Tree water status influences fruit splitting in *Citrus*

Carlos Mesejo\textsuperscript{1}, Carmina Reig\textsuperscript{1}, Amparo Martínez-Fuentes\textsuperscript{1}, Giuliana Gambetta\textsuperscript{2}, Alfredo Gravina\textsuperscript{2}, Manuel Agustí\textsuperscript{1}\textsuperscript{*}

\textsuperscript{1}Instituto Agroforestal Mediterráneo, Universitat Politècnica de València, Camino de Vera s/n, 46022, València, Spain

\textsuperscript{2}Facultad de Agronomía, Universidad de La República, Avenida Garzón 780, 12900, Montevideo, Uruguay

e-mails: carmeco@upv.es, mareiva@prv.upv.es; demarfue@upvnet.upv.es, magusti@prv.upv.es

e-mails: gambetta@fagro.edu.uy, agravina@fagro.edu.uy

\textsuperscript{*}corresponding author: magusti@prv.upv.es
Abstract

Fruit splitting or cracking is a major physiological disorder in fruit trees markedly influenced by environmental conditions, but conclusive data still are required to provide a definite explanation and preventive measures. Changes in climatic conditions critically influence fruit splitting incidence. We studied plant-soil-ambient water relations in splitting-prone citrus grown under 4 contrasting environmental conditions (climate type and soil), in Spain and Uruguay, over a six years period. Automatic trunk and fruit diameter measurements (trunk and fruit growth rate and maximum daily trunk shrinkage), which indicate tree water status, together with factors modifying the tree and fruit water relationship (temperature, ET, rainfall, soil texture, soil moisture, rootstock and xylem anatomy) were studied and correlated with splitting.

A close fruit splitting and soil texture relationship was found, inversely correlated with clay and silt percentages, and positively with those for sand. Under 85%-sand soil conditions, slight changes in soil moisture due to fluctuations in temperature, ET, or rainfall changed trunk and fruit growth rate patterns during few hours and induced splitting. Splitting incidence was higher in trees with larger xylem vessels in the fruit peduncle due to rootstock (‘Carrizo’ and ‘C-35’ citrange being higher than ‘FA-5’, ‘Cleopatra’ and P. trifoliata). Finally, reducing the frequency of irrigation by half increased midday canopy temperatures (~5°C) and splitting (+15%). We conclude that irregularities in the tree water status, due to interactions among soil moisture, rootstock and climatic conditions, leads to a number of substantial changes in fruit growth rate increasing the incidence of fruit splitting.
Key words

Citrus, Climate, Physiological disorder, Splitting, Trunk growth rate, Soil
1. Introduction

Physiological disorders in fruit trees are mainly caused by environmental factors such as climate or soil quality. Changes in the absorption and loss of water can cause most of them (Agustí et al., 2004). **Splitting (or cracking)** is a major pre-harvest physiological disorder in fruit tree species including pome fruits (Kasai et al., 2008), stone fruits (Sekse, 1995), grapes (Clarke et al., 2010), figs (Kong et al., 2013), litchi (Huang et al., 2008), and citrus (Almela et al., 1994). Among the latter, ‘Navel’ and ‘Valencia’ sweet oranges (**Citrus sinensis**) are prone to split (Bar-Akiva, 1975; De Cicco et al., 1988), as are some Clementine mandarins (**Citrus clementina**) (Cronjé et al., 2013), and, specially, mandarin hybrids ‘Nova’, ‘Murcott’ and ‘Ellendale’ (Almela et al., 1994; Barry and Bower, 1997; García-Luis et al., 2001).

Citrus fruit consists of 8-16 clustered carpels that form locules in which juice sacs grow developing the pulp. Ovary walls form fruit rind, which is made up of the spongy internal layer, the albedo (mesocarp), and the external compact layer, the flavedo (exocarp). In citrus, splitting is a consequence of disruption between pulp and rind growth. During the cell enlargement stage the increase in fruit volume is mainly due to pulp growth, and rind thickness progressively diminishes. Although the mesocarp may temporarily alleviate pulp pressure because of its sponginess, the exocarp is more rigid and will eventually crack (Kaufman, 1970). Pressure applied by the rapidly expanding pulp during fruit growth leads to the formation of microcracks in the flavedo and initiation of fruit split (Cronjé et al., 2013).

Fruit splitting has been associated with anatomical, physiological and environmental factors, and their interactions. In citrus, anatomical factors increasing fruit splitting are related to the presence of an open stylar end in the ovary (García-Luis et al., 2001,
or an oblate fruit shape (García-Luis et al., 2001), whereas peel thickness is negatively related to splitting (Almela et al., 1994). It was suggested that splitting occurs when a sudden net influx of water and solutes into the fruit coincides with other factors reducing skin elasticity and strength (Peet, 1992). In apple, the expression in the pulp of expansin \textit{MdEXPA3}, which regulates cell wall extensibility and induces cell expansion, exceeds that in the peel during the cracking period (Kasai et al., 2008). Additionally, low calcium concentrations (soluble, structural or oxalate) in the pericarp and drought conditions, which reduce calcium uptake, also contribute to fruit cracking (Huang et al., 2008).

Fruits on the same tree, individually considered, differ in their response to splitting, indicating that endogenous factors play a crucial role in the incidence of the disorder. By contrast, splitting varies considerably between years and orchards, suggesting a relevant relationship with environmental factors (Almela et al., 1994). Environmental factors associated with fruit splitting include soil moisture, rainfall, relative humidity, temperature and exposure to sunlight (Opara et al., 1997). It is generally assumed that splitting is a result of a sudden increase in the water content of the soil, atmospheric humidity, or temperature (Opara et al., 1997), but conclusive data are still needed in order to obtain a definite explanation. For instance, seasonal water deficit followed by rain during the cell enlargement stage has been linked to splitting in ‘Nova’ mandarin grown in dry hot summers in the Mediterranean basin (Valencia, Spain) (Almela et al., 1990), but not when grown in temperate climate with humid hot summers (Uruguay) (Gravina, unpublished results). Likewise, rainfall did not correlate significantly with splitting in ‘Ellendale’ mandarin grown in hot humid areas (South Africa) (Rabe and Van Rensburg, 1996).
These observations indicate that splitting is a highly complex disorder, which cannot be attributed to one single factor. In this research we tested the hypothesis that variable tree water status induce sudden stressful changes in fruit growth patterns which in turn lead to splitting. We studied plant-soil-ambient water relations in splitting-prone citrus grown under 4 contrasting environmental conditions in Spain and Uruguay over a six years period.

2. Materials and methods

2.1. Experimental design, plant material and orchard characteristics

Four experiments were conducted: 1) study of the relationship between environmental (climate type and soil) conditions and fruit splitting; 2) study of the relationship between tree water status and fruit splitting; 3) study of the relationship between xylem anatomy and fruit splitting; 4) study of effect of the irrigation frequency on fruit splitting.

The first experiment was conducted over a 6 years period (2009-2012; 2014-2015; Table 1) with 10- to 14-year-old ‘Nova’ mandarin trees [Citrus clementina x tangelo ‘Orlando’ (Citrus. reticulata x Citrus. paradisi)], grown in five orchards under contrasting environmental conditions. Three of the five orchards were in Spain, two in Valencia (39º 35’N, 0º 44’W), in the Mediterranean coast, and one in Huelva (37º 25’N, 7º 3’W) in the Atlantic coast. Two more orchards were located in Uruguay, one in Libertad (34º 40’S, 56º 42’W), in the Río de la Plata coast, and the other in Salto (31º 24’S, 57º 50’W), a continental plot. Fruit splitting was measured in 10 trees per orchard every 15 days during 4 months (end of summer to early fall) in each of the 6 years.
studied. Different trees were selected each year according to their uniformity in size and
fruit yield. Split fruits were counted and removed from the tree. Dropped split fruits
were also counted and removed from below the tree. At harvest, the number of fruits
remaining on the tree was recorded. A climatic station (Verdtech Nuevo Campo S.A.,
Madrid, Spain) automatically recorded temperature, rainfall, evapotranspiration, and
soil moisture (see below).

Orchards were selected to obtain a range of soil and climatic conditions. Soils were
classified according to soil texture (USDA; www.nrcs.usda.gov) and soil characteristics
were determined by AGQ Labs and Technological Services S.A (Spain) (for more
details visit www.agq.com.es). In Spain, the Valencia orchards had loamy (48% sand,
16% clay, 36% silt) to sandy-loam (72%, 14%, 14%) soil texture, pH 7.2-7.5, and 2.5-
3.5 % organic matter. The Huelva orchards had sandy-loam (76%, 10%, 14%) to loamy-
sand (85%, 10%, 5%) soil texture, pH 7.3-7.6 and 0.3-0.5 % organic matter. In
Uruguay, the Libertad orchard had silty-clay-loam (16%, 32%, 52%) texture, pH 7.1
and 2.9% organic matter, whereas the Salto orchard had fine-sand (94%, 4%, 2%)
texture, and 0.5 % organic matter. Thus, the orchards differed mainly in terms of soil
texture and organic matter content. According to the Köppen-Geiger climatic
classification (Peel et al., 2007), Valencia and Huelva (in Spain) are Csa climate-type
(temperate, dry summer, hot summer), whereas Libertad and Salto (in Uruguay) are Cfa
climate-type (temperate, without dry season, hot summer). Therefore, the main climatic
difference in the selected orchards is rainfall during summer, coinciding with the fruit
enlargement stage. Average rainfall is 350 mm year\(^{-1}\) in Valencia, 750 mm year\(^{-1}\) in
Huelva and 1200 mm year\(^{-1}\) in Libertad and Salto. In Spain, ‘Nova’ trees were grafted
onto Carrizo citrange (\textit{Citrus sinensis} x \textit{Poncirus trifoliata}) and Forner-Alcaide-5
(\textit{Poncirus trifoliata} x \textit{Citrus reshni}) rootstocks, and onto \textit{Poncirus trifoliata} in Uruguay.
Fertilization, drip irrigation, pruning and pest management were in accordance with optimum commercial practice. Irrigation was applied in order to refill the estimated crop evapotranspiration during the entire season.

The second experiment was carried out with 12-year-old (at the onset of the experiment) ‘Nova’ mandarin trees grafted onto Carrizo citrange rootstock planted in Huelva (orchard Huelva I). The experiment was conducted during six years but only the results for those with higher and lower incidence of fruit splitting (2010 and 2012; see Table 1) are presented. Trunk and fruit diameter variation was automatically measured in three representatives ‘Nova’ mandarin trees. Each tree was equipped with a radial stem dendrometer (Plantsens, Verdtech Nuevo Campo SA, Madrid, Spain), placed about 50 cm from the ground, and a fruit dendrometer (Plantsens, Verdtech Nuevo Campo SA, Madrid, Spain) placed at an average fruit. Measurements were automatically recorded (see below). Fruit splitting and climatic conditions were recorded as previously explained.

In the third experiment, conducted during two years (2011-2012) in Valencia and Huelva (Spain), three splitting-prone varieties and five rootstocks were used: 12-year-old ‘Nova’ mandarin trees grafted onto Carrizo citrange and Forner-Alcaide-5 rootstocks (orchard Huelva I); 10-year-old ‘Clemenrubi’ clementine mandarin (Citrus clementina) trees grafted onto Carrizo citrange and Poncirus trifoliata rootstocks (orchard Huelva II); and 10-year-old ‘Chislett’ navel orange (Citrus sinensis) trees grafted onto Carrizo citrange, C-35 citrange, Forner-Alcaide-5 and ‘Cleopatra’ mandarin (Citrus reshni) (orchard Valencia III). Fruit splitting was measured as previously explained in 10 trees per cultivar and rootstock combinations, and samples of 5 fruits per tree and rootstock combinations were taken to determine peduncle vascular tissue characteristics (see below).
Finally, in the fourth experiment, the effect of irrigation frequency on fruit splitting rate was studied in 12-year-old ‘Nova’ mandarin trees grafted onto Carrizo citrange rootstock (orchard Valencia II, sandy-loam texture). Two irrigation treatments were applied during September 2014: (1) to refill daily estimated crop ET (control treatment), and (2) the same weekly water volume applied every other day. At the end of the experiment, the control trees received 210 l tree\(^{-1}\) week, 30 l tree\(^{-1}\) d\(^{-1}\) every day, whereas the treated trees received 200 l tree\(^{-1}\) week, 50 l tree\(^{-1}\) d\(^{-1}\) every other day. Tree water status was indirectly measured by thermography. Canopy temperature (Tc) was measured as indicated in Ballester et al., (2013) (see below).

2.2. Environmental measurements

Trunk diameter variations were determined by dendrometry in the experiments conducted in Spain. The dendrometers were calibrated individually with a precision micrometer (Verdtech Nuevo Campo SA, Madrid, Spain). The resolution of trunk and fruit diameter measurements was ±5 \(\mu\)m. Trunk diameter variations were used to calculate the Trunk Growth Rate (TGR) and Maximum Daily Shrinkage (MDS) by the difference between the maximum diameter, reached early in the morning, and the minimum diameter, reached during the afternoon. Trunk diameter variations are also a plant water status indicator (Goldhamer and Fereres, 2001).

Soil moisture was determined at 0.1, 0.3 and 0.6 m depth using C-Probe sensors (AquaSpy, Inc., USA); Rain-O-Matic tipping bucket gauges (Pronamic Co. Ltd., Sikeborg, Denmark) recorded precipitation; one combined sensor recorded air temperature and relative humidity (Vaisalya Oyj, Helsinki, Finland). All sensor data were automatically recorded every 30 s using an addWAVE A733GSM remote
telemetry unit (Adcon Telemetry, Austria) for data storage and transmission, programmed to report mean values every 15 min.

In the experiments conducted in Uruguay, a digital tipping bucket rain gauge, with a sensitivity of 1 tip per 0.2 mm (MD523, Pessl Instruments GES.M.B.H., Austria), switched to a reed contact magnetically operated was used. In both locations, every 15 days, three soil samples of 0.5 kg were taken at 0-0.2 m and 0.2-0.4 m depth; they were dried at 105 °C during 48 h, and weighted. Soil moisture was determined as the difference between wet and dry weigh.

Canopy temperature (Tc) was measured by taking frontal images, from a distance of 1.5m, on the SW side of the tree at 10.30h, 12.30h and 14.30h on a sunny day. Measurements were taken with an infrared thermal camera TH9100 WR (NEC Avio Infrared Technologies Co., Ltd., Tokio, Japan). Tc is indirectly related to the tree water status in citrus (Ballester et al., 2013).

2.3. Histological study

Ten cross-sections of average size peduncles from 10 fruits of each scion-rootstock combination were taken for histological analysis. Sections were fixed in FPA (10% formaldehyde, 10% propionic acid, 80% ethanol at 70%). Cross-sections of 10 µm thickness were prepared 5 mm from the calyx with a microtome (Microm HM400R). Preparations were stained in methylene blue for 5 min. The total cross-sectional area of secondary xylem, secondary phloem, cortex and pith were measured together with radial number and diameter of major secondary xylem vessels and secondary phloem cells. Each peduncle section was examined in three replicates. The number of xylem vessels was calculated by multiplying the average number of vessels from 15 xylem
rays by the total number of rays per cross-section. Thirty xylem vessels per cross-section, randomly selected, were used to measure the average diameter. Average phloem cell diameter was calculated by counting the number of cells in a given radial length (75 µm). A Nikon E600 (Japan) light microscope was used for measurements.

2.4. Statistical analysis

Analysis of variance and regression analysis were performed on the data, using the Student-Newman-Keuls’ multiple range test for means separation. Percentages were analysed after arc sin transformation.

3. Results

3.1. Environmental conditions and splitting incidence

The incidence of fruit splitting depended on the orchard and the year. For a given year (2012), the splitting percentage in the ‘Nova’ mandarin varied among orchards from 1.2% to 19.5% (Table 1), and for a given orchard, for instance Huelva I and II, and Valencia III, the splitting percentage varied among years from 8.7%, 8% and 5% to 27.0%, 16% and 14%, respectively (Table 1). Nevertheless, in some cases, no significant differences among years were found for a given orchard. For instance, the splitting incidence in the Libertad orchard was always the same (<2%) (Table 1).

Hence, results suggest a close relationship between environmental factors and splitting. Accordingly, the influence of soil texture, soil moisture (SM %), and climatic conditions [average temperature, \( t_m \) (°C); evapotranspiration, \( ET_0 \) (mm d\(^{-1}\)); and
precipitation, P (mm)] on the incidence of fruit splitting was studied. Soil texture significantly varied between orchards and, thus, in ‘Nova’ mandarin splitting correlated inversely with clay and silt percentages, and positively with sand percentages ($P<0.05$; Figure 1).

Under 85% sand soil conditions (Huelva I orchard, Spain), the highest yearly rate of incidence of split fruit coincided in dates (late September) but not in intensity. Both total splitting incidence and number of fruits split per tree and day were significantly higher in 2010, 27% and 4.8 split fruits tree$^{-1}$ d$^{-1}$ on average, respectively, than in 2012, 8% and 1.2 split fruits tree$^{-1}$ d$^{-1}$, on average, respectively (Figure 2A and 2B). Fruit splitting started at the same phenological fruit growth stage (70% fruit size) in both years and in both the Huelva (Figure 2A and 2B) and Uruguay orchards (data not shown).

Regarding climatic conditions at this critical period (September), no significant differences were found in the time-course or the average $t_m$, $ET_0$ and P between September 2010 and September 2012 in the Huelva I orchard (Figure 2C-2H). In both years, $t_m$ and $ET_0$ diminished progressively over time. Average $t_m$ was 22.7°C and 22.5°C, and average $ET_0$ was 4.1mm d$^{-1}$ and 3.9 mm d$^{-1}$, in September 2010 and September 2012 respectively. The average rainfall was 0.32 mm d$^{-1}$ and 0.31 mm d$^{-1}$ in September 2010 and September 2012, respectively. It rained only on two days in 2010 (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). However, SM significantly differed between years in the Huelva I orchard. In 2010, average SM (8.3%) and minimum SM (7.4%) were significantly lower than in 2012 (9.1% and 8.6%, respectively). But more importantly, average daily SM progressively fell in 2010 but was remained almost constant in 2012. A detailed study of the average daily SM at 10, 30 and 60 cm depth also revealed significant differences between years.
In 2010, average daily SM at 10 cm depth exhibited frequent sharp changes reaching up to a 20% variation, whereas in 2012 changes hardly reached 5% until mid-October, afterwards with changes similar to 2010. Values for daily average SM at 30 and 60 cm depth also varied significantly between years, those for 2010 steadily decreasing until mid-October and increasing afterwards, and those for 2012 varying continuously during the period of the study.

In order to determine the contribution of the climatic factors to the splitting incidence, under 85%-sand soil conditions (Huelva I orchard), a multiple regression analysis was performed including 6 quantitative variables (every 15d recording changes in $t_m$, $ET_0$, P, SM$_{10}$, SM$_{30}$, and SM$_{60}$) and the year as a qualitative variable. Only SM$_{60}$ and year variables were found to be statistically significant $[P<0.01; \text{Splitting} \% = 23.0 + 178.1 \cdot \text{SM}_{60} - 46.9 \cdot \text{Year}]$. This result is reinforced by the relationship between soil moisture and fruit splitting in the two Uruguay orchards in 2015 (Figure 3). During the splitting period (February-April, SH), the orchard located in Libertad (1% fruit splitting, 16% sand) had a significantly higher SM than the orchard located in Salto (7.5% fruit splitting, 94% sand). But more importantly, in Salto, SM was irregular varying from 10% to 37% whereas in Libertad SM varied less (0.7-fold in average) from 53% to 41%. Under these conditions, rainfall was only correlated to fruit splitting in Salto (data not shown). These results suggest a relationship between tree-water status and splitting incidence.

3.2. Tree water status and splitting incidence

TGR, as a tree water status indicator, and FGR were found to be closely correlated for a given tree. In general, both trunk and fruit diameter increased from 5 pm until 9 am (16
h d\(^{-1}\)) the fruit increasing at a rate of 45 \(\mu \text{m h}\(^{-1}\)); on the contrary, both TGR and FGR decreased from 9 am to 5 pm (8 h d\(^{-1}\)) and fruit decreased at a rate of 72 \(\mu \text{m h}\(^{-1}\)). Notwithstanding, this trend varied depending on climatic conditions that temporarily induced notable changes in FGR, TGR and MDS (Figure 4). For instance, on September 16\(^{th}\) and 17\(^{th}\) (2010) two important events were observed (Figure 4A): 1) TGR was significantly less (-7.5 %) at mid-day on September 16\(^{th}\); then, a sudden increase (+11%) in TGR occurred at 4 pm (it rained 3.8 mm) and continued until 10 am on 17\(^{th}\); 2) on the 17\(^{th}\) the trunk did not shrink. As a result, fruit changed its natural daily growing pattern (16 h increase, 8 h decrease) and grew continuously at maximum growth rate (55.9 \(\mu \text{m h}\(^{-1}\) on average) for 46 h. Under these conditions, the fruit splitting rate rose from 6 to 10 split fruits a day.

Changes in TGR and MDS were found to be more intense and much more frequent in 2010 (Figure 4B and 4C) than in 2012 (Figure 4D and 4E), and splitting incidence was significantly higher in the former (Table 1). More importantly, they coincided with pronounced changes in the fruit splitting rate and in \(t_m\), \(E_T\) and \(P\) (Figure 2).

In Citrus, tree water status has been related with the xylem vessels diameter which, in turn, is influenced by rootstock. Thus, it was logical studying the effect of the rootstock on the splitting incidence. The histological study of the peduncles showed that the number of xylem vessels per peduncle did not differ significantly among rootstocks (data not shown), but the average diameter of xylem vessel in peduncles was 8% to 15% larger in the citrange rootstocks than in the Cleopatra mandarin, FA-5, and \(P. \text{ trifoliata}\) (Figure 5B). Indeed, the average proportion of affected fruit in trees grafted onto citrange rootstocks, Carrizo and C-35, varied between 9% and 16% on average, depending on the species (‘Nova’ mandarin > ‘Clemenrubi’ Clementine mandarin > ‘Chislett’ Navel orange), and was significantly higher than in trees grafted onto
Cleopatra mandarin, FA-5 and *P. trifoliata* rootstocks (Figure 5A), and it paralleled the percentage of split fruit.

Accordingly, reducing the frequency of irrigation by half in September, without reducing the total amount of water, significantly modified the tree water status under sandy-loam soil conditions, and increased fruit splitting. Higher midday canopy temperatures (~5°C) and higher rates of fruit splitting were found for trees in these conditions, with the average final splitting incidence being 19% compared to 8% in daily irrigated trees (Figure 6). Fruit from trees irrigated every other day had larger and more micro-cracks at the stylar end compared to fruit from those irrigated every day (data not shown).

### 4. Discussion

Fruit splitting is defined as an extreme form of fruit cracking in which the skin cracks progressively due to pulp pressure (Cronjé et al., 2013; Opara et al., 1997). In pome and stone fruits cracking is easily distinguishable because of their thin skin (Kasai et al., 2008). However, this is not the case in citrus. Nevertheless, the mechanism by which external factors influence fruit splitting seems to be the same regardless of the species: unstable tree water status induces acute changes in the fruit growth pattern which leads to splitting. Our results support this hypothesis since 1) fruit splitting correlates positively to the percentage of sand in the soil, and inversely to that of clay and silt; 2) under sandy-soil conditions slight variations in soil moisture significantly increase splitting incidence; 3) reducing irrigation frequency increases splitting incidence; 4) rootstocks developing larger xylem vessels in fruit peduncle significantly increase splitting incidence regardless of soil conditions or species; 5) sharp changes in TGR and
MDS parallel changes in fruit growth rate, and 6) the greater TGR and MDS variation, the higher splitting incidence.

As a rule, fruit increases in weight during the night-time and early morning whereas it does not increase and even decreases during daytime, when transpiration reaches its maximum (Fishman and Génard, 1998). As turgor pressure drives cell expansion, its diurnal variation in the fruit correlates with fruit fresh mass, showing a sharp decline in the morning and a marked rise in the evening (Fishman and Génard, 1998). However, this is not always observed in natural conditions. In our experiments, fruit from tree growing in extreme sandy-soil conditions (Huelva, Spain) showed significant changes in this pattern (see Fig. 5A), which is due to their high sensibility to tree-water-status variations. Thus, at the end of summer, a single 5 mm rain-day was able to induce continuous fruit growth for more than 40 h (Figs. 3 and 5), resulting in a pulp hydrostatic pressure that might exceed the rind ability to sustain it, causing fruit to split. The increase in splitting took place thereafter. Nevertheless, 100 mm rain in the same period did not induce splitting in fruit from tree growing in silty-clay soil conditions in Libertad, Uruguay (Fig. 4). In apple, splitting mainly occurs while expansins expression (*MdEXPA3*) is lower in the peel than in the pulp (Kasai et al., 2008).

We found a relevant correlation between the degree of MDS and the percentage of split fruit (*y = 7.5867x - 2.8145; r = -0.9046; P<0.05*) during the rapid fruit growth period (until 80% fruit volume was achieved) but not later. This is because xylem flow positively correlates with stem-to-fruit pressure potential gradient during the initial, but not the final part of the developing period, when xylem conductivity appears to be reduced (Morandi et al., 2010). Shrinkage is caused by xylem back flow (Davies et al., 2000). Accordingly, we found a significant correlation due to rootstock differences between xylem vessel area in the fruit peduncle and fruit splitting. Fruits from trees
grafted onto citranges ‘Carrizo’ and ‘C-35’ rootstocks had larger xylem vessels and a higher incidence of splitting regardless of soil conditions or species. Larger xylem vessels appeared to be related to higher hydraulic conductance in roots which gives rise to different mass flows of water to the canopy, strongly influencing tree water relationships (Rodríguez-Gamir et al., 2010). Therefore, the larger the xylem vessels the greater the instability in the tree-water status, giving rise to more pronounced daily fruit shrinkage – expansion. Furthermore, fewer and smaller micro-cracks were found in the stylar end of non-split fruit from regularly irrigated trees.

In our experiments, not every significant variation in daily TGR resulted in a significant variation in fruit splitting rate (measured every 15 d), and the largest TGR variations (during summer) did not produce the greatest increase in fruit splitting rates. The rind thickness time-course may provide a suitable explanation of this as it progressively diminishes from the onset of linear fruit growth stage, early summer, reaching minimum values when fruit stops growing in early autumn (Bain, 1958). Accordingly, spongy mesocarp may alleviate pulp pressure better during the initial, rather than the final, stage of fruit growth. The negative relationship between peel thickness and splitting found in ‘Nova’ mandarin was previously reported (Almela et al., 1994).

In conclusion, fluctuations in the tree water status due to the interaction between soil moisture, rootstock and climatic conditions lead to sharp changes in the fruit growth rate causing fruit splitting.

5. Abbreviations
ET₀: evapotranspiration; FGR: fruit growth rate; MDS: maximum daily trunk shrinkage; P: precipitation; Tc: canopy temperature; TGR: trunk growth rate; tm: average temperature; SM: soil moisture

6. Acknowledgements

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Table 1. Fruit splitting in citrus grown in Spain (Valencia, on the Mediterranean coast, and Huelva, on the Atlantic coast) and Uruguay (Libertad, 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 onto Carrizo citrange rootstock in Spain and *Poncirus trifoliata* rootstock in Uruguay.

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<td>16.1±3.0b 8.0±1.5a</td>
<td></td>
<td></td>
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<tr>
<td>Chislett</td>
<td>Spain</td>
<td>Valencia III</td>
<td>SaL</td>
<td>14.0±2.0b 5.1±1.5a</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 column, respectively, indicate significant differences (P<0.05).
Caption to figures

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

Figure 2. Relationship between fruit splitting and climatic conditions in the Nova mandarin grown under loamy-sand soil (Huelva I orchard, 85% sand), during the years 2010 (A, C, E, G, I) and 2012 (B, D, F, H, J). Data are means ± standard error of 10 trees for splitting evaluation and 20 fruits per tree for diameter evaluation.

Figure 3. Time course of fruit splitting in the ‘Nova’ mandarin (A), precipitation (B) and soil moisture (C) in two orchards with contrasting soil types in Uruguay (Salto, fine-sand soil; Libertad, Silty-clay-loam soil). Data recorded in 2015.

Figure 4. Fruit growth rate (FGR) and trunk growth (TGR) rate patterns (in the same tree) during the maximum splitting period (A, September 13th to 19th 2010). Seasonal pattern of fruit splitting and relative trunk growth rate (TGR) (B and D, 2010 and 2012, respectively), and maximum daily shrinkage (MDS) (C and E, 2010 and 2012, respectively). Data were recorded in the orchard Huelva I (Spain), which has loamy-sand soil. Data are means ± standard error of 10 trees for splitting evaluation.

Figure 5. The influence of rootstock on the incidence of fruit splitting and xylem vessel area in ‘Chislett’ Navel orange, ‘Nova’ mandarin and ‘Clemenrubí’ Clementine mandarin. Results are the average ± of 10 trees per scion-rootstock combination (A) and 10 cross-sections of average size peduncles from 10 fruits of each scion-rootstock combination.
combination (B). CL: Cleopatra mandarin; FA-5: Forner-Alcaide nº 5; C-35: C-35 citrange; CC: Carrizo citrange; *PT: Poncirus trifoliata.*

Figure 6. The influence of irrigation frequency on the percentage of split fruit and canopy temperature in the Nova mandarin. Trees were irrigated daily (210 l tree⁻¹ week⁻¹, 30 l tree⁻¹ d⁻¹) or every other day (alternate irrigation, 200 l tree⁻¹ week⁻¹: 50 l tree⁻¹ d⁻¹ every other day). Data were recorded in Valencia (Spain). Data are means ± standard error of 10 trees for splitting evaluation.