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INFLUENCE OF ENVIRONMENTAL AND CULTURAL FACTORS ON STRUCTURE, COMPOSITION AND AGAMIC PROPAGATION OF TWO MEDITERRANEAN SHRUBS (MYRTUS COMMUNIS L. – PISTACIA LENTISCUS L.)

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Summary

Abstract

The crucial point for the exploitation of Mediterranean species for agronomic purposes relies on the availability of planting material with high physiological quality. As it is known, the plant propagation may be realized by seed or by asexual or vegetative propagation. The latter has an unquestionable advantage for the propagator because it allows obtaining plants that reproduce exactly the genetic and morphological characteristics of the parent plant and, theoretically, it allows to obtain a large number of individuals in a short time.

Among the various types of asexual propagation used in the nursery industry, cutting propagation finds the greater application as it is technically easier to run and economically profitable in respect to all possible alternative methods.

It would be advantageous to set up such propagation method for Mediterranean shrubs that are difficult-to-root, on the one hand selecting superior genotypes and on the other hand developing technical strategies for the improvement of donor physiology and the rooting competence.

The environmental factors, irradiance above all, but also nutrient and water availability in the substrate for growth, influence the physiological, hormonal and nutritional status of stock plants and may influence the cuttings quality and their rooting ability. The ontogenetic age of cutting may also influence the propagating attitude of the species.

As regards myrtle and lentisk, few studies have been done concerning the influence of stock plant physiology on rooting. Crobeddu and Pignatti (2005) studied the rooting results of rejuvenated plants, many other Authors focused on micropropagation but no considerations were done about the physiology of donors.

The primary objective of the present research was to enhance the use as ornamental plants of two typical species of the Mediterranean environment, by means of the optimization of nurseries cycles.

In particular, the study focused on physiological and morphological features potentially related to adventitious rooting in myrtle and lentisk.

The specific objectives aimed at:

- Determining the influence of light availability and rejuvenation on the morphological, chemical and ecophysiological features of mature plants
- Assessing the eventual influences on rooting
- Assessing the influence of intra-specific variability on propagation trials, with the declared aim to look for genotypes offering an economically supportable option to propagate the studied species.

Keywords: myrtle, lentisk, rejuvenation, shading, biomass characteristics

1. MEDITERRANEAN ECOSYSTEMS

1.1. Origins, evolution and present status

The Mediterranean-climate ecosystems, despite the adjective "Mediterranean", can be found in five geographically remote regions of the world (California in North America, South Chile in South America, Western and South Australia, South Africa and Mediterranean basin), distributed between 30° and 40° North and South latitude, characterized by warm, dry summers and cool, wet winters.

The total area occupied by the Mediterranean-climate regions is about 5% of the Earth surface, with the Mediterranean basin occupying over the 70% of the area, but they offer harbourage to about 48250 species, almost the 20% of the world total, with a high number of endemic plants (Cowling *et al.*, 1996).

Table 1. Mediterranean-climate regions, relative areas, native flora and endemic species (modified by Cowling *et al.*, 1996).

Region	Area (km^2)	Number of species in native flora	Number of endemic species (% of native flora)
California	320000	4300	35
Central Chile	140000	2400	23
Mediterranean Basin	2300000	25000	50
South Africa	90000	8550	68
SW Australia	310000	8000	75

The present floristic composition of Mediterranean ecosystems comprises an assortment of species with subtropical characteristics that should be considered as a relic from the subtropical forests that clothed the five regions before the global cooling and aridification in Pliocene, and more recent and drought- and fire-adapted lineages.

Herrera (1984) hypothesized that the families with the main affinity with tropical ones are *Anacardiaceae*, *Santalaceae*, *Oleaceae*, *Rubiaceae* and *Myrtaceae*, while the most recent evolutionary families are *Genistae* and *Cistaceae*. Although the species among

the Mediterranean-climate regions are philologically dissimilar, they show varying degrees of ecological convergence to cope with the summer drought.

The typical vegetation type is evergreen, sclerophyllous shrubland or heathland, but most regions are clothed also by extensive forests and woodlands, and shrublands with xeric, drought-deciduous and semi-succulent species, because of the existence of topographically diverse areas and variability of bioclimatic factors. In fact, the Mediterranean ecosystems extend from 0 to 2700 m above the sea level, in areas with a great variability in annual mean temperature (from 5 to 18 °C) and total annual rainfall (from 300 to 2500 mm) that combines with edaphic factors.

The effects of climate fluctuations, in time and in space, on Mediterranean vegetation are insufficient, anyway, to explain the high variability of these ecosystems. It has been established that the anthropic activity have to be considered one of the key factors in the vegetation development, specialization and evolution of Mediterranean ecosystems, together with the climatic fluctuations (Valladares *et al.*, 2004).

In the Mediterranean Basin in particular, where agriculture and animal-breeding have been practiced for 10000 years, man established a close linkage with the natural resources of Mediterranean environments, harvesting woody species as building material or fuel, and attempting to control or eradicate the species considered useless as, for example, the woody species thought to be poor feed for animals (Papanastasis *et al*., 2008). Now, most of Mediterranean environments are "human-modified" (Blondel and Aronson, 1995).

In the present, the conservation status of Mediterranean ecosystems is very poor (Cowling *et al*., 1996) and, further on, it is threatened by a series of disturbing factors that alter their equilibrium. Humans are responsible for some serious alterations on natural ecosystems, principally because of agricultural intensification, overgrazing, industrial activities, land-use changes, fires, the development of unsustainable tourism and the urbanization, especially in the coastal areas. Western (2000) summarized some ecological consequences of human activity on ecosystem processes (Tab. 2). The human pressure results in an impoverishment of floristic composition, the breaking up of forests, and a general loss of native vegetation, in particular in the semiarid and arid regions of Mediterranean Basin, with the immediate effect of denigration of some soil

properties as soil structure, plant nutrient availability, organic matter content and microbial activity, in other words the reduction of soil fertility.

At a global scale, human pressure on ecosystems contributes to the emission of greenhouse gases in atmosphere, in particular carbon dioxide, accelerating the global warming and the ongoing climate changes that, with a feed-back mechanism, are expected to induce a response in the floristic association of vegetal communities, as the single elements of a vegetation are in equilibrium with the physical and chemical environmental parameters.

Table 2. Some ecological consequences of human activity on ecosystems characteristics (from Western, 2000).

Ecosystem structure	Ecosystem processes	Ecosystem functions	Global processes
- Loss of biodiversity - Structural asymmetry and downsizing of communities - Loss of keystone species and functional groups	- Low internal regulation - High nutrient turnover - High resilience - Low resistance - Low variability - Low adaptability	- High porosity of nutrients and sediments - Loss of productivity - Loss of reflectance	- Modified biogeochemical cycles - Atmospheric change - Accelerated climatic change

The climate forecasting models agree in foreseeing a worsening of the already hot and semi-arid climate of southern Europe that is expected to become warmer and drier, threatening its waterways, agricultural production and timber harvests (IPCC, 2002). Biodiversity forecasting models, although limitative in their effectiveness (Botkin *et al*., 2007), indicate a decline in plant richness.

The scientists have been investigating from a long time about the genetics and biology of until now neglected species, in order to better understand the influence of climatic factors on the life of this plants and to preserve, and possibly to increase, the unevenness of threatened environments. At the same time, researchers' interest has been

focused on the functions and influences of Mediterranean vegetation on the environment and on planning the defence strategy against the disturbing factors.
Now, the ecological role of Mediterranean vegetation, particularly in the areas with a low agricultural value, has been clearly defined:

1. Mediterranean plants contribute to the micro-climate regulation, balancing the seasonal and daily thermal excesses;
2. They are a carbon sink, contributing to mitigate the global climate change;
3. They have a fundamental role in the water balance and in the soil moisture conservation;
4. Vegetation is effective in soil conservation. When, the vegetation is removed, the soil surface is affected by the impacts of rainfall drops and water runoff grows up, increasing soil erosion and then decreasing the ability of soil for maintaining life. Many Authors demonstrated that in a wide range of Mediterranean environments both runoff and sediment loss decreased exponentially as the percentage of vegetation cover increased (Elwell and Stocking, 1976; Lee and Skogerboe, 1985; Francis and Thomes, 1990). Many other demonstrated that the protective effect is due both to the aerial part both to the root system that, with a mixed mechanism, immobilize the soil particles (Bochet *et al.*, 2006; De Baets *et al.*, 2009).
5. Plants are the principal antagonist against water floods;
6. They contribute to the conservation of the local fauna, offering refuge and food to wild animals and migrant birds.

Woody plants in Mediterranean climate perform other important roles too, that go beyond the ecological aspects:

1. They qualify the landscape. They have an aesthetic and recreational value and may be promoted as a touristic destination;
2. They are a potential resource of energy and raw materials (timber, firewood, cork, resins, honey, truffles);
3. They are a potential source of secondary substances of pharmaceutical and cosmetic value, or have potential ornamental features.

Since the Mediterranean environment has been recognized, especially in the last twenty years, as a cultural, historical and ecological inheritance to preserve and to restore where necessary, the international and national politics have gone towards the direction of the conservatorship of local resources, landscapes and natural environment, the sustainable management of natural resources and the saving of traditional knowledge about Mediterranean species. This was aimed both to their protection and to their economic revaluation, when the traditional crops suffered, inducing agriculture to think back to cropping models and to valorise the existing woody patrimony (Vieri, 1994). Moreover, the extension of forest areas by the re-establishment of woody species, after a millenarian history of overexploitation, became a practice that is both environmental desirable and encouraged by the agricultural policies of the European Union (Reg. CEE 1609/89; Reg. CEE 1610/89; Reg. CEE 1611/89; Reg. CEE 1612/89; Reg. CEE 1613/89; Reg. CEE 1614/89; Reg. CEE 1615/89; Reg. CEE 2080/92) and by the global change climate politics that aim to increase the carbon sinks and to reduce the carbon sources in the viewpoint of Kyoto Protocol (1997).

1.2. The shrubs in the Mediterranean vegetation

1.2.1. The shrubs in natural maquis

In Mediterranean environments, shrubs characterize a vegetal association known as maquis. Maquis is considered as a regressive stage of the Mediterranean evergreen tree forest, since the floristic composition of this vegetal association is similar to the evergreen forest, except for the presence of tree individuals.

As it was said previously, the deterioration of Mediterranean tree forest is to attribute to the human pressure, especially to the agricultural use and grazing, but also to fires or frequent cuts. In turn, maquis is interpreted as the result of an environmental pressure, determined by the combined actions of unfavourable edaphic (for example drought) and climatic factors (high temperatures, wind) that maintain the association in a condition called “paraclimax”, preventing the maquis development towards the forest association.

The shrub species that can be encountered in maquis are about 150, but the nucleus consists of 40. Anyway, the Mediterranean maquis assumes a great number of typologies in function of the intensity of human or environmental pressure, and they are classified on the basis of physiognomical criterion or the prevailing species.

The main ecophysiological feature that associates the shrubs species of Mediterranean maquis is their capacity to face the summer drought, guaranteeing the soil cover despite very harsh conditions. This is particularly important along coasts, were maquis is sometimes the only type of allowed vegetation by climate conditions.

Mediterranean shrubs developed two main strategies to cope with unwatered environments: the drought avoidance and the drought tolerance.

Some shrubs developed the ability to avoid drought stress (avoidance strategy) limiting the loss of water by reducing of the number of leaves and the size of leaves and canopy; adopting a rapid closure of stomata and a high sensitivity to water potential variations, low values of stomatic conductance and low transpiration rates or maximizing the water absorption by deep root systems and high root biomass/aerial biomass ratios. Both mechanisms maintain in the plant a high water potential. Two examples of shrubs showing this strategy are the *Genista* spp. and *Pistacia lentiscus* L. known to be thrifty species. Other shrubs, like *Cistus* spp., face drought stress by physiological mechanisms opposing to dehydration, tolerating a low water potential in their tissues. For example, they are able to synthesize and cumulate in their cells a large numbers of compounds, including proline, glutamate, glycine-betaine, carnitine, mannitol, fructans and inorganic ions like K+, that helps cell to maintain their hydrated state, a mechanism known as osmotic adjustment; they have tissues with a high resistance to dehydration; they have very stable photosynthetic pigments; they are able to sprout again in case of death of leaves. In most of shrubs the two strategies coexist and the Mediterranean shrubs acquire a similar appearance, developing small leaves, with a thick blade, a leathery consistency, and with some converging anatomical features: thick cuticles, a considerable palisade parenchyma, sunken stomata and cuticular waxes. These features are known as sclerophylly.

Another interesting characteristic of Mediterranean shrubs is their high water use efficiency (WUE) that is their ability to produce relative high dry matter in relation to the consumed water. This characteristic is one of the most appreciated in forest and

ornamental nurseries were the economical sustainability of productions is one of the main goals.

Mediterranean shrubs are also able to face some perturbations that affect Mediterranean environments, as fire. The fire represents a loss of individuals that release physical space, after which ecosystem triggers a process of recovery.

Sometimes, as in the case of *Quercus* spp., the species developed morphological adaptations that allow survival of the individual. Conversely, some species regenerate after wildfires by resprouting of surviving organs (resprouters), or by post-fire seed germination (seeders). Marked differences exist in resprouting ability among the species that regenerate after fire the vegetative aerial part, and within the same species marked differences exist among ecotypes. Typical resprouters are *Arbutus unedo* L., erica arborea, *Pistacia lentiscus* L. and *Myrtus communis* L. Other species, like *Cistus* spp. and *Rosmarinus officinalis* L., are completely burnt by fire and rely on seeds for survival.

These two models enable fast recovery of communities, which tend to recreate the previous vegetation structures, except in case of close and repeated fires. Generally, seeders are more abundant in earlier stages, while an increase of resprouters is expected in later successional stages. However, both kind of syndromes often coexist, showing the complex interactions between these species and fire regime.

1.2.2. The shrubs in re-naturalized environments

In the new picture of increased awareness of environmental issues, the shrubs, once neglected in the re-forestation works, became to assume the same role of trees. The traditional reforestation techniques in Mediterranean-climate environments, in fact, considered shrubs as competitors against newly planted tree seedlings; the shrubs, in consequence, were cleared before tree plantations.

In recent times, it has been point out that the spatial proximity among plants has a facilitative effect in seedlings colonization in environments such as the Mediterranean-type ecosystems that are characterized by summer drought that limits recruitment of both natural and planted seedlings. Seedlings benefit from the habit amelioration by

shrubs, in terms of limitation of excessive levels of radiation and better moisture content of the soil and nutrient availability, showing both a higher survival and initial growth (Castro *et al.*, 2004; Gòmez-Aparicio *et al*., 2004), confirming the theory that pioneer shrubs benefit the establishment of late-successional species in natural (or re-established) ecosystems. This might be more relevant under the predicted rise in temperatures, dryness and rainfall variability for the Mediterranean region under the global warming (IPCC, 2002).

Moreover, the traditional silvicultural management settled a set of rules aimed at planning growing stocks, ages of harvesting and their spatial and temporal distribution, at promoting regeneration (reforestation), at regulating tree density and structural patterns by thinning and at reducing the impact of multiple uses. If on the one hand, traditional silviculture protected and allowed the maintenance of forests, also restoring cleaned areas, on the other hand, management modified the original structure and composition of forests, towards a strong simplification of ecosystem according to the economic purpose of wood production (Fabbio *et al.*, 2003), with some troubles deriving from mono-specific plantations, as an increased susceptibility to fire and soil impoverishment. Nowadays, forest management is addressed towards the safety of natural ecological equilibrium (Mulas and Deidda, 1998).

1.2.3. Mediterranean shrubs and naturalistic engineering

In the last few years, shrubs assumed a particular interest in the renaturation of degraded environments, like quarries, and in naturalistic engineering works (Vilagrosa *et al.,* 2003). These practices are based upon techniques that use alive plants or their parts, alone or in combination with inert natural materials, in order to reduce the risk of erosion.

Some specific architectural and mechanical plant properties affect the interaction between vegetation and the erosive forces. In particular, the desirable plant traits for soil erosion control in arid and semi-arid regions are a dense and relative deep root system, that traps the earthy particles avoiding removal and reinforce the soil at great depth, and a dense ground cover (high stem density) to increase the surface roughness to reduce the movement of water (De Baets *et al.*, 2009).

Moreover, the plants to be used in a given environment must be harmless to other naturally present species, in respect of the whole ecosystem; they must be pioneer, able to colonize and survive in unfavourable environments. As a rule, the autochthonous and well-adapted species, preferably originating in the environment in which they have to be entered, are to be preferred.

Mediterranean shrubs are the natural candidates for the restoration in the arid and semi-arid environments as the characteristics of their root systems, and especially if they are resprouters, make them very suitable for this purpose. Their high variability would allow a choice of types best suited to different situations.

In the present, the lack of knowledge about the behaviour of root systems and canopies of typical maquis species has been a limiting factor in their use in soil bioengineering techniques in Mediterranean environments (Cocozza Talia *et al.*, 2004). The research state is not till now at an advanced stage but the first findings were encouraging in the use of Mediterranean vegetation in slope stabilisation practices (Mattia *et al*., 2005).

1.2.4. Mediterranean shrubs and production of non-wood products

Mediterranean shrubs have been widely used by Mediterranean people for important purposes related to satisfy many requirements of daily life, from nourishment to the cure of diseases of men and animals, from custom and craft use to dyes extraction. Currently, ethnobotanical uses of Mediterranean species are under investigation in order to seek for new economic applications of the traditional utilizations and there is an impressive bibliography on the matter.

The requests that come from the market for natural products, an actively growing market (CBI Market Survey, 2006a; 2006b; 2006c; ISMEA, 2008), addresses toward the enhancement of the knowledge of:

1. Plants useful for production of active principles for medicinal and cosmetic use (essential oils and oleoresins, vegetable extracts, antioxidants, natural colorants);
2. Plants producing food;
3. Plants with ornamental features.

So far, the use of wild resources has been the most practised, especially in Italy, but in the standpoint of conservation of natural resources and the re-thought of model crops for a sustainable agriculture, these species are under observation as potential new crops. They are particularly attractive for an economic agricultural management as they are drought-resistant and have the ability to grow on poor substrates.

The high levels of intra-specific and inter-specific variability of Mediterranean shrubs allow to retrieve types suited to many different cultivation needs and products (Mulas and Deidda, 2004). Many shrub species have been used in traditional knowledge for a multitude of exploitations and this makes them particularly interesting from the standpoint of multi-functionality. The selection of shrub types is obviously the first step of a long path leading to the domestication of the species.

Actually, some knowledge gaps hamper the introduction in culture of most interesting species, in particular:

1. the development of techniques suited to obtain homogeneous plants is needed (cuttings, micro-propagation, others) (Lai *et al.*, 2004; Fascella *et al.*, 2004);
2. The ability of species and ecotypes to adapt to new environments needs to be studied;
3. The ability of species and ecotypes to adapt to crop management needs to be examined (Azaizeh *et al.*, 2005).

2. LENTISK AND MYRTLE: TWO CASES STUDY

2.1. Taxonomy and botanical characters

2.1.1. Lentisk

Lentisk is a small shrub, sometimes a tree, up to 3-4 m high, with paripinnate leaves consisting of 8-10 lanceolate leaflets, 2-3 cm long. The species is dioecious with unisexual flowers in racemes brought separately by male and female individuals (Camarda and Valsecchi, 1983). As a consequence, lentisk is an obligatory out-crossing species where female flowers are wind pollinated. The inflorescences emerge at the base of one year shoots. The fruit is an ovoid or sub-spherical drupe of 5-7 mm, whose colour is black when ripe. The one-seeded drupes are actively dispersed by birds. The seed, lens-shaped, has a hard coat when ripe.

Bud break occurs in late March-early April and shoot growth continues until the end of June - early July. Generally a vegetative stasis occurs in August, when the high summer temperatures induce a stop in the shoot elongation but a second growth season occurs in September up to the first days of October, although it is weaker than in the spring. Vegetative activity stops completely during the winter.

Flowering occurs between March and April, with male anticipating the female flowering (proterandry). Fruit setting occurs in August and ripe fruits appear in autumn (Mulas *et al.*, 1998). In natural environments lentisk regenerate by seed but seed yield in lentisk is poor in quantity and in quality, since numerous seeds have aborted embryos (unviable seeds) (Almehdi *et al.*, 2002), making difficult the propagation in natural environments and nurseries.

2.1.2. Myrtle

Myrtle is a shrub or a small tree up to 0.5 e 3 m high. Two subspecies are described: *M. communis* subsp. *communis* and *M. communis* subsp. *tarentina* (Picci e Atzei, 1996). The leaves are opposite, sub-sessile, oval or oval-lanceolate shaped.

The species is monoecious, with isolated hermaphrodite flowers in the leaf axil. Nevertheless myrtle is a cross-pollinating species. The flower buds differentiate in the year shoot.

The fruit is an edible berry, with different shapes (spherical, egg-shaped, pyriform), whose colour is black or white-greenish when ripe. The seed, small and kidney-shaped, give to fruits an astringent taste (Mulas *et al.*, 2000).

Bud break occurs in late March-April and an intensive shoot growth occurs until the end of June. A vegetative stasis generated by drought occurs in summer but vegetative activity can continue during autumn in mild and rainy climates.

Fruit setting occurs in June and ripe fruits appear in winter, from November to January. In natural environments myrtle regenerate by seed, produced in large quantities by fruits. Seeds are readily germinable, and germination in natural environments is set out in autumn with the arrival of autumn rains (Scortichini, 1986).

2.2. Ecology, functional roles and distribution

Myrtle and lentisk are typical components of the low altitude and thermophile Mediterranean maquis, whose distribution overlaps to the *Oleo-Lentiscetum* association, of which lentisk is a characteristic element. They can be found in mono-specific populations, less frequently for myrtle (Scortichini, 1986).

Lentisk distribution around the Mediterranean basin extends to the North and Eastern Africa and Madeira Islands (Zohary, 1996).

Lentisk occurs in a wide variety of habitats, from open communities in garrigues to close communities in more mesic and shaded sites, in any soil type but with a preference for siliceous substrates (Camarda, 2004; Mulas *et al*., 1998). Moreover, lentisk is high-resistant to soil salinity and drought (Caravaca *et al.*, 2002). In natural environments lentisk assumes a hemispherical or globose cushion-like canopy that causes the shading of the ground and the litter accumulation, and protects soil against erosion. The basicity of its litter promotes humification (Mulas *et al*., 1988). These elements indicate that lentisk has a great potential in ecological reforestation: the species has been successfully employed in environmental restoration works in areas

affected by drought, poor soil fertility where it plays an important role in the evolution of ecosystems to more complex forms.

The biotechnical characteristics of its rooting system indicate that lentisk is useful to stabilise slope terrain especially in deeper horizons (Mattia *et al*., 2005).

Myrtle can be diffusely found in the hilly and coastal sites, prevailing in patches with a high density of species, in cool and sunny environments and in places sheltered from strong winds. It has a moderately heliophilous behaviour.

Myrtle wild populations are found on acidic and sub-acid soils with a granitic matrix, or on basaltic and alluvial soils with a neutral pH.

Myrtle canopy is bush- or tree-like, with a dense foliage (Camarda and Valsecchi, 1983), readily regenerated after the fire or grazing injuries, so myrtle is actively involved in restoring ecosystems altered by damaging events.

2.3. Uses and economic interest

Myrtle and lentisk have been traditionally employed in a wide variety of uses.

They have interesting features in terms of reforestation, but also a multifunctional use which will allow an additional employment in the production of non-timber forest products, like:

1. Production of substances like cosmetics and medicine: essential oils, resins, antioxidants, antiscavenging substances.

Mastic oil has balsamic, anti-inflammatory, sedative and antiseptic properties on mucosas. *P. lentiscus* oil is used in the perfumery, food and pharmaceutical industries. Lentisk mastic is effective in protecting human LDL from oxidation (Andrikopoulos *et al*., 2003). Currently, the exploitation of the lentisk mastic remains concentrated on the Greek island of Chios and specifically aimed at pharmaceutical industry.

Lentisk has been investigated for the antioxidant and anti-scavenging properties of leaves (Atmani *et al.*, 2008) showing a high and dose-dependent reducing power and a very high scavenging ability against DPPH radical.

Myrtle oil is known for its antiseptic and balsamic properties and it is particularly suitable for respiratory diseases. The oil is used in the formulation of cough syrups and balsamic drops. A cosmetic use is also mentioned for the production of perfumes

(Magherini *et al*., 1988). Myrtle extracts and oil show an interesting antioxidant activity, especially during full flowering stage (Yadegarinia *et al.*, 2006; Gardeli *et al*., 2007).

2. Manufacture of food products (spirits, edible oils).

Lentisk fruit oil represented for Mediterranean population a great resource when olive oil was an inaccessible food (Atzei *et al*., 2004). Actually there is a new interest in the lentisk oil for massage therapy.

Myrtle leaves and berries are used as flavouring essences in cooking, especially with meat, but their most important use is the production of liqueurs (Mulas *et al*., 2002).

Lentisk and myrtle are suitable to be used as food for grazing animals (Rogosic *et al*., 2006), representing an important fodder reserve for livestock in harsh conditions in periods of feed scarcity, especially during summer in Mediterranean region. Lentisk leaves, anyway, have a high tannin content that limits intake and digestibility by grazing animals (Ammar *et al*., 2005).

3. Production of ornamentals (cut green foliage, fronds with fruit, potted flowering plants, plants in Mediterranean-type gardens).

Recently the use of native plants in the design of green areas has been re-discovered since shrubs, especially Mediterranean species, showed a great use potential related to reduced water and maintenance needs, a high adaptation to adverse environments, flexibility in the breeding forms, and resistance to disease and pollution, requiring low technical input levels in the crop management. The ornamental value of Mediterranean shrubs relies on the feature of their foliage, fruits and flowers. Moreover, most species has a high degree of branching, which gives a compact and dwarfish shape to the plant. This is particularly suitable for growing in pots and in small spaces (Cervelli and De Lucia, 2004). Moreover, in some Mediterranean shrubs the decorative appearance persists during all year because they are evergreen. This is the case of myrtle and lentisk. A growing use of lentisk has been carried out in the creation of artificial Mediterranean-type gardens (De Lucia *et al.*, 2004), while the twigs bearing unripe fruits, whose red colour create a pleasant contrast with the green of leaves, are also used for flower arrangements, being the most required foliage in the flower market (Cervelli and De Lucia, 2004). Among the Mediterranean shrubs, lentisk is the most suitable for

environmental restoration and the design of marginal areas, such as sloping or rocking sites (Cervelli, 2005).

Myrtle, as lentisk, is one of the basic species in Mediterranean-type gardens but it shows other employments, as its shoots are used as a green frond, as small size potted plant, and in fitting out ornamental hedges (Cervelli, 2004; Ruffoni, 2004).

2.4. Myrtle and lentisk propagation

In Mediterranean nurseries, myrtle and lentisk cultivation takes place in open air. Generally the propagation materials employed in nurseries come from spontaneous plants growing in natural environment (Morini *et al.*, 2003; Ruffoni and Mascarello, 2007).

Myrtle is generally propagated by seed if seedlings are destined to reforestation or by cuttings for ornamental purposes, while lentisk is propagated exclusively by seed since this species is considered difficult-to-root (Mulas *et al*., 1997). This is particularly negative for ornamental plants were a great homogeneity is required and the obtained seedlings are not true to type. For this reason, it would be advisable to look for superior genotypes with high propagation fitness and to improve the propagation methods.

2.4.1 Propagation by seed

For cultivation aims, seeds are collected from myrtle and lentisk plants when fruits are ripe, in autumn-winter. The pulp removal is an obligate passage as it allows the seeds to be selected by densitometry, separating the embryo-missing seeds from viable seeds before sowing (Gorian, 2003). Manual scarification is a desirable practice for seeds of both species to quicken germination (Mulas *et al.*, 1998; Piotto, 2001). Vernalization is an alternative to scarification for spring sowings.

Both myrtle and lentisk are out-crossing species, thus originating seedlings with a high variability in shape, dimensions, leaf and flowers features.

In natural environments myrtle seeds have no apparent dormancy (Traveset *et al*., 2001) and germination can occur soon after dispersal. The seeds have a high germinability and

fast germination but the different genotypes and ecotypes show accentuated differences in seed germination (Mulas *et al.*, 1998; Cervelli and Giampietro, 2004). Some differences were also found between seeds from plants bearing white berries and plants bearing black berries (Scarpa and Milia, 1999). The germination percentage is about 50-80% (Piotto, 2001).

Lentisk seeds are often nonviable because of parthenocarpy or embryo abortion. The colour of the fruits is strongly associated with seed viability: black fruits contain usually viable seeds whereas red ones contain prevailingly nonviable seeds (Jordano, 1989; Garcia-Fayos and Verdù, 1998). Viable seeds are readily germinable (Verdù and Garcia-Fayos, 1996; Piotto, 2001) but the presence of a woody endocarp reduces the absorption of water delaying germination (Piotto, 1995). In natural populations the seed germination is extremely variable, ranging from 0 to 56% (La Viola *et al.*, 2004; Mulas *et al.*, 1998), but some Authors indicate an average germination rate of 40-80% (Piotto, 2001).

The initial developmental stage of seedlings is quite slow and the transplant, especially for container-grown seedlings, is a critical stage in their establishment, especially in natural environments, where soil water availability represents a major environmental constraint under Mediterranean conditions (Vilagrosa *et al.*, 2003) and the ability to develop rapidly deep and well structured root system is an essential feature after planting out (Green *et al*., 1999; Vallejo *et al.*, 2000). Usually, lentisk seedlings require for these reasons nursery treatments before transferring to the field to modify the root system architecture as, for example, periods of drought or mycorrhizal inoculation to enhance survival where soil microbial activity is reduced (Requena *et al*., 2001; Green *et al*., 2005).

2.4.2 Propagation by cuttings

Myrtle can be propagated by rooting of softwood cuttings but some Authors pointed out that the yields are sometimes low (Canhoto *et al*., 1999). Anyway, the rooting performance of the species is influenced by the characteristics of ecotypes and genotypes. In an experiment conducted in Sardinia by Mulas *et al*. (1998) among 67 ecotypes the rooting percentages ranged between 0 and 89.6%, while De Vita *et al.*

(2004) found some differences in rooting performances and in the characteristics of the adventitious rooting system of cuttings from myrtle plants bearing coloured fruits and white fruits and *M. communis* subsp. *communis* and *M. communis* subsp. *tarentina*. Anyway, myrtle seems to be an easily inducible to root species, as hormonal treatments with IBA or NAA stimulate adventitious rooting but with different intensity in function of the collecting season and genotypes (Morini *et al.*, 2003; Mulas *et al.*, 1998). The most favourable season for adventitious rooting seems to be late summer to winter (Mulas *et al.*, 1998) or late autumn to winter (Klein *et al.*, 2000) for apical cuttings picked from adult plants. On the contrary, Morini *et al.*, (2003) found that the best season to root basal hardwood cuttings was early spring (April) in respect to the beginning of summer (June) or autumn (October). Scarpa and Milia (1999) rooted cuttings from young shoots and suckers of spontaneous shrubs at the beginning of August, obtaining rooting probabilities of 90% as average value. De Vita and Lauro (2004) submitted to pruning three-aged myrtle plants and rooted the semi-hardwood cuttings at the end of June, obtaining rooting probabilities of 60% as average value with different hormonal treatments.

Anyway, the physiological status of cuttings, or better, of the donor plant seems to be of basic importance since young plantlets or resprouts of adult plants gave the best results apart from collecting season (Ruffoni, 2004).

Lentisk is known to be a difficult-to-root species (Mulas, 2002). The propagation of adult plants usually brought to poor results (Morini *et al.*, 2003; La Viola *et al.*, 2004), probably due to the browning of tissues in the basal portion of cutting and to the highly foliar abscission. The best results were obtained with apical than sub-apical cuttings in winter (January) (La Viola *et al*., 2004) or in early summer (June) (Morini *et al*., 2003) with cuttings picked from adult plants, dipping the base of cuttings in IBA (5000 ppm) or IBA-K (3000 ppm). The adventitious root system was constituted by a few number of roots, usually one with few lateral rootlets (La Viola *et al.*, 2004).

Crobeddu and Pignatti (2005) found that adventitious rooting in lentisk and myrtle is influenced by the rooting substrate also. In an experiment where they employed peat and pumice (50%), rice chaff (100%), coconut fiber (100%) and rice chaff, peat and coconut fiber (33%), the best results in adventitious rooting of lentisk were respectively 71%, 19%, 67%, 43% using cuttings collected from 1.5 years old plants, grown in an

unheated greenhouse, frequently pruned to stimulate the emergence of new semi-hardwood shoots. For myrtle, the rooting probability associated to the different substrates were 76% in peat and pumice, 67% in rice chaff, 62% in coconut fiber and 81% in rice chaff, peat and coconut fiber (33%) using cuttings collected from stock plants raised in the same way as lentisk.

The same Authors, in a previous experiment (2004) showed that the use of rejuvenated material obtained by the technique known as 'serial cutting' in comparison with cuttings picked by mature plants gave higher rooting probabilities, especially in August rather than in April (tab. 3). The evidence of this experiment is that the physiological status of cuttings is of basilar importance for a successful rooting of lentisk, as in myrtle.

Table 3. Rooting probability for lentisk and myrtle for a combination of season and material type (modified from Pignatti and Crobeddu, 2005). The values in parenthesis indicate the confidence interval.

Species	April		August	
	Mature	Rejuvenated	Mature	Rejuvenated
M. communis L.	58.3 (40.8-74.5)	86.1 (70.5-95.3)	88.9 (73.9-96.9)	91.7 (77.5-98.2)
P. lentiscus L.	0.0 (0.0-9.7)	22.2 (10.1-39.2)	0.0 (0.0-9.7)	77.8 (60.8-89.9)

As an alternative to propagation by cuttings, *in vitro* propagation techniques have been used for the establishment of rapid multiplication of myrtle and lentisk explants.

Protocols for micropropagation of myrtle through axillary shoot development (Khosh-Khui *et al.*, 1984; Nobre, 1994) and somatic embryogenesis (Parra and Amo-Marco, 1999) have been developed, but other Authors adopted successfully protocols with different hormonal concentrations and combinations (Ruggieri D'Itollo, 1987; Lucchesini *et al.*, 1998; Lucchesini *et al.*, 2001; Morini *et al.*, 2002; Ruffoni *et al.*, 2003; Rigoldi *et al.*, 2004) and culture substrates with different elemental balance: Murashige & Skoog (Ruggieri D'Itollo, 1987; Morini *et al.*, 2002; Damiano *et al.*, 2004; Ruffoni *et al.*, 2003) and McCown Woody Plant medium (Hatzilazarou *et al.*, 2001) in agar substrates and Linsmaier & Skoog liquid substrate (Lucchesini *et al.*, 2000) in perlite as inert medium.

The axillary buds seem to be the most suitable material for an *in vitro* rapid multiplication, as they developed the twice of shoots per month in respect to apical buds in the same experimental conditions (Ruffoni *et al.*, 1993), giving a higher number of potential plantlets in a shorter time.

The preservation of shoots *in vitro* at low temperatures is apparently not a good technique to store propagation material, as the deep-freezing at 4 °C for 20 weeks inhibited the shoot multiplication in subcultures in relation to the use of fresh sprouts (Rigoldi and Satta, 2007). The shoot proliferation usually took place in 3-5 months from the beginning of *in vitro* culture. The specific myrtle genotypes show differences in *in vitro* responses in shooting proliferation and rooting phases and in the subsequent acclimatization stage (Frau *et al.*, 2001).

As it is generally recognized that the quality of plantlets cultured *in vitro* affects the ability of micropropagated plants to develop autotrophy *ex vitro*, Lucchesini *et al.*, (2001) improved the quality of myrtle plantlets enriching with CO_2 the gaseous environment of culture vessels. Plantlets grown in CO_2 enriched vessels showed a higher leaf area and dry mass than plantlets grown in closed vessels and a higher survival probability during acclimatization together with higher stem length, root length and fresh and dry mass.

Lentisk showed to be recalcitrant *in vitro* cultures also. Some Authors pointed out a serious difficult in obtaining sterile explants from spontaneous shrubs and the discharge of brown exudates from explants that implicated a frequent medium renewal to avoid micro-cuttings senescence, with best results obtained with the McCown Woody Plant medium (Fascella *et al.*, 2003; Gatti *et al.*, 2003). The most suitable explants were picked in April but the multiplication and rooting protocols *in vitro* needed to be improved.

3 FACTORS INFLUENCING ROOTING IN WOODY PLANTS

It is well established that the adventitious rooting in plants is affected by the interaction between internal and external factors.

Among the internal factors, the endogenous phytohormones play a key role in rooting initiation, being associated with the differentiation of the root primordia, the division and elongation of meristematic cells and the mobilization of the reserve materials to the site of rooting. In particular the natural auxin, the indole-3-acetic acid (IAA), is known for its promoting effects on adventitious rooting in plants, stimulating root initiation on stem cuttings and lateral root development in tissue culture. Indolacetic acid is produced in shoot apical regions, but also in young leaves and leaf primordia, both from tryptophan (Trp) using Trp-dependent pathways and from an indolic Trp precursor via Trp-independent pathways but actually none of these pathways is fully explained. It is known, however, that plants can also obtain IAA by beta-oxidation of indole-3-butyric acid (IBA), another endogenous auxin, or by hydrolysing IAA conjugates, in which IAA is linked to amino acids (like aspartate), sugars or peptides. The inactivation of natural auxin can be realized by plants through conjugation with other small molecules or through the direct oxidation, principally by IAA peroxidase activity (Woodward and Bartel, 2005).

Anyway, the active IAA (free-IAA) diffuse through apoplasts between cells of shoots and relocates in phloem in basipetal direction, towards roots (or stem base in cuttings) by means of specific proteins.

It has been established, however, that not a single hormone but multiple signals regulate an organ development, and ultimately the plant growth, but the understanding of how these multiple signals and signals pathways relate to one other is largely unknown (Alvey and Harberd, 2005). In a number of plants, a gaseous hormone, the ethylene, promotes adventitious root formation (Visser *et al*., 1996) but in others it may also mediate the effect of rooting factors such as auxin, reducing IAA movement as a result of reduced transport capacity (Bleecker and Kende, 2000). Exogenous abscissic acid (ABA) inhibits root formation too, being antagonistic to auxin (Basu *et al.*, 1970). In grapevine rootstocks, a low level of auxin and a very high amount of GA-like and ABA-like substances characterised hard-to-root rootstocks, while high amount of auxins and a

very low level of GA- and ABA-like substances characterised easy-to-root rootstocks (Kracke *et al.*, 1981).

There's also a debate on the modes of action of hormones on target cells. Two different ways are supposed: a) a dose-response effect, in which the hormone acts on a specific cellular pathway in a manner proportional to its concentration; b) a response due to a change in sensitivity to the hormone or to other informative molecules existing in the cellular environment of target cells. These two modes of action are supposed to coexist in plants, and in cereals they have been demonstrated (Amzallag, 1999).

Many other internal or external substances can promote or inhibit adventitious rooting, for example phenolics and terpenoids and some other single substances (brassinosteroids). Their action is species-genotype-dose dependent. It has been suggested that phenolics, in particular, may carry out a role in the plant acting as enzimatic co-factors. In fact, some o-phenolics inhibit and some monophenols exalt the activity of indolacetic acid oxidase *in vitro*, suggesting an interaction between phenolics and hormonal action. More over, some mono- and dihydro-phenols like quercitin, kampherol, apigenin, may inhibit the polar transport of auxins binding to a membrane protein, the NPA (naphtiltalamic acid) receptor. Since phenols are widely diffuse in the plants, they are suspected to be the natural regulators of polar transport of auxins *in vivo*. A particular category of phenols, the hydrossicinnamic acids, are also the constituents of the cell wall in some monocotyledons and dicotyledons influencing the stretching of the cell wall. They are precursors in the synthesis of lignin.

Most plants answer to exogenous hormones favourable to rooting too, and this is why pre-rooting treatments with exogenous hormones are a common practice in nurseries. The various species show a different answer to the various hormones, doses and type of hormonal treatment, but sometimes they fail with the woody plants, where other factors may exercise a synergic control of rooting. Indole butyric acid (IBA) is the most widely used auxin to stimulate rooting in cuttings because of its ability in promoting root initiation (Weisman *et al*., 1988; Ahmed *et al*., 2002) and its weak toxicity and its high stability compared with naftalene acetic acid (NAA) and indole-3-acetic acid (Blazich, 1988; Hartmann *et al*., 1990). IBA itself or combination with other growth regulators at high concentrations is used to increase or accelerate the rooting of plants that exhibit particular rooting difficulties (Sun and Bassuk, 1991; Ozkan, 2000). It has been

suggested that IBA acts as a source of free IAA but it can also exert a rooting enhancement effect on its own, as demonstrated by van der Krieken *et al.* (1992) in microcuttings of apple. IBA conjugates were also reported to be active in root induction (Wiestnan *et al.*, 1989).

Despite the progress in the field of biology and laboratory techniques, it remains difficult to establish a clear relation between the variation in free and bound auxins and the control of adventitious rooting.

Because of the difficulties in studying hormones and hormonal balances and their effects in modifying cellular biochemistry, organelle development and plant morphogenesis, a great number of studies concerning adventitious rooting consider the influence of indirect aspects, like the presence in plants of some substances (nutritional, reserve) suspected to be concerned with rooting, or some morphological or physiological features of the plant to be propagated and the reaction of plant to the application of exogenous auxins.

In most instances, the plant genotype was found to influence the rooting competence of woody plants (Greenwood and Weir, 1994; Metaxas *et al.*, 2004). Heuser (1976) showed that the ontogenetic growth phase of the mother plant was a critical factor for stem rooting with the difficult-to-root species; in particular, the rooting potential was found to diminish with the aging of the mother shoots. Some other Authors hypothesized that ontogenetic phase may be the most important factor affecting rooting in woody plants (Fishel *et al.*, 2003; Bhardwaj and Mishra, 2005).

During their development, plants pass through a series of phases, where the onset of flowering and seed production marks the transition between pre-reproductive phase of juvenile plants and fully reproductive phase of adult plants (Bond, 2000). Maturation is accompanied by slower growth and morphological changes of leaves, which undergo to reductions in growth, reduction in the photosynthetic rates, and as a consequence of these changes, a lower efficiency in rooting is shown (Greenwood, 1987). At a biochemical level, the explanations for the diminishing in rooting competence of cuttings obtained from mature stock plants rely on the accumulation of rooting inhibiters, the decrease in the endogenous content of auxin and/or root promoters, and in the decreased sensitivity of tissues to auxins with physiological aging of the stock (Greenwood and Weir, 1994; Hartmann *et al.*, 1997).

Many Authors tried to characterise the juvenile and the mature phases of development in plants through morphological, biochemical and anatomical markers.

In *Juglans nigra x Juglans regia*, Jay Allemand *et al.* (1989) reported that juvenility and rejuvenation seemed to be related to high values of the ratio of typical polyphenols during the fist stage of growth after bud burst and to PAL activities. In *Corylus avellana L.,* Rey *et al.* (1992) found that the levels of polyamines were higher in juvenile than in adult leaves and that the ratio putrescine to polyamines correlated with the morphogenic capacity of the plant material. In *Quercus* spp. McGowran *et al*. (1996) evaluated a range of morphological criteria in shoot cultures derived from seedlings, stump sprouts, mature crown scions on grafted plants and old hedged stock plants and found that there was a tendency to thicker stems and plagiotropic growth in shoots derived from mature clones. Valdes *et al.* (2004) found that cytokinins were the major hormones involved in maturation and related processes in conifers. In teak, Husen and Pal (2007) hypothized that wood anatomical features like the number and dimension of vessels and fibres may be used as indicator/markers of juvenility/maturity. But till now, there are no clear universal markers known to indicate whether a plant is juvenile or mature.

Anyway, plants show in nature different ontogenetical phases on the same individuals at the same time. Ontogenetic rejuvenation can be found, for example, in mature trees that produce sprouts from the base of their trunks or from their roots (Del Tredici, 1998).

This fact has the consequence to offer vegetal material with different attitude to propagation, and cuttings taken from the basis of the plants usually root more easily (Fishel *et al.*, 2003; Hartmann *et al.*, 1997). For an operational propagation system, however, it is necessary to arrest maturation in stock plants and maintain juvenile rooting characteristics. “Rejuvenation” is usually used to describe the phenomenon that adult tree organs or mature cell formations recover their juvenile characteristics, such as strong rooting ability. In woody plants it can be achieved by a variety of pruning techniques designed to stimulate stock plants to produce easy-to-root shoots. These techniques include hedging, the annual shearing of shrubs or trees to create a geometrical shape of fixed size; pollarding, the annual pruning of the branches to a fixed point to produce an antler-like crown with vigorous sprouts at the ends of branches; and stooling, the periodic pruning of a woody plant to ground level, inducing a vigorous sprouting from the base.

These cultural treatments proved to be effective in stimulating rooting in many woody plants, for example in lolloby pine (Hamann, 1998) and in Virginia pine (Rosier *et al*., 2006). As alternative, a technique used to improve rooting performances is serial propagation, the repeated taking off of cuttings from a rooted cutting of an initial donor plant (ortet). This technique ensures a better physiological status in the younger green material in comparison with the ortet (Krakowski *et al*., 2005) and proved to be successful for conifers propagation (Clair *et al.*, 1985; Mason *et al.*, 2002), *Platanus occidentalis* L. (Land, 1995) and some Mediterranean species (Pignatti and Crobeddu, 2005). The modern tissue culture techniques (micro-propagation) are a more advanced way to partially reverse mature-stage growth phase to juvenility.

Because phytohormones are the chemical regulators of plant development, it is not surprising that the environmental factors affect, directly or indirectly, cellular levels and signalling processes of various phytohormones, such as auxin, gibberellins, cytokinin, ethylene, abscisic acid, and brassinosteroids, and through the alteration of their balance, the rooting process (Fig. 1). Plants, in fact, face continuously changes in surrounding environment, and in order to complete their life cycle they need to respond to external stimuli with an adaptive response to ensure optimal growth and development, in order to realise their intrinsic developmental programs (Chory and Li, 1997; Jeong *et al.*, 2007).

Bertram reported that increased irradiance to stock plants had different effects on the rooting performance of the cuttings, since in some species rooting was increased, in others it was decreased. The hypothesis that explains this behaviour focuses on the central role of auxins whose activity in the plant may be dependent from an optimal irradiance. The decreased adventitious root formation observed when the irradiance to the stock plants exceed the optimal irradiance may be explained by a decrease in sensitivity to auxin or in a reduced amount of tissues in a less dedifferentiated state, in other words, in a lower potential for dedifferentiation. The presence of higher dedifferentiated tissues in young plants is one of the reasons that explain the better results in propagation of seedlings of many woody plants than in the adult donors. Moreover, the levels of other substances influencing the auxin activity and transport may be influenced by light levels.

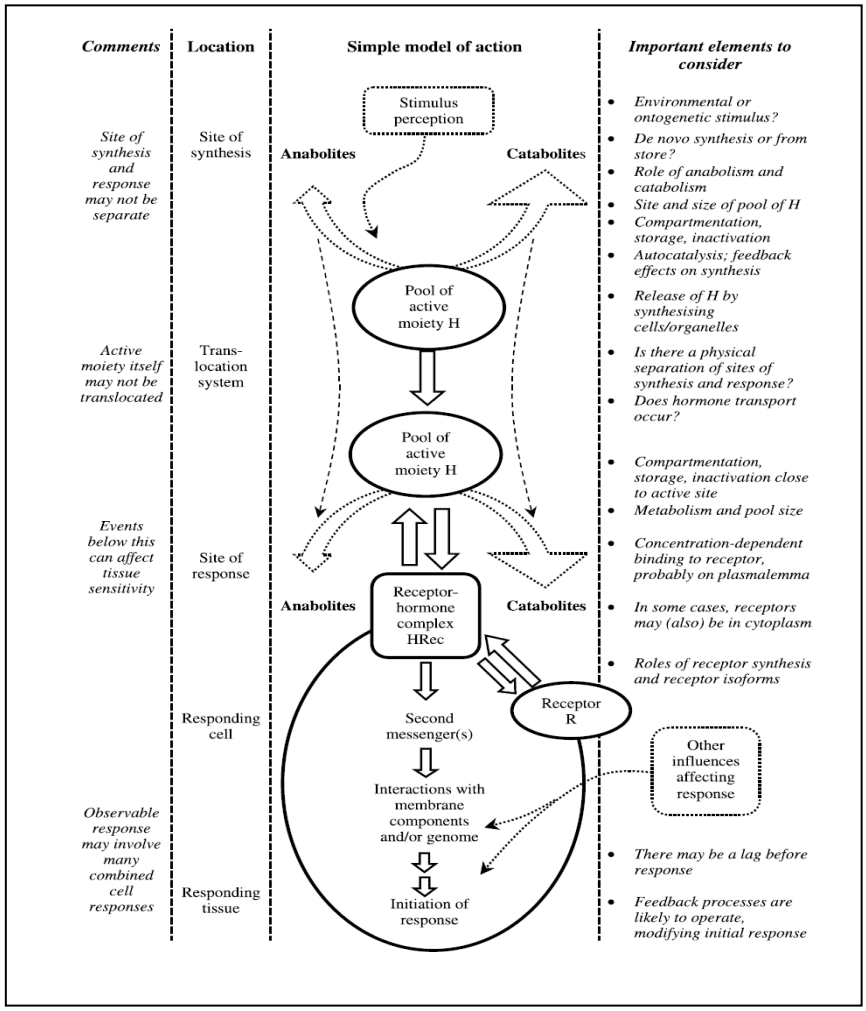


Figure 1. Schematic representation of hormone activation and activities in plants (from Weiers and Paterson, 2001).

Phenols are a category of these substances. The production of phenols in plants generally grows when irradiative levels are high, in accordance with a hypothesis that attributes an ecological significance to phenols. Under high light conditions, in fact, phenols show to be active as a UV filter in the range 280-320 nm, protecting DNA from mutagenesis and NAD or NADP photo-destruction; they have anti-oxidant properties and reduce the probability of photo-oxidation of various categories of compounds in high irradiative levels.

The light manipulation systems commonly adopted in nurseries for the control of plant growth and rooting are etiolation, blanching and shading.

Etiolation is the growing of plants in absence of light or under a heavy shade. Typically, a dormant stock plant is covered by a light barrier and the new growth is made in darkness. Darkness is then gradually reduced after the shoots reach 5-10 cm in length and the shoots allowed to turn green, sometimes with the exception of a portion of the stem, covered with an opaque adhesive band in correspondence of the future base of the cutting (Bassuk and Maynard, 1987).

Blanching is the initial growing of plants in light followed by shading and banding of the basal portion of stems with an opaque band.

Shading is a commonly used practice in ornamental or forestal nurseries both because reduction of irradiance during plants growth is believed to lead to better ornamental features, especially in fronds (Cervelli *et al.*, 2001) and to better physiological features. The shading treatment induces certainly some alterations in the growth and development of the whole plant and single organs inducing morphological, anatomical and physiological adjustments (Conover, 1990; Meletiou-Christou *et al.*, 1994; Kozlowski and Pallardy, 1997) and these alterations facilitate sometimes the adventitious rooting in difficult-to-root species (Bassuk et Maynard, 1987; Pacholczak *et al.*, 2005)).

These light manipulation practices induce a low lignifications in stem tissues, a decreased cell wall thickness and increased protoplasmatic content in cells. The reduction of mechanical properties of stems is sometimes responsible for an increased rooting competence in cuttings (Bassuk et Maynard, 1987).

Other environmental factors known for influencing rooting are water and nutrient availability. Considerable evidence has accumulated that the nutrient status of plant cells can be sensed via the phytohormones and transmitted to the promoters of genes associated with cell division (Beck, 1999). The nutritional status of cuttings, or better, of stock plants have been investigated in many plants and resulted in a great impact of the post-harvest performance of cuttings (Druege *et al*., 2000).

4 OBJECTIVES

The crucial point for the exploitation of Mediterranean species for agronomic purposes relies on the availability of planting material with high physiological quality. As it is known, the plant propagation may be realized by seed or by asexual or vegetative propagation. The latter has an unquestionable advantage for the propagator because it allows obtaining plants that reproduce exactly the genetic and morphological characteristics of the parent plant and, theoretically, it allows to obtain a large number of individuals in a short time.

Among the various types of asexual propagation used in the nursery industry, cutting propagation finds the greater application as it is technically easier to run and economically profitable in respect to all possible alternative methods.

It would be advantageous to set up such propagation method for Mediterranean shrubs that are difficult-to-root, on the one hand selecting superior genotypes and on the other hand developing technical strategies for the improvement of donor physiology and the rooting competence.

The environmental factors, irradiance above all, but also nutrient and water availability in the substrate for growth, influence the physiological, hormonal and nutritional status of stock plants and may influence the cuttings quality and their rooting ability. The ontogenetic age of cutting may also influence the propagating attitude of the species.

As regards myrtle and lentisk, few studies have been done concerning the influence of stock plant physiology on rooting. Crobeddu and Pignatti (2005) studied the rooting results of rejuvenated plants, many other Authors focused on micropropagation but no considerations were done about the physiology of donors.

The primary objective of the present research was to enhance the use as ornamental plants of two typical species of the Mediterranean environment, by means of the optimization of nurseries cycles.

In particular, the study focused on physiological and morphological features potentially related to adventitious rooting in myrtle and lentisk.

The specific objectives aimed at:

- Determining the influence of light availability and rejuvenation on the morphological, chemical and ecophysiological features of mature plants
- Assessing the eventual influences on rooting
- Assessing the influence of intra-specific variability on propagation trials, with the declared aim to look for genotypes offering an economically supportable option to propagate the studied species.

5. MATERIALS AND METHODS

5.1 Study sites

The study was conducted both in Oristano (Sardinia, Italy) and, as far as myrtle is regarded, also in Sanremo (Liguria, Italy).

In Sardinia the experimental field was located within the Experimental Farm at the University of Sassari in Oristano, in the Mid-West coast of Sardinia, Italy (39° 53' N, 8° 37' E, 11 m above sea level, 10 km from the sea) during the years 2006–2008. The mean annual rainfall is about 581 mm, with a large water deficit from May to September and the average annual air temperature is about 17°C.

The site is characterized by an alluvial soil, sub-alkaline. Sand predominates in the first 0.3-m layer, while sand-clay predominates in the 0.3 to 0.6-m layer.

Air temperature and meteorological variables during the experimental period were recorded by the weather station of the Central Office of Agrarian Ecology (UCEA) located in a nearby experimental field located in Santa Lucia (Oristano). Precipitation and air temperatures (minimum and maximum) throughout the years 2006-2008 are summarized in Fig. 1.

In Sanremo the experimental field was located within the Institute of the Research Unit for Floriculture and Ornamental Species (CRA-FSO), in the Western Riviera of Liguria, Italy (43°49'0" N, 7°47'0" E). The site is characterized by an annual rainfall about 680 mm, distributed prevailingly from October to April-May. The annual mean temperature is 16.4 °C, with a temperature range of 14.0 °C. The soil was not characterized as the entire experimental trial was conducted in pots containing a commercial medium with known characteristics. The air temperature and the meteorological variables were recorded by the Institute weather station.

Figure 2. Total precipitation (filled bars) and monthly average air temperatures in the experimental site in Oristano during the experimental period (2006-2008). Source UCEA.

5.2. Plant material

5.2.1. Myrtle plants.

Myrtle plants used for the experimental trials in this work belonged to genotypes selected on the basis of their ornamental feature, in particular for their attitude to be used as fronds or potted plants, by the CRA-FSO - Unit of cultural techniques (clones R, SNM, PA2, I, CG5) and by the Department of Economics and Tree Systems of the University of Sassari (clone CPT5) (Tab. 4).

Myrtle plants were obtained by cutting from stock plants, three years previously the beginning of the experimental trial, and then grown in a cold greenhouse or in open lath house, respectively in Sanremo and in Oristano, and then placed in black polyethylene pots under lath houses or used as bud donors for *in vitro* cultures.

5.2.2 Lentisk plants

Sixteen adult trees of *Pistacia lentiscus* L., ten years aged, were chosen for the tests. The trees were grown from seeds belonging to different lentisk ecotypes growing in the most representative sites in Sardinia Island, selected on the basis of the phenotypic and

reproductive characteristics of superior female donors, estimated as suitable to the use in forest- and ornamental-oriented nurseries (Mulas *et al*., 1997; Mulas and Deidda, 2004). Plants were identified by alphanumerical codes, reporting three letters (the abbreviation of the site of origin of seeds) and one or more numbers (Tab. 5). Plants were grown in full light in a collection orchard in the experimental farm in Oristano. Plants were submitted to water and weed management, while neither fertilizers nor parasiticide were used.

5.3. Experiments with myrtle plants.

5.3.1. Shading

Myrtle plants were submitted to a shading treatment aimed to verify the effect on growth, leaf morphology and some chemical characteristics of plants, used as donors in a rooting experiment.

The experiment was repeated in two sites, Sanremo and Oristano, but in Sanremo the rooting trials were done once, in February 2007. Myrtle plants were placed in pots and then slightly pruned before the shading treatment to induce the growth of new sprouts.

In Oristano, about 50 individuals of *Myrtus communis* L. belonging to the clone CPT5 were grown in pots (50 l), containing soil taken from a field in the farm, enriched with a delayed-release fertilizer (Nitrophoska) to sustain plant growth during the shading treatment, and placed under two shading tunnels, about 17 shrubs per shade treatment, while 14 shrubs were grown in full light conditions and used as reference. The shading rate in tunnels was 65% and 80% in respect of full light conditions as measured by a quantum sensor.

In Sanremo, two selected genotypes (R and SNM) were grown in pots (volume of 85 l) containing a commercial substrate (AG medium) with high organic content and 10% pumice stone 7-12 mm, enriched with a delayed-release fertilizer (Nitrophoska). After a slight pruning, plants were recovered in shading frames where irradiance was reduced to 60% (shading rate of 40%), 40% (shading rate of 60%) and 10% (shading rate of 90%) in respect to full light.

In both sites, plants were conducted by a normal crop management as regards irrigation, nutrients availability and pest and disease control.

5.3.2. Modification of micro-environmental light regimes in in vitro culture

The modification of micro-environmental variables (light intensity, substrate) in an *in vitro* culture of myrtle genotypes was imposed to assess the effect on plant quality and acclimatization performance of cultured plantlets.

Three clones of myrtle ornamental genotypes differing for plant size and leaf shape were used as explants donors. The explants were rinsed in a detergent solution and then sterilized by dipping in 70% (v/v) ethanol for 30 seconds and then in NaClO solution (1.2% of active chlorine) for 20 min and then rinsed three times with sterile distilled water. The initial explants were placed on a pre-incubation medium (Moorashige and Skoog (MS) salts and vitamins) added with agar (7 g l^{-1}), sucrose (30 g l^{-1}) and 6-benzyladenine (BA, 0.3 mg l^{-1}). Ascorbic acid (100 g l^{-1}) and citric acid (10 g l^{-1}) were added in the first two subcultures as antioxidants to avoid the browning of exudates from the base of explants.

The explants were then transferred to a multiplication medium consisting of MS base medium with 0.5 mg l^{-1} BA and 0.2 mg l^{-1} IAA (Ruffoni *et al*., 1994), and then sub-cultured in fresh medium every 28 days. The multiplication phase was performed in a growth chamber at 23±1 °C and a 16 h photoperiod with irradiance of 30 μmol m^{-2} s^{-1}.

At the end of multiplication phase, the surviving explants were cultured onto four different rooting media (MSO Plant Growth Regulator-free or containing IAA 0.5 mg l^{-1} or IBA 0.5 mg l^{-1} or the cytochinin BA 0.1 mg l^{-1} or IAA 0.5 + BA 0.1 mg l^{-1}) and grown in a growth chamber at the same environmental conditions seen before except for irradiance regime, that was established in 25, 50 and 100 μmol m^{-2} s^{-1}. Four replications of 6 plants each were tested for each culture medium and light intensity.

After 40 days, rooted and non rooted plants were evaluated for fresh weight, stem length, rooting probabilities and leaflet chlorophyll content.

The rooted and non-rooted explants after the rooting phase *in vitro* were transferred to a peat-perlite substrate (40/60 v/v), then moved to a greenhouse for acclimatization. Plantlets were placed both under a paper cover or a plastic cover on a bench, and

observed for the rooting development for a 40 days interval. In the greenhouse, the air relative humidity was kept to a constant value of 80% while mean air temperature was about 28 °C. Light levels inside the greenhouse were reduced to 50% in respect to natural irradiation by means of a polyester-aluminium net.

5.3. Experiments with lentisk plants.

Lentisk plants were submitted to two different manipulations aimed to verify their effect on shoot growth, leaf morphology and some chemical characteristics of adult plants, used as donors in subsequent rooting experiments. The first trial aimed to study the effect of a vigorous pruning of the adult plants on some features of the new developed vegetation, and the consequences on the agamic propagation.

The primary branches of ten lentisk plants were pruned to a final height of 80 cm from ground, just before the male blooming, in March 2006, and then the entire plant was cleaned by the surviving stems and leaves. Two adult plants growing nearby the pruned specimens, one per sex, were not pruned and observed as same-age adult controls. After two months, the adventitious buds began to sprout and cuttings were picked from the newly formed shoots at the end of June, when the shoot growth began to slow down and the cuttings were hardened sufficiently to be handled.

The second experiment aimed to verify the effect of an environmental manipulation, the reduction of environmental light levels by means of black shading nets, on shoot growth, leaf morphology and chemical characteristics of lentisk plants used as donors in a rooting experiment performed in two seasons.

Adult plants of *Pistacia lentiscus* L. were partially clothed with shading nets, so that two male and two female plants were covered with black net with a shading rate of 50% in respect to full light conditions. In total, four plants were observed (two per sex).

The choice to shade half canopy of each lentisk plants was induced by the necessity to retain non-shaded reference material with the same genotype of the shaded one.

Table 4. The identification acronyms of the myrtle plants used in the experiments.

Acronym	Ecotype origin	Experimental site
CPT5	Capoterra	Oristano
R	Liguria	Sanremo
SNM	Liguria	Sanremo
PA2	Sicily	Sanremo
Clone I	Liguria	Sanremo
CG5	Liguria	Sanremo

Table 5. The identification acronyms of the lentisk plants used in the experiments. All plants were ten years old and grew in the experimental field in Oristano.

Acronym	**Ecotype origin**	**Plants sex**
ARC2/6	Monte Arci	Female
BOA2	Bosa	Female
MOD1/2	Modolo	Male
OSL1/10	Osilo	Male
OSL2/11	Osilo	Male
OSL2/13	Osilo	Female
PIANTA 1	Pula	Female
PIANTA 10	Pula	Female
PIANTA 11	Pula	Female
PL9	Pula	Male
RUM2/6	Rumanedda	Female
RUM2/12	Rumanedda	Male
RUM2/21	Rumanedda	Male
RUM2/23	Rumanedda	Female
RUM2/31	Rumanedda	Male
RUM3/19	Rumanedda	Male

5.4. Rooting

The rooting trials were conducted for lentisk pruned plants in July and November 2006 and July 2007 and November 2007, for shaded lentisk plants in January and July 2008 and from June 2007 to April 2008 for myrtle plants with two months interval between the rooting runs.

Ten-centimetres long cuttings were picked from the upper of shoots grown in the external side of canopy. A variable number of cuttings (between 45 and 150, divided in three replications) were rooted in each trial for each plant, depending on the shoot availability and the species.

Leaves were removed from lentisk cuttings except for the two-three leaves at the top, which were trimmed to three-six leaflets. The basis of each cutting was dipped in indolbutyric acid (IBA) 0.5% (w/w) dispersed in talc, since in a preliminary experiment this hormonal treatment was the best solution for the rooting of lentisk cuttings.

Leafy myrtle cuttings were dipped one centimetre deep in IBA 1% dispersed in talc (Mulas *et al.*, 1998).

The rooting experiments were conducted in the same conditions for all species in a polycarbonate greenhouse. The cuttings were planted in a medium containing perlite, in a bench under mist system irrigation with sprinkling intervals more frequent in summer. During summer, the greenhouse was covered outside with a black shading net (shading rate 40%) to limit leaf transpiration while during winter a supplemental warming was applied to the rooting medium to keep basal temperature on about 21 °C.

Rooting data were taken at one month intervals and total rooting (percentage of rooted cuttings in respect to the initial number) was calculated by summing up partial values.

5.4.1. Characterization of the rooting system

Number of roots was counted directly from ten rooted cuttings per replication, for a total number of 30 cuttings per treatment. The length of each root was estimated with a meter. The analysis of the root system was possible for myrtle only as in lentisk the

newly developed roots were very fragile and broke during the cutting extraction from perlite.

5.5. Growth pattern observations

Lentisk individuals were observed periodically during the growing season over two years, the rejuvenate plants from March 2006 to September 2008, and the shaded plants from April 2007 to July 2008. Myrtle plants were observed jointly in Oristano, from March 2007 to June 2008.

The growth pattern of plants was observed on the whole canopy in accordance with the elongation rate and the biomass accumulation of annual shoots. Ten shoots were selected on the same individual for detailed measurements. Data were recorded at regular intervals during the growing season in order to determine the shoot elongation rates and biomass accumulation. The determination of the relative growth rate required destructive sampling of newly shoots.

Relative growth rates of shoots, expressed in biomass growth per unit plant biomass (mg g^{-1} day^{-1}) and in elongation (mm cm^{-1} day^{-1}) were calculated according to Hunt (1978).

5.6. Morphological characterization

Fully grown annual shoots and fully expanded leaf samples of shrubs were removed from the upper part of the canopy for morphological, anatomical, chemical and physiological measurements.

The annual final shoot length, the number of internodes and the number of leaves were measured at the end of the growing season, generally between the end of October and December. Mean internodes length was estimated dividing the flush length (cm) and the number of internode units.

Surface and linear dimensions of leaves (10 leaves, 3 repetitions) were measured by means of a computer based analysis system (SkyeLeaf Software, Skye Instruments Ltd, United Kingdom) after the video capturing of the image of the leaf flattened on a light box with fluorescent tubes inside.

5.7. Leaf gas exchange measurements

Photosyntetic active radiation (PAR, μmol photon m^{-2} s^{-2}), net CO_2 assimilation rate (PN, μmol CO_2 m^{-2} s^{-2}), stomatal conductance to water vapour diffusion (g_s, mmol H_2O m^{-2} s^{-2}) and leaf transpiration rate (T, mmol H_2O m^{-2} s^{-2}) and leaf temperature (°C) were measured in three sunny days in the months of June, July and August 2008 with the infrared gas analyser Ciras-1 open system (PP System) equipped with a 2.5 cm^2 area Parkinson Leaf Cuvette (PP Systems, Hitchin Herts, UK). The instantaneous water use efficiency (WUE, μmol CO_2 $mmol^{-1}$ H_2O) was calculated by the ratio of measured PN and E rates.

Leaves were retained in their natural positions during measurements. To maintain a relatively consistent and uniform measurement environment, measurements were taken after the leaf had equilibrated for 45 s in the cuvette. The measures were taken between the 11.30 and 14.30 solar time, using 3-6 completely expanded leaves of the shoot grown in the year, fully exposed to sun, in saturating PAR conditions and clear sky.

5.8. Laboratory analysis

5.8.1. Dry matter determination

Dry weight of stems and leaves was determined after the evaporation of water from an amount of weighted fresh leaves (or stems) in a 80°C forced air oven for at least 48 hours, followed by cooling in a desiccators prior to weighting. The dry matter of the sample was expressed as percent dry weight using the following formula:

Dry Matter %=[100-(Fresh weight-Dry weight)]*100/Fresh Weight

5.8.2. Total phenols determination

Phenols were determined in frozen leaf tissues. The phenols determination was based upon the ability of Folin & Ciocalteu's phenol reagent (1 Hexavalent phosphomolybdic/phosphotungstic acid complexes) to react with phenols to form

chromogens that can be detected spectrophotometrically. The color development is due to the transfer of electrons at basic pH to reduce the phosphomolybdic/phosphotungstic acid complexes to form chromogens in which the metals have lower the metals have lower valence. The Folin-Ciocalteu's reaction is not totally phenol-specific, as it reacts with non-phenolic reducing substances.

Leaf total phenolics were extracted according to Congiu and Franco (1998). Frozen leaves (100 mg/replication) were crushed in a mortar and mixed with about 10 ml of methanol, then put in a refrigerator at 4°C for 24 hours. After filtration, the solution volume was adjusted to exactly 10 ml. The leaf extract (1 ml) was then transferred into a 50 ml volumetric flask, added of Folin-Ciocalteu reagent (2.5 ml) and 20% sodium carbonate (5 ml). After a 70 minutes wait, the time needed for the colour development, the absorbance of the dye solution was measured at 750 nm by a Cary Varian 1E spectrophotometer, using as a reference solution distilled water.

Standard solutions with increasing concentration of pure (+)catechine were used to infer the phenols content of the sample solution.

5.8.3. Chlorophylls and carotenoids in leaf tissue

The chlorophylls and the total carotenoid content were determined according to Lichtenthaler (1987). Ten frozen leaf disks (1.25 cm^2 each) were weighted and crushed in a mortar, added of 10 ml of 80% acetone, and the homogenate preserved in a refrigerator for 12 hours. After the centrifugation, the supernatant volume was adjusted exactly at 10 ml, and then the absorbance of the solution was measured at 470, 647 and 664.5 nm, the absorption peaks respectively for carotenoids, chlorophyll a and chlorophyll b. The global amounts of pigments (mg g^{-1}) were inferred applying the formulas indicated by the Author. A subsequent correction of global amounts of pigments in terms of dry weights was performed after the desiccation of leaf disks.

5.8.4. Minerals (macroelements, microelements, N, P)

Minerals were determined in a solution obtained after grinding, incinerating in a muffle furnace 1 g of dried samples of leaf tissues. After incineration the ashes were solubilised

with 4 N HCl (5 ml), than the solution was filtered and the volume was adjusted to 100 ml. The solution was used to measure microelements (Zn, Cu, Mn, Fe) or, after a dilution, to measure macroelements concentration (Ca, Mg, Na, K) by atomic absorption spectroscopy with a Perkin Elmer (Mod A) atomic absorption spectrophotometer.

5.8.5. Total Nitrogen content

The Kjeldahl method was used to determine total nitrogen in leaves. After the digestion of 1 g of dried sample in concentrated sulfuric acid and selenium as catalyst, the resulting solution containing ammonium sulfate was added of an excess base to convert NH_4 to NH_3. The NH_3 was recovered by distillation in a receiving solution and then quantified by back titration.

5.8.6. Total Phosphorous content

Total phosphorous was determined by spectrophotometry. Phosphate in presence of Ammonium molibdate and hydrazine sulfate, in an acid medium, forms a dye complex whose colour intensity is proportional to the phosphorous amount in the solution. The absorbance of the coloured solution was measured at 650 nm, using as reference absorbance blank a solution containing the same components of dye solutions excluding the sample.

5.8.7. Total starch content in leaf tissue

Starch determination was carried out according to the Megazyme Total Starch Assay procedure, a two-phase enzymic procedure based upon the use of thermostable alfa-amylase and amyloglucosidase. In the first phase, the starch was partially hydrolyzed and totally solubilised. In the second phase the starch dextrins were quantitatively hydrolysed to glucose by amyloglucosidase and then glucose was measured spectrophotometrically at 510 nm. The single steps of the procedure are accurately

described in the booklet accompanying the total starch kit to whom we address for further information.

5.8.8. Leaf colour measurements

Leaf colour measurement were done with a Konica Minolta colorimeter (mod CR-200) using as light source the Standard Illuminant D65. The CIELAB space was used to define colour of leaves. In this colour space, the colour is expressed by three coordinates representing the lightness of the color (L* = 0 yields black and L* = 100 indicates diffuse white), its position between red/magenta and green (a*, negative values indicate green while positive values indicate magenta) and its position between yellow and blue (b*, negative values indicate blue and positive values indicate yellow).

5.8.9. Statistical analysis

All statistical tests were performed using a statistical software package (STATISTICA, StatSoft, USA). Differences in morphological, physiological and chemical traits were determined by analysis of variance (ANOVA) and LSD test for multiple comparisons.
For shading treatments of myrtle, an analysis of variance (ANOVA) with light treatment as the main effect was used to determine effects of the treatments on the measured traits. In lentisk, shade treatment, gender and shade treatment x gender effects were analyzed using the general linear modelling (GLM) procedure.

6. RESULTS AND DISCUSSION

6.1 MYRTLE

6.1 Effects of shading treatments on myrtle.

Results about shading effects on myrtle will be presented separately for the two experimental sites, Oristano and Sanremo.

In Oristano, myrtle (clone CPT5) showed to be vegetative active for the most part of the year. The clone had a rhythmic growth, with a peak in stem elongation in spring (april-may), followed by a vegetative stop during the summer months, when temperatures began to grow up, followed by a weak growth during autumn (Fig. 3). Blooming resulted completely suppressed in the most intensive shade rate. The irradiance reduction did not completely suppressed flowering in 35% shade. Blooming was, anyway, very scarce in relation to the full sunlight environment and the subsequent fructification practically absent.

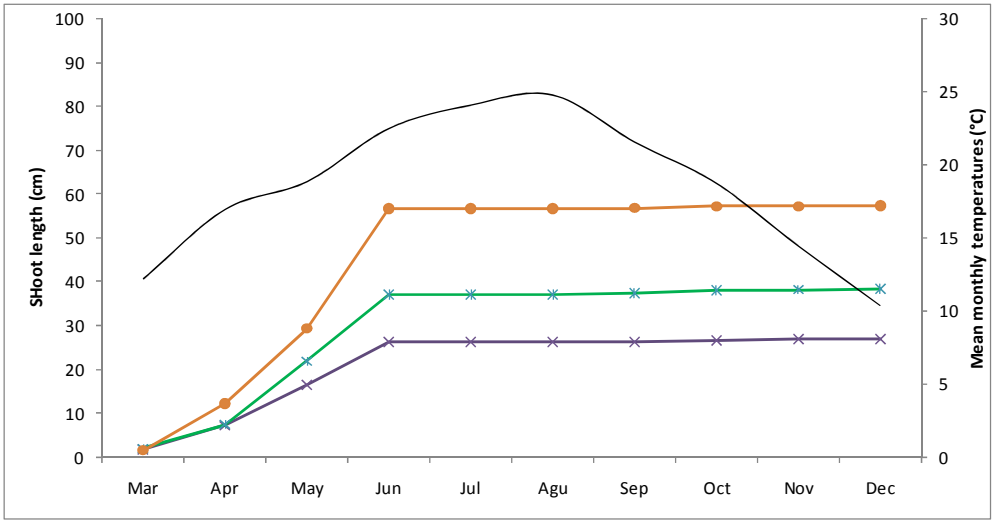


Figure 3. Trends of shoot growth of myrtle plants under differential shade (year 2007). The continuous black line refers to mean values of monthly temperatures, the coloured lines refer respectively: orange – plants under irradiance 20%; green – plants under irradiance 35%; violet – plants in sunlight.

Light environment induced marked alterations in *Myrtus communis* annual stems as well as in leaf morphology and composition. The response of the myrtle plants to light rates was similar to those observed in many other woody species growing under different light regimes (Cardillo and Bernal, 2005; Doley, 1978).

Shoot growth

As far as the shoot growth is regarded, CPT5 showed to be characterized by a great plasticity in adaptation to shade rates. In fact, shoots became longer with the increasing of shade rate and in the most severe light level reduction shoot length reached the maximum value (Fig. 3). This is a common feature in woody plants characterized for having a shade tolerance strategy, aimed to project photosynthetic apparatus towards more favourable light levels. The differences in stem elongation among shade treatments seemed to be attributable to a higher relative growth rate during the favourable season than to differences due to a longer growth season in the different light environments (Tab. 6). In fact, shoots showed to have a well synchronous growth, developing mostly within the month of June.

Table 6. Relative growth rates between 0 and 90 days shown by myrtle shoots developed under different light availability .

	Irradiance		
	100%	35%	20%
RGR (cm cm d^{-1})	0.032 c	0.034 b	0.045 a
RGR (mg g d^{-1})	2.66 c	9.34 b	17.20 a

Shoot structure.

The morphological characteristics of shoots were likewise submitted to differential adaptation. The stem length and the basal diameter of shoots increased linearly from the sunlight conditions to the heaviest shading rate while the number of internodes was lowest in the 35% shade, but it differed statistically only from the number of internodes in the heaviest shade. No statistical differences were assessed between internodes length in shoots grown in shade, but only between shoots grown in sunlight and in any shade rate (Tab.7).

Table 7. Structural characteristics of shoot stems grown under differential light levels. Different letters in the same row indicates values statistically different at ≤ 0,05, LSD test.

	Irradiance		
	100%	35%	20%
Stem length (cm)	25.96 c	42.9 b	57.43 a
Stem diameter (mm)	2 c	3 b	4 a
Number of internodes	23.667 ab	21.267 b	26.867 a
Internode length (cm)	1.077 b	1.963 a	2.071 a
Nr of leaves/stem	46.80 ab	44.533 b	55.733 a
Nr of leaves/unit of stem length	1.802 a	1.031 b	0.964 b
Nr of lateral shoots	0.867 b	4.133 a	5.733 a
Nr of lateral shoots/unit of length of leading shoot	0.033 b	0.100 a	0.095 a

The number of leaves, as a consequence of the differences existing in shoot structure, varied in the single shoots and in the unit of length of shoot. In particular, the stems grown in the heaviest shade showed the highest absolute number of leaves but the lowest leaf density. This behaviour, considering also the highest internode length that characterize the shoots grown in heavy shade in respect to sunlight-grown shoots, indicates an investment which enhance leaf display under reduced irradiance, as shown by many woody plants tolerant to shade like *Quercus* spp. (Banez *et al*., 1999).

The number of lateral sprouts for each stem increased in the shaded plants, especially when growing under 20% irradiance but with no statistical differences between shading rate of 35 and 20%. Sensible differences existed, on the contrary, among fresh and dry weight of shoots in the different light environments. The shoot absolute weight increased from sunlight environment to heavy shade both in terms of fresh and dry values. This is due principally to the great differences in length of shoots, but also to the larger stem diameter under low irradiance.

Biomass and biomass allocation pattern in stems

Marked differences on biomass allocation in newly formed shoots existed among plants developed in the different light rates. Leaves and stem absolute dry weight increased in

shade, but the proportion of leaves, expressed in terms of dry weight percentage in respect to total shoot weight, was higher in full light conditions (Tab.8).

Table 8. Fresh and dry weights of shoots and biomass distribution between stems and leaves. Different letters in the same row indicates values statistically different at ≤ 0,05, LSD test.

	Irradiance		
	100%	35%	20%
Shoot fresh weight (g)	3,20 c	7,56 c	16,56 a
Shoot dry weight (g)	1,33 c	2,92 b	6,86 a
Shoot dry weight (%)	41,47 c	38,82 b	41,35 a
Leaves dry weight /shoot (g)	0,87 c	1,79 b	3,23 a
Stem dry weight/shoot (g)	1,33 c	2,92 b	6,86 a
Leaf dry weight /shoot dry weight (%)	66,11 a	61,75 b	47,99 c

Leaf characteristics.

The leaf size varied widely among treatments. The leaf length, width and area increased with the reduction of light levels. The leaf length/width ratio was lower in leaves developed in sunlight, but it was similar for both shading treatments (Tab. 9). This indicates that in shade leaves linear development is higher in length than in width. Finally, the leaf area increased linearly with the shading rate intensity.

Table 9. Leaf length, leaf width, leaf area and leaf length/leaf width ratio in leaves grown under different light levels. Different letters in the same row indicates values statistically different at ≤ 0,05, LSD test.

	Irradiance		
	100%	35%	20%
Leaf length (cm)	2,840 c	3,587 b	4,523 a
Leaf width (cm)	1,180 c	1,417 b	2,042 a
Leaf length-width ratio	2,231 b	2,533 a	2,417 a
Leaf area (cm^2)	2,295 c	3,523 b	6,318 a

Ecophysiological characterization.

During the month of July 2007, the ecophysiological parameters of myrtle plants were monitored in the middle hours of the day to compare the leaf activities in the different light environments. Data are reported in Tab. 10.

Table 10. Ecophysiological parameters of myrtle leaves grown under different light levels. Different letters in the same row indicates values statistically different at ≤ 0,05, LSD test.

	Irradiance		
	100%	35%	20%
PAR	1861	667	384
Air temperature (°C)	34.8 a	32.2 b	31.2 c
Transpiration (T, mmol H_2O m^{-2} s^{-2})	1.13 a	0.51 b	0.44 b
Stomatal conductance (g_s, mmol H_2O m^{-2} s^{-2})	40.1 a	24.09 b	21.3 b
Leaf temperature (°C)	34.8 a	31.4 b	30.6 c
Net Photoynthesis (PN, μmol CO_2 m^{-2} s^{-2})	3.37 b	5.32 a	3.83 b
WUE (μmol CO_2 $mmol^{-1}$ H_2O)	3.08 b	10.50 a	8.73 a

In the different light environments the air temperature was related to the shade rate, as in sunlight air temperature was higher and in shade it was lower but with different values in 35% shade and 20% shade. This parameter, together with the PAR levels, influenced the transpiration rate of leaves, that was higher in light, and the leaf temperatures, decreasing from sunlight to the heaviest shade environment. No differences where found for stomatal conductance (Gs) in leaves under shade, while sunlit leaves showed the highest Gs value. The different levels in leaf transpiration rate influenced the water use efficiency, the plants grown under shade being the most effective in the water use, without sensible differences between the shading rates.

Total phenols and pigment content in leaves

Both the photosynthetic pigments and the total phenols content in leaves resulted influenced by the light environment (Tab. 11).

In particular, the phenols content, expressed in term of unit fresh weight of leaf tissue, was higher in shade, in particular in 35% shade rate, but no statistical differences

existed between leaves grown in the most intensive shade level and leaves grown in sunlight. A similar trend was observed for phenols content in terms of dry weight.
Under reduced irradiance, plant growth was accompanied by a linear increment of chlorophylls, expressed both in relation to fresh and dry weight of leaves. Moreover, the ratio chlorophyll a/chlorophyll b assumed higher values in leaves grown in full light.

Table 11. Total phenols and chlorophyll content in myrtle leaves grown in differential light levels. The values are the annual mean values. Values with different letters are different at p≤ 0.05 (LSD test).

	Irradiance		
	100%	35%	20%
Total phenols (mg g^{-1} FW)	46,36 b	64,84 a	49,76 b
Total phenols (mg g^{-1} DW)	105.6 b	152.6 a	117.2 b
Chlorophyll a (mg g^{-1} FW)	0,94 c	1,17 b	1,35 a
Chlorophyll a (mg g^{-1} DW)	2,17 c	2,74 b	3,22 a
Chlorophyll b (mg g^{-1} FW)	0,35 c	0,46 b	0,57 a
Chlorophyll b (mg g^{-1} DW)	0,82 c	1,08 b	1,36 a
Chlorophylls ratio a/b	2,66 a	2,53 b	2,38 c

The phenols and photosynthetic pigment content, however, showed a consistent seasonal variation through the year, as expected for leaves in different developmental stages (Penuelas and Estiarte, 1998). The peak in total phenols contents were registered in autumn-winter, in particular in the months of October and December (Fig 4).
The chlorophyll concentration varied among the season too. In particular, the clone was characterized for having higher contents in autumn-winter than in summer months (Fig. 5; Fig. 6)). These results disagree with what reported by Ain-Lhout *et al*. (2004) but partially agree with what reported by Mulas and Melis (2008) that showed a genotype effect on the chlorophyll seasonal trends in some cultivated myrtle clones and a different behaviour in function of the year. The ratio chlorophyll a to chlorophyll b reflected an adaptation of myrtle leaves to reduced light since it diminished significantly among light levels, indicating a relative increment in low light for chlorophyll b higher than that for chlorophyll a. This finding disagree with what found by Mendes *et al.* (2001) in myrtle plants in Portugal that stated that the ratio of chlorophylls was not affected by the light intensities or by the season, presenting a value between 2.3 and 2.5. The plants observed by Mendes *et al*., anyway, were adult and grown in natural light,

unlike the myrtle plants used in this experiment, subjected to a slightly rejuvenation to stimulate the vegetation renewal and grown under shade nets.

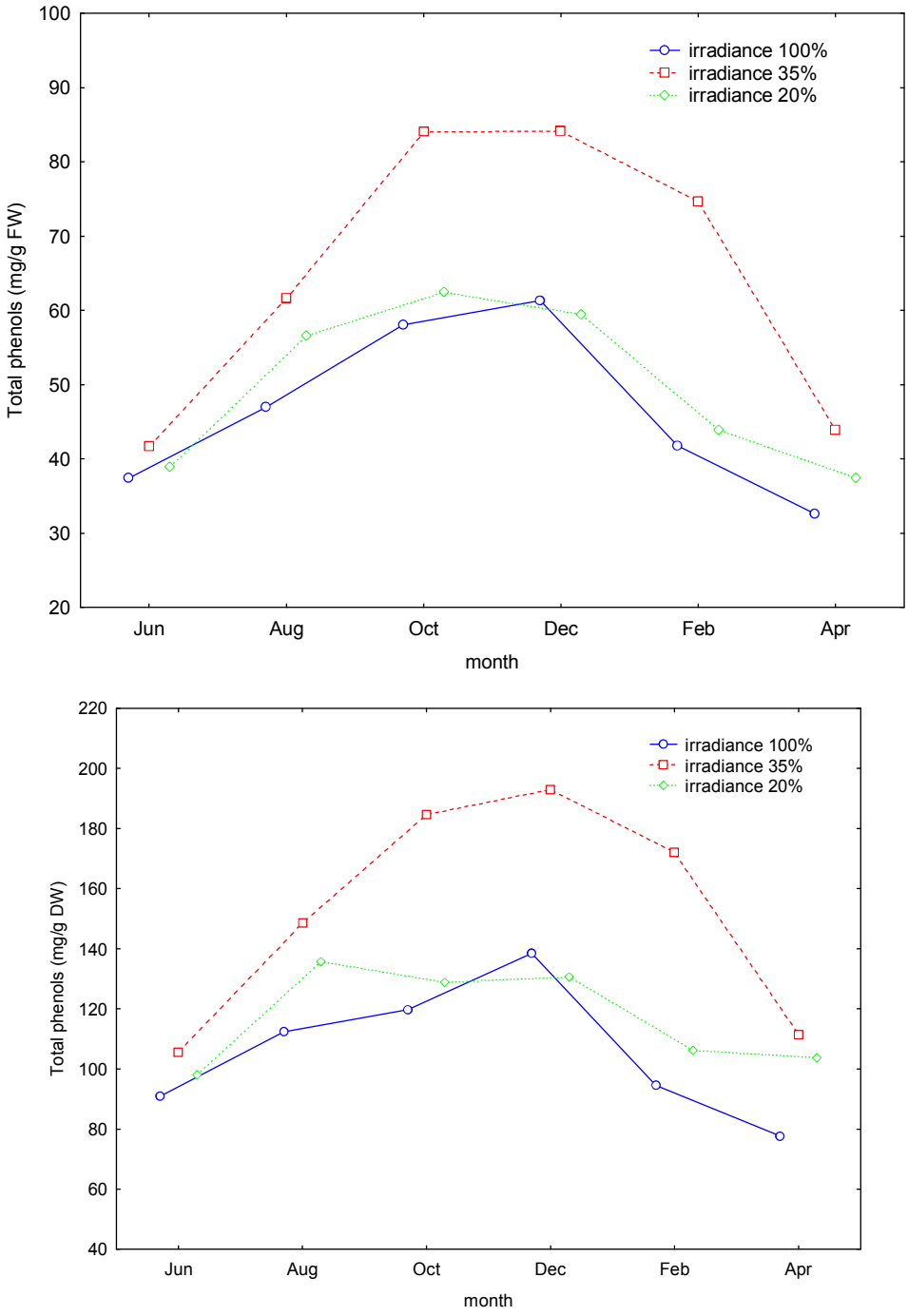


Figure 4. Seasonal trends of total phenols in leaves of Myrtus communis L. grown in different light levels.

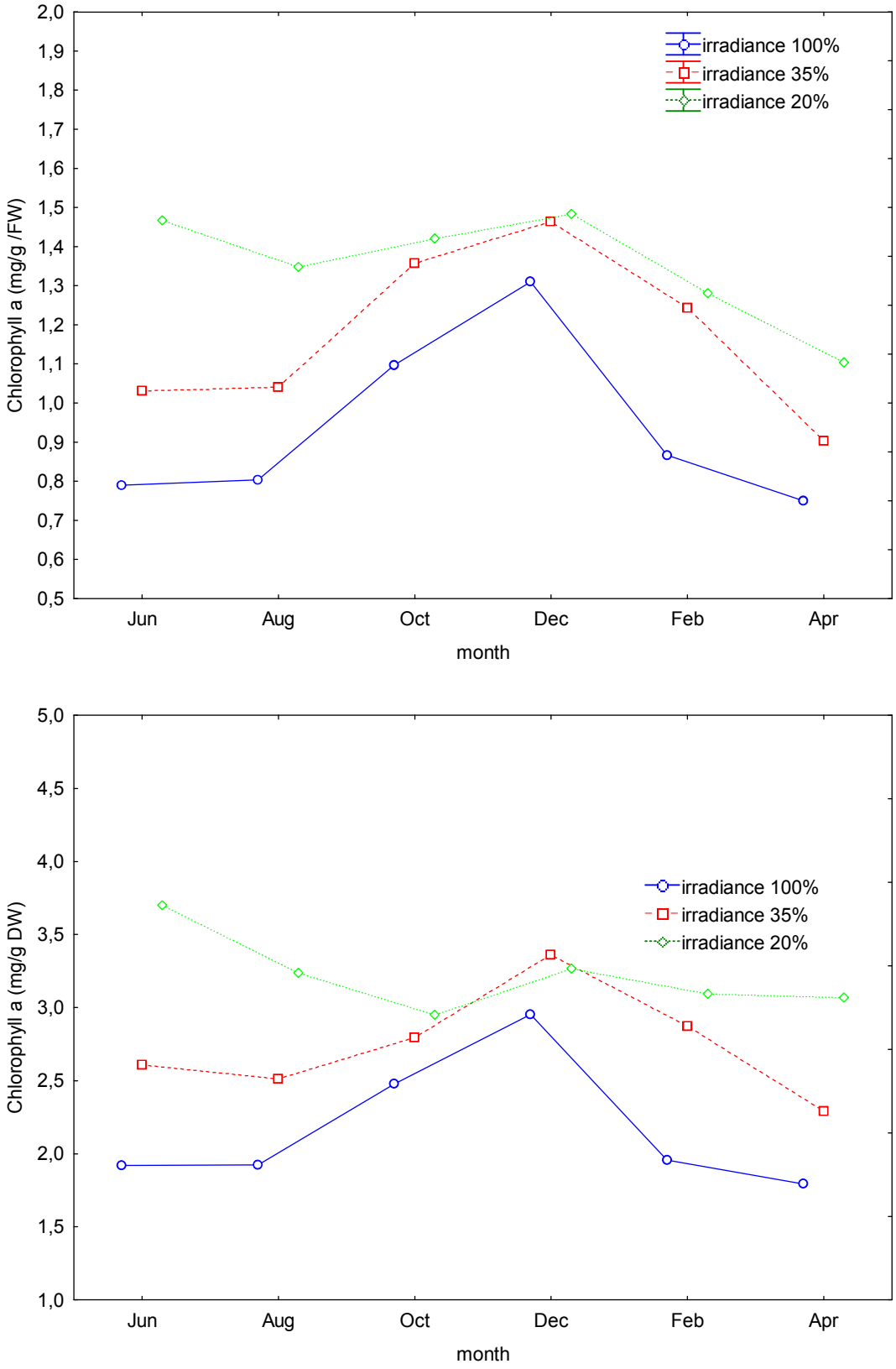


Figure 5. Seasonal trends of chlorophyll a contents in myrtle leaves grown under differential light levels.

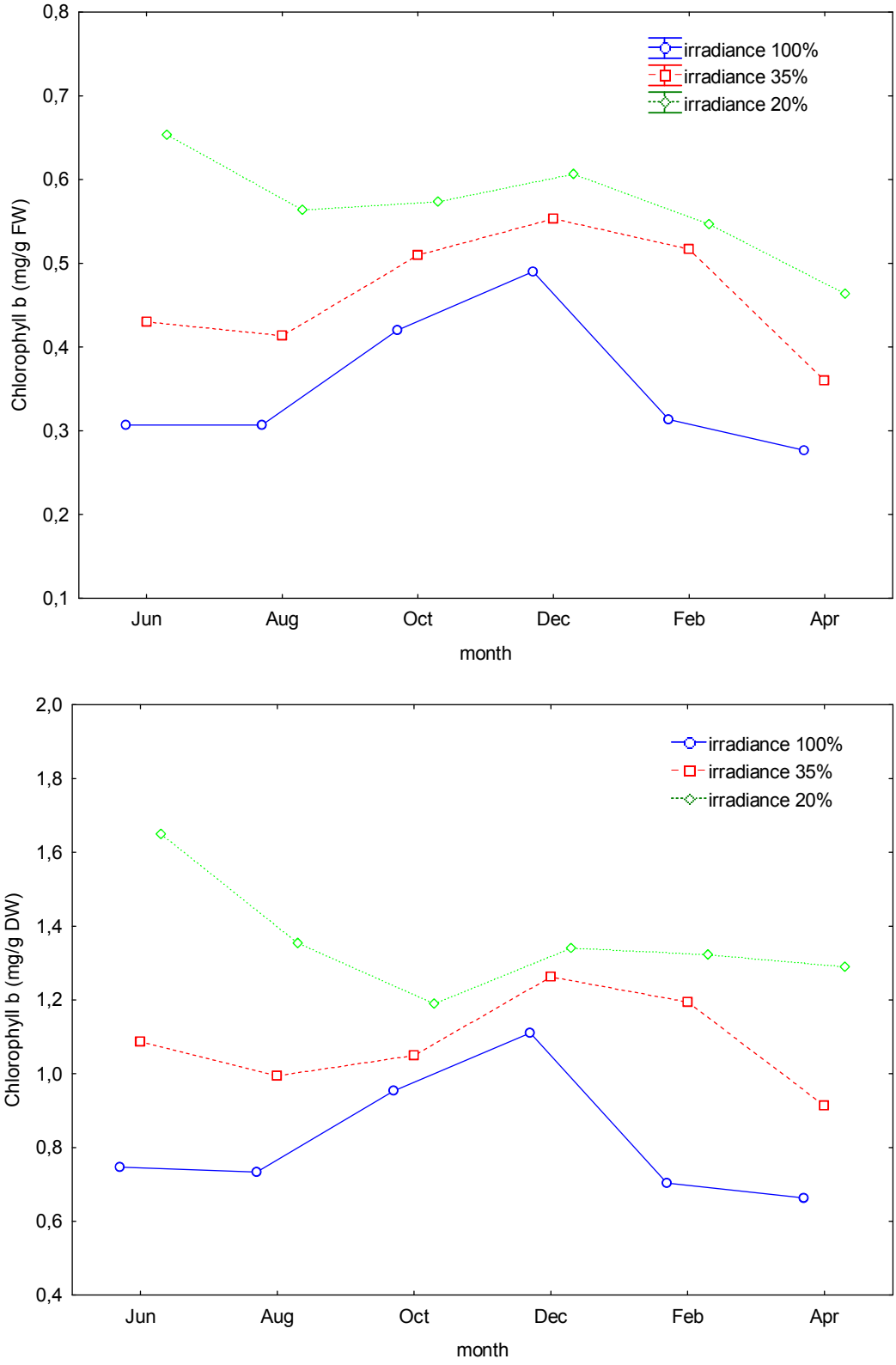
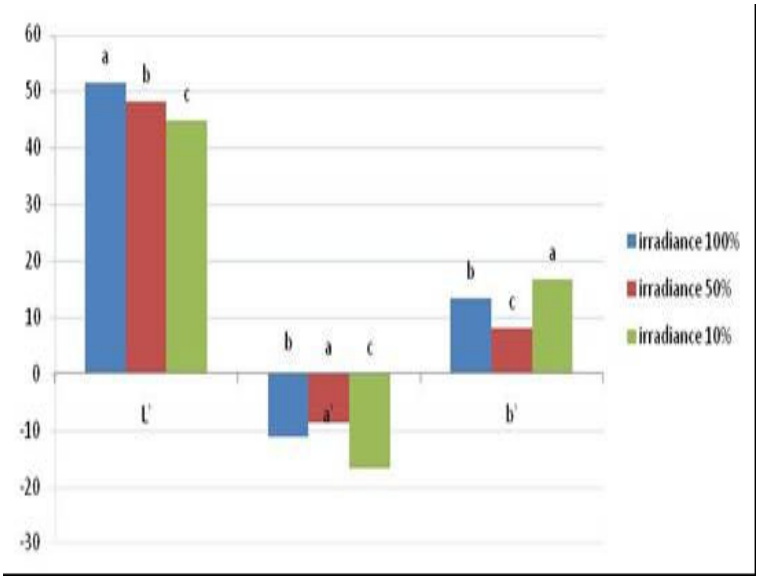


Figure 6. Seasonal trends in chlorophylls content in myrtle leaves grown under differential light levels.

Leaf colour

This datum was registered as a quality parameter for ornamental plants to be used as fronds. The colour of leaves, in fact, is one of the quality parameter used to classify ornamental foliage.

The leaf colour coordinates, depending principally by the chlorophyll content in leaves, were different in leaves grown in shade than in full light, with differences appreciable with the naked eye (Fig. 7).



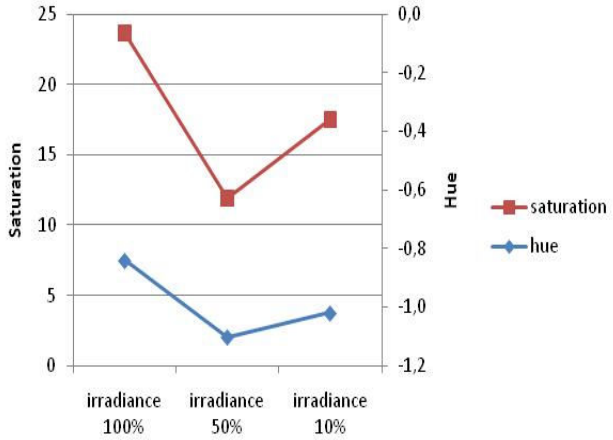


Figure 7. Colour coordinates, hue and saturation of colour measured in myrtle leaves grown under differential irradiance.

Elemental composition of leaves

Myrtle leaves picked from the various light environments showed a different elemental composition relatively to most elements (Tab. 12). Plants grown under reduced irradiance showed highest levels of nitrogen, potassium, sodium, zinc, iron and manganese in respect to sunlit leaves, while plants grown in sunlight showed higher levels of calcium and copper.

Table 12. Annual averages of myrtle leaf elemental composition grown under progressive shading. Different letters in the same row indicates values statistically different at ≤ 0,05, LSD test.

	Irradiance		
Element	**100%**	**35%**	**20%**
N (%)	1.66 c	2.40 a	2.29 b
P (%)	0.50 ab	0.55 a	0.45 b
K (%)	0,69 b	0,80 a	0,86 a
Na (%)	0,19 b	0,26 a	0,29 a
Ca (%)	1,17 a	0,70 b	1,10 a
Mg (%)	0,28 ab	0,26 b	0,29 a
Cu (ppm)	7,56 a	2,67 b	2,33 b
Zn (ppm)	23,22 b	25,94 a	27,56 a
Fe (ppm)	37,83 b	62,89 a	55,22 ab
Mn (ppm)	37,33 b	58,22 a	49,50 ab

The concentrations of most elements differed significantly among the sampling month. However, the seasonal changes were not consistently except for a slighty trend towards minimum values in spring (youngest leaves) and maximum values in autumn-winter (Fig. 8; Fig 9; Fig. 10; Fig. 11; Fig. 12).

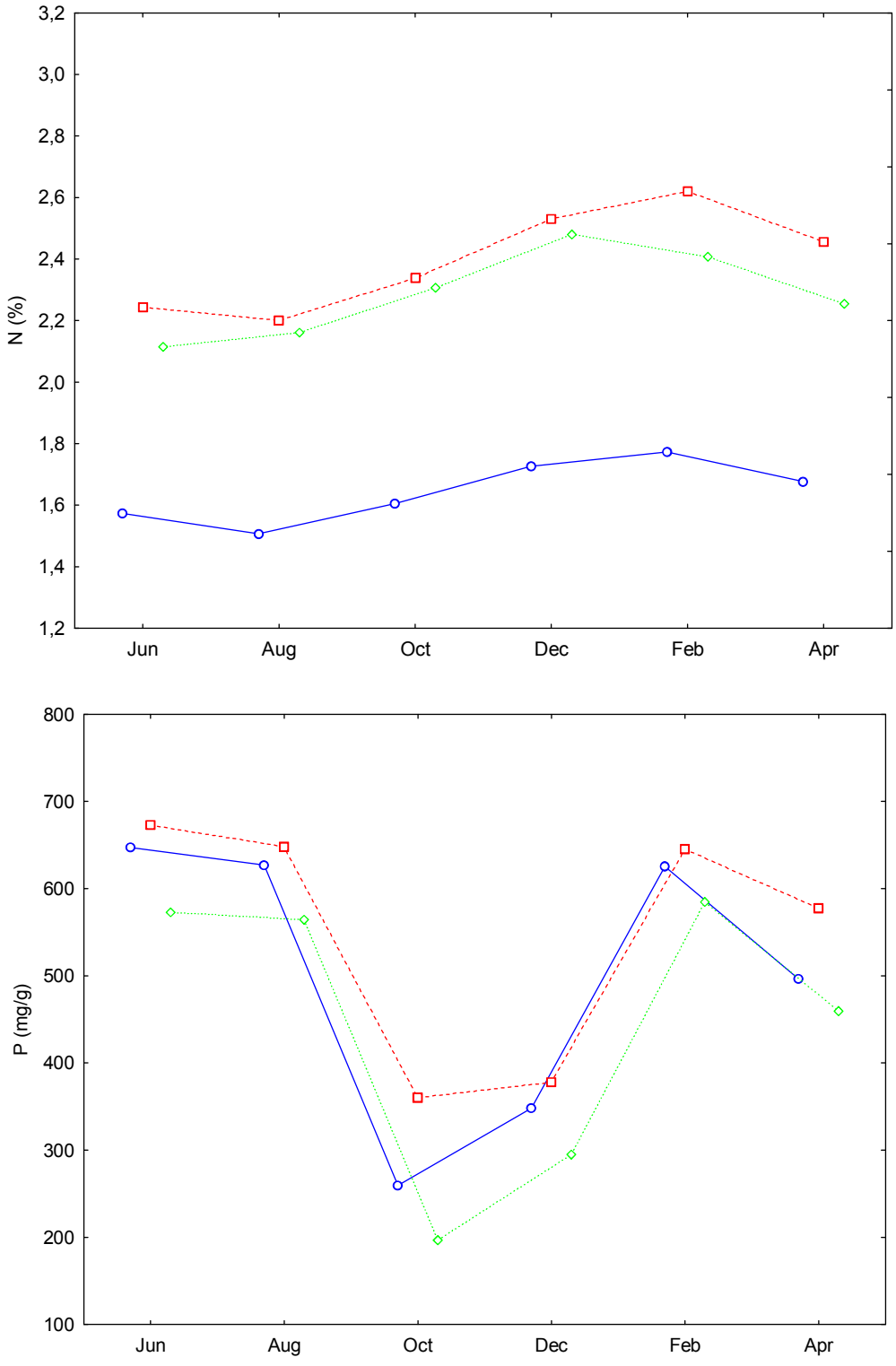


Figure 8. Seasonal courses of macroelements nitrogen and phosphorous in myrtle leaf grown under differential shade. The blue line refers to sunlight environment, the red line to 35% irradiance and the green line to 20% irradiance.

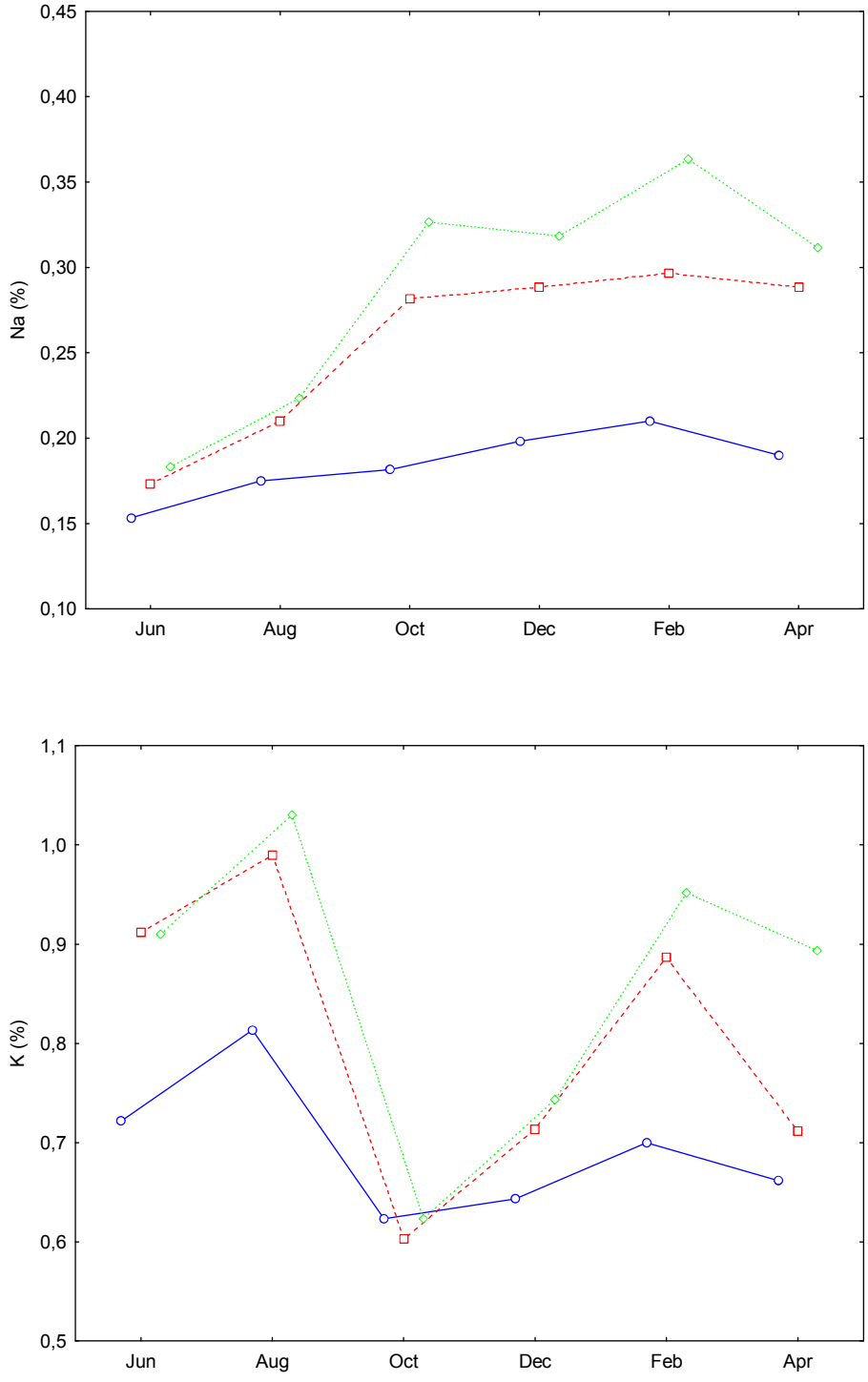


Figure 9. Seasonal courses of macroelements sodium and potassium in myrtle leaf grown under differential shade. The blue line refers to sunlight environment, the red line to 35% irradiance and the green line to 20% irradiance.

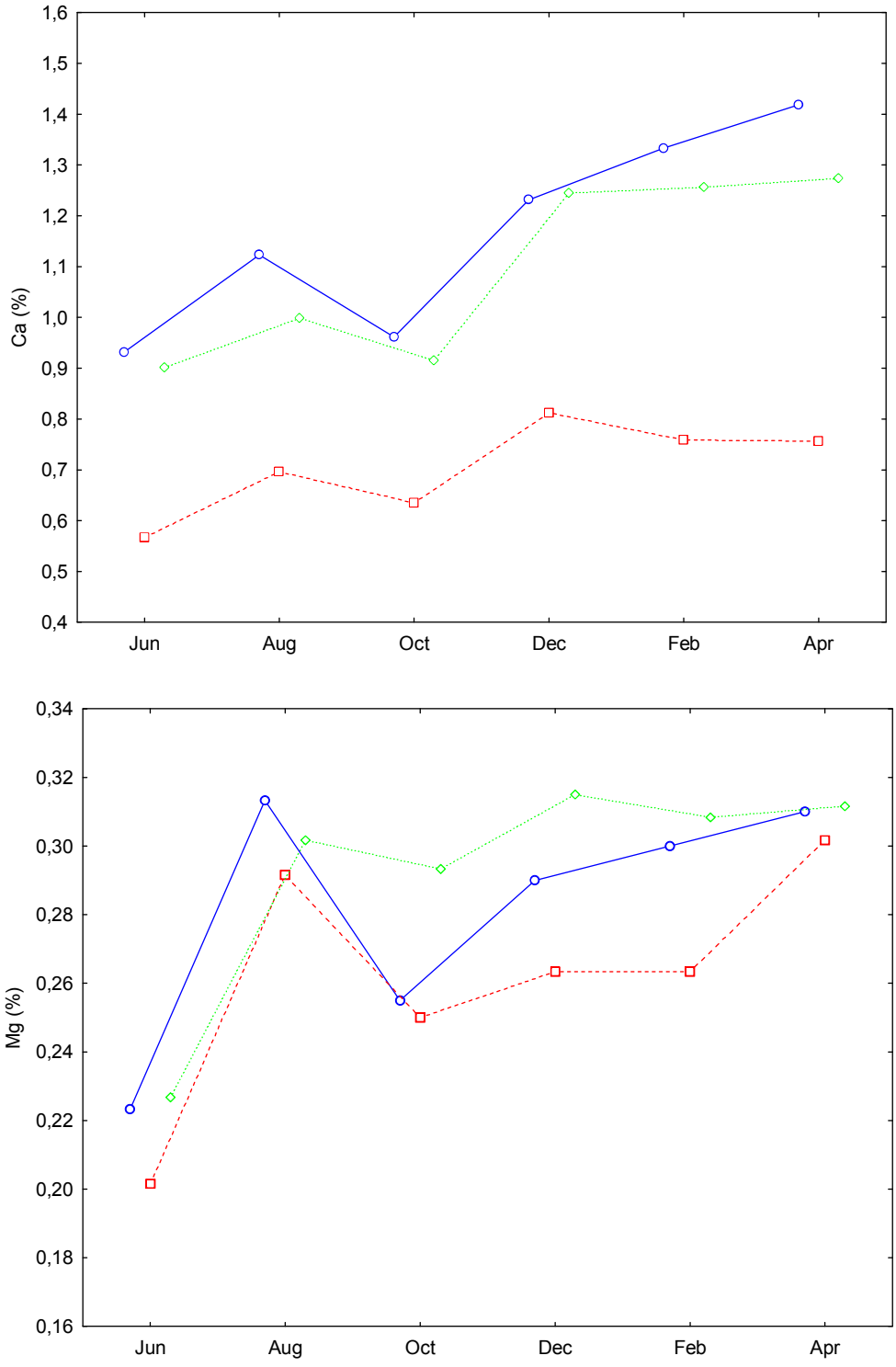


Figure 10. Seasonal courses of macroelements calcium and magnesium in myrtle leaf grown under differential shade. The blue line refers to sunlight environment, the red line to 35% irradiance and the green line to 20% irradiance

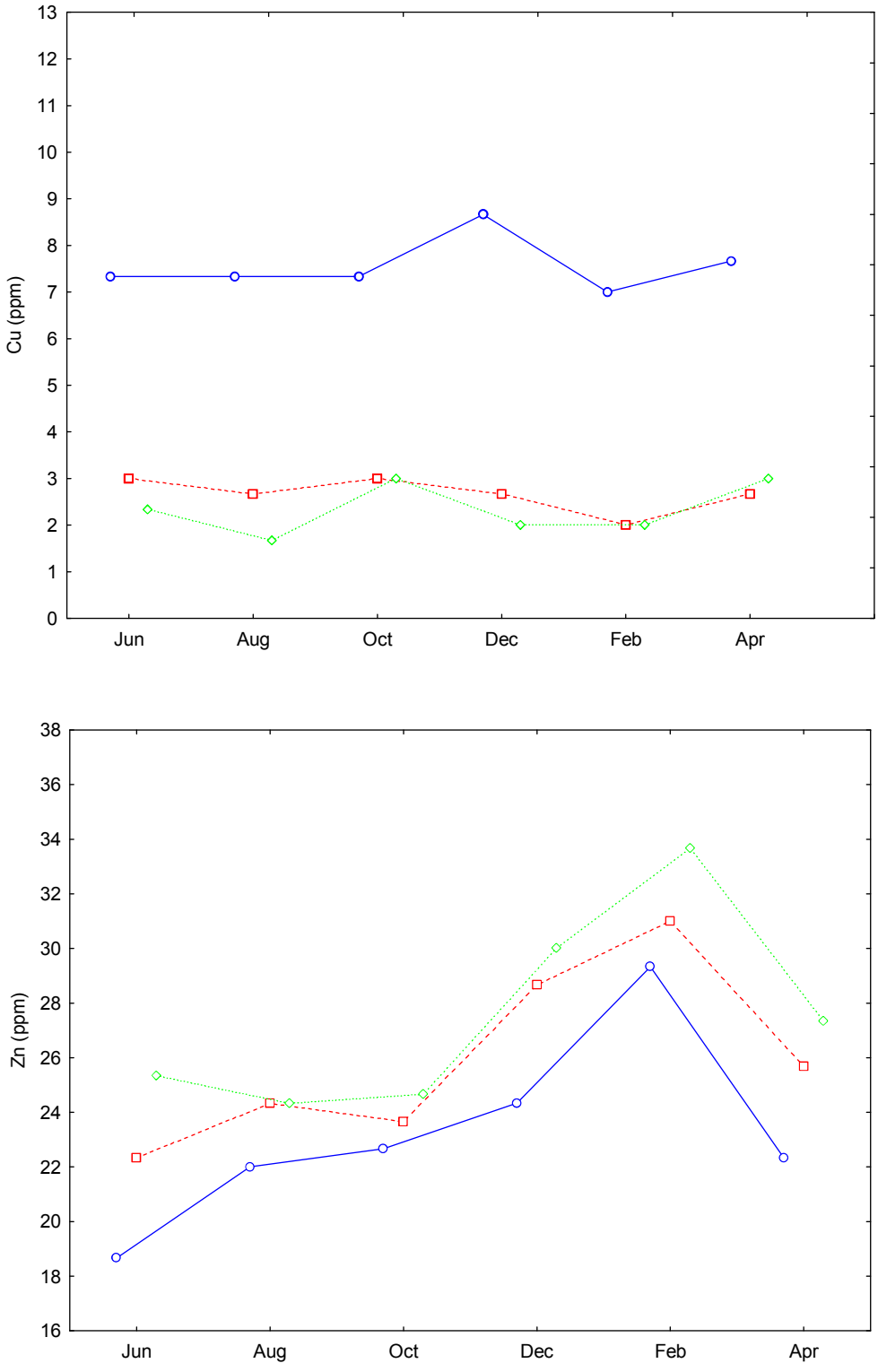


Figure 11. Seasonal courses of microelements copper and zinc in myrtle leaf grown under differential shade. The blue line refers to sunlight environment, the red line to 35% irradiance and the green line to 20% irradiance.

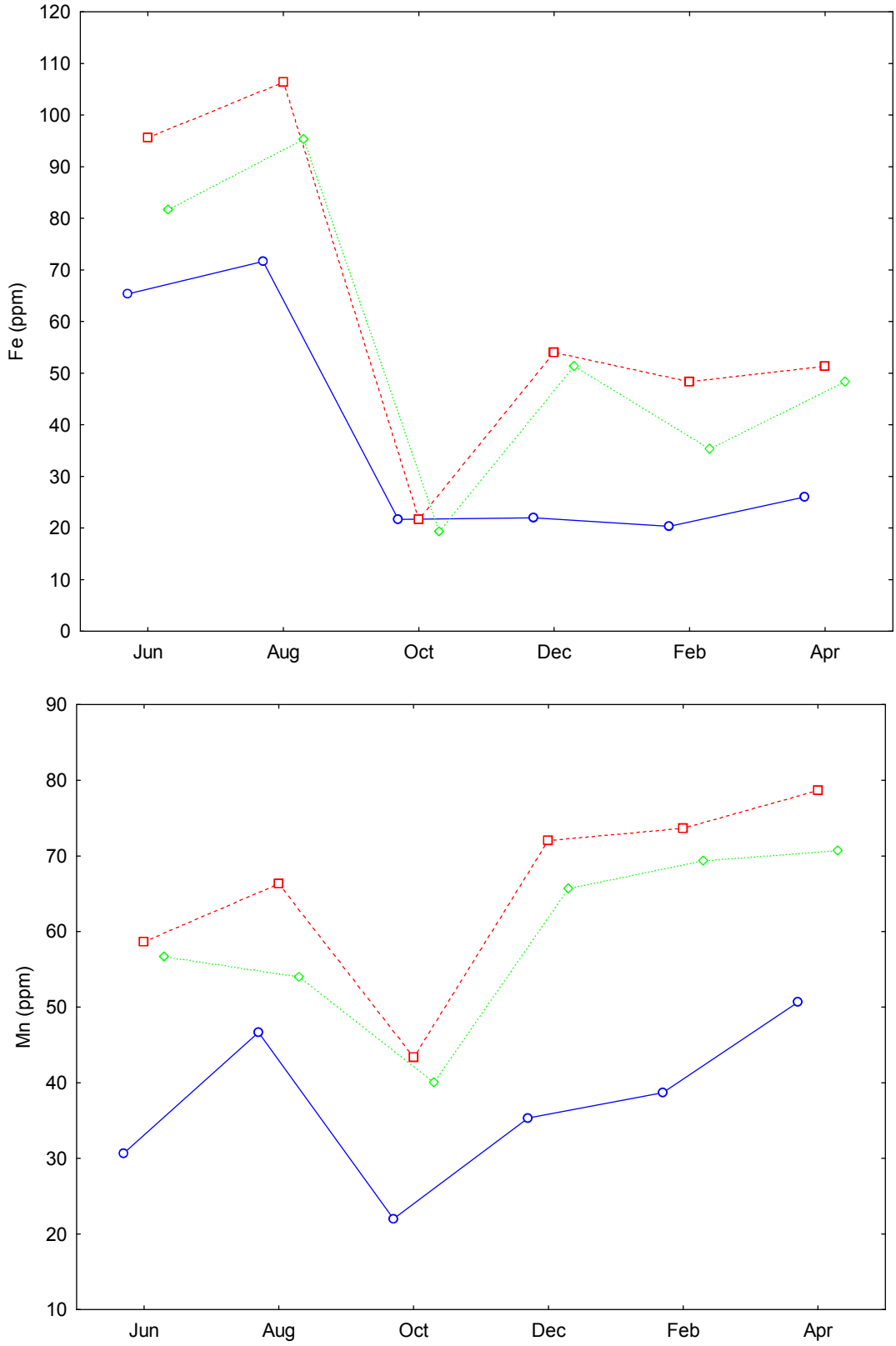


Figure 12. Seasonal courses of microelements iron and manganese in myrtle leaf grown under differential shade. The blue line refers to sunlight environment, the red line to 35% irradiance and the green line to 20% irradiance.

Rooting performances of cuttings

The cuttings collected from the shaded plants resulted the most effective in adventitious rooting, especially the ones collected from stock plants grown under irradiance of 35% (Tab. 13) .The rooting probability of myrtle cuttings picked from mother plants grown in sunlight, 35% and 20% irradiance were respectively 43.6, 62.6 and 43.6% as an annual average value. Anyway, as many other woody plants, myrtle showed to be subjected to a variation in rooting ability during the year, the best results being obtained between August and February, with values ranging from 61% to 72% among the treatments.

These findings partially disagree with the findings of Mulas *et al*., (1998), who stated that the most favourable period for myrtle propagation ranged between August and December. The differences in rooting efficiency during the year, however, depend also from the genotype, which affected in a sensible manner the rooting capability (Klein et al, 2000; Satta and De Pau, 2007)

Table 13. Rooting performances of cuttings from unshaded and shaded plants during 2007-2008.

	Irradiance		
Month	**100%**	**35%**	**20%**
June 2007	28,00±0,053	32,67±0,031	33,33±0,092
August 2007	43,33±0,153	71,67±0,247	66,67±0,176
October 2007	56,67±0,103	90±0,035	63,33±0,114
December 2007	66,67±0,031	89,33±0,023	61,33±0,101
February 2008	61,33±0,023	85,33±0,031	58,67±0,130
April 2008	5,33±0,042	6,67±0,046	1,33±0,012

Correlations

Some positive correlations between rooting performances and some measured parameters in leaves were found, for example between rooting percentage and phenols contents, chlorophylls content, zinc content (Tab. 14). A slightly negative correlation was found between rooting and calcium content. No correlations were found between the morphological characteristics of annual shoots and the rooting probability.

Table 14. Correlations between rooting performances of cuttings and chemical composition of myrtle leaves. Different letters in the same row indicates values statistically different at ≤ 0,05, LSD test.

Element	r	Element	r
Total phenols (FW)	0,82**	K	-0,06ns
Total phenols (DW)	0,76**	Ca	-0,28**
Total Chlorophylls (FW)	0,51**	Mg	-0,07ns
Total chlorophylls (DW)	0,31**	Cu	-0,2ns
N	0,28**	Zn	0,32**
P	-0,24ns	Fe	-0,09ns
Na	0,22ns	Mn	-0,08ns

The same experiment replicated in Sanremo in the month of February 2007 brought to similar results.

The shading treatments induced appreciable differences in the final growth of stems in both clones, but while in SNM the shoot length increased with the shading intensity, in the clone R the longer shoots were those grown in 40% irradiance in respect to sunlight. More intensive shading rates seemed to discourage the shoot growth in this clone. Anyway, the shading rate of 60% showed to be the most favourable for the shoot elongation, leaving out of consideration the clone while no differences existed for the remaining light environments.

The internode number was similar in shoots developed in sunlight and in the 40% shading rate and at the same time no differences were appreciable between internodes number in the remaining light environments. As far as the single clones are regarded, the number of internodes decreased in both clones passing by full light conditions to medium and heavy shading. Contrasting features between clones were shown as far as the internode length is regarded. The clone R differentiated longer internodes in slight shade (irradiance 60%), while SNM differentiated longer internodes in most intensive shading rates. In average, under any shade level internodes resulted significant longer than in sunlight.

The shading treatments induced some variations in the number of leaves, a higher number being favoured in sunlight and in 60% irradiance.

This was partially due both to the reduction of nodes in shade and to a different arrangement of leaves in shoots in sunlight. In fact, both clones differentiate trifoliate verticils in light that disappeared in shade, in favour of a normal opposite arrangement of leaves. In the clone SNM, moreover, the leaf insertion in stems was modified, since in sunlight it tended to be alternate rather than opposite. This behaviour was not present in the clone CPT5 tested in Oristano that retained the natural arrangement of leaves in any light environment.

In both clones, a slight shade seemed to favourite the development of a higher number of lateral sprouts while the increase in light strictness resulted in an unfavourable factor both for the absolute number and the bundling of lateral shoots.

Table 15. Characteristics of annual shoots of two myrtle clones. Different letters in the same row indicates values statistically different at ≤ 0,05, LSD test.

		Irradiance			
Clone	variable	100%	60%	30%	10%
R	Shoot length (cm)	92,56 b	106,06 a	72,87 c	53,99 d
	Shoot diameter (cm)	0.2 b	0.21 b	0.25 ab	0.27 a
	N° internodes/shoot	39,52 a	41,30 a	32,41 b	25,52 c
	Internodes length (cm)	2,20 b	2,68 a	2,27 b	2,02 c
	N° leaves/shoot	80,89 a	89,30 a	67,15 b	51,85 c
	N° sprouts/shoot	28,10 a	35,70 a	11,20 b	6,30 b
SNM	Shoot length (cm)	41,93 c	54,74 ab	58,86 a	60,96 a
	Shoot diameter (cm)	0.26 a	0.23 a	0.19 b	0.2 b
	N° internodes/shoot	46,13 a	45,93 a	36,07 b	40,27 ab
	Internodes length (cm)	0,90 c	1,17 b	1,64 a	1,56 a
	N° leaves/shoot	114,40 a	110,00 a	88,53 b	88,54 b
	N° sprouts/shoot	26,10 b	43,20 a	13,30 c	16,40 c

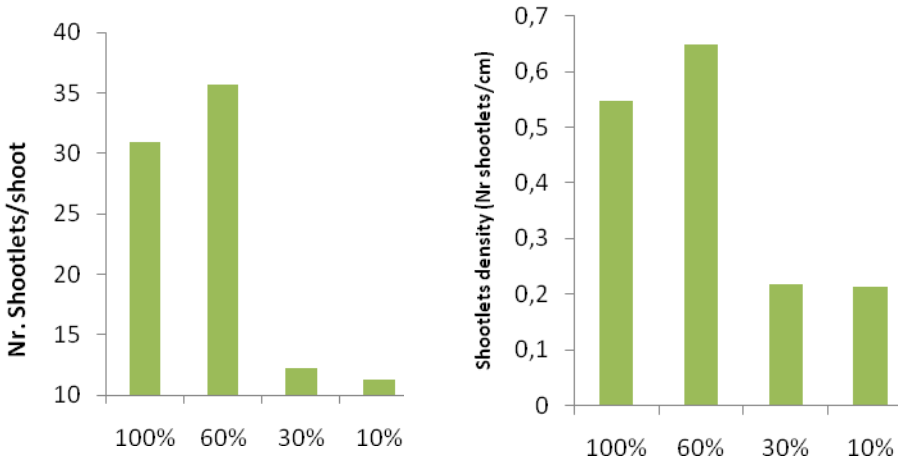


Figure 8. Number of lateral shoots per main shoot and shoot density in stems of myrtle plants growing under differential irradiance.

Biomass allocation in cuttings

The leaf fresh weight per cutting was weakly influenced by the light environment where stock plants grew. Only in the heaviest shade there was a trend toward the diminishing of leaf proportion in terms of absolute fresh and dry weight, but in terms of percentages the proportion of leaves increased in both clones up to the 30% irradiance.

This increase is due to the higher leaf area rather than to the increase in number of leaves. The stem weight, on the contrary, was higher in high light levels and diminished in the most severe shading rates. The leaf and stem dry weight (%) showed a decrease at the increasing of the shading level. This is a well known behaviour in plants acclimatized to low light levels.

Table 16. Allocation plasticity in myrtle cuttings picked from mother plants growing in differential light intensities. Different letters in the same row indicate values statistically different at p≤0.05 (LSD).

		Irradiance			
Clone	variable	100%	60%	30%	10%
R	Leaves/cutting FW (g)	1.48 a	1.46 a	1.61 a	0.53 b
	Leaves/cutting DW (g)	0.64 a	0.63 a	0.62 a	1.20 b
	Leaves/cutting FW (%)	77.48 c	83.13 b	88.62 a	84.87 b
	Leaves/cutting DW (%)	79.97 c	84.45 b	88.20 a	86.97 ab
	Leaf DW (%)	43.27 a	43.57 a	38.68 b	37.67 b
	Stem/cutting FW (g)	0.43 a	0.30 b	0.21 c	0.09 d
	Stem/cutting DW (g)	0.16 a	0.12 b	0.08 c	0.03 d
	Stem DW (%)	37.31 ab	39.54 a	40.30 a	31.95 b
SNM	Leaves/cutting FW (g)	0.70 a	0.69 a	0.55 ab	0.38 b
	Leaves/cutting DW (g)	0.28 a	0.29 a	0.21 a	0.11 b
	Leaves/cutting FW (%)	69.61 a	84.42 b	87.59 a	87.59 a
	Leaf /cutting DW (%)	79.45 b	85.15 ab	90.16 a	85.11 ab
	Leaf DW (%)	40.28 a	42.00 a	38.50 a	31.58 b
	Stem/cutting FW (g)	0.31 a	0.13 b	0.07 b	0.05 b
	Stem/cutting DW (g)	0.07 a	0.05 a	0.02 b	0.02 b
	Stem DW (%)	24.15 b	40.53 a	34.92 ab	39.96 a

Leaf characteristics

Similarly to what shown by the clone CPT5, the leaf area increased linearly with the increasing of shading rates. The leaf length, width and area increased with the reduction of light levels. The leaf length/width ratio was lower in leaves developed in sunlight, but it was similar for both shading treatments (Tab.10). This indicates that in shade leaves linear development is higher in length than in width. At different light levels, the

leaf dry weight (%) and the leaf specific weight (SLW) were higher in light than in shade, as expected.

Table 17. Morphological characteristics of myrtle leaves developed in different light environments. Different letters in the same row indicates values statistically different at ≤ 0,05, LSD test.

		Irradiance			
Clone	variable	100%	60%	30%	10%
R	Leaf area (cm^2)	7,159 c	10,64 b	10,98 ab	11,954 a
	Leaf length (cm)	4,735 b	5,721 a	5,781 a	5,811 a
	Leaf width (cm)	2,350 b	2,920 a	2,889 a	3,071 a
	Length/width ratio	2,024 a	1,971 a	2,015 a	1,90 a
SNM	Leaf area (cm^2)	2,731 c	2,969 c	3,576 b	3,980 a
	Leaf length (cm)	3,039 c	3,432 b	4,345 a	4,141 a
	Leaf width (cm)	1,327 b	1,295 b	1,295 b	1,505 a
	Length/width ratio	2,294 c	2,709 b	3,384 a	2,762 b

Total phenols, photosynthetic pigment and total starch contents in leaves

The myrtle clones differed in average for chlorophylls content in leaf tissues. The differential light environment induced a progressive increase in chlorophyll content in both clones from sunlight to heaviest shade. The chlorophylls ratio decreased gradually from full light to higher shade level, confirming the clone CPT5 behaviour (Tab. 18).

In these clones we measured total carotenoid content too, finding that the clone R was in average richer than SNM in these photo-oxidizing protective compounds.

In R, carotenoids content was unaffected by the progressive increment in shade, while SNM showed a marked and progressive decline in carotenoid content from full light to heavy shade conditions, a behaviour common to many other plants when light levels decrease. As far as the total phenols are regarded, the clone SNM showed to be richer in phenols than R. The reduction in light levels seemed to stimulate the maximum phenols production, in particular under medium light intensities (irradiance reduced to 60% in respect of sunlight) and the starch production in the same conditions.

Table 18. Chlorophylls, total carotenoid, total phenols and total starch contents in myrtle leaves under differential shade. Different letters in the same row indicate values statistically different at p≤0.05 (LSD).

		Irradiance			
Clone	Variables	100%	60%	30%	10%
R	Chlorophyll a (mg/g FW)	2.19 c	2.19 c	3.42 b	3.06 a
	Chlorophyll a (mg/g DW)	3.85 c	3.93 c	7.21 b	8.66 a
	Chlorophyll b (mg/g FW)	0.82 c	0.88 c	1.41 b	1.67 a
	Chlorophyll b (mg/g DW)	1.44 c	1.58 c	2.97 b	3.7 a
	Chlorophylls ratio (a/b)	2.67 a	2.47 ab	2.43 ab	2.34 b
	Total carotenoids (mg/g FW)	3.13 a	3.00 a	2.92 a	2.84 a
	Total carotenoids (mg/g DW)	5.51 a	5.37 a	6.15 a	6.31 a
	Total phenols (mg/g FW)	22.7 c	63.07 a	38.16 b	42.29 b
	Total phenols (mg/g DW)	40.39 c	112.6 a	80.35 b	93.85 ab
	Total Starch (% DW)	0,96 c	1,19 b	1,38 a	-
SNM	Chlorophyll a (mg/g FW)	1.54 b	2.47 ab	2.67 ab	3.29 a
	Chlorophyll a (mg/g DW)	2.85 b	4.93 b	5.84 ab	8.4 a
	Chlorophyll b (mg/g FW)	0.60 b	0.98 ab	1.08 ab	1.4 a
	Chlorophyll b (mg/g DW)	1.10 c	1.96 bc	2.38 ab	3.58 a
	Chlorophyll a/b ratio	2.58 a	2.52 a	2.45 b	2.35 c
	Total carotenoids (mg/g FW)	3,37 a	2,89 b	2,38 c	2,06 d
	Total carotenoids (mg/g DW)	6.26 a	5.78 ab	5.21 b	5.26 b
	Total phenols (mg/g FW)	58.26 a	59.44 a	55.63 a	31.38 b
	Total phenols (mg/g DW)	108.5 ab	118.8 a	122.4 a	80.1 b
	Total Starch (% DW)	1,39 a	1,44 a	1,32 a	0,92 b

Leaf elemental composition

As in the case of CPT5, the shading treatments supported the increment of nitrogen content and that of most elements with the exception of sodium and copper that showed not to be influenced by light levels (Tab.19).

Table 19. Comparative average elemental content in myrtle leaves grown under differential light levels. Different letters in the same row indicates values statistically different at p≤ 0,05, LSD test.

	Irradiance			
	100%	60%	30%	10%
N (%)	1.53 c	1.94 c	2.29 a	2.24 b
P (%)	0.14 bc	0.17 b	0.12 c	0.213 a
Na (%)	0.79 a	0.77 a	0.92 a	1.04 a
K (%)	0.55 b	0.51 b	0.84 a	0.89 a
Ca (%)	0.49 c	1.02 b	1.68 a	1.18 b
Mg (%)	0.14 c	0.20 b	0.27 a	0.22 ab
Cu (ppm)	2.67 a	5.33 a	3.67 a	3.67 a
Zn (ppm)	8.83 b	11.33 b	11.33 b	16 a
Fe	47.17 c	53.50 c	102.67 a	84.33 b
Mn	49.83 c	50.33 c	127.5 a	92.33 b

Table 20. Chemical composition of myrtle R in the different light environments. Different letters in the same row indicate values statistically different at p≤0.05 (LSD).

	Irradiance			
Clone R	100%	60%	30%	10%
N (%)	1,95 d	2,09 c	2,63 a	2,42 b
P (%)	0,13 a	0,16 a	0,06 b	0,05 b
Na (%)	0,50 a	0,43 b	0,41 b	0,62 a
K (%)	0,60 b	0,56 b	0,91 a	0,92 a
Ca (%)	0,26 c	0.49 bc	1.09 a	0.69 b
Mg (%)	0.12 c	0.13 bc	0.20 a	0.15 b
Cu (ppm)	1.00 b	3.67 ab	1.33 ab	4.00 a
Zn (ppm)	6.67 b	8.33 ab	7.33 ab	11.33 a
Fe (ppm)	41.00 b	41.00 b	86.00 a	83.33 a
Mn (ppm)	16.67 b	17.00 b	68.00 a	71.67 a

Table 21. Chemical composition of myrtle SNM in the different light environments. Different letters in the same row indicate values statistically different at p≤0.05 (LSD).

	Irradiance			
Clone SNM	100%	60%	30%	10%
N (%)	1.12 d	1.78 c	1.95 b	2.06 a
P (%)	0.146 b	0.171 b	0.178 b	0.366 a
Na (%)	1.08 a	1.16 a	1.425 a	1.54 a
K (%)	0.50 b	0.46 b	0.77 a	0.87 a
Ca (%)	0.72 c	1.55 b	2.27 a	1.68 b
Mg (%)	0.16 b	0.27 a	0.34 a	0.30 a
Cu (ppm)	4.33 a	7.00 a	6.00 a	3.00 a
Zn (ppm)	11.00 c	14.33 bc	15.33 b	20.50 a
Fe (ppm)	53.33 d	66.00 c	119.33 a	84.50 b
Mn (ppm)	83.00 b	83.67 b	187.00 a	112.50 b

The single clones showed a similar adaptation to light environments, differing basically in adaptation for the single elements to a shading rate than another.

Leaf colour

The two clones showed different hue and saturation values (Tab. 22). R had a medium to dark green leaves in sunlight that become a pronounced dark green in shade. SNM showed in sunlight a light green leaves but in shade the colour become a medium green, with a noteworthy improvement of ornamental feature of leaves.

Table 22. Colorimetric coordinates and hue and saturation values in myrtle leaf grown under different shade conditions. Different letters in the same row indicate values statistically different at p≤0.05 (LSD).

		Irradiance			
Clone	**Variables**	**100%**	**60%**	**30%**	**10%**
R	L	39,85 a	38,03 b	36,34 c	34,40 d
	a	-10,08 b	-9,98 b	- 10,34 b	- 8,11 a
	b	10,08 a	9,67 ab	9,19 b	6,62 c
	hue	-1,02	-1,05 b	-1,13 c	-1,23 d
	saturation	14,26 a	13,90 a	13,84 a	10,47 b
SNM	L	42,03 a	40,91 b	36,00 c	35,90 c
	a	-10,41 a	-11,41 b	-10,31 a	-11,55 b
	b	11,72 a	11,08 a	10,01 b	11,62 a
	hue	-0,90 A	-1,05 C	-1,05 C	-1,00 B
	saturation	15,68 a	15,91 A	14,38 B	16,39 A

Rooting performances of cuttings.

Unlike the rooting performance in the clone CPT5 tested in Oristano, the rooting performances of R and SNM clone were unaffected by the light environments, also if a slighty increment was registered for the heaviest shade conditions in SNM (Tab. 23).

Table 23. Rooting probabilities of myrtle cuttings picked from stock plants grown in different light levels.

	Irradiance			
	100%	60%	30%	10%
R	90.7 ± 6.1	94.0 ± 6.0	88.7 ± 3.1	-
SNM	87.3 ± 3.1	84.7 ± 9.9	88.0 ± 4	94.0 ± 2

The rooting performances of both clones were very high and in contrast with the findings of some authors that obtained the worst results in adventitious rooting of myrtle in February.

Characteristics of adventitious root system

Some differences in the characteristics of the adventitious rooting system were registered in both clones after one month after the rooting induction in cuttings picked from plants grown in different light conditions. Cuttings from plants grown in full light showed a greater number of roots per cutting and a more developed rooting system as far as the total length is regarded (fig 13).

Table 24. Characteristics of the adventitious rooting system in myrtle clones grown under different light levels.

		Irradiance			
		100%	60%	30%	10%
R	Number of roots	21.3±12.37	19.45±12.73	10.9±5.33	-
	Total length (cm)	54.94±24.37	50.93±24.82	39.14±13.13	
	Maximum length (cm)	5.01±1.55	5.23±1.50	6.18±1.54	
SNM	Number of roots	21.6±9.71	17.75±10.18	14.25±6.81	14.35±9.19
	Total length (cm)	55.88±16.93	45.19±22.75	64.42±35.66	52.67±28.86
	Maximum length (cm)	5.17±1.00	4.85±1.37	7.79±1.35	6.76±1.50

Correlations

The correlation test showed that no significant correlations existed among rooting probabilities and the morphological or biometric or chemical features measured in myrtle plants grown in the different light environments.

6.2 Light levels, rooting and acclimatization in myrtle micro-propagated plants

During in vitro culture of myrtle plants, we considered and measured some aspects related to the quality of plantlets, like the shoot multiplication rate, the length and weight of clusters, the number and length of roots and the chlorophylls contents of

leaflets. The shoot multiplication rate was affected both by the myrtle genotype and the growth regulator added in the culture medium (Tab. __). Apart from these factors, light levels showed to influence this parameter too. The most efficient clone in shoot multiplication was clone I, that showed a double multiplication rate per month than the other clones. Most culture medium s allowed a multiplication rate about 2.50 shootlet per month, with the exception of the medium containing the hormone Benziladenine (BA).

High light levels showed to affect negatively the multiplication rate.

The biometrical characteristics of newly shoots were affected by genotype and both light and culture medium. Newly shoots height increased as the photosynthetic photon flux density decreased, confirming that myrtle reacts positively to light. The effect of culture medium on shoot height was not so marked and clearly distinguishable, as only the BA medium showed to inhibit shoot growth.

Table 25. Multiplication rates and shoot length in myrtle micropropagated clones. Different letters in the same row indicate values statistically different at $p \leq 0.05$ (LSD).

	Multiplication rates	Shoot length
Clone		
I	5.52 c	2.18 a
PA2	1.84 a	2.98 b
CG5	2.30 b	2.17 a
Irradiance		
100 mmol	2.8 a	1.5 a
50 mmol	3.45 b	2.24 b
25 mmol	3.41 b	3.59 c
Medium		
MS0	2.56 b	2.56 b
MS0+BA	1.99 a	1.99 a
MS0+IAA	2.65 b	2.65 b
MS0+IAA+BA	2.58 b	2.58 b

The chlorophyll content, on the contrary, was clearly affected by light levels and medium composition. As for myrtle plants grown in the field and under shade, the chlorophylls levels were higher at the lowest irradiance. The medium culture containing BA or the free-growth regulator medium showed to interact positively with light for the chlorophyll content of myrtle leaflets (Tab 26).

Table 26. Interaction among culture mediums and light levels in determining the total chlorophylls content in myrtle clones in *in vitro* cultures under different PPFD. Within each column, values with different letters are different at $p \leq 0.05$ (LSD test).

	Irradiance ($\mu mol\ s^{-1}\ m^2$)		
Culture medium	**100**	**50**	**25**
MS0	0.71 a	1.31 a	3.48 b
MS0+BA	1.34 b	1.91 b	1.91 a
MS0+IAA	1.48 b	1.64 ab	1.79 a
MS0+IAA+BA	1.43 b	1.69 ab	2.04 a

The positive interaction was different for the various clones. The clone I in particular showed a higher reaction to the different combinations of light levels and culture mediums (Tab. 27). The presence of BA or the cohabiting in the medium culture of BA and IAA in cultures under low light levels greatly stimulated the chlorophyll presence in myrtle leaflets. These data confirm a recent report of Yaronskaya *et al*. (2006) on barley seedlings, showing that cytochinins like BA promotes some physiological processes resulting in stimulation of chlorophyll biosynthesis.

Table 27. Interaction among culture mediums and light levels in determining the total chlorophylls content in the clones I, PA2 and CG5 in *in vitro* cultures under different PPFD. Within each column, values with different letters are different at $p \leq 0.05$ (LSD test).

	Irradiance ($\mu mol\ s^{-1}\ m^2$)		
	100	50	25
Clone I			
MS0	0.52 a	1.40 a	3.56 b
MS0+BA	1.28 b	1.89 a	1.47 a
MS0+IAA	0.51 a	1.27 a	1.39 a
MS0+IAA+BA	1.50 b	1.28 a	1.95 a
Clone PA2			
MS0	1.11 a	1.58 a	3.88 c
MS0+BA	1.22 a	1.64 a	2.18 b
MS0+IAA	1.88 a	1.44 a	0.99 a
MS0+IAA+BA	1.50 a	1.54 a	2.37 b
Clone CG5			
MS0	0.50 a	0.97 a	2.99 b
MS0+BA	1.53 b	2.19 b	2.09 ab
MS0+IAA	2.05 c	2.22 b	2.99 b
MS0+IAA+BA	1.29 b	2.25 b	1.78 a

The rooting probability in myrtle cultures showed to be strictly controlled by plant genotype. The clone I, for example, rooted poorly in all the culture mediums, showing no more than 1.7% of plantlets differentiating roots (Tab 28). This is due perhaps to the particular feature of the clone which is characterized by short internodes and a compact habit. In some other plants it has been observed, in fact, that these characteristics are often associated with a poor rooting attitude, limiting the ornamental exploitation of such plants in *in vitro* cultures.

Table 28. Rooting probabilities in myrtle I, PA2 and CG5 in different medium cultures and light levels.

	Irradiance (μmol s^{-1} m^2)		
	100	50	25
Clone I			
MS0	0	4.2	8.3
MS0+BA	0	0	0
MS0+IAA	0	0	0
MS0+IAA+BA	0	0	8.3
Clone PA2			
MS0	0	16.7	33.3
MS0+BA	0	0	0
MS0+IAA	4.2	12.5	0
MS0+IAA+BA	25	62.5	33.3
Clone CG5			
MS0	4.2	0	8.3
MS0+BA	0	0	0
MS0+IAA	0	0	0
MS0+IAA+BA	8.3	20.8	5.4

As expected, the culture mediums containing IAA were the most effective in stimulating root differentiation and development in all the clones. Light reduction contributed in a sensible way to stimulate rooting. In the hard-to-root clone I the lower irradiance was the most effective, simulating the effect of shade treatments in the field to stock plants.

In order to evaluate the abilities of rooted and non-rooted plants grown in the different media and light levels to acclimatize, plantlets were put in the acclimatization greenhouse under two types of covers previously described.

Under plastic cover plantlets survival was reduced up to10% in respect of the paper cover. The paper covering helped in fact to maintain a better microclimate for plantlets guaranteeing suitable air humidity levels. Plantlets were monitored after 60 days from

the beginning of acclimatization and weighted. Most plants, rooted or non-rooted in vitro, showed to have a root system well developed and well connected to shoot system. The acclimatization probabilities for plantlets derived from different culture mediums and light levels are shown in Tab. 29.

Table 29. Acclimatization percentages of myrtle plantlets grown under different culture medium and light environments.

	Irradiance (μmol s^{-1} m^2)		
	100	50	25
Clone I			
MS0	0	0	0
MS0+BA	0	16.7	8.3
MS0+IAA	0	0	0
MS0+IAA+BA	0	16.7	0
Clone PA2			
MS0	0	0	0
MS0+BA	0	7.7	0
MS0+IAA	0	0	0
MS0+IAA+BA	0	0	8.3
Clone CG5			
MS0	0	0	0
MS0+BA	0	0	18.2
MS0+IAA	0	0	0
MS0+IAA+BA	0	0	0

The clusters derived from plantlets grown under 50 and 25 μmol s^{-1} m^2 and in BA medium showed to have the higher fresh weight. The same plants were those with the highest chlorophyll content and resulted the best acclimatized. In accordance with these results, we may assume that the bottleneck represented by acclimatization phase in the success of micropropagation may be get round improving plantlets fresh weight and the total chlorophyll contents rather than acting on rooting emission at the end of the in vitro culture. We may conclude that since in this experiment the light supplied during

the rooting phase in vitro resulted in a best acclimatization of plantlets, this practice could be associated to photoautrophic micropropagation combined with sugar-free culture and CO_2 enrichment as suggested by Zobayed *et al*., (2004) or in traditional micropropagation to increase the chlorophylls content.

6.2. LENTISK

6.2.1 Effects of rejuvenation on lentisk plants

Effect on biomass development

Pollarding treatments were applied to the primary axes of lentisk in April 2006, just before the male flowering and then plants were allowed to resprout. Once the primary shoots had been pruned, the previous year growth and all remaining terminal buds were removed from the remaining branches.

The new vegetation originated from dormant buds in the branches about a month later. The new shoots developed at the end of the branches and were typically grouped in bundles of three-five twigs that showed a very long growth season, with just a brief stop in elongation at the end of July-beginning of August, from May up to November, when the temperatures drop induced the growth stop. No differences were found in growth trends between the two years. As a direct effect of rejuvenation of the vegetation, the blooming resulted completely suppressed both in the year of the pruning treatment and in the following year. The rejuvenated plants showed a different growth rate, reaching in November, at the growth stop, final shoot lengths noticeably different one each other and from the adult control plants (Tab. 31). The growth of the mature shrubs, moreover, was interrupted in early summer, in July. The behaviour of pruned plants was similar to the syndrome shown by lentisk after fire passage (Clemente *et al*., 2005).

Plants indicated as RUM2/23 and OSL 1/10 were chosen as adult control plants.

The first observation about the new vegetation stresses the enormous growth of rejuvenated plants in respect to adult plants. The differences in stem length depended, as said before, both from the prolonged growth season of rejuvenated specimen and the higher relative growth rates in rejuvenated plants in respect to the adult ones. The range of relative growth rates varied among 0.023 for the adult plants, calculated on the basis of the effective numbers of growth (about 81 days) up to 0.026 cm d^{-1} in rejuvenated plants, calculated of the basis of the effective growth season.

Few differences were found among sexes and type of treatments for the considered characteristics.

Figure 9. The pollarding treatment and the subsequent phases of development in lentisk plants.

Table 30. Biometric characteristic of shoots of rejuvenated and adult lentisk plants. Within each column, values with different letters are different at $p \leq 0.05$ (LSD test).

plant	Final shoot length (cm)	Shoot Diameter (cm)	Number of leaves	Number of shootlets	Number of internodes	Internode length (cm)
ARC2/6	66,9 ef	0.64 e	49.67 bcd	0 d	48.67 bc	1.36 f
OSL1/10	8,5 g	0.21 f	6.60 g	0 d	5.60 e	1.58 e
PIANTA 1	82,5 cd	0,96b	48 cd	2.2 c	47 bc	1.78 bcd
PIANTA 10	91,4 bc	1,15 a	47.53 d	3.40 bc	46.53 bc	1.96 b
PIANTA 11	81,4 cd	0.90 bc	45.53 de	5.13 a	44.53 bcd	1.84 bc
PL9	96,4 b	0.84 cd	54.27 bc	0.8 d	53,27 b	1.81 bcd
RUM2/6	65,1 ef	0.66 e	39.80 ef	2.4 c	38.80 cd	1.67 cde
RUM2/12	58,6 f	0.78 d	36.40 f	4.6 ab	35.40 d	1.65 de
RUM2/21	145,2 a	1,15 a	78.20 a	0.4 d	77,2 a	1.89 b
RUM2/23	8,9 g	0.31 f	6.53 g	0 d	5.53 e	1.67 cde
RUM2/31	73,7 de	0.82 cd	35.07f	3.13 c	34.07 d	2.14 a
RUM3/19	66,7 ef	0.65 e	56 b	0.4 d	55 b	1.21 f

Table 31. Relative growth rates of myrtle plants (rejuvenated and adult).

Plant	RGR (cm cm^{-1} d^{-1})
RUM2/21	0.026 a
PL9	0.024 b
PIANTA 10	0.024 b
PIANTA 1	0.023 bc
PIANTA 11	0.023 bc
RUM2/31	0.022 cd
ARC2/6	0.022 de
RUM3/19	0.022 de
RUM2/6	0.022 de
RUM 2/12	0.021 e
RUM2/23	0.023* bc
OSL1/10	0.023* bc

Table 32. Differences among rejuvenated female plants (F), rejuvenated male plants (M) and controls (CF, CM) for some morphological characteristics of annual shoots. Different letters in the same row indicate values differing at $p \leq 0.05$ (LSD test).

	Plant sex and treatment			
	F	**M**	**CF**	**CM**
Final shoot length (cm)	77.47 a	88.12 a	8.90 b	8.54 b
RGR (cm d^{-1})	0.023 a	0.023 a	0.023 a	0.023 a
diameter	0.86 a	0.85 a	0.31 b	0.21 b
Number of leaves	46.11 b	51.99 a	6.53 c	6.60 c
Number of internodes	50.99 a	45.11 b	5.60 c	5.53 c
Number of shootlets	7.63 a	1.87 a	0 b	0 b

The final shoot length, the relative growth rate and the stem diameter were determined by the treatment than by the sex. A statistical difference was found relatively to the number of leaves, that was higher in males, and consequently in the number of internodes. This characteristic is not distinctly attributable to the rejuvenation treatment. Another distinctive characteristic between rejuvenated plants and adult plants was the trend of rejuvenated plant to develop lateral shoots to restablish in a shorter time the lost canopy. This feature was completely absent in shoots of adult plants.

Biomass allocation in cuttings

The allocation of biomass between leaves and stem in cuttings, ten centimetres long each, is reported in Tab. 33. Most of plants showed a similar leaf quantity in cuttings, with the exception of PIANTA1, PIANTA11 and RUM 2/6 that showed a lower amount of fresh leaf biomass and RUM2/23, the female adult, showing the highest amount of leaves in cuttings. The pattern was shown also expressing the leaf weight in terms of dry weight. The stem weight of a cutting tended to be lower in cuttings taken from rejuvenated plants rather than from adults, especially the female control.

Some significant differences were shown among seasons and years relatively to biomass allocation patterns. In 2007, the cuttings showed to have a higher amount of leaf biomass in both seasons of observation (July and November), a symptom of maturation of vegetation, and to grow stems with a higher dry matter (Tab. 34).

Table 33. Allocation patterns of leaves and stems in cuttings. Average values referred to two collecting seasons (July and November 2007). Different letters in the same column indicate different values at $p \leq 0.05$ (LSD test).

	Leaf FW (g)	Leaf DW (g)	Stem FW (g)	Stem DW (g)	Leaf DW %	Stem DW %
RUM 2/21	3.99 ab	1.75 a-c	0.72 ab	0.30 b	41.16 b	41.72 c
PL 9	3.64 ab	1.74 a-c	0.63 a-c	0.28 b	46.17 ab	44.35 ab
PIANTA 10	3.42 ab	1.45 bc	0.52 c	0.22 b	41.15 b	41.29 c
PIANTA 1	2.43 b	1.11 c	0.47 c	0.19 b	46.74 ab	40.55 c
PIANTA 11	2.76 b	1.17 c	0.48 c	0.19 b	41.99 b	39.61 c
RUM 2/31	4.34 ab	2.05 ab	0.57 c	0.24 b	44.57 ab	41.83 c
ARC 2/6	3.40 ab	1.53 bc	0.59 bc	0.26 b	44.03 ab	42.60 bc
RUM 3/19	3.32 ab	1.36 c	0.57 c	0.236	40.77 b	41.55 c
RUM 2/6	2.82 b	1.20 c	0.48 c	0.22 b	41.40 b	41.11 c
RUM 2/12	3.36 ab	1.42 bc	0.53 c	0.22 b	41.20 b	40.77 c
RUM 2/23	4.82 a	2.24 a	0.75 a	0.91 a	46.22 ab	47.30 ab
OSL 1/10	2.98 ab	1.47 bc	0.49 c	0.24 b	49.27 a	48.84 a

Table 34. Leaf and stem weights in each cutting in four sampling dates. Different letters in the same column indicate different values at p≤0.05 (LSD test).

	Leaf/cutting		Stem/cutting	
Date	Total FW (g)	Total DW (g)	FW (g)	DW (g)
July 2006	2.69 c	0.96 c	0.49 c	0.17 b
November 2006	2.28 c	1.04 c	0.58 ab	0.45 a
July 2007	3.30 b	1.49 b	0.55 bc	0.24 b
November 2007	5.48 a	2.66 a	0.64 a	0.31 ab

Leaves of rejuvenated plants were characterized for a lower dry weight than adult plants, with the exception of two rejuvenated genotypes (RUM2/21 e PL9). No statistical differences for this feature were assessed between male and female plants. Some differences were registered, on the contrary, for the stem dry weight in rejuvenated towards adult plants.

Leaf morphology

As far as the morphological characteristics of leaves are regarded, the plants showed a wide range of variation relatively to the petiole length, the linear dimensions of leaves and number and linear dimensions of leaftlets (Tab. 35). No clear differences were found for the leaf dimensions among rejuvenated genotypes and adult plants because of the great dimensions of the leaves of the female control. Anyway, the rejuvenated plants tended to have leaves with higher length and width in respect to male control.

Table 35. Leaf morphological characteristics of lentisk plants subjectedto rejuvenation. The genotypes RUM2/23 and OSL 1/10 are the controls (adult plants not pruned). Different letters in the same column indicate different values at $p \leq 0.05$ (LSD test).

	Petiole length (cm)	Leaf length (cm)	Leaf width (cm)	Leaflet number	Leaflet length (cm)	Leaflet width (cm)	Length/width ratio
RUM 2/21	5.04 cd	8.14 b-d	6.16 a	7.2 b	2.98 ab	1.88 a	1.83 d
PL9	5.5 c	8.5 b	5.85 ab	8.3 b	3.25 a	1.46 bc	2.23 bc
PIANTA 10	5.45 c	8.22 bc	5.84 ab	7.0 b	2.95 ab	1.2 de	2.50 bc
PIANTA 1	4.95 c-e	7.32 ef	5.16 cd	13.6 a	2.7 bc	1.14 de	2.38 bc
PIANTA 11	7.07 a	9.36 a	5.36 bc	10.3 a	2.73 bc	0.86 f	3.21 a
RUM2/31	6.37 b	9.49 a	6.34 a	8.5 b	3.13 a	1.54 b	2.03 cd
ARC2/6	5.13 cd	7.48 d-f	4.8 de	8.7 ab	2.48 cd	1.16 de	2.14 cd
RUM3/19	5.27 cd	7.63 cde	4.61 e	8.7 ab	2.16 d	1.19 de	2.0 cd
RUM2/6	4.8 de	7.42 ef	5.35 bc	7.6 b	2.75 bc	1.32 b-d	2.09 cd
RUM 2/12	5.06	8.26 bc	5.83 ab	8.03 b	3.1 a	1.15 de	2.7 ab
RUM 2/23	6.59 ab	9.7 a	6.08 a	10.10 ab	3.03 ab	1.23 c-e	2.49 bc
OSL1/10	4.34 e	6.81 f	4.91 c-e	7.9 b	2.57 c	1.03 ef	2.52 bc

Ecophysiological features

During the month of July 2007, just before the picking of cutting from mother plants, the ecophysiological parameters of lentisk plants were measured in the middle hours of the day to compare the leaf activities in the different plants (Tab. 36).

The single genotypes presented different features in all the ecophysiological parameters, despite similar exposures to sunlight of the studied leaves.

Table 36. Transpiration rates (T, mmol H_2O m^{-2} s^{-2}), stomatal conductance (gs, mmol H_2O m^{-2} s^{-2}), Leaf temperatures (T_L, °C), Net photosynthesis (PN, μmol CO_2 m^{-2} s^{-2}) and water use efficiency (WUE, mmol CO_2 $mmol^{-1}$ H_2O) in lentisk plants.

	T	g_s,	T_L	PN	WUE
RUM3/19	4,09 a	218,8 a	34,23 abc	12,84 a	3,15 c
ARC2/6	2,24 b	98,67 b	33,66 e	7,74 b	3,48 c
PIANTA10	1,94 bc	78,64 bc	34,00 cde	5,49 bc	2,83 c
PIANTA1	1,93 bc	73,36 bc	33,70 de	6,75 bc	3,78 bc
RUM2/21	1,73 bc	68,82 bcd	34,45 ab	6,70 bc	3,77 bc
RUM2/6	1,47 bc	58,03 cd	34,63 a	7,44 b	5,13 abc
PL9	1,31 bc	54,18 cd	34,03 cde	6,73 bc	5,13 abc
RUM2/23	1,24 bc	47,30 cd	34,53 a	5,09 bc	4,11 bc
RUM2/31	1,165 bc	47,25 cd	34,10 bcd	6,75 bc	6,02 ab
RUM2/12	1,16 bc	48,03 cd	34,53 a	7,21 bc	6,54 a
OSL1/10	3,52 c	40,58 d	33,16 f	3,52 c	3,42 c

The rejuvenated plants showed a greater instantaneous net photosynthesis than adult plants (respectively 7.49 and 4.31 μmol CO_2 m^{-2} s^{-2}) while no difference was assessed as far as the other ecophysiological parameters are regarded. Among rejuvenated plants, no differences were assessed between male and female genotypes.

Total phenols and pigment content in leaves

The chlorophyll a contents in rejuvenated genotypes ranged between 1.05 and 1.32 mg/g fresh weight and between 2.45 and 3.46 mg/g dry weight (Tab. 37) and the chlorophyll b contents between 0.46 and 0.62 mg/g fresh weight and between 1.08 and 1.47 mg/g dry weight. These values are similar to those reported by other Authors for lentisk plants that resprout after an accidental destruction of canopy. The rejuvenated

Table 37. Chlorophyll a and b, chlorophylls ratio ($R_{a,b}$), total carotenoids, total phenols and total starch contents in lentisk rejuvenated genotypes and adult plants. Different letters in the same column indicate different values at $p \leq 0.05$ (LSD test).

	Chlorophyll a		Chlorophyll b		$R_{a,b}$	Carotenoids		Starch	Total phenols	
	(mg/FW)	(mg/g DW)	(mg/g FW)	(mg/g DW)		(mg/g FW)	(mg/g DW)	(%)	(mg/g FW)	(mg/g DW)
RUM2/12	1,25 abc	3,12 abc	0,59 ab	1,47 ab	2,11 c	1,79 ab	4,42 bcd	3,98 cd	8,49 ab	20,39 abc
PIANTA10	1,05 de	2,59 cde	0,46 ef	1,13 cde	2,29 ab	1,99 ab	4,89 abc	3,94 cd	8,93 ab	21,75 ab
PIANTA1	1,11 cd	2,52 de	0,48 def	1,10 def	2,29 ab	1,93 ab	4,41 bcd	3,80 d	7,19 ab	16,46 c
RUM2/6	1,22 bcd	2,87 bcd	0,53 b-e	1,26 bcd	2,28 ab	2,12 a	4,98 ab	4,57 ab	9,63 ab	23,96 a
PIANTA11	1,41 a	3,46 a	0,62 a	1,54 a	2,29 ab	2,31 a	5,65 a	3,91 cd	6,77 b	16,56 c
PL9	1,26 abc	2,80 b-e	0,54 bcd	1,21 cde	2,34 a	1,97 ab	4,37 b-e	4,60 ab	9,58 ab	21,44 abc
RUM2/31	1,06 de	2,45 def	0,46 def	1,08 def	2,28 ab	1,51 b	3,46 e	4,16 bcd	8,19 ab	18,06 bc
ARC2/6	1,14 cd	2,61 cde	0,47 def	1,09 def	2,39 a	2,17 a	5,01 ab	4,38 abc	7,16 ab	16,99 bc
RUM2/21	1,32 ab	3,14 ab	0,57 abc	1,38 abc	2,30 ab	2,03 ab	4,78 a-d	4,63 ab	8,77 ab	19,68 abc
RUM3/19	1,11 cd	2,70 b-e	0,50 cde	1,22 b-e	2,20 bc	1,94 ab	4,72 bcd	4,38 abc	8,79 ab	21,73 ab
OSL1/10	0,93 e	1,93 f	0,41 f	0,85 f	2,29 ab	1,91 ab	3,97 cde	3,94 cd	10,61 a	21,74 ab
RUM2/23	1,07 de	2,32 ef	0,46 ef	1,00 ef	2,33 a	1,81 ab	3,95 de	4,74 a	7,90 ab	17,47 bc

genotypes showed to be richest in chlorophyll a than the adults, showing respectively 1.19 and 1.00 mg/g fresh weight and 2.83 and 2.13 mg/g dry weight of chlorophyll a and 0.53 and 0.44 mg/g fresh weight and 1.25 and 0.93 mg/g dry weight of chlorophyll b. The chlorophyll a values showed to be relatively constant in summer months and in autumn, in fact, no statistical differences were assessed between seasons. On the contrary, the chlorophyll b contents showed to increase in autumn if we refer to fresh weights, but to decrease in terms of dry weights. Similar trends to those of chlorophyll b were shown by total chlorophylls as a consequence of the sums of the two chlorophylls.

The ratio chlorophyll a to chlorophyll b ranged in rejuvenated genotypes between 2.11 and 2.39. No statistical differences were assessed between rejuvenated and control plants. This ratio showed to be influenced by the season, as chlorophylls ratio showed a trend towards an increase in November, assuming values of 2.53 to 2.21 in summer months.

Total carotenoids ranged between 1.51 (3.46) and 2.31 (5.65) mg/g fresh weight (dry weight). Statistical differences attributable to the rejuvenation treatment were found only for the carotenoids content in terms of dry weight, with the rejuvenated genotypes showing higher values towards controls (4.67 to 3.96 mg/g dry weight). Carotenoids content showed a slightly trend to increase in autumn.

As far as the total starch is regarded, the range of values varied between 3.80 and 4.63 % in rejuvenated genotypes but as a mean value, control plants resulted to be richer in starch (4.58% to 4.24%). Higher values were found in November than in July.

Total phenols content ranged between 6.77 (16.46) and 9.63 (21.75) mg/g fresh weight (dry weight) in rejuvenated plants. The rejuvenation treatment seemed to be ineffective in modifying phenols content in plants, as no statistical differences were assessed between rejuvenated and adult plants. As reported for other plants, like myrtle, phenols content showed to increase in winter months in respect to summer months.

Elemental composition of leaves

The average values of macroelements (N, P, Na, K, Ca, Mg,) and microelements (Cu, Zn, Fe, Mn) for control and rejuvenated lentisk plants are reported in Table 38. Rejuvenated plants showed to have statistically different values in respect to control plants relatively for the nitrogen content (respectively 1.32 and 1.13%), phosphorous (0.159 and 0.189%), magnesium (0.25 and 0.21%), copper (2.875 and 0.708 ppm), zinc

Table 38. Elemental composition of rejuvenated and adult plants of lentisk. Different letters in the same column indicate different values at $p \leq 0.05$ (LSD test).

	N (%)	P (%)	Na (%)	K (%)	Ca (%)	Mg (%)	Cu (ppm)	Zn (ppm)	Fe (ppm)	Mn (ppm)
RUM2/12	1,43 a	0,15 bc	0,09 a	0,67 c	1,13 b	0,27 ab	2,91 bc	10,08 a	41,33 ab	59,66 ab
PIANTA10	1,38 a	0,13 c	0,1 a	0,73 c	1,07 b	0,24 abc	2,83 bc	7,33 ab	30,75 c	43,50 ab
PIANTA1	1,37 a	0,14 c	0,09 a	0,92 ab	1,14 b	0,27 ab	2,24 bcd	8,82 ab	34,41 bc	58,16 ab
RUM2/6	1,37 a	0,17 abc	0,097 a	0,77 bc	0,99 b	0,24 bc	2,83 bc	8,58 ab	33,33 bc	58,08 ab
PIANTA11	1,35 a	0,14 c	0,129 a	0,94 a	0,12 b	0,22 c	3,43 ab	7,47 ab	36,75 bc	46,66 ab
PL9	1,31 a	0,16 abc	0,079 a	0,66 c	0,92 b	0,23 bc	2,99 bc	6,74 ab	35,75 bc	52,83 ab
RUM2/31	1,31 a	0,17 a-c	0,091 a	0,66 c	1,07 b	0,24 abc	2,49 bcd	8,49 ab	29,08 c	47,66 ab
ARC2/6	1,31 a	0,18 ab	0,085 a	0,67 c	1,87 a	0,29 a	2,49 bcd	8,66 ab	48,41 a	68,66 a
RUM2/21	1,27 ab	0,17 a-c	0,105 a	0,73 c	1,06 b	0,25 abc	1,66 cd	7,91 ab	33,25 bc	46,41 ab
RUM3/19	1,13 b	0,19 a	0,111 a	0,70 c	0,87 b	0,23 bc	4,83 a	7,83 ab	33,41 bc	33,50 b
OSL1/10	1,13 b	0,19 a	0,099 a	0,67 c	1,13 b	0,22 c	0,083 e	4,24 b	30,33 c	30,75 b
RUM2/23	1,13 b	0,18 ab	0,100 a	0,78 bc	1,15 b	0,21 c	1,33 de	4,16 b	36,25 bc	46,50 ab

(8.22 and 4.20 ppm), and manganese (51.51 and 38.62 ppm). No differences were assessed for the other elements.

In the comparison between sexes, female rejuvenated plants showed, in respect to male ones, higher contents of nitrogen (1.355 to 1.289%), potassium (0.806 to 0.683%) and calcium (1.237 to 1.011%).

Nitrogen, phosphorous and magnesium contents were relatively constant between the two seasons of observations, while sodium, calcium, iron and manganese were higher in November, potassium and copper, zinc in summer months.

Rooting performances

The propagation trials began in the first days of July 2006 and were repeated in November 2006, July and November 2007.

The rooting probabilities associated to the single genotypes were quite different in the different season, ranging from 0% to 98.3%. The average mean value for the entire population of rejuvenated genotypes was about 20%, while the adult plants showed an average rooting percentage of 18%, two very similar values. Among rejuvenated genotypes, male plants showed an average rooting probability about 24%, very similar to those of female control plant (25%), while female genotypes showed a rooting probability of 17%, just slightly higher than the one of male control (12%).

The rejuvenation treatment apparently showed to be ineffective in improving in a sensible manner the rooting ability of lentisk plants, as the rooting percentages of rejuvenated population and control plants were quite similar.

Time sampling affected the rooting efficiency too. The most favourable season for rooting was November than July, when the average rooting probabilities were respectively 26% and 15%. These findings confirm the results obtained by La Viola *et al*. (2004) that assessed a season effect on rooting efficiency in lentisk cutting, with the best results obtained in the winter months.

Table 39. Rooting efficiency in lentisk cuttings collected from rejuvenated stock plants and adult plants. The values are expressed in percentage.

	Year			
	2006		2007	
Genotypes	July	November	July	November
ARC2/6	34.7±4.8	73.2±1.3	10.0±8.7	24.0±10.6
PIANTA 1	0	1.7±1.3	0	0
PIANTA 10	9.0±3.7	1.8±0.5	1.3±2.3	0.7±1.2
PIANTA 11	26.7±6.7	5.0±1.5	0	0
PL 9	6.8±0.3	28.3±4.6	31.3±8.1	0
RUM 2/6	60.5±0.9	66.7±1	6.0±2.0	12.0±6.9
RUM 2/12	34.9±1.4	98.3±1	65.3±10.1	44.0±13.9
RUM 2/21	3.4±3.9	31.7±2	4.0±6.9	14.7±2.3
RUM 2/31	4.4±3.8	75±0	28.0±10.6	1.33±2.3
RUM 3/19	2.2±3.8	3.3±0.6	7.3±6.4	0
RUM 2/23 (CF)	0	41.7±1	4±4	54.0±26.5
OSL 1/10 (CM)	6.2±6.2	40.4±1.6	1.3±2.3	0.7±1.2

Correlations

Some correlations were found between rooting efficiency and some variables measured in the vegetation to coincide with the rooting experiments. Unlike myrtle, total phenols were negatively related with rooting efficiency while the trend for chlorophylls and nitrogen went in the same direction that is a positive trend. No correlations were found between rooting ability and the morphological variables.

Variable	r	Variable	r
Total phenols (FW)	-0.22 **	K	ns
Total phenols (DW)	-0.17**	Ca	ns
Total Chlorophylls (FW)	0.26**	Mg	ns
Total chlorophylls (DW)	0.21**	Cu	ns
N	0.28**	Zn	ns
P	ns	Fe	0.20**
Na	0.21**	Mn	0.23**

Lentisk: effects of shading

Effect on biomass development

Shading treatments were applied to adult plants of lentisk in April 2007, just before the female flowering and then plants were allowed to sprout.

The new vegetation showed a rhythmic growth with a peak in stem elongation in the months of June up to the first days of July, when the stems stopped elongation with the increasing of temperatures in August and no elongation was registered after summer. The growth of sunlit and shaded shoots was synchronous but the shading treatment induced a higher growth rate in the shaded stems, with the final result to increase the final length in shaded twigs (Fig 10). All the plants under examination showed the same behaviour as far as the dynamic of elongation is regarded, except for the relative growth rate (Tab. 40).

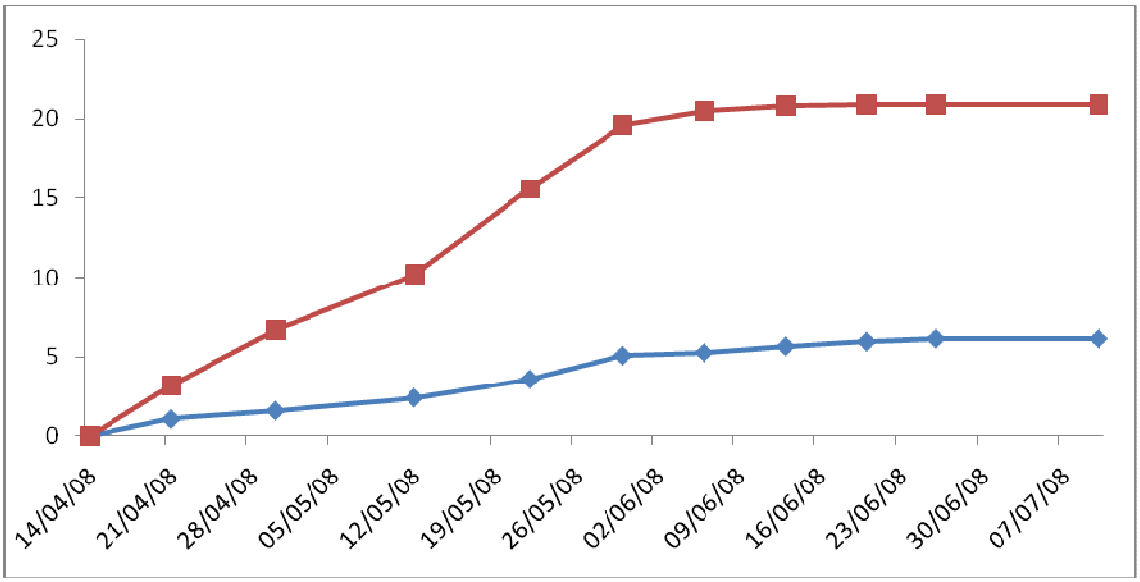


Figure 10. Trend of the shoot growth of lentisk plants partially shaded. The elongation curves refer to the lentisk plants MOD ½. The red line refers to the elongation of shaded shoots and the blue line to the elongation of sunlit shoots.

Tab 40. Relative growth rates of annual shoots of lentisk plants under two different light environment: sunlight (irradiance 100%) and shade (irradiance 50%).

	Irradiance	
	100%	50%
BOA2	0.033	0.037
MOD1/2	0.021	0.035
OSL2/11	0.019	0.028
OSL2/13	0.027	0.034

Shoot structure

The final length of shoots of the various lentisk plants ranged from 29.88 cm in BOA2 to 13.73 cm in OSL2/11. Under shade, the final length of shoots was about 22.23 cm while in sunlight the average shoot length was 17.45 cm. The genotypes showed to adjust shoot elongation under the different light regimes in different manners, as in the genotype BOA2 alone the final shoot length was statistically different from the shaded ones. (Tab. 41). The number of internodes ranged from 9.03 in OSL 2/11 up to 20.03 in BOA2. Under shade the number of internodes was, in average, higher than in light (respectively 14.21 and 12.5 internodes per shoot). The genotypes showed once more a different attitude to adapt to light reduction. The genotype BOA 2 alone showed a clear behaviour, exhibiting an increase in the number of internodes in shade. The clones OSL2/11 and OSL2/13 showed, on the contrary, a slightly tendency toward the reduction of the number of internodes. No statistical differences among genotypes were assessed as far as the mean internodes length is regarded as the range of variation for this variable was very small (1.42 cm in OSL 2/13 to 1.54 in MOD1/2). Anyway, the reduction of light levels induced an extension in internodes mean length (1.52 cm in shade and 1.38 cm in light).

The number of leaves in annual shoots ranged from 10.03 in OSL2/11 to 21.03 in BOA2. As the number of leaves was directly related to the number of internodes, these variables showed the same trend, with a higher number of leaves being differentiated in shade (15.21 in shade and 13.51 in sunlight) and the single genotypes showing a contrasting behaviour. Finally the genotypes showed a similar leaf density, i.e. an equal number of leaves in the unit of length of stem, ranging from 0.72 in BOA2 to 0.80 in

OSL2/12. In light, anyway, the leaf density was higher than in shade, indicating that plants in shade tended to limit the investment in leaves to cope with the light reduction.

Table 41. Differences in annual shoot structure in lentisk shoots grown in sunlight and in shade.

	Irradiance	
	100%	50%
Stem length (cm)		
BOA2	23.13 b	36.63 a
MOD1/2	13.87 de	20.17 bcd
OSL2/11	11.34 d	15.41 cde
OSL2/13	21.49 bc	16.27 b-e
Number of internodes		
BOA2	16.40 b	23.67 a
MOD1/2	10.33 de	12.20 cd
OSL2/11	9.47 de	8.60 e
OSL2/13	13.87 bc	12.40 cd
Internodes mean length (cm)		
BOA2	1.42 bcd	1.53 abc
MOD1/2	1.36 bcd	1.66 ab
OSL2/11	1.20 d	1.79 a
OSL2/13	1.51 abc	1.33 cd
Number of leaves/shoot		
BOA2	17.40 b	24.66 a
MOD1/2	11.33 de	13.20 cd
OSL2/11	10.46 de	9.60 e
OSL2/13	14.86 bc	13.40 cd
Leaf number/cm shoot length		
BOA2	0.75 bc	0.69 bc
MOD1/2	0.82 ab	0.65 c
OSL2/11	0.95 a	0.65 c
OSL2/13	0.78 bc	0.82 ab

Biomass allocation in annual shoots

The variables that identify the biomass allocation between stems and leaves are reported in tab.42.

The single genotypes exhibited a typical stem weight, ranging from 4.8 (2.04) g in OSL2/11 to 13.15 (5.46) in BOA2 in terms of fresh weight (dry weight). The shoot weight, depending from the number and the weight of leaves, resulted higher in shade

than in light, showing values of 9.68 and 7.43 g respectively. Most genotypes showed an increment in shoot weight under reduced light levels, with the exception of OSL2/11 that exhibited an opposite behaviour. The shoot dry weight in percentage was similar among the genotypes, ranging from 41.33 in BOA2 to 46.36 % in MOD1/2. No differences were assessed for the dry weight between light and shade and few differences existed among genotypes in the interaction with light levels.

The proportion of leaves in shoots increased from light (5.87 g fresh weight, 2.54 g dry weight) to shade (7.5 g fresh weight; 3.17 g dry weight) because of the higher leaf number in shade.

Table 42. Biomass allocation in annual shoots of lentisk plants grown under differential irradiance levels.

	Irradiance	
	100%	50%
Shoot fresh weight (g)		
BOA2	11.05 b	15.24 aa
MOD1/2	7.34 bc	11.02 b
OSL2/11	5.1 c	4.51 c
OSL2/13	6.83 c	7.35 bc
Shoot dry weight (g)		
BOA2	4.45 b	6.52 a
MOD1/2	3.58 bc	4.80 b
OSL2/11	1.98 d	2.10 d
OSL2/13	2.91 cd	3.03 cd
Shoot dry weight (%)		
BOA2	39.97 b	42.69 b
MOD1/2	48.90 a	43.82 ab
OSL2/11	43.96 ab	41.27 b
OSL2/13	42.62 b	41.53 b
Leaf/shoot fresh weight (g)		
BOA2	8.41 b	1083 a
MOD1/2	6.04 c	8.84 b
OSL2/11	3.85 d	4.24 cd
OSL2/13	5.20 cd	6.08 c
Leaf/shoot dry weight (g)		
BOA2	3.29 bc	4.62 a
MOD1/2	2.98 bcd	3.84 ab
OSL2/11	1.69 e	1.73 e
OSL2/13	2.21 de	2.49 cde

Leaf morphology

As far as the leaf morphology is regarded, the genotypes showed to be characterized by different petiole length but this variable was not affected by irradiance levels. Some differences between lights were assessed for the leaf length, in fact the shade leaves showed a mean length of 8.35 cm and sunlit leaves of 7.82 cm, and for leaf width that was about 6.36 cm in shade and 5.75 in light. The length to width ratio was unaffected by light levels, being stabilized around 1.33-1.37, and the same was evidenced for the number of leaflets. On the contrary, the length and the width of leaflets were higher in shade than in sunlight, showing respectively values of 3.46 cm and 3.2 cm (length) and 1.20 cm and 1.09 cm (width).

Ecophysiological features

During the month of July 2007, the ecophysiological variables were measured during the central hours of the day. The single genotypes showed different features for all the ecophysiological parameters (Tab. 43) answering in a very confusing manner to irradiance levels.

Total phenols and pigment content

The genotypes differed widely in the phenols contents of leaves, ranging from 7.9 in BOA2 to 10.79 mg/g fresh weight in MOD1/2. Phenols content was similar in sun leaves (9.32 mg/g fresh weight) and in shade leaves (8.86 mg/g fresh weight). A seasonal effect was found for phenols content that tended to be higher in winter than in summer months, as previously seen for rejuvenated lentisk and adult plants, ranging from 4.28 to 6.15 mg/g fresh weight in summer and from 11.31 to 15.44 g in winter. Similar considerations could be done for phenols content in terms of dry weight.

The genotype OSL2/11 resulted the richest in chlorophyll a (1.28 mg/g fresh weight, 2.75 mg/g dry weight), followed by BOA2 (1.10 mg/g; 2.0 mg/g dry weight), MOD1/2 (1.07 mg/g; 2.01 mg/g dry weight) and finally OSL 2/13 (0.89 mg/g; 1.78 mg/g dry weight). No differences were assessed between the two seasons of observation.

As far as the light environment is regarded, the chlorophyll a contents (fresh weight and dry weight) were higher in shade than in light (1.2 and 0.97 mg/g fresh weight and 2.5 to 1.87 mg/g dry weight). Similar trends were found for chlorophyll b, with the

exception of an increase in summer months than in winter. The ratio chlorophyll a to chlorophyll b showed to be higher in winter than in summer (1.94 to 1.82) and in shade than in light (1.92 to 1.84).

Tab. 43. Ecophysiological parameters of sun and shade leaves of lentisk.

	Irradiance	
	100%	50%
Transpiration		
BOA2	0.032 bc	0.022 c
MOD1/2	0.034 bc	0.049 a
OSL2/11	0.037 ab	0.036 ab
OSL2/13	0.024 c	0.038 ab
Stomatal conductance (Gs)		
BOA2	52.58 a	26.41 cd
MOD1/2	27.41 cd	54.14 a
OSL2/11	36.02 bc	49.73 a
OSL2/13	20.57 d	48.80 ab
Leaf temperature (°C)		
BOA2	25.0 b	23.85 c
MOD1/2	26.5 a	24.05 bc
OSL2/11	25.0 b	22.0 d
OSL2/13	25.0 b	22.8 d
Net photosynthesis		
BOA2	4.74 b	3.18 cd
MOD1/2	2.67 d	4.39 b
OSL2/11	5.72 a	3.96 bc
OSL2/13	1.75 e	3.35 cd
Water Use Efficiency		
BOA2	148.40 ab	167.11 a
MOD1/2	81.79 c	92.15 c
OSL2/11	156.24 a	111.63 bc
OSL2/13	75.76 c	87.66c

Elemental composition

Leaves showed to be richer in nitrogen in winter than in summer (2.21 to 1.5%) but no differences were assessed between shade and sun leaves; no differences between winter and summer was assessed for phosphorous content in leaves while the shading treatment induces an increment in shade leaves (0.216%) in respect to light leaves (0.18%); no differences were assessed for sodium, potassium, magnesium, copper contents in different seasons and light levels; calcium levels were influenced only by the shading treatment, with the sun leaves richer than shade leaves (1.40 to 1.33%) similarly to zinc, iron and manganese contents.

Rooting

The rooting trials were completely unsuccessful both in winter and in summer. After three months in bench, just two rooted cuttings were found.

CONCLUSIONS

Light availability is an important factor in determining myrtle morphological and biochemical features. Annual shoots showed an improved growth in low light conditions, a greater final length due to the internodes elongation that compensated for the reduction in number of internodes, a higher leaf area and a tendency towards the increment in relative leaf biomass in respect to total weight of shoots in low light conditions. A genotype effect was found to interact with light conditions. This behaviour was shown also in *in vitro* cultures where an additional interaction was found with the culture medium.

These acclimatization features are similar to those showed by seedlings of *Myrtus communis* L. grown in full light and shade in natural environments (Mendes *et al.*, 2001) and other seedlings of species tolerant to shade like *Quercus* spp. (Cardillo and Bernard, 2006). At a biochemical level, the total chlorophyll and nitrogen content increased from sunlight to heaviest shade rates probably to compensate the light reduction and from higher to low irradiance rates in *in vitro* cultures. This adaptation was found both in myrtle leaves in natural environments and in other ornamental stock plants shaded to various light rates like *Aniba rosaeodora* Ducke (Rosa *et al*., 1998) and in *Cotynus coggyria* (Pacholczak *et al*., 2005). The higher photosynthetic pigment content in shaded myrtle leaves resulted in an increase in net photosynthetic rate under 35% irradiance during summer months. The levels of total phenols too was found to increase under shade, but the clones tested in Oristano and in Sanremo showed a different behaviour relatively to the irradiance rate in which the phenols level was maximized.

The photosynthetic pigment content and the phenols content were positively correlated to rooting abilities of myrtle cuttings. The first correlation could be explained in terms of better photosynthetic conditions that may support the production of sugars and other carbohydrate contents that may favourite the survival of cuttings during the adventitious rooting phase (Druege *et al.*, 2004). As far as the phenols are regarded, they does not stimulate rhizogenesis by themselves but act synergistically or additively to phytohormones, in special way with auxins (Bassuk and Maynard, 1987) but sometimes their presence inhibits adventitious rooting. A positive correlation between phenols and rooting similar to that we found in our experiments was found in *Cotynus coggyria* by

Pacholczak *et al*. (2005). Zinc also was found to correlate positively with rooting and its contents are higher in shade. Zinc was found to be one of the microelements certainly involved in auxin and gibberellins metabolism so it is probably an improvement in phytohormones metabolism in shaded plants.

The shading treatment, as a consequence, resulted effective in bettering the general physiological conditions of myrtle stock plants and in promoting the production of classes of substances and the accumulation of elements that can be related with rooting abilities of cuttings. A strong seasonal effect, however, was found to determine the yield in rooted cuttings, with the best results obtained from August to February.

We conclude that in myrtle the shading treatments can strongly improve the rooting performances of cuttings through the bettering of stock plants physiology, but the "genotype effect" and the seasonality should be studied before to maximize propagation performances of specific clones and the quality of the adventitious root systems.

As far as lentisk is regarded, the shading treatment applied to adult plants in average induced some morphological adaptations to reduced light like the shoot elongation, the increase in internodes length and in leaf linear dimensions (that determined a higher leaf area) and the decrease of leaf density to cope with low light, but the answer was strongly influenced in intensity and in direction by the genotype, so far as the chlorophylls and the phenols contents. The increment in chlorophylls content in shade leaves was not clearly related to photosynthetic rates or to the other ecophysiological measured parameters. The phenols content was apparently not influenced by light levels but only by the season, with higher levels in winter. Anyway, no effects of stock plants physiology on rooting performances of cuttings could be deduced as no rooted cuttings were obtained by shaded and unshaded shoots of adult plants. We could conclude that the reduction of light levels applied to adult lentisk plants leads to adaptations that are not so useful in changing physiology of stock plants and in bettering propagation results.

In lentisk, like in other difficult-to-root species, the ontogenetic phase was, on the contrary, a very important factor in inducing some features of great impact in growth of the stock plants and in the subsequent results in propagation.

The rejuvenated plants showed a high induced vigour in respect to controls (longer shoots), a higher number of leaves and a higher number of lateral twigs that meant a

great photosynthetic area in respect to annual shoots of adult plants and a lower dry weight (reduction of sclerophylly). Moreover, the rejuvenated plants showed higher nitrogen and chlorophylls contents. These features were accompanied by a greater average net photosynthetic rate in rejuvenated plants in respect to adult plants, but with some differences among genotypes, confirming the findings of Clemente *et al*. (2005). The phenols content was apparently not influenced by rejuvenation treatment but only by seasonality. Some differences between rejuvenated and adult plants in elemental composition of leaves indicate that in rejuvenated plants the general metabolism had a great improvement, as elements such phosphorous, magnesium, zinc and manganese are involved in important metabolic processes as enzymatic co-factors or regulators.

As in the case of myrtle, total chlorophylls and nitrogen content were positively correlated with rooting performances, but total phenols were negatively correlated. This indicates that in lentisk phenols, or some categories of phenols to be studied in detail, act as auxin inhibitors. Zinc and irons levels too were positively correlated with rooting performances, indicating that an active metabolism that characterize rejuvenated plants is a key factor in driving adventitious rooting in lentisk, also if strong effects are related to specific genotypes and seasonal factors.

Our results indicate that in lentisk, as in many other woody hard-to-root plants, the ontogenetical phase may be the most important factor in determining the rooting ability, as it influences in a great measure the physiology of donor than other factors, like the environmental ones.

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