

New Forests

Should we use meshes or solid tube shelters when planting in Mediterranean semiarid environments? --Manuscript Draft--

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Corresponding Author:	Juan A. Oliet, Ph.D. Universidad Politécnica de Madrid Madrid, SPAIN	
Corresponding Author Secondary Information:		
Corresponding Author's Institution:	Universidad Politécnica de Madrid	
Corresponding Author's Secondary Institution:		
First Author:	Juan A. Oliet, Ph.D.	
First Author Secondary Information:		
Order of Authors:	Juan A. Oliet, Ph.D.	
	Raúl Blasco	
	Patricio Valenzuela	
	María Melero de Blas	
	Jaime Puértolas	
Order of Authors Secondary Information:		
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Abstract:	<p>Tree shelters in Mediterranean environments have a two-sided effect. They not only protect seedlings from browsing but also ameliorate microclimatic conditions, improving post-planting survival and growth. However, the ecophysiological basis of these effects are poorly understood. A factorial experiment combining light transmissivity and shelter type (solid tube vs mesh wall) was carried out to assess the impact of contrasting microclimatic characteristics on seedling performance and physiological stress levels of shelters in two Mediterranean shrubland species (<i>Quercus coccifera</i> and <i>Rhamnus lycioides</i>) planted in a semiarid site. Even though seedlings in solid tube shelters experienced higher temperature and were slightly more photoinhibited, they had higher predawn water potential and, in general, better survival and growth than in mesh wall shelters. However, these effects were species-specific, with <i>Rh. lycioides</i> more favoured by solid wall shelters than <i>Q. coccifera</i>. However, root growth cannot explain these interactions between species and shelter type on seedling survival. Since light transmission had a marginal effect compared with wall type, we proposed that the observed effects and interaction with species are not dependent on light intensity or temperature but on other microclimatic differences like air velocity or light quality and distribution. Further studies should assess the importance of these factors on post-planting growth and physiological stress levels, which can be critical for matching the correct tree shelters type for each species in plantations in semiarid environments.</p>	
Suggested Reviewers:	Dugald C. Close	

dugald.close@utas.edu.au
Similar studies in Australia with same ecophysiological basis

María Noelia Jiménez
noelia.jimenez.ext@juntadeandalucia.es

Response to Reviewers:

MANUSCRIPT NEFO S 17 00259
Madrid, May 19 2018
COVER LETTER TO Revision #1

Dear Dr. Jacobs

Thank you very much for your email from April 19 concerning the review of the manuscript NEFO 17 00336 Should we use meshes or solid tube shelters when planting in Mediterranean semiarid environments?. We would like to thank the reviewers for their helpful comments, which we believe have improved the clarity and impact of the work. Virtually all the changes proposed by the reviewers have been incorporated in the new version. These changes are highlighted in red font in our revised manuscript, and references to previous or current manuscript position of the changes are presented to facilitate location and checking. Main changes consisted in presenting a different Figure 4 and correspondent post-hoc test in agreement with Reviewer#1 suggestion. Also minor changes in the text have been conducted accordingly. Please note that specific reviewer inquiries are in italics, and our responses are in bold.

COMMENTS FOR THE AUTHOR:

Editor-in-Chief: Thank you for submitting your manuscript, which has now been appraised by an Associate Editor and two expert reviewers. Your manuscript will be acceptable after minor revision based on Associate Editor and reviewer comments below. Please provide a covering letter detailing your specific changes in response to each point raised by the Associate Editor and reviewers. Your revision is due within 6 weeks.

AE: The paper by Oliet et al. (NEFO-D-17-00259) describe a solid study with interesting results. Although much used in practice, not much have been published concerning physiological responses of seedlings to various plant tube materials and designs. Therefore this manuscript is interesting for readers of New Forests and is worth publishing. Both reviewers are positive with some comments that should be addressed before publication.

Thanks a lot for the positive appraisal of the manuscript

Reviewer #1: The paper studies the influence of two types of shelters (mesh/tube) differing in their light transmissivity on the responses of hardwood shrubby species (Rhamnus and kermes oak) planted in a semiarid environment (Central Spain). This topic is indeed of interest for forest and restoration operations in which the early development of seedlings in harsh conditions is a major issue. The authors have carried out a convincing field experiment; the methods used are well detailed and sound; the results are also clearly exposed. Besides, the paper is very well written and the authors have shown a deep knowledge of the literature on this topic. I also found that combining analysis of above- and belowground morphological traits with ecophysiological (predawn potential, fluorescence) and microclimatic measurements is original and shed light on the processes explaining the results obtained. In summary, I really enjoyed reading this paper.

Thanks a lot for your positive inputs!

Despite the undoubted quality of the manuscript, I have relatively minor concerns about some points I would like the authors to consider First, I was not convinced that the authors have always used statistical post-hoc tests in appropriate manner. For instance in Figure 4, post-hoc tests have been made to compare the combined treatments species*shelter*light transmissivity but it is unclear if the interaction is significant or not. If not, please consider to show results in a different way (analyzing

for instance the main effects only as in Fig 1 or interaction of the second order). Same remark for Table 4: the interaction Species*Shelter is only significant for root length (Table 3), so post-hoc tests are not appropriate when analyzing the influence of the treatments on the other variables. Please check this point in your different analyses and correct if necessary.

We agree with the reviewer about the appropriateness of using post-hoc test for species*shelter*light transmissivity combinations if this third order interaction is not significant. We have followed the Reviewer's suggestions in Figure 4 by analyzing the second order interaction for height and diameter, including in this figure the degree of significance of the ANOVA test for each of them. We have reworded the result section accordingly (Lines 320 to 322 of revised version).

With regard to Table 4, we have removed post-hocs letters for all variables except root length. New explanations are given at Table foot.

Second, the duration of the study is somewhat low: 16 months only from February 2014 to June 2015. I acknowledge that the first summer is often of a primary importance for plant survival but it also corresponds to a transplanting shock. To what extent this latter consideration could have influenced your results? Can you add some additional comments on this?

We have added some comments in the Introduction to clarify this question (Lines 100 to 102 of revised version).

Lastly, I have not well understood the method about roots measurements: what do you mean exactly when you indicate that "protruding roots from the plug" were analysed (P5)? I suppose it corresponds to the term "new root" introduced later in the paper. Can you clarify this point?

Some pictures (if available), showing for instance the types of shelter would be helpful for the reader I think.

We refer to the roots that emerge out of the plug. As they do not include new roots formed within the plug, we agree with the reviewer that "new root" can be confusing. Therefore, we have changed "new roots" for "protruding roots" along the text, and explained more the "protruding" issue by adding "out of the plug" (see line 210-211 of revised version).

We do not believe that pictures showing the types of shelter can be too helpful, providing the description given in Mat and Methods are clear enough, as they include transmissivity levels, colors, dimensions of the shelters and net holes dimensions.

Specific remarks

P1L50 benefited = favoured ?

Done

P2L0 leporidae= rabbits and hare? It is easier to understand for the reader

Changed to rabbits and hares

P2L39-51 I particularly appreciated the good analysis of the literature!

Thanks!

P5 Were the protector stabilized by any particular system during the planation? Were there buried in the soil to assure stabilization?

Yes, the protector was stabilized with a stake and buried in the soil. We have added this detail in Mat and Meth.

P5 L41-42 See above remark about the roots

It has been already clarified. See above answer to your remarks.

P7L5 You should call up Fig1 here

Done

P7L17 Table 3 to be replaced by Table 1

You are right. Done

P7L29 Can you indicate how was VPD determined (several formulas are possible)?

VPD was determined using Rosenberg formula:

Rosenberg ND, Blad BL, Berma SB (1983) Microclimate: the biological environment. 2nd Edition. John Wiley and Sons. USA. 495 pp.

We have included this reference.

P7L38 Indicate (Table 2)

Done
P7L49-50 was higher for Rh lycoides (89) than for Q coccifera (80%?)
We have added corresponding value for Q. coccifera survival ($75 \pm 5.8\%$).
P7L57 after "light transmissivity level" you can refer to Fig 4a. I think it's better to split Fig 4 in 4a and 4b
We mention Figure 4A in the following line. We have split Figure 4 in A and B
P8L12 See remark above about root, this parameter "new root length" should be more clearly explained in the M&M section
Done. See response to this comment above.
P8L33 Microclimatic conditions more favourable (T, VPD) inside mesh shelters during...
Added T and VPD in the sentence
P8L43-44 Yes, it's difficult to interpret small differences with fluorescence
P9L4-5 "restricted air movment...reduces foliar water loss and improved hydric water status" Yes, one could have expected a higher RH value inside tubes than inside mesh but this was not observed
P18L54 "among levels of factor" is not very clear. Indicate that letters show significant differences between the type of proctor x and among the transmissivity levels
Done as suggeted
P15 0.081 not in bold
Done, thanks for the thoroughly review.
P20 remove "aa" for solid tube 80%
Done
P21 Fig 4a) heigh and 4b) diameter
Done

Reviewer #2: The work by Oliet et al. was aimed at improving the knowledge of the ecophysiological mechanisms occurring in plants growing in tree-shelter after transplanting in arid environments. They compared the effect of two types of shelter - plastic tube and mesh- in combination with three different gradients of light transmissivity on seedlings of two species usually planted in arid environments for restoration purposes (*Quercus coccifera* and *Rhamnus lycoides*). Despite in international literature the studies on the effect of tree-shelter on early seedling development are pretty spread, most of them are focused on describing the shelter effect against browsing and/or on survival and seedling early morphology. Few experiments are designed to understand the ecophysiological basis to explain such findings. This is the strength point of this study, which was planned and carried out accurately. I enjoyed reading the manuscript by Oliet et al; all sections of the manuscript are well written, materials and methods are correct, and results are interesting and properly presented and discussed. Thus, my recommendation is to accept the manuscript.

Just check citations in the text vs reference list (e.g. in page 2 Dupraz and Bergez 1999 and Mariotti et al. 2015 are cited but not present in reference list).
Thanks a lot for your comments. We have incorporated mentioned references in the list.

[Click here to view linked References](#)

1 **Should we use meshes or solid tube shelters when planting in Mediterranean** 2 **semiarid environments?**

3 Juan A. Oliet¹, Raul Blasco¹, PatricioValenzuela², María Melero de Blas³, Jaime
4 Puértolas⁴

5 1 Departamento de Sistemas y Recursos Naturales, Universidad Politécnica de Madrid,
6 28040 Madrid, Spain. E-mail address: juan.oliet@upm.es. Phone: +34 913366412.

7 ORCID 0000-0001-7719-9327

8 2 Center of Applied Ecology & Sustainability (CAPES), Pontificia Universidad
9 Católica de Chile, 4860 Macul, Santiago, Chile.

10 3 World Wildlife Foundation-España. 28005 Madrid, Spain

11 4 Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK.

12 ORCID 0000-0002-6132-0679

13 **Abstract**

14 Tree shelters in Mediterranean environments have a two-sided effect. They not only
15 protect seedlings from browsing but also ameliorate microclimatic conditions,
16 improving post-planting survival and growth. However, the ecophysiological basis of
17 these effects are poorly understood. A factorial experiment combining light
18 transmissivity and shelter type (solid tube vs mesh wall) was carried out to assess the
19 impact of contrasting microclimatic characteristics on seedling performance and
20 physiological stress levels of shelters in two Mediterranean shrubland species (*Quercus*
21 *coccifera* and *Rhamnus lycioides*) planted in a semiarid site. Even though seedlings in
22 solid tube shelters experienced higher temperature and were slightly more
23 photoinhibited, they had higher predawn water potential and, in general, better survival
24 and growth than in mesh wall shelters. However, these effects were species-specific,
25 with *Rh. lycioides* more **favoured** by solid wall shelters than *Q. coccifera*. **However,**
26 **root growth** cannot explain these interactions between species and shelter type on
27 seedling survival. Since light transmission had a marginal effect compared with wall
28 type, we proposed that the observed effects and interaction with species are not
29 dependent on light intensity or temperature but on other microclimatic differences like
30 air velocity or light quality and distribution. Further studies should assess the
31 importance of these factors on post-planting growth and physiological stress levels,
32 which can be critical for matching the correct tree shelters type for each species in
33 plantations in semiarid environments.

34

35 **Keywords** Afforestation; Restoration; Water potential; Chlorophyll fluorescence;
36 *Quercus coccifera*; *Rhamnus lycioides*

38 **Introduction**

39
40 Animal browsing is an important threat to the successful establishment of planted
41 seedlings (Burney and Jacobs 2018). The incidence of browsing is highly dependent on
42 the ecological characteristics of the reforested area that affects the animal specific
43 composition and their abundance. Landscapes such as cropland matrixes tend to support
44 high amounts of **rabbits, hares** and other generalist species (Calvete et al. 2004) that can
45 be very detrimental for young plantations and result in major economic losses and
46 delays in the restoration process. Among the most common system to protect seedlings
47 is the use of individual tree shelters (Devine and Harrington 2008). Tree shelters are
48 usually plastic tubes enclosing seedlings shoots to preclude browsing. Broadly
49 speaking, two types of tree shelters are commercially available: solid tubes and meshes.
50 Solid tubes are made in a continuous plastic wall, while meshes are open nets that
51 allows free air circulation throughout the seedling. Solid tubes can also be ventilated by
52 several holes. These characteristics have a strong influence on the environmental
53 conditions around the protected plant (Bergez and Dupraz 2009). Therefore, tree
54 shelters not only play a mere physical barrier role, but also can affect plant
55 establishment and growth in additional ways by the changes in temperature, light, vapor
56 pressure deficit or others (Dupraz and Bergez 1999; Oliet and Jacobs 2007; Pemán et al.
57 2010; Puértolas et al. 2010; Mariotti et al. 2015). There are numerous studies from
58 different geographical areas analyzing the response of planted seedlings to the use of
59 solid tube shelters in relation to the micro-environmental conditions inside (Del Campo
60 et al. 2006; Jacobs 2011; Close et al. 2009; Bellot et al. 2002; Bergez and Dupraz,
61 2000). As expected, plant response is species- and environment-specific (Oliet et al.
62 2003; Padilla et al. 2011 Devine and Harrington 2008; Defaa et al. 2015). In semiarid
63 areas, the use of solid tubes has proven to be on average beneficial for survival and
64 growth (Piñeiro et al. 2013). On the contrary, the number of studies analyzing the effect
65 of meshes is much lower (but see Ward et al. 2000; Devine and Harrington 2008),
66 despite this type of protector is broadly used in operational plantations (Taylor et al.
67 2006; Van Lerberghe 2014). Unlike solid tubes, meshes allows air circulation, which
68 precludes greenhouse effect, while reducing radiation incidence on the leaves. Although
69 these effects could be beneficial for plant establishment in harsh areas, we are only
70 aware of two studies addressing plant response to both types of tree shelters under
71 semiarid Mediterranean conditions (Close et al. 2009; Padilla et al. 2011). Results from
72 these studies are opposite, probably due to different site conditions and species, which
73 reinforces the necessity of improving the knowledge of physiological basis for that
74 responses.

75 Constructive characteristics of tree shelters are variable. Apart from different
76 heights to adapt to herbivory size (Van Lerberghe 2014), other characteristics such as
77 ventilation, color or light transmissivity affect micro-environmental conditions inside.
78 Ventilation reduces air overheating during midday (Bergez and Dupraz 2009). Light
79 transmissivity affects the amount of incident radiation, with effects on the intensity of

1 80 stress and on plant growth response that could be crucial for plant survival under harsh
2 81 conditions (Oliet et al. 2003). For example, photoinhibition can occur in shade tolerant
3 82 species when protected by highly transmissive solid wall shelters (Puértolas et al. 2010),
4 83 and some studies reveal a species-specific response of resources allocation to shoot or
5 84 root as a function of shade tolerance (Jiménez et al. 2005; Puértolas et al. 2010;
6 85 Vázquez de Castro et al. 2014). This could explain differences in survival between
7 86 species with contrasted functional traits planted in Mediterranean environments under a
8 87 gradient of light transmissivity (Oliet et al; 2003 and 2015). However, these studies
9 88 have only compared gradients of light transmissivity within solid wall tube shelters, but
10 89 the characteristics of mesh shelters (higher ventilation, different quality of the
11 90 transmitted light) might interact with light transmission to determine the effects on
12 91 survival and performance.

13 92 The objective of our experiment is to compare the effect of both types of tube
14 93 shelters (solid and mesh) on two species (*Quercus coccifera* L. and *Rhamnus lycioides*
15 94 L.) under semiarid Mediterranean conditions. We assessed **first year after planting**
16 95 survival, growth and physiological stress levels (water potential and photochemical
17 96 efficiency) and under a gradient of light transmissivity (40, 60 and 80 %) for both types
18 97 of shelters. Testing a gradient of light transmissivity for both types of guards will help
19 98 to characterize the tree shelter ecophysiological system by assessing the relative
20 99 contribution of different environmental variables on seedling response during
21 100 establishment. **Despite the duration of the study is relatively short, it includes post**
22 101 **planting summer, which under harsh Mediterranean conditions is the most critical**
23 102 **period in terms of survival (Villar-Salvador et al. 2012).** The selected species are
24 103 sprouting shrubs widespread in the western Mediterranean Basin. In semi-arid
25 104 environments, they are considered keystone species affecting community composition
26 105 and ecosystem function (Maestre and Cortina, 2003). *Q. coccifera* is widely used in
27 106 afforestations in the semiarid areas of the Mediterranean basin (Maestre and Cortina,
28 107 2004; Sackali and Ozturk 2004), although frequently accounts for low planting success
29 108 (Baquedano and Castillo 2006). So far, the use of *Rh. lycioides* has been constrained to
30 109 small scale or experimental plantations (Trubat et al. 2011; Chirino et al. 2013).
31 110 Semiarid areas of the Mediterranean Basin are among the most challenging zones for
32 111 the establishment of woody vegetation. Numerous biotic and abiotic factors negatively
33 112 affect survival of planted young trees in these zones. Summer drought combined with
34 113 excess of radiation and high temperatures (Martínez-Ferri et al. 2000; Niinemets and
35 114 Keenan 2014) can reduce post summer survival to very low levels (Villar-Salvador et al.
36 115 2012). Besides, predation by small mammals, birds or ungulates constitutes another
37 116 major source of failure (Leverkus et al. 2013). All these factors dramatically reduce the
38 117 efficiency of restoration efforts. In addition, the current scenario of climate change, with
39 118 higher probabilities of extreme, harsh summers in these areas (Giorgi and Lionello
40 119 2008), suggests that the success of restoration programs in dry Mediterranean
41 120 environments will require improvements in planting techniques (Cortina et al. 2011;
42 121 Vallejo et al. 2012). Our study could contribute to improve establishment success of key
43 122 woody species in these challenging areas by a better management of tree shelters.
44 123

45 124 **Materials and methods**

46 125 *Study site and plant material*

128 The study site is located in an old cropland of central Spain in Toledo province
129 (39°39'8''N, 3°28'5''W, elevation 660 m a.s.l.). The slope of the planting area is North
130 aspect with a moderate 14% steep. Soils are mostly Inceptisols (Gómez-Miguel and
131 Badía-Villas, 2016). In accordance with a sample from study site, soils are deep (0 to
132 110 cm) of mostly loamy texture (22.4-35.2% sand, 44.0-48.7 % silt and 19.7-29.1 %
133 clay) . Horizons are light-colored highly basic (pH from 7.9 to 8.3) and calcic, with
134 organic matter ranging from 2.14% (upper) to 0.86% (deepest horizon). Maximum
135 electric conductivity of deepest horizon is low (170 $\mu\text{S}\cdot\text{cm}^{-1}$). Permeability is high for
136 the first two horizons (0-30 cm) and moderate below this depth, according to Gandullo
137 (1985). The climate is Mediterranean semiarid, with mean annual precipitation of 418
138 mm and mean annual temperature of 14.2°C. Summers are very hot and dry, with
139 drought periods lasting four months, mean maximum temperature of 33°C in July and
140 absolute maximum temperature reaching 43°C. Winters are cold with frequent frosts.
141 Temperature can drop to -11°C and the mean minimum temperature is 0.9°C in January
142 (Ninyerola et al. 2005). During the planting year (2014) annual rainfall was much lower
143 than average (252 mm), with a prolonged dry period from May to September of
144 accumulated rainfall as low as 32 mm (data from National Agency of Meteorology,
145 Agriculture and Environment Department, Spanish Government).
146 Seedlings of *Q. coccifera* and *Rh. lycioides* were raised from seeds of provenance
147 region ES29 Montes de Toledo (Alía-Miranda et al. 2009) and cultivated in 200 cm³
148 cells (plant density 370 m⁻² Plasnor, Spain). After one year in the nursery and prior to
149 planting, seedling height, root collar diameter, total biomass and root biomass were
150 22.1±1.5 cm, 5.5± 0.4 mm, 8.1±0.4 g and 5.0±0.4 g, respectively, for *Q. coccifera*, and
151 22.9±1.5 cm, 3.8± 0.3 mm, 4.7±0.6 g and 1.4±0.2 g, respectively, for *Rh. lycioides*
152 (n=10). These values fall within the recommended ranges for both species according to
153 Pemán et al. 2013.

154 155 156 *Field experiment*

157
158 The site was cross subsoiled prior to planting at a 60 cm depth with two rippers 1 m
159 apart to reduce soil compaction. Subsoiling was conducted following contour lines
160 separated by two meters and perpendicular directions. Seedlings were planted on
161 January 11, 2014 every 1 m along contour lines (spacing was 2 x 1 m), after manually
162 opening holes (0.3 m × 0.3 m × 0.3 m) in the junction of the subsoiling furrows. No
163 weed control were conducted during the experiment, as the seed bank of weeds was
164 weak in this area, specially under the arid conditions of the planting year. Both species
165 were alternated within each planting row, and shelter treatments were randomly
166 assigned to each seedling. The experimental design was a 2×2×3 factorial design, with
167 the following factors and levels: (1) species (*Q. coccifera* vs. *Rh. lycioides*), (2) tree
168 shelter type (solid wall vs. mesh) and (3) light transmissivity of the solid plastic or mesh
169 (values around 40, 60 or 80%). The solid wall tube shelters were made from plastic
170 material supplied by Repsol Química (Spain). Additives were added to the copolymer
171 base to reach the light transmissivities tested in this experiment, maintaining the red/far
172 red ratio around 1 (neutral shade) (Vázquez de Castro et al. 2014). Hand-made tubes
173 using the plastic sheets were circular, single-walled tubes, 50 cm tall × 10 cm wide,
174 with four ventilation holes facing each other of 2.5 cm width and situated at 18 and 36
175 cm in height. Minimum value of 40% light transmissivity was considered as a target
176 when designing shelters that promote biomass allocation to roots and improve water
177 balance of Mediterranean seedlings (Vázquez de Castro et al. 2014). **Solid plastic tubes**

178 were stabilized by fixing a plastic stake with clamps and burying the shelter in the soil.

179 Plastic meshes were chosen among available polyethylene products in the market. Mesh
180 of 80% was a 60 cm tall × 15 cm wide cylindrical blue net with holes 8 × 8 mm (Protec
181 Blaunet model, Projar SA, Spain). 60 % light transmissivity mesh was also a 60 cm tall
182 × 15 cm wide cylindrical black net with holes 2.4 × 2.4 mm (P40 model, Projar S.A.,
183 Spain). And 40 % light transmissivity mesh was a was a 60 cm tall × 12 cm wide
184 cylindrical black net with holes 4 × 3 mm (V8 model, Improfort Limited, Spain).
185 Meshes were stabilized by using two plastic stakes. Actual light transmissivity of solid
186 tubes and meshes under field conditions was determined in several daily cycles of
187 photosynthetically active radiation (PAR) measurements with two or three sensor
188 replicates (QSO-SUN, Onset, USA) per shelter type connected to a U12 data-logger
189 (Onset, USA). Values were registered every 10-15 minutes. Light transmissivity was
190 averaged along the mean daily cycle. Mean transmissivity percentages were 77 (named
191 solid tube 80 %), 58 (named solid tube 60 %) and 36 (named solid tube 40 %) % for
192 solid tubes and 83 (plastic mesh 80 %), 56 (plastic mesh 60 %) and 46 (plastic mesh 40
193 %) % for meshes. A total of 300 seedlings per species were planted, of which 50
194 seedlings were randomly assigned to each combination of type of shelter ×
195 transmissivity. As experimental plot was small and homogeneous, arrangement of
196 treatments was fully randomized, and no blocking or grouping as a mean to control
197 experimental error was necessary.

200 *Monitoring plant response and microclimatic conditions for shelter types*

202 Seedlings survival was measured four times along the study, from June 2014 to
203 February 2015. Some apparently dead seedlings resprouted after measurements and
204 were accounted as live in the following assessment. Height and basal stem diameter
205 were measured on every plant at the end of October 2014. Seedlings biomass and root
206 development were evaluated from five randomly chosen seedlings per treatment and
207 species (60 plants in total) that were destructively harvested on February 28, 2015.
208 Using small hand tools, root systems were carefully excavated from soil up to a depth of
209 70 cm and taking care to retain roots > 1 mm diameter. Shoots were separated from the
210 roots at the root collar and all parts were frozen until processing. Roots protruding out
211 of the plug were excised and washed free from soil with tap water. Leaves and
212 protruding roots were scanned and leaf area and root length measured with an image
213 analyzer (ImageJ V1.48®, National Institutes of Health, USA). After these
214 measurements, dry mass of each component (leaves, stem, plug roots and protruding
215 roots) was determined by oven drying them at 65 °C for 48 h and weighing.

217 Physiological measurements took place in two consecutive sunny days of June (14 and
218 15) and July (15 and 16) 2014 in five seedlings per shelter type, transmissivity and
219 species (60 seedlings in total, 30 per day). A small window was opened in the solid or
220 mesh wall of the shelters to facilitate sampling for water potential and chlorophyll
221 fluorescence measurements; the window was otherwise closed. Shoot xylem water
222 potential was measured at predawn (Ψ_{pd}). A 3-7 cm healthy twig of the upper third of
223 the plant was excised between 05.00 and 07.00 AM, wrapped in aluminum foil, kept in
224 sealed polyethylene bags and stored refrigerated in an ice box. Water potential was
225 measured within 3 h using a pressure chamber (Model 1000®, PMS Instruments
226 Company, USA). To check potential confounding effect of time since twig excising and

227 measurement on water potential, both variables were plotted and no significant
228 correlation was found (data not shown). Chlorophyll fluorescence was evaluated on the
229 same plants. The ratio of variable to maximum fluorescence (F_v/F_m) as a surrogate of
230 maximum photochemical efficiency of photosystem II was measured through the
231 opened window with a fluorometer (FMS, Hansathech Instruments, UK). A fully
232 expanded leaf of the upper third of the seedling was chosen. Prior to F_v/F_m
233 measurements, that were done at predawn (07.00 AM) and midday (13.30 PM) leaves
234 were dark acclimated for 30 min (Kalaji et al. 2014).

235 To assess the effect of light transmissivity of tube shelters on internal microclimate
236 conditions, air temperature and relative humidity (RH) data logger sensors (U23-001
237 Onset, USA) were installed in the shelters from 14 to 25 June 2014. All this period was
238 cloudless. Sensors were randomly installed inside two shelters of each shelter type \times
239 light transmissivity combination (12 sensors in total), attached to a stake at a height
240 between both pairs of ventilation holes for solid tubes and the same height for meshes.
241 Temperature and RH were recorded every 15 min. Vapor pressure deficit (VPD) was
242 calculated from temperature and RH data following Rosenberg et al. (1983) method.

243 244 *Data processing and statistical analysis*

245
246 Post summer (October 2014) and 13 months (February 2015) after planting survival
247 data were analyzed using a generalized lineal model based upon a binomial errors
248 distribution with a logit link function. Full model included shelter type, light
249 transmissivity, species, and all interactions among factors as predictors. Post hoc
250 comparisons among treatments for last measurement (February 2015) were done using
251 Bonferroni correction for paired comparisons.

252 In case of plant development (height, diameter, biomass and root length), a general
253 lineal model with three main fixed factors as per survival were applied by running a
254 three ways analysis of variance (ANOVA). Physiological data were analyzed similarly,
255 although a fourth fixed factor (summer month, June and July) was included in the
256 model. Non-normal data (basal stem diameter in October 2014 and all data from
257 February 2015 plant excavation) were previously converted to logarithmic forms to
258 fulfill normality and variance homogeneity requirements. When ANOVA showed
259 significance, differences among means were identified using a Tukey post-hoc test.
260 Differences were considered statistically significant if $P < 0.05$. Results are given as
261 mean \pm SE throughout the paper.

262 Data from temperature and relative humidity inside tube shelters were averaged per
263 sensor and time to represent a mean daily cycle.

264 All the statistical analyses were performed using software R version 3.1.1 (R Core Team
265 2014 Vienna, Austria). Figures were produced using Sigmaplot, Version 12.0 (Sigma
266 Plot 2012, Inc., San Jose, CA, USA).

267 268 **Results**

269
270 *Physiological response to shelters (maximum photochemical efficiency and water*
271 *potential) and microclimate in summer.*

272
273 Maximum photochemical efficiency (F_v/F_m) at both pre-dawn and midday was
274 significantly affected by all factors of the study during June and July. Besides,

275 significant interactions were found between month of measurement and shelter type
276 (mesh or solid tube) and those two factors and species for predawn F_v/F_m (Table 1).
277 Seedlings growing in solid tubes showed lower values of both predawn and midday
278 F_v/F_m than those in meshes, although differences were lower for predawn (0.02) than for
279 midday (0.06) (Figure 1). A drop in F_v/F_m was found with increasing light
280 transmissivity, with plants growing in lightest tubes (80 %) having lowest values of
281 both pre-dawn and midday maximum photochemical efficiency. In addition
282 fluorescence plants response to light transmissivity was higher for midday F_v/F_m , as
283 seedlings in 60 % light transmissivity shelters had also minimum values as per lightest
284 shelters (Figure 1). On average, F_v/F_m dropped from June to July (data not shown) and,
285 by species, predawn and midday F_v/F_m for *Rh. lycioides* was higher (0.83 ± 0.00 and
286 0.72 ± 0.01) than that of *Q. coccifera* (0.77 ± 0.00) and 0.69 ± 0.01 , respectively).

287 Predawn water potential during summer months was only affected by shelter
288 type (Table 1). Seedlings in meshes were significantly more water stressed, with values
289 of -3.11 ± 0.12 MPa, while plants growing in solid tubes were more hydrated (-2.32 ± 0.11
290 MPa).

291 Averaged temperature in solid wall shelters during the daylight period in late
292 June was higher than that in meshes, with maximum differences (pooling the three light
293 transmissivity levels) reaching 7.3°C at 13:15 h solar time (Figure 2). Temperature
294 differences among light transmissions were minor, with the 80 % shelter tending to
295 have higher temperatures than the rest (1.1°C , data not shown). Differences in RH
296 among type of shelters and light transmissivities were minimum (Figure 2). As a
297 consequence, VPD inside shelters follow the same pattern as temperature, with solid
298 tubes having maximum VPD differences (after averaging by light transmissivity) of
299 1.86 kPa at 13:15 h solar time (Figure 2).

301 *Survival and growth*

302
303 Post summer survival (October 2014) was significantly affected by type of shelter and
304 light transmissivity, although shelter type interacted with species (Table 2). One year
305 after planting (February 2015), the interaction between shelter type and transmissivity
306 became also significant (Table 2). Survival was almost double in solid wall shelters (84
307 ± 3 %) than in meshes (45 ± 4 %) for *Rh. lycioides* (Figure 3). In contrast, for *Q.*
308 *coccifera* only the mesh of 80% light transmissivity had significantly lower survival
309 (Figure 3). In February 2015, this combination of shelter type and transmissivity had the
310 lower survival in both species (Figures 3). Overall survival of *Rh. lycioides* in February
311 2015 was slightly superior to *Q. coccifera* (68 ± 3 versus 60 ± 4 %, respectively), but
312 survival in the most favorable shelter (solid wall tube 60%) was higher for *Rh. lycioides*
313 ($89 \pm 4.6\%$) than for *Q. coccifera* ($75 \pm 5.8\%$).

314 Post summer height was significantly affected by all factors and by almost every
315 second order interactions among them ($P < 0.001$), with the exception of species \times
316 shelter type ($P = 0.07$). Seedlings growing under 40 and 60% light transmissivity grew
317 similarly in height, while a detrimental effect of height growth appears when plants
318 grew under maximum light transmissivity level. Besides, this pattern is much more
319 intense for seedlings growing in meshes and for *Rh. lycioides* (Figure 4A). Thus,
320 differences ranged between almost same height across transmissivities for *Q. coccifera*
321 and plants growing in solid tubes, to a significant depletion in height under 80 %

transmissivity for *Rh. lycioides* and those growing in meshes (Figure 4A). Post-summer basal stem diameter (BSD) was significantly affected by shelter type ($P<0.001$) and by a light transmissivity \times shelter type interaction ($P=0.03$). BSD of plants within solid tubes were on average 18 % larger than that in meshes with no differences between light transmissivity within shelter types except for the 80 % mesh, which were significantly smaller for *Rh.lycioides* and almost significant for *Q. coccifera* (Figure 4B). No significant third order interaction appeared among three factors for height or diameter. Cross values for this three factors combination are presented in Supplementary material.

For all biomass and growth traits measured after plant excavation in February 2015 (13 months after planting), no significant effect was found for light transmissivity (Table 3). However, leaf area and shoot biomass was significantly bigger in solid wall tubes than in meshes (64 and 33 % respectively) (Tables 3 and 4). Protruding roots length was also greater in solid tubes and affected by the interaction of this factor with species: it was 78% longer in *Q. coccifera* and not significantly different from meshes in *Rh. lycioides* (Tables 3 and 4). Shoot:root ratio ($\text{g}\cdot\text{g}^{-1}$), specific leaf area and specific root length of seedlings were unaffected by shelter type or light transmissivity. Most of the evaluated traits from excavation were species-specific: *Q. coccifera* leaf area was 65 % higher, while protruding roots length, shoot:root ratio and specific root length were 36, 60 and 40 % lower than those of *Rh. lycioides*. Nevertheless, data from plant excavation 13 months after planting show high variability levels (Table 4), precluding the declaration of additional significant responses to tested factors.

Discussion

Unexpectedly, seedlings in solid wall tubes showed a higher survival and growth for almost every combination of light transmissivity and species. Microclimatic conditions inside mesh shelters (T, VPD) during the hot Mediterranean summer were expected to decrease heat and water stress compared to solid wall shelters. In fact, chlorophyll fluorescence data suggests a higher degree of photoinhibition in solid tubes with differences deepening from the beginning (June) to midsummer (July). Maximum photochemical efficiency is more sensitive to thermal than to hydric stress, as shown in a wide variety of woody species that presented significant reduction in F_v/F_m under higher temperatures (Matías et al. 2017; Methy et al. 1997). However, values were always close to 0.8, which is considered the optimum value (Bjorkman and Demmig, 1987) and the differences between both shelter types were small. Deactivation of reaction centers of photosystem II is part of the acclimation process to avoid photodamage when photosynthesis is impaired (Demmig-Adams and Adams, 1992). The small changes observed here might only reflect slight differences in this deactivation but with no impact on plant capacity to survive and grow.

In contrast, differences in predawn water potential were in agreement with seedling performance. Average predawn water potential registered in summer for both species in plastic mesh (-3.1 MPa) indicates a moderate level of water stress. *Q. coccifera* shows drastic reduction of assimilation rate with ψ_{pd} values below -2 MPa (Baquedano and Castillo 2006) which implies reduction of root growth. Even though these values are higher than the critical water potential values (-6 MPa) inducing fatal

367 embolism (Vilagrosa et al. 2003), they could impair plant functioning and internal
368 carbon budget, putting plants in higher risk of reaching those critical values.

369 Several hypotheses can be formulated to explain the lower ψ_{pd} in meshes. Air
370 velocity inside solid wall shelters is negligible, even for ventilated tubes (Bergez and
371 Dupraz 2000). Restricted air movement creates a thicker and less conductive boundary
372 layer that reduces foliar water loss and improves hydric status. This effect has been
373 demonstrated in previous works with solid wall shelters under controlled conditions
374 (Kjelgren and Rupp, 1997; Bergez and Dupraz, 1997), where water losses of seedlings
375 in tube shelters was much lower than in unprotected ones. Even though mesh shelters
376 might restrict air movement, they allow some air circulation through leaves. This would
377 decrease leaf-air boundary layer conductance (Lambers 2008), accelerating soil water
378 depletion in the root-zone. Within this hypothesis the increase in transpiration demand
379 due to air movement in meshes would be more intense than the higher VPD inside solid
380 wall shelters provoked by temperature.

381 Alternatively, increased air velocity also induces stomatal closure and hence
382 decreased carbon gain, which along with lower air temperature during spring in mesh
383 shelters could explain the observed reduction in growth in this type of shelters. Besides
384 the effects related to differences in air movement between mesh and tube shelters,
385 growth reduction could be linked to light quality and distribution. Even though the
386 experimental factorial design allowed the comparison of both types of shelters with
387 similar total radiation levels, light inside tubes was exclusively diffuse while in meshes
388 there was a mixture of direct and indirect radiation. Moreover, leaves in meshes were
389 exposed not only to those patches of different light quality and intensity, but also to
390 relatively rapid changes as the sun moved during the day or the leaves were shaken by
391 wind. These rapid changes similar to sunflecks within or beneath canopies can decrease
392 photosynthetic efficiency and water use efficiency, as the capacity of photoacclimation
393 to contrasting light conditions cannot cope with such rapid changes (Townsend et al.
394 2017). Potentially, these two negative effects on carbon gain described above could
395 impair root growth, which is essential for summer survival in dry environments (Padilla
396 and Pugnaire, 2007), and has been linked to survival rates in *Q. coccifera* across a range
397 of different tree shelters (Bellot et al. 2003). However, unlike the latter study, no
398 relationship between root growth and survival was observed here. For *Rh. lycioides*,
399 even though survival was much higher in tubes, root length was similar to mesh
400 shelters, while *Q. coccifera* roots grew less in mesh shelters but only survival was
401 impaired for the 80% transmissivity.

402 This species-specific effect of the type of shelter on seedling performance
403 confirms that the effect of tube shelters on seedling performance depend on the
404 ecophysiological features of the species (Puértolas et al. 2010; Vazquez de Castro et al.
405 2014). These previous studies pointed towards shade tolerance as the main trait
406 explaining plant responses to tree shelters. Shade tolerant species were more benefited
407 from protection, as root growth was less negatively affected by the reduction in light
408 compared to intolerant species. Moreover, light reduction during summer could explain
409 increased survival in *Q. ilex* (Puértolas et al. 2010). Also, *Rh. lycioides* benefits from
410 shade in semiarid plantations (Soliveres et al. 2008). However, our results suggest that
411 other characteristics might also contribute to explain differential effects across species.
412 Lack of knowledge on the physiological characteristics of *Rh. lycioides* makes difficult
413 to understand the basis of these differences. It seems that the higher overall survival

414 rates of this species compared to *Q. coccifera*, which are coincident with previous
415 studies (Trubat et al. 2008, 2011), could be linked to faster root growth. Adults from *Rh.*
416 *lycioides* show higher assimilation rates than *Q. coccifera* during spring, which could
417 explain larger growth and root development (Bellot et al. 2004). The reason for the clear
418 differences in survival between mesh and tube shelters within *Rh. lycioides*, which are
419 less evident than in *Q. coccifera*, are not easy to explain with the current information
420 available. Further studies should investigate which functional attributes other than shade
421 tolerance determine seedling response to tube shelters.
422

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424
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599 Tables

600 Table 1

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602 Table 1. Results from ANOVA (Snedecor F and associated probability P) test for the
 603 effects of Month of measurement (June and July), Species (*Q. coccifera* and *Rh.*
 604 *lycioides*), Shelter type (solid tube and plastic mesh) and Transmissivity (80-60-40 %) during 2014 summer on predawn water potential (Ψ_{pd}) and leaf fluorescence at predawn
 605 ($F_v/F_{m_{pd}}$), midday ($F_v/F_{m_{md}}$).
 606

	Ψ_{pd}			$F_v/F_{m_{pd}}$		$F_v/F_{m_{md}}$	
	df	F	$P>F$	F	$P>F$	F	$P>F$
Month (M)	1	0.02	0.89	6.10	0.01	5.40	0.02
Species (S)	1	1.80	0.18	60.94	<0.001	5.08	0.02
Shelter Type (ST)	1	22.49	<0.001	6.11	0.01	19.80	<0.001
Transmissivity (T)	2	0.15	0.85	6.30	0.002	3.64	0.02
Month \times Species	1	0.11	0.73	0.01	0.93	2.30	0.13
Month \times Shelter Type	1	0.001	0.97	10.83	0.001	0.58	0.44
Species \times Shelter Type	1	0.47	0.49	0.01	0.92	0.40	0.52
Month \times Transmissivity	2	0.17	0.83	0.12	0.88	0.14	0.86
Species \times Transmissivity	2	1.17	0.31	2.64	0.07	0.72	0.48
Shelter Type \times Transmissivity	2	0.01	0.99	0.37	0.68	0.98	0.37
M \times S \times ST	1	0.01	0.93	4.75	0.03	3.49	0.06
M \times S \times T	2	0.250	0.77	0.08	0.91	0.53	0.58
M \times ST \times T	2	0.12	0.88	0.41	0.66	0.11	0.89
S \times ST \times T	2	1.05	0.35	0.71	0.49	0.24	0.78
M \times S \times ST \times T	2	0.16	0.85	1.24	0.29	0.65	0.52

607 Significant values ($P<0.05$) are highlighted in bold

608

609 Table 2

610

611 Table 2. Contrasts of the generalized linear model effects for survival in October 2104
612 and February 2015 of *Q. coccifera* and *Rh. lycioides* species planted with solid tubes or
613 meshes (Shelter type) under three levels of light transmissivity (40- 60- 80%).

	df	October 2014		February 2015	
		Wald χ^2	<i>P</i> > χ^2	Wald χ^2	<i>P</i> > χ^2
Species (S)	1	6.07	0.014	3.04	0.081
Shelter type (ST)	1	31.67	0.000	38.86	0.000
Transmissivity (T)	2	7.25	0.027	12.18	0.002
Species × Shelter Type	1	11.00	0.001	14.12	0.000
Species × Transmissivity	2	1.17	0.556	0.90	0.637
Shelter type × Transmissivity	2	3.76	0.153	7.37	0.025
S×ST×T	2	0.60	0.742	0.21	0.898

614 Significant values (*P*<0.05) are highlighted in bold

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Table 3

Table 3. Results from ANOVA (Snedecor F and associated probability *P*) test for the effects of Species (*Q. coccifera* and *Rh. lycioides*), Shelter type (solid tube and plastic mesh) and Transmissivity (80-60-40 %) on morphological traits of seedlings excavated in February 2015

	df	Leaf Area		Protruding Roots length		Shoot weight		Shoot/Root		Specific Leaf Area		Specific Root Length	
		F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>
Species (S)	1	12.59	0.001	6.68	0.01	2.48	0.12	83.30	0.001	0.007	0.97	16.43	0.001
Shelter type (ST)	1	23.86	< 0.001	3.88	0.05	8.14	0.006	0.81	0.37	1.13	0.29	1.02	0.31
Transmissivity (T)	2	0.57	0.56	0.13	0.87	1.34	0.26	1.83	0.16	0.13	0.87	0.62	0.54
Species × Shelter type	2	0.03	0.86	4.15	0.04	0.03	0.86	0.57	0.45	0.16	0.68	1.29	0.26
Species × Transmissivity	1	0.31	0.73	0.30	0.74	0.06	0.93	0.09	0.91	1.05	0.35	0.46	0.63
Shelter type × Transmissivity	2	1.66	0.19	0.95	0.39	0.45	0.63	2.7	0.07	1.54	0.22	0.27	0.78
S × ST × T	2	2.07	0.13	2.35	0.08	1.43	0.24	1.20	0.30	2.66	0.08	1.5	0.21

Significant values (*P*<0.05) are highlighted in bold

Table 4. Morphological traits by species (*Q. coccifera* and *Rh. lycioides*) as affected by shelter type (plastic mesh or solid tube) of seedlings excavated in February 2015.

	<i>Q. coccifera</i>		<i>Rh. lycioides</i>	
	Plastic mesh	Solid tube	Plastic mesh	Solid tube
Leaf Area (cm ²)	60.60 ± 10.9	127.03 ± 20.43	36.15 ± 10.93	77.25 ± 19.0
Protrud. roots length (mm) ¹	82.93 ± 14.60 ^b	147.50 ± 20.77 ^a	171.47 ± 49.33 ^a	189.30 ± 56.83 ^a
Shoot weight (g)	3.44 ± 0.47	5.11 ± 0.80	2.98 ± 0.93	4.39 ± 1.20
Shoot/Root ratio (g·g ⁻¹)	0.58 ± 0.0	0.59 ± 0.0	1.40 ± 0.23	1.55 ± 0.27
Specific leaf area (cm ² ·g ⁻¹)	74.07 ± 15.37	58.48 ± 5.23	72.84 ± 15.27	63.09 ± 6.5
Specific root length (cm·g ⁻¹)	159.59 ± 25.97	180.80 ± 55.63	323.94 ± 63.80	246.09 ± 46.83

¹ As Species × Shelter type was significant for this variable (see Table 3), different letters following mean values ± SE denote differences among combination treatments after Tukey's post hoc test.

Figures

Figure 1

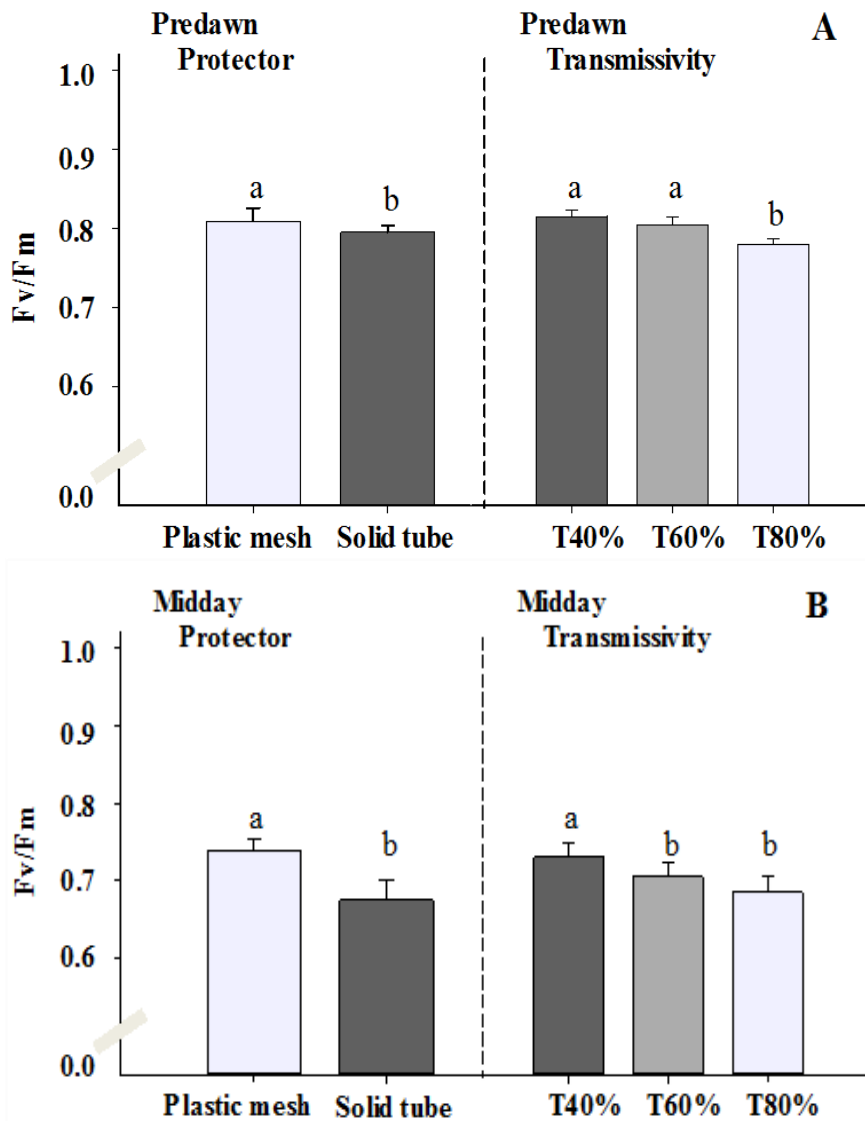


Figure 1. Summer predawn (A) and midday (B) photochemical efficiency (F_v/F_m) as affected by shelter type (plastic mesh or solid tube) and light transmissivity (T40-T60-T80 %). Data are averaged by species and month of measurement (June and July 2014). Different letters **between Shelter type and among Transmissivity levels** denote differences among levels of factors after Tukey's post hoc test. Error bars represent \pm SE.

Figure 2

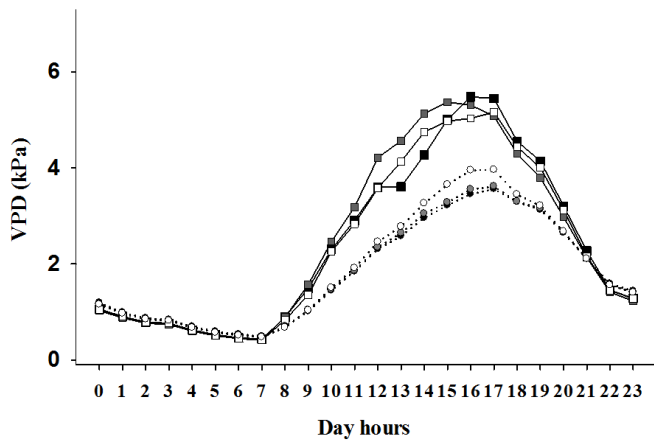
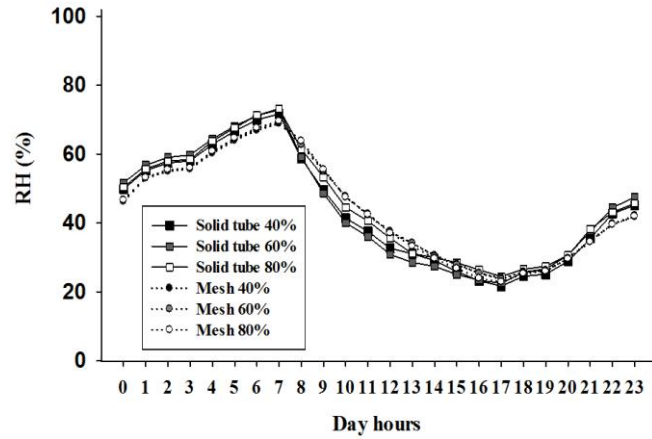
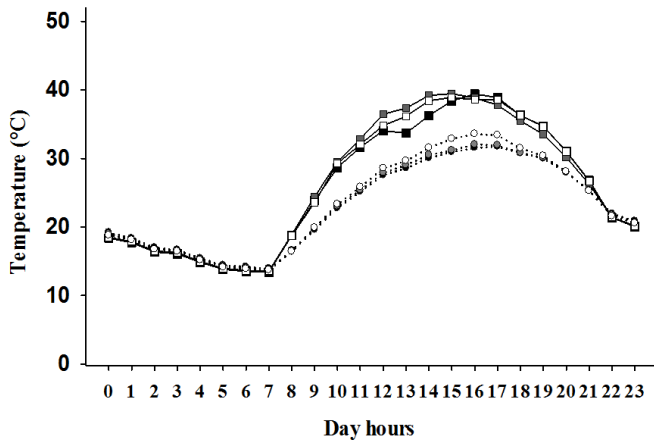


Figure 2. Temperature, relative humidity (RH) and vapor pressure deficit (VPD) during a mean daily cycle (GMT+2) of June 2014 within different combinations of shelters types and light transmissivity. Data from two sensors per shelter type and transmissivity, 11 days of measurements and 15 minutes frequency are averaged to one point per hour.

Figure 3

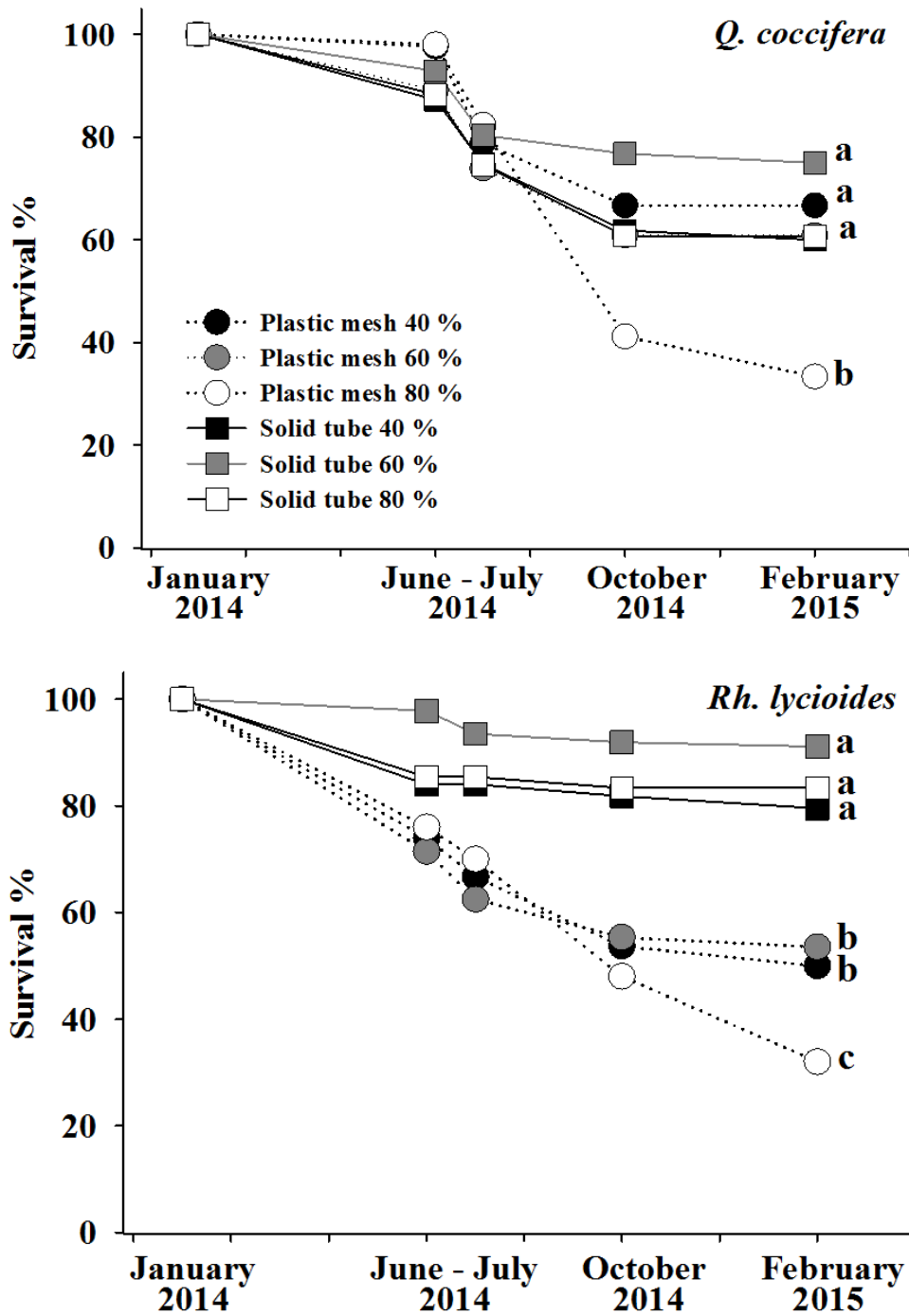


Figure 3. Survival of *Q. coccifera* (top) and *Rh. lycioides* (bottom) along the study period as affected by type of shelter (plastic mesh and solid tube) and transmissivity (40%, 60% and 80%). Letters at the end of each treatment denote significant differences during last measurement (February 2015).

Figure 4

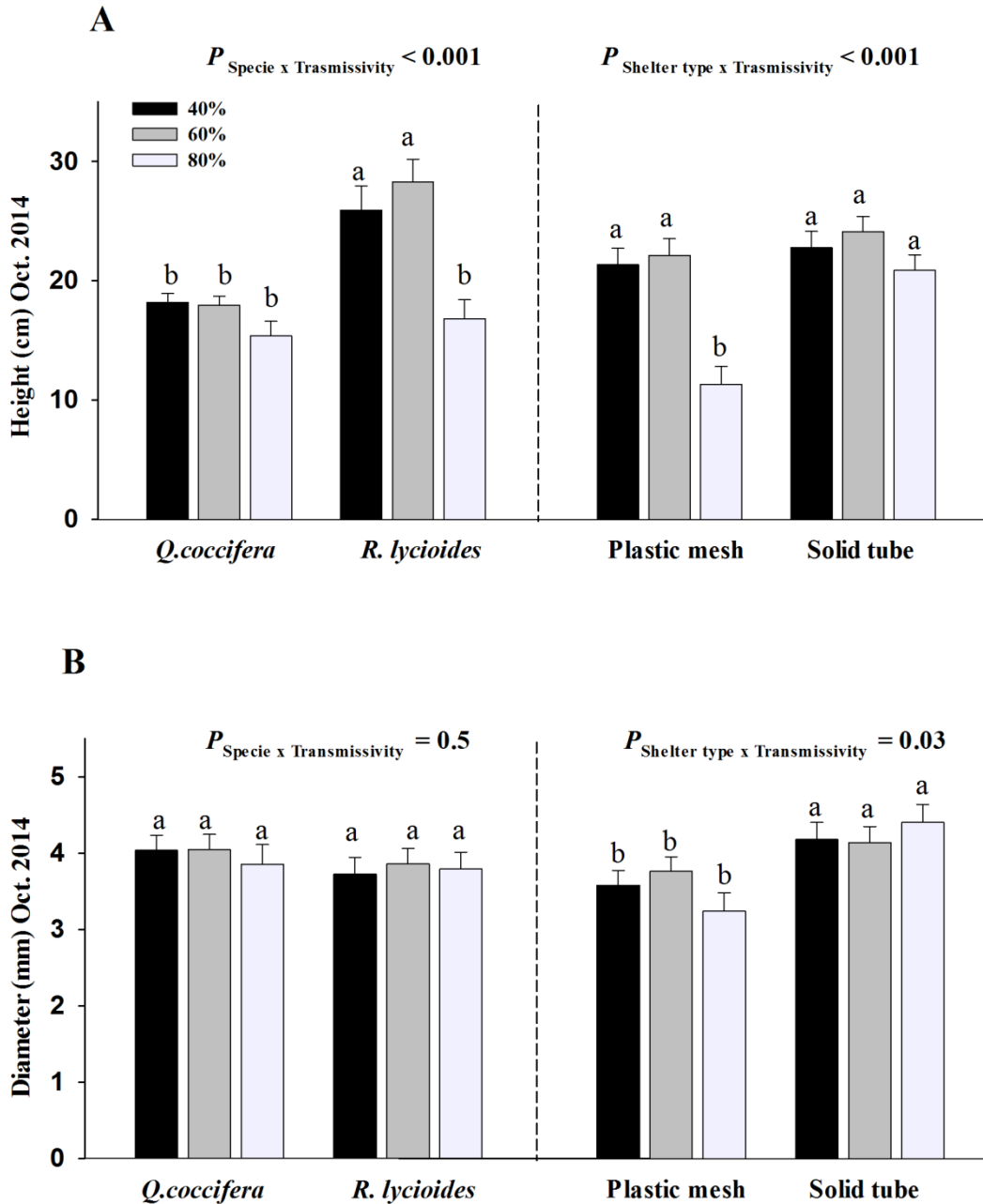
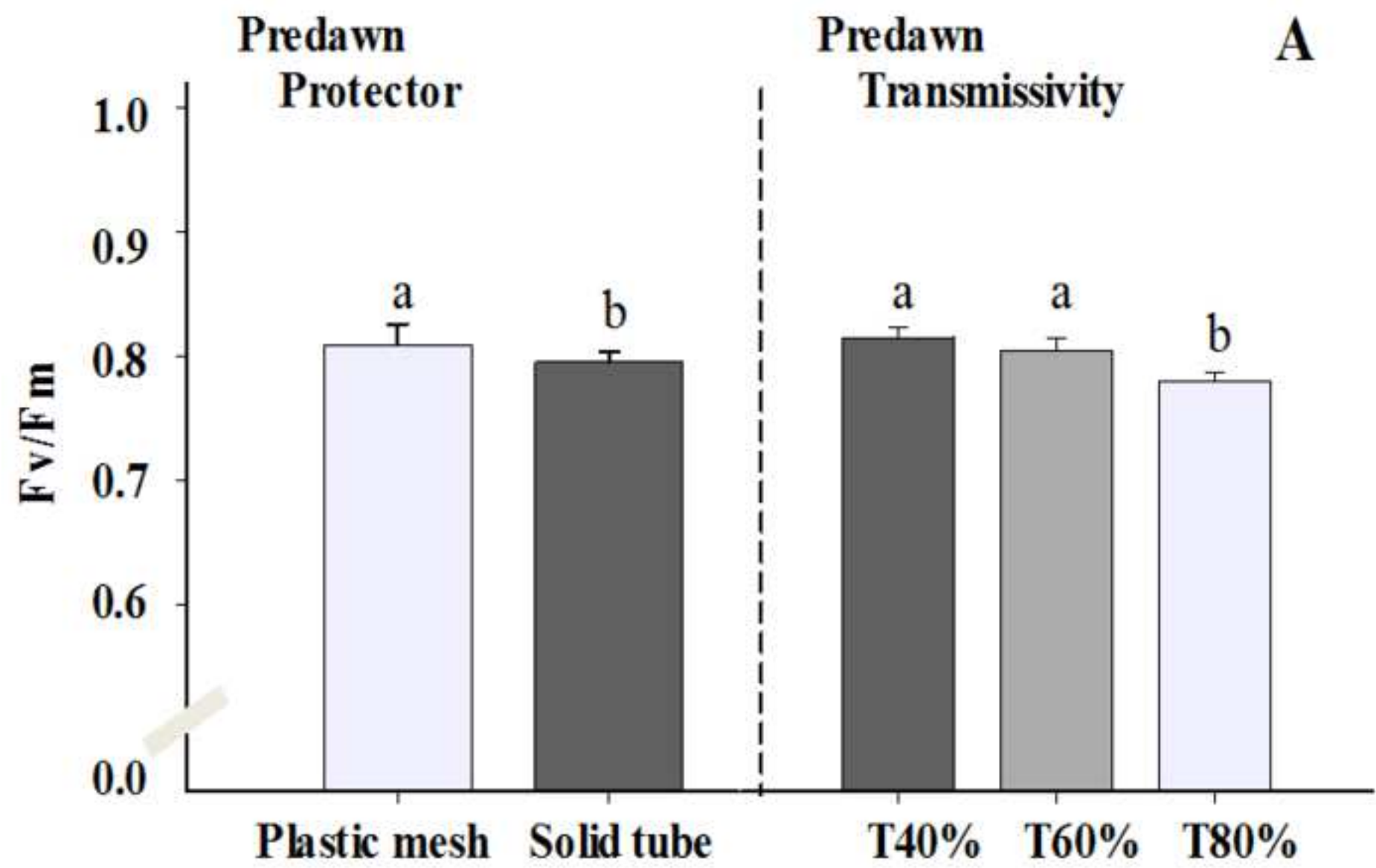
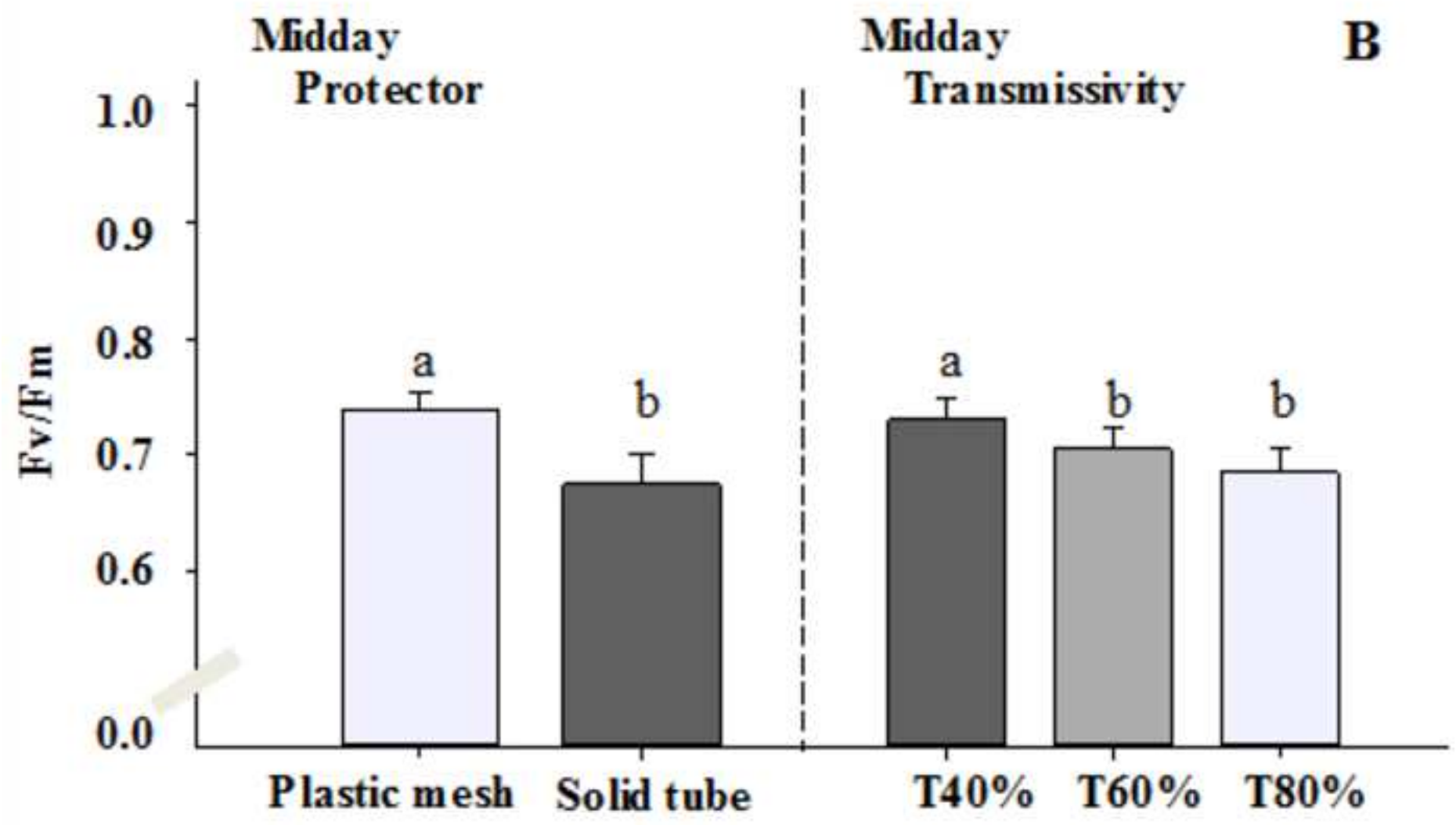
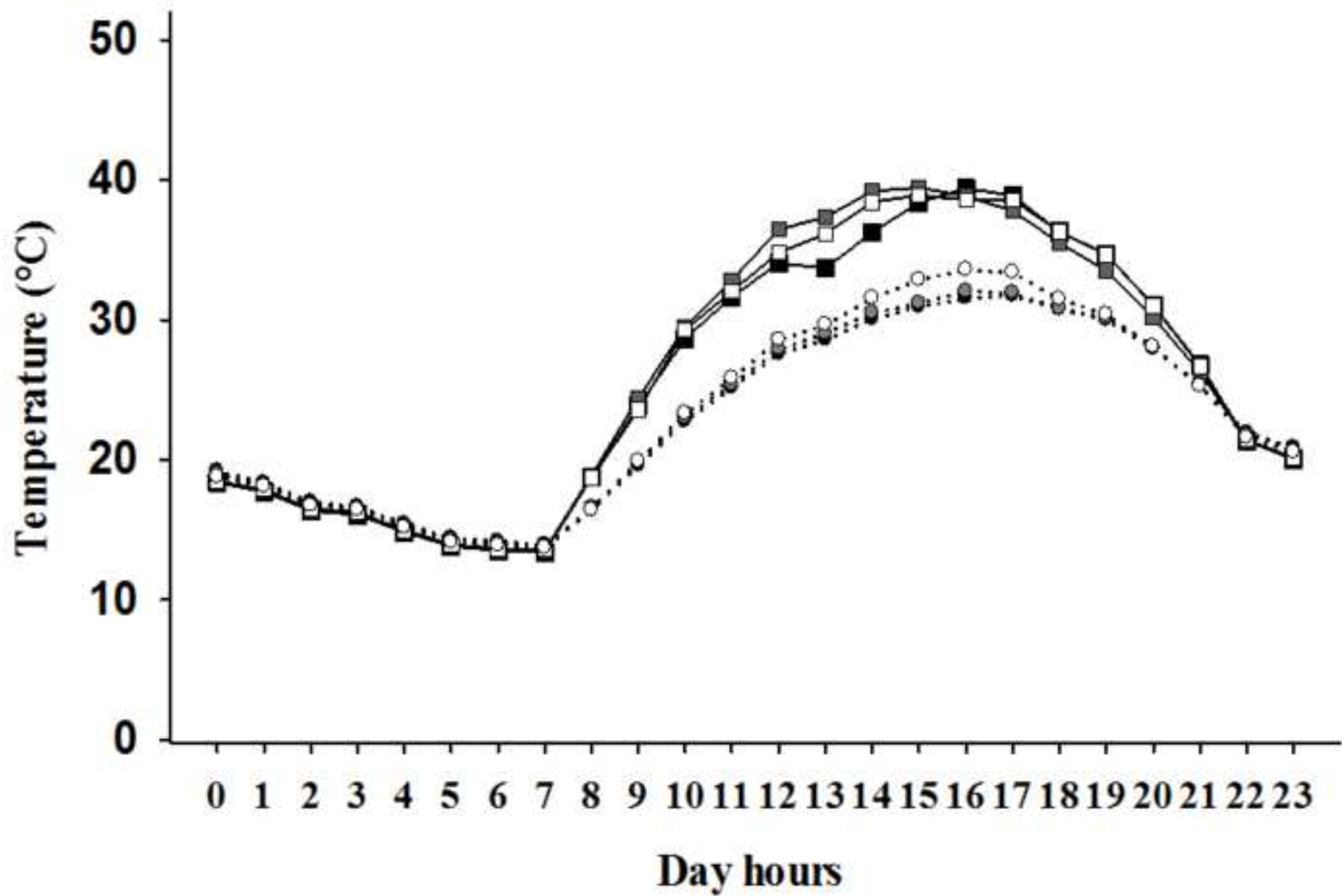
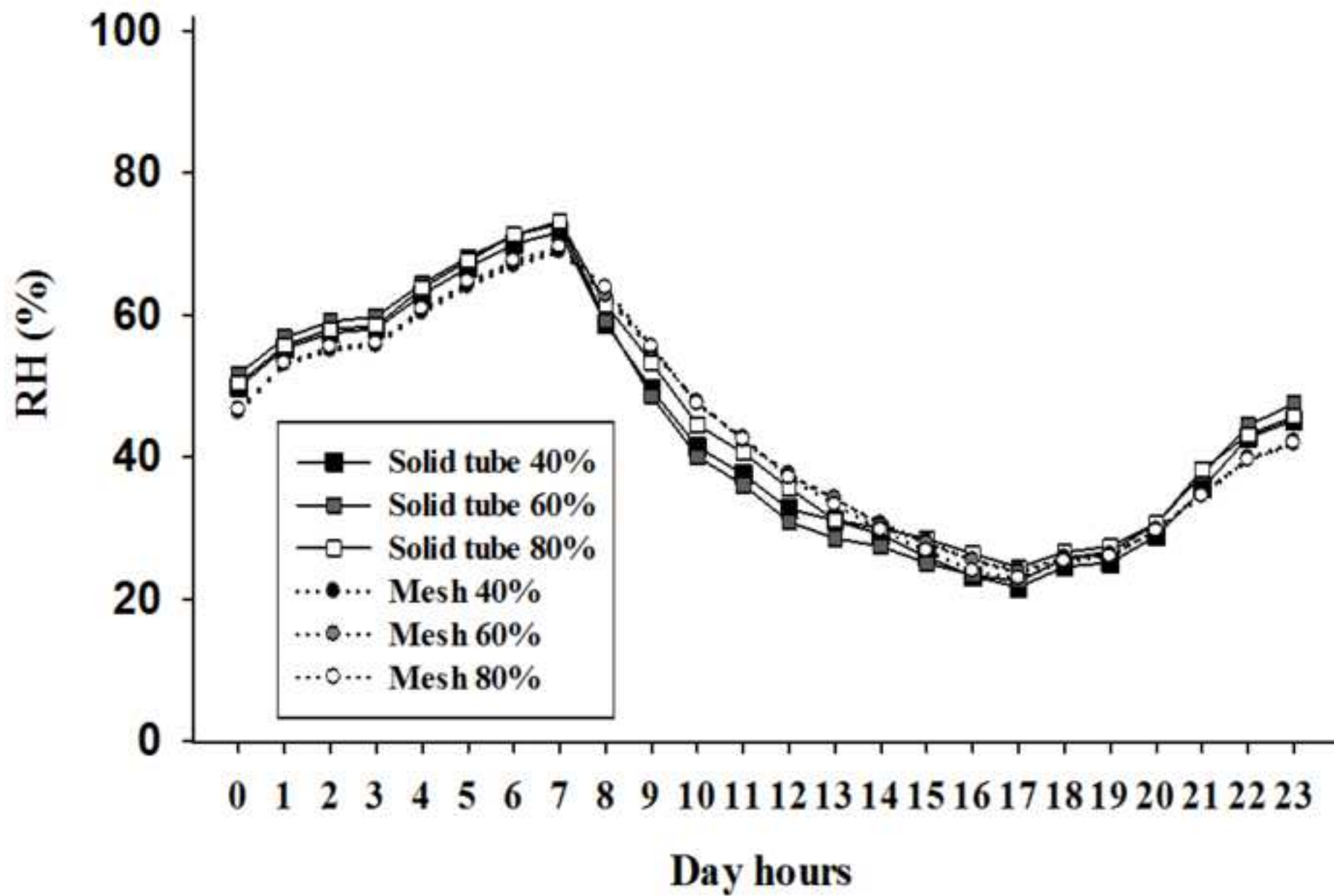


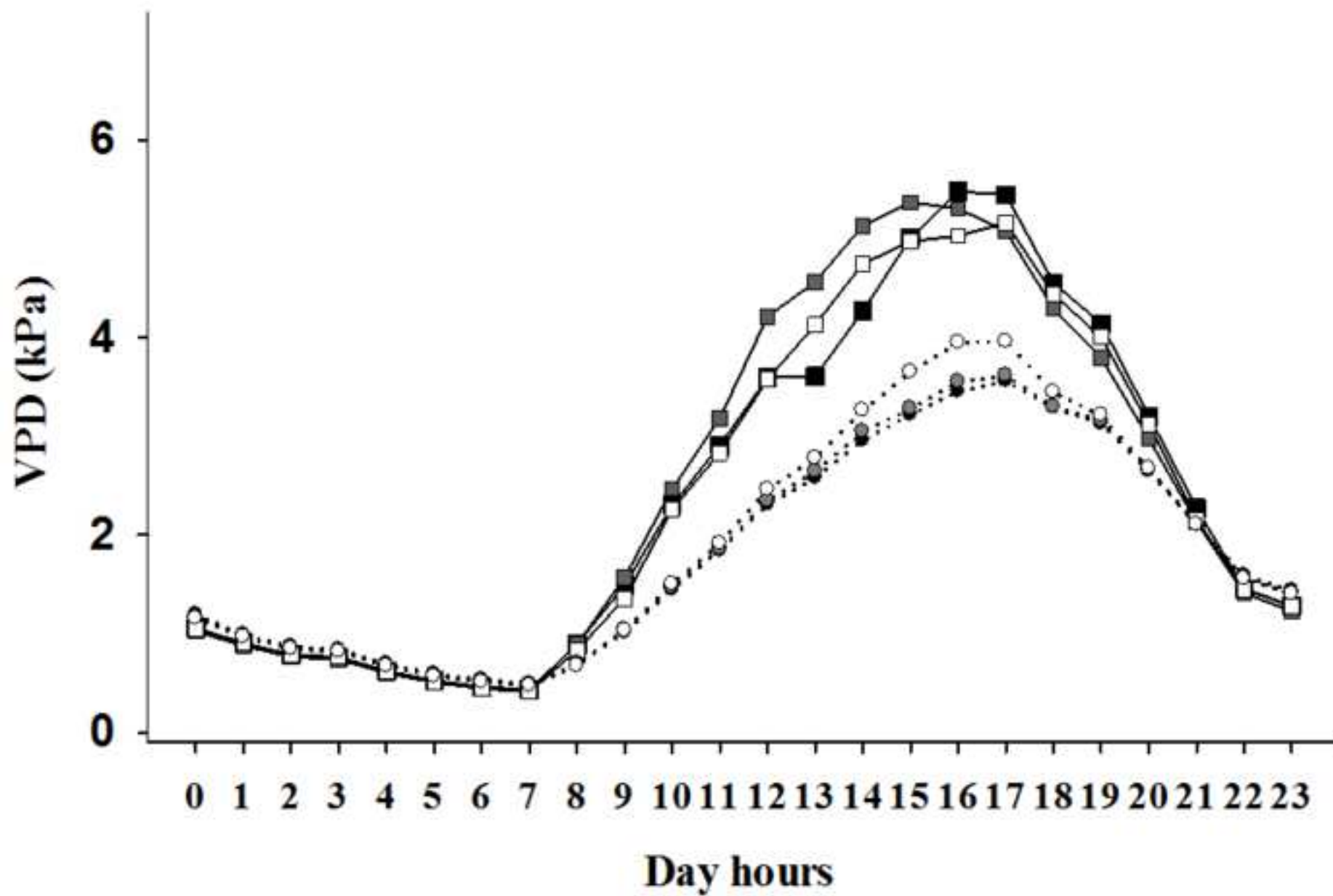
Figure 4. Post summer height (A) and basal diameter (B) of tested species (*Rh. lycioides* and *Q. coccifera*) in October 2014 as affected by shelter type (plastic mesh or solid tube) and light transmissivity (40-60-80 %). Different letters denote differences among levels of treatments after Tukey's post hoc test. Error bars represent \pm SE. **P values for second order interaction are shown on top of each subfigure**

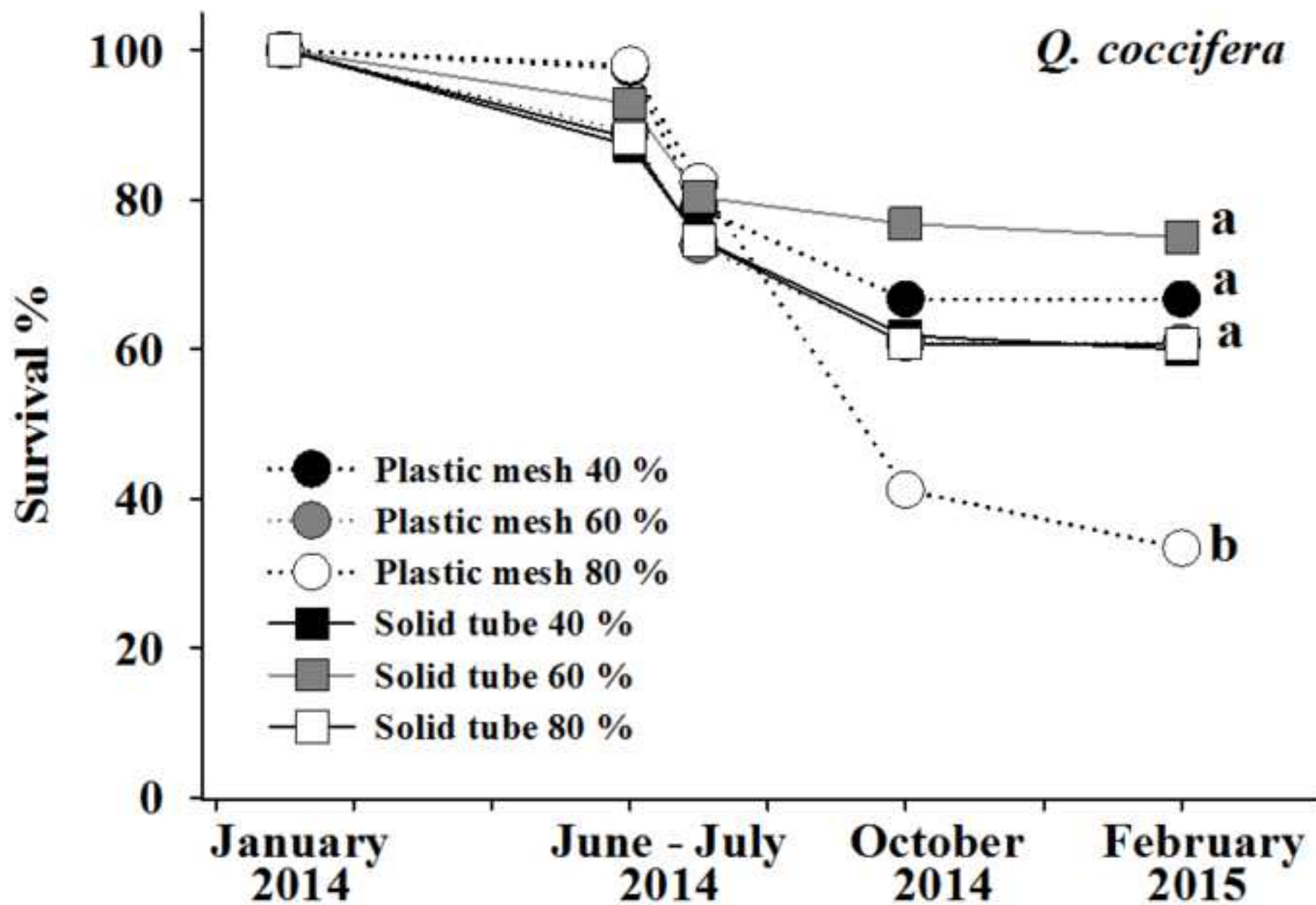


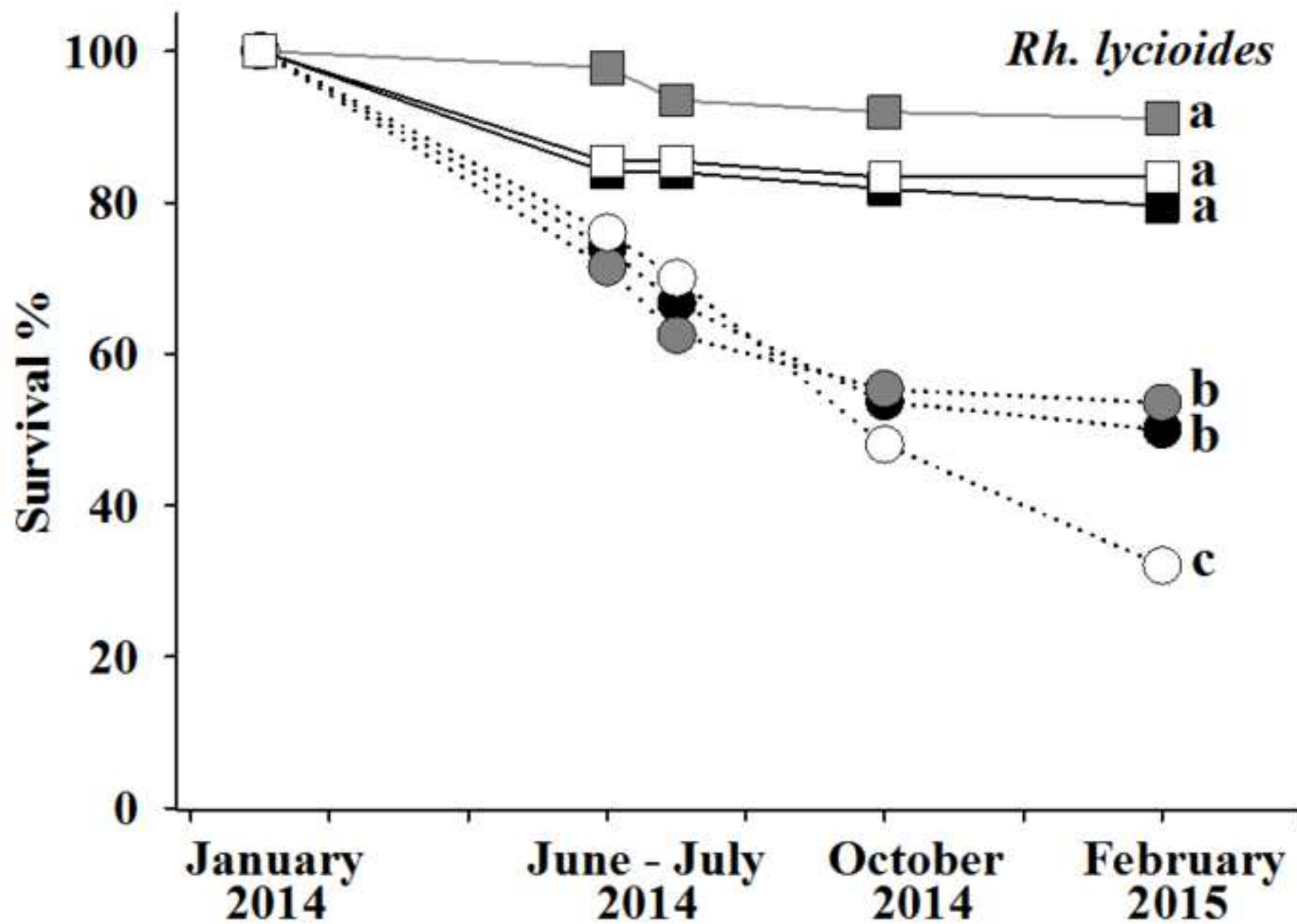


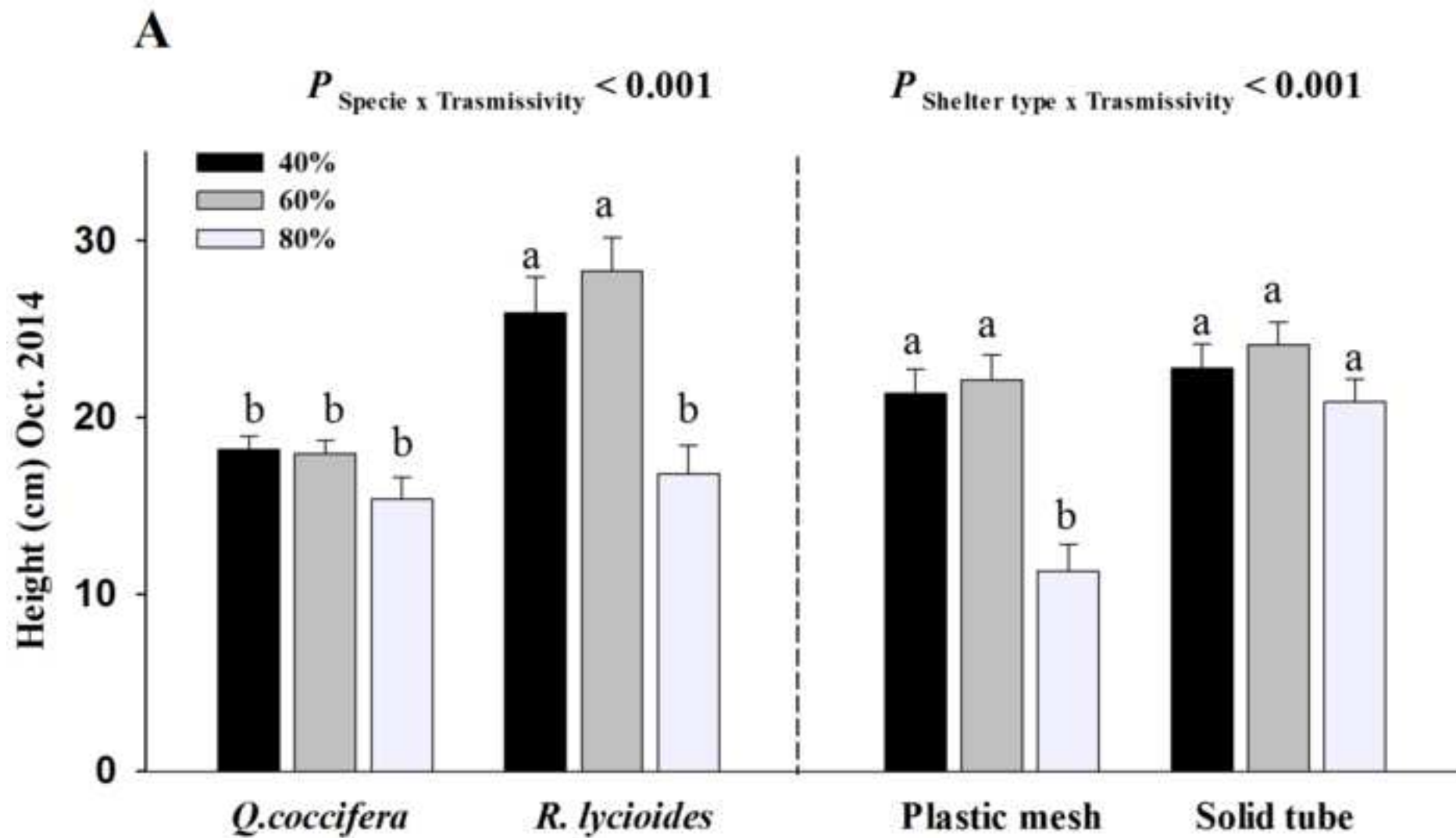


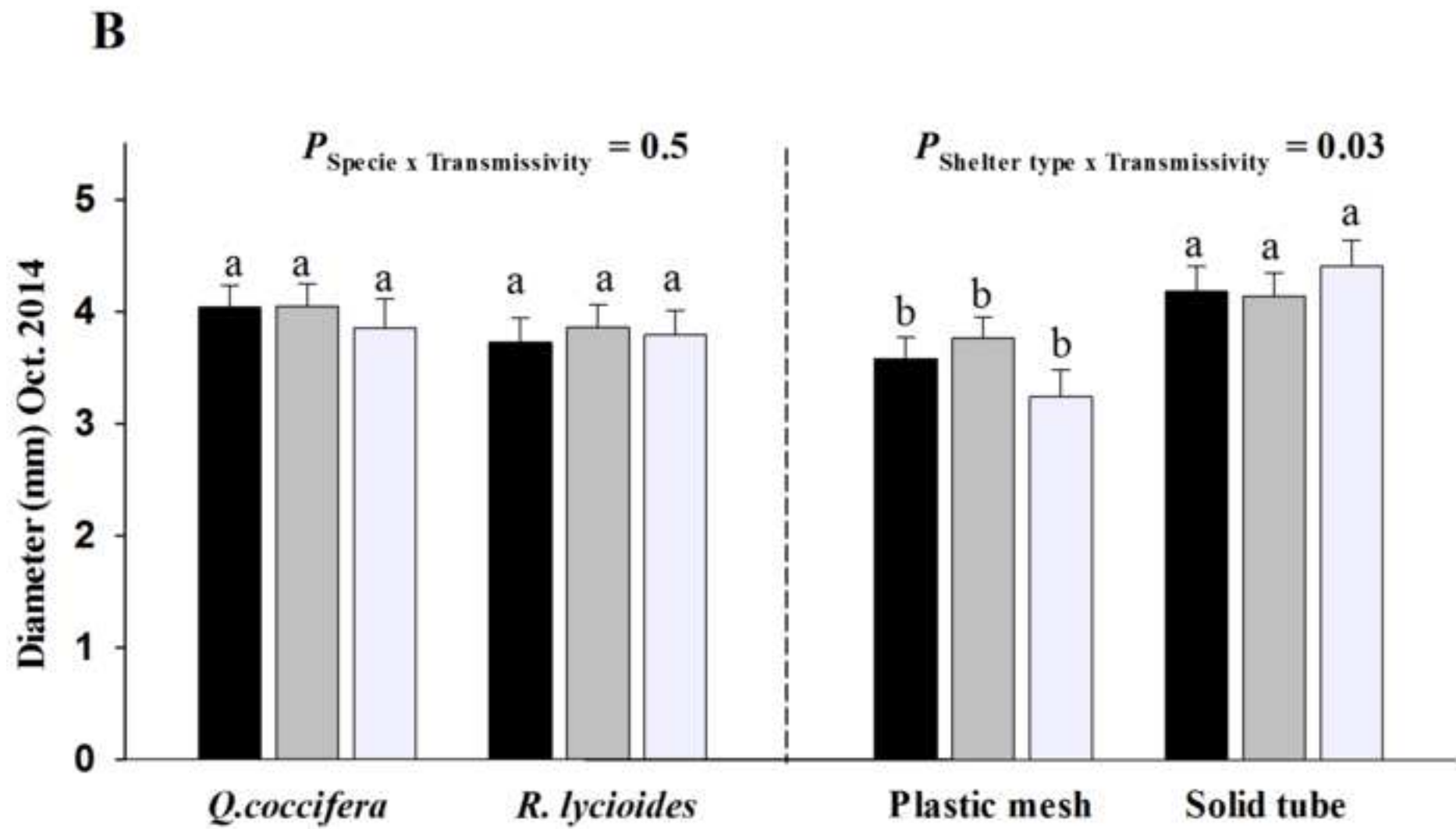












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Title: Should we use meshes or solid tube shelters when planting in Mediterranean semiarid environments?

Juan A. Oliet¹, Raul Blasco¹, PatricioValenzuela², María Melero de Blas³, Jaime Puértolas⁴

¹ Departamento de Sistemas y Recursos Naturales, Universidad Politécnica de Madrid, 28040 Madrid, Spain. E-mail address: juan.oliet@upm.es. Phone: +34 913366412. ORCID 0000-0001-7719-9327. Corresponding author

² Center of Applied Ecology & Sustainability (CAPES), Pontificia Universidad Católica de Chile, 4860 Macul, Santiago, Chile.

³ World Wildlife Foundation-España. 28005 Madrid, Spain

⁴ Lancaster Environment Centre, Lancaster University, Lancaster LA1 4YQ, UK. ORCID 0000-0002-6132-0679

Supplementary material.. Post summer height and basal diameter of tested species (*Rh. lycioides* and *Q. coccifera*) in October 2014 as affected by shelter type (plastic mesh or solid tube) and light transmissivity (40-60-80 %).

	<i>Q.coccifera</i>		<i>Rh. lycioides</i>	
	Plastic Mesh	Solid Tube	Plastic Mesh	Solid Tube
	Height (cm)			
Transmissivity 40 %	17.60 ± 0.72	18.58 ± 0.73	24.91 ± 2.03	26.95 ± 2.03
Transmissivity 60 %	17.52 ± 0.82	18.40 ± 0.70	26.74 ± 1.98	29.81 ± 1.86
Transmissivity 80 %	12.57 ± 1.62	18.17 ± 0.87	10.08 ± 1.39	23.58 ± 1.75
	Diameter (mm)			
Transmissivity 40 %	3.60 ± 0.2	4.47 ± 0.1	3.56 ± 0.1	3.9 ± 0.3
Transmissivity 60 %	3.83 ± 0.1	4.26 ± 0.2	3.70 ± 0.2	4.02 ± 0.2
Transmissivity 80 %	3.31 ± 0.3	4.40 ± 0.2	3.17 ± 0.2	4.41 ± 0.2