Heat tolerance in olive

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Abstract: Heat resistance in shoots and leaves of 10 olive (Olea europaea L.) cultivars was assessed by two different methods. Stem impedance analysis and electrolyte leakage of leaves and shoots were evaluated to determine which method is more reliable for estimating heat injury in olive. A significant decrease in DZ_ratio and increase in electrolyte leakage was found in all genotypes after heat treatment. Results from the two methods showed that both methods were able to detect heat injury in olive. However, electrolyte leakage seemed to work more effectively detecting bigger differences among the heat tolerance of the cultivars tested. It would appear from the present study that shoots are more resistant than leaves to heat injury. On average, leaves and shoots are injured at temperatures around 48 and 50°C, respectively. The lethal temperatures allowed to share out the cultivars in four groups with different tolerance to heat stress. The heat response of these genotypes has been analysed in detail and the limits of artificial heating tests are discussed.

1. Introduction

The most typical kind of stress plants receive from their surroundings is temperature stress. Each plant species has its own optimum temperature for growth, and its distribution is determined to a major extent by the temperature zone in which it can survive (Iba, 2002).

Recently, interest in plant heat stress has increased as a result of changes in the ambient thermal environment and alterations in patterns of precipitation.

Climate change can be defined as the long term fluctuations in temperature, precipitation, wind and all the other aspects of the earth’s climate often related to the term greenhouse effect. The effects of climate change in the Mediterranean area include heavy rains with flash floods, unusually dry winters, cloudy springs and very high summer temperatures. These changes retort on crop yields and quality, producing a tendency for crop production (e.g. olive crops) to move northwards (Maracchi, 2000).

Olive tree is a warm temperature evergreen plant which grows mostly between 30° and 45° latitude in both hemispheres. Usually, for olive trees, low temperatures are more limiting than are high temperature (Mancuso, 2000); however, as the global climate changes, high temperature could jeopardize olive cultivation.

Heat stress often is defined as high temperatures that cause irreversible damage to plant function or development (Hall, 2002). However, definition and quantification of thermal stress is not easy (Mahan et al., 1995). In fact, drought and high temperature are closely associated and it can be very difficult to disentangle the effects of each stress on plants growing in the field. To do this, it is necessary to consider the stresses separately under controlled conditions, for example by studying the influence of high temperature on plants which are sufficiently supplied with water (Fitter and Hay, 1987).

In regions with regular summer drought, heat occurs when the vegetation is already inactivated by water deficiency. In more humid areas, where during summer there are only occasional, short drought periods, heat, as a single stress factor, should be not ignored.

The negative effects of thermal stress on plants are persistent and often result in reduced crop yields (Kramer, 1980; Boyer, 1982).

During the vegetative stage, high temperature can cause injury to components of leaf photosynthesis, reducing carbon dioxide assimilation rates compared...
with environments having optimal temperatures (Al-Khatib and Paulsen, 1989). In particular, at high temperatures, the primary processes of photosynthesis are the first to be repressed following destruction of thylakoid membranes which are particularly sensitive to heat (Berry and Björkman, 1980).

Recent research suggests that global warming will have severe and rapid effects on forests over large areas, all other factors being equal. Murray et al. (1989) found that survival of Picea sitchensis in Britain is improving with the increasing of atmospheric CO₂ concentrations in conjunction with climatic warming, due to reducing the risk of spring and autumn frost damage and lengthening the potential growing season. Also in olive, the effects of climatic warming, if from one side, could result in the extension of cultivation to higher latitude, from another, will lead to an increasing risk of heat stress phenomena influencing the survival and eventually the distribution of trees, because of a lack of adaptation to an altered environment.

The ability of plants to survive under heat stress conditions is one way to define thermal stress resistance. Thus, the determination of the thermal lethal temperature is a useful starting point for studies of the plant’s ability to resist thermal stress. The objective of this study was to evaluate heat stress resistance of Olea europea L. cultivars using two methods: electrolyte leakage and impedance spectroscopy, both based on the concept that injured cells are unable to maintain the chemical composition of their contents and release electrolytes through damaged membranes.

2. Materials and Methods

Plant material and thermal tests

Ten cultivars of Olea europea L.: Carbona, Coratina, Diana, Frantoio, Leccino, Maurino, Moraiolo, Pendolino, Simajca and Urano with different geographic origin were utilized in the present study. The plants, three-years-old, grown in pot, were maintained in greenhouse and brought into a laboratory 24 hr before starting the experiment.

Samples of current year shoots of similar length, diameter and internode length and mature leaves removed from the third node from the top were used for the experiments.

Controlled thermal treatments were carried out in an air programmed chamber. The initial temperature was 25°C, the rate of warming 2°C hr⁻¹ and the maximum temperature was maintained for 4 hr.

Impedance spectroscopy

Electrical impedance spectroscopy (EIS) has been widely used to assess the in vivo condition of animal and plant tissues (Cole, 1972; Lewis et al., 1989). Assessment of heat hardiness by electrical impedance analysis is based on the decrease of the extracellular resistance due to damage in plasma membrane and consequent leaking of the intracellular ions into the extracellular space.

In this method, alternating current is applied to a piece of plant tissue. The proportion of current going through the extracellular and intracellular space of a tissue is dependent on the alternating current frequency and the tissue properties (Repo et al., 1997). With increased frequency, the amount of current that passes through the symplast increases as a result of the decrease of membrane impedance. Impedance measurements made on branch samples at low and high frequencies, therefore, reveal information about extra- and intracellular fluids.

EIS was measured using the technique previously described (Mancuso, 1999). Briefly, two Ag/AgCl electrodes (homebuilt with Ag/AgCl pellets) were placed in contact with samples using a conductive paste (of the type commonly used for electrocardiograms) in order to keep the electrode/tissue interface polarisation to a minimum. The device was calibrated by using OPEN/SHORT circuit correction to eliminate the polarisation impedance of the electrode/paste interface. The absolute impedance value and phase angle were then measured within a frequency range from 100 Hz to 20 KHz at 14 frequency points using an HP 4284A LCR meter. The samples were kept parallel to the direction of electrical current. Input voltage of the sine signal was 30 mV (rms).

In this study the impact of high temperature injury was estimated as the change in impedance ratio (low/high frequency) before and after the thermal treatment:

\[ DZ_{\text{ratio}} = \frac{(Z_{\text{low}}/Z_{\text{high}})_{\text{after}} - (Z_{\text{low}}/Z_{\text{high}})_{\text{before}}}{Z_{\text{low}}/Z_{\text{high}}_{\text{before}}} \]

where \( DZ_{\text{ratio}} \) is the change in the ratio of the impedance to heating, \( (Z_{\text{low}}/Z_{\text{high}})_{\text{after}} \) the ratio of the impedance at 1 and 20 kHz after heating, and \( (Z_{\text{low}}/Z_{\text{high}})_{\text{before}} \) the ratio of the impedance at 1 and 20 kHz before heating.

Electrolyte leakage

For decades, in order to estimate stress damage, measurements have been made of the leakage of electrolytes from plant tissues. The principle of the electrolyte leakage method is based on changes in the cell membrane properties controlling the electrochemical gradient (Steponkus, 1984) that occur during or after exposure to injurious temperature. In fact, electrolyte leakage was used to measure tissue deterioration as a change in membrane permeability.

The electrolyte leakage was measured following the technique already described in a previous work (Fiorino and Mancuso, 2000). In brief, leaf and shoot samples were washed with deionised water and placed in test tubes. Tubes with samples were filled with 20 cc of deionised water after the thermal treatment; after 4 hr the first conductance was measured: \( T_1 \) (treatment), and \( C_1 \) (control) with a conductivity electrode;
the second conductance: \( T_2 \) and \( C_2 \) were measured after the samples were boiled.

Electrolyte leakage was used to calculate the Cell Membrane Stability (CMS) using the following formula:

\[
CMS = \left[1 - \frac{T_1}{T_2}\right] \left[\frac{C_1}{C_2}\right]
\]

the percentage of injury, was, then, calculated as:

\[
\text{In\%} = (100 - CMS).
\]

**Calculation of the LT\(_{50}\)**

Heat hardiness was expressed as LT\(_{50}\) (lethal temperature at which 50% of damage occurs) by fitting the response curves obtained both with the electrolyte leakage and the impedance spectroscopy with the following logistic sigmoid function:

\[
\Delta R = \frac{a}{1 + e^{b(x-c)}} + d
\]

where \( x = \) treatment temperature, \( b = \) slope at inflection point \( c, a \) and \( d \) determine the asymptotes of the function.

### 3. Results

**Impedance spectroscopy**

In accordance with previous works (Mancuso and Rinaldelli, 1996; Mancuso, 1999), the impedance spectra of olive shoots, generated by plotting reactance against resistance for each of the spot frequencies, was composed by an arc in the low frequency domain (Fig. 1). Temperatures of 44°C or lower produced no significant changes in the impedance spectra as shown in figure 1.

By increasing temperature, the tissue’s impedance was reduced. In figure 2 the pattern of the DZ\(_{ratio}\), fitted with a logistic sigmoid function, for the most and the least heat-tolerant cultivar of *Olea europaea* L. resulted by electrical impedance analysis, are shown.

![Fig. 1 - Average (n=10) Cole-Cole plots of shoots of olive tree showing the effect of the high temperature treatment on the impedance spectra of the tissue.](image)

**Electrolyte leakage**

Measurements of electrolyte leakage may be useful when trying to confirm the results obtained using electrical impedance spectroscopy. When electrolyte leakage is high, the apoplast will probably contain high concentrations of electrolytes and impedance will be low, and vice versa (Bauchot et al., 2000).

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>LT(_{50}) shoots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leccino</td>
<td>49.42 ± 3.21</td>
</tr>
<tr>
<td>Frantoio</td>
<td>49.14 ± 3.12</td>
</tr>
<tr>
<td>Maurino</td>
<td>47.30 ± 2.96</td>
</tr>
<tr>
<td>Pendolino</td>
<td>49.14 ± 2.58</td>
</tr>
<tr>
<td>Moraiolo</td>
<td>49.04 ± 3.23</td>
</tr>
<tr>
<td>Carbona</td>
<td>49.36 ± 2.69</td>
</tr>
<tr>
<td>Coratina</td>
<td>48.95 ± 2.54</td>
</tr>
<tr>
<td>Diana</td>
<td>49.18 ± 3.01</td>
</tr>
<tr>
<td>Simjaca</td>
<td>48.00 ± 2.24</td>
</tr>
<tr>
<td>Urano</td>
<td>49.07 ± 2.33</td>
</tr>
</tbody>
</table>

Data are presented as mean (n=10) ± standard deviation.
A clear increase in the electrolyte leakage with increasing temperature was observed in leaves and shoots of all the cultivars tested. Typical curves for leaves (Fig. 3) and shoots (Fig. 4) of two representative cultivars are shown. As usual, the relationship between electrolyte leakage and temperature was sigmoidal. The lethal heat temperatures ($LT_{50}$), calculated by the inflection point, are reported in Table 2. The different organs in olive showed different heat hardness. Thus, leaves resulted more sensitive to thermal treatment than shoots, giving lower $LT_{50}$ values for all the cultivars tested.

‘Leccino’, one of the most important cultivars in Tuscany, resulted the most heat-tolerant cultivar, showing $LT_{50}$ around 50°C for leaves and 53°C for shoots; a similar pattern occurred in ‘Frantoio’, a cultivar diffused in the central regions of Italy and in ‘Coratina’, prevalently cultivated in Puglia. Lethal heating temperature of ‘Maurino’, a Tuscan cultivar, and ‘Diana’, a new variety, are around 49°C for leaves and 52°C for shoots, whereas ‘Moraiolo’ showed $LT_{50}$ around 48°C for leaves and 51°C for shoots.

‘Simjaca’, a cultivar from ex-Jugoslavia, was the most sensitive to heat stress, showing $LT_{50}$ around 46°C both for leaves and shoots.

4. Discussion and Conclusions

Global warming influences olive growth and its distribution. However, it is difficult to determine precise relationships between plant processes and high temperature because of the extreme variability of soil and air temperatures. This difference in temperature makes it very difficult to carry out field studies on the effects of temperature on processes such as photosynthesis and, more generally, growth. In addition, it has been noted that different stages of plant development, and different plant parts, can have different temperature optima (Fitter and Hay, 1987). In the present work, shoots showed a better tolerance to heat if compared with leaves. Accordingly, in olive, twigs were found to be a little more thermotolerant than leaves (Larcher, 2000), whereas no information is available on root thermotolerance of Mediterranean plants. In the complex, however, knowledge about heat resistance of the various plant parts and developmental stages among Mediterranean sclerophylls is greatly lacking.

High temperatures from solar radiation usually last for only a few hours each day. The extreme temperatures measured on plant organs during this time are peak values; the temperatures of leaves and flowers with good heat exchange and low heat capacity quickly equilibrate with that of the surrounding air, so that the peak temperatures usually only affect them for a few minutes. (Larcher, 1995). On the other hand, even in temperate latitudes, the organs near the ground may be exposed to temperatures around 40°C for several hours.

### Table 2: Heat tolerance of leaves and shoots of 10 cultivars of *Olea europaea* estimated by electrolyte leakage

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>$LT_{50}$ leaves</th>
<th>$LT_{50}$ shoots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leccino</td>
<td>50.34 ± 4.20</td>
<td>52.84 ± 4.63</td>
</tr>
<tr>
<td>Frantoio</td>
<td>50.50 ± 3.92</td>
<td>52.32 ± 4.52</td>
</tr>
<tr>
<td>Maurino</td>
<td>49.05 ± 3.89</td>
<td>52.11 ± 4.22</td>
</tr>
<tr>
<td>Pendolino</td>
<td>48.10 ± 4.21</td>
<td>48.75 ± 3.96</td>
</tr>
<tr>
<td>Moraiolo</td>
<td>48.38 ± 4.63</td>
<td>51.63 ± 4.94</td>
</tr>
<tr>
<td>Carbona</td>
<td>50.70 ± 3.65</td>
<td>52.33 ± 4.02</td>
</tr>
<tr>
<td>Coratina</td>
<td>50.74 ± 3.24</td>
<td>51.94 ± 3.69</td>
</tr>
<tr>
<td>Diana</td>
<td>49.79 ± 4.01</td>
<td>52.39 ± 5.12</td>
</tr>
<tr>
<td>Simjaca</td>
<td>46.75 ± 3.96</td>
<td>46.81 ± 3.97</td>
</tr>
<tr>
<td>Urano</td>
<td>46.83 ± 3.66</td>
<td>52.16 ± 5.23</td>
</tr>
</tbody>
</table>

Data are presented as mean (n=10) ± standard deviation.
hours or, for shorter periods, to as much as 50°C. They are thus exposed to a temperature range in which vital processes are severely stressed, and which is dangerously close to the lethal limit.

Concerning the different techniques used, the results obtained from the analysis on leaves using electrolyte leakage technique demonstrated that several of the cultivars have very similar values of heat resistance; the lethal temperatures calculated for the leaves made it possible to assemble the cultivars in four groups: a) ‘Leccino’, ‘Frantoio’, ‘Carbona’ and ‘Coratina’ with lethal temperatures close to 50°C; b) ‘Maurino’ and ‘Diana’ with lethal temperatures close to 49°C; c) ‘Pendolino’ and ‘Moraioio’ with lethal temperatures close to 48°C; and d) ‘Simijaca’ and ‘Uiano’ which resulted the most sensitive to heat showing values close to 46°C. Lethal temperatures calculated for shoots demonstrated that the electrolyte leakage method detects injury at higher temperature than the impedance spectroscopy method.

The measurement of the $DZ_{\text{ratio}}$ was found to be the most sensitive expression of impedance in detecting heat injury. It detected injury at a lower temperature (data not shown) than $DZ_{\text{low}}$ (differences of impedance at 100 Hz before and after treatment) and $DZ_{\text{high}}$ (differences of impedance at 20 KHz before and after treatment). Data in literature are variable and relate to the basis of a full impedance spectrum consisting of as many frequency points as can be measured practically. This type of impedance analysis may allow measurements that are more effective in detecting heat stress temperatures in olive.

Another important point to be considered is that air temperatures do not necessarily constitute a good parameter for the evaluation of thermal stress since the temperature of a plant is not always equal to that of the surrounding air (Mahan et al., 1995). Therefore, dangerously high temperatures of plant tissues are sometimes reached when the air temperatures are not so high. Exposed tree stems, for example, may become very hot in the Mediterranean summer when high temperatures due to strong solar radiation, often combined with water deficiency, can evolve into severe stress for plants. This is particularly true for olive with its evergreen scleromorphous leaves that can develop temperatures higher than air temperatures due to the thickness of the leaves and the low evaporational cooling subsequent the low transpirational rates of the leaves. There is no data on foliage overheating in olive in summer. In Quercus ilex, another Mediterranean sclerophyllous species, temperatures of leaves 4-8°C higher than air temperature have been measured under strong solar radiation in summer (Larcher, 2000). Foliage overheating until 10-15°C above air temperature has been recorded on several Mediterranean species by Lange and Lange (1963).

In conclusion, future changes in the radiative forcing of climate, and hence temperature, will be largely driven by an increase in atmospheric CO$_2$ concentrations (Schimel et al., 1996). This temperature increase is likely to have a significant direct impact on the physiology and structure of vegetation in the Mediterranean basin (Osborne et al., 2000). Thus, heat stress events are plausible to become more and more common in the natural environment of olive. Further studies on the susceptibility of olive to high temperatures (e.g. effects on sexual reproduction, mineral uptake, and photosynthesis) and the interaction with water relations would be most important to understand how this species will react to future climatic scenarios.

References


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