Photosynthesis of white clover (*Trifolium repens* L.) germplasms with contrasting leaf size

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Abstract

In a growth chamber experiment, we determined net photosynthetic rate (P_N) and leaf developmental characteristics of cultivars of a relatively small-, intermediate-, and a large-leaf genotype grown under irradiance of 450-500 μmol(photon) m^{-2} s^{-1} (HI), shade [140-160 μmol(photon) m^{-2} s^{-1}] (LI), and after a shade-to-irradiation (LI →HI) transfer. Differences in physiological responses of the genotypes were more pronounced in HI and LI→HI plants than in LI plants. The small- and intermediate-leaf sizes had greater P_N in the first measured leaf than the large-leaf type by 70 and 63 % in HI plants, and by 23 and 18 % in LI→HI plants, respectively. Similar relationships were observed in the next developed leaf. The LI plants did not differ significantly in P_N. Greater P_N in the small- and intermediate-leaf size genotypes were not associated with greater total dry matter of the plant. Under irradiation, the large-leaf genotype accumulated more total nonstructural saccharides (TNS) and starch than the small- or intermediate-leaf size plants. TNS and starch concentrations in LI plants were about one-half those of HI and LI→HI plants. These results should help to develop management practices that capitalize upon the competitive features of white clover in mixed-species swards.

Additional key words: cultivar; dry matter production; irradiance; leaf development; nitrogen; nonstructural saccharides; starch; stolons.

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Introduction

White clover (*Trifolium repens* L.) is an important forage species because of its high nutritive quality (Thomson and Raymond 1970), ability to fix atmospheric nitrogen in symbiosis with *Rhizobium* spp. (Haystead and Marriott 1978), and wide adaptation to local soil and climatic conditions (Klebesadel 1986). Differences in white clover population responses to photoperiod and temperature can be related to leaf size (Eagles and Othman 1986, 1988); however, leaf size of a genotype can be also modified by environmental (Boller and Nösberger 1983) and management (Brock *et al.* 1988) factors. In a review of factors influencing white clover growth in mixed-species systems, Kessler and Nösberger (1994) noted that management practices which improve radiation conditions in a sward would promote clover growth. Phenotypic plasticity of white clover stolons and leaves contributes to persistence in a range of canopy conditions.

White clover is used principally as a component of grass-clover pastures (Frame and Newbould 1986) where it is subjected to periodic defoliation. Other environmental stresses include inter- and intraspecific competition for water and nutrients in the root zone (Snaydon 1971) and for radiation in the canopy (Stern and Donald 1962, Davies and Evans 1983). White clover leaf morphology would have a deciding effect on competitive ability in terms of photon capture. Small-leaf white clover ecotypes have higher P_N and allocate more dry matter to reserve organs (stolons) than large-leaf ecotypes (Mächler and Nösberger 1977, Boller and Nösberger 1983). Clipping or grazing treatments would impact the persistence of white clover in a mixed-species grassland because of effects on residual leaf area, P_N, leaf appearance, and stolon spreading rates (William and Asiegbu 1982, Brock *et al.* 1988), and subsequent influence on radiation quality (red/far red ratio) and quantity in the sward (Robin *et al.* 1992). Radiation quality within the canopy can be influenced by the botanical composition of the sward as well, which in turn can affect white clover morphology and persistence (Thompson and Harper 1988).

Information about the photosynthetic capacity of white clover cultivars developed in or introduced to North America is rare. The main objective of our controlled environment experiment was to determine the photosynthetic response of three cloned white clover genotypes with contrasting leaf size as a basis for a more detailed field study of the competitive ability of selected white clover cultivars. Growth characteristic responses of the white clover genotypes grown under various irradiances have been studied by Malinowski *et al.* (unpublished). This study focuses on photosynthetic activity of selected white clover cultivars emphasizing activity in simulated 'post-cut' swards. Our results should provide understanding of how differences in the competitive ability of white clover cultivars are realized when grown with associated grass canopies. We can then refine management practices (defoliation intervals, companion grass species, selection of white clover cultivars based upon leaf size) for promoting white clover persistence and production in mixed-species swards in the eastern USA.

Materials and methods

Plants: White clover (*Trifolium repens* L.) plants with distinctly differing leaf characteristics were used: The small-intermediate leaf-size cv. Grasslands Huia (OECD 1984) represented the small leaf size, the intermediate-large type was represented by plant germplasm Brown Loam (intermediate-leaf type), and the large-leaf (Ladino) type was represented by cv. Tillman. Plants were cloned from stolon tips of a genotype selected from each cultivar. Each plant was generated from segments having two nodes and a root, and planted in nursery trays for four weeks to ensure rooting and establishment. The rooting medium was a mixture of fine loamy, siliceous soil from southern West Virginia, USA and sand (3 : 1, volume). Plants were fertilized once a week with 130 cm^3 of 0.5 % solution of *Peters Professional Soluble Fertilizer* (20 : 20 : 20 N : P_2O_5 : K_2O). Plants were supplied with N fertilizer to minimize possible effects of variable N fixation on P_N (Arnott 1984).

After 4 weeks, plants were transplanted into larger pots (11 cm diameter, 820 cm^3 volume) containing the same soil mixture. Plants were placed in a growth chamber (day/night: temperature 23/18 °C, air humidity 75/60 %; photoperiod 15 h), under two irradiances: HI [450-500 μmol(photon) m^{-2} s^{-1}] and LI [140-160 μmol(photon) m^{-2} s^{-1} under shade cloth enclosures]. During this time plants were irrigated with 130 cm^3 of the soluble fertilizer solution (1 %) once a week or with water in amount needed to maintain the field capacity of the soil. After a 6-week growth period, a subset of LI plants was exposed to HI (LI→HI plants) to simulate a change in irradiance in a defoliated sward, and P_N measurements began (day 0).

Development of six consecutive leaves (LD1-LD6) appearing on the main stolon of HI and LI plants was described using the Carlson scale of white clover phenology (Carlson 1966). The first fully developed leaf from the apex (L1) of the main stolon was marked on day 0 (at the time of LI→HI transfer). P_N of L1 was measured on days 0, 2, 4, 6, 9, 14, and 16 for four plants per germplasm and irradiation treatment. P_N of the next developed leaf (L2) was also measured after the leaf reached 1.0 on the Carlson scale. P_N was measured with a portable photosynthesis system (model *LI-6200, LI-COR*, Lincoln, NE, USA) on leaves attached to the plants.

Three destructive harvests of four plants per genotype in each radiation regime were made on days 0, 6, and 16 to monitor herbage (leaves + petioles) dry matter (DM), total DM (shoot and root), and total nonstructural saccharide (TNS), starch, and nitrogen content. TNS and starch contents were determined by the modified method of Smith (Denison *et al.* 1990). Nitrogen content was determined by CHNS Elemental Analyzer *EA 1108* (*Carlo Erba*, Milan, Italy). Leaf and stolon characteristics, DM allocation, and growth characteristics collected at each sequential harvest date will be presented elsewhere.

Statistical analysis: Analyses of variance (split-plot design) were made using the SAS statistical package (Statistical Analyses System, *SAS Institute*, Cary, NC, USA). Irradiation regimes were main plots and genotypes (representing leaf size) were sub-plots. Genotypes were replicated and arranged in a completely random fashion within each radiation regime. Means were compared by the Duncan multiple range test ($p = 0.05$).

Results and discussion

P_N differed among the leaf sizes (Fig. 1). Small- and intermediate-leaf plants had significantly greater P_N than the large-leaf plants when grown in HI and LI→HI regimes. LI plants of the cultivars did not differ in P_N, and maintained distinctly lower P_N than the HI plants. Our observations were similar to previous reports for white clover grown in controlled environment (Dennis and Woledge 1983, Boller and Nösberger 1985, Davidson *et al.* 1990).

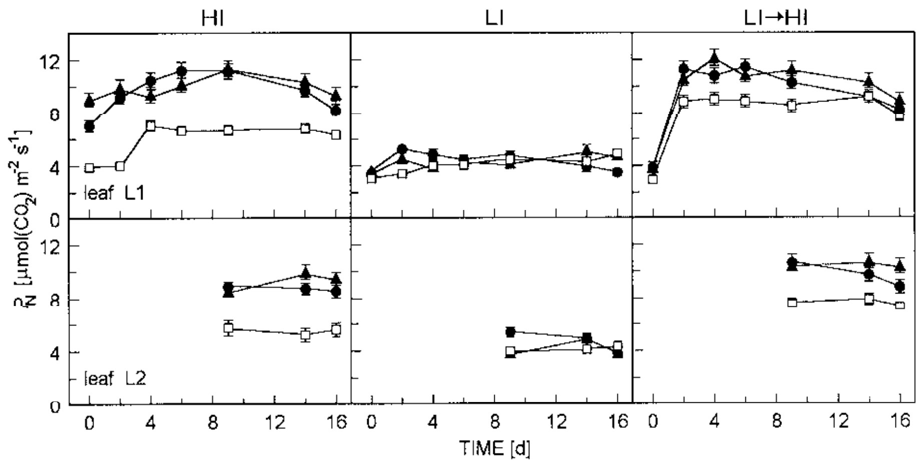

Fig. 1. Net photosynthetic rate (P_N) of the most recent fully developed leaf (L1) and the next one (L2) in three white clover genotypes differing in leaf size: small- (▲), intermediate- (●), and large-leaf plants (□) grown under irradiation (HI), shade (LI), and after LI→HI transfer. Bars indicate ±S.E. (n = 4).

There was a significant interaction between irradiation regimes and white clover genotypes for P_N. Two days after LI→HI transfer, P_N of the most recent, fully developed leaf (L1) in the small- and intermediate-leaf size genotypes was significantly greater than that of comparable leaves on HI plants (Fig. 1). However, leaf P_N of the small- and intermediate-leaf plants grown in HI and LI→HI regimes were similar thereafter. In contrast, large-leaf plants after LI→HI transfer maintained a greater P_N in L1 than the HI plants. A similar response was observed in the second monitored leaf (L2). Change from LI to HI resulted in significantly greater P_N of L2, regardless of leaf size, when compared to HI plants (Fig. 1). However, the P_N of L2 of the intermediate-leaf size plants decreased to the rates of plants grown in HI at the end of the measurement period (day 16). Comparing P_N of LI and HI plants showed that the small- and intermediate-leaf plants had steeper irradiance response curves of P_N than the large-leaf plants (Fig. 2). A greater rate of change in response to irradiation may be an advantage in a dynamic radiation environment found in frequently defoliated canopies. High P_N is a common feature of early successional plants (Sultan and Bazzaz 1993) that take advantage of site disturbance and change. This suggests that large-leaf, low P_N white clover cultivars may be stable components of relatively undisturbed canopies such as long rotation grazing or hay production schemes. Small-leaf, high P_N white clover cultivars may be more adapted to sites that endure frequent disturbances, *i.e.*, continuous or intensive grazing.

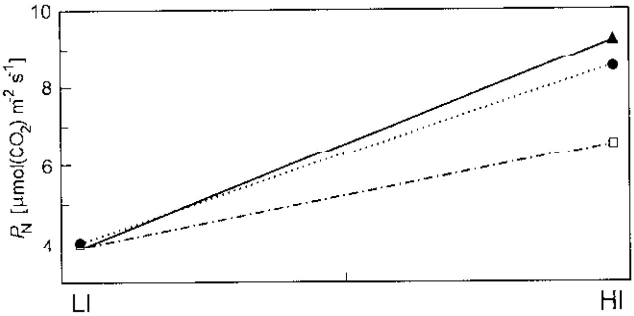

Fig. 2. Dependences of irradiation (HI - high irradiance, LI - shade) of small- (-▲-), intermediate- (-●-), and large-leaf (-□-) white clover genotypes. Points represent averaged P_N values of the most recent fully developed leaf during the measurement period (n = 28).

Dennis and Woledge (1983) found that leaves of white clover responded to increased irradiance up to, or shortly after Carlson stage 0.5 was achieved, where the P_N capacity increased to that of the unshaded leaves. This could explain increased P_N in white clover L2 leaves in our experiment. However, shaded L1 leaves of all germplasms were in Carlson stage 1.0 when they were transfered to HI, and they still responded rapidly to increased irradiance, outperforming P_N in L1 leaves of HI plants. This could be the result of plant shading systems in our study that were different than those in the experiment by Dennis and Woledge (1983) and affected not only the quantity but also quality (R/FR ratio, shading systems) of radiation. The rapid increase in P_N in white clover leaves after transfer to HI might confer a competitive advantage in mixed-species swards after defoliation because a similar response has not been observed in residual leaves of grasses that originated in shade (Prioul *et al.* 1980). The ability to increase P_N in leaves in response to increased irradiance and to position leaves at the top (large-leaf types) or colonize sun flecks in the lower part of the canopy (small- and intermediate-leaf size types) should contribute to improved competitiveness of white clover when interacting with tall-growing grasses (Boller and Nösberger 1985).

The high P_N of small- and intermediate-leaf size white clover plants in HI and LI →HI regimes were not associated with an enhanced total DM (Table 1) which agrees with earlier observations of Nösberger *et al.* (1981). Photosynthates not used for growth or metabolic processes could be accumulated as TNS (Nösberger *et al.* 1981). The TNS and starch concentrations in stolons, however, were greater in the large- than in the small- and intermediate-leaf plants, regardless of irradiation regime. Considering the proportion of stolons in total DM of the plant (Malinowski *et al.* unpublished), the large-leaf plants were able to invest more photosynthates in herbage growth than in reserve organs (stolons), compensating for low P_N with almost twice the individual leaf area (Fig. 3) and leaf area per plant (values not presented) than found in the other white clover genotypes. Higher P_N values in small- and intermediate-leaf size genotypes were associated with greater total N concentrations in herbage (Table 1). Because of greater herbage DM, however, lower

Table 1. Herbage (leaves + petioles) and total (shoot + root) dry matter (DM) [g plant^{-1}] of three white clover genotypes in relation to irradiation conditions at the end of the experiment (HI - high irradiance, LI - shade). Small-, intermediate-, and large-leaf white clover genotypes are represented by cultivars Grasslands Huia, Brown Loam, and Tillman, respectively. S.E., standard error (n = 9).

DM	Leaf size	HI	LI	LI→HI	S.E.
herbage	small	16.8	4.5	8.6	0.41
	intermediate	19.8	6.7	12.4	0.55
	large	22.1	9.3	19.5	0.40
	S.E.	0.26	0.25	0.40	
total	small	20.8	5.2	10.0	0.51
	intermediate	23.7	7.3	13.8	0.61
	large	28.8	10.9	23.4	0.36
	S.E.	0.36	0.46	0.27	

total N concentration in large-leaf plants grown in HI and LI→HI might be simply a ‘dilution effect’. We tried to minimize variation in N supply providing plants with mineral N (Arnott 1984). Total N concentrations in leaves of LI plants were greater than in leaves of HI or LI→HI plants (Table 2). Plants in all irradiation treatments received the same amount of N but LI plants grew less, therefore the N concentration in LI plants was greater. This is in contrast to observations by Dennis and Woledge (1982) who report similar total N concentration in shaded and unshaded field-grown white clover leaves. The difference might be related to form or availability of nitrogen source.

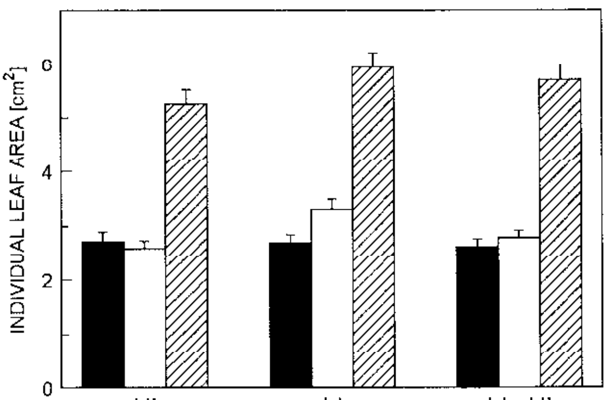

Fig. 3. Individual leaf area of three white clover genotypes [small- (■), intermediate- (□), and large-leaf (▨) differing in leaf size in relation to irradiation (HI - high irradiance, LI - shade). Bars indicate standard errors (n = 4).

Development rate of leaves emerging on the main stolon in HI and LI, as described by the Carlson scale, was lower in the small-leaf genotype than in the other genotypes (Fig. 4). There were marginal differences in this parameter among genotypes for the first and second monitored leaf (LD1 and LD2). These leaves were produced in greenhouse prior to experimental irradiation regimes were imposed.

Table 2. Concentrations [g kg^{-1}(DM)] of nitrogen (N) in herbage (leaves + petioles), and total nonstructural saccharides (TNS) and starch contents in stolons in three white clover genotypes differing in leaf size in relation to irradiation regimes (HI - high irradiance, LI - shade). Small-, intermediate-, and large-leaf white clover genotypes are represented by cultivars Grasslands Huia, Brown Loam, and Tillman, respectively. Values represent mean for three measurements. S.E. indicate standard errors (n = 6).

Irradiation	Leaf size	N	TNS	Starch
HI	small	58.1	171.2	72.1
	intermediate	52.3	214.3	85.4
	large	44.3	260.7	93.9
	S.E.	0.09	13.66	2.77
LI	small	68.4	65.5	33.8
	intermediate	67.2	85.3	48.3
	large	68.2	108.4	52.4
	S.E.	0.24	6.16	2.12
LI→HI	small	56.8	191.0	83.5
	intermediate	56.5	203.8	98.0
	large	44.6	260.5	114.2
	S.E.	0.16	0.02	3.33

Leaves initiated in HI or LI (LD3 to LD6) expressed progressive differences in development rate. The slower leaf development rate of the small-leaf plants, especially when shaded, might have a more negative effect on the competitive ability of plants represented by this genotype than on intermediate- or large-leaf genotypes if maintained under canopy conditions that restrict photon flux (William and Asiegbu

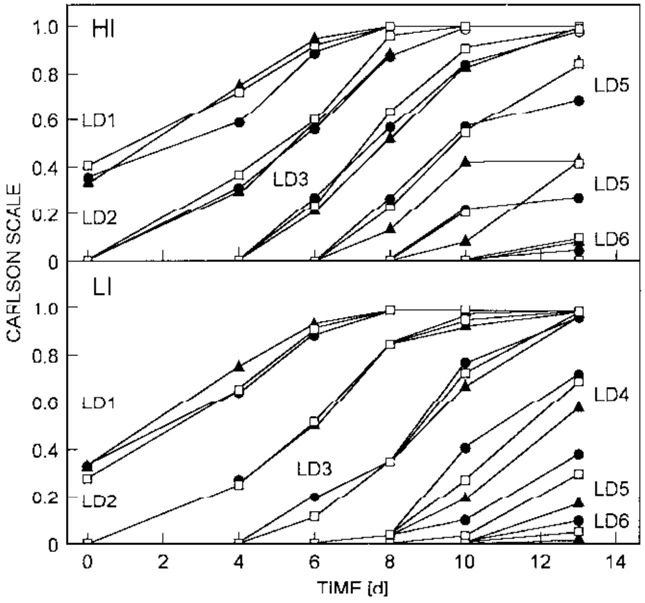

Fig. 4. Leaf development rate as described by the Carlson scale of three white clover genotypes differing in leaf size: small- (-▲-), intermediate- (-●-), and large-leaf (-□-) plants grown under high irradiation (HI) and shade (LI). LD1-LD2 indicated leaves which were produced in greenhouse, prior to experimental irradiation regimes. LD3-LD6 indicated leaves produced under experimental irradiation regimes (n = 4).

1982, Grant and Barthram 1991, Brink 1995). The greater P_N of small-leaf white clover plants, however, could have advantage in 'post-cut' canopies, promoting a rapid regrowth of white clover and increasing its competitive ability.

According to this study, small- and intermediate-leaf genotypes had, in general, greater P_N than the large-leaf genotype in both monitored leaves when grown in HI or when LI→HI moved. In response to continuous shade, however, the genotypes we investigated did not differ in P_N. Greater P_N values in the small- and intermediate-leaf genotypes were not associated with greater total DM, nor with TNS or starch accumulation in stolons. Differences in P_N related to leaf size suggest a potential for various competitive strategies in mixed-species swards. Our results support observation that small- and medium-leaf white clovers may be productive in continuously grazed environments, and suggest that large-leaf white clover cultivars should be considered for extensive grassland management. Our results are part of continuing laboratory and field investigations of the role of white clover leaf size on sward productivity and clover persistence under defoliation in grass-clover mixtures.

References

Arnott, R.A.: An analysis of uninterrupted growth of white clover swards receiving either biologically fixed nitrogen or nitrate in solution. - Grass Forage Sci. **38**: 305-310, 1984.

Boller, B.C., Nösberger, J.: Effects of temperature and photoperiod on stolon characteristics, dry matter partitioning, and nonstructural carbohydrate concentration of two white clover ecotypes. - Crop Sci. **23**: 1057-1062, 1983.

Boller, B.C., Nösberger, J.: Photosynthesis of white clover leaves as influenced by canopy position, leaf age, and temperature. - Ann. Bot. **56**: 19-27, 1985.

Brink, G.E.: White clover growth and morphology under contrasting cutting regimes. - Crop Sci. **35**: 1100-1103, 1995.

Brock, J.L., Hay, M.J.M., Thomas, V.J., Sedloce, J.R.: Morphology of white clover (*Trifolium repens* L.) plants in pastures under intensive sheep grazing. - J. agr. Sci. **111**: 273-283, 1988.

Carlson, G.E.: Growth of clover leaves - developmental morphology and parameters at ten stages. - Crop Sci. **6**: 293-294, 1966.

Davidson, I.A., Culvenor, R.A., Simpson, R.J.: Effect of previous defoliation regime and mineral nitrogen on regrowth in white clover swards: Photosynthesis, respiration, nitrogenase activity and growth. - Ann. Bot. **65**: 665-667, 1990.

Davies, A., Evans, M.E.: Branching in white clover. - Working Group on Detailed Sward Measurement Newsletter 12. Welsh Plant Breeding Station, Aberystwyth 1983.

Denison, R.F, Fedders, J.M., Tong, C.B.S.: Amyloglucosidase hydrolysis can overestimate starch concentration of plants. - Agron. J. **82**: 361-364, 1990.

Dennis, W.D., Woledge, J.: Photosynthesis by white clover leaves in mixed clover/ryegrass swards. - Ann. Bot. **49**: 627-635, 1982.

Dennis, W.D., Woledge, J.: The effect of shade during leaf expansion on photosynthesis by white clover leaves. - Ann. Bot. **51**: 111-118, 1983.

Eagles, C.F., Othman, O.B.: Effect of temperature, irradiance and photoperiod on morphological characters of seedlings of contrasting white clover populations. - Ann. appl. Biol. **108**: 629-638, 1986.

Eagles, C.F., Othman, O.B.: Variation in growth of overwintered stolons of contrasting white clover populations in response to temperature, photoperiod and spring development. - Ann. appl. Biol. **112**: 563-574, 1988.

Frame, J., Newbold, P.: Agronomy of white clover. - Adv. Agron. **40**: 1-88, 1986.

Grant, S.A., Barthram, G.T.: The effects of contrasting cutting regimes on the components of clover and grass growth in microswards. - Grass Forage Sci. **46**: 1-13, 1991.

Haystead, A., Marriott, C.: Fixation and transfer of nitrogen in a white clover-grass sward under hill conditions. - Ann. appl. Biol. **88**: 453-457, 1978.

Kessler, W., Nösberger, J.: Factors limiting white clover growth in grass/clover systems. - In: T'mannetje, L., Frame, J. (ed.): Proc. 15th General Meeting of the European Grassland Federation. Pp. 529-538. Wageningen 1994.

Klebesadel, L.J.: Natural selection may modify introduced white clover toward superior winterhardiness. - Agroborealis **18**: 13-19, 1986.

Mächler, F., Nösberger, J.: Effect of light intensity and temperature on apparent photosynthesis of altitudinal ecotypes of *Trifolium repens* L. - Oecologia **31**: 73-78, 1977.

Nösberger, J., Maechler, F., Boller, B.C.: Photosynthesis and dry matter distribution in altitudinal ecotypes of white clover. - In: Smith, J.A., Hays, V.W. (ed.): Proc. XIV Int. Grassland Congress. Pp. 410-413. Lexington 1981.

OECD: OECD schemes for the varietal certification of seed moving in international trade. List of cultivars eligible for certification under the OECD schemes. - OECD 1984.

Prioul, J.-L., Brangeon, J., Reyss, A.: Interaction between external and internal conditions in the development of photosynthetic features in a grass leaf. I. Regional responses along a leaf during and after low-light or high-light acclimation. - Plant Physiol. **66**: 762-769, 1980.

Robin, C., Varlet-Grancher, C., Gastal, F., Flenet, F., Guckert, A.: Photomorphogenesis of white clover (*Trifolium repens* L.): phytochrome mediated effects on ^{14}C-assimilate partitioning. - Eur. J. Agron. **1**: 235-240, 1992.

Snaydon, R.W.: An analysis of competition between plants of *Trifolium repens* collected from contrasting soils. - J. appl. Ecol. **8**. 687-697, 1971.

Stern, W.R., Donald, C.M.: Light relationships in grass-clover swards. - Aust. J. agr. Res. **13**: 599-614, 1962.

Sultan, S.E., Bazzaz, F.A.: Phenotypic plasticity in *Polygonum persicaria*. I. Diversity and uniformity in genotypic norms of reaction to light. - Evolution **47**: 1009-1031, 1993.

Thomson, D.J., Raymond, W.F.: White clover in animal production. - In: Lowe, J. (ed.): White Clover Research. Pp. 277-284. Brit. Grassl. Soc., Maidenhead 1970.

Thompson, L., Harper, J.L.: The effect of grasses on the quality of transmitted radiation and its influence on the growth of white clover *Trifolium repens*. - Oecologia **75**: 343-347, 1988.

William, D., Asiegbu, E.: The effects of variety, cutting interval and nitrogen application on the morphology and development of stolons and leaves of white clover. - Grass Forage Sci. **37**: 15-27, 1982.