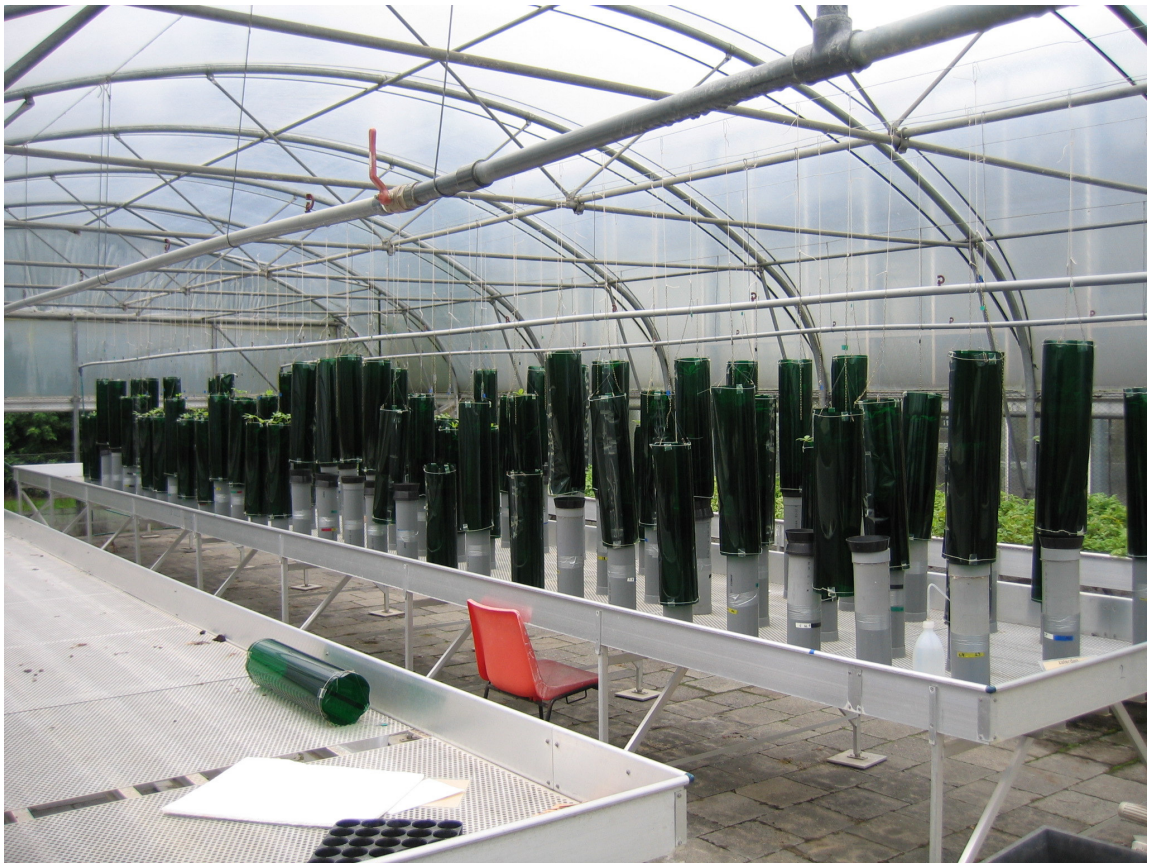
Plastic responses in the competition for light among genotypes of a stoloniferous species



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Plastic responses in the competition for light among genotypes of a stoloniferous species

Fenotypische aanpassingen van verschillende genotypen van een clonale plantensoort in concurrentie om licht

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. J.C. Stoof, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op woensdag 9 april 2008 des ochtends te 10.30 uur

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v.g.w.d.

Content

Chapter 1

General introduction

Coexistence and competition

One of the main goals in plant ecology is to understand the regulation of plant biodiversity. Recently, two models that try to predict the diversity of species have received wide attention, due to the seemingly opposing view they take on how species distributions and abundances are determined. Neutral models assume that all species and individuals are equivalent with respect to reproductive success and mortality, and that dynamics are governed by stochastic processes of random drift, immigration and speciation (Bell 2001; Hubbell 2001; Volkov et al. 2003). On the other end, niche theories assume that dynamics are mainly driven by differences between species in mortality and reproductive success, caused by differences in competitiveness and in the environmental conditions that they compete in (Chase and Liebold 2003). However, by now most ecologists acknowledge that processes from both approaches affect species coexistence (Chave 2004; Gravel et al. 2006; Adler et al. 2007), and an understanding of the underlining mechanisms of both theories is needed.

In this thesis the focus is on the way competition for resources can structure plant communities. In principle, when grown alone species can survive and grow under a wide variety of conditions. However, as was already demonstrated by Tansley (1917) and Gause (1934), when species grow together one species can outcompete the other, and can drive the other species to extinction. This is because when species are competing for similar resources in a similar way, one will always be the superior competitor, a principle Hardin (1960) named the “competitive exclusion principle”. Competition is thus thought to greatly limit the distribution of any species in nature (Pulliam 2000; Leibold 1995).

Within such a competitive setting, coexistence is hypothesized to occur when species specialize to specific conditions in such a way that they are superior to

their competitors (Diamond 1975). Species are forced to specialize through trade-offs, which prevent species of becoming superior in all possible conditions (Silvertown 2004; Miller et al. 2005). Differences between species will then lead to differences in the ability to drive other species to local extinction (Grover 1997; Rastetter and Agren 2002), and the environmental conditions may determine what species will be competitively superior in a fine-scaled pattern (Wilson and Keddy 1986; Miller 1994; Levine et al. 1998; Callaway and Pennings 2000).

Plasticity and the competition for light

Plants themselves can alter their phenotype in response to different environmental conditions. This ability is called plasticity, and in its most general form refers to any change in any plant trait in response to any change in the environment (Bradshaw 1965; Scheiner 1993; Sultan 2000). Not all changes in the phenotype in response to a change in environmental conditions may be beneficial to plant performance. Plasticity may be neutral if the change in phenotype does not result in a change in fitness, injurious or maladaptive if the change leads to a decrease in fitness, and adaptive when it leads to an increase in fitness (Alpert and Simms 2002). In the latter case the plastic response of a plant may enable it to adapt to different and variable environments, which in turn can mitigate the competitive differences between individuals and thus possibly promote coexistence (Mazer and Schick 1991; Sultan and Bazzaz 1993; Van Kleunen et al. 2001; Stoll et al. 2002).
Adaptive plastic responses require reliable signals that can be detected by plants (Novoplansky et al. 1994; Aphalo and Ballaré 1995). One of the most studied plastic responses is the way plants adapt to the cues that signal the presence of aboveground neighbours. Leaves absorb more photons in the wavelength region of red (655-665 nm) than of far-red light (725-735 nm) (Holmes and Smith 1975; Smith and Whitelam 1997), and the change in this ratio can be sensed by phytochrome photoreceptors (Quail et al. 1995; Smith 2000). Similarly, a reduction in blue light can be perceived with blue light receptors (Ballaré and Casal 2000; Casal 2000). Decreased light quantity also plays a role (Grime and Jeffrey 1965; Ballaré et al. 1994), while Pierik et al. (2003) showed that plants can sense ethylene that is omitted by plants. These cues thus provide the signals with which plants can detect possible competitors, even before they are shaded (Ballaré et al. 1990).

In response to these signals plants increase their leaf angles, increase leaf area per unit mass and can elongate their petioles and stems (Dong 1995; Ballaré 1999; Smith and Whitelam 1997). These responses can be adaptive because it may increase light capture in dense vegetation, while it may prevent overinvestment in height growth where density is low (Dudley and Schmitt 1996). In dense herbaceous vegetation, a distinct vertical light gradient occurs (Monsi and Saeki 1953; Fliervoet 1984). In such a gradient plants can increase their light capture by placing their leaves higher up (Leeflang et al. 1998; Weijschede et al. 2006). In addition, they can shade lower placed leaves (Falster and Westoby 2003). Therefore, taller plants may suppress the growth of smaller individuals, leading to the development of large differences in size between a relatively small number of large plants and many small individuals (Weiner 1990). These smaller plants are found to have a higher mortality (Weiner and Thomas 1986; Weiner et al. 2001). The ability to respond quickly to neighbour proximity may thus be a crucial factor in determining the success of an individual within dense vegetation. However, as between species, many authors have found variation in plastic responses to neighbours within species (Donohue et al. 2000; Huber et al. 2004; Weijschede et al. in press), and it is likely that these differences will result in the competitive exclusion of the less plastic genotypes when the genotypes are directly competing.

Game theory

Most studies that have investigated genotypic differences in plasticity have looked at responses when genotypes were not directly competing. The fitness of a genotype will then depend on the environment and the response of the genotype itself (Pronk 2004). While this may give an idea whether plasticity per se is beneficial, it may not predict which level of plasticity may be selected for when plants are interacting. This is because in competition the fitness of a genotype will also depend on the response of all competing individuals. Plants directly influence the light climate of the canopy, and they can therefore alter the environment they and their competitors experience through their plastic responses. Because of this ability to change the light conditions, Donohue (2003) even mentioned canopy formation as an example of “niche construction”. Light availability is of great influence on the photosynthetic rate of an individual, and plants thus strongly affect each others performance (Callaway et al. 2003). Even stands made up of a single species usually contain plants with different genotypes, which differ in their

response to neighbour proximity in the developing canopy. As a result, the light conditions in the developing vegetation will depend on the frequencies of the different genotypes that makeup that canopy (Parker and Maynard Smith 1990; Pronk et al. 2007) and the performance of a genotype may vary accordingly. The analyzes of such frequency-dependent selection processes requires a game-theoretical approach, in which performance is evaluated in relation to the performance of all other competing genotypes (Reichert and Hammerstein 1983).
There are two central questions in such an analysis. First the question is whether a fixed set of genotypes can evolve to a stable state in which only one genotype will remain, or whether a combination of genotypes will coexist (Hammerstein and Selten 1994; Eshel et al. 1998). To put it in other words, to what extent will competition lead to competitive exclusion and/or coexistence. The second, even more theoretical, question is whether such a state is stable. This would mean that the frequencies and identities of the genotypes that will remain will return to that existing state after a new genotype, often termed 'mutant', enters the population. The genotype or combination of genotypes that can not be invaded by a mutant is called an Evolutionarily Stable Strategy/State (Maynard Smith and Price 1973).
The overall production of a stand in an ESS-state is often found to be lower than is theoretically possible with the same level of resources. This is because plants that have plant traits that maximize photosynthetic rate at the stand level can be invaded by mutants that maximize their own photosynthetic rate (Schieving and Poorter 1999; Anten and Hirose 2001). These mutants will increase in frequency, and the production of the whole stand will become lower than its optimal production. This phenomenon is described as "the tragedy of the commons" by Hardin (1968) in a classical paper that has been used in a wide variety of scientific disciplines.

Aim and outline

The basis of this thesis is a competition experiment that was set up in 1998 with ten genotypes of the clonal plant *Potentilla reptans*. After five years of competition, genetic analyzes has shown that one genotype has become abundant (genotype I), several others have slightly increased or remained near the initial frequencies at the start of the experiment (10%), while others have strongly decreased (Stuefer et al. in prep., figure 1.1). This indicates that both competitive exclusion and coexistence have occurred.

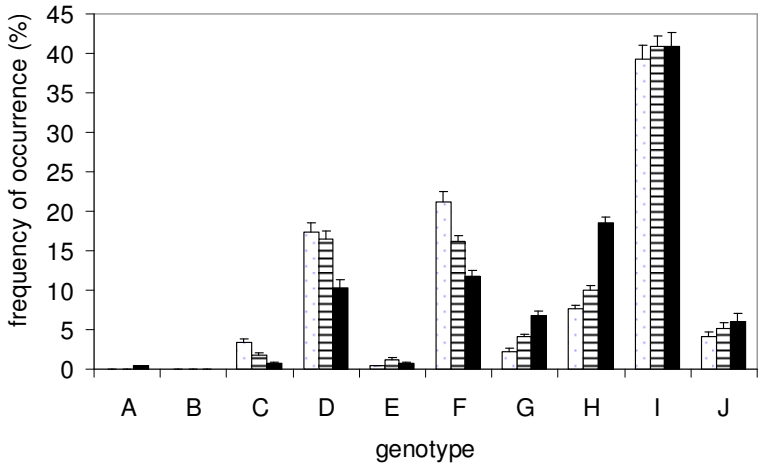

Figure 1.1. Average frequency per plot (% of total leaves + 1 se) of all genotypes in the three harvested layers: left, dotted bars: bottom layer; middle, stripped bars middle layer and right, black bars the top layer. Data are from Stuefer et al. (in prep).

The aim of this thesis is to understand the outcome of competition, and the resulting leaf and canopy characteristics, as a consequence of the genotypic differences in responses to aboveground neighbour proximity and the interplay between these different genotypes.

In chapter 2, the plastic responses of all ten genotypes are investigated in light climates that are fixed during the growth of the plants, to study whether these genotypes differ in their responses to similar cues and whether this can be directly related to the outcome of the competition experiment.

Chapter 3 shows the plasticity of five different genotypes in response to light gradients that increase in height, and explores the possibility that differences in leaf placement between genotypes are caused or enlarged by differences in the ability to keep up with the change in a light gradient during growth, i.e. the height growth of neighbours.

In chapter 4, the genotypes determine their own light climate. The responses to an increase in density in mono-genotypic stands are studied, while asking the question whether genotypes that have a stronger increase in height investment also have a stronger decrease in total production.

Chapters 5, 6 and 7 are based on data from the competition experiment, where the genotypes have formed the light gradient together. Chapter 5 focuses on the light capture of the leaves within different layers of the canopy, and explores whether

the dominant genotype has the highest efficiency in light capture compared to the other genotypes. In chapter 6 a canopy model is introduced to study the photosynthetic characteristics of the different genotypes. This model is used in chapter 7 for a game theoretical approach to see if a “tragedy of the commons” occurs because all genotypes “overinvest” in total lamina area. It also analyzes to what extent the current total lamina area of the different genotypes can be understood using simple optimization. Chapter 8 summarizes all the findings to discuss the mechanisms behind competitive exclusion and coexistence.

Figure 1.2. Growth form of *Potentilla reptans*. Clockwise, starting from top left: Rosette of Genotype H in pot without stolons in November 2005; Rosette of Genotype B grown in the homogeneous shade (see Chapter 2) with newly formed ramets on two stolons; and the washed roots of a Genotype I plant as harvested in November 2005, showing the fine roots and the start of the tap root.

Chapter 2

Shade avoidance responses to homogeneous shade and light gradients

Summary

To test for differences between the ten genotypes in plant traits and plasticity therein, two separate experiments were set up: a homogeneous shading experiment and an experiment were ramets of the ten genotypes were placed in artificial light gradients that differed in length. Also, to assess if these experiments could predict what traits would be selected for in the competition experiment the genotypes were ranked according to the trait and plasticity values, and it was tested if these were correlated to the rank order of abundance in the competition experiment.

Differences between genotypes were found in all trait values. In the homogeneous shade experiment, differences in plasticity between the genotypes were found, but only plasticity in petiole length and in daughter weight were significantly correlated to the abundance in the competition experiment. In the light gradient experiment, no differences between the genotypes in plasticity were found. However, petiole lengths in both gradient lengths were correlated to the abundance in the competition experiment.

Both experiments thus showed that all genotypes display classic shade avoidance responses.

Introduction

The phenotype of plants is strongly affected by the presence of neighboring plants (Pierik 2003). Many plant species respond to crowding with an increase in height growth (Geber 1989; Weiner et al. 1990). At high density, this increased height growth is hypothesized to increase the light interception of a plant (Schmitt and Wulff 1993). However, since increased height growth usually also means an increase in height investment (Givnish 1982; Anten and Hirose 1998), it depends on the balance between costs and benefits whether these plastic responses are beneficial for plant performance.

The costs and benefits in turn depend on the environment the plant is growing in. In the field layer of forests, for instance, there is hardly any vertical light gradient (Grime and Jeffrey 1965; Grime 1966). Most studies on plastic responses are done mimicking this kind of shade through the use of shade cages. In these situations an increase in height investment does not lead to an increase in light capture, and thus this plastic response might even reduce plant performance (Schmitt 1997). However, increases in leaf area or Specific Leaf Area (SLA) might still be relatively beneficial in homogeneous shade because they do increase light capture (Dong 1995; Elemans 2005). In most herbaceous vegetation there is a substantial increase in light availability with increased height (Monsi and Saeki 1953) and thus plants can increase light availability by increasing height growth. Leeflang et al. (1998) indeed found that in *Glechoma hedera* petiole length was higher in the light gradient compared to a homogeneous treatment, and that plants had a higher biomass. In a similar experiment, however, Weijschede et al. (2006) found that *Trifolium repens* plants had lower biomass in the light gradient treatment, despite reaching higher light levels with longer petioles. They argued that the costs of increased height growth might have been larger than the increased light capture, which could be related to the failure of not reaching the top in their experiment. This indicates that plants can show different responses to different light conditions, and that these different conditions can give a different view on the performance and fitness of a plant.

Within many species there is also variation in the way genotypes respond to a change in the environment (Sultan 2005), e.g., in terms of leaf placement and investment in height, which in turn could lead to differences in performance. So far, however, no experiments have tested if differences between genotypes in these traits lead to a shift in abundance over time. Studies that have looked at light interception in dense stands with erect growing, herbaceous species in

monoculture have found that the tallest plants catch more light (Anten and Werger 1996; Anten and Hirose 1998) and have a higher photosynthesis per unit aboveground biomass than shorter plants (Anten and Hirose 2001). Genotypes with low petiole length might not be able to place their leaves at high light availability when competing with genotypes with long petioles, which may result in lower relative performance. One would therefore expect that when conspecific plants of different genotypes are competing in dense vegetation, such as is the case in a long-term competition experiment in which the frequencies of ten genotypes of the clonal plant *Potentilla reptans* were followed over several years (Stuefer et al. in prep.; see also figure 1.1), the genotypes which have the longest petioles and/ or the genotypes with the highest plasticity are the plants that perform the best.

In stoloniferous species such as *Potentilla reptans*, however, height growth might be more costly than in erect-growing species. Each leaf is separately supported, which in terms of biomass use for vertical support is less efficient than the production of a single stem (Liu et al. 2007). Taller genotypes might therefore have high costs, which in turn could lead to a lower growth rate than smaller genotypes. In that case a high trait value or high plasticity might not translate directly into a higher performance in the competition experiment.

We present two experiments to study the plant traits and plasticity of the ten genotypes that were used in the competition experiment (see introduction of thesis, chapter 5,6 and 7, Stuefer et al. in prep.). The first goal of the research reported in this chapter was to see whether the ten genotypes that were used in the competition experiment differ in their traits and their plasticity in these traits in response to shade. A classical homogeneous shading experiment was set up to study the responses of the plants to spectral shading (reduced photo active radiation, PAR, and reduced R:Fr ratio). We expected to find genotypic differences in plastic responses. We also wanted to test the hypothesis that high absolute plasticity was related to large trait values in the high light treatment as was suggested by Pigliucci (2003), which could explain part of the genotypic differences in plasticity. Also, since differences between genotypes could lead to differences in relative performance, we expected to find a genotype * treatment interaction for traits associated with performance, e.g. total biomass and daughter biomass.

To study the responses of the genotypes in a light gradient situation, another independent experiment was set up with the plants growing in two light gradients differing in length. Since an increase in light gradient means a decrease in light

availability at the same height, we expected the plants to show similar responses as in the homogeneous shade experiment: an increase in shade avoidance characteristics with increasing gradient height. We again expected to see genotypic differences in the responses shown at the different treatments. Since a difference in ontogenetic development can affect traits and responses of plants (Birch and Hutchings 1992a; Birch and Hutchings 1992b), we also wanted to see how these traits differ with each newly formed leaf. Furthermore we expected that the genotypes with the longest petioles would show an increase in relative performance in the highest light gradient, because these would be able to reach higher light availability in the 50 cm light gradient sooner than smaller genotypes.
The second goal was to see if these traits and the plasticity in these traits could be related to the outcome of the competition experiment after five years. We wanted to test the hypothesis that the genotypes with the longest petioles and/ or the genotypes with the highest plasticity in these two experiments were the plants that performed the best when competing for five years.

Methods and Materials

Homogeneous shade experiment

Treatments

On the 21st of June 2005, 20 ramets per genotype in the same ontogenetic stage were taken from stock populations maintained at the botanical gardens in Utrecht since 1997. To standardize for size we removed the internodes, cut the roots to a length of 5 cm and removed all leaves except the two youngest fully expanded leaves. The ramets were planted into 13x13x13 cm pots filled with a mixture 2:1 of compost and river sand. Slow-release fertilizer (Osmocote, Grace Sierrra international, Heerlen, the Netherlands) was added to provide nutrition of 5 kg N ha^{-1} wk^{-1}. Plants were watered when needed.
On June 24th, 12 ramets per genotype of similar size were moved to two treatments (six per treatment) in a plastic greenhouse. Six wooden frames of 50 x100 x 50 cm (HxLxW) were constructed, three for the full light treatment (FL) and three for the homogeneous shade treatment. All sides of the full light treatment were covered by transparent plastic, while for the homogeneous-shade (HS) treatment they were covered by green plastic film (Lee colortran # 122 HT fern green). A strip of 5 cm at the top of the two short sides of the frames was left uncovered to allow for sufficient ventilation. For the roofs a frame was made of 100 x100 cm, covered with same

type of material as was used for the sides. This prevented the access of direct light through the 5 cm strips. The PAR in the FL treatment was 80% of the PAR outside the greenhouse (measured with a Licor Li 185), while the R:Fr ratio was 1.12 (Licor 1800 spectroradiometer). For the HS treatment these values where 25% and 0.24 respectively. Earlier measurements in similar cages showed there were no significant differences between the cages in humidity and temperature. The cages were randomly positioned in the plastic greenhouse. Every cage contained 20 randomly placed plants, two per genotype. Every week the plants were moved to a different cage, re-randomized over the cages of the treatment.

After nine weeks the plants were harvested. We separated the petiole and the lamina of the longest petiole, and we measured the Petiole Length and the Lamina Area with a Licor 3100 leaf area meter. The other parts were divided into mother plant (laminas and petioles), daughter plants (ramets and internodes) and roots. The roots were washed free from soil particles. All parts were dried for at least three days at 65ºC, after which biomass was measured. From these data the Specific Lamina Area ($SL_{am}A$, m^2 lamina g^{-1} lamina mass), Specific Petiole Length (SPL, m g^{-1} petiole mass), Petiole Leaf mass Ratio (PLR, g petiole mass g^{-1} total leaf mass, with total leaf mass = petiole mass + lamina mass) and the Root Mass Ratio (RMR, g root mass g^{-1} total plant mass) were calculated. Petiole Length, Lamina Area, $SL_{am}A$, SPL and PLR are thus traits of the longest petiole, while the RMR is based on the whole plant.

Data analysis

We used a two-way Anova, with genotype and treatment as fixed factors in order to test for main effects and interactions. If necessary, data were transformed to meet the demands of normality and homoscedasticity (see tables).

To see whether genotypes with a high trait value in Full light had a higher absolute plasticity, the average trait value for each genotypes was calculated in both treatments for Petiole Length, Lamina Area and the $SL_{am}A$. The absolute plasticity was calculated from the two treatment values by subtraction. We then used regression analysis to see if absolute plasticity was related to the trait value in Full light.

To see if trait values and absolute plasticity were related to the frequency of the genotypes in the competition experiment all averages of the traits, absolute plasticities and the frequencies in the competition experiment were ranked for all

the genotypes, after which we performed a Spearman rank correlation analysis. All analyses were performed in SPPS 11.0.

Light gradient experiment

Figure 2.1. Set up of light gradient experiment. Left: Upper view of a 30 cm light gradient with the plants and the mesh wire removed. Right: Side view of a 50 cm light gradient with two side filter sheets and the right side sheet removed, showing the mesh wire and the plants at the end of the experiment.

Treatments

On the 8th of June 2005, 20 ramets per genotype in the same ontogenetic stage were taken from stock populations maintained at the botanical gardens in Utrecht since 1997. To standardize for size we removed the internodes, cut the roots to a length of 5 cm and removed all leaves except the two youngest fully expanded leaves. The ramets were planted into 8x8x8 cm pots filled with a mixture 2:1 of compost and river sand. Slow-release fertilizer (Osmocote, Grace Sierrra international, Heerlen) was added to provide a nutrition of 5 kg N ha^{-1} wk^{-1}. Plants were watered when needed.

On June 10th 12 ramets per genotype of similar size were moved to two treatments (six per treatment). The two treatments consisted of two light gradients differing in height, a 30 cm gradient and a 50 cm gradient. The gradients were made by attaching four sheets of plastic film (Lee colortran # 122 HT fern green) of 30 cm and 50 cm height respectively vertically to a wooden frame (height 50 cm, width 50 cm length 200 cm). The two inner sheets were 15 cm apart. At 5 cm, 15 cm and 25 cm height a mesh chicken wire of 2.5 cm diameter was placed to provide support for the petioles (see figure 2.1).

This created two different light gradients in the center between the two inner sheets: the 30 cm light gradient (Lg30) and the 50 cm light gradient (Lg50). At the bottom of the 30 cm light gradient the PPFD was about 50% of the light at the top, while the R:Fr ratios were 1.09 at the top and 0.51 at the bottom. For the 50 cm gradient the PPFD at the bottom was reduced to 45%, while the R:Fr ratio was 0.43. Here, however, light availability at the lowest 20 cm increased very slowly (figure 2.2).

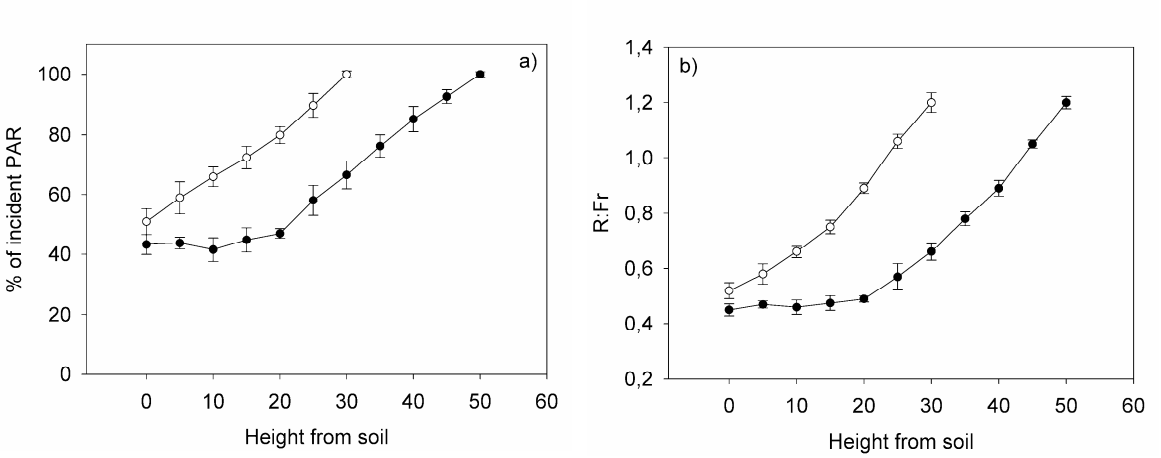

Figure 2.2. Light conditions in the 30 cm light gradient (open circles) and 50 cm gradient (closed circles): a) % (± se) of incident light as compared to the top of the light gradient and b) R:Fr ratio.

Three light gradient cages per treatment were made. The six frames were placed randomly in the greenhouse. Two plants per genotype were randomly positioned in between the two inner sheets, resulting in 20 plants per wooden frame. We thus had six replicas per genotype per treatment and a total of 120 plants for the whole experiment.

Every week the height of the laminas and the number of leaves per plant were measured. The petioles were marked with a thin ring of plastic straw differing in color to be able to follow the development of individual petioles. The pieces of straw were cut open on one side so the petioles would not be limited in their radial growth. All stolons were cut off once a week to prevent them from growing in other pots. After 42 days the height of all the laminas was measured again and the plants were harvested, just at the time when the plants started to shade each other. We measured all individual petioles and laminas of the first four newly formed leaves, because in all plants these had stopped elongating two weeks before the end of the experiment. We therefore assumed that at the time of harvest these leaves had been fully developed. Lamina area was measured with a Licor LI 3100.

Roots were washed free of soil particles, after which all plant material was dried at 65ºC for at least 72 hours and biomass was measured. We also measured the length of all petioles to determine the longest petiole length at harvest (LPL). From the petiole length, lamina area and their biomass the $SL_{am}A$ and SPL were calculated for the four fully developed leaves; the Petiole Mass Ratio (PMR, g total petiole g^{-1} total plant mass) and RMR were calculated on whole plant basis.

Data analysis

Since nor the cages nor the pots were moved during the experiment we used a mixed model approach. First, the mean of squares of the cages, between plants and the remaining (error) sum of squares, was estimated through a two-way Anova with plants nested within the cages. Then a three-way Anova with Treatment (T), Genotype (G) and Leafnumber (L) was performed. Since the variation between cages can be partly attributed to the different gradients, the Treatment effect was analyzed using the mean of squares of between cages from the two-way Anova, and of the Treatment sum of squares from the three-way Anova. The Genotype and Genotype* Treatment interaction were tested using the mean of squares of between plants of the two-way Anova and mean of squares of the G and G*T of the three-way Anova. The L, L*T, L*G and the L*G*T effects were analyzed using the remaining sum of squares in the two-way Anova and the L, L*T, L*G and the L*G*T effects of the 3-way Anova. If necessary, data were transformed to better meet the demands of normality and homoscedasticity (see tables).

Since no Treatment * Genotype interactions were found, we performed the regression analysis of absolute plasticity on trait value in full light only for Lamina Area, because the log transformation might conceal such a relation. To see if trait values were related to the frequency of the genotypes in the competition experiment we performed a similar Spearman rank coefficient analysis as was done for the homogeneous shade experiment. We used the data from the fourth newly formed leaf to rank all leaf traits.

Results

Homogeneous shade

Treatment

Most characteristics were affected by the treatment (figure 2.3, table 2.1). Compared with the plants in the Full Light treatment (FL) the petiole length of the longest petiole increased in the homogeneous shade (HS) treatment, as did the Lamina Area, the Specific Lamina Area ($SL_{am}A$) and the Petiole Leaf mass Ratio (PLR). The Total Weight and the weight in stolons and daughter ramets (daughter weight) decreased. For Specific Petiole Length (SPL) and Root Mass Ratio (RMR) there were no differences among treatments.

Table 2.1. Two-way Anova results (F-values) examining the effect of treatments, genotypes and treatment x genotype interaction in the homogeneous shade experiment. ^{log} means data for this value are log transformed. ns, p>0.10; $, 0.10≥p>0.05; *,0.05≥p>0.01; **, 0.01≥p>0.001; ***, p≤0.001. SLA=Specific Leaf Area (m^2 g^{-1}), SPL= Specific Petiole Length (m g^{-1}), PLR= petiole total leaf mass ratio(g g-1), RMR= Root Mass Ratio (% of total mass in the roots).

Traits	Treatment	Genotype	Treatment x Genotype
d.f.	1	9	9
Petiole length	504.99***	6.93***	3.12**
Lamina area^{log}	245.92***	25.94***	1.33 ns
$SL_{am}A$	182.87***	2.30*	2.36*
SPL	0.70 ns	4.25***	0.46 ns
Total Weight^{log}	467.85***	10.77***	2.82**
Daughter weight^{log}	406.81***	5.59***	2.43*
PLR^{log}	158.11***	2.70**	0.60 ns
RMR	0.92 ns	5.91***	0.97 ns

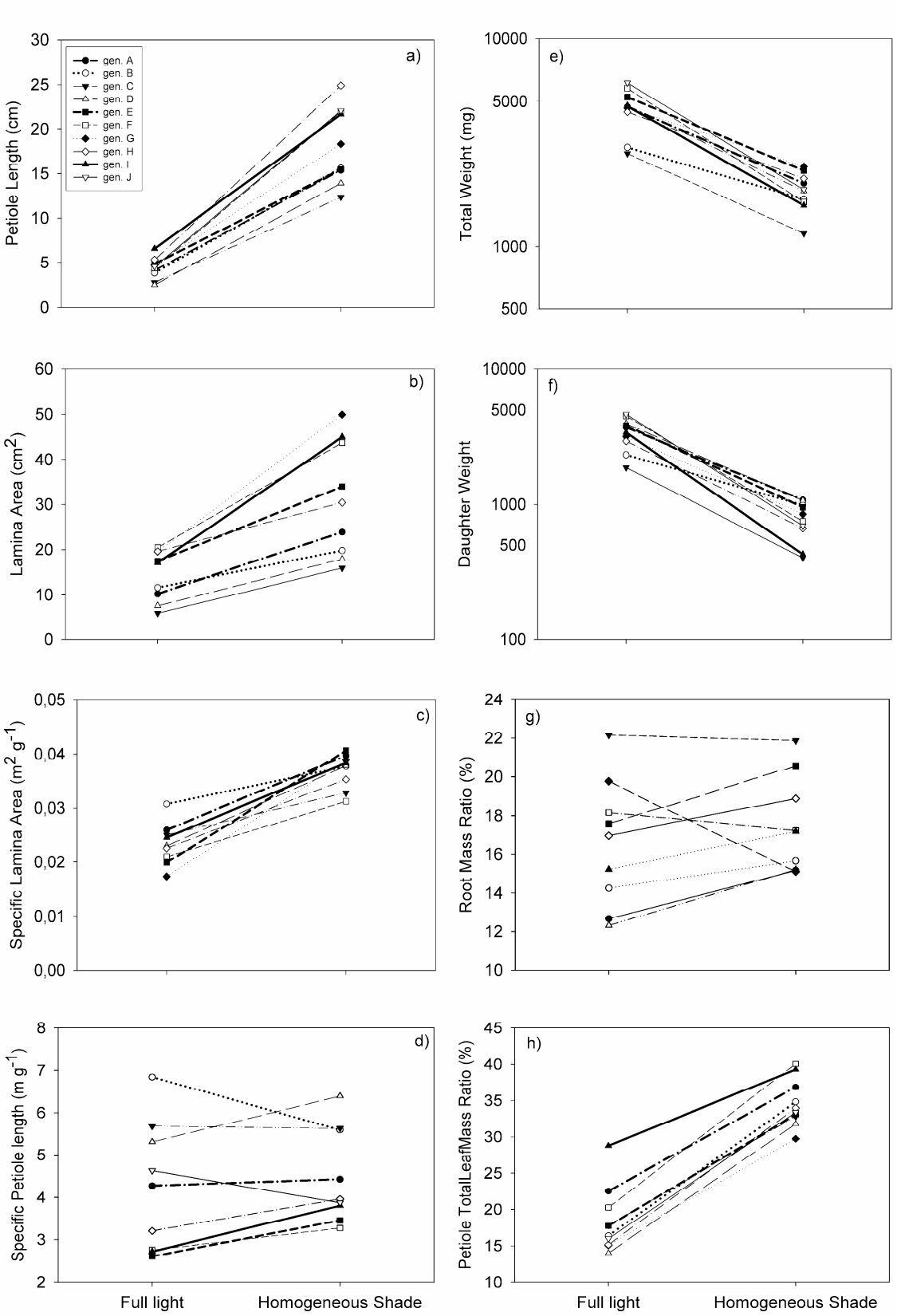

Figure 2.3. Reaction norms of the ten genotypes in the homogenous shade experiment. Y-axis of weight data (E &F) are log transformed. Key is given in figure 2.3a.

Genotypic differences

All traits differed among genotypes (figure 2.3). There were significant differences between genotypes in the way they responded to the treatments in terms of Petiole Length, $SL_{am}A$, Total Weight and Daughter Weight. No significant treatment * genotype interactions were found for Lamina Area, SPL, PLR and RMR (table 2.1). Absolute plasticity seemed to be related to the trait value in Full light for $SL_{am}A$ and Lamina Area (table 2.2). Genotypes that had a high trait value for $SL_{am}A$ under FL conditions had a lower absolute plasticity. Absolute plasticity in Lamina Area was positively related to the values in Full Light. The positive relation between Petiole Length in Full light and absolute petiole plasticity was only marginally significant (table 2.2).

Table 2.2. Results from the regression analyzes testing the relation between absolute plasticity in leaf traits and the value in the 'control' environment for leaf traits which showed a genotype * environment interaction, or were log-transformed, in the analyzes of table 1. Analyzes only done for the homogeneous experiment. β is the unstandardized regression coefficient. Positive values mean a positive relation between leaf trait in Full light (HS experiment) and absolute plasticity; negative values indicate a negative relation. ^{log} means data for this value are log transformed. ns, p>0.10; $, 0.10≥p>0.05; *,0.05≥p>0.01; **, 0.01≥p>0.001; ***, p≤0.001.

Trait	β
Homogeneous shade	
Petiole length	1.624$
Lamina area^{log}	0.679*
$SL_{am}A$	-1.011**

Table 2.3. Results from the Spearman rank correlation analyzes using ranked traits in the homogeneous shade experiment and the ranked abundance data of the genotypes in the competition experiment. In the table the correlation coefficient between the trait in the treatment (Full light, FL and homogeneous shade, HS) or the plasticity of that trait (trait value in HS minus trait value in FL) and the abundance in the competition experiment is given. SLA=Specific Leaf Area ($m^2 g^{-1}$), SPL= Specific Petiole Length ($m g^{-1}$), PLR= petiole total leaf mass ratio), RMR= Root Mass Ratio (% of total mass in the roots). ns, p>0.10; $, 0.10≥p>0.05; *,0.05≥p>0.01; **, 0.01≥p>0.001; ***, p≤0.001.

Trait	FL	HS	plasticity
Petiole length	0.333 ns	0.442 ns	0.600$
Lamina area	0.212 ns	0.224 ns	0.533ns
$SL_{am}A$	-0.467 ns	-0.297 ns	0.261 ns
SPL	-0.261 ns	-0.055 ns	0.552 ns
Total weight	0.430 ns	-0.018 ns	0.406 ns
Daughter weight	0.055 ns	-0.455 ns	0.636*
PLR	0.212 ns	0.180 ns	0.127 ns
RMR	-0.067 ns	-0.224 ns	-0.261 ns

Rank correlation

The rank coefficient correlation analysis showed that only two plasticity traits were related to the frequency of the genotypes in the competition experiment: petiole plasticity (although only marginally) and daughter weight plasticity were both positively related. The frequencies were not related to actual values of any trait in the FL and the HS treatments (table 2.3).

Light gradient experiment

Treatment

With an increase in gradient height the Petiole Length and the $SL_{am}A$ increased significantly (figure 2.5, table 2.4), while the number of leaves decreased (figure 2.4). Longest Petiole Length (LPL) showed the same pattern as Petiole Length (data not shown). Differences in Lamina Area, SPL and Total Weight and PMR were only marginally significant, with Lamina Area, SPL and PMR increasing slightly with gradient height, and Total Weight decreasing (figures 2.5 and 2.6). Root Mass Ratio was not significantly affected by the treatment. In the 50 cm gradient the petiole length increased faster with leaf development than in the 30 cm gradient (steeper slope, figures 2.5a and b). The $SL_{am}A$ in newly formed leaves seemed to change more in the 30 cm gradient, while for the Lamina Area the difference seems to be mainly in the response of the 2nd and 3rd leaf. The SPL showed no significant Leaf * Treatment interaction.

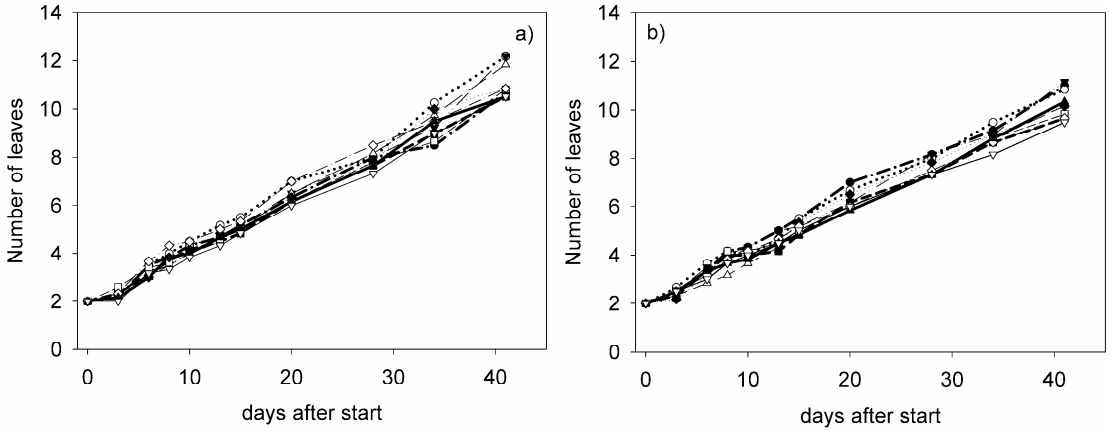

Figure 2.4. Number of leaves (averaged per genotype) during the light gradient experiment. a) 30 cm gradient b) 50 cm gradient. Legend of genotypes is given in figure 2.3a.

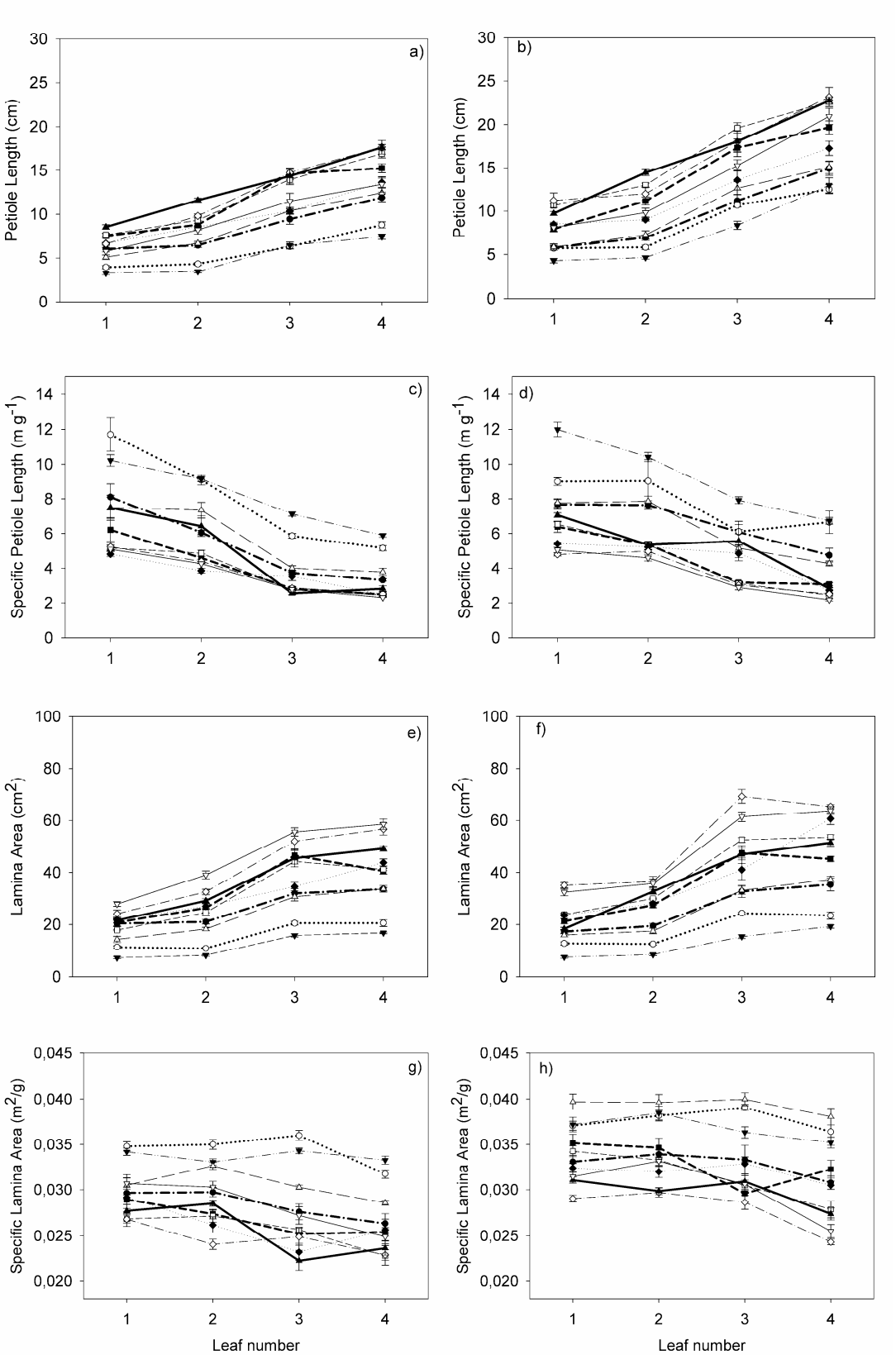

Figure 2.5. Morphological traits of individual leaves for both light gradients for the 30 cm gradient (a, c, e, and g) and the 50 cm gradient (b, d, f, g). Key is given in figure 2.3a.

Table 2.4. Analysis of variance results examining the effect of treatments (T), genotypes (G), leaves (L) and all interactions in the light gradient experiment. ^{log} means data for this value are log transformed. In the table F-values are represented. ns, P>0.10; $, 0.10≥P>0.05; *,0.05≥P>0.01; **, 0.01≥P>0.001; ***, P≤0.001. SLA=Specific Leaf Area (m^2 g^{-1}), SPL= Specific Petiole Length (m g^{-1}), PMR= Petiole Mass Ratio (% of total mass in the petioles, RMR= Root Mass Ratio (% of total mass in the roots), Number of leaves= number of leaves at the end of the experiment, HPL= highest petiole length at harvest.

Trait	Treatment	Genotype	T*G	Leaves	L*T	L*G	L*T*G
d.f.	1	9	9	3	3	27	27
Petiole length	21.46**	15.36***	0.41ns	347.5***	11.14***	2.10**	0.43ns
Lamina area^{log}	5.12$	41.58***	0.75ns	286.4***	2.86*	4.65***	0.48 ns
$SL_{am}A$	91.69***	9.20***	1.05ns	24.3***	3.32*	1.72*	1.35ns
SPL	6.21$	28.25***	0.36ns	97.6***	1.65 ns	1.13ns	1.01ns
Total weight^{log}	5.58$	35.41***	0.67ns				
PMR	5.31$	2.16*	0.76ns				
RMR	3.33 ns	3.56 **	1.39ns				
Number of leaves	7.74*	2.61**	0.56ns				
HPL	27.17**	13.06***	.053ns				

Genotypic differences

All traits did differ significantly among genotypes (figure 2.5, table 2.4). Some genotypes had longest Petiole length of 30 cm in the 50 cm light gradient, while the shortest genotypes had petioles with a maximum of about 18 cm (data not shown). For the leaves that were known to have stopped elongating two weeks before the end of the experiment (first four newly formed leaves) these values were 23 cm an 11 cm respectively (figure 2.5 and b). Genotypes with short petioles did have more leaves, than the taller genotypes. No Treatment * Genotype interactions were found, indicating that absolute plasticity was similar for all genotypes. Regression analysis showed that log-transformed absolute plasticity in Lamina Area was not related to the trait value in the 30 cm gradient (data not shown). No T*G interaction was found for Total Weight, Root Mass Ratio and Petiole Mass Ratio (figure 2.6, table 2.4).

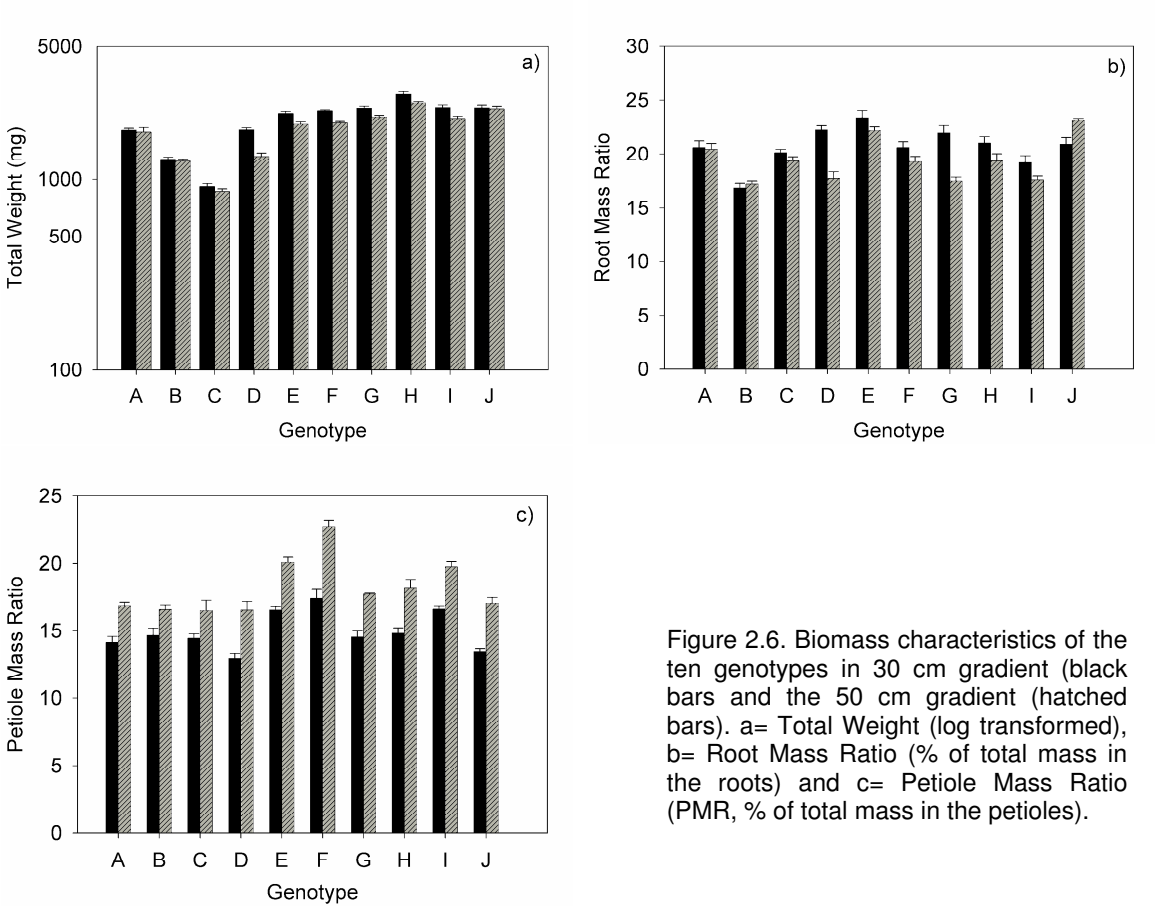

Figure 2.6. Biomass characteristics of the ten genotypes in 30 cm gradient (black bars and the 50 cm gradient (hatched bars). a= Total Weight (log transformed), b= Root Mass Ratio (% of total mass in the roots) and c= Petiole Mass Ratio (PMR, % of total mass in the petioles).

The genotypes did differ in the way several traits changed with each newly formed leaf. The difference in Petiole Length and Lamina Area between leaves increased more for some genotypes than others, while the pattern for the $SL_{am}A$ seemed much more irregular. The SPL, however, showed no Leaf*Genotype interaction. Also, no three-way interactions were found.

Rank correlation

Petiole length of the 4th newly formed leaf in both light gradients was positively related to the frequency of the genotypes in the competition experiment (table 2.5). The Lamina Area, $SL_{am}A$ and Total Weight in the 30 cm gradient were also related, but these same traits in the 50 cm gradient were not linked with frequency.

Table 2.5. Results from the Spearman rank correlation analysis using ranked traits in the light gradient experiment and the ranked abundance data of the genotypes in the competition experiment. In the table the correlation coefficient between the trait in the treatment (30 = 30 cm light gradient, 50 = 50 cm light gradient) and the abundance in the competition experiment is given. ns, P>0.10; $, 0.10≥P>0.05; *,0.05≥P>0.01; **, 0.01≥P>0.001; ***, P≤0.001.

Trait	30	50
Petiole length	0.661*	0.745*
Lamina area	0.588$	0.527 ns
$SL_{am}A$	0.648*	0.467 ns
SPL	-0.358 ns	0.527 ns
Total weight	0.648*	0.467ns
PMR	0.285 ns	0.418 ns
RMR	-0.139 ns	-0.152 ns
Number of leaves	-0.198 ns	-0.359 ns
HPL	0.588 $	0.685*

Discussion

Homogeneous Shade

Shade avoidance

Typical responses to shading among many plants are an increase in stem elongation relative to total biomass, stem diameter and leaf area (Smith 1982, (Schmitt and Wulff 1993), reduced allocation to the roots (Givnish 1982) , and an increase in leaf size and specific leaf area (Björkman 1981; Grime et al. 1986). *Potentilla reptans* showed most of these patterns in response to homogeneous shade: the petioles were longer, Lamina Areas larger and $SL_{am}A$ values higher in than in the full light treatment. The Root Mass Ratio and the Specific Petiole Length, however, did not significantly differ between treatments. This is in contrast with the findings of Huber (1996) and Stuefer & Huber (1998), who did find an relative decrease of allocation to the roots and an increased SPL in *Potentilla reptans* in their homogeneous shade treatments. We can not think of a possible explanation for these contradictions. It is possible that differences between the treatments are due to differences in ontogenetic development. Morphology and allocation patterns can be affected by differences in ontogenetic development (Coleman, McCaughay& Ackerly 1994). In the light gradient experiment, for instance, the SPL decreased with each newly formed leaf. We chose to measure the longest petiole. It is not known if petioles that were measured were of the same developmental stage. Also we do not know if all petioles that were measured had stopped elongation, and therefore some leaves might not have been fully developed. This could have affected the analysis. However, the increase in petiole length while total biomass decreased in the shade treatment shows that this

response can not be the result of a difference in ontogenetic development. Our data and the findings of Huber (1996) and Stuefer & Huber (1998) also confer with the generally found responses of other plants to shade. This thus shows that *Potentilla reptans* reacts to homogeneous shade with typical shade avoidance responses.

Genotypic differences

The genotypes differed in all traits that were measured. Also, there was a Treatment * Genotype interaction, showing that there were differences in plasticity among genotypes. The absolute difference in trait values of genotypes between the high-light and low-light treatments was correlated with the trait value itself in the high-light treatment: genotypes with a high trait value in the full light for Petiole Length and Lamina Area had higher absolute plasticity, while genotypes with a high SLA value were less plastic. Where Pigliucci et al. (2003) found only positive relations, we also found a negative relation for $SL_{am}A$. This shows that trait values can be related to trait values, but that a high trait values doesn't always mean high plasticity. Also, there is still some variation within this general trend: not all genotypes with a high trait values in full light had high absolute plasticity. This suggests that the difference in plasticity between the genotypes can not be explained by trait values alone, supporting the notion that plasticity can evolve separately from the trait value (Scheiner 1993a; Scheiner 1993b).The rank order of the genotypes in terms of total mass also changed from one treatment to the other. This suggests that some genotypes perform best in full light conditions, such as occur in the beginning of the season, while other genotypes do better than the other ones in shade conditions. In general, the genotypes differed in their responses, suggesting that different light climates can select for different genotypes.

Rank correlations

The rank correlations showed that only two plasticity traits were related to the frequency of the genotypes in the competition experiment: plasticity in petiole length and in daughter weight. None of the trait values in Full light and the homogeneous shade correlated with the genotype frequency. As was argued in the introduction, homogeneous shade might not mimic the light conditions in the competition experiment very well, because an increase in height does not lead to an increase in light availability. This might cause the genotypes to respond

differently than they would in the dense vegetation of the competition experiment. The related plasticity traits might then represent the ability of the genotypes to change these traits, investing more in the petioles and possibly less in offspring in order to reach higher light availability, which then leads to better performance in the up growing vegetation of the competition experiment.

Light gradient

Shade avoidance

As expected, plants in the 50 cm gradient showed similar characteristics as plants in the homogeneous shade. For instance, with an increase in gradient height the petiole length also increased. With each newly formed leaf, the length of the new petiole was higher in the 50 cm gradient than that of the corresponding leaf in the 30 cm gradient, showing that this increase in height growth continues during plant development. With increasing gradient length $SL_{am}A$ also increased, and, although only marginally significant, Lamina Area and Specific Petiole Length (SPL) increased as well. Again we didn't find significant effects of the treatment on Root Mass Ratio. This could be because each week the newly formed stolons were cut, or because the number of replicas was relatively low compared to the loss of root mass while washing them. But in general, the similar responses of the plants in this experiment in comparison with the homogeneous shade experiment show that *Potentilla reptans* reacts to an increase in light gradient height with shade avoidance responses.

Genotypic differences

As in the homogeneous shade experiment, the genotypes differed in all traits. For instance, the genotypes differed in the petiole lengths of the first four newly formed leaves. This difference increased with each newly formed leaf. Genotypes with a low Petiole Length did make more leaves, but the differences in number of total leaves between genotypes were small. So although petiole length increased with each newly formed leaf, genotypes which had shorter petioles in the first 4 newly formed leaves also had lower longest petiole length at harvest. The larger number of leaves of smaller genotypes thus didn't allow them to reach similar heights as genotypes with a higher petiole length of the first four newly formed leaves.

In contrast with the HS experiment, no significant Treatment* Genotype interactions were found. This suggests that all genotypes had similar absolute

plasticity in response to variation in height of the light gradient, as was also found by Weijschede et al. (2006) for *Trifolium repens*, although for Leaf Area this could not be properly tested because of the heterogeneity of the non-transformed data. Again this indicates that high plasticity is not related to high trait values. Plasticity still could be related to high trait value if having a high trait value allows a plant to increase its plasticity in the more extreme parts of the environmental gradient, where plants that have lower values might have already reached there maximum plasticity. However, within the environmental range of both our experiments we find no evidence for this. In contrast with our expectations the genotypes with the longest petioles did not increase their relative performance. Possibly, the benefits of reaching the increasing part of the gradient earlier were not high enough. In dense vegetation, however, longer petioles have another advantage besides a higher light availability: they can shade leaves of genotypes with shorter petioles. Where in this experiment the harvest took place at the onset of interference between the plants, the longer petioles can increase the relative performance of these genotypes in competition by decreasing the light availability of shorter competitors. This way genotypes with longer petioles can exclude other genotypes.

Rank correlations

The possible higher performance of genotypes with long petioles is supported by the relation between the petiole lengths in both gradients and the frequency of the genotypes in the competition experiment. Petiole length can therefore be an important trait explaining the relative performance of the plants in the competition for light. The correlation is also an indication that a light gradient might be a better predictor of competitive strength than a homogeneous shade experiment, because the leaf traits were more directly related to the genotypic performance in the competition experiment.

Conclusions

In general, these experiments again show that *Potentilla reptans* responds to lower light levels with shade avoidance responses and that these responses also can occur in light gradients. Since the genotypes differed in many traits, genotypes will perform differently if selection occurs on these traits. In accordance with this, the rank correlation analysis indicates that the selection that was found in the competition experiment is in part due to differences in petiole length.

Figure 2.6. Capacity of leaves to carry their own weight after top 2 mesh wire support structures are removed.

Chapter 3

Height convergence in response to neighbour growth: genotypic differences in the stoloniferous plant *Potentilla reptans*

Published with Niels P.R. Anten, Feike Schieving, Marinus J.A. Werger, & Heinjo J. During
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Summary

Using a new experimental set up, the way in which height growth of stoloniferous plants is adjusted to that of their neighbours, as well as differences between genotypes in their ability to keep up with neighbour height growth were tested.

Five *Potentilla reptans* genotypes inherently differing in petiole length were subjected to three experimental light gradients, involving light intensity and red:far-red ratio. Each plant was placed in a vertically adjustable cylinder of green foil, and the treatments differed in the speed of cylinder height increase and final height.

Total weight of plants decreased from the Slow to the Fast treatment, while petiole length increased. Leaves reaching the top of the cylinder stopped petiole elongation, resulting in similar final heights for all genotypes in the Slow treatment. In the Fast treatment only the fastest growing genotype maintained its position in the top of the cylinder and genotypes differed strongly in final height within the cylinders.

Plants adjust their height growth to that of the surrounding vegetation leading to height convergence in short light gradients that slowly increase. These adjustments and genotypic differences in ability to keep up with fast-growing neighbours can influence the outcome of competition for light.

Introduction

Game theoretical models predict that with increasing density plants should invest more mass in height in order to maintain their position in the canopy and prevent being shaded (Givnish 1982; Iwasa et al. 1985; Givnish 1995). The fitness benefits of this response have been experimentally demonstrated in various studies (Schmitt et al. 1995; Dudley and Schmitt 1996; Pierik et al. 2003). These game theoretical models also predict that plants should not grow much taller than the surrounding vegetation as the minimal increase in light capture would not compensate the additional cost. This suggests that in dense stands a so called height convergence will occur, where taller plants in the canopy have about the same height irrespective of other size measures and associated growth potential. Indeed studies in dense stands of erect plants have documented such height convergence patterns (Weiner and Thomas 1992; Nagashima and Terashima 1995). This implies that plants should be able to adjust their rate of vertical spacer elongation to different rates of height increment of the surrounding vegetation.

Many studies have investigated the mechanisms through which plants respond to their neighbours. Generally, a reduced R:Fr ratio of light reflected by neighbours is believed to be a prominent cue (Ballaré et al. 1997; Casal et al. 2003), but other factors such as neighbour-produced ethylene (Pierik et al. 2006) and wind shielding (Telewski and Jaffe 1986; Anten et al. 2005) are also thought to play a role. Plants adjust their height growth using these cues, which can differ in strength and quality depending on the vertical position of the plant. Individuals that are positioned deeper in the canopy will experience strong photomorphogenetic signals that induce height growth (Weiner and Fishman 1994; Yokozawa and Hara 1995; Berntson and Wayne 2000). At the top of the vegetation these signals are less strong, except for wind force, and further height growth is reduced (Vince-Prue et al. 1976; Casal and Smith 1988; Lötscher and Nösberger 1997; Anten et al. 2005). As a result plants that are initially shaded by taller plants may realize a stronger height growth than plants at the top, and these plastic responses work to mitigate the variation in height between individuals (Ballaré 1999; Aphalo et al. 1999). If the ability to respond plastically is reduced, for instance through a mutation in the phytochrome, blue light photoreceptor or ethylene sensing, height inequality within the vegetation increases (Ballaré et al. 1994; Ballaré and Scopel 1997; Pierik et al. 2004).

Many plant species show intra-specific differences in height growth and the plasticity therein, suggesting genotypic differences in height growth potential

(Dudley and Schmitt 1995; Turkington 1996; Van Kleunen and Fischer 2001). If plants grow in vegetation that grows inherently slow, genotypes with a low height growth potential or low height growth plasticity can probably still reach the top of the canopy, and height convergence may occur. Plants of such genotypes will not be able to position their leaves in a high-light environment, however, in vegetation that grows rapidly. Since a small lag in height growth can result in low light availability when plants are growing in crowded vegetations (Ballaré et al. 1988), genotypic differences in height growth plasticity and height growth of the surrounding plants can have large effects on plant performance.

Most studies that have experimentally investigated plasticity in height growth in response to shade have been carried out in a vertically homogeneous light environment using shade cages. Under such conditions height growth is probably maladaptive as it does not place the leaves in a better light climate (Schmitt 1997; Leeflang et al. 1998), and thus such studies do not properly evaluate the benefits of increased height. Others have used light gradients of fixed length (Huber and Wiggerman 1997; Leeflang et al. 1998; Weijschede et al. 2006), mimicking the instantaneous light gradient that occurs in most dense herbaceous vegetation (Monsi and Saeki 1953; Grime and Jeffrey 1965). In these situations, however, the performance of the plants only depends on the response of the plant itself and the experiment does not test the effects of a change in the light environment around the plant. The benefits of height growth are thus overestimated, as even a minimal height increment places the leaves in better light climate. In a dense vegetation of hemicryptophytes or annuals, all plants tend to increase in height together early in the season (Fliervoet 1984), and the pay-off in terms of light capture to individuals increasing in height will depend on the general increase in height of the canopy as a whole. Therefore the experimental setting should mimic the surrounding vegetation's increase in height with time. Such a set up would also allow to directly test the hypothesis that plants can adjust their rate of vertical spacer elongation to different rates of height increment of the surrounding vegetation, by manipulation of the speed with which the light gradients increase in height.

Fine-tuning of the vertical positioning of leaves within upgrowing vegetation might be especially important for stoloniferous plants. Whereas in erect-growing plants increased investment in stem can be seen as an investment for future height of later-formed leaves, each new leaf of a stoloniferous plant has to start at the bottom of the light gradient. Height growth of such species might therefore be more costly than that of erect plants, because each leaf is supported separately from

ground level upwards, which in terms of biomass use for vertical support is less efficient than the production of a single stem bearing several leaves (Liu et al. 2007). Because the ability to elongate the petiole decreases with leaf age and does not come back once the lamina has reached high light conditions (Leeflang 1999), the height of a lamina is more or less fixed once the petiole has stopped elongating. This means that when the vegetation around it is getting taller over time, existing leaves are shaded and new leaves will have to be formed which need to exhibit stronger elongation to reach the top of the canopy.

We used the stoloniferous plant *Potentilla reptans* in a new experimental set up in which surrounding vegetation with different speeds of height growth was mimicked through the use of vertically adjustable cylinders of light-filtering plastic sheet. The experimental period was equal for all treatments, so the treatments differed not only in the rate of height increase, but also in the final length of the cylinders. Genotypic differences in responses were studied by using five genotypes that were known to differ in maximum petiole length in a fixed vertical light gradient (chapter 2).

Our goal was to investigate whether the plants adjusted their height growth to that of the surrounding light gradient. We expected that if the rate of cylinder height increase was relatively slow, and thus the resulting light gradient short, all genotypes would be able to put new leaves at the top of the gradient throughout the experiment but would not outgrow it, i.e. show height convergence despite differences in biomass and morphological traits such as the number of leaves, the root mass ratio, specific petiole length and specific lamina area. We also expected that when the shading gradient increased more rapidly in height, not all genotypes would succeed in reaching the top of the light gradient and that a height hierarchy would develop among them.

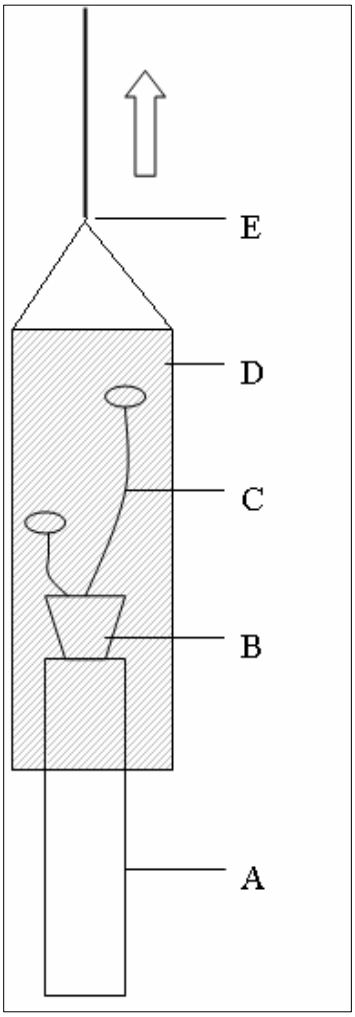
Methods

Plant material

Potentilla reptans is a stoloniferous herb found at moderately disturbed, productive pastures, mown grasslands, lake and river shores, road margins and several other man-made habitats (Van der Meijden 2005). The plant produces sympodially growing stolons with rooted rosette-forming ramets on its nodes. In the absence of physical disturbance the ramets remain interconnected throughout one growing season (Stuefer et al. 2002). Each leaf consists of 5-7 palmately arranged leaflets

borne on a vertically orientated petiole attached to the ground rosette. The height of an individual leaf is thus the result of the length of its petiole (Huber 1996; Stuefer and Huber 1998).

A set of ten genotypes was collected in 1997 from ten different locations in the Netherlands and kept in the botanical gardens of Utrecht University. From this set five genotypes were selected in 2006, on the basis of their potential petiole length: two genotypes with the shortest petioles (genotypes B and C), two genotypes with long petioles (genotypes F and I), and one genotype with an intermediate petiole length (genotype D). These characteristics had been determined in two previous experiments: an experiment with a light gradient of fixed length (Chapter 2) and an experiment with a vertically homogeneous light environment using shade cages (Liu et al. 2007). Identification letters of the genotypes are the same as used in other experiments (Stuefer et al. in prep.).



Experimental set up

On the 6th of July 2006, 30 young ramets of similar size from each of these five genotypes were taken from the stock populations. All leaves but the two youngest unfolded leaves were removed and the roots were cut to a length of 5 cm. The ramets were planted in pots with a diameter of 13 cm and height of 11 cm, filled with a 1: 1 mixture of river sand and compost, with slow release fertilizer (Osmocote plus, Grace Sierra International, Heerlen, The Netherlands) to provide an added 13 mg N wk-1per pot. On the 10th of July, 24 pots with ramets of similar size per genotype were selected. Six were used for the measurement of start biomass. The other eighteen were randomly assigned to 3 treatments: light gradients that increase in height slowly (S), at medium speed (M), or fast (F).

Figure 3.1.
Experimental setup. (A) Column, on which pot (B) is standing, (C) petiole of plant, (D) Green filter cylinder around the plant, (E) chain, which is attached to the cable hanging above the table.

The pots were placed on a water tray placed on 50 cm high columns, which were standing in three rows on a table in a plastic greenhouse (Photosynthetic Photon Flux Density, PPFD, 80% of outside PPFD, no change in R:Fr) in the botanical gardens. Around each column a 58.5 cm long, 14 cm diameter cylinder of green filter (Lee colortran international, Andover, UK; #139 HT primary green) was placed. Each cylinder was attached to a chain, which was hanging from a cable above the set up (figure 3.1). At the start of the experiment the top of the cylinder was 4.5 cm above the surface of the pots and the leaves reached to 1.5 cm beneath the top of the cylinder. All pots were randomly positioned in one of the rows on the table.

Starting on the 12th of July, each cylinder was moved upwards three times a week, by moving the cylinder along the chain, placing the top 0.75 cm (Slow treatment), 1.5 cm (Medium treatment) or 2.25 cm (Fast treatment) higher above the soil surface. The light gradients thus increased in height at a speed of 2.25, 4.5 and 6.75 cm per week. Measurements in a competition experiment with the full set of ten genotypes had shown that height growth early in the season is ± 4.5-6 cm per week, with an average maximum height of 32 cm (Vermeulen, unpublished data). As the experimental period was equal for the three treatments, this resulted in light gradients that differed in final length. Relative PPFD inside the cylinders was measured using a Licor Li-185A photometer, with a ceptometer (Delta-T Devices,

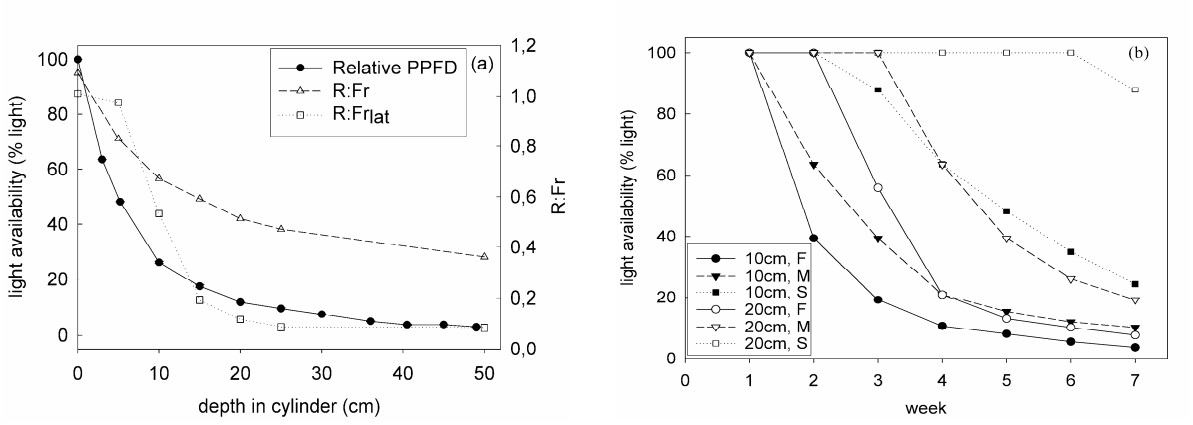


Figure 3.2. Light characteristics of the light gradient with increasing depth in the shade cylinder.

(a) Light availability (as % of light measured at the top of the light gradient), R:Fr ratio with sensor facing upwards and R:Fr ratio measured side ways (R:Fr$_{lat}$). (b) Decrease of light availability over time of a hypothetical lamina placed at 10 cm height and 20 cm height for the three treatments Slow (S), Middle (M),and Fast (F). Note: figure a is based on depth, measured from the top of the cylinder downwards, figure b on height, measured from the soil surface.

Cambridge, UK) to simultaneously measure light at the top of the light gradient. The relative PPFD declined steeply to about 22% over the first 10 cm from the top of the cylinders. Close to the bottom of the cylinders, at 50 cm depth, relative PPFD was on average 2.8% (figure 3.2a). The different speeds of the cylinders thus created three light gradients that differed in the rate at which the light availability at a certain height changed over time (figure 3.2b). R:Fr ratio in the gradient was measured using a Licor 1800 spectroradiometer connected to a remote cosine receptor. R:Fr ratio was 1.09 at the top of the gradient, while it was 0.36 at the bottom (figure 2a). We also measured the R:Fr ratio of horizontally directed radiation inside the cylinders. This lateral R:Fr ratio (R:Frlat) decreased strongly below the top 5 cm to very low values lower down in the gradient (figure 3.2a). The R:Fr gradient showed similar differences over time between treatments as the relative PPFD did (data not shown).

The pots were watered daily. Each week the length of all petioles was measured (apart from week six). Stolons produced by the rosettes were cut off to prevent crowding effects within the cylinder and dried. The experiment was harvested after seven weeks. Of each individual leaf we measured the petiole length, and the lamina area using a Licor LI-3100 leaf area meter. We washed the roots free from soil particles. All parts were dried at 65 ºC for at least three days and then their mass was measured. From the individual leaf characteristics and the root biomass we calculated the total petiole mass ratio (PMR, total petiole mass/ total plant mass), the lamina mass ratio (LamMR, total lamina mass/ total plant mass), the root mass ratio (root mass/ total plant mass), and the stolon mass ratio (total stolon mass produced during the experiment/ total plant mass). From the total lamina area and the total lamina mass we calculated the Specific Lamina Area (SLamA, m2 lamina area g-1 lamina mass) and from the petiole characteristics the Specific Petiole Length (m petiole length g-1 petiole). All these characteristics are thus mean whole-plant trait values and will indicate how the treatments affected the allocation and morphology of the plants.

Statistics

Analysis of the start biomass, using a one-way Anova with genotype as fixed factor, showed no significant differences between the genotypes. Also, no difference between the genotypes in the height above the soil surface at the start of the experiment was found. Repeated measures Anovas were performed to test for differences in the length of the tallest petiole over time, one two-way repeated

Anova with treatment and genotype as fixed factors (the genotypes were chosen on the basis of their height growth characteristics from a pool of 10 genotypes), and repeated Anovas for each separate treatment. Tallest petiole length is defined as the length of the tallest petiole at each census. The tallest petiole was a different, later-formed petiole every week.

To test for differences between treatments and genotypes and the genotype * treatment interaction a two-way Anova with both genotype and treatment as fixed factor was used for the five characteristics measured at the end of the experiment. Since the mass ratios are interdependent, we adjusted the significance levels in separate two-way Anovas with the four mass ratios as dependent variable, using a Bonferroni correction. To test if, within treatments, the genotypes differed in their tallest petiole length at final harvest, 3 separate one-way Anovas were performed with genotype as fixed factor. If necessary data were transformed to meet the demands of normality and homoscedasticity (see tables).

In our set up, differences between treatments may be caused by the confounding effect of the differences in both the rate of height increase and the absolute height. Therefore, in order to separate the effects of height growth rate of the cylinders from the absolute height, a two-way Anova was performed, with tallest petiole length at common cylinder height as dependent variable. This tested whether the genotypes responded differently to the rate of height increase alone. For the Medium treatment the tallest petiole length at week 7 was used for this analysis,

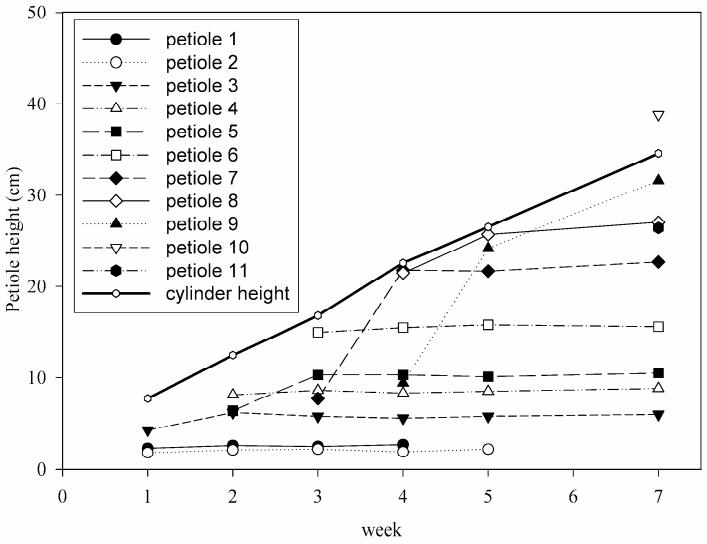


Figure 3.3. Petiole height development through time of a genotype 6 plant in the Medium treatment. Order of petioles is the order in which they were formed. The tallest petiole at each census of this plant are 3, 4, 6, 7, 8, and 10, respectively.

while for the Fast treatment the length at week 4 was used. A one-way Anova showed that at these two different points in time the height of the cylinder was not significantly different between the two treatments (F=2.61, P=0.101). It was not possible to include the Slow treatment in such a comparison, because there were no time points at which the cylinder height in all three treatments was not significantly different.

Results

All genotypes followed the same general pattern of leaf placement in response to the three treatments (see figure 3.3 for an example). Petiole elongation of a single leaf lasted between 1 to a little over 2 weeks. With time, newly formed leaves were placed above the older leaves. As a result, the tallest petiole was a different petiole at every census. The tallest petiole of the previous week had more or less stopped elongating by the time the next census took place and did not restart elongation when the shading cylinder was moved up.

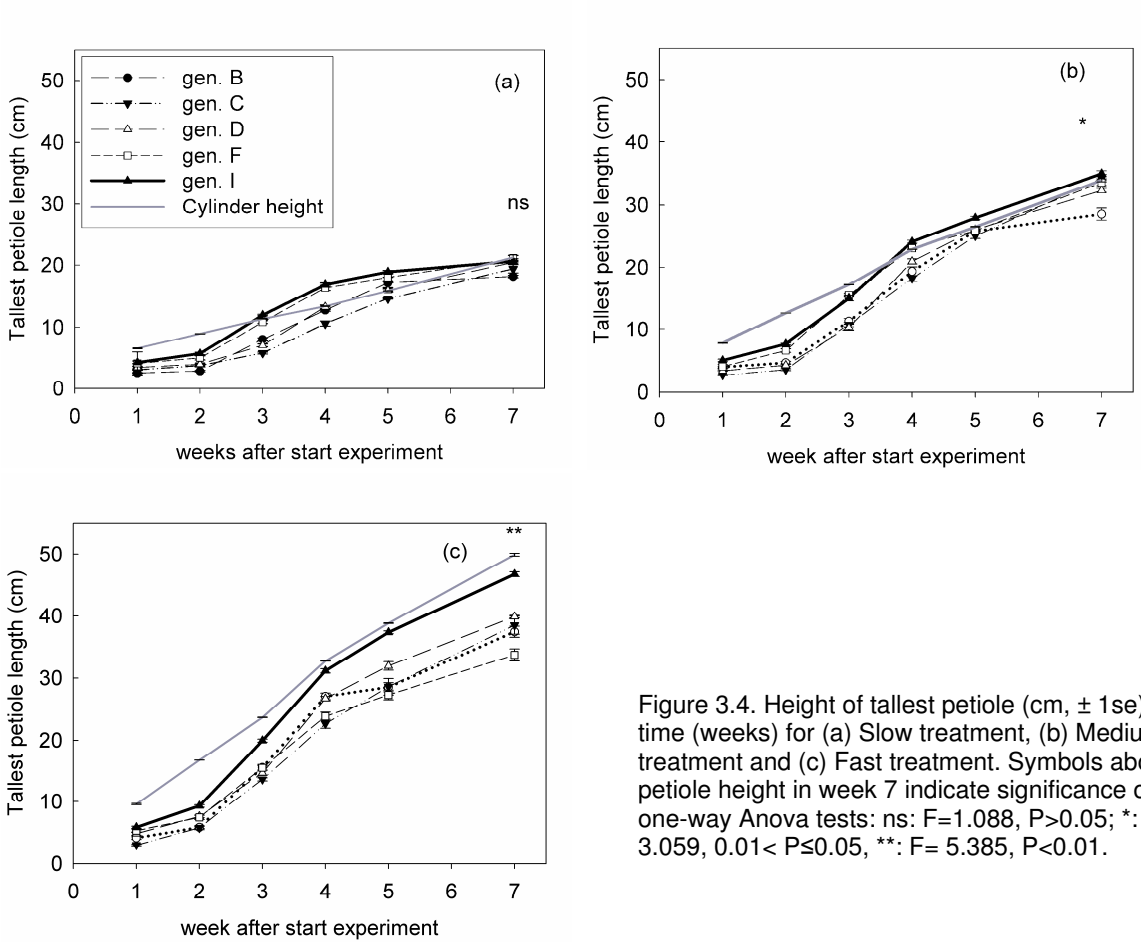


Figure 3.4. Height of tallest petiole (cm, ± 1se) in time (weeks) for (a) Slow treatment, (b) Medium treatment and (c) Fast treatment. Symbols above petiole height in week 7 indicate significance of the one-way Anova tests: ns: F=1.088, P>0.05; *: F= 3.059, 0.01< P≤0.05, **: F= 5.385, P<0.01.

Table 3.1. Results of the two-way Anovas examining the effects of treatment and genotype and their interaction. Table shows three separate analyses: two-way Anova, the two-way Anova with Bonferroni correction, and two-way Anova at similar cylinder height. In the table F-values and their significance are presented: ns, P>0.05; *, 0.05≥ P > 0.01; **, 0.01≥ P > 0.001; ***, P< 0.001; bns (Bonferroni corrected significant level), P>0.0125. ^{log} indicates log-transformed data.

Analysis	trait	Treatment	Genotype	Treatment* Genotype	error
Two way Anova	d.f.	2	4	8	75
	Total plant mass^{log}	40.22***	20.68***	2.37*	
	Number of leaves	25.18***	5.58**	2.35*	
	Specific Petiole length	19.87***	20.84***	2.17*	
	Specific Lamina Area	35.93***	4.88**	1.96ns	
	Tallest Petiole at harvest	184.7***	6.43***	2.94**	
Two way Bonferroni	Petiole Mass Ratio	49.18***	4.61**	2.04bns	
	Lamina Mass Ratio	18.04***	3.93**	2.32bns	
	Root Mass Ratio	58.24***	2.97bns	2.20bns	
	Stolon Mass Ratio	25.36***	8.74***	0.57bns	
Two way at similar cylinder height	Tallest petiole length	53.41***	4.43**	4.45**	

In general, at final harvest of the plants petioles were tallest in the Fast treatment, and petioles were taller in the Medium treatment than in the Slow treatment (figure 3.4, table 3.1). The tallest petiole at harvest of genotype F, however, did not differ in length between the fast and medium treatment. Total plant weight decreased from the Slow to the Fast treatment (fig. 3.5a), as did the number of leaves that were produced during the experiment (fig. 3.5b). Plants invested relatively more biomass in petioles and laminas, and less in roots and stolons from the Slow to the Fast treatment (fig. 3.5, table 3.1). Specific Petiole Length (SPL, fig. 3.5g) and Specific Lamina Area (SLamA, fig. 3.5h) were also higher in the Fast treatment.

The repeated measures Anova on tallest petiole lengths showed a significant time*treatment*genotype interaction (table 3.2), showing that treatment had an effect on the difference between genotypes in the height at which they placed their leaves with time. Analysis of the tallest petiole length at final harvest showed that the genotypes differed in response to the different treatments (significant treatment*genotype interaction, table 3.1). Further analysis in separate one-way Anovas showed no significant difference between the genotypes for the tallest petiole length at final harvest in the Slow treatment (figure 3.4). In the Medium and Fast treatment, however, there was a significant difference between genotypes. The separate repeated Anovas showed that in all three treatments there was a significant time*genotype interaction for tallest petiole length (table 3.2), indicating that in all treatments genotypes differed in their ability to place their leaves higher up in the light gradient with time.

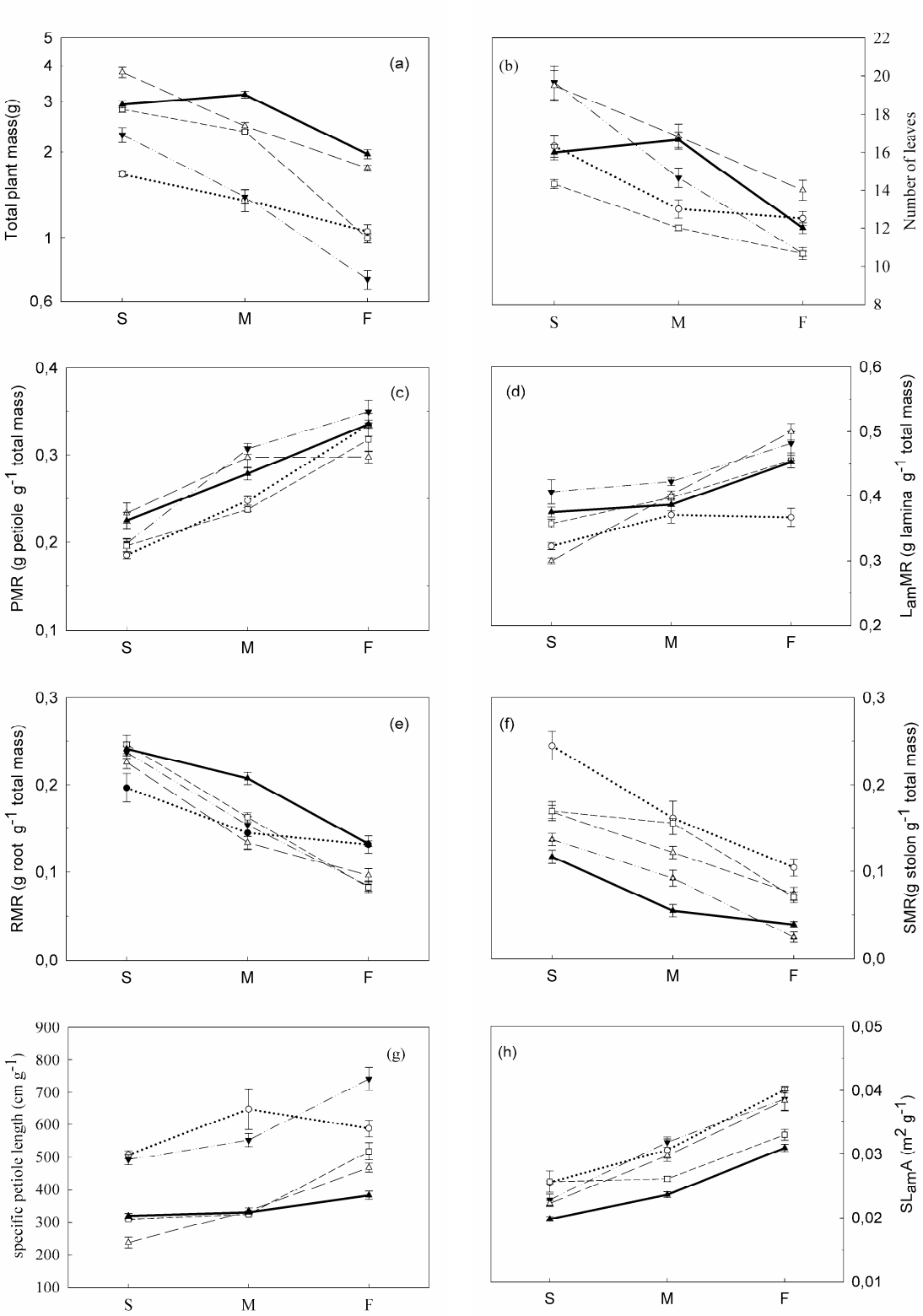


Figure 3.5. Morphological characteristics of the five genotypes in response to the treatments. (a) total plant mass. (b) total number of leaves produced at week 7 (c) Petiole Mass Ratio. (d) Lamina Mass Ratio (e) Root Mass Ratio. (f) Stolon Mass Ratio (g) Specific Petiole length (h) Specific Lamina Area. Treatments: S, Slow; M, Medium; F, Fast. Note the differences in scales, with figure (a) log-transformed. Symbols of genotypes can be found in the key of fig. 3.4a.

Table 3.2. Results of the repeated measurement Anovas examining the effects of treatment, genotype and time and their interactions on the tallest petioles. In the two way analysis, time was the within subject factor, while treatment and genotype were treated as fixed between factors. In the one way repeated analysis done separately per treatment, time was the within subject factor with genotype a fixed factor. In the table F-values and their significance are presented: ns, P>0.05; *, 0.05≥ P > 0.01; **, 0.01≥ P > 0.001; ***, P< 0.001

Analysis		Treatment	Genotype	Treatment* Genotype	Time	Time * Treatment	Time* Genotype	Time* Treatment* Genotype	error
2 way repeated	d.f.	2	4	8	5	10	20	40	75
	F	225.43***	17.16***	2.76*	2056***	75.99***	4.14***	1.85**	
One way	d.f.		4		5		20		25
Slow	F		8.21***		508.5***		2.53**		
Medium	F		7.62***		996.4***		2.56**		
Fast	F		7.59***		682.7***		2.67**		

The analysis of tallest petiole length at similar cylinder height showed that genotypes responded differently to a higher rate of cylinder increase (table 3.1). More variation seemed to occur between the genotypes in the Fast treatment at week 4 than in the Medium treatment at week 7 (figure 3.4). Overall, tallest petiole length was higher in the Medium treatment at week 7 than in the Fast treatment at week 4.

The genotypes also differed in all other measured parameters (fig. 3.5, table 3.1). A significant treatment * genotype interaction was found for most plant characteristics, except for SLamA, where the interaction was only marginally significant (table 1). The ratios also did not show significant treatment * genotype interactions. The total mass of the plants did show a treatment* genotype interaction, with the rank order of the genotypes changing with treatments (table 3.1, fig. 3.5a).

Discussion

Our set up is a valuable tool for studying plastic responses in a light environment that changes while plants are developing. The benefit of this set up is that growth differences between genotypes at final harvest resulted both from the ability of the genotype to place newly formed leaves at high light levels, and the ability of these leaves to perform at lower light levels once they were shaded later in time. Also, in our experiment the plants started at high-light levels. This means that light conditions did not strongly change when the plants were moved from precultivation to the experimental conditions. It is important to note that the differences between treatments reflect both the rate of cylinder height increase and the cylinders'

absolute final height, since our setup confounds these two aspects of neighbor canopy height. This resembles height growth in natural stands, where an increase in height growth rate will lead to taller vegetation, and to a faster decrease of light availability of lower placed leaves.

From the Slow to the Fast treatment plants showed responses that are also found as crowding gets more intense (Geber 1989; Ballare et al. 1991; Schmitt and Wulff 1993): an increased allocation to height growth, i.e. to the petioles, a decreased allocation to the roots and higher values of the Specific Lamina Area.

Plants increased the length of their petioles from the Slow to the Fast treatment. Total mass of the plants, however, decreased, while the number of leaves that was produced and the amount of stolon mass that was cut off during the experiment was lower. This shows that an increase in speed of elevating the vertical shade gradient and the resulting increase in absolute height reduced the production of biomass, delayed the formation of new leaves, and increased the allocation to the petioles at the expense of allocation to the roots and stolons.

From the Slow to the Fast treatment, the petiole length per unit mass, the specific petiole length (SPL), increased. This is in contrast with the findings of Huber and Wiggerman (1997) and Leeflang et al. (1998) in light gradients of fixed length. They argued that in order to place a lamina higher up in the canopy an increase in mass per unit petiole length, i.e. a decrease in the length per unit mass, is necessary to support the weight of the lamina. Liu et al. (2007) showed that *Potentilla reptans* produces shorter, more flexible petioles in response to mechanical stress, a response that is likely to decrease the chance of buckling. The higher SPL that was found in our study indicates that the increased elongation in response to the increased height of the cylinders may have reduced the mechanical stability of the petioles.

In all our treatments, the significant genotype*time interaction for tallest petiole indicates that genotypes differ in their ability to quickly place their new leaves at the top of the light gradient. The differences between genotypes in tallest petiole length at harvest in the medium and fast treatment also show that they differ in height growth potential. In the Slow treatment, however, the length of the tallest petiole at harvest was similar for all genotypes, while they did differ in biomass, allocation patterns, specific lamina area and other morphological characteristics. This indicates that when height growth of surrounding vegetation is slow, and the resulting light gradient relatively short, height convergence of clonal plants can occur, despite differences between genotypes in height growth potential.

Ballaré (1999) and Aphalo et al. (1999) argued that photomorphogenic signaling among neighbours can buffer the development of differences in height between plants, because shorter plants experience a stronger signal and thus have a stronger height growth. The responses of the genotypes in our Slow treatment support this view. After an initial period in which leaves were located in shade below the top of the cylinder, some genotypes had grown new, elongated petioles which placed their laminas at the top of the light gradient at the third census. The other, initially shorter genotypes had their laminas at lower light levels, experienced stronger signals and thus had a stronger height growth later in time. As a result all genotypes had placed leaves at similar height at final harvest in the slow treatment, and thus at similar light levels.

Elongation stopped once the laminas were in high-light conditions, while petioles of the same age became taller in the fast treatment as compared to the medium and slow treatment. Several studies working with stoloniferous species have shown that petiole elongation slowed down or even stopped once the plants reached the top of a light gradient (Price and Hutchings 1996; Lötscher and Nösberger 1997; Leeflang et al. 1998). This indicates that the detection mechanism for these stoloniferous plants is located near the lamina, as was found by Thompson (Thompson 1995) for *Trifolium repens*, and that the sensing of radiation reflected by neighbours works in a similar way as found in erect species (Ballaré 1999). So far, however, no study has investigated the effect of cues such as ethylene and blue light on stoloniferous plants. Also, the fact that elongation of the petioles starts at the bottom of the light gradient, and that elongation, and thus meristem activity, stops, suggests that the regulation of height growth in stoloniferous plants could be different from that of the stems of erect plants.

Whether a genotype can place its laminas at the top of the vegetation can be influenced by both the absolute length of the light gradient and its rate of increase. Leaves that start at ground level need to elongate the length of the initial light gradient, plus the elongation rate of the vegetation during leaf development in order to place their laminas at the top. Genotypic differences in leaf positioning thus can result from differences in elongation rate, the time period a petiole elongates and a possible maximum petiole length a genotype can reach. In the medium and fast treatment there was a clear differentiation between genotypes as regards to the height they reached and the time they needed to reach that height. Despite the stronger photomorphogenic signals that the shorter genotypes experienced, they could not place their leaves at similar height as taller genotypes

did. Our results thus suggest that if height growth of surrounding vegetation is fast and results in tall vegetation, differences between genotypes in height growth potential will result in leaves of different genotypes occupying different positions in the vertical light gradient of the stand. Moreover, our analysis of the longest petiole, at similar cylinder height in the Medium and Fast treatments, indicates that the rate of height change of the light gradient alone can induce these genotypic differences.
The fact that petioles were longer in the Medium treatment at week 7 than in the Fast treatment at week 4 suggests that petiole length is limited by the production capacity of the plants, as plants in the Fast treatment were smaller. This could in part explain why genotype F, which was chosen because of its long petioles in other experiments, had the lowest values for tallest petiole length in the Fast treatment. Analyses of leaves in the stock populations revealed that it had higher dark respiration than the other genotypes (chapter 6), indicating a lower carbon gain at low light levels. The fast decrease in light availability of lower placed leaves may have resulted in low carbon gain, which in turn may have limited its ability to place leaves at the top of the cylinder.
Differences between individuals in leaf positioning at the beginning of the season may affect the positioning later on. In even-aged stands of herbaceous plants rank correlation in height of plants is fixed shortly after canopy closure, as differences in final height between plants are determined at an early stage of canopy development and plants that fall behind will not be able to catch up (Ford 1975; Anten and Werger 1996; Nagashima 1999; Xiao et al. 2006). Our results indicate that the same applies for competition between genotypes of a stoloniferous plant in a fast growing canopy. This can strongly affect the outcome of competition between these genotypes.
In dense stands, either mono- or multi-specific, smaller plants can be excluded from the vegetation as they are shaded by neighbours and as a result develop a lower biomass use efficiency for light harvesting than their taller neighbours (Anten and Hirose 1998; Werger et al. 2002; Hikosaka et al. 2003). Similarly, since the differences in length of the tallest petioles between genotypes in our study increased from the Slow to the Fast treatment, rapid height growth of the surrounding neighbours, leading to taller vegetation, may lead to competitive exclusion of the shortest or least plastic genotypes. Our results also show that more genotypes are able to place their leaves at the top of the light gradient when height growth of the vegetation is low. This would suggest that in such vegetations

more genotypes can coexist for longer time periods in the top of the canopy than in faster growing, taller vegetation.

In conclusion, our results show that although stoloniferous plants have the disadvantage of starting new leaves from ground level, they can well adjust their height growth to that of the surrounding vegetation. Our findings confirm that height convergence can occur between different genotypes of stoloniferous plants. Our results also indicate that depending on height growth of the canopy, genotypes may differ in their abilities to keep up with neighbouring plants. Genotypes that are limited in height growth potential can only link up with potentially higher growing genotypes in a light gradient with a restricted height increase. Since leaf positioning affects the light harvesting efficiency of a plant, height growth and plasticity therein can be an important factor explaining the outcome of competition between different genotypes.

Chapter 4

Genotype-density interactions in a stoloniferous plant: costs of increased height growth?

Summary

Game theoretical models predict that in dense vegetation an increase in height growth investment may lead to a decreased plant production, as it potentially trades off with investment in other plant organs such as the leaves. We tested whether genotypes with a stronger increase in height investment in response to higher density showed a stronger decrease in total plant mass, using the stoloniferous plant *Potentilla reptans*. As shade avoidance at the ramet level may trade-off with clonal expansion, we also tested whether a stronger increase in mother ramet investment would lead to a stronger decrease in investment the vegetative propagation.

To increase the differences between plants in height investment, ten genotypes that were known to differ in plasticity in height growth investment were grown in mono-genotypic stands at two different densities.

Genotypes differed in their increase of petiole investment in response to an increase in density, but not in their decrease in total plant mass or root mass. Also, total lamina area did not significantly differ between the two densities, nor did the relative biomass allocation to laminas. Both a change in vegetation height and a change in petiole investment were not significantly negatively correlated to a change in total plant mass. The genotypes did differ in the change of allocation to the mother ramet: a stronger increase in investment in the mother ramet was correlated to a stronger decrease in vegetative propagation.

These findings show that a stronger increase in height investment did not lead to a stronger decrease in biomass production, because there were alternatives to the decrease in total lamina investment or root mass, notably a decrease in vegetative propagation. This suggests that in homogeneous conditions, stoloniferous plants have the ability to adapt to an increase in density at relatively low cost. In addition, genotypic differences in the response to density may lead to differences between mono-genotypic stands in life history evolution.

Introduction

Height growth of an individual plant is strongly regulated by the signals that the plant receives from the surrounding vegetation. At higher densities cues such as the photo-active radiation (PAR), the R:Fr ratio, blue light and volatiles such as ethylene change, and many plants increase their height growth (Schmitt and Wulff 1993; Smith 2000; Pierik et al. 2004), which may cause a shift in biomass allocation. For instance, with an increase in density the total biomass of an individual plant decreases, but the investment (in this chapter defined as the absolute investment in grams) in vertical spacers increases, while investment in other plant parts such as the roots decreases (Ballaré et al. 1987; Geber 1989; Weiner and Thomas 1992).

An individual increasing its investment in height can increase its fitness relative to that of neighbours responding less strongly to these cues, because a taller plant shades shorter neighbours (Dudley and Schmitt 1996; Schmitt et al. 2003; Anten et al. 2005). Since this higher investment to height growth will go at the expense of investment in other plant organs such as the leaves, the biomass production capacity of the plant could be reduced (King 1990; Falster and Westoby 2003; Weiner 2003). Game theoretical models thus predict that the competition for light may lead to a “tragedy of the commons” (Hardin 1968) , i.e. individuals with height growth responses that would optimize photosynthesis at the stand level will be outcompeted by plants that have a higher height investment, thus leading to a lower production of the vegetation as a whole (Givnish 1982; Iwasa et al. 1985; Givnish 1995; Anten 2005).

Many studies have shown that there are genotypic differences in the strength of the response to signals of neighbour proximity (Schlichting 1986; Sultan and Bazzaz 1993; Sultan 2000). This suggests that with an increase in density, genotypes can differ in the increase in investment in height growth, which in turn can lead to differences in biomass production. However, in multi-genotypic canopies plants that lag behind in height will receive stronger signals, and thus may elongate to similar height as surrounding plants, thereby reducing the differences in height (Ballaré 1999; Aphalo et al. 1999). On the other hand, the added investment of plants that keep placing their leaves above those of others may be compensated by the increased light availability. This makes it difficult to detect costs of increased height investment. In mono-genotypic vegetations, however, all plants will have the same response to neighbour proximity. The height investment then depends on the characteristics of the genotype itself, and thus

differences between mono-genotypic stands in the response to an increase in density will be more pronounced. Also, as all surrounding plants in a mono-genotypic stand will be able to place their leaves at similar height, an increase in height will not be compensated by increased light availability. Following the assumptions in game theoretical models, mono-genotypic stands that strongly increase height investment have less biomass to invest in other structure, such as the leaves, which will reduce its photosynthetic rate. Therefore, a stand consisting of a genotype that responds to neighbour proximity with a strong increase in height investment will have a stronger decrease in total biomass than monostands of genotypes with a less strong increase in height growth investment.

Stoloniferous plants may form such mono-genotypic stands, as they can propagate vegetatively (DeKroon and van Groenendaal 1997). Increased height growth is achieved by increasing the investment in the petioles, which potentially can enhance the light capture of the laminas by placing them higher up in the canopy (Leeflang et al. 1998; Weijschede et al. 2006). Huber and Wiggerman (1997) found that an increase in vegetation height resulted in more biomass investment in the petioles of the stoloniferous plant Trifolium repens, but also in a reduction of the formation of secondary ramets. They argued this to be circumstantial evidence for a trade-off between shade avoidance at the ramet level and clonal expansion at the fragment level. If this were correct, genotypes showing a strong increase in investment in the above ground parts of the mother ramet should show a strong decrease in investment in vegetative propagation.

The main goal of this chapter was to see whether stands that strongly increased in height investment in response to increased density would show a stronger decrease in total production. We therefore set up an experiment with the stoloniferous plant *Potentilla reptans*, where a target clone fragment was surrounded by either zero or eight neighbours, thus creating two densities. The focal plant was used to represent the changes of the whole stand. Ten genotypes were used that are known to differ in height investment and in their response to shade in homogeneous shade cages (Liu et al. 2007), and that have been used in a long-lasting competition experiment (Stuefer et al. in prep.).

As most studies determine changes in investments to different plant parts in relation to the total plant mass (% of total biomass invested in specific plant part, in this chapter defined as allocation), we first wanted to see if an increase in density would lead to differences between genotypes in the way allocation patterns changed. We expected that an increase in density would lead to a decrease in

biomass of the target clone fragment. Biomass allocation to the petioles was expected to increase, while the allocation to the roots and laminas was expected to decrease.
Secondly, we also tested whether these differences between genotypes in the change in allocation patterns would lead to differences in the way investment in the different plant parts would change.
This would allow us to test 1) if mono-genotypic stands with a stronger density-induced increase in petiole investment would show a stronger decrease in biomass production and 2) if mono-genotypic stands with a stronger density-induced increase in investment in the aboveground part of the mother ramet would show a stronger decrease in investment in vegetative propagation.

Materials and methods

Potentilla reptans is a stoloniferous herb found at moderately disturbed, productive pastures, mown grasslands, lake and river shores, road margins and several other man-made habitats (Van der Meijden 2005). The plant produces sympodially growing stolons with rooted rosette-forming ramets connected trough internodes. In the absence of physical disturbance the ramets remain interconnected throughout one growing season (Stuefer et al. 2002). Each leaf consists of five to seven palmately arranged leaflets borne on a vertically orientated petiole attached to the ground rosette. Height growth is achieved by elongating this petiole (Huber 1995), and the laminas of new leaves are placed above older leaves (see also chapter 3).
A set of ten genotypes was collected in 1997 from ten different locations in the Netherlands and kept in the botanical gardens of Utrecht University. On June 13th 2006 160 trays were prepared with nine pots each. The pots had a diameter of 4.8 cm and were 18 cm long, and were filled with a 1:1 volumetric mixture of compost and river sand, with slow release fertilizer (Osmocote plus, Grace Sierra international, Heerlen, The Netherlands) added to provide 0.5g N m^{-2} wk^{-1}per pot. One week later, 80 ramets of similar size for each of the ten genotypes were taken from the stock population that was maintained in the botanical gardens of Utrecht University. All stolons were removed and the roots were cut to a length of 5cm. These ramets were defined as the mother ramets. Eight of these ramets per genotype were used for measurements at the start of the experiment and were dried at 65ºC for at least three days.

The pots were placed in a 3*3 arrangement. For each genotype two densities were created. In the low density treatment one plant was planted in the middle pot of the tray, and the surrounding pots stayed empty (LD, 44.4 ramets m^{-2}). In the high density treatment all pots in the 3*3 arrangement were planted with similar mother ramets of the same genotype (HD, 400 ramets m^{-2}), with the middle plant being the target plant. Root cloth was put on the bare surface to prevent rooting of other ramets in each pot. There were eight replicate trays for each combination of treatment and genotype. The trays were randomly placed on two tables in a plastic greenhouse of the botanical gardens (80% light of full light, no change in R:Fr).

Black plastic, attached to four sticks of 75cm high at each corner, was put around the tray to limit the area of each "arena" to 15 cm by 15 cm. The height of this barrier was adjustable up to a height of 50 cm, but at the start of the experiment it was positioned at soil level. Twice a week the black plastic was moved up to the level of the highest lamina of the surrounding plants bordering the target plant, to enclose the arena and to prevent light entering from the side into the canopy. The plants were watered daily. Once a week, the height of the vegetation was measured, defined as the height of the highest lamina above the soil surface of the target plant.

After nine weeks the target plants were harvested. The plants were separated into seven parts: laminas and petioles of the mother ramet; the internodes between ramets; laminas and petioles of the daughter ramets (defined as all ramets that were formed during the experiment); all dead material (laminas and petioles) pooled together, and roots. Roots were washed free from soil particles. Most daughter ramets did not form roots. However, some of the newly formed ramets of the target plants did root in pots of the surrounding plants. This was limited to 25 ramets in total, and the fraction of their root biomass never exceeded 8% of total root biomass. This root mass of the daughter ramets was also harvested and added to the total root mass of the target plant. Analysis showed that removing this secondary root mass did not alter the direction of the results. The total lamina area of the leaves from the mother ramet (La_m) and the lamina area of all the leaves of the daughter ramets (La_d) were measured using a Licor LI-3100 leaf area meter. All parts were dried at 65 ºC for at least three days and then their mass was measured.

From these data we calculated the total lamina mass ratio (LMR, total lamina mass/ total plant mass), the total petiole mass ratio (PMR, total petiole mass/ total plant mass), the internode mass ratio (IMR, total internode mass/ total plant mass),

the root mass ratio (root mass/ total plant mass) and dead mass ratio (DMR, total dead mass/ total plant mass). The LMR and PMR were further divided into the mother lamina mass ratio (LmMR, lamina mass of mother ramet/ total plant mass), the daughter lamina mass ratio LdMR (lamina mass of daughter ramets/ total plant mass), mother petiole mass ratio (PmMR, petiole mass of mother ramet/ total plant mass), and the daughter petiole mass ratio (PdMR, petiole mass of daughter ramets/ total plant mass). Aboveground mother mass is the sum of the mother petiole mass and the mother lamina mass. Vegetative propagation is the sum of the daughter petiole mass, daughter lamina mass and the internodes. Total living mass is the total plant mass minus the dead mass.

Statistics

Analysis of the start biomass, using a one-way Anova with genotype as fixed factor, showed no significant differences between the genotypes. Therefore starting mass was left out of further analyses.

Because differences in absolute biomass allocation may be due to size differences (Poorter and Nagel 2000), we tested the change in allocation relative to the total

Table 4.1. Results of the two-way Anova examining the effects of the density and genotype and their interaction on plant characteristics. Treatment and genotype were treated as fixed effects. ^{log} indicates log transformed data. In the table F-values and their significance are presented: ns, P>0.05; *, 0.05≥ P>0.01; **, 0.01≥ P >0.001; ***, P≤0.001.

Trait	Subpart	Density	Genotype	Density* Genotype	error
d.f.		1	9	9	134
Vegetation height		2418.18***	55.49***	34.83***	
Number of ramets		128.84***	19.65***	0.85ns	
Mass ^{log}	Total	52.79***	2.89**	0.85ns	
	Total living	56.17***	2.83**	0.98ns	
	Total Petiole	32.143***	4.091***	2.365*	
	Total lamina	45.136***	5.017***	1.118ns	
	Dead	0.543ns	10.167***	3.602***	
	Root	71.216***	4.466***	1.142ns	
	Aboveground mother	0.249ns	33.402***	8.174***	
	Vegetative propagation	105.501***	4.234***	2.364*	
Lamina area	Total	1.94ns	5.59***	0.96ns	
	Mother	6.67*	10.21***	3.36**	
	Daughter	9.53**	4.85***	2.67**	

plant mass. Therefore a two-way covariance analysis was performed, with the mass of the focal plant part as dependent variable (log transformed), genotype and treatment as fixed factors and total plant mass as Covariable (also log transformed). No significant slope effects were found (no significant Covariance * treatment, Covariance*Genotype or Covariance*Treatment*Genotype interactions), so only the F values for the intercept are presented.

To test if an increase in density had lead to a significant change in total plant biomass, and in the absolute biomass invested in the different plant parts, two-way Anova's were performed, with both genotype and density as fixed factors, as the genotypes were chosen because they differed in height growth investment in a experiments using homogenous shade cages (Liu et al. 2007). The biomass data were log-transformed to meet the assumptions of Anova, but untransformed data are presented in the figures for clarity. A significant Density* Genotype interaction would indicate that genotypes differed in their response to density. Similar analyses were performed to test for the effects of density and genotype on height of the vegetation and total lamina area.

Table 4.2. Results of two way analysis of covariance (ANCOVA) with total plant mass as covariable. Density and genotype were treated as fixed effects. No slope effects were found, so only intercept results are given. All data is log transformed. In the table F-values and their significance are presented: ns, P>0.05; *, 0.05≥ P>0.01; **, 0.01≥ P >0.001; ***, P≤0.001. Degrees of freedom are:covariable 1, density 1, genotype 9, density*genotype 9, error 134.

Dependent	Covariable	Density	Genotype	Density* Genotype
Root mass	142.56***	15.22***	4.91***	1.30ns
Dead mass	22.12***	3.34ns	11.04***	4.23***
Internode mass	178.20***	40.29**	10.97***	4.95***
Petiole mass	345.47***	362.07***	6.23***	3.25**
Petiole mother mass	4.07*	65.07***	20.41***	5.73***
Petiole daughter mass	173.69***	46.44***	8.79***	2.08*
Lamina mass	450.07***	0.35ns	5.06***	1.72ns
Lamina mother mass	4.64*	0.37ns	18.73***	5.05***
Lamina daughter mass	181.47***	8.32**	14.59***	3.97***
Total mother mass	23.61***	25.52***	19.36***	8.32***
Vegetative propagation	347.67***	3.31ns	12.07***	4.72***

Then correlation tests were performed to see if a stronger increase in height, or a stronger investment in height growth (i.e. petiole mass), was correlated to a stronger decrease in total plant mass. First, for each genotype in both treatments, the average value for height, petiole mass and total plant mass was calculated. Then for each genotype, the average value in High Density was divided by the value in Low Density, to get the relative change. This was done to correct for size differences between genotypes. Then Pearson's correlation analysis was used to test if the relative change in height or relative change in petiole mass was significantly related to the relative change in total plant mass. As petiole mass is included in the total plant mass, the null hypothesis is that there is a positive correlation between the relative change in petiole mass and the relative change in

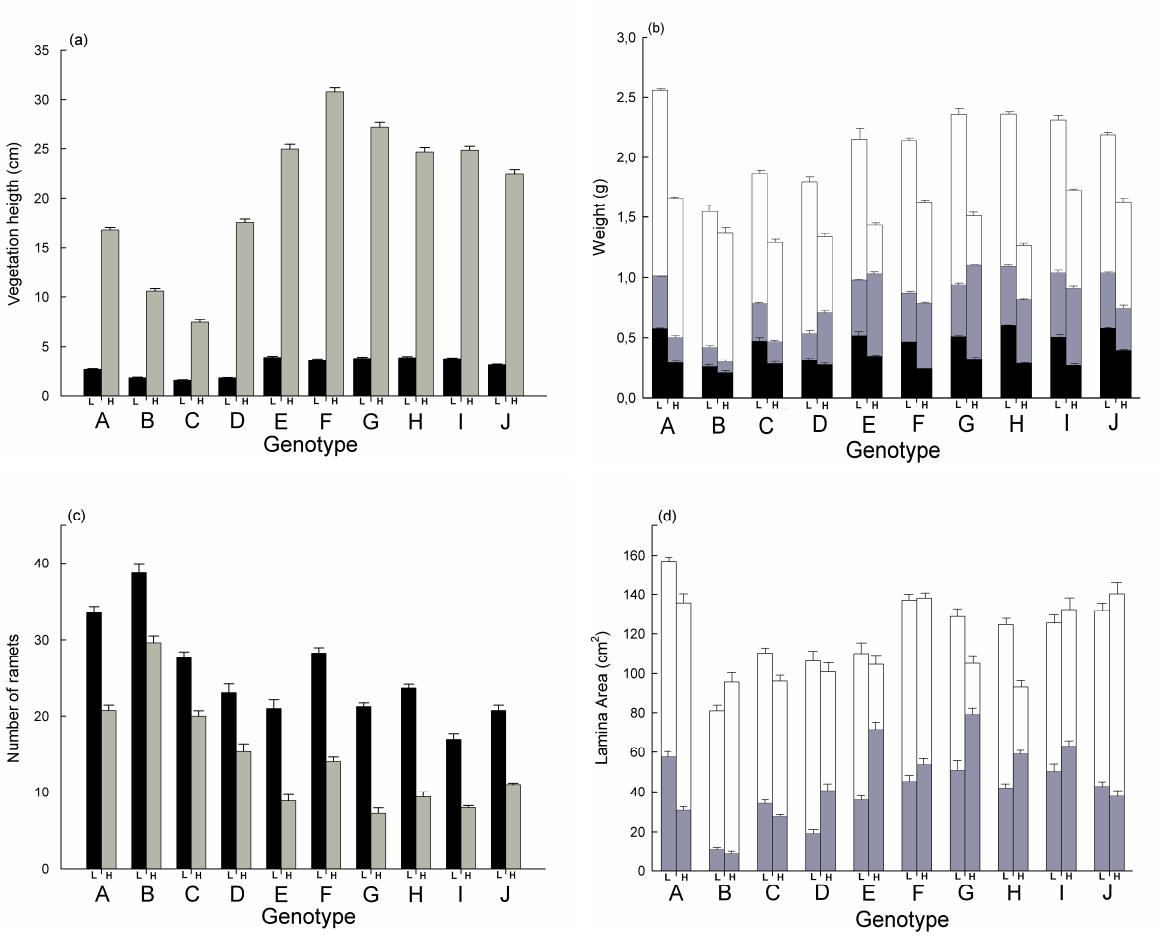


Figure 4.1. Characteristics (+ 1 se) of the ten genotypes at low density (L) and at high density (H). a) Mean vegetation height of the ten genotypes b) total living plant mass, divided into root mass (black), aboveground mother mass (grey) and vegetative propagation (white). c) Total number of newly formed ramets. d) Total lamina area, divided into mother ramet (grey) and total lamina area of the newly formed ramets (white).

total plant mass. But we expect, based on the game theory discussed in the introduction, that a stronger increase in petiole mass is related to a stronger decrease in total plant mass, i.e. a negative correlation is expected. Analyzes with total plant mass and total living mass gave similar results, and only those using total plant mass are shown.
A similar procedure was used to test if a change in aboveground mother mass was related to a change in vegetative propagation, to a change in root mass, or to a change in dead mass. This time, no mass term of one plant part is included in the calculation of the other. The null hypothesis is that plant parts decrease or increase in a similar way, i.e. are positively correlated. But the alternative hypothesis is based on the expectation that an increase in investment in above ground mother mass is correlated to a decrease in vegetative propagation, i.e. again a negative correlation is expected.

Results

At high density, the vegetation was taller than at low density. The total plant dry mass, the total mass of living tissue and the total number of ramets per plant at high density were lower than at low density. Total lamina area, however, did not differ between densities (figure 4.1, table 4.1). Allocation to the petioles increased with increasing density, while allocation to the roots decreased. Allocation to the laminas was not significantly different between densities (figure 4.2, table 4.2).
The change in allocation to the petioles differed between genotypes, with some genotypes showing a stronger increase than others. The change in percentage of dead mass also showed a difference between genotypes. In some genotypes the amount of dead material decreased with increasing density, while in others the reverse was found. No difference between genotypes was found for the change in allocation to the roots and laminas (figure 4.2, table 4.2).
However, when allocation to the mother and vegetative propagation was analyzed separately genotypes differed strongly in their response to density. Some genotypes strongly increased the allocation to the mother ramets, and strongly decreased allocation to vegetative propagation at high density, while other genotypes did the reverse (figure 4.1b, table 4.2). Genotypes that increased allocation to the vegetative propagation hardly showed a decrease in allocation to the internodes and the laminas of the daughter ramets, while the allocation to the petioles of the daughter ramets increased (figure 4.2, table 4.2). Also, these

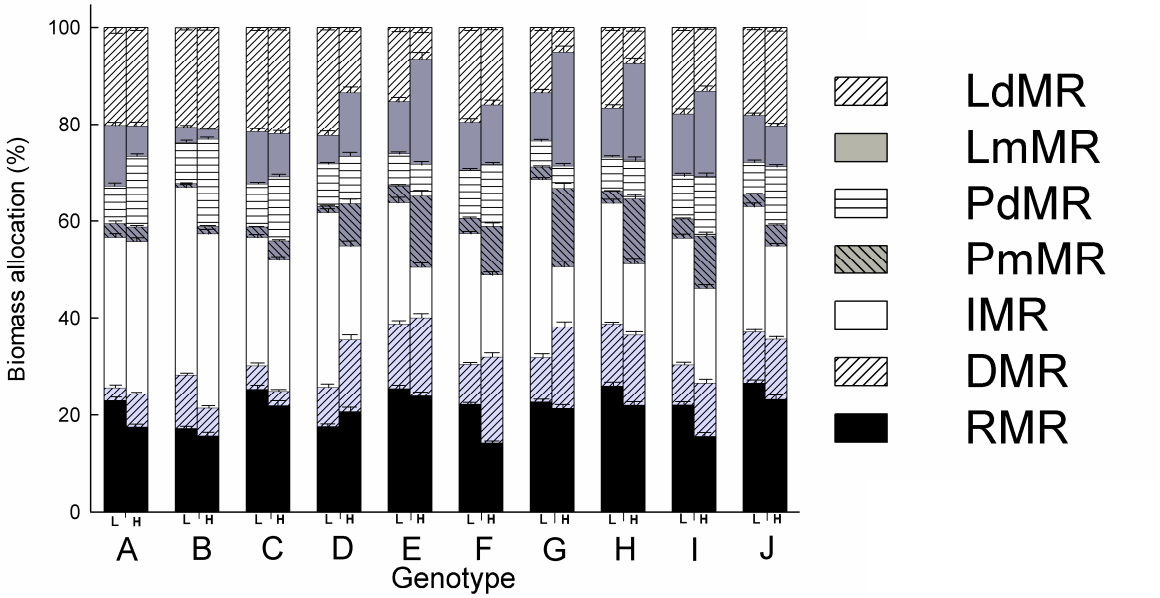


Figure 4.2. Proportional biomass allocation (+ 1se) to plant organs for the different genotypes at low (L) and high density (H).From top to bottom: LdMR= daughter lamina mass ratio, LmMR= mother lamina mass ratio, PdMR= daughter petiole mass ratio, PmMR= mother petiole mass ratio, IMR= internode mass ratio, DMR= dead mass ratio, RMR= root mass ratio.

genotypes showed a decrease in the total lamina area of the mother ramet and an increase in the lamina area of the daughter ramets, while for other genotypes this pattern was reversed (figure 4.1d, table 4.1).

Responses of vegetation height to density differed greatly among genotypes (significant density*genotype interaction): some genotypes showed a stronger height increase with increasing density than others. However, responses of total plant mass, total living mass (table 4.1, figure 4.1), number of ramets, or total lamina area to density did not differ among genotypes (table 4.1, figure 4.1). Responses of petiole mass to increased density did differ between genotypes (significant Genotype*Density interaction, table 4.1). Such genotypic differences in response to density were also found for aboveground mother mass, vegetative propagation and dead mass. No significant Genotype*Density interaction was found for root mass and total lamina mass (Table 4.1, figure 4.1b).

The correlation analysis showed that a change in vegetation height and a change in petiole mass was not significantly correlated to a change in total plant mass (figures 4.3a). A change in total petiole mass was marginally positively related to a change in total living mass (figure 4.3b). A change in aboveground mother ramet

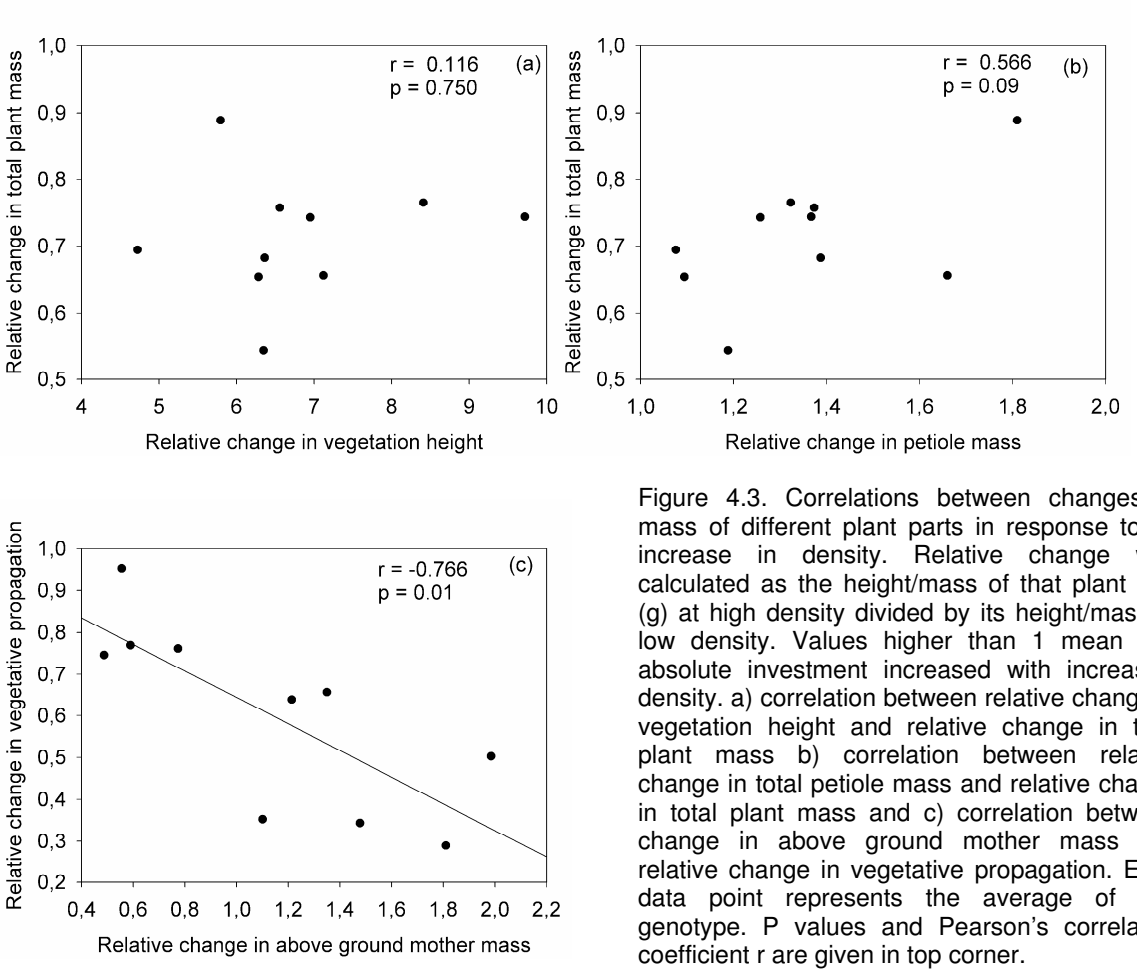


Figure 4.3. Correlations between changes in mass of different plant parts in response to an increase in density. Relative change was calculated as the height/mass of that plant part (g) at high density divided by its height/mass at low density. Values higher than 1 mean that absolute investment increased with increasing density. a) correlation between relative change in vegetation height and relative change in total plant mass b) correlation between relative change in total petiole mass and relative change in total plant mass and c) correlation between change in above ground mother mass and relative change in vegetative propagation. Each data point represents the average of one genotype. P values and Pearson's correlation coefficient r are given in top corner.

mass was negatively related to a change in vegetative reproduction (figure 4.3c), but not significantly correlated to either a change in root mass (p=0.448) or a change in dead mass (p=0.293).

Discussion

In this experiment, we increased the density of monostands of ten individual genotypes of *Potentilla reptans* nine-fold. As expected, total plant mass and total living mass per plant decreased with increasing density, the height of the vegetation increased, petiole mass increased and roots mass decreased in the high density treatment. The allocation to the laminas was not significantly different between densities, however, and the total lamina area also was not significantly affected. Thus an increase in allocation to the petioles did not decrease the allocation towards the photosynthetic tissue, nor did it reduce the total lamina area

of the whole plant. This is in contrast with the general notion that increasing height investment trades-off with investment in the light harvesting apparatus (Givnish 1984; Hirose and Werger 1995; Givnish 2002). Yet, at high densities the total leaf area per unit ground area (LAI) was approximately 4 to 6 m^2 m^{-2}, compared to about 0.5 at low density, indicating that a large part of the leaves was severely shaded. This may explain why total biomass was lower at high density.

The setup was chosen based on the expectation that mono-genotypic stands would lead to large differences in height between the genotypes in the high density treatment. The results confirm this idea: the genotypes showed large differences in vegetation height at high density, while results from a five year long competition experiment between these 10 genotypes showed that they converge to similar height when competing (Stuefer et al. in prep.). As the mono-genotypic stands also differed in the increase in investment to the petioles, our set up proved to be suitable for testing the main hypothesis: that a stronger increase in petiole investment in response to an increase in density would lead to a stronger decrease in total biomass production.

But in contrast to our expectation, the mono-genotypic stands did not differ in the way the total plant mass or total living mass changed. Also, the change in total plant mass was not significantly correlated to the change in vegetation height. In addition, in contrast to our expectations the change in petiole mass was marginally positively correlated to a change in total plant mass. Thus, the prediction that mono-genotypic stands with a stronger increase in petiole investment would show a stronger decrease in biomass production could not be confirmed.

Our findings suggest that this was because mono-genotypic stands with a stronger increase in petiole investment did not show a stronger decrease in investment and allocation to the laminas, nor a stronger decrease in root investment. This indicates that the production capacity of the plants that increased investment in the petioles was not more strongly reduced. These findings thus do not show that increased height growth is costly because it reduces investment in the roots and leaves, as has often been found (Casal and Smith 1989; Schmitt and Wulff 1993; Cipollini and Schultz 1999). However, Weijschede et al. (2006) showed that in vertically homogeneous light conditions, genotypes with larger petioles did not have reduced total biomass, while Ballaré et al. (1991) even found that plants with a higher investment in stems had a higher total biomass than plants whose height growth were suppressed by light with a high R:Fr ratio. The latter authors gave two suggestions as to how plants with a higher investment in height growth would be

able to achieve at least similar biomass production: 1) the elongated plants captured light more efficiently and 2) they had higher light use efficiencies.

Our results indicate that at least for stoloniferous plants there is a third possibility: a reduced vegetative reproduction. Where some studies have found a decrease in vegetative reproduction with increasing density (Abrahamson 1975; Holler and Abrahamson 1977; Rautiainen et al. 2004), or no significant difference (Eriksson 1985), our results indicate that a change in allocation to newly formed ramets is strongly genotype-dependent: some genotypes increased allocation to the daughter ramets and decreased allocation to the mother ramet, while others did the reverse. Increased investment in new ramets implies the differentiation of axillary meristems into new vegetative apices (Geber 1990; Huber and During 2001), leading to the production of additional leaf area at some distance of the mother ramet. In homogeneous, dense vegetation, as mimicked by our setup, however, this additional leaf area will not be placed in more favourable conditions, and the added investment in internodes will not pay off. Rather, if height growth of surrounding vegetation is high, a reduction of ramet production and increased biomass allocation to existing ramets may consolidate growth of the ramets that have already been established (Geber et al. 1992). In accordance with this argument, we found that a strong increase in investment to the mother ramet was significantly correlated with a strong decrease in investment in vegetative propagation. The finding that these genotypes can do so without greatly reducing their biomass production suggests that the stoloniferous species *Potentilla reptans* has the ability to adjust its allocation pattern at relatively low cost.

In more heterogeneous and/or more disturbed environments, however, increased investment the mother ramet and the resulting decrease in investment in the daughter ramets may be more costly. The production of a larger number of ramets can enable a genet to rapidly colonize open spaces (Fahrig et al. 1994). It also increases the possibility of increasing genet performance through the specialization of individual ramets to different, locally abundant resources, i.e. division of labour (Stuefer et al. 1994; Stuefer et al. 1996). Furthermore, increased numbers of ramets are thought to spread the risk of genet death in case of local disturbances (Eriksson and Jerling 1990), while storage in internodes increases the survival probability of ramets (Stuefer and Huber 1999; Suzuki and Stuefer 1999). Therefore, genotypes with a larger investment in newly formed ramets hold an advantage in environments where disturbances regularly create open spaces (Stuefer et al. 2002). Thus while increased investment to the mother ramet may

maintain the performance of this ramet, it may reduce the overall performance of the clone fragment in heterogeneous environments.

Intraspecific competition may play an important role in the life history evolution of a clonal plant, as an increase in density can affect the balance between sexual and vegetative reproduction (Van Kleunen et al. 2001; Van Kleunen and Fischer 2003; Rautiainen et al. 2004). Although not many flowers were found in the course of our experiment, our results show that if a genotype responds to an increased density with an increase in investment in the mother ramet, the investment to the vegetative propagation decreases. Ultimately, in very dense vegetation the investment in the mother ramets may be so strong that investment in new ramets is completely stopped. This mechanism may partly explain why clonal plants seem to control their ramet density (Hutchings 1979; De Kroon 1993; De Kroon and Kalliola 1995). The genotypic differences that were found in this experiment therefore suggest that the way density affects life history evolution differs between genotypes.

In conclusion, a stronger increase in height investment did not inevitably lead to a stronger decrease in biomass production, because there were alternatives to the expected decrease in total lamina investment or root mass, notably a decrease in vegetative propagation. A stronger increase in mother ramet investment led to a stronger decrease in daughter ramet investment, which suggests that 1) *Potentilla reptans* can adjust its allocation to increasing density at low cost in homogeneous vegetation and that 2) genotypic differences in the response to density may lead to differences between mono-genotypic stands in life history evolution.

Chapter 5

Leaf investment and light partitioning among leaves of different genotypes of the clonal plant *Potentilla reptans* in a dense stand after five years of competition

Summary

Differences between genotypes in leaf biomass allocation, leaf characteristics and the resulting light capture were studied within the competition experiment to see 1) whether different genotypes would do better in different layers of the canopy, thereby promoting coexistence and 2) whether patterns in light capture paralleled the observed shifts in abundance after five years of competition.

The genotypes differed in specific lamina area, lamina mass ratio and lamina area ratio for leaves placed at the same light levels. However, genotypes with a high specific lamina area had a low lamina mass ratio and vice versa, and the difference between genotypes in lamina area ratio were small. Genotypes did not significantly differ in light capture per unit mass (Φ_{mass}) for leaves with the laminas placed at the same light levels. Φ_{mass} values increased for all genotypes with increasing light availability. The most abundant genotype had average light capture per unit mass values on plot level (plot Φ_{mass}) compared to the other genotypes.

Results suggest that coexistence was not the result of different genotypes being most efficient at different layers of the canopy. Although these results indicate that within species competition for light should result in selection for height growth, the differences in instantaneous light capture efficiency did not explain the frequency of the genotypes.

Introduction

Plants in nutrient-rich environments are thought to compete predominantly for light (Goldberg and Miller 1990; Tilman and Pacala 2006). Because light is a unidirectional resource successful competitors are usually described as having "traits leading to overtopping of the neighbours" (Aerts 1999). These taller individuals can increase their fitness directly by increasing their light capture, and indirectly by making the resource unavailable to competitors (Falster and Westoby 2003). As a result taller plants may catch a disproportional share of light, i.e. they can catch more light per unit biomass than smaller individuals, also called asymmetrical competition (Schwinning and Weiner 1998; Anten and Hirose 1998; Berntson and Wayne 2000).

Increased height growth, however, occurs at a cost. To maintain mechanical stability tall plants invest disproportionally more in stems and relatively less in leaves (Ballaré et al. 1987). Therefore the leaf mass ratio (LMR, g invested in leaves g^{-1} total biomass) generally decreases with plant height (Givnish 1982; Givnish 1995). Plants also increase the leaf area per unit leaf mass invested in leaves (specific leaf area, SLA, m^2 g^{-1} leaf biomass) in response to shade (Corré 1983a; Corré 1983b). Consequently, tall plants that have their leaves placed at higher light availability in general have a low leaf area per unit plant mass (LAR; LAR= SLA*LMR; (Hirose and Werger 1995). The performance of a plant within the vegetation thus should depend on the amount of light it captures in relation to the biomass needed to capture it, relative to what the neighbouring plants do.

To analyze the benefits (light capture) and costs (aboveground biomass) of different plants within a dense canopy, Hirose and Werger (1995) developed an approach in which they calculated the light captured per unit biomass (Φ_{mass}), with Φ_{mass} being the product of the light interception per unit of leaf area (Φ_{area}) and the LAR of a plant. They showed that within a multi-species grassland, tall dominant species captured more light per unit of leaf area than subordinate ones. In another study shifts in dominance from one species to another in a successional sequence could be explained by increased height growth of the winning species, which led to a decrease in Φ_{area} and/or Φ_{mass} of the other species (Werger et al. 2002). This indicates that shorter plants can be excluded from the population by taller plants. However, both these studies also showed that shorter subordinate species could persist in the lower parts of the canopy, because compared to the dominants they had relatively high LMR, SLA and thus LAR values, resulting in similar or even higher Φ_{mass}. Thus partly because of their contrasting intrinsic architectures,

different species can occupy different layers of the canopy yet be similarly efficient in capturing light per unit biomass. This might contribute to their coexistence (Hirose and Werger 1995; Anten and Hirose 1999; Werger et al. 2002).

Within species however, the variation in SLA and LMR might not be large enough to allow subordinate individuals to persist in the lower layers of the vegetation, as was argued by Anten and Hirose (1998). They found that in dense mono-specific *Xanthium canadense* stands the tallest dominant individuals had higher Φ_{mass} values than subordinate individuals. Later Hikosaka et al. (2003) found the same result for mono-specific stands of *Chenopodium album*. Thus it could be argued that within species selection should favour genotypes that have a high height growth, enabling them to capture a disproportional amount of light relative to their size.

However, selection within a canopy consisting solely of one stoloniferous species could still favour different height growth strategies, because of the growth form. In general, meristems are placed in the axils of leaves (Bell 1991). The activity of these meristems are suppressed by low light and low R:Fr ratio (Schmitt and Wulff 1993; Bonser and Aarssen 2003). Light levels and R:Fr ratio increase with height in the vegetation (Ballaré et al. 1990; Schmitt et al. 2003). Through stem elongation an erect-growing plant can place its meristems at these higher levels, which in turn could reduce the apical dominance, allowing the plant to branch, and consequently to increase its growth by placing more leaves at more favourable light conditions. This will increase the benefits of increased height growth. But since stoloniferous plants can only increase height through the elongation of the petioles (Dong 1995; Huber 1996; Huber et al. 1998), they have to invest relatively much in height for every new leaf in order to place it at the top of the canopy. This in turn could allow subordinate stoloniferous plants with smaller investment in petioles to reach similar Φ_{mass} values as the tall individuals.

In 1998 an experiment was started with ten genotypes of the clonal stoloniferous plant *Potentilla reptans*, all growing in competition in one arena. Analysis of the relative frequency of these genotypes after five years using ISSR markers revealed that one genotype had become the most abundant genotype (± 40% of all leaves), while several others were still present in approximately the same frequency as at the start of the experiment (Stuefer et al. in prep.). Other genotypes had declined in frequency, which might indicate that selection had occurred.

We used data collected from this experiment to compare leaf biomass allocation, leaf characteristics and the resulting light capture between genotypes. We wanted

to see if the patterns in light capture paralleled the observed shifts in abundance after five years of competition. We expected that the most abundant genotype would have higher Φ_{mass} values than the genotypes that had declined in frequency. We hypothesized that the genotypes that had more or less maintained their initial frequency would have similar Φ_{mass} values as the most abundant genotype, as they might have been more efficient at capturing light in the lower parts of the canopy. This would mean that the tallest genotypes were not necessarily disproportionally successful in capturing light.

Materials and methods

Plant material

Potentilla reptans is a stoloniferous herb found at moderately disturbed, productive pastures, mown grasslands, lake and river shores, road margins and other man made habitats (Van der Meijden 1996). The plant produces sympodially growing stolons with rooted ramets on its nodes. In the absence of physical disturbance the ramets remain interconnected throughout one growing season (Stuefer et al. 2002).

Ten genotypes of *P.reptans* were collected in a wide range of habitats in The Netherlands. The sites included river shores, mown pastures, parking lots and relatively undisturbed grasslands. Differences between genotypes thus represent within species variation. The genotypes were propagated at the botanical gardens of Utrecht University. In shading experiments these genotypes differed in several traits, such as SLA, LMR and petiole length (Stuefer et al. in prep., Chapters 2, 3 and 4 in this thesis).

Experimental setup

A more detailed description of the experimental set up can be found in Stuefer et al. (in prep.). Here a short description is given.

In the botanical gardens of Utrecht University 16 plots of 2 by 2 meters were established in the spring of 1998. In these plots 100 planting points were positioned on a regular grid. For each plot ten similar sized juvenile ramets per genotype were taken from the stock population and randomized over these planting points. Every genotype thus started with an initial frequency of 10%. Two treatments were applied: a disturbance treatment and a control treatment. The data

necessary to calculate light capture were only measured in the control plots. Therefore we only report data from these eight plots.
At the beginning of July 2003 100 leaves in each plot were harvested at randomly chosen grid points in the vegetation. At each sample point the vegetation was visually divided into three layers, and the layer from which the leaf had to be sampled was drawn randomly. The first leaf that was hit in this layer was sampled. Since the plots were part of an ongoing experiment, we sampled only leaves, and left stolons and roots intact.

Leaf measurements

The leaf is defined as the petiole plus the palmate lamina, which consists of five to seven leaflets. For each leaf the height of the lamina above the ground and the height of the vegetation at the position of the sampled leaf were measured. From these two measurements we calculated the depth of the vegetation at the height of the lamina.
The lamina was separated from the petiole after which the lamina was cut in two. Then the lamina area (LA) of both halves was measured using a Licor LI-3100 leaf area meter. One half was used to determine the identity of the genotype using ISSR (Stuefer et al. in prep.). The other half was used to measure dry weight. This lamina half and the petiole were dried for at least three days at 65ºC.
Dry weight was then determined (accuracy 0.1 mg), after which the Specific lamina Area ($SL_{am}A$, $m^2\ g^{-1}$) of the dried lamina part was calculated. The total lamina weight was then calculated using this SLA and the total leaf area of both halves together.
We then calculated the other parameters:
Total Leaf Weight (TLW, g) = lamina weight + petiole weight
Lamina Mass Ratio ($L_{am}MR$, $g\ g^{-1}$) = total lamina weight/ total leaf weight
Lamina Area Ratio ($L_{am}AR$, $m^2\ g^{-1}$) = total lamina area/ total leaf weight.

Light capture

Every plot was divided into four subplots, in each of which a light profile was measured under an overcast sky. Starting at the top of the vegetation two measurements were made at 5 cm intervals using a Delta-T ceptometer. Photosynthetic Photon Flux Density (PPFD) above the canopy ($PPFD_o$) was measured simultaneously using a Licor Li 185A photometer. The relative PPFD (rPPFD) was calculated for each point of measurement and the two values

obtained per point were averaged. The rPPFD within the interval between two measurement heights was estimated by means of interpolation. Because we were interested in relative differences in light capture, an accurate measurement of the daily PPFD above the canopy was not necessary. Therefore we calculated the daily light availability for each height from the rPPFD assuming an average day of 12 hours and an average light availability above the vegetation of 1000 µmol m^{-2} s^{-1}.

Daily light availability at the height of the lamina ($PPFD_h$, mol m^{-2} day^{-1}) was taken from the light profile of the subplot in which the leaf was collected using the depth of the vegetation at lamina height. Leaf angle was not taken into account since the variation between the genotypes seemed small. To obtain the light extinction coefficient (K) and the leaf area per layer, for every plot a stratified clipping in a 30 by 30 cm plot was done. Every 5 cm the relative light was measured in the same way as in the subplots. Leaf area was determined by taken a subsample from the laminas that were cut from the 5cm layer, and by calculating the SLA of the subsample. Total leaf area of the layer was then calculated using this SLA and the total lamina weight. The extinction coefficient (K) was then calculated following equation (16) from Anten and Hirose (2001):

$$K = \frac{\ln(PPFD_b / PPFD_0)}{L_c} \quad (1)$$

with $PPFD_b$ the light at the bottom of the canopy, $PPFD_0$ the light above the canopy and L_c the cumulative LAI, which ranged between 3.8 and 6.1. K was found to be 0.83, a normal value for a dicotyl species (Monsi and Saeki 1953).

Daily light capture per lamina (Φ_d, mol day^{-1}) was calculated using the PPFDh, the lamina area (LA, m^2) and the leaf absorbance (α) and the K:

$$\Phi_d = PPFD_h * LA * \alpha * K \quad (2)$$

Leaf absorbance was taken to be 0.8 following Goudriaan (1977).

Light capture per unit biomass (Φ_{mass}, mol g^{-1} day^{-1}) was calculated adjusting the formula from Hirose and Werger (1995), using the total leaf weight (TLW):

$$\Phi_{mass} = \frac{\Phi_d}{TLW} \quad (3)$$

Note that leaf area, biomass and light acquisition are defined at the level of individual leaves and not of whole plants.

Next we calculated the plot Φ_{mass} ($_p\Phi_{mass}$, mol g^{-1} day^{-1}) of each genotype as a measure of the light capture efficiency of the genotypes within each plot, taking into account that the number of leaves that were harvested came from layers with a different number of total leaves. For each layer, we calculated the layer Φ_{mass} per genotype ($_L\Phi_{mass}$) by dividing the summarized Total Light Capture by the summarized Total Leaf Weight. Then we estimated the leaf area per layer using the data from the stratified clipping. The total number of leaves per m^2 of each layer (LN_L), thus for all genotypes combined, was estimated using the LAI of that layer (LAI_L) and the average leaf area for all leaves harvested within that layer ($_ALA_T$):

$$LN_L = \frac{LAI_L}{{}_A LA_T} \qquad (4)$$

The total leaves per layer per genotype (LN_{LG}) can then be calculated through the proportion of leaf area from the harvested leaves that belonged to the genotype:

$$LN_{LG} = LN_L * \frac{LA_{LG}}{LA_L} \qquad (5)$$

with LA_{LG} the summarized leaf area of all leaves of that genotype within the layer and LA_L the total leaf area of all leaves measured in that layer.

Then the weighted $_p\Phi_{mass}$ for each genotype was calculated as:

$${}_p\Phi_{mass} \frac{\sum({}_L\Phi mass * LN_{LG})}{\sum LN_{LG}} \qquad (6)$$

Statistics

Genotype 1 was left out of the analyses because only one leaf was found in all 8 plots together.

We used two-way covariance analyses (ANCOVA) with plot as a block factor, genotype as a random factor and rPPFD as the covariate to test for differences between genotypes for $SL_{am}A$, $L_{am}MR$, $L_{am}AR$ and Φ_{mass}. All data were log transformed to better meet demands of normality and homoscedasticity.

To see whether light capture increased disproportionally with Total Leaf Weight we fitted a linear regression line following Anten and Hirose (1998), with light capture

(log transformed) as dependent variable and Total Leaf Weight (log transformed) as predictor:

$$\log \Phi_d = \log a + \beta \log TLW \quad (7)$$

We did this for every plot, since plot was a significant factor in the covariance analysis (see table 1). If the coefficient β was larger than one, light capture increased exponentially and thus disproportionally with Total Leaf Weight. Genotypic differences in ${}_p\Phi_{mass}$ were tested in an ANOVA with plot as a block factor and genotype as a random factor. For all analyzes we used SPSS version 12.1.

Table 5.1. Results of two way analysis of covariance (ANCOVA). All values are P values. All data has been log transformed. * indicates significant effects P<0.05, *** = P<0.001.

Dependent	Covariate	Factor	Among slopes	Among intercepts
Specific Lamina area	Relative PPFD ^{log} ***	Genotype	.458	.012*
		Plot	.205	.167
		G*P	.794	.795
Lamina mass ratio	Relative PPFD ^{log} ***	Genotype	.196	.035*
		Plot	.932	.293
		G*P	.423	.878
Lamina area ratio	Relative PPFD ^{log} ***	Genotype	.500	.022*
		Plot	.226	.499
		G*P	.916	.909
Total leaf weight	Relative PPFD ^{log} ***	Genotype	.435	.000*
		Plot	.106	.019*
		G*P	.752	.099
Φmass	Relative PPFD ^{log} ***	Genotype	.486	.099
		Plot	.690	.401
		G*P	.660	.787
Φ	Tdw ^{log}***	Genotype	.431	.070
		Plot	.103	.003*
		G*P	.291	.915

Results

Frequency of genotypes

Figure 1.1 shows the frequency of the ten genotypes in the vegetation after 5 years of competition (adapted from Stuefer et al. in prep.). One genotype (Genotype I) was most abundant in all three layers of the vegetation. No leaves were found of Genotype B. Of the other genotypes some had a frequency close to or higher than their initial abundance of 10% (genotypes D, F and H), while the other genotypes had decreased in frequency after 5 years. All remaining genotypes except genotype one were present in all three layers, but they differed in their frequency of leaves in the different layers, with some having relatively more leaves in the lower layer (genotypes C, D en F) while others had more leaves in the top layer

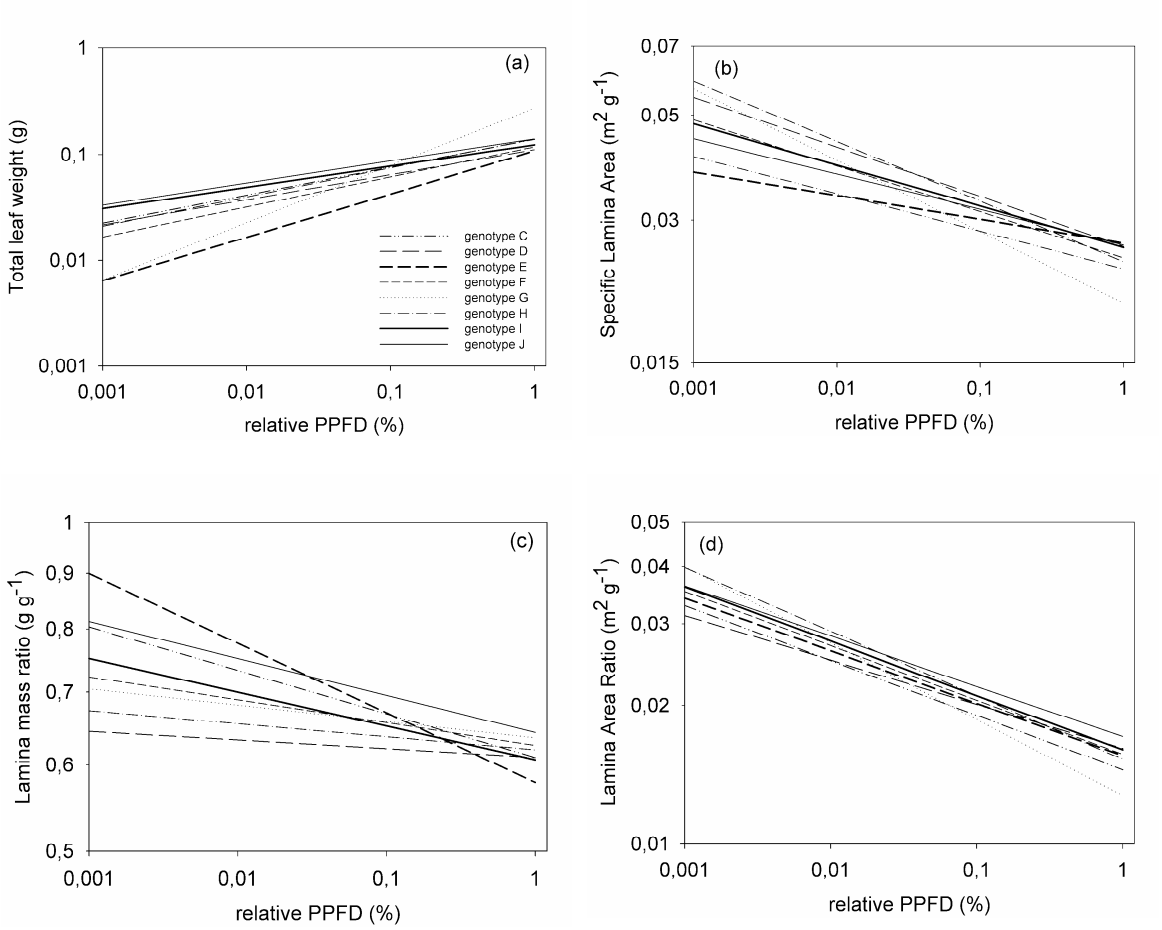


Figure 5.1. Allometric relations between leaf characters of 8 genotypes of *Potentilla reptans* and relative PPFD at the position of each leaf lamina: A) Total Leaf Weight (g), B) Specific Lamina Area (m^2 g^{-1}), C) Lamina Mass Ratio (g g^{-1}) and D) Lamina Area Ratio (m^2 g^{-1}). Lines represent linear regression lines of the genotypes based on log transformed data of all plots pooled together. Legend is given in figure A. Covariance analysis is given in table 5.1.

(genotypes seven, eight and ten). Genotypes five and nine had a more or less even distribution of leaves over the three layers (see figure 5.1).

Leaf architecture

All characteristics changed with increasing available PPFD. Total leaf weight increased while SLamA, LamMR and LamAR all decreased with increasing light availability (figure 5.1). No interaction was found between the morphological changes of the genotypes and the changes in PPFD in any characteristic (table 5.1, among slopes). The genotypes, however, did differ in all leaf characteristics (table 5.1, among intercepts). Although genotypes differed in their LamAR, the variation in this trait between genotypes appeared to be smaller than that in SLamA and LamMR. The three genotypes with the highest SLamA had the lowest LamMR while for the genotypes with the lowest $SL_{am}A$ the reverse was true. The most abundant genotype in general had average leaf characteristics values.

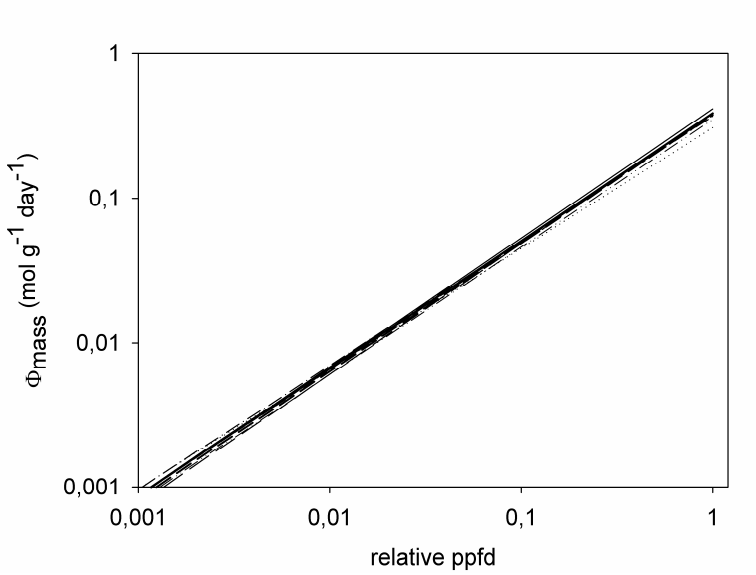
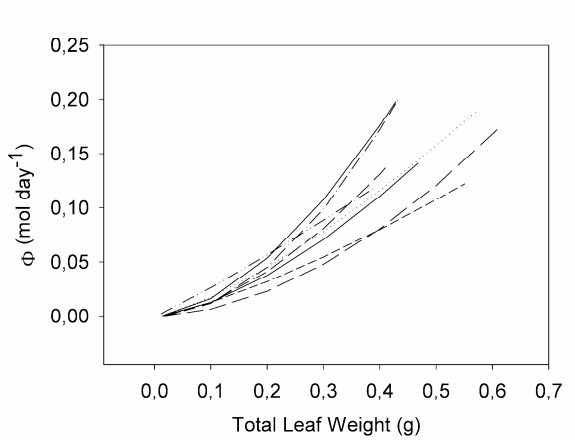


Figure 5.2. Relation between light capture efficiency (Φmass, mol g^{-1} day^{-1}) and relative PPFD. Lines represent linear regression lines. For key of genotypes see legend in figure 2A. Note log scales. For statistics see table 5.1.



Plot	Adjusted R^2	β	95% confidence interval
1	.366 ***	1.556	1.114-1.999*
2	.437 ***	1.375	1.036-1.713*
3	.524 ***	1.325	1.059-1.591*
4	.774 ***	1.050	.973-1.237
5	.497 ***	1.811	1.430-2.193*
6	.542 ***	1.931	1.571-2.291*
7	.587 ***	1.678	1.386-1.970*
8	.688 ***	1.715	1.474-1.975*

Left: Figure 5.3.Power relation between light capture of individual leaves (mol day^{-1}) and total leaf weight (g). Different lines indicate different plots. Coefficients of fitted power functions are given in table 2.

Right: table 5.2. Regression analysis per plot with dependent log transformed light capture (Φ) and predictor log transformedTotal Leaf Weight (TLW). β is the regression coefficient in the linear expression: log Φ_d= log a+ β logTLW , with β> 1 indicating a disproportional increase of φ with total leaf weight. *** indicates significant regression (P <0.001). * indicates lower part of 95% confidence interval to be significantly larger than 1.

Light capture efficiency

For all genotypes Φ_{mass} increased with increasing PPFD (fig. 5.2) and Φ increased disproportionally with increasing total leaf mass (fig. 5.3, table 5.2). No significant differences in the light capture efficiency (Φ_{mass}) of leaves positioned at the same light availability were found between genotypes (table 5.1, among intercepts). We also did not find an interaction between the genotypes and increasing light availability (among slopes).

Genotypes with relatively more leaves in the upper layer of the vegetation had higher ${}_p\Phi_{mass}$ values (fig. 5.4). However, the most abundant genotypes did not have the highest ${}_p\Phi_{mass}$ values, nor did all genotypes that had declined in frequency have lower ${}_p\Phi_{mass}$ values.

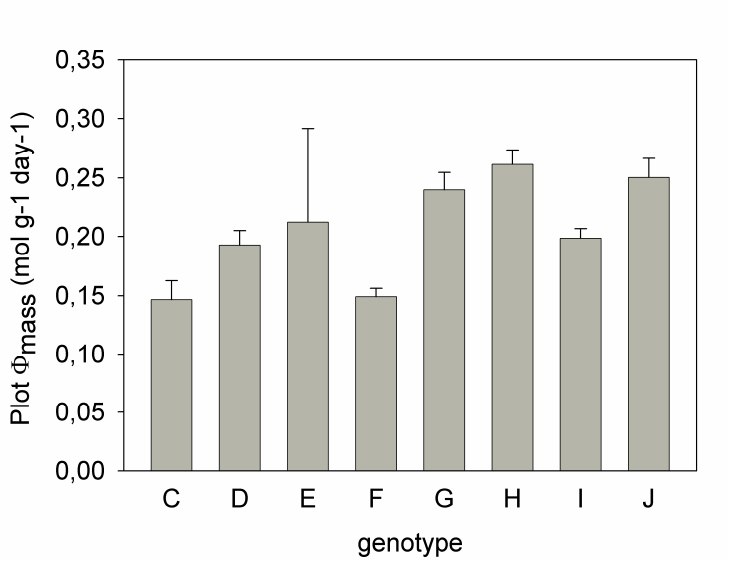


Figure 5.4. Plot Φ_{mass} (mol g^{-1} day^{-1}) for the 8 genotypes (+ se) as calculated from all leaves within a plot. Statistics are given in table 5.3.

Discussion

De Kroon et al. (2005) argued that the fitness of a modular organism is a product of the response of individual plant parts to the individual growth conditions they experience. We therefore expected that the performance of the genotypes would depend on the positioning of the leaves and the efficiency with which these leaves captured light per unit of biomass (Φ_{mass}). In contrast to our expectations however, the light capture efficiency was not related to the observed shifts in frequency.

The genotypes did differ in all leaf characteristics for leaves that were placed at the same light availability, including $SL_{am}A$ and $L_{am}MR$. This however did not result in differences in Φ_{mass}. In general SLA is found to be an important factor in determining differences in the relative growth rate between species, which in turn is linked with plant performance; the LMR usually is unrelated (Poorter and Remkes 1990; Westoby et al. 2002; Reich et al. 2003; Shipley 2006). In our study the most abundant genotype did not have the highest $SL_{am}A$. More remarkably, the genotypes with the highest $SL_{am}A$ had the lowest $L_{am}MR$. As a consequence the variation in $L_{am}AR$ between the genotypes was small. Because the genotypes did not differ in leaf angle (pers. obs.), this in turn means that the genotypes differed little in Φ_{mass} for leaves placed at the same height. Since all genotypes were present in all layers of the canopy, they had similar Φ_{mass} values throughout the

vegetation. Therefore the coexistence of the genotypes was not the result of different genotypes being most efficient at different places in the canopy.

We offer two explanations for genotypes with a high $SL_{am}A$ having a low $L_{am}MR$. Firstly, it might reflect a difference between the genotypes in shade avoidance, where a stronger response to shade might mean both a higher $SL_{am}A$ and a relatively higher investment in height. Secondly, leaves that are placed later in time might experience lower light levels because of the growth of the vegetation, and therefore might have stronger shade avoidance characteristics. Differences between genotypes in leaf characteristics might then reflect differences in the timing of leaf positioning.

Since leaves placed at the same light availability did not differ in Φ_{mass}, differences between genotypes in light capture efficiency per plot ($_p\Phi_{mass}$) thus depend on the amount of leaves placed in the upper layers of the vegetation. We found that with increasing light availability the Φ_{mass} values of individual leaves increased for all genotypes. We also found a disproportional increase in Φ_{mass} with increasing Total Leaf Weight, while Total Leaf Weight increased with increasing light availability. This shows that it is most efficient to place leaves at the top of the canopy. Therefore genotypes that had relatively more leaves placed in the top layer were on average more efficient in light capture and thus captured disproportionally more light per unit biomass.

Game theoretical models predict that taller plants can displace shorter individuals if the gain in light capture is higher than the construction costs (Givnish 1982, Iwasa 1985, Falster and Westoby 2003). Therefore one would have expected that in our arenas the genotypes with relatively more leaves in the higher layers had become the most abundant. This, however, was not the case. The genotype with the highest frequency did not have the highest $_p\Phi_{mass}$ values. Also, some of the genotypes whose frequencies had declined had $_p\Phi_{mass}$ values similar to or higher than the most abundant genotype. Therefore Φ_{mass} is not the trait that can explain why genotype I became so abundant.

There may be several reasons why a disproportional advantage in light capture might not lead to abundance. Firstly, the Φ_{mass} we used is a measure of efficiency at one point in time, and does not take into account the life span of a leaf. In general, leaves will stop elongation when the lamina is placed in full light (Vince-Prue et al. 1976; Leeflang et al. 1998). As the canopy increases in height after the plants emerge in spring, these older leaves are shaded by younger ones. Differences between genotypes in $_p\Phi_{mass}$ values might therefore be a reflection of

differences in leaf placement. As the LAI also increases in the beginning of the growing season, height growth might not result in a disproportional advantage throughout the whole season. Aan et al. (2006) found that the advantage of being tall increased with stand LAI, showing that at low LAI there was no disproportional increase of light capture with mass. Anten et al. (1999) showed that shorter species appeared to be able to use the earlier part of the season for efficient light capture, while taller species were more efficient in the later part of the season. Therefore genotypes that invest in many short leaves might hold an advantage early in the season, while genotypes that invest in taller leaves might be more efficient later. The life time efficiency of leaves, which includes these growing patterns and leaf senescence (Harper 1989; Poorter 1994; Westoby et al. 2000), might therefore give a better understanding of the frequency pattern after five years.

Secondly, although Φ_{mass} might give a good indication of the success of a plant in the competition for light, the growth of individuals is also determined by the efficiency with which they use the captured light for photosynthesis (Hikosaka et al. 1999). Anten and Hirose (2003) showed that dominant species in a multi-species canopy achieved higher rates of photosynthesis per unit of absorbed light while they had similar Φmass values as subordinate species. Differences in light use efficiency also occur within species (Inthapanya et al. 2000; Green et al. 2001). Differences in abundance could therefore be the result of differences between the genotypes in light use efficiency.

Alternatively, interaction between different carbon sinks within the clone fragments could play an important role. The allocation to daughter ramets might affect the competitive ability of clonal plants in dense vegetation (Stuefer et al. 2002). Also, clonal plants store carbon in roots and stolons, which could be used for plastic responses to temporal changes in the growing conditions of a plant, or for survival and emergence after winter (Suzuki and Stuefer 1999). The ten genotypes that were used in this study differed in these allocation patterns (Stuefer et al. in prep., Vermeulen unpublished data). The Φ_{mass} values of the genotypes we used here are only based on leaves and do not take into account the biomass put in roots and stolons. Incorporating these carbon sinks might show that the genotypes differ much more in Φ_{mass} values than we have found on the basis of leaves alone.

In conclusion, our data show that the differences and similarities in frequency of the genotypes were not caused by differences in instantaneous light capture efficiency. All remaining genotypes were present throughout the canopy and differed little in Φ_{mass} values of individual leaves, despite differences in leaf characteristics. Light

capture did increase disproportionally with increasing weight, and thus genotypes with relatively more taller leaves had higher plot Φ_{mass} values. Also, coexistence was not the result of different genotypes being most efficient at different layers of the canopy. Although these results indicate that within species competition for light should result in selection for height growth, the differences in instantaneous light capture efficiency did not explain the frequency of the genotypes. This might indicate that the advantages of being taller not necessarily have to lead to the exclusion of smaller plants.

Figure 5.5. Overview of the competition experiment in the first week of April 2006.

Chapter 6

Carbon gain and the competition for resources between genotypes of a stoloniferous plant

Summary

With a canopy model the photosynthetic rate of the different genotypes were calculated within different layers of the canopy, to explore possible mechanisms of the competitive exclusion and coexistence that has occurred between the genotypes.

Results showed that genotypes differed in both the relation between maximum photosynthetic rate (Pmax) and nitrogen per unit area (Narea) and the dark respiration and Narea. The most abundant genotype I had lower Pmax and lower Rd values for a given Narea than most genotypes, while in the competition experiment it had intermediate Narea values compared the other genotypes. Consequently the most abundant genotype had intermediate photosynthetic rate per unit mass (Pmass) in the top layers of the canopy, but relatively high Pmass values at the bottom. Genotype I had overall Pmass values that were intermediate compared to the other genotypes.

This would support the notion that the dominant genotype is a superior competitor because it can survive the lowest light levels. It is suggested that a longer leaf life span may play an important role. On the other hand, coexistence may have occurred because other genotypes were more efficient at the top of the canopy.

Introduction

Nitrogen and light are considered as two important resources, as they limit photosynthetic production in many types of vegetation (Mooney and Gulman 1979; Chapin et al. 2004). As carbon gain is often considered as a proxy for plant fitness, the efficiency with which plants acquire and use these resources for photosynthesis is an important factor determining the outcome of competition (Hirose 2005).

Resource competition theory predicts that when only one nutrient is limiting in well mixed, constant environments, the species with the lowest critical nutrient requirement (R*) will exclude the other species (Stewart and Levin 1973; Armstrong and McGehee 1980). In other words, the species that can survive the lowest resource level will be the best competitor (Tilman 1982). This has been experimentally demonstrated for microbial competition (Hansen and Hubbell 1980; Smith 1993), competition between phytoplankton (Sommer 1989), algae (Tilman 1976; Tilman 1977; Grover 1991) and terrestrial plants (Tilman and Wedin 1991; Wedin and Tilman 1993).

In this respect competition for light is fundamentally different from competition for other resources. This is because in dense vegetation a light gradient is formed by the plant themselves (Monsi and Saeki 1953), which makes it rather difficult to assign a critical requirement value that is similar to R*. Where roots have to deal with the low nutrient levels they have created themselves, leaves shade the leaves below them but not the other way around, and thus taller plants can have a competitive advantage (Ford 1975; Weiner et al. 1990; Schmitt and Wulff 1993). Still, resource models predict that if plants do not differ in biomass distribution over the light gradient, the species that can survive lower light levels deep inside the vegetation (i.e. have a low critical light level I*) will outcompete the other species(Tilman 1988; Weissing and Huisman 1994; Huisman and Weissing 1994).

Miller (2005) argued in a review that many hypotheses of the resource theory still lack sufficient experimental support, especially for terrestrial plants. Yet Dybzinksi and Tilman (2007) showed that competitive outcome between species could be relatively well predicted based on the R* for nitrogen and I* for light measured in their monocultures. In a long term study on the effects of both light and nitrogen availability on competition between grasslands species, they showed that in six out eight pairings the species with both the lowest R* and I* displaced the other species, while in two pairings where one species had lowest R* and the other the lowest I* they seemed to coexist. These findings thus supported the “resource ratio theory”, sensu Tilman (1986).

However, pinpointing the cause why competitive exclusion or coexistence occurs will be difficult with this approach. Firstly, the predictions are made on the basis of measurements in respective monocultures. This ignores the fact that species are plastic in changing their growth form to that of the competitor (Huber and Wiggerman 1997; Schmitt et al. 1999; Lepik et al. 2005). Thus while resource models show that the species which can survive lower light levels deep inside the vegetation will exclude the other species, the same models also predict that species which have a higher I* can still outcompete the species with lower I* if they have a better position in the light gradient (Tilman 1988; Weissing and Huisman 1994). As a result, the R* and I* values measured in monocultures may not necessarily be important in the outcome of competition. Additionally, by using the R* and I* one assumes that they are both independent values that could explain the difference in fitness between the plants. This ignores the fact that nitrogen and light both affect photosynthetic rate and that the required ratio of these two may vary between species. A method with which one can asses the photosynthetic performance of competing plants may shed more light on the mechanisms behind competitive exclusion or coexistence.

Canopy modeling can be a way of addressing this problem. It uses the relation between nitrogen and light to calculate the net photosynthesis of plants within the different layers of the canopy (Barnes et al. 1990; Goudriaan and Laar 1994; Hikosaka et al. 1999) and can thus assess why some plants may perform better than others. Analyses in multi-species canopies have indicated that species which make up a large part of the vegetation's biomass are dominant because they are taller (Fliervoet 1984; Hirose and Werger 1995; Anten and Werger 1996) and have photosynthetic characteristics for high carbon gain at high light levels, resulting in high photosynthetic rate per unit mass (Pmass, Anten and Hirose 2003). Higher light interception has also been found to explain the replacement of dominant species in succession in abandoned grasslands (Werger et al. 2002) and along a nitrogen gradient (Aan et al. 2006), indicating that species exclusion can occur when winning species have characteristics that allow for high carbon gain. Yet, analysis in multi-species stands also show that plants with low investment in height growth can persist low in the canopy when they have a high light capture efficiency per unit biomass, suggesting that coexistence between plants with different photosynthetic characteristics can occur (Hirose and Werger 1995; Anten and Hirose 1999). Within species however, such coexistence is hypothesized not to

occur, because the variation in traits within species may be too small (Anten and Werger 1996; Anten 2005, chapter 5).

An interesting experimental setup by Stuefer et al. (in prep.), with ten competing genotypes of the stoloniferous plant *Potentilla reptans* at high nitrogen availability, may provide a good test of the different predictions. In dense stands this fast growing plant reproduces almost exclusively vegetatively, which makes it possible to run long term experiments without the traits of the ten competitors changing. Analyses of leaf frequency after five years of competition showed that one genotype had become abundant (>40% of leaves, genotype I), while others had increased slightly (F, D) or remained around their initial 10% (H), had declined in frequency (C,E,G,I) , or became rare (A, 1 occurrence out of 800 leaves) or extinct (B). Both competitive exclusion and coexistence thus seem to have occurred (see figure 1.1).

We wanted to use this experiment to test the predictions from the resource ratio theory and canopy models. Therefore, a canopy model was constructed to calculate the net photosynthesis of the genotypes in the different layers of the canopy. We expected that, following the resource theory, the most abundant genotype would have characteristics that would allow it to survive at low light levels, i.e. have a positive carbon gain at the bottom of the canopy, where others might show a negative carbon gain. We also expected that it would have higher photosynthetic gain per unit mass than declining genotypes. As a possible explanation for coexistence, some of the genotypes that have increased or have remained near their initial frequency were expected to have slightly lower photosynthetic rate per unit mass (Pmass) as the dominant genotype, but also to differ in characteristics from the dominant genotype, which would allow them to have higher photosynthetic gains in different layers of the canopy.

Methods

Potentilla reptans

Potentilla reptans is a stoloniferous herb found at moderately disturbed, productive pastures, mown grasslands, lake and river shores, road margins and other man-made habitats (Van der Meijden 1996). The plant produces sympodially growing stolons, which can form a long string of interconnected ramets on its nodes. In the absence of physical disturbance the ramets remain interconnected throughout one growing season (Stuefer et al. 2002). Leaves consist of a petiole and a lamina, and

height growth is achieved by elongating the petioles (Huber 1995; 1996). Leaves emerge in spring from the tap roots, and per plant new leaves are placed above older leaves (see Chapter 3). This height growth continues until early summer. Throughout the season, leaf turnover is high (P.J. Vermeulen, unpublished data, 2006).

Frequency harvest

A more detailed description of the experimental setup can be found in Stuefer et al. (in prep.). Here a short description is given. In the botanical gardens of Utrecht University 16 plots of 2 by 2 meters were established in the spring of 1998. In these plots 100 planting points were positioned on a regular grid. For each plot ten similar sized juvenile ramets per genotype were taken from the stock populations and randomized over these planting points. Every genotype thus started with an initial frequency of 10%. Two treatments were applied: a disturbance treatment and a control treatment. The data necessary to calculate light capture were only measured in the control plots. Therefore we only report data from these eight plots.

At the beginning of July 2003, every plot was divided into four subplots, in each of which a light profile was measured under an overcast sky. Starting at the top of the vegetation two measurements were made at 5 cm intervals using a ceptometer (Delta-T Devices, Cambridge, UK). Photosynthetic Photon Flux Density (PPFD) above the canopy ($PPFD_o$) was measured simultaneously using a Licor Li 190 quantum sensor. Then, 100 leaves in each plot were harvested at randomly chosen grid points in the vegetation: at each sample point the vegetation was visually divided into three layers, and the layer from which the leaf had to be sampled was drawn randomly. The first leaf that was hit in this layer was sampled. Since the plots were part of an ongoing experiment, we sampled only leaves, and left stolons and roots intact.

The leaf is defined as the petiole plus the palmate lamina, which consists of five to seven leaflets. For each leaf the height of the lamina above the ground and the height of the vegetation at the position of the sampled leaf were measured. From these two measurements we calculated the depth of the vegetation at the height of the lamina. On all laminas one measurement with a SPAD 502 meter (Minolta, Japan) was performed; these values were used to calculate chlorophyll contents (see below). Then the lamina was split in two parts: one for the ISSR analysis to determine its genotype (see Stuefer et al. (in prep.)), and the other for weight and nitrogen measurements. For both parts the lamina area was measured. From the

latter half the lamina dry mass was measured, together with the petiole dry mass. Total lamina mass was calculated using the specific lamina area (m^2 g^{-1}) of this latter part, and the combined lamina area of the two parts.
To get sufficient material for nitrogen analyzes, all laminas halves of each genotype with similar depth were pooled per plot. Total N (Nt_i, as %gN of total lamina mass) was analyzed on homogenized dry material with an elemental analyzer (Carlo Erba, Model EA NA 1110, Milan, Italy).

Canopy structure and light extinction
Because the plots were used for subsequent sampling (Stuefer et al. in prep.), destructive measurement of total LAI of the plots was not possible and LAI had to be estimated indirectly. The LAI and leaf area distribution in each subplots were estimated from the measured light distributions and extinction coefficient for light (K) by rewriting Beer's law for light distribution (Monsi and Saeki 1953):

$$L\_C = \ln(I / I_o) / K \quad (1)$$

with L_c the cumulative LAI above a given point in the canopy and I and I_o the light intensity at that point and above the canopy, respectively. LAI is estimated by substituting I by the light intensity below the canopy I_b. In a subsequent year (July 2005) the light distribution was measured again in the same way as described above. Subsequently all the laminas were clipped in each 5 cm horizontal layer and their area was measured with a leaf area meter (LI-3100 LiCor). K was then calculated using equation 1. K was taken as the average of all eight plots, and was found to be 0.84 ±0.008 (1se), a normal value for a dicotyledonous species (Monsi and Saeki 1953). This approach assumes that the extinction coefficient for light did not change significantly between 2003 and 2005, which is reasonable given the fact that K depends mostly on leaf angle distribution which did not differ between genotypes. In addition, the estimated average LAI per plot of 2003 (4.97±0.08) was very similar to the average LAI in 2005 (4.96±0.12). Because several genotypes were rare and did not occur in many of the subplots, the data of the four subplots of each plot were pooled.
Lamina absorbance (α) was calculated as a function of lamina chlorophyll content (chl, µmol m^{-2}) following Evans (1993):

$$\alpha = Chl / (Chl + 76) \quad (2)$$

The chlorophyll content in turn was estimated from the SPAD measurements that had been done on each lamina sampled (see above) using a calibration line made for separate sets of leaves (chl = 27.66*SPAD + 21.88, R^2 = 0.88). Chlorophyll content was determined with a spectrophotometer on a 2 cm^2 fresh sample extracted in dimethylformamide (Inskeep and Bloom 1985). The thus estimated α values did not differ significantly between genotypes nor between laminas from different depths, and was set to 0.83, the average that was found for all laminas.

Leaf gas exchange measurements

For each genotype gross photosynthesis at saturating light (Pm_i) and dark respiration (Rd_i) were measured on 15 leaves, which were taken from different layers, and since these stands also had high density thus from different light levels, in their stock populations. Petioles were cut and put in water. Then the petioles were cut again under water, to prevent air blocking the water supply to the laminas. A gas-exchange measuring system was used with leaf chambers with a 69x67 mm window (Pons and Welschen 2002). An infrared gas analyzer (LI-6262, LI-COR) was used to measure CO_2 and H_2O partial pressure. Lamina temperature was maintained at 25°C; lamina-to-air vapor pressure difference was approximately 1 kPa, and CO_2 partial pressure of the air entering the leaf chambers was 38 Pa. Light availability was kept at 2000 µmol m^{-2} s^{-1} for laminas taken from the top of the canopy and 1000µmol m^{-2} s^{-1} for laminas taken from the lower parts, the latter to prevent photo-inhibition from taking place. Net rates of photosynthesis Pnet were calculated according to von Caemmerer and Farquhar (1981). Dark respiration rates (Rd_i) were measured after 20 minutes in the dark. Pm_i is the sum of $Pnet_i$ and Rd_i. The lamina area enclosed in the chamber was measured, after which the nitrogen content was measured in the same way as described above. From these data the relation between the Pm and Narea and Rd and Narea for each genotype i can be found following Hirose and Werger (1987):

$$Pm_i = a_{pi} Narea_i + b_{pi} \quad (3a)$$

$$Rd_i = a_{ri} Narea_i + b_{ri} \quad (3b)$$

Model

We calculated net daily photosynthesis at the leaf, genotype and stand level using a canopy based on previous models (Hirose and Werger 1987; Hikosaka et al. 1999) with modifications to account for specific experimental aspects of our study.

Leaf mass, lamina area and nitrogen distribution within the canopy

We have two datasets with different dimensions: the frequency harvest data, with genotype frequency as the number of leaves that were found out of 100 sampled leaves per plot, and the canopy structure data, giving the lamina area (m^2) within a 5cm layer. From these data, a model was constructed for each plot, with canopy layers of 2.5 cm thick, an intermediate between the canopy structure data of 5cm and the frequency data of individual leaves, whose positions in the canopy were measured at the 1cm scale. To calculate the total lamina area within these model layers, the lamina area in each 5 cm layer from the canopy structure data was divided by two, while the lamina area below a depth of 20 cm was pooled into one model layer. The individual leaves from the frequency harvest were assigned to these model layers according to their measured depth within the vegetation.

The total number of leaves of a given genotype i in layer j of a plot NL_ij was calculated as:

$$NL\_ij = NL\_cns\_ij * CVj \quad (4a)$$

where NL_cns_ij are the number of leaves of that genotype sampled from the layer during the census of the frequency harvest. The factor CVj is a multiplier to relate the lamina area per layer sampled in the frequency harvest to the total lamina area in that layer:

$$CVj = LA\_j / LA\_CNS\_j \quad (4b)$$

with LA_j, the total lamina area in layer j estimated from the light distribution and LA_cns_j the total lamina area sampled from this layer during the frequency harvest.

The average lamina area (Lav_{ij}, m^2), lamina mass (g), petiole mass (g) and total leaf mass (g m^{-2} ground layer, lamina mass + petiole mass) of a single leaf of genotype i in layer j was calculated from the frequency data. Petiole Mass is the mass of the whole petiole, i.e. the mass that is invested to put the lamina at the

height it was found. Total lamina mass (Mla_{ij}, g m^{-2} ground layer), total petiole mass (g m^{-2} ground layer) and total leaf mass (M_{ij}, g m^{-2} ground layer) of genotype i in layer j were then be found by multiplying the average mass values by the number of leaves NLij. Similarly, the total lamina area (L_{ij}, m^{-2} m^{-2} ground layer) can be found using the average lamina area and the number of leaves.

$$L_{ij} = NL_{ij} * Lav_{ij} \tag{5}$$

The total lamina area in layer L_j is the sum of the lamina area of all genotypes within this layer.

The average nitrogen content per unit lamina mass of genotype i in layer j (N_{ij}, mmol N g^{-1} lamina mass) was taken as the average of the nitrogen content (Nt_i, see methods) of the laminas of genotype i assigned to the model layer j. Nitrogen per unit lamina area of genotype i in layer j ($Narea_{ij}$, mmol N m^{-2}) can be found as:

$$Narea_{ij} = \frac{N_{ij} * Mla_{ij}}{L_{ij}} \tag{6}$$

Light interception within the canopy

The photon flux density (PPFD) incident on the laminas in layer j was calculated assuming Beer's law following (Hikosaka 2003):

$$I_j = \frac{I_o K}{\alpha} \exp(-K(L_{jt} + 0.5 * L_j)) \tag{7}$$

where I_o is the average PPFD above the canopy, L_{jt} the average cumulative LAI above layer j, L_j the LAI of that layer, K the light extinction coefficient and α the lamina absorbance. This calculation assumes that the position of the laminas in a layer can be represented by the median height of that layer (e.g. for layer 0 to 5 cm that would be 2.5 cm). It further assumes that the optical and geometric characterstics of leaves do not differ between genotypes. The distribution of light above the canopy (Io) is assumed to follow a sinusoidal pattern (Hirose and Werger 1987):

$$I_{oh} = I_{oo} \sin^2\left[\frac{\pi(T-6)}{12}\right] \qquad (6 \le T < 18) \tag{8}$$

$$I_{oh} = 0 \qquad (0 \le T < 6, 18 \le T < 24)$$

Where I_{oo} is the noon PPFD above the canopy set at 2000 µmol m^{-2} s^{-1} and T the average solar time within the one hour interval (i.e. 6.5, 7.5 etc).

Genotypic and whole canopy photosynthesis

For each genotype i within layer j, a non-rectangular hyperbola was used to characterize the light response of net leaf photosynthesis within a one hour interval (PLijh, µmol m^{-2} s^{-1}):

$$P_{Lijh} = \frac{(Pm_{ij} + \Phi I_{jh}) - \left[(Pm_{ij} + \Phi I_{jh})^2 - 4\Phi\theta Pm_{ij} I_{jh}\right]^{0.5}}{2\theta} - R_{dij} \quad (9)$$

Where I_{jh} is the absorbed PPFD, Pm_{ij} (µmol m^{-2} s^{-1}) is the light-saturated rate of gross photosynthesis and R_{dij} is dark respiration (µmol m^{-2} s^{-1}) of genotype i in layer j, and Φ and θ the quantum yield (mol mol^{-1}) and curvature factor, respectively (Marshal and Biscoe 1980). Pm_{ij} and Rd_{ij} can be found by substituting $Narea_i$ in equations 3a and 3b by $Narea_{ij}$. Φ was taken to be 0.05 (Ehleringer and Björkman 1977) and for θ 0.8 was assumed as an average of previous studies (Hirose et al. 1997).

Daily net photosynthesis for genotype i in layer j can than be found by integration over the day:

$$P_{ijh} = \int_0^{24} P_{Lijh}\, L_{ij}\, dT \quad (10)$$

where L_{ij} is the total lamina area (m^2) of genotype i in layer j. Consequently, the daily photosynthesis of genotype i in layer j (P_{ij}, mol day^{-1}) and total daily photosynthesis of genotype i (P_i, mol day^{-1}) can be found as:

$$P_{ij} = \sum_h P_{ijh} \quad (11)$$

and

$$P_i = \sum_j P_{ij} \quad (12)$$

Because the genotypes differ strongly in frequency and thus in total photosynthesis, we used the daily photosynthesis per unit aboveground mass of genotype i ($Pmass_i$, mol g^{-1} day^{-1}) as performance measure, following Anten and Hirose (2001):

$$Pmass_i = P_i / M_i \quad (13)$$

Statistics

To test whether genotypes differed in the relation between Pmax and Rd with increasing Narea, a one way covariance analysis was performed, with Pmax and Rd as dependent variables, genotype as fixed variable and Narea as covariable. A two way covariance analysis was used with nitrogen content N per unit lamina mass and Narea as dependent variables, genotype as random factor and plot as a fixed factor, with depth as covariable, to test if the genotypes differed in their decrease of N content and Narea with increasing depth in the canopy.

Table 6.1. Results of two way analysis of covariance (ANCOVA). All values are F values. Log and arcsinsqrtmeans data have been log transformed and arcsinsquareroot transformed respectively. Ns: P>0.05, *: 0.01≤P<0.05, **: 0.001≤P<0.01, ***: P<0.001.

Dependent	Covariate	Factor	Among slopes	Among intercepts
N content^{arcsinsqrt}	depth^{ns}	Genotype	2.357*	
		Plot	1.880^{ns}	
		G*P	1.197^{ns}	
Narea^{log}	Depth***	Genotype	1.600ns	2.938**
		Plot	0.553ns	.980ns
		G*P	1.073ns	
Pmass	Narea***	Genotype	.1.082^{ns}	3.446**
Rd	Narea***	Genotype	1.353^{ns}	7.945***
Pmass	Layer***	Genotype	4.066***	
		Plot	1.663ns	
		G*P	0.888ns	
Pdg^{log}	Layer***	Genotype	1.425ns	
		Plot	2.883**	
		G*P	0.671ns	
Tdw^{log}	Layer***	Genotype	2.375*	
		Plot	3.554**	
		G*P	1.035^{ns}	

A two way covariance with Pmass, net photosynthesis and total mass as dependent variables, genotype as random factor, plot as fixed factor and layer as covariable, was used to test for differences between genotypes for these data calculated by the model. Finally, a two way Anova with genotype as random factor and plot as fixed factor was performed to test for differences in overall Pmass.

Results

The averages of the morphological traits in the model were taken from the data presented in chapter 5. More details can be found there. In order to make the model outcome more clear, a brief summary of these results is given in the following paragraph. The specific lamina area increased with increasing depth in the canopy. The most abundant genotype I had, together with genotype J, relatively high specific lamina area when traits where compared at similar positions in the canopy. Both genotypes had, however, a low lamina mass ratio, resulting small differences between genotypes in lamina area ratio, and thus in small differences between genotypes for light interception per unit mass (Φ_{mass}). Differences between genotypes in overall light interception were dependant on the relative amount of leaves placed at the top of the canopy, because the Φ_{mass} values of leaves placed higher up were always higher than leaves placed lower down. Genotype I had intermediate overall Φmass values.

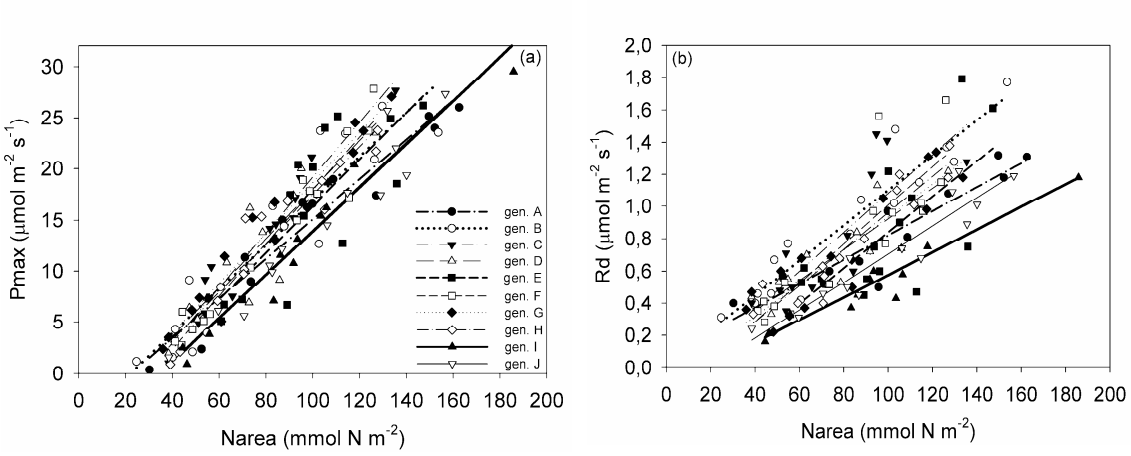


Figure 6.1. Relation between Pmax (a) and Rd with N_{area} (b) for the 10 genotypes in their monoculture

Leaf traits

Both maximum photosynthesis (Pmax, µmol m^{-2} s^{-1}) and dark respiration (Rd, µmol m^{-2} s^{-1}) increased with increasing nitrogen content per unit area (Narea, figure 6.1, table 6.1). Genotypes differed in both the relation between Pmax and Narea and Rd and Narea. Two of the genotypes that have strongly declined abundance over time, genotypes B and C, had a relatively high Rd. Genotype I had lower Pmax and lower Rd values for a given Narea than most genotypes.

Nitrogen content in competition experiment

Overall, nitrogen content per unit mass (Nmass) was not strongly related to the position in the canopy (figure 6.2a). The relationship, however, differed between genotypes (significant genotype*depth interaction, table 6.1). In some genotypes, including genotype I, Nmass decreases with depth in the canopy, but for most there was no relationship. When nitrogen was expressed per unit lamina area (Narea, mmol N m_{-2}), however, a strong decrease with increasing vegetation depth was found (figure 6.2b). Genotypes differ in Narea at a given depth (table 6.1, among intercepts, but no difference can be found between genotypes in the way Narea decreases (among slopes). The winning genotype I has intermediate Narea. Plot did not interact with any of these variables.

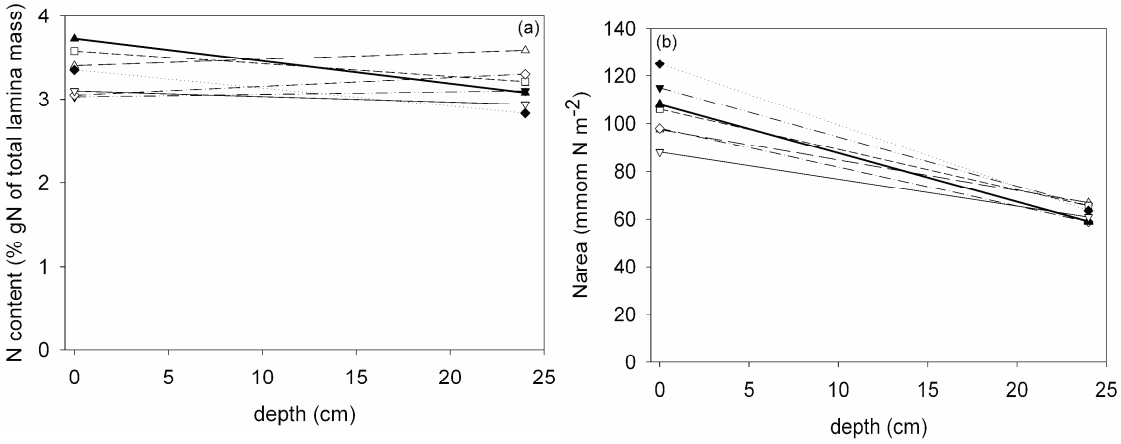


Figure 6.2. Relation between N content (gN g^{-1} total lamina mass) (a) and nitrogen per unit are (Narea, mmol N m_{-2}) (b) with depth for the different genotypes in the competition experiment. Lines represent linear regression lines of the genotypes based on log transformed data of all plots pooled together.

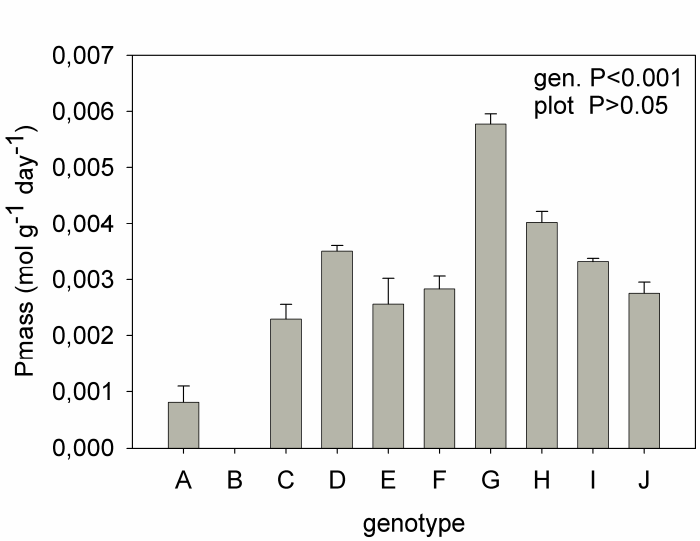


Figure 6.3. Average overall Pmass (mol g^{-1} day^{-1}, +se) for each genotype. P values of Anova analysis are given in top right corner. Bars indicate standard errors.

Carbon gain

Genotypes differed with respect to whole genotype carbon gain per unit mass (Pmass), without there being a plot effect (figure 6.3). Genotypes G and H had a high Pmass values, while genotype I had Pmass values that were intermediate between those of the other genotypes. When analyzing the components that make

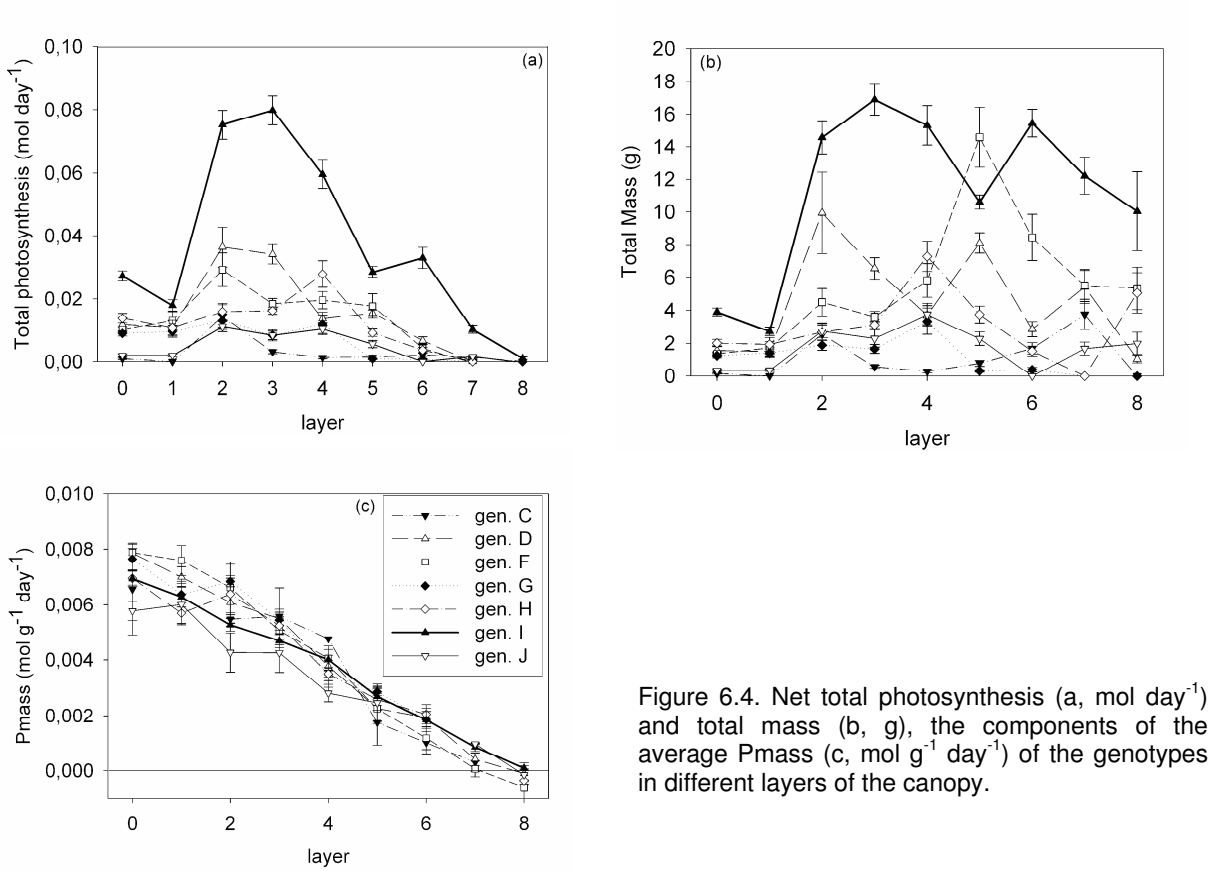


Figure 6.4. Net total photosynthesis (a, mol day^{-1}) and total mass (b, g), the components of the average Pmass (c, mol g^{-1} day^{-1}) of the genotypes in different layers of the canopy.

up Pmass separately, genotype I had relatively high biomass in the lower layers, where little photosynthesis takes place, while the genotypes with high Pmass values had relatively few or no biomass in the lower layers (figure 6.4a and b, significant genotype*layer interaction, table 6.1). Plot did show an interaction with both total biomass and total photosynthesis, probably as a result of differences in frequency. However, no such interaction was found in the way Pmass changes with layer. Genotype I had intermediate Pmass values at the top of the vegetation, but had the highest Pmass values in the bottom layers, where most other genotypes that are present showed a negative carbon gain (figure 6.4c, significant genotype*layer interaction, table 6.1). Genotypes D, and F, that also have increased in frequency, had high Pmass in the first layers of the canopy.

Discussion

One prediction of the resource ratio hypothesis is that the species that can survive at the lowest level of a limiting resource will be the best competitor. The result from our canopy model seems to support this prediction for competition between different genotypes of the clonal species *Potentilla reptans*. The genotype that has become dominant after five years of competition could reach higher carbon gain than others at low light levels, and most other genotypes had negative carbon gains or were absent in the lower layers of the canopy. This suggests that the dominant genotype has characteristics which allow it to survive low light conditions. Passarage (2006) found that in a phytoplankton competition study with both light and phosphorous as possible limiting factors the winning species could capture more light per unit biomass than its competitor. But Anten and Hirose (2003) concluded that the differences between plant species in carbon gain per unit mass were more related to differences in leaf physiology. Our results indicate that among genotypes of *P. reptans* the latter applies. Light capture data from our competition experiment showed that genotype I did not have the highest light capture per unit mass, and that light capture efficiency was quite similar between genotypes for leaves placed at the same height (chapter 5). Leaf physiology, however, did differ between genotypes. As found by other studies (Hirose and Werger 1987; Anten et al. 1995), both maximum photosynthesis and the dark respiration increased with increasing Narea. In this competition experiment, genotype I did show a gradient from the top to thelower layers in N content per mass basis, while other genotypes hardly changed their contents. The winning genotype thus had an N content, which

can lead to high photosynthesis at the top, and low respiration at the bottom of the canopy. However, because it also had a high SLA (chapter 5), the resulting Narea of the winning genotype is intermediate compared to the other genotypes. But it did have lower Pmax and lower dark respiration per Narea than the other genotypes, which allowed genotype I to have a positive carbon gain at the bottom of the canopy. This suggests that with the same N content it can survive lower light levels.

However, differences in height could influence the outcome of competition (Wilson 1988; Anten and Werger 1996; Werger et al. 2002). Our results show that laminas which are placed higher up in the canopy are more efficient in photosynthesis on a mass basis (Pmass) than lower placed laminas, similar to what is found in other studies (Anten and Hirose 2001; Hikosaka et al. 2003). This demonstrates why genotypes should place their new leaves at the top of the canopy, and confirms that height growth plasticity plays an important role in competition. It also shows that relatively more leaves at the top of the vegetation will lead to a higher overall Pmass. But the genotypes with relatively more biomass at the top of the canopy, and thus a high overall Pmass (G and H) are not the genotypes that increased in frequency. The winning genotype even has an opposite distribution: relatively more laminas in layers where net photosynthesis is low.

This apparent discrepancy can be explained by the instant nature of the Pmass values, as it does not take the dynamics of leaf loss into account. *Potentilla reptans* is a fast growing plant and the maximum photosynthesis (Pmax) it can reach is fairly high. High maximum photosynthetic rate is assumed to be correlated to short leaf longevity because it drives fast growth and consequently the shading of older leaves (Meziane and Shipley 2001). These older leaves should be shed in order to increase total plant photosynthetic gains, either because N reallocation to the top leaves is more efficient or because those leaves do not have a positive carbon gain (Hikosaka 2003; Oikawa et al. 2006; Boonman et al. 2006). We found in our experiment that on average about six out of eight newly formed leaves were dropped within a three month period, though we could not measure this for separate genotypes (P.J. Vermeulen, unpublished 2006 data). Genotypes with a high dark respiration (Rd) may lose their leaves sooner, as they will reach the point where shedding the leaves becomes beneficial at higher light levels. Because laminas lower in the canopy have low Pmass values, the overall Pmass will increase by shedding these leaves. Our winning genotype had relatively more leaves at the bottom of the vegetation and therefore a low overall Pmass.

This would support the notion that the decline of losing competitors takes place because they can not survive the low light levels (Tilman 1988; Huisman and Weissing 1994; Werger et al. 2002) that occur at the bottom of the canopy. However, the most abundant genotype did not have the highest Pmass at the top of the canopy. Therefore, the abundance of genotype I can only be explained if the benefits of longer carbon gain of leaves at low light will be higher than the benefits of higher photosynthesis at the top of the canopy of other genotypes. This in turn will depend on the height growth rate of the vegetation itself, which determines the length of time before laminas are shaded (chapter 3), and thus the period of positive carbon gain.

Not all genotypes have declined in frequency, however, which suggests that coexistence between several genotypes occurs. This may be partly explained by the different photosynthetic characteristics of the different genotypes. Genotypes D and F, which have also increased in frequency in five years, had high Pmass values at the top of the canopy, while they had a Pmass that was lower than genotype I at the bottom. It seems that there is a trade-off between high photosynthetic rates at the top and at the bottom of the canopy. This trade-off may allow coexistence if the benefits of higher photosynthetic rates at the top are equal to the benefits of a longer leaf life span, or if the net photosynthetic rates of leaves at the bottom of the vegetation stay positive and there are many more leaves in these layers.

The coexistence could be temporal in the sense that if one genotype continues to increase it could still outcompete all the others. In that case the resource theory would predict that genotype I would be the only one left in the long run, because it had in general shade tolerant characteristics. An instantaneous measurement of Pmass will not provide the definite proof for this statement. But five years of competition under a regime of sufficient nutrient supply seems quite sufficient to realize such a monotypic survival, or at least sufficient to show a decline in all genotypes but one, and we therefore think we can reject this suggestion.

Figure 6.5. One plot of the competition experiment. Top: picture taken in the first week of April 2006; and bottom: same plot four weeks later.

Chapter 7

A game theoretical approach to optimal total lamina area in a five year old multi-genotypic stand

Summary

The canopy model presented in chapter 6 was used to explore 1) whether a reduction of the total lamina area would increase the photosynthetic rate of the canopy as a whole; 2) to what extent the success of the different genotypes can be explained by simple optimization and 3) to what extent the canopy is evolutionarily stable.

In this study, a reduction of total lamina area of all genotypes simultaneously and a reduction of the total lamina area of all genotypes separately both led to an increase in the photosynthetic rate of the canopy as whole. The current total lamina area of the most abundant genotype was higher than the total lamina area that would maximize its absolute photosynthetic rate at the genotype level, while it did not significantly differ from the total lamina area that would maximize its relative share of the photosynthetic rate of the whole canopy. Several other genotypes had current lamina areas that were higher than the lamina area that would maximize either their absolute photosynthetic rate at the genotype level or their relative share. Although mutants of the most abundant genotype could not significantly outperform their “mother genotype”, mutants of other genotypes could.

Results thus suggest that a tragedy of the commons has occurred in terms of total lamina area production. Also, the success of the most abundant genotype can be partly explained because it had a total lamina area that was close to the total lamina area that would maximize its relative share of whole canopy photosynthetic rate, where other genotypes had higher current total lamina areas. Finally it is concluded that the vegetation is not evolutionarily stable, as mutants from the less abundant genotypes can invade this canopy.

Introduction

Photosynthesis provides the structural substrates for growth and reproduction. Plants with a high photosynthetic carbon gain should have the greatest amount of resources with which to compete for additional water, light and nutrients (Givnish 1982). In view of this, many authors (Monsi and Saeki 1953; Saeki 1960; Kuroiwa 1971; Givnish 1982; Hirose and Werger 1987; Gutschick and Wiegel 1988) have argued that maximization of photosynthesis has been an important driving force determining the evolution of leaf and canopy characteristics in plants. Accordingly, these traits would have to be optimized to allow for a maximization of photosynthetic traits.

However, the question then is how to evaluate “optimality”. Early studies that analyzed traits of plants growing in vegetation stands used frequency independent optimization, where a trait value is considered optimal when whole-stand carbon gain is maximized. These studies included analyses of optimal leaf angle (Kuroiwa 1971; Hara 1985), nitrogen allocation among leaves (Field 1983; Hirose and Werger 1987), leaf area per unit mass (SLA) (Gutschick and Wiegel 1988) and leaf area production (Anten et al. 1995; Goudriaan 1995). The assumption underlying frequency independent optimization is that the optimal trait values of individuals are independent of those of their neighbours (Parker and Maynard Smith 1990). This, however, does not apply to dense vegetation stands as plants strongly affect each other’s light and nutrient availability. Therefore it is more appropriate to analyze the adaptive significance of plant traits in dense vegetation assuming frequency dependence, for instance using a game theoretical approach (Falster and Westoby 2003; Anten 2005; Pronk et al. 2007)

Game theoretical studies have shown that an optimal vegetation structure for maximum whole stand productivity is not evolutionarily stable. Stands with optimal leaf angle distribution, leaf area index or plant height can be invaded by a mutant that produces more horizontally projected leaves (Hikosaka and Hirose 1997), more leaf area (Anten and Hirose 2001) with a larger SLA (Schieving and Poorter 1999), or grows taller (Givnish 1982; Iwasa et al. 1985). This results in stands that are not maximally productive in terms of photosynthesis and growth, i.e. a so-called ‘tragedy of the commons’ (Hardin 1968) occurs. These studies using competitive optimization have generally resulted in predictions of plant traits closer to field values than studies using simple optimization (Anten 2002).

The underlying idea of an evolutionarily stable vegetation structure is that the phenotypes which form the canopy have evolved to a state where, following the

definition of Maynard Smith (1982), no mutant can successfully invade a stand of competing individuals. However, several assumptions are made that may not apply to many natural vegetations. Firstly, while the analyzes are often applied to organisms that reproduce sexually, it is implicitly assumed that the traits are passed on only through vegetative reproduction (Nowak and Sigmund 2004; Vincent and Brown 2005). This does not take into account the constraints sexual reproduction can place on trait evolution (Weissing 1996; Hammerstein and Selten 1994; Hammerstein 2005). Secondly, the mentioned ESS approaches assume that the resident population consists of plants with the same traits, i.e. consist of a single genotype. But even clonal plant populations usually consist of several genotypes (Ellstrand and Roose 1987; Widén et al. 1994; Verburg et al. 2000). In such a multi-genotypic stand, the success of a mutation in a single trait will depend on the other traits of the genotype in which the mutation occurs, and the effect could thus be different for each separate genotype. These assumptions will make it difficult to accurately predict ESS trait values in a multi-genotypic stand (Weissing 1996). Even so, the key idea of an evolutionary game is to search for strategies that would be maintained by selection once they are established (Hammerstein 2005), and therefore is a valuable tool to analyze the values of current traits of different genotypes in the context of competing plants.

In this chapter, the lamina area of genotypes of the clonal plant *Potentilla reptans* that were remaining after five years of competition among originally ten genotypes was analyzed in relation to the vegetation structure and productivity. Using this stoloniferous plant in game theoretical analyses has several advantages. Establishment from seed is unlikely (Stuefer et al. in prep.) and in practice only vegetative reproduction occurs. One thus knows which fixed genotypes, and thus which traits and the phenotypic plasticity in these traits, occur within the vegetation. Because each new lamina is supported by a separate single petiole which has to start at the bottom of the canopy, the pay-offs of the different traits can easily be analyzed. In addition, as *P. reptans* places its ramets away from the mother ramet, the different genotypes can be considered to be well mixed in the five year old stands, an assumption that is widely used in game theoretical approaches (Xiao et al. 2006; Schieving and Poorter 1999).

Analyzes using ISSR primers revealed that strong shifts in abundance have occurred within the five year period: one genotype has increased to over 40%, two other genotypes also have higher frequency than at the start of the experiment, one genotype has not significantly changed, five other genotypes have decreased

in frequency, while one seems to have been excluded (Stuefer et al in prep.). This indicates that the differences in trait values between genotypes lead to selection.
Data from this experiment were used in the construction of a canopy model, with which we calculated the effect of change in lamina area on plant photosynthesis. We focused on lamina area because it strongly influences photosynthetic gains through its effect on light capture and maximum photosynthetic capacity. These two components trade-off as the latter is positively related to the nitrogen per unit leaf area (Narea), and an increase in lamina area results in a decrease in Narea. Hence the LAI of a stand is closely linked with the total nitrogen that is available (Hikosaka 2003).
With the model, the following questions were analyzed:

- Is the real LAI larger than the optimal one for maximization of whole-stand productivity, i.e. is there a tragedy of the commons? And if so, do all genotypes contribute to this 'overinvestment'? If the latter holds true, the total lamina area of each single genotype should be higher than the total lamina area that, while keeping the total lamina area of other genotypes constant, would maximize canopy photosynthesis.
- To what extent can the success of the different genotypes be explained by simple optimization? Or can the total lamina be better explained by competitive optimization?
- To what extent are these trait values stable, in the sense that no mutant of the remaining genotypes, differing only in lamina area, can invade this stand?

Methods

Potentilla reptans

Potentilla reptans is a stoloniferous, rosette forming herb found at moderately disturbed, productive pastures, mown grasslands, lake and river shores, road margins and other man-made habitats (Van der Meijden 1996). The plant produces sympodially growing stolons, which can form a long string of interconnected ramets on its nodes. These internodes can reach an average length of 9-14cm in shade cages (Huber 1995; 1996; Huber et al. 1998). Because the initial space between planted ramets at the start of the experiment was 10cm (Stuefer et al. in prep.), the ramets of the different genotypes can be assumed to be well mixed after five years of growth. In the absence of physical disturbance the ramets remain interconnected

throughout one growing season, after which the internode dies off (Stuefer et al. 2002). Most ramets that are established after five years will therefore be fully independent. Leaves consist of a petiole and a lamina, and height growth is achieved by elongating the petioles (Huber 1995; Huber 1996). Self shading within a single ramet is not likely to take place, as the ramets in the competition experiment had an average of only three leaves at the same time (P.J. Vermeulen, unpublished 2006 data), and more importantly, long petioles that can place the laminas away from each other, which will minimize self shading (Hikosaka et al. 2001). Leaves die off in autumn. Leaves emerge in spring from the tap roots, and per plant new leaves are placed above older leaves (see Chapter 3). This height growth continues until early summer. Throughout the season, leaf turnover is high (P.J. Vermeulen, unpublished data from 2006).

Frequency harvest

A more detailed description of the experimental setup can be found in Stuefer et al. (in prep.). Here a short description is given. In the botanical gardens of Utrecht University 16 plots of 2 by 2 meters were established in the spring of 1998. In these plots 100 planting points were positioned on a regular grid. For each plot ten similar sized juvenile ramets per genotype were taken from the stock population and randomized over these planting points. Every genotype thus started with an initial frequency of 10%. Two treatments were applied: a disturbance treatment and a control treatment. The data necessary to calculate light capture were only measured in the control plots. Therefore we only report data from these eight plots.

At the beginning of July 2003, every plot was divided into four subplots, in each of which a light profile was measured under an overcast sky. Starting at the top of the vegetation two measurements were made at 5 cm intervals using a ceptometer (Delta-T Devices, Cambridge, UK). Photosynthetic Photon Flux Density (PPFD) above the canopy ($PPFD_o$) was measured simultaneously using a Licor Li 190 quantum sensor. Then, 100 leaves in each plot were harvested at randomly chosen grid points in the vegetation. At each sample point the vegetation was visually divided into three layers, and the layer from which the leaf had to be sampled was drawn randomly. The first leaf that was hit in this layer was sampled. Since the plots were part of an ongoing experiment, we sampled only leaves, and left stolons and roots intact.

The leaf is defined as the petiole plus the palmate lamina, which consists of five to seven leaflets. For each leaf the height of the lamina above the ground and the

height of the vegetation at the position of the sampled leaf were measured. From these two measurements we calculated the depth of the vegetation at the height of the lamina. On all laminas one measurement with a SPAD 502 meter (Minolta, Japan) was performed, and these values were used to calculate chlorophyll contents (see below). Then the lamina was split in two parts: one for the ISSR analysis to determine its genotype (see Stuefer et al in prep.), and the other for weight and nitrogen measurements. For both parts the lamina area was measured. From the latter half the lamina dry mass was measured, together with the petiole dry mass. Total lamina mass was calculated using the specific lamina area ($m^2\ g^{-1}$) of this latter part, and the combined lamina area of the two parts.
To get sufficient material for nitrogen analyzes, all laminas halves of each genotype with similar depth were pooled per plot. Total N (Nt_i, as %gN of total lamina mass) was analyzed on homogenized dry material with an elemental analyzer (Carlo Erba, Model EA NA 1110, Milan, Italy).

Canopy structure and light extinction
Because the plots were used for subsequent sampling (Stuefer et al. in prep.), destructive measurement of total LAI of the plots was not possible and LAI had to be estimated indirectly. The LAI and leaf area distribution in each subplots were estimated from the measured light distributions and extinction coefficient for light (K) by rewriting Beer's law for light distribution (Monsi and Saeki 1953):

$$L\_C = \ln(I/I_o)/K \qquad (1)$$

with L_c the cumulative LAI above a given point in the canopy and I and I_o the light intensity at that point and above the canopy, respectively. LAI is estimated by substituting I by the light intensity below the canopy I_b. In a subsequent year (July 2005) the light distribution was measured again in the same way as described above. Subsequently all the laminas were clipped in each 5 cm horizontal layer and their area was measured with a leaf area meter (LI-3100 LiCor). K was then calculated using equation 1. K was taken as the average of all 8 plots, and was found to be 0.84 ±0.008 (1se), a normal value for a dicotyledonous species (Monsi and Saeki 1953). This approach assumes that the extinction coefficient for light did not change significantly between 2003 and 2005, which is reasonable given the fact that K depends mostly on leaf angle distribution which did not differ between genotypes. In addition, the estimated average LAI per plot of 2003 (4.97±0.08) was

very similar to the average LAI in 2005 (4.96±0.12). Because several genotypes were rare and did not occur in many of the subplots, the data of the four subplots of each plot were pooled.
Lamina absorbance (α) was calculated as a function of lamina chlorophyll content (chl, µmol m–2) following Evans (1993):

$$\alpha = Chl/(Chl + 76) \quad (2)$$

The chlorophyll content in turn was estimated from the SPAD measurements that had been done on each lamina sampled (see above) using a calibration line made for separate sets of leaves (chl = 27.66*SPAD + 21.88, R^2 = 0.88). Chlorophyll content was determined with a spectrophotometer on a 2 cm^2 fresh sample extracted in dimethylformamide (Inskeep and Bloom 1985). The thus estimated α values did not differ significantly between genotypes nor between laminas from different depths, and was set to 0.83, the average that was found for all laminas.

Leaf gas exchange measurements
For each genotype gross photosynthesis at saturating light (Pm_i) and dark respiration (Rd_i) were measured on 15 leaves, which were taken from different layers in their stock populations. Petioles were cut and put in water. Then the petioles were cut again under water, to prevent air blocking the water supply to the laminas. A gas-exchange measuring system was used with leaf chambers with a 69x67 mm window (Pons and Welschen 2002). An infrared gas analyzer (LI-6262, LI-COR) was used to measure CO_2 and H_2O partial pressure. Lamina temperature was maintained at 25°C; lamina-to-air vapor pressure difference was approximately 1 kPa, and CO_2 partial pressure of the air entering the leaf chambers was 38 Pa. Light availability was kept at 2000 µmol m^{-2} s^{-1} for laminas taken from the top of the canopy and 1000µmol m^{-2} s^{-1} for laminas taken from the lower parts, the latter to prevent photo-inhibition from taking place. Net rates of photosynthesis Pnet were calculated according to Von Caemmerer and Farquhar (1981). Dark respiration rates (Rd_i) were measured after 20 minutes in the dark. Pm is the sum of $Pnet_i$ and Rd_i. The lamina area enclosed in the chamber was measured, after which the nitrogen content was measured in the same way as described above. From these data the relation between the Pm and Narea and Rd and Narea for each genotype i can be found following Hirose and Werger (1987):

$$Pm_i = a_{pi} Narea_i + b_{pi} \quad (3a)$$

$$Rd_i = a_{ri} Narea_i + b_{ri} \quad (3b)$$

Model

We calculated net daily photosynthesis at the leaf, genotype and stand using an approach based on previous models (Hirose and Werger 1987; Hikosaka et al. 1999) with modifications to account for specific experimental aspects of our study.

Leaf mass, lamina area and nitrogen distribution within the canopy

We have used two datasets with different dimensions: the frequency harvest data, with genotype frequency as the number of leaves that were found out of 100 sampled leaves per plot, and the canopy structure data, giving the total lamina area (m^2) within a 5cm layer. From these data, a model was constructed for each plot, with canopy layers of 2.5 cm thick,. The total lamina area within these model layers was found by dividing the lamina area in each 5 cm layer from the canopy structure data by two. The lamina area of the deepest model layer was found as the sum of all lamina area below a depth of 20 cm. The individual leaves from the frequency harvest were assigned to these model layers according to their measured depth within the vegetation.

The total number of leaves of a given genotype i in layer j of a plot NL_ij was calculated as:

$$NL\_ij = NL\_cns\_ij * CVj \quad (4a)$$

where NL_cns,ij are the number of leaves of that genotype sampled from the layer during the census of the frequency harvest. The factor CVj is a multiplier to relate the lamina area per layer sampled in the frequency harvest to the total lamina area in that layer:

$$CVj = LA\_j / LA\_CNS\_j \quad (4b)$$

with LA_j, the total lamina area in layer j estimated from the light distribution and LA_cns_j the total lamina area sampled from this layer during the frequency harvest.

The average lamina area (Lav_{ij}, m^2), lamina mass (g), petiole mass (g) and total leaf mass (g m^{-2} ground layer, lamina mass + petiole mass) of a single leaf of genotype i in layer j was calculated from the frequency data. Petiole Mass is the mass of the whole petiole, i.e. the mass that is invested to put the lamina at the height it was found. Total lamina mass (Mla_{ij}, g m^{-2} ground layer), total petiole mass (g m^{-2} ground layer) and total leaf mass (M_{ij}, g m-2 ground layer) of genotype i in layer j were then be found by multiplying the average values by the number of leaves NL_{ij}. Similarly, the total lamina area (L_{ij}, m^{-2} m^{-2} ground layer) can be found using the average lamina area and the number of leaves. However, a variable (Xla) was added that allows for scenarios with different total lamina area:

$$L_{ij} = NL_{ij} * Lav_{ij} * X_{la} \quad (5)$$

For analyzes of current lamina area Xla is set to 1 (see also scenario's). Than the total lamina area in layer L_j is the sum of the lamina area of all genotypes within this layer.

The average Nitrogen content of genotype i in layer j (N_{ij}, mmol N g^{-1} lamina mass) was taken as the average of the nitrogen content (Nt_i, see methods) of the laminas of genotype i assigned to the model layer j. Nitrogen per unit lamina area of genotype i in layer j ($Narea_{ij}$, mmol N m^{-2}) can be found as:

$$Narea_{ij} = \frac{N_{ij} * Mla_{ij}}{L_{ij}} \quad (6)$$

Light interception within the canopy

The photon flux density (PPFD) incident on the laminas in layer j was calculated assuming Beer's law following Hikosaka (2003):

$$I_j = \frac{I_o K}{\alpha} \exp(-K(L_{jt} + 0.5 * L_j)) \quad (7)$$

where I_o is the average PPFD above the canopy, L_{jt} the average cumulative LAI above layer j, L_j the LAI of that layer, K the light extinction coefficient and α the lamina absorbance. This calculation assumes that the position of the laminas in a layer can be represented by the median height of that layer (e.g. for layer 0 to 5 cm that would be 2.5 cm). It further assumes that the optical and geometric

characteristics of leaves do not differ between genotypes. The distribution of light above the canopy (I_o) is assumed to follow a sinusoidal pattern (Hirose and Werger 1987):

$$I_{oh} = I_{oo} \sin^2\left[\frac{\pi(T-6)}{12}\right] \qquad (6 \le T < 18)$$
$$I_{oh} = 0 \qquad (0 \le T < 6, 18 \le T < 24) \qquad (8)$$

Where I_{oo} is the noon PPFD above the canopy set at 2000 µmol m^{-2} s^{-1} and T the average solar time within the one hour interval (i.e. 6.5, 7.5 etc).

Genotypic and whole canopy photosynthesis

For each genotype i within layer j, a non-rectangular hyperbola was used to characterize the light response of net leaf photosynthesis within a one hour interval (PL_{ijh}, µmol m–2 s–1):

$$P_{Lijh} = \frac{(Pm_{ij} + \Phi I_{jh}) - \left[(Pm_{ij} + \Phi I_{jh})^2 - 4\Phi\theta Pm_{ij} I_{jh}\right]^{0.5}}{2\theta} - R_{dij} \qquad (9)$$

Where I_{jh} is the absorbed PPFD, Pm_{ij} (µmol m^{-2} s^{-1}) is the light-saturated rate of gross photosynthesis and Rd_{ij} is dark respiration (µmol m^{-2} s^{-1}) of genotype i in layer j, and Φ and θ the quantum yield (mol mol–1) and curvature factor, respectively (Marshal and Biscoe 1980). Pm_{ij} and Rd_{ij} can be found by substituting $Narea_i$ in equations 3a and 3b by $Narea_{ij}$. Φ was taken to be 0.05 (Ehleringer and Björkman 1977) and for θ 0.8 was assumed as an average of previous studies (Hirose et al. 1997). Daily net photosynthesis for genotype i in layer j can than be found by integration over the day:

$$P_{ijh} = \int_0^{24} P_{Lijh}\, L_{ij}\, dT \qquad (10)$$

where L_{ij} is the total lamina area (m^2) of genotype i in layer j. Consequently, the daily photosynthesis of genotype i in layer j (P_{ij}, mol day^{-1}) and total daily photosynthesis of genotype i (P_i, mol day^{-1}) can be found as:

$$P_{ij} = \sum_{h} P_{ijh} \tag{11}$$

and

$$P_{i} = \sum_{j} P_{ij} \tag{12}$$

Daily photosynthesis per unit aboveground mass of genotype i ($Pmass_i$, mol g^{-1} day^{-1}) can be found, following Anten and Hirose (2001):

$$Pmass_i = P_i / M_i \tag{13}$$

$$Pmass_i = P_i / M_i \tag{14}$$

Scenarios

Through equation (4) the total lamina area (TLA) and thus the light interception of individual genotypes can be altered. Xla is set to 1 for current total lamina area, a 10% increase is modelled by setting Xla to 1.1%, and 10% reduction by setting it to 0.9 (Anten and Hirose 2001). Xla is similar for all layers, thus an increase in Xla means an increase in lamina area in all layers simultaneously. Since the mass of the laminas remains the same, increasing the lamina area can be seen as increasing the specific lamina area (SLamA).

Increasing the total lamina area has several consequences in addition to changing the light interception. Firstly, the total lamina area in each layer (Lj) will increase. This will decrease the average light availability within each layer through equation 7 and 8. Secondly, through equation 5, the Narea of a genotype will decrease, which will result in decreasing Pmax, but also in a decrease in the dark respiration, through equations 3a and b.

Five scenarios are run with this model. An overview of the different scenarios can be found in table 7.1. In the first scenario, the total lamina area of all genotypes was changed simultaneously (i.e. Xla was equal for all genotypes), and the optimal lamina area was defined as the total lamina area where photosynthetic gain for the whole vegetation is maximized. This will test whether the current lamina area is

Table 7.1. Overview of the different scenarios

Scenario	Changes	Maximizes	Optimal lamina area represented by
Scenario1	Lamina area of all genotypes simultaneously	Absolute photosynthetic gain of the whole vegetation	Xla
Scenario 2	Lamina area of one genotype, while keeping other lamina area's at current levels	Absolute photosynthetic gain of the whole vegetation	Xla_veg
Scenario 3	Lamina area of one genotype, while keeping other lamina area's at current levels	Absolute photosynthetic gain of focal genotype	Xla_gen
Scenario 4	Lamina area of one genotype, while keeping other lamina area's at current levels	% of total canopy photosynthesis of the focal genotype	Xla_rel
Scenario 5	Lamina area of one mutant of a focal genotype, while keeping other lamina area's at current levels	Absolute photosynthetic gain of mutant of focal genotype	Xla_mut

higher than the total lamina area at which whole canopy photosynthetic rate is maximized, i.e. if the Xla of the latter is significantly smaller than 1.

In the second scenario, the total lamina area was changed for the focal genotypes only. Like in the first scenario, the optimal lamina was defined as the total lamina area where photosynthetic gain of the whole vegetation is maximized. This will analyze whether the current lamina area of each genotype is higher than would be optimal for whole canopy photosynthesis, i.e. whether all genotypes contribute to this "overinvestment' in lamina area at the canopy level. In this case the Xla where whole canopy photosynthesis is maximized (Xla_veg) should be lower than 1 for all genotypes.

In the third scenario, the optimal lamina area was defined as the total lamina area that maximizes the photosynthesis of the focal genotype. The Xla is changed for the focal genotype only while keeping the lamina area of the other genotypes constant. This tests whether the current lamina area of the focal genotype (i.e. Xla=1) differs from the lamina area that maximizes its absolute photosynthetic gain (represented by Xla_gen)

A change in lamina area will change the light conditions of all genotypes, and some genotypes may profit more than the focal genotype. To analyze this we ran the third scenario again, only this time the optimal lamina area was defined as total the lamina area that maximizes the relative photosynthetic gain of the focal genotype,

calculated as the highest % of total canopy photosynthesis the focal genotype could theoretically achieve by changing its lamina area (represented by Xla_rel). The Xla of the focal genotype is changed, while the lamina area of other genotypes was held constant by keeping their Xla at 1 (scenario 4).
In the fifth scenario, an evolutionarily game theoretical approach was used: a rare invader was introduced, with a frequency so low that it would not significantly affect the light climate (Schieving and Poorter 1999). The total amount of leaves within each layer for genotype i (NLij) was divided by 10.000. Then its traits were calculated using the average traits values of genotype i (see equation 4 and the paragraph above). This creates a genotype that is identical in all traits including the relative distribution of its leaves over the model layers and its Pmass, but that occurs with very low frequency: it will not shade itself, nor will it influence the light climate of other genotypes. Then the optimum lamina area of this mutant is defined as the total lamina area that maximizes its photosynthesis. This tests if the Xla values where its photosynthesis is maximized (Xla_mut) differs from 1. If this is true, the mutant will perform better than its “mother” genotype. One “mutant” of a focal genotype at a time was analyzed.

Statistics
To test if a change of the total lamina area of all genotypes simultaneously resulted in higher photosynthesis for the vegetation as a whole, we performed a t-test to check whether the Xla of the optimum canopy photosynthesis differed significantly from 1 (were Xla=1 represents the current lamina area). The eight plots thus function here as the replicas.
For the other 4 scenarios, the following two tests were performed. First, to test if the genotypes differed in their optimal Xla, a 2 way Anova was performed with the optimal Xla , defined differently for the four scenario’s, as dependent variable, plot as random factor and genotype as fixed. Then separate t-tests were performed to test if the Xla from individual genotypes differed significantly from 1.
It should be noted that genotype C did occur in most plots, but only with one or two leaves, making the estimation of true lamina distribution within a plot highly inaccurate. Similarly, genotype E occurred in only three plots, making the statistical power of the t-test very low. Removing these genotypes from the two way Anova analysis did not change the direction of the results. So while these values are given in the graphs, these genotypes are ignored in the interpretation of the data.

Results

Canopy structure and genotypic traits

Detailed information about the canopy structure and the way genotypes differ in traits with increasing vegetation depth can be found in chapters 5 and 6. Here only a brief description is given. LAI of the different plots ranged from 3.6 to 6.3 m^2 m^{-2}. Genotypes differed in their specific lamina area ($Sl_{am}A$), mass invested in lamina per gram total leaf mass ($L_{am}MR$) and their Narea values. However, they did not differ significantly in the way these values changed with increasing canopy depth. The most abundant genotype had relatively high $Sl_{am}A$ values, relatively low $L_{am}MR$ values and intermediate Narea. The genotypes differed in the relation between Pmax and Rd with Narea, with genotype I having a low Pmax and Rd at a given Narea. The photosynthesis per unit invested mass, Pmass, decreased with increasing canopy depth. It differed between genotypes and the genotypes also differed in the way Pmass changed with canopy depth. Genotype I had intermediate Pmass values at the top of the canopy, but higher Pmass values than other genotypes at the bottom.

Analyses of leaf frequency after five years of competition show that one genotype has become abundant (>40% of leaves, genotype I), while others have increased slightly (F, D) or remained around their initial 10% (H,J), had declined in frequency (C,E,G,) , or became rare (A, one occurrence out of 800 leaves) or extinct (B, see figure 1.1).

Scenario results

When decreasing the total lamina area of all genotypes simultaneously (scenario 1), the photosynthesis of the whole canopy increased, until total lamina area was on average 47% (±0.04) of the current total lamina area. The optimum lamina area which maximized canopy photosynthesis was significantly smaller than 1 (figure 7.1).

Similarly, when the total lamina area of each genotype was changed separately, without changing the current lamina area of the other genotypes (scenario 2), the current lamina area was significantly higher for all genotypes than the total lamina area at which canopy photosynthetic gain was maximized (Xla-veg, figure 7.2a). The genotypes did differ in their Xla_veg (table 7.2), indicating that the relative overinvestment of some genotypes, such as the dominant genotype I, was larger than that of other genotypes, such as genotype F, who's Xla_veg was closer to to 1.

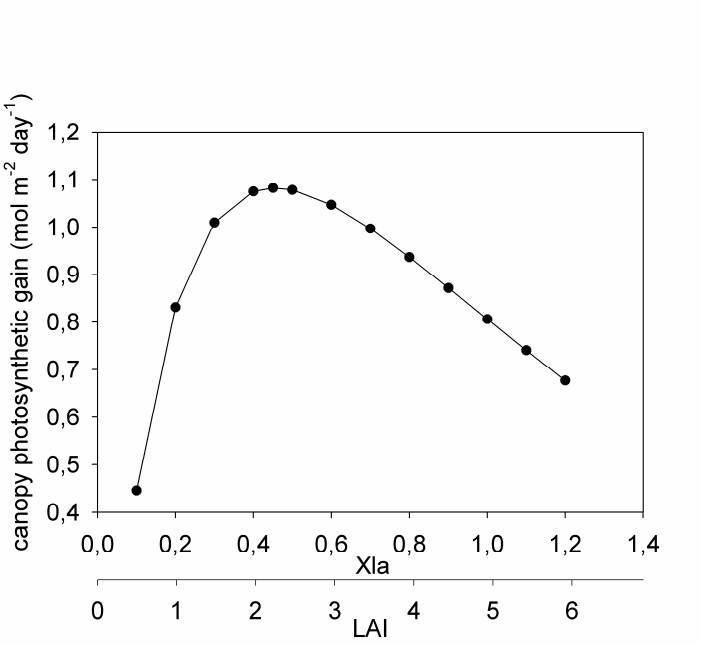


Figure 7.1. Whole canopy photosynthesis as a function of increasing total lamina area. Curve represents an average of all eight plots. Top x-axis indicates the parameter Xla, where the current total lamina area is given at Xla=1. Bottom x-axis shows the calculated lamina area index that follows from the Xla parameter. Xla at which photosynthetic gain of the whole canopy is maximized is significantly larger than 1 ($t=-37.738$, $P<0.001$).

When optimum lamina area was defined as the total lamina area at which a genotype reached its highest absolute photosynthetic gain with the lamina area of the other genotypes kept constant at current levels (Xla_gen, scenario 3), the genotypes differed in the Xla_gen (table 7.2), showing that some genotypes had current values closer to this optimum lamina area than others. The current lamina area of the most abundant genotype I was significantly higher than its optimum as defined above (figure 7.2b), indicating that a lower total lamina area would increase its absolute photosynthetic gain. Genotype J had a total lamina area that was marginally significantly lower than its calculated optimum, while genotypes D and G had marginally significantly higher total lamina area than their calculated optima.

However, the current lamina area of the most abundant genotype I did not differ significantly from its relative optimum lamina area (scenario 4; figure 7.2c). In other words genotype I could not significantly increase its share of the total carbon gain of all genotypes combined by changing its total lamina area. The same result was found for genotype J. Nevertheless, genotypic differences were found (table 7.2). The current lamina areas of genotypes D, G and H were significantly lower than their relative optima, showing that their percentage of whole canopy photosynthetic gain could be higher if they had a higher total lamina area.

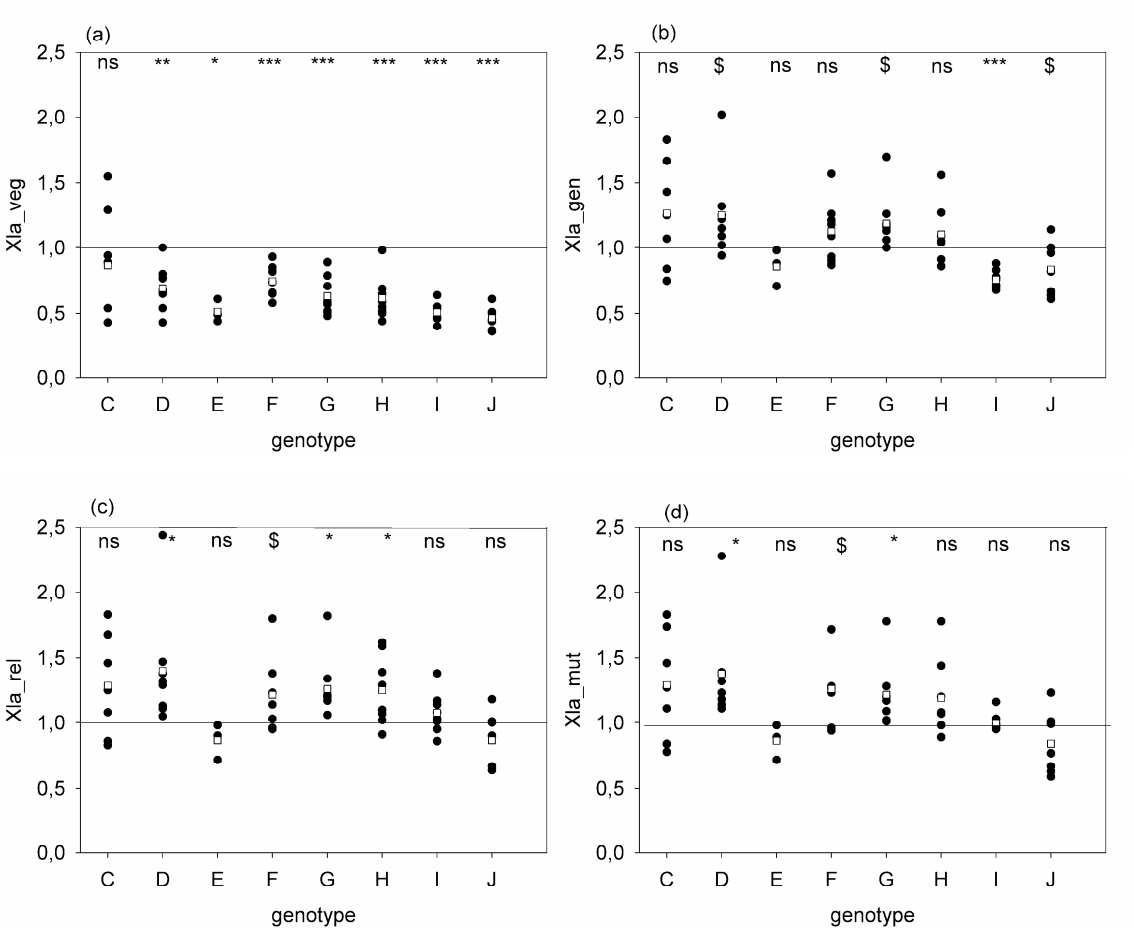


Figure 7.2. Xla of the focal genotype, or lamina area in relation to current lamina area (at y-axis) at which the photosynthesis is optimized, defined as a) maximized photosynthesis for the whole canopy, Xla_veg b) maximized photosynthesis for the focal genotype itself, Xla_gen c) maximized relative photosynthesis, i.e. the highest % of the total canopy photosynthesis the focal genotype can reach, Xla_rel and d) maximized for a small, mutant invader (Xla_inv). Black dots represent the different plots, the white square the average. Symbols above indicate that the average value is significantly different from 1 (t-test): ns: P>0.10; $: 0.05<P≤0.1 *: 0.01< P≤0.05, **: 0.001<P≤0.01, ***:P≤0.001. N.B. only the Xla of the focal genotype is changed, the lamina area of the rest is kept at current levels.

The optimum lamina area of the small rare mutant, with the traits of the abundant genotype I (scenario 5), was very close to the current lamina area of genotype I (figure 7.2d). The average optimal sensitivity parameter (Xla_mut) for this genotype was closer to 1 (0.998) than that of the other genotypes. Again, genotypic differences were found (table 7.2). The mutants of genotypes D, F and G did have significantly higher optimal lamina areas, showing that a mutant with a higher total lamina area would be more efficient than its “mother genotype”.

Table 7.2. Results of the two-way Anovas examining the effects of genotype and plot and on the parameter Xla that optimizes photosynthesis. Xla_veg is the Xla that optimizes photosynthesis of the whole stand; Xla_gen optimizes photosynthesis of the focal genotype; Xla_rel the % of whole stand photosynthesis and Xla_inv the photosynthesis of an invading mutant. In the table F-values and their significance are presented: ns, P>0.05; *, 0.05≥P>0.01.

	genotype	plot	error
d.f.	7	7	42
Xla_veg	3.124*	1.195^{ns}	
Xla_gen	3.985*	0.975^{ns}	
Xla_rel	2.594*	1.694^{ns}	
Xla_mut	2.731*	1.042^{ns}	

Discussion

In dense canopies the LAI is predicted to be higher than the LAI that maximizes the photosynthesis of the whole vegetation, because rather than optimizing canopy photosynthesis an individual is predicted to optimize its own photosynthetic rate (Anten 2002; Schieving and Poorter 1999; Finnoff and Tschirhart 2007). Our results show that such a tragedy of the commons indeed occurs in a canopy consisting of different genotypes of a stoloniferous plant. Simultaneously reducing the lamina area of all genotypes, thereby decreasing the total LAI of the stand, would considerably increase the photosynthesis of the whole canopy. Maximum photosynthesis for the stands was calculated to occur at a LAI that was on average 53% lower than current LAI, which is in line with several other studies that found that actual LAI was greater than the optimal values (Gutschick and Wiegel 1988; Werger and Hirose 1991; Schieving et al. 1992; Anten et al. 1995; Hirose et al. 1997).

Our results also show that all genotypes contributed to this overinvestment in lamina area. The current total lamina area was higher for all genotypes than the lamina area that would maximize the photosynthesis of the whole stand, keeping the lamina area of other genotypes constant (scenario 2). Also, the lamina area that would maximize the photosynthesis of the genotypes themselves (scenario 3) was always higher than the lamina area that maximized canopy photosynthesis (scenario 2). This clearly demonstrates that for all genotypes it is beneficial within the current stand to maximize their own photosynthesis rather than that of the canopy. This confirms the conclusion from other studies (Anten and Hirose 2001; Anten 2002) that plants maximizing canopy photosynthesis will be outcompeted by genotypes that maximize their own photosynthesis, therefore leading to a tragedy of the commons. While these previous studies assumed vegetation stands to consist of plants that are physiologically identical, which is an unrealistic

assumption, we show that a tragedy of the commons also arises in multi-genotypic stands.

Game theoretical models argue that photosynthetic gain should be maximized at the level of the individual (Anten and Hirose 2001; Anten 2005). The question then arises at what level the total lamina area should be maximized in clonal plants. In *Potentilla reptans*, the connection between ramets disintegrates within at the end of one growing season (Stuefer et al. 2002), and therefore after five years most established ramets will be fully independent (Eriksson and Jerling 1990). Selection may then work on the ramet level rather than at the whole genet level (Tuomi and Vuorisalo 1989; Pedersen and Tuomi 1995; Pan and Price 2001), and thus photosynthetic rate may be maximized at the ramet level rather than at the genet level. In agreement with this argument the current lamina area of the most abundant genotype was significantly higher than the total lamina area that would have maximized its carbon gain at the genet level.

Potentially, a genotype could benefit from a reduced total lamina area. Anten (2005) found, following Hikosaka and Hirose (1997), that a higher degree of self shading should lead to lower the optimal LAI. An abundant genotype will experience a high level of self shading within the genotype. Our results show that reducing the total lamina area of the most abundant genotype would result in higher absolute photosynthetic gain at the genotype level. Yet our data also showed that an overall reduction of the lamina area of the most abundant genotype increased the relative amount of carbon gain of the less abundant genotypes. It would thus be beneficial if a genet could shade neighbours but not itself, i.e. to have the ability to discriminate between self and non-self. Studies that have shown that self/non-self discrimination does occur in clonal plants below ground have indicated that connection between the ramets is crucial (Gruntman and Novoplansky 2004; Falik et al. 2003; Falik et al. 2006). Though this mechanism may hold for the present year's ramets which are still interconnected, most of the ramets in the plots are since long disconnected, and would not discriminate between self and non-self. The benefice is therefore expected to be found at the ramet level and not at the genet level.

Spatial dispersion of ramets may influence the total lamina area at which photosynthesis is maximized. Clonal plants can clump or disperse their newly formed ramets (guerrilla versus phalanx growth form, sensu Doust (1981). Based on the higher chance of self-competition within clones, Semchenko et al. (2007) predicted that plants with a phalanx growth form should respond less strongly to

neighbours. In addition, the more clumped growth form may also decrease the level of mixing of the genotypes. In that case selection may favour genotypes with a less competitive strategy (Xiao et al. 2007). Consequently, species that have a phalanx growth form are predicted to have lower optimal total lamina area than guerrilla species.

As *P.reptans* has a guerrilla growth form with long internodes, the ramets of the different genotypes can be assumed to be well mixed after five years of growth. This is conform the assumptions made in most evolutionarily game theoretical analyzes (Nowak and Sigmund 2004). The current lamina area of the most abundant genotype is close to the lamina area that would maximize its share of the total carbon gain of all genotypes combined. This may explain the success of this genotype.

While the scenarios discussed above are more in the line of a sensitivity analyzes of the current total lamina area, evolutionary game theory analyses whether the resident population has a fitness advantage over rare invading mutants strategies that do not notably affect the environmental conditions in the communities (Maynard Smith and Price 1973; Dieckmann and Metz 2006). When introducing a rare mutant of genotype I, its average optimal lamina area was almost identical to the current lamina area. Both the sensitivity analyzes and this latter approach thus suggest that genotype I is relatively stable in the sense that a mutation of this genotype causing it to make more or less lamina area will not lead to an increase in photosynthesis of this genotype, and consequently not to a change in frequency.

In contrast to the most abundant genotype, mutations in the lamina area of other genotypes can result in higher performance of these genotypes. For several genotypes optimum lamina areas of rare mutants were higher than the current lamina area, including genotypes that have increased in abundance after 5 years. A mutant of these genotypes could thus increase in frequency, at least relative to its “parent” genotype, thereby changing the interactions within the vegetation and thus possibly the abundance of the currently dominant genotype. The vegetation as a whole is thus not stable against new strategies.

Our model is based on several assumptions. For instance, we implicitly assume that the abundances of the different genotypes are near equilibrium, a state that is considered to be appropriate for analyzing newly invading genotypes (Eshel and Feldman 1984; Hammerstein and Selten 1994; Eshel et al. 1998). This seems quite reasonable after five years of competition. Furthermore it should be noted that it assumes constant high light conditions while in reality these will change over

the season and over different growth seasons. This makes it tricky to interpret the results in terms of future development. However, lowering light levels does not alter the general trend that the current lamina area of most genotypes is more different from the total lamina area that maximizes their photosynthetic gain than that of the most dominant genotype. Rather, lower light levels are beneficial for the most abundant genotype as it is rather shade tolerant (chapter 6), and its relative share of the total canopy photosynthetic gain would increase with a decrease in light availability.

One of the most interesting aspects of this study is that by applying a game theoretical approach to a rather simple canopy model, new insights can be gained about the processes within an existing plant population. It demonstrates that game theory is a useful tool to analyze plant traits in a competitive setting. Our analyzes also support the conclusions of Pedersen and Tuomi (1995) that while analyzing consequences of changes in clonal plant traits it is important to know the level at which selection operates.

Chapter 8

General discussion and summary

The central idea of this thesis was to link plastic responses of individual genotypes to their performance in competition, where the outcome depends on the plasticity and performance of all other genotypes. Therefore, several experiments were set up to study the plastic responses of the genotypes separately to different light conditions (Chapters 2, 3 and 4), while through canopy modeling the effects of genotypic differences in morphological traits and physiological traits were assessed by calculating the light interception and photosynthetic rates in a five year old competition experiment (Chapters 5 and 6). Finally, a game theoretical approach was used in combination with the canopy model, to study the effects of total lamina area on genotypic performance (Chapter 7). By synthesizing these data in this final Chapter, some general patterns on the mechanisms of competitive exclusion arise.

Height growth investment and coexistence

In dense herbaceous stands, light availability decreases with increasing depth in the canopy (Monsi and Saeki 1953). Taller plants are thought to have an advantage in such canopy, because they have leaves that are placed in a high light environment, while they also directly shade shorter plants (Ford 1975; Schmitt and Wulff 1993; Anten and Werger 1996). Taller plants are found to have higher relative growth rates than smaller plants (Weiner and Thomas 1986; Weiner 1990; Hara 1992) and taller plants are found to produce 200 to 800 times more seeds (Nagashima 1995). Therefore, smaller genotypes may be excluded from the population.

Plasticity in height growth is often assumed to reduce this competitive exclusion. A plastic increase in height growth in response to neighbours can reduce the differences between individual plants in height, therefore reducing the difference in growth rate (Casal and Smith 1989; Ballaré et al. 1990; Schwinning and Weiner 1998; Stoll et al. 2002). A reduced ability to plastically respond to neighbours may cause shading by neighbouring plants, leading to growth suppression (Ballaré et al. 1994; Ballaré et al. 1997; Pierik et al. 2004). It has therefore been hypothesized that “small-scale diversity is positively related to mean shoot morphological plasticity of locally coexisting species” (Lepik et al. 2005).

This implies that species competing in dense stands should always show a response that increases height growth. However, coexistence may also occur if some species show a less strong increase in height investment. Increased height growth requires an increase in investment in the vertical spacers (Huber et al. 1998; Leeflang 1999), and whether or not a plastic increase in height is adaptive depends on the balance between these costs and the potential higher light capture (Anten et al. 1995), or the potential higher ability to shade neighbours (Falster and Westoby 2003). If these costs outweigh the benefits, the response can be considered mall adaptive. In multi-species stands species with low investment in height had similar or even higher light capture per unit mass (Φmass) than the tallest species (Hirose and Werger 1995; Anten and Hirose 1999; Werger et al. 2002). This indicates that, if these species have shade tolerant photosynthetic characteristics, they can persist in the lower layers of the canopy. Pronk et al. (2007) showed that in order for this coexistence to occur, the difference in height investment between species should be rather large. In other words, some species should thus respond less plastically to neighbours.

Within species, however, the differences in plastic response may not be large enough to allow such coexistence to occur between different genotypes (Anten 2005). Limited differences in plasticity will result in small differences in height investment, and thus different genotypes may not be able to specialize on different positions within the canopy. In agreement with this argument, all remaining genotypes in the competition experiment had similar light capture per unit mass at a given depth in the canopy. In addition, all remaining genotypes had at least one lamina that was placed in the top layer of the canopy (Chapter 5). The other experiments also demonstrate that even the genotypes that may seem “small”, such as genotypes B and C, were highly plastic. In Chapter 4, all genotypes were grown in mono-genotypic stands at two different densities. The differences between the genotypes in response to increased density between the mono-genotypic stands were quite large, resulting in large differences in height in the high density stands, with genotypes B and C forming stands of low height. But when these two genotypes were placed in an artificial setting in which the light gradient strongly increased in height, mimicking fast height growth of surrounding competitors (Chapter 3), these smaller genotypes strongly increased their height. In that sense all genotypes are typical shade avoiders, responding strongly to shading. Therefore the coexistence between different genotypes which seems to have occurred is not the result of different genotypes occupying different positions in the canopy.

Height growth plasticity

Placing laminas at the top of the canopy thus seems equally important for all genotypes. Similar to what is found in other mono-specific stands (Anten and Hirose 1998; Hikosaka et al. 1999) the leaves which had the laminas placed at the top of the canopy had higher light capture per unit mass than lower placed laminas (Chapter 5), and the photosynthetic rate per unit mass was also higher in top layers than in bottom layers (Chapter 6). This was true for all genotypes. Therefore, if differences in plasticity lead to differences between genotypes in leaf positioning, it may explain the decline of some of the genotypes. This is supported by the correlation between rank order in the highest petiole in the fixed light gradient treatments, and the rank order in abundance of the genotypes in the competition experiment (Chapter 2).

In Chapter 4 it was argued that genotypic differences in leaf positioning could be the result of a theoretically maximum petiole length. However, all remaining

genotypes were able to place laminas in the top layer in the competition experiment. Also, in the experiment where neighbour growth was mimicked using cylinders of light filters that increased in height (Chapter 3), even in the fast increasing cylinders did the genotypes continue to place new leaves above older ones at the time when the experiment was harvested. It is likely that had the increase of the height treatment stopped, all genotypes would have reached the top. Therefore it is unlikely that some genotypes had declined in frequency in the competition experiment because they intrinsically could not reach the top layer.

Rather, the time it takes for a genotype to reach the top of the canopy, and the subsequent ability to keep up with it, could play an important role. It is generally accepted that initial size differences between plants will result in the overtopping of smaller plants, causing slower growth of these smaller plants (Ford 1975; Weiner 1985). At high densities, this may lead to high mortality among these subordinate individuals (Weiner and Solbrig 1984; Weiner and Thomas 1986; Weiner et al. 2001). The rank order of plants becomes fixed shortly after canopy closure, and thus the fate of a plant in crowed vegetation can be determined relatively early in the growing season (Nagashima 1999). Likewise, a reduced ability to quickly place leaves at the top may lead to competitive exclusion.

Chapter 3 clearly shows that there are differences between genotypes in this ability. Interestingly, it also demonstrates that the speed at which the surrounding vegetation increases in height, combined with differences that are created during height growth in the length of the light gradient, influences this ability. In the experiment were competitors surrounding a focal plant were mimicked using cylinders made of light filters (Chapter 3), all five genotypes that were used could place their laminas at the top when the cylinders increased in height slowly, but in the fast treatment clear differences in leaf positioning were found. This suggests that the fate of a genotype can also be determined by the identity of the surrounding genotypes. The mono-genotypic stands in Chapter 4 differed not only in final height, but also in height growth rate (figure 8.1). In stands dominated by genotypes with a high response to increased neighbour density, less genotypes will be able to reach high light conditions, and thus the competitive exclusion will be much faster than in slow growing stands of other genotypes.

The experiment with the light gradients increasing in height with time also demonstrated that the rank order in leaf positioning can change with increasing height growth rate and the subsequent longer length of the light gradient. Interestingly, it was a genotype with long petioles in the both the slow and medium

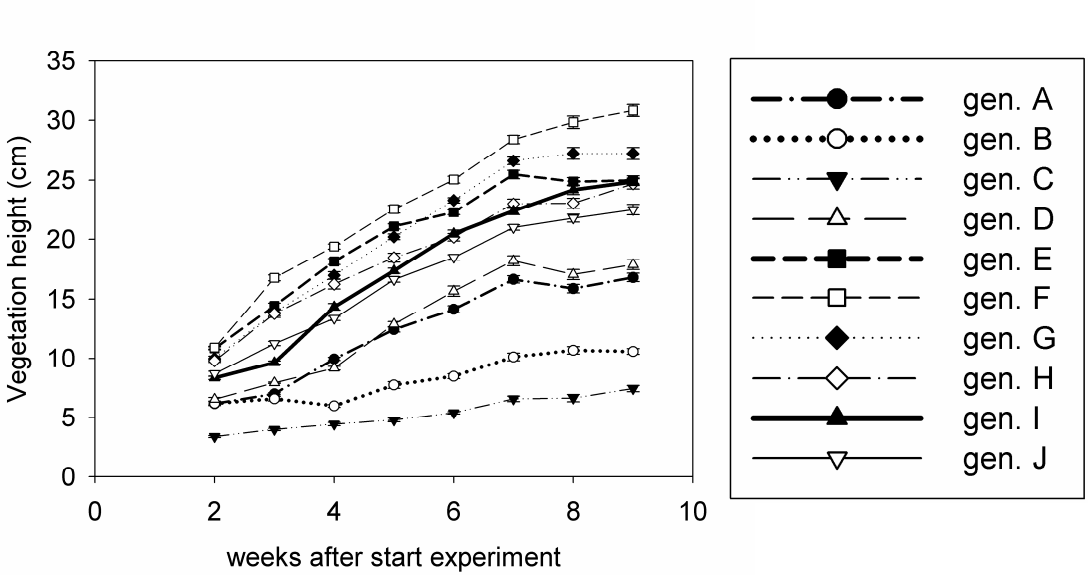


Figure 8.1. Development of height (cm) in mono-genotypic stands with high density. Height is defined as the height of the highest lamina above the soil surface of the target plant. For description of experimental set up see Chapter 4.

treatment that strongly decreased in rank order of both tallest petiole length and in total biomass in the fast treatment (Genotype F). The distribution of laminas of this genotype in this treatment somewhat resembles the distribution of laminas in the competition experiment: relatively few laminas at the top of the canopy. This may suggest that this lower abundance in the top layer may have been caused by a reduced ability to place newly formed leaves at the top because of the fast growth rate of the surrounding plants.

As hypothesized in Chapter 3, this may have been caused by the physiological traits of this genotype. Several genotypes (i.e. B, C and F) are shown to have a high maximum photosynthetic rate, but also higher dark respiration for a given Nitrogen content per unit area (Narea). In the competition experiment, this resulted in negative carbon gain in the lowest layers of the canopy (Chapter 6). This may indicate they are rather shade intolerant. Initially, new laminas can be placed in high light conditions, where a high maximum photosynthetic rate is beneficial (Küppers 1985; Hirose and Werger 1995; Anten and Hirose 1999). But once the petiole stops elongating, the lamina's position will be fixed (Chapter 3). Light availability then depends on the growth rate of the surrounding vegetation: the faster this will be, the faster the light availability of this lamina will decline (see also figure 3.2). Because of the high dark respiration, this will quickly result in zero or even negative carbon gain. The life-time carbon gain of the leaf may therefore be low, and there will not be enough carbon available to place new laminas at the top.

As a consequence, its light capture per unit area will decrease. This may either extend the time it takes for the genotype to catch up with the top of the canopy, or may result in fast exclusion. Height growth plasticity may thus be affected by the physiological traits of the genotype, in combination with the growth rate of the surrounding vegetation.

Several authors have shown that plants are more plastic in their height range than in their proportional allocation to leaf area (Leeflang et al. 1998; Huber and Wiggerman 1997; Werger et al. 2002). Similarly in our studies, differences between genotypes were larger in height growth plasticity than in the lamina area ratio, which was in fact very similar for leaves for the laminas placed at the same height in the competition experiment (Chapter 5). However, differences in overall light capture per unit mass ($_p\Phi_{mass}$) and photosynthetic rate per unit mass (Pmass), which were mainly caused by the difference in relative number of laminas a genotype had placed at the top of the canopy, could not be readily related to the abundances or coexistence of the genotypes. The most abundant genotype had average $_p\Phi_{mass}$ and Pmass values compared to other genotypes. Some genotypes that had declined had relatively more laminas placed at the top of the canopy, and thus higher Pmass and $_p\Phi_{mass}$ values. Clearly, while the ability to reach the top is important for achieving high photosynthetic rates, the outcome of competition can not be explained by the differences in biomass allocation and height growth plasticity alone.

Photosynthetic traits and optimization

The ten genotypes of *Potentialla reptans* differed in the maximum rate of photosynthesis (Pm) at a given Narea (Chapter 6). Surprisingly, the most abundant genotype I had a low Pm. Additionally, it had a low dark respiration rate at a given Narea. This suggests that rather shade tolerant characteristics may have been favoured in the competition experiment. Consequently, it had higher carbon gain than others at low light levels, and most other genotypes had negative carbon gains or were absent in the lower layers of the canopy. The results from the canopy model thus seem to support a hypothesis put forward by the resource ratio theory: that the plant that can survive the lowest light levels will be the best competitor (Tilman 1988, Huisman 1994, Dybzinksi and Tilman 2007).

No direct measurements were made on leaf turnover, but it may the mechanism that has lead to the dominance of genotype I. Leaves should be shed in order to increase total plant photosynthetic gains, either because N reallocation to the top

leaves is more efficient in terms of carbon gain or because the lower leaves do not have a positive carbon gain (Hikosaka 2003; Oikawa et al. 2006; Boonman et al. 2006). As most genotypes had a higher dark respiration than genotype I (Chapter 6), leaf turnover may be higher for these genotypes as they sooner reach the point where shedding of leaves becomes beneficial than the most abundant genotype. As a result, these genotypes will have relatively more leaves at the top of the canopy. These leaves are more efficient per unit mass in both light capture and photosynthetic rate. Therefore, shedding lower leaves will result in high overall $_{p}\Phi_{mass}$ and Pmass values, which in turn may explain why the most abundant genotype does not have the highest values. Thus the relative higher amount of laminas that some declining genotypes had in the top of the canopy may not be the result of a higher plasticity, but simply of a higher lamina turnover at the bottom layers.

Game theory implies that even if the light capture per unit mass is not high, a high total light capture may still be beneficial for an individual if it negatively affects the performance of other plants (Falster and Westoby 2003). As lower light levels will lead to a high leaf turnover of most other genotypes, high total light capture will benefit the most abundant genotype I. It had a total current lamina area that was larger than the total lamina area that would maximize its photosynthetic rate at the genet level (Chapter 7). While this higher lamina area lowers its own photosynthetic rate, it lowers the photosynthetic rate of others more because of its effect on the light level inside the canopy. In agreement with this argument its current total lamina area was close to the total lamina area that would maximize its share of carbon gain of the whole vegetation.

Arntz and Delph (2001) argued that selection for physiological traits may work indirectly via correlation with other traits. Because different genotypes have different Pmax and Rd traits, maximization of photosynthetic rates may require different total lamina areas for the different genotypes within the canopy. All genotypes did have total current lamina areas that were higher than the total lamina area that would have maximized whole canopy photosynthetic rate. All genotypes thus contributed to the overinvestment in lamina area, i.e. in the tragedy of the commons (Hardin 1968). This suggests that for all genotypes the total lamina area can not be explained by simple optimization of photosynthesis at the whole canopy level.

However, as was shown in Chapter 7, not all genotypes had lamina areas that maximized their photosynthetic rate. As a result, the vegetation is not stable which

means it can be invaded by mutants of less abundant genotypes. The invasion of such a new genotype will start the process of competitive exclusion again, until a new, possibly different equilibrium between the genotype frequencies is reached (Eshel and Feldman 1984; Hammerstein and Selten 1994; Eshel et al. 1998).
While the above discussion focuses on the success of the most abundant genotype, several other genotypes have managed to maintain themselves in the stands after five years. Other traits besides the shade tolerant traits of the most abundant genotype may contribute to this coexistence. As argued in Chapter 6, there seems to be a trade-off between relatively high photosynthetic gains either at the top of the canopy or at the bottom of the vegetation. This could allow less abundant genotypes to achieve a high life-time carbon gain, despite the hypothesized shorter leaf life spans. Also, light availability fluctuates during the season, which may allow coexistence of different species that can reach high carbon gain at different times in the season (Anten and Hirose 1999). Similarly, genotypes with high maximum photosynthetic rate may have a higher carbon gain early in the season when the competition for light is not so severe. It may also pay off to have many small leaves when competition is low, while having few tall leaves might be more beneficial when the canopy is dense (Chapter 5). Finally, differences in root storage may affect the ramet growth rate and ramet survival (Suzuki and Stuefer 1999). There are some indications that the ten genotypes differ in the amount of lager roots they make, and in the seasonal allocation of resources to these parts (Elena Caballero Jiménez, unpublished data), but no effect of genotypic differences in root storage on plant performance has been shown yet.

Clonal plants

Clonal plants are organized at many levels of integration. There has been a large debate at which level selection will operate (Tuomi and Vuorisalo 1989; Eriksson and Jerling 1990; Pan and Price 2001) and this thesis shows that the level of organization within plants and populations affects the way traits should be maximized. De Kroon et al. (2005) even argued that phenotypic plasticity is expressed at the sub-individual level, such as in individual leaves and branches, and that the whole plant reaction is a by-product of the responses of these parts and the way they interact. In this thesis, the responses of the genotypes were scaled from the individual leaf level up until population level, and thus it seems desirable to briefly discuss some aspects of clonal growth in this light.

The light capture of an individual leaf is determined by its position and its leaf area. However, where much information exists about the effects of light quantity and quality on petiole elongation (Huber 1995; 1999; Leeflang 1999) little is known about how leaf area is controlled (Solangaarachchi and Harper 1987; Heraut-Bron et al. 1999). Heraut-Bron et al. (2001) found that the lamina area of *Trifolium repens* was unaffected by low R:Fr ratio at the buds, which may indicate that laminas adapt to the local light quantity they experience when height growth stops. But for the erect growing species *Chenopodium album* it has been shown that the light climate of mature leaves plays an important role in the leaf anatomy of newly formed leaves (Yano and Terashima 2001). It is not yet known if and how cues received by other plant parts, such as older leaves, may affect the lamina area in stoloniferous plants.

Petiole lengths of individual leaves seemed strongly regulated at the individual leaf level. Several studies with stoloniferous plants have shown that petiole elongation stopped once plants reach the top of a light gradient (Price et al. 1996; Lötscher and Nösberger 1997; Leeflang et al. 1998). Thompson (1995) demostrated that *Trifolium repens* can detect changing light quality with the tip of the lamina, without it changing the stolon length or stolon branching, showing that the response is mostly influenced by local cues. Petioles of *Potentilla reptans* elongate over the whole length, but elongation is strongest directly near the top of the lamina (Petri, 2005). This suggests that a meristem may be located near the position where the change in light quality can be detected. Also, petioles of all genotypes stopped elongating shortly after they outgrew the top of the cylinders in the experiment of Chapter 3. It is therefore unlikely that possible cue interception by older, already shaded laminas directly affects the final length of newly formed petioles.

Petiole elongation may, however, be limited by the available biomass. In Chapter 3 all genotypes could not place the laminas of the firstly formed leaves at the top of the light gradient. This may have been caused by the small size of the ramets at that time. At larger ramet size resource allocation may then supply the young petioles with the biomass needed to place the laminas at the top. In Chapter 4, an increase in investment in the mother ramet was correlated to a decrease in vegetative reproduction. This suggests that an increase in petiole investment of the mother ramet went at the expense of investment in newly formed ramets (Huber and Wiggerman 1997; Weijschede et al. 2007). This in turn suggests that assimilates were allocated to the apices rather than to the axillary buds (Robin et al. 1992; Thompson 1995; Heraut-Bron et al. 2001). As outgrowth of axillary buds

or tillers can be delayed or suppressed under low radiation and R:Fr ratios (Lötscher and Nösberger 1997; Evers et al. 2007), the overall response of the ramet can be influenced by the sensing of different signals from different plant parts.

Interestingly, no strong decrease in total biomass was found for genotypes that showed a higher investment in the petioles upon crowding in the mono-genotypic stands (Chapter 4). Similar results have been found for *Trifolium repens* (Huber and Wiggerman 1997). This suggests that a stronger decrease in the ability to explore space does not result in reduced performance in dense vegetation, and that the increased petiole elongation allows a genet to keep up its growth rate.

Physiological integration will be important in shaping traits such as the total lamina area. Correlated growth responses may prevent intra-clonal shoot competition (De Kroon and Schieving 1990). Studies that have suggested that such prevention of competition with self occurs below ground have found that the physical connection is crucial (Gruntman and Novoplansky 2004; Falik et al. 2006). Such belowground detection can also occur in *Potentilla reptans* (Joost Keuskamp, Msc. thesis, Vermeulen, unpublished data). However, it has not been shown that, following Gersani's model (2001), ramets can sense the presence of multiple neighbours (Benitez Lopez, 2005). Also, aboveground discrimination between self and non-self has not yet been demonstrated (Petri, 2005). As the connection between ramets of *Potentilla reptans* disintegrates at the end of the growing season, most established ramets will be fully independent after 5 years, and therefore it is unlikely that competition between self is minimized through a self/non-self detection mechanism.

However, Semchenko et al. (2007) argued that growth form may still select for less competitive traits, if new ramets are placed close by the mother ramets, i.e. if species have a phalanx growth form. This would imply that when neighbour density is high, these species should have less investment in height growth, and a lower total lamina area than guerrilla species. Thus the total lamina area of a genotype with phalanx growth form would be closer to the total lamina area that maximizes photosynthetic rate at the lamina level (Chapter 7).

Potentilla reptans has a guerrilla growth form, at least compared to the initial distance of the ramet at the start of the competition experiment (Chapter 7). The fact that the current total lamina area of genotype I was higher than the total lamina area that would maximize photosynthetic rate at the genet level could thus be caused by the fact that photosynthetic rate is maximized at the ramet level

(Pedersen and Tuomi 1995). This will result in a lower photosynthetic rate at the genet level in a similar way as maximization at the genotype level leads to lower photosynthetic rate at the whole canopy level. The total lamina area of the smallest unit that was analyzed in Chapter 7, the rare mutant of genotype I, was almost identical to the total lamina area of its mother genotype, which may confirm this view. However, this mutant is so small it does not shade others, and the competitive game between this mutant and the other ramets is still infinitesimally small. Possibly, an optimal plastic response may not be the response that results in the maximization of the absolute photosynthetic rate of an individual ramet, but a response that maximizes the relative share of carbon gain. As this would reduce the growth rate of an individual plant, analyzes that test if a trait or a plastic response in this trait is adaptive based on individual responses, such as for instance the use of selection gradients, may not predict the adequate level of response that would lead to a high performance when plants are directly competing.

Conclusions

This thesis illustrates that differences between genotypes in plastic responses, and therefore differences in performance, are influenced by the growth of the surrounding vegetation. Therefore, the success of a genotype not only depends on its own response, but also on the genotypes that make up the canopy. Furthermore, the results suggest that in vegetations where light levels at a certain height strongly change during the growing season, rather shade tolerant characteristics may be selected for, in addition to the ability to reach the top of the canopy. The understanding of the process of competitive exclusion, and thus also the understanding of mechanisms behind coexistence, would greatly benefit if both morphological plastic responses and physiological traits are studied, in combination with the temporal changes that occur during the growing season.

Figure 8.2. Markings on petiole, showing the higher elongation of the part closest to the lamina.

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Samenvatting in het Nederlands

Concurrentie om licht is een belangrijke factor, die mede bepaalt welke planten zich in een dichte vegetatie kunnen handhaven. Doordat licht vanuit één richting komt, is de verticale positionering van de bladeren bepalend voor de hoeveelheid licht die een plant kan onderscheppen. Bladeren bovenin de vegetatie staan in een omgeving met een hoge lichtintensiteit en onderscheppen een deel van het licht, waardoor er in de vegetatie een gradiënt van hoge lichtbeschikbaarheid aan de top van de vegetatie naar lage beschikbaarheid onderin ontstaat. De balans tussen de investering, die nodig is voor hoogtegroei, en de resulterende beschikbaarheid van licht bepaalt, in combinatie met de omzetting van het onderschepte licht naar biomassa, of de plant binnen de vegetatie een positieve groei kan halen of niet.

De meeste plantensoorten kunnen de verdeling van biomassa over de verschillende delen actief aan verschillende omstandigheden aanpassen. Deze mogelijkheid tot het vertonen van meerdere fenotypen door één genotype wordt plasticiteit genoemd en kan een grote rol spelen in de ontwikkeling van een dichte vegetatie. Zo kunnen planten de aanwezigheid van buurplanten detecteren en daarop reageren door onder andere langer te worden. Dit zou er toe kunnen leiden, dat ze hun bladeren in een omgeving met een hoger lichtniveau plaatsen, of dat ze beschaduwing door die andere planten voorkomen. Binnen één soort verschillen genotypen echter in hoogtegroei en de plasticiteit hierin. Hierdoor kunnen er tussen planten verschillen ontstaan in hun verticale positie in de vegetatie en ontstaan er dus verschillen in lichtonderschepping. Dit zou tot het verdwijnen van sommige genotypen uit de populatie kunnen leiden.

In dit proefschrift wordt van een langlopende concurrentie proef tussen 10 genotypen van *Potentilla reptans* (Vijfvingerkruid), een kruipende clonale plant, gebruikt gemaakt. Genetische analyse liet zien, dat na 5 jaar sommige genotypen in frequentie waren afgenomen, of zelfs niet meer aanwezig waren. Er was één genotype dominant geworden, terwijl zich ook andere genotypen hadden weten te handhaven (zie figuur 1.1). Het doel van dit proefschrift is om te begrijpen hoe de genotypische verschillen in reactie op buurplanten en de daaraan gekoppelde concurrentie om licht tot deze uitkomst kunnen leiden, om daardoor inzicht in de eigenschappen van de daaruit voortvloeiende vegetatie te krijgen.

De data uit hoofdstukken 2, 3 en 4 in dit proefschrift tonen aan, dat de genotypen in veel kenmerken verschillen, en de meeste proeven in die hoofdstukken laten zien dat ze ook verschillen in de mate waarin die kenmerken in reactie op buurplanten worden aangepast. Toch reageren de genotypen over het algemeen gezien hetzelfde op signalen dat er buurplanten zijn. Hoofdstuk 3 laat zien, dat zelfs de genotypen die een lage vegetatie vormen wanneer ze door buurplanten met hetzelfde genotype worden omringd (hoofdstuk 4), sterk in hoogte toenemen als de lichtgradiënt tijdens het groeiseizoen sterk in hoogte toeneemt. Ook bleek uit het concurrentie-experiment dat alle genotypen die na 5 jaar nog aanwezig zijn de toplaag van de vegetatie konden bereiken (figuur 1.1; zie ook hoofdstuk 5). Alle genotypen reageren dus met een sterke toename in hoogte als de hoogtegroei van de buren hoog is. De oorzaak voor coëxistentie van deze genotypen is dus niet dat sommige genotypen door weinig in hoogtegroei te investeren laag in de vegetatie blijven en daar eenzelfde hoeveelheid licht per gram geïnvesteerde biomassa kunnen onderscheppen als genotypen die hun bladeren bovenin de vegetatie plaatsen, zoals bij vegetaties bestaande uit verschillende soorten wel het geval is.
Als gevolg hiervan is het plaatsen van de bladeren bovenin de vegetatie van groot belang. Bladeren die in het concurrentie-experiment bovenin waren geplaatst vingen meer licht per eenheid geïnvesteerde biomassa dan bladeren die lager waren geplaatst (hoofdstuk 5). Ook de fotosynthese per eenheid geïnvesteerde biomassa was hoger in de toplagen (hoofdstuk 6). Verschillen tussen genotypen in hun vermogen om bladeren aan de top te kunnen plaatsen kunnen dus tot verschillen in lichtonderschepping en fotosynthesesnelheid leiden, en dus tot verschillen in groeisnelheid.
Hoofdstuk 3 laat zien, dat genotypen verschillen in hun vermogen om de top van een lichtgradiënt, die tijdens de groei in de lengte toeneemt, bij te houden. Nog belangrijker is het, dat dit aantoont dat het van de snelheid en daarmee van de grotere lengte van de lichtgradiënt afhangt, welke genotypen hun bladeren aan de top kunnen plaatsen. De snelheid waarmee een vegetatie in de hoogte groeit, hangt weer af van de genotypen waaruit de vegetatie is samengesteld. De monogenotypische vegetaties in hoofdstuk 4 verschilden sterk in hoogtegroei (zie ook hoofdstuk 8), al leidde de sterkere toename van hoogtegroei in reactie op een hogere dichtheid van sommige genotypen niet tot een sterkere afname in totale biomassa. Dit suggereert, dat het afhangt van de frequentie van de genotypen in de vegetatie, welke genotypen zich zullen handhaven.

Omdat bladeren die bovenin de vegetatie zijn geplaatst meer efficiënt in lichtonderschepping zijn, en een hogere fotosynthesesnelheid per eenheid geïnvesteerde biomassa hebben, zou men verwachten dat, in het concurrentie-experiment het dominante genotype relatief gezien meer bladeren boven in de vegetatie zou hebben gehad. Na 5 jaar was het meest dominante genotype echter niet het genotype dat het meeste licht per eenheid geïnvesteerde biomassa onderschepte (hoofdstuk 5), noch had het de hoogste fotosynthesesnelheid per eenheid geïnvesteerde biomassa (hoofdstuk 6). Een mogelijke verklaring hiervoor is dat de uitkomsten van het fotosynthesemodel in hoofdstuk 6 laten zien, dat het dominante genotype relatief gezien schaduwtolerante fotosynthese-eigenschappen heeft. Die zorgen ervoor, dat het een positieve fotosynthesebalans in de diepste delen van de vegetatie kan behalen, waar andere genotypen niet meer aanwezig zijn of een negatieve fotosynthesebalans hebben. Dat zou het relatief grote aantal bladeren in de diepere lagen, en dus de relatief lage lichtonderschepping en fotosynthesesnelheid per eenheid geïnvesteerde biomassa, van dit genotype kunnen verklaren. Ook suggereert dit dat een lager bladverlies ervoor zorgt dat het genotype dominant wordt. Coëxistentie zou dan kunnen optreden, omdat andere genotypen een hogere fotosynthesesnelheid bovenin de vegetatie halen, hetgeen het hogere bladverlies zou kunnen compenseren.

Naast hoogte is de hoeveelheid bladoppervlak van een genotype van belang voor de lichtonderschepping en de fotosynthesesnelheid. Nadere analyse van het lichtmodel in hoofdstuk 7 laat zien, dat het totale bladoppervlak van de gehele vegetatie in het concurrentie-experiment groter was dan het totale bladoppervlak dat de fotosynthese van de hele vegetatie zou maximaliseren. Dit blijkt uit het feit, dat een individueel genotype een hogere fotosynthesesnelheid kan bereiken door een hoger totaal bladoppervlak aan te maken, ook al gaat dit ten koste van de fotosynthesesnelheid van de vegetatie als geheel.

Opvallend is dat het totale bladoppervlak van het meest dominante genotype groter was dan het totale bladoppervlak dat de fotosynthesesnelheid van het eigen genotype zou maximaliseren. Het verschilde daarentegen niet significant van het bladoppervlak dat het relatieve aandeel van het genotype aan de totale fotosynthesesnelheid van de vegetatie zou maximaliseren. Dus een verlaging van het bladoppervlak zou weliswaar zijn eigen totale fotosynthesesnelheid verhogen, maar die van de andere genotypen nog meer. Dit suggereert dat dit genotype dominant is geworden, omdat het door zijn relatief schaduwtolerante fotosynthese-

eigenschappen minder last heeft van de voor zijn eigen totale fotosynthesesnelheid te hoge bladoppervlak dan de andere genotypen.
Ook laat hoofdstuk 7 zien dat, alhoewel een mutatie in het bladoppervlak van het dominante genotype niet tot een hogere fotosynthesesnelheid van deze mutant zal leiden, de vegetatie van het concurrentie-experiment zelf niet stabiel is, aangezien bepaalde mutanten van sommige andere genotypen wel een hogere fotosynthesesnelheid zullen hebben dan het genotype waar ze van af stammen. Een mutant van één van de minder abundante genotypen zou de vegetatie dus binnen kunnen dringen, hetgeen een verandering in de concurrentieverhoudingen tussen de huidige genotypen zou kunnen betekenen.
De conclusies van dit proefschrift zijn in het kort dat verschillen tussen genotypen in plasticiteit en dus in biomassaproductie door the groei van de omringende vegetatie worden beïnvloed. Het succes van een genotype hangt dus niet alleen van zijn eigen eigenschappen af, maar ook van de andere genotypen die de vegetatie vormen. De resultaten suggereren ook dat, in vegetaties waarin de lichtbeschikbaarheid onderin tijdens het groeiseizoen snel afneemt, het belangrijk is om de bladeren bovenin te plaatsen, in combinatie met het hebben van relatief schaduwtolerante fotosynthese eigenschappen. Het laat zien dat een speltheoretische benadering in de analyse van bestaande vegetaties inzicht in de concurrentie om licht kan geven.

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Curriculum Vitae

Peter Johannes Vermeulen was born on September 8th, 1977 in Bleijswijk, The Netherlands. From 1989 until 1995 he attended secondary school at the Kruisheren Kollege in Uden. He started his study in Biology at Wageningen University in 1995. He specialized in plant ecology, through a dissertation on the effects of nutrients on different peat bog species, and a dissertation on plant species richness in the Alaskan tundra. He did an internship at another Long Term Ecological research Station, The Short Grass Steppe LTER, through Colorado State University, in 2001. In the same year he received his MSc. degree. In 2003 he was hired as a research scholar for the summer at the SGS LTER station, to continue the work of his internship on the use of NDVI in monitoring vegetation development.

In 2004 he started with his PhD position at Utrecht University, on a project that was originally called “Game-theoretical analysis of phenotypic plasticity in plants”. This has resulted in the publication of this thesis.

Thesis overview. Top left: Chapter 2, with 30 cm light gradient in front and the homogeneous shade treatments in the background; Top right: detail of the moving light gradients at the end of the experimental period, as described in Chapter 3; Bottom left: the monocultures of Chapter 4. Bottom right: one plot of the competition experiment, described in Chapters 5, 6 and 7.