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Research article

Selection of shade-adapted subterranean clover species for cover cropping in orchards

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Abstract – The environmental side effects of intensive agriculture have underlined the need to develop sustainable farming systems. In particular, the use of cover cropping in orchards is a means of improving cash crop yield and of reducing the quantity of applied fertilisers. In the Mediterranean environment, subterranean clover species could be the best choice for cover cropping, but they are only poorly adapted to the heavily shaded conditions characteristic of modern high-density orchards. The plant traits needed to improve adaptation of subterranean clover are not well understood. Therefore, in a two-year experiment we studied the effects of four shading levels, of 0%, 40%, 60% and 90% reduction of photosynthetic active radiation, on phenology, growth and development of two subterranean clover species: *Trifolium brachycalycinum* cv. 'Clare' and *T. subterraneum* ecotype 'Ragalna'. Our results show that shading progressively delayed seedling emergence by up to 21 days, the initiation of flowering by up to 27 days, and the end of flowering by up to 25 days. Shading also lengthened the life cycle from 237 to 267 days. Shading reduced both soil cover by up to 38.2% and cover crop density by up to 39.7%. Shading lowered both the quantity of above-ground dry biomass by up to 820 g m^{-2} and photosynthetically active surface area by up to 213 cm^2 $plant^{-1}$. *Trifolium brachycalycinum* 'Clare' was more productive in terms of above-ground dry biomass yield, but *T. subterraneum* 'Ragalna' was better adapted to shading in terms of rapid emergence, earliness and the time taken to achieve soil cover. These species differences suggest that the breeding targets for improving the adaptation of subterranean clover to heavy shading are the ability to maintain earliness and the capacity to quickly break down hard-seededness under conditions of partial shade. A rapid initial increase in photosynthetically active surface area is particularly needed for maximising light harvesting during the early growth period.

***Trifolium brachycalycinum* / *T. subterraneum* / cover crop / orchard / low irradiance**

1. INTRODUCTION

Over recent decades, the intensification of agriculture has brought with it the depletion and contamination of natural resources, a loss of soil fertility and the accumulation of pesticide residues in food. These negative side effects of increased productivity have stimulated the quest for more sustainable agronomic practices, aimed at retaining crop productivity and improving end-use quality, while at the same time reducing the requirement for external inputs (Lu et al., 2000). In this context, cover cropping (the mono- or intercropping of herbaceous plants over either part of a year, or over an entire year) has attracted renewed attention (Campiglia, 1999). Its potential to increase sustainability derives from its reduction in soil erosion (Mitchell et al., 1999) and nitrogen leaching (Wyland et al., 1996), its enrichment of soil organic matter and nitrogen content (especially when leguminous cover crops are grown), its enhancement of soil microbial populations, its release of previously non-available soil phosphorus (Kamh et al., 1999), its encouragement of generalist predator arthropod populations and heartworm communities, and its reduction of pest and weed pressure toward the cash crops (Bugg et al., 1990; Enache and Ilnicki, 1990; Hiltbrunner et al., 2007; den Hollander et al., 2007; Pelosi et al., 2009). The current shift towards zero/reduced tillage farming systems also favours the deployment of cover crops (Lu et al., 2000). In the Mediterranean environment, subterranean clovers have been suggested as the most desirable leguminous species for cover cropping purposes (Knight et al., 1982; Campiglia, 1999; Caporali and Campiglia, 2001). These are annual, cool-season, predominantly autogamous, self-reseeding pasture species, native to the Mediterranean Basin and temperate Western Europe (Morley, 1961). Among the four species within the taxon, *Trifolium subterraneum* L. and *T. brachycalycinum* Katzn. et Morley are the most widely distributed (Pecetti and Piano, 1998, 2002). Both exploit burr burial for self-reseeding (particularly effective in *T. subterraneum*), a device which

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allows the species to produce long-lasting swards in natural pastures (Piano et al., 1993). This property, along with its ability to thrive in poor soils, high nitrogen-fixing capacity and adaptation to the Mediterranean sub-arid climate, has sustained their role as cover crops (Caporali and Campiglia, 2001). Self-reseeding is one of the key characters for economically viable cover cropping, given that it ensures self-regeneration over a number of growing seasons (Campiglia, 1999). Cover cropping in high-density fruit orchards requires plants which are able to adapt to low levels of radiation. Indeed, photon flux density affects many aspects of plant growth, including petiole length, specific leaf area, growth rate, dry biomass accumulation and allocation, flowering time, and seed set across a range of species (e.g. Tester et al., 1986; Steinger et al., 2003; Weijschedé et al., 2006). In self-reseeding species, many of these physiological traits are key for rapid establishment and development, as well as for their effectiveness in achieving soil cover, with resulting implications for both the technical and economic viability of cover cropping. Nevertheless, to date, no specific breeding programme has been directed to improving the performance of subterranean clover under conditions of low light. Cultivars specifically constituted for low light environments would extend subterranean clover cover cropping into orchard production systems, which, conventionally, require substantial quantities of external inputs to remain economically productive. The aim of the present research was to investigate the effects of shading on the growth and development of two subterranean clover accessions, focusing particularly on traits which are important in determining effectiveness for cover cropping.

2. MATERIALS AND METHODS

2.1. Site, climate and soil

One field experiment was conducted over two growing seasons (2004/2005 and 2005/2006) at the coastal plain of South-eastern Sicily (37° 03'N, 15° 18'E, 15 m a.s.l.), in an area where lemon orchards and table-grape vineyards are common. The local climate consists of mild, wet winters, and warm, rainless summers. Winter frost is rare (two events in 30 years). The January mean maximum day and mean minimum night temperatures over the period 1959–1988 were, respectively, 15.3 and 7.5 °C, and the average annual rainfall over the same period was 447 mm. The moderately deep soil is a Calcixerollic Xerochrepts (USDA, Soil Taxonomy), which, at the onset of the experiment, was assessed as being composed of 28% clay, 25% silt, 45% sand, 2% organic matter, 1.8‰ total nitrogen, 78 Kg ha^{-1} assimilable P_2O_5 and 37 Kg ha^{-1} exchangeable K_2O. The soil pH was 8.4, its moisture capacity was 29% (0.34 m^3 m^{-3}) of dry soil, and its wilting point 11% (0.13 m^3 m^{-3}).

2.2. Experimental design, plant material and management practices

A randomised split-plot design with four replications was applied, with four shading levels (0, 40, 60 and 90% of photosynthetic active radiation reduction; these are hereafter referred to as S_0, S_{40}, S_{60} and S_{90}, respectively) as the main plots, and the two subterranean clover entries, *T. brachycalycinum* cv. 'Clare' and *T. subterraneum* ecotype 'Ragalna', as sub-plots. These varieties were chosen because of their rapid growth and ability to generate dense swards (Pardini et al., 1995; Mauromicale et al., 1997). *T. brachycalycinum* is a pure line cultivar released in Australia in 1950 (Caporali and Campiglia, 2001). *T. subterraneum* seeds were obtained from the germplasm collection held at the Department of Agronomical, Agrochemical and Animal Production Sciences of the University of Catania, the original accession having been collected from a natural stand in the Mount Etna area (Mauromicale et al., 1997). Shading levels were imposed to simulate the environment of orchards varying in plant density and/or age, and the maximum shading level imposed – which was intended to simulate the conditions within an adult table-grape vineyard or citrus orchard – was slightly higher than the ~80% reduction in photosynthetic active radiation experienced in an adult hazelnut orchard (Campiglia et al., 1989). Shading was achieved by the erection, at ~1.8 m above ground level, of four black polypropylene nets differing in their mesh density. The main plots were oriented in an E-W direction, with the netting at each end sloping to 0.3 m above ground level to avoid lateral irradiation and to homogenise the microclimate within the plots. The effectiveness of the shading was monitored monthly with a solarimetric bar (LI-COR Line Quantum LQA, One Metre Sensing Length, manufactured by LI-COR Inc., Lincoln, Nebraska, USA). The sub-plot area was 16 m^2 (4 × 4 m) and the main plots were separated from one another by 3 m, to give a net experimental area of 512 m^2. The soil was shallow-tilled shortly before sowing by hand in September 2004 with 500 germinable seeds per m^2. The germination percentages, as obtained following Margot and Tattersfield (1970), of 'Clare' and 'Ragalna' were, respectively, 74.7% and 79.3%. Prior to seeding, 50 kg ha^{-1} P_2O_5 and 20 kg ha^{-1} N were incorporated into the soil to supplement the fertility and optimise plant growth. After sowing, the plots were harrowed and uniformly rolled. As there was a history of *Trifolium* spp. cultivation at the site, no *Rhizobium* supplement was given. To promote germination, the plots were sprinkler-irrigated to field capacity on September 28, 2004 and September 22, 2005. Otherwise, the only interventions involved some minor hand weeding during the course of the trial.

2.3. Meteorological measurements

Meteorological data were recorded on a CR 21 data logger (Campbell Scientific Inc., UK) sited at a meteorological station about 15 m from the experimental plot. Air temperature (minimum, maximum and mean), relative humidity (minimum, maximum and mean), soil temperature at 20 cm (minimum, maximum and mean), wind direction and speed, global radiation, photosynthetically active radiation (PAR), rainfall, and evaporation were recorded every half hour. Under the shading nets, the mean maximum and minimum day temperatures were recorded once a day, using conventional minimum and maximum thermometers.

2.4. Phenological measurements

To measure phenological variables, four randomly chosen 0.25 m^2 micro-areas per sub-plot were non-destructively sampled each day. The following stages were timed in days post the initial irrigation: the beginning of emergence, taken as the day when at least 20 seedlings per m^2 were visible; beginning of flowering, when at least 40 inflorescences per m^2 were visible; and the end of flowering, when all the flowers had either set seed or had aborted. The length of the life cycle was defined as the period between the beginning of emergence and the time when all the plants within a sub-plot had completely dried up.

2.5. Plant growth and development measurements

From each of the randomly sampled 0.25 m^2 micro-areas, on a monthly basis between 30 and 210 days after the initial irrigation, both the percentage soil surface covered by the crop and cover crop density were assessed. The former represented the mean of two independent estimates, and the latter the number of plants per m^2, calculated from a count of all the plants present within the micro-areas. At flowering, a sample of plants was cut at ~1 cm above ground level and weighed. The photosynthetically active surface area per plant was determined using an Area Measurement System (Delta – T Devices Ltd., Burwell, Cambridge, England). A sub-sample of plants was re-weighed and oven-dried at 105 °C, until a constant weight had been reached. This allowed for the calculation of the above-ground dry biomass.

2.6. Data analysis

Bartlett's test was used to test for homoscedasticity, following which the data were subjected to a two-way Analysis of Variance for split-plots, considering the growing season as a random variable (Gomez and Gomez, 1984). Means were separated on the basis of Fisher's protected Least Significant Difference test ($P \leq 0.05$). Soil cover and cover crop density values were normalised, by, respectively, Bliss' and square-root transformation (actual data are discussed), and their temporal progress described by the logistic regression $Y = A + \{B / [1 + (X/t_{50})^D]\}$, where A and B represent, respectively, the lower and upper asymptotes, t_{50} the number of days required to reach half of the inter-asymptote distance (the inflection point), and D the slope of the function. The pooled values of the phenological and agronomic variables in response to shading level were subjected to polynomial regression analysis.

3. RESULTS AND DISCUSSION

3.1. Weather conditions

Total rainfall in the 2004–2005 season fell mostly between October and March (355 out of 454 mm), as is typical for the Mediterranean climate. The 2005–2006 season was rather unusual, in that the rainfall amount was high (572 mm), and 81% of it fell between October and January. Shading progressively reduced both the maximum and minimum mean monthly maximum and minimum temperatures by, respectively, 0.2–4.4 °C and 0.1–3.9°C, with the highest reduction affecting the period from June to August. All temperatures lay within the optimal range for subterranean clover growth, since across the whole two-year period, the mean maxima ranged from 22.9 to 26.7 °C during emergence (October), and from 19.4 to 21.6 °C during flowering (April), while in January, the minima were above 5.8 °C.

3.2. The phenological response to shading

Shading significantly modified phenology, but its effect, in most cases, was season-dependent (Tab. I). In the second season, the beginning of emergence was markedly delayed by shading, increasing from 5 days under S_0 to 8 days under S_{60} and 26 days under S_{90}, an overall delay of three weeks between S_0 and S_{90} (Fig. 1A). Similarly, in the second season, flowering initiation was 27 days later in S_{90} compared with S_0 (Fig. 1B), while the end of flowering was delayed by 25 days (Fig. 1C). Thus, the length of the life cycle was significantly extended by shading, increasing from 237 days under S_0 to 267 days under S_{90}, with a significant trend in relation to the quadratic component of the polynomial regression (Tab. II). The phenology of the two entries was similar in the first season, but in the second season 'Ragalna' emerged more rapidly than 'Clare' (9 vs. 14 days), and completed flowering sooner (214 vs. 220 days) (Fig. 2A–B). As a consequence, in the 2005–2006 season, the life cycle of 'Ragalna' was significantly shorter than that of 'Clare' (239 vs. 249 days) (Fig. 2C).

Under the experimental conditions experienced, the shift in emergence time was probably the most critical effect induced by shading, since this trait has a high impact on the effectiveness of a cover crop to protect the orchard during the autumn (the period during which most of the rainfall can be expected in the Mediterranean climate) from run-off, N leaching and weed establishment. During plant emergence, the mean temperature maxima and minima ranged, respectively, from 22.1 (S_0) to 24.2 °C (S_{90}), and from 16.6 (S_0) to 18.4 °C (S_{90}), which are well within the optimum range for subterranean clover (Turner et al., 2001). Thus it is probably shading per se, rather than any slowing in germination, which either increased hard-seededness at the end of the first season, or altered the dormancy break pattern during the summer, or both. This effect became significant at S_{60} and above. It is difficult to ascertain which of the possible mechanisms was involved; however, Collins et al. (1976) and Taylor (1976) have shown that an increase in hard-seededness of subterranean clover can

Table I. *F*-values of the main factors and their interactions for the phenological and agronomical variables, with the significance resulting from the analysis of variance. (Sh): shading; (G): genotype; (Se): season. NS = not significant; *, **, *** significant at $P \leqslant 0.05$, 0.01 and 0.001, respectively.

Source of variation / Variable	Shading	Genotype	(Sh) × (G)	(Sh) × (Se)	(G) × (Se)
Degrees of freedom	3	1	3	3	1
Begin. of emergence time	17.6***	NS	NS	14.1***	5.9*
Begin. of flowering	31.1***	NS	NS	8.9**	NS
End of flowering	41.0***	NS	NS	7.9*	9.7**
Length of life cycle	34.8***	NS	NS	NS	15.3***
Soil cover trend					
t_{50}^{1}	47.0***	58.2***	NS	20.7***	98.6***
Maximum soil cover[2]	17.4***	NS	NS	11.9***	NS
Cover crop density trend					
t_{50}^{1}	196.1***	NS	NS	85.0***	15.4***
Maximum cover crop density[2]	29.2***	90.7***	NS	19.4***	31.5***
Aboveground dry biomass	169.0***	73.0***	NS	77.1***	21.3***
Photosynthetically active surface area	119.3***	36.4***	NS	NS	57.2***

[1] Estimated parameters.
[2] Observed values at the last measurement date (210 days after starter irrigation).

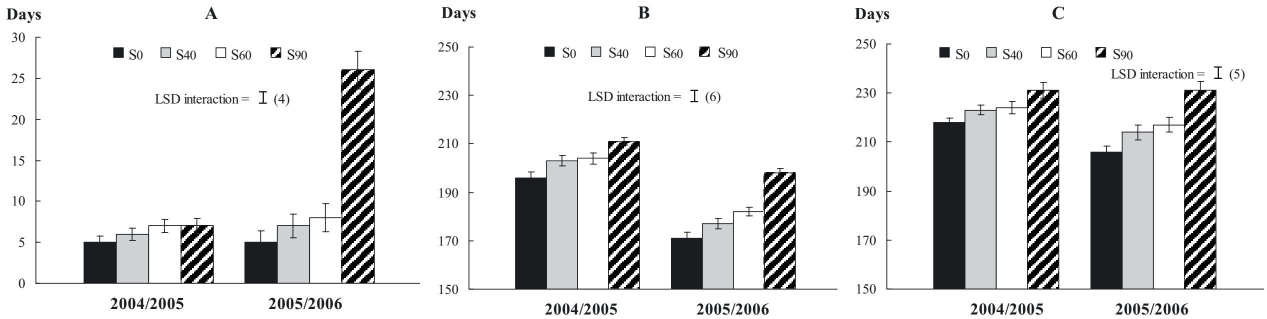

Figure 1. Beginning of emergence (A), flowering initiation (B) and end of flowering (C) as affected by 'shading × season' interaction. Vertical bars indicate the standard error.

occur in response to light deprivation. On the other hand, shading progressively delayed flowering by about 4 weeks, thereby causing a shift in the timing of burr formation. As a result, shading reduced the period of exposure to environmentally-induced softening of the seed. Secondly, the shade-induced lengthening of the cover crop life cycle shifted the timing of full senescence from early May to late June. Low light levels have been claimed to lengthen the period of vegetative growth, while retarding reproductive development (Cockshull, 1988). The use of early-flowering cover cropping cultivars is one of the favoured means for avoiding significant orchard yield losses, but the current data suggest that shading tends to lengthen the period during which the cover crop and the cash crop overlap their life cycle, so competing with one another for moisture. Of the two entries, 'Ragalna' was the more able to buffer the negative effects of shading, particularly during the re-seeding season. Both entries have a different origin ('Clare' is a selected cultivar while 'Ragalna' is a Sicilian ecotype): in-depth studies would need to elucidate possible differences in their population structure, and their role in conferring such a rapid adaptative response against light deprivation. Our data suggest that under highly shaded conditions, adapted material needs to be selected for high earliness and rapid germination, a property which can be achieved by ensuring the rapid breakdown of hard-seededness during the summer months.

3.3. The effect of shading on soil cover and cover crop density

The logistic model fitted the temporal trend of soil cover and cover crop density in both seasons (data not shown). Both shading treatment and entry exerted a significant season-dependent effect on both these traits (Tab. I). The time taken to achieve 50% soil cover (t_{50}) was less in the second season than in the first, both under S_{40} (from 73 to 52 days) and S_{60} (from 90 to 51 days), but was markedly longer under S_{90} (from 92 to 124 days) (Tab. III). This delay had a major effect on the maximum soil cover achieved, which was 61.8% under S_{90}, but was 100% under the other shading levels (Tab. III). The cover crop density t_{50}, i.e. the time taken to achieve 50% emerged plants, was significantly delayed by shading in the second season, especially under S_{60} (increasing from 8 to 60 days) and S_{90} (from 27 to 146 days) (Tab. IV). In the second

Table II. Phenological variables affected by shading and genotype (main effects). Different letters within each factor's column indicate significance according to Fisher's protected least significant difference (LSD) test ($P \leqslant 0.05$). NS: not significant; *, **, ***: significant at $P \leqslant 0.05$, 0.01 and 0.001, respectively; L: linear; Q: quadratic; C: cubic.

Treatment	Begin. of emergence time	Begin. of flowering	End of flowering	Length of life cycle
		Days after initial irrigation		
Shading level				
S_0	5 b	183 c	212 c	237 c
S_{40}	7 b	190 b	218 b	242 bc
S_{60}	7 b	193 b	221 b	248 b
S_{90}	17 a	204 a	231 a	267 a
Trend	C^{***}	L^{*}	Q^{**}	Q^{**}
Genotype				
T. brachycalycinum 'Clare'	9 a	191 a	221 a	250 a
T. subterraneum 'Ragalna'	8 a	194 a	220 a	247 a

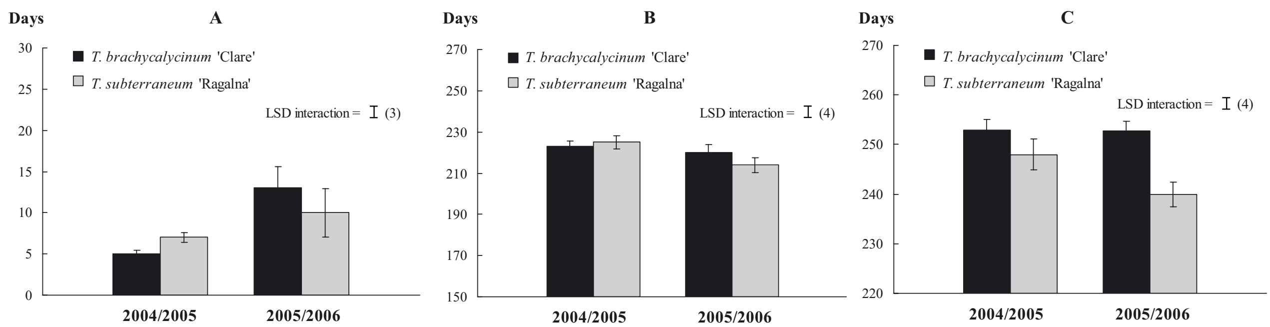

Figure 2. Beginning of emergence (A), end of flowering (B) and length of life cycle (C) as affected by 'genotype × season' interaction. Vertical bars indicate the standard error.

season, the maximum cover crop density was less affected under S_{90} (from 250 to 661 plants per m^2) than under the other shading treatments (on average from 305 to 1109 plants per m^2) (Tab. IV). Comparing the two entries, and passing from the first to second growing season, 'Ragalna' strongly accelerated the time course of soil cover (t_{50} passed from 109 to 65 days) unlike 'Clare' (in which t_{50} passed from 51 to 73 days), and showed, also, the lowest increase in the t_{50} of the cover crop density (from 16 to 77 days in 'Ragalna' and from 10 to 78 days in 'Clare') (Tabs. III and IV). In contrast, 'Clare' showed the highest increase among seasons in maximum cover crop density (from 335 to 1183 plant m^{-2}) in comparison with 'Ragalna' (from 240 to 817 plant m^{-2}) (Tab. IV).

The rapid soil cover achieved under medium shade in the second season reflected a high level of seedling establishment, although its effect only became apparent when the exponential increase phase of seedling emergence started at about one month after the initial irrigation. Under heavy shade, this phase was shifted from early autumn to the beginning of spring, so that most of the seed stayed in the ground for several additional months before germination. Consequentially, there was a long gap during which the soil remained unprotected, corresponding to the period when many weed species could exploit residual nitrogen, mineralised senesced vegetation and early autumn rainfall. The delay in the emergence pattern limited the growing period, making it less likely that the crop could achieve complete soil cover, despite the large population of plants present. The differences in the maximum cover crop density recorded at the time of re-seeding suggest in addition that heavy shading has a large effect on the seed set of both entries, consistent with the observations of Balocchi and Phillips (1997), who noted that light levels below a third of the normal levels were associated with reductions in burr (and seed) formation in *T. subterraneum* cv. 'Mount Barker'. Hence, provided that the cover crop can produce adequate amounts of seed, fast and uniform seedling emergence is a key factor in determining the ability of subterranean clover to rapidly achieve complete ground cover.

3.4. The effect of shading on above-ground dry biomass and photosynthetically active surface area

Shading significantly affected the above-ground dry biomass of both entries, with the extent of the effect being season-dependent (Tab. I). Passing from the first to second growing season, the above-ground dry biomass rose from 476 to 949 g m^{-2} under S_0 (+120%), from 432 to 764 g m^{-2} under S_{40} (+77%), and from 432 to 645 g m^{-2} under S_{60} (+49%); on the contrary, under S_{90} the above-ground dry biomass fell from 315 to 129 g m^{-2} (–59%) (Tab. V). Thus, the above-ground dry biomass difference between the extreme shading levels increased markedly from 161 g m^{-2} in the first season

Table III. Effect of the studied factors on t_{50} soil cover and maximum soil cover of subterranean clover. Different letters within each factor's column indicate significance according to Fisher's protected least significant difference (LSD) test ($P \leq 0.05$). Sh: shading; G: genotype; Se: season. NS: not significant; *, ***: significant at $P \leqslant 0.05$ and 0.001, respectively; Q: quadratic; C: cubic.

	t_{50} (days after initial irrigation)			Maximum soil cover (percentage of plot surface)		
	2004–2005	2005–2006	Mean	2004–2005	2005–2006	Mean
Shading level						
S_0	65	50	58 c	100	100	100 a
S_{40}	73	52	63 c	100	100	100 a
S_{60}	90	51	70 b	100	100	100 a
S_{90}	92	124	108 a	95.0	61.8	78.4 b
Trend			Q^{*}			C^{***}
Genotype						
T. brachycalycinum 'Clare'	51	73	62 b	100	98.8	99.4 a
T. subterraneum 'Ragalna'	109	65	87 a	96.3	91.5	93.9 a
LSD interaction ($P \leq 0.05$)						
(Sh) × (Se)	17			5		
(G) × (Se)	8			NS		
(Sh) × (G)	NS			NS		

Table IV. Effect of the studied factors on t_{50} cover crop density and maximum cover crop density of subterranean clover. Different letters within each factor's column indicate significance according to Fisher's protected least significant difference (LSD) test ($P \leqslant 0.05$). Sh: shading; G: genotype; Se: season. NS: not significant; **: significant at $P \leqslant 0.01$. Q: quadratic.

	t_{50} (days after initial irrigation)			Maximum cover crop density (N. plant m^{-2})		
	2004–2005	2005–2006	Mean	2004–2005	2005–2006	Mean
Shading level						
S_0	9	49	29 c	314	1,097	706 ab
S_{40}	7	56	32 bc	315	1,159	737 a
S_{60}	8	60	34 b	286	1,070	678 b
S_{90}	27	146	86 a	250	661	456 c
Trend			Q^{**}			Q^{**}
Genotype						
T. brachycalycinum 'Clare'	10	78	44 a	335	1,183	759 a
T. subterraneum 'Ragalna'	16	77	47 a	240	817	529 b
LSD interaction ($P \leq 0.05$)						
(Sh) × (Se)	11			93		
(G) × (Se)	5			76		
(Sh) × (G)	NS			NS		

to 820 g m^{-2} in the second season (Tab. V). Shading significantly reduced the photosynthetically active surface area of both entries as well, from 307 cm^2 per plant under S_0 to 93 cm^2 per plant under S_{90} (Tab. V). The behaviour of the two entries differed significantly between the two seasons in relation to both above-ground dry biomass and photosynthetically active surface area (Tab. I). Indeed, passing from the first to second season, the above-ground dry biomass increased from 519 to 651 g m^{-2} in 'Clare' (+26%), while it increased from 309 to 593 g m^{-2} in 'Ragalna' (+91%) (Tab. V). In contrast, 'Clare' showed the strongest reduction of photosynthetically active surface area among seasons (from 283 to 149 cm^2 $plant^{-1}$) compared with 'Ragalna' (from 185 to 149 cm^2 $plant^{-1}$) (Tab. V).

Overall, the data suggest that the loss in above-ground dry biomass in response to heavy shading was due to both a reduction in the number of plants per unit area, and a change in plant morphology. Both entries responded to shading by a loss in photosynthetically active surface area, so reducing their light harvesting capacity and growth potential. However, they did appear able to partially offset the detrimental effects of medium shading on above-ground dry biomass, at least for both the highest number of seedlings per unit area and earliest seedling emergence date, which allowed for a more efficient and longer period of radiation interception. Thus, under highly shaded conditions, in addition to maintaining a rapid rate of emergence, adapted subterranean clover genotypes need to be capable of rapidly expanding their photosynthetic area, in

Table V. Effect of the studied factors on above-ground dry biomass and photosynthetically active surface area of subterranean clover. Different letters within each factor's column indicate significance according to Fisher's protected least significant difference (LSD) test ($P \leq 0.05$). Sh: shading; G: genotype; Se: season. NS: not significant. *: significant at $P \leqslant 0.05$. L: linear; Q: quadratic.

	Aboveground dry biomass ($g\,m^{-2}$ dry matter)			Photosynthetically active surface area (cm^2 $plant^{-1}$)		
	2004–2005	2005–2006	Mean	2004–2005	2005–2006	Mean
Shading level						
S_0	476	949	713 a	366	248	307 a
S_{40}	432	764	598 b	225	164	195 b
S_{60}	432	645	539 b	193	148	170 b
S_{90}	315	129	222 c	152	35	93 c
Trend			Q*			L*
Genotype						
T. brachycalycinum 'Clare'	519	651	585 a	283	149	216 a
T. subterraneum 'Ragalna'	309	593	451 b	185	149	167 b
LSD interaction ($P \leq 0.05$)						
(Sh) × (Se)	71			40		
(G) × (Se)	59			19		
(Sh) × (G)	NS			NS		

order to optimise the light harvesting and overall crop growth performances.

4. CONCLUSION

Cover cropping can enjoy a central role in enhancing the sustainability of farming systems. Essential for its success is the selection of plant types able to adapt their growth to the targeted environment. Our experiment, designed to simulate the conditions found in high-density orchards, showed that low light levels progressively altered the growth and development of subterranean clover, particularly during the re-seeding season. Under heavily shaded conditions, the effectiveness of both entries was limited to the first season. The major phenological modifications induced by shading were a delay in seedling emergence and the lengthening of the overall plant life cycle. The delay in seedling emergence and the lower emergence rate were the primary causes for the longer period required to achieve full soil cover, but also effectively reduced the available growing period, thereby compromising the crop's growth potential. The lengthening of the plants' life cycle had a negative effect on earliness, a characteristic upon which the economic viability of cover cropping in the Mediterranean climate relies rather heavily. The two entries differed in their ability to buffer the negative effect of shading, with 'Ragalna' showing the highest earliness, achieving ground cover more rapidly and increasing its above-ground dry biomass over seasons more than 'Clare'. The latter two traits are probably a consequence of 'Ragalna''s more rapid seedling emergence, which gave it a longer light-harvesting period and better overall growth performances. This behaviour indicates that the rate with which hard-seededness is broken down during summer months is an important determinant of successful re-seeding under conditions of heavy shade. The three most important characteristics that an adapted subterranean clover genotype needs to have, therefore, are fast emergence, rapid early growth under shaded conditions and the ability to retain earliness under shade. These can ensure that maximum use is made of incoming photosynthetically active radiation (particularly during the early growth stages), so allowing the maximisation of above-ground dry biomass accumulation, hastening the achievement of ground cover, and maintaining a sufficient temporal separation in growing period between the cover crop and the cash crop. A component of the first of the three key traits is the speed with which hard-seededness is broken down, particularly under heavy shading. In relation to the second trait, an improved understanding of photosynthetic behaviour under shaded conditions is needed in order to identify suitable selection criteria for enhanced photosynthesis under low light. The attainment of these objectives may be complicated by the association between earliness and hard-seededness which has evolved to allow subterranean clover to cope with the high temperatures and low rainfall characteristic of the Mediterranean sub-arid climate. However, in hilly Mediterranean areas, where neither rainfall nor high temperatures are limiting, natural populations of subterranean clover have been identified as being highly polymorphic for the relevant adaptive traits and their linkages (Piano et al., 1996). These populations therefore represent a sensible starting point for the identification of early flowering individuals, amongst which selection can be applied for favourable dormancy traits.

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