

## Effects of different nursery conditions on the plant development and some leaf characteristics in Chestnuts (*Castanea sativa* Mill.)

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### Abstract

The aim of this study was to determine the influence of different nursery conditions on plant development (the length and diameter of graft shoot) and some leaf characteristics (leaf number per plant, mean leaf area and total leaf area per plant) in the chestnut. The study was carried out in the open field and under shaded (50%) and unshaded greenhouse conditions. The inverted radicle grafting method was used with scions of genotype SE 3-12 and Marigoule on seedlings of 554-14 genotype. Grafting was performed during the second week of May in the first year and during the last week of April in the second year. Significant differences in plant development and leaf characteristics were found in different nursery conditions. The shoot length and the mean and total leaf area obtained under shaded greenhouse conditions were higher ( $P < 0.01$ ) than that from other conditions; also leaf numbers obtained under unshaded greenhouse conditions were higher ( $P < 0.01$ ) than those from other conditions. The highest correlation ( $r = 0.925$ ) amongst the plant growth parameters was determined to be that between shoot length and plant total leaf area ( $P < 0.01$ ). These results indicate that the chestnut genotypes used in this study are capable of surviving in a shadier environment and that under these conditions the length and diameter of the graft shoot is maximum at the end of the vegetation period.

**Keywords:** chestnut seedling, greenhouse, shoot length, leaf area, shading

**Abbreviations:** SEM: Standard Error of the Mean

### Introduction

Many factors, such as genetic and environmental, affect plant growth and development. Plants are able to modify their growth, development and physiology according to their environment. The ability of plants to do this plays a key role in determining their tolerance to stress and their maintaining efficient growth (Murchie and Horton, 1997; Walters et al., 2003). Therefore environmental conditions, such as light and temperature, are the most important factors on plant growth and development. Temperature has a considerable influence throughout the development of a plant too (Koorneef et al., 2002), stimulating developmental processes such as plant growth, seed dormancy and release, germination and vernalization (Heggie and Halliday, 2005). Chestnut tree species are moderately thermophilic, being well adapted to ecosystems with an average temperature between 8-15°C (Gomes-Laranjo et al., 2006). Light is not only an energy source for photosynthesis but also a stimulus that regulates numerous developmental processes, from seed germination to the onset of flowering. Light intensity, quality and duration are also critical factors affecting the early survival and growth of tree seedlings, because of their importance in determining the morphological and anatomical structure of plants (Christie, 2007). Therefore the light intensity, quality and duration, as well as temperature should be adjusted with shading material. Shade tolerance of the American chestnut (*Castanea dentata*) has been investigated by measuring morphological and physiological leaf parameters (Joesting et al., 2009). Researchers have reported that seedlings, saplings, and mature trees have high maximum rates of photosynthesis and a low leaf mass per unit area in shade tolerant species; and furthermore that these species also have other features to

compensate for low light levels. Groninger et al. (1996) noted that total biomass reduced with shading of seedlings of the loblolly pine (*Pinus taeda* L.), white pine (*Pinus strobus* L.), red maple (*Acer rubrum* L.), and yellow-poplar (*Liriodendron tulipifera* L.). The net photosynthetic rate, plant height, diameter and total biomass increased with increasing light intensity, but the specific leaf area significantly increased with higher levels of shading in the American chestnut (*C. dentata*) (Wang et al. (2006). Additionally, field studies have noted a greater survival of chestnut sprouts and seedlings under moderate shade compared to full-sun (Anagnostakis, 2007), although a greater initial seedling growth is typically measured in high-light environments (McNab, 2003). The environmental conditions during and following grafting are factors influencing the healing of the graft union and plant growth (Hartmann et al., 1990). The highest rates for graft success and plant survival were obtained from shaded greenhouse conditions in the European chestnut (*C. sativa*) (Ozturk et al., 2009). Although there is some information on the healing of the graft union and plant growth in chestnuts, there is much that is still unknown. Little research has been published on the physiological ecology of chestnuts, such as how they respond to light, humidity, and other environmental conditions. The SE 3-12 genotype is a promising cultivar candidate for fresh consumption (Serdar et al., 2009). The Marigoule cultivar grows very well in the Black Sea Region (Serdar and Macit, 2010). Grafted plant production is currently being planned with these chestnut genotypes. However, there is no evidence on whether growing the SE 3-12 chestnut genotype (Serdar, 1999) and Marigoule (Chapa

and Verlhac, 1978) in shaded or unshaded greenhouse conditions affect plant development (the length and diameter of graft shoot) and some leaf characteristics (leaf number per plant, leaf area and total leaf area per plant). Nor, whether the length and diameter of the graft shoot which depend on the leaf number, mean and total leaf areas per plant of chestnuts, are influenced by the nursery conditions. The objective of this study was to investigate different nursery conditions on the plant development (shoot length and diameter) and some leaf characteristics (leaf number, and mean and total leaf areas per plant) in the Chestnut. Accordingly, in the study reported here, the changes in the length and diameter of the graft shoot, leaf number, and the mean and total leaf areas per plant of two chestnut genotypes (SE 3-12 and Marigoule), influenced by shaded, unshaded and open field nursery conditions, were investigated. It was also determined whether the length and diameter of the graft shoot of chestnuts depend on the leaf number, mean and total leaf areas per plant when influenced by the nursery conditions.

## Results and discussion

### *Plant Development*

The length and diameter of shoots in chestnuts grown in the shaded and unshaded greenhouse, and open conditions with respect to the year and cultivars are presented in Table 1. Nursery conditions affected the shoot length in used chestnut genotypes ( $P < 0.01$ ). The length and diameter of shoots were higher ( $P < 0.01$ ) in the shaded greenhouse than the other nursery conditions (Table 2). The chestnut genotypes that were studied are capable of surviving in a shadier environment and of maximizing growth in an environment with less available light. Also, the results indicate that these genotypes are shade tolerant: particularly like the intermediate shade tolerant genotypes of the American chestnut studied by McCament and McCarthy (2005); Anagnostakis (2007) and Joesting et al., (2007). Moreover, the results from the studies of these authors (McCament and McCarthy, 2005; Anagnostakis, 2007; Joesting et al., 2007) support the suggestion of King (2003) who stated that the general response of the American chestnut to light is that of intermediate shade tolerance. There was a significant effect of the cultivar upon shoot length ( $P < 0.01$ ) but not on shoot diameters (Table 1). The shoots of the SE 3-12 genotype were shorter ( $P < 0.01$ ) than the Marigoule. This supports the idea that some plant and phenological characteristics of chestnut genotypes from the Black Sea Region of Turkey differ from those of the Marigoule, a European x Japanese hybrid cultivar (Serdar et al., unpublished data). On the other hand, the interaction between the cultivar and the year had a significant effect on shoot length ( $P < 0.05$ ). Also there were significant effects of the nursery conditions x cultivar x year interaction on shoot length ( $P < 0.05$ ). There were significant effects of the year on studied shoot characteristics ( $P < 0.01$ ) (Table 1). Plant development in the second year was stronger than that in the first year ( $P < 0.01$ ). The effect of the year on the shoot characteristics of the cultivars studied may be the result of higher temperatures during the nursery conditions (Figure 1) or a relation between seed planting time and seedling growth in the chestnut. Furthermore, Bilgener and Serdar (1995) reported that chestnut seeds planted at the beginning of April had better seedling growth than those

planted at the end of April. Whilst in the present study the vegetation period in the first year was shorter (by 12 days) than that in the second year; in the study of Bilgener and Serdar (1995) the difference between the experimental years was 30 days. Therefore it is likely that our results with respect to the year effect indicate that differences in the shoot characteristics of the cultivars studied between experimental years depend on differences of temperature rather than differences of the vegetation period between experimental years. In the present study the chestnuts grown in the shaded greenhouse had 40% longer internodes than those in the other nursery conditions, confirming the suggestion of Kevseroglu (1999). The present study also observed that this increase in the distance between internodes may cause a semi-recumbent development of grafted seedlings in the chestnut. There were significant effects of nursery condition and year interactions on the length and diameter of shoots ( $P < 0.01$ ) (Table 1). The highest shoot length was obtained from the plants grown in shaded greenhouse conditions in the second year (71.7 cm). Groninger et al. (1996) reported that stem height growth in yellow-poplar increased by 89% in the shade. Unlike shoot length, the highest shoot diameter was obtained from the plants grown in the open field in the second year (6.87 mm) (Figure 4). In the first year, from grafting (10th May) to the end of the vegetation period (December) shaded, unshaded greenhouse and open conditions had a mean temperature of 19.8, 20.2, and 17.7°C, a minimum of 6.8, 6.5 and 7.8°C, a maximum of 26.2, 28.0 and 32°C, and a relative humidity of 80.1, 76.3 and 77.3%, respectively. For the second year, the corresponding values were a mean temperature of 22.7, 22.9, and 20.4°C, minimum of 6.8, 6.5 and 7.8°C, maximum of 26.2, 28.0 and 32°C, and relative humidity of 78.5, 75.3 and 75.8%, respectively (Figures 1 and 2). The mean temperature of the open field in the second year was higher than even the unshaded greenhouse in the first year (20.4 and 20.2°C respectively). Shoot diameter had a lower correlation with the mean or total leaf area than shoot length (Table 3). Higher light intensity was measured in the open field than in the greenhouse conditions in both years (Figure 3). The higher shoot diameter may have been because of higher carbohydrate accumulation in the open field in the second year. This may explain why plant development and some leaf characteristics in chestnuts may change as influenced by the nursery conditions, regardless of the cultivar.

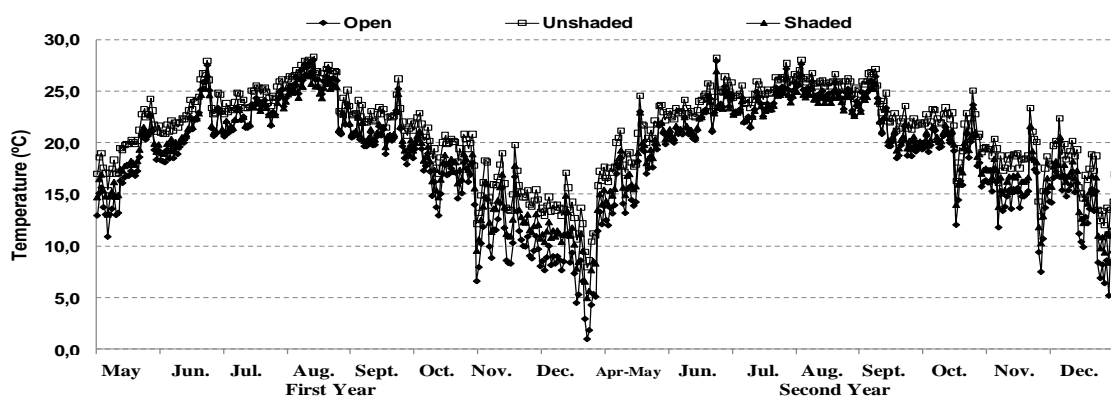
### *Leaf Characteristics*

The leaf number, mean and total area of the leaf in chestnuts grown in the shaded and unshaded greenhouse, and open conditions with respect to years and cultivars are presented in Table 1. Nursery conditions affected the leaf characteristics of used chestnut genotypes ( $P < 0.01$ ). The mean and total leaf area (which are important characteristics for plant development) of chestnuts grown in a shaded greenhouse were higher (60.2 cm<sup>2</sup> and 1270.1 cm<sup>2</sup> respectively) ( $P < 0.01$ ) than both those grown in an unshaded greenhouse (35.2 cm<sup>2</sup> and 819.0 cm<sup>2</sup> respectively) and those grown in the open (33.3 cm<sup>2</sup> and 589.1 cm<sup>2</sup> respectively) (Table 2). Also, the total leaf area of chestnuts grown in an unshaded greenhouse was higher ( $P < 0.01$ ) than that of those grown in the open. Lambers et al. (1998) reported that increases in the area and number of leaves are a common response of leaves that are adapted to shadier conditions and of species that are shade

**Table 1.** The length and diameter of shoot and the number, mean area and total area of leaf in chestnuts grown in shaded and unshaded greenhouse and open conditions with respect to years and cultivars

| Nursery Conditions                   | Genotypes | Shoot       |               | Leaf   |                         |                               |
|--------------------------------------|-----------|-------------|---------------|--------|-------------------------|-------------------------------|
|                                      |           | Length (cm) | Diameter (mm) | Number | Area (cm <sup>2</sup> ) | Total Area (cm <sup>2</sup> ) |
| First Year                           |           |             |               |        |                         |                               |
| Shaded                               | Marigoule | 59.6        | 6.18          | 16.7   | 61.9                    | 1036.1                        |
|                                      | SE 3-12   | 61.0        | 5.99          | 20.3   | 50.3                    | 1018.4                        |
| Unshaded                             | Marigoule | 41.9        | 5.52          | 19.4   | 35.1                    | 675.2                         |
|                                      | SE 3-12   | 44.6        | 5.34          | 25.5   | 20.6                    | 525.8                         |
| Open                                 | Marigoule | 19.3        | 4.12          | 10.3   | 31.0                    | 322.2                         |
|                                      | SE 3-12   | 16.5        | 3.65          | 10.0   | 18.5                    | 179.0                         |
| Second Year                          |           |             |               |        |                         |                               |
| Shaded                               | Marigoule | 79.8        | 6.59          | 23.9   | 67.3                    | 1606.2                        |
|                                      | SE 3-12   | 63.7        | 5.70          | 23.1   | 61.5                    | 1419.4                        |
| Unshaded                             | Marigoule | 57.4        | 5.78          | 23.5   | 53.0                    | 1246.8                        |
|                                      | SE 3-12   | 43.2        | 5.68          | 26.2   | 32.0                    | 828.3                         |
| Open                                 | Marigoule | 45.7        | 6.87          | 19.9   | 47.6                    | 947.5                         |
|                                      | SE 3-12   | 42.7        | 6.79          | 25.2   | 36.0                    | 907.6                         |
| Pooled SEM*                          |           | 0.770       | 0.092         | 0.358  | 0.704                   | 17.1                          |
| Main effect of                       |           |             |               |        |                         |                               |
| Nursery Conditions                   |           | **          | *             | **     | **                      | **                            |
| Cultivar                             |           | **          | N.S           | **     | **                      | **                            |
| Year                                 |           | **          | **            | **     | **                      | **                            |
| Nursery Conditions x Cultivar        |           | N.S         | N.S           | N.S    | *                       | NS                            |
| Nursery Conditions x Year            |           | **          | **            | **     | *                       | *                             |
| Cultivar x Year                      |           | *           | N.S           | N.S    | N.S                     | NS                            |
| Nursery Conditions x Cultivar x Year |           | *           | N.S           | *      | N.S                     | NS                            |

\*:Pooled SEM. pooled standard error of the mean. NS. P > 0.05; \*, P < 0.05; \*\*, P < 0.01.



**Fig 1.** The temperature of nursery conditions with respect to years

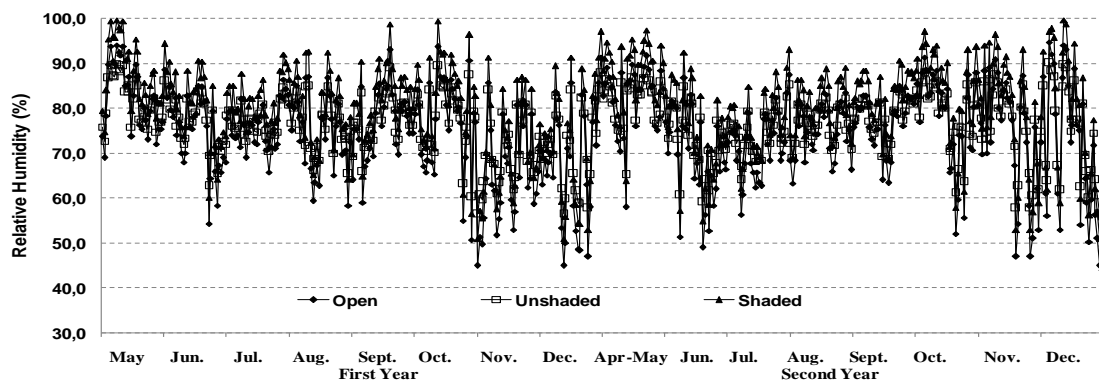
tolerant or intermediate in shade tolerance. These findings may be due to specific leaf area (cm<sup>2</sup>/g) and leaf area ratio (cm<sup>2</sup>/g) increasing with increasing shade, as has been seen in the American chestnut (Wang et al., 2006). More leaves grew on chestnuts grown in an unshaded greenhouse compared to those grown in the other conditions (Table 2). Demirsoy et al. (2007) reported that leaf numbers in the strawberry were higher in plants grown in unshaded greenhouses than those grown in shaded greenhouses or open fields. Fletcher et al. (2002) also studied the strawberry and noted that leaf numbers reduced with increasing shading levels (31%, 48% and 63%). Some scientific articles on the development of grafted seedlings grown in different nursery conditions also contain physiological data such as: rates of photosynthesis and respiration, daily carbon gain, the light compensation point, quantum efficiency, leaf mass per area and nitrogen

content per unit area. These physiological traits were not investigated in the present study. Our results with respect to leaf numbers do not confirm Lambers et al. (1998) in their suggestion that (in shade tolerant and semi shade tolerant types of the American chestnut) leaf numbers increase with increasing shade. The length and diameter of graft shoots were dependent on the leaf number, mean and total leaf areas per plant in chestnuts as influenced by the nursery conditions (Table 3). However the highest correlation ( $r=0.925$ ,  $P<0.01$ ) was found between shoot length and total leaf area per plant in the present study (Table 3). Therefore, our results indicate that plant development in chestnuts depends on total leaf area per plant rather than mean leaf area or leaf number as influenced by the nursery conditions. Table 1 shows that the

**Table 2.** The length and diameter of shoot and the number, mean area and total area of leaf in chestnuts grown in shaded and unshaded greenhouse and open conditions

| Nursery Conditions | Shoot        |               | Leaf           |                         |                               |
|--------------------|--------------|---------------|----------------|-------------------------|-------------------------------|
|                    | Length (cm)  | Diameter (mm) | Number (piece) | Area (cm <sup>2</sup> ) | Total Area (cm <sup>2</sup> ) |
| Shaded             | 66.0 ± 9.9 a | 6.11 ± 0.5 a  | 21.0 ± 3.3 b   | 60.2 ± 8.1 a            | 1270.1 ± 146.2 a              |
| Unshaded           | 46.8 ± 7.7 b | 5.58 ± 0.4 b  | 23.6 ± 3.7 a   | 35.2 ± 12.4 b           | 819.0 ± 155.1 b               |
| Open               | 31.0 ± 14.0c | 5.35 ± 1.6 b  | 16.4 ± 6.9 c   | 33.3 ± 11.3 b           | 589.1 ± 197.1 c               |
| Significance       | **           | *             | **             | **                      | **                            |

a.b Means with different letters in the same column were significantly different (\*\*P < 0.01, \*P<0.05).



**Fig 2.** The relative humidity of nursery conditions with respect to years

cultivar has a significant effect upon leaf characteristics ( $P < 0.01$ ) and also that there were significant effects of the year on studied leaf characteristics ( $P < 0.01$ ). The leaf area and leaf numbers per plant of the SE 3-12 genotype were smaller ( $P < 0.01$ ) and higher ( $P < 0.01$ ) respectively than that of the Marigoule. Serdar and Kurt (2011) reported that genotype and the year had a significant effect on some leaf dimensions in the chestnut. There were significant effects of the nursery condition and the year upon the leaf number ( $P < 0.01$ ), and the mean and total leaf areas ( $P < 0.05$ ) (Table 1). Also there were significant effects of nursery condition x cultivar x the year upon leaf numbers ( $P < 0.05$ ). However, the results with respect to plant development and leaf characteristics show that no significant interaction between chestnut genotype and nursery conditions was observed, except for the mean leaf area (Table 1). This result is very important for using this method in the production of grafted chestnut trees safely. Photosynthesis is very dependent on temperature, light intensity and relative humidity (Koorneef et al., 2002; Gomes-Laranjo, et al., 2006). It has been reported that for photosynthesis the optimal temperature range is between 26-30°C, with a decrease of about 50% when the temperature increases to 32-34°C; the decrease being higher than 80% when temperatures reach 38°C (Gomes-Laranjo et al., 2006). In deciduous fruit trees growth and development slows at both low and high temperatures, because low temperatures (<20°C) reduce photosynthesis and high temperatures (>35°C) increase respiration (Went, 1953). Consequently this study suggests using greenhouse conditions for grafted chestnut tree production. For the chestnut, a higher graft shoot length and diameter were obtained in greenhouse conditions than in open conditions in potted grafted tree production (Serdar and Soyulu, 2005; Duman and Serdar,

2006). On the other hand, some scion shoots may die due to the harmful effects of high temperatures in unshaded greenhouses (Duman and Serdar, 2006; Ozturk et al., 2009). Therefore, there is a need for shading in grafted tree production in greenhouses. Although the results suggest using greenhouses rather than the open for grafted chestnut tree production, the results do not show that development of grafted chestnut seedlings in shaded greenhouses is superior to that of those in unshaded greenhouses or in the open.

## Materials and methods

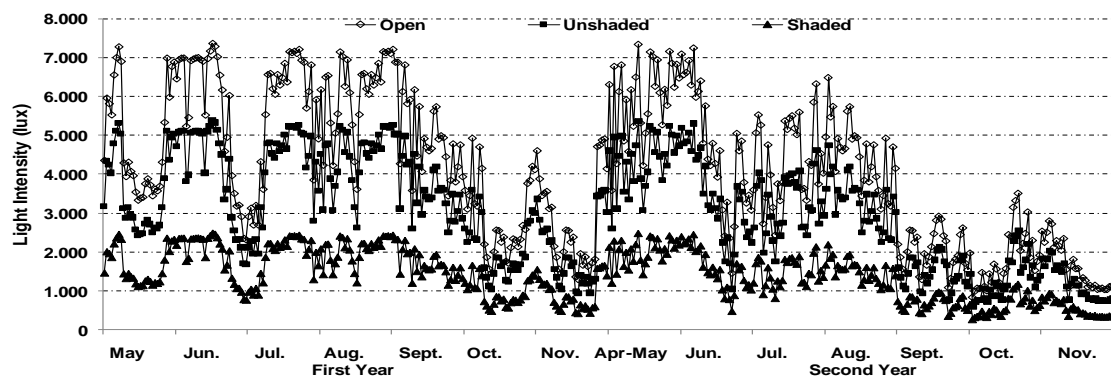
### Plant Material

This study was carried out in Samsun, Turkey using the SE 3-12 genotype (Serdar, 1999) and the Marigoule cultivar (Chapa and Verlhac, 1978) as scions. The scions were taken from an orchard in the province of Samsun, in February and were stored at  $4 \pm 0.5^\circ\text{C}$  until they were used for grafting. Newly germinated seeds of chestnut genotype 554-14 (Soyulu and Serdar, 2000) were used as rootstock. Two or three bud scions were used for inverted radicle grafting. The inverted radicle graft was made on 10<sup>th</sup> May in the first year and 29<sup>th</sup> April in the second year (Ozturk et al., 2009). Grafted seeds were planted in pots (30 x 40 cm) filled with a medium containing 3/4 soil + 1/4 ground pine needles and manure. The growing mediums were clay loam with 3.03% and 1.62% organic matter and pH 7.30 and pH 7.82 in the 2 experimental years, respectively. This research is a continuation of the study entitled "The influence of different nursery conditions on graft success and plant survival using the inverted radicle grafting method on the chestnut" made by Ozturk et al., (2009).

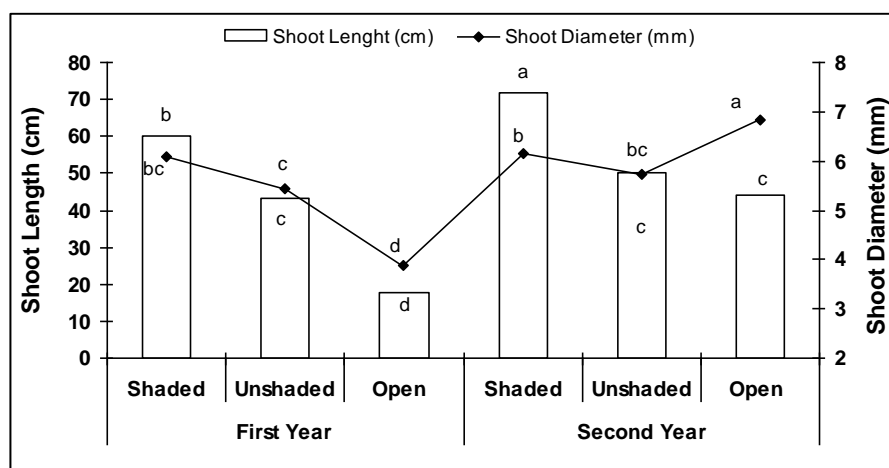
**Table 3.** Correlations amongst the plant development and some leaf characteristics

|                       | Shoot Length | Shoot Diameter | Leaf Number | Leaf Area |
|-----------------------|--------------|----------------|-------------|-----------|
| Shoot Diameter        | 0.687**      |                |             |           |
| Leaf Number           | 0.590**      | 0.602**        |             |           |
| Leaf Area             | 0.849**      | 0.579**        | 0.232       |           |
| Plant Total Leaf Area | 0.925**      | 0.670**        | 0.601**     | 0.899**   |

\*\*Correlation is significant at the 0.01 level (2-tailed).



**Fig 3.** The light intensity of nursery conditions with respect to years



**Fig 4.** Length and diameter of shoot in chestnuts grown in shaded and unshaded greenhouses and in the open in 2 different years (a,b Means with different letters in the same column were significantly different at  $P < 0.01$ )

### Study Design

The study was carried out in the open field and under shaded and unshaded greenhouse conditions. Shading was carried out with netting having a light transmission of 50% and the samples were immediately covered with this netting after grafting. The temperature, relative humidity and light intensity of the nursery conditions were measured using a data logger (Kimo KT100, France). Data of the nursery conditions measured during the experimental period are presented in Figures 1-3.

### Growth Measurement

In this study plant development was measured in terms of shoot length and diameter. Leaf numbers per plant were counted and the length and width of the leaf lamina for all leaves of the plant were measured in order to determine mean leaf area in November of both years. Estimation was used to determine leaf area by following the method of Serdar and Demirsoy (2006). The total leaf area was calculated by

multiplication of the mean leaf area and leaf numbers per plant. All measurements were taken over two years.

### Data Analyses

Plant development and leaf characteristics of the genotypes, including the effects of nursery conditions, genotypes, the year and their interactions were studied by three factored ANOVA. Therefore, the data was analyzed in a randomized block design as a factorial arrangement (3 x 2 x 2) of treatments. Five plants were used for plant development and leaf characteristics per replication. For data on leaf characteristics, individual leaves were considered to be the experimental unit ( $n = 60$  per treatment). When the F test was significant, differences were determined by Duncan's multiple range tests. All analyses were performed using the SPSS statistical package (SPSS, 2004). Results are presented as means and a pooled SEM. Correlation analysis was applied to assess whether the length and diameter of graft

shoots depend on the leaf number, mean and total leaf areas per plant in chestnuts as influenced by the nursery conditions.

## Conclusions

The results of this study show that the chestnut genotypes used are capable of surviving in a shadier environment, and of maximizing the length and diameter of graft shoots in an environment with less available light from the healing of the graft union to the end of the vegetation period. Since shaded greenhouses have an adverse effect on the development of shoot diameter, the distance between internodes and leaf size in chestnut plants in some years and the use of shaded greenhouses to maximize the length and diameter of graft shoots in chestnuts should be treated with caution. Therefore, it is suggested that new studies should be undertaken with a net having a higher light transmission value than 50% and different humidity levels. Also, the shading material (netting 50%) should be taken down at the end of the summer to allow enough carbohydrate accumulation and lignifications.

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