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Tree water status influences fruit splitting in *Citrus*

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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 are a tree water status indicator, 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 *Poncirus 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.

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).

Abbreviations: ET_0, evapotranspiration; FGR, fruit growth rate; MDS, maximum daily trunk shrinkage; P, precipitation; Tc, canopy temperature; TGR, trunk growth rate; t_m, average temperature; SM, soil moisture.

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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.,1994, 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 *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* × tangelo 'Orlando' (*Citrus reticulata* × *Citrus paradisi*)], grown in five orchards under contrasting environmental conditions. Three of the five orchards were in Spain, two in Valencia (39° 35′N, 0° 44′W), in the Mediterranean coast, and one in Huelva (37° 25′N, 7° 3′W) in the Atlantic coast. Two more orchards were located in Uruguay, one in Libertad (34° 40′S, 56° 42′W), in the Rio de la Plata coast, and the other in Salto (31° 24′S, 57° 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.gob) 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 (*Citrus sinensis* × *Poncirus trifoliata*) and Forner-Alcaide-5 (FA–5) (*Poncirus trifoliata* × *Citrus reshni*) rootstocks, and onto *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 FA–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, FA–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