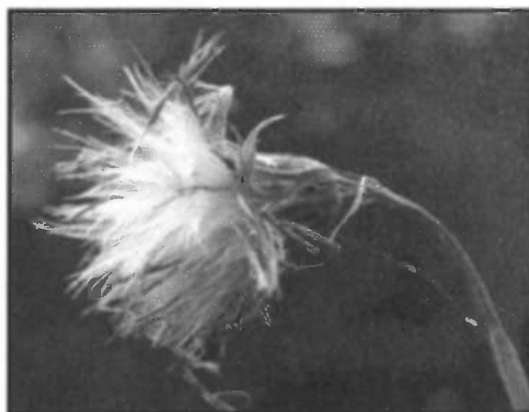
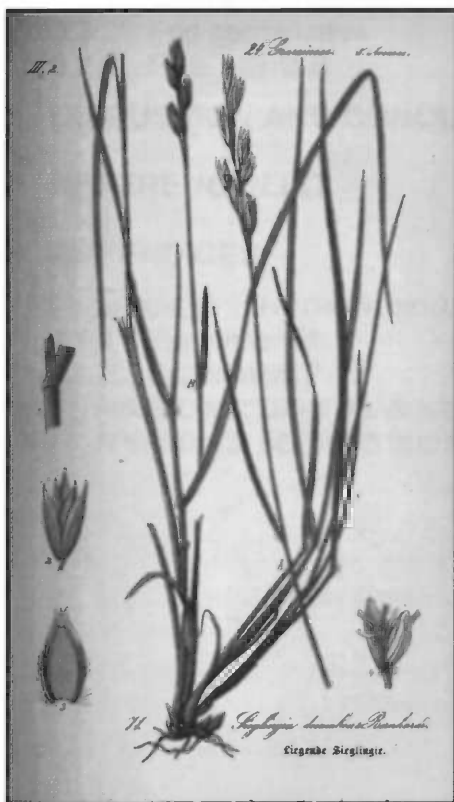


# Germination and growth of three *Cirsio-Molinietum* species in a *Holcus lanatus* sward at different nitrogen levels



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## Abstract

Since halfway the last century heathlands and species-rich grasslands have been becoming increasingly rare. The reason for this decrease is mainly due to the transformation of nature areas into intensive farming areas, especially by the use of artificial fertilisers. In recent years there have been attempts to restore the former low production vegetation types. This is often done by topsoil removal, resulting in a decrease of soil nutrients and in a situation with a limited productivity. There are a number of areas where monitoring of the vegetation has been taking place after topsoil removal. At the start of monitoring it was determined that the abiotic conditions in most of the sites were favourable for the regeneration. However, after nine years, the establishment of target species of these low production communities has hardly occurred. Two possible reasons for a lack of re-establishment of certain species can be that either there is no seed source and/or that the conditions for germination/establishment is not favourable.

In order to gain more knowledge about whether the presence of an existing vegetation (as is the case in many of the nature development areas now) and if increasing levels of nitrogen (as could be the case in the future) can affect the germination and establishment of target species plants, two experiments were carried out. In these experiments seeds and seedlings of target species were placed in an existing *Holcus lanatus* vegetation at different densities and at different nitrogen levels. The planting of *H. lanatus* in different densities did not lead to a significant difference in *H. lanatus* dry weight or light transmitted in either the growth or the germination experiment. The different nitrogen additions did, however, lead to significantly different *H. lanatus* dry weights and light conditions.

In the first experiment the growth<sup>of</sup> seedlings of three target species of the Junco-Molinia alliance; *Cirsium dissectum*, *Danthonia decumbens* and *Juncus conglomeratus* was determined by using the relative growth rate and the final dry weight. The cumulative RGR curves for the control seedlings showed a large amount of growth during the experiment, with an influence of nitrogen. The seedlings grown in the presence of *H. lanatus* showed very little to no growth. This was the same for all the different nitrogen treatments. The final dry weights of the seedlings showed the same pattern as for the RGR, *C. dissectum* and *J. conglomeratus* control seedlings were significantly influenced by nitrogen, for *D. decumbens* this influence was not significant. There was no correlation between seedling dry weight and the percentage light transmitted. The seedlings in competition showed an enormous repression of growth by the vegetation, the percentage dry weight in competition/ dry weight without competition was the following: *C. dissectum* 14%, *D. decumbens* 16% and *J. conglomeratus* 4%.

In the second experiment the germination of *C. dissectum* and *D. decumbens* in an established *H. lanatus* vegetation of varying density and at different nitrogen additions were examined. Both *C. dissectum* and *D. decumbens* showed normal germination curves. There were no significant differences in germination percentages for any of the treatments for both *C. dissectum* and *D. decumbens*.

At the end of this experiment the seedling dry weights were also measured. They showed a similar result to the first experiment: a large difference between the control plants and the competition plants with a significant effect of nitrogen on the control plants but not on the competition plants. The fact that this occurred even at very low *H. lanatus* densities suggests that a mechanism such as allelopathy may also be involved.

From these results it can be concluded that although germination of the examined species was independent of an existing vegetation, seedling growth was very heavily influenced by the *H. lanatus* vegetation. It can therefore be expected that if there is an existing (dense) vegetation in a nature restoration area the chance that a target species will be able to establish will be very small. It would probably be better to attempt to get target species established before the more common species become established in large numbers by sowing seeds or by planting seedlings of the target species.

# 1. Introduction

## 1.1 Background

Since halfway the last century heathlands and species-rich grasslands have been becoming increasingly rare. Populations which were once plentiful are under increasing pressure. The reason for this decrease is mainly due to the transformation of nature areas into intensive farming areas. Before the application of artificial fertilisers and large scale drainage projects many of these areas were either too infertile or too wet to be able to sustain large amounts of crops or livestock and these areas were left mainly to themselves or left for grazing by a small herd of livestock. Further, large scale atmospheric nitrogen deposition and changes of groundwater tables, as a result of current agriculture practice, also affects nature areas surrounding the agricultural lands. These indirect changes lead to the diminishment of the remaining nature areas as the characteristic species of heathland and species-rich grasslands are out-competed by more general species which are invading these previously oligotrophic-species rich areas (Klooker *et al.* 1999).

In recent years there have been attempts to restore the former low production vegetation types. Restoration (or creation) of the nature areas is often done by topsoil removal, resulting in a decrease of soil nutrients and in a situation with a limited productivity. Nitrogen is especially efficiently removed and Klooker *et al.* (1999) have shown that in some of the areas where topsoil removal has taken place nitrogen has become the limiting nutrient for plant production.

There are a number of areas where monitoring of the vegetation has been taking place after topsoil removal. At the start of monitoring it was estimated that the abiotic conditions in most of the sites were favourable for the regeneration of heathland and species-rich grassland (Klooker *et al.* 1999). During the first nine years the abiotic conditions remain suitable for these communities (Verhagen *et al.* 2001). However, after nine years, the establishment of target species<sup>1</sup> of these low production communities has hardly occurred. Most of the target species have returned very slowly in small numbers only and several target species are completely absent (Verhagen *et al.* 2001). Instead, a number of more common species, such as *Agrostis capillaris*, *Holcus lanatus* and *Juncus effusus* have invaded the areas and are among the dominant species.

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<sup>1</sup> species which are characteristic for a certain type of vegetation

Two possible reasons for a lack of re-establishment of certain species can be that either (1) there is no seed source and/or (2) the conditions for germination/establishment are not favourable.

First of all, the seed source. Seeds will have to establish in an area either from the seedbank or from a source in the neighbourhood of the development area.

The seedbank is believed to be especially important for colonisation in the first years after restoration measures are taken. Removal of the topsoil leads to a large reduction in the amount of seeds in the soil. However, after many years of intensive agricultural exploitation (in most cases more than fifty years), it is unlikely that there would be a large amount of target seeds in the seed bank (Bekker 1998). Sampling showed that most of the seedbank consists of seeds of non-target species (Klooker *et al.* 1999). So, considering the amount of time of agricultural exploitation, the chance of a successful restoration will be very small if the only source for seeds is the seedbank.

That leaves the neighbourhood as a potential source for seeds for re-establishment. Most of the studied sites had a source for seeds of the target species nearby, however most of the seeds reaching the nature development areas were from very common pioneer species, resulting in a large amount of competition for germination sites (Klooker *et al.* 1999). In general seeds will only be dispersed over a small distance and most seedlings in an undisturbed vegetation will originate from the local seed rain (Bakker *et al.* 1980).

Introduction of a number of characteristic species in the first years after topsoil removal (by means of sowing and planting of seedlings) has resulted in a reasonably successful establishment. This shows that the lack of propagules reaching the area is at least one of the limiting factors for the colonisation of the topsoil removed areas (Klooker *et al.* 1999; Verhagen *et al.* 2001).

It has been proposed that because seed dispersal seems to be a limiting factor in target species re-establishment, it would be possible to introduce the missing species by active introduction (Verhagen *et al.* 2001). But, even if seeds were to enter the areas, would they be able to germinate and establish?

The main factors that determine whether or when a seed will germinate are dormancy, moisture, light and temperature (Schütz 2000), but nitrate and nitrite are also believed to play a significant part (Baskin and Baskin 1998). The presence of a dense vegetation will markedly affect the amount of light and more importantly the composition of this light reaching the seeds. The low red:far-red ratios caused by light filtering through leaves have been shown to both inhibit and stimulate germination (Fenner 1978; Tooren and Pons 1988; Rees and Brown 1991; Baskin and Baskin 1998). This dichotomy is also true for nitrate and/or nitrite, which can also both stimulate and inhibit germination (Baskin and Baskin 1998).

Seedlings can be said to be established when they are no longer dependent on their seed reserves and capable of generating their own food (Ryser 1993). Whether a species, once it has germinated successfully, will establish is dependent on a number of factors but most important is the competitive ability of the plant.

The competitive ability of a plant will be determined by its surroundings. As several years after topsoil removal large numbers of more general species characteristic of eutrophic areas have invaded the restoration areas, the seeds of the target species that reach the areas after germination have to compete with mainly adult plants of these species for light, water, nutrients and space. An experiment by Foster and Gross (1998) has shown that an established vegetation can lead to inhibition of seedling establishment.

There are two important elements to competition in plants: root competition and shoot competition. Root competition is believed to be important when there is a limitation of nutrients, while shoot competition for light becomes more and more important with increasing nutrient availability. In the case of root competition the ability of a plant to profit from the available nutrients is very important to its survival, whereas in the case of shoot competition the relative growth rate is an important element (Wilson 1988).

In eutrophic conditions, plants that grow and produce seeds quickly usually dominate the vegetation, whereas in oligotrophic areas plants which are economical with their nutrients will be more successful than the rapid growers (Berendse and Elberse 1990). Biomass increases with improvement in the nutrient supply, which leads to a decrease in the total number of species (Vermeer and Berendse 1983). Considering that in most topsoil removed areas productivity is limited by nitrogen, an important aspect of the competitive balance will be the availability of nitrogen.

## 1.2 Hypothesis

In order to gain more knowledge about whether the presence of an existing vegetation (as is the case in many of the nature development areas nowadays) and whether increasing levels of nitrogen (as could be the case in the future) can affect the germination and establishment of target species plants, I have carried out two experiments. In the first experiment the influence of an existing sward on the *germination* of target species at different levels of nitrogen availability was examined. The influence of an existing sward on the *establishment and growth* of target species at different levels of nitrogen availability was examined in the second experiment. In these experiments I have varied the density and height of the existing sward in order to vary the amount of shoot competition and have varied the amount of nitrogen added to influence the root competition.

The hypothesis is that at the low nitrogen levels and low *Holcus lanatus* densities and heights the target species would compete better than at the higher nitrogen and *Holcus lanatus* densities and heights.

## 2 Materials and method

### 2.1 General introduction

In the experiments species characteristic of the Junco-Molinion alliance, one of the target vegetation types for the nature restoration areas, were used. The Junco-Molinion alliance includes only one association; the *Cirsio dissecti-Molinietum*. *Cirsio-Molinietum* meadows are moderately wet and nutrient-poor and occur on weakly acidic to neutral sand or peat soils. The soils are characterised by a low phosphate and potassium content and the meadows are very sensitive to changes in the ground water table (Schaminée *et al.* 1996). The following species were used in the experiments: *Cirsium*

*dissectum*, *Danthonia decumbens* and *Juncus conglomeratus* (for the establishment experiment only). These three species were chosen because they represent three different functional groups; the herbs, grasses and rushes, respectively. Growth form is an important aspect of competitive response (as mentioned in (Leishman 1999)), therefore it might be expected that different functional groups will react differently to different treatments.

*Cirsium dissectum* is a herb that flowers at the beginning of the summer, it often produces shoots from its rhizomes causing it to grow in clusters. It was reasonably common until approximately 50 years ago in sandy areas in the Netherlands and occurs on relatively low lying, nutrient-poor, mildly acidic soil, influenced mainly by rainwater. It is especially sensitive to a decrease in winter ground water levels (Weeda *et al.* 1991). *C. dissectum* is a diagnostic species for the *Cirsio dissecti-Molinietum* association (Schaminée *et al.* 1996).

*Danthonia decumbens* is a strong character species for the sub-association *nardetosum* of the *Cirsio dissecti-Molinietum* association (Schaminée *et al.* 1996). This grass forms dense turfs, can tolerate light shade and flowers at the beginning of summer. It is a characteristic inhabitant of nutrient-poor meadows and thrives especially on phosphate-poor, weakly acidic, moist to reasonably dry soil. Just as *C. dissectum*, the occurrence of *D. decumbens* has also strongly declined in recent years (Weeda *et al.* 1994).

*Juncus conglomeratus* is characteristic for the whole Junco-Molinion vegetation type and therefore has a broader occurrence than *C. dissectum* and *D. decumbens* (Schaminée *et al.* 1996). It is a rush which forms dense tussocks that flower early in the summer. It can tolerate disturbances in the ground water levels but is reasonably intolerant of fertilisation and shade (Weeda *et al.* 1994).

As a representative of an established vegetation in which target species have to invade, *Holcus lanatus* was used in the experiments. *H. lanatus* occurs in large numbers in most of the nature areas. It is a perennial member of the Poacea family with a very broad occurrence, as long as the soil is humous and moist enough. It is fibrous-rooted and forms dense stands. *H. lanatus* is often a coloniser of moist, open land. It can be very difficult to decrease its dominance and its presence can last up to twenty years (Weeda *et al.* 1994).



Seeds for both experiments were collected in the summer of 2000 in the following nature areas: Eexterveld (*D. decumbens*, *C. dissectum* and *H. lanatus*), Hullenzand, Bakkeveen (*H. lanatus*) and Delleburen (*J. conglomeratus*). The seeds were kept in dry storage at 4 °C until the start of the experiments.

The soil used in both experiments was taken on two separate occasions (in December 2000 and June 2001) from a part of the Hullenzand nature area where about 30 cm of the topsoil had been recently removed. The following soil properties were found:

pH(H<sub>2</sub>O): 5.0 - 5.75                      pH(KCl): 4.15 - 4.73

N<sub>tot</sub>: 0.020 - 0.022 (mg N/g)                      C/N: 23 - 27

These values indicate a moderately acidic, mesotrophic soil with a low nitrogen content.

At the end of the second experiment the pH(H<sub>2</sub>O) was measured again resulting in values ranging from 4.92 to 5.29.

Both experiments were performed in the greenhouse at the Biological Centre in Haren, the Netherlands. The establishment experiment started in February 2001 and lasted until May 2001. The germination experiment was carried out in the period July-September 2001. The plants received a minimum of 12 hours daylight throughout the experiments, during the winter period the plants received extra light from 400-Watt lamps. The minimum temperature was 25 °C /15 °C day/night, with a maximum of around 35 °C on exceptionally hot days in the summer. The pots were watered automatically twice a day with demineralised water.

Every week during both experiments the pots were fertilised with a 'general' nutrient solution containing PK and microelements but without nitrogen (for the exact composition see appendix 1).

### 2.1.1 Statistical analysis

Regression was carried out on the following sets; {plant surface area-end biomass} and {light measurement-*H. lanatus* dry weight}.

ANOVA's were carried out on the following parameters: target species final dry weight and the percentage of seeds germinated. Nitrogen levels and *H. lanatus* density were set as fixed factors, these were checked separately and for interactions. ANOVA's have a prerequisite that the underlying data has a homogeneous variance. Using Levene's test for equality of variances, each dependent variable across all level combinations of the between-subjects factors was tested. Data that did not show an equal variance was subsequently transformed using either a log transformation or, in the case of data based on proportions (# germination and light transmitted), an arcsine/ $\sqrt{p}$  transformation. ( Zar 1984; Heath 1995). Tuckey's b test was performed to group the significantly different subsets.

All statistical tests were performed using SPSS 11.0 for Windows.

## 2.2 Experiment 1: seedling growth

### 2.2.1 Experimental design

*H. lanatus* seeds were sown in pots of 17cm x 17cm in two densities. They were thinned to densities of 10 and 25 plants per 100 cm<sup>2</sup> (code L and H, respectively) approximately two weeks after germination. There were two series of different *H. lanatus* plant heights; in one series the plants were kept at 10 cm throughout the experiment (code 10), in the other series the *H. lanatus* plants were left uncut (code 30). This left four different *H. lanatus* treatments: control (no *H. lanatus*, code CON), L10, H10 and H30. There was no L30 treatment as this would be too similar to the H10 treatment.

Three weeks after the *H. lanatus* seeds germinated, nitrogen fertilisation was started with a 1:1 mixture of NH<sub>4</sub>:NO<sub>3</sub> to a final concentration of 10, 20, 40 or 80 kg N/ha/year (code 10N, 20N, 40N and 80N, respectively) (see appendix 1). Each treatment included four replicates, leaving a total of 48 pots per species. The four replicates were kept separate, the pots were randomly placed within each density.

Seeds from the three target species were sown at different times (to co-ordinate germination as best as possible) on a 5-cm thick bed of sifted, sterile peat/sand mixture. As soon as all the seedlings from one species were large enough (2 to 3 cm in height) they were transplanted to the *H. lanatus* pots, at five target individuals per pot.

During the experiment two light measurements were performed, once in March and once in May. The measurements were taken using a light sensitive resistor coupled to a datalogger, the details of which are in appendix 2. One light measurement per pot was taken. The light meter was first held at 20 cm above the plants, the readout on the datalogger was noted after 5 seconds, and then held on the soil surface and again the readout was noted. The amount of W\*m<sup>-2</sup> was calculated from a calibration line (in appendix 2) and then the proportion of light reaching the ground was calculated per pot ( $\frac{W_{\text{soil surface}}}{W_{\text{above}}} * 100\%$ ).

Every two weeks the surface area of each seedling was measured.

At the end of the experiment all above ground parts of all the plants were harvested, dried in a stove at 70 °C for 48 hours and weighed. For the target species the dry weight of each individual was determined, for the *H. lanatus* this was done per pot.

## 2.2.2 Plant measurements

### 2.2.2.1 The Relative Growth Rate

It has been proposed that the relative growth rate (RGR) is one of the most fundamental seedling traits affecting establishment (Schütz 2000). The RGR is a measurement of the speed at which plants are growing through an increase in their biomass over a certain period of time. It is known that species from infertile habitats have low relative growth rates, whereas fast-growing species are mainly from more fertile and disturbed habitats (Grime and Hunt 1975; Ryser and Wahl 2001).

The relative growth rate (RGR) was determined using the following equation:

$$\text{RGR} = \Delta \ln(\text{length} \cdot \text{width} \cdot \# \text{ leaves}) / \Delta \ln(\text{time}) \text{ (Hunt 1990)}$$

However, when the RGR is measured over a daily or weekly time-span, it can vary considerably. To smooth out the weekly growth fluctuations the cumulative relative growth rate was used according to the following equation:

$$\text{RGR}_{\text{cum}}(t_x) = \text{RGR}(t_x) + \text{RGR}_{\text{cum}}(t_{x-1})$$

In the first experiment a non-destructive method to measure the biomass of the seedlings was used. Every two weeks the length and width of the largest leaf and the total number of leaves of each target plant were measured. For *J. conglomeratus* the diameter of the largest stem was taken instead of the width.

However, to determine the relative growth rate it is more common to use the dry weight. Therefore, to check that the measured area of the non-destructive method was (directly) proportional to the dry weight of each plant species, leaf surface area and dry weight per plant species was measured at the beginning and at the end of the experiment. For all three species a highly significant, positive relationship ( $r^2$  ranging from 0.88 to 0.95 and  $P < 0.001$ ) was found (Figure 1). This suggests that in this experiment it is allowed to use the leaf area as a measure of the plant biomass.

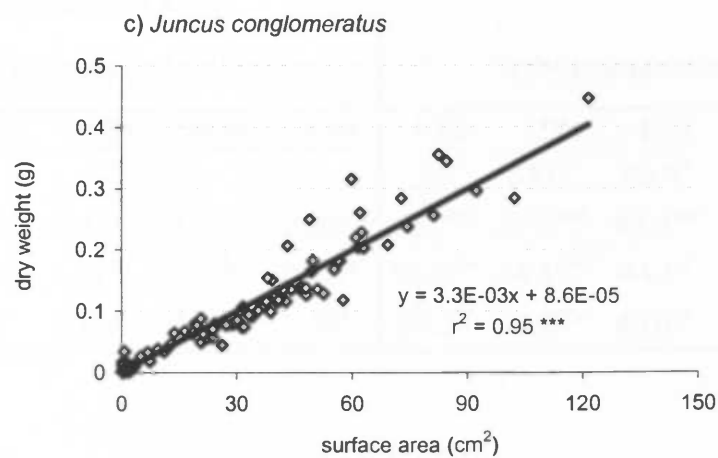
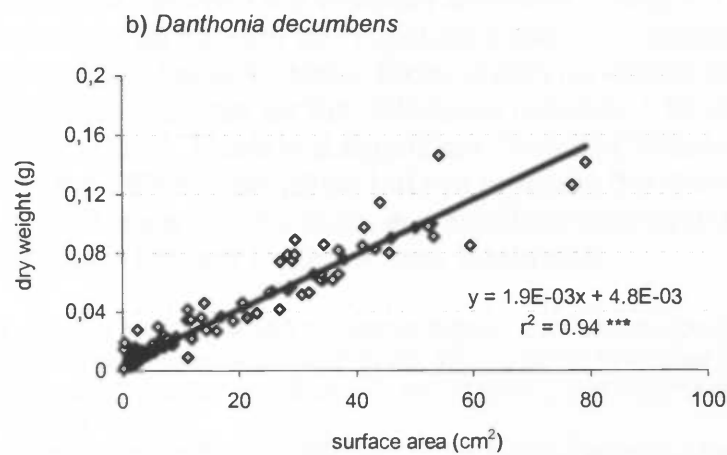
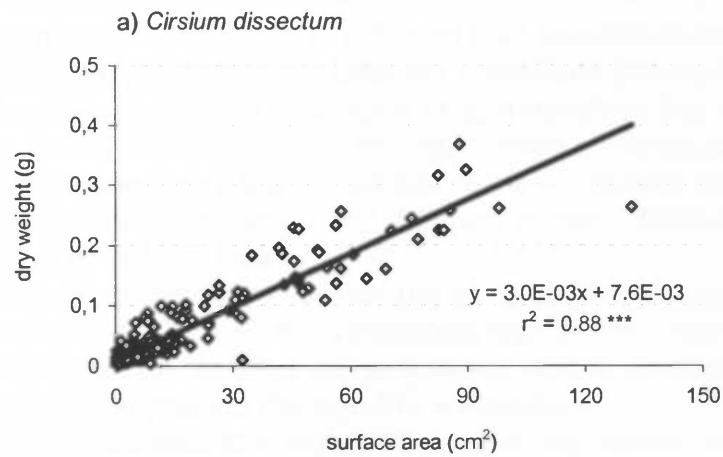


Figure 1: Dry weights of *C. dissectum* (a), *D. decumbens* (b) and *J. conglomeratus* (c) related to their surface areas. The regression line, regression equation and coefficient of determination ( $r^2$ ) are also shown. \*\*\* =  $P < 0.001$

2.2.3 *Holcus lanatus* dry weight and light measurements

In order to estimate the influence of different light conditions on the seedlings it is necessary to establish that the different conditions (nitrogen levels and *H. lanatus* densities) caused a difference in light reaching the target plants/seeds. During the experiment two light measurements were taken and at the end of the experiment the dry weight of the *H. lanatus* plants was measured to quantify the effects of the different nitrogen treatments and densities on the *H. lanatus* plants.

A highly significant correlation between the *H. lanatus* dry weight and the percentage of light left through the vegetation was found (Figure 2).

Therefore, changes in *H. lanatus* dry weight will lead to changes in the amount of light that is passed through the vegetation.

Higher nitrogen levels lead to a higher *H. lanatus* dry weight, but there is no significant difference between the different densities (Table 1).

The percentage light let through the vegetation shows a similar trend as the *H. lanatus* dry weight (Table 1). Here, there is also no significant difference for the different densities, except for the difference between L10 and H30 in the 80N treatment ( $P<0.05$ ). There is a significant ( $P<0.05$ ) difference between the control and the different densities but not between the different densities themselves. Within each density there is a significant difference between the different N treatment (except for the control treatment).

Table 1: *H. lanatus* dry weight and the percentage of light let through by the vegetation at different nitrogen levels (10, 20, 40 and 80 kg N/ha/year) and *H. lanatus* densities (10 and 25 plants per 100 cm<sup>2</sup> (L and H resp.)) and heights of 10 cm and uncut (10 and 30 resp.)).  
Different letters show significantly different values (using Tuckey's b test).  
For each treatment n = 4.

<i>Holcus lanatus</i> dry weight (g)					light transmitted (%)				
	10N	20N	40N	80N	10N	20N	40N	80N	
CON	-	-	-	-	79.0 <sup>a</sup>	84.0 <sup>a</sup>	80.5 <sup>a</sup>	83.2 <sup>a</sup>	CON
L10	1.14 <sup>a</sup>	1.14 <sup>a</sup>	2.12 <sup>c</sup>	3.03 <sup>d</sup>	66.7 <sup>bc</sup>	57.0 <sup>bcd</sup>	45.3 <sup>def</sup>	36.3 <sup>fg</sup>	L10
H10	1.41 <sup>a</sup>	1.57 <sup>ab</sup>	2.03 <sup>bc</sup>	3.27 <sup>de</sup>	52.6 <sup>cde</sup>	54.6 <sup>cde</sup>	44.1 <sup>ef</sup>	25.4 <sup>g</sup>	H10
H30	1.14 <sup>a</sup>	1.48 <sup>a</sup>	2.20 <sup>c</sup>	3.59 <sup>e</sup>	66.5 <sup>b</sup>	54.0 <sup>cde</sup>	45.0 <sup>def</sup>	31.9 <sup>g</sup>	H30

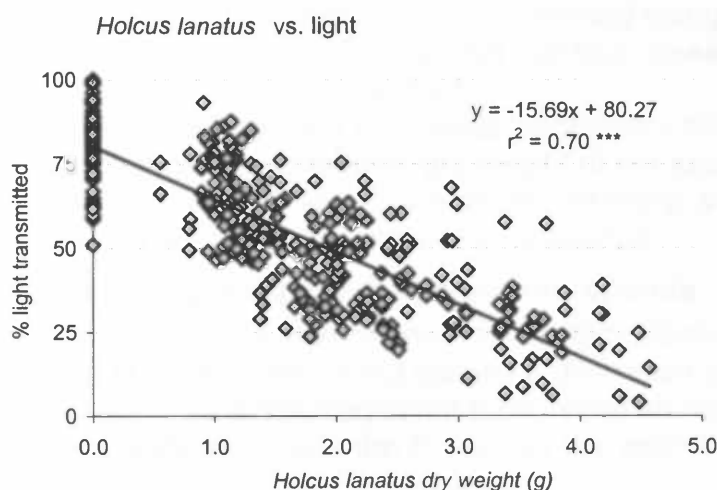


Figure 2: *H. lanatus* dry weight and light in experiment 1:  
*H. lanatus* dry weight related to the percentage of light transmitted by the vegetation.  
 The regression line, regression equation and coefficient of determination are shown.  
 \*\*\* =  $P < 0.001$

## 2.3 Experiment 2: germination

### 2.3.1 Experimental design

After experiment 1 a large number of the *H. lanatus* plants were 'recycled' for the second experiment. The largest plants were taken out of their pots, the roots washed and were randomly planted in new pots (30cm x 30 cm) at the following densities: 1, 4 or 10 plants/ 100 cm<sup>2</sup> and a 'no *H. lanatus*' control (codes 1, 4, 10 and CON). There were four replicates per treatment. The placement of the pots was completely randomly.

Nitrogen fertilisation was started immediately with the same 1:1 NH<sub>4</sub>:NO<sub>3</sub> mixture, this time to a final concentration of 10 and 40 kg N/ha/year (codes 10N and 40N) (see appendix 1).

About three weeks after transplanting the *H. lanatus* plants, seeds of *C. dissectum* and *D. decumbens* were sown into the pots. 50 *D. decumbens* seeds and 39 *C. dissectum* seeds sown. The seeds were covered with a thin layer of sifted sand from the 'Hullenzand' to prevent them from drying out. At the beginning of the experiment the automatic watering system was turned off because the pots were getting too wet; instead the pots were watered with demineralised water whenever the topsoil seemed too dry.

As soon as germination started the amount of seeds that germinated was counted every other day and once the main germination peak was over, twice a week. At the end of the experiment the proportion of germinated seeds were determined by the following formula: # seeds germinated/# seeds sown.

Seeds that had germinated but had subsequently died were not taken into account.

In this experiment one light measurement was performed using the same method as described in paragraph 2.2.1, except that four measurements per pot were taken because of the larger pot size.

The dry weights of the seedlings and *H. lanatus* plants were also measured at the end of this experiment. The combined dry weight of the seedlings in each pot was determined and the average dry weight per seedling was determined by dividing the total dry weight by the number of seedlings.

### 2.3.2 *Holcus lanatus* dry weight and light measurements

As in the first experiment it was determined whether the different conditions (nitrogen levels and *H. lanatus* densities) caused a difference in light reaching the target plants/seeds. The percentage light transmitted shows a highly significant, negative relationship with the *H. lanatus* dry weight with a  $r^2$  of 0.69 (Figure 3).

In this experiment, unlike in the first, there is a (slightly) significant difference between *H. lanatus* density and *H. lanatus* dry weight. Here, the difference between the different N treatments is highly significant ( $P < 0.001$ ) and the differences between the different *H. lanatus* densities is less significant ( $P < 0.05$ ).

In the second experiment the densities were altered somewhat resulting in a highly significant difference (for all densities  $P < 0.001$ ) in light reaching the seeds/seedlings. Unlike the first experiment there was not a significant effect of the N treatment (except for density 4 where the % of light left through in the 10N is significantly ( $P < 0.05$ ) higher than that of the 40N treatment). The results for the Tuckey's b test for *H. lanatus* dry weight and for the percentage of light let through the vegetation are shown in Table 2.

Table 2: The effect of nitrogen levels (10 and 40 kg N/ha/year) and *H. lanatus* density (0, 1, 4 and 10 plants/ 100 cm<sup>2</sup>) on the *H. lanatus* dry weight and the percentage of light let through the vegetation.

Different letters show significantly different values (using Tuckey's b test).

For each treatment n = 4.

	<i>Holcus lanatus</i> dry weight (g)		light transmitted (%)		
	10N	40N	10N	40N	
CON	-	-	93.5 <sup>a</sup>	90.5 <sup>a</sup>	CON
1	2.94 <sup>a</sup>	5.14 <sup>c</sup>	72.6 <sup>b</sup>	65.6 <sup>bc</sup>	1
4	3.59 <sup>ab</sup>	6.31 <sup>d</sup>	64.4 <sup>bc</sup>	53.9 <sup>cd</sup>	4
10	4.24 <sup>b</sup>	6.42 <sup>d</sup>	51.1 <sup>d</sup>	46.1 <sup>d</sup>	10

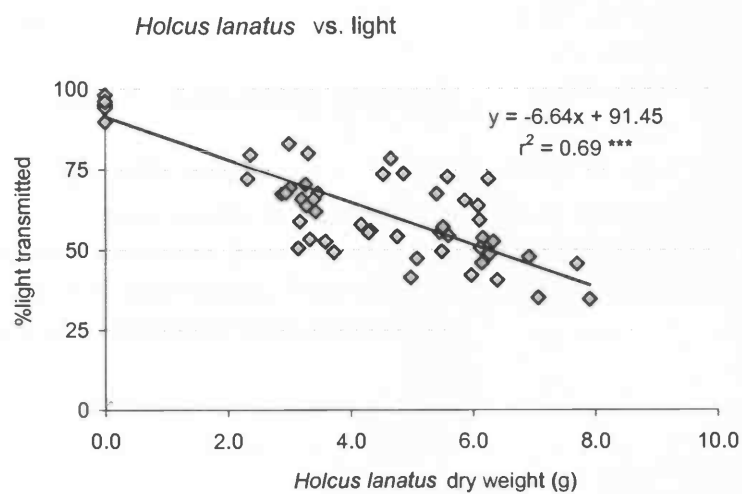


Figure 3: *H. lanatus* dry weight and light in experiment 2:  
*H. lanatus* dry weight related to the percentage of light transmitted by the vegetation.  
 The regression line, regression equation and coefficient of determination are shown.  
 \*\*\* =  $P < 0.001$



### 3 Results

#### 3.1 Experiment 1: seedling growth

##### 3.1.1 Relative growth rate

The  $RGR_{cum}$ 's of those plants in competition with *H. lanatus* (L10, H10 and H30) were pooled because the difference between the different density treatments was non-significant. The nitrogen treatments were significantly different and were therefore kept separate.

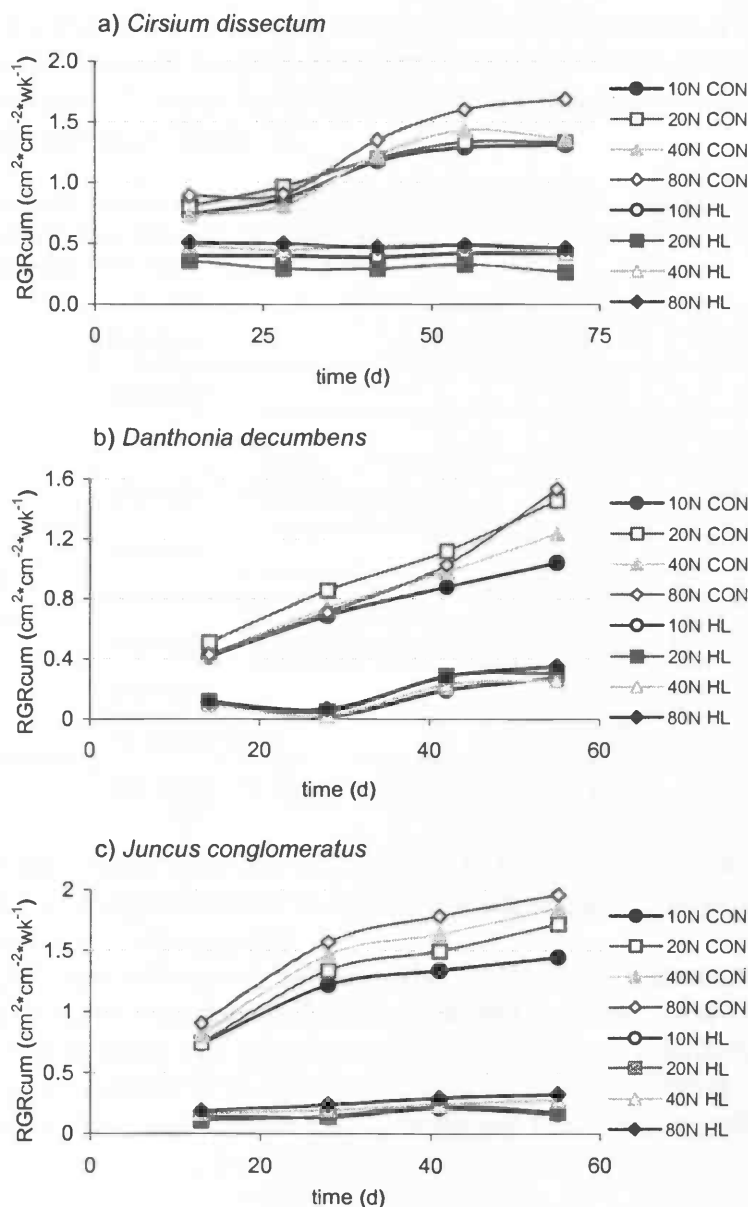


Figure 4: the average cumulative relative growth rate of *C. dissectum* (a), *D. decumbens* (b) and *J. conglomeratus* (c) in the course of the experiment. CON stands for the control series (no *H. lanatus*) and HL is the pooled *H. lanatus* series (L10, H10 and H30). 10N, 20N, 40N and 80N are the different nitrogen treatments.

It is clear that for all three target species in competition with *H. lanatus* there is hardly any growth. This is true for the different *H. lanatus* densities as well as for the different N concentrations (Figure 4). It is worth noting though that there was also very little seedling mortality.

In the absence of competition, however, all three species show a well developed growth curve with an effect of nitrogen on the growth. The curves for *D. decumbens* and *J. conglomeratus* are still rising, indicating increasing growth rates whereas the curve for *C. dissectum* is levelling off which shows a stabilisation of the growth rate (Figure 4).

### 3.1.2 Final biomass

For the plants that were in competition with *H. lanatus* there is no significant effect of the different *H. lanatus* densities or nitrogen concentration on the final biomass after 70 days for *C. dissectum* and 55 days for *D. decumbens* and *J. conglomeratus* (Table 3).

Table 3: ANOVA results on the final biomass of *C. dissectum*, *D. decumbens* and *J. conglomeratus*. The different densities were CON, L10, H10 and H30, the N treatments were 10N, 20N, 40N and 80N.

species	Factor	d.f.	F	significance
<i>Cirsium dissectum</i>	density	3	181.1	P < 0.0001
	N treatment	3	0.697	P = 0.554 (n.s.)
	density * N treatment	9	1.851	P = 0.059 (n.s.)
<i>Danthonia decumbens</i>	density	3	117.6	P < 0.0001
	N treatment	3	2.385	P = 0.069 (n.s.)
	density * N treatment	9	1.816	P = 0.065 (n.s.)
<i>Juncus conglomeratus</i>	density	3	1005	P < 0.0001
	N treatment	3	11.97	P < 0.0001
	density * N treatment	9	5.77	P < 0.0001

The significant results from the ANOVA for N treatment and density\*N treatment for *J. conglomeratus* is due to the significant difference in the *J. conglomeratus* control series.

A large, significant difference between the absence or presence of *H. lanatus* for all three species was found, as was also the case with the  $RGR_{cum}$ 's (Figure 5). It is evident that the nitrogen levels do have a significant effect on the control plant growth of *C. dissectum* and *J. conglomeratus*. For *D. decumbens* there is a similar trend, however this is not significant.

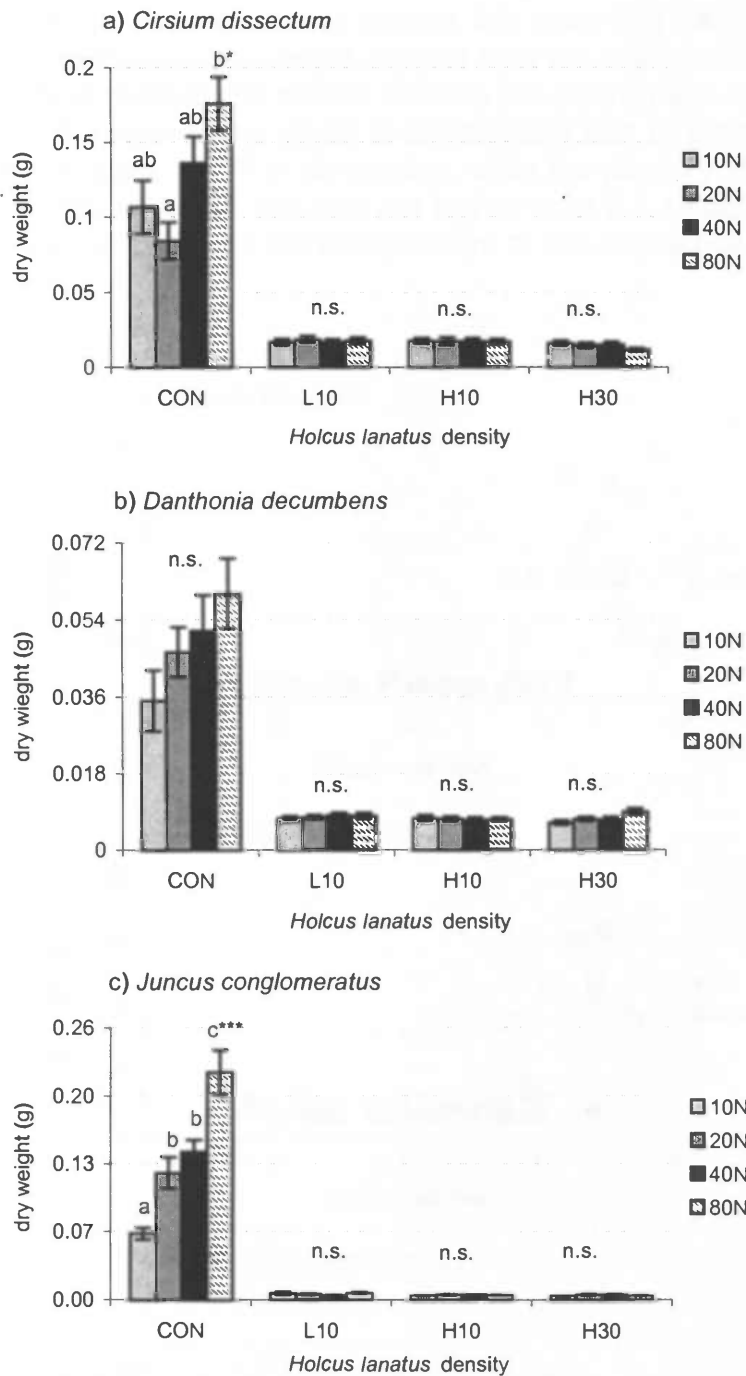


Figure 5: the final dry weight (mean  $\pm$  S.E.) of *C. dissectum* (a), *D. decumbens* (b) and *J. conglomeratus* (c) set out against the different *H. lanatus* densities (CON, L10, H10 and H30) at different nitrogen treatments (10N, 20N, 40N and 80N). Different letters indicate dry weights that are significantly different from each other within the treatment. n.s. = not-significant, \* =  $P < 0.05$ , \*\*\* =  $P < 0.0001$

Of the three species *J. conglomeratus* seems the most affected by the competition, the average dry weight of the (pooled) plants in competition is only 4% of the plants without competition, for *C. dissectum* it is 14 % and for *D. decumbens* it is 16%. These differences can be clearly seen in the photographs in appendix 3.

### 3.1.3 Seedling dry weight under different light conditions

The seedlings in competition were pooled. It is clear that there is no correlation between the dry weight and the light the plants received in the case of plants in competition with *H. lanatus* but also for the control seedlings (Figure 6). In the case of the plants in competition with *H. lanatus* the  $r^2$  for the correlation is less than 0.05 in all species, while the plants in the control pots showed a slightly higher  $r^2$ , but also not higher than 0.3. In both cases it can be said that the dry weights are independent of the amount of light they have received.

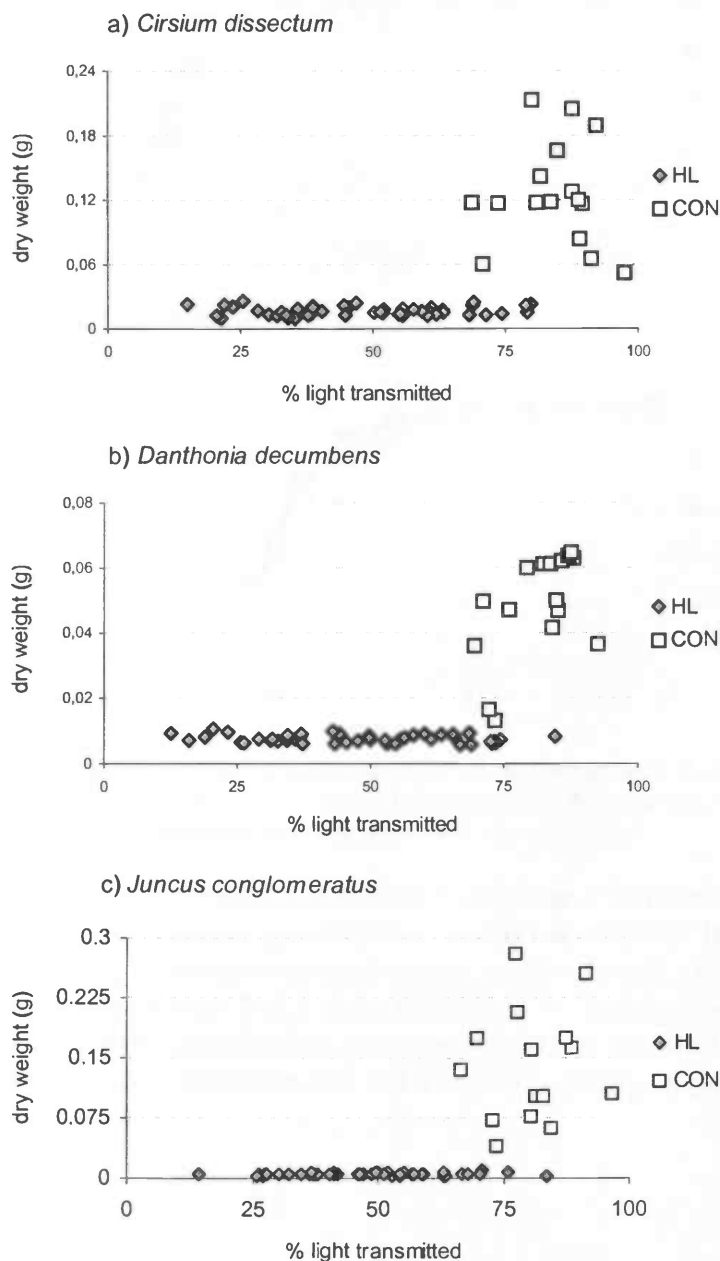


Figure 6 : the dry weights of the different target species set out against the measured percentage of light let through by the *H. lanatus* vegetation.  
CON= control plants, HL = plants in competition with *H. lanatus*

## 3.2 Experiment 2: germination

### 3.2.1 Germination curve

No significant differences between the two different nitrogen treatments were found and therefore they were pooled.

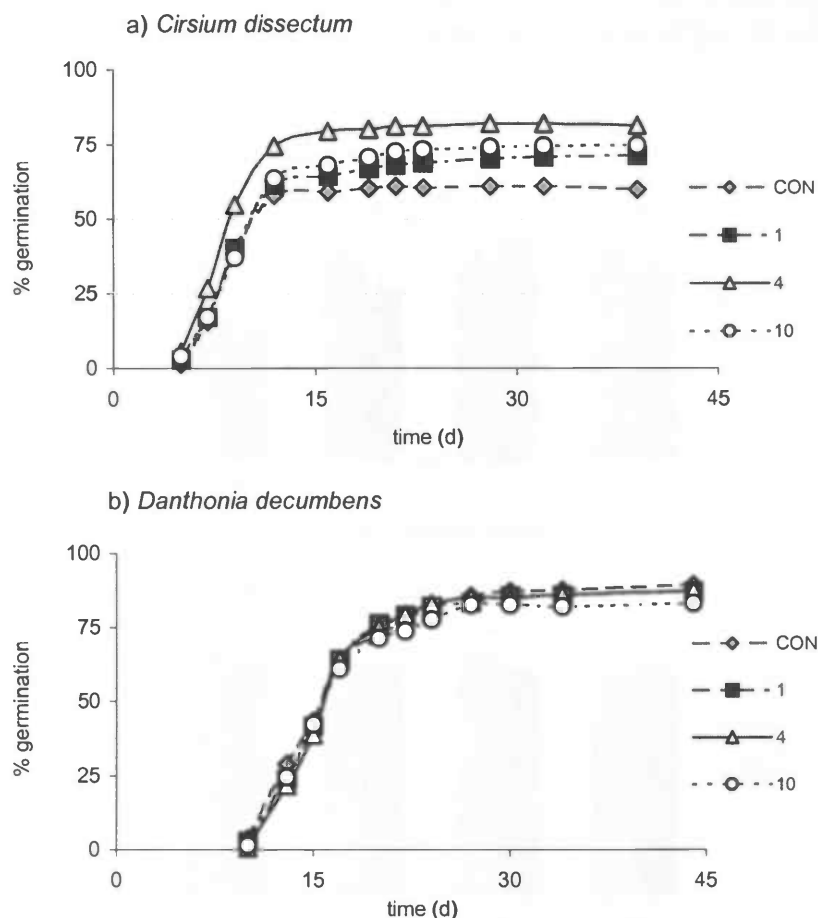


Figure 7: the percentage of germinated seeds of *C. dissectum* and *D. decumbens* during the experiment at different *H. lanatus* densities (CON = control, 1, 4 and 10 plants/100 cm<sup>2</sup>). The N treatments (10N and 40N) were grouped.

Germination started for *C. dissectum* after 5 days and levelled off about 7 days later. For *D. decumbens* germination started somewhat later (after 10 days) and the main germination period lasted a little longer, about 15 days (Figure 7). For *C. dissectum* the 4 plants/100 cm<sup>2</sup> *H. lanatus* densities showed the highest percentage germination, the control plants showed the lowest. The different *H. lanatus* densities did not affect the timing of germination.

### 3.2.2 End germination

There is no significant effect either from a variation of *H. lanatus* density or from a difference in nitrogen treatment on the final percentage germination of either *C. dissectum* or *D. decumbens* (Figure 8). The *C. dissectum* seeds seem to show a higher germination at the 4 plants/100 cm<sup>2</sup> and 40N treatment and a lower germination for the control, but this is not significant. In Table 4 the ANOVA values for the average percentage of germinated seeds are shown.

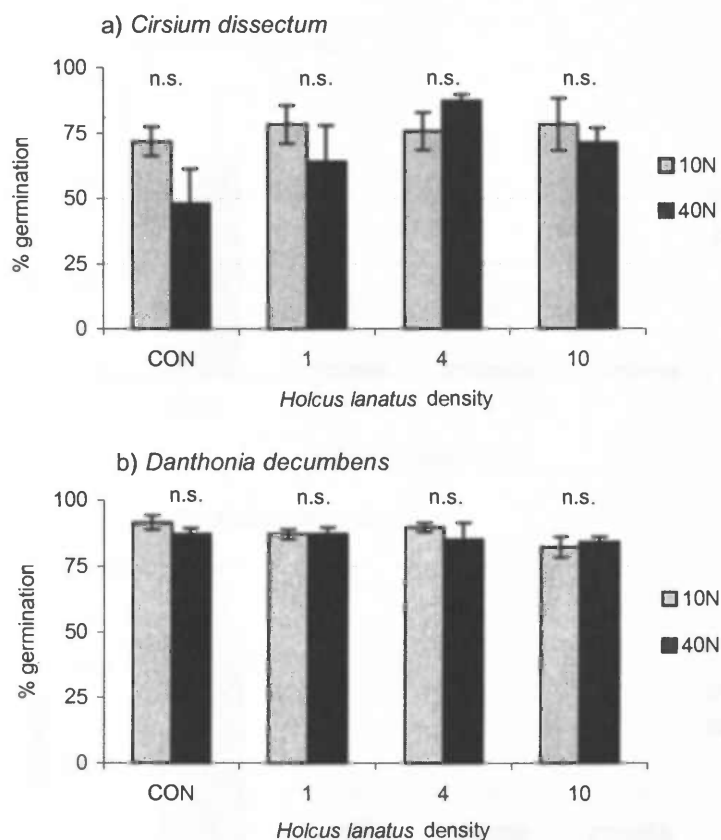


Figure 8: the percentage (mean  $\pm$  S.E.) of germinated seeds at the end of the experiment set out against the different *H. lanatus* densities (CON = control, 1, 4 and 10 plants/100 cm<sup>2</sup>) at the two different nitrogen treatments (10N and 40N). n.s. = not-significant.

Table 4: ANOVA values for the percentage of germinated seeds at the end of the experiment. The different densities were CON, 1, 4 and 10, the different N treatments were 10N and 40N.

species	Factor	d.f.	F	significance
<i>Cirsium dissectum</i>	density	3	1.454	P = 0.240 (n.s.)
	N treatment	1	1.969	P = 0.146 (n.s.)
	density * N treatment	3	1.202	P = 0.330 (n.s.)
<i>Danthonia decumbens</i>	density	3	0.592	P = 0.449 (n.s.)
	N treatment	1	1.461	P = 0.250 (n.s.)
	density * N treatment	3	0.484	P = 0.696 (n.s.)

### 3.2.3 Final biomass

As in experiment 1 there is a highly significant ( $P < 0.0001$ ) effect of the competition from the *H. lanatus* plants on the dry weight of the seedlings, even with lower *H. lanatus* densities. There was no effect of the different densities or different nitrogen treatments on the growth of the seedlings which were in competition with *H. lanatus* (Figure 9).

There was again a large effect of nitrogen treatment on the biomass of the seedlings of both species in the control series, with the 40N treatment significantly larger.

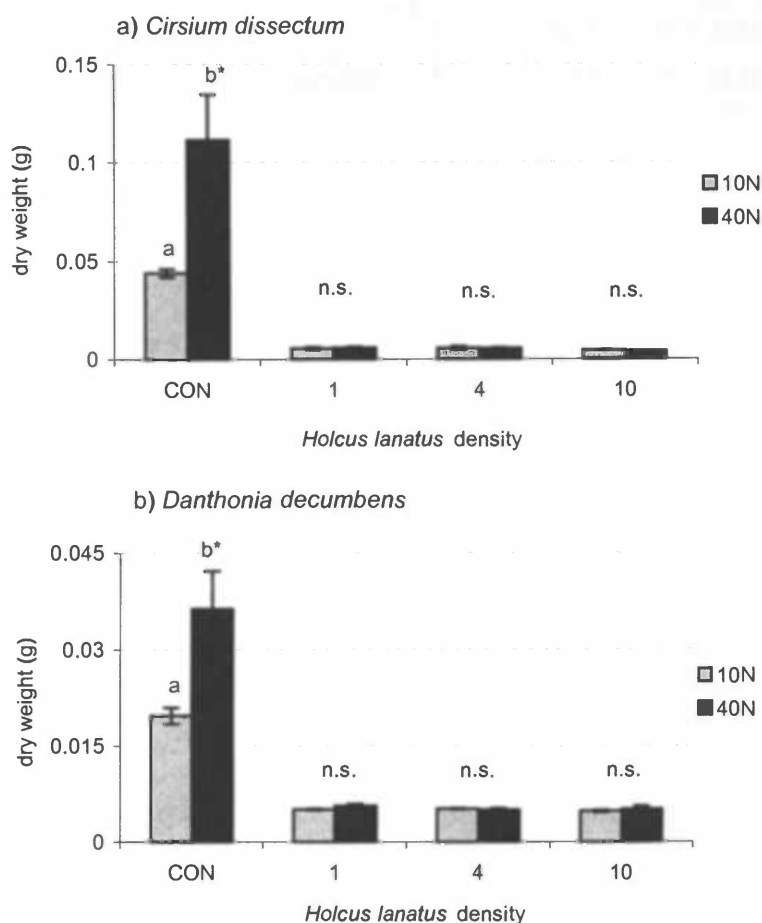


Figure 9: average dry weight (mean  $\pm$  S.E.) of germinated *C. dissectum* (a) and *D. decumbens* (b) seedlings at the end of the second experiment. The *H. lanatus* densities were as follows: control (CON), 1, 4 and 10 plants/100 cm<sup>2</sup> at N treatments of 10 or 40 kg N/ha/year. n.s. = not-significant, \* =  $P < 0.05$

The influence of the competition was in contrast to the first experiment much larger on *C. dissectum*, in competition the biomass was only 7% that of the biomass with no competition. For *D. decumbens* this number was about the same as in the first experiment, namely, 18%.

Table 5: ANOVA values for the final biomass of germinated seeds at the end of the experiment. The different densities were CON, 1, 4 and 10, the different N treatments were 10N and 40N.

species	Factor	d.f.	F	significance
<i>Cirsium dissectum</i>	density	3	223.69	P > 0.0001.
	N treatment	1	6.409	P > 0.05
	density * N treatment	3	6.791	P > 0.005
<i>Danthonia decumbens</i>	density	3	176.56	P > 0.0001
	N treatment	1	7.621	P > 0.05
	density * N treatment	3	4.887	P > 0.01



## 4 Discussion and conclusion

Using the measured surface area per plant instead of their dry weights worked very well for all three species. Only a small percentage of the variation in the dry weight could not be explained by the surface area, the  $r^2$  for the three species ranged from 0.88 to 0.95. It was therefore, in this experiment, allowed to use the surface area per plant as a measure of biomass for the relative growth rate. A large advantage to using this method is that fewer seedlings needed to be used. More important however, by using this method individual plants can be monitored non-destructively throughout the experiment. This can give a more accurate viewpoint of the growth of the plants. Drawbacks to this method is that it is a labour intensive procedure and that the measurement of the plants can be inaccurate, especially with small seedlings and for plants with less clearly defined leaves like *J. conglomeratus*. In both experiments two different factors were tried to be manipulated; that is competition for nutrients (different densities for *H. lanatus* and fertilisation) and light (different densities and light). In the first experiment the different levels of added nitrogen had a significant effect on the dry weight of the *H. lanatus* plants at higher levels, however, this was not the case between the two lower levels. At the 10 and 20 N treatment the *H. lanatus* plants looked more yellow and thinner, a clear symptom of nitrogen limitation (Bennett 1993), than those of the higher fertility treatments. The light measurements showed a similar pattern to the dry weights for the nitrogen treatments (except that there was also a slightly significant difference at the lower nitrogen levels), the different nitrogen additions had a profound effect on the *H. lanatus* plants and led to clearly different conditions for the seedlings.

The planting of *H. lanatus* in different densities did not lead to the expected differences in *H. lanatus* dry weight. There were no differences in dry weight between the different densities or heights. This could be due to the densities being too high and the differences between heights not being large enough. The *H. lanatus* plants at a higher density seemed cramped, they were thin and did not grow out much, there seemed to be a large amount of competition between them. The low-density plants grew out a lot more, forming a higher number of leaves and all together seemed to take up roughly the same amount of space as the high-density plants. This was confirmed by the light measurements, which showed no differences in light reaching the soil surface at the different densities or heights. In his experiment looking at competition between *Festuca rubra* and ruderals or closed-turf species, Fenner (1978) used heights of 1 and 8 cm to determine the effect of competition from an established grass sward. The grass height of 8 cm severely reduced the RGR of all the seedlings, but no effect was observed for the 1 cm height. In the present experiment it is quite possible that the 'low' height of 10 cm was simply too high to create significantly different conditions from the uncut plants. There were therefore only two different density conditions for light; competition and no competition.

In the second experiment the conditions were altered because of the results of the first experiment. There were only two different nitrogen treatments and three different densities (with densities much lower than in the first experiment) and no differences in *H. lanatus* height. Again there was a significant difference of nitrogen on the end dry weight of the *H. lanatus* plants, however this was not reflected in the light measurements where there really was no difference. And again there was not a significant difference between the dry weight of the *H. lanatus* at the different densities even though they were much lower than in the first experiment. In this case though there was an effect of the density on the amount of light left through by the vegetation. This inconsistency between the effect of the *H. lanatus* density on the dry weight and the amount of light let through could be due to the reasonably large time span between light measurement and harvesting the *H. lanatus* plants for the dry weights (unlike in the first experiment). The effect of the nitrogen may not have been as pronounced in the beginning, when the light measurement was done, as in the end when the dry weight was determined. In contrast, the difference in density would only be visible in the beginning because the *H. lanatus* plants had a tendency to grow into the space they were given, possibly due to having less intraspecific competition. The idea behind the use of different *Holcus lanatus* heights and densities was to create different conditions so that root and shoot competition could be distinguished. Considering that the results in *H. lanatus* dry weight and the light measurements were inconclusive as far as the height and density was concerned, it was not possible to say whether the found competition was due to root or shoot competition.

Although the germination of many species is affected by both light and nitrogen (Karssen and Hilhorst 1992; Baskin and Baskin 1998), this does not seem to be the case for *C. dissectum* and *D. decumbens*. For both species no significant differences in amount or time of germination were found. For *C. dissectum* a lower but not significant maximum germination for the control plants was measured. The end germination for the control plants also showed a difference for the nitrogen treatment, with the 40N treatment causing a lower percentage of germination, but again this was not significant. For the *D. decumbens* seeds there was not any detectable difference between the different treatments in the germination curves or in the end germination percentage.

The hypothesis for the growth experiment was that there would be better seedling growth at the lower *H. lanatus* density and heights and at low N levels than at the higher densities and heights. However, the growth of the seedlings was too low to be able to compete under any circumstance. The average RGR ( $\text{g} \cdot \text{g}^{-1} \cdot \text{d}^{-1}$ ) of the *C. dissectum* control plants over the study period was 0.027 and in competition it was 0.0054, for *D. decumbens* this was 0.047 and 0.011 and for *J. conglomeratus* it was 0.062 and 0.0079, in comparison *H. lanatus* has a RGR of between 0.05 and 0.25 depending on the amount of added nitrogen (Hunt 1982; Poorter *et al.* 1995).

Analysis of the final dry weight shows a similar result to the RGR, there is no difference between any of the competition levels. This is the case throughout the experiment. At the end of the second experiment there was also no difference in final dry weights of any of the plants in competition. Of the three species *J. conglomeratus* seemed to be the most affected by the competition from *H. lanatus*. Faster-growing plants can be relatively more affected by competition from tall turf (Fenner 1978), this could be the reason that *J. conglomeratus* was more affected.

An interesting point is that despite the seemingly harsh conditions there was very little seedling death. Perhaps the seedlings were just able to survive but unable to grow under the circumstances, and were 'waiting' for better circumstances. Fenner (1978) found that the seedlings he used in a competition experiment were able to live without making any growth and even after 8 months were still very small but looked reasonably healthy.

Chippindale (as mentioned in (Fenner 1978)) showed that stunted *Festuca pratensis* seedlings could survive in a *Lolium italicum* turf for 10 months, and that subsequently they could develop normally when released from competition for light and nutrients. The present growth experiment did not last long enough to give a definite conclusion. A more long-term experiment would need to be performed to confirm the results of Fenner (1978).

Even at the extremely low *H. lanatus* densities of the second experiment there was a severe inhibition of target species growth. A possible explanation for this phenomenon is that allelopathy by *H. lanatus* on the seedlings has played a role in the competition. Allelopathy is the direct or indirect harmful effect by one species on another through the production of chemical compounds that escape into the environment (Rice 1974). In several experiments, looking at the role of allelopathy in old-field succession, Rice (1974) showed that (at least in part) the dominance of species was due to allelochemicals. These chemicals came from both the roots and shoots of either dominant species themselves or from the pioneer species of an earlier succession stage and inhibited both the germination and growth of several other species. However, in the present experiment no such effect on the germination was observed. Unfortunately, the extent to which allelopathy can limit the growth of neighbouring plants is not clear nor is it evident precisely how significant it is in the competition between species under field conditions (Harborne 1997).

Although experiments performed in the greenhouse are in no way the same as experiments under field conditions it is nonetheless of importance to draw practical conclusions as they can give helpful insights into the relationship between plants and help in determining where potential problems in the field may lie. The successful restoration of many types of nature areas can be very costly and depends to a large extent on the proper starting conditions and management practice. It therefore makes sense to adopt only those conditions and practices that have a reasonable chance of success. From the experiments described in this report and other experiments it is clear that the presence of an existing vegetation probably will not hinder the germination of the target species but could play an important role in the inhibition of seedling establishment in nature restoration areas (Haugland and Tawfiq 2001; Isselstein *et al.* 2002). The competition from the adult population of *H. lanatus* plants did not allow any seedling growth at any *H. lanatus* density or nitrogen addition. Whether the target seedlings would be capable of resuming their growth if the adult vegetation were to be removed or diminished is not clear but it is a possibility. This means that in areas where there is already a well developed vegetation of common species some way of removing or diminishing this population will be useful. An important way of achieving this is by keeping the nitrogen content of the soil low. From the present experiments it is apparent that nitrogen does have an effect at least on the *H. lanatus* plants and that a nitrogen limitation on the *H. lanatus* plants could cause them to eventually die and possibly open up the way for the 'dormant' target seedlings. Nitrogen deposition should therefore be kept to a minimum and preferably a way of further lowering of soil nitrogen content after top-soil removal should be used, for instance mowing or extensive grazing by herds of livestock. The advantage to using grazing is that it also creates gaps in the vegetation where target seedling could get the opportunity to establish without too much competition (Isselstein *et al.* 2002). However, the chances of (full) restoration of the *Cirsio-Molinietum* meadows will probably be severely hindered once a dominant species such as *H. lanatus* is established and it could quite possibly take a long time and a lot of effort to change this situation. Alternative methods of restoring nature areas, such as the sowing of target species or small-scale and strategic seedling planting, have been shown to be effective (Tallowin and Smith 2001). In an experiment looking at the effect of sowing on the establishment of calcareous grasslands Stevenson *et al.* (1995) found that the sowing of even low amounts of locally collected seeds significantly increased the establishment of the desired species. However, Warren *et al.* (2002) found that it was a difference in management regimes (grazing by either cattle or sheep and mowing) rather than the sowing of a seed mixture which significantly changed the succession towards the restoration of a semi-natural community. In both experiments seeds were sown onto a bare soil (ploughed) and it would seem to be the best time to sow directly after topsoil removal. A more effective method of ensuring the rapid return of target species would be the planting of seedlings but because it is more impractical this would probably be best used when seeds are hard to obtain (Tallowin and Smith 2001) or if sowing is ineffective and the area threatens to become overgrown by more common species.

## 5 Reference list

- Bakker, J.P., Dekker, M. & Vries, Y.d. (1980) *The effect of different management practices on a grassland community and the resulting fate of seedlings*. *Acta Botanica Neerlandica*, **29**, 469-482.
- Baskin, C.C. & Baskin, J.M. (1998) *Seeds - Ecology, Biogeography, and Evolution of Dormancy and Germination*. Academic Press, San Diego.
- Bekker, R.M. (1998) *The ecology of soil seed banks in grassland ecosystems*. Rijksuniversiteit Groningen.
- Bennett, W.F. (1993) *Nutrient Deficiencies & Toxicities In Crop Plants*. The American Phytopathological Society, Minnesota.
- Berendse, F. & Elberse, W.Th. (1990) Competition and Nutrient Availability in Heathland and Grassland Ecosystems. *Perspectives on Plant Competition* (Grace, J. B. and Tilman, D.), pp. 93-116. Academic Press Inc., San Diego.
- Fenner, M. (1978) *A comparison of the abilities of colonizers and closed-turf species to establish from seed in artificial swards*. *Journal of Ecology*, **66**, 953-963.
- Foster, B.L. & Gross, K.L. (1998) *Species richness in a successional grassland: Effects of nitrogen enrichment and plant litter*. *Ecology Washington D.C.*, **79**, 2593-2602.
- Grime, J.P. & Hunt, R. (1975) *Relative growth-rate: it's range and adaptive significance in a local flora*. *Journal of Ecology*, **63**, 393-422.
- Harborne, J.B. (1997) Plant Secondary Metabolism. *Plant Ecology* (Crawley, M. J.), pp. 154-155. Blackwell Science Ltd, Oxford.
- Haugland, E. & Tawfiq, M. (2001) *Root and shoot competition between established grass species and newly sown seedlings during spring growth*. *Grass and Forage Science*, **56**, 193-199.
- Heath, D. (1995) *An Introduction to Experimental Design and Statistics for Biology*. UCL Press Limited, London.
- Hunt, R. (1982) Growth analysis of individuals. *Plant Growth Curves The Functional Approach to Plant Growth Analysis* pp. 16-33. Edward Arnold (Publishers) Limited, London.
- Hunt, R. (1990) Relative growth rates. *Basic Growth Analysis* pp. 25-34. Unwin Hyman Ltd, London.
- Isselstein, J., Tallowin, J.R.B. & Smith, R.E.N. (2002) *Factors Affecting Seed Germination and Seedling Establishment of Fen-Meadow Species*. *Restoration Ecology*, **10**, 173-184.

- Karssen, C.M. & Hilhorst, H.W.M. (1992) Effect of Chemical Environment on Seed Germination. *Seeds, The Ecology of Regeneration in Plant Communities* (Fenner, M.), pp. 328-335. CAB International, Oxon.
- Klooker, J., Diggelen, R. van, and Bakker, J. P. Natuurontwikkeling op minerale gronden: *Ontgronden: nieuwe kansen voor bedreigde plantensoorten*. 1999.
- Leishman, M.R. (1999) *How well do plant traits correlate with establishment ability? Evidence from a study of 16 calcareous grassland species*. *New Phytologist*, **141**, 487-496.
- Poorter, H., Vijver, C.A.D.M.v.d., Boot, R.G.A. & Lambers, H. (1995) Growth and carbon economy of a fast-growing and a slow-growing grass species as dependent on nitrate supply. *Plant and Soil*, **171**, 217-227.
- Rees, M. & Brown, V.K. (1991) *The effect of established plants on recruitment in the annual forb Sinapis arvensis*. *Oecologia*, **87**, 58-62.
- Rice, E.L. (1974) *Allelopathy*. Academic Press, Inc., New York.
- Ryser, P. (1993) *Influences of neighbouring plants on seedling establishment in limestone grassland*. *Journal of Vegetation Science*, **4**, 195-202.
- Ryser, P. & Wahl, S. (2001) *Interspecific Variation in RGR and the Underlying Traits among 24 Grass Species Grown in Full Daylight*. *Plant biol.*, **3**, 426-436.
- Schaminée, J.H.J., Stortelder, A.H.F. & Weeda, E.J. (1996) Deel 3: Plantengemeenschappen van graslanden, zomen en droge heiden. *De Vegetatie van Nederland* Opulus Press, Uppsala & Leiden.
- Schütz, W. (2000) *The importance of seed regeneration strategies for the persistence of species in the changing landscape of Central Europe*. *Zeitschrift für Ökologie und Naturschutz*, **9**, 73-83.
- Tallowin, J.R.B. & Smith, R.E.N. (2001) *Restoration of a Cirsio-Molinietum Fen Meadow on an Agriculturally Improved Pasture*. *Restoration Ecology*, **9**, 167-178.
- Tooren, B.F.v. & Pons, T.L. (1988) *Effects of temperature and light on the germination in chalk grassland species*. *Functional Ecology*, 303-310.
- Verhagen, R., Klooker, J., Bakker, J.P. & van Diggelen, R. (2001) *Restoration success of low-production plant communities on former agricultural soils after top-soil removal*. *Applied Vegetation Science*, **4**, 75-82.
- Vermeer, J.G. & Berendse, F. (1983) *The relationship between nutrient availability, shoot biomass and species richness in grassland and wetland communities*. *Vegetatio*, **53**, 121-126.

Warren,J., Christal,A., Wilson,F. (2002) *Effects of sowing and management on vegetation succession during grassland habitat restoration. Agriculture, Ecosystems and Environment*, **93**, 393-402.

Weeda,E.J., Westra,R., Westra,Ch. & Westra,T. (1991) *nederlandsche oecologische Flora, deel IV*. IVN in samenwerking met de VARA en de Vewin.

Weeda,E.J., Westra,R., Westra,Ch. & Westra,T. (1994) *nederlandsche oecologische Flora, deel V*. IVN in samenwerking met de VARA en de Vewin.

Wilson,J.B. (1988) *Shoot competition and root competition. Journal of Applied Ecology*, **25**, 279-296.

Zar,J.H. (1984) *Biostatistical Analysis*. Prentice-Hall Inc., New Jersey.

## 6 Appendices

### 6.1 Appendix 1: Nutrient solutions

#### 6.1.1 Experiment 1:

General fertilisation: twice weekly per pot with 50 ml 0 %-N nutrient solution:

STOCKSOLUTION	ml stock./60 l	concentration/100 ml (mM)
1M KH <sub>2</sub> PO <sub>4</sub>	27	0.045
0,5M K <sub>2</sub> SO <sub>4</sub>	9	0.015
1M KCl	63	0.105
1M CaCl <sub>2</sub>	54	0.09
1M MgSO <sub>4</sub>	135	0.225
1M NaCl	9	0.015

Add per 60 litre:

1.8 ml stock solution spore elements (Pegtel '86):

0.1 M MnSO<sub>4</sub>·H<sub>2</sub>O (17.09 g/l)      0.03 M ZnSO<sub>4</sub>·7H<sub>2</sub>O (8.79 g/l)

5 mM CuSO<sub>4</sub>·5H<sub>2</sub>O (1.26 g/l)      5 mM Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O (1.21 g/l)

0.2 M H<sub>3</sub>BO<sub>3</sub> (12.60 g/l)

3.6 ml 5% Fe-raxenol

Concentrations have been calculated on the basis of an N:P:K proportion of 7:1:3.5 and with an N-concentration of 80 kg N/ha/year.

80 kg N/ha/year = 153.85 mg N/m<sup>2</sup>/week = 10.99 mM N/m<sup>2</sup>/week

1 pot is 17 cm x 17 cm = 0.0289 m<sup>2</sup>

thus 80 kg N/ha/year => 0.317 mM N/pot/week (=0.635 mM N/pot/2 weeks)

=> 0.0453 mM P/pot/week

=> 0.159 mM K/pot/week

N fertilisation: once every **two weeks** per pot with 50 ml of the appropriate solutions:

TREATMENT	Solutions*	concentration/50 ml (mM)
80 kg N/ha/yr.      ①	76.2 ml 1M NH <sub>4</sub> NO <sub>3</sub> in 6 litres	0.635
40 kg N/ha/yr.      ②	3 litre ① + 3 litre H <sub>2</sub> O	0.317
20 kg N/ha/yr.      ③	2.5 litre ② + 2.5 litre H <sub>2</sub> O	0.159
10 kg N/ha/yr.      ④	1.5 litre ③ + 1.5 litre H <sub>2</sub> O	0.0793

\* freshly made



## 6.1.2 Experiment 2

General fertilisation: twice weekly per pot with 100 ml 0 %-N nutrient solutions:

STOCKSOLUTION	ml stock/60 l	concentration/100 ml (mM)
1M KH <sub>2</sub> PO <sub>4</sub>	84	0.14
0,5M K <sub>2</sub> SO <sub>4</sub>	29	0.05
1M KCl	195	0.33
1M CaCl <sub>2</sub>	173	0.28
1M MgSO <sub>4</sub>	420	0.70
1M NaCl	29	0.05

Add per 60 litre:

5.6 ml stock solution spore elements (Pegtel '86):

0.1 M MnSO<sub>4</sub>·H<sub>2</sub>O (17.09 g/l)      0.03 M ZnSO<sub>4</sub>·7H<sub>2</sub>O (8.79 g/l)  
5 mM CuSO<sub>4</sub>·5H<sub>2</sub>O (1.26 g/l)      5 mM Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O (1.21 g/l)  
0.2 M H<sub>3</sub>BO<sub>3</sub> (12.60 g/l)

11.2 ml 5% Fe-rexanol

Concentrations have been calculated on the basis of an N:P:K proportion of 7:1:3.5 and with an N-concentration of 40 kg N/ha/year.

40 kg N/ha/year = 153.85 mg N/m<sup>2</sup>/2 weeks = 10.99 mM N/m<sup>2</sup>/2 weeks

1 pot is 30 cm x 30 cm = 0.09 m<sup>2</sup>

thus 40 kg N/ha/year => 0.989 mM N/pot/2 weeks

=> 0.141 mM P/pot/2 weeks

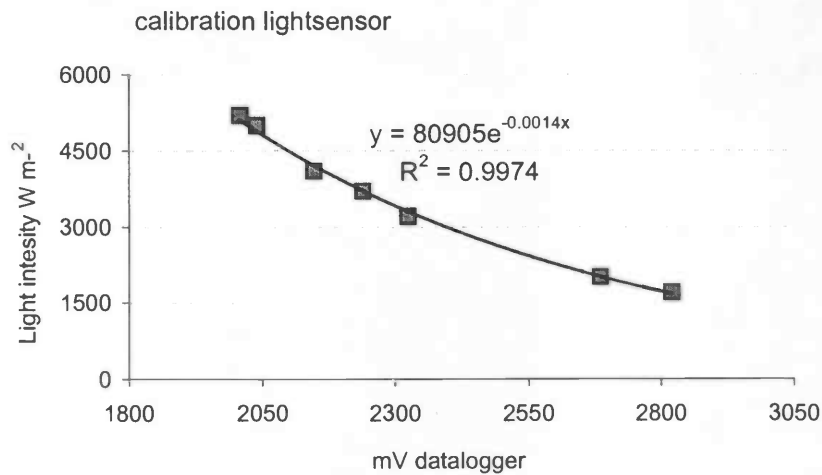
=> 0.495 mM K/pot/2 weeks

N fertilisation: once every **two weeks** per pot with 150 ml of the appropriate solutions:

TREATMENT	solutions*	concentration/150 ml (mM)
40 kg N/ha/yr.    ①	23 ml 1M NH <sub>4</sub> NO <sub>3</sub> in 7 litre H <sub>2</sub> O	0.99
10 kg N/ha/yr.    ②	1.25 litre ① + 3.75 litre H <sub>2</sub> O	0.25

\* freshly made

## 6.2 Appendix 2: light measurement



The formula used to calculate the light intensity above and below the vegetation is:

$$y = 80905e^{-0.0014x}$$

where  $x$  is the recording from the datalogger (in mV), and  $y$  gives the corresponding light intensity (in  $\text{W/m}^2$ ).

The datalogger is the 21x micrologger from Campbell Scientific Ltd. with the following settings:

execution time: 1 second

programme nr.: 4, EX-DEL-SE

number of repeats: 1

range of the measurements: 5 V

measurement delay: 0.05 seconds

pulse voltage: 5 V

The light sensor is a light sensitive resistance made in the laboratory of the Plant Ecology Group of the university of Groningen.

### 6.3 Appendix 3: seedling photographs



*Cirsium dissectum* 10N



*Cirsium dissectum* 80N



*Danthonia decumbens* 10N



*Danthonia decumbens* 80N



*Juncus conglomeratus* 10N



*Juncus conglomeratus* 80N