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Growth and water relations of walnut trees (*Juglans regia* L.) on a mesic site in central Italy: effects of understorey herbs and polyethylene mulching

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Abstract

The establishment of trees and associated herbaceous understorey vegetation during the afforestation of former arable lands can decrease soil erosion, increase soil fertility and diversify plantation income. This study reports on the five-year results from experimental plots of common walnut (*Juglans regia* L.) established in association with two different herbaceous understoreys in 1994 in central Italy. Treatments included: (i) walnut established with plastic film mulching in association with subclover (*Trifolium subterraneum* L.); (ii) walnut with subclover; (iii) walnut with a spontaneous herbaceous cover (grassing treatment); (iv) clean-cultivated walnut (control). Stem growth rates and the periodical changes in predawn and midday leaf water potentials of walnut, as well as the annual sward dry matter production, were measured. Over the five-years, the understorey vegetation was competitive towards trees, negatively affecting their leaf water status relative to the control, especially during mid-summer observations, with the onset of summer drought. Tree growth in the grassing treatment was slightly but significantly ($p < 0.05$) reduced in height in comparison to unmulched trees with subclover. Subclover competitiveness towards walnut was completely masked by the plastic mulching, so that mulched walnut with subclover had the highest cumulative stem diameter and height (+20% than control treatment). This was associated with water potentials that were never higher than the control. The subclover-mulched treatment, due to its three main advantages (highest cumulative stem growth, an annual dry matter fodder production of 6.3 t/ha, and soil erosion protection), appears to be a promising cultural model for walnut cultivation in areas without marked drought.

Introduction

In Italy, during the 1990s almost 100,000 ha of forest tree plantations were established on former agricultural lands with grants from the European Union (E.U. Reg. 2080/'92) (Mercurio 2003). In a large percentage of these plantations (40–50%, Minotta 2002), the common walnut tree (*Juglans regia* L.) was planted as the main species, due to the high value of walnut wood in the European market (600 €/m^3, Fady et al. 2003). Current cultural models of walnut plantation forestry in Italy do not utilise tree association either with an herbaceous cover or with intercropping. The second option is not allowed by the E.U. Reg. 2080/'92, despite the fact that the cultivation of an herbaceous understorey amongst the walnut trees can be a very favourable option with several ecological and economic benefits (Dupraz 1994; Dupraz and Newman 1997; Paris et al. 2001). A permanent herbaceous cover can increase soil fertility and reduce the rate of soil erosion, as is evident from research in commercial orchards (Haynes 1980; Loreti and Pisani 1986). In the case of cash or fodder crops (agroforestry cultural models) their early returns can partially offset the long cultivation cycle of trees (Dupraz 1994). On the other hand, associated herbs can strongly compete with young trees for site resources (water and/or soil nutrients) (Nambiar and Sands 1993). These competitive interactions can dramatically reduce the early growth of common walnut trees (Paris et al. 1995, 1998). In order to avoid or reduce competitive interactions, specific cultural treatments and species combinations may be required that are appropriate for the site conditions.

The tree–crop interface is a key factor in any modern agroforestry cultural model in plantation forestry (Dupraz and Newman 1997). Although herbicides are commonly used to reduce the competition between herbaceous vegetation and newly established trees, mulching around the trees may be a more suitable option. Polyethylene mulching spread out along the tree row was shown to be a highly effective means of decreasing the competitiveness of intercropped alfalfa (*Medicago sativa* L) towards young walnut trees in a Mediterranean site in central Italy (Paris et al. 1995, 1998). Other techniques, such as tree root pruning (Ong et al. 2002) and a polyethylene root barrier along the soil profile (Jose et al. 2000), can be used to separate the root systems of intercropped species in agroforestry systems. The latter systems were successfully used in North America for separating intercropped maize (*Zea mais* L.) and black walnut (*Juglans nigra* L.) roots, and was found to be more favourable for increasing the soil moisture available for the intercrop.

The competition intensity of herbaceous crops towards trees can also be strongly affected by the species composition of the herbaceous understorey (Alley et al. 1999); and it has been suggested that cool-season herbs may be less competitive than warm-season ones, from research with young black walnut (*Juglans nigra* L.) trees in continental North America (Garrett et al. 1991). Permanent cool-season clovers (*Trifolium* spp.) are evidently a promising cover crop for use in association with seedlings in newly established plantations, for suppressing weed growth and protecting the soil from erosion (living mulch) in North America (Alley et al. 1999).

Subclover (*Trifolium subterraneum* L.) is an annual cool-legume that may be appropriate as a cover crop in Mediterranean countries. Subclover is native to Mediterranean Europe, Asia and Africa and it is well adapted to summer drought. Its vegetative and reproductive growth occurs between autumn and spring with rainy conditions. In summer, when conditions are warm and dry, subclover typically dies, with its dead phytomass possibly acting as a mulch. Subclover regrows from seed in the autumn with the first rains. As a legume, subclover may also increase soil fertility, and is highly regarded as a forage species, as a pure sward or mixed with other forage species in silvopastoral systems.

It is hypothesized that subclover would be only slightly competitive for nitrogen and water when grown in close proximity to newly established walnut trees, and might therefore be successfully incorporated into walnut silvicultural systems for soil erosion control, and possibly as a commercial intercrop in areas with a mediterranean climate that exhibits summer drought. The objectives of this research were to analyse: (i) the influence of subclover on stem growth and the water status of young walnut trees, both mulched and unmulched with polyethylene film; and (ii) to compare the effect of subclover intercrops on walnut growth and water status with that of a spontaneous permanent cover and clean cultivation.

Materials and methods

Site description

Experimental plots (1.10 ha) were located in the experimental fields of the CNR- *Istituto di Biologia Agro-ambientale e Forestale* (CNR-IBAF), in a hilly volcanic area of central Italy (*Biagio* locality, *Umbria*, latitude 42°43′ N, longitude 12°02′ E; ca. 55 km from the Tyrrenian Sea coast). The climate of the area is transitional between the mediterranean climate, typical of the coastal areas of the peninsular part of Italy, and the cool humid climate typical of the inner hilly areas of central Italy. Long-term average climatic parameters of the study site are not available. Some climatic data for the site collected during the study period are listed in Table 1. During the dry summer months the soil moisture, related to precipitation, is the main limiting factor for walnut growth. Annual total precipitation varied between a minimum of 619 mm (first year) to a maximum of 1054 mm (third year). Annual average temperatures of the site varied between 11.8 and 13.1. This compares with the annual average temperature range of 10–17 °C required by common walnut (Mercurio and Minotta 2000). As reported by the same authors, walnut cultivation for wood production needs at least 5 months with the average temperature not lower than 10 °C, and during the study period the months of May through October had an average temperature higher than that limit. Absolute extreme temperatures recorded during the study period were −7.6 °C and 35 °C, in December 1996 and August 1998, respectively.

Plots were set out across a hillside with a south-easterly aspect of moderate slope (average slope of 19.6%), at about 500 m a.s.l. The site has a 'land capability' (USDA 1975) of between Class II and III. The soil has a rock-free profile depth of 50–70 cm, lying above a soft stratum of sedimentary volcanic rock, and soil texture is sandy-loam (Table 2). Formerly the field was used for agricultural cropping with a typical wheat-clover rotation.

Site preparation, plot maintenance and experimental layout

In February 1992 the soil was deeply scarified to a depth of 1 m, and phosphorous was applied at the rate of 100 kg P_2O_5/ha. The field was then managed as a meadow for fodder production.

In March 1994, the soil was tilled to a depth of 0.3 m, and one-year-old bare rooted seedlings of the Italian walnut cv. '*Feltre*' were planted in hand-made holes at a spacing of 4 m × 4 m. The plantation was then divided in 12 rectangular plots each containing 56 trees. Plots were grouped in three blocks of four plots having homogeneous slope and aspect. In April 1994, black polyethylene (PE) film (2 m width and 0.2 mm thick) was spread out along all tree rows in one randomly selected plot of each of the three blocks. The PE film was centered along tree rows and about 20 cm of each lateral side of the film was inserted into the soil. This left a 3.6 m alley free of PE film between the tree rows. The plantation floor in all 12 plots was maintained free of herbaceous vegetation by two surface roto-tillings in May and July. In unmulched plots, the tree base was maintained free of competing vegetation by two hand weedings.

In early autumn of the first growing season (October, 1994), subclover (cv. '*Trikkala*') was sowed, at the rate of 50 kg/ha, in mulched plots and in three other randomly selected plots (one for each block). Subclover was sown in autumn as is usual for most of the clovers in Italy. In the same period other plots were randomly selected to leave the spontaneous herbaceous vegetation to cover the soil (grassing treatment). Herbaceous vegetation in this treatment was made up of a mixture of the following species, in order of abundance: *Lolium perenne* L., *Trifolium pratense* L., *Dactylis glomerata* L., *Festuca rubra* L., *Rumex acetosa* L., *Asplenium tricomanes* L, *Sinapsis alba*, *Capsella bursa-pastoris* Moench. The remaining three plots of the experimental plantation were kept as clean

Table 1. Climate data for the experimental site during the study period, at Biagio, Umbria, central Italy.

Year	Annual average temperature (°C)	Average of minimum temperature of January (°C)	Average of maximum temperature of July (°C)	Annual total precipitation (mm)	July–August Precipitation	
					(mm)	% of total precipitation
1994	13.1	3.0	28.9	619	19	3.1
1995	11.9	0.3	28.8	745	122	16.4
1996	11.8	3.3	26.2	1054	91	8.6
1997	12.7	3.6	26.9	961	48	5.0
1998	12.4	2.8	28.8	799	50	6.3
Average (1994–1998)	12.4	2.6	27.9	835	66	7.9

Table 2. Soil characteristics for the study site in the first 60 cm profile, at Biagio, Umbria, central Italy.

Sand (%)	77.6
Silt (%)	14.4
Clay (%)	8
Total porosity (%)	11
Bulk density (g/cm^3)	1.12
Organic carbon (%)	1.0
$CaCO_3$	n.d.
pH	6.6
CEC	22.1
Mineralogy	Ac^3, Ct^3, I^3, Ag^2, Qu^2, Fk^2, Pl^1, Sm^1
Classification (USDA soil taxonomy)	Andic distrochrept

Ac = analcime; Ag = augite; Ct = chlorite; Fk = k-feldspar; I = Illite; Pl = (Ca–Na)-feldspar; Qu = quartz; Sm = smecite; 3 = moderate (50–10%), 2 = small (10–5%), 1 = trace (< 5%).
n.d. = not detectable.

cultivated treatment (control). Any control plus PE mulching treatment of walnut tree was included in this experiment. This because it was already well known the strong positive effect of plastic mulching on the early growth of weeded trees of common walnut at the same site (Paris et al. 1995, 1998).

During the subsequent four growing seasons, the control treatment was maintained free of weed vegetation by surface roto-tillings, carried out in the period March to September. Three tillings in each year were sufficient to control the competing vegetation during the walnut growing season. In the subclover-mulched and subclover plots the sward was periodically cut and removed from the plots. In the spontaneous grassing plots, the herbaceous cover was periodically mowed during the growing season. Subclover was harvested twice (in early May and June end/early July) in all years of the intercropping with the exception of the second plantation year when a third harvest was done in mid-October. The spontaneous sward in the grassing treatment was mowed three times (approximately in early May, June end/early July and mid-October) during each year.

The unmulched tree intra-row in the control, subclover and spontaneous grassing plots was kept free of competing vegetation by a mix of chemical and mechanical treatment (glyphosate in mid-April, at a rate of 0.1152 g/ha, plus local mechanical weeding) applied in late spring and followed by a mechanical weeding in mid-summer. Herbicide contact with trees was carefully avoided by the operator using a man-operated spray pump.

Stem growth

At the end of each growing season stem diameters (15 cm above soil level) were recorded with a calliper (precision of 1/10 mm) on 30 inner test trees per plot. Total stem height of those trees was also measured with a metric pole (precision of 1 cm).

Leaf water potentials

During the second, third, fourth and fifth growing seasons, at approximately 15-day intervals, the leaf water potential of the walnut tree was measured at predawn (Ψ_{pd}) and again at midday (Ψ_{md}) using the pressure chamber technique (Turner 1988). Measurements were initiated in June, with the end of walnut leaf extension, and continued through September, until the recovery of tree water status after the summer drought with the late-summer precipitation. Fully expanded, mature and intact apical leaflets were collected from the upper 1/3 of the crown for each observation. For each treatment four leaflets were sampled from four randomly selected trees in each of the three replicates. Leaves were sampled from the same trees in each pair of midday and predawn observations.

Sward yield

The sward yield of the subclover and spontaneous herbaceous cover treatments was measured only during the second and fifth plantation years. Standing vegetation was harvested from three 1 m^2 subplots uniformly distributed along the plots in a north-west/south-east diagonal in all three replicates of subclover-mulched, subclover and grassing treatments. One subplot was located at the plot centre, while the other two were at two opposite corners at 6 m distance from the corner borders. Standing vegetation of the above-mentioned treatments was sampled three times (in early May, June end/early July, and October) each year. A representative sub-sample for each treatment was used for the determination of dry yield after oven drying at 60 °C.

Statistical analysis

Mean plot values were used in the analyses of all data. Sward yield data were analysed using a year repeated measures approach with ANOVAR with the understorey treatments and the years as the independent variables. A three-way ANOVAR was used to analyse walnut leaf water potentials, with the observation days as an independent variable in addition to the understorey treatments and the years. A two-way ANCOVAR was used in the analyses of tree growth data, with understorey treatments and years as independent variables, and tree height of the observation year^{-1} ($H_y{}^{-1}$) as covariate variable. For the planting year, seedling heights were used as data of the covariate variable. ANCOVAR was used after that a significant linear regression was found between tree stem height and diameter values and the $H_y{}^{-1}$, with a R^2 of 0.96*** for both tree dimensions. Planned contrasts were used for comparisons among treatment means and differences were considered significant at $p < 0.05$.

Results

The daily precipitation during the growing seasons of the second through the fifth years is reported in the predawn leaf water potential (Ψ_{pd}) graphs (Figures 2 and 3). Mid-summer precipitation varied between years: August of the second year was the wettest with 115 mm of rain; August of the third and fourth year had identical amounts of precipitation (65.4 mm); August of the fifth year was the driest with 37.2 mm of rain.

There was a highly significant effect of the understorey treatments on tree height and diameter, with $p < 0.001$ for both parameters (Figure 1). The interaction between these independent variables was not significant nor for tree diameter neither for tree height. Over the five-year measurement period as a whole, the walnut trees in the subclover-mulched treatment were significantly larger in diameter and height than the other three understorey treatments ($p < 0.05$). Trees in the control treatment were significantly larger in diameter than that ones with subclover without plastic mulching ($p < 0.05$), but the difference in tree height between these treatments was not significant. Trees in the grassing down treatment had

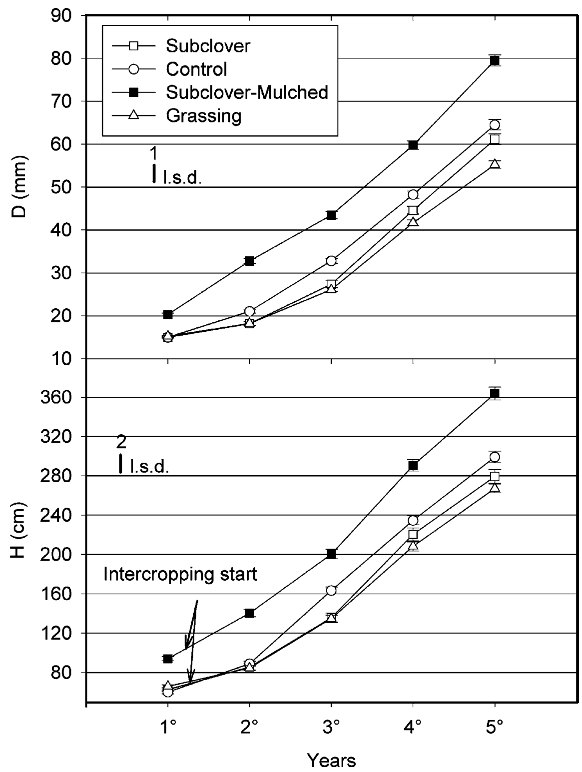

Figure 1. Cumulative growth in stem basal diameter (*D*) and height (*H*) of common walnut under the four understorey treatments during the five years of the study period at Biagio, Umbria, central Italy ($n = \pm 90$). L.s.d. = [1,2] least significant difference between means of any treatment × year combination at $p < 0.05$ for *D* and *H*, respectively. Vertical bars represent ±SE. Bars, when not visible, are smaller than the symbol.

the lowest significant diameter ($p < 0.05$), but their diameter was not significantly different than in the subclover treatment.

There was any significant effect of the understorey treatments on the walnut leaf water predawn potentials, while the interaction between the understorey treatments and the years was highly significant ($p < 0.01$). Over the four-year observation period as a whole, the mulched-subclover walnut trees had never significant higher predawn values than ones of subclover, control and grassing down treatments (Figures 2 and 3). For the midday values, the effect of the understorey treatments over the four-year period was highly significant ($p < 0.001$), and also the two-way interactions of this treatment with the years and the observation day were both highly significant ($p < 0.001$). Differences in midday values among the understorey treatments were concentrated during

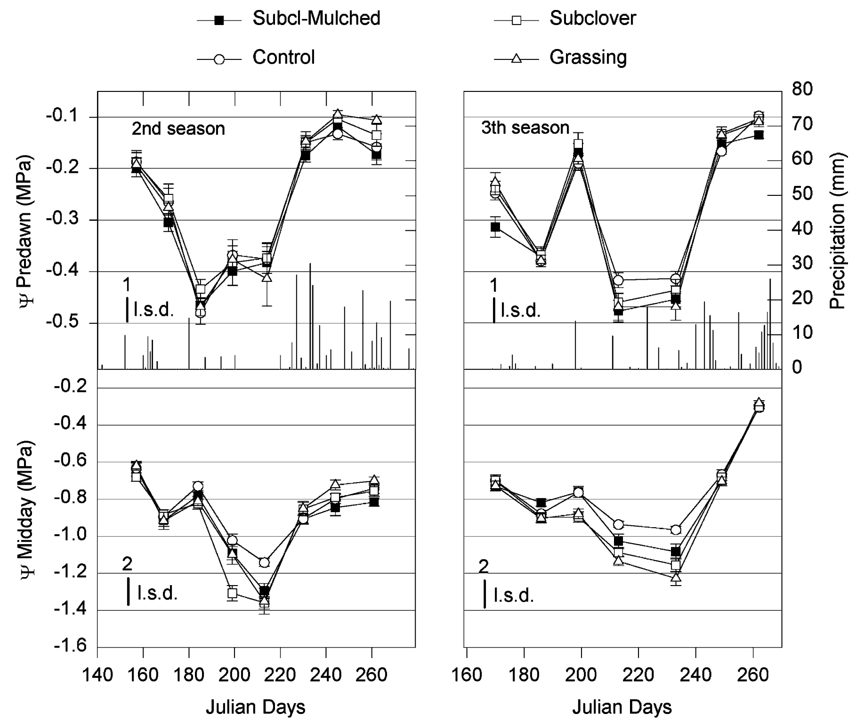

Figure 2. Periodical changes in leaf water potentials of the common walnut under the four understorey treatments during the observed periods of the second and third growing seasons, at Biagio, Umbria, central Italy ($n = \pm 12$). Leaf water potentials were measured at predawn and midday (Ψ_{pd} and Ψ_{md}, respectively). L.s.d. = [1,2] least significant difference between means of any treatment × year × observation day combination at $p < 0.05$ for Ψ_{pd} and Ψ_{md}, respectively. Vertical bars represent ±SE. Bars, when not visible, are smaller than the symbol. In Ψ_{pd} graphs are also reported daily precipitation (vertical bars).

mid-summer observation days, with the control trees having the highest values, with the exception of the fifth season (Figures 2 and 3). Mid-summer lowest values of walnut leaf water potentials were commensurate with the onset of summer drought. (Figures 2 and 3).

There were any significant differences among understorey treatments for the sward dry yield, nor for the years and neither for the interaction between years and the understorey treatments (Figure 4).

Discussion

Mulched walnut associated with subclover (subclover-mulched treatment) had the highest cumulative stem dimensions during the entire study period (Figure 1). This result is in contrast with previous published research for temperate agroforestry systems, which suggest that growth of young trees associated with crops or herbs is always lower than sole-weeded trees (Gordon and Williams 1991; Dupraz and Newman 1997). Although the results suggest that subclover competes less with young trees than the spontaneous grasses, much of the difference observed between the mulch treatment and the control was probably due to growth in the first year prior to the subclover being introduced. The mulched trees had benefited from the positive influence of the plastic mulch for almost the entire first season. In fact, at the end of the first year walnut stem dimensions of the mulched walnut trees were already ca. +35% and +54% larger in stem diameter and height, respectively, compared with the unmulched trees (Figure 1). This result confirms what is already known about the positive effect of PE mulching on the early growth of trees (Traux and Gagnon 1993; Mariano and Sperandio 1994) and, more specifically, on common walnut (Paris et al. 1995, 1998; Piccini and Sperandio 1996).

It is likely that the growth responses of mulched walnut in association with subclover could have

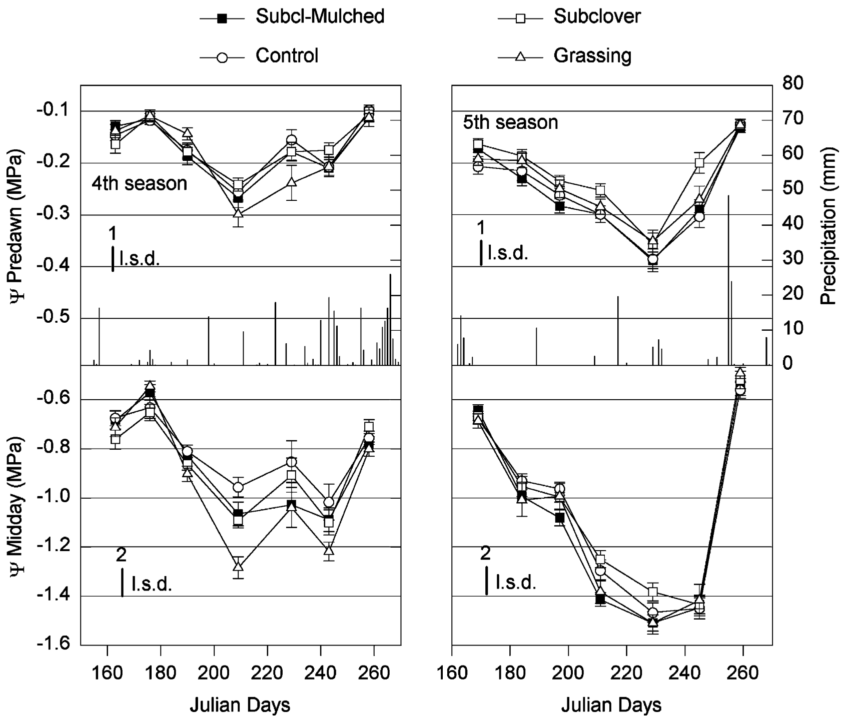

Figure 3. Periodical changes in the leaf water potentials of the common walnut under the four understorey treatments during the observed periods of the fourth and fifth growing seasons, at Biagio, Umbria, central Italy ($n = \pm 12$). Leaf water potentials were measured at predawn and midday (Ψ_{pd} and Ψ_{md}, respectively). L.s.d. = [1,2] least significant difference between means of any treatment × year × observation day combination at $p < 0.05$ for Ψ_{pd} and Ψ_{md}, respectively. Vertical bars represent ±SE. Bars, when not visible, are smaller than the symbol. In Ψ_{pd} graphs are also reported daily precipitation (vertical bars).

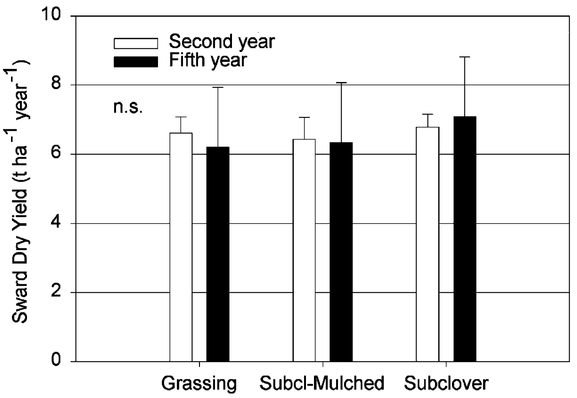

Figure 4. Annual dry yield of subclover and spontaneous swards in the understorey treatments during the second and fifth year, at Biagio, Umbria, central Italy. Vertical bars represent ±SE. N.s. = not significant difference.

been positively affected by the first season of growth, which provided the basis for higher growth increments in the following years. In fact, tree growth follows the law of compound interest, especially in the juvenile phase: the higher is the tree size (capital), the higher is the growth increment (interest). This is because the higher tree size is associated with larger leaf area (and thus larger photosynthesising surface) and root system (Cannel 1989). Actually, a higher crown volume of walnut in the subclover treatment in comparison with the other treatments was recorded during the fifth season (Paris et al. in preparation).

Mulched walnut was somewhat negatively affected by the presence of subclover from the third year onwards. The percent ratio of stem dimensions for the subclover-mulched treatment fell from approximately +55%, relative to the control, in the second season, to approximately +20% of the fifth year (data not shown). Furthermore, this treatment did not generally have higher values of leaf water potentials, as seasonal means, compared with either the control or unmulched subclover treatment. Only during the mid-summer observations of the second and third years did the mulched walnut have higher Ψ_{md}

values than the unmulched walnut with subclover (Figures 2 and 3).

The subclover treatment showed a low competitiveness towards walnut stem diameter growth. The trees grown in the unmulched subclover treatment were significantly larger in tree height than trees in the grassing treatment ($p > 0.05$) (Figure 1). Furthermore, during the fourth and fifth season the mid-summer Ψ_{md} values of walnut in the subclover treatment were slightly higher than in the grassing one (Figures 2 and 3). Thus, water competition seems to be one of the components of the higher competitiveness of the spontaneous grassing in comparison with the subclover. Other causes could be associated with water competition, or with such effects as nutrient competition or allelopathy. Subclover is a nitrogen fixing legume and therefore it could be possible that the trees associated with the subclover benefited from an improved nitrogen status. Although this was not assessed in this trial, previous research has shown that hybrid walnut (*Juglans nigra* × *J. regia* NG23) and black walnut benefit from improved soil nutrition when associated with legume intercrops (Dupraz et al. 1999). Both species, when intercropped with alfalfa or sainfoin (*Onbrychis sativa* L.), had leaflet nitrogen contents higher than trees associated with tall fescue (*Festuca arundinacea* Schr.) or with spontaneous weeds (Dupraz et al. 1999).

Allelopathic qualities, negatively affecting black walnut growth, have been reported for the tall fescue intercrop (Rink and Van Sambeek 1985), although a similar response has not been observed for *J. regia* L.

Conclusions

At the end of the five-year experiment, and after four seasons of association of young walnut trees with either subclover or spontaneous herbaceous cover, our results show that both ground covers reduce tree growth and negatively affect tree leaf water status relative to the control of clean cultivation. The PE mulching of walnut proved to be an effective means of increasing final stem dimensions of the trees intercropped with subclover. These trees had an increase in stem diameter and height growth of approximately +20% in comparison with the control trees at the end of the study period. Fodder production of subclover associated to mulched walnut averaged 6.3 t/ha year in dry matter, a level similar to that of subclover and spontaneous cover without mulching.

These early results are extremely important for the development of management systems for young walnut plantations in Italy. In order to avoid problems of plantation failure, due to competition between the young walnut trees and associated herbs, it is generally suggested to avoid any association of the young trees with herbaceous vegetation in most of the areas of peninsular Italy, with varying degrees of Mediterranean climate. Our results were obtained at a site with a meso-Mediterranean climate, with average precipitation of 835 mm/year. The lower limit indicated in Italy for the successful establishment of walnut plantations is 700–800 mm/year (Mercurio and Minotta 2000). This means that the results of this experiment can be extrapolated to include most of the climatic site conditions suitable for walnut plantation forestry in Italy.

These early results support the idea that a judicious choice of intercropping can bring much more to the deployment of sustainable and successful walnut plantation forestry in Italy than the current monospecific plantations so far established by farmers.

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