

Variation in petiole and internode length affects plant performance in *Trifolium repens* under opposing selection regimes

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Abstract We studied the effects of genotypic and plastic variation in vertical and horizontal spacer lengths on plant performance in a stoloniferous herb subjected to opposing selection regimes. We hypothesized that longer vertical structures are beneficial if plants are subjected to competition, but they should negatively affect plant performance if plants are exposed to aboveground disturbance. To test these hypotheses we subjected 34 genotypes of *Trifolium repens* to competition and disturbance treatments. Competition was imposed by a grass canopy consisting of *Lolium perenne*, and disturbance was simulated by regularly clipping the target plants and all the surrounding vegetation at 1 cm above soil level. Conform to our hypothesis, genotypes with longer vertical structures (petioles) produced fewer ramets than genotypes with shorter petioles in the disturbance treatment. However, genotypes with longer petioles did not perform better under competition than genotypes with shorter petioles. Genotypes with highly plastic vertical structures tended to produce more shoot mass under competition, and they produced fewer ramets if subjected to disturbance. Unexpectedly, horizontal structures (stolon internodes) expanded in response to competition which, furthermore, was associated with enhanced plant performance. However, producing longer internodes is inherently associated with costs in terms of increased resource allocation to the longer structures, but not to benefits in terms of increased resource capture. Positive correlations among the length and plasticity of vertical and horizontal structures may explain the apparent positive effect of producing longer internodes on plant performance. Our data thus support the notion that trait correlations may weaken selective forces acting on a focal trait in a specific environment if opposing selection pressures act on genetically correlated traits.

Keywords Competition · Disturbance · Shade avoidance · Phenotypic plasticity · Vertical and horizontal spacers

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Introduction

Plants are often exposed to multiple selective forces. Consequently, species can display considerable variation in morphological traits both among and within genotypes (Galloway 1995; Via and Lande 1985). Factors contributing to temporal and spatial heterogeneity in pastures are variation in soil conditions, irregular disturbance due to herbivory and human activity such as mowing (Farley and Fitter 1999; Jackson and Caldwell 1993; Waite 1994). Herbaceous vegetations can be characterized as dynamic mosaics of different microhabitats ranging from sites with low levels of disturbance, and severe competition for light, to more open spots with low above ground competition resulting from high levels of canopy disturbance by grazing or mowing (Evans and Turkington 1988; Marcovitz and Turkington 2000).

Plants have evolved several mechanisms to escape or buffer potentially negative effects of competition (Schlichting and Smith 2002; Schmitt and Wulff 1993; Schmitt et al. 1999; Schmitt et al. 2003; Smith 1982; Smith and Whitelam 1997). Long vertical structures allow for the positioning of leaves in more favourable light conditions thereby increasing light harvesting and reducing the negative effects of low light availability (Aphalo and Ballare 1995; de Kroon and Hutchings 1995; Donohue et al. 2000; Geber and Griffen 2003; Huber et al. 1998; Sultan 1995). In competitive sites plants capable of producing long vertical structures perform better than smaller or less plastic plants (Ballare et al. 1994; Dudley and Schmitt 1996; Griffith and Sultan 2006; Schmitt et al. 1995; Weijsschede et al. 2006; Weinig 2000a). However, plants with longer vertical structures can be at a disadvantage in grazed or mown sites, because they lose relatively more biomass than plants with shorter vertical structures. Under such conditions investment in long or highly plastic vertical structures can negatively affect plant performance, as the investment into long structures will be associated with costs, but not with benefits (DeWitt et al. 1998; Dorn et al. 2000; Lande and Arnold 1983; Poulton and Winn 2002; Weinig 2000b). Grazing or mowing is therefore expected to favour plants with shorter and less plastic vertical structures, while competition should favour plants with longer and more plastic vertical structures (Stuefer et al. 2002).

As competition for light can mainly be avoided in the vertical direction, plants in dense canopies may prioritize the production of long vertical structures at the expense of horizontal expansion (Hirose and Werger 1995; Thompson and Harper 1988). Indeed, comparative studies involving plants with horizontal and vertical stems have revealed that shade-induced spacer elongation is mainly expressed in a vertical direction (Huber and Hutchings 1997; Huber et al. 1998). In contrast to results obtained under greenhouse conditions, different stoloniferous species have been shown to elongate their horizontally oriented internodes in dense natural canopies, thereby positioning their offspring ramets further away from the parent ramets (Cain 1994; Hutchings et al. 1997; Kleijn and van Groenendael 1999; Waite 1994). As patterns of light availability are usually less predictable in a horizontal than in a vertical direction, elongation of horizontal structures may not result in increased light capture of newly produced ramets. If increased internode length does not lead to enhanced light capture, the resources needed for internode elongation are lost while they could have been used for other plant functions. It can therefore be expected that plasticity of horizontal structures will be disfavoured in canopies characterized by a low spatial predictability in the horizontal direction and a strong predictable light gradient in vertical direction.

General responses to shading have been investigated thoroughly and the selective advantage of shade avoidance has been shown in a number of studies (Ballare et al. 1991;

Dudley and Schmitt 1996; Huber et al. 1998; Schmitt 1997; Schmitt and Wulff 1993; Schmitt et al. 2003; Thompson 1993; Weinig 2000a). Nevertheless, experiments evaluating plasticity and fitness consequences under multiple contrasting selection regimes are still scarce (but see Anten et al. 2005; Callahan and Pigliucci 2002; Huber et al. 2004; Weinig et al. 2004). In this study we aim at testing the relationship between plasticity and performance of plants subjected to opposing selection regimes. We expect that genotypes with longer vertical structures or higher density-induced plasticity will perform better in high canopies created by a natural competitor than genotypes with shorter vertical structures or lower density-induced plasticity. Regular disturbance (grazing or mowing) involving the loss of above-ground biomass is expected to disfavour genotypes with longer vertical structures and higher density-induced plasticity. Horizontal structures are expected to remain shorter under competition and—due to resource loss—under grazing or mowing compared to control conditions.

Materials and methods

Plant material and pre-treatment conditions

During the summer of 2001, 107 *Trifolium repens* plants were randomly collected from a natural meta-population in a riverine grassland close to the river Waal near Ewijk (51°52'54" N, 5°45'00" E, The Netherlands). Due to the activity of cows and horses the herbaceous vegetation consists of a mosaic of different microhabitats ranging from sites with low levels of disturbance and dense vegetation to more open sites where the vegetation has been disturbed or removed (H. van de Steeg and J. Weijsschede, personal observation). In summer 2002, all plants were screened for morphological traits, including petiole lengths and internode lengths. A total of 34 genotypes were selected which expressed a wide range (1.9–6.8 cm) of petiole lengths under common garden conditions. Molecular fingerprinting techniques (AFLP, four primer combinations, 145 markers) were used to confirm the genetic identity of the collected plants. In a previous experiment we had shown that all 34 genotypes express various degrees of petiole plasticity in response to a vertical light gradient (Weijsschede et al. 2006). This elongation response did not depend on the petiole length expressed under control conditions (Weijsschede et al. 2006). All 34 genotypes were clonally propagated in a heated greenhouse. A total of 408 Cuttings (12 per genotype) were taken from the stock material and transplanted into $0.29 \times 0.19 \times 0.19$ m trays (one cutting per tray), filled with a 2:1 mixture of sand and potting soil. All trays were placed outside the greenhouse on an empty field. Every cutting consisted of one ramet with a well-developed root system and a lateral stolon with 3–5 ramets.

Experimental treatments

On June 6th 2003, all 34 selected genotypes were subjected to the following four treatments:

- (1) No competition and no clipping (referred to as *control conditions*);
- (2) No competition and clipping (referred to as *clipping*);
- (3) Competition and no clipping (referred to as *competition*);
- (4) Competition and clipping (referred to as the *clipping + competition* treatment).

Under control conditions plants were allowed to grow in an undisturbed manner and without competitors. Plants assigned to clipping treatments were subjected to a simulated grazing regime in which all leaf, but no stolon biomass was clipped 1 cm above the soil level and removed. Clipping treatments left apical and lateral meristems of all plants intact. Clipping was applied on the 12th, the 18th, and the 32nd day after the onset of the experiment. Plants subjected to competition were grown together with *Lolium perenne* (Ken Ken, Unifarm, Wageningen, The Netherlands). A total of 310 mg *L. perenne* seeds (app. 220 seeds) were sown per plot 28 days prior to the start of the experiment. When the *T. repens* cuttings were placed in the trays, *L. perenne* plants were about 6 cm high and covered homogeneously the surface of the trays. *T. repens* plants were not able to avoid or escape competition through horizontal expansion. Under undisturbed conditions the grass reached an above ground dry mass density of $173.0 \pm 5.6 \text{ g m}^{-2}$. In the clipping + competition treatment, *T. repens* was subjected to the same clipping regime as in the clipping treatment and to the same competition regime as in the competition treatment. In the clipping + competition treatment, all *L. perenne* biomass higher than 1 cm was removed together with the leaves of *T. repens*. At harvest, the grass had an above ground dry mass density of $56.7 \pm 1.5 \text{ g m}^{-2}$ in the clipping + competition treatment.

Immediately after planting, ramet number was assessed for each genotype to correct for initial size differences. All genotypes were represented once in each treatment, and treatments were replicated in three temporal blocks. A total of 408 plants were used in the experiment. For practical reasons, blocks (representing every treatment once) were temporally separated by one week intervals.

Harvest

Plants were harvested after 48 days. Roots were not collected because it was impossible to separate the *T. repens* roots from the *L. perenne* roots. For all *T. repens* plants, we measured the length of the primary stolon, counted the number of ramets on the primary stolon, the number of branches on the primary stolon and the total number of ramets. For each plant, the 4th ramet counted from the apex on the primary stolon was used to measure the petiole length and internode length. Only undamaged leaves were used to measure petiole lengths. Dry mass of these structures was determined after plant parts were dried at 110°C for 48 h.

Statistical analysis

To test for overall treatment effects, we performed a mixed model ANCOVA (using the GLM procedure in SAS), with genotype, competition and clipping as main factors. Genotype was considered a random factor and competition and clipping were considered fixed factors. Blocks were added as a random factor to the model. This analysis shows how genotype, competition, clipping and their interactions affect various plant traits.

In order to test for the effects of traits on performance, we followed two approaches. First, we used multiple regression analyses based on genotypic means to test for the effects of petiole lengths under high light conditions and competition induced petiole length plasticity on shoot biomass and ramet number in the four treatments separately (see DeWitt et al. 1998; Scheiner and Berrigan 1998; van Kleunen and Fischer 2005; van Tienderen 1991). The same multiple regression model was applied to data on internode length and its

plasticity. The absolute differences in petiole and internode length expressed under competitive and control conditions were used to calculate trait plasticities. Genotypic trait values were standardized to the means per treatment to allow for direct comparisons of different regression coefficients. For details about the analyses see Weijsschede et al. (2006). We used separate correlation analyses to calculate correlation coefficients of the genetic mean values among the four morphological traits included in the selection analyses.

Thereafter we performed a mixed model ANCOVA (using the MIXED procedure in SAS) with competition and clipping treated as fixed effects and genotype as random effect. The genotypic mean (using least square means to correct for block effects) values of the trait of interest were added to the model as a covariate to test for the effects of traits on plant performance. A significant effect of the covariate indicates that, in addition to genetic variation in performance, plant performance was also affected by the covariate (trait length or trait length plasticity). Interactions between the covariate and the treatments were added to the model to test for differential treatment effects of the covariate on plant performance. In other words, this analysis shows whether genotypic differences in a trait (e.g., petiole length) affect plant performance and whether the consequences associated with a given petiole length differ between treatments. A block effect was added to the model to correct for variation among the three temporal blocks. This analysis was performed using mean petiole length and mean internode length produced under high light conditions and mean competition induced petiole length plasticity and mean internode length plasticity. Non significant 3-way interactions were removed from the model. SAS (version 9.1) was used for all statistical analyses.

Results

Overall treatment effects

Genotypes differed in trait expression. However, due to high variation within genotypes and the low number of replicates we failed to detect significant differences in the response of genotypes to the treatments (Table 1). Competition reduced total shoot biomass of *T. repens* by 56% and clipping reduced total shoot biomass up to 32% compared to control conditions (Fig. 1). The combination of competition and clipping reduced total shoot biomass by 62% (significant competition \times clipping interaction). Competition reduced total ramet numbers by 59% and clipping by 12% (Fig. 1). The combination of competition and clipping reduced the total ramet number by 47%, indicating that clipping reduced the strong negative effects of competition on the total ramet numbers (significant competition \times clipping interaction). Genotypes tended to respond differently to the combination of clipping and competition (marginally significant genotype \times clipping \times competition interaction).

On average, petioles elongated by 111% in response to competition (Fig. 1). In the clipping treatment, petioles were 16% shorter than under control conditions. In the combined competition + clipping treatment, petioles were 14% longer as compared to control conditions (significant competition \times clipping interaction, Table 1). Internodes elongated on average by 18% in response to competition, while clipping did not change internode lengths. The combination regime reduced internode length by up to 14 % (significant competition \times clipping interaction, Table 1).

Table 1 Results of ANCOVAs examining effects of genotype, competition and clipping on total shoot dry weight, total ramet number, petiole length and internode length

Source	df	Total shoot dry mass	Total ramet number	Petiole length	Internode length
Genotype	33	1.65*	4.51***	3.31***	2.10***
Clipping	1	18.74***	0.26 ns	150.01***	30.14***
Competition	1	98.64***	208.46***	161.53***	0.17 ns
Clipping × genotype	33	1.23 ns	0.99 ns	0.60 ns	0.78 ns
Competition × genotype	33	1.05 ns	1.12 ns	1.08 ns	1.02 ns
Competition × clipping	33	8.00**	16.67***	21.66***	5.79*
Genotype × clipping × competition	33	1.37 ^s	1.17 ns	1.00 ns	0.98 ns
Block	3	5.85**	2.95 ^s	7.52***	32.10***
Initial ramet number	1	6.86**	12.17***	0.60 ns	0.73 ns
Error	265				

Initial ramet number was added as a covariate to the model. All traits were log transformed to meet ANCOVA assumptions. *F*-values and their significances are presented. Significance levels are as follows: ns, $P > 0.10$; ^s0.10 $\geq P > 0.05$; *0.05 $\geq P > 0.01$; **0.01 $\geq P > 0.001$; *** $P < 0.001$

Genetic correlations among morphological traits

Petiole length expressed under control conditions was not significantly correlated with competition induced plasticity in petiole length ($r = -0.14$, $P = 0.412$). Internode length under control conditions was negatively correlated with internode length plasticity ($r = -0.51$, $P = 0.002$), indicating that genotypes with longer internodes under control conditions exhibited lower levels of internode elongation than genotypes with shorter internodes. Internode and petiole lengths were positively correlated ($r = 0.66$, $P < 0.001$) under control conditions. Competition-induced plasticity in petiole length was positively correlated with competition-induced internode plasticity ($r = 0.48$, $P = 0.002$)

Effects of petiole length and plasticity on plant performance

Longer petioles were generally associated with the production of fewer ramets, but had no effects on shoot weight (Table 2a, Fig. 2a, b). Under control conditions long petioles tended to be associated with increased dry weights (Fig. 2a). The ability to elongate petioles in response to competition did not significantly affect plant performance under control conditions (Table 2b, Fig. 2c, d).

Petiole length did not affect performance of plants grown in competitive environments (Table 2a, Fig. 2a, b). The negative effect of petiole length of plants grown under control conditions on ramet number was thus diminished under competitive conditions. This indicates that benefits associated with producing long petioles in competitive environments buffered costs associated with long petioles apparent in the absence of competition (Fig. 2b). For plants subjected to competition high degrees of petiole plasticity tended to be positively associated with increased shoot dry mass, but not with ramet numbers (Table 2b, Fig. 2c, d).

Mixed model Ancova revealed a significant interaction in the effects of clipping and petiole length on plant performance (Table 2a, b). Genotypes with longer petioles

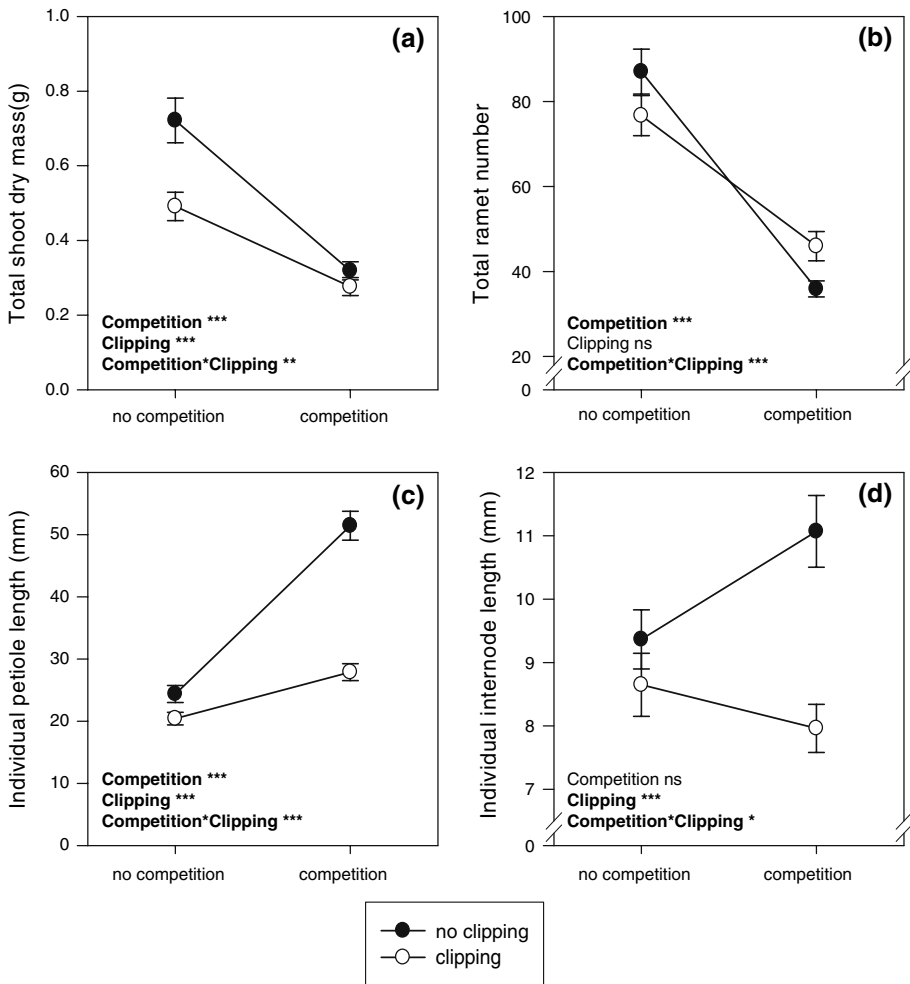


Fig. 1 Responses of (a) total shoot dry mass, (b) total ramet number, (c) petiole length and (d) internode length to grass competition and clipping. Significances of the main effects competition, clipping and competition + clipping on plant characters are inserted in the figures (see Table 1 for the complete statistical). Significant treatment effects are highlighted in bold and are indicated as follows: ns, $P > 0.05$; $*0.05 \geq P > 0.01$; $**0.01 \geq P > 0.001$; $***P < 0.001$. Values are means (± 1 se) per treatment

produced less biomass and fewer ramets if subjected to disturbance, but not if grown under control conditions (Fig. 2a, b). Selection analyses revealed that under disturbance regimes (both, with and without concurrent competition) petiole length expressed under control conditions had a significant negative effect on ramet production (Fig. 2b). The potential to elongate petioles under competitive environments tended to have a negative effect on ramet production if plants were simultaneously subjected to clipping as well, indicating that the production of long petioles and high degrees of petiole length plasticity will be selected against in disturbed environments (Fig. 2d).

Table 2 Results of mixed model of ANCOVAs examining the effects of competition and clipping (fixed effects) and the covariates (a) control petiole length, (b) petiole length plasticity, (c) control internode length and (d) internode length plasticity on plant performance (shoot biomass and ramet production)

Source	Total shoot drymass	Total ramet number	Source	Total shoot drymass	Total ramet number
a					
Competition	3.85 [§]	23.78***	Competition	33.15***	57.13***
Clipping	0.52 ns	2.94 [§]	Clipping	0.74 ns	1.75 ns
Competition × Clipping	7.16**	14.02***	Competition × Clipping	7.08**	14.02***
Petiole length	0.73 ns	5.72*	Petiole length plasticity	1.40 ns	0.95 ns
Competition × Petiole length	1.93 ns	0.00 ns	Competition × Petiole plasticity	3.16 [§]	2.07 ns
Clipping × Petiole length	5.75*	2.82 [§]	Clipping × Petiole plasticity	1.46 ns	1.61 ns
Block ^a	0.94 ns	0.92 ns	Block ^a	0.93 ns	0.92 ns
Genotype ^a	1.47 [§]	2.92**	Genotype ^a	1.40 [§]	3.05**
Residual ^a	13.48***	13.44***	Residual ^a	13.48***	13.43***
c					
Competition	0.04 ns	4.43*	Competition	118.54***	224.09***
Clipping	0.45 ns	1.70 ns	Clipping	18.78***	0.67 ns
Competition × Clipping	4.06*	0.03 ns	Competition × Clipping	15.86***	19.75***
Internode length	1.35 ns	3.44 [§]	Internode length plasticity	3.53 [§]	0.12 ns
Competition × Internode length	9.43**	4.10*	Competition × Internode plasticity	17.24***	11.92***
Clipping × Internode length	4.21*	1.54 ns	Clipping × Internode plasticity	0.01 ns	1.04 ns
Competition*Clipping*Internode length	8.30**	0.78 ns	Competition × Clipping × Internode plasticity	11.94***	5.31*
Block ^a	0.94 ns	0.92 ns	Block ^a	0.94 ns	0.93 ns
Genotype ^a	1.46 [§]	2.99**	Genotype ^a	1.36 [§]	3.10**
Residual ^a	13.45***	13.42***	Residual ^a	13.45***	13.42***

^a Z-values and significances are given for random effects

Internode and petiole length plasticity were calculated as the absolute difference of internode and petiole length in control as compared to competition treatment. Genotype and block were added as random effects. F-values and significances are presented. For significance levels see Table 1

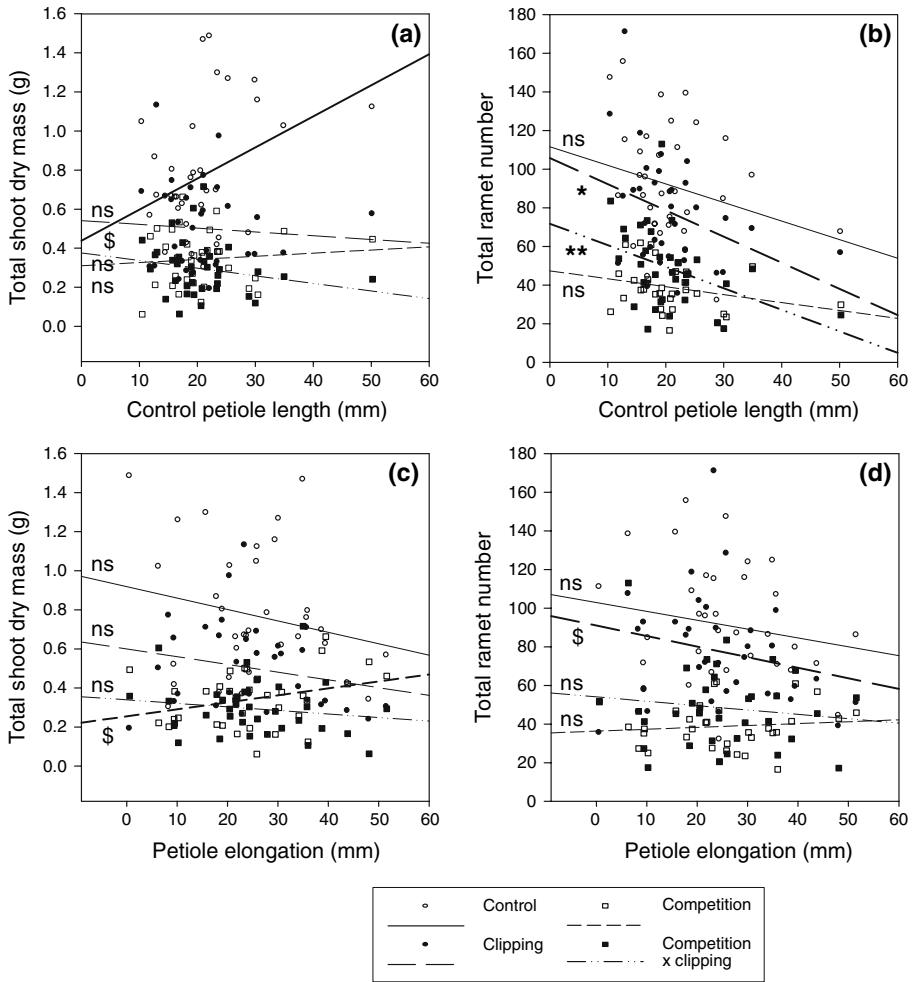


Fig. 2 The relationship between petiole length found under control conditions (*x*-axis) and (a) total shoot biomass and (b) ramet number in the four treatments. Graphs (c) and (d) show relations between petiole elongation (measured as the absolute difference in petiole length between the competition treatment relative to control conditions (*x*-axis)) and total shoot biomass (c) and ramet number (d) per treatment. Bold lines indicate a significant or marginally significant effect of the trait on performance as indicated by selection analyses (multiple regression on performance, see Materials and method section) revealed an effect of petiole and petiole plasticity on plant performance. For significance levels see Table 1

Effects of internode length and plasticity on plant performance

Internode length had a slight negative effect on ramet production, but not on shoot dry mass. Plasticity in internode length had a slight overall negative effect on shoot dry mass, but not on ramet production. Under control conditions, longer internodes tended to be associated with increased shoot dry mass (Fig. 3a), while the ability to produce longer internodes under competition had negative effects on dry mass and ramet production under control conditions (Table 2d, Fig. 3c, d).

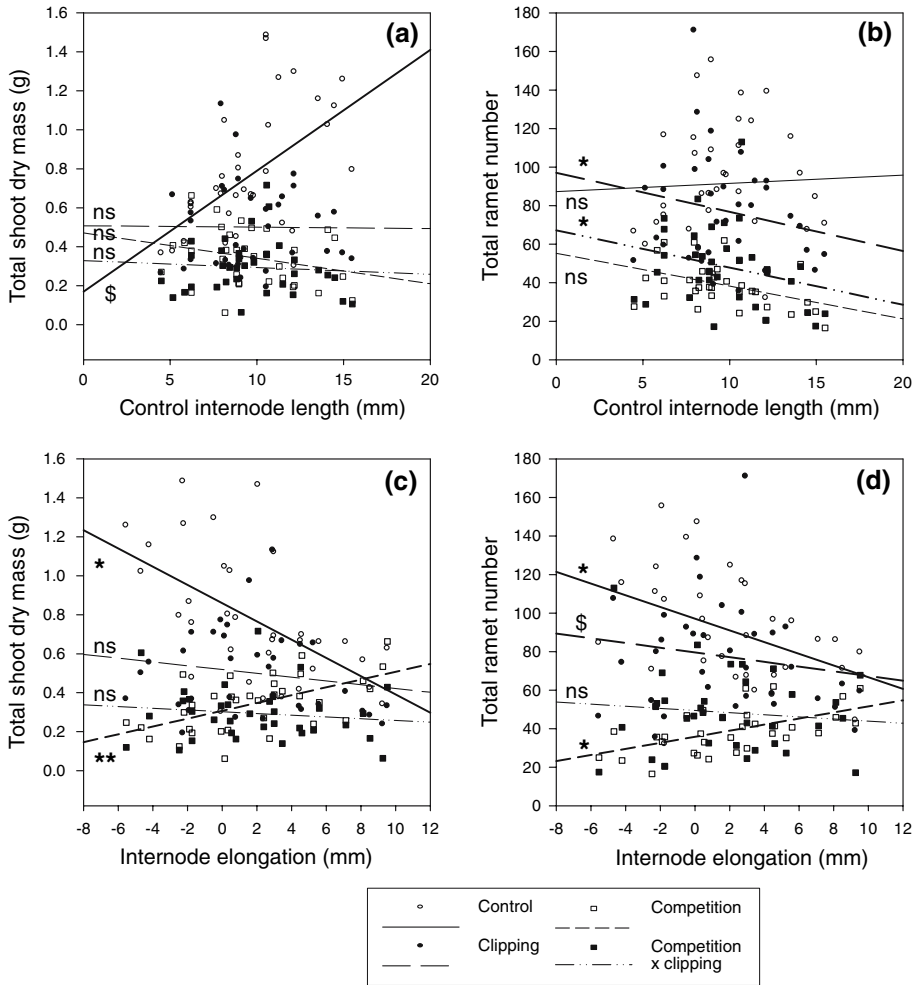


Fig. 3 The relationships between internode length found under control conditions (x-axis) and total shoot biomass (a) and ramet number (b) per treatment. Graphs (c) and (d) show relations between internode elongation (measured as the absolute difference in internode length between the competition treatment relative to control conditions (x-axis)) and total shoot biomass (c) and ramet number (d) per treatment. Bold lines indicate that selection analyses revealed a significant and marginal significant effect of internode length and internode plasticity on performance. Significance levels are as follows: ns, For significance levels see Table 1

These relationships reversed if plants were grown in competition with *Lolium perenne*. Mixed model Ancova revealed a significant interaction between competition and internode length, indicating that genotypes producing longer internodes performed relatively worse if grown in competition than if grown under control conditions (Table 2c, Fig. 3a, b). However, there was no direct selection on internode length in competitive environments (Fig. 3a, b). Genotypes responding to competition by shortening their internodes performed significantly worse if subjected to competition than if grown in competition-free environments (Table 2d, Fig. 3c,d).

In contrast to control conditions, plants could not benefit from producing longer internodes if subjected to clipping (Table 2c, Fig. 3a). Selection analyses revealed a negative effect of internode length and internode length plasticity on ramet production (Fig. 3b, d). Increased internode length was also associated with reduced ramet production in plants subjected to concurrent competition and clipping (Fig. 3b), indicating that under disturbed conditions internode length and competition induced internode length plasticity will be selected against.

Discussion

Longer vertical structures may buffer negative effects of light limitation within herbaceous canopies (Schmitt and Wulff 1993; Schmitt et al. 2003; Van Hinsberg 1997). Conversely, grazing or mowing should select against the production of long vertical spacers. Extension of horizontal structures should be disfavoured by both, competition and clipping as producing longer horizontal structures is unlikely to enhance light capture and biomass production, while it incurs costs in terms of inefficient biomass allocation (Thompson and Harper 1988). Contrary to our expectations genotypes with long petioles under control conditions were not favoured under competition. As predicted, higher degrees of petiole plasticity tended to increase plant performance under competition. Regular clipping disfavoured genotypes with longer petioles and genotypes expressing higher degrees of petiole plasticity were marginally disfavoured. Longer internodes were disfavoured under clipping but did not negatively affect plant performance under competition. By contrast, plastic internode elongation in response to competition was associated with enhanced plant performance. This was unexpected as plastic internode elongation did not result in enhanced light harvesting in this experiment.

Consequences of genotypic trait differences

The length and competition-induced plasticity of vertical and horizontal structures were highly correlated in our experiment. We also found strong positive relationships between total shoot dry mass, petiole length and internode length under control conditions. In a previous experiment we showed that petiole length was also positively correlated with leaf area (Weijsschede et al. 2006). Natural populations of *T. repens* genotypes consist thus of a continuum of morphologies ranging from genotypes with large modules (longer petioles, longer internodes, and larger leaf areas), higher biomass production and a more linear morphology (less branching) to genotypes with smaller modules (shorter petioles, shorter internodes, and smaller leaf areas), lower biomass production and a more branched morphology. Our data explore the consequences of these genotypic trait differences for plant performance. Genotypes with longer petioles and internodes under high light conditions were disfavoured under clipping regimes. In contrast to our hypothesis, however, producing long petioles under high light conditions was not beneficial if plants were subjected to competition. Since petiole and internode length were positively correlated, the expected benefits of producing longer petioles under competitive, low light conditions may have been counteracted by ineffective resource allocation to the production of longer internodes (note the similarity between Figs. 2 and 3). These trait correlations may hence explain the apparent discrepancy between our prediction and results. Our data support the notion that trait correlations may weaken selective forces acting on a focal trait in a specific

environment if opposing selection pressures act on genetically correlated traits (Garland and Kelly 2006; Pigliucci and Kolodynska 2002; Pigliucci et al. 1998)

Internode elongation

In contrast to our hypothesis, plants subjected to competition produced 18% longer internodes than plants grown alone. These results are in line with some observations reported in the literature (de Kroon and Hutchings 1995; Thompson 1995; van Kleunen and Fischer 2001; Waite 1994) but are contradictory to many others (Huber et al. 1998; Leeftang 1999; Solangaarachchi and Harper 1987; Thompson and Harper 1988) including our previous work with the same *T. repens* genotypes (Weijsschede et al. 2006) in which low levels of plasticity or a shortening of horizontal structures were observed under low light conditions. Our data obtained on the same set of genotypes grown in different experimental conditions suggest that internode elongation may not only be triggered by decreased light availability, a reduction of the red to far-red ratio (Schmitt and Wulff 1993) or a reduction in blue light (Gautier et al. 1998). Plant resource status and other cues intercepted by plants under field conditions like for example, ethylene concentrations (Pierik et al. 2003) or relative humidity (Price and Hutchings 1996) may interact in determining final internode length.

Consequences of plastic responses

In line with our hypothesis, genotypes which expressed higher degrees of plasticity in vertical structures performed (marginally) better under competition compared to less plastic genotypes. Expressing higher degrees of petiole elongation was associated with higher shoot biomass (but not increased vegetative propagation) under competition. Genotypes which produced the highest shoot dry mass under competition were also characterized by the highest degrees of internode elongation. The positive genetic correlation among internode and petiole length plasticity may explain the unexpected positive relationship between internode elongation and shoot biomass production, as internode length plasticity does not directly affect resource capture and can thus also not affect biomass production. Pleiotropic effects may have caused the positive relationship between internode and petiole length plasticity. Alternatively, the increased resource capture in genotypes characterized by high petiole length plasticity may also be allocated to the internodes, leading to increased internode growth.

Similar to our earlier results on petioles (Weijsschede et al. 2006) genotypes that expressed higher degrees of shade induced internode plasticity produced lower shoot biomass and fewer ramets under control conditions. We found that the potential to express higher degrees of internode plasticity was associated with decreased plant performance under conditions in which plasticity was not induced. Observations in both studies suggest that the potential to express shade-induced elongation *per se* is costly for plants that grow in non-inductive environments (DeWitt et al. 1998; van Tienderen 1991).

Implications for selection

If trait correlations constrained the benefits of plastic responses, selective pressures would consequently be weakened. Shade-avoidance responses should result in benefits under poor

light conditions but pleiotropic effects of correlated traits may lower these benefits. Other trait correlations such as changes in root-shoot allocation between treatments or genotypes may also have reduced the expected benefits of petiole length and petiole length plasticity (Cahill 2002, 2003; Zobel and Zobel 2002). This suggests that selection on shade-avoidance traits may be weaker or virtually absent in some systems, while they are undoubtedly prevalent in a number of other systems (Dorn et al. 2000; Dudley and Schmitt 1996; Schmitt et al. 1999; Schmitt et al. 2003). In *T. repens* the evolutionary consequences of trait correlations will depend on the spatial and temporal scale of environmental heterogeneity. In homogeneous grasslands, escaping the shade of neighbouring plants can only be achieved in the vertical direction and concurrent length increase of correlated horizontal structures may constrain the benefits of producing longer vertical structures. Alternatively, in highly patchy environments plants are likely to frequently encounter different microsites during their life span. In this case, escape in both vertical and horizontal direction will be beneficial and increased plasticity in the length of vertical and horizontal structures will result in enhanced plant performance (Cain 1994; Waite 1994). Under these conditions, trait correlations may not counteract the benefits associated with longer or more plastic vertical structures.

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