Classification models for automatic identification of daily states from leaf turgor related
measurements in olive

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Abstract: The leaf patch clamp pressure (LPCP) probe is being used to remotely assess leaf
turgor pressure. Recently, different shapes of the LPCP daily curves have been suggested as
potential water stress indicators for irrigation scheduling. These curves shapes, called states,
have been studied and related to different water stress levels for olives. To our knowledge, the
only way to differentiate these curves shapes or states is through the visual observation of the
dynamics of the LPCP records during the day, which is highly time-consuming and reduces its
potential to automatically schedule irrigation. The aims of this study were: (i) to obtain a random
forest model to automatically identify the states from daily LPCP curves recorded in olive trees,
by using visually identified states to train the model; (ii) to improve the identification of state II
through a second random forest model, relating this state to the midday stem water potential,
and; (iii) to obtain a random forest model to identify the states based on ranges of stem water
potential. We used LPCP daily curves collected in a commercial olive orchard from 2011 to
2015. The states were visually identified for the days on which concomitant measurements of
stem water potential and leaf stomatal conductance were made. We had a data set of 307 LPCP
daily curves, being 157 curves in state I, 78 in state II and 71 in state III. The two biggest

inflection points of the LPCP curves were used to adjust the models through the use of the R package “randomForest”, using the Leave-p-Out Cross-Validation method. With the first model, which was obtained from the whole dataset, its data regarding the inflection points and the visually identified states, we obtained an overall accuracy of 94.37%. With the second model, obtained with the use of the data regarding curves visually identified as state II only, the overall accuracy was of 88.64%. This model was adjusted to be used after the first model, to narrow the stem water potential range of state II curves. Finally, the third model was obtained using the whole dataset and the states established from ranges of stem water potential. This last model did not consider the visual identification, and yielded an overall accuracy of 88.08%. Our results facilitate the use of LPCP probes, since it allows for the automatic identification of the states related to leaf turgor pressure, a key information to schedule irrigation.

Keywords: Random forest, leaf patch clamp pressure probe, supervised classification, inflection points, Leave-p-out cross validation

1. Introduction

Deficit irrigation (DI) is applied in fruit tree orchards to ensure production in areas with low irrigation water availability. A reliable monitoring of the tree water status has been suggested as an appropriate option to sustainably manage DI strategies. This is definitely necessary in regulated deficit irrigation (RDI), one of the most effective strategies for fruit orchards, in which severe water stress is avoided with enough irrigation during phases of the growing period sensitive to water stress (Fernández, 2014a), while water supplies are reduced on periods when the crop is less sensitive to drought.

50 A variety of methods based on plant measurements have been successfully used to monitor
51 water status in olive trees (Cuevas et al., 2013; Fernández et al., 2013; Girón et al., 2015;
52 Padilla-Díaz et al., 2016), apple and pear trees (Fernández et al., 2008), and vineyards (Rüger
53 et al., 2011), among other woody crops. However, the use of these methods is still very limited
54 in commercial orchards, mainly because they are expensive and both their installation and data
55 interpretation require training (Fernández, 2014b). One of these methods relies on the use of the
56 leaf patch clamp pressure (LPCP) probe (Zimmermann et al., 2008). This is a user-friendly
57 method suitable to monitor water stress and to schedule irrigation in commercial orchards
58 (Fernández et al., 2011; Padilla-Díaz et al., 2016). The LPCP probe contains a miniaturized
59 pressure sensor integrated into a small metal piece. After being clamped in a leaf, the LPCP
60 probe measures the attenuated pressure (P_p) response of the leaf patch upon the application of
61 a constant clamped pressure (P_{clamp}). Values of P_p are inversely proportional to the leaf turgor
62 pressure, P_c (Ehrenberger et al., 2012). A commercial version of the probe, called ZIM probe
63 (YARA ZIM Plant Technology, Hennigsdorf, Germany) uses a magnet, instead of a clamp, to
64 apply P_{clamp} on the leaf.

65 From the shape of the daily P_p curves recorded with LPCP probes in olive trees under
66 different water status, Fernández et al. (2011) and Ehrenberger et al. (2012) described three
67 different states, related to the water stress level of the monitored trees: state I accounted for low
68 water stress, state II for moderate water stress, and state III for severe water stress. They both
69 reported that state I corresponded, for olive, to $\psi_{\text{stem}} > -1.2$ MPa (being ψ_{stem} the midday stem
70 water potential), state II to $-1.2 > \psi_{\text{stem}} > -1.7$ MPa and state III to $\psi_{\text{stem}} < -1.7$ MPa. These ψ_{stem}
71 ranges for States I to III may be different in orchards with different cultivars and locations
72 (Marino et al., 2016). Findings by Fernández et al. (2011) and Ehrenberger et al. (2012), and a
73 recent work by Padilla-Díaz et al. (2016), confirm that the ranges reported above are valid for
74 'Arbequina' trees and for our orchard conditions.

Both Fernández et al. (2011) and Ehrenberger et al. (2012), and also Padilla-Díaz et al. (2016), analyzed the correlation between the state determined from a visual analysis of the daily P_p curves and the stem water potential (ψ_{stem}) measured with a Scholander-type pressure chamber. Padilla-Díaz et al. (2016) used the biggest data set and found that 68.3% of the trees in state I showed $\psi_{\text{stem}} > -1.2$ MPa and that 81.8% of those days in state III presented $\psi_{\text{stem}} < -1.7$ MPa. However, only 32.8% of the trees in state II days showed values of ψ_{stem} between -1.2 and -1.7 MPa. Their results agree with findings already stated by Fernández et al. (2011), in the sense that that states I and III are easily defined from a visual analysis of the curves, and that both states are reasonably well correlated to ψ_{stem} . The correlation of state II with ψ_{stem} , however, is low, which could be partly explained by this state being more difficult to be visually identified by the user. A proper identification of state II, however, is important when using the LPCP probes for scheduling irrigation, as it represents a moderate stress level between state I and III. Thus, the appearance of state II may advice for modifying the irrigation dose or irrigation frequency (Fernández et al., 2011; Padilla-Díaz et al., 2016).

An option to improve the identification of states is to use classification methods usually applied for automatic sensed data. Among them, decision tree classification techniques have substantial advantages for automatically sensed data classification problems because of their flexibility, intuitive simplicity and computational efficiency (Fayyad and Irani, 1992; Hampson and Volper, 1986). Moreover, decision trees are strictly nonparametric and do not require assumptions regarding the distribution of the input data. In addition, decision trees handle nonlinear relations between features and classes, allow missing data and are capable of handling both numeric and categorical inputs in a natural fashion (Fayyad and Irani, 1992; Hampson and Volper, 1986). To improve the prediction accuracy, random forests have been used as a classification method because they are based on a specific algorithm that uses hundreds of decision trees to achieve a better prediction. Thus, we hypothesize that the

100 identification of states from daily P_p curves can be substantially improved using the random
101 forest classification method. Other classification methods imply some assumptions, such as data
102 normality, and some even require that independent variables are identically distributed. The
103 linear discriminant analysis (LDA), support vector machine (SVM) and artificial neural networks
104 (ANNs) could also be used but they imply relevant assumptions and this curtails their
105 applicability.

106 This work had three main objectives: (i) to adjust a random forest model to automatically
107 identify the daily states from P_p daily curves recorded in a commercial olive orchard; (ii) to
108 improve the identification of state II curves through a second random forest model; and (iii) to
109 adjust a random forest model to identify the states based on Ψ_{stem} ranges only, without any
110 visual identification. We used a large data set of P_p daily curves collected along five years from
111 the olive orchard in which Fernández et al. (2011) and Padilla-Díaz et al. (2016) worked, and
112 identified characteristic points and tendencies of the P_p variations during the day as predictors to
113 train the model, together with the states visually identified.

114

115 **2. Material and Methods**

116

117 **2.1. Experimental orchard and irrigation treatments**

118

119 Measurements were made by Fernández et al. (2011) and Padilla-Díaz et al. (2016) in a
120 super-high-density olive orchard (*Olea europaea* L., cv. Arbequina), located at 25 km to the
121 south-east of Seville (37°15'N, -5°48'W). It had trees planted at 4 m x 1.5 m (1667 trees ha⁻¹), at
122 the top of 0.4 m high ridges oriented N-NE to S-SW. The annual average precipitation (P) and
123 potential evapotranspiration (ET_o) in the area are 501.2 mm and 1498.1 mm, respectively
124 (period 2002-2016). The olive trees were five years old in 2011, when the experiments started.

125 In 2011 and 2012 the olive trees were submitted to three irrigation treatments: (i) full irrigation
126 (FI), in which trees were irrigated for the whole irrigation season to replace 100% of the irrigation
127 needs (IN); (ii) 60% regulated deficit irrigation (60RDI), in which the trees were irrigated with
128 60% of the total IN, varying depending on the phenological phase water requirements, and; (iii)
129 30% regulated deficit irrigation (30RDI), in which the trees were irrigated with 30% of the total
130 IN, varying depending on the phenological phase water requirements.

131 From the results of 2011 and 2012, Fernández (2014b) concluded that the best irrigation
132 strategy should be between 30 and 60% of the IN. Therefore, from 2013 there were two
133 treatments: (i) full irrigation (FI), in which trees were daily irrigated for the whole irrigation season
134 to replace 100% of the irrigation needs (IN), and; (ii) a regulated deficit irrigation treatment
135 (45RDI_{CC}), in which the total water supplied along the season was aimed to replace 45% of IN.
136 Both treatments were scheduled with the crop coefficient approach (Allen et al., 1998). See
137 Fernández (2014b) for details. In 2014 and 2015, the olives were also submitted to FI and to
138 45RDI_{CC}, but in addition we had a 45RDI_{TP} treatment, for which we also used the 45RDI strategy
139 but scheduled from leaf turgor pressure related measurements, i.e. from the outputs of LPCP
140 probes. See Padilla-Díaz et al. (2016) for details.

141 The treatments were distributed in a randomized block design with four 12 m x 16 m plots per
142 treatment. Each plot contained eight central trees surrounded by 24 border trees. All
143 measurements were taken in the central trees of each plot. The irrigation seasons lasted from
144 May until the end of October or mid-November (see next section for details).

145

146 **2.2. Plant water status assessment**

147

148 During the five experimental irrigation seasons, one leaf per tree from two representative
149 trees in three plots per irrigation treatment ($n = 8$) were sampled for measurements of midday

150 stem water potential (Ψ_{stem}). The leaves, close to a main branch of tree, were covered with
151 aluminum foil ca. 2h before measurement with the Scholander-type pressure chamber (PMS
152 Instrument Company, Albany, Oregon, USA) at 12.00-13.00 hours (GMT). These measurements
153 were taken all along the irrigation seasons (May to October): from DOY 165 to 293 in 2011; from
154 DOY 130 to 292 in 2012; from DOY 133 to 301 in 2013; from DOY 116 to 324 in 2014, and; from
155 DOY 98 to 298 in 2015.

156 Maximum leaf stomatal conductance ($g_{s,\text{max}}$) was measured in one leaf per tree from two
157 representative trees in four plots per irrigation treatment ($n = 8$). The leaves sampled were the
158 4th or 5th leaf from the apex of current-year shoots, from the outer part of the canopy, facing S-E
159 at ca. 1.5 m above ground. We used an open flow single pass gas exchange system with a
160 standard 2 cm x 3 cm chamber (Li-6400, Li-Cor Inc., Lincoln, NE, USA), at 8.00 - 9.00 hours
161 (GMT) which, according to Fernández et al. (1997) is the hour of $g_{s,\text{max}}$. The measurements were
162 made in the same days than those of Ψ_{stem} .

163 The measurements of both Ψ_{stem} and $g_{s,\text{max}}$ were performed in trees considered
164 representative of those in each plot in terms of canopy size, leaf area, water status and gas
165 exchange. For both variables (Ψ_{stem} and $g_{s,\text{max}}$) the average value of the two leaves was
166 considered.

167 One tree in three plots per irrigation treatment was monitored with LPCP probes to monitor
168 the state. As mentioned before, the output of these probes was used to schedule irrigation
169 during the 2014 and 2015 irrigation periods. To monitor water status with the LPCP probes, one
170 central tree was chosen, being representative to the plot, regarding size, leaf area, water status
171 and gas exchange. In the chosen trees, a fully expanded leaf was chosen from a current-year
172 branch at an approximate height of 1.5m above ground at the east side of the canopy. The
173 LPCP probes were installed at DOY 153, 103, 115, 97 and 126 for the years of 2011, 2012,

174 2013, 2014 and 2015, respectively. In total, there were nine LPCP probes in 2011, 2014 and
175 2015; eight probes in 2012 and six probes in 2013.

176 From the irrigation periods of the five experimental years, we obtained 307 P_p daily curves
177 corresponding to the days of concomitant measurements of LPCP probes (P_p), ψ_{stem} and $g_{s,\text{max}}$.
178 The 307 P_p daily curves collected along the five irrigation seasons were observed to identify the
179 state, according to Fernández et al. (2011) and Ehrenberger et al. (2012). Typical P_p curves for
180 each state are shown in Fig. 1. As stated by Fernández et al. (2011) and Ehrenberger et al.
181 (2012), state I corresponds to a P_p curve with maximum values at then central hours of the day
182 and minimum values during the night (Fig. 1a). State II is characterized by a P_p curve (Fig. 1b)
183 with a decrease in P_p values by early afternoon and an increase in late afternoon, i.e. with two
184 peaks during the day. This curve is also known as half-inverted curve. And state III is
185 characterized by a fully inverted curve, with higher values during the night and minimum values
186 during the day (Fig. 1c). To obtain the random forest models we have provided the known state
187 for each day, i.e. the state identified by visual observation of the P_p daily curves. This was
188 necessary because random forest models are obtained through a type of analysis known as
189 supervised classification or supervised machine learning, which requires teaching the model the
190 pattern to be predicted or imitated.

191

Fig. 1: Typical daily P_p curves for each state: (a) state I (low water stress); (b) state II (moderate water stress) and; (c) state III (severe water stress). The grey areas represent the night. P_p – the output pressure sensed by the LPCP probe, which is inversely proportional to the leaf turgor pressure.

2.3 P_p daily curves analysis

Through the observance of daily P_p curves for each state, we identified different indicators that could be used to characterize and differentiate the state. These indicators allowed us to derive numbers from the P_p curves, which could be used as predictors of the random forest models. The indicators, represented in Fig. 2, were: (i) the slope of the linear regression of the values between the maximum and minimum P_p (slope1); (ii) the slope of the linear regression of the P_p values between 16.15 GMT and the end of the day (slope2) which comprehends the afternoon hours when vapor pressure deficit (D_a) decreases and the leaves recover its turgor pressure. This time was established by observing the overall behavior of the curves, in most cases, the change in the slope of the curve corresponding to the last part of the day occurred around that time, and 16.15 GMT was the closest to the actual average value; (iii) the moment of the day when started the biggest fall of P_p value (max1); (iv) the duration of the fall starting at

210 max1 (pmax1); (v) the ratio between the beginning and the end of the fall of P_p values starting at
 211 max1 and lasting pmax1 (rat1); (vi) the moment of the day when started the second biggest fall
 212 of P_p value (max2); (vii) the duration of the fall starting at max2 (pmax2), and; (viii) ratio between
 213 the beginning and the end of the fall on P_p values starting at max2 and lasting pmax2 (rat2).
 214

215
 216 Fig. 2: Graphical representation of predictors for the use in random forest models, as usually seen in state I (a),
 217 state II (b) and state III (c) P_p daily curves. The tangents of the dashed and dotted lines represent slope1 and
 218 slope2, respectively; points A and C represent the beginning of the inflection ('max'); pmax is the horizontal distance
 219 between points A and B and between C and D, and; the ratio between P_p values in A and B and between C and D
 220 is 'rat', i.e. the ratio between the P_p value at the beginning and at the end of the inflection. P_p – the output pressure
 221 sensed by the LPCP probe, which is inversely proportional to the leaf turgor pressure.
 222

223 2.4. Statistical analysis

224
 225 For each day, the data from the LPCP probes were filtered using the moving average method
 226 (Wei, 2006), with the use of 20 data points, ten before and ten after, for the calculation of each
 227 moving average. For all data mining and statistical procedures the R 3.2.2[®] (R Core Team,

2015) was used. The R package randomForest (Liaw and Wiener, 2002) was used to adjust the random forest models, using 1000 trees for each Random Forest model obtained.

To fulfill the three objectives of this study and obtain the best model, the Leave-p-Out Cross Validation method (LpO CV, Arlot and Celisse, 2010; Shao, 1993) was used. This method consists on randomly dividing the data set into three sets (1, 2 and 3), using two of them to obtain a model and the third one to validate it. This procedure was repeated for three times, each with a different pair of sets of data (1 and 2; 2 and 3, and 1 and 3). From the resulting three models we chose the one with the best overall accuracy and tested it against the whole dataset.

The LpO CV was used with all the 307 P_p daily curves, its predictors (Fig. 2) and the state visually identified, in order to obtain a random forest model capable of identifying the states in an automatic way, as similar to the visual identification as possible. The chosen model in this part will be named as "rf1", to facilitate future explanation of results.

Secondly, the LpO CV was used to adjust a random forest model specifically for the P_p daily curves that were visually identified as state II. Some of these curves presented physiological characteristics (ψ_{stem} and $g_{s,\text{max}}$) typical of state I or III. We wanted to obtain a random forest model able to clearly differentiate state II curves with ψ_{stem} and $g_{s,\text{max}}$ values typical of that state, from the rest of state II curves with ψ_{stem} and $g_{s,\text{max}}$ values normally found in curves of states I or III. For this, the data of state II days was separated into three groups: (i) group II.1 with $\psi_{\text{stem}} > -1.5$ MPa and $g_{s,\text{max}} > 0.2$ mol m⁻² s⁻¹, which physiologically should correspond to state I but were visually identified as state II; (ii) group II.2 with ψ_{stem} between -1 and -2 MPa and $g_{s,\text{max}}$ between 0.1 and 0.2 mol m⁻² s⁻¹, which physiologically should correspond to state II and were indeed visually identified as state II, and; (iii) group II.3 with $\psi_{\text{stem}} < -3$ MPa and $g_{s,\text{max}} < 0.1$ mol m⁻² s⁻¹, which physiologically should correspond to state III but were visually identified as state II. The total data obtained in these three groups were 44 curves, 17 from group II.1, 18 from group II.2 and nine from group II.3. The ψ_{stem} and g_s ranges used to split state II were used as a trial to

253 narrow the variety of curves in each group, this is why they are different from the ranges
254 mentioned by Ehrenberger et al. (2012) and Fernández et al. (2011). The same predictors that
255 were used for rf1 (Fig. 2) were also used to obtain this random forest model. The model with
256 higher overall accuracy was chosen, being named "rf2". Both rf1 and rf2 were then used
257 together, first rf1 was applied to all the data and following rf2 was applied for the curves
258 predicted as state II by rf1.

259 Finally, we applied the LpO CV method to the whole dataset, not using the states visually
260 identified but with the states being established as ranges of ψ_{stem} . The ψ_{stem} ranges to establish
261 the states were: (i) State I $\psi_{\text{stem}} > -1.2$; (ii) State II ψ_{stem} between -1.2 and -1.7 MPa, and; (iii)
262 State III $\psi_{\text{stem}} < -1.7$ MPa. For this we used the whole data set of 307 curves, the same used for
263 rf1, using the same predictors as for rf1 and rf2 (Fig. 2). We obtained 121 curves with ψ_{stem} in
264 the range characteristic of state I, 67 in the range of state II and 118 in the range of state III.
265 These ψ_{stem} ranges were used to follow the results and conclusions obtained by Ehrenberger et
266 al. (2012) and Fernández et al. (2011) as mentioned before. The obtained model was used to
267 predict states for the same data set, and then plotted its ψ_{stem} and $g_{s,\text{max}}$ with different colors for
268 each state. The chosen model will be named "rfp". In Fig. 3 there is a graphical representation of
269 the rf1, rf2 and rfp models as a workflow of these three random forest models.

270 From the dataset of physiological variables we have plotted ψ_{stem} versus $g_{s,\text{max}}$ in different
271 symbols according to the state visually identified and predicted both by rf1 and rf2 used together
272 and by rfp. These plots helped us to calculate what we called as percentage of agreement for
273 each model, i.e. how each random forest model was able to match the states and its
274 characteristic ψ_{stem} ranges (Ehrenberger et al., 2012; Fernández et al., 2011).

275

276

277 Fig. 3: Representation of the rf1, rf2 and rfp models as workflows. The input data are the data supplied to obtain the
 278 model, the output data is the data (states or groups) predicted by each model. ψ_{stem} – stem water potential; $g_{s,\text{max}}$ –
 279 maximum leaf stomatal conductance ($\text{mol m}^{-2} \text{ s}^{-1}$); slope1 – the slope of a linear regression line between the

280 maximum and minimum P_p value of each day; slope2 – the slope of a linear regression line between the P_p values
281 from 16.15 GMT and the end of the day; max1 and max2 – the points where the two biggest inflection points in
282 each day begin; pmax1 and pmax2 – the duration of the inflections which started at max1 and max2 (respectively);
283 rat1 and rat2 – the ratio between the P_p values between the beginning and the end of the inflections starting at
284 max1 and max2 (respectively). Group II.1 constituted by P_p daily curves identified as state II but with physiological
285 values characteristic of state I. Group II.2 is constituted by P_p daily curves identified as state II and with
286 physiological values characteristic of state II curves. And group II.3 is constituted by P_p daily curves identified as
287 state II but with physiological values characteristic of state III curves. P_p – the output pressure sensed by the LPCP
288 probe, which is inversely proportional to the leaf turgor pressure.

289

290 **3. Results**

291

292 **3.1. States and physiological variables**

293

294 The ψ_{stem} vs. $g_{s,\text{max}}$ values, measured on days with different states visually identified, are
295 shown in Fig. 4.

296

297

298 Fig. 4: Scatter plot between stem water potential and maximum leaf stomatal conductance according to the states
 299 visually identified through the P_p daily curves. State I curves as black dots, state II as gray squares and state III as
 300 white triangles. P_p – the output pressure sensed by the LPCP probe, which is inversely proportional to the leaf
 301 turgor pressure.

302

303 From the analysis of the ψ_{stem} ranges for each state (Figure 4) we obtained that 57.3% of the
 304 state I curves correspond to $\psi_{\text{stem}} > -1.2$ MPa, 24.4% of the state II curves correspond to ψ_{stem}
 305 in the range from -1.2 to -1.7 MPa, and 87.3% of the state III curves correspond to $\psi_{\text{stem}} < -1.7$
 306 MPa. The average of these percentages weighted by the number of curves of each state was
 307 55.9%. This number could be considered as an indicator of the accuracy of the states' visual
 308 identification.

309

310 3.2. Random forest model based on visual identification of the states

311

312 We used the predictors of the 307 curves and the visual identification of the states to obtain a
 313 random forest model (rf1 model) through the LpO CV method. The rf1 model, when used with
 314 the whole dataset resulted in the confusion matrix shown in Table 1.

315

316 Table 1: Confusion matrix and accuracies, for each state, for the rf1 model when tested against the whole data set
 317 of the 307 P_p daily curves from the experimental trees. Data in the table show the number of curves in which the
 318 state determined through visual analysis (Reference) agreed with the state predicted by the model (Prediction) as
 319 well as the number of curves in disagreement between Reference and Prediction.

Prediction	Reference		
	I	II	III
I	152	3	0
II	3	71	7
III	1	3	62
Accuracies	97.4%	92.2%	89.9%

320

321 The rf1 model presented an overall accuracy of 94.4% (Table 2), with accuracies of 97.4,
 322 92.2, and 89.9% for states I, II and III, respectively (Table 1). For the three states the rf1 model
 323 presented a high accuracy level, approximately 90%.

324

325 Table 2: Random forest models' datasets, overall accuracies, error rates and percentages of agreement. In the case
 326 of the percentage of agreement shown for model rf2, it was obtained with the joint use of models rf1 and rf2
 327 together.

Model	Dataset	Overall accuracy	Error rate	% of agreement
rf1	307 P_p daily curves	94.4%	5.6%	55.9%
rf2	44 P_p daily curves	88.6%	11.4%	61.9%
rfp	307 P_p daily curves	88.1%	11.9%	84.4%

328

339 3.3. Random forest model for state II curves

330

331 The confusion matrix of the rf2 model obtained with the data from state II splitted into II.1, II.2
332 and II.3 groups when tested with the data of the three groups is shown in Table 3.

333

334 Table 3: Confusion matrix and overall accuracy for the rf2 model. The II.1 to II.3 groups are the groups in which the
335 state II curves were split to obtain the model. A total of 44 curves were considered in the analysis. Data in the table
336 show the number of curves in which the group determined according to the Ψ_{stem} and g_s values (Reference) agreed
337 with the group predicted by the model (Prediction), as well as the number of curves in disagreement between
338 Reference and Prediction.

	Reference		
Prediction	II.1	II.2	II.3
II.1	17	2	0
II.2	0	14	1
II.3	0	2	8
Accuracy	100%	77.8%	88.9%

339

340 The rf2 model presents an overall accuracy of 88.6% (Table 2), and accuracies of 100, 77.8
341 and 88.9% for the groups II.1, II.2 and II.3, respectively (Table 3). It is evident that the model
342 presented better accuracy for the groups II.1 and II.3 than for group II.2. Actually it showed no
343 error for the group II.1 (Table 3).

344 The states predicted by rf2 and its corresponding Ψ_{stem} and $g_{s,\text{max}}$ values were plotted in
345 Figure 5a. We have also applied the rf2 model to all the state II data, regardless of the range of
346 Ψ_{stem} and $g_{s,\text{max}}$ (Fig. 5b).

347

348

349 Fig. 5: Scatter plot between stem water potential and maximum leaf stomatal conductance obtained with data
 350 predicted by the random forest model trained with the state II data. (a) regarding the data about the groups II.1, II.2
 351 and II.3; (b) regarding all state II data. Being group II.1 constituted by P_p daily curves identified as state II but with
 352 physiological values characteristic of state I (black dots). Group II.2 is constituted by P_p daily curves identified as
 353 state II and with physiological values characteristic of state II curves (gray squares). And group II.3 is constituted by
 354 P_p daily curves identified as state II but with physiological values characteristic of state III curves (white triangles).
 355 P_p – the output pressure sensed by the LPCP probe, which is inversely proportional to the leaf turgor pressure.

356

357 The data predicted by the rf2 model agreed with the expected results: points from group II.1
 358 corresponds the highest ψ_{stem} and $g_{s,\text{max}}$ values; points from group II.2 were in the central area
 359 of the plot and points from group II.3 corresponded to the lowest ψ_{stem} and $g_{s,\text{max}}$ values.

360 The rf2 model was then applied for the curves identified as state II by the rf1 model (Fig. 6a).
 361 Figure 6b shows the same data than Fig. 6a, but after grouping the results from the rf1 and rf2

models, i.e. after considering the points from group II.1 as state I and those from group II.3 as state III. The points from group II.2 are represented as state II.

Fig. 6: Stem water potential and maximum leaf stomatal conductance according to the states predicted by the two random forest models: (a) splitting state II into three groups, state I as black dots, group II.1 represented as diamonds, group II.2 as gray squares, group II.3 as gray inverted triangles and state III represented as white triangles; (b) representing group II.1 as state I (black dots), group II.3 as state III (white triangles), and group II.2 as state II (gray squares). Being group II.1 constituted by P_p daily curves identified as state II but with physiological values characteristic of state I curves. Group II.2 is constituted by P_p daily curves identified as state II and with physiological values characteristic of state II curves. And group II.3 is constituted by P_p daily curves identified as state II but with physiological values characteristic of state III curves. P_p – the output pressure sensed by the LPCP probe, which is inversely proportional to the leaf turgor pressure.

The use of both rf1 and rf2 (Fig. 5) enabled a better ψ_{stem} and $g_{s,\text{max}}$ range definition for the three turgor states than the visual identification (Fig. 3) or the use of rf1 alone. Data from group

378 II.1 and II.3 (predicted by the rf2 model) were close to the ranges of state I and state III,
379 respectively (Fig. 5a). Most of the data identified as state II by the use of both rf1 and rf2 are in a
380 range of Ψ_{stem} from -1 to -3 MPa, and correspond to $g_{s,\text{max}}$ values below $0.2 \text{ mol m}^{-2} \text{ s}^{-1}$ (Figure
381 5b). It is a considerable improvement of the range of the physiological variables of state II when
382 compared to the visual identification (Figure 4).

383 Through comparison between results from Fig. 6 (predicted by the rf2 model) and the Ψ_{stem}
384 measurements, we obtained that 56.0% of the state I curves corresponded to $\Psi_{\text{stem}} > -1.2 \text{ MPa}$,
385 31.4% of the state II curves corresponded to Ψ_{stem} in the range from -1.2 to -1.7 MPa, and
386 80.9% of the state III curves had $\Psi_{\text{stem}} < -1.7 \text{ MPa}$. Calculating the average of these
387 percentages weighted by the number of curves of each state we obtained an overall percentage
388 of agreement of 61.9%, from the joint use of rf1 and rf2.

389 It is important to remember that the rf1 model was trained based on the visual identification of
390 the states. The groups used to train rf2 were split according to the Ψ_{stem} of the curves that were
391 visually identified as state II.

392 393 **3.4. Random forest model based on states as Ψ_{stem} ranges**

394
395 The model obtained by the use the states defined by ranges of Ψ_{stem} (rfp) resulted in the
396 confusion matrix shown in Table 4. The rfp model presented a better performance for states I
397 and III (90.8 and 95.7%, respectively), than for state II (69.2%).

398
399 Table 4: Confusion matrix and accuracies for the rfp model. Data in the table show the number of curves in which
400 the state determined according to the Ψ_{stem} values (Reference) agreed with the group predicted by the model
401 (Prediction), as well as the number of curves in disagreement between Reference and Prediction. A total of 307
402 curves were considered in the analysis.

	Reference		
Prediction	I	II	III
I	109	15	4
II	5	45	1
III	6	5	112
Accuracy	90.8%	69.2%	95.7%

403

404 Fig. 7 shows the scatter plot between ψ_{stem} and $g_{\text{s,max}}$ with different icons to the states as
405 predicted by the rfp model. As shown in Tables 2 and 4, the overall accuracy was 88.1%, with
406 accuracies of 90.8, 69.2 and 95.7% for the states I, II and III, respectively.

407

408

409 Fig. 7: Stem water potential and maximum leaf stomatal conductance of the states as predicted by rfp model. State
410 I is represented as black dots, state II as gray squares and state III as white triangles.

411

412 The analysis of Fig. 6 showed that 81.1% of the state I curves correspond to $\Psi_{\text{stem}} > -1.2$
413 MPa, 85.5% of the state II curves correspond to Ψ_{stem} in the range from -1.2 to -1.7 MPa, and
414 87.4% of the state III curves have $\Psi_{\text{stem}} < -1.7$ MPa. Calculating the average of these
415 percentages weighted by the number of curves of each state we obtained 84.4% as an overall
416 percentage of agreement of the rfp model. These percentages of agreement were greater than
417 those obtained by the rf1model alone (Fig. 4, Table 2), and than those obtained with the joint
418 use of the rf1 and rf2 models (Fig. 5b, Table 2). The percentages of agreement were also better
419 than the ones from the visual analysis of the daily P_p curves (Fig. 3).

420 The results obtained by the rfp model helped us to understand how well this model is capable
421 of restricting the range of Ψ_{stem} for each state.

422

423 4. Discussion

424

425 The LPCP probes and related system may be useful for both assessing plant water status
426 and scheduling irrigation in an automatic, continuous and remote way. This is based on the
427 close relation between the states and the level of water stress suffered by the instrumented
428 plant. As already mentioned, however, identifying the state of the curve from visual analysis is
429 subjected to uncertainties. In the present study we have obtained random forest models that
430 allow the automatic identification of the state related to the leaf turgor pressure. Our approach
431 therefore, avoid the visual analysis of the daily P_p curves, which reduces time, effort and
432 uncertainties and, consequently, increases the potential for the LPCP probes and related system
433 to be used with the purposes mentioned above.

434 The results on the visual identification of the states (Fig. 3) are slightly different from those
435 reported by Padilla-Díaz et al. (2016) regarding the amount of data in the ranges of Ψ_{stem}
436 suggested by Fernández et al. (2011) and Ehrenberger et al. (2012) for each state (Fig.4 and

section 3.1). Padilla-Díaz et al. (2016) reported that 68.3, 32.8 and 81.8% of the daily P_p curves identified as states I, II and III (respectively) were in the corresponding ψ_{stem} ranges. However, we have obtained 57.3, 24.4 and 87.3% for the states I, II and III (respectively), i.e. lower percentages for states I and II and a higher percentage for state III. These differences found between the present study and the results reported by Padilla-Díaz et al. (2016) are likely due to the period analyzed in each study. We have analyzed data from 2011 through 2015 (including both years), while Padilla-Díaz et al. (2016) considered the 2010-2014 period. The relation found between $g_{s,\text{max}}$ and ψ_{stem} (Fig. 4) has also been reported by Erel et al. (2014) in olive trees, with a greater range of $g_{s,\text{max}}$ when ψ_{stem} is above -1.5 MPa (approximately) and with a significant decrease when ψ_{stem} is lower than -1.5 MPa.

The rf1 model, obtained based in the states visually identification, was able to imitate the data from the visual identification, being an automatic and fast way to predict the states, predicting similar results than those obtained by visual identification, with an overall accuracy of 94,4% (Table 2). Therefore, there was a high correlation between the shape of the P_p curves and the states predicted by the model since it was taught to imitate the visual patterns of the states. It also means that the percentages of data inside the respective ψ_{stem} ranges would also be predicted by the model, i.e., the low correlation between each state and its ψ_{stem} range will be reproduced by the model. However, the rf1 model is still an alternative for automatic identification of the states, being faster and more user-friendly than the visual identification.

Differently from rf1, the rf2 model was obtained with the use of only state II P_p daily curves splitted by ranges of ψ_{stem} and $g_{s,\text{max}}$ values. The joint use of rf1 and rf2 increased in 6% the data from the states in its respective ψ_{stem} ranges in comparison to the isolated use of rf1 model and also to the visual identification. Therefore, the joint use of both rf1 and rf2 is recommended as a way to obtain an identification of the states with an increased correspondence with the ranges of ψ_{stem} . It is important to consider that the states predicted by the joint use of the rf1 and

462 rf2 models may not be so correlated to the shape of curves as the use of the rf1 model, as
463 splitting the data into three groups was performed regardless of the shapes of the curves.

464 The rfp model, as mentioned, was obtained with the states defined by Ψ_{stem} ranges, as
465 suggested by Fernández et al. (2011) and Ehrenberger et al. (2012). In other words, the rfp
466 model was obtained by trying to get the best relation between the P_p daily curves' predictors and
467 Ψ_{stem} ranges. In contrast to the rf1 model, the rfp model predicts the states differently from the
468 visual identification. The percentage of agreement for the rfp model was 27.1 and 22.5% higher
469 (Table 2) than the isolated use of rf1 and the joint use of rf1 and rf2 (respectively). It was able to
470 find characteristics of the P_p daily curves' predictors that are related to the ranges of Ψ_{stem}
471 values correspondent to each state. Thereby, the data predicted by the rfp model does not
472 necessarily correspond to the visual aspects that would be considered in the visual identification;
473 however, it presented the highest percentage of agreement (84.4% – Table 2) among the states
474 identification models obtained in the present study. Caution must be taken when assessing
475 States I to III from the measured Ψ_{stem} values before using the rf2 and rfp models, since there
476 are evidence on the Ψ_{stem} ranges corresponding to States I to III since being cultivar and
477 location dependent (Fernández et al., 2011; Marino et al., 2016).

478 As mentioned, the isolated use of rf1 and rfp models and the joint use of rf1 and rf2 are
479 different approaches to the issue of identifying the states from P_p daily curves. The isolated use
480 of rf1 is recommended when aiming at predicting the states as similar to the visual identification
481 as possible, with a high correspondence between the shapes of P_p daily curves and the
482 predicted states. It is important to consider that the same mistakes or errors that a user would
483 commit will occur in the states predicted by rf1. The joint use of rf1 and rf2 models and the use
484 of the rfp model is recommended when higher importance is given to the relation between the
485 states and Ψ_{stem} ranges. The difference between the joint use of rf1 and rf2 and the use of rfp is
486 that the former identifies state I and III similarly to the visual identification, using the Ψ_{stem} ranges

487 just for the state II identification, however the rfp model uses the Ψ_{stem} ranges for all the states.
488 As a general consideration, all three models (rf1, rf1+rf2 and rfp) are unbiased mathematical
489 methods to predict the states from P_p daily curves, which are much faster than the visual
490 identification, being able to process big datasets in a matter of seconds.

491 The random forest methodology was suitable to handle both non-parametric data and data
492 from different ranges of scales. Their capacity to consider a big number of input variables, as
493 that used in the present study, and the lack of requirements for any assumption on the data
494 distribution, proved to be useful features to shorten and facilitate the data analysis.

495

496 **5. Conclusions**

497

498 The adjusted rf1 random forest model proved to be reliable for the analysis and automatic
499 identification of the state of daily P_p curves in leaves of olive trees from a super high density
500 orchard. A total of 307 curves were considered in the analysis. Moreover, the obtained rf2 model
501 was successful on relating the shape of state II P_p curves and the corresponding Ψ_{stem} and g_s
502 ranges. Finally, the rfp model trained for the Ψ_{stem} ranges was capable of a robust identification
503 of the state of daily P_p curves. The good performance of the three models was due, to a high
504 extent, to their ability to handle non-parametric and non-normalized data. Despite of being rarely
505 use in plant physiological studies, our findings suggest a great potential of the use of random
506 forest models to better assess plant water stress. The results obtained in this study are relevant
507 for the use of LPCP probes for irrigation scheduling of commercial farms and orchards, since it
508 allows the automatic identification of the state of daily P_p curves. This eliminates the uncertainty
509 derived from the visual analysis of the P_p curves by the user, with automatic data processing a
510 matter of seconds.

511

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513

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518

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