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Additional Information

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1 **Tree water status influences fruit splitting in *Citrus***

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27 **Abstract**

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29 Fruit splitting or cracking is a major physiological disorder in fruit trees markedly

30 influenced by environmental conditions, but conclusive data still are required to provide

31 a definite explanation and preventive measures. Changes in climatic conditions

32 critically influence fruit splitting incidence. We studied plant-soil-ambient water

33 relations in splitting-prone citrus grown under 4 contrasting environmental conditions

34 (climate type and soil), in Spain and Uruguay, over a six years period. Automatic trunk

35 and fruit diameter measurements (trunk and fruit growth rate and maximum daily trunk

36 shrinkage), which indicate tree water status, together with factors modifying the tree

37 and fruit water relationship (temperature, ET, rainfall, soil texture, soil moisture,

38 rootstock and xylem anatomy) were studied and correlated with splitting.

39 A close fruit splitting and soil texture relationship was found, inversely correlated with

40 clay and silt percentages, and positively with those for sand. Under 85%-sand soil

41 conditions, slight changes in soil moisture due to fluctuations in temperature, ET, or

42 rainfall changed trunk and fruit growth rate patterns during few hours and induced

43 splitting. Splitting incidence was higher in trees with larger xylem vessels in the fruit

44 peduncle due to rootstock ('Carrizo' and 'C-35' citrange being higher than 'FA-5',

45 'Cleopatra' and *P. trifoliata*). Finally, reducing the frequency of irrigation by half

46 increased midday canopy temperatures (~5ºC) and splitting (+15%). We conclude that

47 irregularities in the tree water status, due to interactions among soil moisture, rootstock

48 and climatic conditions, leads to a number of substantial changes in fruit growth rate

49 increasing the incidence of fruit splitting.

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51 **Key words**

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53 Citrus, Climate, Physiological disorder, Splitting, Trunk growth rate, Soil

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76 **1. Introduction**

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78   Physiological disorders in fruit trees are mainly caused by environmental factors such

79 as climate or soil quality. Changes in the absorption and loss of water can cause most of

80 them (Agustí et al., 2004). *Splitting* (or *cracking*) is a major pre-harvest physiological

81 disorder in fruit tree species including pome fruits (Kasai et al., 2008), stone fruits

82 (Sekse, 1995), grapes (Clarke et al., 2010), figs (Kong et al., 2013), litchi (Huang et al.,

83 2008), and citrus (Almela et al., 1994). Among the latter, 'Navel' and 'Valencia' sweet

84 oranges (*Citrus sinensis*) are prone to split (Bar-Akiva, 1975; De Cicco et al., 1988), as

85 are some Clementine mandarins (*Citrus clementina*) (Cronjé et al., 2013), and,

86 specially, mandarin hybrids 'Nova', 'Murcott' and 'Ellendale' (Almela et al., 1994;

87 Barry and Bower, 1997; García-Luis et al., 2001).

88   Citrus fruit consists of 8-16 clustered carpels that form locules in which juice sacs

89 grow developing the pulp. Ovary walls form fruit rind, which is made up of the spongy

90 internal layer, the albedo (mesocarp), and the external compact layer, the flavedo

91 (exocarp). In citrus, splitting is a consequence of disruption between pulp and rind

92 growth. During the cell enlargement stage the increase in fruit volume is mainly due to

93 pulp growth, and rind thickness progressively diminishes. Although the mesocarp may

94 temporarily alleviate pulp pressure because of its sponginess, the exocarp is more rigid

95 and will eventually crack (Kaufman, 1970). Pressure applied by the rapidly expanding

96 pulp during fruit growth leads to the formation of microcracks in the flavedo and

97 initiation of fruit split (Cronjé et al., 2013).

98   Fruit splitting has been associated with anatomical, physiological and environmental

99 factors, and their interactions. In citrus, anatomical factors increasing fruit splitting are

100 related to the presence of an open stylar end in the ovary (García-Luis et al., 2001,

101 1994) or an oblate fruit shape (García-Luis et al., 2001), whereas peel thickness is

102 negatively related to splitting (Almela et al., 1994). It was suggested that splitting

103 occurs when a sudden net influx of water and solutes into the fruit coincides with other

104 factors reducing skin elasticity and strength (Peet, 1992). In apple, the expression in the

105 pulp of expansin *MdEXPA3*, which regulates cell wall extensibility and induces cell

106 expansion, exceeds that in the peel during the cracking period (Kasai et al., 2008).

107 Additionally, low calcium concentrations (soluble, structural or oxalate) in the pericarp

108 and drought conditions, which reduce calcium uptake, also contribute to fruit cracking

109 (Huang et al., 2008).

110 Fruits on the same tree, individually considered, differ in their response to splitting,

111 indicating that endogenous factors play a crucial role in the incidence of the disorder.

112 By contrast, splitting varies considerably between years and orchards, suggesting a

113 relevant relationship with environmental factors (Almela et al., 1994). Environmental

114 factors associated with fruit splitting include soil moisture, rainfall, relative humidity,

115 temperature and exposure to sunlight (Opara et al., 1997). It is generally assumed that

116 splitting is a result of a sudden increase in the water content of the soil, atmospheric

117 humidity, or temperature (Opara et al., 1997), but conclusive data are still needed in

118 order to obtain a definite explanation. For instance, seasonal water deficit followed by

119 rain during the cell enlargement stage has been linked to splitting in 'Nova' mandarin

120 grown in dry hot summers in the Mediterranean basin (Valencia, Spain) (Almela et al.,

121 1990), but not when grown in temperate climate with humid hot summers (Uruguay)

122 (Gravina, unpublished results). Likewise, rainfall did not correlate significantly with

123 splitting in 'Ellendale' mandarin grown in hot humid areas (South Africa) (Rabe and

124 Van Rensburg, 1996).

125   These observations indicate that splitting is a highly complex disorder, which cannot

126   be attributed to one single factor. In this research we tested the hypothesis that variable

127   tree water status induce sudden stressful changes in fruit growth patterns which in turn

128   lead to splitting. We studied plant-soil-ambient water relations in splitting-prone citrus

129   grown under 4 contrasting environmental conditions in Spain and Uruguay over a six

130   years period.

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132   **2. Materials and methods**

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134   *2.1. Experimental design, plant material and orchard characteristics*

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136   Four experiments were conducted: 1) study of the relationship between environmental

137   (climate type and soil) conditions and fruit splitting; 2) study of the relationship

138   between tree water status and fruit splitting; 3) study of the relationship between xylem

139   anatomy and fruit splitting; 4) study of effect of the irrigation frequency on fruit

140   splitting.

141   The first experiment was conducted over a 6 years period (2009-2012; 2014-2015;

142   Table 1) with 10- to 14-year-old 'Nova' mandarin trees [*Citrus clementina* x tangelo

143   'Orlando' (*Citrus.reticulata* x *Citrus. paradisi*)], grown in five orchards under

144   contrasting environmental conditions. Three of the five orchards were in Spain, two in

145   Valencia (39º 35'N, 0º 44'W), in the Mediterranean coast, and one in Huelva (37º 25'N,

146   7º 3'W) in the Atlantic coast. Two more orchards were located in Uruguay, one in

147   Libertad (34º 40'S, 56º 42'W), in the Rio de la Plata coast, and the other in Salto (31º

148   24'S, 57º 50'W), a continental plot. Fruit splitting was measured in 10 trees per orchard

149   every 15 days during 4 months (end of summer to early fall) in each of the 6 years

150   studied. Different trees were selected each year according to their uniformity in size and

151   fruit yield. Split fruits were counted and removed from the tree. Dropped split fruits

152   were also counted and removed from below the tree. At harvest, the number of fruits

153   remaining on the tree was recorded. A climatic station (Verdtech Nuevo Campo S.A.,

154   Madrid, Spain) automatically recorded temperature, rainfall, evapotranspiration, and

155   soil moisture (see below).

156   Orchards were selected to obtain a range of soil and climatic conditions. Soils were

157   classified according to soil texture (USDA; www.nrcs.usda.gob) and soil characteristics

158   were determined by AGQ Labs and Technological Services S.A (Spain) (for more

159   details visit www.agq.com.es). In Spain, the Valencia orchards had loamy (48% sand,

160   16% clay, 36% silt) to sandy-loam (72%, 14%, 14%) soil texture, pH 7.2-7.5, and 2.5-

161   3.5 % organic matter. The Huelva orchards had sandy-loam (76%, 10%, 14%) to loamy-

162   sand (85%, 10%, 5%) soil texture, pH 7.3-7.6 and 0.3-0.5 % organic matter. In

163   Uruguay, the Libertad orchard had silty-clay-loam (16%, 32%, 52%) texture, pH 7.1

164   and 2.9% organic matter, whereas the Salto orchard had fine-sand (94%, 4%, 2%)

165   texture, and 0.5 % organic matter. Thus, the orchards differed mainly in terms of soil

166   texture and organic matter content. According to the Köppen-Geiger climatic

167   classification (Peel et al., 2007), Valencia and Huelva (in Spain) are Csa climate-type

168   (temperate, dry summer, hot summer), whereas Libertad and Salto (in Uruguay) are Cfa

169   climate-type (temperate, without dry season, hot summer) . Therefore, the main climatic

170   difference in the selected orchards is rainfall during summer, coinciding with the fruit

171   enlargement stage. Average rainfall is 350 mm year^{-1} in Valencia, 750 mm year^{-1} in

172   Huelva and 1200 mm year^{-1} in Libertad and Salto. In Spain, 'Nova' trees were grafted

173   onto Carrizo citrange (*Citrus sinensis* x *Poncirus trifoliata*) and Forner-Alcaide-5

174   (*Poncirus trifoliata* x *Citrus reshni*) rootstocks, and onto *Poncirus trifoliata* in Uruguay.

175 Fertilization, drip irrigation, pruning and pest management were in accordance with

176 optimum commercial practice. Irrigation was applied in order to refill the estimated

177 crop evapotranspiration during the entire season.

178   The second experiment was carried out with 12-year-old (at the onset of the

179 experiment) 'Nova' mandarin trees grafted onto Carrizo citrange rootstock planted in

180 Huelva (orchard Huelva I). The experiment was conducted during six years but only the

181 results for those with higher and lower incidence of fruit splitting (2010 and 2012; see

182 Table 1) are presented. Trunk and fruit diameter variation was automatically measured

183 in three representatives 'Nova' mandarin trees. Each tree was equipped with a radial

184 stem dendrometer (Plantsens, Verdtech Nuevo Campo SA, Madrid, Spain), placed

185 about 50 cm from the ground, and a fruit dendrometer (Plantsens, Verdtech Nuevo

186 Campo SA, Madrid, Spain) placed at an average fruit. Measurements were

187 automatically recorded (see below). Fruit splitting and climatic conditions were

188 recorded as previously explained.

189   In the third experiment, conducted during two years (2011-2012) in Valencia and

190 Huelva (Spain), three splitting-prone varieties and five rootstocks were used: 12-year-

191 old 'Nova' mandarin trees grafted onto Carrizo citrange and Forner-Alcaide-5

192 rootstocks (orchard Huelva I); 10-year-old 'Clemenrubi' clementine mandarin (*Citrus*

193 *clementina*) trees grafted onto Carrizo citrange and *Poncirus trifoliata* rootstocks

194 (orchard Huelva II); and 10-year-old 'Chislett' navel orange (*Citrus sinensis*) trees

195 grafted onto Carrizo citrange, C-35 citrange, Forner-Alcaide-5 and 'Cleopatra'

196 mandarin (*Citrus reshni*) (orchard Valencia III). Fruit splitting was measured as

197 previously explained in 10 trees per cultivar and rootstock combinations, and samples of

198 5 fruits per tree and rootstock combinations were taken to determine peduncle vascular

199 tissue characteristics (see below).

200 Finally, in the fourth experiment, the effect of irrigation frequency on fruit splitting

201 rate was studied in 12-year-old 'Nova' mandarin trees grafted onto Carrizo citrange

202 rootstock (orchard Valencia II, sandy-loam texture). Two irrigation treatments were

203 applied during September 2014: (1) to refill daily estimated crop ET (control treatment),

204 and (2) the same weekly water volume applied every other day. At the end of the

205 experiment, the control trees received 210 l tree^{-1} week, 30 l tree^{-1} d^{-1} every day,

206 whereas the treated trees received 200 l tree^{-1} week, 50 l tree^{-1} d^{-1} every other day. Tree

207 water status was indirectly measured by thermography. Canopy temperature (Tc) was

208 measured as indicated in Ballester et al., (2013) (see below).

209

210 *2.2. Environmental measurements*

211

212 Trunk diameter variations were determined by dendrometry in the experiments

213 conducted in Spain. The dendrometers were calibrated individually with a precision

214 micrometer (Verdtech Nuevo Campo SA, Madrid, Spain). The resolution of trunk and

215 fruit diameter measurements was ±5 μm. Trunk diameter variations were used to

216 calculate the Trunk Growth Rate (TGR) and Maximum Daily Shrinkage (MDS) by the

217 difference between the maximum diameter, reached early in the morning, and the

218 minimum diameter, reached during the afternoon. Trunk diameter variations are also a

219 plant water status indicator (Goldhamer and Fereres, 2001).

220 Soil moisture was determined at 0.1, 0.3 and 0.6 m depth using C-Probe sensors

221 (AquaSpy, Inc., USA); Rain-O-Matic tipping bucket gauges (Pronamic Co. Ltd.,

222 Sikeborg, Denmark) recorded precipitation; one combined sensor recorded air

223 temperature and relative humidity (Vaisalya Oyj, Helsinki, Finland). All sensor data

224 were automatically recorded every 30 s using an addWAVE A733GSM remote

225   telemetry unit (Adcon Telemetry, Austria) for data storage and transmission,

226   programmed to report mean values every 15 min.

227   In the experiments conducted in Uruguay, a digital tipping bucket rain gauge, with a

228   sensitivity of 1 tip per 0.2 mm (MD523, Pessl Instruments GES.M.B.H., Austria),

229   switched to a reed contact magnetically operated was used. In both locations, every 15

230   days, three soil samples of 0.5 kg were taken at 0-0.2 m and 0.2-0.4 m depth; they were

231   dried at 105 °C during 48 h, and weighted. Soil moisture was determined as the

232   difference between wet and dry weigh.

233   Canopy temperature (Tc) was measured by taking frontal images, from a distance of

234   1.5m, on the SW side of the tree at 10.30h, 12.30h and 14.30h on a sunny day.

235   Measurements were taken with an infrared thermal camera TH9100 WR (NEC Avio

236   Infrared Technologies Co., Ltd., Tokio, Japan). Tc is indirectly related to the tree water

237   status in citrus (Ballester et al., 2013).

238

239   *2.3. Histological study*

240

241   Ten cross-sections of average size peduncles from 10 fruits of each scion-rootstock

242   combination were taken for histological analysis. Sections were fixed in FPA (10%

243   formaldehyde, 10% propionic acid, 80% ethanol at 70%). Cross-sections of 10 μm

244   thickness were prepared 5 mm from the calyx with a microtome (*Microm HM400R*).

245   Preparations were stained in methylene blue for 5 min. The total cross-sectional area of

246   secondary xylem, secondary phloem, cortex and pith were measured together with

247   radial number and diameter of major secondary xylem vessels and secondary phloem

248   cells. Each peduncle section was examined in three replicates. The number of xylem

249   vessels was calculated by multiplying the average number of vessels from 15 xylem

250 rays by the total number of rays per cross-section. Thirty xylem vessels per cross-

251 section, randomly selected, were used to measure the average diameter. Average

252 phloem cell diameter was calculated by counting the number of cells in a given radial

253 length (75 μm). A *Nikon E600* (Japan) light microscope was used for measurements.

254

255 *2.4. Statistical analysis*

256

257 Analysis of variance and regression analysis were performed on the data, using the

258 Student-Newman-Keuls' multiple range test for means separation. Percentages were

259 analysed after arc sin transformation.

260

261 **3. Results**

262

263 *3.1. Environmental conditions and splitting incidence*

264

265 The incidence of fruit splitting depended on the orchard and the year. For a given year

266 (2012), the splitting percentage in the 'Nova' mandarin varied among orchards from

267 1.2% to 19.5% (Table 1), and for a given orchard, for instance Huelva I and II, and

268 Valencia III, the splitting percentage varied among years from 8.7%, 8% and 5% to

269 27.0%, 16% and 14%, respectively (Table 1). Nevertheless, in some cases, no significant

270 differences among years were found for a given orchard. For instance, the splitting

271 incidence in the Libertad orchard was always the same (<2%) (Table 1).

272 Hence, results suggest a close relationship between environmental factors and splitting.

273 Accordingly, the influence of soil texture, soil moisture (SM %), and climatic

274 conditions [average temperature, t_m (ºC); evapotranspiration, ET_0 (mm d^{-1}); and

275   precipitation, P (mm)] on the incidence of fruit splitting was studied. Soil texture

276   significantly varied between orchards and, thus, in 'Nova' mandarin splitting correlated

277   inversely with clay and silt percentages, and positively with sand percentages ($P<0.05$;

278   Figure 1).

279   Under 85% sand soil conditions (Huelva I orchard, Spain), the highest yearly rate of

280   incidence of split fruit coincided in dates (late September) but not in intensity. Both

281   total splitting incidence and number of fruits split per tree and day were significantly

282   higher in 2010, 27% and 4.8 split fruits tree^{-1} d^{-1} on average, respectively, than in 2012,

283   8% and 1.2 split fruits tree^{-1} d^{-1}, on average, respectively (Figure 2A and 2B). Fruit

284   splitting started at the same phenological fruit growth stage (70% fruit size) in both

285   years and in both the Huelva (Figure 2A and 2B) and Uruguay orchards (data not

286   shown).

287   Regarding climatic conditions at this critical period (September), no significant

288   differences were found in the time-course or the average t_m, ET_0 and P between

289   September 2010 and September 2012 in the Huelva I orchard (Figure 2C-2H). In both

290   years, t_m and ET_0 diminished progressively over time. Average t_m was 22.7ºC and

291   22.5ºC, and average ET_0 was 4.1mm d^{-1} and 3.9 mm d^{-1}, in September 2010 and

292   September 2012 respectively. The average rainfall was 0.32 mm d^{-1} and 0,31 mm d^{-1} in

293   September 2010 and September 2012, respectively. It rained only on two days in 2010

294   (5.6 and 3.8 mm d^{-1}) and on 3 days in 2012 (1.6, 5.2, 1.6 mm d^{-1}) (Figure 2G and 2H).

295   However, SM significantly differed between years in the Huelva I orchard. In 2010,

296   average SM (8.3%) and minimum SM (7.4%) were significantly lower than in 2012

297   (9.1% and 8.6%, respectively). But more importantly, average daily SM progressively

298   fell in 2010 but was remained almost constant in 2012. A detailed study of the average

299   daily SM at 10, 30 and 60 cm depth also revealed significant differences between years

300   (Figure 2I and 2J). In 2010, average daily SM at 10 cm depth exhibited frequent sharp

301   changes reaching up to a 20% variation, whereas in 2012 changes hardly reached 5%

302   until mid-October, afterwards with changes similar to 2010. Values for daily average

303   SM at 30 and 60 cm depth also varied significantly between years, those for 2010

304   steadily decreasing until mid-October and increasing afterwards, and those for 2012

305   varying continuously during the period of the study.

306   In order to determine the contribution of the climatic factors to the splitting incidence,

307   under 85%-sand soil conditions (Huelva I orchard), a multiple regression analysis was

308   performed including 6 quantitative variables (every 15d recording changes in t_m, ET_0, P,

309   SM_{10}, SM_{30}, and SM_{60}) and the year as a qualitative variable. Only SM_{60} and year

310   variables were found to be statistically significant [$P<0.01$; Splitting (%) = 23,0 +

311   178,1·SM_{60} - 46,9·Year]. This result is reinforced by the relationship between soil

312   moisture and fruit splitting in the two Uruguay orchards in 2015 (Figure 3). During the

313   splitting period (February-April, SH), the orchard located in Libertad (1% fruit splitting,

314   16% sand) had a significantly higher SM than the orchard located in Salto (7.5% fruit

315   splitting, 94% sand). But more importantly, in Salto, SM was irregular varying from

316   10% to 37% whereas in Libertad SM varied less (0.7-fold in average) from 53% to

317   41%. Under these conditions, rainfall was only correlated to fruit splitting in Salto (data

318   not shown). These results suggest a relationship between tree-water status and splitting

319   incidence.

320

321   *3.2. Tree water status and splitting incidence*

322

323   TGR, as a tree water status indicator, and FGR were found to be closely correlated for a

324   given tree. In general, both trunk and fruit diameter increased from 5 pm until 9 am (16

325 h d^{-1}) the fruit increasing at a rate of 45 μm h^{-1}; on the contrary, both TGR and FGR

326 decreased from 9 am to 5 pm (8 h d^{-1}) and fruit decreased at a rate of 72 μm h^{-1}.

327 Notwithstanding, this trend varied depending on climatic conditions that temporarily

328 induced notable changes in FGR, TGR and MDS (Figure 4). For instance, on September

329 16th and 17th (2010) two important events were observed (Figure 4A): 1) TGR was

330 significantly less (-7.5 %) at mid-day on September 16th; then, a sudden increase

331 (+11%) in TGR occurred at 4 pm (it rained 3.8 mm) and continued until 10 am on 17th;

332 2) on the 17th the trunk did not shrink. As a result, fruit changed its natural daily

333 growing pattern (16 h increase, 8 h decrease) and grew continuously at maximum

334 growth rate (55.9 μm h^{-1} on average) for 46 h. Under these conditions, the fruit splitting

335 rate rose from 6 to 10 split fruits a day.

336 Changes in TGR and MDS were found to be more intense and much more frequent in

337 2010 (Figure 4B and 4C) than in 2012 (Figure 4D and 4E), and splitting incidence was

338 significantly higher in the former (Table 1). More importantly, they coincided with

339 pronounced changes in the fruit splitting rate and in t_m, ET_0 and P (Figure 2).

340 In Citrus, tree water status has been related with the xylem vessels diameter which, in

341 turn, is influenced by rootstock. Thus, it was logical studying the effect of the rootstock

342 on the splitting incidence. The histological study of the peduncles showed that the

343 number of xylem vessels per peduncle did not differ significantly among rootstocks

344 (data not shown), but the average diameter of xylem vessel in peduncles was 8% to 15%

345 larger in the citrange rootstocks than in the Cleopatra mandarin, FA-5, and *P. trifoliata*

346 (Figure 5B). Indeed, the average proportion of affected fruit in trees grafted onto

347 citrange rootstocks, Carrizo and C-35, varied between 9% and 16% on average,

348 depending on the species ('Nova' mandarin > 'Clemenrubi' Clementine mandarin >

349 'Chislett' Navel orange), and was significantly higher than in trees grafted onto

350   Cleopatra mandarin, FA-5 and *P. trifoliata* rootstocks (Figure 5A), and it paralleled the

351   percentage of split fruit.

352   Accordingly, reducing the frequency of irrigation by half in September, without

353   reducing the total amount of water, significantly modified the tree water status under

354   sandy-loam soil conditions, and increased fruit splitting. Higher midday canopy

355   temperatures (~5ºC) and higher rates of fruit splitting were found for trees in these

356   conditions, with the average final splitting incidence being 19 % compared to 8 % in

357   daily irrigated trees (Figure 6). Fruit from trees irrigated every other day had larger and

358   more micro-cracks at the stylar end compared to fruit from those irrigated every day

359   (data not shown).

360

361   **4. Discussion**

362

363   Fruit splitting is defined as an extreme form of fruit cracking in which the skin cracks

364   progressively due to pulp pressure (Cronjé et al., 2013; Opara et al., 1997). In pome and

365   stone fruits cracking is easily distinguishable because of their thin skin (Kasai et al.,

366   2008). However, this is not the case in citrus. Nevertheless, the mechanism by which

367   external factors influence fruit splitting seems to be the same regardless of the species:

368   unstable tree water status induces acute changes in the fruit growth pattern which leads

369   to splitting. Our results support this hypothesis since 1) fruit splitting correlates

370   positively to the percentage of sand in the soil, and inversely to that of clay and silt; 2)

371   under sandy-soil conditions slight variations in soil moisture significantly increase

372   splitting incidence; 3) reducing irrigation frequency increases splitting incidence; 4)

373   rootstocks developing larger xylem vessels in fruit peduncle significantly increase

374   splitting incidence regardless of soil conditions or species; 5) sharp changes in TGR and

375 MDS parallel changes in fruit growth rate, and 6) the greater TGR and MDS variation,

376 the higher splitting incidence.

377   As a rule, fruit increases in weight during the night-time and early morning whereas it

378 does not increase and even decreases during daytime, when transpiration reaches its

379 maximum (Fishman and Génard, 1998). As turgor pressure drives cell expansion, its

380 diurnal variation in the fruit correlates with fruit fresh mass, showing a sharp decline in

381 the morning and a marked rise in the evening (Fishman and Génard, 1998). However,

382 this is not always observed in natural conditions. In our experiments, fruit from tree

383 growing in extreme sandy-soil conditions (Huelva, Spain) showed significant changes

384 in this pattern (see Fig. 5A), which is due to their high sensibility to tree-water-status

385 variations. Thus, at the end of summer, a single 5 mm rain-day was able to induce

386 continuous fruit growth for more than 40 h (Figs. 3 and 5), resulting in a pulp

387 hydrostatic pressure that might exceed the rind ability to sustain it, causing fruit to split.

388 The increase in splitting took place thereafter. Nevertheless, 100 mm rain in the same

389 period did not induce splitting in fruit from tree growing in silty-clay soil conditions in

390 Libertad, Uruguay (Fig. 4). In apple, splitting mainly occurs while expansins expression

391 (*MdEXPA3*) is lower in the peel than in the pulp (Kasai et al., 2008).

392   We found a relevant correlation between the degree of MDS and the percentage of

393 split fruit (y = 7.5867x - 2.8145; r = -0.9046; P<0.05) during the rapid fruit growth

394 period (until 80% fruit volume was achieved) but not later. This is because xylem flow

395 positively correlates with stem-to-fruit pressure potential gradient during the initial, but

396 not the final part of the developing period, when xylem conductivity appears to be

397 reduced (Morandi et al., 2010). Shrinkage is caused by xylem back flow (Davies et al.,

398 2000). Accordingly, we found a significant correlation due to rootstock differences

399 between xylem vessel area in the fruit peduncle and fruit splitting. Fruits from trees

400 grafted onto citranges 'Carrizo' and 'C-35' rootstocks had larger xylem vessels and a

401 higher incidence of splitting regardless of soil conditions or species. Larger xylem

402 vessels appeared to be related to higher hydraulic conductance in roots which gives rise

403 to different mass flows of water to the canopy, strongly influencing tree water

404 relationships (Rodríguez-Gamir et al., 2010). Therefore, the larger the xylem vessels the

405 greater the instability in the tree-water status, giving rise to more pronounced daily fruit

406 shrinkage – expansion. Furthermore, fewer and smaller micro-cracks were found in the

407 stylar end of non-split fruit from regularly irrigated trees.

408 In our experiments, not every significant variation in daily TGR resulted in a

409 significant variation in fruit splitting rate (measured every 15 d), and the largest TGR

410 variations (during summer) did not produce the greatest increase in fruit splitting rates.

411 The rind thickness time-course may provide a suitable explanation of this as it

412 progressively diminishes from the onset of linear fruit growth stage, early summer,

413 reaching minimum values when fruit stops growing in early autumn (Bain, 1958).

414 Accordingly, spongy mesocarp may alleviate pulp pressure better during the initial,

415 rather than the final, stage of fruit growth. The negative relationship between peel

416 thickness and splitting found in 'Nova' mandarin was previously reported (Almela et

417 al., 1994).

418 In conclusion, fluctuations in the tree water status due to the interaction between soil

419 moisture, rootstock and climatic conditions lead to sharp changes in the fruit growth

420 rate causing fruit splitting.

421

422 **5. Abbreviations**

423

424 ET$_0$: evapotranspiration; FGR: fruit growth rate; MDS: maximum daily trunk shrinkage;

425 P: precipitation; Tc: canopy temperature; TGR: trunk growth rate; tm: average

426 temperature; SM: soil moisture

427

428 **6. Acknowledgements**

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433

434 **7. References**

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517 **Tables**

518 Table 1. Fruit splitting in citrus grown in Spain (Valencia, on the Mediterranean coast, and Huelva, on the Atlantic coast) and Uruguay (Libertad,

519 on the Rio de La Plata coast, and Salto, a continental plot). Data are means ± standard error of 10 trees per orchard and year. Trees were budded

520 onto Carrizo citrange rootstock in Spain and *Poncirus trifoliata* rootstock in Uruguay.

521

Variety	Country	Orchard	Soil	2009	2010	2011	2012	2014	2015
Nova									
	Spain	Valencia I	L				5.2±2.1 b B	1.5±0.1 a A	
	Spain	Valencia II	SaL				15.0±3.0 b C	8.0±1.1 a B	
	Spain	Huelva I	LSa	19.5±3.1 b	27.0±4.0 c	11.2±2.5 a	8.7±2.0 a B	20.7±2.2 b C	17.2±2.0 b C
	Uruguay	Libertad	SiC				1.2±0.2 A		1.6±0.2 A
	Uruguay	Salto	S						7.7±0.2 B
Clemenrubí	Spain	Huelva II	SaL			16.1±3.0b	8.0±1.5a		
Chislett	Spain	Valencia III	SaL			14.0±2.0b	5.1±1.5a		

522   LSa: Loamy-sand; L: Loamy; SaL: Sandy-loam; SiC: Silty-clay-loam; S: Sand. Different minor and major case letters in the same row and

523   column, respectively, indicate significant differences ($P<0.05$).

524 **Caption to figures**

525

526 Figure 1. Relationship between soil texture and percentages of split fruits per tree in the

527 Nova mandarin.

528

529 Figure 2. Relationship between fruit splitting and climatic conditions in the Nova

530 mandarin grown under loamy-sand soil (Huelva I orchard, 85% sand), during the years

531 2010 (A, C, E, G, I) and 2012 (B, D, F, H, J). Data are means ± standard error of 10

532 trees for splitting evaluation and 20 fruits per tree for diameter evaluation.

533

534 Figure 3. Time course of fruit splitting in the 'Nova' mandarin (A), precipitation (B)

535 and soil moisture (C) in two orchards with contrasting soil types in Uruguay (Salto,

536 fine-sand soil; Libertad, Silty-clay-loam soil). Data recorded in 2015.

537

538 Figure 4. Fruit growth rate (FGR) and trunk growth (TGR) rate patterns (in the same

539 tree) during the maximum splitting period (A, September 13th to 19th 2010). Seasonal

540 pattern of fruit splitting and relative trunk growth rate (TGR) (B and D, 2010 and 2012,

541 respectively), and maximum daily shrinkage (MDS) (C and E, 2010 and 2012,

542 respectively). Data were recorded in the orchard Huelva I (Spain), which has loamy-

543 sand soil. Data are means ± standard error of 10 trees for splitting evaluation.

544

545 Figure 5. The influence of rootstock on the incidence of fruit splitting and xylem vessel

546 area in 'Chislett' Navel orange, 'Nova' mandarin and 'Clemenrubí' Clementine

547 mandarin. Results are the average ± of 10 trees per scion-rootstock combination (A) and

548 10 cross-sections of average size peduncles from 10 fruits of each scion-rootstock

549   combination (B). CL: Cleopatra mandarin; FA-5: Forner-Alcaide nº 5; C-35: C-35

550   citrange; CC: Carrizo citrange; *PT: Poncirus trifoliata*.

551

552   Figure 6. The influence of irrigation frequency on the percentage of split fruit and

553   canopy temperature in the Nova mandarin. Trees were irrigated daily (210 l tree^{-1} week^{-1}

554   ^{1}, 30 l tree^{-1} d^{-1}) or every other day (alternate irrigation, 200 l tree^{-1} week^{-1}: 50 l tree^{-1} d^{-}

555   ^{1} every other day). Data were recorded in Valencia (Spain). Data are means ± standard

556   error of 10 trees for splitting evaluation.

557