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Attached fruits of *Actinidia deliciosa* were subjected to Low (L) and High (H) *VPD*. Their transpiration was measured from early after fruit-set to day 157 after full bloom (DAFB). Fruits were picked at 70, 130 and 157 DAFB for Ca and K determinations and for water budget analysis.

Cumulative transpired water was ~70 g and ~16 g H_2O f^{-1} in H_{VPD} and L_{VPD}, respectively. Calcium accumulated linearly (R^2 = 0.71) with cumulative transpiration when *VPD* was high, while correlation was weaker (R^2 = 0.24) under L_{VPD}. Under low *VPD* the fraction of xylem stream destined to transpiration declined to 40-50%.

Results suggest that Ca accumulation is coupled to cumulative transpiration under high *VPD* because under that condition cumulative transpiration equals xylem stream (which carry the nutrient). At L_{VPD}, Ca gain by fruit is uncoupled from transpiration because ~60% of the xylemic water is needed to sustain fruit growth. Results will apply to most fruits (apples, tomatoes, capsicum, grapes etc) since most suffer Ca deficiency disorders and grow in changing environments with variable *VPD*, also they could be supportive for the implementation of fruit quality models accounting also for mineral compositions and for a reinterpretation of certain field practices aimed at naturally improve fruit Ca content.

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Abstract

Accumulation of Ca in several fleshy fruit is often supposed to depend, among others, by climatic variables driving fruit transpiration. This study tests the whole causal chain hypothesis: *VPD* → *fruit transpiration* → *Ca accumulation*. Also there are evidences that relationship between fruit transpiration and Ca content is not always clear, hence the hypothesis that low *VPD* reduces the fraction of xylemic water destined to transpiration was tested by examining the water budget of fruit.

Attached fruits of *Actinidia deliciosa* were subjected to Low (L) and High (H) *VPD*. Their transpiration was measured from early after fruit-set to day 157 after full bloom (DAFB). Fruits were picked at 70, 130 and 157 DAFB for Ca and K determinations and for water budget analysis.

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Results suggest that Ca accumulation is coupled to cumulative transpiration under high *VPD* because under that condition cumulative transpiration equals xylem stream (which carry the nutrient). At L_{VPD}, Ca gain by fruit is uncoupled from transpiration because ~60% of the xylemic water is needed to sustain fruit growth. Results will apply to most fruits (apples, tomatoes, capsicum, grapes etc.) since most suffer Ca deficiency disorders and grow in changing environments with variable *VPD*, also they could be supportive for the implementation of fruit quality models accounting also for mineral compositions and for a reinterpretation of certain field practices aimed at naturally improve fruit Ca content.

Keywords: fruit water budget, nutrient transport, microenvironment, phloem, *VPD*, water loss, xylem.

Abbreviations: DAFB, day after full bloom; E, daily transpiration rate; G, fruit growth; H_{VPD}, high vapour pressure deficit; L_{VPD}, low vapour pressure deficit; P, phloem water inflow; T, cumulative transpiration; X, xylem water inflow.

Introduction

Many fleshy fruits (e.g. apple, avocado, tomato, grape) including kiwifruit suffers physiological disorders during storage and, sometimes, also pre-harvest. Such disorders can be associated with low calcium (Ca) content (Saure, 1996; Ferguson and Watkins, 1989; Cutting and Bower, 1989; Witney et al., 1990; Ho and White, 2005; Thorp et al., 2003; Ferguson et al., 2003, Ciccarese et al., 2013). Optimal Ca content of fruit and vegetables at harvest is generally found to improve their storability, probably because of its role in maintaining tissue mechanical strength (Hirschi, 2004). Improved storage is associated with reduced costs along the supply chain and therefore application of Ca-based foliar spray (field) or adoption of specific preconditioning protocol (packhouse) are common in fruit industry (Crisosto et al., 1997; Alcaraz-Lopez et al., 2003; Gerasopoulos and Drogoudi 2005), even though natural friendly increased Ca is highly desirable.

Internal and external factors affecting the accumulation of Ca in fleshy fruit have been reported by Saure (2005). More recently, a review examined the factors (and their interactions) operating along the soil-to-fruit pathway in kiwifruit (Montanaro et al., 2014). In the light of the causal chain hypothesis: *weather* → *fruit transpiration* → *Ca accumulation* (Montanaro et al., 2012), among the external factors affecting fruit Ca accumulation in kiwifruit are the meteorological variables affecting fruit transpiration. Predominantly, these are those contributing to atmospheric vapour pressure deficit (*VPD*) - namely temperature and humidity.

Vapour pressure deficit may also affect fruit mineral nutrition indirectly through its effect on leaf transpiration (Lang and Volz, 1998). For example, studies have shown that reducing leaf transpiration by reducing *VPD* can also reduce the import of Ca by leaves. Assuming a constant uptake rate of Ca by the plant from the soil, a reduced leaf import, may favour an increased fruit import (Guichard et al., 2005; De Freitas et al., 2011).

The period of early fruit developmental is the most critical for Ca accumulation in most fleshy fruit including in kiwifruit. Principally, this seems to be because Ca is xylem mobile but phloem immobile (Bukovac and Wittwer, 1957), so it is the xylem sap inflow rate to the fruit, that determines the Ca import rate. In most fleshy fruits, the inflows of xylem sap and phloem sap are fairly similar in the early season (Lang 1990),

whereas the total sap inflow in the late season is strongly dominated by the phloem, with xylem sap inflow being negligible because of strongly reduction of transpiration (Saure, 2005; Montanaro et al. 2014). Thus, during the early stages of kiwifruit development (roughly from day 10 to day 50 after fruit set) a reduction in atmospheric *VPD*, reduces fruit transpiration, fruit xylem sap inflow, and supposedly Ca import (Morandi et al., 2012).

It appears that concurrent measurements of fruit transpiration and of fruit Ca accumulation along a relatively wide range of water loss have not been made. Therefore, this paper tests the hypothesis that if the *VPD* is the principle driver of fruit transpiration (Montanaro et al., 2012) and if fruit transpiration is the principle determinant of fruit Ca import, then fruit Ca import will be increased by rises in the cumulative fruits' transpiration as driven by increased *VPD*. Meanwhile, the xylem- and phloem-mobile nutrient potassium (K) will be much less affected by the increased *VPD*. To test this hypothesis, fruit transpiration, and the imports of Ca and K were assessed in fruit grown under high (H_{VPD}) and low *VPD* (L_{VPD}) conditions.

Although for transpiring organs, their transpiration probably remains the main driver for Ca accumulation, within-plant transport and partitioning of Ca and other minerals are also known to be affected by "transpiration-independent" factors. For example, calcium demand by an organ and the physicochemical features of the conducting tissues (e.g. ion adsorption and desorption occurring at exchange sites along the walls of the xylem pathway) are likely to influence the metabolic movement of Ca (McLaughlin and Wimmer, 1999). Also the action of specialised transfer cells and the higher expression of Ca transporters and Ca binding proteins may promote accumulation of Ca into fruit independently of its transpiration (Pate and Gunning 1972; Park et al., 2005).

This anticipates that Ca accumulation could be uncoupled from transpiration and encourages a slight reinterpretation of the *transpiration → Ca accumulation* element of the earlier causal chain relationship, at least for low-transpiring organs such as fruit. Here, to explain uncoupled Ca we would evoke the water budget (*sensu* Lang 1990) of fruit as possibly modulated by *VPD*.

The various components of the fruit water budget (xylem, phloem, transpiration and growth) have been analysed in detail (Lang and Thorpe, 1989; Fishman and Genard,

1998) also in relation to the functionality of the vasculature (Clearwater et al., 2012) and, interestingly, to the *VPD* of the surrounding environment even if with contrasting conclusions. For example, in tomato increased *VPD* reduced fruit xylem inflows while phloem flow was almost unaffected, similarly, in young kiwifruit, increased *VPD* increased xylem and transpiration, but does not affect phloem imports (Guichard et al., 2005; Morandi et al., 2012). In peach fruit, both phloem and xylem fluxes were reduced under low *VPD* (Morandi et al., 2010b). Unfortunately, the physiological significance in terms of Ca accumulation of that *VPD*-induced variations on water budget was not explored.

Considering that (*i*) Ca is carried by the water sourced by xylem which is then evaporated (transpiration) or alternatively stored to allow fruit growth, and (*ii*) that the partitioning amidst transpiration and growth may change under different *VPD*, it is proposed that assessing the components of fruit water budget under different *VPD* may help to explain the non-linearity existing between Ca and transpiration. There is a general consensus that growth of fruit integrates fluxes *in* and *out* of fruit (Lang and Thorpe 1989) and that reduced *VPD* doesn't impact neither fruit growth nor phloem inflow (Guichard et al. 2005; Morandi et al., 2012). Therefore, it was hypothesised that under reduced *VPD* to adequately sustain fruit growth, the fraction of xylem flux destined to transpiration is reduced causing Ca accumulation to be uncoupled from transpiration.

To test this hypothesis, sources (xylem, phloem) and destiny (transpiration, growth) of the components of fruit water balance were analysed in fruit grown under H_{VPD} and L_{VPD} conditions.

Materials and Methods

Study site and plant material

The experiment was carried out during the 2011 season in southern Italy (N 40° 25' 19.24''; E 16° 44' 3.03'') on mature, own-rooted Hayward kiwifruit vines (*Actinidia deliciosa* var. *deliciosa*, C.F. Liang et A. R. Ferguson) planted at 625 plants ha^{-1} and trained to a pergola. The orchard was managed to local commercial practice. Nitrogen

144 was supplied through fertigation approximately every 20-30 days from April to July to
145 reach a total of about 50 kg ha^{-1} N, additional 50 kg ha^{-1} N are supplied through organic
146 fertiliser distributed in winter. No phosphorous, potassium or calcium were applied
147 because the soil was already sufficient in these elements. The plants were regularly drip-
148 irrigated (2 drippers per plant, 10 L h^{-1} each) during the season on an approximately
149 weekly basis. Bloom occurred during the last week of May (full bloom on May 24,
150 2011) and natural bee pollination ensured normal fruit-set.

151

152 *Differentiating VPD*

153 On day 7 after full-bloom (DAFB), when fruit had a length (±SE) of about 13±0.3 mm,
154 100 attached fruit were enclosed with a closable (Ziploc®) transparent polyester bag
155 (80×120 mm, 0.06 mm thick, CarloErba Reagents, Italy), an additional clip was used to
156 ensure bag closure (see Fig. 1). Fruit were selected on 50 terminating fruiting canes
157 from 15 randomly chosen vines.

158 To create two levels of *VPD*, half of the bagged fruit were loaded with 5 silica-gel
159 sachets (Carlo Erba, cod. 345702, 93 × 37 mm) each containing 5 g of desiccant. Silica
160 gel can absorb up to 30% of its weight of water while maintaining the relative humidity
161 of the atmosphere well below 80%, hence this treatment was labelled High-*VPD* (H_{VPD}).
162 In bagged fruit without silica gel, the relative humidity of the air surrounding the fruit
163 was near saturation (Montanaro et al., 2010), therefore that treatment was labelled Low-
164 *VPD* (L_{VPD}). To minimise shoot-to-shoot variability, pairs of L_{VPD} and H_{VPD} fruit were
165 selected on the same fruiting shoots. Figure 1 reports the bag treatment design.

166 Temperature and relative humidity inside the bags (×2 per treatment) were measured
167 through a digital probe (mod. CS215, Campbell Scientific Inc., Utah, USA) connected
168 to a datalogger (CR10X, Campbell Scientific Inc., Utah, USA), which was
169 programmed to record at 60 s intervals and to compute and store averages at 15 min
170 intervals. To avoid possible direct contact of the probe with droplets of condensed water
171 vapour, the probe was shielded within a plastic tube open at the bottom. The *VPD* was
172 calculated from air temperature and relative humidity values according to Goudriaan
173 and van Laar (1994).

174

175 *Fruit transpiration*

176 During the first 80 DAFB, the fruit transpiration was measured every 4-7 days, two
177 additional measurements were made late in the season. The bags were weighed (w_1)
178 (with or without silica gel) just before installation in the field (t_1). After a short time (t_2)
179 (4-7 days), these were replaced by "new" pre-weighed bags. The removed "old" bags
180 were promptly transferred in lab and weighed (w_2). This not only maintained the
181 treatment effect (lowered RH in the bags with silica gel, before the silica gel become
182 water-saturated), but it also allowed the 'old' bags to be weighed (w_2) so providing an
183 integrated measurement of fruit transpiration over the previous interval time *t* period (t_2-
184 t_1) by the bag weight difference. All bag exchanges were carried out between 10 and
185 11am and weighings employed a 3-point (1 mg sensitivity) balance (Sartorius, Expert
186 Series ED 323S, Göttingen, Germany). Daily fruit transpiration rate was calculated as
187 follow:

188 $$E = \frac{w_2 - w_1}{t_2 - t_1} - k \qquad (g\ f^{-1}\ d^{-1})$$

189 where w_1 is the bag weight (g) (including 5 dry silica gel sachets for the H_{VPD} treatment)
190 just before field installation at time t_1 and w_2 is the weigh (g) of bag measured at t_2 i.e.
191 at the end of the standing period in the field (t_2- t_1 days) and the average E values were
192 referred to the mid-point of that period. The coefficient *k* accounts for moisture
193 absorption by the PVC bags (Vogt et al., 2003). The values of *k* were determined by
194 means of 4-5 bags of each treatment hanging within the canopy without fruit inside for
195 4-7 days in three occasions through the experiment; their weight variation per day was
196 then calculated. Values were averaged separately for the bags of each treatment and
197 values of 0.11 (L_{VPD}) and 0.14 (H_{VPD}) g f^{-1} d^{-1} were used. In both treatment the
198 cumulative transpiration (T) (g f^{-1}) was calculated by the summation of consecutive
199 daily transpiration values.

200

201

Fruit water budget

Fruit water budget was modelled at days 70, 130 and 157 after full bloom on the same fruit collected for minerals determinations (see below) on fruit grown under H_{VPD} and L_{VPD} according to Lang (1990). This method allows to quantify the magnitude of the three pathways for water uptake and loss assuming fruit growth (G) as the result of the algebraic sum of phloem (P), xylem (X) and transpiration (T) flows:

$$G = X - T + P \qquad (g\ f^{-1}).$$

According to Montanaro et al. (2006) and Clark and Smith (1988), values of G ($g\ f^{-1}$) were obtained as the difference between fresh and dry weight measured just after fruit have been collected (see Chemical analysis section), values of T ($g\ f^{-1}$) were calculated by the summation of daily transpiration (E), and P ($g\ f^{-1}$) values were calculated from the fruit carbon budget considering the flow required to support fruit respiration and growth. Briefly, this carbon budget assumes that approx. 48% of total carbon (C) received by fruit remains in fruit dry matter (DM) (g) and that part of C is lost by fruit respiration (C_r) at a daily rate decreasing from 1.4 to 0.1 mmol $CO_2\ g^{-1}$ DM per day, following an exponential decay pattern throughout the season:

$$C = DM\ (0.48 + C_r) \qquad (g\ f^{-1})$$

then the amount of P was calculated adopting a mean phloem carbon concentration C_p of 85 mg C g^{-1} H_2O.

$$P = C/C_p \qquad (g\ f^{-1})$$

Values of xylem fruit water inflow (X) were then calculated as:

$$X = G + T - P \qquad (g\ f^{-1}).$$

Finally, the fraction (%) of the xylem fruit water inflow destined to transpiration was calculated as $T/X \times 100$.

Chemical analyses

At days 70, 130 and 157 after full bloom, 20 fruits per treatment (10 at 157 DAFB) were collected for Ca and K determination. Fruits were promptly transferred to the laboratory, weighed (fresh weight), sliced and separately dried to constant weight in a ventilated oven at 110°C for a minimum of 72 h. Each dried entire fruit except for the fruit stalk and the floral residues (the desiccated sepals, anthers and styles) was milled to a fine powder in a grinder with steel blades. A sample (~1 g) was removed from the well-mixed powder, weighed, ashed in a muffle furnace (550°C for 18 h) and dissolved in 10 mL HCl 1 M and adjusted to 50 mL with distilled water and the mineral content determined through the inductively coupled plasma optical emission spectrometry analysis (ICP-OES, Thermo Fisher Corporation, iCAP 6000 Series, Cambridge, UK).

Statistical analysis

Comparison between treatments were carried out by Student's *t*-test at the 0.05 or 0.001 probability levels using Microsoft® Office Excel 2003; data processing and curve fitting were by OriginPro 9.1.0 (OriginLab Corporation, Northampton, MA 01060 USA).

Results

Air temperature and humidity (*RH*) inside the bag showed the expected day/night oscillations in both H_{VPD} and L_{VPD} treatments, with the presence or absence of the silica gel sachets effectively creating distinctly different microclimates inside the bags with respect to *RH*, while the temperature values were similar (see inset of Fig. 2). After the installation of the H_{VPD} bags, the silica gel gradually saturated as it absorbed transpired water, so that *RH* increased to ~70% after about 4 d then it fell rapidly to a base value when the sachets were replaced, by contrast under the L_{VPD} treatment, *RH* was roughly constant at 95-100% (see inset of Fig. 2) and *VPD* very low, ranging between 0.1 and

0.5 kPa (over the whole season), in contrast *VPD* ranged between 0.2 and 4.8 kPa in the H_{VPD} treatment (Fig. 2).

At the beginning of the trial, a considerable increase in daily fruit transpiration rate was recorded in both high and low *VPD* treatments (Fig. 3A). Within the early period (to 25 DAFB) daily transpiration per fruit (f) reached ~0.3 and ~1.3 g H_2O f^{-1} d^{-1} under L and H_{VPD}, respectively (Fig. 3A). Transpiration under H_{VPD} conditions peaked at 1.7±0.04 (±SE) g H_2O f^{-1} d^{-1} at 28 DAFB. During the following four weeks, transpiration was fairly stable under both H_{VPD} and L_{VPD} conditions, but it declined thereafter toward a minimum value recorded at 114 DAFB approaching 0.06 and 0.16 g H_2O f^{-1} d^{-1} under L_{VPD} and H_{VPD} conditions, respectively (Fig. 3A). Cumulative fruit transpiration increased progressively under both conditions reaching ~16 (L_{VPD}) and ~70 (H_{VPD}) g H_2O f^{-1} d^{-1} at approx. 60 and 80 DAFB, respectively, thereafter transpiration was fairly stable till the end of the experimental period (Fig. 3B).

Concentrations of Ca and K are shown in Fig. 4. Calcium in the H_{VPD} fruit was significantly higher (Student's *t*-test, P=0.05) than in the L_{VPD} ones on each of the three sampling dates, while K concentrations were not significantly different, except for the sample at 130 DAFB. Fruit dry weight was similar between the *VPD* treatments at 70 DAFB, 6.1±0.23 (±SE) g, but by 130 DAFB it had risen to 12.07±0.59 g (H_{VPD}) and 9.16±1.13 g (L_{VPD}). By the last sampling on 157 DAFB, fruit dry weight had risen to 16.28±1.04 g and 14.98±1.12 g, under H_{VPD} and L_{VPD}, conditions, respectively (Fig. 4).

Correlations between cumulative fruit transpiration and cumulative import of Ca and K (mass per fruit) are presented in Fig. 5. For the high transpiring fruit in the high *VPD* treatment, a reasonably good relationship (R^2 up to 0.74) were found over the range of 45-95 g of transpired water per fruit. The correlation was weaker in the low *VPD* treatment over the range 5-32 g H_2O per fruit (Fig. 5). However, cumulative import of K at low *VPD* was better correlated with cumulative fruit transpiration than Ca.

The analysis of the fruit water budget reveals that different *VPD* did not differentiated the overall phloem fluxes neither after 70 nor 130 DAFB being ~35 and ~ 65 g f^{-1} in both H and L_{VPD} fruit (Fig. 6). Later, at 157 DAFB phloem flow was close to 95 (H_{VPD}) and 80 (L_{VPD}) g f^{-1} (Fig. 6). Similarly, amount of water that grow fruit (G) did not differed between treatments, it increased from approx. 60 at the earlier analysis up to

100 g f^{-1} at 157 DAFB. Transpiration was 3-4-fold higher in H_{VPD} than L_{VPD} and xylem flux was on average 2-fold higher in fruit under H_{VPD} than that of L_{VPD} (Fig. 6).

Discussion

The effects of weather variables on fruit transpiration have been the subject of much recent interest in several fruit species even though its physiological impact on nutrient transport was not adequately explored (Montanaro et al., 2010 and 2012; Morandi et al., 2010b; Léchaudela et al., 2013). The present study extends that findings to the physiology of Ca transport in fruit through testing the whole causal chain hypothesis: *weather →(i)→ fruit transpiration →(ii)→ fruit calcium*. Results show that fruit transpiration couples Ca accumulation (the second step *→(ii)→* in that hypothesis), at least for higher values of cumulative transpired water. Nevertheless the idea is difficult to discuss against a literature background as there is limited information on this, and especially so for fruit. Most researchers have examined the model *VPD* and/or *irradiance→transpiration* (Leonardi et al., 2000, Montanaro et al., 2006), where the balance between irradiance and *VPD* depends not only on the organ being considered (i.e. isolated organ or closed canopy) (Jarvis 1985). Meanwhile others have examined the model *VPD* and/or *irradiance→Ca,* which contains the embedded assumption that fruit Ca accumulation might be driven mainly by transpiration (de Freitas et al., 2010). In an earlier study we have already established through a mechanistic model that *VPD* (not irradiance) is the dominant driver of transpiration for a developing kiwifruit (Montanaro et al., 2012), so this paper integrates previous knowledge examining both links in the causal chain *VPD→transpiration→Ca*.

Microclimatic condition and transpiration

As predicted by Ohm's low *sensu* Nobel (2005), fruit water loss will be significantly affected by changes in the *VPD* of the surrounding air. The *VPD* over the whole experimental period for L_{VPD} fruit was ~10% of that of H_{VPD} one, and in turn daily fruit transpiration was as low as ~25% of that of H_{VPD} fruit (Fig. 2, 3A). The effect of bagging treatment in reducing transpiration is similar to observations in peach fruit enclosed in plastic bag (Morandi et al., 2010b). Interestingly, daily fruit transpiration (g H_2O f^{-1} d^{-1}) measured here by bag weight for H_{VPD} is comparable to that obtained

previously in kiwifruit using a gas exchange method (Montanaro et al., 2006) and a mass balance approach (Morandi et al., 2012).

The increase of fruit transpiration rate early in the growing season (Fig. 3A) reflects the fruit enlargement due to cell division occurring at this stage (Hopping et al., 1976), while its decline toward a minimum value detected after 7-8 weeks after full bloom could be associated with changes in certain anatomical and functional traits of fruit such as increased hydraulic resistance of the xylem and the development of a suberized periderm (see Montanaro et al., 2014 for review). Consequently, cumulative transpiration asymptoted to a maximum at around 80 DAFB (H_{VPD}) or ~3 weeks earlier (L_{VPD}) according to what reported for attached fruit (Fig. 3B) (Montanaro et al., 2006). The low-transpiring fruit lost ~16 g H_2O over the whole experiment, even though they were subjected to near saturating conditions. This could be explained considering mainly the persistent (even minimal) *VPD*. In addition the not-insignificant permeability of the PVC Ziploc® bags and the water evolved during the respiration of sugars may have partly contributed to that transpiration (Clark and Smith 1988; Ulutan and Balköse, 1996).

For a long time, manipulation of the fruit microenvironment through bagging treatments have been used in research as well as in commercial fruit production to obtain certain physiological responses in terms of colour, blemishes, cracking, mineral composition, size, etc. (Hofman et al., 1997; Amarante et al., 2002 and references therein; Montanaro et al., 2010; Morandi et al., 2010b). However, the effects of bagging on the key microclimatic variables (including temperature and relative humidity) of the air surrounding the fruit have not been examined in much detail - often they've been merely assumed. The present study, therefore, represents a significant design improvement on these issues, in that the micrometeorological variables most affecting air *VPD* were recorded. In addition, the use of in-bag, silica gel sachets further allowed a *VPD* treatment to be imposed (by drying the air), while not at the same time creating a number of other, confounding, micrometeorological differences experienced between a bagged and an un-bagged fruit – differences in temperature, insolation and air flow (to name the dominant ones likely to affect fruit).

Dry weight, potassium and calcium accumulation

Microclimatic condition (e.g. light, temperature, *VPD*) may affect functional and metabolic parameters/processes of fruit including antioxidant content, enzyme activities, transpiration and mineral composition (Montanaro et al., 2006; Léchaudela et al., 2013). However, with respect to the effect of *VPD* on nutrients accumulation it would ultimately depend on the within-plant mobility of the nutrients. For example, K is primarily a phloem mobile, with K^+ being the dominant cation in phloem sap in most plants, contributing to phloem sap osmolarity about equally with sucrose (Patrick, 1997). Thus any putative reduction in K delivery to the fruit in the xylem sap as a result of *VPD*-induced reduced transpiration (and thus reduced xylem influx) would be masked by the delivery of K in the phloem sap. This would explain the minor effects of low *VPD* on fruit K compare to Ca (phloem immobile) (Fig. 5) as already documented for apple fruit (Tromp and Van Vuure, 1993).

Over time different transpiration affected fruit dry weight accumulation which was in L_{VPD} treatment significantly 8% lower than that of H_{VPD} treatment (see older fruit in Fig. 4). Similar reduction is reported for low-transpiring apricot and peach fruit (Montanaro et al., 2010; Morandi et al., 2010b). In the present study, differences in dry matter content could not be attributable to temperature-induced increased respiration (Walton and De Jong, 1990) because temperature was similar in both treatments (see inset of Fig. 2). To explain low dry matter content in low-transpiring peach fruit, it has been evoked the reduction of fruit sink for carbohydrates which in turn limited net photosynthesis of leaves surrounding the fruit (Li et al., 2001). That reduced sink strength was associated to the reduced enzymes activity due to the plastic-induced increased temperature of the fruit (Li et al., 2001). This would not be the case with the present experiment since both H_{VPD} and L_{VPD} were bagged and, again, temperature was not affected by treatment. The mass balance model may be useful to track phloem unload and in turn dry matter accumulation. The model reveals that although a tenuous greater phloem fraction in H_{VPD} fruit could be envisaged in older fruit (Fig. 6), phloem unload was substantially unaffected by different *VPD* which fits with fluxes measured in kiwifruit and tomato (Guichard et al., 2005; Morandi et al., 2012). To explain differences in dry matter accumulation both bulk and passive phloem unload (*sensu* Patrick 1997) would be evoked. Considering that fruit transpiration is proportional to

fruit surface conductance and to a driving force (e.g. difference between water vapour inside the fruit and the surrounding air) (Montanaro et al., 2012), assuming that changed *VPD* did not affect fruit conductance, the higher fruit transpiration (Fig. 3) conceivably induced a lower turgor and more negative water potential in H_{VPD} fruit which may have increased bulk phloem unload and in turn assimilates partitioning (Lang and Thorpe 1986). The higher dry matter content in highly transpiring fruit (Fig. 6) could be also attributable to increased phloem sap sugar concentration to help to maintain the water potential gradient and turgor in sieve tube (Cernusak et al., 2003), however further efforts are required to test it.

The general plausibility of the idea that Ca import by a transpiring organ might be proportional to its transpiration suggests that the more xylem water passes into an organ, the more Ca will be left behind as it evaporates. The partitioning of fruit fluxes of high-transpiring fruit over a relatively long time period (i.e. 70-157 days) shows a close correspondence between transpiration and xylem stream (Fig. 6) as reported for daily flows in fruit of similar age (Morandi et al., 2012). Accordingly, Ca accumulation rises roughly linearly (R^2 0.71) with increasing fruit transpiration behind ~60 g H_2O per fruit, however, transpiration seems to be ineffective in driving Ca at a lower range namely 5-60 g H_2O (Fig. 5). In that lower range, Ca did not increased with increasing transpiration and its variability (from 6 to 30 mg H_2O f^{-1}) remained within that observed at field scale (Ferguson et al., 2003). This observation is in line with White and Broadley (2003) who stress that there is a close positive relationship between transpiration and Ca delivery to a transpiring organ when transpiration is high.

Fruit water budget and uncoupled Ca

The uncertainty of the effect of *VPD* and in turn of transpiration on Ca level in fruit was highlighted in apple by Tromp and Van Vuure (1992 and reference therein) who postulated the *"absence of any clear humidity effect on Ca influx into fruits"* because of opposing results. Pooled data covering a relative wide range of cumulative transpiration (from ~5 up to 95 g) confirms that Ca accumulation does not bear a simple linear relationship to cumulative transpired water (Fig. 7). That distribution shows an initial lag phase during which increasing fruit transpiration does not proportionally increase Ca content. A similar result was previously documented by Dichio et al. (2007) focusing the effect of windspeed on Ca accumulation in kiwifruit. In that study fruit Ca remained

almost constant despite an increased windspeeds (and presumably transpiration) till a certain threshold, Ca rose in response to wind only when windspeeds blow >1.5 m s^{-1}.

Explanations for that non-linear dependency have been indirectly proposed in several fruit species evoking so called non-transpirational (or transpiration-independent) Ca transport mechanisms. For example, the 'metabolic Ca demand' and the chemical aspects of the conductive tissues (e.g. adsorption and desorption along the walls of the xylem pathway) and the mutual relationship between polar basipetal auxin transport and acropetal Ca transport (Stahly and Benson, 1970; Bangerth, 1976 and 1979; Banuelos et al. 1987; Cutting and Bower, 1989; Pomper and Grusak, 1997; McLaughlin and Wimmer, 1999). Also the action of specialised transfer cells and the higher expression of Ca transporters and Ca binding proteins may promote accumulation of Ca into fruit independently of its transpiration (Pate and Gunning 1972; Park et al., 2005).

The present study was not design to ascertain any of these transpiration-independent mechanisms, however the mechanistic approach sitting behind the fruit water budget analysis used to track the destiny of xylem stream (i.e. transpiration or growth) may help to explain that poor Ca:transpiration relationship. That is, at unrestricted transpiration the evaporative destiny of xylem stream was close to 80-100% (Fig. 6) allowing the transpiration to be coupled to Ca accumulation as the roughly good linear relationship documents (R^2 = 0.71) (Fig. 5). According to the initial hypothesis, under reduced *VPD* the destiny of the xylem flow would change in favour of fruit growth leading to the reduction of the evaporative fraction. Indeed, the water budget analysis show that the fraction of water sourced by xylem and lost through transpiration in low-transpiring fruit declined to approx. 40-55% (Fig. 6). A similar 50% reduction of the evaporative destiny of the daily water entering the fruit upon reduced *VPD* has been observed in low-transpiring tomato fruit (Leonardi et al., 2000; Guichard et al., 2005) who changed *VPD* through misting technique. Reduction of the evaporative destiny at lowered *VPD* agreed with previous observation in kiwifruit (Morandi et al., 2010a). Clearwater et al. (2012) compared over a 30-day period the effect of microclimate (dry or humid) of the growing environment on water budget in a ripening fruit of a closely related species (*A. chinensis*) showing that at the wetter environment transpirational water losses were a less dominant feature of the water balance. It is unfortunate that in these papers the physiological implication of changed water budget on fruit Ca

accumulation was not examined. The present study was designed to test the whole causal chain *VPD→transpiration→Ca*, and results gained allow the conclusion that at low transpiration the slow movement of xylem sap into the fruit mainly sustains fruit growth rather than the evaporative flux, this *de facto* makes transpiration uncoupled to Ca accumulation.

The main fruit quality traits focussed by carbon and water balance models relate to size, dry matter and sugars content, and fraction of edible tissue sometimes under contrasting environmental (temperature, air vapour pressure deficit) and plant conditions (fruit load and fruit position) (Lescourret and Génard 2005, Liu et al., 2007; Clearwater et al., 2012). In that context, the importance of water potential, osmotic potential and turgor pressure of fruit and environmental conditions as driving force of water import have been emphasized along with variations of hydraulic resistance of fruit and stalk and skin conductance (Génard et al., 2007; Montanaro et al., 2012; Mazzeo et al., 2013). However, modelling of water transport and transpiration remains highly desirable because of its significance on mineral composition (Génard et al., 2007). The present study enhances our understanding of the physiology of Ca accumulation by fruit in relation to its transpiration focussing a variable destiny of the xylem stream depending on the level of transpiration. The evidence that in low-transpiring fruit increasing cumulative transpiration did not induced any significant increase in Ca accumulation (Fig. 7) -which remained within the vine-to-vine variability- reveals Ca accumulation to be uncoupled to transpiration. While in high transpiring fruit Ca accumulation became proportional to fruit water loss because amount of xylem stream and its evaporative destiny tend to converge. This information could be supportive for the implementation of virtual fruit models accounting also for mineral compositions.

Within the frame of fruit mineral transport in responses to environmental variables, results gained in this paper will apply to most fruits (apples, tomatoes, capsicum, grapes, avocado etc.) since most suffer Ca deficiency disorders (Cutting and Bower, 1989; Ferguson, 1989; Witney et al., 1990; Saure, 1996; Ho and White, 2005) and grow in changing environments with variable *VPD*. Results could open up the reinterpretation of certain techniques suggested at field scale to improve fruit quality. For example, summer pruning is aimed at naturally increase Ca content through increased fruit transpiration (Gerasopoulos and Drogoudi, 2005; Montanaro et al., 2014), however it

could be envisaged that such practice would be less effective in higher humidity environment having low *VPD* where Ca accumulation is expected to be uncoupled from transpiration.

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633 **Figure legends**

634 **Fig. 1.** Schematic and photograph of the bag treatment. The temperature and relative
635 humidity probe was shielded by a plastic tube open at the bottom (dashed cylinder).
636 Note that the silica gel sachets were loaded only into the high *VPD* treatment bags.

637 **Fig. 2.** Vapour pressure deficit (*VPD*, kPa) of the air surrounding fruit under (A) High
638 and (B) Low *VPD* conditions throughout the experiment. In the inset (panel B) are the
639 daily pattern of relative humidity (RH, %) and temperature (°C) of the air surrounding
640 bagged fruit under high and low *VPD* conditions over an arbitrary five-day period (grey
641 strip), bag replacement occurred at times indicated by the arrows.

642 **Fig. 3.** Variation of (A) daily fruit transpiration (g H_2O f^{-1} d^{-1}) and (B) cumulative fruit
643 transpiration (g H_2O f^{-1} d^{-1}) measured (bag weight gain) for bagged fruit grown under
644 low (○) and high (●) *VPD* conditions. Each point is the mean (±SE) recorded for 50 (10-
645 67 DAFB), 30 (68-130 DAFB) and 10 (157 DAFB) fruits. Comparing values for high
646 and low *VPD* over the same period * indicates significant differences (Student's *t*-test,
647 *P*=0.001).

648 **Fig. 4.** Concentration of (A) Ca and (B) K (%DW), and (C) dry weight (g f^{-1}) measured
649 in fruit grown under low and high *VPD* conditions at three times during the season. Bars
650 are SE, DAFB = day after full bloom; comparison of treatments at the same time (*)
651 indicates significant differences (Student's *t*-test, $P = 0.05$).

652 **Fig. 5.** Relationships between cumulative transpiration (g H_2O f^{-1}) and (A) K and (B)
653 Ca content (mg f^{-1}) under low (○) and high *VPD* (●) conditions.

654 **Fig. 6.** Water budget (g f^{-1}) partitioning in fruit grown under low and high *VPD*
655 identifying source (X=xylem, P= phloem) and destiny (G=growth, T=transpiration) of
656 water fluxes. The inset shows the transpiration as % of xylem (±SE). Comparison of
657 treatments at the same time and for the same water pattern * indicates significant
658 differences, n.s. not significant (Student's *t*-test, $P = 0.05$).

659 **Fig. 7.** Relationship between Ca and cumulative fruit transpiration highlighting the
660 dominance of filling or evaporative destiny of xylem flow; data are redrawn from Fig.
661 5-B.

Figure 1 revised B/W

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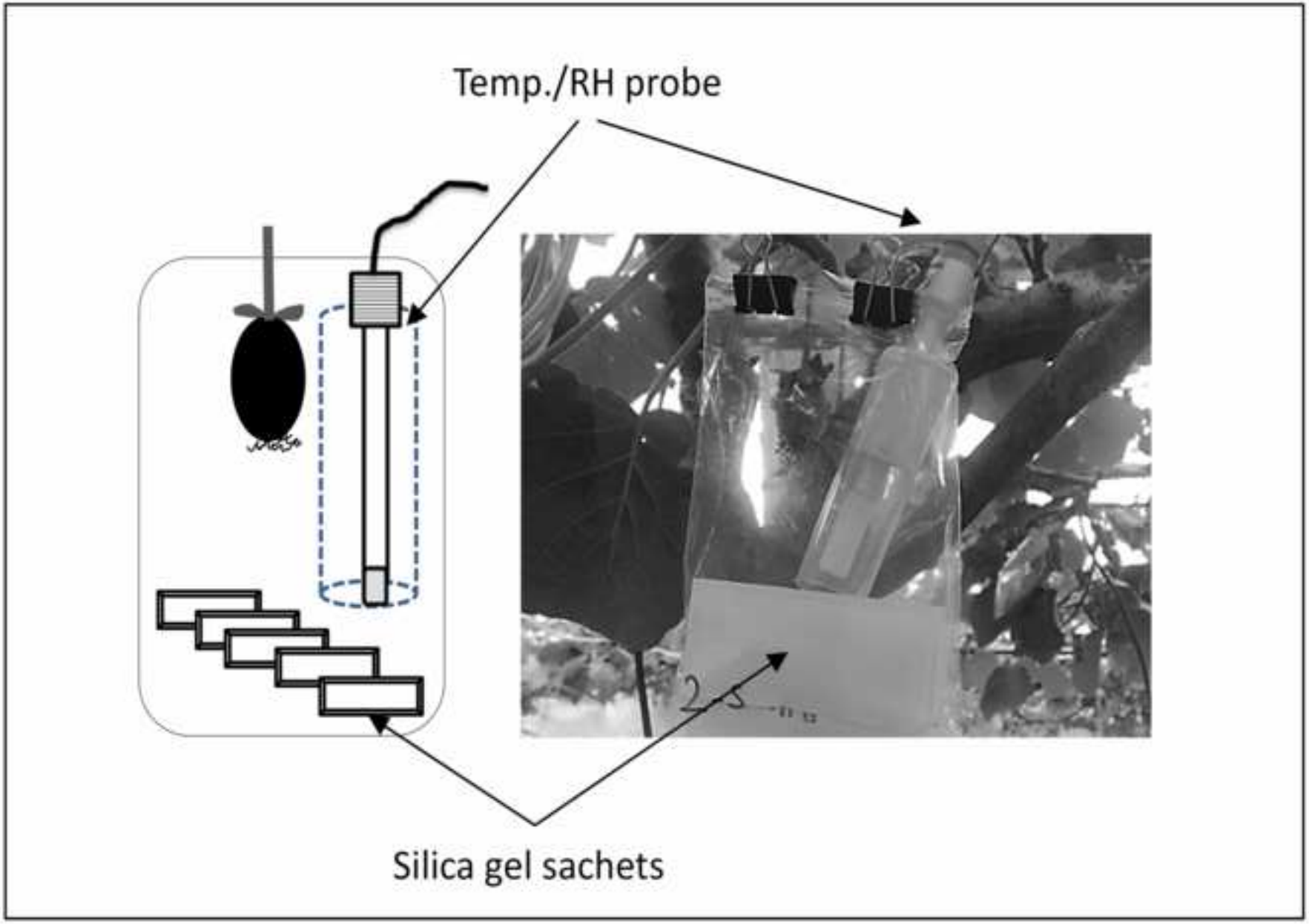

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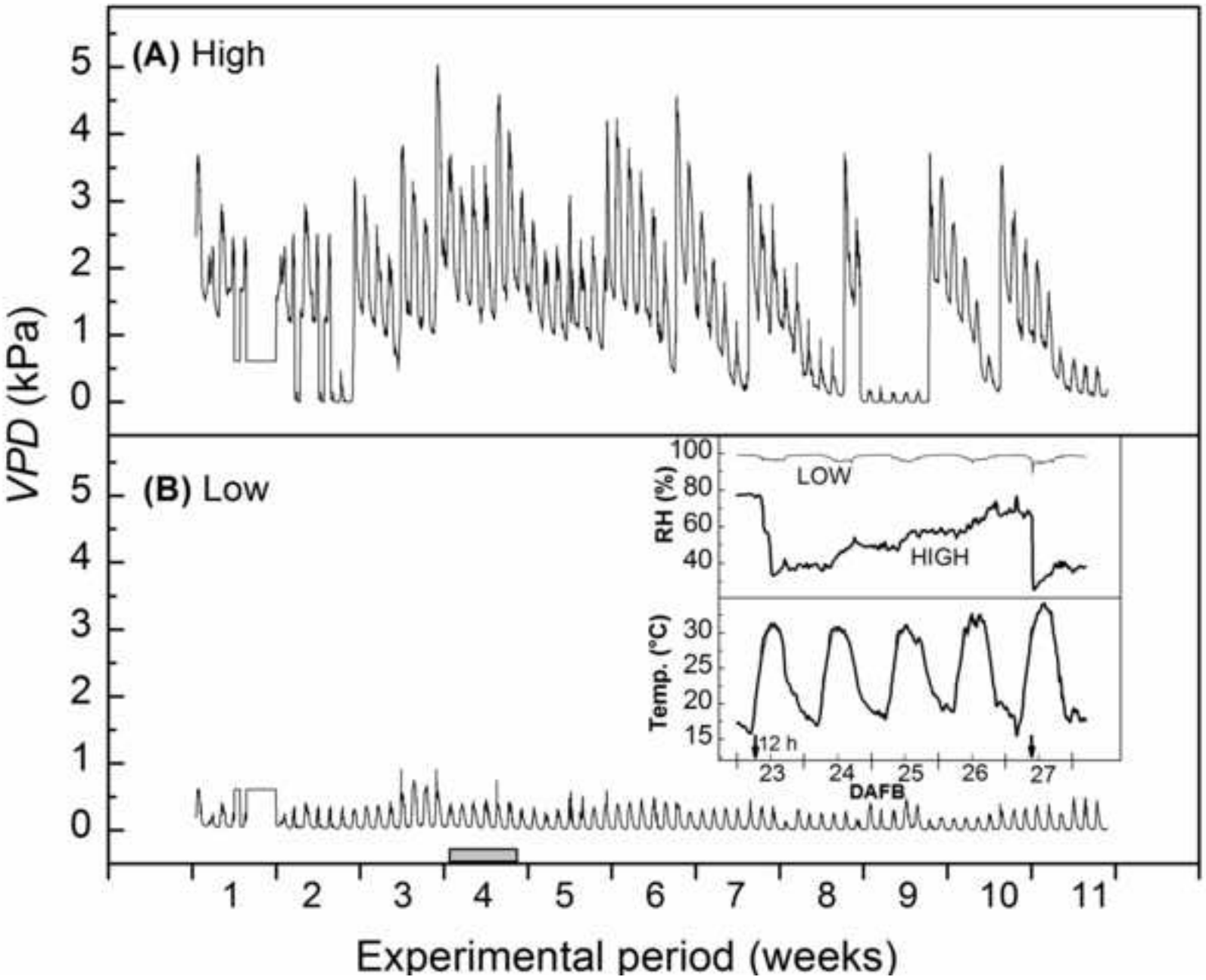

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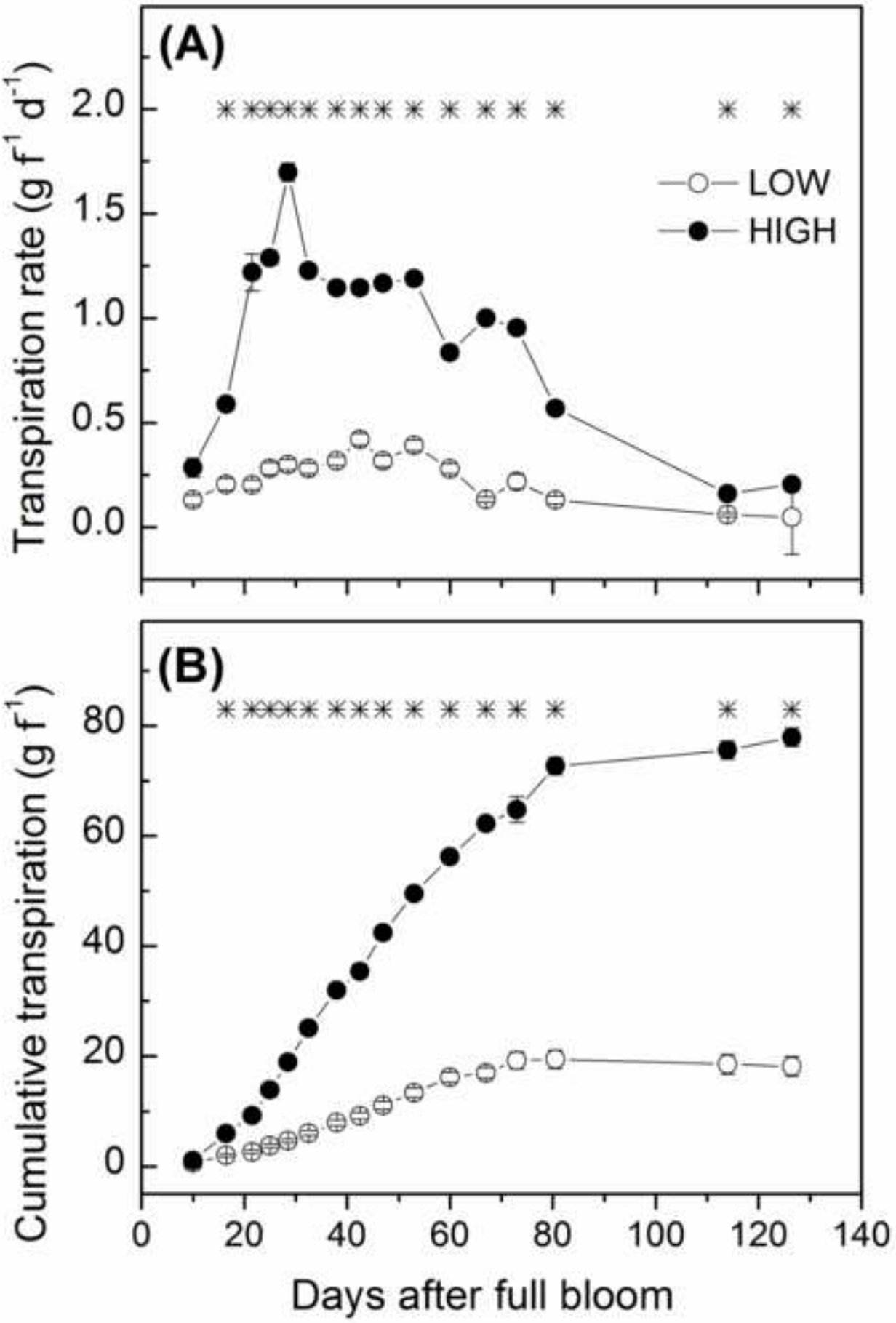

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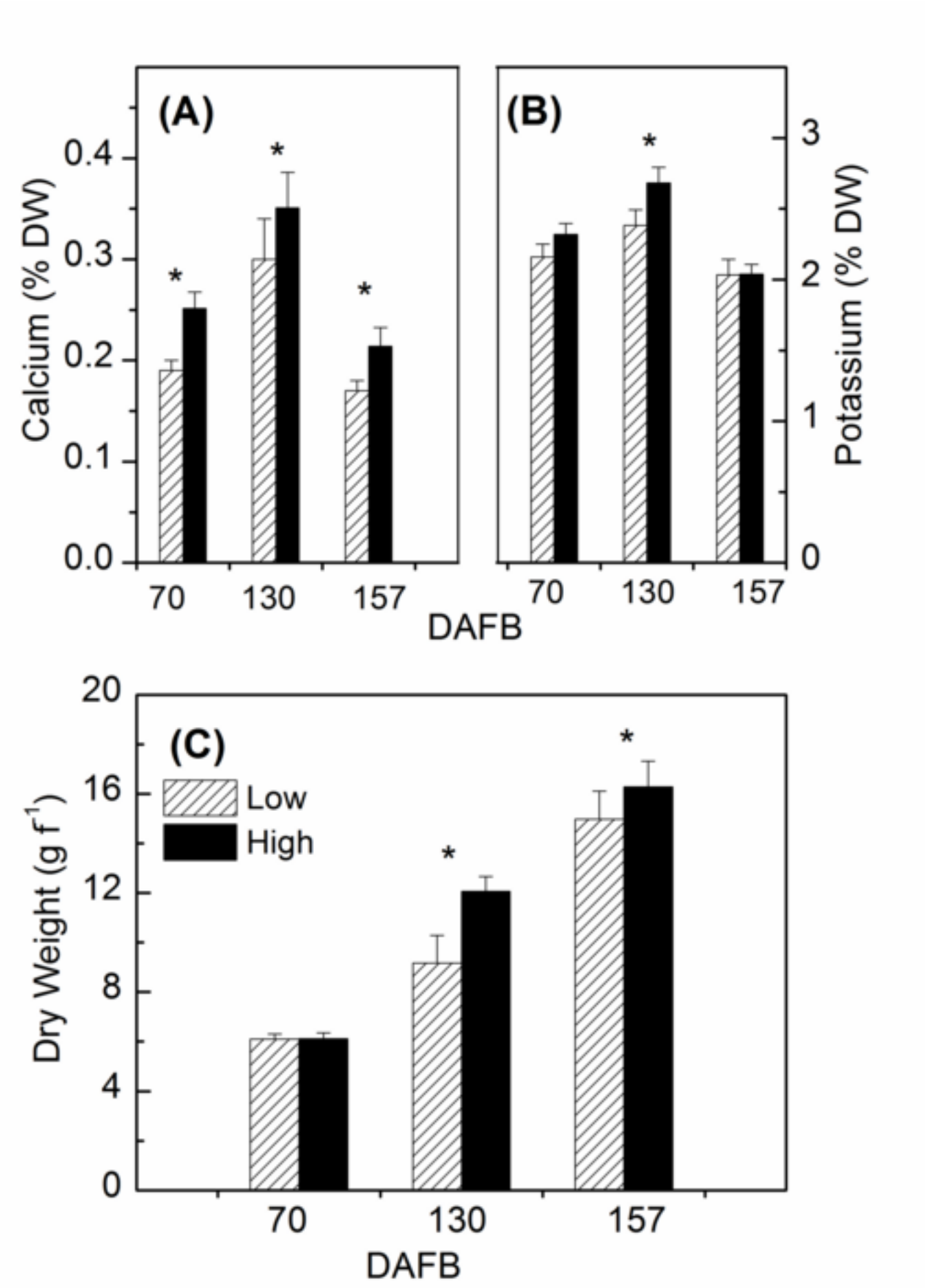

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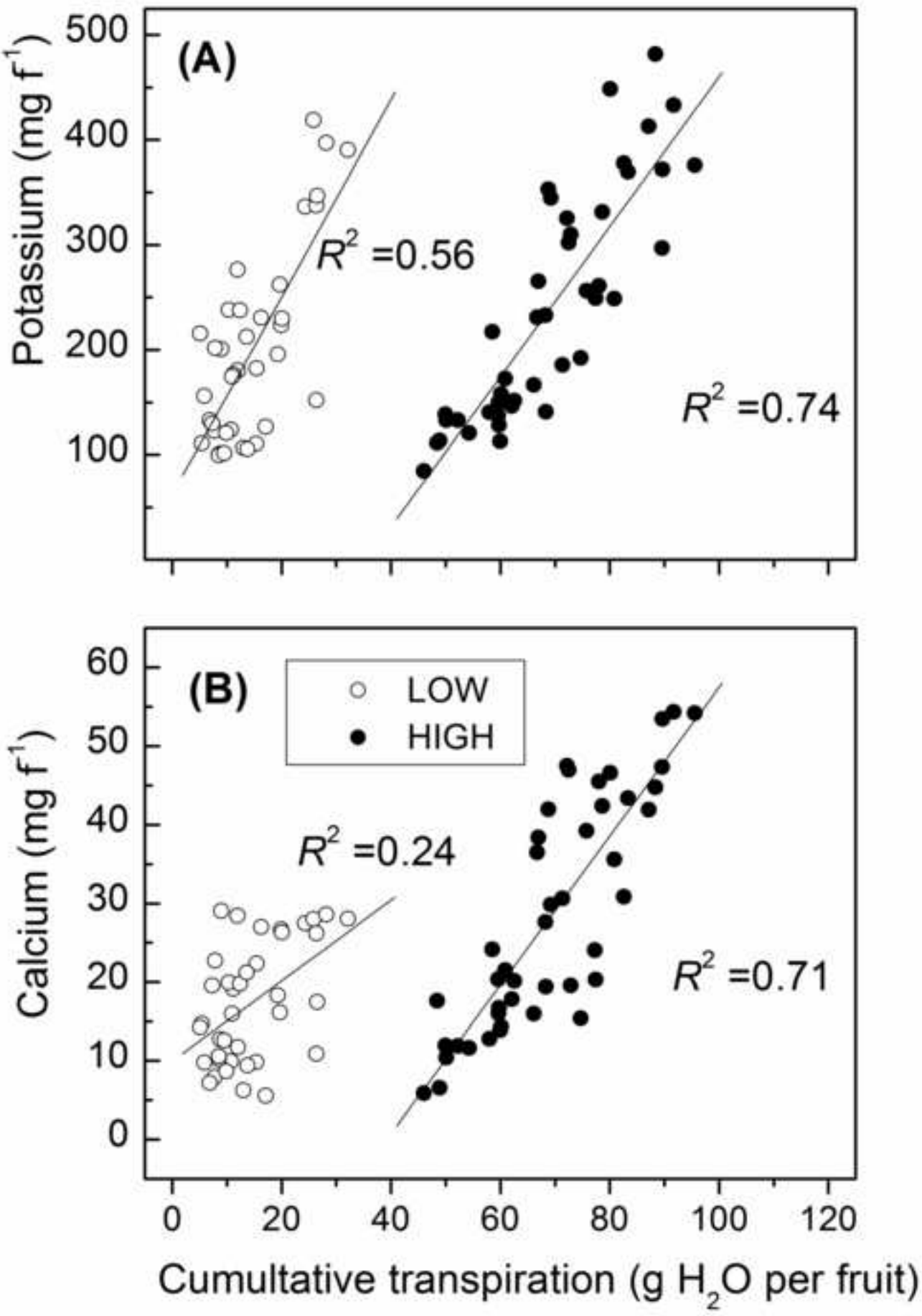

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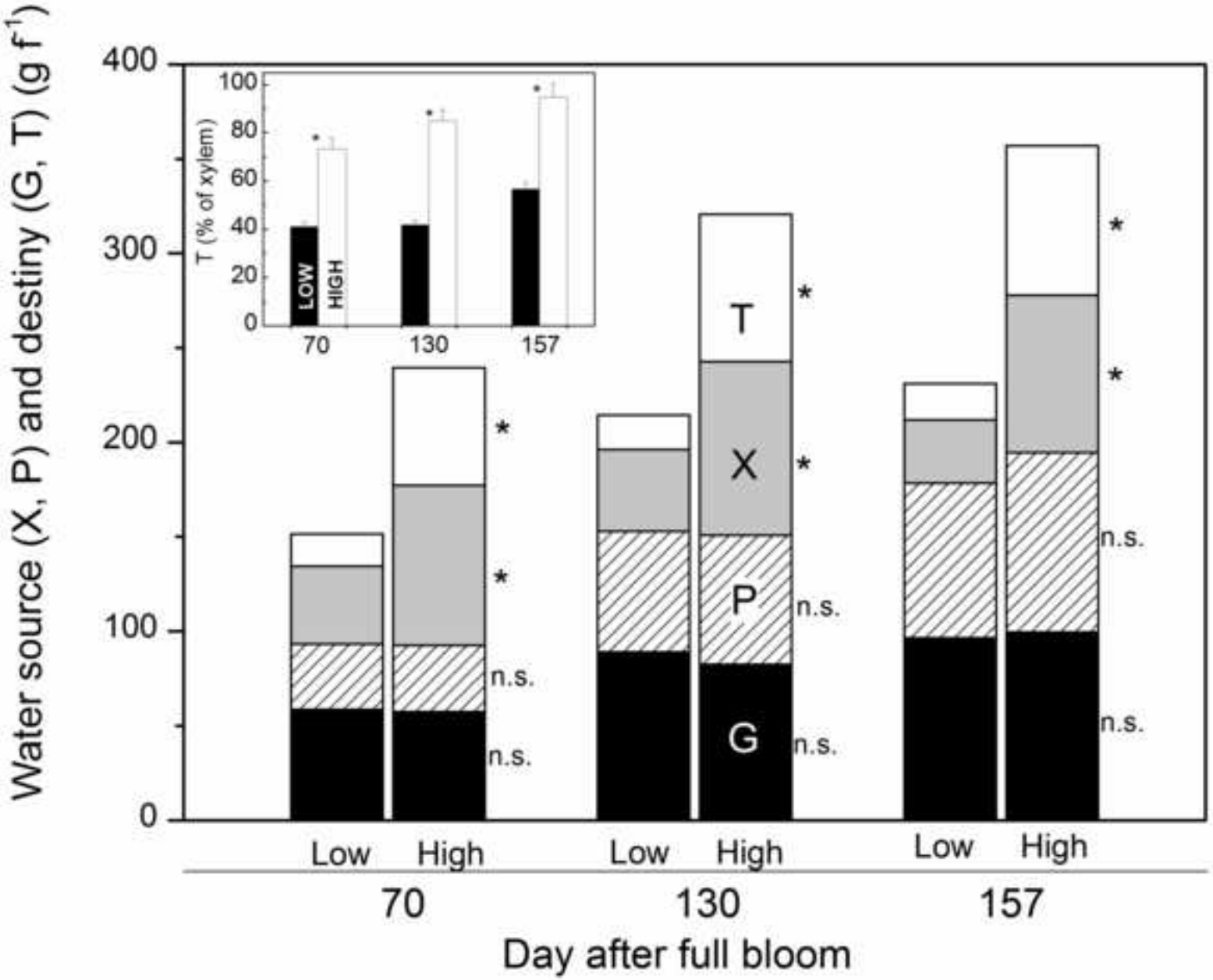

Figure 7

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