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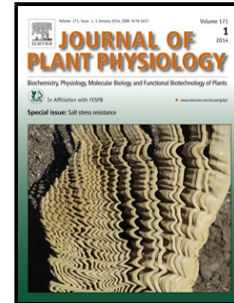Please cite this article as: Grappadelli LC, Morandi B, Manfrini L, O'Connell M, Apoplasmic and simplasmic phloem unloading mechanisms: do they co-exist in Angeleno plums under demanding environmental conditions?, *Journal of Plant Physiology* (2019), https://doi.org/10.1016/j.jplph.2019.04.005

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Apoplasmic and simplasmic phloem unloading mechanisms: do they co-exist in Angeleno plums under demanding environmental conditions?

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Abstract

Biophysical fruit growth depends on a balance among the vascular and transpiration flows entering/exiting the fruit via phloem, xylem and through the epidermis. There is no information on vascular flows of Japanese plums, a species characterized by high-sugar content of its fruit at harvest. Vascular flows of Angeleno plums were monitored by fruit gauges during late fruit development, under the dry environment of the Goulburn Valley, Victoria, Australia. Phloem, xylem flows and skin transpiratory losses were determined, as well as diurnal leaf, stem and fruit pressure potentials. Fruit seasonal development, skin conductance and dry matter accumulation were also monitored. Fruit grew following a double-sigmoid pattern, but fruit size increased only 3.1 g over the last 3 weeks of development. Fruit grew very little in the morning, primarily due to phloem inflows (0.05 g fruit^{-1} hr^{-1}), while water left the fruit via the xylem. Negligible skin transpiration was recorded for vapor pressure deficit (VPD) values below 3 kPa. This growth pattern, in the absence of skin transpiration, suggests apoplastic phloem unloading. However, at VPD values over 3 kPa (e.g. from early afternoon to a peak around 18:00 hour), transpiratory losses through the skin (up to 0.25 g fruit^{-1}hr^{-1}) caused fruit to shrink, leading to enhanced phloem and xylem inflows (ca. 0.15 g fruit^{-1}hr^{-1}), a scenario that would correspond to symplastic phloem unloading. Over 24 hours the fruit showed a slightly negative total growth, consistent with fruit growth measured *in situ* during the season at weekly intervals. A few fruit species are known to alter their phloem unloading mechanism, switching from symplastic

to apoplastic during the season. Our data support the coexistence in Japanese plum of different phloem unloading strategies within the same day.

Keywords: Japanese plum, Fruit growth, Vascular flows, Dry matter, Water potentials

1. Introduction

Angeleno is the most widely grown Japanese plum worldwide. The success of this cultivar is related to its high yields, sweetness and appearance, late ripening time and the extended storage/shelf life. However, this cultivar is prone to insufficient final fruit size, particularly in relation to high cropping levels; although industry-wide production data for Italy range between 25-35 t ha^{-1} (Palmieri and Pirazzoli, 2014)□, anecdotal evidence of yields in excess of 100 t ha^{-1} has been reported. Because of the susceptibility to small final fruit size and the economic relevance of this cultivar, it is of interest to try and understand the physiological basis underpinning the fruit growth dynamics of Japanese plums.

The biophysical process of fruit growth is the result of its vascular flows. In recent years, these have been elucidated for several species, including grapes (Greenspan et al., 1994)□, apple (Lang, 1990)□, peach (Morandi et al., 2007b)□, kiwifruit (Clearwater et al., 2012; Morandi et al., 2010b), European pear (Morandi et al., 2014)□, sweet cherry (Brüggenwirth et al., 2016; Winkler et al., 2016). Fruit growth depends on its gain in water and dry matter imported from the tree. Water enters the fruit via the phloem and xylem vascular systems, while carbohydrates only via the phloem. It is possible for fruit to lose water either via xylem backflow driven by pressure potential gradients between the fruit and the tree (Jones and Higgs, 1982; Tromp, 1984), or via transpiratory losses through fruit skin (Fishman and Génard, 1998; Morandi et al., 2007b). In addition to their scientific relevance, these studies provide the foundation for innovative crop management strategies, since growing practices, such as irrigation management, should be geared according to the specific fruit growth physiology of the individual fruit species.

Phloem unloading into the fruit parenchyma can occur either through apoplastic (active) or symplastic (passive) mechanisms (Patrick, 1997)□. In the first pathway, sugars reach sinks through mass flow in the phloem and are transferred to the apoplast by membrane bound transporters. From the apoplast, carbohydrates enter the fruit flesh cells by a similar, apoplastic mechanism. In symplastic unloading, transpiratory losses through the skin can help establish higher osmotic concentrations in the fruit parenchyma cells, leading to more negative fruit pressure potentials, a condition favoring carbohidrate fluxes (Li et al., 2001; Morandi et al., 2010a), in accordance with the Münch pressure flow model (Münch, 1930)□.

The 'active' vs 'passive' terminology may be misleading. Apoplastic unloading requires metabolic energy to fuel the process; symplastic unloading can also require energy-dependent conversion of phloem-transported moieties to storage carbohydrates (Berüter, 1985)□, or active transport across the tonoplast for storage in the vacuole (e.g. sorbitol to sucrose in peach, Lo Bianco and Rieger, 2002; Vizzotto et al., 1996). In both cases, a concentration gradient can be established, which can favour diffusion from the vascular system towards the parenchyma cells, where it might be used to fuel cell growth, or to be stored (Patrick, 1997)□. Uniport membrane channels have been characterized, the 'SWEET' family (Eom et al., 2015)□, which appear involved in trans-membrane transport along concentration gradients, facilitating diffusion-driven vacuolar compartmentation in apoplastic phloem-unloading in sugar cane and sugar beet.

Lacking an apoplastic barrier separating phloem from storage cells, elevated osmotic concentrations in fruit cells unloading symplastically would impede rather than enhance flows through the symplastic pathway as predicted by the Münch pressure flow hypothesis (Milne et al., 2018)□. However, it is agreed that transpiratory losses will reduce the fruit water potential which could, in the absence of any osmotic adjustment by fruit cells, lower their turgor potentials to enhance Münch pressure flow through a symplastic unloading route into the fruit cells, which can occur passively, as shown in peach by Morandi et al. (2007b)□. Never-the-less, fruit expansion will cease if transpiration fluxes depress fruit turgor below the yield threshold of the fruit cell walls. To sustain growth under these conditions osmotic adjustment would be required to restore cell turgor potential above the yield threshold or, as it has been demonstrated in several fruit species, turgor can be restored subsequently, following an increase in xylem flow which re-hydrates the fruit tissue after its typical mid-day shrinkage (young apple, Lang, 1990; pear, Morandi et al., 2014; kiwfruit, Morandi et al., 2010b; peach, Morandi et al., 2007b)□.

This trait appears genetically determined: apples are considered apoplastic phloem unloaders (Zhang, 2004)□, while in peaches symplastic phloem unloading is known to contribute for the majority of fruit growth (Fishman and Génard, 1998; Morandi et al., 2007b), though apoplastic unloading of carbohydrates into the fruit has also been shown (Zanon et al., 2015)□, suggesting the coexistence of the two mechanisms. In kiwifruit, *Actinidia deliciosa* (A. Chev.) C.F. Liang & A.R. Ferguson var. *deliciosa* 'Summerkiwi' (Morandi et al., 2010b)□ and 'Qinmei' (Chen et al., 2017)□, fruit growth is likely driven by a symplastic mechanism of phloem unloading during the first stage of rapid fresh mass growth, while the second stage of slow growth is apparently fueled only by apoplastic phloem unloading, in the presence of a progressive xylem dysfunctionality. However, vascular flows and phloem unloading may change depending on the species/cultivar. In fact, Clearwater et al. (2012)□ have reported, for *Actinidia chinensis* (Planch) var. *chinensis* 'Hort16A', phloem and xylem functionality during the last stages of fruit growth, until harvest. Interestingly, the fruit of this cultivar

exhibited different growth patterns when growing in cool and moderately wet conditions in New Zealand as compared to much warmer and drier conditions in California, where fruit exhibited *nose shriveling*, a physiological disorder attributed to the high fruit-to-air vapor pressure deficit in California which caused fruit to lose water through the skin (transpiration losses) in proximity of harvest.

While the two mechanisms are not in theory mutually exclusive, functional and anatomical features required for apoplastic unloading are not easy to create in a mature cell. However, Choat et al. (2009) stipulate for wine grapes that loss of xylem contribution to fruit growth late in the season does not necessarily imply loss of xylematic functionality, as control of water flows out of the fruit during instances of strong stem-fruit pressure gradients may reside elsewhere, for example at the level of plasma membrane-bound aquaporins.

Elucidating the vascular flows supporting fruit growth can open novel approaches to crop management, as it can inform physiological mechanisms, such as the complex interactions between light interception, air and leaf temperature, tree water status, fruit quality and production outcomes. Because fruit trees loose photosynthetic potential under very strong light intensities (Jifon and Syvertsen, 2003), shading (e.g. by hail nets) can alleviate photo-inhibitory stress (Losciale et al., 2011). Irrigation water can be saved thanks to lower temperatures and vapour pressure deficits caused by reduced irradiation. In apple, 50% shading did not reduce photosynthesis, yield and fruit quality, but allowed water savings up to 50% of the fully illuminated control (Lopez et al., 2018). These interactions are mediated by the water potential gradients that are established among stems, leaves and fruit. These gradients are dynamic and depend on time of day, soil water content, and evaporative demand by the air. These complex interactions can too be exploited to favor fruit growth: in kiwifruit, delaying by a few hours the start of irrigation increased fruit xylem inflow, since the late irrigation allowed the creation of a more favorable fruit-to-stem water potential gradient. At the same time, in the control vines, leaves had a much more pronounced stem-to-leaf gradient and were depriving fruit from water needed for growth (Torres-Ruiz et al., 2016).

This study was carried out in the Goulburn Valley, Victoria, Australia under a temperate climate where Angeleno is widely grown, and issues related to efficient water use and premium fruit production are relevant for growers. Our aim was to elucidate the vascular flows of the fruit of this cultivar towards the end of the stage of cell expansion in fruit development (stage III), i.e. in late summer, when high temperatures and vapor deficit conditions may strongly limit fruit growth. Our expectation was that, from the scientific knowledge obtained, some insights may be gained to improve irrigation management practices in commercial orchards.

2. Materials & Methods

2.1. Plant Material

The experiment was carried out at the Profitable Stonefruit Systems Orchard at Tatura, Victoria, Australia (Lat. -36.435774, Long. 145.269262, elev. 114 m) in the 2016-17 growing season. Trees of the Japanese plum (*Prunus salicina* L.) cv. Angeleno, grafted on Myrobalan H29C, were planted in winter 2014 at 4.5 x 1.0 m spacings, and trained to Tatura Trellis (Chalmers and van den Ende, 1975)□, at a planting density of 2,222 trees ha^{-1}. Rows were N-S oriented. Trees were managed according to standard pruning, fertilization, thinning and irrigation. Climate is temperate in the region, and rainfall is evenly distributed throughout the year (Table 1); irrigation is used to counter the high evaporative demand experienced in summer months. Irrigation volumes were determined using a weather-based evapotranspiration FAO-56 approach with a crop coefficient adjusted for tree size, measured as the fractional photosynthetically active radiation (PAR) interception (f_{PAR}). Irrigation water was of high quality (C_1S_1 class, Wilcox, 1948)□ and applied daily via a single drip line comprising of in-line pressure compensating emitters (1.6 l/h discharge, 0.5 m spacing). Full bloom, assessed as 80% flowers open, was recorded on Sept. 5, 2016. Fruit were harvested on March 16, 2017. The study took place between February 22 and March 1, 2017. Environmental data were obtained for the duration of the study from an automatic weather station located less than 1 km from the experimental site, which was recording at 10-min intervals.

2.2. Seasonal fruit growth

Beginning on Nov. 3rd, 2016, the equatorial diameter of 60 fruit was measured weekly with a set of digital calipers fitted with a memory module (Calibit, Horticultural Knowledge srl, Bologna, Italy). The fruit were randomly chosen on the trial trees and individually tagged. At each recording, a sample of 15 fruit of average size was collected and rapidly transferred to the laboratory, where fresh mass and the equatorial diameter were recorded. These fruits were further used for the skin conductance determination (see below). The diameter (D, mm) of each of the 60 fruit monitored over the season was converted to mass (M, g) using the following equation:

$$M\ (g) = a \times D^b\ (mm) \tag{1}$$

where a and b were 0.000863 and 2.8788, respectively, with R^2 = to 0.996. This equation was obtained by plotting D and M of the 315 fruit collected during the season. Equation 1 was used to convert diameter readings to mass values for the fruit used to determine diurnal vascular flows (see below).

2.3. Water potentials

On February 22, 2017, the day prior to the start of detailed fruit growth measurements, stem, leaf and fruit water potentials were determined by a Scholander pressure chamber (Soilmoisture Equipment Corp., Santa Barbara, CA, USA), at 06:00 (pre-dawn), 09:00, 11:00, 13:00 and 16:00 hours. At each time, 4 random samples were measured for each category. Stem water potentials were determined on leaves that had been covered with aluminum foil at least 90 min. prior to measurement to allow equilibration with the stem (Turner and Long, 1980)□. All fruit were removed with about 50mm of lignified twig and the cut surface on one side of the twig was immediately covered with glue while the other side was inserted in the chamber lid for measurement. For each time of recording, values were subjected to ANOVA, followed by means separation by Tukey's HSD test, using R software, version 3.2.3.

2.4. Fruit surface conductance and dry mass

Surface conductance (g_c) was determined according to Gibert et al. (2005)□. During the growing season, the fruit used to determine the diameter/mass conversion equation were also utilized for skin conductance. Immediately after mass and diameter determinations, they were placed for 24 hours in a constant temperature room, held at 25°C. Temperature and relative humidity in the room were monitored by a data logger (Tiny Tag, Gemini Data Loggers Ltd, UK). After 24 hours fruit were weighed again and g_c (hourly conductance, m h^{-1}) was calculated according to the following equation:

$$gc = \frac{Tf(h)}{Sfx\frac{Mw}{RxTemp}xP*x(Hf-Ha)x100} \qquad (2)$$

Where h is time since sampling (h), T_f is the transpiration per unit time (g h^{-1}), which is equal to the weight loss, S_f is the fruit surface area at $h = 0$ (cm^2), M_w is the molecular mass of water (18 g mol^{-1}), R is the gas constant (83 cm^3 bar mol^{-1} K^{-1}), $Temp$ is the temperature (K), P* is the saturation vapour pressure (bar) [depending on temperature following the equation of Fishman and Genard (1998)□: $P^* = 0.008048$ x exp(0.0547 x $Temp$ -273.15)], H_f is the relative humidity within the fruit (assumed to be equal to 100%), and H_a is the relative humidity of the atmosphere.

After g_c determinations fruit were placed in a fan-forced oven held at 65°C for 5 days until constant (Morandi et al., 2010a)□weight. Fruit were then weighed again to determine dry mass. Dry matter % was then calculated from the fresh and dry mass of each fruit.

2.5. Fruit vascular flows

Vascular flows subtending fruit growth (phloem inflow, xylem in/outflow and transpiration outflow) were determined following Lang (1990)☐ and Morandi et al. (2010a)☐. The diameter of 9 randomly chosen fruit was recorded every 15 min using self-built fruit gauges described by Morandi et al. (2007a)☐, connected to a wi-fi network (Winet srl, Cesena, Italy) installed in the orchard. All fruit were measured for at least 48 hours in each of the following conditions: "intact" (normal vascular connections), "girdled" (phloem connection severed) and "detached" (all vascular connections severed). All fruit were subjected to the same treatment on the same day. The time elapsed in each condition ranged from 6 days in the "intact" condition, to 4 days in the "girdled" and to 2 days in the "detached" state. From these periods, days with similar weather conditions were chosen for the calculations presented. In "detached" fruit, the peduncle surface was covered with glue to avoid any water loss, and fruit were hung in their original positions using thin strand plastic webbing, which did not reduce in a significant way the possibility to exchange water vapour with the atmosphere.

At each recording time (t), phloem contribution (P_t) was calculated, following Lang (1990)☐, as the difference between the diameters in normal (N_t) and girdled (G_t; Eq. 3); the same was done for the xylem (X_t; Eq. 4) by subtracting the diameters in detached (D_t; Eq. 5) fruit from the girdled ones. Fruit diameter at each time was converted to mass using the conversion equation derived from the weekly determinations of dry matter and skin conductance.

$$P_t = N_t - G_t \qquad\qquad (3)$$

$$X_t = G_t - D_t \qquad\qquad (4)$$

$$T_t = D_t \qquad\qquad (5)$$

Average growth, phloem and xylem flows and transpiration rates per hour were calculated for each fruit, and were expressed as absolute growth rates (AGR, g fruit^{-1}hr^{-1}). For each data point, a total of 4 readings per fruit per hour were used (n=36).

3. Results & Discussion

3.1. Fruit seasonal growth

Angeleno fruit grew following the double-sigmoid pattern typical of stone fruit (Fig. 1). After a first stage of growth ending about 80 days after full bloom (DAFB), and a short (14 days) stage II (stone hardening), fruit entered rapid cell expansion (stage III), which began mid-December and lasted for about 3 months, until fruit were harvested mid-March. When this study was carried out (end of February – beginning of March) daily fruit growth had slowed considerably (Fig. 1). Fruit mass, determined from the diameter data by equation 1, followed a very similar double-sigmoid pattern (Fig.1).

3.2. Environmental data

Night temperatures remained relatively high in the three days chosen for fruit vascular growth analysis (February 23rd and 27th, March 1st) reaching a minimum value of 16.2 °C at 07:00 hour on February 23rd ('fruit intact'), while daytime temperatures peaked above 30°C, reaching a maximum of 34.7 °C (at 18:00 hour on March 1st; 'fruit detached'; Fig. 2). Corresponding VPD values where 0.6 and 4.4 kPa, respectively. On February 22nd, the day when water potentials were measured, a broader range in both T and VPD was encountered (11.5 and 35.6 °C; 0.25 and 4.96 kPa, respectively; Fig. 2). The time when maximum temperature and VPD values were reached was late in the day (around 18:00 hour) in all instances.

3.3. Diurnal water potentials

Pre-dawn, fruit had the least negative water potential, while leaves and stems were more negative, and similar between each other (Fig. 3). At this time, stem water potentials ranged between -0.9 and -1.3 MPa, somewhat negative considering that the trees received a full irrigation. At 09:00 hour, potentials were rapidly becoming more negative (below -1.5 MPa), in response to tree transpiratory losses, with stems less negative than leaves, and fruit intermediate. From late morning (11:00 hour) onward, values had dropped below -2.4 MPa. Fruit and leaves had similar values, lower than stems. Stem water potentials started a slow recovery from mid-day, but remained below -2.0 MPa, whereas both leaves and fruit remained at lower levels, trending towards more negative values as the afternoon continued. At 16:00 hour, fruit were near -3.0 MPa, although not different from leaves. However, as maximum VPD was recorded after the time of the last water potential readings (Fig. 2), it is possible that this separation would have continued – and even increased – later in the afternoon. It is worth noting that the maximum value reached for VPD on this day was ca 5 kPa, a value that is considered extreme in other parts of the World where plums are grown, but that is not considered unusual at the site of this experiment.

3.4. Seasonal skin conductance

Skin conductance showed a negative curvilinear trend, with higher values scored early in the season (Fig. 4). Similar seasonal skin conductance responses have been observed in apple (Jones and Higgs, 1982) and peach (Gibert et al., 2005). However, plum conductance was about 25% lower than the early season data reported for the other two species. The skin of plums is characterized by wax deposition (Skene, 1963) which starts early in the season. Skin waxes contribute to regulating the loss of water through the skin (Mukhtar et al., 2014). The decrease in fruit skin conductance over the season is consistent with an apoplastic phloem unloading mechanism, as reported for apple (Lang, 1990), or kiwifruit (Morandi et al., 2010b). Fruit with very low skin conductance are usually characterized by this mechanism of phloem unloading due to their difficulty to reduce their turgor pressure and create the necessary gradient for symplastic phloem unloading to occur (Morandi et al., 2016). However, conductance values never quite reached zero, remaining at very low, but positive levels up to harvest (Fig. 4).

3.5. Fruit vascular flows

Fruit vascular flows are depicted in figure 5, which presents data collected during Feb. 23rd, 27th, and March 1st; the relative environmental conditions (T and VPD) are shown in figure 2, panels B), C) and D), respectively. The VPD patterns in the three days were not dissimilar from those observed for the day when water potentials were measured (Feb. 22nd; Fig. 2, A). In general, VPD reached its lowest daily values (around 0.5 kPa) after midnight, until it started to increase around 07:00, and continued to increase until reaching its maximum in late afternoon; from this moment onward, it declined steadily (Fig. 2). Maximum VPD values did not reach 4 kPa on Feb. 23rd and 27th, but obtained 4.5 kPa on March 1st ('fruit detached'), conditions closer to the maximum of 4.96 kPa recorded on Feb. 22nd, the water potential measurement day.

Intact fruit grew very little from midnight until 07:00 hour, when their AGR increased to positive values (albeit low, in the order of 0.05 g hr^{-1}; Fig. 5) following an equivalent increase in phloem inflow, suggesting a flow of current photosynthates reaching the fruit. With a delay that might be justified by the need to establish proper pressure potential gradients between stem and leaves on one side, and the fruit on the other, xylem flow to the fruit appeared, consistent with the increased fruit-stem pressure gradient recorded at this time (Fig. 3). Phloem flow was maintained at these low levels until early afternoon but, since the xylem inflow was reduced during this time, total fruit growth was reduced to almost no growth at 13:00 hour.

Until this time of day, no transpiratory losses appeared. Absence of transpiratory fluxes and an absence of a water potential gradient between fruit and stem suggests the operation of an apoplastic phloem unloading mechanism. However, in biophysical terms, if the fruit cells are capable of deformation (i.e. volume growth), then phloem unloading through a symplastic route could still occur passively in the absence of transpiration. Our data show that phloem transport into fruit can occur in the absence of transpiratory losses. This could be accounted for either by symplastic or apoplastic phloem unloading mechanisms.

Total fruit growth was accounted for by the contribution of phloem and xylem until the latter was reduced to approximately zero (in absence of transpiratory losses from the fruit), indicating a possible xylem backflow, in response to putative increased transpiratory losses from leaves, and the lowering of their water potential. It is only in the morning therefore, that the fruit appear capable of growth, however small it may be.

Beginning in early afternoon, with VPD values above 3 kPa on March 1st, fruit transpiration began and steadily increased, reaching rates of 0.25 g fruit^{-1} hr^{-1} (Fig. 5) and thus causing a shrinkage and a further decrease in fruit water potential. At this time, leaf transpiration may have reduced, due to stomatal closure; in turn, this may have caused stem water potential to stop its decline, and to start a slow recovery (Fig.3), which may have benefited the fruit, as fruit water potential trended to more negative values than leaves from this point on (although not significant). The strong fruit transpiration fluxes likely reduced both xylem and phloem water potentials. The decreased xylem water potential, in the absence of any osmotic adjustment by the fruit, would lead to reductions in fruit phloem and storage cell hydrostatic pressure potentials. This could drive a sustained increased Munch flow into a non-growing fruit through a symplastic unloading route if the rates of imported phloem were dissipated through transpiratory rates that were equal or greater than those of phloem imported water. In response to these decreased potentials, both xylem and phloem increased their import to the fruit, to values of approximately 0.15 g hr^{-1}, three times higher than in the morning. This scenario also reveals how the xylem of plum remains functional until this late stage of the season. When VPD values began to decrease in the early evening, transpiration losses were reduced, and phloem and xylem inflows to the fruit also decreased (Fig. 5).

3.6. Daily fruit growth

The cumulative total of losses and gains over the 24 hours shows a negative balance, as indicated by the growth of the intact fruit in figure 5, that do not record a gain in mass over the entire day. This response is in line with the non-destructive *in situ* fruit size measurements reported in Figure 1, of essentially zero fruit growth for Angeleno fruit towards the end of the growing season. This is also

reflected in the total daily contribution to fruit growth of each of its components: with a phloem contribution of 0.406 g d^{-1} and 0.709 g d^{-1} from the xylem, in the presence of a transpiratory loss of 1.212 g d^{-1}, these fruit lost 0.023 g d^{-1}. Plums are known to achieve high dry matter content, compared to other stone fruits. Morandi et al. (2016)□ reported for peaches values around 13% for "Red Gold" nectarine 1 week before harvest. In this study, values of about 19% were reached one month before, and maintained until, harvest (Fig. 6). Li et al. (2001)□ and Morandi et al. (2010a)□ found a positive effect of transpiration on the capacity of peaches to accumulate dry matter during the stage of rapid fruit growth by cell expansion. These studies indicated a reduced fruit pressure potential (resulting from transpiratory losses) as a promoting factor in symplastic phloem unloading, since reduced turgor would facilitate both carbon translocation towards the fruit and phloem unloading at the sink. In winegrapes, strong accumulation of sugars in the fruit after veraison occurs in the presence of low carbohydrate concentrations in the phloem (Choat et al., 2009)□. These authors stipulate that phloem water may be lost to the plant via xylem, thus allowing such an increase in apoplastic storage of sugars in the fruit. In our study, the loss of more water via transpiration than what was imported during the day through phloem and xylem might promote high concentrations of sugar to occur. However, this is purely speculative, and would need further investigation.

That xylem inflows could not replenish transpiratory losses in the afternoon is an indication of the tree undergoing water shortage at this time of the day. This suggests the possibility to innovate irrigation practices by targeting water application more precisely – in terms of its timing – in order to maintain appropriate water levels in the tree when they are more necessary. In green-fleshed kiwifruit, Torres et al. (2016)□ were able to positively influence berry growth by simply shifting the timing of irrigation from 03:00 to 11:00 hour.

3.7. Interaction between growth and environmental conditions

The data presented here provide for the first-time insights into the vascular flows of a Japanese plum. Although they only refer to one time in the season (stage III), they depict an interesting situation in which the two putative mechanisms of phloem unloading seem to co-exist and work in synchronicity to support the growth of the fruit. In the first half of the day growth proceeds at very low rates, that are compatible with an apoplastic phloem unloading mechanism, and appear in line (around 0.6 g fruit^{-1} day^{-1}, assuming a 12 hour growth period at this rate) with previously published data for green-fleshed kiwifruit by Morandi et al. (2010b)□, who showed a gain of ca 0.7 g fruit^{-1} day^{-1} at a similarly advanced stage in the season, when fruit of this species are considered to grow by an apoplastic mechanism. Later in the day and in presence of strong VPDs, fruit transpiration begins and reaches a relatively high level, causing a loss of 1.2 g fruit^{-1} day^{-1}, i.e. much higher than apple or green-fleshed

kiwifruit at a comparable point in the season (Lang, 1990; Morandi et al., 2010b) In fact, this value represents between 15-40% of water losses by peach (a symplastic unloader) at this stage (Morandi et al., 2010a)☐. During the season, similar VPD values are not uncommon at the site of the trial; on the other hand, they are certainly higher than those that are normally encountered elsewhere, for example in the Po Valley of Italy (where Angeleno is widely grown), where VPDs between 2 and 3 kPa are commonly observed. The strong evaporative demand at the site of the study is therefore responsible for this response, since plum skin conductance reported here is about 10% that reported for peach by Gibert et al. (2005)☐. Certainly, the epidermal waxes that cover the skin of plums have an important role in reducing transpiration; however, they still allow a significant amount of water to be lost to the atmosphere. The more so if one considers that, even though the trees were receiving a standard irrigation regime, the predawn water potentials were rather negative, between -0.9 and -1.3 MPa, an indication of a dry environment. Considering the above, these transpiratory losses must be considered relatively strong, to the point in fact, that they are not replenished by the concurrent inflows through the phloem and xylem, and the fruit total mass gain over 24 hours results in a slightly negative fruit growth outcome. Several studies have reported shifts between vascular flow patterns supporting fruit growth, regarding the contribution of xylem in the apoplastic phloem unloading pattern. For grapes (Choat et al., 2009; Greenspan et al., 1994), apple (Lang, 1990)☐ and green-fleshed kiwifruit(Chen et al., 2017; Morandi et al., 2010b) these changes occur over the season, with fruit transiting from one model of growth where xylem contributes to growth, to one where it loses importance. In yellow-fleshed kiwifruit, Clearwater et al. (2012)☐ have demonstrated not only a different mechanism from the green-fleshed species, whereby xylem and phloem remain functional throughout the season although in the presence of an apoplastic unloading mechanism, but they also have demonstrated the effect of environmental conditions, as they tackled the causes of a fruit growth disorder that appears in warmer, drier climates (e.g. California, Central and Southern Italy), but not in the cooler and less arid (New Zealand) growing conditions. In our study the warm and very dry environmental conditions, leading to high VPDs, appear conducive to the utilization by plums of both the apoplastic and symplastic mechanisms of fruit growth during the same day, although at different times. This coexistence on a daily basis appears closely coupled to the environment and should be studied under a different environment, with lower VPD values.

4. Conclusions

In this study we report vascular flows compatible with the co-existence of the apoplastic and symplastic mechanisms of phloem unloading over a very short time span (e.g. hours). To our knowledge, this is the first study detailing this daily co-existence in a fruit species. Growth of plums

appears to be able to switch from one mechanism of phloem flow to the other, in adaptation to, or to exploit, the changing environmental conditions encountered daily. The fact that the whole process does not yield a positive gain in fruit size over one day confirms the importance of these crop physiological studies from a grower's point of view, since we detail the mechanisms by which there can be days when fruit are shrinking due to climate, even in the presence of apparently tolerable temperatures and sufficient irrigation. It must be stressed once again that similar conditions with very high VPDs would not be so common elsewhere; however, they are at the site of this work, in one of Australia's most important fruit growing regions. In this sense, this study provides an example of GxE interaction, whereby the same genotype would react differently to different environments: it is in fact possible that the co-existence of the two phloem unloading mechanisms illustrated here might not appear as clearly under less demanding conditions, such as, for example, the quite milder climate normally found in the Italian region of Emilia-Romagna.

CRediT roles statement:

Luca Corelli Grappadelli: Conceptualization, Supervision, Investigation, Formal Analysis, Validation, Writing Original Draft. **Luigi Manfrini**: Investigation, Data curation, Review & Editing. **Brunella Morandi**: Conceptualization, Methodology, Review & Editing. **Mark O'Connell**: Conceptualization, Resources, Investigation, Review & Editing.

Declarations of interest: none.

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Figure Captions

Figure 1. Seasonal growth (± SE) of Angeleno fruit. Diameter values were converted to mass by Eq. 1. SE bars smaller than symbols not visible. DAFB: Days After Full Bloom.
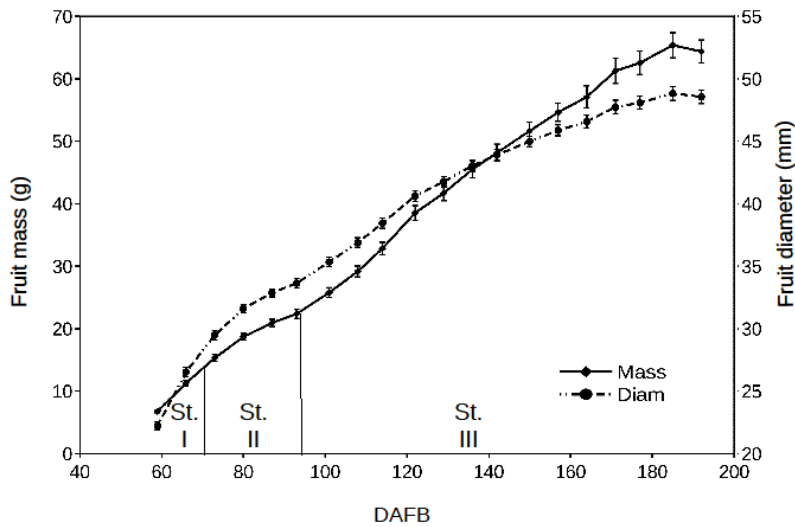

Figure 2. Daily hourly average (± SE) of T (°C) and VPD (kPa) on the days of the experiment: A) Feb 22nd, day of water potentials determination; B) Feb 23rd, 'fruit intact'; C) Feb 27th, 'fruit girdled' ; D) March 1st, 'fruit detached'. n=6. SE bars smaller than symbols not visible.
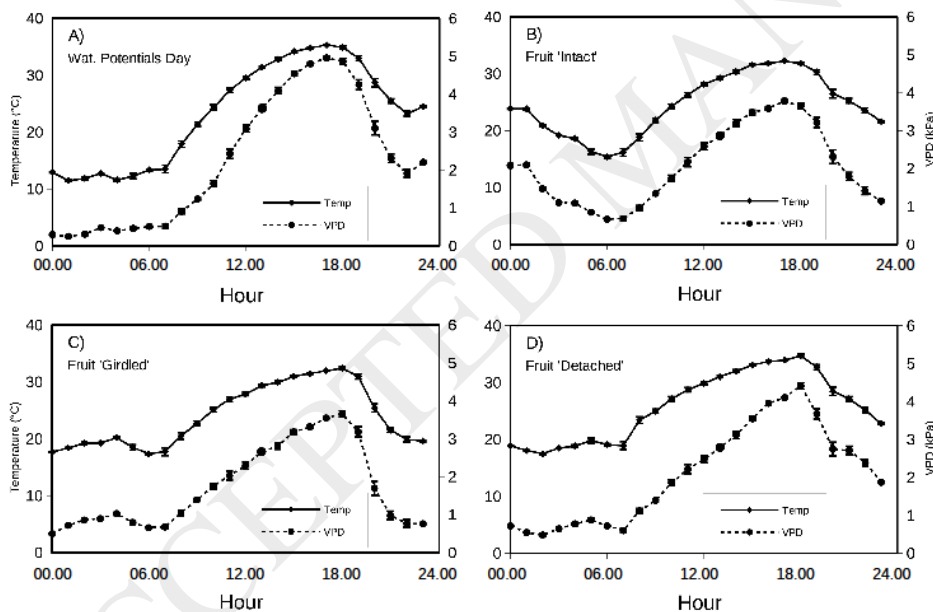

Figure 3. Diurnal trends of leaf, stem and fruit water potentials of Angeleno plum, measured on Feb 22nd. Means separation by Tukey's HSD. $P<0.05$. SE bars smaller than symbols not visible.
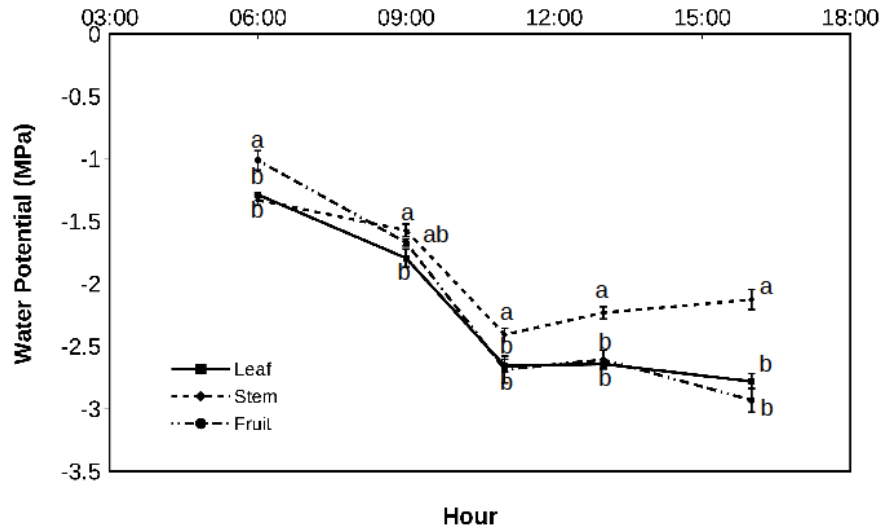
Figure 4. Seasonal pattern of Angeleno fruit skin conductance. Each point average of 15 fruit. SE bars smaller than symbols not visible. DAFB: Days After Full Bloom.
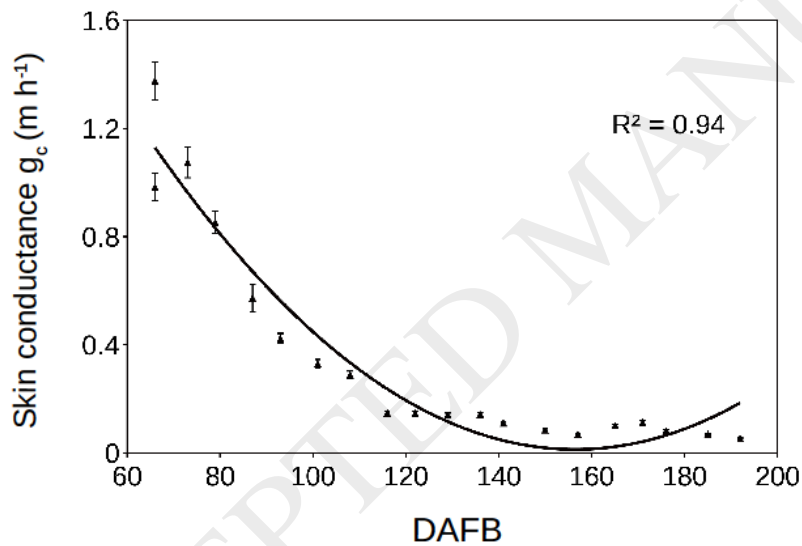

Figure 5. Intact fruit growth and rates of vascular flows of Angeleno fruit ($\pm$ SE) and VPD on March 1st ('fruit detached). SE bars smaller than symbols not visible.
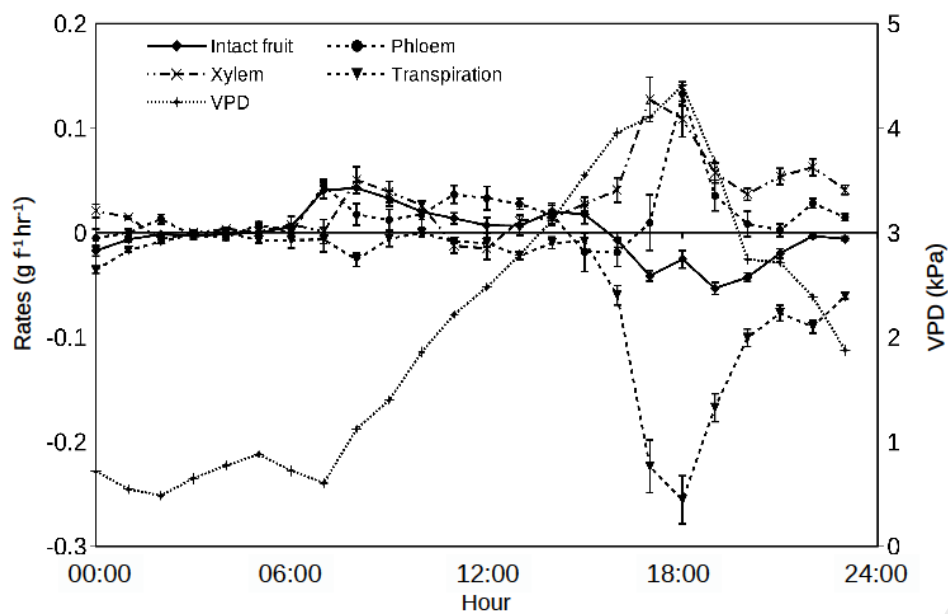
Figure 6. Seasonal dry matter (% fresh mass) of fruit of Angeleno plum. For each date, 15 fruit were sampled. DAFB: Days After Full Bloom.
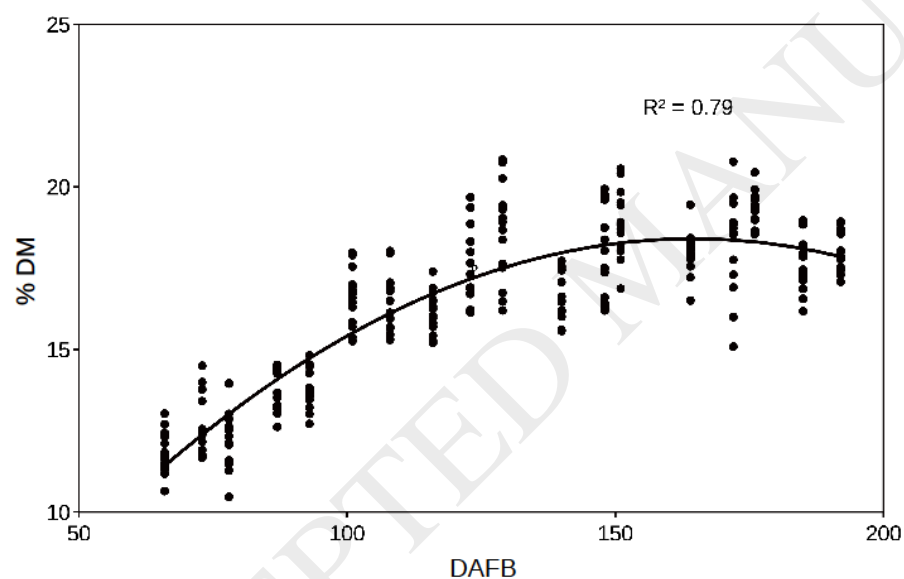
Table Captions

Table 1. Long-term meteorological data for the site of the experiment. (Source: Australian Bureau of Meteorology).

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Annual
		Mean Temperature (°C)											
Max.	29.8	29.6	26.4	21.6	17.3	14.0	13.0	14.7	17.5	21.2	24.8	27.6	21.5
Min.	14.3	14.5	11.9	8.4	5.8	3.7	3.0	3.8	5.4	7.6	10.3	12.4	8.4
		Mean Rainfall (mm)											
	33.3	32.9	33.9	34.4	44.3	43.7	47.5	46.8	42.7	46.1	40.2	35.5	481.2
		Mean number of days of rain ≥ 1 mm											
	3.4	2.9	3.8	4.6	6.5	7.2	8.6	8.5	6.8	6.8	5.2	4.3	68.6
		Mean daily sunshine (hours)											
	10.1	9.8	8.7	7.4	5.1	4.2	4.2	5.5	6.6	8.0	9.0	9.6	7.4