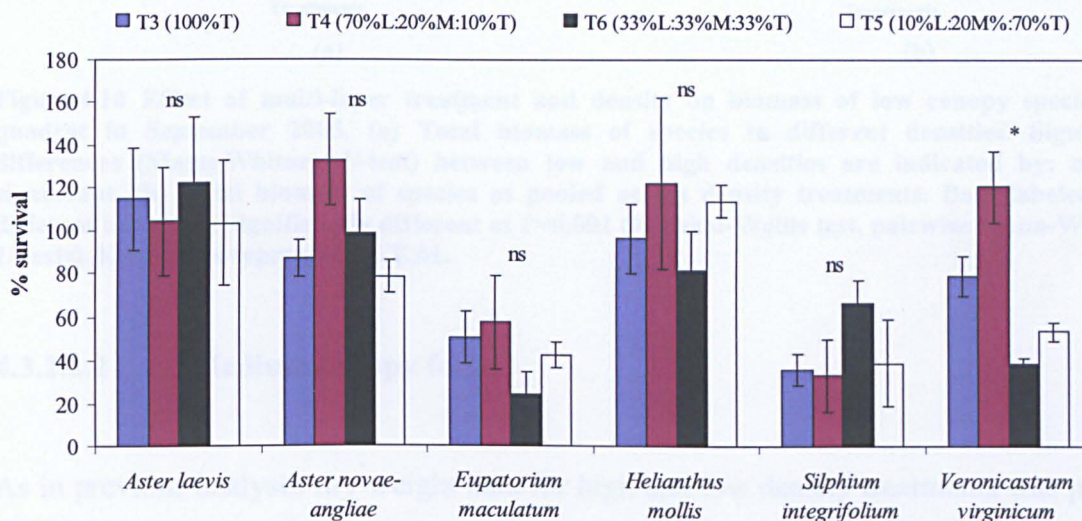


Figure 4.9 Survival of individual tall canopy species in response to different multi-layer treatments. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between treatments are indicated by: \*  $P = 0.05$ ; ns, not significant.

#### 4.3.2.2 Growth (above ground dry weight) of species in September 2005

##### 4.3.2.2.1 Low canopy, understorey forbs

As in previous analyses dry weight data for high and low density treatments was pooled. As shown in Figure 4.10b, the biomass of understorey forbs was significantly higher ( $P = 0.001$ ) when not mixed with either medium or tall canopy species T4 (Mix, understorey dominant), T5 (Mix, tall canopy dominant) and T6 (Mix equivalent understorey, medium, tall). This is to be expected given that these latter mixes contained fewer plants of understorey species than T1 (Understorey).

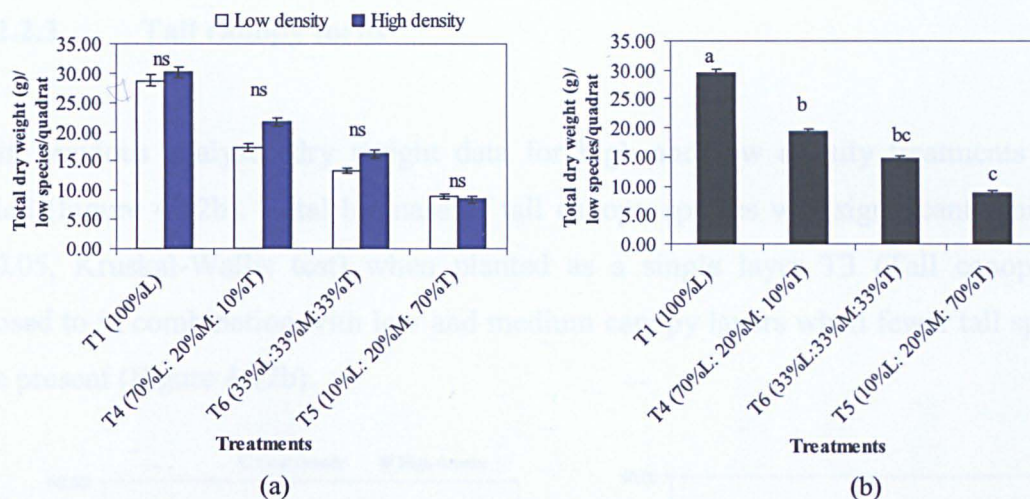


Figure 4.10 Effect of multi-layer treatment and density on biomass of low canopy species per quadrat in September 2005. (a) Total biomass of species in different densities. Significant differences (Mann-Whitney *U*-test) between low and high densities are indicated by: ns, not significant. (b) Total biomass of species as pooled across density treatments. Bars labeled with different letters are significantly different at  $P=0.001$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-tests). Error bars represent 1 S.E.M.

#### 4.3.2.2.2 Medium canopy forbs

As in previous analyses dry weight data for high and low density treatments was pooled (Figure 4.11a). As with understorey forbs, dry weight of medium canopy species was greatest ( $P=0.001$ , Kruskal-Wallis test) when not mixed with other canopy layers (Figure 4.11b) than involved a diminution in the number of medium canopy plants present.

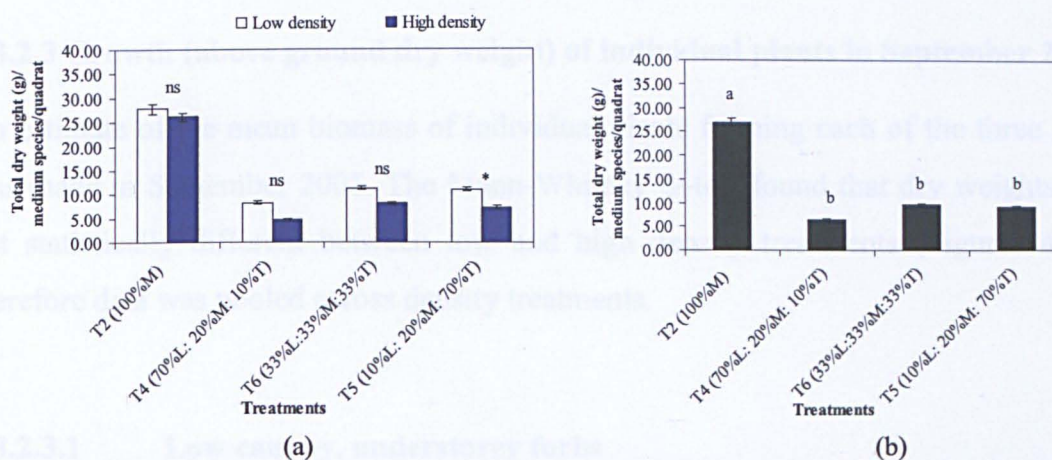
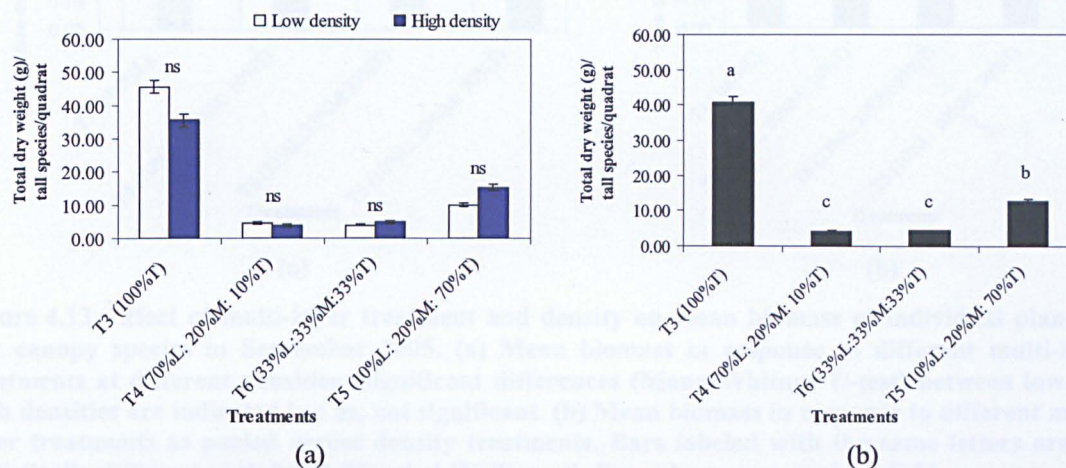


Figure 4.11 Effect of multi-layer treatment and density on biomass of medium canopy species per quadrat in September 2005. (a) Total biomass of species in different densities. Significant differences (Mann-Whitney *U*-test) between low and high densities are indicated by: \*,  $P=0.05$ ; ns, not significant. (b) Total biomass of species as pooled across density treatments. Bars labeled with different letters are significantly different at  $P=0.001$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-tests). Error bars represent 1 S.E.M.

### 4.3.2.2.3 Tall canopy forbs

As in previous analyses dry weight data for high and low density treatments were pooled (Figure 4.12b). Total biomass of tall canopy species was significantly highest ( $P=0.05$ , Kruskal-Wallis test) when planted as a single layer T3 (Tall canopy) as opposed to in combination with low and medium canopy layers when fewer tall species were present (Figure 4.12b).



**Figure 4.12** Effect of multi-layer treatment and density on biomass of tall canopy species per quadrat in September 2005. (a) Total biomass of species in different densities. Significant differences (Mann-Whitney *U*-test) between low and high densities are indicated by; \*  $P=0.05$ ; ns, not significant. (b) Total biomass of species as pooled across density treatments. Bars labeled with different letters are significantly different at  $P=0.001$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-tests). Error bars represent 1 S.E.M.

### 4.3.2.3 Growth (above ground dry weight) of individual plants in September 2005

An estimate of the mean biomass of individual plants forming each of the three layers was made in September 2005. The Mann-Whitney *U*-test found that dry weights were not statistically different between low and high density treatments (Figures 4.13a), therefore data was pooled across density treatments.

#### 4.3.2.3.1 Low canopy, understorey forbs

There was no significant difference at  $P=0.259$  (Kruskal-Wallis test) in mean biomass of individual low canopy layer plants in composition between the six communities, T1 (Understorey) – T6 (Mix equivalent understorey, medium, tall) (Figure 4.13b). The

biomass of low canopy layer individuals was greatest in T5 (Mix, tall canopy dominant).

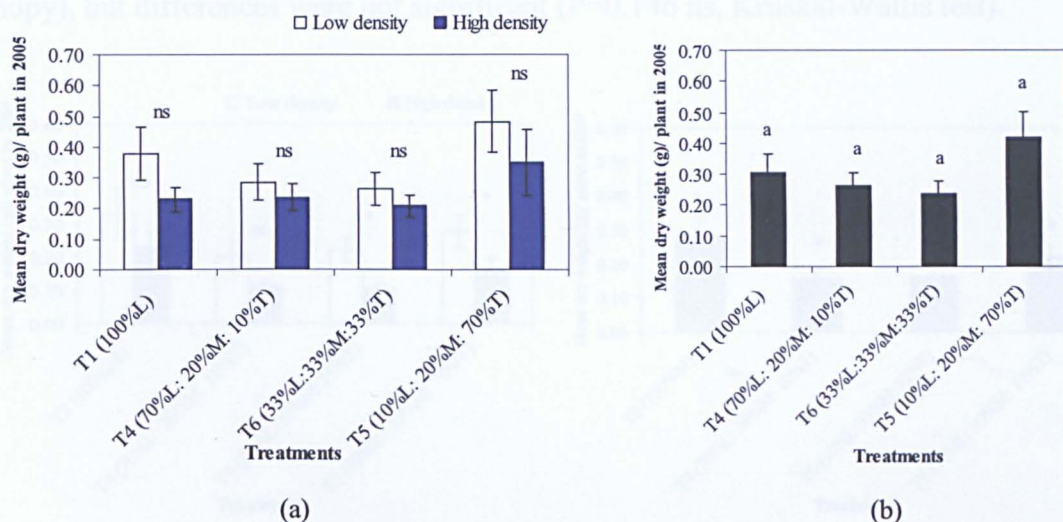


Figure 4.13 Effect of multi-layer treatment and density on mean biomass of individual plants in low canopy species in September 2005. (a) Mean biomass in response to different multi-layer treatments at different densities. Significant differences (Mann-Whitney  $U$ -test) between low and high densities are indicated by: ns, not significant. (b) Mean biomass in response to different multi-layer treatments as pooled across density treatments. Bars labeled with the same letters are not statistically different at  $P=0.259$  (Kruskal-Wallis test). Error bars represent 1 S.E.M.

Most understorey species were also typically largest in T5 (Mix, tall canopy dominant) (Figure 4.14). Size of individual species (g/plant) differed significantly ( $P<0.05$ , Kruskal-Wallis test) between treatments for *P. divaricata* ( $P=0.013$ ), *P. reptans* ( $P=0.001$ ), *P. veris* ( $P=0.001$ ) and *P. vulgaris* ( $P=0.001$ ).

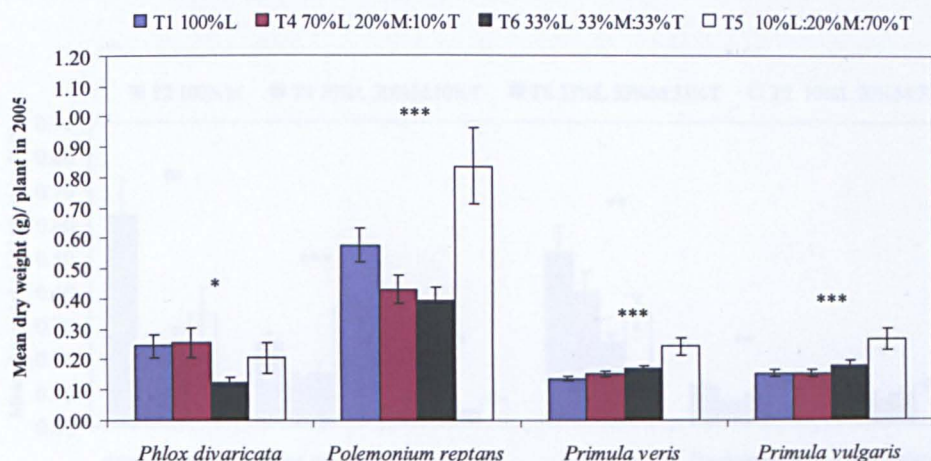


Figure 4.14 Mean biomass of individual species (g/plant) in response to composition of sowing mix. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between multi-layer treatments are indicated by: ns, not significant. *Primula elatior* and *Dodecatheon meadia* were excluded from this analysis due to insufficient data.

### 4.3.2.3.2 Medium canopy forbs

As shown in Figure 4.15b, the medium layer dry weight was greatest in T3 (Tall canopy), but differences were not significant ( $P=0.146$  ns, Kruskal-Wallis test).

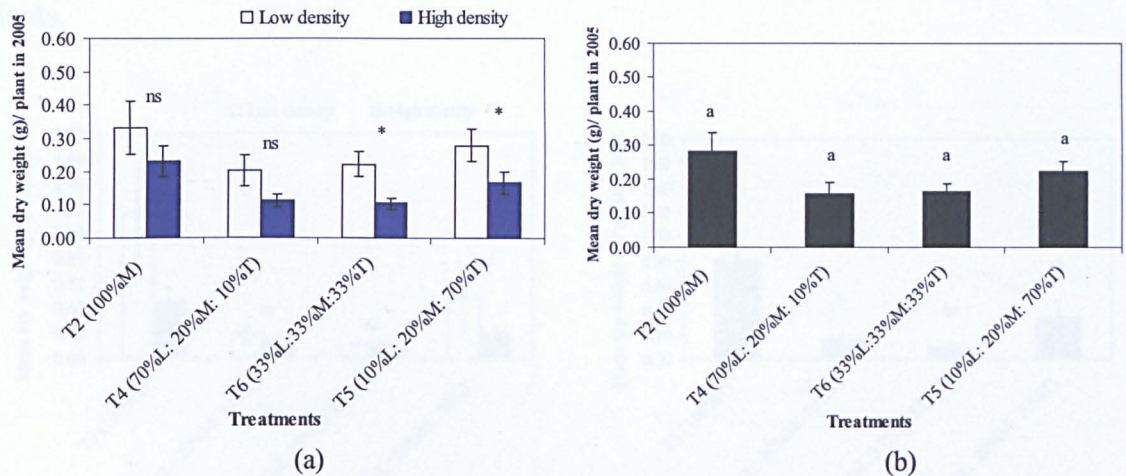


Figure 4.15 Effect of multi-layer treatment and density on mean biomass of individual plants in medium canopy species in September 2005. (a) Mean biomass in response to different multi-layer treatments at different densities. Significant differences (Mann-Whitney  $U$ -test) between low and high densities are indicated by; \*  $P=0.05$ ; ns, not significant. (b) Mean biomass in response to different multi-layer treatments as pooled across density treatments. Bars labeled with the same letters are not statistically different at  $P=0.146$  (Kruskal-Wallis test). Error bars represent 1 S.E.M.

The greatest individual species dry weights were achieved (*A. azureus* and *S. speciosa*) in T2 (Figure 4.16). Dry weight of individual species (g/plant) was significantly different between treatments ( $P<0.05$ , Kruskal-Wallis test) in *Phlox pilosa*, *P. maculata*, *S. speciosa*, *T. ohioensis* and *Z. aptera*. Dry weight was not significantly different in *A. azureus* ( $P=0.052$ ).

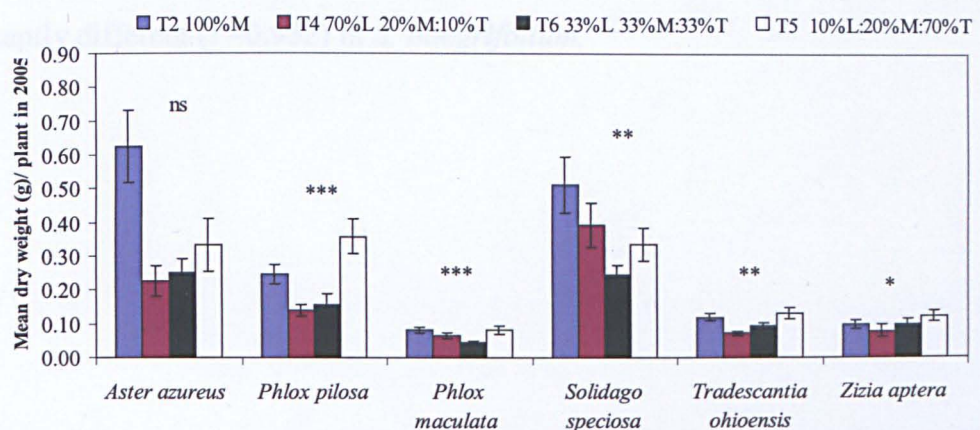
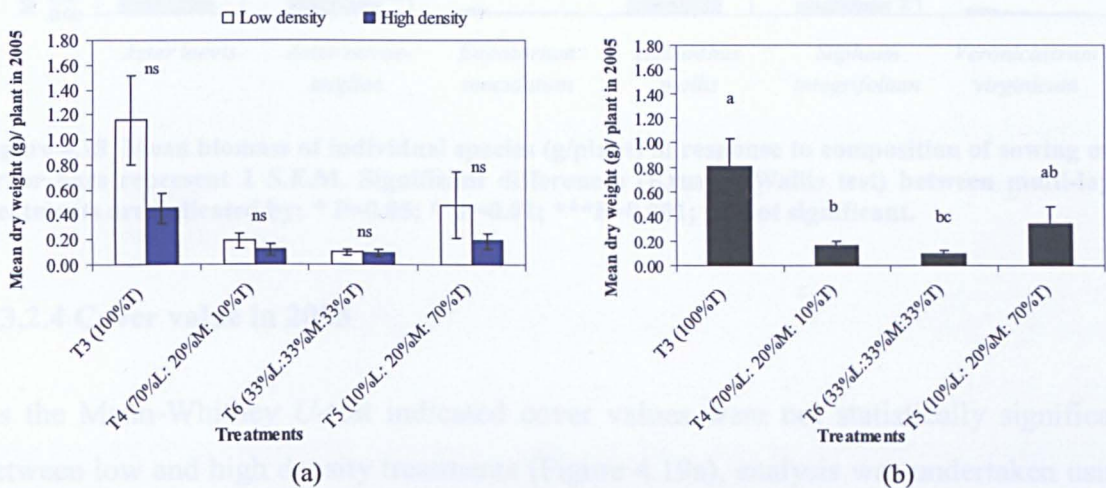


Figure 4.16 Mean biomass of individual species (g/plant) in response to composition of sowing mix. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between multi-layer treatments are indicated by: \*  $P=0.05$ ; \*\* $P=0.01$ ; \*\*\* $P=0.001$ ; ns, not significant.

### 4.3.2.3.3 Tall canopy forbs

As shown in Figure 3.17b, the greatest dry weight of tall layer individuals was achieved in T3 (Tall canopy) when other layers were absent ( $P=0.001$ , Kruskal-Wallis test). The presence of other layers caused a decline in the size of individual tall canopy layer plants.



**Figure 4.17** Effect of multi-layer treatment and density on mean biomass of individual plants in tall canopy species in September 2005. (a) Mean biomass in response to different multi-layer treatments at different densities. Significant differences (Mann-Whitney  $U$ -test) between low and high densities are indicated by; ns, not significant. (b) Mean biomass in response to different multi-layer treatments as pooled across density treatments. Bars labeled with different letters are significantly different at  $P=0.001$  (Kruskal-Wallis test, pairwise Mann-Whitney  $U$ -tests). Error bars represent 1 S.E.M.

Within tall canopy species, the largest dry weights were recorded for *Aster laevis* and *H. mollis* in T3 (Tall canopy) (Figure 4.18). Dry weight of each individual species (g/plant) was significantly different ( $P<0.05$ , Kruskal-Wallis test) in *A. laevis*, *A. novae-angliae*, *E. maculatum*, *H. mollis* and *V. virginicum* between treatments. Dry weight was not significantly different ( $P=0.952$ ) in *S. integrifolium*.



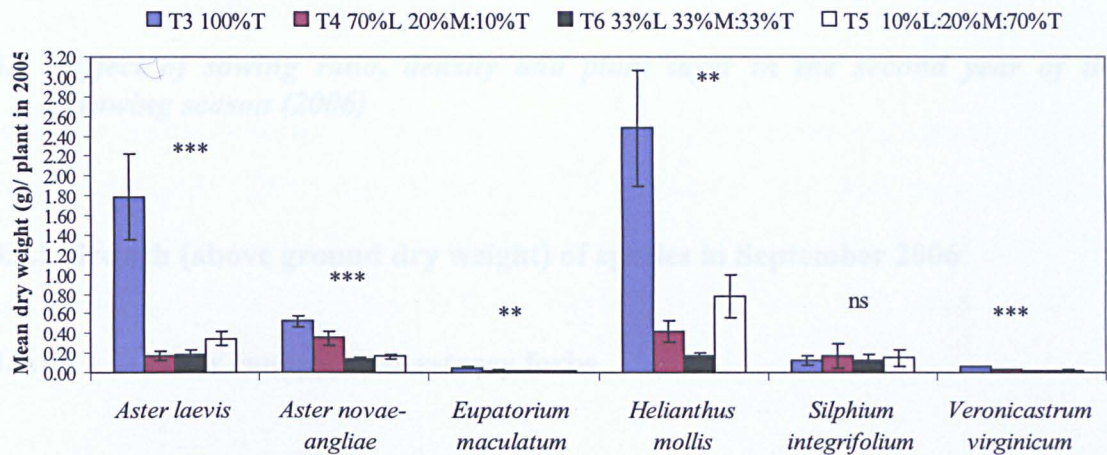


Figure 4.18 Mean biomass of individual species (g/plant) in response to composition of sowing mix. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between multi-layer treatments are indicated by: \* P=0.05; \*\*P=0.01; \*\*\*P=0.001; ns, not significant.

#### 4.3.2.4 Cover value in 2005

As the Mann-Whitney *U*-test indicated cover values were not statistically significant between low and high density treatments (Figure 4.19a), analysis was undertaken using pooled coverage values. The low canopy layer, T1 (Understorey) had significantly higher cover values ( $P=0.001$ , Kruskal-Wallis tests) in September 2005 (Figure 4.19b). Typically the presence of an understorey layer significantly improved cover values in medium and tall prairie vegetation.

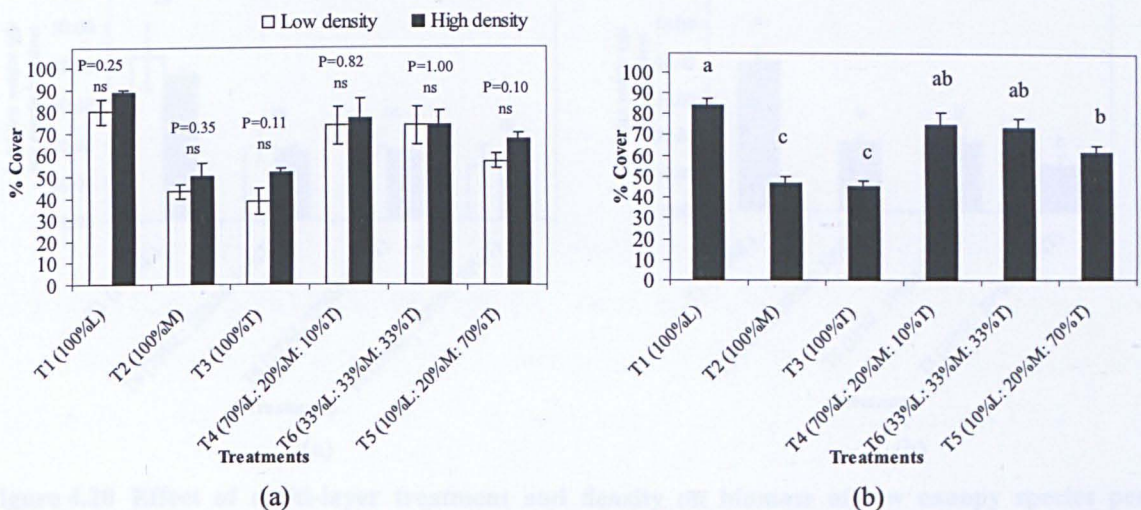


Figure 4.19 Effect of seed ratios and sowing density on cover values (a) low v high density (b) mean values for both density in September 2005. Bars labeled with different letters are significantly different at  $P=0.001$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-test). Error bars represent 1 S.E.M.

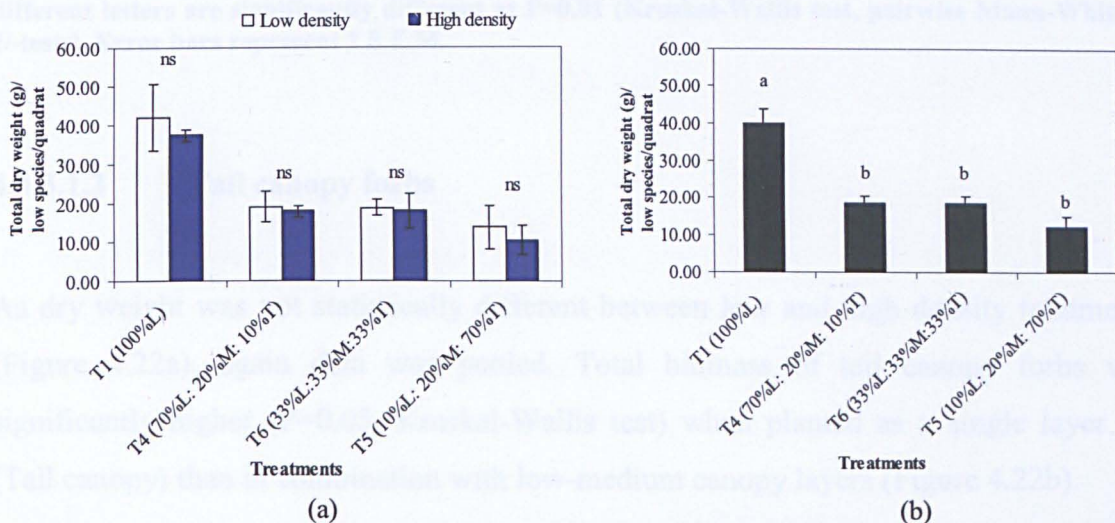
### 4.3.3 Effect of sowing ratio, density and plant layer in the second year of the growing season (2006)

#### 4.3.3.1 Growth (above ground dry weight) of species in September 2006

##### 4.3.3.1.1 Low canopy, understorey forbs

As a result of the early disappearance of some individual low canopy species at harvest date (September 2006) due to dormancy, the plants were harvested as a total biomass to generate growth data.

The Mann-Whitney *U*-test indicated that since there was no significant difference between low and high density treatments (Figure 4.20a), hence analysis was undertaken using pooled data. As shown in Figure 4.20b, as expected, the biomass of understorey forbs in T1 (undercanopy layer) was significantly higher ( $P=0.002$ ) than when combined with medium-tall canopy layer treatments T4 (Mix, understorey dominant), T5 (Mix, tall canopy dominant) and T6 (Mix equivalent understorey, medium, tall).



**Figure 4.20** Effect of multi-layer treatment and density on biomass of low canopy species per quadrat in September 2006. (a) Total biomass of species in different densities. Significant differences (Mann-Whitney *U*-test) between low and high densities are indicated by: ns, not significant. (b) Total biomass of species as pooled across density treatments. Bars labeled with different letters are significantly different at  $P=0.001$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-tests). Error bars represent 1 S.E.M.

### 4.3.3.1.2 Medium canopy forbs

Again, the Mann-Whitney *U*-test found that dry weight was not statistically different between low and high density treatments (Figure 4.21a), and data were pooled for analysis. Medium canopy forbs planted as a single layer T2 (Medium canopy) had significantly greater dry weight ( $P=0.01$ , Kruskal-Wallis test) than those planted in combination with medium-tall canopy layers (Figure 4.21b).

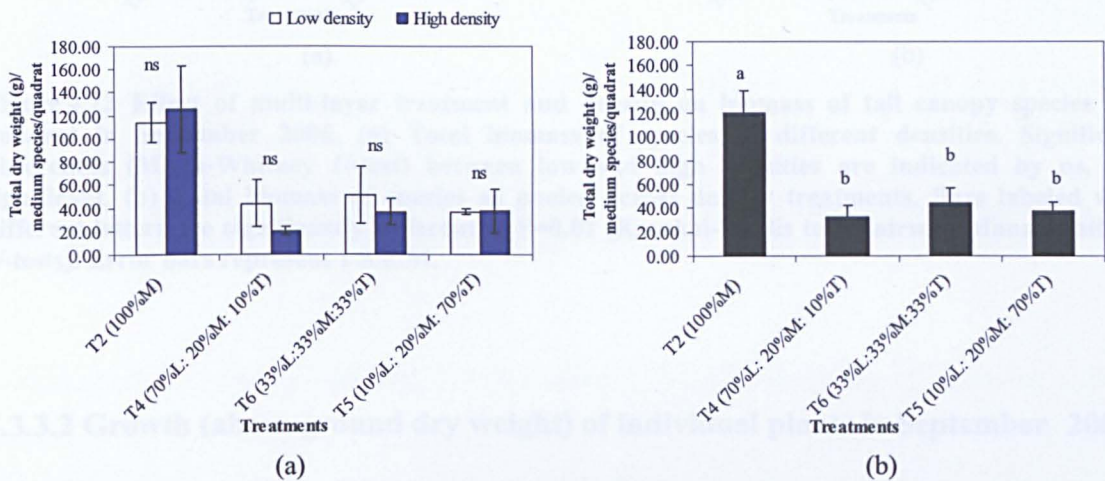


Figure 4.21 Effect of multi-layer treatment and density on biomass of medium canopy species per quadrat in September 2006. (a) Total biomass of species in different densities. Significant differences (Mann-Whitney *U*-test) between low and high densities are indicated by: ns, not significant. (b) Total biomass of species as pooled across density treatments. Bars labeled with different letters are significantly different at  $P=0.01$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-tests). Error bars represent 1 S.E.M.

### 4.3.3.1.3 Tall canopy forbs

As dry weight was not statistically different between low and high density treatments (Figure 4.22a), again data was pooled. Total biomass of tall canopy forbs was significantly higher ( $P=0.05$ , Kruskal-Wallis test) when planted as a single layer T3 (Tall canopy) than in combination with low-medium canopy layers (Figure 4.22b).

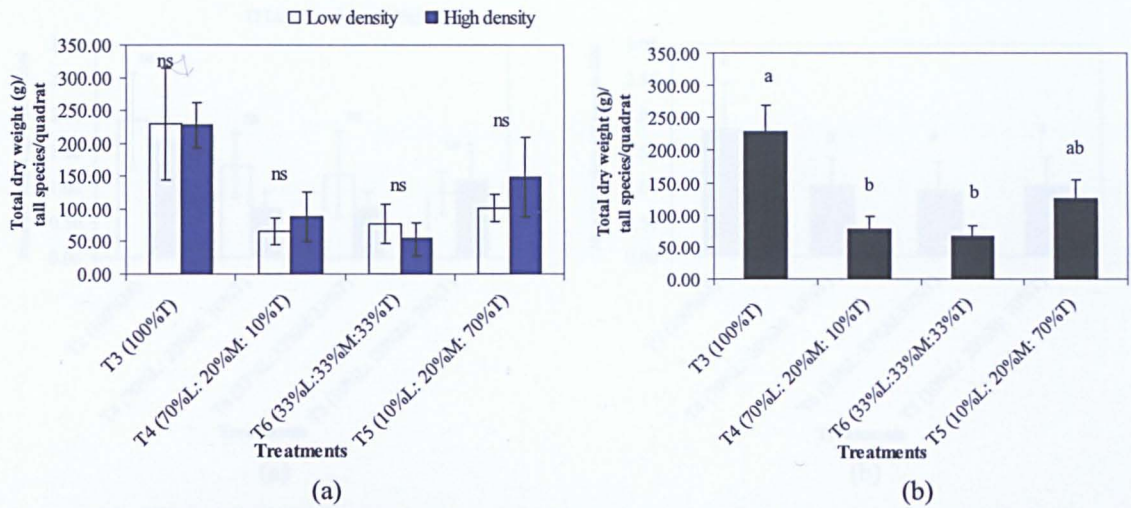


Figure 4.22 Effect of multi-layer treatment and density on biomass of tall canopy species per quadrat in September 2006. (a) Total biomass of species in different densities. Significant differences (Mann-Whitney *U*-test) between low and high densities are indicated by ns, not significant. (b) Total biomass of species as pooled across density treatments. Bars labeled with different letters are significantly different at  $P=0.01$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-tests). Error bars represent 1 S.E.M.

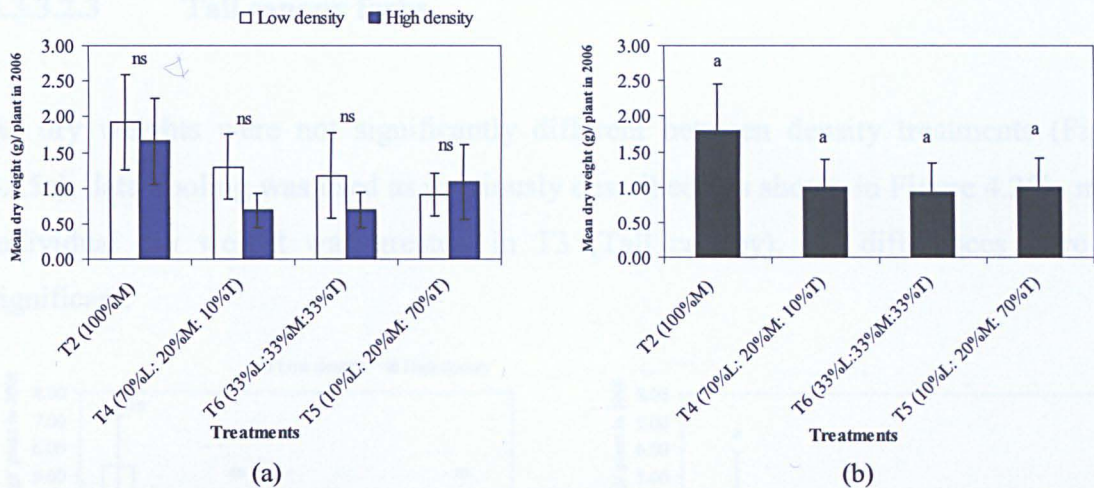
#### 4.3.3.2 Growth (above ground dry weight) of individual plants in September 2006

##### 4.3.3.2.1 Low canopy, understorey forbs

No data was available due to “layer only” harvesting in September 2006.

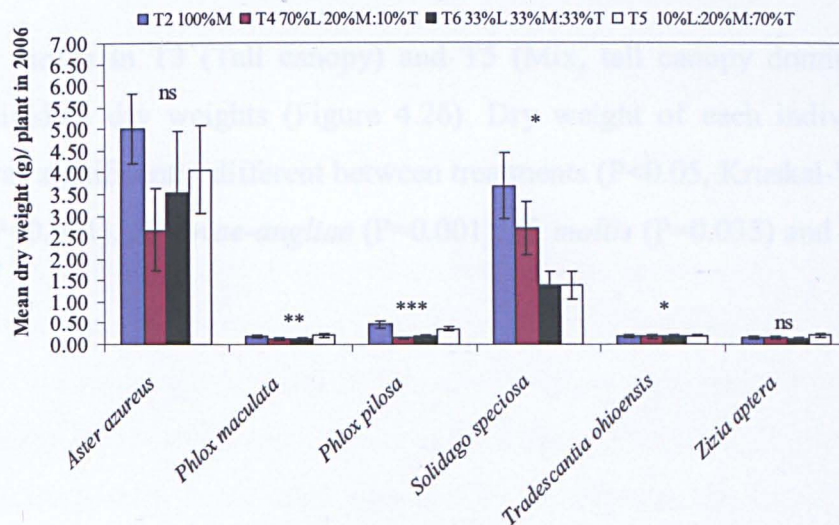
##### 4.3.3.2.2 Medium canopy forbs

As dry weights were not statistically different between low and high density treatments (Figure 4.23a), data was pooled. As shown in Figure 4.23b, individual dry weights were greatest in T2 (Medium canopy) when plants were not mixed with other canopy layers, however these differences were not significant ( $P=0.414$  ns, Kruskal-Wallis test).



**Figure 4.23** Effect of multi-layer treatment and density on mean biomass of individual plants in medium canopy species in September 2006. (a) Mean biomass in response to different multi-layer treatments at different densities. Significant differences (Mann-Whitney  $U$ -test) between low and high densities are indicated by; ns, not significant. (b) Mean biomass in response to different multi-layer treatments as pooled across density treatments. Bars labeled with different letters are not significantly different at  $P=0.05$  (Kruskal-Wallis test, pairwise Mann-Whitney  $U$ -tests). Error bars represent 1 S.E.M.

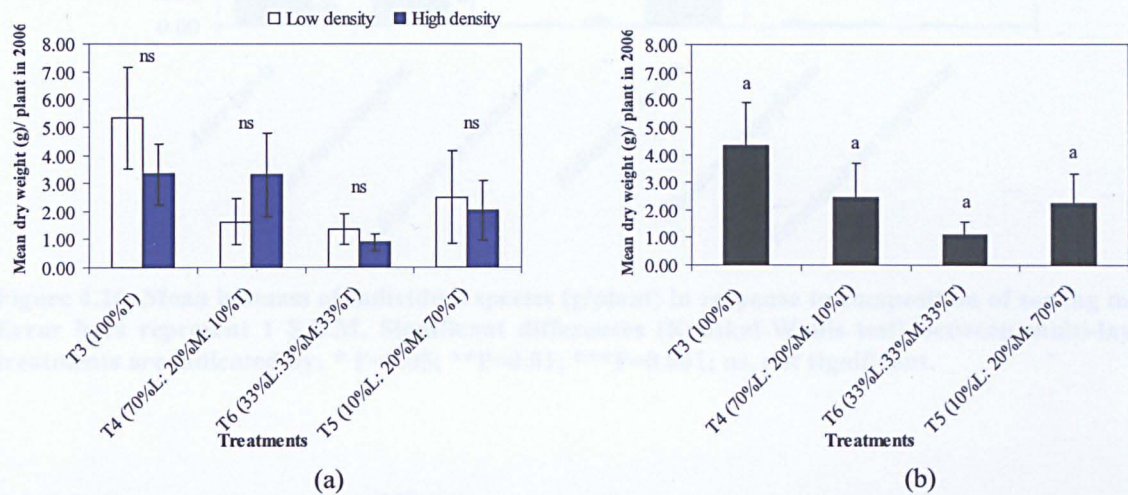
Within medium canopy species, the highest dry weights were for *A. azureus* and *S. speciosa* in T2 (Medium canopy) (Figure 4.24). Dry weights were significantly different between treatments ( $P<0.05$ , Kruskal-Wallis test) for *P. maculata* ( $P=0.009$ ), *Phlox pilosa* ( $P=0.000$ ), *S. speciosa* ( $P=0.011$ ) and *T. ohioensis* ( $P=0.027$ ).



**Figure 4.24** Mean biomass of individual species (g/plant) in response to composition of sowing mix. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between multi-layer treatments are indicated by: \*  $P=0.05$ ; \*\*  $P=0.01$ ; \*\*\*  $P=0.001$ ; ns, not significant.

### 4.3.3.2.3 Tall canopy forbs

As dry weights were not significantly different between density treatments (Figure 4.25a), data pooling was used as previously described. As shown in Figure 4.25b, mean individual dry weight was greatest in T3 (Tall canopy), but differences were not significant.



**Figure 4.25** Effect of multi-layer treatment and density on mean biomass of individual plants in tall canopy species in September 2006. (a) Mean biomass in response to different multi-layer treatments at different densities. Significant differences (Mann-Whitney  $U$ -test) between low and high densities are indicated by; ns, not significant. (b) Mean biomass in response to different multi-layer treatments as pooled across density treatments. Bars labeled with the same letters are not significantly different at  $P=0.05$  (Kruskal-Wallis test). Error bars represent 1 S.E.M.

*Helianthus mollis* in T3 (Tall canopy) and T5 (Mix, tall canopy dominant) had the largest individual dry weights (Figure 4.26). Dry weight of each individual species (g/plant) was significantly different between treatments ( $P<0.05$ , Kruskal-Wallis test) in *A. laevis* ( $P=0.001$ ), *A. novae-angliae* ( $P=0.001$ ), *H. mollis* ( $P=0.035$ ) and *V. virginicum* ( $P=0.001$ ).

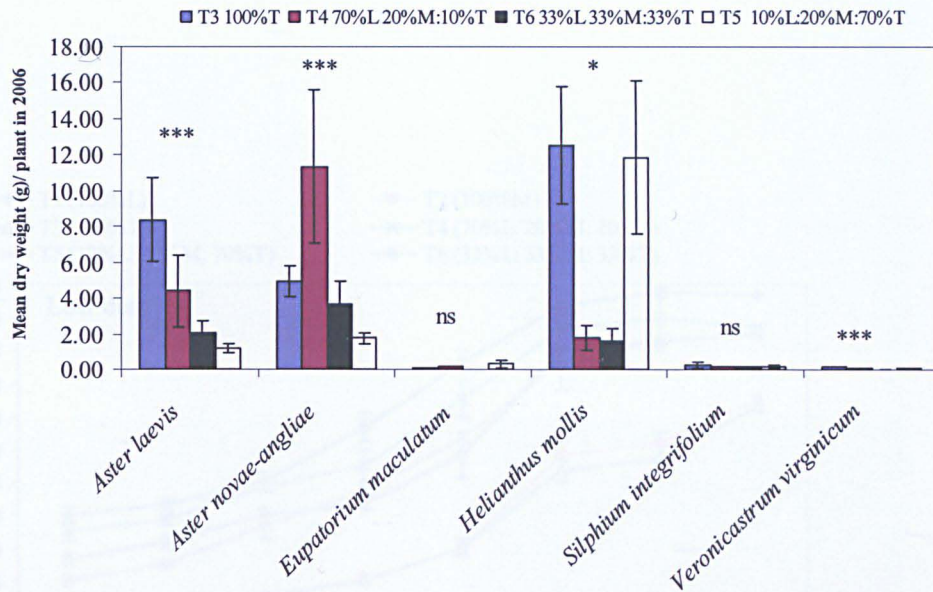
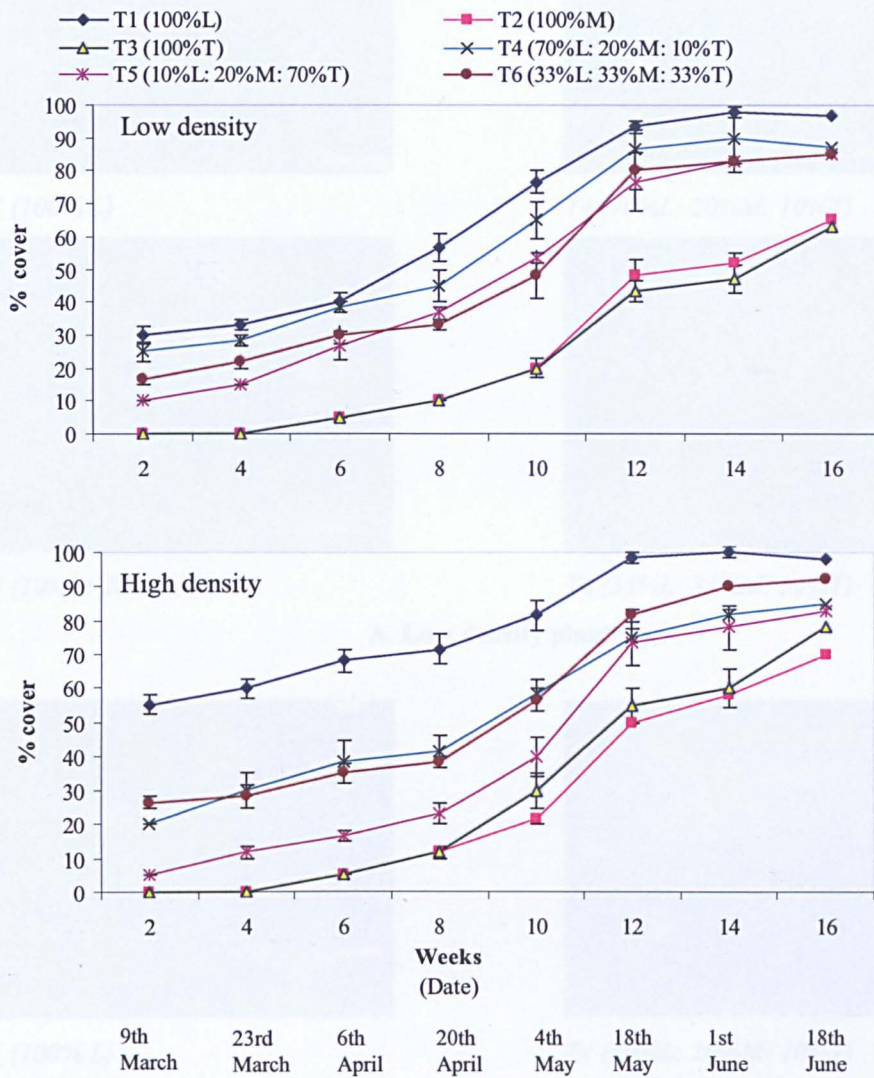


Figure 4.26 Mean biomass of individual species (g/plant) in response to composition of sowing mix. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between multi-layer treatments are indicated by: \*  $P=0.05$ ; \*\*  $P=0.01$ ; \*\*\*  $P=0.001$ ; ns, not significant.

#### 4.3.3.3 Cover value in year 2006

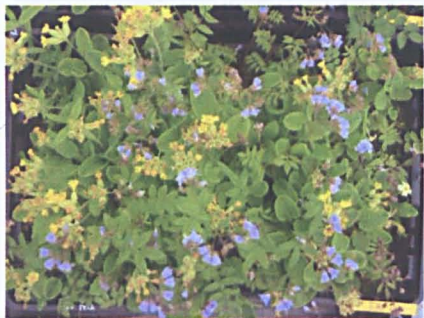
Cover values were recorded at two weeks interval during the development of the plant communities (March to June 2006) (Figure 4.27). After 12 weeks, T1 (Understorey), T4 (Mix, understorey dominant), T5 (Mix, tall canopy dominant) and T6 (Mix equivalent understorey, medium, tall) (all containing some understorey forbs) covered more than 70% of the trays (Figure 4.28a). Cover values in T2 (Medium canopy) and T3 (Tall canopy) were lower at less than 60% at 12 weeks (Figure 4.28b).

To assist data interpretation, cover values recorded in spring (4th May 2005) were analysed for comparison between the treatments. Data for analysis were pooled as cover values were not significantly different between densities treatments (Figure 4.29a). As shown in figure 4.29b, these were significantly different ( $P=0.001$ , Kruskal-Wallis test) between the treatments. In common with the first year results, the highest cover values in multi-layer plant communities in the second year growing were associated with the present of low canopy species. This was true for low and high density planting. It shows that by mixing understorey forbs in composition, high cover value ( $>70\%$ ) was achieved in June 2006. Perhaps more importantly cover values are much higher earlier in the year in treatments when an understorey layer is present, potentially restricting invasion of weedy species during this time.



**Figure 4.27** Effect of weeks and multi-layer herbaceous plant communities treatments on cover in March-June 2006. Error bars represent 1 S.E.M.





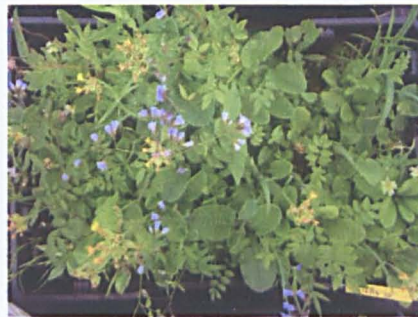
*T1 (100% L)*



*T4 (70%L: 20%M: 10%T)*



*T5 (10%L: 20%M: 70%T)*

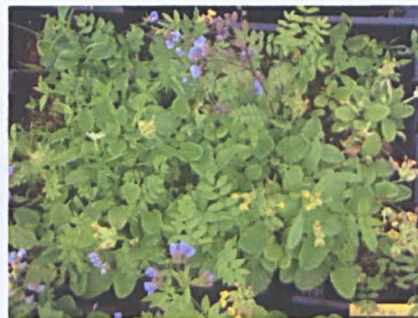


*T6 (33%L: 33%M: 33%T)*

**A. Low density planting**



*T1 (100% L)*



*T4 (70%L: 20%M: 10%T)*



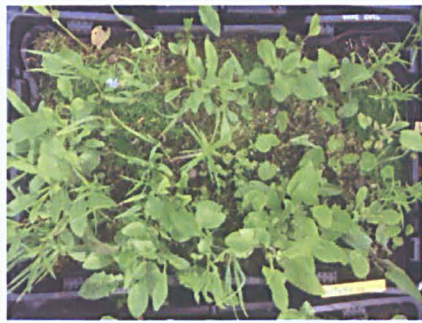
*T5 (10%L: 20%M: 70%T)*



*T6 (33%L: 33%M: 33%T)*

**B. High density planting**

(a)



T2 (100%M)



T3 (100%T)

A. Low density planting



T2 (100%M)



T3 (100%T)

B. High density planting

(b)

Figure 4.28 (a) Cover values (>70%) representing T1 (Understorey), (T4 (Mix, understorey dominant), T5 (Mix, tall canopy dominant) and T6 (Mix equivalent understorey, medium, tall) in this study (18<sup>th</sup> May 2006). (b) Cover values (<60%) representing T2 (Medium canopy) and T3 (Tall canopy) in this study (18<sup>th</sup> May 2006). This coverage may allow weed seedlings to invade.

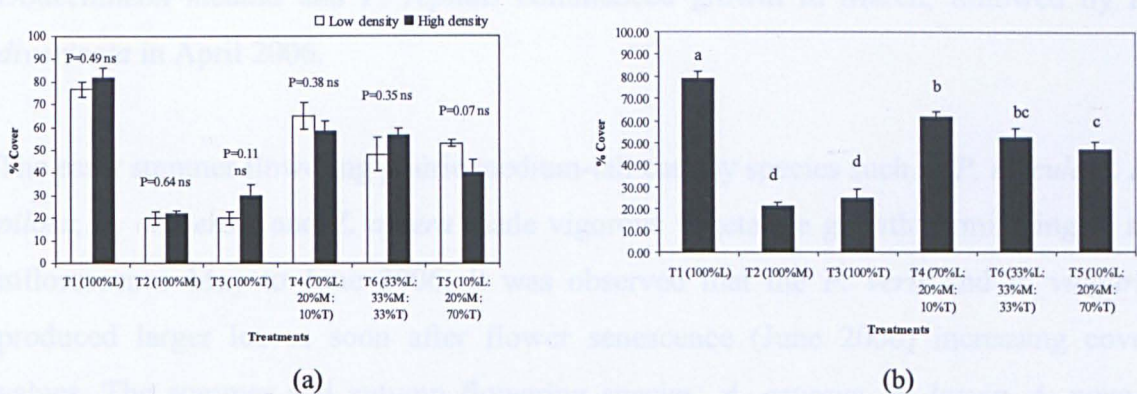


Figure 4.29 Effect of seed ratios and sowing density on cover values (a) low v high density (b) mean values for both density in May 2005. Bars labeled with the same letters are significantly different at P=0.001 (Kruskal-Wallis test). Error bars represent 1 S.E.M.

At the final harvest, data for analysis was pooled as cover values were not significantly different between densities treatments (Figure 4.30a). As shown in figure 4.30b, there is no significant difference between the treatments. This indicates that cover value

(approximately 100%) was achieved during early autumn in year two which suggests that cover values were associated with the presence of medium-tall canopy layer species which grow vigorously during that season. This is true for low and high density planting in all treatments which score high coverage (>80%) in September 2006.

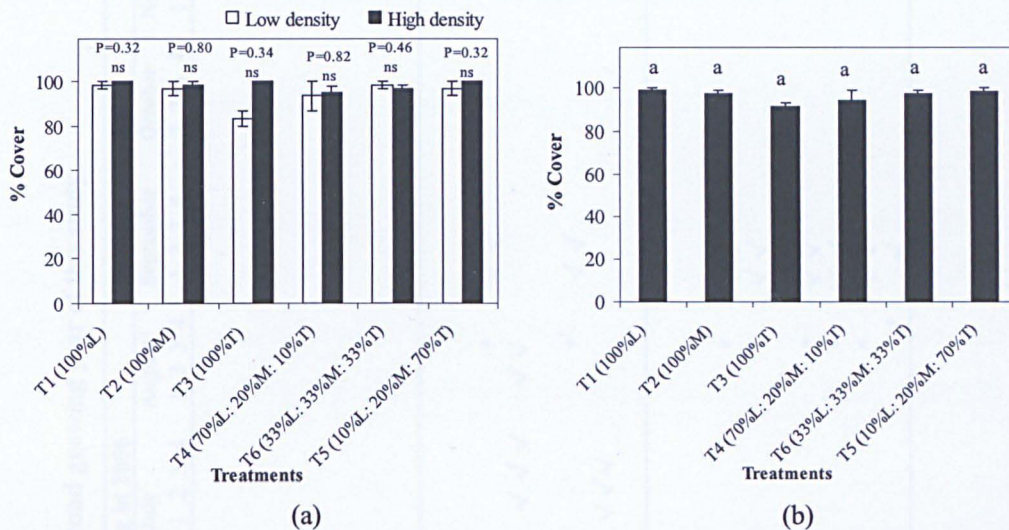


Figure 4.30 Effect of seed ratios and sowing density on cover values (a) low v high density (b) mean values for both density in September 2006. Bars labeled with the same letters are not significantly different ( $P=0.493$ , Kruskal-Wallis test). Error bars represent 1 S.E.M.

#### 4.3.3.4 Phenology of the species by 2006

The evergreen shade tolerant understorey forbs (*P. veris* and *P. vulgaris*) started to produce new leaves in February 2006 and flowered from April to May 2006. *Dodecatheon meadia* and *P. reptans* commenced growth in March, followed by *P. divaricata* in April 2006.

The early summer flowering prairie medium-tall canopy species such as *P. maculata*, *P. pilosa*, *T. ohioensis* and *Z. aptera* made vigorous vegetative growth terminating in an inflorescence May to June 2006. It was observed that the *P. veris* and *P. vulgaris* produced larger leaves soon after flower senescence (June 2006) increasing cover values. The summer and autumn flowering species; *A. azureus*, *A. laevis*, *A. novae-angliae*, *E. maculatum*, *H. mollis*, *S. integrifolium*, *S. speciosa* and *V. virginicum* produced erect vegetative shoots terminating in an inflorescence from mid-June to August 2006. The leaves of these species started to die back from October onwards. All species in this study were blooming at different times and in different patterns (Table 4.6), thus providing a long lasting dramatic impact throughout the year.

Table 4.6 Flowering phenology of each species under multi-layer communities in the second growing year of this study.

Species	Months Weeks	Plant Flowering in 2006																																																			
		January				February				March				April				May				June				July				August				September				October				November				December							
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4								
<b>Low canopy (L)</b>																																																					
<i>Dodecatheon meadia</i>																																																					
<i>Phlox divaricata</i>																																																					
<i>Polemonium reptans</i>																																																					
<i>Primula elatior</i>																																																					
<i>Primula veris</i>																																																					
<i>Primula vulgaris</i>																																																					
<b>Medium canopy (M)</b>																																																					
<i>Aster azureus</i>																																																					
<i>Phlox maculata</i>																																																					
<i>Phlox pilosa</i>																																																					
<i>Solidago speciosa</i>																																																					
<i>Tradescantia ohioensis</i>																																																					
<i>Zizia aptera</i>																																																					
<b>Tall canopy (T)</b>																																																					
<i>Aster laevis</i>																																																					
<i>Aster novae-angliae</i>																																																					
'Septemberrubin'																																																					
<i>Eupatorium maculatum</i>																																																					
<i>Helianthus mollis</i>																																																					
<i>Silphium integrifolium</i>																																																					
<i>Veronicastrum virginicum</i>																																																					

Key: \* Start producing flower buds

√ More than 5 plants blooming

### 4.3.4 Effect of sowing ratio, density and plant layer in the third year of the growing season (2007)

#### 4.3.4.1 Growth (above ground dry weight) of species in May 2007

##### 4.3.4.1.1 Low canopy, understorey forbs

As a result of the complete dormancy or partial foliage senescence of individual low canopy species by the harvest date in the second growing year (September 2006) these species were harvested individually in May 2007 at peak standing biomass in order to gain a more realistic assessment of their contribution to community function.

Analysis was undertaken using a total mean biomass for both densities as this was not significantly different (Figure 4.31a). As shown in Figure 4.31b, the biomass of understorey forbs in T1 (low canopy layer only) was significantly higher ( $P=0.008$ ) than when combined with medium-tall canopy layers T5 (Mix, tall canopy dominant) and T6 (Mix equivalent understorey, medium, tall).

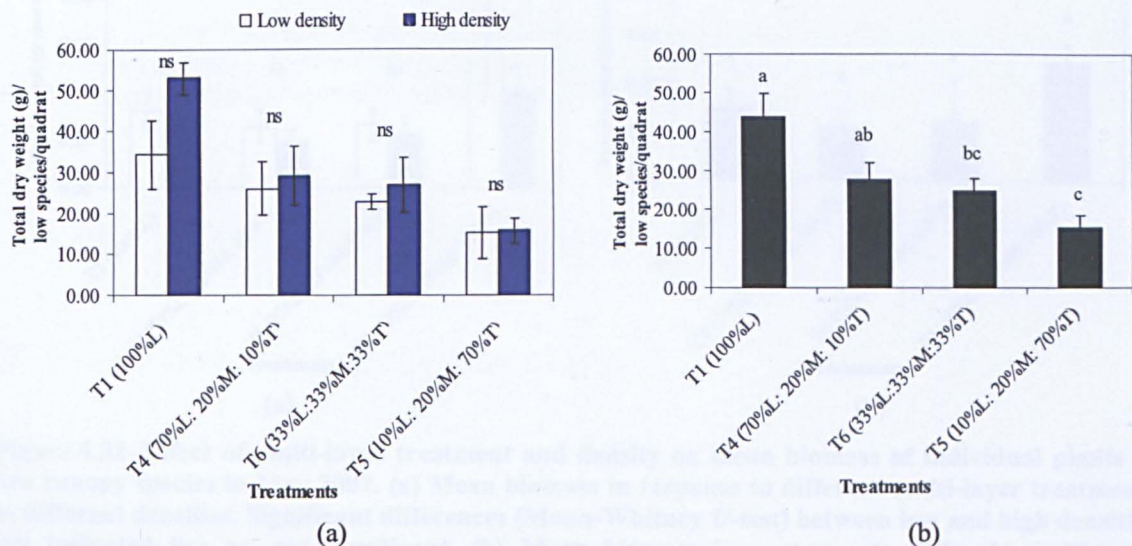


Figure 4.31 Effect of multi-layer treatment and density on biomass of low canopy species per quadrat in May 2007. (a) Total biomass of species in different densities. Significant differences (Mann-Whitney  $U$ -test) between low and high densities are indicated by: ns, not significant. (b) Total biomass of species as pooled across density treatments. Bars labeled with different letters are significantly different ( $P<0.01$ ; Kruskal-Wallis test, pairwise Mann-Whitney  $U$ -tests). Error bars represent 1 S.E.M.

#### 4.3.4.1.2 Medium and tall canopy forbs

As these were just commencing growth in May 2007 these were not harvested, rather their biomass in September 2006 was used as a final estimate.

#### 4.3.4.2 Growth (above ground dry weight) of individual plants in May 2007

##### 4.3.4.2.1 Low canopy, understorey forbs

An estimate of the mean biomass of the individual plants (g per plant as a mean for all species) was made in May 2007. Data for the two densities were pooled as the Mann-Whitney *U*-test found that data was not statistically different between density treatments (Figure 4.32a). The largest dry weights of low canopy layer individuals was achieved in T5 (Mix, tall canopy dominant) (Figure 4.32b) but not of the differences were statistically significant.

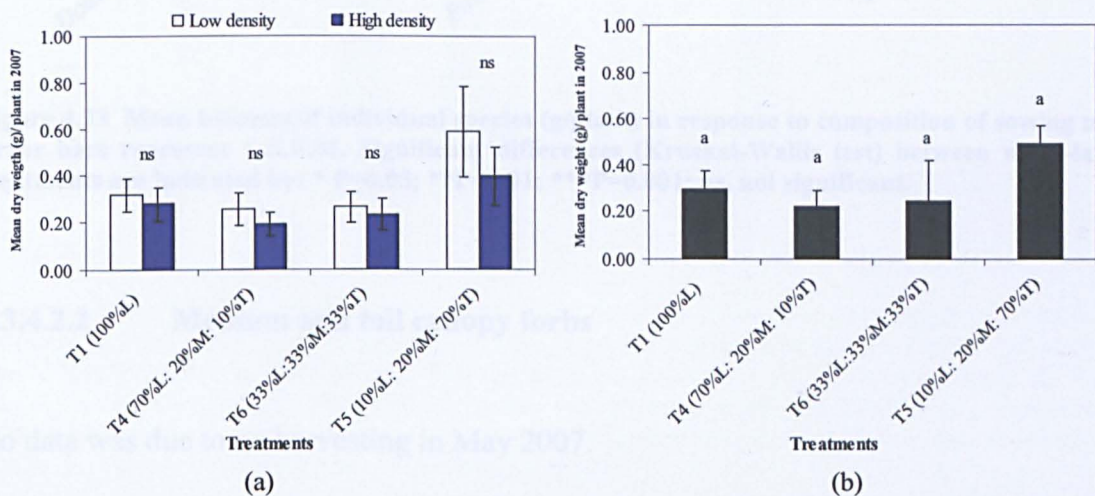


Figure 4.32 Effect of multi-layer treatment and density on mean biomass of individual plants in low canopy species in May 2007. (a) Mean biomass in response to different multi-layer treatments at different densities. Significant differences (Mann-Whitney *U*-test) between low and high densities are indicated by; ns, not significant. (b) Mean biomass in response to different multi-layer treatments as pooled across density treatments. Bars labeled with different letters are not significantly different at  $P=0.05$  (Kruskal-Wallis test, pairwise Mann-Whitney *U*-tests). Error bars represent 1 S.E.M.

Within undercanopy species plants of *P. veris* recorded the highest biomass and were significantly larger in T5 (Mix, tall canopy dominant) (Figure 4.33). Plant weight differed significantly ( $P < 0.05$ , Kruskal-Wallis test) in response to community type in *D. meadia* ( $P = 0.006$ ), *P. reptans* ( $P = 0.001$ ), *P. veris* ( $P = 0.001$ ) and *P. vulgaris* ( $P = 0.030$ ).

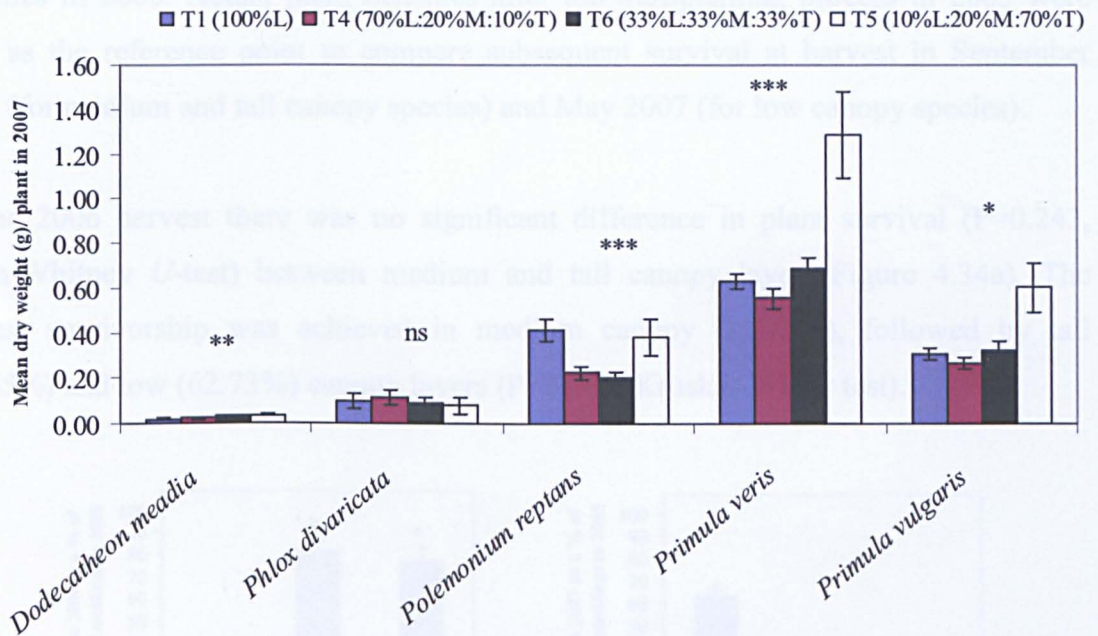


Figure 4.33 Mean biomass of individual species (g/plant) in response to composition of sowing mix. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between multi-layer treatments are indicated by: \*  $P = 0.05$ ; \*\*  $P = 0.01$ ; \*\*\*  $P = 0.001$ ; ns, not significant.

#### 4.3.4.2.2 Medium and tall canopy forbs

No data was due to no harvesting in May 2007.

### 4.3.5 Effect of sowing ratio and density for the multi-layer community as a whole (2005-2007)

#### 4.3.5.1 Survival of sown forbs as a percentage of those present in 2005

This parameter was used to eliminate potentially misleading losses associated with low seedling emergence and the transplanting process used to achieve target seedling densities in 2005. Actual plant densities after the transplanting process in 2005 were used as the reference point to compare subsequent survival at harvest in September 2006 (for medium and tall canopy species) and May 2007 (for low canopy species).

At the 2006 harvest there was no significant difference in plant survival ( $P=0.243$ , Mann-Whitney  $U$ -test) between medium and tall canopy layer (Figure 4.34a). The highest survivorship was achieved in medium canopy (81.35%), followed by tall (76.65%) and low (62.73%) canopy layers ( $P=0.006$ ; Kruskal-Wallis test).

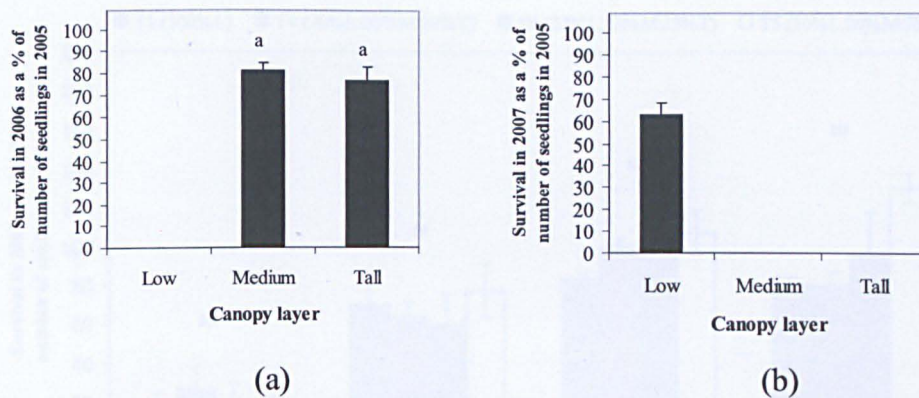


Figure 4.34 (a) Survival of different canopy layer plants in 2006 as a percentage of number of seedlings in 2005. Error bars represent 1 S.E.M. Low layer plant was excluded from this analysis due to insufficient data. Bars labeled with the same letters are not significantly different ( $P=0.243$  ns, Mann-Whitney  $U$ -test). (b) Survival of low canopy layer plants in 2007 as a percentage of number of seedlings in 2005. Medium and tall canopy layer plants were excluded from this analysis due to insufficient data. Error bars represent 1 S.E.M.

#### 4.3.5.1.1 Low canopy, understorey forbs

No significant difference ( $P=0.795$ , Kruskal-Wallis test) was found between treatments on the survivorship of low canopy species in 2007 (Figure 4.35). Two species, *P. veris* and *P. vulgaris* were more numerous in T5 in 2007 than in 2005, suggesting seedling



recruitment had occurred. *Phlox divaricata* showing the lowest plant survival (<25%) in all treatment. Data for all species are presented in Figure 4.36.

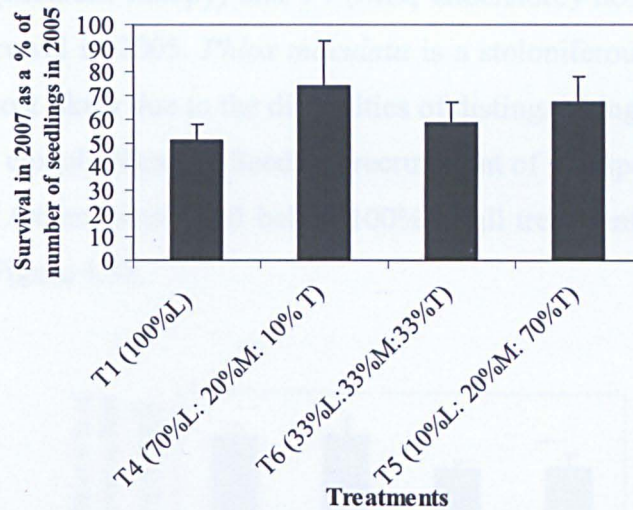


Figure 4.35 Effect of multi-layer treatments on survival of low species in 2007 as a percentage of number of seedlings in 2005. Error bars represent 1 S.E.M. ( $P=0.795$  ns, Kruskal-Wallis test).

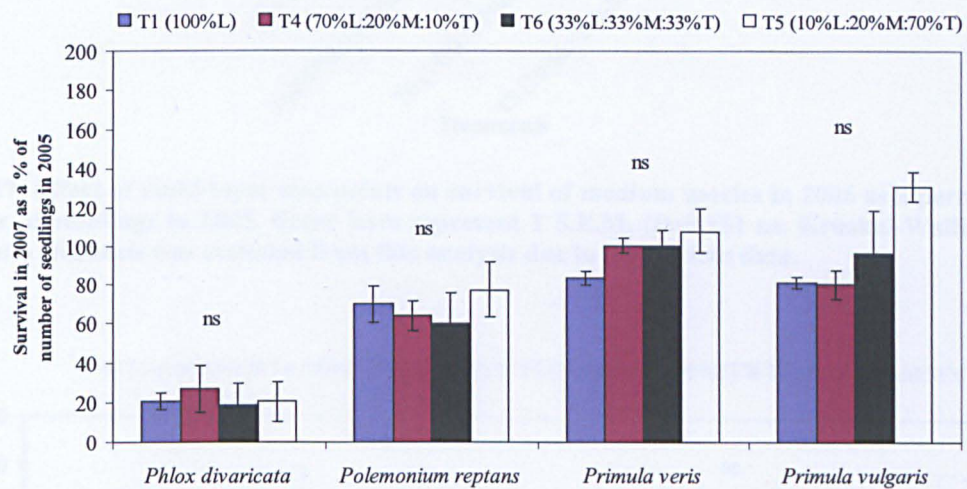


Figure 4.36 Survival of individual low canopy species in response to different multi-layer treatments. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between treatments are indicated by: ns, not significant. *Dodecatheon meadia* was excluded from this analysis due to insufficient data.

#### 4.3.5.1.2 Medium canopy forbs

No significant difference ( $P=0.151$ , Kruskal-Wallis test) was found between the survivorship of medium canopy species in 2006 across the treatments (Figure 4.37) as

mean of all species. However, *P. maculata* showed a significant increase ( $P=0.04$ ) in T4 (Mix, understorey dominant) compared to the other treatment. In 2006, only *P. maculata* in T2 (Medium canopy) and T4 (Mix, understorey dominant) exceeded their original plant survival in 2005. *Phlox maculata* is a stoloniferous species and some of this increase is most likely due to the difficulties of distinguishing between the shoots of parent plants and clonal offspring. Seedling recruitment of this species was not observed during the study. Other species fell below 100% in all treatments. Data for all species are presented in Figure 4.38.

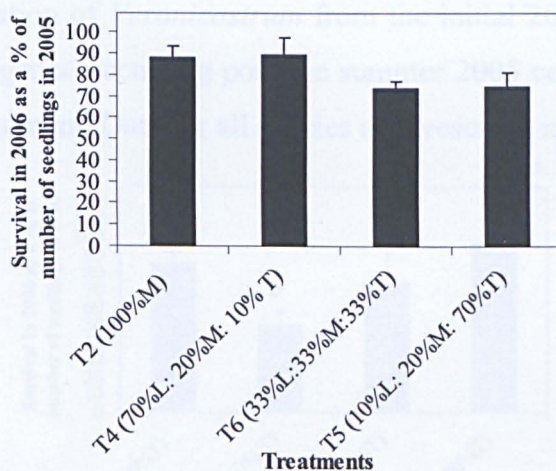


Figure 4.37 Effect of multi-layer treatments on survival of medium species in 2006 as a percentage of number of seedlings in 2005. Error bars represent 1 S.E.M. ( $P=0.151$  ns, Kruskal-Wallis test). *Tradescantia ohioensis* was excluded from this analysis due to insufficient data.

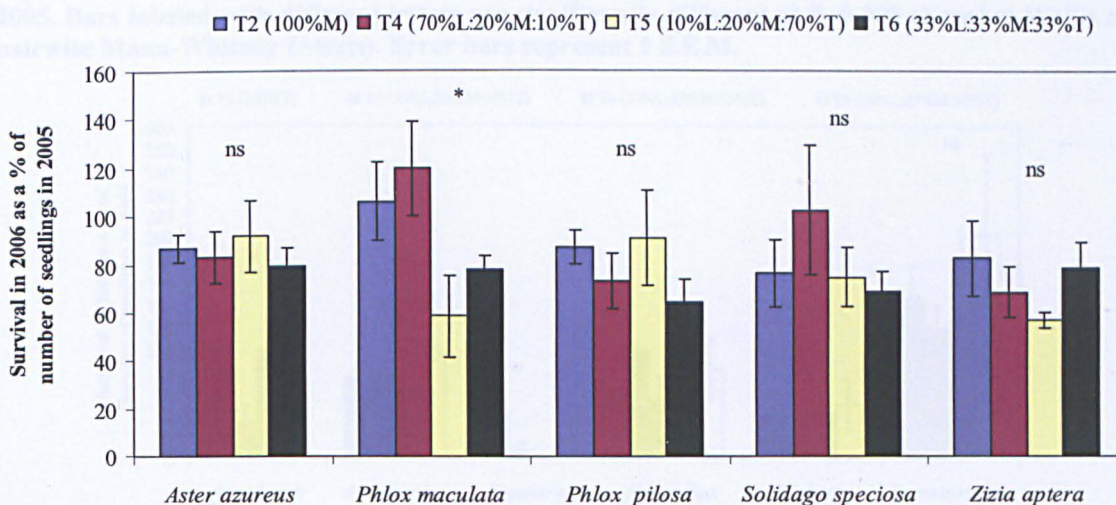


Figure 4.38 Survival of individual medium canopy species in response to different multi-layer treatments. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between treatments are indicated by: \*  $P=0.05$ ; ns, not significant. *Tradescantia ohioensis* was excluded from this analysis due to insufficient data.

### 4.3.5.1.3 Tall canopy forbs

Survival of tall canopy species was significantly less ( $P=0.008$ , Kruskal-Wallis test) in 2006 in T4 (dominated by understorey species) than in T3 (tall species only) (Figure 4.39). *Aster laevis* with the exception in T5 (Mix, tall canopy dominant) and *E. maculatum* showed a significant decrease ( $P<0.05$ ) across the treatments. In 2006, only *V. veronicastrum* (in all treatments) and *A. laevis* in T3 (Tall canopy) and T5 (Mix, tall canopy dominant) exceeded their original survivorship in 2005. This suggests that these two species recruited from seed during the course of the study. There is however some evidence that germination of *Veronicastrum* from the initial 2005 sowing was delayed with substantial emergence occurring post the summer 2005 census. Other species fell below 100% in all treatment. Data for all species are presented in Figure 4.40.

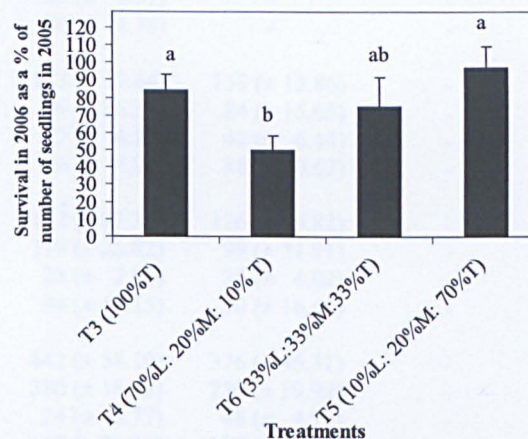


Figure 4.39 Effect of multi-layer treatments on survival of tall species in 2006 as a percentage of 2005. Bars labeled with different letters are significantly different at  $P=0.008$  (Kruskal-Wallis test, pairwise Mann-Whitney  $U$ -tests). Error bars represent 1 S.E.M.

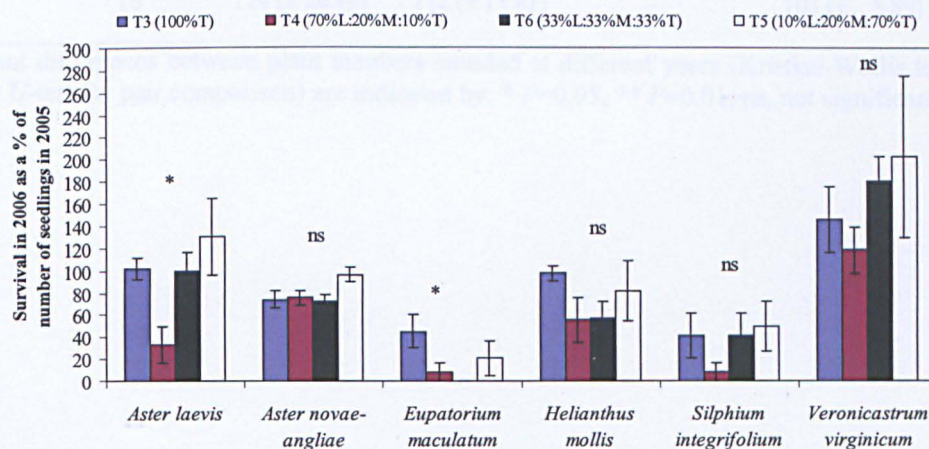


Figure 4.40 Survival of individual tall canopy species in response to different multi-layer treatments. Significant differences (Kruskal-Wallis test) between treatments are indicated by: \*  $P=0.05$ ; ns, not significant. Error bars represent 1 S.E.M.

## 4.3.5.2 Density of seedlings between 2005 and 2007

The density of individual forbs species (for both low and high densities) for all 3 years studied is shown in Table 4.7, 4.8 and 4.9, and again reflects the overall trend in survivorship of each species in different treatment mixes. To aid interpretation, values in these tables have been expressed as seedlings/m<sup>2</sup> rather than seedling/quadrat.

**Table 4.7** Density of low species at different counted years; 2005, 2006 and 2007. *P*-values refer to the difference in seedling numbers between July 2005 and the final census date (May 2007).

Species	Treatment mixes	Mean initial number of seedling/m <sup>2</sup> July 2005	Mean number of seedling/m <sup>2</sup> in			<i>P</i> -value
			Sept. 2005	Sept. 2006	May 2007	
<i>Dodecatheon meadia</i>	T1	538 (± 65.10)	-	-	17 (± 10.34)	0.004 **
	T4	323 (± 26.55)	-	-	36 (± 9.43)	0.004 **
	T5	23 (± 1.57)	-	-	47 (± 12.44)	0.012 *
	T6	86 (± 14.38)	-	-	56 (± 11.92)	0.108 ns
<i>Phlox divaricata</i>	T1	173 (± 17.64)	138 (± 13.86)	-	28 (± 5.65)	0.002 **
	T4	99 (± 16.36)	84 (± 15.65)	-	22 (± 13.14)	0.013 *
	T5	27 (± 4.27)	42 (± 6.44)	-	7 (± 2.38)	0.002 **
	T6	56 (± 4.01)	88 (± 20.62)	-	21 (± 14.99)	0.036 *
<i>Polemonium reptans</i>	T1	152 (± 22.37)	126 (± 19.82)	-	80 (± 8.99)	0.057 ns
	T4	119 (± 26.82)	99 (± 31.91)	-	66 (± 27.76)	0.103 ns
	T5	23 (± 2.88)	34 (± 4.02)	-	29 (± 7.28)	0.309 ns
	T6	84 (± 12.15)	90 (± 16.42)	-	44 (± 11.81)	0.083 ns
<i>Primula veris</i>	T1	442 (± 58.19)	376 (± 46.31)	-	309 (± 33.82)	0.231 ns
	T4	280 (± 16.72)	230 (± 19.92)	-	226 (± 17.38)	0.302 ns
	T5	24 (± 3.77)	48 (± 4.71)	-	51 (± 6.97)	0.012 *
	T6	113 (± 21.20)	177 (± 14.01)	-	173 (± 16.16)	0.062 ns
<i>Primula vulgaris</i>	T1	319 (± 63.44)	248 (± 37.35)	-	199 (± 27.46)	0.264 ns
	T4	221 (± 25.89)	196 (± 16.41)	-	153 (± 11.73)	0.108 ns
	T5	22 (± 2.33)	33 (± 5.43)	-	40 (± 6.42)	0.072 ns
	T6	128 (± 26.98)	112 (± 15.81)	-	103 (± 8.89)	0.905 ns

Significant differences between plant numbers counted at different years (Kruskal-Wallis test or Mann-Whitney *U*-test for pair comparison) are indicated by: \* *P*=0.05, \*\* *P*=0.01; ns, not significant.

Table 4.8 Density of medium species at different counted year; 2005, 2006 and 2007. *P*-values refer to the difference in seedling numbers between July 2005 and the final census date (May 2007).

Species	Treatment mixes	Mean initial number of seedling/m <sup>2</sup> July 2005	Mean number of seedling/ m <sup>2</sup> in			<i>P</i> -value
			Sept. 2005	Sept. 2006	May 2007	
<i>Aster azureus</i>	T2	131 (± 11.53)	110 (± 8.13)	96 (± 10.43)	-	0.104 ns
	T4	48 (± 5.04)	44 (± 8.01)	38 (± 8.94)	-	0.616 ns
	T5	36 (± 9.32)	47 (± 5.96)	40 (± 4.49)	-	0.399 ns
	T6	93 (± 28.18)	68 (± 6.32)	54 (± 7.58)	-	0.354 ns
<i>Phlox maculata</i>	T2	193 (± 31.69)	183 (± 33.19)	174 (± 28.73)	-	0.851 ns
	T4	48 (± 10.03)	42 (± 9.05)	44 (± 8.22)	-	0.890 ns
	T5	38 (± 3.67)	47 (± 7.33)	30 (± 7.70)	-	0.156 ns
	T6	74 (± 6.89)	92 (± 11.35)	70 (± 8.50)	-	0.140 ns
<i>Phlox pilosa</i>	T2	130 (± 20.30)	101 (± 15.81)	88 (± 13.96)	-	0.089 ns
	T4	51 (± 10.98)	43 (± 5.91)	31 (± 5.13)	-	0.386 ns
	T5	32 (± 1.97)	29 (± 5.85)	29 (± 8.19)	-	0.657 ns
	T6	80 (± 10.19)	53 (± 11.09)	34 (± 9.24)	-	0.024 *
<i>Solidago speciosa</i>	T2	82 (± 7.21)	79 (± 10.43)	60 (± 13.85)	54 (± 6.33)	0.134 ns
	T4	46 (± 5.05)	38 (± 5.88)	34 (± 5.37)	32 (± 4.75)	0.397 ns
	T5	38 (± 9.45)	58 (± 7.17)	43 (± 9.58)	30 (± 5.34)	0.173 ns
	T6	94 (± 11.17)	79 (± 10.61)	54 (± 9.45)	42 (± 8.37)	0.014 *
<i>Tradescantia ohioensis</i>	T2	179 (± 23.23)	161 (± 21.72)	58 (± 7.30)	104 (± 12.54)	0.003 **
	T4	57 (± 5.19)	70 (± 4.50)	24 (± 8.01)	41 (± 3.55)	0.002 **
	T5	37 (± 4.42)	47 (± 5.80)	9 (± 3.71)	26 (± 6.69)	0.002 **
	T6	91 (± 15.07)	121 (± 14.91)	30 (± 8.59)	74 (± 14.72)	0.002 **
<i>Zizia aptera</i>	T2	156 (± 14.36)	108 (± 17.96)	78 (± 9.05)	-	0.009 **
	T4	49 (± 10.98)	58 (± 12.12)	38 (± 10.09)	-	0.326 ns
	T5	31 (± 3.73)	48 (± 7.44)	27 (± 4.22)	-	0.095 ns
	T6	64 (± 13.42)	54 (± 12.86)	42 (± 9.48)	-	0.380 ns

Significant differences between plant numbers counted at different years (Kruskal-Wallis test) are indicated by: \* *P*=0.05, \*\* *P*=0.01; ns, not significant.

**Table 4.9** Density of tall species at different counted year; 2005, 2006 and 2007. *P*-values refer to the difference in seedling numbers between July 2005 and the final census date (May 2007).

Species	Treatment mixes	Mean initial number of seedling/m <sup>2</sup> July 2005	Mean number of seedling/ m <sup>2</sup> in			<i>P</i> -value
			Sept. 2005	Sept. 2006	May 2007	
<i>Aster laevis</i>	T3	33 (± 6.91)	33 (± 3.42)	36 (± 6.60)	-	0.962 ns
	T4	14 (± 4.01)	13 (± 3.87)	7 (± 3.42)	-	0.283 ns
	T5	22 (± 3.35)	21 (± 4.69)	23 (± 5.19)	-	0.936 ns
	T6	14 (± 2.62)	17 (± 3.70)	16 (± 3.28)	-	0.970 ns
<i>Aster novae-angliae</i> 'Septemberrubin'	T3	257 (± 33.72)	223 (± 32.42)	161 (± 21.75)	143 (± 18.19)	0.051 ns
	T4	42 (± 4.77)	56 (± 8.69)	41 (± 5.88)	37 (± 3.79)	0.141 ns
	T5	413 (± 31.80)	329 (± 47.15)	313 (± 49.15)	208 (± 32.45)	0.029 *
	T6	153 (± 14.62)	143 (± 14.20)	102 (± 11.67)	61 (± 8.35)	0.001 ***
<i>Eupatorium maculatum</i>	T3	96 (± 16.05)	52 (± 15.65)	20 (± 6.94)	-	0.011 *
	T4	36 (± 11.35)	9 (± 2.77)	1 (± 1.17)	-	0.003 **
	T5	50 (± 8.94)	20 (± 6.04)	3 (± 2.25)	-	0.002 **
	T6	62 (± 23.68)	18 (± 10.33)	0 (± 0.00)	-	0.003 **
<i>Helianthus mollis</i>	T3	32 (± 3.64)	31 (± 5.59)	30 (± 5.15)	-	0.912 ns
	T4	7 (± 2.98)	11 (± 4.10)	8 (± 4.05)	-	0.655 ns
	T5	22 (± 5.55)	26 (± 6.23)	20 (± 5.96)	-	0.811 ns
	T6	27 (± 7.05)	26 (± 6.33)	18 (± 5.88)	-	0.521 ns
<i>Silphium integrifolium</i>	T3	8 (± 3.16)	4 (± 2.20)	3 (± 1.57)	-	0.549 ns
	T4	6 (± 2.62)	3 (± 2.25)	1 (± 1.17)	-	0.396 ns
	T5	6 (± 3.19)	3 (± 1.57)	3 (± 1.57)	-	0.951 ns
	T6	4 (± 2.20)	4 (± 2.20)	3 (± 1.57)	-	0.953 ns
<i>Veronicastrum virginicum</i>	T3	294 (± 48.11)	228 (± 42.97)	296 (± 47.71)	284 (± 41.06)	0.758 ns
	T4	44 (± 7.22)	54 (± 12.76)	57 (± 9.14)	54 (± 7.79)	0.705 ns
	T5	203 (± 38.01)	91 (± 16.32)	147 (± 27.07)	158 (± 26.91)	0.080 ns
	T6	206 (± 17.33)	79 (± 10.88)	113 (± 24.50)	127 (± 8.92)	0.002 **

Significant differences between plant numbers counted at different years (Kruskal-Wallis test) are indicated by: \* *P*=0.05, \*\* *P*=0.01; \*\*\* *P*=0.001; ns, not significant.

#### 4.3.5.3 Growth (above ground dry weight) of species at final harvest as a percentage of harvest weight in 2005

Tall canopy layer showed the highest percentage increase in dry weight ( $P=0.001$ , Kruskal-Wallis test) across the study at the 2006 harvest (Figure 4.41a). The 2007 harvest provides a more meaningful assessment of growth of low canopy species (Figure 4.41b).

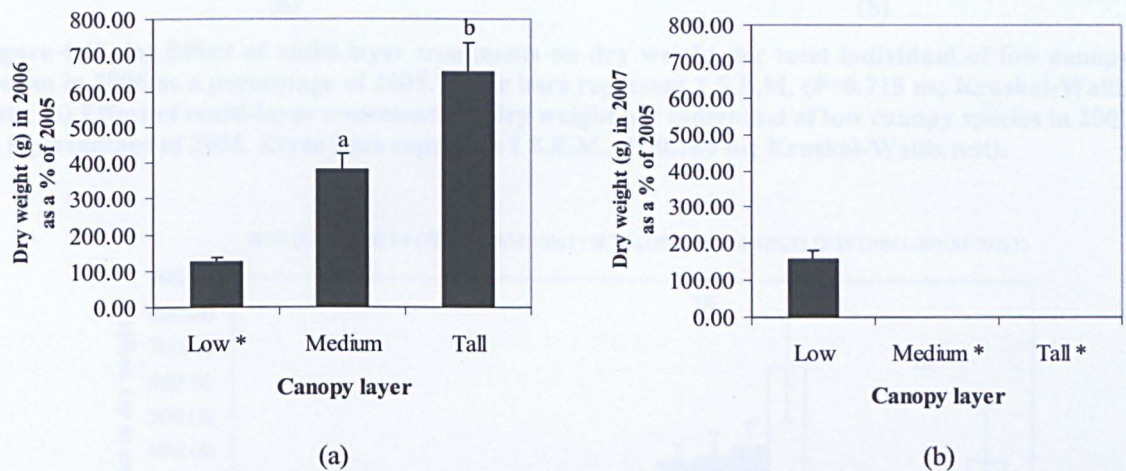


Figure 4.41 (a) Dry weight of different canopy layer plants in 2006 as a percentage of their survival in 2005. \* Dry weight of low canopy layer was excluded from statistical analysis as it does not produce a realistic assessment of canopy mass due of loss foliage in these species by harvest time. Error bars represent 1 S.E.M. Bars labeled with different letters are significantly different at  $P=0.001$  (Kruskal-Wallis test, pairwise Mann-Whitney  $U$ -test). (b) Dry weight of low canopy layer plants in 2007 as a percentage of their survival in 2005. \* Dry weight of medium and tall canopy layers was excluded from statistical analysis as it does not produce a realistic assessment of canopy mass in these species by harvest time.

##### 4.3.5.3.1 Low canopy, understorey forbs

In all cases, no significant difference was found for dry weights between treatments for low canopy species in either 2006 or 2007 (Figures 4.42a and 4.42b). In both these years all treatments that included a low canopy layer exceeded their original dry weight in 2005. In the third year (2007), the highest dry weight was achieved in T5 (Mix, tall canopy dominant) and the lowest in T1 (Understorey). As shown in Figure 4.43, *P. veris* and *P. vulgaris* showing greater growth in T5 (Mix, tall canopy dominant) than the other species and exceeded their original dry weight in 2005 in all treatment. *Phlox divaricata* and *P. reptans* fell below that in 2005 in all treatment mixes.

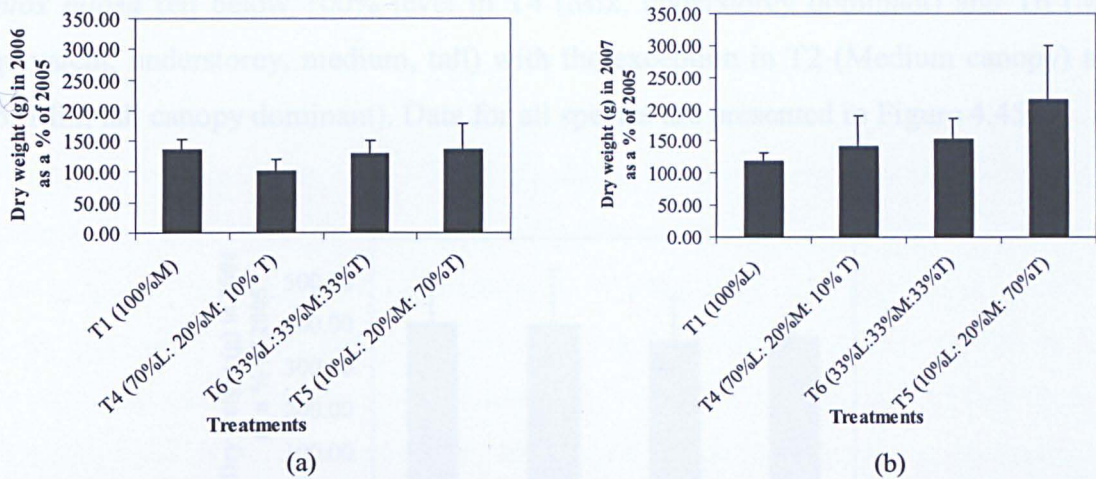


Figure 4.42 (a) Effect of multi-layer treatments on dry weight per total individual of low canopy species in 2006 as a percentage of 2005. Error bars represent 1 S.E.M. ( $P=0.715$  ns; Kruskal-Wallis test). (b) Effect of multi-layer treatments on dry weight per individual of low canopy species in 2007 as a percentage of 2005. Error bars represent 1 S.E.M. ( $P=0.789$  ns; Kruskal-Wallis test).

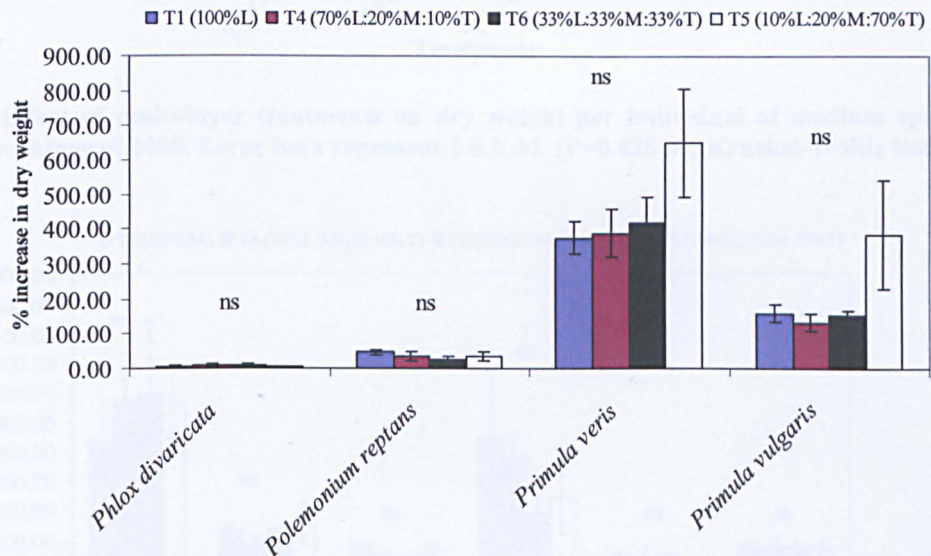


Figure 4.43 Dry weight of individual low canopy species in response to different multi-layer treatments. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between treatments are indicated by ns, not significant.

#### 4.3.5.3.2 Medium canopy forbs

In all cases, no significant difference was found between treatments on the mean dry weight per individual for medium canopy species in 2006 (Figure 4.44). Nor was mean dry weight per individual significantly different across the treatments for individual species. *Aster azureus* showed the highest percentage increase in dry weight, followed by *S. speciosa* in all treatment. In 2006, only four species, *A. azureus*, *P. maculata*, *S. speciosa* and *Z. aptera* exceeded their original dry weight in 2005 for all treatment.



*Phlox pilosa* fell below 100% level in T4 (Mix, understorey dominant) and T6 (Mix equivalent, understorey, medium, tall) with the exception in T2 (Medium canopy) and T5 (Mix, tall canopy dominant). Data for all species are presented in Figure 4.45.

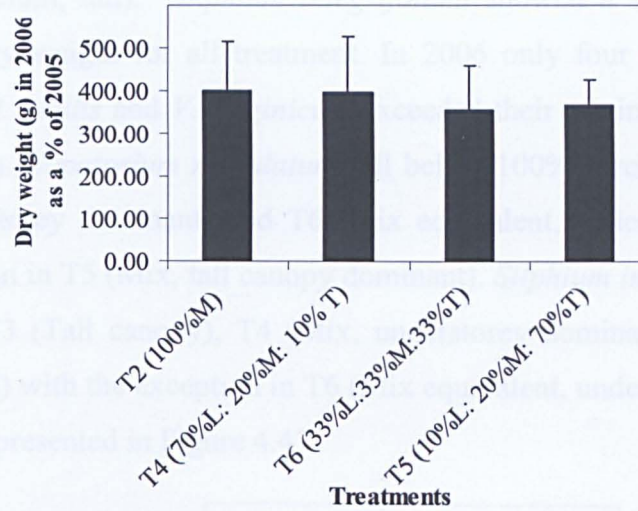


Figure 4.44 Effect of multi-layer treatments on dry weight per individual of medium species in 2006 as a percentage of 2005. Error bars represent 1 S.E.M. ( $P=0.826$  ns; Kruskal-Wallis test).

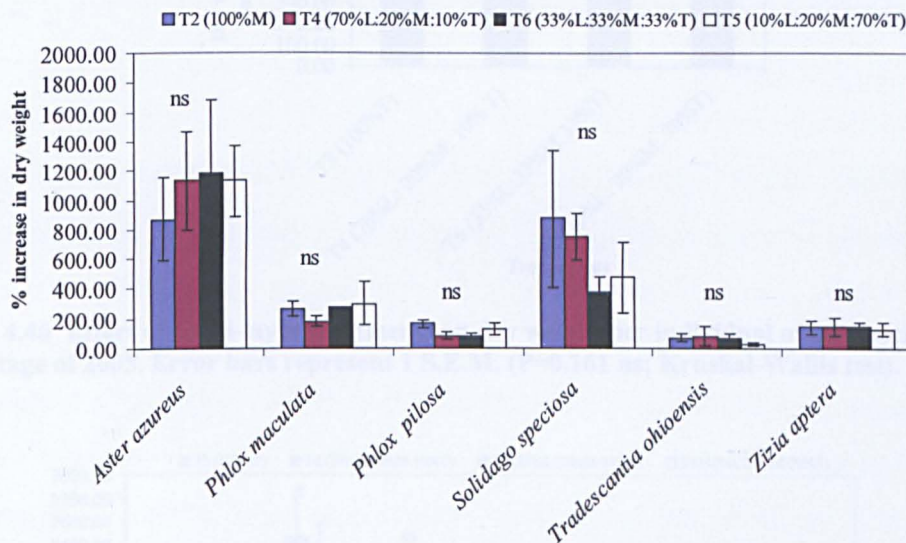


Figure 4.45 Dry weight of individual medium canopy species in response to different multi-layer treatments. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between treatments are indicated by ns, not significant.

#### 4.3.5.3.3 Tall canopy forbs

In all cases, no significant difference was found between treatments for the mean dry weight of individual plants of tall canopy species in 2006 (Figure 4.46). There were

significant differences ( $P < 0.05$ , Kruskal-Wallis test) in dry weight per plant amongst individual species in response to treatments. Most individual species were larger when mixed with other canopy types. The largest individual plants in the experiment were with *A. novae-angliae* in T4 (Mix, understorey dominant) and T6 (Mix equivalent, understorey, medium, tall). *Silphium integrifolium* showed a significant decrease in percentage of dry weight for all treatment. In 2006 only four species, *A. laevis*, *A. novae-angliae*, *H. mollis* and *V. virginicum* exceeded their original dry weight in 2005 for all treatments. *Eupatorium maculatum* fell below 100% level in T3 (Tall canopy), T4 (Mix, understorey dominant) and T6 (Mix equivalent, understorey, medium, tall) with the exception in T5 (Mix, tall canopy dominant). *Silphium integrifolium* fell below 100% level in T3 (Tall canopy), T4 (Mix, understorey dominant) and T5 (Mix, tall canopy dominant) with the exception in T6 (Mix equivalent, understorey, medium, tall). Data for 2006 is presented in Figure 4.47.

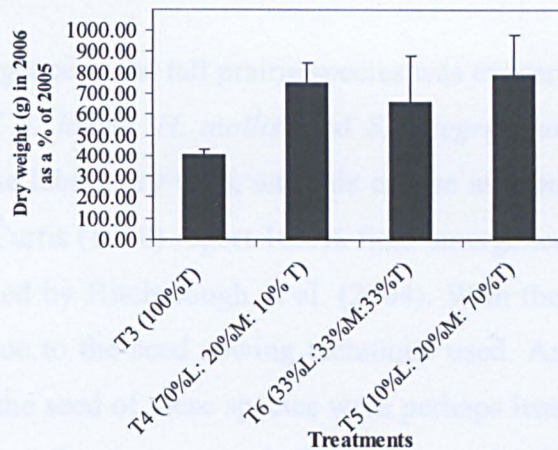


Figure 4.46 Effect of multi-layer treatments on dry weight per individual of tall species in 2006 as a percentage of 2005. Error bars represent 1 S.E.M. ( $P = 0.161$  ns; Kruskal-Wallis test).

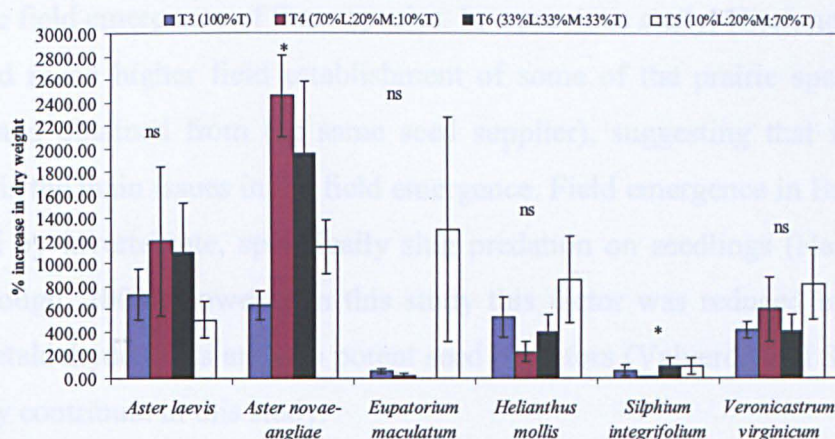


Figure 4.47 Dry weight of individual tall canopy species in response to different multi-layer treatments. Error bars represent 1 S.E.M. Significant differences (Kruskal-Wallis test) between treatments are indicated by: \*  $P = 0.05$ ; ns, not significant.

## 4.4 Discussion



### 4.4.1 Seedling emergence in the microcosm

Emergence in the microcosm was much higher in understorey species than in medium-tall prairie species, suggesting that conditions were more favourable for the latter species, and that higher seed rates would be necessary for the prairie species to achieve the same establishment densities. This study shows how important it is to factor in field emergence data when formulating seed sowing mixes for semi-naturalistic planting to achieve target densities of individual species. Within the two plant/habitat groups there is however very considerable variation in field emergence of the individual species, suggesting that any apparent trends are probably caused by different sampling of the species chosen to represent these groups.

Low mean field emergence in the tall prairie species was exacerbated by the particularly poor performance of *A. laevis*, *H. mollis*, and *S. integrifolium*. The aster performed similarly poorly in the laboratory tests, and this can be ascribed primarily to low seed quality. Greene and Curtis (1950) report 16.0% field emergence in this species, a value similar to that recorded by Hitchmough et al. (2004). With the other two species poor emergence may be due to the seed sowing technique used. As the two largest seeded species in the study, the seed of these species were perhaps least well incorporated post sowing by the “raking in” technique used. Seed of many *Silphium* were evident on the soil surface in spring and most probably died whilst attempting to germinate. A combination of superior quality and more effective soil incorporation post sowing may improve field emergence of these species. In a previous study, Hitchmough et al. (2004) recorded much higher field establishment of some of the prairie species used in this study (and obtained from the same seed supplier), suggesting that inconsistent seed quality is the main issues in the field emergence. Field emergence in Britain is normally reduced by invertebrate, specifically slug predation on seedlings (Hanley et al. 1995; Hitchmough, 2003), however in this study this factor was reduced by regular baiting with metaldehyde. Ants are also potent seed predators (Valverde and Silvertown, 1995) and may contribute in this study.

Whilst two of the three native species (*P. veris* and *P. vulgaris*) had very high emergence, and some of the North American species had poor emergence, seed quality and sowing technique appear to be more potent factors in successful seed emergence than the “nativeness” of the species used in the microcosm.

#### 4.4.2 The multi-layer herbaceous plant communities as a whole

##### 4.4.2.1 Effect of sowing ratio

Individual species performance results suggest that different sowing ratios affected capacity to survive and grow. The success of species depended upon the initial ratio of species present, and the different layers present. Slow growing understorey species such as *D. meadia* and to some degree *P. divaricata* are more adversely affected by other understorey species such as the near evergreen *P. veris* and *P. vulgaris* than by taller species. This is shown by the differences in decline say of *D. meadia* in T1 (Understorey) (approx 97% decline in numbers) (see table 4.7) compared with a 34% decline in T6 (Mix equivalent, understorey, medium, tall) and an increase in T5 (Mix, tall canopy dominant). To overcome this in practice, seeding rates used in shade tolerant species need to be adjusted accordingly. By reducing the rates of *Primula* species in the sowing mix survival and persistence of *D. meadia* could be improved in the longer term.

Tall species, for example *A. novae-angliae* and *H. mollis* were larger when mixed with understorey and medium species than with other tall canopy species (Figure 4.26). The growth of *A. novae-angliae* (in terms of biomass production) increases when the ratio of tall went from 70% to 33% to 10%. This suggests that a high ratio of tall canopy species in mixture adversely affects the growth of individual plants of this species.

The layers and ratios present in community composition also affected the survivorship of individual species across the experiment. This study showed that understorey layer species showed the highest survival and growth in the first year in T5 (Mix, tall canopy dominant) as only 10% of the spaces in this treatment were occupied by understorey plants. Hence there was space and other resources available. In the third year however, *P. veris* and *P. vulgaris* showed greater survival in T5 (Mix, tall canopy dominant) than

the other species in all treatment mixes, suggesting seedling recruitment had occurred in the previously under-utilised spaces under the medium-tall canopy. Both *Primula* show a similar trend in response to the mixes, i.e. a dry weight spike in T5 (Mix, tall canopy dominant) whereas the other species show no such spike which suggests the other understorey species were not able to compete for the initial ground level space in T5 (Mix, tall canopy dominant) as effectively as the two *Primula* species.

Cover values are substantially affected, especially in spring by the presence of understorey forbs layer. This is true for low and high density planting. It shows that adding low canopy layers (understorey species) significantly improved cover values (>60%) in multi-species communities of medium and tall prairie in spring. This is likely to have a significant impact on resistance to weed invasion.

Many species did well and survived well irrespective of layer mix and ratio. One of the reasons for this is that they are all relatively well fitted to cultivation in Northern Britain. However, this experiment was conducted in a microcosm environment without a substantial guard row around each quadrat, the tall species did not have such a detrimental shading effect on the medium and possibly lower species, than will happen in the field experiment described in **Chapter 5**. There were too many edges and the quadrats were too small to generate deep shade, and this is a limitation in the study.

#### 4.4.2.2 Effect of sowing density

The choice of sowing rate is an important factor in creating semi-naturalistic herbaceous plant communities, influencing plant density and the rate of seedling establishment. Based on experience in agricultural crops, plant density affects canopy development, radiation interception, biomass production, weed competition and the development of pests and diseases (Lopez-Bellido et al., 2005). However, it has been reported that a high plant density reduced competition from weeds in the plant community (Stevenson et al., 1995). According to Hitchmough (2006), prairie plant densities approximating to 50 plants/m<sup>2</sup> allow herbaceous plant communities and compete effectively with many invading weeds. In landscape practice, plant establishment by high density sowing may potentially lead to the elimination of slower growing species by faster growing species (Hitchmough and de La Fleur, 2006). Hence the ratio of fast to slow growing species is important when creating sowing mixes.

Plant density in multi-layer herbaceous plant communities created by direct sowing in this experiment was much higher than in conventional plantings. Analysis in each canopy layer showed there to be no significant difference between low (900 seedling/m<sup>2</sup>) and high (1500 seedling/m<sup>2</sup>) sowing densities in terms of plant survival and growth.

In the second and third growth season, some of the species in this study reduced their density through process of self-thinning. It is normal for the number of plants to decline especially at high densities (Lopez-Bellido et al., 2005; Hitchmough and de La Fleur, 2006). In general, plant communities self-thin to an optimum plant density which depends upon the size of individual plants in the community. Although process of self-thinning appears to have occurred, two species, *P. veris* and *P. vulgaris* in T5 were more numerous suggesting that self-sown had occurred. This suggests that any thinning that occurred was at the expense of other understorey species.

This study has also demonstrated that the medium canopy species used in the study seem well fitted to the UK climate. They seem remarkably stable irrespective of the community multi-layer composition. Sowing density did not have an obvious effect of survival of medium species across the study.

#### **4.4.3 Growth and survival of species in relation to 2005**

The present study showed that standing biomass of the three canopy layers used was tall>medium>low (Figure 4.41). To aid interpretation, the summed total biomass for each plant layer in multi-layer communities (low + medium + tall) and mono-layer have been estimated (Table 4.10).

**Table 4.10** Total biomass (g) for each plant layer groups per quadrat, based on data shown in Figures 4.10, 4.11,4.12, 4.20,4.21,4.22 and 4.31. Dry weight at the peak of standing biomass is indicated by bold type.

Harvest time		Treatment mixes					
September 2005		T1	T2	T3	T4	T5	T6
Layer group		(100%L)	(100%M)	(100%T)	(70%L:20%M:10%T)	(10%L:20%M:70%T)	(33%L:33%M:33%T)
	Low	29.47	-	-	19.45	8.72	14.76
	Medium	-	26.95	-	6.53	9.18	9.73
	Tall	-	-	40.63	4.29	12.63	4.45
<i>Total</i>	(Low+Medium+Tall)	29.47	26.95	40.63	30.27	30.53	28.94
<hr/>							
September 2006							
	Low	39.75	-	-	18.49	12.17	18.39
	Medium	-	<b>119.83<sup>a</sup></b>	-	<b>32.05<sup>b</sup></b>	<b>37.10<sup>b</sup></b>	<b>44.18<sup>b</sup></b>
	Tall	-	-	<b>228.81<sup>a</sup></b>	<b>77.54<sup>b</sup></b>	<b>125.07<sup>ab</sup></b>	<b>65.74<sup>b</sup></b>
<i>Total</i>	(Low+Medium+Tall)	39.75	119.83	228.81	128.08	174.34	128.31
<hr/>							
May 2007							
	Low	<b>43.62<sup>a</sup></b>	-	-	<b>27.58<sup>ab</sup></b>	<b>15.45<sup>c</sup></b>	<b>24.75<sup>bc</sup></b>
	Medium	-	-	-	-	-	-
	Tall	-	-	-	-	-	-
<i>Total</i>	(Low+Medium+Tall)	43.62	-	-	-	-	-
<hr/>							
<b>Total mono-layer*</b>		<b>43.62</b>	<b>119.83</b>	<b>228.81</b>			
<b>Total multi-layer **</b> (Low+Medium+Tall)					<b>137.17</b>	<b>117.62</b>	<b>134.67</b>

**Key:** \* Total dry weight at the peak of standing biomass. \*\*Total dry weight base on adding up the biomass of the component (as indicated by bold type) at split harvest. Within rows, values followed by the same letter are not significantly different at P=0.01 (Kruskal-Wallis test).

When additional layers were added to 100% understorey layer (T1) total biomass was substantially increased. The same was true, although less markedly for medium species. The opposite was true for tall species, the total biomass of these declines when other layers were added. These results partially support the ecological theory (Tilman, 1999; Grime, 2001) that predicts the biomass of complex multi-layers is greater than a simple mono-layer (Whale, 1984). The biomass of a single layer of tall prairie species was almost double the next highest biomass of multi-layer communities. This suggests that the dense packing of tall upright stems is a more efficient means of supporting high biomass than more complex spatial arrangements. Similar parallels can be drawn with the extremely high biomasses associated with structurally simple plant communities such as reed-swamp.

Although the addition of layers may reduce the total biomass per unit area of tall canopy species, this does not necessarily mean the capacity of these to restrict invasion from the outside, will be reduced. The presence of a layer of understorey foliage in spring may, for example, outweigh the negative of reduced total biomass.

*Phlox divaricata*, *P. pilosa*, *P. reptans*, *E. maculatum* and *S. integrifolium* declined (as evidenced by dry weight and survivorship) between 2005 and 2007. In the context of the conditions in the microcosm these species appear to be decreaser species. This suggests that the capacity of these species to establish depends on favourable combinations factors such as availability of water, nutrients and lights. From the viewpoint of capacity to be successful as a multi-layer community in urban spaces, low-medium-tall canopy species vary from being decreasers to increasers.

Species that behaved as “increasers” in this study included: the understorey forbs; *P. veris* and *P. vulgaris*, medium forbs; *A. azureus* and *S. speciosa* and tall canopy forbs; *A. laevis*, *A. novae-angliae* and *V. virginicum*. All were on average larger in 2007 than they were in 2005 in each treatment mixes, but these differences were only statistically significant ( $P=0.05$ ) for *A. novae-angliae*. This suggests that all of these species are well suited to the UK climates. One explanation for this is that survival of understorey forbs (*P. veris* and *P. vulgaris*) was significantly improved by the shading of medium-tall shade intolerant forbs, as shown in treatments T4 (70%L: 20%M: 10%T) and T5 (10%L: 20%M: 70%T). Both *P. veris* and *P. vulgaris* are shade tolerant plants, and



often grow slowly at very low light levels in summer (Whale, 1984; Valverde and Silvertown, 1995) beneath canopy trees.

In this experiment, 'decreaser' species were represented by forbs with declining dry weight and low survival under the environment of the experiment, for example, the understorey forbs, *P. divaricata* and *P. reptans* were poorly fitted below environment in medium-tall canopy prairie forbs. In the USA these species are understorey plants in both dry and wet prairie habitats (Curtis, 1959). In the experimental microcosm *Phlox* maybe sensitive to intense competition for water and nutrients, but given it occurs in woodlands shading is probably not a major factor.

#### 4.4.4 Aesthetic aspects of the study

This study has shown that in terms of aesthetics in, a combination of understorey and prairie forbs in multi-layer communities creates a long season of visual interest. Flowers occurred from March to October. In the early year, flowering with a significant impact was produced by low canopy layer forbs such as *P. reptans*, *P. veris* and *P. vulgaris* from May to June, followed by prairie species (medium-tall canopy layer) such as *Z. aptera* (mid-May to June), *Phlox* spp. (June to July), *T. ohioensis* (June to July) and *Aster* spp (late July to October). In landscape practice, these changing flowers potentially create a beautiful and varied aspect in urban parks and green spaces. In addition, the understorey species such as *P. veris* and *P. vulgaris* maintain some leaves throughout the year creating an 'evergreen' effect during winter.

#### 4.5 Conclusion

This (microcosm) study has shown that the concept of mixing of shade tolerant understorey forbs and shade intolerant medium-tall forbs into a multi-layer herbaceous plant community is possible. Some of the species used were problematic however; *P. elatior* failed to germinate and *D. meadia* and *P. divaricata* were largely eliminated due to inter-species competition by the third year growing.

The hypothesis that tall canopy forbs demonstrated a greater survivorship than medium canopy forbs; medium canopy forbs demonstrated a greater survivorship than low canopy species is not supported in all species (see Figure 4.34). Typically standing biomass production is proportional to canopy height; tall canopy forbs demonstrated a higher biomass production than medium canopy forbs and medium canopy forbs demonstrated a higher biomass production than low canopy forbs (see Figure 4.41).

The main findings of this research are as follows:

- The multi-layer communities created generally performed satisfactorily and the experiments suggest this is a viable vegetation type.
- The species *P. veris* and *P. vulgaris* showed the greatest growth of all understorey species in all communities. They achieved maximum size under medium-tall prairie vegetation (T5).
- The medium canopy species chosen seem remarkably stable irrespective of the community under multi-layer composition.
- The tall canopy layer showed the highest total biomass of the 3 canopy layers.
- This study shows that high cover values in spring multi-layer plant communities were associated with the presence of a low canopy layer.
- This study has also shown that very few weeds established in the communities during the course of the experiment.

## CHAPTER 5: FIELD EXPERIMENT INTO MULTI-LAYER COMMUNITY DEVELOPMENT

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### 5.1 Introduction

Chapter 4 explored the establishment, survival and growth of multi-layer plant communities in response to different sowing ratios and density. These microcosm experiments showed that it was possible to create multi-layer communities, at least under the relatively controlled conditions of a microcosm. This chapter deals with an experiment that sought to test whether multi-layer communities can be created in the field by sowing *in situ*, under “near to practice” conditions. Some of the key research questions underpinning this experiment were as follows:

- What effect does type of substrate have on the competitive relationships between different layer “guilds” and individual species?
- What effect does predation, particularly from slugs and snails have upon layer structure and species persistence?
- Is it possible to create sophisticated multi-layer herbaceous vegetation by seed sowing at the scale associated with landscape practice?

Seeding rates/density (Martin et al., 2004; Fischbach et al., 2006), competition (Hitchmough and de La Fleur, 2006) and environmental factors such as soil type (Hitchmough and de La Fleur, 2006), light and nutrients (Elemans, 2004) and mollusc predation (Inouye et al., 1980; Clarke and Davison, 2004) affect the development and structuring of a plant community. These factors are likely to be particularly important when creating a multiple layer plant community the constituent species of which originate from highly contrasting ecological habitats, in this case woodland understorey and tallgrass prairie.

Recent research has revealed that seeding rates affect species diversity and biomass production (Fischbach et al., 2006). Increasing seeding rates typically increases productivity, at least in the short term (Stevenson et al., 1995; Fischbach et al., 2006), but too high a seedling density may decrease species diversity (Launchbaugh and Owensby, 1970; Stevenson et al., 1995). In landscape research, sowing densities used in

the field often vary from 60-800 seeds/m<sup>2</sup> per species in sowing mixtures (Hitchmough et al., 2004; Martin et al., 2004). By increasing plant density it is possible to suppress weedy species through competition, particularly at the initial stage of community establishment (Stevenson et al., 1995). To achieve this, seed mixes must be broadcast evenly on the surface sown to ensure that weeds do not escape competition with the sown species. Weiner et al. (2001) provide evidence in annual agricultural crops (*Triticum aestivum*) sown at high density weed invasion was significantly reduced.

In the field, at high density planting, individual seedlings in sown communities compete for resources thus causing the 'self thinning' process (Ellison, 1989; Weller, 1991) that reduces the number of plants to more sustainable densities. It has been reported by Hitchmough (2006) that self thinning was more intense at high density planting for mesic sown prairie. In this particular research a sustainable post thinning density approximated to 50 plants/m<sup>2</sup>. Generally, as sown communities mature and seedlings become adult their above ground mass will potentially reduce weed colonization. Multi-layered structures can potentially assist this process by reducing dramatic loss of foliage cover during the winter and spring months.

Competition in multi-layer plant communities may be affected by site productivity. On a highly productive site the most intense plant competition is experienced (Buckland and Grime, 2000). Schwinning and Weiner (1998) report that elimination of a species by its neighbour arises where competition is asymmetrical, i.e. there are differences in the size and vigour of the competing individuals. Vigorous seedlings may eliminate small, slow growing seedlings, hence frustrating attempts to establish the desired species and preferred diversity.

Conversely, on a low fertility, unproductive substrate, the intensity of plant competition decreases and the species richness and the number of individual plants increases (Buckland and Grime, 2000; Hitchmough and de La Fleur, 2006). Interestingly, several research papers (Mahmoud and Grime, 1976; Hitchmough et al., 2003) reported that biomass production of the less competitive species increased relative to the most competitive species. Thus less competitive, slow growing species are less likely to be eliminated from multi-layer communities under unproductive conditions increasing

structural complexity and plant diversity. This pattern is more likely to persist in the long term.

In the microcosm studies discussed in **Chapter 4**, mollusc predation was reduced by the nature and location of the experiment. In landscape practice in Britain and elsewhere in Western Europe, slug and snail predation is a major factor in shaping plant communities (Hanley et al., 1996). Slug grazing reduces photosynthetic success and prevent individual seedlings from competing with unpalatable species, hence resulting in the disappearance of certain species from a community vegetation type (Scheidel and Bruelheide, 1999). In long-term community development, Hitchmough and de La Fleur (2006) found that some prairie forbs (*Monarda fistulosa* and *Ratibida pinnata* for example) had the lowest persistence due to being highly palatable to slug grazing.

Mulching plant communities with granular mineral materials such as sand has a dramatic impact on reducing slug predation (Hitchmough and de La Fleur, 2006). These mulches have also proved useful as a means to reduce emergence of weed seedlings from the underlying soil when creating vegetation from field sowing (Dunnett and Hitchmough, 2001; Hitchmough et al., 2004). Mulching comprises a 50 mm deep layer of coarse sand (weed seed free substrate) that is spread across the area to be sown over which the seed mix is then evenly broadcast (Hitchmough et al., 2004). The sand mulch is maintained during the April germination period (Hitchmough and de La Fleur, 2006).

The effects of mulch and soil type (productivity), sowing densities, seeding ratios and mollusc predation effects on the development of multi-layer plant communities in a large scale experiment are described in this chapter. This involved investigating two groups of forbs; i) European and North American woodland understorey , and ii) North American medium to tall prairie used to create multi-layer vegetation.

### 5.1.1 Species selection

Based on the review in **Chapter 2**, and studies conducted in **Chapter 3** and **4** plus observation in the field, more than 30 species of herbaceous plants with low, medium and tall canopy characteristics were considered for use in this third stage of the research. The 26 native and exotic forb species finally selected are shown in Table 5.1. Species

were selected on the basis of criteria which were described in section 3.2 in **Chapter 3**. Seed was obtained in November and December 2005 from Jelitto Seeds, Schwarmstedt, Germany for European and some prairie species and Prairie Moon Nursery, Winona, MN, USA, for most prairie species. Moreover, seeds which were not available commercially were collected locally from experimental seed plots in Sheffield in September 2006. This included *Phlox amplifolia*, *Phlox glaberrima* and *Veronicastrum virginicum*. Seed was dry stored at approximately 4°C in the fridge prior to sowing. Detailed characteristics of the species selected in this experiment are shown in Appendix Table A4.1.

**Table 5.1** Understorey and prairie forbs, and prairie grass species used in the study.

Species	Typical habitat	Plant canopy / layers*
<i>Dodecatheon meadia</i>	understorey forb	low
<i>Lathyrus vernus</i>	understorey forb	low
<i>Phlox divaricata</i>	understorey forb	low
<i>Phlox pilosa</i>	understorey forb/prairie forb	low-medium
<i>Polemonium reptans</i>	understorey forb	low
<i>Primula elatior</i>	understorey forb	low
<i>Primula vulgaris</i>	understorey forb	low
<i>Zizia aptera</i>	understorey forb	low-medium
<i>Aster azureus</i>	prairie forb	medium
<i>Echinacea purpurea</i>	prairie forb	medium
<i>Gillenia trifoliata</i>	prairie forb	medium
<i>Phlox glaberrima</i>	prairie forb	medium
<i>Penstemon digitalis</i>	prairie forb	medium
<i>Phlox maculata</i>	understorey forb/prairie forb	medium
<i>Rudbeckia speciosa</i>	prairie forb	medium
<i>Silene regia</i>	prairie forb	medium
<i>Solidago speciosa</i>	prairie forb	medium
<i>Andropogon gerardii</i>	prairie grass (C4)	tall
<i>Aster novae-angliae</i> 'Septemberrubin'	prairie forb	tall
<i>Eupatorium maculatum</i>	prairie forb	tall
<i>Helianthus mollis</i>	prairie forb	tall
<i>Helenium autumnale</i>	prairie forb	tall
<i>Phlox amplifolia</i>	prairie forb	tall
<i>Rudbeckia subtomentosa</i>	prairie forb	tall
<i>Silphium integrifolium</i>	prairie forb	tall
<i>Veronicastrum virginicum</i>	understorey forb/prairie forb	tall

\*Under typical garden condition; low = < 450 mm, medium = 450-900 mm and tall = >900 mm in height

### 5.1.2 Objectives

The specific objectives of this study were:

- 1) To assess the effect of sowing mulch (sand v deep subsoil) on seedling emergence, survival and growth in year 1 and 2.
- 2) To assess the effect of sowing mix on cover values in year 1 and 2.
- 3) To assess the effect of underlying productive topsoil v unproductive subsoil on the cover values and survival of the communities in year 1 and 2.

## 5.2 Materials and methods

The experiment was conducted in Lower Walkely, a suburb of Sheffield (53°N24', 1°W30'), United Kingdom, on soil previously planted by various species of prairie vegetation. Climatic data for the site is described in Hitchmough et. al. (2004). During site preparation, in September 2005, weed and other prairie vegetation were sprayed with a glyphosate herbicide and removed manually once to achieve plant free conditions at sowing. The top soil in the experimental site can be classified as a well-drained clay loam, sited on a coarse clay subsoil. Physical and chemical analyses for the soils used in the study are given in Table 5.2.

**Table 5.2 Physical and chemical properties of the soil types/mulch used in the experiment (source from Hitchmough et. al. (2004)).**

	pH	Plant available N (NO <sub>3</sub> - + NH <sub>4</sub> <sup>+</sup> ) (ppm)	Plant Available P (ppm)	Plant Available K (ppm)	Percentage particles (<0.05 mm)	Percentage particles (0.05-1.0 mm)	Percentage particles (>1/0 mm)
Sand	6.8	<0.2	<0.2	9	2	26	72
Subsoil	7	39.6	11.3	141.4	60	29	11
Topsoil	6.2	111.6	65.7	499	51	37	12

A full factorial, balanced, randomised split-plot experiment involving 4 replicates of each treatment was set-up in December 2005. The experiment involved a total of 16, 3 x 2 m treatment blocks (main plot), arranged randomly in 3 rows and 6 columns as illustrated in Figure 5.1.

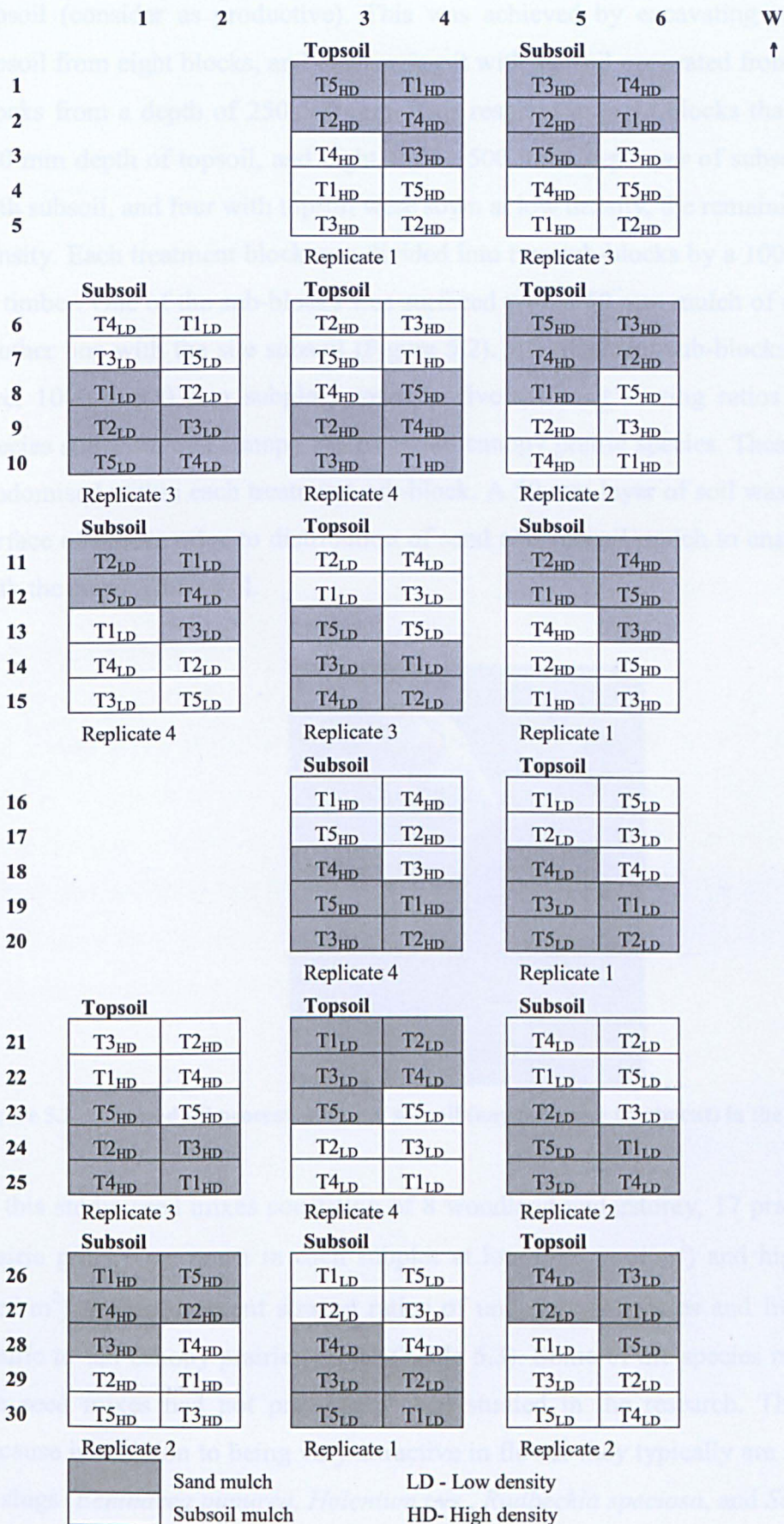


Figure 5.1 Arrangement of the treatment plot in the productivity experiment.



Eight treatment blocks were subsoil (unproductive) whereas the remaining eight were topsoil (consider as productive). This was achieved by excavating a 250 mm deep topsoil from eight blocks, and exchanging it with subsoil excavated from a further eight blocks from a depth of 250-500 mm. This resulted in eight blocks that consisted of a 500 mm depth of topsoil, and eight with a 500 mm deep layer of subsoil. Four blocks with subsoil, and four with topsoil were sown at low density, the remaining eight at high density. Each treatment block was divided into two sub-blocks by a 100 x 25 mm piece of timber. One of the sub-blocks was surfaced with a 50 mm mulch of coarse sand and another one with the site subsoil (Figure 5.2). All treatment sub-blocks were split into five; 1000 x 600 mm subplots sown in five different sowing ratios of understory species and from mid canopy prairie to tall canopy prairie species. These subplots were randomised within each treatment sub-block. A 50 mm layer of soil was scraped off the surface of blocks prior to distribution of sand and subsoil mulch to ensure it was flush with the surrounding soil.



**Figure 5.2** Cut and fill process to obtain subsoil (unproductive treatment) in the study.

In this study, seed mixes consisting of 8 woodland understory, 17 prairie forbs and 1 prairie grass were sown in each subplot at low ( $100 \text{ seed/m}^2$ ) and high density ( $200 \text{ seed/m}^2$ ) in five different sowing ratios of understory species and from mid canopy prairie to tall canopy prairie species (Table 5.3). Some of the species incorporated into the seed mixes had not previously been studied in the research. They were added because in addition to being very attractive in flower they typically are highly palatable to slugs (*Echinacea pupurea*, *Helenium cvs.*, *Rudbeckia speciosa*, and *Silene regia*), and

hence were used to provide an indication of how the treatments might influence the intensity of predation experienced.

**Table 5.3** Different sowing ratios for each plant functional group tested in this study.

Sowing Mixes (Treatment)	Herbaceous species with different canopy height (ratio L:M:T)		
	Low species (L)	Medium species (M)	Tall species (T)
T1	1	1	3
T2	3	1	1
T3	1	1	1
T4	1	3	3
T5	3	3	1

The estimated number of seed sown was mark-up in certain species to ensure that enough numbers of seedlings per species be established in each subplot achieved (Table 5.4). When making up the various sowing mixes for the experiment, seed was counted rather than weighed.

Seed mixes for each treatment were mixed with fine sand to aid distribution and carefully broadcast as two passes at right angles to one another. Plywood frames was used to prevent seed sown from being distributed in other subplots (Figure 5.3a). Each subplot was raked to incorporate seed and lightly firmed. Sowing was completed on 12th January 2006 to allow between 90-120 days of natural chilling. An overall view of the experimental site is shown in Figure 5.3b.



**Figure 5.3** Experimental site at the Lower Walkley, Sheffield, United Kingdom; (a) Plywood frames was used to prevent 'seed blow' during sowing, (b) Overall views of treatment plots.

Table 5.4 Estimation of seed numbers for each species with different combination treatments and densities per subplot (0.6m<sup>2</sup>).

Species	Seed weight		Approximate no of seed sown/ 0.6m <sup>2</sup>									
	Mean	SE	T1 (1L: 1M: 3T)		T2 (3L: 1M: 1T)		T3 (1L: 1M: 1T)		T4 (1L: 3M: 3T)		T5 (3L: 3M: 1T)	
			LD	HD	LD	HD	LD	HD	LD	HD	LD	HD
<b>Low canopy (L)</b>												
<i>Dodecatheon meadia</i>	0.236 *	0.37	5 (1.5)	10 (3)	15 (4.5)	30 (9)	8 (2.5)	17 (5)	4 (1.1)	7 (2.2)	11 (3.2)	22 (6.4)
<i>Lathyrus vernus</i>	17.522 *	9.07	8 (1.5)	15 (3)	23 (4.5)	45 (9)	13 (2.5)	25 (5)	5 (1.1)	11 (2.2)	16 (3.2)	32 (6.4)
<i>Phlox divaricata</i>	2.380 *	1.62	5 (1.5)	10 (3)	15 (4.5)	30 (9)	8 (2.5)	17 (5)	4 (1.1)	7 (2.2)	11 (3.2)	22 (6.4)
<i>Phlox pilosa</i>	1.388 *	3.25	8 (1.5)	15 (3)	23 (4.5)	45 (9)	13 (2.5)	25 (5)	5 (1.1)	11 (2.2)	16 (3.2)	32 (6.4)
<i>Polemonium reptans</i>	1.269 *	1.51	5 (1.5)	10 (3)	15 (4.5)	30 (9)	8 (2.5)	17 (5)	4 (1.1)	7 (2.2)	11 (3.2)	22 (6.4)
<i>Primula elatior</i>	0.903 *	1.23	4 (1.5)	8 (3)	11 (4.5)	23 (9)	6 (2.5)	12 (5)	3 (1.1)	5 (2.2)	8 (3.2)	16 (6.4)
<i>Primula vulgaris</i>	0.936 *	0.69	12 (1.5)	24 (3)	11 (4.5)	23 (9)	12 (2.5)	24 (5)	9 (1.1)	15 (2.2)	8 (3.2)	16 (6.4)
<i>Zizia aptera</i>	1.538 *	3.10	5 (1.5)	10 (3)	15 (4.5)	30 (9)	8 (2.5)	17 (5)	4 (1.1)	7 (2.2)	11 (3.2)	22 (6.4)
<b>Medium canopy (M)</b>												
<i>Aster azureus</i>	0.318 *	0.43	8 (1.5)	15 (3)	8 (1.5)	15 (3)	13 (2.5)	25 (5)	16 (3.2)	32 (6.4)	16 (3.2)	32 (6.4)
<i>Echinacea purpurea</i>	4.348 *	-	8 (1.5)	15 (3)	8 (1.5)	15 (3)	13 (2.5)	25 (5)	16 (3.2)	32 (6.4)	16 (3.2)	32 (6.4)
<i>Gillenia trifoliata</i>	2.500 *	-	8 (0.8)	15 (1.5)	8 (0.8)	15 (1.5)	13 (1.3)	25 (2.5)	16 (1.6)	32 (3.2)	16 (1.6)	32 (3.2)
<i>Phlox glaberrima</i>	4.000 *	-	4 (0.8)	8 (1.5)	4 (0.8)	8 (1.5)	6 (1.3)	13 (2.5)	8 (1.6)	16 (3.2)	8 (1.6)	16 (3.2)
<i>Penstemon digitalis</i>	0.278 *	-	15 (1.5)	30 (3)	15 (1.5)	30 (3)	25 (2.5)	50 (5)	32 (3.2)	65 (6.4)	32 (3.2)	65 (6.4)
<i>Phlox maculata</i>	2.478 *	1.36	15 (1.5)	30 (3)	15 (1.5)	30 (3)	25 (2.5)	50 (5)	32 (3.2)	65 (6.4)	32 (3.2)	65 (6.4)
<i>Rudbeckia speciosa</i>	1.042 *	-	15 (1.5)	30 (3)	15 (1.5)	30 (3)	25 (2.5)	50 (5)	32 (3.2)	65 (6.4)	32 (3.2)	65 (6.4)
<i>Silene regia</i>	1.220 *	-	15 (1.5)	30 (3)	15 (1.5)	30 (3)	25 (2.5)	50 (5)	32 (3.2)	65 (6.4)	32 (3.2)	65 (6.4)
<i>Solidago speciosa</i>	0.244 *	0.50	24 (1.5)	45 (3)	24 (1.5)	45 (3)	26 (2.5)	50 (5)	16 (3.2)	32 (6.4)	16 (3.2)	32 (6.4)
<b>Tall canopy (T)</b>												
<i>Andropogon gerardii</i>	2.857 *	-	45 (4.5)	90 (9)	15 (1.5)	30 (3)	25 (2.5)	50 (5)	32 (3.2)	65 (6.4)	11 (1.1)	22 (2.2)
<i>Aster novae-angliae</i> 'Septemberrubin'	0.434 *	0.21	11 (4.5)	23 (9)	12 (1.5)	24 (3)	12 (2.5)	24 (5)	8 (3.2)	16 (6.4)	9 (1.1)	15 (2.2)
<i>Eupatorium maculatum</i>	0.314 *	0.62	24 (3.7)	49 (7.4)	8 (1.2)	16 (2.5)	14 (2)	27 (4.1)	18 (2.6)	36 (5.2)	6 (0.9)	12 (1.8)
<i>Helianthus mollis</i>	3.094 *	3.40	23 (4.5)	45 (9)	8 (1.5)	15 (3)	13 (2.5)	25 (5)	16 (3.2)	32 (6.4)	5 (1.1)	11 (2.2)
<i>Helenium autumnale</i>	0.250 *	-	45 (4.5)	90 (9)	15 (1.5)	30 (3)	25 (2.5)	50 (5)	32 (3.2)	65 (6.4)	11 (1.1)	22 (2.2)
<i>Phlox amplifolia</i>	10.000 *	-	8 (0.8)	16 (1.6)	3 (0.3)	5 (0.5)	5 (0.5)	9 (0.9)	6 (0.6)	11 (1.2)	2 (0.2)	4 (0.4)
<i>Rudbeckia subtomentosa</i>	0.625 *	-	90 (4.5)	180 (9)	30 (1.5)	60 (3)	50 (2.5)	100 (5)	65 (3.2)	130 (6.4)	22 (1.1)	43 (2.2)
<i>Silphium integrifolium</i>	14.646 *	57.04	23 (4.5)	45 (9)	8 (1.5)	15 (3)	13 (2.5)	25 (5)	16 (3.2)	32 (6.4)	5 (1.1)	11 (2.2)
<i>Veronicastrum virginicum</i>	0.031 *	0.12	45 (4.5)	90 (9)	15 (1.5)	30 (3)	25 (2.5)	50 (5)	32 (3.2)	65 (6.4)	11 (1.1)	22 (2.2)
<b>TOTAL</b>			478 (60)	948 (120)	354 (60)	699 (120)	429 (60)	852 (120)	463 (60)	926 (120)	374 (60)	750 (120)

\* Mean of three replicates. \* Estimated values from the seed suppliers. ( ); Values in the bracket is the actual target plant number of each species in this study.

The actual number of seeds sown to achieve the target densities was based on seedling emergence in the previous microcosm experiments, plus other ongoing research within the Departmental research group examining related topics.

Seedling emergence occurred from mid March to May 2006. To reduce the impact of slug predation on seedling emergence, plots were baited post-sowing with metaldehyde containing pellets at approximately 40 pellets/m<sup>2</sup>. Slug pellets were re-applied at approximately 2 weekly intervals until the end of June 2006. From 13 to 15 June 2006, emerged seedlings within a permanent quadrat (800 x 400 mm), which was set 200 mm from the outside edges within each treatment sub-plot and marked by wires, were identified and counted using one. To achieve the target seed ratios as shown in table 5.2, the numbers of each species were corrected by removal (thinning) of existing seedlings, or addition of new seedlings, depending on the emerged numbers of each species per subplot. As germinated seedlings were too low on subsoil, the transplanting of new seedlings was undertaken, based upon 'random distribution' with at least one plant per species per quadrat present in each treatment subplot. This approach was designed to achieve a uniform spatial distribution of species as specified in Table 5.4. Transplanting and thinning of new seedlings was completed on 30th July 2006, with approximately 56 seedlings/quadrat ( $\approx 170$  seedlings/m<sup>2</sup>) for low density and 84 seedlings/quadrat ( $\approx 260$  seedlings/m<sup>2</sup>) for high density.

### 5.2.1 Data collection

One permanent quadrat (800 x 400 mm) within each subplot was the sampling frame. Percentage emergence data is derived from the number of seedling emergents counted in mid-May 2006. Canopy cover was estimated in September 2006 at the peak of standing biomass. The values were estimated visually using a Sykes (1983) method, for the previously described permanent quadrat. In the second growing season, the numbers of plants in each quadrat were counted in April 2007. The cover values were also estimated at weekly intervals from April to May 2007. Thirty two slug shelters (130 mm plastic plantpot saucers); 16 dishes on sand mulch + 16 dishes on subsoil mulch) were placed on the soil surface as an under canopy layer to estimate slug numbers in June 2007. Digital images of the subplots were taken throughout the experiment.

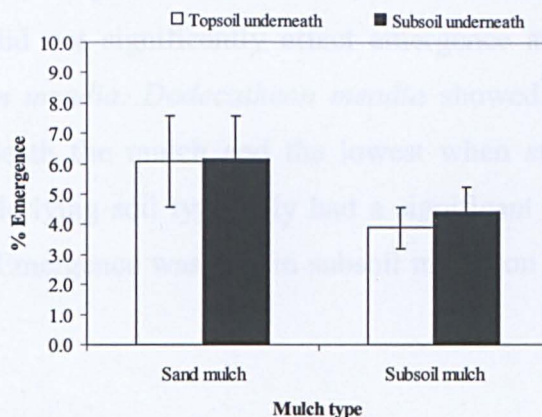
## 5.2.2 Statistical analysis

As Kolmogorov-Smirnov tests indicated that count data was non-normal and could not be adequately improved by transformation, analysis was undertaken using a non-parametric test (Dytham, 2003). Statistical analysis was undertaken using *SPSS version 12* for Windows. The Mann-Whitney *U*-test was used in lieu of *t*-tests for paired comparisons. This test was used to compare the significant difference between low and high density sowing. The Kruskal-Wallis test was used for one way ANOVA. Where a Kruskal-Wallis test gave significant results ( $P < 0.05$ ), a Mann-Whitney *U*-test was undertaken to allow comparison and ranking of means. The Scheirer-Ray-Hare test was used for the two-way ANOVA analysis. Mean in figures and tables that are significantly different ( $P < 0.05$ ) are indicated by the use of suffix subscript letters.

## 5.3 Results

### 5.3.1 Effect of mulch and underlying soil type on seedling emergence

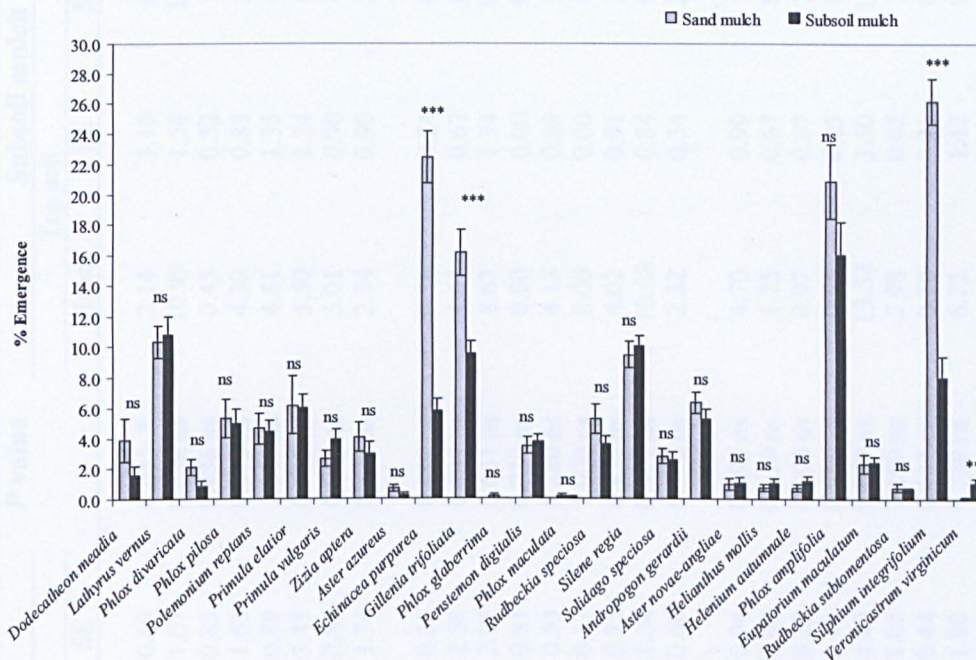
Percentage emergence in sowing mix across all species (Figure 5.4) was greater on sand mulch (6.10%) than on subsoil mulch (4.14%), but not significantly different. This pattern was consistent for both treatments of topsoil and subsoil underneath. A Scheirer-Ray-Hare test found that soil type underneath the mulch did not have a significant effect on emergence ( $P = 0.658$ ) nor was interaction significant ( $P = 0.930$ ). Overall, emergence values were lower than had been experienced in previous sowings with these species in the past.



**Figure 5.4** Summary of seedling emergence (mean of all species) in response to mulch type and soil underneath. Error bars represent 1 S.E.M.

### 5.3.2 Effect of mulch and underlying soil type on seedling emergence of individual species

The effect of mulch type on the emergence of individual species in the sowing mix was statistically analysed using the Mann-Whitney  $U$ -test (Figure 5.5). Species that showed significantly greater emergence ( $P < 0.01$ ) in sand than in subsoil mulch were *E. purpurea*, *Gillenia trifoliata* and *Silphium integrifolium*. Other species did not show any significant difference in emergence in sand and subsoil mulch.



**Figure 5.5** Percentage emergence of individual sowing mix species in response to mulching type. Error bars represent 1 S.E.M. Significant differences (Mann-Whitney  $U$ -test) between sand and subsoil mulch are indicated by; \* $P=0.05$ ; \*\* $P=0.01$ ; \*\*\* $P=0.001$ ; ns, not significant.

A comparison between emergence in sand and subsoil mulch on the two different soil types was also analysed using the Mann-Whitney  $U$ -test (Table 5.5). On sand mulch, underlying soil type did not significantly effect emergence at  $P=0.05$  in all species except in *Dodecatheon meadia*. *Dodecatheon meadia* showed the highest emergence when topsoil was beneath the mulch and the lowest when subsoil was present. On subsoil mulch, the underlying soil type only had a significant effect on emergence in *Helianthus autumnale*. Emergence was low in subsoil mulch on top of both subsoil and topsoil.

Table 5.5 Percentage emergence of individual species in response to soil type underneath when sown in sand and subsoil mulch.

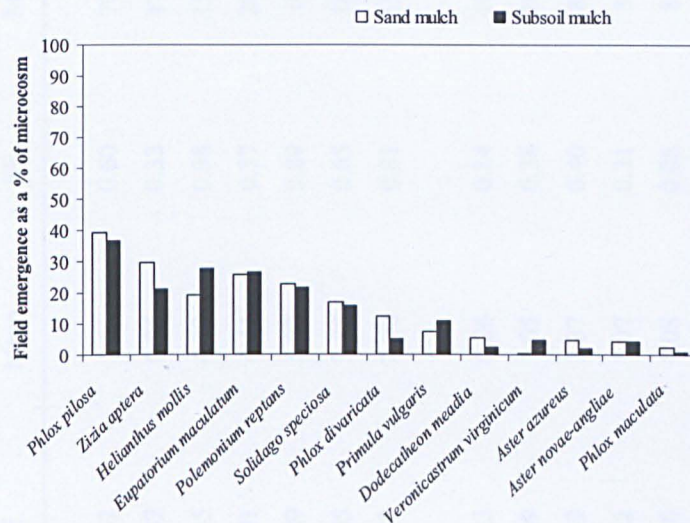
	Sand mulch				P value	Subsoil mulch				P value
	Top soil		Subsoil			Top soil		Subsoil		
	Mean	SE	Mean	SE		Mean	SE	Mean	SE	
<b>Low canopy</b>										
<i>Dodecatheon meadia</i>	6.71	2.52	1.08	0.69	0.035 *	2.14	1.10	0.98	0.48	0.631 ns
<i>Lathyrus vernus</i>	8.39	1.60	12.26	1.07	0.089 ns	10.99	1.54	10.57	1.88	0.971 ns
<i>Phlox divaricata</i>	1.54	0.57	2.62	0.85	0.436 ns	0.45	0.32	1.26	0.58	0.393 ns
<i>Phlox pilosa</i>	2.90	1.39	7.71	1.63	0.075 ns	4.39	0.83	5.46	1.82	0.912 ns
<i>Polemonium reptans</i>	5.31	1.84	3.95	0.89	1.000 ns	4.81	1.35	4.00	0.80	0.971 ns
<i>Primula elatior</i>	5.75	1.76	6.56	3.45	0.739 ns	5.50	1.34	6.45	1.23	0.579 ns
<i>Primula vulgaris</i>	2.29	0.68	2.96	0.89	0.684 ns	3.01	0.90	4.75	0.90	0.165 ns
<i>Zizia aptera</i>	3.05	0.92	5.07	1.74	0.529 ns	2.74	0.90	3.11	1.39	0.912 ns
<b>Medium canopy</b>										
<i>Aster azureus</i>	0.23	0.23	1.10	0.36	0.089 ns	0.36	0.24	0.17	0.17	0.739 ns
<i>Echinacea purpurea</i>	23.72	2.42	21.18	2.58	0.739 ns	4.61	0.67	6.94	1.36	0.247 ns
<i>Gillenia trifoliata</i>	16.58	2.13	15.71	2.31	0.631 ns	8.63	1.34	10.41	0.86	0.579 ns
<i>Phlox glaberrima</i>	0.00	0.00	0.31	0.31	0.739 ns	0.00	0.00	0.00	0.00	1.000 ns
<i>Penstemon digitalis</i>	3.32	0.97	3.62	0.53	0.280 ns	4.18	0.69	3.23	0.66	0.353 ns
<i>Phlox maculata</i>	0.16	0.11	0.21	0.17	1.000 ns	0.00	0.00	0.17	0.17	0.739 ns
<i>Rudbeckia speciosa</i>	6.56	1.76	3.96	0.75	0.436 ns	4.02	0.91	3.16	0.50	0.529 ns
<i>Silene regia</i>	9.50	1.39	9.39	1.04	0.971 ns	10.08	0.84	9.96	1.09	0.684 ns
<i>Solidago speciosa</i>	2.72	0.74	2.83	0.66	0.912 ns	2.32	0.34	2.83	0.98	0.739 ns
<b>Tall canopy</b>										
<i>Andropogon gerardii</i>	6.02	1.09	6.63	0.94	0.393 ns	4.70	0.99	5.80	0.83	0.481 ns
<i>Aster novae-angliae</i>	0.57	0.33	1.39	0.69	0.529 ns	1.25	0.67	0.84	0.38	0.971 ns
<i>Helianthus mollis</i>	1.09	0.37	0.40	0.22	0.190 ns	0.97	0.37	1.18	0.50	0.971 ns
<i>Helenium autumnale</i>	0.47	0.18	1.03	0.41	0.436 ns	0.64	0.25	1.81	0.42	0.043 *
<i>Phlox amplifolia</i>	21.65	2.53	20.07	4.40	0.280 ns	13.52	3.50	18.45	2.48	0.089 ns
<i>Eupatorium maculatum</i>	1.41	0.47	3.18	1.05	0.280 ns	2.98	0.62	1.75	0.47	0.165 ns
<i>Rudbeckia subtomentosa</i>	0.54	0.13	1.03	0.44	0.631 ns	0.70	0.21	0.58	0.22	0.529 ns
<i>Silphium integrifolium</i>	26.75	2.52	25.60	1.80	0.739 ns	6.72	1.42	9.24	2.35	0.529 ns
<i>Veronicastrum virginicum</i>	0.00	0.00	0.17	0.09	0.280 ns	1.11	0.47	0.96	0.39	0.796 ns

Significant differences (Mann-Whitney U-test) between soil type underneath are indicated by asterisks; ns: not significant. \* P=0.05

### 5.3.3 Comparison of field emergence with the previous microcosm experiment

Field emergence of shade tolerant understorey forbs commenced in April 2006, once the average soil temperature exceeded 5°C (approximately 3 months post sowing). Field emergence of shade intolerant medium-tall forbs (prairie species) typically commenced 2 weeks later. Germination of the sown species typically commences in March-early April. March 2006 was extremely cold with night time minima as low as -12°C. This appeared to delay emergence into a period of very dry unseasonally warm weather.

To assist data interpretation, field emergence in sand and subsoil mulch were compared with data from the microcosm experiment (**Chapter 4**). Field emergence was considerably lower for most species than the maximum germination values recorded in microcosm experiment (Figure 5.6 and Table 5.6).



**Figure 5.6** Field emergence of species as a percentage of their maximum emergence in the microcosm experiment.

Mean emergence of medium-tall shade intolerant species in sand mulch was 4.24% as opposed to 4.11% in shade tolerant, understorey species ( $P=0.04$ , Mann-Whitney  $U$ -test).

### 5.3.4 Number of seedlings after adjustment

After emergence, seedling numbers were corrected by removal and addition of plants. The numbers of forbs present in all treatments at the end of this process were recorded (Table 5.7).



Table 5.6 Comparison of field emergence with microcosm experiment.

Species	Field				Microcosm		P-value	P-value
	Percentage emergence in sowing mix (sand mulch)		Percentage emergence in sowing mix (subsoil mulch)		(Percentage emergence in sowing mix)		Field emergence (sand mulch) vs. microcosm	Field emergence (subsoil mulch) vs. microcosm
	Mean	SE	Mean	SE	Mean	SE		
<b>Shade tolerant understorey forbs</b>								
<i>Dodecatheon meadia</i>	3.90	1.42	1.56	0.60	70.33	7.54	0.000 ***	0.000 ***
<i>Phlox divaricata</i>	2.08	0.52	0.85	0.33	17.14	2.75	0.000 ***	0.000 ***
<i>Phlox pilosa</i>	5.30	1.25	4.92	0.98	13.53	2.38	0.004 **	0.001 ***
<i>Polemonium reptans</i>	4.63	1.01	4.41	0.77	20.39	2.20	0.000 ***	0.000 ***
<i>Primula elatior</i>	6.16	1.89	5.97	0.89	0.00	0.00	0.000 ***	0.000 ***
<i>Primula vulgaris</i>	2.62	0.55	3.88	0.65	36.21	3.90	0.000 ***	0.000 ***
<i>Zizia aptera</i>	4.06	0.98	2.92	0.81	13.69	2.48	0.001 ***	0.000 ***
<b>Shade intolerant medium-tall forbs</b>								
<i>Aster azureus</i>	0.66	0.23	0.26	0.14	14.67	1.74	0.000 ***	0.000 ***
<i>Aster novae-angliae</i> 'Septemberrubin'	0.98	0.39	1.05	0.38	24.25	2.86	0.000 ***	0.000 ***
<i>Eupatorium maculatum</i>	2.30	0.59	2.37	0.40	8.99	2.52	0.013 *	0.013 *
<i>Helianthus mollis</i>	0.74	0.22	1.07	0.31	3.88	2.95	0.003 **	0.009 **
<i>Phlox maculata</i>	0.18	0.10	0.08	0.08	8.40	1.31	0.000 ***	0.000 ***
<i>Silphium integrifolium</i>	26.17	1.51	7.98	1.37	0.76	0.47	0.000 ***	0.000 ***
<i>Solidago speciosa</i>	2.77	0.48	2.57	0.51	16.29	1.23	0.000 ***	0.000 ***
<i>Veronicastrum virginicum</i>	0.08	0.05	1.03	0.29	22.65	5.36	0.000 ***	0.000 ***

Significant differences (Mann-Whitney *U*-test) between field and microcosm emergence are indicated by asterisks; ns: not significant; \* P=0.05; \*\*P=0.01

Table 5.7 Comparison of target number of plants per replicate and those actually present (in parentheses) in August 2006.

## a) Topsoil underneath + sand mulch

Species	Approximate no of seedling after adjustment/quadrat									
	T1 (1L: 1M: 3T) (R1, R2, R3, R4)		T2 (3L: 1M: 1T) (R1, R2, R3, R4)		T3 (1L: 1M: 1T) (R1, R2, R3, R4)		T4 (1L: 3M: 3T) (R1, R2, R3, R4)		T5 (3L: 3M: 1T) (R1, R2, R3, R4)	
	LD	HD	LD	HD	LD	HD	LD	HD	LD	HD
<b>Low canopy (L)</b>										
<i>Dodecatheon meadia</i>	1.5 (1, 1, 1, 1)	3 (2, 1, 1, 1)	4.5 (3, 1, 2, 1)	9 (6, 4, 2, 5)	2.5 (2, 2, 2, 1)	5 (3, 4, 1, 1)	1.1 (1, 1, 1, 1)	2.2 (1, 5, 1, 1)	3.2 (3, 3, 1, 1)	6.4 (4, 4, 1, 6)
<i>Lathyrus vernus</i>	1.5 (1, 1, 3, 1)	3 (2, 4, 1, 3)	4.5 (2, 1, 3, 2)	9 (3, 3, 5, 6)	2.5 (3, 2, 2, 2)	5 (3, 1, 1, 2)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)	3.2 (3, 3, 2, 3)	6.4 (1, 2, 4, 2)
<i>Phlox divaricata</i>	1.5 (1, 1, 1, 1)	3 (1, 1, 1, 1)	4.5 (2, 1, 2, 1)	9 (2, 2, 2, 1)	2.5 (2, 1, 1, 1)	5 (1, 1, 1, 1)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)	3.2 (3, 3, 1, 1)	6.4 (2, 1, 1, 2)
<i>Phlox pilosa</i>	1.5 (1, 1, 2, 2)	3 (2, 1, 2, 1)	4.5 (4, 1, 5, 7)	9 (1, 5, 3, 1)	2.5 (2, 2, 2, 3)	5 (5, 4, 4, 5)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)	3.2 (3, 3, 3, 3)	6.4 (1, 6, 3, 4)
<i>Polemonium reptans</i>	1.5 (2, 4, 1, 1)	3 (1, 1, 5, 1)	4.5 (11, 10, 6, 2)	9 (1, 1, 12, 2)	2.5 (4, 6, 2, 1)	5 (2, 1, 5, 1)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 2, 1)	3.2 (3, 3, 4, 2)	6.4 (1, 1, 5, 1)
<i>Primula elatior</i>	1.5 (2, 1, 1, 1)	3 (3, 3, 3, 6)	4.5 (4, 5, 9, 14)	9 (25, 16, 10, 11)	2.5 (2, 2, 5, 3)	5 (5, 4, 7, 10)	1.1 (1, 1, 1, 1)	2.2 (4, 1, 2, 3)	3.2 (3, 3, 7, 5)	6.4 (17, 9, 10, 12)
<i>Primula vulgaris</i>	1.5 (2, 1, 1, 2)	3 (5, 3, 3, 3)	4.5 (4, 5, 6, 6)	9 (12, 15, 10, 13)	2.5 (2, 2, 4, 3)	5 (5, 4, 7, 7)	1.1 (1, 1, 1, 1)	2.2 (2, 1, 2, 3)	3.2 (3, 3, 5, 8)	6.4 (9, 7, 7, 8)
<i>Zizia aptera</i>	1.5 (1, 1, 1, 2)	3 (1, 3, 1, 1)	4.5 (4, 10, 1, 1)	9 (1, 5, 7, 12)	2.5 (2, 2, 1, 5)	5 (4, 9, 2, 1)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 2, 1)	3.2 (3, 3, 1, 1)	6.4 (1, 6, 5, 1)
<b>Total</b>	<b>12 (11,11,11,11)</b>	<b>24 (17,17,17,17)</b>	<b>36 (34,34,34,34)</b>	<b>72 (51,51,51,51)</b>	<b>20 (19,19,19,19)</b>	<b>40 (28,28,28,28)</b>	<b>9 (8, 8, 8, 8)</b>	<b>18 (12,12,12,12)</b>	<b>26 (24,24,24,24)</b>	<b>52 (36,36,36,36)</b>
<b>Medium canopy (M)</b>										
<i>Aster azureus</i>	1.5 (1, 1, 1, 1)	3 (2, 1, 1, 1)	1.5 (1, 1, 1, 1)	3 (2, 1, 1, 1)	2.5 (1, 2, 1, 1)	5 (1, 1, 1, 1)	3.2 (1, 3, 1, 1)	6.4 (2, 1, 1, 1)	3.2 (1, 3, 1, 1)	6.4 (4, 1, 3, 1)
<i>Echinacea purpurea</i>	1.5 (1, 1, 1, 1)	3 (2, 3, 5, 3)	1.5 (1, 1, 1, 2)	3 (3, 3, 3, 2)	2.5 (4, 3, 2, 3)	5 (6, 9, 6, 5)	3.2 (5, 3, 3, 6)	6.4 (7, 3, 6, 9)	3.2 (7, 3, 4, 7)	6.4 (4, 6, 4, 6)
<i>Gillenia trifoliata</i>	0.8 (2, 2, 2, 2)	1.5 (3, 3, 3, 2)	0.8 (2, 2, 1, 1)	1.5 (2, 4, 1, 6)	1.3 (3, 2, 2, 6)	2.5 (2, 5, 5, 5)	1.6 (3, 3, 3, 4)	3.2 (5, 10, 8, 5)	1.6 (3, 3, 2, 3)	3.2 (6, 7, 8, 5)
<i>Phlox glaberrima</i>	0.8 (1, 1, 1, 1)	1.5 (1, 1, 1, 1)	0.8 (1, 1, 1, 1)	1.5 (1, 1, 1, 1)	1.3 (1, 1, 1, 1)	2.5 (1, 1, 1, 1)	1.6 (1, 2, 1, 1)	3.2 (2, 1, 1, 1)	1.6 (1, 1, 1, 1)	3.2 (2, 1, 1, 1)
<i>Penstemon digitalis</i>	1.5 (1, 1, 1, 2)	3 (2, 1, 2, 3)	1.5 (2, 1, 2, 1)	3 (2, 4, 2, 2)	2.5 (1, 2, 5, 1)	5 (7, 6, 3, 6)	3.2 (3, 3, 4, 4)	6.4 (6, 9, 4, 4)	3.2 (2, 3, 3, 2)	6.4 (6, 5, 3, 6)
<i>Phlox maculata</i>	1.5 (1, 1, 1, 1)	3 (1, 1, 1, 1)	1.5 (1, 1, 1, 1)	3 (1, 1, 1, 1)	2.5 (1, 1, 1, 1)	5 (2, 1, 1, 1)	3.2 (2, 2, 1, 1)	6.4 (2, 1, 1, 1)	3.2 (1, 1, 1, 1)	6.4 (2, 2, 1, 1)
<i>Rudbeckia speciosa</i>	1.5 (1, 1, 2, 1)	3 (2, 5, 1, 2)	1.5 (1, 1, 2, 2)	3 (3, 1, 1, 1)	2.5 (4, 3, 2, 4)	5 (4, 1, 2, 2)	3.2 (3, 3, 3, 5)	6.4 (5, 2, 7, 5)	3.2 (5, 3, 4, 2)	6.4 (4, 7, 7, 9)
<i>Silene regia</i>	1.5 (2, 2, 1, 1)	3 (2, 1, 1, 3)	1.5 (1, 2, 1, 1)	3 (2, 1, 5, 2)	2.5 (3, 3, 4, 1)	5 (4, 3, 8, 6)	3.2 (5, 3, 7, 1)	6.4 (6, 8, 6, 9)	3.2 (3, 4, 6, 6)	6.4 (4, 5, 8, 6)
<i>Solidago speciosa</i>	1.5 (1, 1, 1, 1)	3 (2, 1, 2, 1)	1.5 (1, 1, 1, 1)	3 (1, 1, 2, 1)	2.5 (1, 2, 1, 1)	5 (1, 1, 1, 1)	3.2 (1, 2, 1, 1)	6.4 (1, 1, 2, 1)	3.2 (1, 3, 2, 1)	6.4 (4, 2, 1, 1)
<b>Total</b>	<b>12 (11,11,11,11)</b>	<b>24 (17,17,17,17)</b>	<b>12 (11,11,11,11)</b>	<b>24 (17,17,17,17)</b>	<b>20 (19,19,19,19)</b>	<b>40 (28,28,28,28)</b>	<b>25 (24,24,24,24)</b>	<b>51 (36,36,36,36)</b>	<b>25 (24,24,24,24)</b>	<b>51 (36,36,36,36)</b>
<b>Tall canopy (T)</b>										
<i>Andropogon gerardii</i>	4.5 (4, 4, 3, 3)	9 (6, 8, 8, 5)	1.5 (1, 2, 2, 1)	3 (2, 2, 2, 2)	2.5 (2, 2, 1, 2)	5 (3, 4, 5, 3)	3.2 (4, 3, 1, 1)	6.4 (4, 5, 7, 5)	1.1 (1, 1, 1, 1)	2.2 (3, 1, 2, 2)
<i>Aster novae-angliae</i>	4.5 (5, 4, 5, 2)	9 (6, 5, 3, 2)	1.5 (1, 1, 1, 1)	3 (2, 2, 1, 1)	2.5 (2, 2, 1, 1)	5 (3, 2, 1, 3)	3.2 (2, 3, 2, 2)	6.4 (6, 1, 2, 2)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)
<i>Eupatorium maculatum</i>	3.7 (5, 6, 6, 8)	7.4 (8, 6, 9, 5)	1.2 (1, 1, 1, 1)	2.5 (2, 2, 1, 1)	2 (2, 2, 1, 2)	4.1 (5, 1, 6, 5)	2.6 (2, 5, 5, 4)	5.2 (3, 5, 5, 1)	0.9 (1, 1, 1, 1)	1.8 (1, 2, 1, 1)
<i>Helianthus mollis</i>	4.5 (1, 1, 1, 1)	9 (5, 1, 1, 5)	1.5 (1, 1, 1, 1)	3 (1, 1, 1, 1)	2.5 (1, 2, 1, 1)	5 (2, 1, 1, 1)	3.2 (2, 1, 1, 1)	6.4 (2, 1, 1, 1)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)
<i>Helenium autumnale</i>	4.5 (5, 4, 2, 8)	9 (4, 5, 9, 7)	1.5 (1, 1, 1, 2)	3 (2, 2, 3, 1)	2.5 (2, 2, 2, 2)	5 (4, 3, 1, 3)	3.2 (2, 3, 3, 2)	6.4 (5, 5, 5, 5)	1.1 (1, 1, 1, 1)	2.2 (1, 2, 1, 1)
<i>Phlox amplifolia</i>	0.8 (2, 1, 4, 1)	1.6 (4, 2, 2, 6)	0.3 (2, 1, 1, 1)	0.5 (1, 1, 1, 1)	0.5 (1, 2, 2, 2)	0.9 (2, 2, 3, 1)	0.6 (1, 2, 2, 3)	1.2 (2, 5, 1, 5)	0.2 (1, 1, 1, 1)	0.4 (1, 1, 1, 1)
<i>Rudbeckia subtomentosa</i>	4.5 (3, 4, 4, 4)	9 (6, 6, 8, 5)	1.5 (1, 1, 1, 1)	3 (1, 2, 2, 1)	2.5 (1, 2, 3, 2)	5 (2, 5, 2, 4)	3.2 (2, 3, 1, 1)	6.4 (4, 4, 1, 1)	1.1 (1, 1, 1, 1)	2.2 (1, 2, 1, 1)
<i>Silphium integrifolium</i>	4.5 (5, 6, 5, 3)	9 (7, 16, 7, 9)	1.5 (1, 2, 2, 1)	3 (4, 3, 5, 8)	2.5 (6, 2, 5, 5)	5 (3, 5, 5, 5)	3.2 (7, 2, 7, 4)	6.4 (5, 7, 9, 11)	1.1 (1, 1, 1, 1)	2.2 (2, 1, 3, 3)
<i>Veronicastrum virginicum</i>	4.5 (4, 4, 4, 4)	9 (5, 2, 4, 7)	1.5 (2, 1, 1, 2)	3 (2, 2, 1, 1)	2.5 (2, 3, 3, 2)	5 (4, 5, 4, 3)	3.2 (2, 2, 2, 6)	6.4 (5, 3, 5, 5)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)
<b>Total</b>	<b>36 (34, 34, 34, 34)</b>	<b>72 (51, 51, 51, 51)</b>	<b>12 (11, 11, 11, 11)</b>	<b>24 (17, 17, 17, 17)</b>	<b>20 (19, 19, 19, 19)</b>	<b>40 (28, 28, 28, 28)</b>	<b>25 (24, 24, 24, 24)</b>	<b>51 (36, 36, 36, 36)</b>	<b>10 (9, 9, 9, 9)</b>	<b>18 (12, 12, 12, 12)</b>
<b>~Seedling ratios/replicate</b>	<b>1L : 1M : 3T</b>		<b>3L : 1M : 1T</b>		<b>1L : 1M : 1T</b>		<b>1L : 3M : 3T</b>		<b>3L : 3M : 1T</b>	

Key: LD=Low density; HD=High density; R=Replicate

## b) Topsoil underneath + subsoil mulch

Species	Approximate no of seedling after adjustment/quadrat									
	T1 (1L: 1M: 3T) (R1, R2, R3, R4)		T2 (3L: 1M: 1T) (R1, R2, R3, R4)		T3 (1L: 1M: 1T) (R1, R2, R3, R4)		T4 (1L: 3M: 3T) (R1, R2, R3, R4)		T5 (3L: 3M: 1T) (R1, R2, R3, R4)	
	LD	HD	LD	HD	LD	HD	LD	HD	LD	HD
<b>Low canopy (L)</b>										
<i>Dodecatheon meadia</i>	1.5 (1, 1, 2, 1)	3 (2, 1, 1, 1)	4.5 (3, 4, 4, 1)	9 (6, 5, 5, 3)	2.5 (2, 2, 2, 1)	5 (3, 2, 1, 1)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)	3.2 (3, 3, 2, 1)	6.4 (4, 3, 2, 1)
<i>Lathyrus vernus</i>	1.5 (2, 1, 1, 3)	3 (3, 2, 5, 1)	4.5 (5, 1, 1, 3)	9 (7, 10, 3, 5)	2.5 (2, 2, 4, 1)	5 (3, 2, 1, 2)	1.1 (1, 1, 1, 1)	2.2 (2, 1, 1, 2)	3.2 (3, 1, 3, 1)	6.4 (2, 2, 4, 1)
<i>Phlox divaricata</i>	1.5 (1, 1, 2, 1)	3 (1, 1, 1, 1)	4.5 (2, 4, 2, 1)	9 (2, 3, 2, 1)	2.5 (2, 2, 1, 1)	5 (3, 1, 1, 1)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)	3.2 (3, 3, 2, 1)	6.4 (2, 2, 1, 1)
<i>Phlox pilosa</i>	1.5 (1, 1, 1, 1)	3 (2, 2, 3, 4)	4.5 (4, 4, 5, 5)	9 (6, 5, 2, 8)	2.5 (2, 2, 4, 4)	5 (4, 4, 1, 5)	1.1 (1, 1, 1, 1)	2.2 (1, 2, 2, 1)	3.2 (3, 3, 2, 2)	6.4 (5, 5, 6, 6)
<i>Polemonium reptans</i>	1.5 (2, 4, 2, 1)	3 (3, 2, 2, 3)	4.5 (8, 6, 3, 1)	9 (10, 12, 4, 8)	2.5 (5, 5, 3, 2)	5 (6, 8, 1, 7)	1.1 (1, 1, 1, 1)	2.2 (3, 1, 2, 2)	3.2 (3, 3, 5, 1)	6.4 (6, 8, 2, 7)
<i>Primula elatior</i>	1.5 (2, 1, 1, 2)	3 (3, 4, 2, 2)	4.5 (4, 5, 10, 6)	9 (13, 6, 14, 15)	2.5 (2, 2, 2, 6)	5 (5, 4, 15, 6)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 2, 3)	3.2 (3, 5, 4, 10)	6.4 (8, 7, 14, 10)
<i>Primula vulgaris</i>	1.5 (1, 1, 1, 1)	3 (2, 3, 2, 3)	4.5 (4, 7, 8, 10)	9 (6, 1, 11, 10)	2.5 (2, 2, 2, 3)	5 (3, 4, 6, 5)	1.1 (1, 1, 1, 1)	2.2 (2, 4, 2, 1)	3.2 (3, 5, 4, 7)	6.4 (8, 7, 6, 9)
<i>Zizia aptera</i>	1.5 (1, 1, 1, 1)	3 (1, 2, 1, 2)	4.5 (4, 3, 1, 7)	9 (1, 9, 10, 1)	2.5 (2, 2, 1, 1)	5 (1, 3, 2, 1)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)	3.2 (3, 1, 2, 1)	6.4 (1, 2, 1, 1)
<b>Total</b>	<b>12 (11,11,11,11)</b>	<b>24 (17,17,17,17)</b>	<b>36 (34,34,34,34)</b>	<b>72 (51,51,51,51)</b>	<b>20 (19,19,19,19)</b>	<b>40 (28,28,28,28)</b>	<b>9 (8, 8, 8, 8)</b>	<b>18 (12,12,12,12)</b>	<b>26 (24,24,24,24)</b>	<b>52 (36,36,36,36)</b>
<b>Medium canopy (M)</b>										
<i>Aster azureus</i>	1.5 (1, 1, 1, 1)	3 (1, 1, 1, 1)	1.5 (1, 1, 1, 1)	3 (1, 1, 1, 1)	2.5 (2, 2, 1, 1)	5 (3, 1, 1, 1)	3.2 (1, 2, 1, 1)	6.4 (4, 1, 1, 1)	3.2 (2, 2, 1, 1)	6.4 (4, 1, 1, 1)
<i>Echinacea purpurea</i>	1.5 (2, 1, 1, 1)	3 (3, 1, 1, 3)	1.5 (2, 1, 1, 1)	3 (2, 1, 1, 3)	2.5 (2, 2, 6, 1)	5 (2, 6, 2, 7)	3.2 (4, 3, 8, 3)	6.4 (4, 5, 6, 5)	3.2 (3, 3, 1, 2)	6.4 (4, 6, 6, 7)
<i>Gillenia trifoliata</i>	0.8 (1, 2, 1, 3)	1.5 (2, 2, 1, 2)	0.8 (1, 2, 1, 2)	1.5 (2, 2, 3, 1)	1.3 (2, 3, 2, 6)	2.5 (3, 4, 2, 6)	1.6 (1, 3, 2, 3)	3.2 (6, 8, 9, 6)	1.6 (2, 4, 1, 6)	3.2 (5, 5, 6, 7)
<i>Phlox glaberrima</i>	0.8 (1, 1, 1, 1)	1.5 (1, 1, 1, 1)	0.8 (1, 1, 1, 1)	1.5 (1, 1, 1, 1)	1.3 (2, 2, 1, 1)	2.5 (2, 1, 1, 1)	1.6 (1, 2, 1, 1)	3.2 (4, 1, 1, 1)	1.6 (1, 2, 1, 1)	3.2 (3, 1, 1, 1)
<i>Penstemon digitalis</i>	1.5 (1, 1, 2, 1)	3 (2, 2, 2, 3)	1.5 (2, 1, 2, 1)	3 (1, 2, 1, 4)	2.5 (3, 2, 2, 3)	5 (3, 5, 5, 4)	3.2 (5, 3, 2, 5)	6.4 (4, 5, 5, 6)	3.2 (4, 3, 4, 5)	6.4 (4, 8, 6, 4)
<i>Phlox maculata</i>	1.5 (1, 1, 1, 1)	3 (1, 1, 1, 1)	1.5 (1, 1, 1, 1)	3 (1, 1, 1, 1)	2.5 (2, 2, 1, 1)	5 (3, 1, 1, 1)	3.2 (1, 2, 1, 1)	6.4 (2, 1, 1, 1)	3.2 (2, 2, 1, 1)	6.4 (1, 1, 1, 1)
<i>Rudbeckia speciosa</i>	1.5 (1, 1, 1, 1)	3 (3, 2, 3, 3)	1.5 (1, 1, 1, 1)	3 (3, 6, 1, 1)	2.5 (2, 2, 1, 3)	5 (4, 4, 5, 1)	3.2 (3, 3, 4, 4)	6.4 (4, 4, 7, 6)	3.2 (4, 3, 7, 3)	6.4 (7, 8, 5, 6)
<i>Silene regia</i>	1.5 (2, 2, 2, 1)	3 (3, 6, 2, 2)	1.5 (1, 2, 1, 2)	3 (5, 2, 4, 4)	2.5 (2, 2, 4, 2)	5 (6, 5, 6, 5)	3.2 (6, 3, 4, 5)	6.4 (4, 10, 3, 9)	3.2 (4, 3, 6, 4)	6.4 (6, 5, 8, 8)
<i>Solidago speciosa</i>	1.5 (1, 1, 1, 1)	3 (1, 1, 5, 1)	1.5 (1, 1, 2, 1)	3 (1, 1, 4, 1)	2.5 (2, 2, 1, 1)	5 (2, 1, 5, 2)	3.2 (2, 3, 1, 1)	6.4 (4, 1, 3, 1)	3.2 (2, 2, 2, 1)	6.4 (2, 1, 2, 1)
<b>Total</b>	<b>12 (11,11,11,11)</b>	<b>24 (17,17,17,17)</b>	<b>12 (11,11,11,11)</b>	<b>24 (17,17,17,17)</b>	<b>20 (19,19,19,19)</b>	<b>40 (28,28,28,28)</b>	<b>25 (24,24,24,24)</b>	<b>51 (36,36,36,36)</b>	<b>25 (24,24,24,24)</b>	<b>51 (36,36,36,36)</b>
<b>Tall canopy (T)</b>										
<i>Andropogon gerardii</i>	4.5 (4, 4, 3, 5)	9 (7, 9, 8, 10)	1.5 (1, 1, 1, 2)	3 (2, 1, 2, 5)	2.5 (1, 2, 1, 3)	5 (3, 5, 3, 3)	3.2 (3, 3, 3, 1)	6.4 (6, 7, 3, 7)	1.1 (1, 1, 1, 1)	2.2 (2, 1, 2, 2)
<i>Aster novae-angliae</i>	4.5 (4, 7, 1, 5)	9 (6, 3, 3, 3)	1.5 (1, 1, 1, 1)	3 (2, 1, 2, 1)	2.5 (2, 1, 1, 2)	5 (3, 2, 2, 2)	3.2 (4, 3, 1, 4)	6.4 (3, 2, 1, 2)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)
<i>Eupatorium maculatum</i>	3.7 (6, 3, 7, 5)	7.4 (7, 2, 7, 8)	1.2 (2, 2, 2, 1)	2.5 (2, 1, 2, 1)	2 (2, 3, 3, 2)	4.1 (4, 5, 4, 6)	2.6 (3, 4, 4, 5)	5.2 (6, 5, 6, 5)	0.9 (1, 1, 1, 1)	1.8 (1, 2, 1, 2)
<i>Helianthus mollis</i>	4.5 (4, 1, 1, 1)	9 (1, 1, 1, 2)	1.5 (1, 1, 1, 1)	3 (1, 1, 1, 1)	2.5 (2, 2, 1, 1)	5 (3, 1, 1, 1)	3.2 (1, 1, 1, 1)	6.4 (2, 1, 1, 1)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)
<i>Helenium autumnale</i>	4.5 (4, 4, 3, 6)	9 (6, 8, 8, 2)	1.5 (1, 1, 1, 1)	3 (2, 3, 3, 1)	2.5 (2, 3, 5, 3)	5 (4, 3, 5, 4)	3.2 (1, 3, 5, 4)	6.4 (2, 6, 8, 5)	1.1 (1, 1, 1, 1)	2.2 (1, 2, 1, 2)
<i>Phlox amplifolia</i>	0.8 (1, 1, 1, 1)	1.6 (3, 3, 3, 1)	0.3 (1, 1, 1, 1)	0.5 (1, 1, 1, 2)	0.5 (2, 1, 1, 1)	0.9 (2, 1, 2, 1)	0.6 (2, 1, 1, 2)	1.2 (3, 1, 1, 1)	0.2 (1, 1, 1, 1)	0.4 (2, 1, 2, 1)
<i>Rudbeckia subtomentosa</i>	4.5 (2, 4, 8, 3)	9 (6, 9, 7, 8)	1.5 (1, 1, 1, 2)	3 (2, 3, 1, 1)	2.5 (3, 2, 1, 3)	5 (3, 5, 4, 5)	3.2 (4, 3, 3, 2)	6.4 (4, 6, 5, 5)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)
<i>Silphium integrifolium</i>	4.5 (4, 4, 2, 3)	9 (9, 8, 6, 9)	1.5 (1, 1, 2, 1)	3 (2, 2, 3, 1)	2.5 (2, 2, 1, 1)	5 (3, 2, 2, 1)	3.2 (4, 2, 1, 1)	6.4 (4, 3, 5, 4)	1.1 (1, 1, 1, 1)	2.2 (2, 1, 2, 1)
<i>Veronicastrum virginicum</i>	4.5 (5, 6, 8, 5)	9 (6, 8, 8, 8)	1.5 (2, 2, 1, 1)	3 (3, 4, 2, 4)	2.5 (3, 3, 5, 3)	5 (3, 4, 5, 5)	3.2 (2, 4, 5, 4)	6.4 (6, 5, 6, 6)	1.1 (1, 1, 1, 1)	2.2 (1, 2, 1, 1)
<b>Total</b>	<b>36 (34,34,34,34)</b>	<b>72 (51,51,51,51)</b>	<b>12 (11,11,11,11)</b>	<b>24 (17,17,17,17)</b>	<b>20 (19,19,19,19)</b>	<b>40 (28,28,28,28)</b>	<b>25 (24,24,24,24)</b>	<b>51 (36,36,36,36)</b>	<b>10 (9, 9, 9, 9)</b>	<b>18 (12,12,12,12)</b>
<b>~Seedling ratios/replicate</b>	<b>1L : 1M : 3T</b>		<b>3L : 1M : 1T</b>		<b>1L : 1M : 1T</b>		<b>1L : 3M : 3T</b>		<b>3L : 3M : 1T</b>	

Key: LD=Low density; HD=High density; R=Replicate

## c) Subsoil underneath + sand mulch

Species	Approximate no of seedling after adjustment/quadrat									
	T1 (1L: 1M: 3T) (R1, R2, R3, R4)		T2 (3L: 1M: 1T) (R1, R2, R3, R4)		T3 (1L: 1M: 1T) (R1, R2, R3, R4)		T4 (1L: 3M: 3T) (R1, R2, R3, R4)		T5 (3L: 3M: 1T) (R1, R2, R3, R4)	
	LD	HD	LD	HD	LD	HD	LD	HD	LD	HD
<b>Low canopy (L)</b>										
<i>Dodecatheon meadia</i>	1.5 (1, 1, 1, 1)	3 (1, 1, 2, 2)	4.5 (4, 1, 3, 3)	9 (3, 3, 6, 6)	2.5 (2, 1, 1, 1)	5 (1, 1, 3, 4)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)	3.2 (3, 1, 1, 1)	6.4 (3, 1, 4, 3)
<i>Lathyrus vernus</i>	1.5 (1, 2, 1, 1)	3 (2, 4, 2, 3)	4.5 (2, 4, 1, 3)	9 (12, 2, 1, 6)	2.5 (2, 2, 2, 2)	5 (5, 2, 2, 3)	1.1 (1, 1, 1, 1)	2.2 (1, 2, 1, 2)	3.2 (2, 2, 2, 4)	6.4 (5, 5, 3, 5)
<i>Phlox divaricata</i>	1.5 (1, 1, 1, 1)	3 (1, 1, 2, 2)	4.5 (2, 1, 1, 1)	9 (2, 2, 6, 5)	2.5 (2, 1, 1, 1)	5 (1, 1, 3, 3)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)	3.2 (2, 2, 1, 1)	6.4 (1, 1, 4, 2)
<i>Phlox pilosa</i>	1.5 (1, 1, 1, 1)	3 (3, 1, 2, 2)	4.5 (3, 5, 4, 4)	9 (10, 3, 6, 6)	2.5 (1, 1, 2, 2)	5 (6, 5, 3, 4)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 2)	3.2 (4, 5, 5, 5)	6.4 (7, 5, 4, 5)
<i>Polemonium reptans</i>	1.5 (2, 2, 2, 2)	3 (1, 1, 3, 2)	4.5 (2, 9, 8, 6)	9 (1, 1, 1, 10)	2.5 (1, 5, 5, 2)	5 (2, 1, 1, 5)	1.1 (1, 1, 1, 1)	2.2 (3, 1, 5, 2)	3.2 (1, 6, 6, 3)	6.4 (2, 2, 1, 7)
<i>Primula elatior</i>	1.5 (2, 1, 2, 2)	3 (4, 3, 2, 2)	4.5 (11, 6, 6, 10)	9 (14, 24, 15, 6)	2.5 (8, 3, 2, 5)	5 (6, 7, 8, 3)	1.1 (1, 1, 1, 1)	2.2 (2, 2, 1, 1)	3.2 (5, 3, 4, 5)	6.4 (11, 13, 11, 4)
<i>Primula vulgaris</i>	1.5 (2, 1, 2, 2)	3 (4, 4, 2, 2)	4.5 (8, 5, 5, 6)	9 (8, 14, 14, 6)	2.5 (2, 4, 3, 5)	5 (6, 8, 6, 3)	1.1 (1, 1, 1, 1)	2.2 (2, 2, 1, 1)	3.2 (4, 3, 3, 4)	6.4 (6, 8, 7, 4)
<i>Zizia aptera</i>	1.5 (1, 2, 1, 1)	3 (1, 2, 2, 2)	4.5 (2, 3, 6, 1)	9 (1, 2, 2, 6)	2.5 (1, 2, 3, 1)	5 (1, 3, 2, 3)	1.1 (1, 1, 1, 1)	2.2 (1, 2, 1, 2)	3.2 (3, 2, 2, 1)	6.4 (1, 1, 2, 6)
<b>Total</b>	<b>12 (11,11,11,11)</b>	<b>24 (17,17,17,17)</b>	<b>36(34,34,34,34)</b>	<b>72(51,51,51,51)</b>	<b>20(19,19,19,19)</b>	<b>40(28,28,28,28)</b>	<b>9 (8, 8, 8, 8)</b>	<b>18(12,12,12,12)</b>	<b>26(24,24,24,24)</b>	<b>52(36,36,36,36)</b>
<b>Medium canopy (M)</b>										
<i>Aster azureus</i>	1.5 (1, 1, 1, 1)	3 (1, 1, 1, 1)	1.5 (1, 1, 1, 1)	3 (1, 1, 1, 1)	2.5 (2, 1, 1, 1)	5 (1, 1, 3, 2)	3.2 (2, 1, 1, 1)	6.4 (1, 1, 1, 3)	3.2 (2, 1, 1, 1)	6.4 (1, 1, 4, 3)
<i>Echinacea purpurea</i>	1.5 (2, 1, 1, 1)	3 (2, 2, 4, 4)	1.5 (2, 2, 2, 1)	3 (4, 3, 3, 1)	2.5 (2, 3, 4, 4)	5 (4, 8, 4, 3)	3.2 (3, 2, 5, 4)	6.4 (14, 7, 4, 8)	3.2 (5, 9, 3, 3)	6.4 (8, 3, 4, 9)
<i>Gillenia trifoliata</i>	0.8 (1, 2, 3, 2)	1.5 (1, 3, 2, 4)	0.8 (1, 1, 2, 1)	1.5 (2, 2, 3, 6)	1.3 (3, 2, 4, 1)	2.5 (5, 5, 3, 5)	1.6 (4, 5, 4, 2)	3.2 (5, 9, 7, 5)	1.6 (5, 2, 5, 4)	3.2 (5, 8, 7, 6)
<i>Phlox glaberrima</i>	0.8 (1, 1, 1, 1)	1.5 (2, 1, 1, 1)	0.8 (1, 1, 1, 1)	1.5 (1, 1, 1, 1)	1.3 (2, 1, 1, 1)	2.5 (1, 1, 1, 1)	1.6 (1, 1, 1, 1)	3.2 (1, 1, 1, 1)	1.6 (1, 1, 1, 1)	3.2 (1, 1, 1, 1)
<i>Penstemon digitalis</i>	1.5 (2, 2, 1, 1)	3 (2, 1, 2, 1)	1.5 (2, 2, 1, 1)	3 (2, 3, 2, 3)	2.5 (2, 3, 2, 5)	5 (6, 2, 3, 6)	3.2 (4, 5, 5, 4)	6.4 (6, 5, 4, 3)	3.2 (3, 4, 4, 6)	6.4 (6, 1, 4, 2)
<i>Phlox maculata</i>	1.5 (1, 1, 1, 1)	3 (1, 1, 2, 1)	1.5 (1, 1, 1, 1)	3 (1, 1, 2, 1)	2.5 (2, 1, 1, 1)	5 (1, 1, 3, 2)	3.2 (2, 1, 1, 1)	6.4 (1, 1, 4, 2)	3.2 (1, 1, 1, 1)	6.4 (1, 1, 4, 1)
<i>Rudbeckia speciosa</i>	1.5 (1, 1, 1, 1)	3 (3, 2, 2, 2)	1.5 (1, 1, 1, 2)	3 (1, 3, 2, 1)	2.5 (2, 3, 2, 2)	5 (3, 2, 5, 2)	3.2 (3, 3, 4, 4)	6.4 (4, 6, 7, 4)	3.2 (3, 1, 1, 3)	6.4 (5, 2, 4, 1)
<i>Silene regia</i>	1.5 (1, 1, 1, 2)	3 (4, 4, 1, 2)	1.5 (1, 1, 1, 2)	3 (4, 2, 2, 2)	2.5 (3, 4, 3, 2)	5 (4, 6, 3, 5)	3.2 (3, 5, 2, 6)	6.4 (3, 3, 4, 9)	3.2 (2, 4, 5, 4)	6.4 (8, 18, 4, 10)
<i>Solidago speciosa</i>	1.5 (1, 1, 1, 1)	3 (1, 2, 2, 1)	1.5 (1, 1, 1, 1)	3 (1, 1, 1, 1)	2.5 (1, 1, 1, 2)	5 (3, 2, 3, 2)	3.2 (2, 1, 1, 1)	6.4 (1, 3, 4, 1)	3.2 (2, 1, 3, 1)	6.4 (1, 1, 4, 3)
<b>Total</b>	<b>12 (11,11,11,11)</b>	<b>24 (17,17,17,17)</b>	<b>12(11,11,11,11)</b>	<b>24(17,17,17,17)</b>	<b>20(19,19,19,19)</b>	<b>40(28,28,28,28)</b>	<b>25(24,24,24,24)</b>	<b>51(36,36,36,36)</b>	<b>25(24,24,24,24)</b>	<b>51(36,36,36,36)</b>
<b>Tall canopy (T)</b>										
<i>Andropogon gerardii</i>	4.5 (2, 2, 5, 2)	9 (9, 7, 5, 7)	1.5 (1, 3, 1, 1)	3 (1, 2, 2, 2)	2.5 (2, 1, 4, 3)	5 (3, 2, 2, 5)	3.2 (2, 2, 2, 6)	6.4 (6, 3, 4, 4)	1.1 (1, 1, 1, 1)	2.2 (1, 2, 1, 2)
<i>Aster novae-angliae</i>	4.5 (1, 2, 1, 1)	9 (5, 3, 5, 5)	1.5 (1, 1, 1, 1)	3 (1, 3, 2, 1)	2.5 (2, 1, 1, 1)	5 (2, 2, 3, 3)	3.2 (2, 2, 1, 1)	6.4 (5, 2, 4, 4)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)
<i>Eupatorium maculatum</i>	3.7 (7, 5, 1, 5)	7.4 (6, 9, 8, 6)	1.2 (1, 1, 1, 2)	2.5 (2, 3, 2, 3)	2 (3, 3, 2, 1)	4.1 (3, 5, 3, 3)	2.6 (1, 2, 3, 1)	5.2 (4, 9, 7, 4)	0.9 (1, 1, 1, 1)	1.8 (3, 1, 1, 1)
<i>Helianthus mollis</i>	4.5 (2, 1, 5, 1)	9 (1, 2, 1, 2)	1.5 (1, 1, 1, 1)	3 (1, 1, 1, 1)	2.5 (1, 1, 1, 1)	5 (1, 1, 3, 2)	3.2 (1, 1, 1, 1)	6.4 (1, 1, 1, 1)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)
<i>Helenium autumnale</i>	4.5 (5, 5, 2, 5)	9 (5, 5, 9, 5)	1.5 (1, 1, 1, 2)	3 (2, 1, 2, 2)	2.5 (2, 3, 1, 2)	5 (3, 3, 3, 3)	3.2 (2, 5, 4, 5)	6.4 (1, 4, 7, 5)	1.1 (1, 1, 1, 1)	2.2 (1, 2, 1, 1)
<i>Phlox amplifolia</i>	0.8 (1, 2, 7, 1)	1.6 (3, 3, 3, 3)	0.3 (1, 1, 1, 1)	0.5 (1, 1, 1, 1)	0.5 (1, 1, 1, 1)	0.9 (1, 1, 2, 1)	0.6 (2, 3, 1, 2)	1.2 (3, 3, 1, 3)	0.2 (1, 1, 1, 1)	0.4 (1, 2, 3, 1)
<i>Rudbeckia subtomentosa</i>	4.5 (3, 7, 2, 9)	9 (4, 7, 4, 5)	1.5 (1, 1, 1, 1)	3 (2, 1, 2, 2)	2.5 (2, 2, 2, 3)	5 (3, 3, 3, 2)	3.2 (3, 3, 2, 1)	6.4 (3, 5, 4, 4)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)
<i>Silphium integrifolium</i>	4.5 (9, 3, 6, 5)	9 (14, 7, 9, 12)	1.5 (3, 1, 3, 1)	3 (5, 3, 3, 3)	2.5 (4, 4, 5, 4)	5 (9, 7, 3, 6)	3.2 (7, 1, 8, 4)	6.4 (8, 2, 3, 8)	1.1 (1, 1, 1, 1)	2.2 (2, 1, 2, 3)
<i>Veronicastrum virginicum</i>	4.5 (4, 7, 5, 5)	9 (4, 8, 7, 7)	1.5 (1, 1, 1, 1)	3 (2, 2, 2, 2)	2.5 (2, 3, 2, 3)	5 (3, 4, 6, 3)	3.2 (4, 5, 2, 3)	6.4 (5, 7, 5, 3)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)
<b>Total</b>	<b>36 (34,34,34,34)</b>	<b>72 (51,51,51,51)</b>	<b>12(11,11,11,11)</b>	<b>24(17,17,17,17)</b>	<b>20(19,19,19,19)</b>	<b>40(28,28,28,28)</b>	<b>25(24,24,24,24)</b>	<b>51(36,36,36,36)</b>	<b>10(9, 9, 9, 9)</b>	<b>18(12,12,12,12)</b>
~Seedling ratios/replicate	1L : 1M : 3T		3L : 1M : 1T		1L : 1M : 1T		1L : 3M : 3T		3L : 3M : 1T	

Key: LD=Low density; HD=High density; R=Replicate

## d) Subsoil underneath + subsoil mulch

Species	Approximate no of seedling after adjustment/quadrat									
	T1 (1L: 1M: 3T) (R1, R2, R3, R4)		T2 (3L: 1M: 1T) (R1, R2, R3, R4)		T3 (1L: 1M: 1T) (R1, R2, R3, R4)		T4 (1L: 3M: 3T) (R1, R2, R3, R4)		T5 (3L: 3M: 1T) (R1, R2, R3, R4)	
	LD	HD	LD	HD	LD	HD	LD	HD	LD	HD
<b>Low canopy (L)</b>										
<i>Dodecatheon meadia</i>	1.5 (1, 1, 1, 1)	3 (1, 1, 2, 2)	4.5 (4, 1, 1, 1)	9 (5, 1, 6, 6)	2.5 (2, 1, 1, 1)	5 (2, 1, 3, 1)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)	3.2 (2, 1, 1, 1)	6.4 (3, 4, 4, 5)
<i>Lathyrus vernus</i>	1.5 (3, 1, 1, 2)	3 (3, 2, 1, 2)	4.5 (1, 4, 1, 7)	9 (5, 9, 4, 6)	2.5 (2, 1, 1, 3)	5 (3, 1, 2, 3)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 2)	3.2 (2, 2, 2, 2)	6.4 (4, 3, 4, 5)
<i>Phlox divaricata</i>	1.5 (1, 1, 1, 1)	3 (1, 1, 1, 2)	4.5 (4, 1, 2, 2)	9 (3, 1, 2, 6)	2.5 (2, 1, 1, 1)	5 (2, 1, 1, 3)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)	3.2 (2, 1, 1, 2)	6.4 (3, 1, 4, 1)
<i>Phlox pilosa</i>	1.5 (1, 2, 2, 1)	3 (2, 3, 2, 2)	4.5 (4, 9, 7, 4)	9 (7, 6, 3, 6)	2.5 (2, 2, 3, 6)	5 (7, 5, 3, 3)	1.1 (1, 1, 1, 1)	2.2 (2, 2, 1, 1)	3.2 (3, 1, 2, 3)	6.4 (5, 4, 3, 4)
<i>Polemonium reptans</i>	1.5 (2, 2, 2, 2)	3 (3, 1, 4, 3)	4.5 (2, 4, 7, 3)	9 (9, 2, 15, 9)	2.5 (2, 5, 4, 1)	5 (6, 1, 1, 10)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 2)	3.2 (1, 5, 6, 4)	6.4 (4, 1, 1, 9)
<i>Primula elatior</i>	1.5 (1, 2, 1, 1)	3 (3, 6, 3, 2)	4.5 (11, 4, 6, 6)	9 (13, 23, 8, 6)	2.5 (5, 2, 3, 3)	5 (3, 10, 10, 2)	1.1 (1, 1, 1, 1)	2.2 (3, 3, 2, 1)	3.2 (9, 4, 5, 5)	6.4 (10, 16, 10, 4)
<i>Primula vulgaris</i>	1.5 (1, 1, 2, 2)	3 (3, 2, 2, 2)	4.5 (7, 6, 7, 8)	9 (8, 8, 8, 6)	2.5 (3, 3, 3, 3)	5 (3, 7, 6, 3)	1.1 (1, 1, 1, 1)	2.2 (2, 1, 3, 2)	3.2 (4, 4, 5, 6)	6.4 (6, 6, 6, 4)
<i>Zizia aptera</i>	1.5 (1, 1, 1, 1)	3 (1, 1, 2, 2)	4.5 (1, 5, 3, 3)	9 (1, 1, 5, 6)	2.5 (1, 4, 3, 1)	5 (2, 2, 2, 3)	1.1 (1, 1, 1, 1)	2.2 (1, 2, 2, 2)	3.2 (1, 6, 2, 1)	6.4 (1, 1, 4, 4)
<b>Total</b>	12 (11,11,11,11)	24 (17,17,17,17)	36(34,34,34,34)	72(51,51,51,51)	20(19,19,19,19)	40(28,28,28,28)	9 (8, 8, 8, 8)	18(12,12,12,12)	26(24,24,24,24)	52(36,36,36,36)
<b>Medium canopy (M)</b>										
<i>Aster azureus</i>	1.5 (1, 1, 1, 1)	3 (1, 1, 1, 1)	1.5 (1, 1, 1, 1)	3 (1, 1, 1, 1)	2.5 (1, 1, 1, 1)	5 (1, 1, 3, 2)	3.2 (2, 1, 1, 1)	6.4 (1, 1, 4, 3)	3.2 (2, 1, 1, 1)	6.4 (1, 1, 4, 3)
<i>Echinacea purpurea</i>	1.5 (1, 1, 1, 1)	3 (3, 4, 2, 2)	1.5 (1, 1, 1, 2)	3 (3, 3, 2, 1)	2.5 (3, 2, 3, 3)	5 (6, 7, 3, 4)	3.2 (2, 2, 5, 5)	6.4 (3, 9, 4, 4)	3.2 (5, 5, 5, 5)	6.4 (4, 7, 4, 6)
<i>Gillenia trifoliata</i>	0.8 (2, 1, 2, 1)	1.5 (3, 3, 2, 3)	0.8 (2, 2, 1, 1)	1.5 (2, 2, 2, 3)	1.3 (1, 4, 3, 2)	2.5 (2, 8, 2, 4)	1.6 (4, 4, 5, 4)	3.2 (3, 4, 7, 4)	1.6 (3, 5, 5, 4)	3.2 (6, 7, 4, 4)
<i>Phlox glaberrima</i>	0.8 (1, 1, 1, 1)	1.5 (1, 1, 2, 1)	0.8 (1, 1, 1, 1)	1.5 (1, 1, 1, 1)	1.3 (1, 1, 1, 1)	2.5 (1, 1, 3, 1)	1.6 (2, 1, 1, 1)	3.2 (1, 1, 1, 2)	1.6 (2, 1, 1, 1)	3.2 (1, 1, 4, 2)
<i>Penstemon digitalis</i>	1.5 (1, 1, 1, 2)	3 (1, 2, 2, 2)	1.5 (1, 2, 2, 1)	3 (1, 1, 2, 2)	2.5 (1, 2, 2, 4)	5 (8, 5, 3, 5)	3.2 (6, 4, 1, 3)	6.4 (7, 5, 4, 7)	3.2 (3, 4, 2, 3)	6.4 (6, 5, 4, 6)
<i>Phlox maculata</i>	1.5 (1, 1, 1, 1)	3 (1, 1, 2, 2)	1.5 (1, 1, 1, 1)	3 (1, 1, 1, 2)	2.5 (1, 1, 1, 1)	5 (1, 1, 3, 1)	3.2 (1, 1, 1, 1)	6.4 (1, 1, 4, 2)	3.2 (2, 1, 1, 1)	6.4 (1, 1, 4, 2)
<i>Rudbeckia speciosa</i>	1.5 (1, 2, 1, 1)	3 (3, 1, 2, 2)	1.5 (1, 1, 2, 1)	3 (1, 3, 2, 2)	2.5 (1, 4, 5, 1)	5 (5, 1, 3, 5)	3.2 (3, 3, 5, 5)	6.4 (3, 6, 4, 4)	3.2 (2, 4, 2, 5)	6.4 (6, 8, 4, 5)
<i>Silene regia</i>	1.5 (2, 2, 2, 1)	3 (3, 3, 3, 2)	1.5 (2, 1, 1, 2)	3 (6, 4, 5, 4)	2.5 (5, 2, 2, 3)	5 (3, 3, 5, 5)	3.2 (2, 6, 4, 3)	6.4 (15, 8, 4, 7)	3.2 (2, 2, 6, 3)	6.4 (10, 4, 4, 6)
<i>Solidago speciosa</i>	1.5 (1, 1, 1, 2)	3 (1, 1, 1, 2)	1.5 (1, 1, 1, 1)	3 (1, 1, 1, 1)	2.5 (5, 2, 1, 3)	5 (1, 1, 3, 1)	3.2 (2, 2, 1, 1)	6.4 (2, 1, 4, 3)	3.2 (3, 1, 1, 1)	6.4 (1, 2, 4, 2)
<b>Total</b>	12 (11,11,11,11)	24 (17,17,17,17)	12(11,11,11,11)	24(17,17,17,17)	20(19,19,19,19)	40(28,28,28,28)	25(24,24,24,24)	51(36,36,36,36)	25(24,24,24,24)	51(36,36,36,36)
<b>Tall canopy (T)</b>										
<i>Andropogon gerardii</i>	4.5 (4, 5, 5, 7)	9 (8, 9, 5, 6)	1.5 (1, 1, 2, 1)	3 (3, 1, 4, 1)	2.5 (2, 1, 3, 3)	5 (4, 3, 5, 4)	3.2 (4, 3, 6, 3)	6.4 (5, 6, 3, 7)	1.1 (1, 1, 1, 1)	2.2 (3, 1, 3, 2)
<i>Aster novae-angliae</i>	4.5 (4, 3, 3, 1)	9 (5, 2, 5, 6)	1.5 (1, 1, 1, 1)	3 (2, 3, 2, 2)	2.5 (2, 1, 3, 1)	5 (3, 1, 3, 3)	3.2 (1, 2, 2, 1)	6.4 (5, 2, 4, 3)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)
<i>Eupatorium maculatum</i>	3.7 (7, 4, 8, 6)	7.4 (8, 10, 8, 7)	1.2 (2, 1, 1, 2)	2.5 (2, 2, 2, 2)	2 (1, 5, 3, 1)	4.1 (5, 6, 3, 6)	2.6 (2, 5, 2, 5)	5.2 (6, 6, 4, 5)	0.9 (1, 1, 1, 1)	1.8 (2, 1, 1, 1)
<i>Helianthus mollis</i>	4.5 (2, 1, 1, 1)	9 (1, 1, 5, 3)	1.5 (1, 1, 1, 1)	3 (1, 1, 1, 2)	2.5 (2, 1, 1, 1)	5 (1, 1, 2, 1)	3.2 (1, 1, 1, 1)	6.4 (1, 1, 4, 1)	1.1 (1, 1, 1, 1)	2.2 (1, 2, 1, 1)
<i>Helenium autumnale</i>	4.5 (5, 6, 3, 2)	9 (6, 5, 5, 6)	1.5 (1, 2, 1, 1)	3 (1, 4, 2, 2)	2.5 (1, 2, 1, 3)	5 (4, 2, 2, 2)	3.2 (3, 3, 1, 4)	6.4 (3, 6, 5, 4)	1.1 (1, 1, 1, 1)	2.2 (1, 3, 1, 1)
<i>Phlox amplifolia</i>	0.8 (1, 1, 3, 2)	1.6 (5, 4, 1, 2)	0.3 (1, 1, 1, 1)	0.5 (2, 1, 1, 2)	0.5 (3, 1, 1, 1)	0.9 (2, 3, 1, 1)	0.6 (2, 1, 1, 3)	1.2 (1, 2, 1, 2)	0.2 (1, 1, 1, 1)	0.4 (1, 1, 1, 3)
<i>Rudbeckia subtomentosa</i>	4.5 (4, 6, 2, 6)	9 (7, 7, 5, 9)	1.5 (1, 1, 1, 1)	3 (2, 2, 2, 2)	2.5 (2, 2, 3, 3)	5 (1, 6, 3, 3)	3.2 (2, 1, 5, 3)	6.4 (6, 5, 4, 4)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)
<i>Silphium integrifolium</i>	4.5 (5, 3, 3, 4)	9 (2, 4, 7, 6)	1.5 (1, 1, 1, 1)	3 (2, 1, 2, 2)	2.5 (4, 2, 1, 4)	5 (5, 3, 3, 4)	3.2 (5, 3, 2, 1)	6.4 (1, 3, 7, 4)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 1, 1)
<i>Veronicastrum virginicum</i>	4.5 (2, 5, 6, 5)	9 (9, 9, 10, 6)	1.5 (2, 2, 2, 2)	3 (2, 2, 1, 2)	2.5 (2, 4, 3, 2)	5 (3, 3, 6, 4)	3.2 (4, 5, 4, 3)	6.4 (8, 5, 4, 6)	1.1 (1, 1, 1, 1)	2.2 (1, 1, 2, 1)
<b>Total</b>	36 (34,34,34,34)	72 (51,51,51,51)	12(11,11,11,11)	24(17,17,17,17)	20(19,19,19,19)	40(28,28,28,28)	25(24,24,24,24)	51(36,36,36,36)	10(9, 9, 9, 9)	18(12,12,12,12)
~Seedling ratios/replicate	1L : 1M : 3T		3L : 1M : 1T		1L : 1M : 1T		1L : 3M : 3T		3L : 3M : 1T	

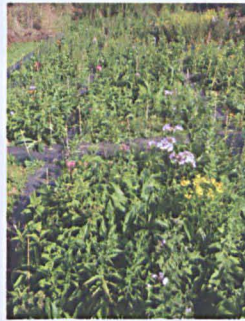
Key: LD=Low density; HD=High density; R=Replicate

### 5.3.5 Growth in the first year (2006)

After planting/removal adjustments to achieve target treatment, the growth of the multi-layer community was monitored. The vegetation in this study grew vigorously especially, after the heavy rains in August 2006. June and July were very hot and dry. The experiment was very colourful when flowering occurred between late August and October (Figure 5.7). Most of the medium-tall canopy species started to grow and compete with each other to create multiple-layered communities (Figure 5.8). Tall canopy species providing shade for lower species. The grass *Andropogon gerardii* was up to 150 cm in height by the end of summer (Figure 5.9).



A. July 2006



B. Early September 2006



C. End September 2006

**Figure 5.7** Creating multi-layer communities on different productivity and mulches during the first year of growing season; (A) the plot was treated with sand mulch and subsoil mulch; showing seedling start to growth and (B,C) the same site in September 2006, showing most of prairie species start to producing flowers.



**Figure 5.8** The same experiment in the first year of growing season, showing how medium-tall shade intolerant plant compete with each other to develop attractive multi-layer communities.



**Figure 5.9** *Andropogon gerardii* reached up to 150 cm in height (October 2006).

### 5.3.5.1 Impact of flowering in 2006

The photographs below (Figure 5.10) show some of the forbs species were producing flowers in the first growing year. These species gave a mass of colour in the experimental site.

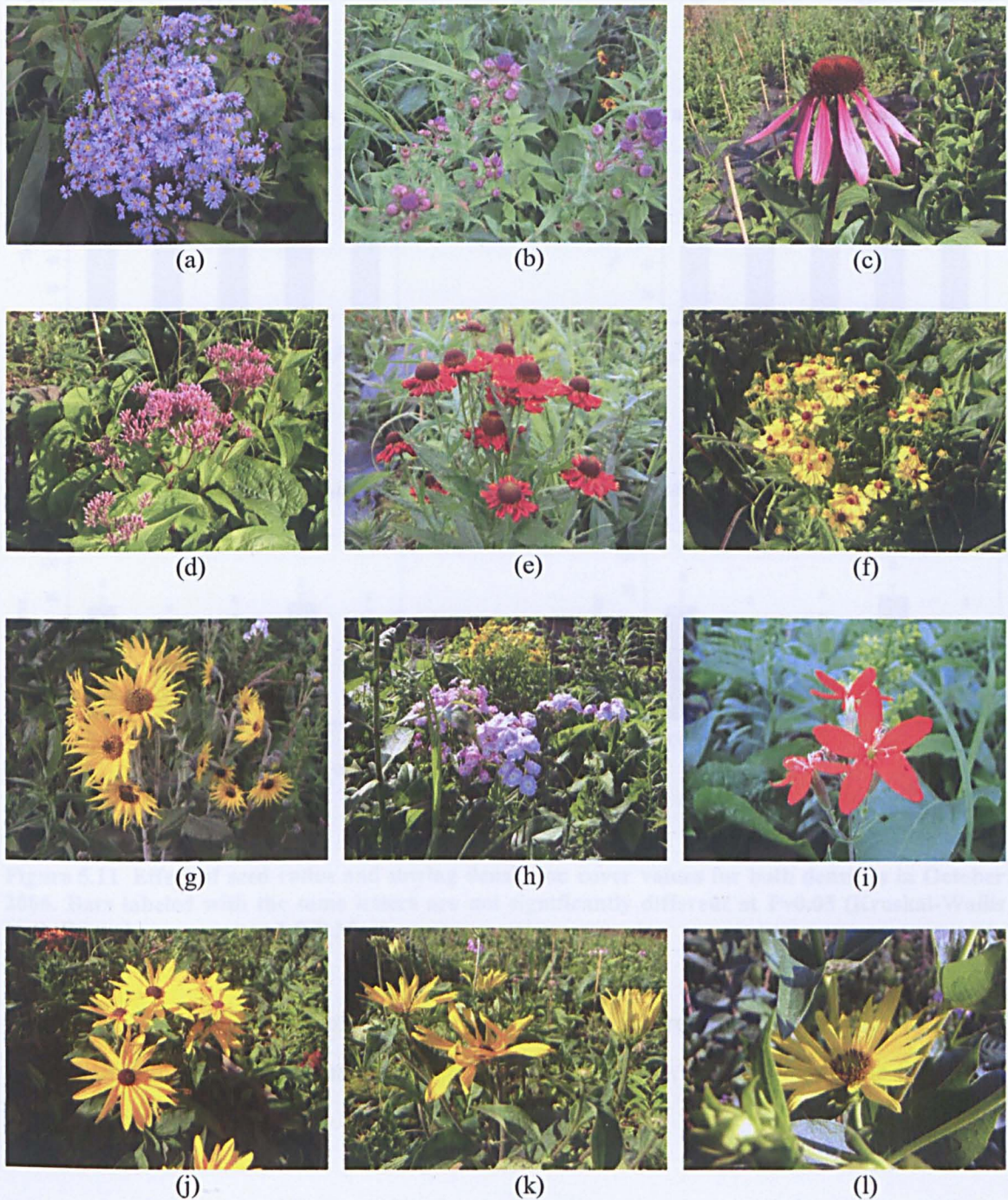
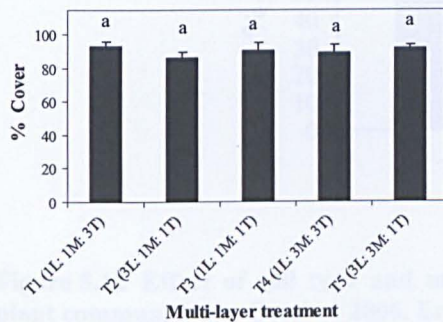


Figure 5.10 Some of the prairie forbs were producing flowers in year one; (a) *Aster azureus*, (b) *Aster novae-angliae*, (c) *Echinacea purpurea*, (d) *Eupatorium maculatum*, (e, f) *Helianthus autumnale*, (g) *Helianthus mollis*, (h) *Phlox amplifolia*, (i) *Silene regia*, (j, k) *Rudbeckia* spp., (l) *Silphium integrifolium*. All images were taken in September 2006.

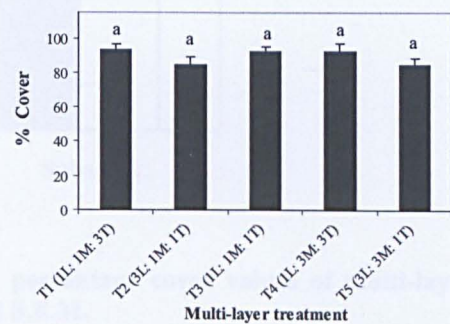
### 5.3.5.2 Cover value in year 2006

The development of plant growth in terms of percentage foliage cover was recorded in October 2006. The Mann-Whitney  $U$ -test indicated that in all cases, density was not statistically significant ( $P=0.05$ , ns) between low and high density treatment (see Appendix Figure A5.1), hence analysis was undertaken using mean coverage values for both densities (Figure 5.11).

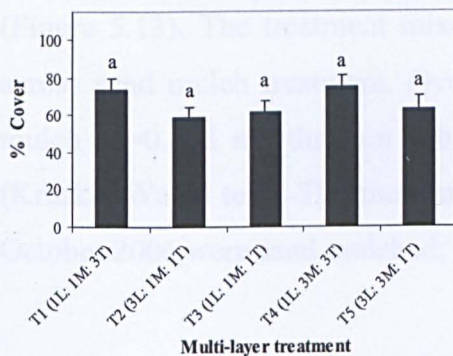
#### 1) Sand mulch + Topsoil underneath



#### 2) Sand mulch + Subsoil underneath



#### 3) Subsoil mulch + Topsoil underneath



#### 4) Subsoil mulch + Subsoil underneath

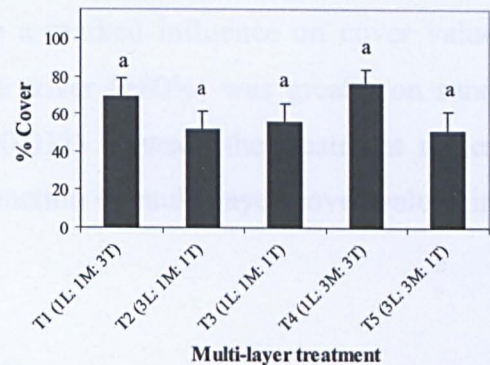


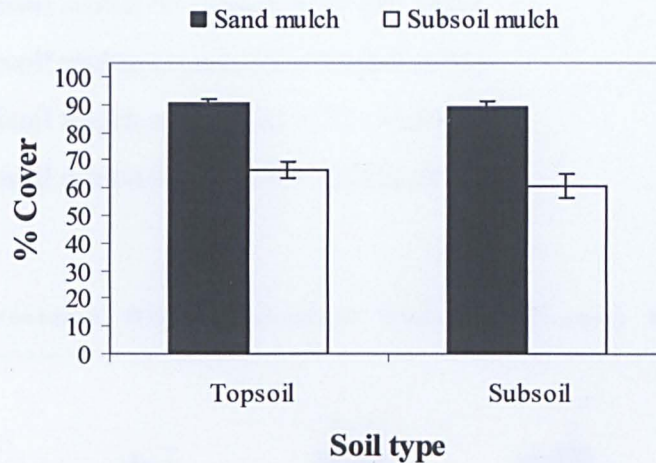
Figure 5.11 Effect of seed ratios and sowing density on cover values for both densities in October 2006. Bars labeled with the same letters are not significantly different at  $P=0.05$  (Kruskal-Wallis test). Error bars represent 1 S.E.M.

Percentage cover (as mean of all treatment mixes) was greater on sand mulch than on subsoil mulch ( $P=0.001$ , Kruskal-Wallis test; Figure 5.12). This involved the following combination;

- sand mulch + topsoil underneath (90.25%)
- sand mulch + subsoil underneath (89.38%)
- subsoil mulch + topsoil underneath (66.38%)
- subsoil mulch + subsoil underneath (61.00%).



A Scheirer-Ray-Hare test found that cover values in 2006 were more affected by mulch type ( $P=0.001$ ) than soil underneath ( $P=0.967$ , ns), the interaction was not significant ( $P=0.967$ ).



**Figure 5.12** Effect of soil type and mulch treatments on percentage cover values of multi-layer plant communities in October 2006. Error bars represent 1 S.E.M.

To assist data interpretation, mean cover value was analysed in each treatment mix (Figure 5.13). The treatment mixes did not have a marked influence on cover value across sand mulch treatment. Overall, percentage cover ( $>80\%$ ) was greater on sand mulch ( $P=0.148$  ns) than on subsoil mulch ( $P=0.032$ ) between the treatment mixes (Kruskal-Wallis test). The most successful combination of multi-layer cover values in October 2006 were sand mulched;

- sand mulch on topsoil + T1 (93.13%)
- sand mulch on subsoil + T1 (93.13%)
- sand mulch on subsoil + T3 (92.5%)
- sand mulch on subsoil + T4 (92.5%)
- sand mulch on topsoil + T5 (91.88%)
- sand mulch on topsoil + T3 (90.63%)
- sand mulch on topsoil + T4 (89.38%)
- sand mulch on topsoil + T2 (86.25%)
- sand mulch on subsoil + T2 (84.38%)
- sand mulch on subsoil + T5 (84.38%)

The least successful treatment combinations (<65% cover) were subsoil mulched;

- subsoil mulch on topsoil + T5 (63.13%)
- subsoil mulch on topsoil + T3 (61.25%)
- subsoil mulch on topsoil + T2 (58.75%)
- subsoil mulch on subsoil + T3 (56.25%)
- subsoil mulch on subsoil + T2 (51.88%)
- subsoil mulch on subsoil + T5 (51.25%)

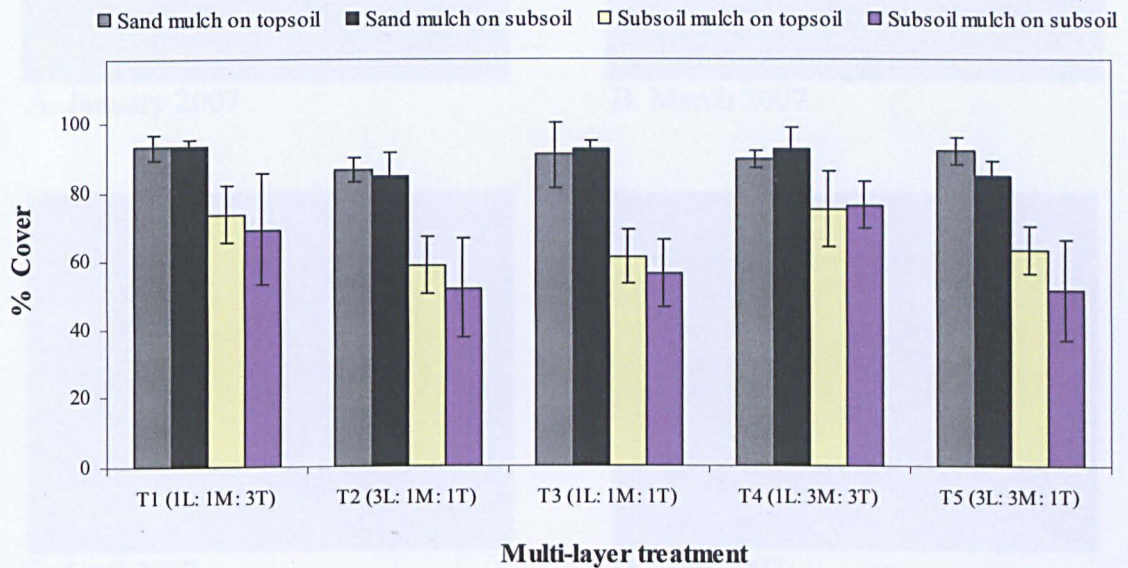
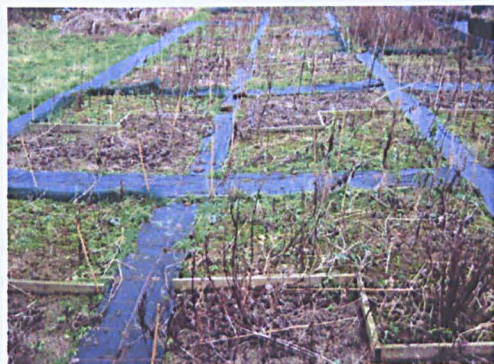


Figure 5.13 Effect of multi-layer treatments and mulch on topsoil/ subsoil on multi-layer cover in October 2006. Error bars represent 1 S.E.M.

### 5.3.6 Effect of the second year growth (2007) on the development of multi-layer community

The first species to flower in 2007 were the understory forbs *P. elatior* and *Primula vulgaris* in early March. *Lathyrus vernus* and *Polemonium reptans* flowered in mid April and were followed in May by *Zizia aptera* and *Phlox divaricata/pilosa*. The photographs below (Figure 5.14) show the changes in the experimental site from January to June 2007.



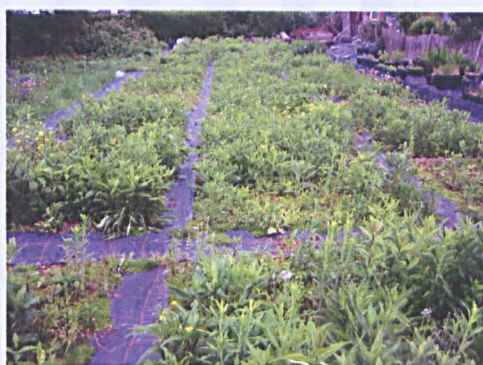
A. January 2007



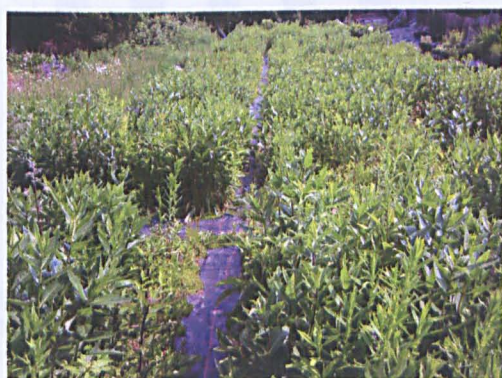
B. March 2007



C. April 2007



D. May 2007



E. June 2007

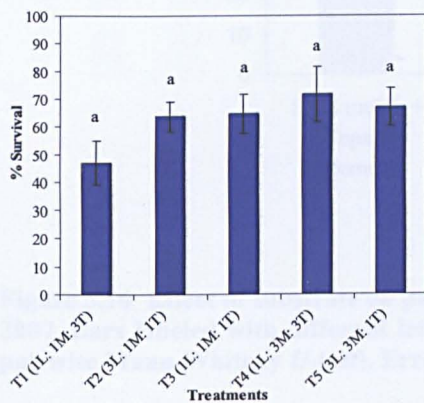
**Figure 5.14** The experimental plot in the second growing season; (A) most of the forbs are dormant in winter, (B,C) the same site showing some of the understorey forbs start to grow and flower in April 2007 and (D,E) most of the prairie forbs grow vigorously from May to June 2007.

### 5.3.6.1 Survival of sown forbs in April 2007 as a percentage those present in September 2006

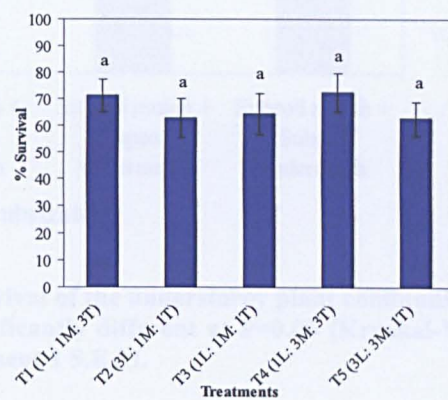
#### 5.3.6.1.1 Low canopy, understorey forbs

The Mann-Whitney  $U$ -test found that data was not statistically different between low and high density treatments (see Appendix Figure A5.2), so data was pooled across density treatments (Figure 5.15).

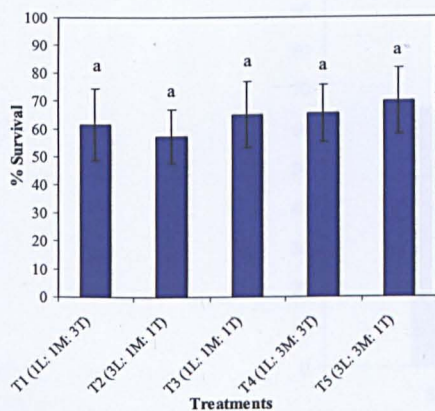
#### 1) Sand mulch + Topsoil underneath



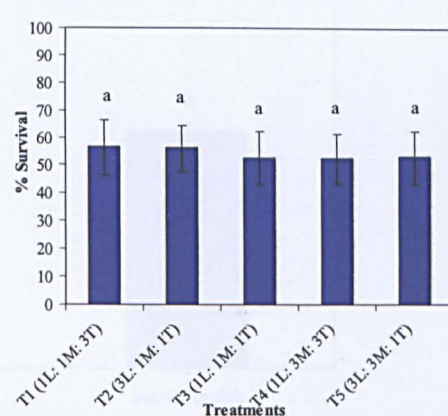
#### 2) Sand mulch + Subsoil underneath



#### 3) Subsoil mulch + Topsoil underneath



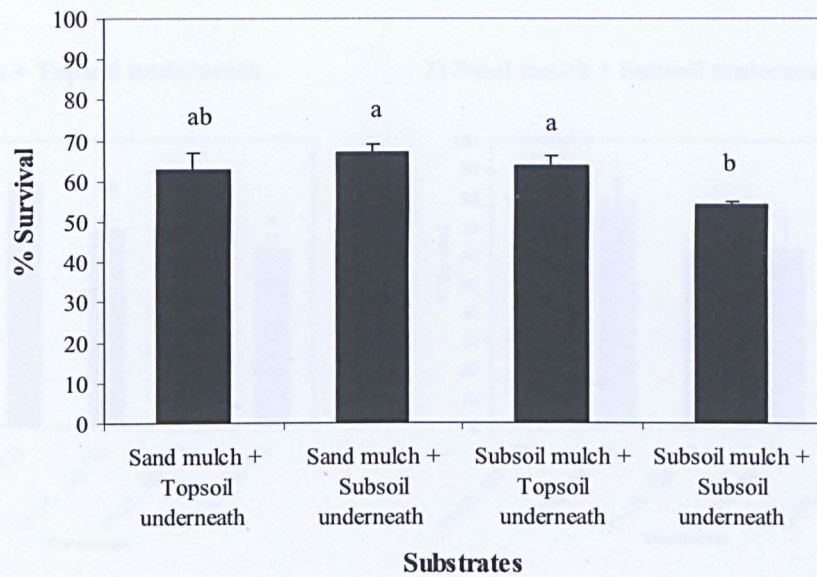
#### 4) Subsoil mulch + Subsoil underneath



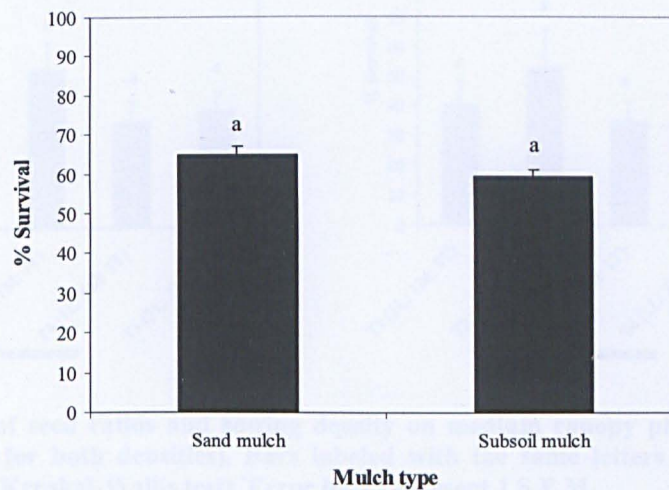
**Figure 5.15** Effect of seed ratios and sowing density on low canopy plant survival in April 2007 (mean values for both densities). Bars labeled with the same letters are not significantly different at  $P=0.05$  (Kruskal-Wallis test). Error bars represent 1 S.E.M.

In all cases, there was no statistical significance ( $P=0.05$ , Kruskal-Wallis test) between treatment mixes across different substrates when tested for the “understorey” layer. To assist data interpretation, percentage survival (as mean of all treatment mixes) was analysed within each productivity gradient (Figure 5.16). Substrate had a marked influence on survivorship of low canopy forbs ( $P=0.039$ ; Kruskal-Wallis test). Seedling

survival (as a mean of all treatment mixes) (Figure 5.17) was greater on sand mulch (64.70%) than subsoil mulch (59.05%) ( $P=0.07$ , Mann-Whitney  $U$ -test).



**Figure 5.16** Effect of substrate on percentage survival of the understorey plant community in April 2007. Bars labeled with different letters are significantly different at  $P=0.05$  (Kruskal-Wallis test, pairwise Mann-Whitney  $U$ -test). Error bars represent 1 S.E.M.



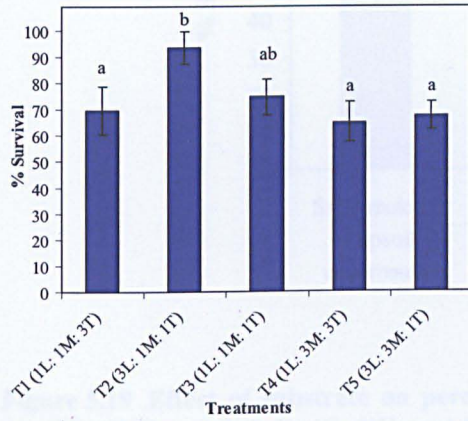
**Figure 5.17** Effect of mulching type on percentage survival of the understorey plant community in April 2007. Bars labeled with the same letters are not significantly different at  $P=0.05$  (Mann-Whitney  $U$ -test). Error bars represent 1 S.E.M.

### 5.3.6.1.2 Medium canopy forbs

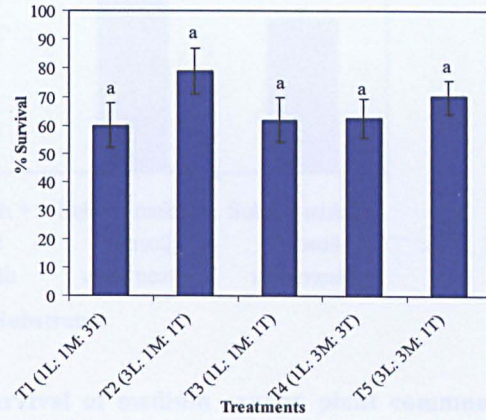
The Mann-Whitney  $U$ -test found that data was not significantly different between low and high density treatments (see Appendix Figure A5.3), so data was pooled across

density treatments (Figure 5.18). In all cases (with the exception in sand mulch + topsoil treatment), there was no statistical significance ( $P=0.05$ , Kruskal-Wallis test) within treatment mixes across different substrates tested.

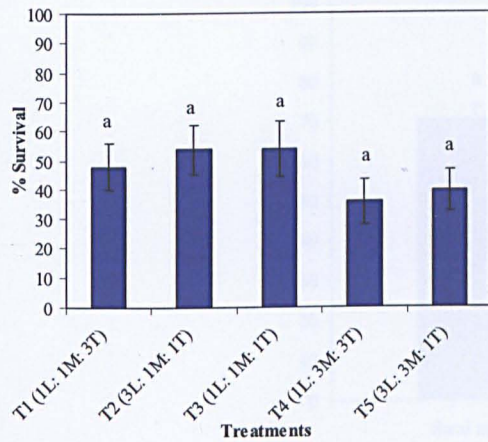
### 1) Sand mulch + Topsoil underneath



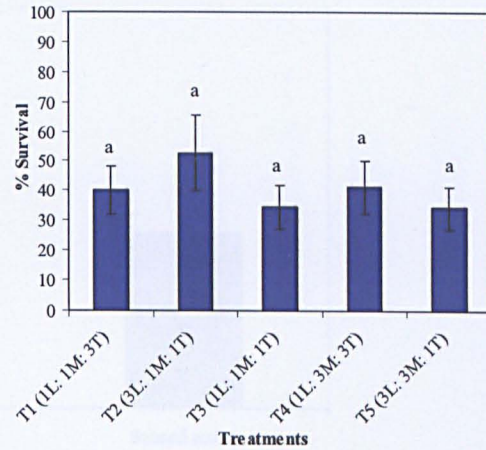
### 2) Sand mulch + Subsoil underneath



### 3) Subsoil mulch + Topsoil underneath

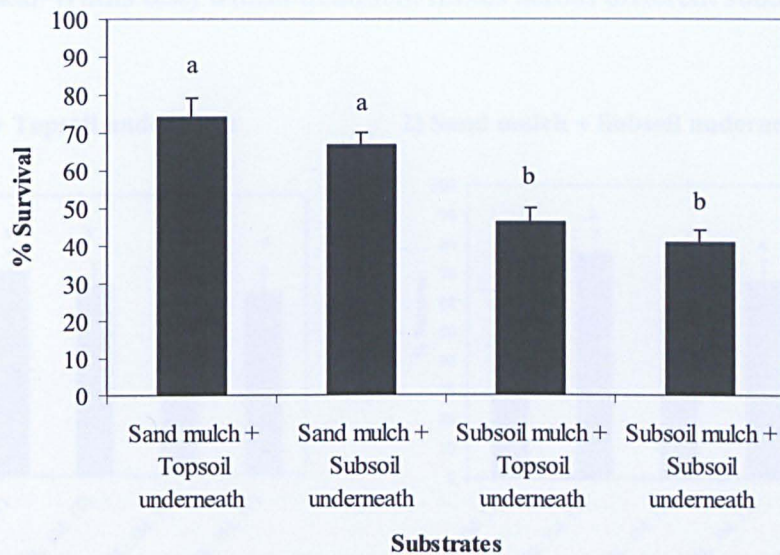


### 4) Subsoil mulch + Subsoil underneath

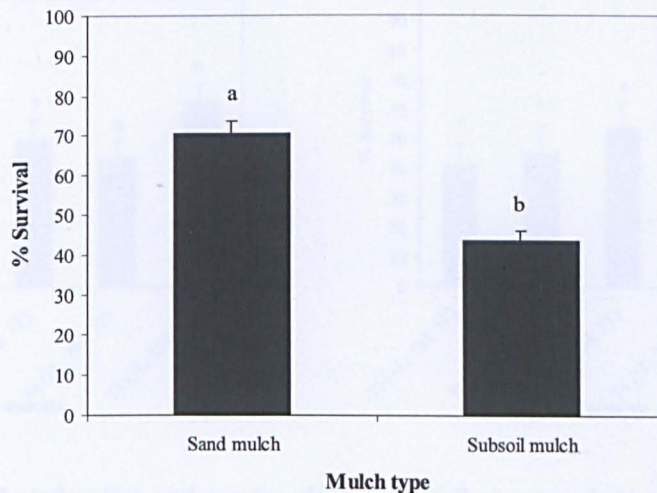


**Figure 5.18** Effect of seed ratios and sowing density on medium canopy plant survival in April 2007 (mean values for both densities). Bars labeled with the same letters are not significantly different at  $P=0.05$  (Kruskal-Wallis test). Error bars represent 1 S.E.M.

To assist data interpretation, percentage survival (as mean of all treatment mixes) was analysed in each substrate (Figure 5.19). Substrate had a marked influence on survivorship of medium canopy forbs ( $P=0.002$ ; Kruskal-Wallis test). Seedlings survival (as mean of all treatment mixes) (Figure 5.20) was greater on sand mulched (70.36%) than subsoil mulched (43.42%) ( $P=0.001$ , Mann-Whitney  $U$ -test).



**Figure 5.19** Effect of substrate on percentage survival of medium canopy plant communities in April 2007. Bars labeled with different letters are significantly different at  $P=0.01$  (Kruskal-Wallis test, pairwise Mann-Whitney  $U$ -test). Error bars represent 1 S.E.M.



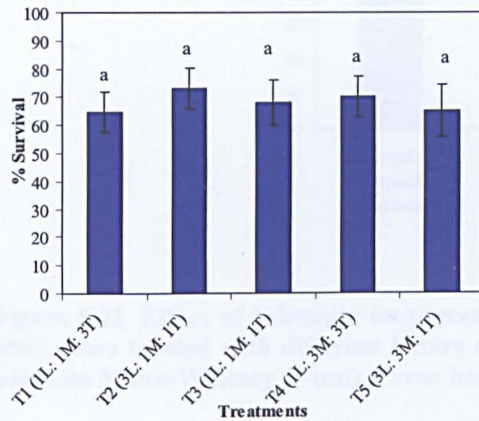
**Figure 5.20** Effect of mulching type on percentage survival of medium canopy plant communities in April 2007. Bars labeled with different letters are significantly different at  $P=0.001$  (Mann-Whitney  $U$ -test). Error bars represent 1 S.E.M.

### 5.3.6.1.3 Tall canopy forbs

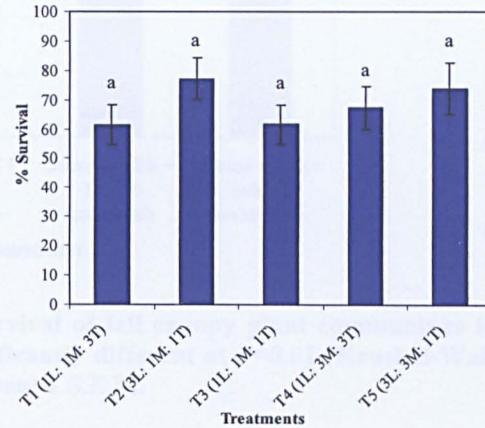
The Mann-Whitney  $U$ -test found that data was not statistically different between low and high density treatments (see Appendix Figure A5.4), so data was pooled across

density treatments (Figure 5.21). In all cases, there was no statistical significance ( $P=0.05$ , Kruskal-Wallis test) within treatment mixes across different substrates tested.

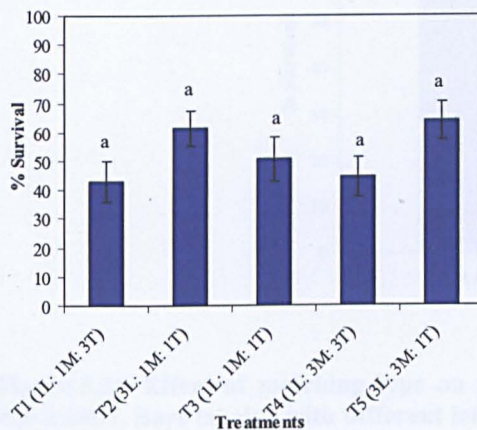
### 1) Sand mulch + Topsoil underneath



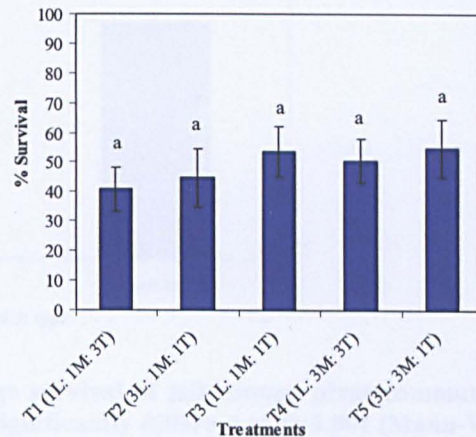
### 2) Sand mulch + Subsoil underneath



### 3) Subsoil mulch + Topsoil underneath



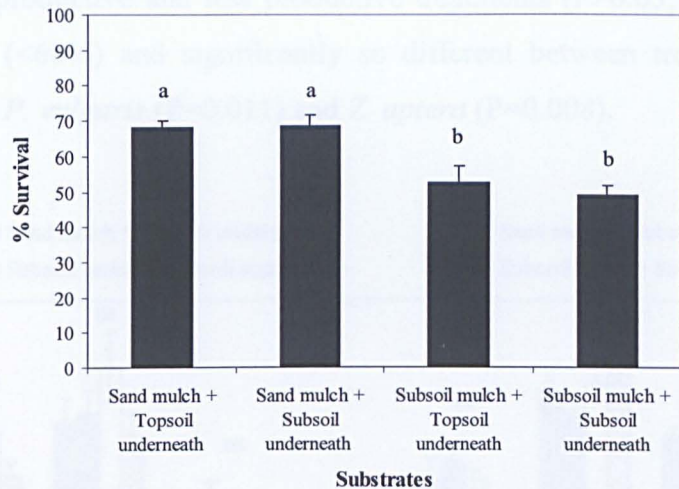
### 4) Subsoil mulch + Subsoil underneath



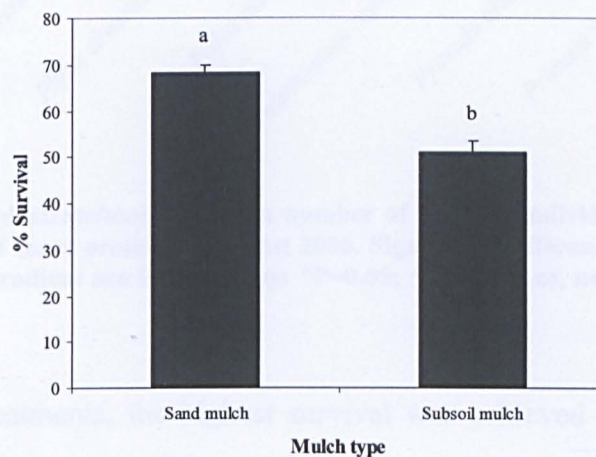
**Figure 5.21** Effect of seed ratios and sowing density on tall canopy plant survival in April 2007 (mean values for both densities). Bars labeled with the same letters are not significantly different at  $P=0.05$  (Kruskal-Wallis test). Error bars represent 1 S.E.M.

To assist data interpretation, percentage survival (as mean of all treatment mixes) was analysed in each substrate (Figure 5.22). Substrate had a marked influence on survivorship of tall canopy forbs ( $P=0.004$ ; Kruskal-Wallis test). Seedlings survival (as mean of all treatment mixes) (Figure 5.23) was greater on sand mulch (68.30%) than subsoil mulch (50.89%) ( $P=0.001$ , Mann-Whitney  $U$ -test).





**Figure 5.22** Effect of substrate on percentage survival of tall canopy plant communities in April 2007. Bars labeled with different letters are significantly different at  $P=0.01$  (Kruskal-Wallis test, pairwise Mann-Whitney  $U$ -test). Error bars represent 1 S.E.M.



**Figure 5.23** Effect of mulching type on percentage survival of tall canopy plant communities in April 2007. Bars labeled with different letters are significantly different at  $P=0.001$  (Mann-Whitney  $U$ -test). Error bars represent 1 S.E.M.

### 5.3.6.2 Survival of individual sown forbs in April 2007 as a percentage of those present in September 2006

#### 5.3.6.2.1 Low canopy, understory forbs

Substrate had a significant effect in many understory forbs in terms of number of plants present in quadrat by April 2007 compared with September 2006 (Figure 5.24). Survival was generally highest (>60%) in *D. meadia*, *L. vernus*, *P. elatior* and *P.*

*vulgaris* in both productive and less productive treatments ( $P > 0.05$ , ns). Survival was generally lowest ( $< 60\%$ ) and significantly so different between treatments in *Phlox pilosa* ( $P = 0.006$ ), *P. vulgaris* ( $P = 0.011$ ) and *Z. aptera* ( $P = 0.008$ ).

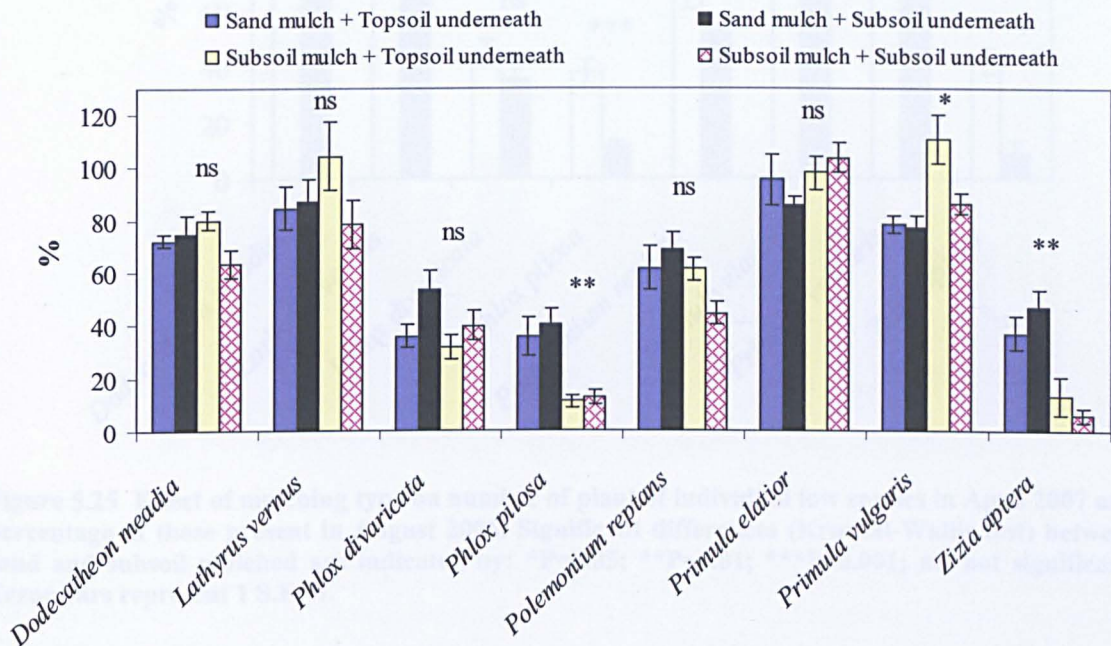


Figure 5.24 Effect of substrate/mulch type on number of plant of individual low species in April 2007 as a percentage of those present in August 2006. Significant differences (Kruskal-Wallis test) between productivity gradient are indicated by: \* $P < 0.05$ ; \*\* $P < 0.01$ ; ns, not significant. Error bars represent 1 S.E.M.

Within mulching treatments, the highest survival was achieved by using sand mulch significantly ( $P < 0.05$ ) in 4 out of 8 species (Figure 5.25). Other species which showed high survival ( $> 90\%$ ) on subsoil mulch were *L. vernus*, *P. elatior* and *P. vulgaris*. *Primula elatior* showed 'survival' in excess of 100% due to active recruitment from initial seed-sown on a subsoil mulched plot.

In all cases (with the exception of *P. vulgaris* in subsoil mulch + topsoil underneath), there was no statistically significant differences ( $P = 0.05$ , Kruskal-Wallis test) within treatment mixes between the two productivity treatments (see Appendix Figure A5.5).

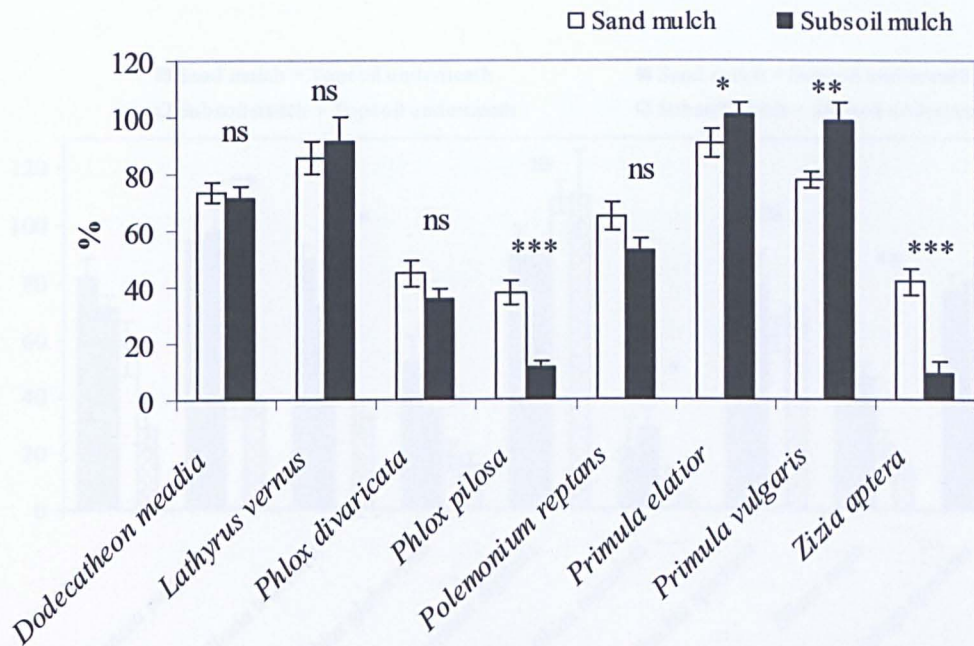


Figure 5.25 Effect of mulching type on number of plant of individual low species in April 2007 as a percentage of those present in August 2006. Significant differences (Kruskal-Wallis test) between sand and subsoil mulched are indicated by: \* $P=0.05$ ; \*\* $P=0.01$ ; \*\*\* $P=0.001$ ; ns, not significant. Error bars represent 1 S.E.M.

### 5.3.6.2.2 Medium canopy forbs

Substrate treatment had a significant effect in many medium prairie forbs in terms of number of plant present in quadrats by April 2007 compared with September 2006 (Figure 5.26). Survival was generally highest (60%) on sand mulch, significantly so ( $P<0.05$ ), in 7 out of 9 species (mainly on topsoil underneath). Survival was generally lowest (<60%) in all species (with the exception of *Penstemon digitalis* and *R. speciosa*) on subsoil mulch.

Within mulching treatments, highest survival was achieved by using sand mulch (with the exception of *P. digitalis*) significantly so ( $P<0.05$ ), in 8 out of 9 species (Figure 5.27). *Penstemon digitalis* showed 'survival' in excess of 100% due to active recruitment from initial seed-sown on subsoil mulched plots.

In all cases (with the exception of *Phlox maculata* in sand mulch + topsoil underneath and *R. speciosa* in sand mulch + subsoil underneath), there was no statistical significance ( $P=0.05$ , Kruskal-Wallis test) within treatment mixes in the two productivity treatments (see Appendix Figure A5.6).

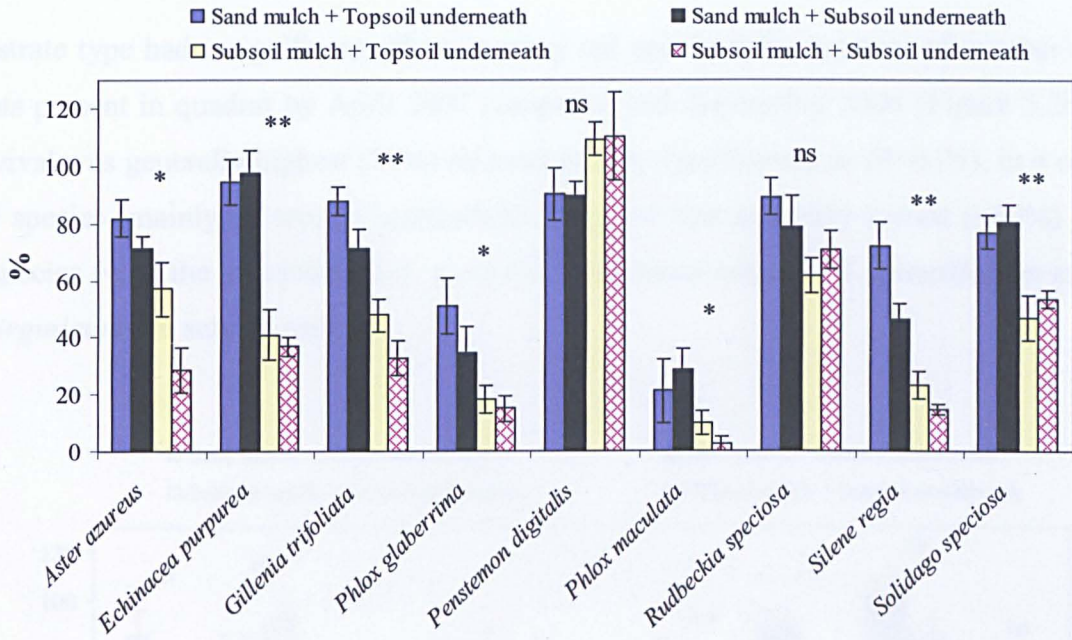


Figure 5.26 Effect of substrate type on number of plant of individual medium species in April 2007 as a percentage of those present in August 2006. Significant differences (Kruskal-Wallis test) between productivity gradient are indicated by: \*P=0.05; \*\*P=0.01; ns, not significant. Error bars represent 1 S.E.M.

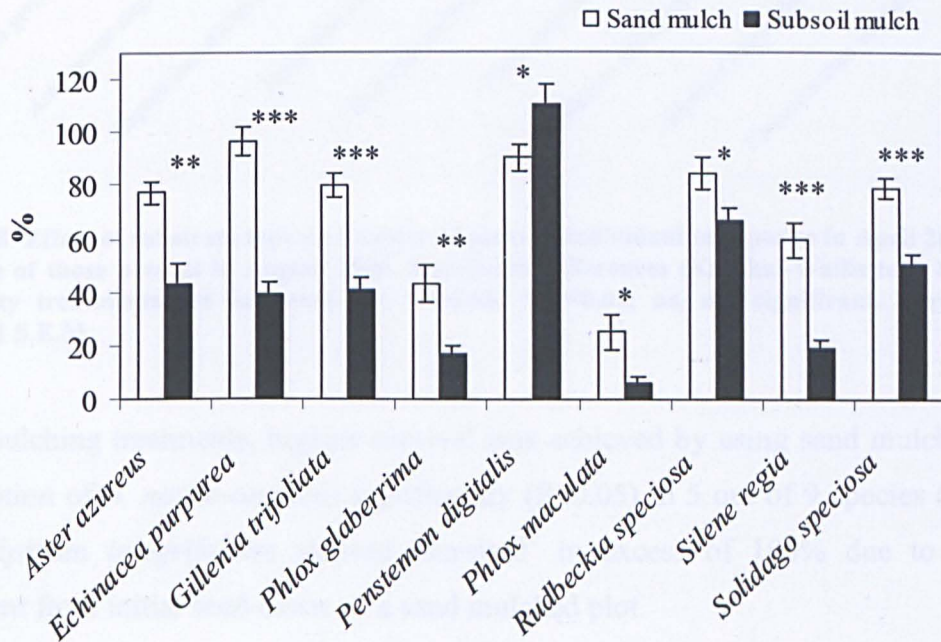


Figure 5.27 Effect of mulching type on number of plant of individual medium species in April 2007 as a percentage of those present in August 2006. Significant differences (Kruskal-Wallis test) between sand and subsoil mulched are indicated by: \*P=0.05; \*\*P=0.01; \*\*\*P=0.001; ns, not significant. Error bars represent 1 S.E.M.

### 5.3.6.2.3 Tall canopy forbs

Substrate type had a significant effect in many tall prairie forbs in terms of number of plants present in quadrat by April 2007 compared with September 2006 (Figure 5.28). Survival was generally highest (75%) on sand mulch, significantly so ( $P < 0.05$ ), in 4 out of 9 species (mainly on topsoil underneath). Survival was generally lowest ( $< 60\%$ ) in all species (with the exception of *A. gerardii*, *Aster novae-angliae*, *S. integrifolium* and *V. virginicum*) on subsoil mulch.

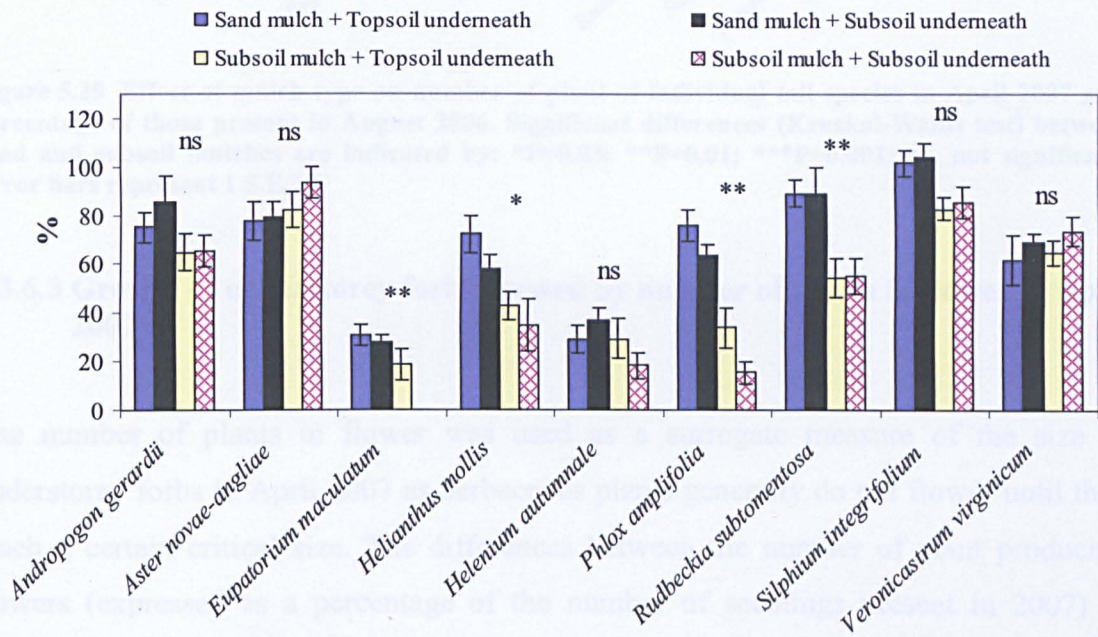


Figure 5.28 Effect of substrate type on number of plant of individual tall species in April 2007 as a percentage of those present in August 2006. Significant differences (Kruskal-Wallis test) between productivity treatments are indicated by: \* $P=0.05$ ; \*\* $P=0.01$ ; ns, not significant. Error bars represent 1 S.E.M.

Within mulching treatments, highest survival was achieved by using sand mulch (with the exception of *A. novae-angliae*) significantly ( $P < 0.05$ ) in 5 out of 9 species (Figure 5.29). *Silphium integrifolium* showed 'survival' in excess of 100% due to active recruitment from initial seed-sown on a sand mulched plot.

In all cases (with the exception of *Rudbeckia subtomentosa* in sand mulch + subsoil underneath), there was no statistically significant differences ( $P=0.05$ , Kruskal-Wallis test) within treatment mixes in the two productivity treatments (see Appendix Figure A5.7)

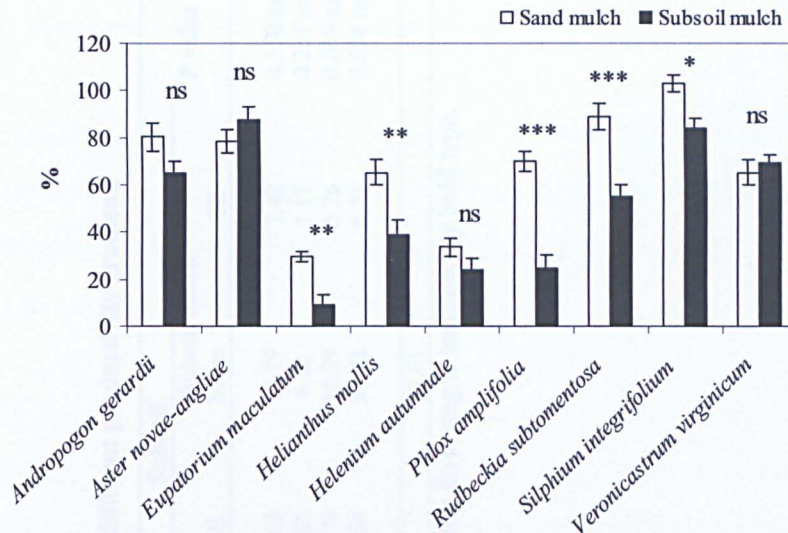


Figure 5.29 Effect of mulch type on number of plant of individual tall species in April 2007 as a percentage of those present in August 2006. Significant differences (Kruskal-Wallis test) between sand and subsoil mulches are indicated by: \* $P=0.05$ ; \*\* $P=0.01$ ; \*\*\* $P=0.001$ ; ns, not significant. Error bars represent 1 S.E.M.

### 5.3.6.3 Growth of understorey forb assessed by number of plants in flower in April 2007

The number of plants in flower was used as a surrogate measure of the size of understorey forbs in April 2007 as herbaceous plants generally do not flower until they reach a certain critical size. The differences between the number of plant producing flowers (expressed as a percentage of the number of seedlings present in 2007) in topsoil and subsoil with sand/subsoil mulch were analysed. The Mann-Whitney  $U$ -test found that data was not statistically different between low and high density treatments (see Appendix Table A5.1), so data was pooled across density treatments as shown in Table 5.8. The percentage of flowering plants (as mean of all species in all treatment mixes) was greater on subsoil mulch + topsoil underneath (18.30%) than sand mulch + subsoil underneath (12.37%), but not significantly different ( $P=0.248$ , Mann-Whitney  $U$ -test). Nor were they significantly different ( $P=0.618$ , Kruskal-Wallis test) between the productivity treatments. These tests involved the following combinations:

- subsoil mulch + topsoil underneath (18.30%)
- subsoil mulch + subsoil underneath (17.61%).
- sand mulch + topsoil underneath (13.33%)
- sand mulch + subsoil underneath (12.37%)

**Table 5.8** Mean percentage of plants in flower (pooled across density treatments) of understory forb in response to different productivity gradient.

Species	Topsoil					Subsoil				
	Sand mulch		Subsoil mulch		<i>P</i> value	Sand mulch		Subsoil mulch		<i>P</i> value
	Mean	SE	Mean	SE		Mean	SE	Mean	SE	
<i>Lathyrus vernus</i>	16.75	5.06	<b>23.63</b>	5.11	0.156 ns	10.42	4.03	19.79	7.42	0.330 ns
<i>Polemonium reptans</i>	<b>20.77</b>	5.93	12.42	4.22	0.455 ns	14.81	4.85	6.82	3.11	0.231 ns
<i>Primula elatior</i>	6.74	3.02	<b>11.93</b>	3.70	0.132 ns	4.25	2.79	10.39	3.76	0.069 ns
<i>Primula vulgaris</i>	10.00	4.21	25.22	5.59	0.382 ns	20.00	5.56	<b>33.42</b>	6.21	0.098 ns
Mean across species	13.33		<b>18.30</b>			12.37		17.61		

Significant differences (Mann-Whitney *U*-test) between soil plus mulch type are indicated by asterisks; ns: not significant. Greatest flowering is indicated by bold type.

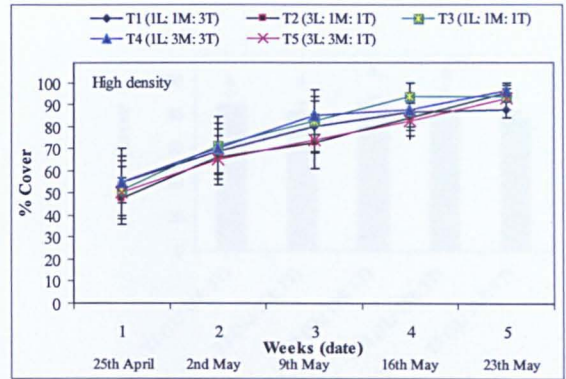
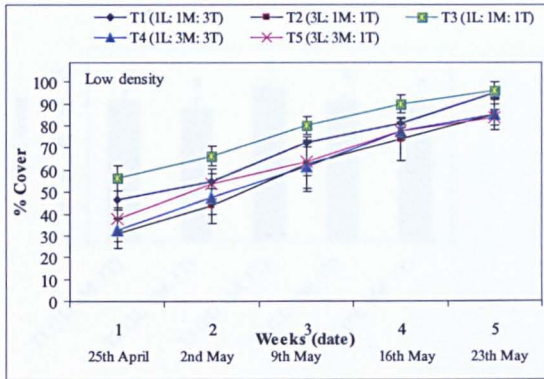
#### 5.3.6.4 Cover values in the second year (2007).

Cover values were recorded at weekly intervals between April to May 2007 and the results are presented in Figure 5.30. After 5 weeks observation, both in low and high density treatments, plants on the productive site (sand mulch + topsoil underneath) in all treatment mixes covered more than 70% of the area.

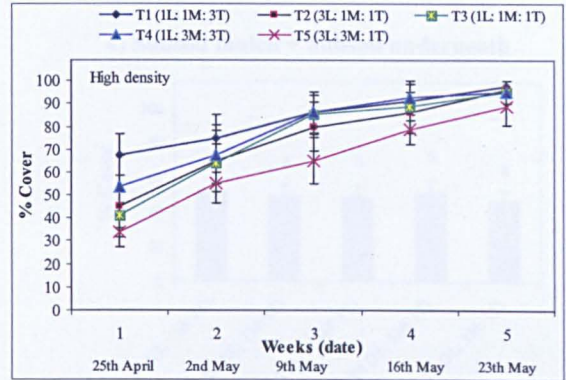
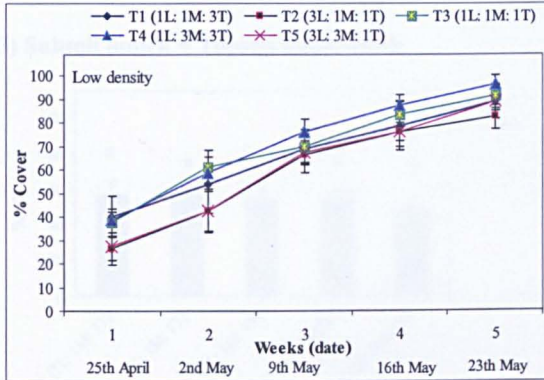
To assist data interpretation, cover values for 16th May 2007 were analysed for the substrate treatments. The Mann-Whitney *U*-test indicated that density was not statistically significant ( $P > 0.05$ , ns) between low and high density treatment (see Appendix Figure A5.8), hence analysis was undertaken using mean coverage values for both density (Figure 5.31). There were no significant differences between treatments.



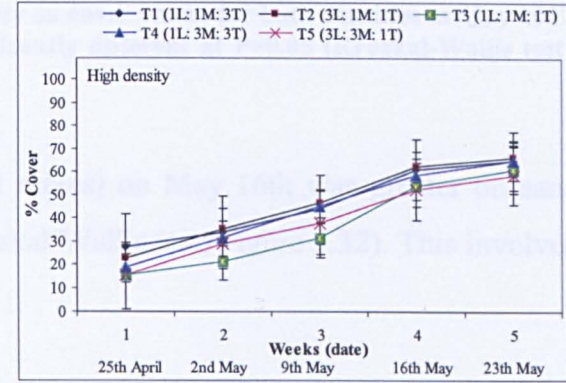
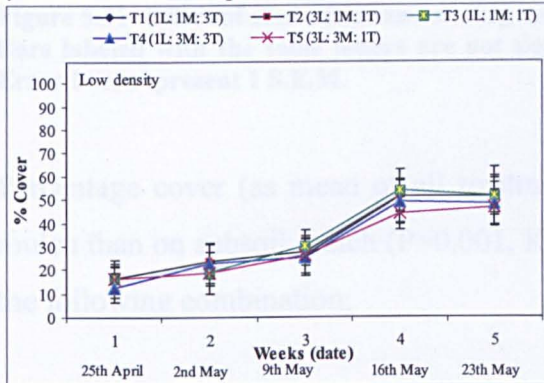
1) Sand mulch + Topsoil underneath



2) Sand mulch + Subsoil underneath



3) Subsoil mulch + Topsoil underneath



4) Subsoil mulch + Subsoil underneath

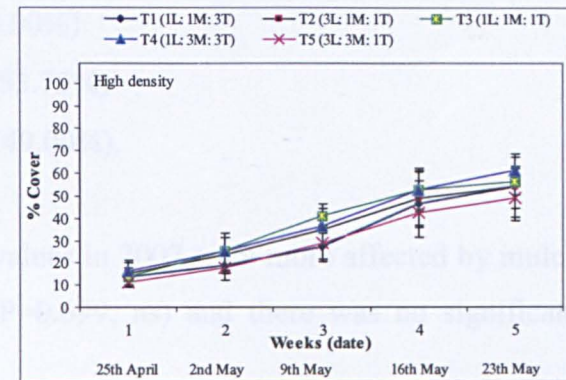
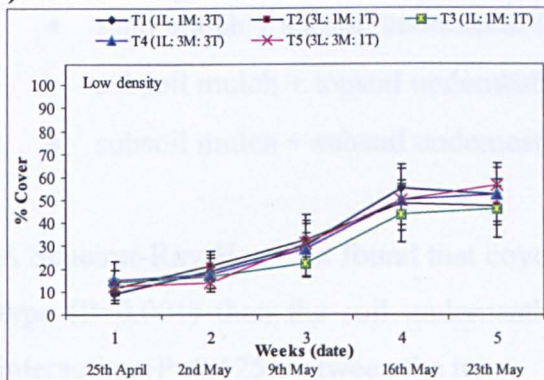
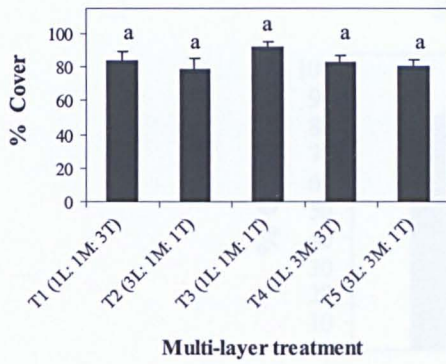
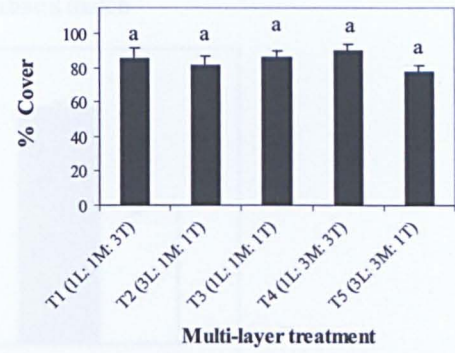


Figure 5.30 Effect of weeks and treatment mixes on plant coverage in April-May 2007. Error bars represent 1 S.E.M.

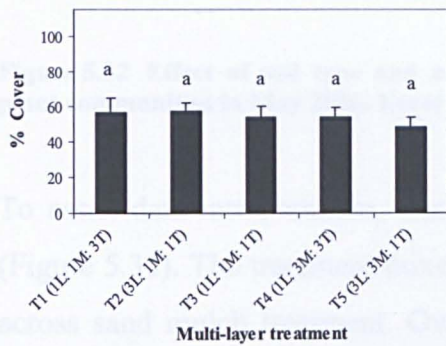
## 1) Sand mulch + Topsoil underneath



## 2) Sand mulch + Subsoil underneath



## 3) Subsoil mulch + Topsoil underneath



## 4) Subsoil mulch + Subsoil underneath

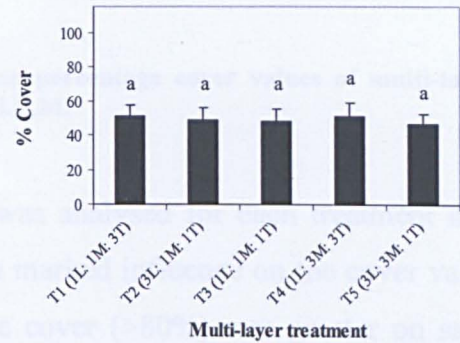
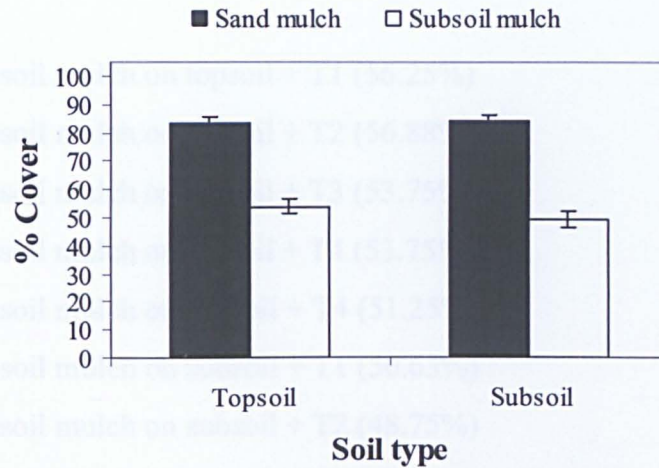


Figure 5.31 Effect of seed ratios and sowing density on cover values for both densities in May 2007. Bars labeled with the same letters are not significantly different at  $P=0.05$  (Kruskal-Wallis test). Error bars represent 1 S.E.M.

Percentage cover (as mean of all treatment mixes) on May 16th was greater on sand mulch than on subsoil mulch ( $P=0.001$ , Kruskal-Wallis test; Figure 5.32). This involved the following combination;

- sand mulch + topsoil underneath (83.38%)
- sand mulch + subsoil underneath (84.00%)
- subsoil mulch + topsoil underneath (53.75%)
- subsoil mulch + subsoil underneath (49.00%).

A Scheirer-Ray-Hare test found that cover values in 2007 were more affected by mulch type ( $P=0.001$ ) than the soil underneath ( $P=0.699$ , ns) and there was no significant interaction ( $P=0.625$ ) between the two.



**Figure 5.32** Effect of soil type and mulch treatments on percentage cover values of multi-layer plant communities in May 2006. Error bars represent 1 S.E.M.

To assist data interpretation, mean cover value was analysed for each treatment mix (Figure 5.33). The treatment mixes did not have a marked influence on the cover value across sand mulch treatment. Overall, percentage cover (>80%) was greater on sand mulch ( $P=0.108$ , ns) than on subsoil mulch ( $P=0.887$ , ns) within the treatment mixes (Kruskal-Wallis test). The most successful combination of multi-layer cover values in May 2007 involved sand mulch;

- sand mulch on topsoil + T3 (91.88%)
- sand mulch on subsoil + T4 (90.00%)
- sand mulch on subsoil + T3 (86.25%)
- sand mulch on subsoil + T1 (85.00%)
- sand mulch on topsoil + T1 (83.75%)
- sand mulch on topsoil + T4 (82.50%)
- sand mulch on subsoil + T2 (81.25%)
- sand mulch on topsoil + T5 (80.00%)

The least successful treatment combinations (<65% cover) involved subsoil mulch;

- subsoil mulch on topsoil + T1 (56.25%)
- subsoil mulch on topsoil + T2 (56.88%)
- subsoil mulch on topsoil + T3 (53.75%)
- subsoil mulch on topsoil + T4 (53.75%)
- subsoil mulch on subsoil + T4 (51.25%)
- subsoil mulch on subsoil + T1 (50.63%)
- subsoil mulch on subsoil + T2 (48.75%)
- subsoil mulch on topsoil + T5 (48.13%)
- subsoil mulch on subsoil + T3 (48.13%)
- subsoil mulch on subsoil + T5 (46.25%)

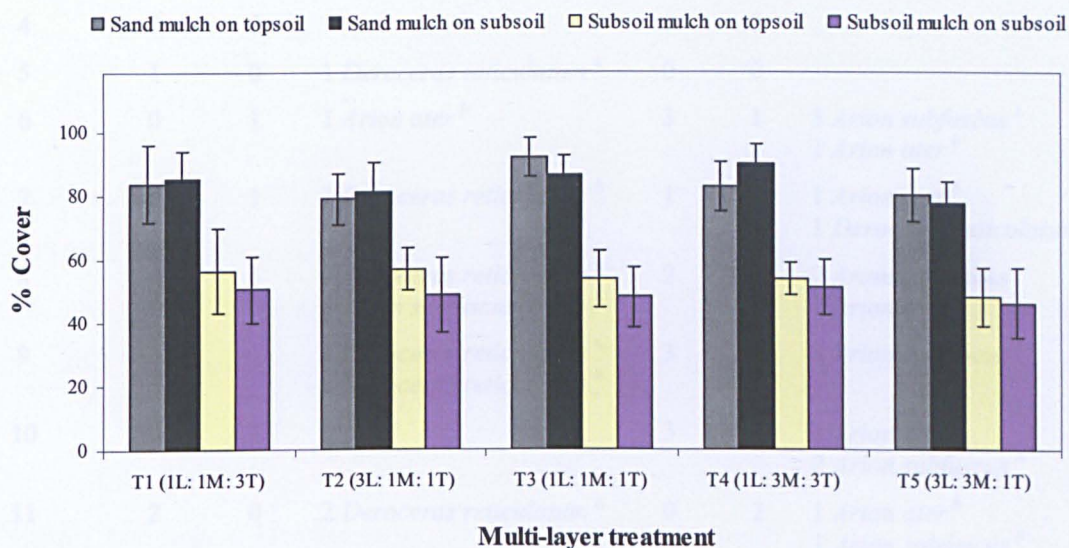


Figure 5.33 Effect of multi-layer treatments and mulch on topsoil/ subsoil on multi-layer cover in May 2007. Error bars represent 1 S.E.M.

### 5.3.6.5 Effect of slug grazing by June 2007

The influence of vegetation establishment was further studied by evaluating the effect of slug grazing on sand and subsoil mulch in each treatment plot. Counts of the number of slugs present in shelters placed in treatment plots confirmed that the distribution of slugs were associated with mulch type (Table 5.9). Slug density was higher on subsoil

mulch than on sand mulch, significantly different ( $P < 0.05$ ) in dry weather. The effect of the higher intensity of slug predation on subsoil mulch plots was dramatic; by June 2007 the most palatable species had almost disappeared. On adjacent sand mulch plots, the same species were largely ungrazed (Figure 5.34).

**Table 5.9** Numbers of slug on the experiment plot on a wet and a dry day in June 2007.

Sampling area (across treatment plot)	No of slugs in shelters on sand mulch		Species	No of slugs in shelters on subsoil mulch		Species
	Dry	Wet		Dry	Wet	
1	0	1	1 <i>Deroceras reticulatum</i> <sup>b</sup>	2	0	2 <i>Arion intermedius</i> <sup>a</sup>
2	0	1	1 <i>Deroceras reticulatum</i> <sup>b</sup>	2	0	1 <i>Arion ater</i> <sup>a</sup> 1 <i>Arion ater</i> <sup>a</sup>
3	0	0		1	2	1 <i>Arion ater</i> <sup>a</sup> 2 <i>Arion subfuscus</i> <sup>b</sup>
4	0	0		0	0	
5	1	0	1 <i>Deroceras reticulatum</i> <sup>a</sup>	0	0	
6	0	1	1 <i>Arion ater</i> <sup>b</sup>	3	1	3 <i>Arion subfuscus</i> <sup>a</sup> 1 <i>Arion ater</i> <sup>b</sup>
7	0	1	1 <i>Deroceras reticulatum</i> <sup>b</sup>	1	1	1 <i>Arion ater</i> <sup>a</sup> 1 <i>Deroceras reticulatum</i> <sup>b</sup>
8	2	1	2 <i>Deroceras reticulatum</i> <sup>a</sup> 1 <i>Arion subfuscus</i> <sup>b</sup>	2	1	2 <i>Arion subfuscus</i> <sup>a</sup> 1 <i>Arion ater</i> <sup>b</sup>
9	2	1	2 <i>Deroceras reticulatum</i> <sup>a</sup> 1 <i>Deroceras reticulatum</i> <sup>b</sup>	3	0	3 <i>Arion subfuscus</i> <sup>a</sup>
10	0	0		3	0	1 <i>Arion ater</i> <sup>a</sup> 2 <i>Arion subfuscus</i> <sup>a</sup>
11	2	0	2 <i>Deroceras reticulatum</i> <sup>a</sup>	0	2	1 <i>Arion ater</i> <sup>b</sup> 1 <i>Arion subfuscus</i> <sup>b</sup>
12	0	1	1 <i>Arion subfuscus</i> <sup>b</sup>	1	1	1 <i>Arion subfuscus</i> <sup>a</sup> 1 <i>Arion ater</i> <sup>b</sup>
13	0	0		2	1	1 <i>Arion ater</i> <sup>a</sup> 1 <i>Arion subfuscus</i> <sup>a</sup> 1 <i>Arion ater</i> <sup>b</sup>
14	0	0		1	0	1 <i>Arion subfuscus</i> <sup>a</sup>
15	1	0	1 <i>Deroceras reticulatum</i> <sup>a</sup>	2	0	1 <i>Arion ater</i> <sup>a</sup> 1 <i>Arion ater</i> <sup>a</sup>
16	0	0		0	0	
<b>TOTAL</b>	<b>8</b>	<b>7</b>		<b>23</b>	<b>9</b>	

<sup>a</sup> Dry condition; <sup>b</sup> wet condition. Slug numbers on sand and subsoil mulch are significantly different at  $P = 0.012$  in dry and not significantly different at  $P = 0.764$  in wet conditions (Mann-Whitney  $U$ -test).



Figure 5.34 The effect of predation during the establishment of plants in the second year growing (May 2007); plants growth is much more abundant on sand mulch (left view) than on subsoil mulch (right view) in part due to slug grazing.

## 5.4 Discussion

### 5.4.1 Effect of mulch and topsoil v subsoil on overall percentage emergence in the first year growth (2006)

This study showed that after winter sowing, percentage emergence was greater on sand mulch (6.10%) than subsoil mulch (4.14%). However, the percentage emergence was considerably lower than reported by Hitchmough (2004) for winter sown seed on sand mulch (25.1%), possibly due to poorer seed quality and less favourable environmental conditions. Emergence is strongly affected by seed quality (Kolasinska et al., 2000; Lehtila and Ehrlen, 2005) and environmental factors such as rainfall/irrigation (Pelaez et al., 1996), soil type (Forcella et al., 2000), predation (Clarke and Davison, 2004) and temperature (Forcella et al., 2000; Hardegree and Van Vactor, 2000; Shimono and Washitani, 2004). In this study attempts were made to minimise predation by regular baiting with metaldehyde. This suggests that lower emergence was substantially due to weather in March to May 2006. Due to a very late cold March, germination was pushed back into late spring coinciding with a period of hot and dry weather, leading to either death of seedlings at germination, or a period of induced dormancy, as has previously been commented on for *P. digitalis*. Hitchmough (2004) proposed that the different emergence levels between sand and subsoil mulch are due to soil moisture stress. In establishing prairie vegetation in commercial landscape practice (Hitchmough, 2007) sand mulches are covered with jute erosion matting to reduce the rate of drying out after

rain or irrigation, but this was not undertaken in this study.

Although subsoil mulch has the highest water holding capacity and is able to maintain good seed-soil contact to increase emergence (see Table 5.2), there was a lower emergence recorded in this study. The penetrative resistance of the subsoil clay used as a mulch was observed to become very high upon drying, and this was probably a significant issue during the hot dry weather experienced. As the subsoil dried it cracked heavily creating an extensive habitat for herbivorous invertebrates. Even though the site was baited with metaldehyde pellets at regular intervals, there were clear signs of ongoing seedling predation.

The soil below the mulch had little apparent effect on seedling emergence. According to Wilson and Gerry (1995); soil moisture stress is the most potent factor influencing the success or failure emergence of prairie seedlings. In this study the moisture supplying capacity of the understorey soil probably was insufficiently different to have a marked effect on the mulch layer above. Overall, this study agrees that sowing mulches (Hitchmough et al., 2004) and soil moisture stress (Tobe et al., 2005; Colbach et al., 2006) are key factors in successful seedling emergence.

#### 5.4.2 Effect of mulch and topsoil v subsoil on emergence of individual species

The emergence of *E. purpurea*, *G. trifoliata* and *S. integrifolium* was significantly affected by mulch type with emergence greatest on sand mulched plots. As can be seen from Figure 5.5 *E. purpurea* and *G. trifoliata* survival was significantly increased by sand mulching suggesting that they are highly palatable to slugs. The same data shows that *Silphium* is not palatable to slugs as an adult, (although it may be palatable at the cotyledon stage). The large seeds of *Silphium* may have difficulties in emerging on the compacted subsoil clay. An alternative hypothesis is that these species demonstrate superior emergence on the driest substrates (sand), however it seems likely that mollusc predation is the key factor.

The rest of the species in this study showed no significant difference in emergence in response to mulch type, with low percentage emergence which suggests that several factors such as low seed viability, soil moisture stress and pre-chilling regimes might

contribute to low species emergence. Ahmad and Hitchmough (2007) found that germinability of some of the species used in this study derived from the North American prairie seed industry, was very low.

In the current study, the underlying soil only had a significant effect on seedling emergence in *D. meadia* on sand mulch, and *H. autumnale* on subsoil mulch. This suggests that generally the soil underneath has little effect on individual emergence. There are no published data specifically on soil requirement for emergence in *D. meadia* and *H. autumnale*, however, it has been reported that emergence of *D. meadia* was studied in woodland soil habitat and influenced by temperature and chilling period (Turner and Quarterman, 1968) but these are clearly not critical factors in this study.

#### **5.4.3 Effect of mulch and topsoil v subsoil on growth of multi-layer community in the first year growth (2006)**

In the first growing season, plant growth (in terms of cover values in 2006) as a whole was affected by mulch type. Surprisingly the soil underneath the mulch did not have a significant effect on plant cover. Cover values were greatest on sand mulch with topsoil underneath than those growing in subsoil. This highlights that the seedlings were able to respond to some degree to the productive topsoil where weed competition was prohibited by sand mulching. The response was not however significantly greater than that occurring on subsoil. However, individual plants grown on subsoil were considerably slower and smaller compared to those on sand mulch with topsoil laid underneath. They were able to grow on the unproductive subsoil without demonstrating symptoms of stress or nutrient deficiency. This result is similar to that recorded in previous studies on prairie vegetation (Hitchmough and de La Fleur, 2006). By October 2006 the most vigorous prairie plants were between 300 and 800 mm tall and many *H. autumnale*, *P. amplifolia*, *R. speciosa*, *R. subtomentosa*, *S. integrifolium* plus individuals of *Aster azureus*, *A. novae-angliae*, *E. purpurea*, *Eupatorium maculatum*, *Helianthus mollis* and *S. regia* were flowering.



#### 5.4.4 Effect of mulch and topsoil/subsoil on plant survival in the second year (2007)

In the second growing season, the combination of mulching and soil underneath had a significant effect on percentage plant survival (as mean of all species). The combination of sand mulch + topsoil/subsoil underneath was most likely to improve plant survival in understorey forbs and medium-tall prairie forbs. Survival of these forbs on sand mulch with topsoil underneath was essentially the same as subsoil with sand mulch suggesting that survival is primarily associated with the mulch.

By May 2007, survival of both medium and tall prairie forbs was significantly greater ( $P=0.001$  Mann-Whitney  $U$ -test) on sand mulched plots. The same was true for understorey (Figure 5.17). The most compelling explanation for this is that sand mulch reduced slug grazing. In horticultural practice, coarse sands have been used as a physical barrier around garden plants that are palatable to slug predation (Halstead, 1999). Slugs are deterred by a gritty, granular element on the soil surface. In a previous study, Hitchmough (2006) found that most of the prairie plants did not exhibit mollusc damage on sand mulched plots, suggesting that mulch type and predation are key issues in establishing plants in the field.

Survival of individual species largely reflects the trends shown for each group of forbs tested. The highest survival in understorey forbs was achieved with 5 out of the 8 species (Figure 5.25), although not always statistically so, on sand mulch. Again on sand mulch, highest survival was achieved with 8 out of the 9 species in medium canopy forbs, significantly ( $P<0.05$ ) in all species (Figure 5.27) and 7 out of 9 species in tall canopy forbs (Figure 5.29). As a whole, understorey species; *D. meadia*, *L. vernus*, *P. elatior*, and *P. vulgaris*, and medium-tall prairies; *P. digitalis*, *A. novae-angliae* and *S. integrifolium* achieved high survival (>65%) both on sand and subsoil mulch, suggesting that they are either tolerant to mollusc grazing or highly unpalatable. In contrast to sand, subsoil mulch provides no deterrent to slugs particularly in spring because of its high water holding capacity.

There was also low survival (<40%) however on sand mulched treatments, for example in the case of medium prairie species *P. pilosa* and *P. maculata*, and tall prairies *E.*

*maculatum* and *H. autumnale*, suggesting that such losses are due to overwintering/erosion, or grazing of early leaves (as in the case of the *Phlox*) that are produced early in the year when the chances of the sand surface remaining moist are high.

Although the overall trend was for sand mulch to increase survival, there was a substantial variation in the response in each plant group. This is summarised in Table 5.10. Understorey forbs typically performed well on both sand and subsoil mulch, which suggests that in contrast to prairie species they are relatively unpalatable to slugs and snails.

**Table 5.10** Categorisation of forbs in terms of treatment that best aided survival between 2006 and 2007, based on statistical significance shown in Figures 5.13, 5.16 and 5.19.

Treatment best	Plant group		
	Understorey forb	Medium forb	Tall forb
Sand mulch	√	√	√
Subsoil mulch	√		

#### 5.4.5 Effect of mulch and topsoil/subsoil on growth and flowering of multi-layer community in the second year growing season (2007)

In terms of cover values in 2007 plant growth was affected by mulch type. The underlying soil did not have a significant effect on plant cover. Cover values were greatest on sand mulch and topsoil. This highlights that generally the communities responded to the productive substrate. This pattern is similar with the results recorded in 2006.

In April 2007, plants growth on subsoil was considerably slower and smaller compared to those on sand mulched with topsoil underneath. The most vigorous prairie plants were less than 300 mm tall, compared with tall canopy species on sand mulched plots. At this time, some individual understorey forbs of *L. vernus*, *P. elatior*, *P. reptans* and *P. vulgaris* flowered most successfully on subsoil mulch, although there was no

significant difference on sand mulch. Subsoil seems to appear as a suitable mulch for growth and flowering of understorey forbs that are unpalatable to molluscs.

Response in terms of flowering as a measure of growth in 2007 in each plant group is summarised in Table 5.11.

**Table 5.11** Categorisation of forbs in terms of treatment that best aided establishment, based on plant flower in Table 5.9, plus statistical significance shown in Figures 5.13, 5.16 and 5.19.

Treatment	Plant group		
	Understorey forb (not palatable)	Medium forb (palatable)	Tall forb (palatable)
Sand mulch		√	√
Subsoil mulch	√		

#### 5.4.6 Effect of slug predation in 2007

This study suggests that survival and growth cannot be fully explained by the direct effects of mulch, soil types and plant competition, and highlights the major impact of predation. Sih et al. (1985) noted that '*predation includes any interaction in which energy flows from one organism to another*'. Some of the species used in this study such as *Echinacea*, *Helenium* and *Silene* are highly palatable to slugs and snails. As a result, the leaves of those species showed evidence of high grazing but more so for plants in subsoil mulch, than those in sand mulch plot. Measurement of slug densities across the experimental plot showed higher slug numbers on *Arion ater*, *Arion subfuscus*, and *Deroceras reticulatum* on subsoil mulch than on sand mulch (Table 5.9) particularly in dry if compared to wet conditions. This suggests that slug predation is one of the key factors in the decline in survival and growth in subsoil mulch.

On sand mulch treatment, less damage was observed on highly palatable species (with the highest survival and growth achieved as shown in Figures 5.20 and 5.32). This suggests that sand is a good mulching agent to prevent slug activities when creating multi-layer plant communities. This finding supports the previous studies by Hitchmough and de La Fleur (2006) that observed less plant damage from slug grazing with coarse sand mulching, thus improving plant persistence in the long term. Sand

mulch acts as an unfavourable surface for mollusc activities due to being deterred by gritty and/or granular materials.

#### **5.4.7 Effect of sowing ratios and density on seedling survival and growth (2006-2007)**

In the first year of growth, it was not possible to establish a significant statistical link between different sowing ratios in treatment mixes. This study showed that mean density on low and high after seedlings adjustment were; 170 and 260 seedlings/m<sup>2</sup> respectively. By October 2006, there was no statistical significance between low and high density treatments on seedling growth (in terms of cover values) in each treatment mix, across the productivity gradient. This suggests that the ranges of seedling densities used in the study are above the minimum threshold required to “close canopy”, and secondly are insufficiently different to have a significant impact on the measured parameters. Hitchmough and de la Fleur (2006) reported that high seedling density increased competitive elimination of slow growing, shade intolerant species. Since all of the understorey and some of the medium species possess shade tolerance, rapid changes in the study under discussion are unlikely to occur except when driven by predation. Several researchers reported that high sowing rates increase the establishment of sown species (Tilman, 1997; Fischbach et al., 2006), influence community composition (Zamfir and Goldberg, 2000) and reduce weed invasion (Stevenson et al., 1995). Generally, in the first growing year, resources such as light, water and nutrient have not yet been fully utilised by the tallest most vigorous plants. This “lag” effect has been described by Hitchmough and de La Fleur (2006). As competition for these resources increases in subsequent years it is anticipated that density effect will be more apparent.

### **5.5 Conclusion**

The results of this study show that despite the sensitivity of direct seeding to soil moisture stress during emergence this technique can be successfully used as an option to create multi-layer communities in urban parks and green spaces. The emergence estimates on which the seed mixes were formulated were based on responses in the previous microcosm experiment which involved a soil based substrate that was probably irrigated more frequently than was possible in this study. This, in combination with low quality seed of some species resulted in low establishment which was adjusted by

transplanting nursery grown seedlings.

The most significant treatment variable in terms of emergence and seedling survival in the first growing season was mulch type. Sand mulching was a key factor in improving emergence and establishment, probably through reduced weed competition, increased soil moisture content in the underlying soil due to the surface of the sand mulches strong physical discontinuity with the soil water phase, minimisation of slug predation and loss of small seedlings over-winter by rain erosion.

As in previous work with North American prairie species, this study has shown that in stark contrast to the understorey species (and particularly the European species) these are typically highly palatable to slugs. The long terms impact of this factor on the development of the multi-layer communities will be revealed in assessments over the next five years.

Although the vegetation developed as part of this study is still immature, the first year suggests that it is possible to successfully create a multi-layer communities from European and North American woodland understorey species beneath taller North American prairie species.

## CHAPTER 6: OVERALL DISCUSSION AND CONCLUSION

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### 6.1 Discussion

The work in this thesis has examined the development of multi-layer plant communities in urban parks and green spaces which can potentially provide strong visual impact through flowers in spring, summer and autumn. This final chapter reviews the findings of all the experiments that have been undertaken in this study including seed germination studies in response to different chilling treatments, microcosm studies into seedling survival in different sowing ratios and species mixtures, and field experiments into multi-layer community development on different substrate treatments. Based on these results, overall aspects of the development of multi-layer communities in urban parks and green spaces are discussed structured around a combination of the research questions and objectives specified in **Chapter 1** of this thesis and potential areas for future research suggested.

#### 6.1.1 Is it possible to create multi-layer herbaceous vegetation by sowing?

In many ways this is the fundamental question to answer. Whilst this question involves many different dimensions, overall the work in this thesis has shown that it is possible to create multi-layer herbaceous vegetation by sowing using species of shade tolerant and shade intolerant forbs. The long term success of established multi-layer plant communities is initially based on the capacity of seeds to germinate. As demonstrated in this study, under laboratory conditions (**Chapter 3**) many of these species have some form of endogenous dormancy and chilling is required to achieve satisfactory germination in all species except for the understorey forbs; *Montia sibirica* and *Polemonium reptans*. To achieve satisfactory germination in the field requires seed to be sown in winter to allow natural chilling of seed to occur. When this is not possible seed must be fridge chilled in moist sand prior to sowing. Of the 31 species tested, 18 species showed greatest germination after 60 and 90 days chilling. Highest germination under laboratory conditions was obtained in *Dodecatheon meadia* (96.67%). Fridge chilling is in some ways problematic in practice especially when dealing with mixtures of species some of which require lengthy and others brief chilling. Many understorey

species tend to germinate in the fridge at 4°C when chilled for longer than 60 day. Medium and tall canopy species of more open habitats were much less likely to do this. The strategy of germinating at low temperatures in the fridge is associated with the tendency to germinate in their habitat in early spring to facilitate establishment prior to the closure of the canopy of taller species. Not all understorey species responded in this way; *M. sibirica* and *P. reptans* were non-dormant, suggesting germination may occur in late summer after seed dispersal in their natural habitat.

In the laboratory experiment, chilling imbibed seed at 4°C was found to be an efficient techniques for seed germination in over 50% of the species tested, and 60 days chilling was the best compromise between breaking dormancy and preventing germination in the fridge. Thus, in practice to create multi-layer herbaceous vegetation by sowing, when natural chilling is not available, chilling should be restricted to 60 days to avoid germination within the fridge and the subsequent death of germinants when sown in landscape sites.

An alternative strategy to avoid the risk of germination within the chilling environment is to chill in batches of species with similar chilling requirements, co-ordinating timing of placement in the fridge to ensure that all seed can be removed from the fridge for sowing on the same day.

This study supplies the first published scientific data on chilling requirements for breaking dormancy and enhancing the germination of understorey and medium-tall prairie forbs under laboratory conditions hence allowing this latter strategy to be pursued. Emergence clearly varies substantially between species and some species are much more economic propositions for use in practice than others.

Coming back to the question of “Is it possible to create multi-layer herbaceous vegetation by sowing?”, whilst emergence was, given suitable pre-chilling generally satisfactory in the laboratory studies, it was far more sporadic in the microcosm study. Moving from the lab to microcosm involves reduced capacity to control environmental factors such as substrate type, rainfall/irrigation and moisture stress; key factors in determining successful seed emergence. Variability of seed quality also contributed to the capacity of seeds to germinate. These factors are typically most important at the

scale of field sowing, and this proved to be the case in this study with emergence in the field experiment considerably reduced from that in the microcosm/laboratory. Had the field experiment been undertaken in a real urban landscape in practice, it would have been considered to be a partial failure. Poor performance appears to be associated with the soil moisture stress, overly optimistic estimates of field emergence (based on performance in the less moisture stressed microcosms, and poor seed quality. Although the site was irrigated during emergence, the low water pressure at the site restricted this to watering cans, and the volume applied was probably too low bring the substrate to field capacity at each watering. Temperatures were unseasonably high during this time period, exacerbating this problem. Nor was jute erosion matting used in the study; Hitchmough (pers comm.) has found this improves reliability of emergence in practice. Given the large numbers of species involved in the mixes, the numbers of seed of each species sown into each treatment plots was often very low, with little buffering capacity given adverse climatic conditions. Higher sowing rates and more pessimistic emergence estimates would have improved success in the field experiment. Laboratory and microcosm tests are a poor indicator of field emergence.

As has been established by Ahmad and Hitchmough (2007) that quality of seed from the North American prairie industry is often variable and this confounds overly optimistic estimates of seed emergence in practice. Additional research is required on these factors before multi-layer prairie-woodland vegetation can be established with confidence in public or commercial landscapes. Given this however, the future for this type of vegetation seems positive.

### **6.1.2 Do fast growing species typically eliminate low, slow growing species?**

In the microcosm experiment (Chapter 4), survivorship of many of the understorey species declined significantly from 2005 to 2007 across all treatment mixes (see Figure 4.34). Mortality of low species within the medium and tall dominated communities was much less marked, with significant reductions in seedling numbers across the period restricted to a few species only. This suggests that decline is more marked when plants are subject to competition from within the low growing layer itself, and particularly the two community dominants *P. veris* and *P. vulgaris*. In the case of the understorey species *D. meadia* this very slow growing species was gradually eliminated by the early



spring presence of the leaf rosettes of the two dominant understorey species; *P. veris* and *P. vulgaris* during the first growing season. Hence it appears that tall fast growing species do not necessarily eliminate low growing species, that are shade tolerant. By the third growing season however, *Phlox divaricata* was largely eliminated ( $P < 0.05$ ) due to competition within all treatment mixes (Table 4.6). There was no evidence that predation played a significant role in the microcosm experiment. This suggests that this species was relatively poorly fitted to the competition regime within the experiment. The microcosms were however very uniform (within each treatment) in terms of species composition and density, and in a field situation this situation is less likely to occur, leading to patchier, more heterogeneous plantings with greater opportunities for subordinate species such as the *Phlox* and *Dodecatheon*.

To achieve successful multi-layer communities requires that high densities of large, fast growing species with dense basal foliage are avoided, especially during the first year. The potentially most competitive species (*S. integrifolium*) in the studies failed to establish in most of the microcosms and hence (with the exception of *D. meadia* and *P. divaricata*) most species competed and persisted effectively in the multi-layer communities in the microcosm, and also in the initial development of the field experiment.

In the microcosm experiment (**Chapter 4**), the hypothesis that survivorship is in the order; tall canopy forbs > medium canopy forbs > low canopy species, was not supported (Figure 4.34). Clearly in any layer on a given site some of the chosen species are going to be better fitted than others, leading to dominants and subordinates. The most important factor in creating sustainable multi-layer communities to ensure that at least a percentage of each layer is sufficiently well fitted to the site and sufficiently tolerant of competition with other species in the same and other layers to persist. In the longer term, in face of weed species invading from the outside, a low canopy layer which generates high cover values in spring, followed by medium-tall prairie forbs prairie which greatly increases standing biomass from summer to autumn will be crucial.

In the field experiment (**Chapter 5**), it was not possible to determine any significant statistical links between survival and competition with first and second year of growing season data. These effects may be able to be seen much more clearly after several years

of growth, as for example in work on prairie vegetation by Hitchmough and De La Fleur (2006). To date this study suggests that plant survival was primarily influenced by mulching type, with the nature of the soil laid underneath far less significant in terms of survival, rather than the composition of the plant community. In this study, in terms of application to practice, the most successful substrate treatments used to achieve high survival and initial growth were sand mulch + either topsoil or subsoil underneath. Subsoil mulch was much less satisfactory. Overall, treatment mixes achieved much faster coverage (in terms of cover values) on sand mulch, particularly with topsoil laid underneath, than on subsoil mulch. This suggests that plant competition was less intense on subsoil mulch (unproductive site) with slower coverage in these treatment mixes. This directly supports the hypothesis of Grime (1973) that competition intensity is much lower in unproductive than in productive sites. This may in turn prove to be beneficial in the future as a greater diversity of species may ultimately be able to survive under these less productive conditions.

On subsoil mulch, the poor survival of some species - especially highly palatable species such as *Echinacea purpurea* and *Silene regia* - were due to heavy slug grazing in spring. The surface of the subsoil remained moister for longer especially in spring or after rainfall, and this favoured slug grazing. As reported by Hitchmough et al. (2001) slug grazing reduced survival of seedlings and was the major factor affecting plant growth and survival in the sown community.

Although most species showed higher survival on sand mulch in the second growing season, survivorship varied from >100% for *S. integrifolium* to only 25% for *Phlox maculata*, with other species intermediate, suggesting that mortality was due to overwintering/rain erosion as has previously been reported by Hitchmough et al (2004).

#### 6.1.2.1 Effect of sowing ratios

Different sowing ratios of forbs did not affect the survival and growth of a given layer of plants; however in some cases the individual species was significantly affected. This suggests that the sowing ratios of low, medium and tall canopy layers used in the microcosm experiment were insufficiently 'stretched' to result in significant changes

between the layers. The limited range of ratios that were chosen is a reflection of the resources available to the experimenters. When considering all types of forbs used in this study, high survival and successful growth of many species in each canopy layer were achieved, especially where shade tolerant species (with the exception of *D. meadia*) were grown under a canopy of medium-tall species. The shade cast by taller canopy layers improves survival and growth of many understorey species in multi-layer community.

### 6.1.2.2 Effect of density

In Chapter 4 and 5, both high and low density mixes were studied. Over the course of these two studies there was no significant difference between these (in terms of seedling survival, biomass production and/or cover values) in each treatment mix, both in the microcosm and field experiments. This suggests that the higher of the two densities used in each experiment, (900 and 1500 seedlings/ m<sup>2</sup> in the microcosm and 170 and 260 seedlings/m<sup>2</sup> in the field experiment) were insufficiently high to result in significant changes in the previously mentioned community parameters. This is not surprising in the field experiment which was only in its second growing season and densities relatively low. Explaining why the much higher density microcosm experiments did not have obvious effects is more difficult. A possible explanation for this is that self thinning did occur but was masked by the expansion of individual plants and the difficulties of distinguishing between individuals and the multiple stems of clonal patches. Another contributor to the lack of apparent self thinning in the microcosm may be that the roots of the species grew through the membrane in the bottom of the tray and through the weed membrane beneath into the underlying soil. The tension in these membranes restrict root radial thickening and hence imposed substantial stress on plants and restricted individual biomass resulting in cohorts of “dwarfed” individuals to persist. Similar responses to this have been observed in other experiments which involve rooting through a membrane (Hitchmough, unpublished data).

Substantial regrowth appears to have occurred after the initial density correction thinning in April 2005 from decapitated ramets and also from delayed seedling emergence. This is reflected in survival of some species being >100%. In the field

experiment the effects of density are likely to be more marked as the vegetation develops and dominance comes into play. These effects will be monitored in the future.

### **6.1.3 Are multi-layer plant communities visually attractive, low maintenance and sustainable over a longer period?**

The multi-layer herbaceous vegetation created in this study was colourful for a substantial percentage of the time between spring to autumn. Significant impact was produced by low canopy layer forbs which flowered from March to the end of May, followed by a medium-tall canopy layer from mid-May to October, although most of the latter flowered, and were most dramatic between July and September. The multiple layer nature of the community was most obvious in early summer as the medium and tall species were emerging through the underlying layer of woodland species. Before and after this the community appears as a single layer, as the viewers eye reads the top of the predominant foliage canopy. With the shade tolerant species growing successfully under the canopy of medium-tall species, this structural and taxonomic arrangement creates a sound basis for sustainable plant community over a longer period.

Understorey species such as *Primula veris* and *Primula vulgaris* are evergreen and cover the soil during the winter and early spring, increasing cover values at this time of year. This is likely to make it much more difficult for weeds to invade, and establish as more of the potential gaps are occupied. Understorey layers appears to have a significant role in terms of suppressing weeds through plant coverage during the course of the experiment. Although no formal assessment was made of weed invasion into the microcosms, it was surprisingly limited, given the site was surrounded by dense populations of weedy native species. Plant density appears to have successfully reduced invasion of weeds. Evidence from the agricultural crop (*Triticum aestivum*) showed that significant weed suppression was achieved by increasing density from 200 to 600 seed/m<sup>2</sup> (Weiner et al., 2001). An assessment of weed invasion in the field experiment over the next 3 years will allow for a testing of these density effects.

One of the most interesting insights to emerge from the study was that the above ground biomass of the 100% tall community was always substantially greater than that of any other combination. Having more layers present did not allow the biomass of the tallest

community to be exceeded. This is somewhat contrary to popular and even scientific notions that increasing structural diversity increases the total biomass present through more complete utilisation of resources such as light, water and nutrients. It remains to be seen whether the lesser biomass of the multi-layer communities will be more or less effective in the longer run in restricting invasion from outside than a larger biomass of tall species.

A multiple layer demonstration planting (4 tall prairie and 4 woodland understorey forbs) was established by Carolyn Ross (an MA student) in 2000 and this is still extant. Despite little maintenance beyond cutting down and removal of the dead stems in late winter, this has persisted extremely well and weed invasion has been very limited, despite being surrounded by many weeds.

A pre-requisite for long term sustainability of herbaceous vegetation is unpalatability of adults and seedlings. The combined effects of mulching type and slug predation are shown in Table 5.9. This highlights that the use of sand mulching is likely to facilitate long-term persistence of understorey and prairie forbs, even when these are highly palatable to species such as *E. purpurea* and *S. regia*. Where sand layers are absent, as in the case of the field experiment mulched with subsoil, slug grazing is likely to lead to poor survival and establishment.

## 6.2 Conclusions

In conclusion, the results presented in this study have lead to a better understanding of germination, emergence, growth and establishment of two groups of forbs; i) those that form an understorey in European and North American woodland or under taller herbaceous vegetation, ii) medium to tall forbs that form part of the dominant strata in North American prairie vegetation. It has been demonstrated that these two groups of forbs can be used to create a multiple-layered plant community which consists of three layers vegetation of summer-autumn flowering prairie species emerging out of an understorey of shade tolerant, vernal forbs.

### 6.3 Recommendation for future research

This study provides a starting point for the use of understorey and prairie forbs to create an attractive multi-layer plant community which imitates semi-natural vegetation. Many of the forbs have been observed and recorded growing successfully in multiple-layered communities in urban landscapes. However, further work is required to explore the effect of soil productivity, and mulch type on the survival and growth of individual species in the long term. It is desirable to know which species might fail or persist after several years of growth. The field experiment has been retained and will be monitored over the next 3-5 years. This will provide a much better understanding of factors affecting successful long term management of multi-layers communities in urban parks and green spaces.

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## APPENDICES

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**Table A4.1** Ecological characteristics of the species used in the experiment (Curtis, 1959; Lang, 1985; Hitchmough et al., 2001; Hendy, 2005; Nearctica, 2005; Prairiemoon, 2005; USDA, 2006), plus the observations of the author. Ecological strategy assessment derived from Grime (2001).

Species (Common name)	Family	Life form/ Ecological strategy <sup>b</sup>	Typical habitat/ distribution	Flower colour and season (in Britain)
<b>Low Canopy</b>				
<i>Dodecatheon meadia</i> (Shooting Star)	Primulaceae	Stress tolerator?	Dry to wet prairie, also in meadows and open woodlands. Western Minnesota to New York, south to Florida and Texas	Pink, early summer (April to June)
<i>Lathyrus vernus</i> (Spring Pea)	Papilionaceae	Stress tolerator?	Wet-mesic species, open woodland or wasteland in temperate regions of America.	Reddish-purple (March to April)
<i>Phlox divaricata</i> (Blue Phlox)	Polemoniaceae	Stress tolerator?	Deciduous woods. Minnesota to Quebec, southward to Florida, Louisiana and Texas	Pale violet, late spring to early summer (April to June)
<i>Polemonium reptans</i> (Greek Valerian)	Polemoniaceae	Stress tolerator?	Wet woods and bottomlands. Minnesota to Southern New England, south to Georgia and Oklahoma	Violet, early summer (April to June)
<i>Primula elatior</i> (Oxlip)	Primulaceae	Stress tolerator?	Eurasian wet meadow. In Britain, moist woods on chalky boulder clay	Pale yellow, spring (April to May)
<i>Primula veris</i> (Cowslip)	Primulaceae	Stress tolerator?	European dry meadow.	Yellow peach, spring (April to May)
<i>Primula vulgaris</i> (Primrose)	Primulaceae	Stress tolerator?	Western Europe; damp/wet meadow. In Southern Britain; woods and shady banks	Yellow, spring (March to June)

Species (Common name)	Family	Life form/ Ecological strategy <sup>b</sup>	Typical habitat/ distribution	Flower colour and season (in Britain)
<b>Medium Canopy</b>				
<i>Aster azureus</i> (Sky Blue Aster)	Asteraceae	Stress tolerator- CSR.	Dry-wet prairie, Western New York to Minnesota, south to Texas	Violet-blue daisies, summer (mid-August to September or October)
<i>Echinacea purpurea</i> (Purple Coneflower)	Asteraceae	Stress tolerator- CSR?	Moist prairie, Iowa, Michigan, Ohio and east to Maryland.	Pink-purple daisies, summer (July to September)
<i>Gillenia trifoliata</i> (Bowman's Root)	Rosaceae	Stress tolerator- CSR?	Prairie forb, Eastern US	White, summer (June to August)
<i>Penstemon digitalis</i> (Foxglove Beardtongue)	Scrophulariaceae	Stress tolerator- CSR?	Found in field and in open woods, Eastern and central North America.	White, summer (June to July)
<i>Phlox glaberrima</i> (Marsh Phlox)	Polemoniaceae	Stress tolerator- CSR?	Found in woods and thickets, Wisconsin, Virginia, Florida and Louisiana.	Pink, summer, (June to September)
<i>Phlox maculata</i> (Wild Sweet William)	Polemoniaceae	Stress tolerator- CSR?	Low forest, fringing lakes and rivers, Minnesota to Southern New England, southward to Florida and Mississippi	Pink, early summer (May to September)
<i>Phlox pilosa</i> (Downy Phlox)	Polemoniaceae	Stress tolerator- CSR?	Dry prairie, open, dry woods and pine barrens, Eastern and Central North America	Pink, early summer (May to June)

Species (Common name)	Family	Life form/ Ecological strategy <sup>b</sup>	Typical habitat/ distribution	Flower colour and season (in Britain)
<b>Medium Canopy</b>				
<i>Rudbeckia speciosa</i> (Showy Black-eyed Susan)	Asteraceae	Stress tolerator- CSR?	Prairie; Eastern and Central North America	Yellow, summer (August to September)
<i>Silene regia</i> (Royal Catchfly)	Caryophyllaceae	Stress tolerator- CSR?	Prairie, Illinois to Ohio and Georgia	Red, summer (July to August)
<i>Solidago speciosa</i> (Showy Goldenrod)	Asteraceae	Stress tolerator- CSR?	Dry-mesic prairie, Massachusetts to Ontario, south to Texas	Canary-yellow, autumn (September to October)
<i>Tradescantia ohioensis</i> (Ohio Spiderwort/ Bluejacket)	Commelinaceae	Stress tolerator- CSR?	Dry-mesic prairie and open woods, Northern USA	Blue, late spring to mid- summer (May to July)
<i>Zizia aptera</i> (Heart-leaf Golden Alexandras)	Apiaceae	Stress tolerator- CSR?	Dry-mesic prairie, Northern USA	Yellow, spring to early summer (April to May)

Species (Common name)	Family	Life form/ Ecological strategy <sup>b</sup>	Typical habitat/ distribution	Flower colour and season (in Britain)
<b>Tall canopy</b>				
<i>Andropogon gerardii</i> (Big Bluestem)	Poaceae	Stress tolerator competitor?	Moist prairie, Northern US	Yellow, summer (July to August)
<i>Aster laevis</i> (Smooth Blue Aster)	Asteraceae	Stress tolerant competitor?	Dry-wet prairie, Maine to Ontario south to Alabama	Violet-blue daisies, autumn (late August to October)
<i>Aster novae-angliae</i> <i>Septemberrubin</i> <sup>c</sup> (New England Aster)	Asteraceae	Competitor?	Wet prairie and damp scrub, Quebec to Alberta. 'Septemberrubin' is a German cultivar	Violet purple, autumn (September to October)
<i>Eupatorium maculatum</i> (Spotted Joe-pye-weed)	Asteraceae	Competitor?	Wet meadows/clearings, Eastern USA	Purple-pink, summer (July to September)
<i>Helenium autumnale</i> (Sneezeweed)	Asteraceae	Stress tolerant competitor?	Wet prairie, North or Central US	Yellow, summer (August to October)
<i>Helianthus mollis</i> (Downy Sunflower)	Asteraceae	Stress tolerant competitor?	Dry prairie and open woods, Michigan to New England, south to Georgia and Texas	Golden yellow; late summer-autumn (August to October)
<i>Phlox amplifolia</i> (Largeleaf Phlox)	Polemoniaceae	Stress tolerator- CSR?	Dry-prairie, Indiana, Virginia, Alabama and Missouri	Pink, summer (July to August)
<i>Rudbeckia subtomentosa</i> (Sweet Black-eyed Susan)	Asteraceae	Stress tolerant competitor?	Moist prairie; Wisconsin to Texas	Yellow, late summer- autumn (August to October)

Species (Common name)	Family	Life form/ Ecological strategy <sup>b</sup>	Typical habitat/ distribution	Flower colour and season (in Britain)
<b>Tall canopy</b>				
<i>Silphium integrifolium</i> (Rosinweed)	Asteraceae	Stress tolerant competitor?	Moist prairie, Northern USA	Yellow, summer (July to September)
<i>Veronicastrum virginicum</i> (Culver's Root)	Scrophulariaceae	Stress tolerant competitor?	Wet prairie, Ontario to Georgia	White, summer (Jun or July to August)

<sup>a</sup> Under typical garden condition.

<sup>b</sup> Strategy assessment based on habitat type and ecological role.

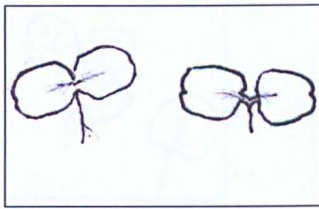
Table A5.1 Mean percentage of number of plant flower across all treatment mixes of understorey forb in response to different productivity gradient at different densities.

Species	Topsoil										Subsoil													
	Sand mulch					P-value	Subsoil mulch					P-value	Sand mulch					P-value	Subsoil mulch					
	LD		HD		Mean		SE	LD		HD			Mean	SE	LD		HD		Mean	SE	LD		HD	
Mean	SE	Mean	SE	Mean		SE		Mean	SE	Mean	SE	Mean			SE	Mean	SE	Mean			SE	Mean	SE	Mean
<i>Lathyrus vernus</i>	21.67	8.39	11.83	5.69	0.547	27.50	8.29	19.75	6.08	0.677	17.50	7.50	3.33	2.29	0.153	29.17	14.20	10.42	3.90	0.959				
					ns					ns					ns					ns				
<i>Polemonium reptans</i>	25.83	9.40	15.71	7.30	0.581	11.17	5.78	13.67	6.28	0.711	13.79	6.56	15.83	7.30	0.929	10.42	5.63	3.21	2.56	0.349				
					ns					ns					ns					ns				
<i>Primula elatior</i>	1.87	1.31	11.62	5.76	0.178	8.25	3.65	15.62	6.44	0.346	1.00	1.00	7.50	5.47	0.515	16.50	7.07	4.25	2.04	0.459				
					ns					ns					ns					ns				
<i>Primula vulgaris</i>	10.83	6.87	9.17	5.05	0.948	18.80	6.62	31.64	8.94	0.259	23.75	8.49	16.25	7.32	0.483	38.93	9.52	27.92	8.03	0.437				
					ns					ns					ns					ns				

Significant differences (Mann-Whitney *U* test) between low and high densities are indicated by asterisks; ns: not significant. LD=Low density, HD= High density.



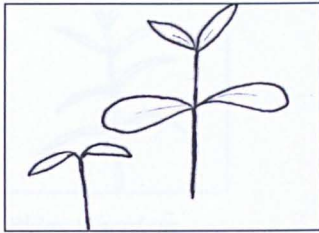
**LOW CANOPY, UNDERSTOREY FORBS**



*Dodecatheon meadia*



*Image on the 4<sup>th</sup> May 2005*



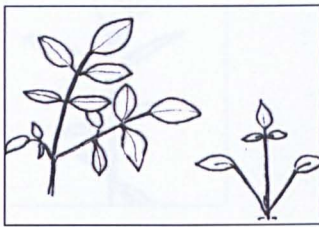
*Phlox divaricata*



*Image on the 5<sup>th</sup> April 2005*



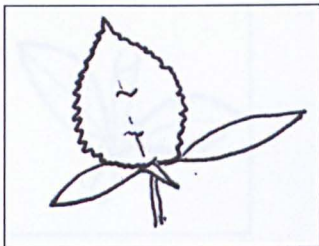
*Image on the 4<sup>th</sup> May 2005*



*Polemonium reptans*



*Image on the 28<sup>th</sup> April 2005*



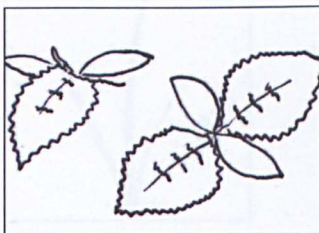
*Primula veris\**



*Image on the 4<sup>th</sup> May 2005*



*Image on the 12<sup>th</sup> May 2005*



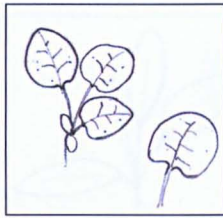
*Primula vulgaris\**



*Image on the 4<sup>th</sup> May 2005*

**Figure A4.1** The illustrated of understorey forb seedlings at early establishment in the microcosm experiment.

**MEDIUM CANOPY FORBS**



*Aster azureus*



Image on the 11<sup>th</sup> May 2005



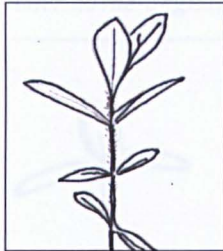
*Phlox maculata*



Image on the 4<sup>th</sup> May 2005



Image on the 28<sup>th</sup> May 2005



*Phlox pilosa*



Image on the 4<sup>th</sup> May 2005



Image on the 28<sup>th</sup> May 2005



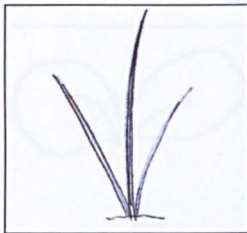
*Solidago speciosa*



Image on the 11<sup>th</sup> May 2005



Image on the 28<sup>th</sup> May 2005



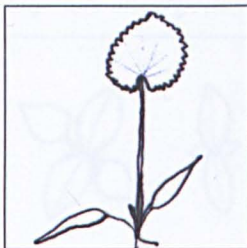
*Tradescantia ohioensis*



Image on the 4<sup>th</sup> May 2005



Image on the 28 May 2005



*Zizia aptera*



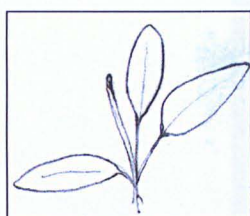
Image on the 4<sup>th</sup> May 2005



Image on the 28<sup>th</sup> May 2005

**Figure A4.2** The illustrated of medium canopy seedlings at early establishment in the microcosm experiment.

**TALL CANOPY FORBS**



*Aster laevis*



Image on the 19<sup>th</sup> May 2005

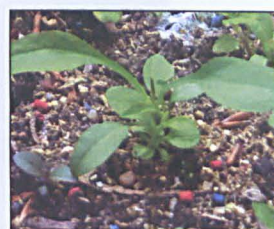
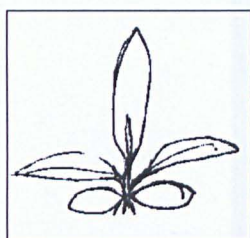


Image on the 28<sup>th</sup> May 2005



*Aster novae-angliae* 'Septemberrubin'

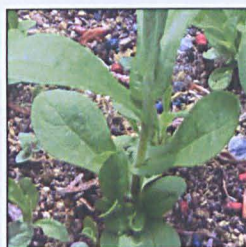
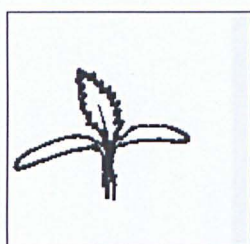


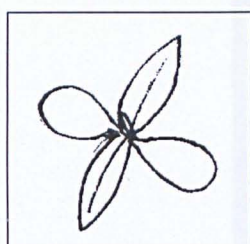
Image on the 8<sup>th</sup> May 2005



*Eupatorium maculatum*



Image on the 28<sup>th</sup> May 2005



*Helianthus mollis*

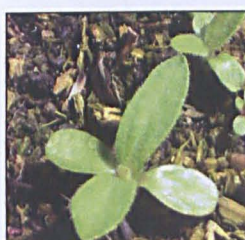


Image on the 5<sup>th</sup> May 2005

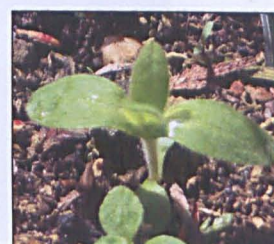
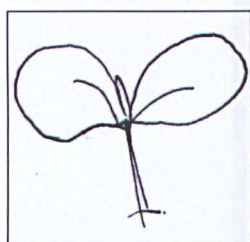


Image on the 21<sup>st</sup> May 2005



*Silphium integrifolium*



Image on the 5<sup>th</sup> May 2005

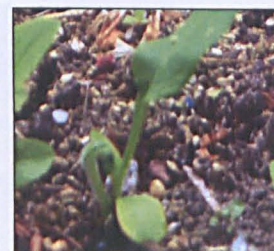
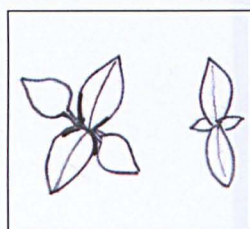


Image on the 28<sup>th</sup> May 2005



*Veronicastrum virginicum*



Image on the 12<sup>th</sup> May 2005



Image on the 28<sup>th</sup> May 2005

**Figure A4.3** The illustrated of tall canopy seedlings at early establishment in the microcosm experiment.



T1 (Understorey)



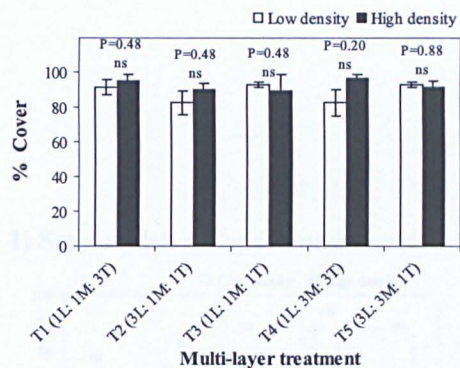
T4 (Mix, understorey dominant)



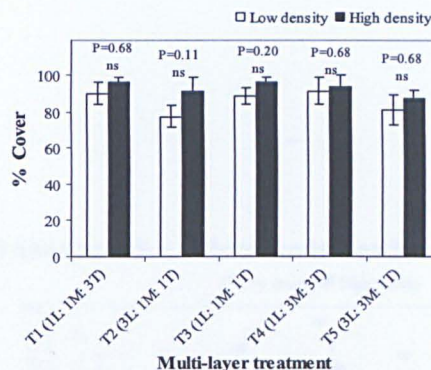
T6 (Mix, equivalent understorey, medium, tall)

Figure A4.4 Some of the emerged seedlings of high density treatment (4000 seed/m<sup>2</sup>) at early establishment in the microcosm experiment. Site photographs on the 9th May 2005.

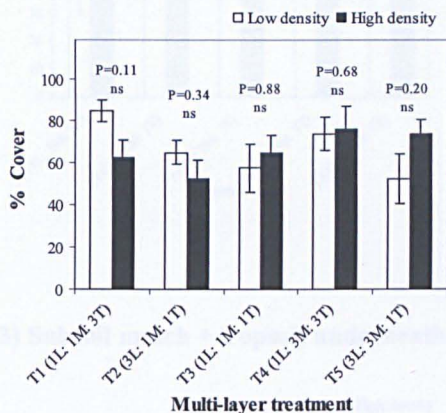
1) Sand mulch + Topsoil underneath



2) Sand mulch + Subsoil underneath



3) Subsoil mulch + Topsoil underneath



4) Subsoil mulch + Subsoil underneath

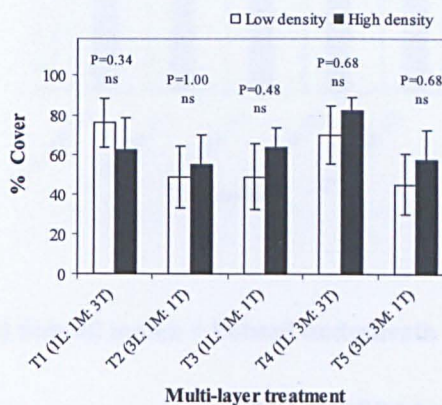
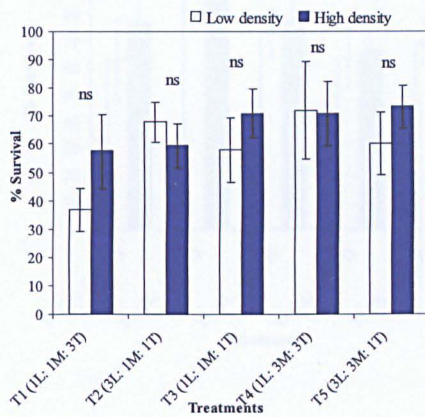
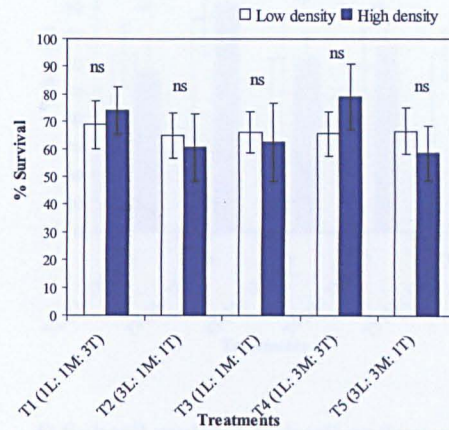


Figure A5.1 Effect of seed ratios and sowing density on cover values; low v high density in October 2006. Bars labeled with the same letters are not significantly different (Mann Whitney U-test,  $P > 0.05$ ). Error bars represent 1 S.E.M.

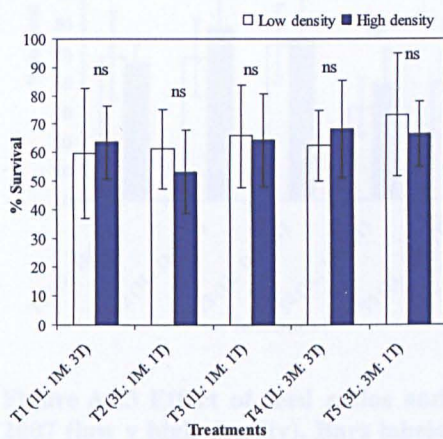
1) Sand mulch + Topsoil underneath



2) Sand mulch + Subsoil underneath



3) Subsoil mulch + Topsoil underneath



4) Subsoil mulch + Subsoil underneath

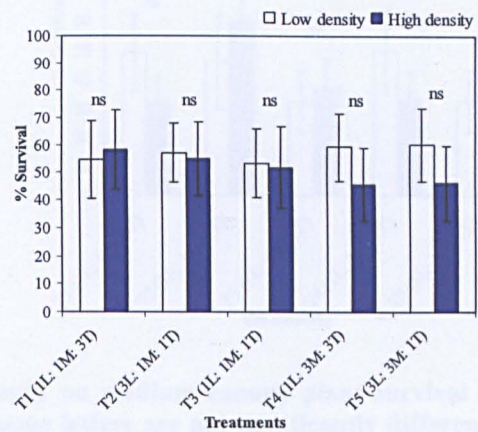
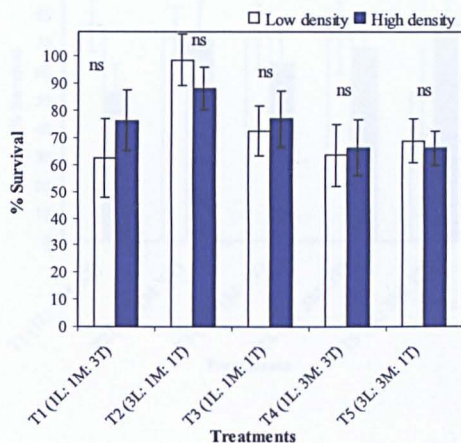
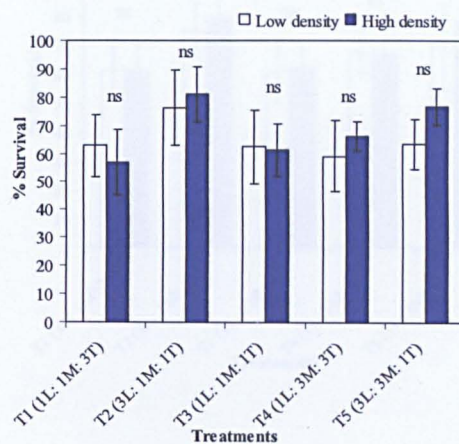


Figure A5.2 Effect of seed ratios and sowing density on low canopy plant survival in April 2007 (low v high density). Bars labeled with the same letters are not significantly different (Mann Whitney *U*-test,  $P > 0.05$ ). Error bars represent 1 S.E.M.

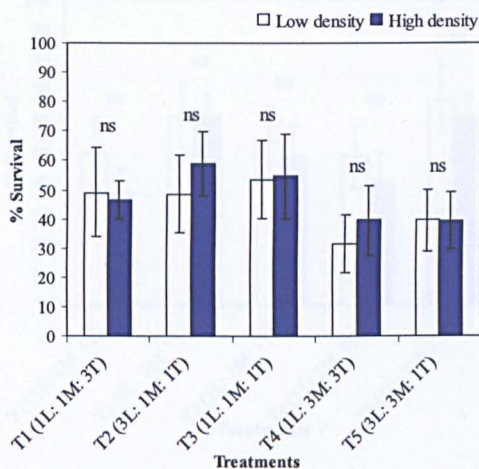
1) Sand mulch + Topsoil underneath



2) Sand mulch + Subsoil underneath



3) Subsoil mulch + Topsoil underneath



4) Subsoil mulch + Subsoil underneath

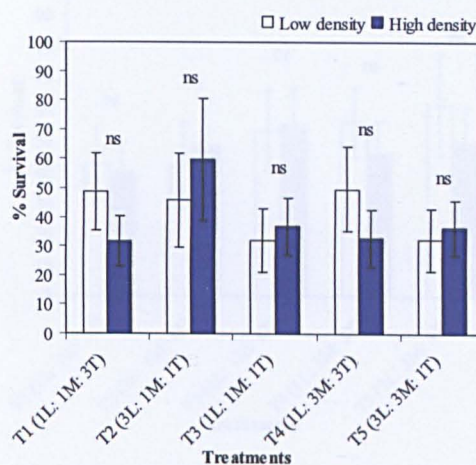
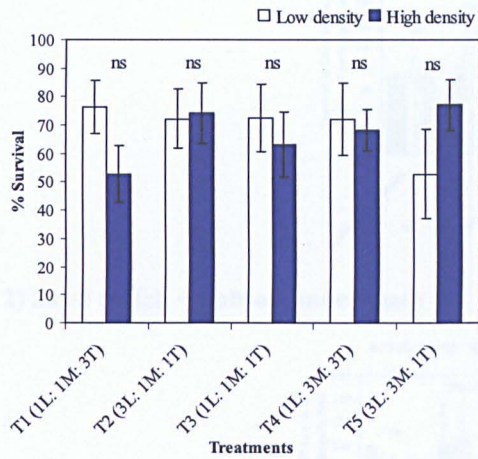
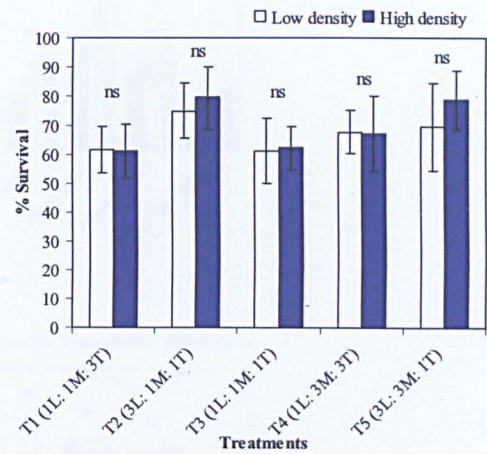


Figure A5.3 Effect of seed ratios and sowing density on medium canopy plant survival in April 2007 (low v high density). Bars labeled with the same letters are not significantly different (Mann Whitney *U*-test,  $P > 0.05$ ). Error bars represent 1 S.E.M.

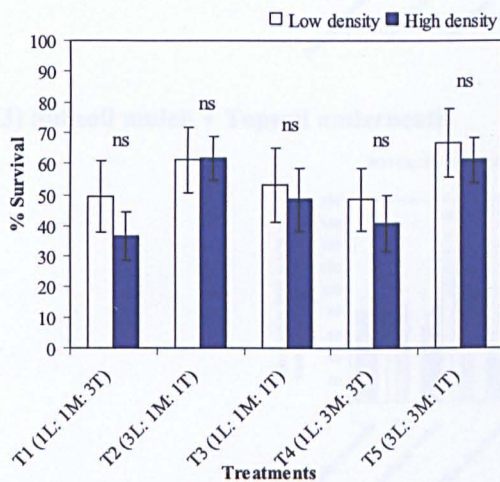
1) Sand mulch + Topsoil underneath



2) Sand mulch + Subsoil underneath



3) Subsoil mulch + Topsoil underneath



4) Subsoil mulch + Subsoil underneath

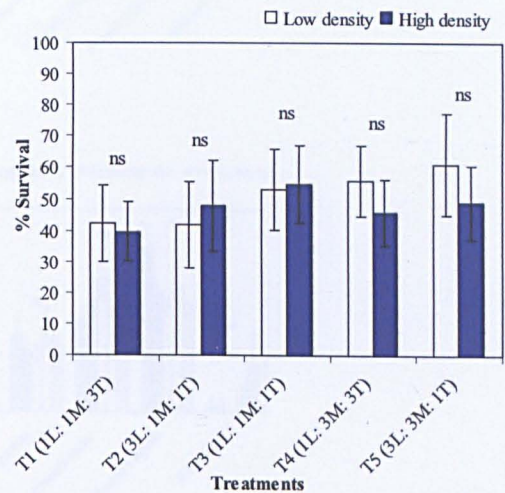
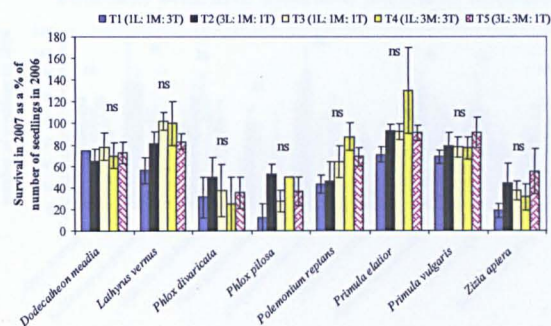


Figure A5.4 Effect of seed ratios and sowing density on tall canopy plant survival in April 2007 (low v high density). Bars labeled with the same letters are not significantly different (Mann Whitney *U*-test,  $P > 0.05$ ). Error bars represent 1 S.E.M.

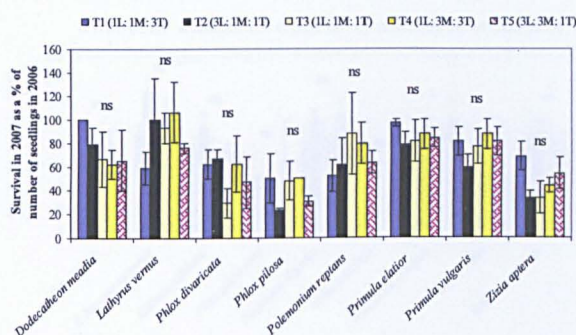


### Low canopy, understory forbs

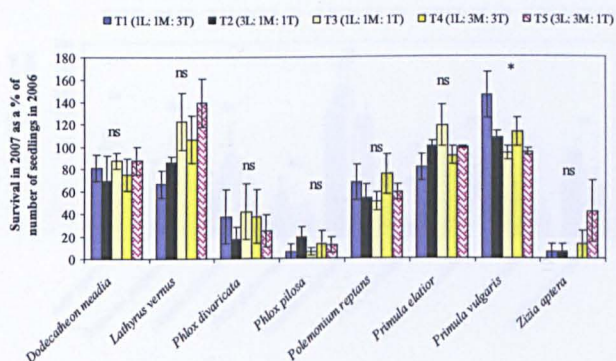
#### 1) Sand mulch + Topsoil underneath



#### 2) Sand mulch + Subsoil underneath



#### 3) Subsoil mulch + Topsoil underneath



#### 4) Subsoil mulch + Subsoil underneath

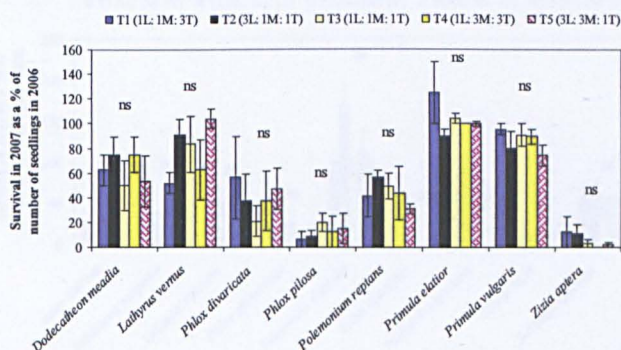
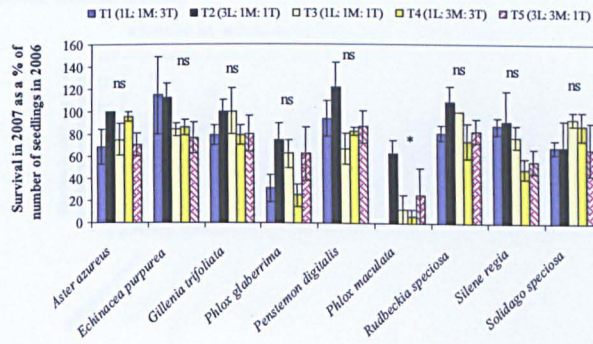


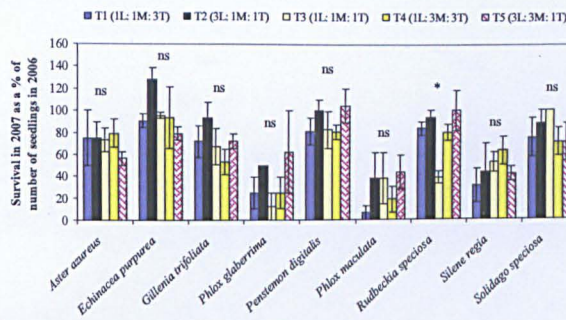
Figure A5.5 Effect of substrate type on number of plant of individual low species in April 2007 as a percentage of those present in August 2006. Significant differences (Kruskal-Wallis test) between treatment mixes are indicated by: \*P=0.05; ns, not significant. Error bars represent 1 S.E.M.

## Medium canopy forbs

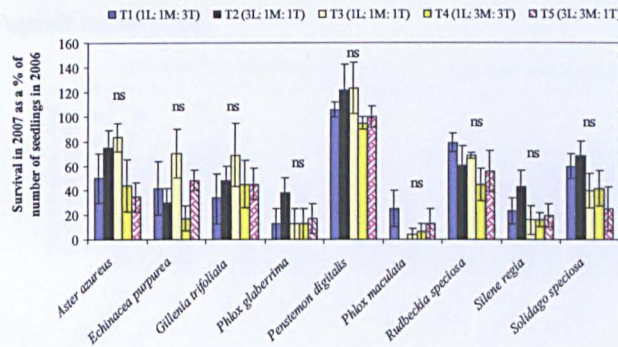
### 1) Sand mulch + Topsoil underneath



### 2) Sand mulch + Subsoil underneath



### 3) Subsoil mulch + Topsoil underneath



### 4) Subsoil mulch + Subsoil underneath

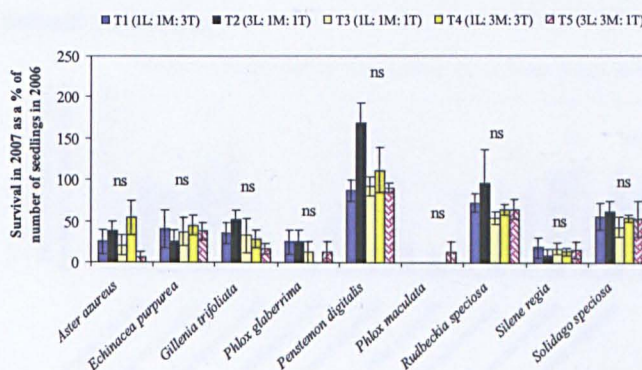
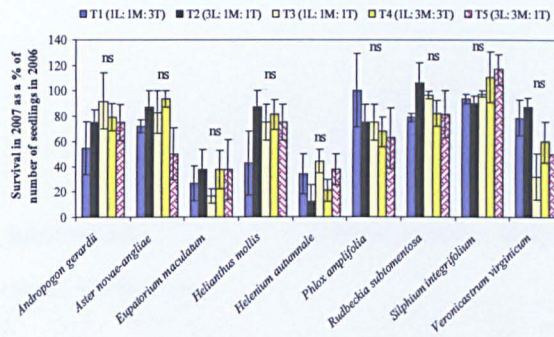


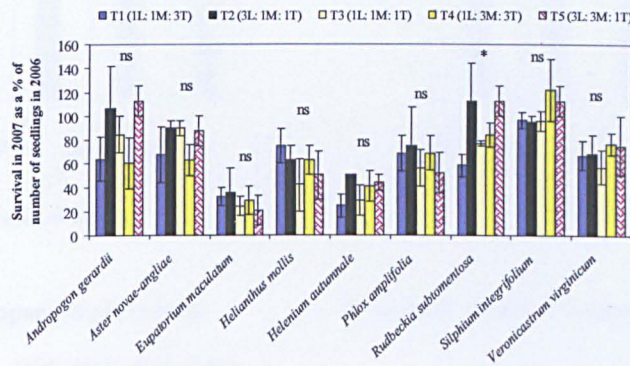
Figure A5.6 Effect of substrate type on number of plant of individual medium species in April 2007 as a percentage of those present in August 2006. Significant differences (Kruskal-Wallis test) between treatment mixes are indicated by: \*P=0.05; ns, not significant. Error bars represent 1 S.E.M.

## Tall canopy forbs

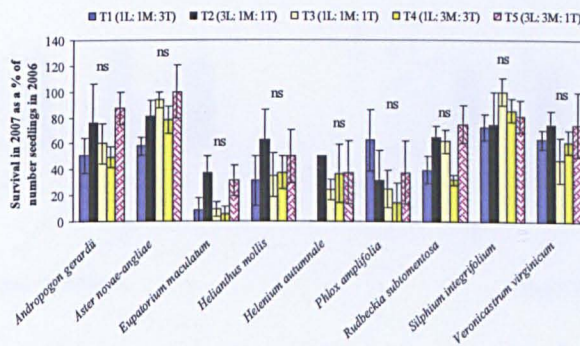
### 1) Sand mulch + Topsoil underneath



### 2) Sand mulch + Subsoil underneath



### 3) Subsoil mulch + Topsoil underneath



### 4) Subsoil mulch + Subsoil underneath

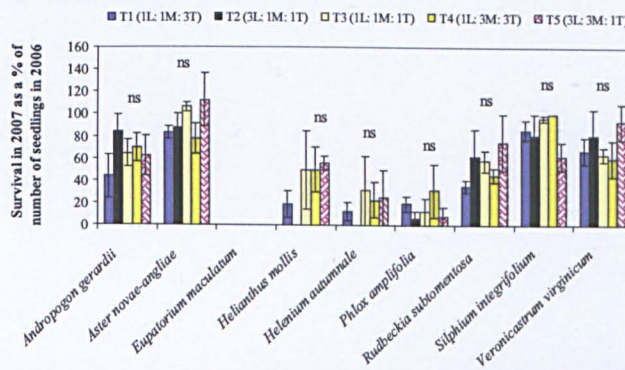
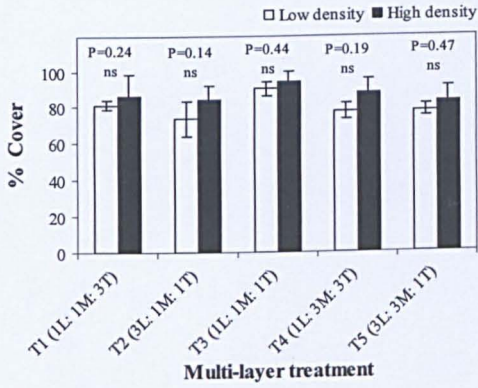
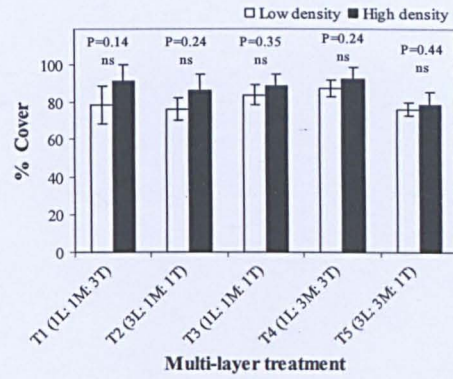


Figure A5.7 Effect of substrate type on number of plant of individual tall species in April 2007 as a percentage of those present in August 2006. Significant differences (Kruskal-Wallis test) between treatment mixes are indicated by: \*P=0.05; ns, not significant. Error bars represent 1 S.E.M.

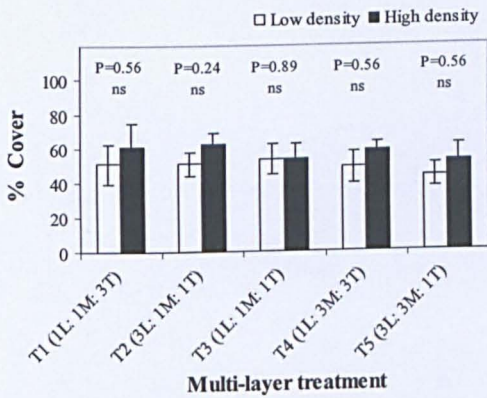
1) Sand mulch + Topsoil underneath



2) Sand mulch + Subsoil underneath



3) Subsoil mulch + Topsoil underneath



4) Subsoil mulch + Subsoil underneath

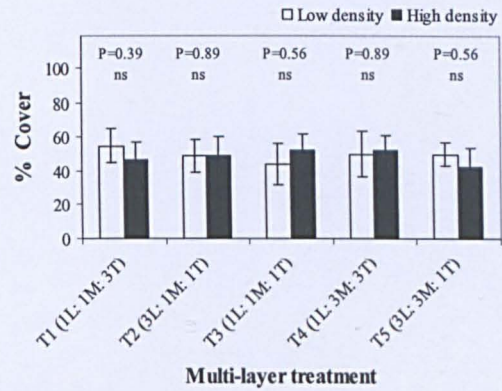


Figure A5.8 Effect of seed ratios and sowing density on cover values; low v high density in May 2007. Bars labeled with the same letters are not significantly different (Mann Whitney *U*-test,  $P > 0.05$ ). Error bars represent 1 S.E.M.