



# Simulated fire injury: effects of trunk girdling and partial defoliation on reproductive development of apple trees (*Malus domestica*)

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## Abstract

Fire damage can significantly impact fruit productivity in orchards. However, the effects of nonlethal fire injuries on the reproductive development of apple trees remain poorly understood. To investigate these effects, we implemented three treatments: trunk girdling to simulate fire injury to xylem, defoliation of a third of the canopy (simulated crown fire injury), and a combined treatment (simulated surface fire injury), alongside a control. The experiment was conducted during the 2021–22 growing season using a randomised block design with four biological replicate plots. Girdling was less effective than crown and surface fire treatments in influencing fruit composition during the current growing season, and flowering and fruiting in the following season. The crown and surface fire treatments induced localised detrimental effects on fruit sugar and titratable acidity while stimulating peel blush. Additionally, these treatments led to reduced starch reserves by harvest, which likely disrupted subsequent flowering and crop load near the previously defoliated sections of the canopy. When surface fires damage leaves near the base of the canopy in addition to the trunk, fruit production in the lower part of the canopy is more likely to be compromised in the following season. Crown fires, which cause leaf loss near the apex of the canopy, appear to be particularly detrimental to tree productivity, as the top defoliation treatment impaired carbohydrate reserves in shoot terminals and roots. In conclusion, fire-induced loss of leaf area during fruit growth alters fruit composition in the current growing season and may lead to lower yields in the subsequent season.

**Keywords** Carbohydrate dynamics · Defoliation · Fruit maturation · Girdling · Orchard productivity · Wildfires

## Introduction

Bushfires in Australia have recently inflicted significant damage on apple (*Malus domestica*) orchards, leading to immediate crop losses and long-term reductions in productivity (Idowu et al. 2023a). Extreme bushfires, which

are associated with climate change, are becoming increasingly prevalent in various agricultural regions worldwide, including Australia, Southeast Asia, and North America (Sun et al. 2019; Idowu et al. 2023a). Fire injury to fruiting trees may damage the perennial structure and vascular system, possibly resulting in cambium and phloem necrosis and a loss of xylem hydraulic conductivity (Bär et al. 2019). This restricts the transport of sugars, nutrients, and water between roots and the canopy. Moreover, fires may diminish leaf area, thereby reducing photosynthesis and further restricting carbohydrate availability. The growth of carbon sinks, including fruits, can consequently be impaired, as a limited carbohydrate supply hinders the reproductive and vegetative development of trees (Wünsche et al. 2010; Breen et al. 2020).

Although the effects of fires on forest ecosystems have been extensively studied (Michaletz and Johnson 2007; Bär et al. 2019; Varner et al. 2021), less attention has been given to the impacts of bushfires on agricultural systems, including fruit orchards. Of particular concern are surface

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and crown fires, as they can easily damage or destroy tree crops (Weise et al. 2018). Surface fires may take the form of smouldering embers burning on the orchard floor and consuming dry vegetation such as grasses and cover crops. This kind of fire often impacts the trunk and lower canopy of trees, damaging the vascular system and destroying the leaves near the base of the canopy, respectively (Idowu et al. 2023a). In contrast, crown fires burn through tree canopies, causing severe leaf area loss, especially near the apex of trees (Bär et al. 2019).

In horticulture, girdling is used to disrupt the phloem-based translocation of sugars, nutrients, and other metabolites between the canopy and roots (Goren et al. 2003). Trunk girdling, to some extent, mimics the detrimental impacts of fire injury on sugar distribution in plants, as smouldering surface fires can induce effects comparable to those caused by girdling (Bär et al. 2019; Idowu et al. 2023a). Girdling has been found to restrict vegetative growth but potentially increases fruit yields, soluble solid content, acidity, and peel colour development in apples (Goren et al. 2003). Moreover, trunk girdling of apple trees in a single growing season appears sufficient to increase fruit size in the current season and yield in the following season (Falahi et al. 2018). Although girdling may have favourable effects on fruit productivity and quality in the short term, its continuous long-term application may be less favourable. For instance, continuous trunk girdling of jujube (*Ziziphus jujuba*) trees over multiple growing seasons has been shown to be detrimental to fruit sugar and protein contents, as well as size (Ran et al. 2022). Persistent inhibition of phloem function, coupled with permanent phloem damage and injury to the xylem surrounding the girdling wound, poses additional challenges, ultimately resulting in tree mortality.

Partial defoliation is commonly used to investigate source–sink interactions in fruiting crops (Iglesias et al. 2002; Zhou and Quebedeaux 2003; Rossouw et al. 2017b). Leaves are the primary source of carbohydrates, supplying assimilates to fruit (Bairam et al. 2019), and experiments involving partial defoliation have shown increased photosynthesis rates in the remaining leaves (Zhou and Quebedeaux 2003). However, the reduced canopy leaf area resulting from defoliation can lead to inferior starch and sugar accumulation in fruit (Toldam-Andersen and Hansen 1995). A decrease in canopy leaf area in apple trees may significantly reduce nonstructural carbohydrate reserves in woody tissues, particularly roots, resulting in lower starch reserve levels at the beginning of the following growing season (Breen et al. 2020). Some reserves may also be depleted in trunks and branches following defoliation (Loescher et al. 1990). Nonetheless, root reserves play a crucial role in early respiration, vegetative growth, and reproductive development. The sensitivity of root reserves to late-season stresses such as defoliation may disrupt plant performance and

yield, particularly by interfering with flowering and early fruit development in the subsequent season.

Starch reserves play a crucial role in balancing carbohydrate supplies with the demands of developing sinks. These reserves are mobilised from roots and woody tissues when assimilate supply falls short of meeting sink requirements (Yoshioka et al. 1988; Loescher et al. 1990; Stitt and Zeeman 2012). The concept of potential branch autonomy is also pertinent for understanding the distribution of sugars between sources and sinks (Sprugel et al. 1991), particularly when manipulating source-sink biomass in specific areas of the canopy. While full branch autonomy involves synthesising all carbon required by sinks without importing sugars from other parts of the tree, the degree of autonomy varies across tree species and phenological stages. In apple trees, carbohydrates can be imported from sources outside a branch when there is an imbalance between source supply and sink demand (Belhassine et al. 2019). Strong sinks, such as rapidly growing fruit, draw carbon from sources regardless of distance, while weaker sinks, such as younger fruitlets, rely more on nearby sources.

This study aimed to address three primary objectives. First, we examined the effects of trunk girdling and partial defoliation of the canopy during fruit development on fruit retention, weight, and composition at harvest. Second, we investigated whether conditions resulting in reduced carbohydrate supply during fruit growth hinder the accumulation of nonstructural carbohydrates in various apple tree tissues, including the reserves in roots, shoots, and leaves. Third, we evaluated the carry-over effects of trunk girdling and partial defoliation in the current growing season on the subsequent cropping of apple trees, including flowering and fruit yield, the following season.

By applying treatments that simulate possible fire-related injuries, our aim was to elucidate the physiological implications of bushfire-induced damage to apple trees and to determine the effects of partial canopy defoliation and trunk girdling on fruit development, composition, and carbohydrate dynamics. This study offers a novel and systematic evaluation of the potential impacts of fire injury on fruit productivity in apple trees. To the best of our knowledge, no prior studies have conducted a controlled examination of such effects. By simulating fire damage in apple trees, this research fills a crucial gap in our understanding of the response of fruit-bearing trees to environmental stressors. This research provides valuable insights for orchard management in fire-prone regions of the world.

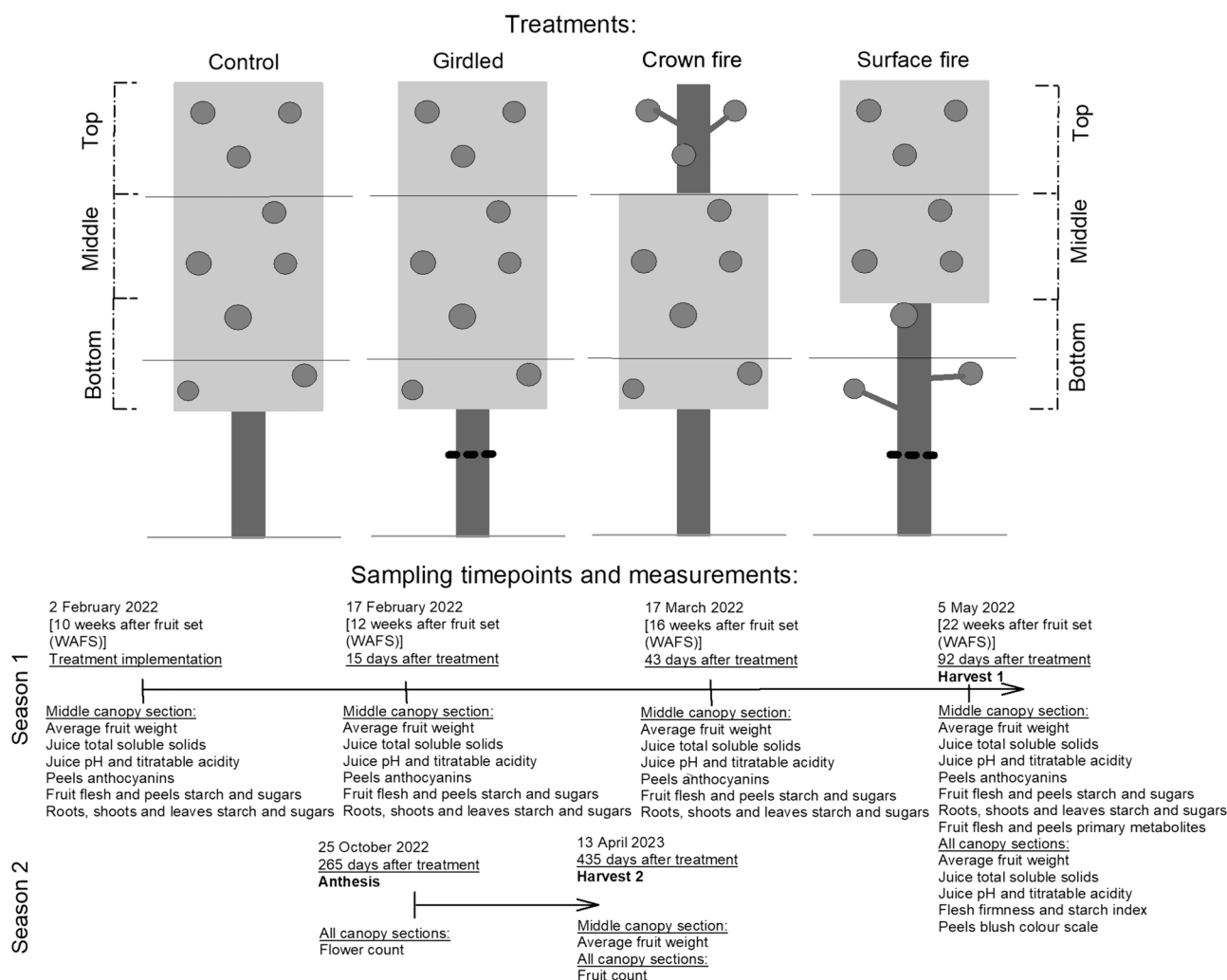
## Materials and methods

### Experimental design and treatments

To simulate potential fire injuries, we implemented three types of treatments during fruit growth: trunk girdling, partial canopy defoliation, and a combination (Fig. 1). These treatments aimed to mimic the effects of surface fires (trunk girdling with or without defoliation of the lower part of the canopy) or crown fires (defoliation of the upper part of the canopy). Trunk girdling was intended to disrupt the phloem-based translocation of sugars, nutrients, and metabolites between the canopy and roots. In treatments involving partial canopy defoliation, all leaves from the top or bottom thirds of the canopy were removed.

The defoliation of the bottom third of the canopy was combined with trunk girdling.

The treatments were conducted during the 2021–2022 growing season using 10 years-old 'Cripps pink' apple trees grafted onto the 'MM106' rootstock. The trees were planted with row and tree spacings of 4 and 1.5 m, respectively. The experiment was conducted at the New South Wales Department of Primary Industries' Orange Agricultural Institute in Orange, New South Wales, Australia (33°33' S; 149°08' E). The trees were trained to a single leader on a two-wire trellis system, with wires installed at heights of 85 cm and 170 cm above ground level. Conventional spray and irrigation practices were followed for orchard management, and the rows were oriented in a north–south direction. No crop thinning was performed prior to the experiment. The site is characterised by ferrosols soil, which is known for its good



**Fig. 1** Schematic representation of the applied treatments. The lines flanking the trees indicate the division of the canopy into top, middle, and bottom sections. Gaps in the canopy represent regions where defoliation occurred, while lines on the trunks indicate girdling appli-

cations. The horizontal lines across the canopies represent the trellis wires. The text at the bottom of the figure outlines the various sampling dates and the corresponding measurements taken

drainage and clay-loam texture, with a clay content exceeding 45% in the deep profile (Isbell 2016). The subsoil is rich in free iron oxide, and the soil contains high levels of organic matter. Naturally, the soil is mildly acidic.

A total of 48 trees, all of similar size, were selected from six rows, each comprising 20 trees. These trees were organised into 16 plots, with each plot containing three adjacent trees. To ensure randomness and reduce bias, a randomised block design was used, and the plots were divided into four replicates. Within each replicate, four plots were established and distributed across the rows to ensure an even distribution. Additionally, buffer trees were present at the end of rows and between plots within a row to minimise any potential edge effects.

The experimental treatments comprised three treatment groups and a control (Fig. 1). The treatments were implemented on 2 February 2022, approximately 10 weeks after fruit set, when the fruit juice had an average total soluble solid concentration of 8.1 °Brix, a pH of 3.2, and a titratable acidity of 10.9 g/L. The trees had an average height of 2.8 m, and the functional canopy was approximately 40 cm above ground level, resulting in a vertical foliage layout stretching approximately 2.4 m. Using the trellis wires as a reference, the canopy of each tree was visually divided into three horizontal sections of 80 cm each.

The first treatment involved trunk girdling by making a 6 mm wide cut to remove an entire ring of bark around the trunk, within 15 to 30 cm above the graft union, using a girdling knife. This treatment aimed to disrupt phloem transport between the canopy and roots. In the second treatment, we conducted complete defoliation of the top third of the canopy to simulate damage associated with crown fire injury. The third treatment combined girdling, as in treatment 1, with complete defoliation of the bottom third of the canopy to simulate injury related to surface fire. The control trees were left unaltered. The treatments are henceforth referred to as girdled, crown fire and surface fire, respectively.

To maintain the effects of girdling, the process was repeated monthly (Rainer-Lethaus and Oberhuber 2018). Therefore, two additional girdling procedures were conducted, on 2 March and 6 April 2022. Each time, a new girdle was created 5 cm above or below the original girdle. For the defoliation treatments, any new vegetative growth relevant to the defoliated section was removed monthly, coinciding with the girdling.

### Fruit development and basic composition

The first sampling occurred just before the implementation of the treatments on 2 February 2022 [(i.e., approximately 10 weeks after fruit set (WAFS)], followed by additional sampling on 17 February (approximately 12 WAFS) and 17 March (approximately 16 WAFS) (Fig. 1). The final

samples were collected at harvest on 5 May (approximately 22 WAFS). For each sampling event, six random, representative apples were selected from the middle section of the canopy for each plot, two per tree, with three apples taken from each side of the row. At harvest, an additional six apples were collected from both the bottom and top sections of the canopy, for each plot. The apples were weighed to determine the average fresh weight per apple. At harvest, the total number of fruits on each tree located at the centre of every plot was counted.

For each apple sampled from the middle section, a subsample was immediately collected for carbohydrate analysis at all sampling points and for primary metabolite analysis at harvest. A 1 cm wide wedge was cut from the outside to the centre of the apple on both the blushed and non-blushed sides. The peels were then carefully removed from the flesh using a vegetable peeler, and the subsamples from different apples were combined for each plot. The peel and flesh subsamples were immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  until further processing. Subsequently, the frozen peel and flesh subsamples were ground to a fine powder under liquid nitrogen using an A11 basic analytical mill (IKA, Selangor, Malaysia) and freeze-dried using a Gamma 1–16 LSC freeze dryer (Christ, Osterode am Harz, Lower Saxony, Germany).

Juice extraction and analysis were conducted for each sampling date and plot. The remaining entire fruit sample per plot, with the subsamples removed as described above, was juiced using a benchtop juicer (BJE200, Breville, Sydney, Australia). The total soluble solid concentration (°Brix) of the juice was measured using a digital refractometer. The pH and titratable acidity (TA) were determined by titration with 0.1 mol of sodium hydroxide using an automatic titrator (fully automated 59 place Titrando system, Metrohm, Herisau, Switzerland) until the pH reached 8.2. TA was quantified as the tartaric acid equivalent and converted to the malic acid equivalent using a factor of 0.89 (Jolicœur 2011).

At harvest, additional fruit quality parameters, including firmness, starch index, and colour (percentage blush) were assessed. Firmness measurements were taken in the middle of each apple on both the blushed and non-blushed sides using a hydraulically operated laboratory penetrometer mounted on a drill press after removing a small section of peel. The colour scale was determined by grading the surface coloration of each sampled apple based on the percentage of coloured surface area (< 20%, 20–40%, 40–60%, 60–80%, or > 80%). To estimate the starch pattern index, the fruit was sliced horizontally through the equatorial plane, stained with iodine-potassium iodide solution (Brookfield et al. 1997), and visually scored against a six-point starch pattern index chart (ENZA International Ltd., Hastings, New Zealand). The higher the starch pattern index score is, the less starch is present.

## Fruit nonstructural carbohydrates (NSC) and anthocyanins

Starch and soluble sugar concentrations, encompassing sucrose, glucose, and fructose, were assessed in 20 mg freeze-dried and finely ground subsamples of apple peels and flesh using enzymatic assays (K-TSTA and K-SUFRG, Megazyme International, Bray, Ireland) following the procedures outlined in Smith and Holzapfel (2009). Given that sucrose, glucose, and fructose are the primary soluble sugars in apple fruit (Karadeniz and Ekşi 2002; Ma et al. 2015), their combined concentrations were reported as the total sugar concentration. Sorbitol, a significant transport polyol (sugar alcohol) in apple trees, was not included in the quantification of total fruit sugar concentration, as its levels in mature fruit typically constitute less than 10% of total sugars (Aprea et al. 2017). Nonetheless, given the importance of sorbitol as a carbohydrate in apple trees, its abundance was evaluated via primary metabolite analysis, as detailed later.

The anthocyanin concentration in apple peels was determined by analysing a 100 mg freeze-dried and finely ground subsample, following the method described by Kubasek et al. (1992). Briefly, samples were mixed with 1 mL of 1% hydrochloric acid (HCl) in methanol before extraction of the anthocyanins in the dark for 24 h. The samples were then centrifuged for 20 min at 10,000 rpm. The absorbance of the supernatants at 530 and 657 nm was measured using a Synergy HTX Multi-Mode Reader (Bio-Tek, Winooski, VT, USA), and the total anthocyanin content was calculated as  $A_{530} - 0.25 \times A_{657}$  to account for the contribution of chlorophyll and its degradation products to the absorption at 530 nm (Rabino and Mancinelli 1986).

## Fruit primary metabolites

For the samples collected on the final sampling date (harvest, 22 WAFS) in the middle part of the canopy, an untargeted analysis was performed to assess the abundance of primary metabolites in freeze-dried and finely ground apple skin and flesh samples (100 mg). The methods described in Rossouw et al. (2019) were utilised with minor amendments. The analysis was conducted using an Agilent system, comprising a 7890A gas chromatograph and a 5975C mass spectrometer equipped with an electron impact ionisation source and a quadrupole analyser (Agilent Technologies, Santa Clara, CA, USA). The injection volume was 1 µL in split and splitless mode (200:1). The helium carrier gas flow rate was set to 1.0235 mL/min. For the splitless mode, the column temperature program was set at 60 °C for 1 min, followed by a 10 °C/min ramp to 300 °C, where it was held for 10 min. For the split mode, the column temperature program was set at 80 °C, followed by a 15 °C/min ramp to 300 °C, where it was held for 10 min. Compound identification was

achieved through spectral deconvolution, and semiquantification was performed by normalising the data to an internal standard (adonitol) and tissue dry weight.

## Root, shoot and leaf nonstructural carbohydrates (NSC)

Concurrently with the fruit sampling, root, shoot, and leaf samples were collected. Root sampling involved dividing the area under each tree into four 90 degree sectors radiating from the trunk. The sampling events alternated between these sectors and included the collection of live roots measuring 4 to 10 mm in diameter from the vicinity of the tree base, within 20 cm of the soil surface. After collection, the roots were washed with distilled water, trimmed to remove soil and dead tissues, blotted dry and immediately frozen in liquid nitrogen. Frozen samples were stored at -80 °C until further processing. The processing involved grinding the frozen samples into a fine powder under liquid nitrogen, similar to the process used for the fruit samples described earlier, followed by freeze-drying. Starch and total soluble sugar (sucrose, glucose and fructose) concentrations in a 20 mg freeze-dried root tissue sample were determined using the same methods as those described earlier for fruit.

Shoot samples from the middle section of the canopy were obtained by taking a 5 cm long section from the junction of the terminal and subterminal growth units, with equal portions collected from each part. The shoot samples were frozen and stored at -80 °C before being freeze dried and ground to a fine powder using an ultracentrifugal mill (Retsch ZM200, Hann, NRW, Germany). The grinding process involved passing the samples through a 4 mm sieve initially, followed by further grinding to a fine powder using a 0.12 mm sieve. Starch and total soluble sugar concentrations were determined using previously described methods.

Ten fully expanded leaves positioned on fruiting terminal shoots in the middle section of the canopy were collected, with five leaves collected from each side of the row. The leaves were collected between 8.30 and 10.30 am, and frozen before being stored at -80 °C until processing. The frozen samples were then ground into a fine powder under liquid nitrogen before being freeze-dried. Starch and total soluble sugar concentrations were determined using previously described methods. However, for sugar analysis, the leaf extracts were subjected to a decolorization step using activated charcoal as described by Dayer et al. (2016).

## Return flowering and fruiting

On 25 October 2022, during the anthesis stage of the following growing season (2022–2023), the number of flowers on the upper, middle, and lower sections of each tree located at the centre of each plot was counted. Similarly, at the

subsequent harvest on 13 April 2023, the number of mature fruits each tree in the centre of each plot was counted for each horizontally divided section. During the same harvest, a sample of fruit per plot was collected from the middle section of the canopy and weighed. Five fruits were collected from each side of the row per tree, totalling thirty fruits across the three trees per plot.

## Statistical analysis

Statistical analysis was performed using Statistica 14 software (Tibco, Palo Alto, CA, USA). The analysis focused on evaluating treatment differences at individual time (sampling) points. Additionally, for applicable cases, the changes in treatments over time were assessed. The residual variance at each time point (sampling event) could vary, and the interventionist nature of the treatments might lead to reduced residual variance for some treatments. Therefore, in line with the randomised block experimental design aimed at addressing potential sources of variability, univariate analysis of variance (ANOVA) was performed for each sampling date and for each treatment where data were collected at multiple time points (all sampling dates). Significant differences between means were determined using Fisher's least significant difference (LSD) test ( $P < 0.05$ ). The results section outlines these significant differences between treatments or the significant changes observed for a specific treatment over the study period. Artwork was created using SigmaPlot 15 software (Systat, Grafitti, Palo Alto, CA, USA).

## Results

### Basic fruit compositional development

The average fresh weight per fruit sampled from the middle section of the canopy increased across all groups during the experiment (Table 1). However, no significant differences in fresh weight were observed between any of the groups at any stage. The fruit juice soluble solid (TSS) concentration ( $^{\circ}$ Brix) also increased for all groups (Table 1), with juice from both the control and girdled treatment demonstrating greater TSS than that from the crown and surface fire treatments at 16 WAFS.

The pH of the juice increased during the experiment (Table 1). No significant differences in pH were observed between the groups, except for the surface fire treatment, which exhibited a higher pH than the girdled treatment at 10 WAFS. This disparity was noted before treatment implementation, suggesting inherent variation in juice pH among the trees. In contrast, the juice titratable acidity (TA) decreased throughout the experiment (Table 1). TA was lower for the control than for the girdled and crown fire treatments at 10

WAFS, indicating pre-existing variability before treatment implementation.

Peel anthocyanin concentrations increased between 16 and 22 WAFS (Table 1). In terms of treatment disparities, simulated crown fire resulted in greater peel anthocyanin concentrations than did the control and girdled treatment at 16 and 22 WAFS.

### Basic fruit composition at harvest

No significant differences were detected in average fruit fresh weight among the groups when comparing fruits from the lower, middle, or upper sections of the canopy at harvest (22 WAFS) (Fig. 2a). Similarly, the number of fruits present per tree did not vary at harvest among the groups (Online Resource 1). Fruit from the surface fire treatment had a higher level of juice TSS in the top section of the canopy than in the bottom of the same trees (Fig. 2b). In the bottom section, fruits from the control and girdled treatments exhibited greater TSS than did those from the crown and surface fire treatments. Conversely, in the top section, the crown fire treatment corresponded to the lowest TSS.

Regarding juice pH, fruits from the control and surface fire treatment exhibited lower pH values at the top of the canopy than at the bottom (Fig. 2c). In the girdled treatment, fruits at the top had a lower pH than those at the middle and bottom. At the top, the pH was greater in the apples from the crown fire treatment than in those from the surface fire and girdled treatments. Additionally, the control had a higher juice pH than did the girdled treatment at the top.

For the crown fire treatment, fruits from the bottom exhibited greater juice TA than did those from the top (Fig. 2d). Conversely, for the surface fire treatment, fruit from the top had a greater juice TA concentration than did those from the bottom. At the bottom, fruits from the girdled treatment displayed a greater TA than did those from the crown and surface fire treatments. Furthermore, fruits from the control group had greater TA than did those from the surface fire treatment group. At the top, fruits from the crown fire treatment group had the lowest TA.

For the control, fruits from the top had reduced firmness compared to those from the middle and bottom (Fig. 2e). In the surface fire treatment, fruits from the top exhibited greater firmness than those from the bottom. At the bottom, fruits from the control treatment had greater firmness than those from the girdled treatment. In the middle, fruits from the surface fire treatment showed greater firmness than those from both the girdled and crown fire treatments. At the top, fruits from the surface fire treatment group exhibited greater firmness than those from the control and girdled treatment groups. Additionally, at the top, fruits from the crown fire treatment group had greater firmness than those from the control group.

**Table 1** Influence of control, trunk girdling, simulated crown fires, and simulated surface fires on fruit weight, juice total soluble solids, pH and titratable acidity, and peel anthocyanin concentration at the various sampling timepoints [weeks after fruit set (WAFS)] (mean  $\pm$  SE;  $n = 4$ )

Treatment	10 WAFS	12 WAFS	16 WAFS	22 WAFS	LSD <sub>0.05</sub>								
Fruit weight (g/apple)	Control	A	101.7 $\pm$ 3.4	A	125.5 $\pm$ 7.7	b	A	189.4 $\pm$ 8.9	a	20.16			
	Girdled	A	85.6 $\pm$ 5.2	A	99.4 $\pm$ 4.9	c	A	131.1 $\pm$ 6.7	b	17.59			
	Crown fire	A	85.1 $\pm$ 3.2	A	100.0 $\pm$ 3.3	c	A	129.0 $\pm$ 3.8	b	15.81			
	Surface fire	A	88.3 $\pm$ 4.0	A	102.6 $\pm$ 3.4	c	A	122.2 $\pm$ 5.5	b	19.67			
LSD <sub>0.05</sub>													
Juice TSS ( $^{\circ}$ Brix)	Control	A	9.5 $\pm$ 0.1	A	10.3 $\pm$ 0.1	d	A	11.1 $\pm$ 0.2	b	A	12.9 $\pm$ 0.3	a	0.04
	Girdled	A	9.7 $\pm$ 0.1	A	10.4 $\pm$ 0.2	c	A	11.2 $\pm$ 0.2	b	A	12.5 $\pm$ 0.4	a	0.06
	Crown fire	A	9.6 $\pm$ 0.1	A	9.9 $\pm$ 0.1	d	A	10.6 $\pm$ 0.1	b	A	12.0 $\pm$ 0.1	a	0.03
	Surface fire	A	9.3 $\pm$ 0.3	A	9.9 $\pm$ 0.1	c	A	10.4 $\pm$ 0.1	b	A	12.2 $\pm$ 0.2	a	0.07
LSD <sub>0.05</sub>													
Juice pH	Control	AB	3.25 $\pm$ 0.01	A	3.24 $\pm$ 0.01	c	A	3.32 $\pm$ 0.02	b	A	3.42 $\pm$ 0.01	a	0.04
	Girdled	B	3.20 $\pm$ 0.01	A	3.24 $\pm$ 0.01	c	A	3.35 $\pm$ 0.01	b	A	3.43 $\pm$ 0.03	a	0.06
	Crown fire	B	3.23 $\pm$ 0.01	A	3.26 $\pm$ 0.01	d	A	3.32 $\pm$ 0.01	b	A	3.41 $\pm$ 0.01	a	0.03
	Surface fire	A	3.30 $\pm$ 0.02	A	3.24 $\pm$ 0.02	bc	A	3.35 $\pm$ 0.01	ab	A	3.41 $\pm$ 0.03	a	0.07
LSD <sub>0.05</sub>													
Juice TA (g/L)	Control	B	10.4 $\pm$ 0.2	A	9.8 $\pm$ 0.4	a	A	8.3 $\pm$ 0.3	b	A	5.9 $\pm$ 0.4	c	0.97
	Girdled	A	11.2 $\pm$ 0.2	A	10.3 $\pm$ 0.3	a	A	8.5 $\pm$ 0.4	b	A	6.2 $\pm$ 0.4	c	1.09
	Crown fire	A	11.3 $\pm$ 0.2	A	9.5 $\pm$ 0.2	a	A	7.7 $\pm$ 0.1	c	A	5.2 $\pm$ 0.1	d	0.51
	Surface fire	AB	10.6 $\pm$ 0.3	A	9.5 $\pm$ 0.2	a	A	7.4 $\pm$ 0.2	c	A	5.5 $\pm$ 0.4	d	0.94
LSD <sub>0.05</sub>													
Peel anthocyanin (Abs/g)	Control	A	4.3 $\pm$ 0.2	B	3.8 $\pm$ 0.1	b	B	5.1 $\pm$ 0.2	b	A	27.0 $\pm$ 4.1	a	6.35
	Girdled	A	4.3 $\pm$ 0.2	B	4.4 $\pm$ 0.4	b	B	5.1 $\pm$ 0.3	b	A	26.9 $\pm$ 2.6	a	4.02
	Crown fire	A	4.4 $\pm$ 0.2	A	5.7 $\pm$ 0.3	b	A	7.3 $\pm$ 0.5	b	A	33.0 $\pm$ 3.1	a	4.89
	Surface fire	A	4.8 $\pm$ 0.4	AB	4.8 $\pm$ 0.5	b	AB	6.2 $\pm$ 0.6	b	A	25.7 $\pm$ 3.2	a	5.13
LSD <sub>0.05</sub>													
			0.88		1.04		1.31		10.15				

Treatments were implemented at 10 WAFS. Means were separated within columns using Fisher's LSD test, and significant differences are indicated at  $P < 0.05$ . When the same capital and lowercase letters appear in a column and row, respectively, the values do not significantly differ

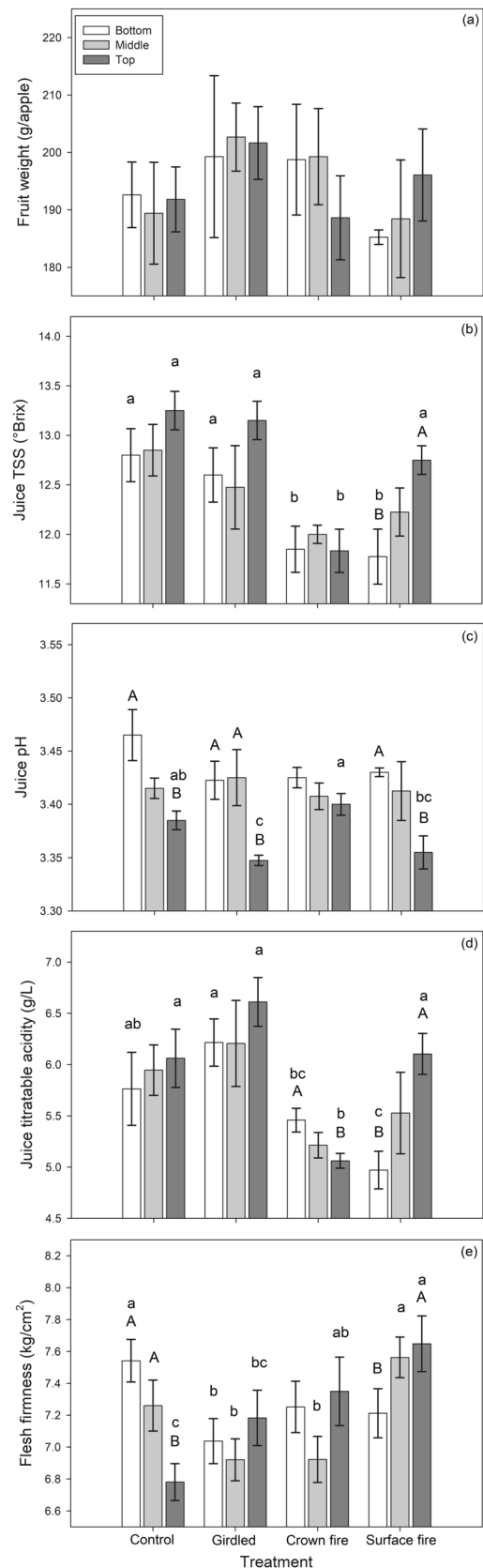
**Fig. 2** Effects of the control, trunk girdling, simulated crown fire and simulated surface fire treatments on the average weight per fruit (a), juice soluble solid concentration (b), pH (c), titratable acidity (d) and flesh firmness (e) at harvest [22 weeks after fruit set (WAFS)] for the bottom, middle and top of the canopy. The different upper and lower-case letters above the bars represent significant differences ( $P < 0.05$ ) among canopy positions within a treatment and among treatments within a canopy position, respectively (mean  $\pm$  SE;  $n = 4$ )

At harvest, fruits were categorised into starch indices of 4, 5, or 6, with indices of 4 and 6 indicating higher and lower starch contents, respectively (Table 2). At the bottom of the canopy, a larger proportion of fruits from the control had a starch index of 6 compared to 4. For the crown fire treatment, the smallest proportion of fruit at the bottom had a starch index of 4. Additionally, at the bottom, no fruit from the surface fire treatment had a starch index of 4, although a larger proportion had an index of 6 compared to 5. In the middle, no fruits from the crown and surface fire treatments had a starch index of 4. In the upper section, for the crown fire treatment, no fruits had a starch index of 4. For both the crown and surface fire treatments, the majority of fruits had a starch index of 6 at the top. Among the fruits with a starch index of 6, those in the girdled treatment had a reduced proportion compared to those in the crown and surface fire treatments at the top.

Regarding fruit peel colour scoring, at the bottom of the canopy, for the surface fire treatment, no fruit blushed less than 40%, and the highest proportion were greater than 80% blushed (Table 2). In the middle, none of the fruits from the crown fire treatment showed less than 20% blush. For the surface fire treatment in the middle, more fruit exhibited more than 80% blush compared to those with less than 40%. Additionally, in the middle, the control group had more fruits displaying less than 20% blush than did the crown and surface fire treatment groups. In the upper section, for the crown fire treatment, all fruits had a colour scale greater than 80% blush.

### Fruit nonstructural carbohydrates (NSC)

The fruit flesh starch concentrations decreased for all groups during the experiment, particularly between 16 and 22 WAFS (Fig. 3a). At 16 and 22 WAFS, fruits from control and the girdled treatment, respectively, had greater starch levels than did those from the crown and surface fire treatments. In terms of peel starch concentrations, all groups experienced decreases between 16 and 22 WAFS, reaching their lowest levels at harvest (Fig. 3b). At 22 WAFS, the control group exhibited greater peel starch levels than did the crown and surface fire treatment groups, while the girdled treatment group had greater peel starch levels than did the crown fire treatment.

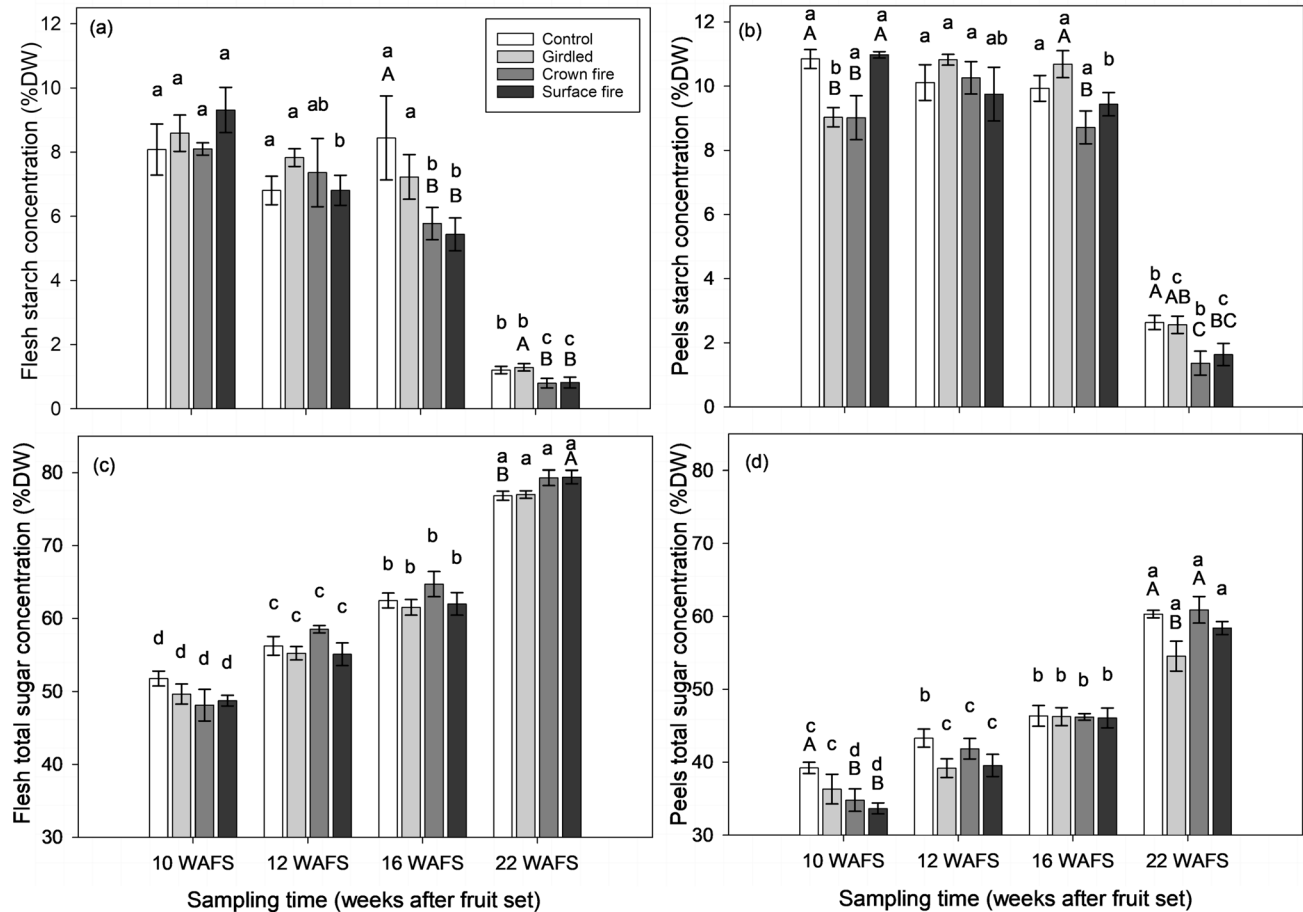




**Table 2** Influence of the control, trunk girdling, simulated crown fire and simulated surface fire treatments on the flesh starch index and peel surface blush development for fruits in the bottom, middle and top canopy sections at harvest [22 weeks after fruit set (WAFS)]

Section	Treatment	Starch index						Colour scale						LSD <sub>0.05</sub>													
		4	5	6	LSD <sub>0.05</sub>	<20%	20–40%	40–60%	60–80%	>80%	LSD <sub>0.05</sub>																
Bottom	Control	A	8±8	b	A	38±8	ab	A	54±14	a	33.81	A	8±5	b	A	21±4	b	A	42±11	a	A	13±8	b	B	4±4	b	20.76
	Girdled	A	8±5	a	A	58±16	a	A	33±15	a	41.70	A	21±11	a	A	17±7	a	A	33±15	a	A	25±11	a	B	4±4	a	30.76
	Crown fire	A	4±4	b	A	46±11	a	A	50±12	a	30.14	A	17±17	a	A	17±7	a	A	33±7	a	A	29±13	a	B	4±4	a	31.44
	Surface fire	A	0	c	A	42±5	b	A	58±5	a	12.59	A	0	c	A	0	c	A	13±8	bc	A	42±11	ab	A	46±19	a	30.76
	LSD <sub>0.05</sub>		16.16		32.74		37.61		31.23		16.15		32.94		32.73		30.56										
Middle	Control	A	8±8	b	A	67±14	a	A	25±8	a	33.25	A	21±8	a	A	29±4	a	A	21±8	a	A	17±12	a	A	13±4	a	23.38
	Girdled	A	8±5	b	A	63±14	a	A	29±14	ab	38.24	AB	13±4	bc	A	29±8	ab	A	33±7	a	A	21±4	abc	A	4±4	c	17.16
	Crown fire	A	0	b	A	54±13	a	A	46±13	a	32.65	B	0	c	B	4±4	bc	A	13±4	abc	A	46±19	a	A	38±17	ab	34.93
	Surface fire	A	0	b	A	46±14	a	A	54±14	a	37.15	B	4±4	c	B	4±4	c	A	13±4	bc	A	50±10	a	A	29±8	b	19.46
	LSD <sub>0.05</sub>		14.82		42.10		38.68		15.28		16.57		18.53		37.43		30.56										
Top	Control	A	13±4	b	A	58±11	a	AB	29±13	ab	31.42	A	0	b	A	13±4	ab	A	42±14	a	AB	33±15	ab	B	13±4	ab	29.37
	Girdled	A	17±10	b	A	67±12	a	B	17±12	b	35.35	A	0	b	B	0	b	A	25±14	b	A	58±11	a	B	17±12	b	29.01
	Crown fire	A	0	b	AB	44±6	a	A	56±6	a	15.73	A	0	b	B	0	b	A	0	b	B	0	b	A	100	a	-
	Surface fire	A	17±7	b	B	25±5	b	A	58±8	a	21.77	A	0	c	A	13±4	bc	A	21±8	b	A	46±4	a	B	21±8	b	17.16
	LSD <sub>0.05</sub>		21.12		28.95		32.97		-		9.96		37.05		32.28		25.07										

The proportion of fruits categorised according to the starch index or blush colour scale is shown for each treatment (mean ± SE;  $n=4$ ). Means were separated within columns using Fisher's LSD test, and significant differences are indicated at  $P<0.05$ . When the same capital and lowercase letters appear in a column and row, respectively, the values do not significantly differ



**Fig. 3** Effects of the control, trunk girdling, simulated crown fire and simulated surface fire treatments on starch concentrations in fruit flesh (a) and peels (b) and total sugar concentrations in flesh (c) and peels (d) for fruit collected from the middle canopy section at 10, 12, 16 and 22 weeks after fruit set (WAFS). Treatments were imple-

mented at 10 WAFS. The different upper and lowercase letters above the bars represent significant differences ( $P < 0.05$ ) among treatments at a given sampling time and among sampling times for a treatment, respectively (mean  $\pm$  SE;  $n = 4$ )

The total sugar concentration in the flesh increased between each sampling date for all groups (Fig. 3c). At 22 WAFS, the fruits in the surface fire treatment group exhibited greater flesh sugar concentrations than did the control group. Similarly, the total sugar concentration in peels also increased during the experiment (Fig. 3d). At 22 WAFS, both the control and the crown fire treatment had higher peel sugar concentrations than did the girdled treatment.

### Fruit primary metabolites

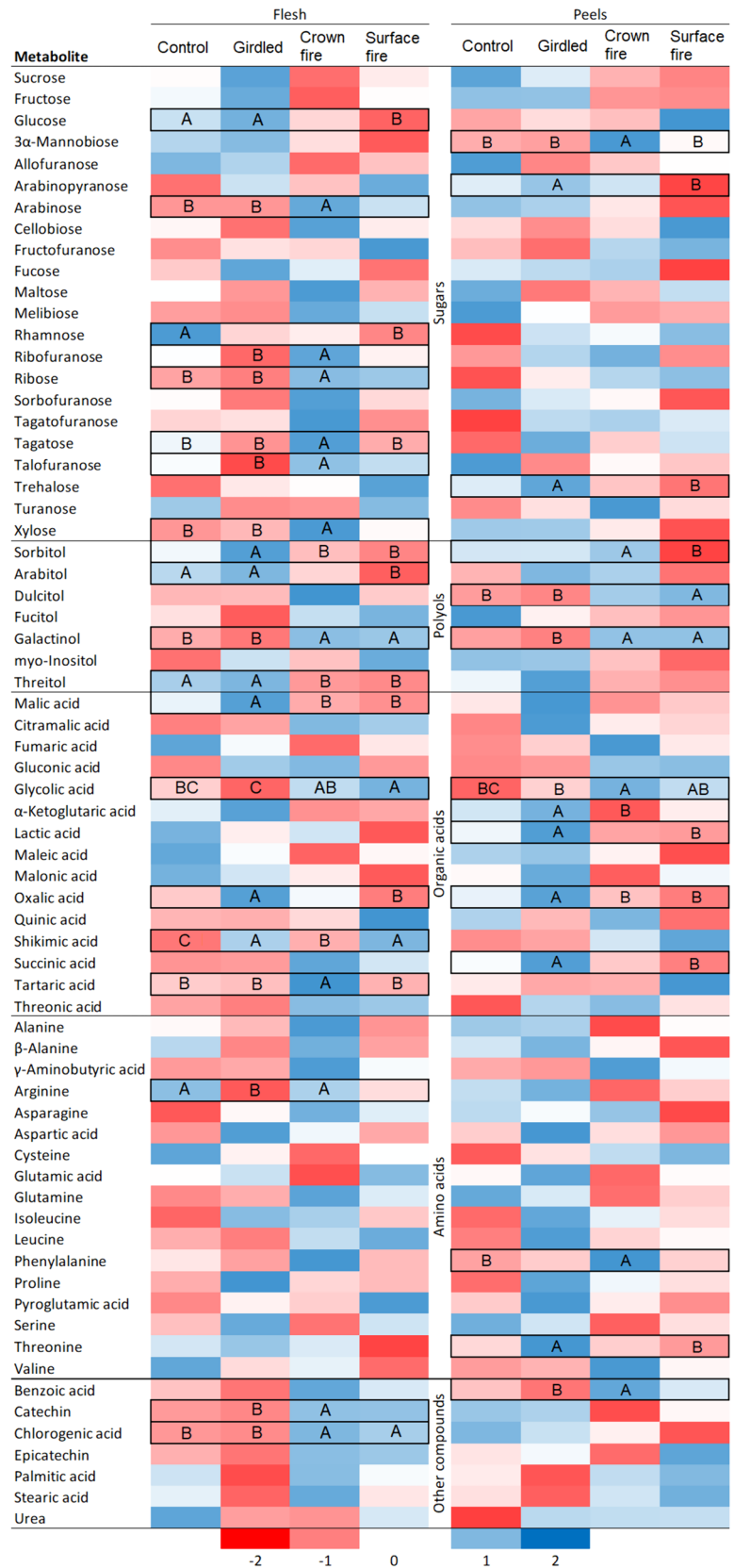
The primary metabolites of the fruit flesh and peels at harvest were categorised into carbohydrates (sugars and polyols, also known as sugar alcohols), amino acids, organic acids, phenolic compounds (polyphenols and phenolic acids), and other compounds (nitrogenous compounds and fatty acids). Twenty-two sugars, seven polyols, 15 organic acids, 17 amino acids, four phenolic compounds, one nitrogenous

compound, and two saturated fatty acids were identified through GC/MS analysis. Detailed information on the abundance and mass spectra of all identified metabolites can be found in Online Resource 2. The metabolites that exhibited significant treatment effects are described below (Fig. 4).

Among the major sugars, sucrose and fructose did not show significant treatment effects at harvest in fruit flesh. However, glucose levels were notably greater in both the control and girdled treatment than in the surface fire treatment. Several less abundant (minor) sugars, including arabinose, rhamnose, ribofuranose, ribose, tagatose, talofuranose, trehalose, and xylose, had significant treatment effects. A discernible trend emerged, with specific minor sugars being favoured by either the control and girdled treatment or by the crown and surface fire treatments.

In addition to glucose, the control displayed higher levels of rhamnose than did the surface fire treatment. Compared with those in the control and girdled treatment, the levels

**Fig. 4** Effects of the control, trunk girdling, simulated crown fire and simulated surface fire treatments on primary metabolite abundance in fruit flesh (left) and peels (right) for fruit collected from the middle canopy section at harvest [22 weeks after fruit set (WAFS)]. Metabolites are classified according to their chemical properties [sugars, polyols, organic acids, amino acids and other compounds (phenolic compounds, saturated fatty acids and nitrogenous compounds)]. The average abundance of metabolites is depicted using colour-coded scales at the base of the figure. Different uppercase letters represent significant differences ( $P < 0.05$ ) between treatments



of arabinose, ribose, and in the crown fire treatment group were greater. Furthermore, the crown fire treatment group had higher levels of ribofuranose and talofuranose than did the girdled treatment group, as well as higher levels of tagatose than did all other groups. The surface fire treatment had greater levels of ribose compared to control and the girdled treatment, greater levels of talofuranose compared to the girdled treatment, and increased levels of trehalose compared to control.

In fruit flesh, the girdled treatment had greater levels of sorbitol than did the crown and surface fire treatments. Both the control and the girdled treatment exhibited more arabitol than did to the surface fire treatment, while the threitol levels in both the control and the girdled treatment were greater than those in the other two treatments. Conversely, the galactinol levels were greater in the crown and surface fire treatments than in the control and the girdled treatment.

Malic acid levels were greater in fruit flesh from the girdled treatment than in that from the crown and surface fire treatments, while greater levels of oxalic acid were also detected in the girdled treatment than in the surface fire treatment. Additionally, the girdled and surface fire treatments had more shikimic acid than did the control and the crown fire treatment, while the control had more shikimic acid than did the crown fire treatment. The surface fire treatment had more glycolic acid than did the control and the girdled treatment, with the crown fire treatment exhibiting greater glycolic acid levels than did to the girdled treatment. Tartaric acid levels were highest in the crown fire treatment group.

Regarding amino acids, arginine levels were greater in fruit flesh from the control and the crown fire treatment than in that from the girdled treatment. In terms of phenolic compounds, catechin exhibited greater levels in the crown fire treatment than in the girdled treatment, while chlorogenic acid was present in greater levels for the crown and surface fire treatments than in the control and the girdled treatment.

In fruit peels, the major sugars sucrose, fructose, and glucose did not significantly differ among the groups, nor did most other sugars. However, arabinopyranose and trehalose were more abundant in the girdled treatment than in the surface fire treatment. Conversely, the disaccharide 3 $\alpha$ -mannobiose was more abundant in peels from the crown fire treatment group than in those from all the other groups. Sorbitol levels were greater for the crown fire treatment than for the surface fire treatment. Higher levels of dulcitol were detected in the surface fire treatment group than in the control and girdled treatment groups. Both the crown and surface fire treatments resulted in greater levels of galactinol than did the girdled treatment.

The girdled treatment had greater levels of lactic and succinic acids compared to the surface fire treatment and greater levels of oxalic acid compared to both the crown

and surface fire treatments. For  $\alpha$ -ketoglutaric acid, the girdled treatment had greater levels than did the crown fire treatment. Furthermore, glycolic acid levels were greater in the crown and surface fire treatments than in the control, with the crown fire treatment also having greater glycolic acid levels than the girdled treatment.

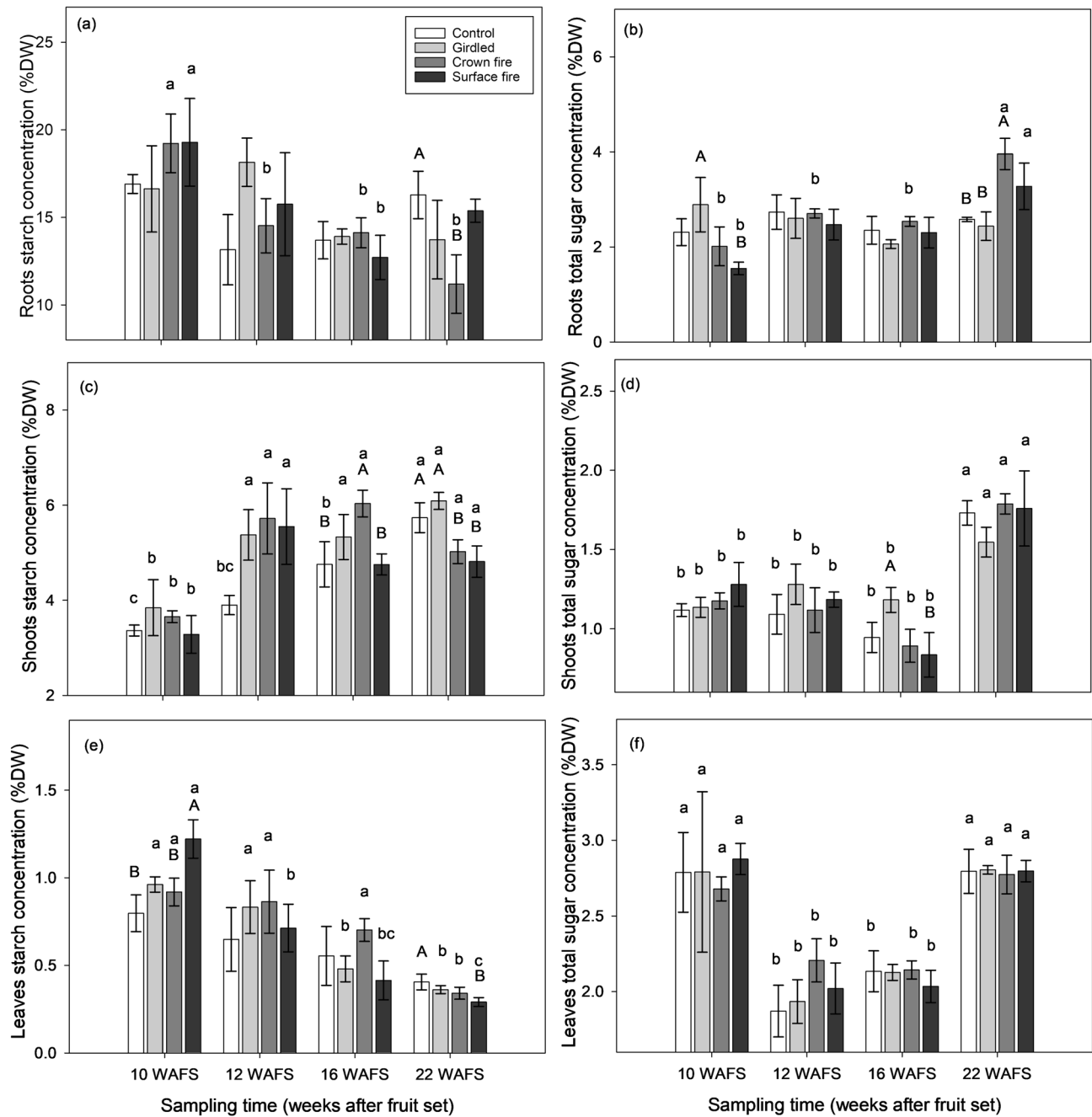
Among the amino acids, threonine and phenylalanine showed notable differences among the treatments. The girdled treatment group displayed greater threonine levels than did the surface fire treatment group. Conversely, for phenylalanine, the crown fire treatment group exhibited higher phenylalanine levels than the control group. Among the phenolic compounds, benzoic acid levels were greater in the crown fire treatment group than in the girdled treatment group.

### Root, shoot and leaf nonstructural carbohydrates (NSC)

The crown fire treatment decreased the amount of root starch between 10 and 12 WAFS, which was lower than that at the initial sampling for the subsequent samplings (Fig. 5a). Similarly, the surface fire treatment reduced the amount of root starch between 10 and 16 WAFS. At 22 WAFS, the control had a greater root starch concentration than did to the crown fire treatment. The root total sugar concentration increased in response to the crown fire treatment between 16 and 22 WAFS (Fig. 5b). The surface fire treatment increased the amount of root sugar between 10 and 22 WAFS. At 22 WAFS, the trees in the crown fire treatment had greater root sugar concentrations than did the control and girdled treatment.

At the junction of the terminal and subterminal growth units of fruiting shoots in the middle of the canopy, starch concentrations increased for all groups between 10 and 22 WAFS (Fig. 5c). At 16 WAFS, the crown fire treatment group exhibited greater shoot starch than the control and surface fire treatment groups. However, at 22 WAFS, the control and girdling treatment had greater shoot starch concentrations than did the other two treatments. The total sugar concentration in shoots increased for all groups between 16 and 22 WAFS (Fig. 5d). At 16 WAFS, shoots from the girdled treatment had a greater total sugar concentration than did those from the surface fire treatment.

In leaves from the middle of the canopy, starch concentrations decreased for all groups during the experiment (Fig. 5e). At 22 WAFS, the control had a greater leaf starch concentration than did the surface fire treatment. The leaf total sugar concentration decreased for all groups between 10 and 12 WAFS, albeit increasing again to baseline levels by 22 WAFS (Fig. 5f).



**Fig. 5** Effects of the control, trunk girdling, simulated crown fire and simulated surface fire treatments on starch and total sugar concentrations in roots (**a** and **b**), shoots (**c** and **d**), and leaves (**e** and **f**) at 10, 12, 16 and 22 weeks after fruit set (WAFS). Treatments were imple-

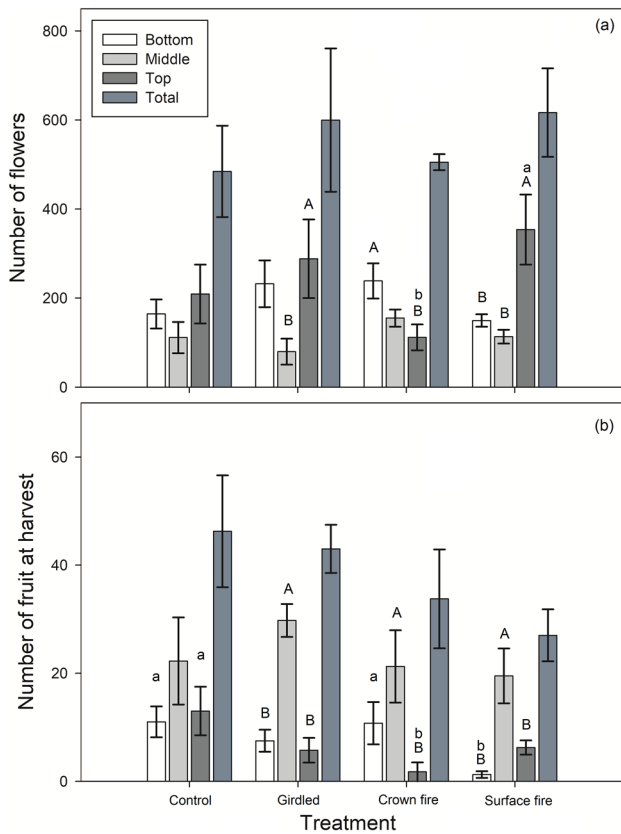
mented at 10 WAFS. The different upper and lowercase letters above the bars represent significant differences ( $P < 0.05$ ) among treatments at a given sampling time and among sampling times for a treatment, respectively (mean  $\pm$  SE;  $n = 4$ )

### Return flowering and fruiting

During the anthesis stage of the following (2022–23) growing season, the top section of trees in the surface fire treatment had more flowers than did the crown fire treatment (Fig. 6a). Additionally, the girdled treatment had more flowers in the top section than in the middle section. The surface

and crown fire treatment groups displayed more flowers in the top sections compared to the bottom and middle sections, and in the bottom sections compared to the top sections, respectively. However, there were no significant differences in the total number of flowers per tree among the groups.

At harvest, in the growing season following the treatment period, variations in fruit presence were observed at the top of



**Fig. 6** Effects of the control, trunk girdling, simulated crown fire and simulated surface fire treatments on flower count at anthesis (a) and fruit count at harvest (b) in the growing season after the treatment period, across the different canopy regions (bottom, middle, and top) as well as cumulatively per tree. The different upper and lowercase letters above the bars represent significant differences ( $P < 0.05$ ) among canopy positions (bottom, middle or top) within a treatment and among treatments within a canopy position, respectively (mean  $\pm$  SE;  $n = 4$ )

the canopy (Fig. 6b). Control trees had more fruit compared to the crown fire treatment, which had very few fruits in this part of the canopy. At the bottom, the surface fire treatment group had fewer fruits than the control and crown fire treatment groups. For the crown fire treatment, the middle of the trees had more fruit than did the top. For the girdled and surface fire treatments, the middle had the highest fruit count. However, there were no significant differences in total fruit numbers per tree among the treatment groups. The average weight per apple in the middle of the canopy also did not significantly differ among groups (Online Resource 3).

## Discussion

This study aimed to simulate the effects of surface and crown fires on ‘Pink Cripps’ apple trees by implementing defoliation, girdling, and combined treatments during

the 2021–22 growing season. This approach allowed us to investigate the impact of these treatments on reproductive development and nonstructural carbohydrate (NSC) reserves, with a focus on fruit composition. Given the timing of the experiment during the summer danger period for bushfires in southern Australia (Sharples et al. 2016), this study sought to provide insights into how potential fire-related injuries could affect apple tree physiology.

The treatments, including defoliation of specific canopy sections and girdling, were designed to mimic the effects of surface and crown fires (Fig. 1). Defoliation targeted either the upper or lower thirds of the canopy, with the latter combined with girdling to simulate surface fire injury. The middle section of the canopy remained untreated for comparison among the groups. Fruit development and composition were primarily assessed in this central portion, with additional evaluations conducted on fruit from the top and bottom sections of the canopy during harvest. The experimental design also allowed for the examination of carry-over effects into the subsequent growing season (2022–23) by assessing flowering and fruiting patterns.

## Fruit size

The treatments did not significantly affect fruit size, although there was an increase in fruit weight over time (Table 1). Traditional girdling tends to increase fruit size in various horticultural crops such as grapevine (*Vitis vinifera*), peach (*Prunus persica*), and avocado (*Persea americana*), by enhancing the allocation of photosynthates to fruit (Davie et al. 1995; Goren et al. 2003). Based on the current results, the effectiveness of trunk girdling, similar to mild fire damage to phloem conductivity, in influencing apple fruit size appears to be limited (Miller 1995; Arakawa et al. 1998; Goren et al. 2003).

The lack of an apparent defoliation effect on fruit weight development aligns with findings by Matsumoto et al. (2018). However, it is worth noting that early and severe defoliation, particularly around flowering and exceeding 60% of canopy leaf area, has been shown to lead to smaller fruits (Bound 2021). The current results suggest that fruit size may remain largely unaffected when the trunk phloem is damaged by fires or when up to a third of the canopy’s leaf area is burned during apple fruit growth.

It is important to consider the differences between the temporary phloem damage caused by girdling and the longer-term damage and progressive injury caused by smouldering surface fires, which can even lead to tree death (Goren et al. 2003; Idowu et al. 2023a).

## Fruit carbohydrates

Trunk girdling did not notably impact the fruit juice total soluble solids (TSS) concentration at harvest (Fig. 2). This outcome aligns with the findings of previous research, showing that the effect of trunk girdling on TSS concentration can vary based on factors such as girdle position, severity, timing, and plant species (Goren et al. 2003). For example, some studies have shown that trunk girdling only modestly increases the TSS of apple juice, with no significant effect noted in subsequent growing seasons (Fallahi et al. 2018).

Other factors, such as rootstock and tree structure, appear to have a more significant influence on TSS. Interestingly, severe damage from smouldering fires has been noted to enhance apple maturation, including sugar accumulation (Idowu et al. 2023b). However, based on the current results, when only mild damage occurs to trunk vascular tissues within the final 13 weeks of fruit development, it seems unlikely that the juice TSS concentration will be significantly affected.

A temporary slowing of fruit sugar accumulation was observed in the middle canopy following the simulated crown and surface fire treatments (Table 1). However, the proximity to the defoliation zone influenced fruit sugar accumulation at harvest, with the crown fire treatment showing the lowest TSS levels, 11% and 7% lower in the top and bottom canopy sections, respectively, than those in the control (Fig. 2). In the bottom section, the crown and surface fire treatments led to a 7% and 8% decrease in TSS, respectively. This suggests a localised disruption of fruit sugar accumulation due to defoliation.

The observed effects may be attributed to decreased canopy-produced sugars and limited fruit carbohydrate allocation in the defoliation zone (Rossouw et al. 2018). Additionally, trunk girdling in the surface fire treatment would have restricted the mobilisation of starch reserves from the roots to the canopy, potentially further limiting the fruit carbohydrate supply (Goren et al. 2003; Rossouw et al. 2017a). These findings suggest a certain autonomy between branches concerning carbohydrate depletion's impact on apple fruit maturation (Sprugel et al. 1991). Therefore, in instances of fire damage to certain foliage segments during fruit development, fruits in or near those sections may experience reduced TSS compared with those in unaffected areas.

Starch concentrations decreased in the flesh as fruit matured (Fig. 3), which is consistent with the natural degradation of starch in apples (Brookfield et al. 1997; Li et al. 2012). This degradation is accompanied by an increase in sugar concentration (Fig. 3), indicating the accumulation of sugars resulting from starch degradation. Trunk girdling alone did not significantly affect the fruit starch index at harvest relative to control (Table 2), consistent with the findings of Elfving et al. (1991). However, compared to that in the

girdled trees, the loss of leaf area in the top section of the canopy seemingly accelerated starch degradation, irrespective of the defoliation location (Fig. 3).

Crown and surface fire treatments also led to lower fruit starch concentrations in the middle of the canopy, suggesting that the loss of leaf area during fruit growth can hasten starch degradation in apples (Bound 2021). Compared with the control group, the surface fire treatment resulted in a greater total sugar concentration at harvest on a dry weight basis, corresponding to the accelerated starch degradation observed (Fig. 3).

As the fruit approached maturity, there was a notable decrease in peel starch, indicating the degradation of starch not only in fruit flesh but also in peels (Fig. 3). This effect was more pronounced in the crown fire treatment. The accelerated breakdown of starch following defoliation coincided with higher concentrations of total sugars. Overall, these results suggest that loss of leaf area following crown fires is likely to significantly alter the starch-to-sugar ratios in apples during maturation.

## Fruit acidity, colour and firmness

By harvest, the girdling treatment had a minor enhancing effect on the juice titratable acidity (TA) (Fig. 2), which is consistent with the findings of Arakawa et al. (1998). This effect was particularly noticeable in fruit found at the canopy's top. Conversely, both the crown and surface fire treatments negatively impacted TA, especially in fruits nearer to the defoliation zone. Increased fruit exposure to sunlight and intensive respiration of malic acid may contribute to lower TA levels following defoliation (Bergqvist et al. 2001; Acimovic et al. 2016). Therefore, damage to the trunk vascular system and loss of canopy leaf area during fruit maturation have repercussions for fruit acidity, which likely affects the sensory and quality perception of apples (Corollaro et al. 2014).

Contrary to the findings of Fallahi et al. (2018), girdling did not significantly influence apple peel anthocyanins (Table 1). It is possible that factors such as the timing of girdling and sugar accumulation could determine the stimulation of anthocyanin production following girdling, given the crucial roles sugars and carbon availability play in anthocyanin biosynthesis (Goren et al. 2003; Das et al. 2012; Durán-Soria et al. 2020). Thus, the timing of surface fire damage to apple tree trunk phloem may be crucial for influencing not only fruit size and sugar accumulation but also fruit peel colour development.

Notably, compared with the control group, the crown fire treatment increased the peel anthocyanin concentration in the middle by 50% and 43% respectively, at 12 and 16 WAFS (Table 1), which was consistent with the visual colour scores at harvest (Table 2). Light has been proven to stimulate the

synthesis and accumulation of anthocyanins in apple peels (Saure 1990; Takos et al. 2006; Jaakola 2013), suggesting that the crown fire treatment's enhancement of red colouration in apple peels may be due to improved light conditions. Interestingly, the effects of crown and surface fire treatments on peel colour scores were localised across different canopy sections at harvest. In the lower section, surface fire treatment yielded a greater percentage of fruits exhibiting more than 80% blush compared to the other groups, while at the top, all fruits from the crown fire treatment showed more than 80% blush, outperforming the other treatments. These localised effects highlight the significant role of light exposure in the development of apple fruit peel colour.

Trunk girdling suppressed fruit firmness, particularly at the bottom of the canopy (Fig. 2), consistent with the findings of Mostafa (2002). In contrast, Elfving et al. (1991) showed that girdling can help retain firmness in apples. This suggests that the impact of girdling, or surface fire damage to the trunk, on apple firmness is influenced by factors such as the location of the fruit within the canopy, cultivar and the timing of the girdling effect (DeEll et al. 2001). Compared with the control treatment, the crown and surface fire treatments resulted in greater fruit firmness at the top of the canopy. Some studies have reported no effect of defoliation on firmness (Matsumoto et al. 2018), while others have suggested slight increases in firmness but only with severe defoliation of approximately 50 to 75% of the total canopy leaf area (Bound 2021). However, these studies did not specifically assess fruit from distinct sections of the canopy as in our case.

### Primary metabolites in fruit flesh

At harvest, the treatments had a significant impact on less than half of the sugars and polyols identified through GC–MS analysis in the fruit flesh from the untreated middle section of the canopy (Fig. 4). The girdling treatment failed to induce a notable influence on sugars or polyols. However, sorbitol, which, in apple trees, is the primary carbohydrate transported through the phloem from sources such as photosynthesising leaves to sinks such as growing fruit (Loescher et al. 1982; Tijero et al. 2021; Idowu et al. 2023a), benefitted more from the girdling treatment than from the crown and surface fire treatments. This suggests that relative to girdling, a loss of leaf area may hamper the supply of sorbitol from the canopy, likely hindering its supply to fruit. Moreover, defoliation may accelerate the conversion of sorbitol into sugars such as fructose, a process that occurs naturally as apples mature (Tijero et al. 2021).

The crown and surface fire treatments also had detrimental effects on flesh arabinol, while the surface fire treatment had a detrimental effect on the abundance of threitol. A reduction in leaf area and the consequent impact on the

synthesis or transport of these polyols could have resulted in their lower abundance in fruit. The surface fire treatment also suppressed the accumulation of glucose and rhamnose, indicating that a decrease in leaf area in the lower part of the canopy coupled with trunk girdling has a detrimental impact on the synthesis of certain sugars in apples.

In contrast to the inhibitory effects of the crown and surface fire treatments on some carbohydrates in fruit, it appears that these treatments had beneficial effects on the abundance of certain other sugars and polyols. Sugars and polyols are known to contribute to the tolerance of plants and plant organs to abiotic stresses such as drought and high salinity (Sami et al. 2016). They play roles in cellular osmotic regulation and osmoprotection, and act as signalling molecules that modulate stress responses to control gene expression and developmental processes in plants (Moing 2000; Rontein et al. 2002; Valluru and Van den Ende 2011; Krasensky and Jonak 2012). The higher levels of certain sugars, including arabinose, ribose, xylose, trehalose, tagatose, and the polyol, galactose, in fruits from the middle canopy after a loss of leaf area, potentially resulted from a stress induced response.

As discussed earlier, the crown and surface fire treatments had a negative impact on juice TA, and the metabolic profiling analysis revealed that these variations appear to be attributed to differences in malic acid levels. Malic acid is the primary organic acid found in apples (Suni et al. 2000; Wu et al. 2007), and it is likely that increased sunlight exposure on the fruit in the middle canopy accelerated the breakdown of malic acid after defoliation, particularly compared to girdling alone. In contrast to malic acid, tartaric acid concentrations were stimulated by crown fire treatment, possibly also resulting from improved sunlight exposure. This finding aligns with research on grapes indicating that sunlight exposure may increase the fruit tartaric acid concentration but is detrimental to malic acid (Smart 1985; Reshef et al. 2017).

The girdling and surface fire treatments appeared to promote the accumulation of shikimic acid, indicating a stimulatory effect of trunk girdling on this metabolite. Conversely, crown fire treatment had an inhibitory effect on shikimic acid. The crown and surface fire treatments favoured the accumulation of glycolic acid, while the girdled treatment resulted in higher levels of oxalic acid than did to the crown fire treatment. Overall, these findings indicate that both girdling, and defoliation, similar to different types of fire injury, can alter the organic acid composition of apple flesh, although their effects are variable.

The effects of the treatments on amino acids in apple fruit flesh were generally not significant, except for arginine, which showed a noticeable response. Girdling seemed to have a suppressive effect on arginine levels. Arginine is a major long-distance transport amino acid in the phloem of apple trees (Tromp and Ova 1971; Lea et al. 2007). These



results imply that girdling-induced phloem damage might hinder the accumulation of arginine in apples. However, when girdling was combined with defoliation for surface fire treatment, this negative effect on arginine levels was not observed.

Compared with the girdling treatment, the crown fire treatment resulted in higher levels of the polyphenol catechin. Furthermore, both the crown and surface fire treatments led to greater concentrations of the phenolic acid chlorogenic acid than did the control and the girdling treatment. These findings suggest that a decrease in leaf area may have a beneficial impact on specific phenolic compounds in apple fruit flesh. This observation is consistent with the stimulating effect of defoliation, particularly crown fire treatment, on the accumulation of peel anthocyanins, another type of phenolic compound. Taken together, these results emphasise the potential of defoliation to increase the content of various phenolic compounds in apple fruit, likely by improving sunlight exposure. It is known that sunlight exposure stimulates the expression of genes involved in the synthesis of phenolic compounds in apples (Feng et al. 2013; Li et al. 2013). These compounds can contribute to UV-B protection under conditions of high sunlight exposure (Solovchenko and Schmitz-Eiberger 2003).

### Primary metabolites in fruit peels

The effects of the treatments on apple peel carbohydrate metabolites were limited, with only three sugars and three polyols showing significant changes (Fig. 4). However, girdling had a stimulatory effect on the levels of various organic acids in the peel. These findings suggest that trunk girdling may enhance the accumulation of organic acids involved in primary carbon metabolism, including the tricarboxylic acid cycle (Igamberdiev and Eprintsev 2016).

Additionally, crown fire treatment stimulated the accumulation of the organic acid phenylalanine in the peel. This aligns with a previous study on grapes, where defoliation was found to be associated with increased phenylalanine levels in red berries (Rossouw et al. 2018). Phenylalanine serves as a precursor for the synthesis of flavonoids, including anthocyanins, through the phenylpropanoid pathway (Fanyuk et al. 2022). The increased exposure to sunlight after defoliation may have had a stimulating effect on phenylalanine concentrations, potentially contributing to the enhanced biosynthesis of anthocyanins as observed in the peels.

### Carbohydrate reserves

The root starch reserves decreased following the crown and surface fire treatments and were significantly lower in the crown fire treatment group than in the control group at

harvest (Fig. 5). In contrast to starch, the crown fire treatment induced higher sugar concentrations than did the control at harvest (Fig. 5). Root sugar accumulation in conjunction with starch depletion suggests a potential reallocation of carbohydrates to support fruit development when the leaf area is insufficient relative to the crop load (Rossouw et al. 2017a). With deciduous crops such as apple if root starch reserves fail to recover during the postharvest period prior to leaf fall or if leaf functioning remains inadequate, it could impact fruit yields in subsequent growing seasons (Loescher et al. 1990; Smith and Holzapfel 2009; Holzapfel et al. 2010).

In fruiting shoots collected from the middle of the canopy, a reduction in canopy leaf area hindered the accumulation of starch reserves by harvest, regardless of the presence of trunk girdling (Fig. 5). This limitation in reserve accumulation can be attributed to the increased demand for carbohydrates within nearby woody tissues to support fruit growth (Candolfi-Vasconcelos et al. 1994; Rossouw et al. 2017a; Sharma et al. 2019). Consequently, low starch reserves in shoots after harvest may restrict interannual fruit bearing, affecting productivity in subsequent fruiting cycles (Jackson 2003; Sharma et al. 2019; Capelli et al. 2021).

For the surface fire treatment, the combination of leaf loss at the bottom of the canopy and trunk girdling led to decreased starch storage in the middle leaves (Fig. 5). This phenomenon can be ascribed to an escalated demand for carbon export from the remaining apple leaves when the overall leaf area is diminished (Zhou and Quebedeaux 2003). Additionally, the restriction imposed by girdling on the remobilisation of starch reserves from roots to the canopy may exacerbate this effect.

### Return productivity

In the following spring, trees previously subjected to crown fire treatment showed a 53% reduction in the number of flowers in the top third of the canopy compared to those in the bottom section (Fig. 6). On the other hand, the surface fire treatment was associated with a decreased number of flowers at the bottom and middle parts of the canopy compared to at the top, with reductions of 58% and 68%, respectively. This indicates that the treatments involving defoliation, irrespective of the presence of trunk girdling, had a negative impact on flowering in the subsequent growing season, with localised effects observed near the zone of defoliation. A loss in leaf area may curtail the replenishment of carbohydrate reserves in the perennial structure, often in a localised fashion, meaning woody tissues near the zone of defoliation may enter the next growing season with low reserves (Sprugel et al. 1991). These depleted reserves likely contributed to the reduction in flowering observed in the following growing season.

Compared with those of the control, the crown and surface fire treatments resulted in a substantial reduction of 87% and 89%, respectively, in the average number of fruits in the top and bottom parts of the canopy (Fig. 6). These findings underscore the impact that leaf area loss may have on fruiting in the subsequent growing season, particularly in the areas of the canopy where defoliation originally occurred. In addition to reduced flowering, the decrease in fruit number likely results from disrupted fruit set and retention (Breen et al. 2020; Bound 2021).

Biennial or alternate bearing is a phenomenon characterised by a high production growing season followed by a lighter crop load in the subsequent year. This pattern has been documented in various fruit crops, including apple, and is thought to be related to fluctuations in carbohydrate availability (Jackson 2003; Smith and Samach 2013; Sharma et al. 2019). Based on our findings, it can be inferred that erratic bearing in apple trees may occur after leaf area loss, and this phenomenon may be localised, specifically when a section of the canopy experiences defoliation. Hence, the relative impacts of surface and crown fires on tree carbohydrate reserves are likely to vary, affecting fruit distribution across the canopy in the following growing season.

## Conclusions

A study was conducted to investigate the effects of simulated fire damage on the carbohydrate dynamics and reproductive development of apple trees by simulating injuries through trunk girdling, partial defoliation, and a combination treatment during fruit maturation. Damage to the trunk phloem and partial leaf loss had negligible effects on fruit size, while trunk girdling alone did not significantly impact fruit starch, sugar, nor anthocyanin levels. However, partial defoliation led to localised negative effects on fruit sugar accumulation and accelerated starch degradation. Girdling enhanced fruit acidity, while defoliation had a depressing effect, particularly in the defoliated areas. Defoliation also increased fruit peel anthocyanin levels, particularly in fruit near the defoliation zone. Moreover, defoliation negatively affected carbohydrate reserves in the roots, particularly when leaf loss occurred near the apex, with implications for flowering and crop load in the following growing season. Overall, while mild damage to trunk vascular tissues, such as girdling, appears to have relatively minor detrimental effects on apple tree reproductive development, severe smouldering fires resulting in long-term damage to vascular tissues could have more pronounced effects. Additionally, defoliation of approximately one-third of the canopy can have significant repercussions on various aspects of fruit development including fruit compositional changes and loss of productivity in the subsequent growing season. This study underscores

the probable differential adverse effects of surface and crown fires on apple fruit development and productivity over two consecutive growing seasons.

## Author contributions

GCR, BPH and OI conceptualised the study; GCR designed the experiment and methodology, acquired the funding, administered the project, provided supervision, analysed the data, and wrote the first manuscript draft; GCR, BPH, OI and AG conducted the experiment and collected data; BPH, OI and AG reviewed and contributed to the manuscript.

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**Data availability** Data are available at the discretion of the corresponding author upon reasonable request.

## Declarations

**Competing interests** The authors have no relevant financial or non-financial interests to disclose.

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