Short communication

In situ study of deep roots of Capparis spinosa L. during the dry season: Evidence from a natural “rhizotron” in the ancient catacombs of Milos Island (Greece)

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A B S T R A C T

The growing period of the deep-rooted, winter-deciduous Capparis spinosa (caper) coincides with the dry season, in the Mediterranean Basin. Roots of wild shrubs of C. spinosa penetrated cracks of a pumice substrate and lengthened in the subterranean environment of the ancient catacombs of Milos Island (Greece); therein, they reached depths of up to 20 m and exploited water present in deep soil layers. Elevated water potential (Ψ) was measured in root segments (Ψr = -0.35 ± 0.06 MPa), a few centimetres from the root apices of C. spinosa found in the catacombs during the dry season. Also, estimates of foliar predawn water potential and gas exchange components, as well as of nocturnal foliar water potential of C. spinosa grown on slopes above the subterranean monument were high and reflected the efficiency of roots in retaining acquisition of water from deep soil layers, during the dry season.

1. Introduction

The catacombs of Milos Island (24° 25’ 33” E, 36° 44’ 21” N; altitude 70.5 m) in Greece (https://www.google.com/maps/place/Catacombs+of+Milos) were excavated in loose, volcanic rock of pumice tuff and consist of corridors (length: 184 m) and chambers (width: 1.2–5.5 m and height: 1.6–2.5 m). This was a place of worship, a burial site and a refuge in times of persecution of early Christians (1st–6th century). The catacombs of Milos (www.catacombs.gr/map), officially revealed in the 19th century (Sotiriou, 1928), are among the most important discovered worldwide along with the catacombs of Rome and the Holy Land. Also, it may be worth noting that near the catacombs and inside a buried niche, the Venus of Milos—one of the most famous works of art of all time— was found in the 19th century (Curtis, 2004).

The natural vegetation on slopes and abandoned terraces at approximately 10–25 m above the catacombs (Fig. 1) consists of Artemisia absinthium L. (Asteraceae), Capparis spinosa L. (Capparidaceae), Ficus carica L. (Moraceae), Phlomis fruticosa L. (Lamiaceae), Pistacia lentiscus L. (Anacardiaceae) and Salsola L. spp. (Chenopodiaceae). We observed lateral spread of roots at shallow depths in the pumice horizons, roots grown in fissures and soil-rock interfaces and roots expanded under stones. Also, we observed roots entering the catacombs and elongating for many meters laterally attached to the ceiling of chambers and corridors, in cohesiveness with the pumice medium. Roots were growing along paths, picking their way through stony particles, decaying fibre and the texture of the volcanic substrate (Andronopoulos and Tzitziras, 1988). It is well known that the physical characteristics of the substrate affect the development of roots; it seems likely that root penetration of the roof of the Milos catacombs was greatly influenced by the compaction of the pumice tuff. Roots growing over large distances horizontally and vertically, within chambers and corridors, most probably responding to moisture gradient and gravity vector, eventually reached the floor of the catacombs.

2. Materials and methods

The expansion rate of ten tips of roots grown in the catacombs was measured using a ruler and marks made with small paint brushes.

The water potential (Ψ) was measured in ten root segments, collected a few centimetres above the same root apices used for

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elongation measurements, using C-52 sample chambers (Wescor, Inc. Logan, Utah, USA) attached to a Dew point psychrometer microvoltmeter (Wescor, HR-33T, Inc. Logan, Utah, USA). Also, predawn $\Psi$ was measured periodically on discs (6 mm in diameter) of fully expanded leaves (three leaves per shrub at the 4th to 5th position from shoot apex), as well as nocturnal $\Psi$ of expanded flower petals (Rhizopoulou et al., 2006) collected from five shrubs of *C. spinosa* L. (Capparaceae), grown on slopes approximately 18–20 m above the catacombs of Milos (Fig. 1), during the summer flowering period of *C. spinosa*.

Root samples were cut from ten roots found elongating in different sites in the catacombs of Milos, and used for elongation and water potential measurements; the samples were carefully transferred to the laboratory, where they were cut in pieces and fixed in 3% glutaraldehyde in Na-phosphate buffer at pH 7, at room temperature, for 2 h. The plant material was washed three times by immersion in buffer for 30 min each time; then, it was post fixed in 1% OsO$_4$ in the same buffer at 4 °C for 5 h and dehydrated in acetone solutions. Dehydrated tissues were embedded in SPURR (Serva) resin. Semi-thin sections of embedded tissues (LKB Ultratome III microtome) were stained in Toluidine Blue ‘0’, in 1% borax solution, photographed and digitally recorded using a Zeiss Axioplan light microscope (Carl Zeiss Inc., Thornwood, N.Y.) equipped with a digital camera (Zeiss AxioCam MRc5).

Net CO$_2$ assimilation and transpiration were obtained from ten fully expanded leaves of selected from shrubs of *C. spinosa* grown above the catacombs of Milos (Fig. 1) using a portable gas analyzer (Li-6400, Li-Cor Inc., Lincoln, NE, USA) equipped with a light source (6200-02B LED, Li-Cor), at midday, throughout July. Conditions in the leaf cuvette consisted of a photosynthetic photon flux density of 1600 μmol m$^{-2}$ s$^{-1}$, leaf temperature was maintained at 25 °C, leaf to air water vapour pressure deficit was kept <1.1 kPa, and the ambient CO$_2$ concentration was 340 μmol mol$^{-1}$ air; measurements were made after steady state conditions were obtained, which required around 2 min (Meletiou-Christou and Rhizopoulou, 2012).

Soluble sugars were extracted from the dried (48 h at 80 °C), powdered plant material collected from shrubs of *C. spinosa* grown on the slopes above the catacombs of Milos, as well as from roots expanded within the catacombs, and used for physiological and anatomical estimates, and were quantified in triplicate samples, according to the colorimetric method of Dubois et al. (1956); D-glucose (Serva) solutions were used for the standard curve.

Values of monthly precipitation and air temperature were obtained from a meteorological enclosure in the research site, over 15 years. Microclimatic variables in the indoor air of the hypogaeal network of the catacombs of Milos were measured using a portable Li-6400 system (Li-Cor, Lincoln, NE, USA), throughout a year.

3. Results and discussion

In the indoor air of the hypogaeal network of the catacombs of Milos elevated CO$_2$ concentration (0.05–0.07 %), enhanced relative humidity (60–80 %) and temperatures in the range of 15–25 °C were detected.
We identified those roots grown in the dark and humid microenvironment of the catacombs as belonging to *C. spinosa* according to Psaras and Sofroniou (1999), based on quantitative features of root xylem and vessel distribution (Psaras and Sofroniou, 1999; Selmeier, 2005; Gan et al., 2013). It is likely that quantitative traits of *C. spinosa* such as vessel diameters are highly diagnostic (Selmeier, 2005). The mean diameter of root vessels was approximately 90 ± 6 μm; the conducting capacity of a capillary is proportional to the 4th power of its radius (Hagen-Poiseuille law) (Zimmermann, 1983). The conducting elements of the well developed root xylem constitute a pathway for long-distance water transport and may be considered an adaptation of *C. spinosa* to the arid environment (Rhizopoulou, 1990; Gan et al., 2013). The xylem reflects the root segment history, i.e. its development in relation to the environment (Lobet et al., 2014). *C. spinosa* (caper) is a winter-deciduous, herbaceous, perennial, branched bush growing close to the ground and in crevices and walls, creeping along steep rocky cliffs and stony slopes, in dry and arid environments (Sozzi, 2001), and widely distributed in arid and semi-arid landscapes in the Mediterranean region and adjacent areas. The pickled flowers of this species are well known as caper appetizers and the commercial spice caper. Although water scarcity limits plant productivity in the Mediterranean region through a dry period, the growth of the above ground tissues and the completion of the life-cycle of *C. spinosa* (from May to September) coincide with the dry season (Rhizopoulou and Psaras, 2003; Gan et al., 2013), which is considered the most stressful for plant life in this region. In particular, during the study period the average daily mean air temperature and average monthly rainfall was 24 ± 2 °C and 2 ± 0 mm in June, 25 ± 5 °C and 0 ± 0 mm in July, and 27 ± 4 °C and 1 ± 0 mm in August, respectively. The vegetative activity of *C. spinosa* starts by forming new expanding stems and leaves in the beginning of the dry period (Rhizopoulou et al., 1997), i.e. in May, when average daily mean air temperature and monthly rainfall are 22 ± 8 °C and 13 ± 3 mm, respectively. The aboveground parts of *C. spinosa* wilt in the beginning of the wet period in October, when average daily mean air temperature and monthly rainfall are 18 ± 2 °C and 43 ± 4 mm, respectively. Another striking feature of *C. spinosa* is the nocturnal, large, short-lived flowers that expand during the summer drought (Rhizopoulou et al., 2006; Chirona et al., 2012).

Elevated values of water potential (Ψ) were obtained from root segments (Ψf = −0.35 ± 0.06 MPa) located a few centimetres from the root apices of *C. spinosa* found in the catacombs, during the dry season (Table 1). Also, although roots expanding in the catacombs cannot be directly linked with aboveground plant tissues, predawn Ψ of fully expanded leaves (Ψ1 = −1.18 ± 0.05 MPa) and nocturnal Ψ of expanded flower petals (Ψf = −0.82 ± 0.03 MPa) from shrubs of *C. spinosa* (Table 1) growing on slopes approximately 18–20 m above the catacombs of Milos (Fig. 1) indicated access to water sources.

The expansion rate of root tips of *C. spinosa* growing in the catacombs was found to be approximately 2.5 mm h⁻¹ during the drought summer season (Table 1). Also, enhanced soluble sugar content was estimated in roots (18 mg g⁻¹ d.w.) collected from the catacombs, as well as in leaves (53 mg g⁻¹ d.w.) and shoots (30 mg g⁻¹ d.w.) of shrubs of *C. spinosa* growing above the catacombs (Table 1). The expansion of plant tissues depends on carbohydrate supply and sugar translocation from source (i.e. distant photosynthetic tissues) to sink indicates transport activity (Yili et al., 2006); increased allocation to roots will increase the probability of reaching deeper water stores necessary for survival through a dry period.

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Transpiration and net CO₂ assimilation rates (Table 1) of the adaxial (8.2 mmol m⁻² s⁻¹ and 17.5 μmol m⁻² s⁻¹, respectively) and the abaxial (5.6 mmol m⁻² s⁻¹ and 11.8 μmol m⁻² s⁻¹, respectively) leaf surfaces of *C. spinosa* grown above the catacombs are consistent with earlier results (Rhizopoulou et al., 1997; Levizou et al., 2004; Gan et al., 2013).

Plant rooting depth and functional root morphology are key-elements of plant response to water deficit conditions (White et al., 2013). It is likely that long roots of *C. spinosa*, extending deep into the catacombs of Milos (total length > 50 m) and reaching depths of approximately 20 m below the above-ground plant parts (Fig. 1), tap water from very deep soil layers, enabling the plant to

**Table 1**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Roots</th>
<th>Shoots</th>
<th>Leaves</th>
<th>Petals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water potential (MPa)</td>
<td>−0.35 ± 0.06</td>
<td>−1.18 ± 0.05</td>
<td>−0.82 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Elongation rate (mm h⁻¹)</td>
<td>2.50 ± 0.20</td>
<td>30.00 ± 0.40</td>
<td>53.00 ± 0.80</td>
<td></td>
</tr>
<tr>
<td>Soluble sugars (mg g⁻¹ d.w.)</td>
<td>18.00 ± 0.60</td>
<td>30.00 ± 0.40</td>
<td>53.00 ± 0.80</td>
<td></td>
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<table>
<thead>
<tr>
<th>Leaf surface</th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Adaxial</td>
<td>17.5 ± 0.40</td>
<td>11.8 ± 0.60</td>
<td>5.6 ± 0.30</td>
<td></td>
</tr>
<tr>
<td>Abaxial</td>
<td>8.2 ± 0.80</td>
<td>5.6 ± 0.30</td>
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**Fig. 2** Representative light micrographs of transverse sections of roots (left) grown in the catacombs of Milos and (right) belonging to wild shrubs of *C. spinosa* according to Psaras and Sofroniou (1999).
sustain leaf and floral water demand during the prolonged dry period. *C. spinosa* has evolved adaptations to water stress during the progressive drought, as the soil water reserve is not refilled during its growing season, and is gradually restricted to deeper soil layers. Roots of wild shrubs of *C. spinosa*, initially tracing their way through upper soil horizons, penetrated cracks in pumice substrate and exhibited horizontal and vertical spread in the catacombs of Milos, allowing plants to effectively exploit larger volumes of the pumice substrate and facilitating acquisition of water from deep soil layers.

Despite recent technological advances and innovations, the study of deep roots in natural ecosystems is still fragmentary and remains time-consuming, technically demanding and costly (Maeght et al., 2013). Excavation and coring techniques used to obtain data concerning deep root distribution are particularly labour intensive. The cost of establishing deep trenches lead researchers to use available soil profiles created by road cuts, exposed stream cut-banks or landslides, in order to determine vertical rooting pattern. Also, roots of plants are regularly found in caves and mine shafts; however, such observations have merely been mentioned in the literature as curiosities. Rhizotrons, promising a rooting pattern. Also, roots of plants are regularly found in caves and mine shafts; however, such observations have merely been mentioned in the literature as curiosities. Rhizotrons, promising tools for laboratory studies and relatively small soil volumes, remain of limited use for deep root observation; while, sampling depths are often decided arbitrarily and set to values that are too shallow to allow reliable estimates of rooting depth, under ambient conditions. In other words, research on roots is mostly confined to the uppermost soil horizons, while research on deep roots remains laborious.

The particularly deep roots of *C. spinosa* found in the catacombs of Milos draw water from soil layers, where direct sampling of root specimens and measurements of root water status are reported for the first time. In this respect, the study of roots of *C. spinosa* has been facilitated by *in situ* monitoring of root development in the ancient monument.

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**References**


