Water relation response to soil chilling of six olive (*Olea europaea* L.) cultivars with different frost resistance

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Abstract

The relationship between the water relations of six olive cultivars exposed to different soil temperatures (14 ± 0.1, 9.9 ± 0.1 and $5.8 \pm 0.2°C$) and their inherent frost resistance (as determined by two different methods) was investigated. Soil chilling was achieved by introducing pots of olive plants into water baths. The water relations of these plants were compared to those of plants kept under conditions of room temperature. The cultivars Frantoio, Picual and Changlot Real began to show significant dehydration below 14°C, while Cornicabra, Arbequina and Ascolana Tenera showed this below 10°C. This response is probably due to delayed stomatal closure. Only Cornicabra and Picual showed a significant reduction in leaf conductance (below 10°C and 6°C respectively). This absence of stomatal control led to a significantly greater dehydration in Ascolana Tenera. These variations in response to the soil chilling temperature suggest that different mechanisms may be at work, and indicate that would be necessary to study the influence of rootstock in the frost resistance of olive plants. The variations recorded grouped the cultivars as either resistant (Cornicabra), tolerant (Picual, Ascolana Tenera and Arbequina), or sensitive (Frantoio and Changlot Real). This classification is in line with the frost resistance reported for these cultivars in the literature, and with the results obtained in the present work using the stomatal density and ion leakage methods of determining such resistance.

Additional key words: chilling-induced dehydration, frost resistance, leaf conductance, root signal, water potential.

Resumen

Respuesta de las relaciones hídricas al frío en el suelo de seis variedades de olivo (*Olea europaea* L.) con diferente resistencia al frío

Se ha investigado la relación existente entre el estado hídrico de 6 variedades de olivo expuestas a diferentes temperaturas del suelo y su resistencia al frío (determinada por dos métodos). El frío del suelo fue conseguido introduciendo las macetas de las plantas de olivo en baños de agua. Se comparó el estado hídrico de estas plantas con el de plantas en condiciones de temperatura ambiente. Las variedades Frantoio, Picual y Changlot Real comenzaron a mostrar una deshidratación significativa por debajo de 14°C, mientras que para Cornicabra, Arbequina y Ascolana Tenera lo fue por debajo de 10°C. Esta respuesta es posiblemente debida a un retraso en el cierre estomático. Solamente Cornicabra y Picual mostraron una reducción significativa de la conductancia de la hoja (por debajo de 10°C y 6°C respectivamente). Esta ausencia de control estomático condujo a una significativa mayor deshidratación en Ascolana Ternera. Estas variaciones en respuesta al frío en el suelo sugieren diferentes mecanismos de actuación, e indican que la utilización de un portainjerto puede dar resistencia al frío en plantas de olivo. Los datos agruparon las variedades como resistentes (Cornicabra), tolerantes (Picual, Ascolana Tenera y Arbequina) o sensibles (Frantoio y Changlot Real). Esta clasificación está en consonancia con la resistencia a frío que se recoge en la literatura para estas variedades, y con los resultados obtenidos en este trabajo usando los métodos de densidad estomática y liberación de iones para determinar dicha resistencia.

Palabras clave adicionales: conductancia estomática, deshidratación inducida por frío, potencial hídrico, resistencia al frío, señal de raíz.

Abreviations used: C (ratio between water potential of the bath trees and the room temperature trees), EC_1 (electrical conductivity of the solution after temperature treatment), EC_F (electrical conductivity of the solution after the sterilizer treatment), EC_R (relative electrical conductivity), g (leaf conductance), LT_{50} (lethal freezing temperature at which 50% ion leakage occurs), RT (room temperature), SWP (stem water potential), VPD (vapour pressure deficit).

Introduction

Olive trees (*Olea europaea* L.) are mainly cultivated in the Mediterranean, though in recent years the high price of olive oil has encouraged the planting of new orchards in America and Australia. The traditional latitude range for olive cultivation is from 30°N (lower altitudes are too warm) and 45°N (higher latitudes are too cold) (Bongi and Palliotti, 1994). However, the increase in the area devoted to olive production has seen new orchards planted in areas with appreciable frost risk. Temperatures below –12°C are commonly associated with serious damage (Bongi and Palliotti, 1994).

The optimum temperature for growth in olive tree is 20-30°C. The rate of metabolic processes such as respiration are slightly diminished in the 10-25°C range (Rinaldelli and Mancuso, 1994; Rinaldelli and Bandinelli, 1999), and below 10°C there is a sharp reduction. The critical temperature for respiration and the temperature required to maintain the potential difference of the cell membrane varies from 7.5 to 12.5°C, depending on the cultivar and the tissue (Gucci *et al*., 2003).

Under field conditions, cold temperatures induce dehydration in olive trees (Antognozzi *et al*., 1994; Moriana and Fereres, 2002) and other sensitive crops (McWilliam *et al*., 1982). This dehydration is associated with an imbalance between water absorption and transpiration (McWilliam *et al*., 1982). In cultivar Picual plants, Pavel and Fereres (1998) report 10°C to be the temperature at which chilling-induced dehydration begins, while leaf conductance is reduced from 5°C. The roots of olive trees are the most sensitive organs to low temperatures (Mancuso, 2000); indeed, the potential difference of the cell membrane in cultivar Frantoio root cells is significantly affected by temperatures below 15°C. Chilling-induced dehydration is therefore probably related to a reduction in the absorption of water by the root with no compensatory effect on transpiration.

The risk of frost limits the expansion of olive tree cultivation in several places, where would be interesting to know which cultivar is more frost resistant. Several methods are available for evaluating the frost resistance of cultivars, and most of the main types (mostly Spanish and Italian) have been characterized (Palliotti and Bongi, 1996; Mancuso, 2000; Barranco *et al*., 2005a,b). However, little is known about frost resistance mechanisms. Under field conditions, Antognozzi *et al*. (1994) reported that olive tree frost resistance increased from Autumn until January, and that this pattern was related to the reduction in water potential induced by chilling. However, Bartolozzi *et al*. (2002) reported no significant differences (except in March) between olive plants hardened by exposure to chilling temperatures and non-hardened plants. In other fruit trees, the mechanism involved in hardiness has been related to the chilling temperature and photoperiod (Irving and Lanphear, 1967; Greer *et al*., 1989). Hardiness is likely related to the biochemical processes that occur during winter. During this period, olive trees show a reduction in their starch reserves and an increase in their soluble sugar content (Drossopoulos and Niavis, 1988), though not all the soluble sugars detected are probably involved in hardiness (Bartolozzi *et al*., 2002). In addition, the maximum intracellular and minimum extracellular resistances of olive tree shoots have been recorded during winter (Mancuso, 1998).

Mancuso (2000) suggests the electrical resistance of the tissues at different temperatures to be an indicator of olive tree frost resistance. The temperature at which electrical resistance increased was above 0°C in all the cultivars this author tested, although it increased with cultivar sensitivity. The chilling temperature at which the water potential becomes reduced in plants of cultivar Picual (a frost resistant cultivar) is reported to be similar (Pavel and Fereres, 1998).

Frost protection induced by applying plant hormones (rice: Lee *et al*., 1996) or by ensuring drought conditions (wheat: Tyler *et al*., 1981; cabbage: Sasaki *et al*., 1998) is commonly explained by a reduction in leaf conductance. Stomatal control may be involved in this. Boese *et al*. (1997) reported a reduction of chilling-induced dehydration in bean, cucumber and corn —chilling-sensitive species— in conditions of elevated CO_2 designed to reduce transpiration rates. The same type of response was also reported by McWilliam *et al*. (1982) for chilling-resistant crops. Palliotti and Bongi (1996) suggest that mefluidide treatment of olive trees induces water stress conditions that increases frost resistance in cultivar Frantoio.

The aim of the present work was to study the effect of soil chilling on the water relations of olive seedling of different cultivars (frost sensitive and resistant). The working hypothesis was that, under such conditions, these water relations differ depending on inherent frost resistance. The degree of stomatal control and the temperature at which chilling-induced dehydration begins may differentiate cultivars.

Material and methods

Cultivars examined

Six olive cultivars (Cornicabra, Picual, Arbequina, Ascolana Tenera, Frantoio and Changlot Real) were selected to reflect a range of economic interest and frost resistance. Cornicabra, Picual and Arbequina are the most planted in Spain (Barranco, 1995) and are considered frost resistant (Barranco *et al.*, 2005a). Frantoio and Ascolana Tenera are Italian cultivars, and are respectively frost sensitive and resistant (Mancuso, 2000). Changlot Real is the least economically important cultivar and is frost sensitive (Barranco *et al.*, 2000).

Water relations at different soil-chilling temperatures

The following experiment was performed at the El Chaparrillo Research Station, Ciudad Real, Spain (3° 56' W 39° 0' N; altitude 640 m). Between September and October 2004, 60 one year-old olive plants of each cultivar, each in a 5-L regularly irrigated pot, were subjected to three different soil temperatures: 14, 10 and 6°C. In each experiment, ten plants were used to reach a soil temperature and ten were left at room temperature (RT). The root temperature of trees at RT in all experiments was around 20°C. Soil temperatures were obtained by immersing the pots into water baths (dimensions $400 \times 600 \times 250$ mm) containing a solution of 1.5% polyethylene glycol (v/v) (bath trees). All the pots were wrapped with plastic bags to avoid contamination from this solution. The level of the solution was always around 5 cm below the base of the plant. Prior to the immersion of the bath trees, the solution was brought to the required temperature by two refrigerating units (Frigedor 3001214, PSelecta, Barcelona, Spain). To obtain a homogenous temperature distribution, two pumps (D-21V, Dinko, Barcelona, Spain) were used to induce flow. The baths were covered with white polyethylene 4 cm thick. The temperature was measured using thermistors (T-107, Campbell Sci, UK) on each bath tree and in the solution. Measurements were taken every 30 seconds using a datalogger (CR10 X, Campbell Sci, UK) programmed to calculate 15 min averages. When the temperature in the bath was slightly lower than that desired, the plants were introduced. The intensity of the refrigerating units was then adjusted

according to the temperature of the soil in the pots. The desired soil temperature was considered reached when the temperature of all the plants were ± 1°C around this value. Measurements for water relation analysis were taken when the plants had been at the target temperature for 1 h. The soil temperature of the RT trees was measured with a thermometer.

Stem water potential (SWP) measurements were used to evaluate tree water status. Fully expanded leaves were covered with aluminium foil for at least 30 min before removal; the water potential was measured with a pressure chamber (Soil Moisture Equip, Santa Barbara, Calif, USA). At the same time, abaxial leaf conductance (g) measurements were taken in two leaves per seedling using a permanent porometer (LICOR 1600, UK).

The water potential and leaf conductance were analysed using one way ANOVA employing Statistic v. 8.0 software. To further characterise the effect of soil temperature on water relations, the ratios (C) between the SWP of the bath trees and RT trees were calculated. When the values of SWP were significantly different, the regression between C and soil temperature was obtained for each seedling and the results compared.

Frost resistance as measured by stomatal density

Leaves were taken from 1-yr-old olive plants (in 5-L regularly irrigated pots) and from young olive trees (3-4 yr old) belonging to the olive cultivar collection of the Centro Agrario El Chaparrillo. The spacing of the latter trees (grown in an irrigated orchard) was 7×7 m.

Roselli *et al.* (1989) suggested determining the stomatal density as a means of evaluating frost resistance. The protocol used in the present work is similar to that used by these authors. On different days of the winter of 2004, when no growth was detected in the trees, 20 leaves from the midshoot of the last growth period were randomly selected from 5 trees per cultivar, and examined on the same day. In olive trees the stomata are located mainly (around 95%) on the abaxial leaf surface which is covered with stellate hairs (Rapoport, 1995). These were removed from each leaf by stripping with adhesive tape. A thin film of nail varnish was then painted on the abaxial surface. This was allowed to dry at RT and then peeled off. The film from each leaf was mounted on a glass microscope slide and the

stomata counted with the aid of a binocular microscope (25x objective, field 0.024 mm^2). All counts were made at the apex area of the leaf; four counts were made per leaf.

To compare the stomatal densities of the cultivars and the influence of their source, the results were subjected to one-way ANOVA and a Tukey multiple comparison test. All calculations were made using Statistic v.8.0 software.

Frost resistance as measured by ion leakage

This experiment was performed during November and December 2004. Leaf samples were obtained from five trees of each cultivar from the olive tree collection mentioned above. Twelve leaves were randomly cut from the mid shoot of the last season's growth. For each test temperature (a range of 30 temperatures between –4 and –20°C), 10 leaf discs 7 mm in diameter were punched (including the area of the major veins) and introduced into a test tube that was then covered with aluminium foil. These were then introduced into one of the stainless steal water baths containing a 50% polyethylene glycol solution (v/v). This solution covered the first centimetres of the test tube; the level of the solution was always higher than the leaf discs. The solution was brought to the target temperature in the same way as above. Measurements were taken (three replications) every 10 s; the datalogger (CR10 X, Campbell Sci, UK) was programmed to calculate 5 min averages. The test tubes were extracted from the bath after 1 h at the target temperature and introduced into a refrigerator at 4°C for 1 h to thaw. When this time had elapsed, 8 mL of deionised water were added to the tubes, which were then placed in a shaker (Rotabit, Pselecta, Barcelona, Spain) for 16 h at 120 cycles per min at RT. The electrical conductivity (EC$_I$) of the solution was measured using a digital conductivity meter (Basic 30, Crison, Carpi, Italy) to estimate the ion leakage associated with each temperature. The tubes were then covered with cotton wool and aluminium foil and introduced into a sterilizer for 1 h at 120°C and 1 atmosphere of pressure to destroy the tissue. The samples were then shaken for 2 h at 200 cycles per min at RT and the electrical conductivity (EC$_F$) measured again. The percentage of ion leakage at each temperature was represented by the relative electrical conductivity (EC$_R$):

$$EC_R = (EC_I / EC_F) * 100$$

Response curves were produced using the following sigmoid function:

$$EC_R (\%) = \frac{a}{1 + e^{b(T^a - c)}} + d$$
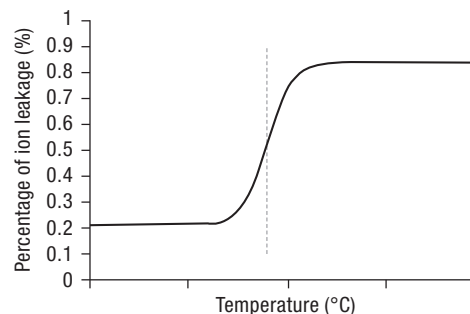
where *a* is the range of EC$_R$ from the minimum to maximum temperature tested, *b* is the slope coefficient (at the temperature of the inflection point), *c* is the temperature at the inflection point, and T^a the absolute temperature (°C); the latter variables were estimated using the SPSS v.12.0 software (SPSS, 2003) non-linear linear regression curve fitting routine. The inflection point of the sigmoid response curve (*c*) predicts the lethal freezing temperatures (LT$_{50}$) corresponding to the subzero temperature at which 50% ion leakage occurs. The slope of the curve at this point (coefficient *b*) is an indicator of the ion leakage velocity. The ion leakage values of the different cultivars were compared by one-way ANOVA and the Tukey multiple comparison test. These calculations were performed using Statistic 8.0 software.

Results

Water relations at different soil-chilling temperatures

Figure 1 shows the stem water potential (SWP) for the RT and bath trees at the three soil temperatures selected. Significant differences were seen between the RT and bath tree representatives of cultivars. Changlot Real, Frantoio and Picual; those of Cornicabra, Arbequina and Ascolana Tenera only showed significant differences below 10°C. The lowest SWP values were recorded at 6°C for all cultivars. Frantoio bath trees showed the lowest at –2.5 MPa; that of the Frantoio RT trees was –0.8 MPa. Cornicabra and Picual RT trees showed the highest SWP with around –1.5 MPa and –0.7 MPa respectively. The SWP values obtained in all

the experiments with RT trees were similar, and varied from –0.6 to –1.0 MPa. Only in Arbequina and Ascolana Tenera trees at 14°C was SWP any lower, probably because of the high vapour pressure deficit (VPD) of the day on which the experiment was performed (around 3 KPa).
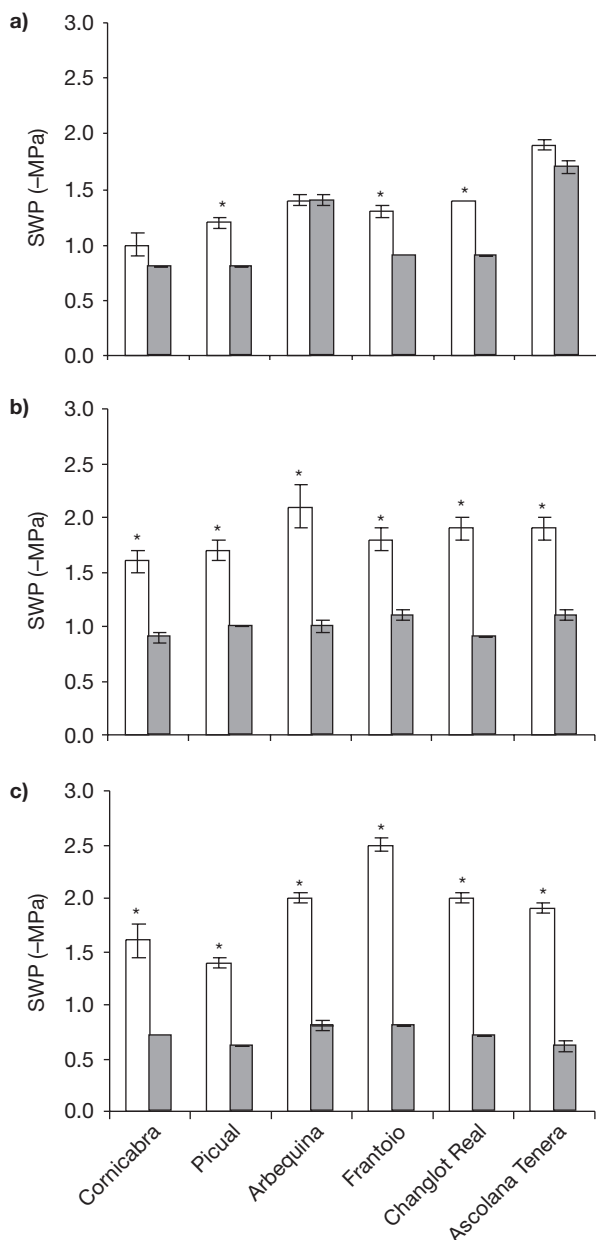

To estimate the rate of dehydration at different soil temperatures, the ratio between the SWP values of the bath trees and the mean of the RT trees (C) was compared with the root temperature of each tree (Table 1). The values of C varied from 1 (no differences between bath and RT trees) to 3.5. The cultivars Cornicabra, Arbequina and Picual showed maximum C values of around 2.3, while in the remaining cultivars these values reached as high as 3.5. A linear relationship between C and soil temperature was seen; only after the temperature at which differences between bath and RT trees were significant a breakdown in this relationship was seen (Table 1). The regression line for this relationship provides an estimate of the rate of dehydration for each cultivar (Table 1). The regression values obtained when C = 1 are shown and the soil temperature at which chilling-induced dehydration started is estimated (Table 1). The regression line for Cornicabra was not significant and was therefore not compared with the rest. The rate of dehydration was only significantly different in Ascolana Tenera (–0.32 °C^{-1}), the remaining cultivars all showed values of around –0.15°C^{-1}. The differences between regressions gathered the cultivars into four groups: Ascolana Tenera, which was clearly different from the rest, Picual and Arbequina, with no significant differences between them, and Frantoio and Changlot Real (Table 1).

Leaf conductance (g) was also affected by soil temperature (Fig. 2). The reduction in g was only significant from 10°C in cultivar Cornicabra and 6°C in cultivar Picual. The g of the bath trees was significantly lower (in fact around 50% lower) than that of the RT trees. These differences were detected on days when the VPD was not high (1.6, 1 and 0.5 kPa) and when the value for g for other cultivars under the same conditions were similar. Therefore, the reductions in g in the bath trees was probably due to the low soil temperature. The remaining cultivars showed reductions of g of around 30% compared to the values for RT trees at 10°C (significant reductions were recorded for Arbequina and Ascolana Tenera). However, these reductions in g were not seen at 6°C.

Frost resistance as measured by stomatal density

Stomatal density varied with the source of the sample. In the plants, stomatal density varied from 346 stomata mm^{-2} in Changlot Real to 646 stomata mm^{-2} in Corni-

Figure 1. Stem water potential (SWP) of seedlings of the six olive cultivars under chilling (□) and room temperature (■) conditions. The soil temperatures for the chilled seedlings were 14.0 ± 0.1°C (a), 9.9 ± 0.1°C (b) and 5.8 ± 0.2°C (c). Each histogram is the average of five measurements. Vertical lines represent the standard error. Asterisks denote significant differences between chilled and RT conditions (*P* < 0.05).

Table 1. Regression analysis involving soil temperature (X) and the C (Y) ratio (SWP_{bath}/SWP_{RT}) for each plant. The table shows the regression coefficient (R^2), number of data considered (n), slope of the equation (a) and temperature (RT) at which chilling-induced dehydration started (according to the regression). Statistical differences between the regressions are also shown (PI: Picual. AR: Arbequina. FR: Frantoio. CR: Changlot Real. AT: Ascolana Tenera)

Cultivar	Significant differences with	Equation	R^2	n	a	RT
Ascolana Tenera	PI, AR, FR, CR	Y = −0.32X + 4.88	0.72	10	−0.32[b]	12.1
Picual	AT, FR, CR	Y = −0.10X + 2.81	0.38	15	−0.10[a]	15.3
Arbequina	AT, CR	Y = −0.17X + 3.39	0.53	10	−0.17[a]	14.1
Frantoio	AT, PI	Y = −0.18X + 3.77	0.75	15	−0.18[a]	15.4
Changlot Real	AT, PI, AR	Y = −0.14X + 3.55	0.82	15	−0.14[a]	17.8
Cornicabra	—	Y = −0.11X + 2.91	0.13[ns]	10	—	—

Different letters in the slope column (a) indicate significant differences ($P < 0.05$). ns: not significant.

cabra (Fig. 3). Statistical analysis grouped the cultivars into three sets: The first one was Cornicabra and Picual varieties (646 and 642 stomata mm^{-2} respectively; no significant difference), the second one was formed by Arbequina (538 stomata mm^{-2}) and Ascolana Tenera (496 stomata mm^{-2}) and finally Frantoio (371 stomata mm^{-2}) and Changlot Real (346 stomata mm^{-2}).

The results obtained were slightly different for young trees grown in field conditions (Fig. 3). Stomata density varied from 625 stomata mm^{-2} (Changlot Real) to 892 stomata mm^{-2} (Cornicabra). Cornicabra had the highest stomatal density of all ($P < 0.05$), Picual (796 stomata mm^{-2}), Ascolana Tenera (796 stomata mm^{-2}) and Frantoio (771 stomata mm^{-2}) formed an intermediate group, and Arbequina (667 stomata mm^{-2}) had the lowest with a value significantly lower than those of the last group but higher than Changlot Real. The values for these young trees were significantly greater than their corresponding plants in pots. The greatest differences were seen in Frantoio and Changlot Real, the plants of which showed a 50% lower stomatal density than the trees. Picual and Arbequina plants had a 20% lower stomatal density than their corresponding young trees.

Frost resistance as measured by ion leakage

Reducing the temperature of the leaf samples led to progressive breakage of the cells and an increase in the relative conductivity of the medium (EC_R). In all the cultivars the relationship between EC_R and temperature was described by a sigmoid curve (Table 2). The minimum and maximum values of EC_R were similar for all the cultivars (around 20% and 80% respectively). In cultivar Cornicabra, EC_R increased from −9°C, reaching a maximum at around −20°C. The increase of EC_R in cultivar Picual started at around −6°C and was again maximal at around −20°C. Arbequina showed a sharp increase in EC_R from −11°C and a maximum at around −19°C. The EC_R of Frantoio increased at around just −5°C; the maximum value was reached at around −17°C. That of cultivar Changlot Real increased sharply from −10°C with a maximum around −14°C. Finally, the EC_R of Ascolana Tenera started to increase from -8°C and reached a maximum at around −17°C.

Table 2 shows the values of the parameters of the regression equation for each cultivar. Coefficient *c*, which corresponds to the LT_{50}, divided the cultivars into two groups: Picual (−13.2°C), Cornicabra (−14.0°C)

Table 2. Parameters for the sigmoid curve involving relative electrical conductivity (EC_R) and chilling temperature

Parameter	Picual	Cornicabra	Arbequina	Frantoio	Ascolata Tenera	Changlot Real
c (°C)	−13.2[a]	−14.0[a]	−14.2[a]	−11.6[b]	−11.5[b]	−11.5[b]
a (%)	61.20[ab]	62.88[a]	62.81[a]	60.20[ab]	59.44[ab]	48.66[b]
b	0.56	1.61	0.72	0.74	0.85	1.78
d (%)	18.14	21.47	20.54	18.56	16.54	24.01
n	90	90	90	90	90	90
R^2	0.88	0.83	0.83	0.86	0.87	0.85

Different letters for the same parameter indicate significant differences ($P < 0.05$).
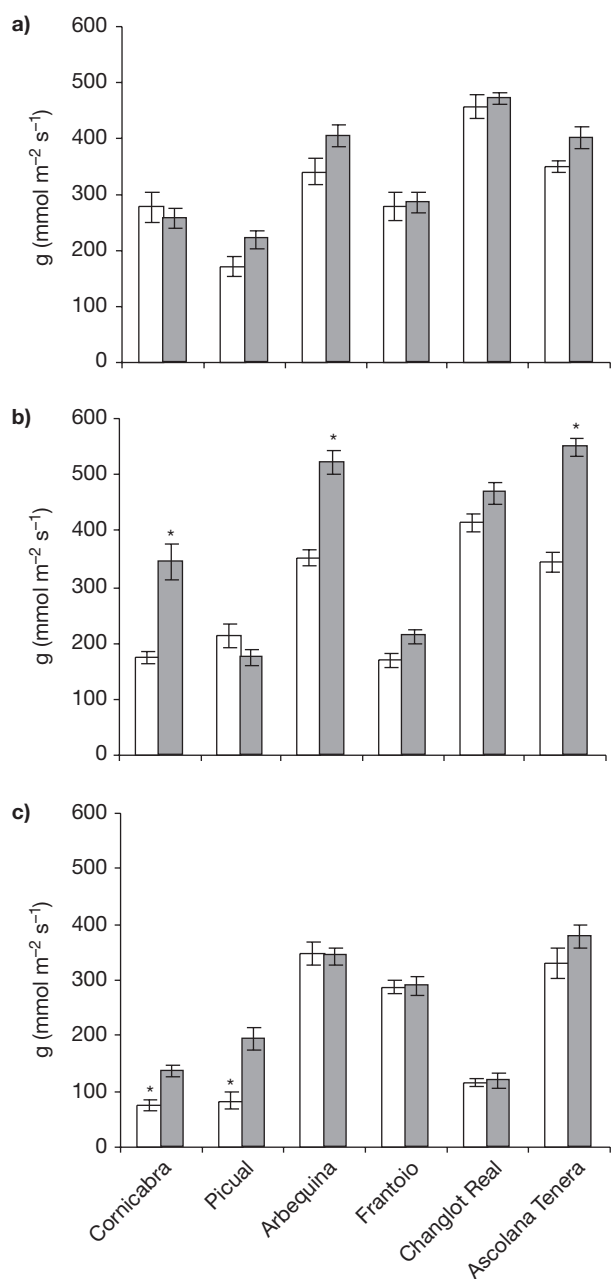
a)

b)

c)

Figure 2. Leaf conductance (g) of the six olive cultivar plants in chilled (□) and room temperature (■) conditions. The soil temperatures for the chilled plants were $14.0 \pm 0.1°C$ (a); $9.9 \pm 0.1°C$ (b) and $5.8 \pm 0.2°C$ (c). Each histogram is the average of five measurements. Vertical lines = RT conditions ($P < 0.05$).

and Arbequina ($-14.2°C$) with no significant differences, and Frantoio ($-11.6°C$), Ascolana Tenera ($-11.5°C$) and Changlot Real ($-11.5°C$) (significant higher LT_{50} values than those of the last group). The remaining parameters showed no significant differences between cultivars.
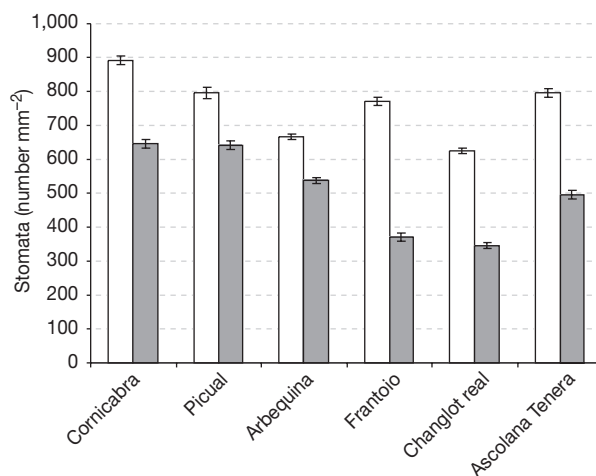

Figure 3. Stomatal density of the six different olive cultivars. The sources of the samples were: plants in pots (■) and young olive trees (□). Each histogram is the average of 80 data; vertical lines represent the standard error.

Discussion

Water relations at different soil-chilling temperatures

Reducing the soil temperature led to significant dehydration in all cultivars. This response has also been described in a similar experiment involving cultivar Picual (Pavel and Fereres, 1998) and under field conditions (Antognozzi *et al.*, 1994; Moriana and Fereres, 2002). Dehydration is likely caused by an imbalance between the absorption of water by the root and transpiration at the leaf. This reduction in root absorption may be related to a general reduction in root activity (Rinaldelli and Mancuso, 1994) or the increase in water viscosity at lower temperatures (Pavel and Fereres, 1998). McWilliam *et al.* (1982) reported chilling-induced dehydration in chilling-sensitive crops to be due to stomatal closure being less complete than in chilling-resistant species. Pavel and Fereres (1998) reported similar results in olive plants (cultivar Picual); significant dehydration began to occur at around 10°C, but no reduction in stomatal conductance was seen until a soil temperature of 5°C was reached. Though the present results confirm the above findings, the responses of the cultivars were different. Some of these cultivars showed significant dehydration at lower temperatures (Cornicabra, Ascolana Tenera and Arbequina; Fig. 1) but no significant reduction in leaf conductance even at the lowest soil temperature (Fig. 2). Cultivar Ascolana Tenera showed the

greatest dehydration rate (Table 1), thus indicating to have poor stomatal control. The cultivars affected at the highest soil temperatures (Picual, Frantoio and Changlot Real) did not respond in the same way. Picual showed a significant reduction in leaf conductance while Frantoio and Chaglot Real did not. These results suggest that other mechanisms may be involved in the response to chilling-induced dehydration. An increase in cell elasticity has been suggested as a common response to drought conditions in olive trees (Dichio *et al*., 2003) and as a frost resistance mechanism in this (Mancuso, 1998) and other crops (Rajashekar and Burke, 1996; Wanner and Junttila, 1999).

The present results also suggest the important influence of root system activity on chilling-induced dehydration: water absorption at the roots may alter and these organs may even exert some control over leaf conductance. In olive (Mancuso, 2000) and other fruit trees (Calmé *et al*., 1994) the roots are reported to be the organ most sensitive to chilling temperature. Wahbi *et al*. (2005) suggest that root signals have significant influence on leaf conductance in olive trees under field conditions. Therefore, the rootstock selected may affect the response of olive cultivars to chilling-induced dehydration and probably their sensitivity to frost.

The response of the cultivars to soil chilling may be used as an indicator of their frost resistance. Three indicators of this were examined: the temperature at which chilling-induced dehydration starts, the response of leaf conductance to temperature, and rate of dehydration at different temperatures. The most resistant cultivar (Cornicabra) showed a reduction in leaf conductance at a relatively high temperature and a low dehydration induction temperature. The moderately resistant cultivars Arbequina, Picual and Ascolana Tenera showed less stomatal control in chilling conditions; however, other mechanisms may also be involved. These showed signs of chilling-induced — and more severe— dehydration at higher temperatures. Finally, the sensitive cultivars Frantoio and Changlot Real showed no significant reduction in leaf conductance as temperature fell, and, therefore suffered the most severe chilling-induced dehydration which began at the highest temperatures. This classification agrees with that obtained with other methods by other authors (Antognozzi *et al*., 1994; Barranco, 1995; Palliotti and Bongi, 1996; Barranco *et al*., 2000, 2005a; Mancuso, 2000); it may therefore be used to complement them.

Frost resistance as measured by stomatal density

Stomatal density of the plants was influenced by their source. It is very sensitive to environmental conditions (Hetherington and Woodward, 2003). However, little *in vivo* work has been performed in this topìc. Drought is probably the most important limiting factor to the success of olive orchards. Water stress apparently increases the stomatal density and reduces the size of stomata in cvs. Koroneki and Mastoidis (Bosabalidis and Kofidis, 2002). However, Bacelar *et al*. (2004) indicate drought resistance to be unrelated to stomatal density. The age of the tree is probably also an important source of variation; several authors suggest that stomatal density increases with age. In cultivar Koroneki, experiments with plants recorded values of around 110 stomata mm^{-2} (Soleimani *et al*., 2003) while 3-4 years-old trees showed some 380-400 stomata mm^{-2} (Bosalidis and Kofidis, 2002; Hagidimitriou and Pontikis, 2005). In addition 1-2 years-old trees of cvs. Frantoio and Leccino have densities of some 300-380 mm^{-2} (Bongi *et al*., 1987; Gucci *et al*., 2002) while mature trees show values of 515-600 mm^{-2} (Roselli *et al*., 1989). These variations are similar to those detected in the present work. Thus, the absolute stomatal density values of mature trees would probably not reflect frost resistance well.

The most frost-resistant cultivars did, however, have the highest stomatal densities. Traditionally this technique has indicated exactly the opposite pattern (*i.e.,* a low stomatal density has been taken to indicate greater frost resistance) (Roselli *et al*., 1989; Roselli and Venora, 1990). However, the cultivars of warmer climates tend to have lower stomatal densities than those of cooler places (Bongi *et al*., 1987; Hagidimitriou and Pontikis, 2005). Nevertheless, the present cultivars with the highest (Cornicabra, Picual and Ascolana Tenera) and lowest (Changlot Real) stomatal densities have been concluded as resistant and sensitive respectively —independent of the source of the sample— by other authors (Mancuso, 2000; Barranco *et al*., 2000, 2005b; Barranco, 2004). In the present work, Arbequina and Frantoio showed the most sample-source variation. The results obtained with the plants that suggested Arbequina to be resistant and Frantoio sensitive, are similar to those reported in the literature (Antognozzi *et al*., 1994; Palliotti and Bongi, 1996; Barranco *et al*., 2000; Mancuso, 2000; Bartolozzi *et al*., 2002; Barranco, 2004).

Frost resistance as measured by ion leakage

This methodology only separated the cultivars into two groups with respect to LT_{50}. The results are similar to those obtained by other authors using the same technique. Barranco *et al.* (2005a) reported no significant differences between Arbequina, Picual and Cornicabra, though they were the most resistant cultivars of the eight tested. The results obtained for Ascolana Tenera and Frantoio are somewhat contradictory. Fiorino and Mancuso (2000) indicate Ascolana Tenera to be more resistant than Frantoio, but Bartolozzi and Fontanazza (1999) reported no significant differences between these cultivars.

The absolute values of LT_{50} obtained in the present work are, however, somewhat different to those reported in the literature. The absolute values differ depending on the study consulted. Traditionally, $-12°C$ is considered to be the temperature at which the crown is affected (Bongi and Palliotti, 1994) though some authors regard $-10°C$ to be more accurate (Bartolozzi and Fontanazza, 1999). In the literature (Fiorino and Mancuso, 2000; Barranco *et al.*, 2005a), the Spanish frost resistant cultivars showed absolute resistance values close to that of cultivar Frantoio (a sensitive Italian cultivar), although they were found to be more resistant in the present work (Table 2). However, Ascolana Tenera (an Italian frost resistant cultivar) showed no significant differences to Frantoio and Changlot Real. An explanation for the contradictory results between different works can be the use of different plant material with the same name. This is likely with Frantoio, widely used in Italy, where it only means that their olives are used to produce oil.

In conclusion, the response of olive cultivars to chilling-induced dehydration is different and may be related to frost resistance. The control exercised by the root in this process in the most resistant varieties is very important; the selection of an appropriate rootstock may therefore be of great importance. Studies to evaluate the influence of rootstock in frost resistance scion are necessaries. Stomatal densities can be strongly influenced by the environment, and the results obtained may be affected by the age and location of the trees examined. The use of this index for estimating frost resistance is therefore limited. Ion leakage did not differentiate the cultivars.

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