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Abstract The negative effects of fruit production during one cycle on reproduction during the following cycle are generally explained by two complementary processes: hormone synthesis and carbohydrate mobilization. Our study focused on mango (*Mangifera indica* L.) for which it has been shown that reproduction decreases and delays vegetative bud outgrowth. This, in turn, affects flowering and fruiting in the following cycle. Vegetative growth therefore plays a pivotal role in irregular fruit production patterns across consecutive years. Our aim was to decipher the respective roles of hormones and carbohydrates on the negative effects of reproduction on vegetative growth. We analyzed the changes in various hormone (auxin, cytokinin, abscisic acid) and carbohydrate (glucose, sucrose, starch) concentrations in terminal axes with vegetative and reproductive fates of two mango cultivars, Cogshall and José, characterized by different bearing patterns across consecutive phenological periods during a growing cycle. Auxin concentrations were high in inflorescences, fruit peduncles and axes bearing inflorescences or fruit, suggesting auxin-induced inhibition of vegetative bud outgrowth in the flowering and fruiting axes. Moreover, growing fruits, which are strong sink organs, depleted carbohydrates from non-fruiting axes. During vegetative growth, this starch depletion probably contributed to decreasing the probability of and to delaying vegetative bud outgrowth of reproductive axes for Cogshall, and of reproductive and non-reproductive axes for José. Starch dynamics in quiescent and flowering GUs during early fruit growth and their starch concentrations at fruit maturity differed between the two cultivars, presumably in relation to the observed contrasted crop loads and/or to differences in photosynthetic capacity or carbohydrate allocation. These differences between the two cultivars in terms of starch concentration in terminal axes during vegetative growth could partly explain their different bearing patterns.

Abbreviations: ABA: abscisic acid; CK: cytokinin; IAA: indole-3-acetic acid; ZR: zeatin-riboside.

Introduction

Heavy fruit load reduces flowering and fruiting in the following year in several fruit tree species (Muñoz-Fambuena et al. 2011, Guitton et al. 2012, Samach and Smith 2013). This phenomenon is referred to as alternate or irregular bearing, depending on the bearing pattern over consecutive years (Monselise and Goldschmidt 1982, Goldschmidt and Sadka 2021). This could be a direct effect of current fruit load on floral induction when the latter occurs during fruit growth, i.e., in most temperate fruit trees (Chan and Cain 1967, Wilkie et al. 2008, Samach and Smith 2013). However, it could also be an indirect effect through vegetative growth (Wilkie et al. 2008, Smith and Samach 2013, Capelli et al. 2016), as explained below. This indirect effect is more likely to occur in species for which floral induction is not concomitant with current fruit growth, such as tropical evergreen species like the mango tree, *Mangifera indica L.* (Ramírez and Davenport 2010).

Reproduction has negative effects on vegetative growth in various temperate and tropical fruit tree species (Lauri and Térouanne 1999, in apple; Costes et al. 2000, in apricot; Berman and DeJong 2003 in peach; Lovatt 2010 in avocado; Capelli et al. 2016, in mango), with a cultivar-dependent effect (Capelli et al. 2016, Normand et al. 2016). On the other hand, flower and fruit production is positively related to the quantity of previous vegetative growth (Lovatt 2010, Capelli et al. 2016, Normand et al. 2016). Moreover, the structural (Lauri and Trottier 2004, Normand et al. 2009, Gaaliche et al. 2011) and/or temporal (Wilkie et al. 2008, Dambreville et al. 2013) characteristics of vegetative growth may affect flowering. These findings on mango as well as on some other species (Hasegawa and Takeda 2001, in forest trees; Lauri and Trottier 2004, in apple) strongly support the idea that vegetative growth

between two consecutive reproductive periods plays a pivotal, but insufficiently known, role in irregular bearing.

Previous results on mango highlight three negative effects of reproduction on the outgrowth of lateral vegetative buds, leading to less vegetative growth: (1) Inflorescence and fruit inhibit lateral bud outgrowth as long as they are present on the bearing axis (Singh and Singh 1969); (2) Flowering and fruiting decrease the probability of vegetative bud outgrowth at the axis scale compared to quiescent axes (Capelli et al. 2016); and (3) Fruiting delays vegetative bud outgrowth when it occurs (Dambreville et al. 2013). Two hypotheses, possibly complementary, are generally put forward to explain the negative effects of high fruit load on vegetative growth: (1) hormone synthesis by the reproductive organs that inhibit vegetative growth; and (2) carbohydrate mobilization by fruit, which reduces their availability for vegetative growth (Smith and Samach 2013, Martinez-Alcántara et al. 2015). The aim of this study was to simultaneously explore these two hypotheses in order to better understand the three above-mentioned negative effects of reproductive organs on vegetative bud outgrowth in the mango tree.

The hormonal hypothesis holds that the reproductive parts of the plant produce hormones that negatively affect vegetative growth. The most extensively studied hormone is auxin (IAA). Inflorescences and seeds growing in fruits have been reported to produce a basipetal auxin flux (Friml and Palme 2002, Wolbang et al. 2004) that inhibits the outgrowth of lateral buds located lower down on the axis (Ferguson and Beveridge 2009, Waldie et al. 2010, Smith and Samach 2013) in the same way that apical dominance does (Cline 1994, Wilson 2000). Other hormones may also play a role in vegetative bud outgrowth. Abscisic acid (ABA) is produced by roots, leaves and seeds. It can negatively affect vegetative development in response to various environmental stresses (Seo and Koshiba 2002), and likewise contributes to the inhibition of bud outgrowth (Knox and Wareign 1984, Shimizu-Sato and

Mori 2001, Nguyen and Emery 2017). This hormone is also reported to induce flowering in several fruit species in which its content transiently increases at this phenological stage (Sheng-Hui et al. 2011, in pineapple; Li et al. 2021, in apple). It is also involved in fruit ripening (Davies 2010, Zaharah et al. 2012). In contrast, cytokinins (CKs), produced by roots and axes, stimulate vegetative bud outgrowth (Davies 2010). As a whole, it is very likely that vegetative growth is affected by the interplay of these various hormones with, for example, the inhibition of local cytokinin biosynthesis in the axes by auxin (Tanaka et al. 2006, Ferguson and Beveridge 2009).

The carbohydrate hypothesis holds that high fruit load depletes a large proportion of the carbohydrates, locally in branches and/or at the whole tree scale (Smith 1976 in 'Murcott' mandarin; Goldschmidt and Golomb 1982, in 'Wilking' mandarin; Van Vuuren et al. 1997, in avocado; Normand et al. 2006, in mango; Spann et al. 2008, in pistachio), that are required for further vegetative growth (Goldschmidt and Golomb 1982, Goldschmidt 1999, Zapata et al. 2004, Rohla et al. 2007).

Based on these results, we could expect that both hormones and carbohydrates negatively affect vegetative bud outgrowth in terminal mango axes, depending on their reproductive or quiescent fate and on the following sequence. During vegetative rest, we could anticipate a high ABA concentration in quiescent axes in relation to the lack of irrigation and water stress. During flowering and fruit growth, we could expect a high IAA concentration in the inflorescence-bearing axes and in the fruit-bearing axes, probably synthesized by these organs. We could also expect a decrease of carbohydrate concentration, more pronounced in fruit-bearing axes than in inflorescence-bearing and non-reproductive axes. After harvest, we can assume that carbohydrate concentration increases in terminal axes, with higher concentration in non-reproductive axes that were less depleted during fruit growth. IAA concentration would drop after flowering in the flowering axes that did not set fruit, and after

harvest in fruit-bearing axes. ABA concentration would remain higher in quiescent axes than in other axes with, however, an increase or relatively high concentration in flowering axis during flowering and in fruiting axes at the end of fruit development. CK concentration could then increase in these axes due to the removal of CK synthesis inhibition by IAA. If we assume a positive role of carbohydrates and CKs, and a negative role of IAA and ABA on vegetative bud outgrowth, this sequence could then explain the three negative effects of reproduction on vegetative bud outgrowth in the mango tree presented above.

To explore these expected effects, we examined the dynamics of hormone and carbohydrate concentrations in relation to vegetative and reproductive phenology in the terminal axes of two mango cultivars, Cogshall and José, characterized by different bearing patterns. Cogshall is an irregular cultivar and José is an alternating cultivar. These patterns resulted from contrasted negative effects of the reproductive effort on the vegetative growth of the axes (Capelli et al. 2016).

Materials and methods

Plant material, experimental design and data collection

The experiment was carried out in two orchards located at the CIRAD (French Agricultural Research Center for International Development) research station in Saint-Pierre, Reunion Island (21°19'21 S, 55°29'25 E). The first orchard was composed of ten mango cultivars, with 13 trees per cultivar, including ‘José’, grafted onto the polyembryonic rootstock ‘Maison Rouge’, and planted in October 2007. The second orchard was composed of 153 trees of the cultivar ‘Cogshall’, grafted onto the polyembryonic rootstock ‘Maison Rouge’, and planted in May 2004. ‘Cogshall’ is a Floridian cultivar with an irregular bearing trend, which represents about 25% of the mango production in Reunion Island. ‘José’ is a local cultivar with an alternate bearing trend (Capelli 2017). It represents more than 50% of the mango production

in Reunion Island (Vincenot 2004, Hoarau et al. 2009). The fruit production of individual trees was recorded each year in the two orchards. An automatic weather station close to the orchard recorded temperature and rainfall (Figures 1A and B).

The scale of study was the growth unit (GU), the portion of the axis developed during an uninterrupted period of growth (Hallé and Martin 1968, Barthélémy and Caraglio 2007). In particular, we considered terminal GUs, i.e., GUs in the distal position on the axes, which may bear inflorescences, fruits, or future GUs during the following period of vegetative growth.

The experiment was carried out at five dates, from June 2014 to April 2015, corresponding to five phenological periods (Figure 1D): vegetative rest period (VR, in June); flowering (F, in September); mid-fruit growth (MFG, in November); harvest, i.e., fruit maturity (FM, in January); and vegetative growth (VG, in April). Vegetative growth is the period during which new GUs appear in the trees. Vegetative growth may begin at the end of flowering or during fruit growth, but it mainly occurs after harvest during the hot and rainy season. Flowering is terminal in the mango tree. Inflorescences develop from the apical bud and possibly from the lateral buds of the terminal GUs. Only some inflorescences set fruits. Mango fruit growth lasts for about 4 months, from inflorescence flowering to fruit maturity (Joas and Léchaudel 2009; Figure 1C).

During each phenological period, the distal part of terminal GUs with four different fates (quiescent, flowering, fruiting and fruiting with fruit removal at mid-fruit growth; Figure 1E) was sampled. Quiescent GUs were terminal GUs that had no vegetative or reproductive activity during the experiment. They were sampled during the five phenological periods. Flowering GUs corresponded to terminal GUs that set one or several inflorescence(s) during flowering but did not set fruit. They were sampled during four phenological periods, from flowering onward. After flowering, these GUs had no more inflorescences but were still

sampled as long as they did not produce new GUs. Fruiting GUs corresponded to terminal GUs that flowered and set fruit. They were sampled during three phenological periods, from mid-fruit growth onward. During vegetative growth, fruiting GUs that bore one or more fruits several weeks before were sampled, although fruit was no longer present on these GUs. Fruiting GUs with fruit removal corresponded to GUs that flowered and set fruit, and where the fruit was removed at mid-fruit growth. They were sampled during three phenological periods of the experiment, from mid-fruit growth (10 days after fruit removal), to vegetative growth (five months after fruit removal). The purpose of this manipulation was to examine the rapid changes (10 days) in hormones and carbohydrates in the GUs when fruit was removed at mid-fruit growth.

We labeled all the necessary flowering GUs at flowering and all the necessary fruiting GUs at mid-fruit growth on five trees per cultivar in order to be able to sample GUs with the different fates identified up to the last sampling date when reproductive organs were no longer present on GUs. At each sampling date, three GUs per fate (Figure 1) were sampled on five trees for hormone analyses, and on three out of these five trees for carbohydrate analyses. Leaves were removed from the GU stem. Several organs were also sampled for hormone analyses when they were present on the GUs: inflorescences, inflorescence axes bearing the fruit, referred to as 'peduncles' hereafter, fruit pulp and seeds. All samples were immersed just after sampling in liquid nitrogen and freeze-dried. They were then finely ground (< 1 µm) using bowls previously immersed in liquid nitrogen, and stored in airtight pillboxes at −20°C. They were sent to the Institut Jean-Pierre Bourgin in Versailles, France (INRAE/AgroParisTech – UMR 1318; http://www-ijpb.versailles.inra.fr/fr/plateformes/Observatoire-du-vegetal.html) for hormone analysis, and to the INRAE Research Center in Avignon, France (PSH unit; http://www6.paca.inra.fr/psh) for carbohydrate analysis.

Hormone analysis

For each sample, 10 mg of dry powder were extracted with 0.8 mL of acetone/water/acetic acid (80/19/1 v/v/v). Stable labeled isotopes of abscisic acid (ABA), zeatin-riboside (ZR, an active form of cytokinin (CK)), and indole-3-acetic acid (IAA; an active form of auxin), used as internal standards, were prepared as described in Le Roux et al. (2014). A quantity of 2 ng of each standard was added to the sample. The extract was vigorously shaken for 1 min, sonicated for 1 min at 25 Hz, shaken for 10 min at 10°C in a Thermomixer (Eppendorf®), and then centrifuged at 8.00 g and 10°C for 10 min. The supernatant was collected and the pellet was re-extracted twice with 0.4 mL of the same extraction solution, then vigorously shaken (1 min) and sonicated (1 min; 25 Hz). After centrifugation, the three supernatants were pooled and dried. Each dry extract was dissolved in 100 µL of acetonitrile/water (50/50 v/v), filtered, and analyzed using a Waters Acquity ultra-performance liquid chromatograph coupled to a Waters Xevo Triple quadrupole mass spectrometer TQS (UPLC-ESI-MS/MS). The compounds were separated on a reverse-phase column (Uptisphere C18 UP3HDO, 100*2.1 mm*3 µm particle size; Interchim, France) at a flow rate of 0.4 mL min^{-1} and a binary gradient: (A) 0.1% acetic acid in water (v/v), and (B) acetonitrile with 0.1% acetic acid; the column temperature was 40°C. We used the following binary gradient (time, %A): (0 min, 98%), (3 min, 70%), (7.5 min, 50%), (8.5 min, 5%), (9.6 min, 0%), (13.2 min, 98%), (15.7 min, 98%). Mass spectrometry was conducted in electrospray and Multiple Reaction Monitoring scanning mode (MRM mode), in positive ion mode for the IAA, and in negative ion mode for the other hormones. Relevant instrumental parameters were set as follows: capillary 1.5 kV (negative mode), source block and desolvation gas temperatures: 130°C and 500°C, respectively. Nitrogen was used to assist the cone and desolvation (150 L h^{-1} and 800 L h^{-1}, respectively), argon was used as the collision gas at a flow rate of 0.18 mL min^{-1}. The

IAA quantification threshold in GUs was 45.85 ng g^{-1} at rest and flowering, 34.40 ng g^{-1} at fruit growth and harvest, and 22.90 ng g^{-1} at vegetative growth. The ABA quantification threshold in GUs was 46.60 ng g^{-1} at rest and flowering, 44.57 ng g^{-1} at fruit growth, 61.39 ng g^{-1} at harvest, and 0.21 ng g^{-1} at vegetative growth. In inflorescences and peduncles, quantification thresholds were 26.34 ng g^{-1} for IAA and 0.31 ng g^{-1} for ABA. In fruit pulp and seeds, quantification thresholds were 9.86 ng g^{-1} for IAA and 16.50 ng g^{-1} for ABA.

Carbohydrate analysis

Before analysis, sample powder was dried at a moderate temperature (50°C) in an oven and then brought back to room temperature in a desiccator before the assay samples were weighed (Gomez et al. 2007). The first step was the extraction and purification of soluble components. Subsequently, the enzymatic assay method with microplates (MP) was used. Samples were diluted to obtain an appropriate final sugar concentration that was inclusive of the calibration standard (0 to 0.066 g L^{-1} for each soluble sugar, glucose, fructose and sucrose). Enzymatic reactions were directly performed in each MP well. Glucose, fructose and sucrose concentrations were successively quantified by measuring the production of NADH. The enzyme produced phosphoglucose from phosphofructose, and the resulting production of NADH in the presence of G6PDH was proportional to the initial fructose content in the extract. Sugar molecular weights were used to calculate the initial sucrose concentration from the glucose produced (Gomez et al. 2007). Starch was analyzed using a method adapted from that described by Gomez et al. (2007). After dispersing starch by autoclaving (2b, 120°C, 2 h), starch was hydrolyzed into glucose with amyloglucosidase (6 IU/tube) in a water bath (56°C, 1h30). Glucose was determined in the supernatant using the previous MP method (Gomez et al. 2007).

Data analysis

For a given date and GU fate, a sample was composed of the three GUs sampled per tree. The sample number was therefore the number of sampled trees, i.e., n=5 for hormone analyses and n=3 for carbohydrate analyses. One-way analysis of variance followed by Tukey's comparison of means test was used to separately analyze the effects of the three factors, GU fate, sampling date and cultivar, on hormone and carbohydrate concentration at the GU scale. The significance level was set at P=0.05. Statistical analyses were performed with R software (R Core Team 2015).

Results

Fruit production of the sampled trees

Except for six Cogshall trees that had a low production (< 20 kg.tree^{-1}) and were sampled at flowering, crop load was high on the sampled trees during the 2014-2015 cycle. It varied between 2.2 kg and 91.1 kg for Cogshall, and between 33.6 kg and 143.1 kg for José. Crop load was an average of two times higher on José than on Cogshall trees (Table 1). Crop load for trees sampled at flowering, i.e., about four months before harvest, indicated the importance of flowering on these trees since poor crop load is generally related to poor flowering in the mango tree. Crop load for trees sampled at vegetative growth, i.e., after harvest, indicated the importance of the reproductive effort undergone by these trees one month and a half before the sampling date.

Hormones in terminal growth units

For both cultivars, abscisic acid (ABA) concentration in terminal GUs of the different fates showed significant differences among phenological periods, with a major decrease from VR

to VG where it reached a minimum (Figures 2A and B). The decrease was regular for Cogshall quiescent GUs. In José quiescent GUs, ABA concentration increased, although not significantly, from VR to F where it reached a maximum. ABA concentration in Cogshall flowering and fruiting GUs remained constant up to FM, before markedly and significantly decreasing. ABA concentration also showed significant differences among GU fates at F and MFG for Cogshall, and at F, MFG and FM for José. The greatest difference, common to the two cultivars, occurred at F where ABA concentration was more than two times higher in quiescent GUs than in flowering GUs. It is noteworthy that ABA concentration was remarkably low for all GU fates and for both cultivars at VG.

Auxin (IAA) concentration was high in flowering GUs at F, and in fruiting GUs at MFG and FM for both cultivars, indicating that IAA concentration remained high as long as the GU bore an inflorescence or a fruit (Figures 2C and D). IAA concentration in flowering GUs at F and in fruiting GUs at MFG and at FM were not significantly different for both cultivars. Similarly, IAA concentration in flowering GUs at F was not significantly different between Cogshall and José, nor was IAA concentration in fruiting GUs at MFG and FM. For all other GU fates and phenological periods, IAA concentration was consistently low, under the quantification thresholds.

Due to a technical problem, zeatin-riboside (ZR) concentration in fruiting GUs was not available at MFG, but only for the two last sampling dates, FM and VG. ZR concentration in quiescent GUs significantly decreased from VR for Cogshall, and from F for José, to FM where it reached a minimum for the two cultivars (Figure 2E and F). ZR concentration in fruiting GUs with fruit removal was maximal at MFG and then reached a minimum at FM for both cultivars. ZR concentration tended to increase between FM and VG in all GU fates and for both cultivars. However, the differences were not significant (except for José flowering GUs) because of the large variability observed in the data at this period. ZR concentration

also showed significant differences among GU fates at MFG and FM for Cogshall, and at F, MFG and FM for José. José flowering GUs had significantly lower ZR concentration than quiescent GUs at F. At MFG, fruiting GUs with fruit removal had significantly higher ZR concentration than flowering and quiescent GUs for both cultivars. ZR concentration was significantly higher in José fruiting GUs than in other GUs at FM.

Hormones in inflorescences, peduncles, pulp and seeds

The cultivar did not affect the ABA and IAA concentrations in inflorescences (data not shown). Mean ABA concentration in inflorescences (21.7 ± 5.6 ng g^{-1}) was much lower than in GUs with different fates, except at VG (Figures 2A and B). Mean IAA concentration in inflorescences (63.8 ± 32.2 ng g^{-1}) was not significantly different than in flowering GUs at F, and in fruiting GUs at MFG and FM (Figures 2C and D). ZR concentration was significantly higher in José inflorescences (131.5 ± 22.0 ng g^{-1}) than in Cogshall inflorescences (80.9 ± 17.5 ng g^{-1}). These concentrations were higher than in flowering GUs at F, but not significantly for Cogshall (Figures 2E and F).

ABA concentration in fruit pulp significantly increased between MFG and FM for both cultivars (Table 2). ABA concentration in seeds significantly decreased between MFG and FM for Cogshall. In contrast, it increased for José, but not significantly, probably because of the large variability between data at FM. ABA concentrations in peduncles at MFG and at FM were dramatically lower than in pulp, seeds and inflorescences at F for both cultivars.

IAA concentrations in seeds and pulp were under the quantification threshold, and were notably lower than IAA concentration in the peduncle. There was no significant difference in IAA concentration in the peduncle between MFG and FM for Cogshall, as observed in the corresponding fruiting GUs (Figure 2C). For José, a pronounced and marginally significant reduction of IAA concentration in the peduncle was shown between MFG and FM, paralleling

what was observed in the corresponding fruiting GUs (Figure 2D). Furthermore, IAA concentration in the peduncle was not significantly different to that in fruiting GUs at MFG and FM for José (Figure 2D), and at FM for Cogshall (Figure 2C). However, IAA concentration in the peduncle was significantly higher than in fruiting GUs at MFG for Cogshall (Figure 2C).

ZR was measured in the seed and the peduncle, but not in the pulp for both cultivars. There was no significant difference between MFG and FM in ZR concentration in the seed and the peduncle for both cultivars. For both cultivars, ZR concentration in the peduncle was significantly higher than in fruiting GUs at FM (Figures 2E and F).

Carbohydrates in terminal growth units

The dynamics of starch concentration was similar between Cogshall and José, except for the quiescent GUs (Figures 3A and B). The general trend was that starch concentration was high at F and low at FM and VG, in particular in GUs that bore a fruit. However, major differences can be observed between GU fates and between cultivars. Starch concentration in Cogshall quiescent GUs regularly and significantly increased from VR to MFG where it reached a maximum, and then decreased at FM and VG with a value similar to that at VR. For José quiescent GUs, starch concentration increased from VR to F and then decreased to a low value at MFG, FM and VG. For both cultivars, starch concentration in flowering GUs was maximal at F and then markedly and significantly decreased up to FM. It then significantly increased between FM and VG for Cogshall. For both cultivars, starch concentration in fruiting GUs was maximal at MFG. It markedly and significantly decreased up to FM, and remained low at VG. Starch concentration in fruiting GUs with fruit removal was constantly low at MFG, FM and VG. Starch concentration also showed significant differences among GU fates at F and MFG for Cogshall, and at F and FM for José. For both cultivars, it was

significantly higher in flowering GUs than in quiescent GUs at F. An interesting difference between the cultivars was at MFG: starch concentration remained high in quiescent, flowering and fruiting GUs for Cogshall, whereas it was already low in quiescent and flowering GUs for José, and was high only in fruiting GUs. Starch concentrations in the different GU fates were low and similar for José at VG. In contrast, starch concentration was about two times higher in Cogshall quiescent and flowering GUs, but the differences were not significant. This was presumably due to the wide variability among the data.

The dynamics of glucose concentration in terminal GUs differed between Cogshall and José (Figures 3C and D). Glucose concentration in Cogshall quiescent and flowering GUs increased more than two-fold from F to FM where they reached a maximum (Figure 3C). Glucose concentration in fruiting GUs increased similarly from MFG to FM where it reached approximately the same value as quiescent and flowering GUs. For all GU fates, it then significantly dropped between FM and VG where it reached values similar to those at VR, F and MFG. Glucose concentration in fruiting GUs with fruit removal was high at MFG and FM, and then dropped at VG as it did for the other GU fates. Changes in glucose concentration were of far lower amplitude for José GUs (Figure 3D). Glucose concentration in quiescent GUs was low and stable from VR to FM, and then significantly decreased at VG where it had the lowest values. On the contrary, glucose concentration in flowering GUs increased from F to MFG and then remained constant up to VG. Similarly, glucose concentration in fruiting GUs with or without fruit removal did not significantly vary between MFG and VG. Glucose concentration showed significant differences among GU fates only at MFG for both cultivars. At this date, fruiting GUs with fruit removal had the highest glucose concentration. The dynamics of fructose concentration in terminal GUs was similar to the one of glucose concentration and is not presented here.

For both cultivars, only quiescent and flowering GUs showed significant differences in sucrose concentration between phenological periods. In contrast, the high variability of sucrose concentration in fruiting GUs with or without fruit removal prevented the detection of significant differences (Figures 3E and F). However, the dynamics of sucrose concentration differed between the two cultivars. Sucrose concentration in Cogshall quiescent GUs was highest at VR, lowest at F, and then remained more or less constant with intermediate values between MFG and VG (Figure 3E). For José quiescent GUs, it remained constant and high at VR and F, and then regularly and significantly decreased up to VG (Figure 3F). Sucrose concentration in Cogshall flowering GUs was low and constant at F and MFG, and high and constant at FM and VG. For José flowering GUs, it significantly decreased from F to FM, and then significantly increased at VG where it reached a slightly higher value than at VR. No significant difference in sucrose concentration was observed between GU fates at each phenological period for both cultivars.

Discussion

It is assumed that inflorescences, and more notably fruits, play a major role in regulating the outgrowth of lateral buds along the bearing axis (Smith and Samach 2013). Two hypotheses involving hormones or carbohydrates are generally put forward to explain this role, and we explored these hypotheses in this study on the mango tree. Although sugars and hormones can interact (León and Sheen 2003, Barbier et al. 2015, Li et al. 2021) and play a role in reproduction and vegetative growth (Smith and Samach 2013), they are rarely considered together. Our study showed that the relationships between reproduction and vegetative bud outgrowth in mango were better explained by the dynamics of hormones and carbohydrates together rather than by one or the other considered independently. Their roles, suggested by the results, are synthesized in Figure 4 and discussed below.

The inhibition of vegetative growth on flowering and fruiting GUs appears to be predominantly driven by IAA

The inhibition of vegetative growth on flowering and fruiting GUs between flowering and fruit maturity appeared to be predominantly driven by IAA, and carbohydrates might play a role at fruit maturity. Flowering and fruiting GUs had significantly higher IAA concentration than quiescent GUs for both cultivars as long as inflorescences and fruit were present on GUs (Figures 2C and D). Ten days after fruit removal, IAA concentration in fruiting GUs with fruit removal was very low and similar to that of quiescent GUs. In apple and tomato, drops in IAA concentration are also observed after fruit removal, sometimes as quickly as after one day (Gruber and Bangerth 1990). It has been reported in many species, from herbaceous plants to fruit trees, including mango, that IAA is produced by developing inflorescences and fruit (Chacko et al. 1970, Lee 1988, Wolbang et al. 2004). IAA appears to move basipetally with a high export rate from those organs to the bearing shoot through the polar auxin transport system (Goldsmith 1977, Bangerth 1992, Friml and Palme 2002). Our results agree with this hypothesis: high and similar IAA concentrations were measured in inflorescences, in fruit peduncles (Table 2) and in flowering and fruiting GU axes (Figures 2C and D). Compared to the results reported for other species (Ferguson and Beveridge 2009, Waldie et al. 2010, Smith and Samach 2013), our results suggest a basipetal flux of IAA from the inflorescence and fruit that may inhibit vegetative bud outgrowth of reproductive GUs. The very low IAA concentrations measured in seeds (Table 2) could probably be explained by our protocol. The whole seed, i.e., mostly cotyledons, was sampled, and not just the embryo where IAA is produced (Locascio et al. 2014), leading to IAA dilution in the samples.

Starch and glucose concentrations varied across phenological stages in flowering and fruiting GUs, as shown in previous studies (Léchaudel et al. 2005, Normand et al. 2006). The

pattern of starch concentration changes in flowering and fruiting GUs was similar between the two cultivars (Figures 3A and B). At flowering, starch concentration in flowering GUs was significantly higher than in quiescent GUs for both cultivars, suggesting that a higher terminal GU starch concentration promotes mango flowering, as observed in *Citrus sinensis* (Goldschmidt et al. 1985) and *Arabidopsis thaliana* (Bernier et al. 1993). Furthermore, our results showed an accumulation of starch in GUs before flowering, followed by a decrease between flowering and fruit maturity in all sampled GUs, as found in previous studies on mango (Davie and Stassen 1997, Davie et al. 2000, Léchaudel et al. 2005) and other fruit species (Hubbard et al. 1991, Marchal and Folliot 1992). This decrease could be explained by the presence of growing fruits that are strong sinks for carbohydrates. A source of carbohydrates could be starch, which is stored in different woody parts of the tree (Van Vuuren et al. 1997, Normand et al. 2006), and photosynthesis, whose capacity is higher in leaves close to growing mango fruit (Urban et al. 2003, 2004). Although sampled when the fruit growth rate, i.e., the sink strength, was maximal (Figure 1C), starch concentration in fruiting GUs of both cultivars was high at mid-fruit growth, whereas it was low at the same period in fruiting GUs with the fruit removed 10 days before (Figures 3A and B). This suggests that the higher photosynthetic capacity (Urban et al. 2003, 2004) or the increased net photosynthesis with fruit demand (Léchaudel et al. 2005) documented in Cogshall fruiting GUs was able to maintain a high starch level in these GUs, at least up to mid-fruit growth in both cultivars. In contrast, the starch reserve was already partially depleted in flowering GUs, and in quiescent GUs for José, at that time. The decrease in starch concentration in flowering and quiescent GUs up to harvest suggests a strong mobilization of carbohydrates in non-fruiting terminal GUs, necessary to support fruit growth from an early stage. The different starch dynamics in quiescent and flowering GUs during early fruit growth in the two cultivars, and their different starch concentrations at fruit maturity (Figures 3A and B) were presumably

related to tree crop load, which was two times higher for José than for Cogshall (Table 1). These different starch dynamics could also result from different partitioning patterns of carbohydrate resources, or from differences in photosynthetic capacity between the two cultivars, as shown for other cultivars by Lu et al. (2012). Further investigations are required to explore these hypotheses.

Bud outgrowth is highly dependent on carbohydrates (Zapata et al. 2004). It depends on the accumulation of starch in shoot and bud tissues, and on the importation and use of nutrients from the shoot and the roots (Greer et al. 2002, Greer and Wünsche 2003, Zapata et al. 2004). In this way, starch accumulation in *Picea abies* buds and shoots is considered as an indication of the onset of lateral bud development (Hejnowicz and Obarska 1995). Starch concentrations in flowering GUs at flowering and in fruiting GUs at mid-fruit growth were higher than in quiescent GUs at vegetative growth. This suggests that starch was not a limiting factor for vegetative growth in these GUs during early fruit growth, supporting the dominant role of IAA. At fruit maturity, starch concentration was low in most of the GUs, except in quiescent GUs in Cogshall, and was more likely a limiting factor for vegetative growth at that period.

The roles of ZR and ABA on vegetative growth inhibition in flowering and fruiting GUs was less clear. Low ZR concentrations and high ABA concentrations between flowering and fruit maturity tended to show an unfavorable context for vegetative growth on terminal GUs during this period (Shimizu-Sato and Mori 2001, Davies 2010). This context was, however, not specific to flowering and fruiting GUs since it concerned quiescent GUs as well. The reported positive effect of sucrose (Barbier et al. 2015) and negative effect of IAA (Tanaka et al. 2006) on CK synthesis in stem tissue were not clearly revealed by our results. The higher ABA concentration in GUs during vegetative rest and flowering were in accordance with the role of ABA in inhibiting vegetative growth through an upstream control of axillary bud

outgrowth (Barbier et al. 2019). The role of ABA in promoting flowering has been suggested in several studies. However, lower concentrations were observed in mango flowering GUs. This could imply that bud break competency is reached below a specific threshold of ABA concentration (Vimont et al. 2020), enabling reproductive (Sheng-Hui et al. 2011, Li et al. 2021) but not vegetative growth.

Interestingly and independently of the question of vegetative bud outgrowth, fruiting GUs with the fruit removed 10 days before had the highest ZR and ABA concentrations at mid-fruit growth in both cultivars (Figure 2). We could hypothesize that the higher ZR concentration in these GUs was related to the removal of the fruit, the assumed source of basipetal IAA flux. This is consistent with the results of several studies showing that stem CK concentration rapidly increases after shoot decapitation (Bangerth 1994, Li et al. 1995, Turnbull et al. 1997). It is reported that ABA is produced by mature leaves and by roots, and migrates to the fruit where it leads to ripening (Vendrell and Palomer 1997, Davies 2010, Zaharah et al. 2012). Accordingly, we showed a significant increase of ABA concentration in mango pulp between mid-fruit growth and fruit maturity for both cultivars, indicating the accumulation of this hormone at the end of fruit growth (Table 2). The high ABA concentration in fruiting GUs 10 days after fruit removal was probably related to the temporary accumulation of ABA in the bearing GU following the removal of this ABA sink.

Carbohydrates appeared to be crucial to the lower probability and the delay of vegetative bud outgrowth after harvest in fruiting GUs

The large carbohydrate sinks represented by fruit vanished between fruit maturity and vegetative growth. However, starch concentration in previously fruiting GUs stagnated (Cogshall) or decreased (José) between fruit maturity and vegetative growth. On the other hand, an increase in starch concentration, although not always significant, was observed in

quiescent GUs and previously flowering GUs for both cultivars (Figures 3A and B). This increase suggests that photosynthates were distributed to these GUs and refilled carbohydrate reserves after the depletion of resources related to fruit growth (Davie et al. 2000). If we assume that vegetative bud outgrowth is positively linked to starch concentration in GUs, as shown in previous studies (Goldschmidt 1999, Marquat et al. 1999, Greer et al. 2002, Zapata et al. 2004, Smith and Samach 2013), then the trend toward low starch concentration in mango fruiting GUs at vegetative growth could partly explain the prevention of bud outgrowth for these fruiting GUs compared to the flowering and quiescent GUs. Moreover, the slower increase in starch concentration in fruiting GUs, when it occurs, suggests that photosynthates were preferentially distributed to major zones of carbohydrate storage in the tree, such as roots (Kozlowski 1992, Normand et al. 2006). This result could explain the delay of bud outgrowth of fruiting GUs when it occurs, presumably related to the delay needed to recover higher starch concentration in fruiting GU stem tissues.

After vegetative rest for Cogshall, and after flowering for José, ABA concentration of quiescent GUs dramatically and significantly decreased up to vegetative growth (Figures 2A and B). This decrease was probably related to both the decrease of abiotic stress (Giday et al. 2014, Shalom et al. 2014) since trees were irrigated from flowering to fruit growth and temperatures increased as of flowering (Figures 1A and B), and to fruit removal on trees during harvest, which were important sinks for ABA (Table 2). During the period of vegetative growth, the increase in ZR and the very low IAA and ABA concentrations (Figure 2) suggest that there was no hormonal limitation for bud outgrowth of GUs, including the previously fruiting GUs, strengthening the result of a limitation by local carbohydrate availability.

Could cultivar-specific hormones and carbohydrate dynamics explain the bearing pattern of each cultivar?

Similar changes in ABA and IAA concentrations across phenological phases were observed for both cultivars, indicating that the differences in vegetative bud outgrowth of fruiting GUs between each other (Capelli et al. 2016) were not explained by these hormones. On the other hand, the two cultivars revealed differences in GU starch concentration during the period of vegetative growth and in glucose and sucrose concentration at fruit maturity. These results suggest that the low probability of bud outgrowth in fruiting GUs that leads to low vegetative growth and potentially low fruit production in the following year (Capelli et al. 2016) could be more specifically related to carbohydrate availability than to hormone concentrations.

During the period of vegetative growth, quiescent and previously flowering GUs had higher starch concentrations than previously fruiting GUs. The difference was larger for Cogshall than for José (Figures 3A and B). For Cogshall, an increase in glucose and sucrose concentrations was observed for all GUs at harvest. In contrast, the glucose and sucrose concentrations in José GUs were quite low and stable for all GUs during the entire cycle, especially in quiescent GUs (Figures 3D and F).

Irregular bearing leads to various production patterns depending on the cultivar (Jonkers 1979, on apple and pear; Schaffer et al. 1985, on citrus; Chacko 1986, on mango). The higher starch concentration of Cogshall quiescent and flowering GUs (3.28% DM, Figure 3A) than that of fruiting GUs (0.76% DM, Figure 3A) suggests a higher probability of vegetative bud outgrowth on the former than on the latter GUs, supported by previous results (Capelli et al. 2016). At the tree scale, the proportion of non-fruiting vs. fruiting terminal GUs within the canopy would then define vegetative growth and the potential for flowering and fruit production during the following cycle, leading to an irregular bearing pattern for this cultivar.

In contrast, the low and quite similar starch concentration in all José terminal GUs during the period of vegetative growth (1.75% DM, Figure 3B) would negatively affect vegetative bud outgrowth. At the tree scale, this result implied that all terminal GUs, regardless of their fate, have the same behavior within the canopy, leading to poor vegetative growth and, consequently, to poor flowering and fruiting potential for the following cycle. This contributes to a typical alternate bearing pattern with an 'on' year followed by an 'off' year (Capelli et al. 2016). This result must however be qualified since the differences in starch concentration observed at vegetative growth between the two cultivars could be related to their differences in tree crop load (Table 1).

In conclusion, our results suggest that both hormones and carbohydrates play a role in the negative effect of reproduction on vegetative bud outgrowth at the scale of the mango terminal GU. The effects of hormones and carbohydrates were limited spatially and in time, corresponding to the presence of fruit and inflorescences as hormone sources and fruit as carbohydrate sinks at the GU scale (GU fate), and to the phenology at the whole tree scale, respectively (Figure 4). In this way, lateral vegetative bud outgrowth of flowering and fruiting GUs was inhibited by high IAA concentration, probably produced by inflorescences and fruit, whereas starch concentration remained high in fruiting GUs, at least up to mid-fruit growth. At fruit maturity, starch concentration was low in most terminal GUs and was undoubtedly limiting for bud outgrowth. After harvest, hormones were no longer a limiting factor. At that moment, the low starch concentration in previously fruiting GUs was probably responsible for their lower probability of vegetative bud outgrowth. The lower starch concentration in fruiting GUs compared to non-fruiting GUs possibly leads to a longer time to refill local carbohydrate reserves, inducing a delay in vegetative bud outgrowth. The differences between the two

cultivars in carbohydrate dynamics and in starch concentration in fruiting and non-fruiting GUs could partly explain their alternate (José) and irregular (Cogshall) bearing patterns.

In the future, the use of molecular tools such as gene activity (Barbier et al. 2015, Vimont et al. 2020) and transcriptome profiling (Kebrom and Mullet 2016) would be interesting to help us to better understand the respective roles of hormones (including other hormones such as gibberellins or strigolactones), carbohydrates, and of their interactions, in the negative effect of reproduction on vegetative growth at the GU scale in mango.

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Figure legends

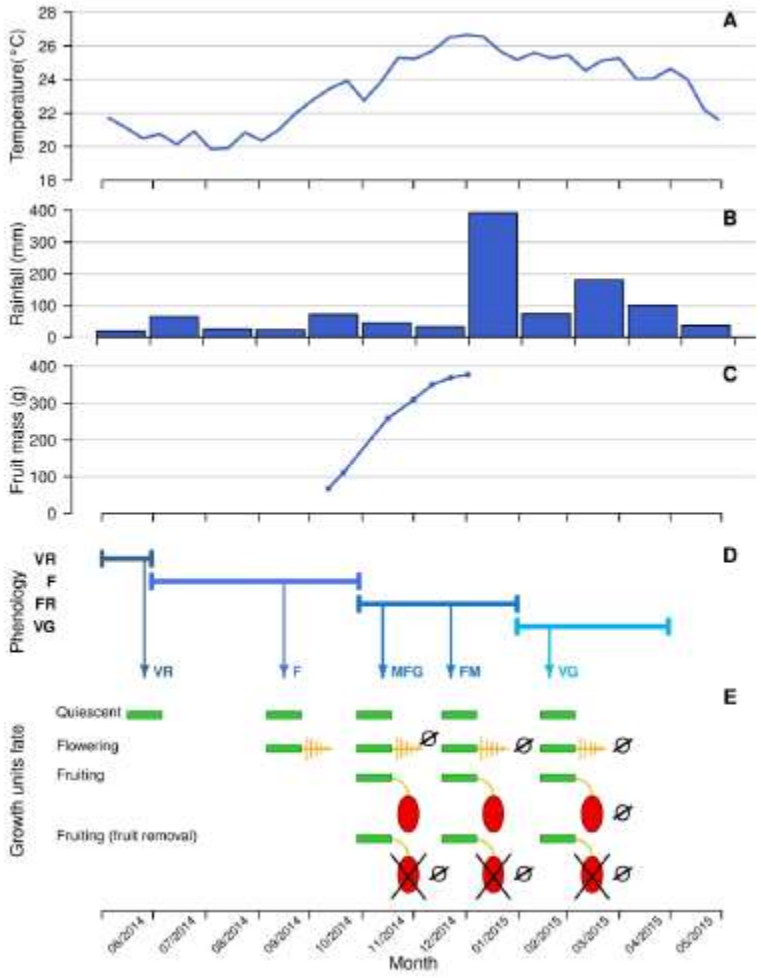

Figure 1. Mean decadal temperature (A) and monthly rainfall (B) at the experimental orchards from 1 June 2014 to 31 May 2015; changes in fruit mass during the fruit growth season (C); sampling dates (downward vertical arrows) during the four main mango tree phenological periods (VR: vegetative rest; F: flowering; FR: fruit growth (sampling dates at mid-fruit growth (MFG) and fruit maturity (FM)); VG: vegetative growth) (D); and the sampled growth unit (GU) fates (quiescent, flowering, fruiting, fruiting with fruit removal) at each date (E). The different GU fates are schematized. Terminal GUs are represented by green rectangles. Inflorescences are represented by orange fishbone-like symbols, and fruit by red ellipses. Fruit with a cross represent fruit removed at mid-fruit growth. The symbol Ø indicates that

inflorescence or fruit was no longer present on the flowering or fruiting GUs at these sampling dates.

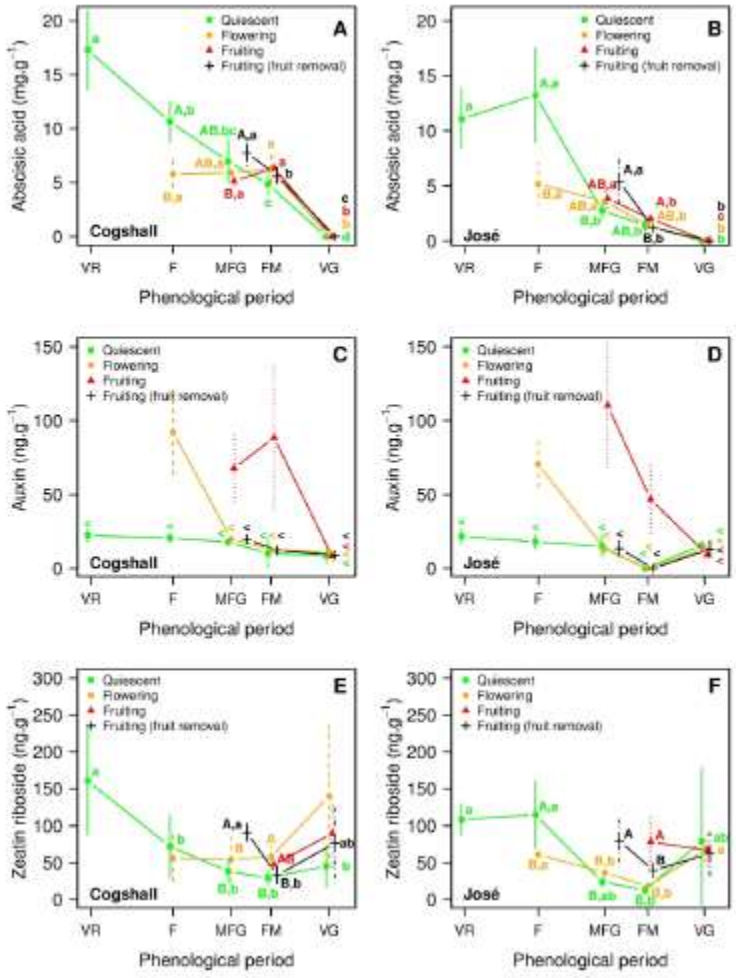

Figure 2. Changes in abscisic acid (ABA; A, B), auxin (IAA; C, D) and zeatin-riboside (ZR; E, F) concentrations (mean ± SD; ng.g^{-1} of dry matter) in terminal mango growth units (GUs) with different fates (quiescent; flowering; fruiting; fruiting with fruit removal) across five phenological periods (vegetative rest (VR); flowering (F); mid-fruit growth (MFG); fruit maturity (FM); vegetative growth (VG)) for two mango cultivars, Cogshall (A, C, E) and José (B, D, F). The distance between phenological periods on the x-axis is proportional to the number of days between each sampling date. For each date, a noise is introduced to avoid a superposition of means and standard deviation bars, except for fruiting GUs with fruit removal at MFG that were sampled 10 days after the other GUs. Upper-case letters, when

present, indicate significantly different mean values between GU fates at a given phenological period. Lower-case letters, when present, indicate significantly different mean values between phenological periods for the same GU fate (analysis of variance followed by Tukey's test; n=5; P<0.05). The '<' symbol indicates that values are under the quantification threshold.

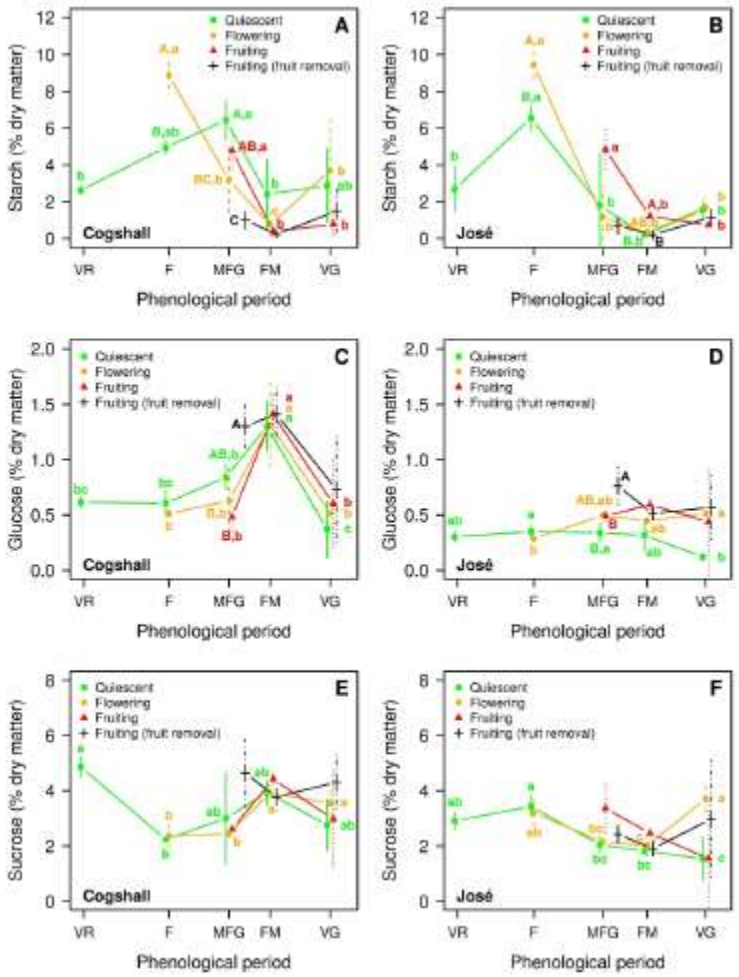

Figure 3. Changes in starch (A, B), glucose (C, D), and sucrose (E, F) concentrations (mean ± SD; % of dry matter) in mango terminal growth units (GUs) with different fates (quiescent; flowering; fruiting; fruiting with fruit removal) across five phenological periods (vegetative rest (VR); flowering (F); mid-fruit growth (MFG); fruit maturity (FM); vegetative growth (VG)) for two mango cultivars, Cogshall (A, C, E) and José (B, D, F). The distance between phenological periods on the x-axis is proportional to the number of days between each

sampling date. For each date, a noise is introduced to avoid a superposition of means and standard deviation bars, except for fruiting GUs with fruit removal at MFG, which were sampled 10 days after the other GUs. Upper-case letters, when present, indicate significantly different mean values between GU fates at a given phenological period. Lower-case letters, when present, indicate significantly different mean values between phenological periods for the same GU fate (analysis of variance followed by Tukey's test; n=3; $P<0.05$).

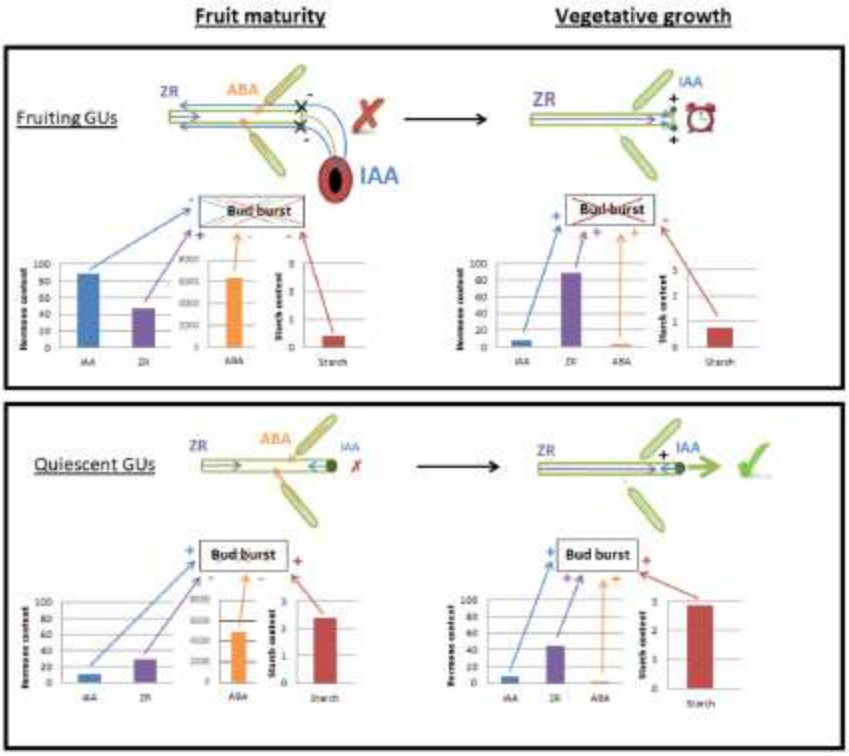

Figure 4. Synthesis of the effects of hormones and carbohydrates on vegetative bud outgrowth at fruit maturity and during the period of vegetative growth on mango terminal fruiting and quiescent growth units (GUs) of Cogshall cultivar. Supposed auxin (IAA) and abscisic acid (ABA) fluxes are represented by blue and orange arrows, respectively. Zeatin-riboside (ZR) concentration in the GU stem is represented by purple arrows. IAA, ZR, ABA and starch concentrations are those measured in our study. Symbols '+' and '-' represent positive and negative effects, respectively, on vegetative bud outgrowth. The big red cross means that the probability of bud outgrowth is very low. The small red cross means that the probability of

vegetative bud outgrowth is low to medium. The green arrow and the green check represent the possibility of bud outgrowth. The clock represents a delay in bud outgrowth. Green circles represent the buds. The red ellipse represents the mango fruit, and the black ellipse the seed inside.

Tables

Table 1. Mean (± SD, kg) crop load during the 2014-2015 harvest for the trees sampled for hormone (n=5 trees) and carbohydrate (n=3 trees) analyses at flowering, mid-fruit growth, fruit maturity and vegetative growth phenological stages for two mango cultivars, Cogshall and José.

Cultivar	Nature of analyses performed on the trees	Sampling date			
		Flowering	Mid-fruit growth	Fruit maturity	Vegetative growth
Cogshall	Carbohydrates	9.0 ± 10.5	50.6 ± 39.1	59.0 ± 27.9	59.0 ± 27.9
	Hormones	6.7 ± 8.1	40.2 ± 32.1	50.7 ± 22.8	50.7 ± 22.8
José	Carbohydrates	111.0 ± 19.4	120.0 ± 19.7	114.0 ± 13.2	135.1 ± 9.6
	Hormones	100.9 ± 41.7	114.4 ± 18.5	93.6 ± 36.0	105.5 ± 45.5

Table 2. Abscisic acid (ABA), auxin (IAA) and zeatin-riboside (ZR) concentrations (mean ± SD; ng g^{-1} dry matter) in the pulp, seed and peduncle of mango fruit at mid-fruit growth and at fruit maturity for two cultivars, Cogshall and José. Mean values followed by different letters are significantly different between mid-fruit growth and fruit maturity for the same organ and cultivar (analysis of variance; n=5; $P<0.05$). nq: data below the quantification threshold. '-': data not available.

Hormone	Organ	Cogshall			José		
		Mid-fruit growth	Fruit maturity	*P*	Mid-fruit growth	Fruit maturity	*P*
Abscisic acid	Pulp	527.8 ± 206.3 b	3061.3 ± 1924.9 a	**0.04**	765.9 ± 205.7 b	3149.0 ± 1375.9 a	**0.01**
	Seed	2086.7 ± 517.6 a	525.8 ± 206.5 b	**<0.001**	640.5 ± 159.7	1630.1 ± 1471.9	0.23
	Peduncle	5.8 ± 0.8 b	8.2 ± 1.6 a	**0.02**	3.6 ± 0.7 a	2.3 ± 1.0 b	**0.04**
Auxin	Pulp	5.3 ± 1.4 nq	5.1 ± 2.1 nq	-	8.1 ± 1.9 nq	3.6 ± 1.6 nq	-
	Seed	2.8 ± 0.8 nq	1.9 ± 0.7 nq	-	5.8 ± 2.1 nq	1.7 ± 1.1 nq	-
	Peduncle	93.2 ± 14.5	85.0 ± 35.2	0.64	96.8 ± 30.4	49.6 ± 35.0	0.05
Zeatin-riboside	Pulp	-	-	-	-	-	-
	Seed	111.0 ± 106.6	80.4 ± 42.8	0.57	116.9 ± 29.2	47.0 ± 65.0	0.08
	Peduncle	177.4 ± 36.2	174.6 ± 25.6	0.89	219.4 ± 33.4	246.5 ± 73.0	0.47