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ORIGINAL ARTICLE

Variation in constitutive and induced chemistry in the needles, bark and roots of young *Pinus radiata* trees

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Abstract

Key message **In *Pinus radiata*, both primary and secondary compounds may play a role in defence against herbivory. Allocation of constitutive and induced primary and secondary compounds varies among needles, bark and roots. Responses to stresses are stronger in primary than secondary compounds and some responses of root chemistry to above-ground stressors were detected.**

Abstract The capacity of trees to cope with pests and pathogens depends in part on the variation of constitutive and induced chemical defences within the plant. Here we examined the constitutive and induced variation of primary (sugars and fatty acids) and secondary (mono-, sesqui- and di- terpenoids as well as volatile phenolics) metabolites in the needles, bark and, for the first time, roots of 2-year old *Pinus radiata*. A total of 81 compounds were examined. The plant parts differed significantly in constitutive levels of individual sugars, fatty acids, mono-, sesqui- and di- terpenoids as well as volatile phenolics. Overall, the bark had more compounds and a higher amount of most secondary compounds and the levels of compounds in the roots differed from that of the needles and bark. For example, glucose was the dominant sugar in the needles and bark whereas fructose dominated in the roots. Of the fully identified secondary compounds, monoterpenoids dominated in all plant parts but with different qualitative patterns. Following methyl jasmonate and bark stripping treatments, a marked reduction in sugars but weaker changes in secondary compounds were detected in the needles and bark. Responses in the roots were minor but the few that were detected were mostly in response to the bark stripping treatment. Changes in correlations among chemicals within plant parts and between the same compound across the different plant parts were also detected after stress treatments. Overall, results showed that the constitutive composition in the roots differs from that of the bark and needles in *P. radiata* and inducibility is stronger in the primary than secondary metabolites and differs between plant parts. This detailed assessment of *Pinus radiata* chemistry in the needles, bark and roots, including the compounds that respond to simulated biotic stress will potentially facilitate the identification of related chemical defence traits.

Keywords Plant defence · Primary chemistry · Roots · Bark stripping · Methyl jasmonate

Introduction

The chemistry of different parts of a plant may not be homogeneous given the different roles they play in plant life functions and plant–environment interactions. In conifers, differences in chemical composition and concentration between plant parts of individual trees of the same species have been well studied from the perspective of defences (Moreira et al. 2012a; Tomlin et al. 2000). The main secondary metabolites (also known as specialized natural products), the terpenes, phenolics and alkaloids (Franceschi et al. 2005), have been implicated in resistance to various pests and pathogens in the needles and bark (Moreira et al. 2012a; Reglinski et al.

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2017). Chemical defences that are constitutive or induced locally at the site of damage or across the plant/tissues (i.e. systemically), can differ among plant parts (Franceschi et al. 2005; Iason et al. 2011; Moreira et al. 2012a, 2013a). The timing of induced defences following herbivore damage or exogenous application of chemical elicitors, such as methyl jasmonate, has also been shown to differ for different compounds and between plant parts (Lewinsohn et al. 1991; Miller et al. 2005). Various theories explain the spatial and temporal distribution of chemistry in plants. Within an individual, the optimal defence hypothesis predicts that defences will be concentrated in parts or tissues that are at higher risk of herbivory and/or tissues that are more valuable (McKey 1974). In conifers, most studies have focussed on above-ground parts showing differential allocation of chemistry between needles, bark or xylem (Lewinsohn et al. 1991; Miller et al. 2005). However, plant-wide variation in constitutive and induced chemistry that includes the chemistry of roots has been comparatively less studied.

Roots can be equally at risk from pests and pathogens as above-ground parts, especially in the juvenile stages (Hernandez-Escribano et al. 2018; Moreira et al. 2012a; Senior et al. 2018), and consequently might also be well defended. Roots can also be involved in above-ground responses to stresses by their direct effects on water and nutrient acquisition, and through correlation or trade-offs with the bark and/or needles (Huber et al. 2005; Moreira et al. 2012b). However, few studies have investigated conifer root defences and the relationship between above- and below-ground defences is poorly understood (Huber et al. 2005; Moreira et al. 2012b). Huber et al. (2005) indicated that there is variability in root secondary chemistry, and this can change in response to different treatments. This study also showed that application of methyl jasmonate to the roots of *Pseudotsuga menziesii* Mirb. caused significant changes in the above-ground tissues (Huber et al. 2005). Similarly, in response to above-ground treatment with methyl jasmonate, Moreira et al. (2012a) showed changes in root physiological traits in *Pinus pinaster* Aiton. In *Pinus radiata* D. Don, a link between above- and below-ground defences has been signalled by the elevated above-ground resistance to stem infections following root exposure to *Fusarium circinatum* which causes pitch canker (Swett and Gordon 2017). Root defences are particularly relevant given the importance of chemical communication at the root–soil interface (Senior et al. 2018), the effects of plant-derived metabolites on the soil microbiome assemblage (Senior et al. 2018), and the possible influence of this microbiome on plant resistance (Mhlongo et al. 2018). Various studies and reviews involving non-conifer species have reported significant differences between above- and below-ground defences (De Coninck et al. 2015; Kaplan et al. 2008; Kleine and Müller 2013; Tsunoda and van Dam 2017).

While secondary metabolites are often implicated in plant defence, there is increasing evidence that sugars, such as glucose, fructose and sucrose, play direct and indirect roles in tolerance and resistance in many herbivore–plant systems. Sugars are the primary chemical substrate for structural material in plants other than lignin, but as part of the pool of non-structural carbohydrates (Hartmann and Trumbore 2016) they provide energy for defence responses and may also act as signal molecules (Schwachtje and Baldwin 2008; Tauzin and Giardina 2014). The biosynthesis of terpenes has a metabolic cost (Gershenzon 1994), in which case, a high supply of sugars should lead to increased resistance (Clancy 1992), supporting a positive correlation between primary and secondary chemistry which has been shown in some conifers (Sampedro et al. 2011; Villari et al. 2014). Studies also indicate that reallocation of sugars in stressed plants is a major consequence of the induced response to herbivory. Reduction of sugars in the stem tissues following application of methyl jasmonate was, for example, reported in *P. pinaster* (Sampedro et al. 2011), which may explain the increase in biomass in fine roots (Moreira et al. 2012b). In other trees, evidence of herbivore-induced resource reallocation to roots in response to herbivory, including increased transport of sugars, has been demonstrated (Babst et al. 2008). The direction of resource movement may depend on the kind of herbivory (Frost and Hunter 2008). Nevertheless, roots are a key sink for sugars (Babst et al. 2005), and this sink of non-structural carbohydrates is important for enhancing tolerance (Zhou et al. 2015). The importance of sugars in defence may also be linked to their potential as a food source for herbivores, in which case they may attract herbivores in contrast to their positive roles in resistance and tolerance. Although no studies show that sugars alone are sufficient to differentiate resistant and susceptible hosts, there is a clear indication that sugars play a role in host selectivity (Kurek et al. 2019; Snyder 1992). However, few studies have also considered the distribution of primary metabolites like the sugars in conifers (Cranswick et al. 1987).

Of particular interest is the response of bark chemistry to bark stripping. Bark stripping by mammalian herbivores has become a major problem in many *Pinus* species (Arhipova et al. 2015), including in *P. radiata* plantations in Australia (Miller et al. 2014; Page et al. 2013). In Australia, bark stripping in *P. radiata* plantations occurs in autumn and winter on trees between ages of 1–6 years (Miller et al. 2014; Page et al. 2013). The chemical defence of *P. radiata* against bark stripping by mammals has not yet been investigated. In other conifer species, few studies have documented defences against mammalian herbivores and the role of terpenes and phenolics remains unclear (Bucyanayandi et al. 1990; Ilse and Hellgren 2007; Snyder 1992; Stolter et al. 2009; Zhang and States 1991). In *P. radiata*, a single study has implicated sugars. Page et al. (2013) attributed bark stripping

to higher sugars in the bark compared to surrounding food sources. However, the interaction of sugars with secondary compounds to explain the observed variation in mammalian bark stripping has not yet been investigated.

Here we examined the induced chemical responses to bark stripping in *P. radiata* and compared these responses to the comparatively well-documented responses to methyl jasmonate. We asked: 1) Are there qualitative and quantitative differences in constitutive primary (sugars and fatty acids) and secondary chemistry (mono-, sesqui- and di-terpenoids and volatile phenolics) between the needles, bark and roots of *Pinus radiata*? 2) What chemical changes occur in the different plant parts following artificial bark stripping and above-ground application of methyl jasmonate? 3) Do compounds quantitatively correlate between and within plant parts?

Materials and methods

Study species

The conifer *Pinus radiata* (Pinales: Pinaceae) is native to California (Axelrod 1988) and is one of the most widely planted commercial timber trees in temperate regions of the world, mainly because of its fast growth rate and wood quality (Burdon et al. 2017). The needles, bark and roots of *P. radiata* are attacked by numerous insects, fungi and animals (Mead 2013; Miller et al. 2014; Nantongo et al. 2020), with up to 600 pests and pathogens recorded (Brockerhoff and Bulman 2014). While the constitutive and induced chemistry of *Pinus radiata* needles and bark have been reported (Bonello et al. 2001; Moreira et al. 2012a), there are no comparative studies which include roots. There is also limited understanding of the timing of induction across plant parts.

Experimental design

In 2015, 6-month-old seedlings of 18 full-sib families of *Pinus radiata* (D. Don) originating from the Radiata Pine Breeding Company deployment population in New Zealand were obtained from a commercial nursery. Seedlings were transferred into 145 mm × 220 mm pots containing 4L of basic potting mix (composted pine bark 80% by volume, coarse sand 20%, lime 3 kg/m^3 and dolomite 3 kg/m^3) and raised outside in a fenced area (to protect against animals) at the University of Tasmania, Hobart. At the onset of winter (May–June, average temperature ~ 15.6 °C), when plants were 2 years of age (average height = 130 cm), 25 mM Methyl jasmonate (Moreira et al. 2013a) was applied to plants of 6 families, bark stripping treatments were applied to another 6 families, and the remaining 6 families were kept as controls (control). Methyl jasmonate (MJ) was applied by spraying the stem and needles with a fine mist from a hand sprayer until 'just before run-off'. The treated plants were sprayed in a well-ventilated area away from untreated plants to avoid cross contamination (Moreira et al. 2013a). For bark stripping (strip), plants were artificially stripped by removing a 30 cm vertical strip, beginning 2 cm from the ground and covering 50% of the stem circumference. The area removed was representative of the common upper threshold for browsing observed in natural field conditions. The three treatment groups (control, strip and MJ) were arranged in a randomized block design of 3 blocks in a shade house, each block comprising a treatment plot of two families (Fig. 1). Watering was done once every evening using a manual hose. Plants of each family were linearly arranged in a family plot. Families were randomly allocated to treatments, blocks, and position within treatment plots. The treatment plots were separated within each block to minimise any potential interference from the MJ and bark strip treatments. Sampling was done in winter to coincide with the peak times for bark stripping by mammalian herbivores in this system (Page et al. 2013).

Sample processing and phytochemical extractions

Each family had a minimum of 4 plants from which one seedling was randomly sampled weekly from T0–T28 (Fig. 1). T0 represents the time immediately before treatment application. T7, T14, T21 and T28 represents respective sampling times at 7, 14, 21 and 28 days after treatment application. For constitutive estimates, one plant from each family ($n = 18$) was destructively harvested just before application of treatments (T0). Thereafter, one plant from each family (3 treatments × 6 families = 18 plants) was destructively harvested at T7, T14, T21 and T28 to investigate induced changes in chemistry (Fig. 1). At harvesting, the plant was divided into 3 parts; the upper part with most needles for needle collection, the middle stem for bark collection and the stump in the soil for root harvesting. Because of the small size of the trees (average height = 130 cm), most of the needles, bark and roots on the plant were collected. Needles were cut off the upper stem using scissors. From the middle stem, the bark was carefully peeled avoiding the wood using a knife. The knife and scissors were cleaned with ethanol after every harvest to avoid cross contamination. For the roots, each stump was carefully removed from the pot and soil gently separated from the roots. The roots were further shaken and rinsed in water to remove any remaining soil and both fine and coarse roots were cut from the stump. Needles, bark and roots from each plant were kept separate and immediately put in a cooler and transported to a − 20 °C freezer for storage until chemical extraction. A subsample of each tissue was freeze dried.

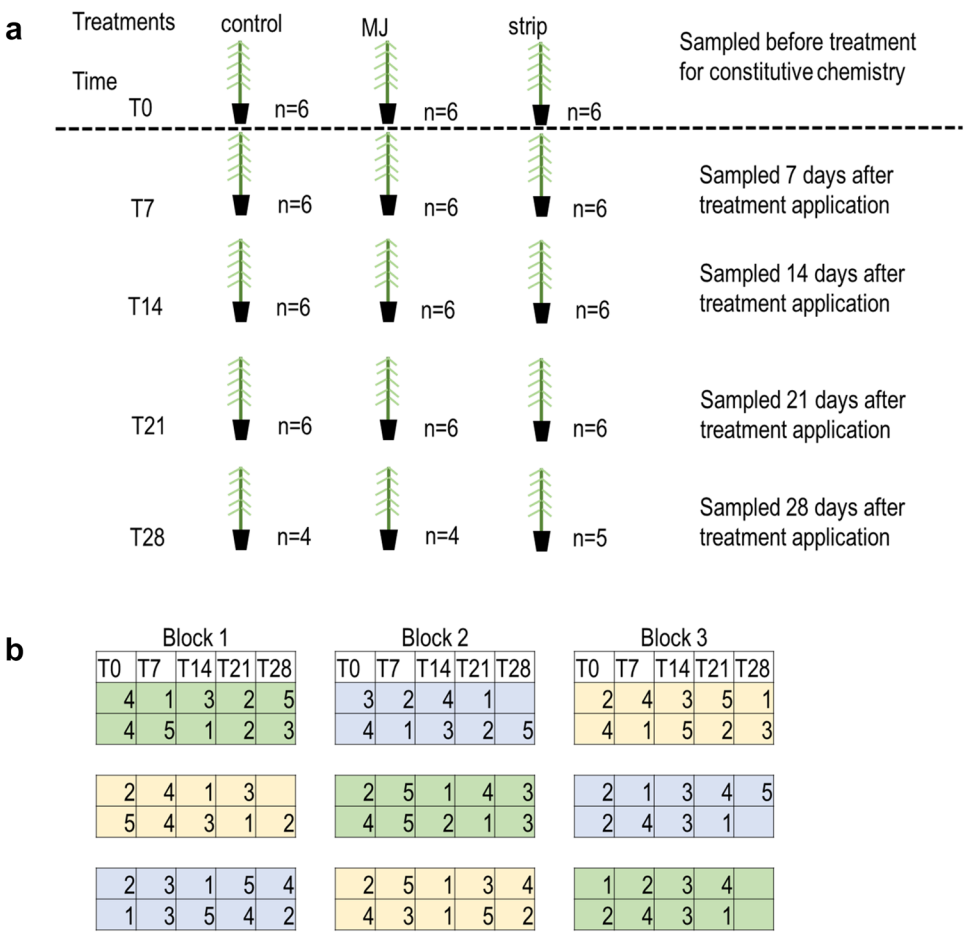

Fig. 1 Experimental lay-out in the shade house. **a** At T0, plants were destructively harvested for needle, bark and root samples prior to treatment application. At T7, T14, T21 and T28 days after treatment, one plant from each family (total number of seedlings per sampling time = 18, equivalent to the number of families and $n = 6$ are seedlings sampled for each treatment) were destructively harvested. **b** The experimental design of the treatments. Each set of squares is a table where we placed plants and represents a replicate block. Each row represents a different family. Each treatment plot comprised two family plots in which plants of the same family were arranged in a row and a seedling sampled at random for studying temporal changes in chemistry. Each square represents a plant and the number represents the order in which they were sampled. Squares of the same colours received the same treatment i.e. yellow = MJ treated, blue = strip and green = control. The blank squares represent missing individuals

Before analysing the target samples an initial screening of secondary metabolites was performed on tissues from mature and young *P. radiata* to understand the range of metabolites expected in the final screening. The initial screening indicated that although tannins, phenolics and terpenoids were abundant in the mature tissues, tannins and most phenolics were below detection thresholds in the tissues from the young *P. radiata* plants. Additionally, after a thorough literature search, the extraction protocols were not optimised for alkaloids as these have not previously been documented in *P. radiata*.

Chemical extractions (targeting terpenes, volatile phenolics and sugars) were undertaken randomly and separately from each of the three plant parts (needles, bark and roots) from each plant sampled. Dichloromethane (DCM) and acetone were used to extract polar and semipolar components, respectively (Jones et al. 2002; Kajdzanoska et al. 2011; Sasidharan et al. 2011). For DCM-extracted compounds, frozen material was cut into smaller sizes and 1.5 g of tissue was weighed and extracted in 10 mL of 99.9% dichloromethane (Jones et al. 2002). An internal standard (n-heptadecane) was added to dichloromethane at the concentration of 100 ppm. Acetone extracts were made from 50 mg of freeze dried, ground material in 10 mL of 95% acetone, and sugars were extracted from 50 mg of freeze dried, ground material in 10 mL of hot water (Jones et al. 2002). Rutin was used as the internal standard for the acetone extracts and final concentration of 0.2 mg/L was added to each sample. Acetone extracts were then reconstituted by mixing 600 µL of the extract with 600 µL of 98:2 acetonitrile/water and then 1 mL of each extract was transferred into a vial and stored at − 20 °C until analysis. An extra subsample of each tissue per individual was weighed, dried in the oven at 110 °C for 72 h and reweighed to convert samples from fresh to dry weights. The DCM extracts that comprised the mono-, sesqui- and diterpenoids and volatile phenolic compounds were then analysed by gas chromatography–mass spectrometry (GC–MS). The diterpenoid resin acids, although present in the DCM extracts, were ultimately quantified by ultra-high-performance liquid chromatography–mass spectrometry (UHPLC–MS) of the acetone extracts which also contained the fatty acids. The sugars were also quantified by UHPLC–MS in a separate analysis.

GC–MS analyses

Gas chromatography–mass spectrometry (GC–MS) analyses were carried out on a Varian 3800 GC coupled to a Bruker-300 triple quadrupole mass spectrometer, using helium carrier gas at 1.2 mL/min in constant flow mode. The column was an Agilent DB-5 column (30 m × 0.25 mm internal diameter and 0.25 µm film). Injections of 1 µL were made using a Varian CP-8400 autosampler and a Varian 1177 split/splitless injector in split mode with a 4:1 split ratio. The injector temperature was 250 °C. The column oven was started at 60 °C then ramped to 290 °C at 15 °C /min with a 1-min hold at the final temperature. The ion source was held at 220 °C, and the transfer line at 290 °C. Electron ionisation mass spectra at 70 eV were acquired over the range *m*/*z* 35 to 400 over 130 ms, with additional Selected Ion Monitoring (SIM) channels in 4 different time windows, all with 15 ms dwell time per channel. Window 1 from 0 to 8 min included *m*/*z* 41, 68, 69, 71, 93, 104, 135, 148, 151, 178, window 2 from 8 to 9.7 min included *m*/*z* 81, 107, 121, 162, window 3 from 9.7 to 14.5 min included *m*/*z* 69, 91, 109, 137, 177, 229.1, 239.2, 240.2 and window 4 from 14.5 min to the end included *m*/*z* 81, 109, 134 and 221.1. These ions were chosen based on initial full scan analyses to select compounds to target. Peak areas of relevant characteristic ions were measured using Bruker Workstation MS Data Review version 7.0, then scaled up to the equivalent total ion current based on measurements of the proportion the diagnostic ion was of the full spectrum (based on a good quality spectrum). All adjusted peak areas were finally expressed as ratios of the total ion current for each compound to the internal standard, n-heptadecane. The preliminary identification of compounds was based on the comparison of the mass spectra with standard libraries with the National Institute of Standards and Technology mass spectra library (NIST 2014). Most compounds gave ions that were structurally characteristic. Example chromatograms have been provided in Supplementary Figure S1. The DCM components were expressed as milligrams of heptadecane equivalents (HE) per gram of dry weight of the sample (mg HE/g dw).

LC–MS analyses

To analyse the acetone extracts, 12 µL aliquots were injected using a Waters Acquity H-series UHPLC coupled to a Waters Xevo triple quadrupole mass spectrometer operating MassLynx 4.1 software. A Waters Acquity UHPLC BEH C18 column (2.1 × 100 mm × 1.7 micron particles) was used, with 1% acetic acid (Solvent A) and acetonitrile (Solvent B) at a flow rate of 0.35 mL/min and, after an initial hold for 30 s at 85% A:15% B, a linear gradient was followed to 45% A:55% B at 10 min, then a further linear gradient to 5% A:95% B at 15 min with a 1-min hold at the final value, before re-equilibration to starting conditions for 4 min. The mass spectrometer was operated in negative ion electrospray mode. The ion source temperature was 150 °C, the desolvation gas was nitrogen at 950 L/h, and the desolvation temperature was 450 °C and needle voltage 2.7 kV. Based on some trial full scan analyses, a range of target ions were included in Selected Ion Monitoring (SIM) mode with dwell time of 27 ms per channel, as well as a full scan from *m*/*z* 120 to 1200 over 250 ms. The SIM channels chosen (with cone voltages in brackets) were 277 (20), 285 (25), 289 (25), 297 (C19 fatty acid standard) (25), 303 (25), 317 (30), 319 (30), 333 (30), 349 (30), 365 (30), 405 (30), 465 (35), 481 (35), 495 (40), 561 (30), 575 (30), 577 (30), 579 (30), 609.1 (rutin standard) (40), 709.2 (40), 739.2 (40), 863.2 (45). Data were analysed with MassLynx and TargetLynx software. The LC–MS analytes were expressed as milligrams of rutin equivalents (RE) per gram of dry weight of the sample (mg RE/g dw).

To further characterise the diterpenoid resin acids, some of the acetone extracts above were evaporated to dryness. The residue was redissolved in methanol/ chloroform/ HCl (10:1:1) and heated at 80 °C for 1 h (methylated). The methylated diterpenoid resin acids were then extracted with hexane/chloroform (4:1) and the resulting extract analysed by GC–MS. To verify the retention times for final identification of the resin acids by UHPLC–MS, standards of abietic acid, neoabietic acid, dehydroabietic acid, palustric acid, levopimaric acid, pimaric acid and isopimaric acid were purchased from Santa Cruz Biotechnology and analysed by UHPLC–MS.

For sugars, 1 µL aliquots were injected using the instrument described above for the acetone extracts. A Waters Acquity UHPLC BEH Amide column (2.1 × 50 mm × 1.7-micron particles) was used, with 0.4% ammonia in water (Solvent A) and acetonitrile (Solvent B) at a flow rate of 0.37 mL/min, with a gradient from 20% A:80% B to 28% A:72% B at 2 min, before immediate re-equilibration to initial conditions for 2.5 min. The mass spectrometer was operated in negative ion electrospray mode, and selected ion monitoring was used to detect the specific sugars, which were quantified by external calibration. The ion source temperature was 150 °C, the desolvation gas was nitrogen at 1000 L/h, and the desolvation temperature was 450 °C and needle voltage 2.7 kV. The $[M–H]^-$ ion was monitored for monosaccharides and inositol at *m*/*z* 179.1 and for sucrose and other disaccharides at *m*/*z* 341.1. Cone voltages were 17 V for monosaccharides and 24 V for disaccharides. Standard curves were created over the range 0–500 ppm with standards at 0, 10, 25, 50, 100, 250 and 500 ppm. Non-linear (second-order) equations were used for sucrose (typical $R^2 = 0.99996$) while linear fits were used for the monosaccharides (typical $R^2 = 0.9992$). Due to potential drift in MS

response, the full standard curve was repeated after every tenth sample, and a 250 ppm QC was run in the middle of each set of ten. Under these conditions, fructose eluted at 1.11 min, glucose at 1.38 min, sucrose at 2.09 min and inositol at 2.49 min. In a typical large set of analyses, with 18 250 ppm QC samples run, the average values reported for fructose, glucose and sucrose were 249 ppm, 248 ppm and 250 ppm, respectively, with relative standard deviations of 2.0%, 1.5% and 1.8%, respectively. Accurate mass data for molecular formula assignment of major unknowns were acquired by direct infusion on a Thermo Orbitrap mass spectrometer operating at a resolution of 15,000.

Compounds that were quantified by the GC–MS were summed according to functional groups and expressed in their relevant units. In addition to individual compounds/groups, analysis was made of total compound classes, i.e. total monoterpenoids, sesquiterpenoids, GC–MS diterpenoids and volatile phenolics. For LC–MS compounds, only total sugars were derived as the sum of individual sugars, since these were quantified in absolute amounts. The rest of the compounds analysed by the LC–MS i.e. the fatty acids and the LC–MS diterpenoids were not summed. One group of resin acids ($C_{20}H_{30}O_2$ resin acids [39]) that eluted together was also considered in the total compound classes. All amounts were expressed relative to the internal standard except the sugars that were measured in absolute amounts. For both the GC–MS and LC–MS compounds, focus was on the compound groups (terpenoids, volatile phenolics, sugars and fatty acids), that have been identified in *Pinus radiata* by other studies. The terpenoids and phenolics are broadly categorised under secondary metabolites while the sugars as well as fatty acids are primary compounds. All individual compounds that were identified have been given a unique identifier for ease of location in the tables.

Statistical analyses

All statistical analyses were conducted using the software R (version 3.6.0) (R Core Team 2018). Principal components analysis (PCA), using FactoMinerR version 1.41 (Lê et al. 2008) was used to visualise the overall difference between samples from the three plant parts. The PCA was based on the correlation matrix among all identified chemical compounds.

To further characterize the differences in the plant parts, arithmetic means and standard errors (se) were calculated for all compounds that were detected. For compounds detected in more than one plant part, differences among plant parts were tested with Kruskal–Wallis (KW) one-way analysis of variance, and where significant results were obtained, a Dunn's test (Dn) was performed to identify the parts which were significantly different. Bonferroni adjustments (Bf) were made to account for multiple tests for individual compounds. Significant p values were considered at 0.05/n, where n is the number of statistical tests (McDonald 2009), for example, a p value of 0.05/48 = 0.001 was considered significant for pairwise tests between plant parts for individual monoterpenoids, where 48 = 16 monoterpenoids X 3 plant parts. To detect induced changes, the mean amounts of compounds in each plant part for the plants allocated to different treatments (control, strip and MJ) was first compared at T0 using KW to check if there are inherent differences. Since no differences between treatments were detected at T0 (results not presented), then the mean amounts of compounds after treatment (induced; T7–T28) were compared. Induced changes for each time were detected by comparing the MJ and strip treatments to the control for that time (Fig. 1) (Morris et al. 2006).

To examine the correlation of the level of each constitutive compound and total compound class across plant parts, Spearman's rank correlations were calculated. These were calculated for all compounds that were detected in more than one plant part. To examine the stability of these correlations in the induced chemistry, they were also calculated for the methyl jasmonate treatment for each sampling time (T7–T28). Spearman's rank correlations were also calculated among the levels of the total compound classes (total mono-, sesqui- and GC–MS diterpenoids, volatile phenolics and sugars, as well as the LC–MS resin acid group) within each plant part for the constitutive and methyl jasmonate T7 sample. Absolute p values are reported for the various Spearman's rank correlations calculated.

Results

Across all 255 samples from 3 plant parts and 85 plants of 2-year old *P. radiata*, a total of 81 compounds/groups, that had detectable peaks were quantified. Full or partial identification was achieved for 52 of these, which included 15 monoterpenoids, 20 diterpenoids, 3 sesquiterpenoids, 7 phenolic compounds, 4 sugars and 3 fatty acids (Supplementary Table 1). The 28 unidentified compounds/groups had molecular weights ranging between 104 and 770, and where group allocation was possible these included terpenoids and sugars (Supplementary Table 1). The major $C_{20}H_{30}O_2$ [39] diterpenoid resin acids (Supplementary Table 1) that had very close retention times by LC–MS were measured as a group. All the 81 compounds/groups were given a unique number for ease of identification in the tables.

Constitutive differences between plant parts

Overall differences

Of the 81 compounds, 62 were detected in the bark, 45 in the needles and 35 in the roots (Supplementary Table 1). While 20 of these compounds were detected in all the

three plant parts, 22, 13 and 5 compounds were detected only in the bark, needles and roots, respectively (Supplementary Table 1).

The constitutive (T0) data for all plant parts was then visualised using the principal components (PC) analysis plot, where PC1, PC2 and PC3 explained 37.2%, 20.2% and 7.3% of the chemical variation, respectively. The three plant parts were clearly differentiated in the two-dimensional space defined by the first (PC1) and second (PC2) principal components (Supplementary Figure S2). PC1 differentiated the needles and bark but did not differentiate between needles and roots. The root samples were differentiated from the needle samples along PC2. The third principal component further separated the roots from the bark and needles but not needles from bark.

Differences between plant parts for total compound classes

Plant parts differed in the amounts of total compound classes. These differences are summarised in Fig. 2. In summary, total GC–MS diterpenoids were lower in the roots than the bark ($p < 0.01$; *Dn, Bf*) and needles ($p < 0.001$; *Dn, Bf*) but were not significantly different between needles and bark. Sesquiterpenoids were not detected in the roots and were not significantly different between the bark and needles. Total volatile phenolics were almost absent in the roots hence levels were lower than in the bark ($p < 0.001$; *Dn, Bf*) and needles ($p < 0.01$; *Dn, Bf*). The levels of total volatile phenolics were significantly higher in the bark than needles ($p < 0.05$; *Dn, Bf*). Total sugars were lower in the roots than in the needles or bark ($p < 0.001$; *Dn, Bf*) with no significant difference between needles and bark. The LC–MS $C_{20}H_{30}O_2$ resin acids [39] that eluted as a group were higher in the bark ($p < 0.001$; *Dn, Bf*) than the needles, with the roots

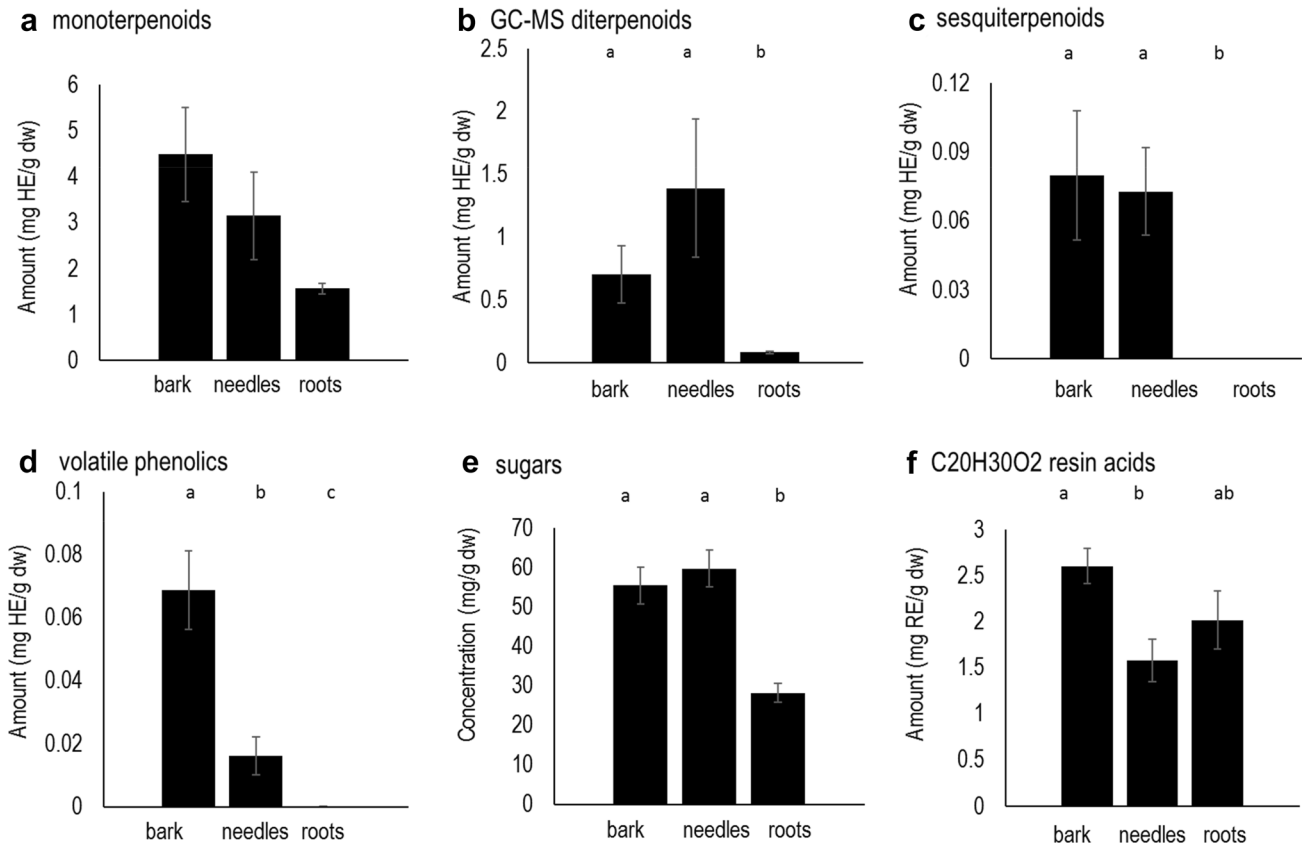

Fig. 2 Relative amounts (mg/g ± SE) of total compound classes: **a** monoterpenoids, **b** GC–MS diterpenoids, **c** sesquiterpenoids, **d** volatile phenolics, **e** sugars and **f** $C_{20}H_{30}O_2$ resin acids in the bark, needles and roots of *Pinus radiata* plants at T0. No sesquiterpenoids were found in the roots. Different letters adjacent to the plot indicate significant differences ($p < 0.05$) between plant parts based on Dunn's test for post hoc evaluations with Bonferroni adjustments for multiple compound classes. The resin acid group was not derived by summation of individual resin acids quantified by the LC–MS. This class represents a group of LC–MS resin acids that eluted together. The terpenoids and phenolics are expressed as mg heptadecane equivalents (HE)/g dw and resin acids are expressed as mg rutin equivalents (RE)/g dw, as no absolute quantitation was carried out on these analytes. The absolute amounts of sugars are quantified in mg/g dw

intermediate and not significantly different from the bark or the needles. Total monoterpenoids did not differ significantly between plant parts.

Differences between plant parts for dominant individual compounds

Within the total compound groups, there were some individual compounds that were dominant, and these varied with plant part (Supplementary Table 1, Fig. 3). The monoterpenoids were dominated by β/α-pinene [4,1] followed by β-phellandrene [3] in all three plant parts (Fig. 3). The GC–MS diterpenoids were dominated by agatholal [21] and agathadiol [20] both in the bark and needles and by methyl levopimarate [25] in the roots. The sesquiterpenoids in the needles were dominated by the unknown sesquiterpenoid alcohol [19] and those in the bark dominated by trans-farnesol [18]. No sesquiterpenoids were detected in the roots. The volatile phenolics were dominated by trans-coniferyl alcohol [58] in the needles and the bark. Ethyl 4-ethoxybenzoate [54] was the only phenolic compound detected in the roots. Glucose [61] dominated the sugars in the needles and bark, but fructose [60] dominated in the roots (Fig. 3). Sucrose was not detected in the needles (Supplementary Table 1). Of the fatty acids, palmitic acid [69] dominated in the needles and linoleic acid [67] in the bark and the roots. Overall, based on the dominant compounds the needles and the bark had a more similar chemical profile relative to the roots.

Constitutive phenotypic correlations

To examine the degree to which the amounts of compounds in the needles, bark and roots are correlated, Spearman rank correlations (r_s) of the variation in constitutive chemistry (T0) between plant parts were calculated for total compound classes and individual compounds (Table 1). The amounts of sesquiterpenoids in the needles and bark positively correlated among individuals ($r_s = 0.55$, $p < 0.05$). Total volatile phenolics also correlated between the needles and the roots ($r_s = 0.48$, $p < 0.05$). The total sugars of the roots and needles were also highly positively correlated ($r_s = 0.94$, $p < 0.01$). No other significant correlation between plant parts was detected for the total compound classes.

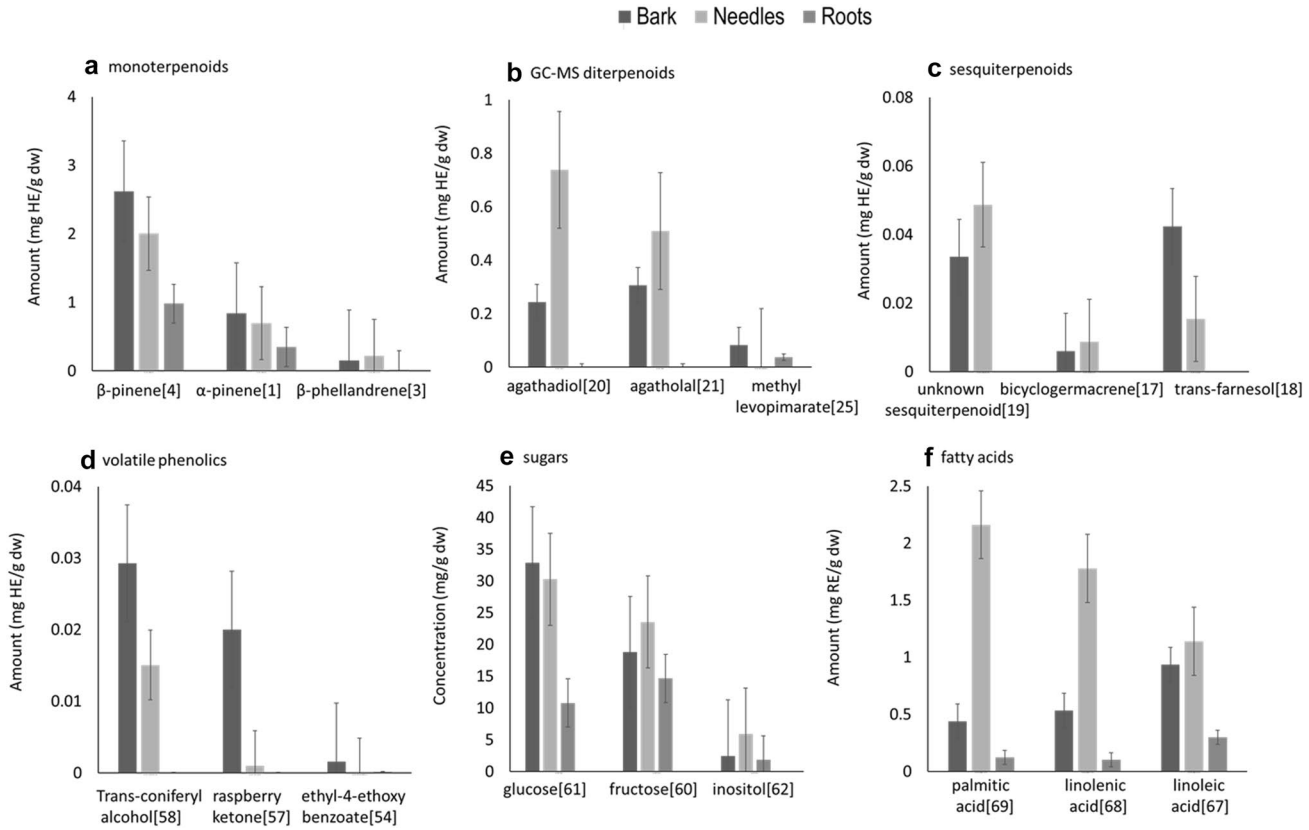

Fig. 3 Mean constitutive amounts of the dominant individual: **a** monoterpenoids, **b** diterpenoids, **c** sesquiterpenoids, **d** volatile phenolics, **e** sugars and **f** fatty acids at T0, showing differences in the dominant compounds of the bark, needles and roots of *Pinus radiata*. The terpenoids and phenolics are expressed as mg heptadecane equivalents (HE)/g dw and fatty acids are expressed as mg rutin equivalents (RE)/g dw, as no absolute quantitation was carried out on these analytes. The absolute amounts of sugars are quantified in mg/g dw

Table 1 Spearman rank correlations between the bark [B], needles [N] and roots [R] of individual and total compounds that were identified in more than one plant part, where B/R for example signifies the correlation between bark (B) and roots (R)

ID	Compound/Part	Correlations at T0			Correlations at time			
		Bark (B) and Needles (N)	Bark(B) and Roots (R)	Needles (N) and Roots (R)	T7	T14	T21	T28
	Compound classes							
	Total monoterpenoids	0.29	− 0.08	0.06				
	Total GC-MS diterpenoids	0.12	− 0.10	0.38				
	Total sesquiterpenoids	0.55*			0.83(B/N)*			
	Total volatile phenolics	− 0.36	0.06	0.48*				
	Total sugars	0.49	0.43	0.94**				
	Individual compounds/groups							
1	α-pinene	0.31	− 0.14	0.18				
3	β-phellandrene	0.30	0.28	0.14	1.0 (B/R)*** 0.9(B/N)*0.9(R/N)*	0.9 (R/N)*		0.75 (B/R)**0.56 (B/N)*
4	β-pinene	0.28	− 0.03	− 0.07				
5	Camphene	0.21						
10	Limonene	0.64**	− 0.20	− 0.18				
13	Sabinene	0.37	− 0.14	0.05	0.94 (R/N)**			
15	Terpinolene		− 0.04					
16	Unknown Mol Wt 150	− 0.08						
17	Bicyclogermacrene	0.57*						
18	Trans-farnesol	0.41						
19	Unknown sesquiterpenoid alcohol	0.61**						
20	Agathadiol	0.13						
21	Agatholal	0.19						
23	Levopimaral		0.01					
24	Methyl dehydroabietate	0.13	0.16	0.38				
25	Methyl levopimarate		0.09					1.00 (B/R)***
27	Dehydroabietic acid	0.12	− 0.14	0.2				
30	Unknown diterpenoid-3	0.21	0.66**	− 0.07				
31	Unknown m/z 109 A		0.25					
32	Unknown m/z 109 B	0.15						
35	Unknown m/z 239			− 0.27				
39	$C_{20}H_{30}O_2$ resin acids	0.11	0.05	0.46	0.94 (B/R)**			0.90(B/R)*
44	Unknown $C_{20}H_{30}O_3$	− 0.26	0.36	0.13				
45	Unknown $C_{20}H_{30}O_3$ A	0.14	0.48	− 0.42			0.58 (B/N)**	0.66 (B/N)*

Table 1 (continued)

ID	Compound/Part	Correlations at T0			Correlations at time			
		Bark (B) and Needles (N)	Bark(B) and Roots (R)	Needles (N) and Roots (R)	T7	T14	T21	T28
48	Unknown $C_{20}H_{30}O_4$	0.09	0.03	0.25				− 1.00(N/R)***
49	Unknown $C_{20}H_{30}O_5$		− 0.06		0.84 (B/R)*		− 0.88(B/R)*	
54	Ethyl 4-ethoxy-benzoate		− 0.24		0.83 (B/R)*	− 0.88(B/N)*		
57	Raspberry ketone	0.23			0.48 (B/N)*			
58	Trans-coniferyl alcohol	− 0.28						
60	Fructose	− 0.37	0.08	0.49				− 0.90 (N/R)*
61	Glucose	0.66	0.43	0.77				
62	Inositol	0.09	− 0.26	0.54				
63	Sucrose		− 0.14					
64	Unknown disaccharide A	− 0.20	− 0.13	− 0.65				
65	Unknown disaccharide B	0.49	− 0.15	− 0.88*				0.89 (N/R)***
66	Unknown monosaccharide	0.42						
67	Linoleic acid	− 0.53*	0.02	0.06		0.98 (B/R)***		
68	Linolenic acid	− 0.26	− 0.25	− 0.21				
69	Palmitic acid	− 0.27	− 0.18	0.04		− 0.94 (B/R)**		− 0.9 (B/N)*
74	Unknown Mol Wt 274		− 0.09					
78	Unknown Mol Wt 406 A		0.07					

Correlations were estimated before treatment (T0). For simplicity of illustration, the comparable correlations evident in the treatments from T7–T28 are shown only for the methyl jasmonate samples. T7, T14, T21 and T28 represents 7, 14, 21 and 28 days after treatment application, respectively. The unadjusted p values that the correlations are different from zero are indicated as $*p<0.05$, $**p<0.01$ and $***p<0.001$. All compounds were given a unique identifier (ID) for ease of identification

For individual compounds between plant parts for T0, only 6 significant correlations were detected (Table 1). Three of these involved positive correlations between amounts of limonene [10], bicyclogermacrene [17] and unknown sesquiterpenoid alcohol [19] in the needles and the bark ($r_s = 0.57$ to 0.64). The other significant positive correlation was the amount of unknown diterpenoid-3 [30] in the bark and roots ($r_s = 0.66$, $p < 0.01$). Significant negative correlations were detected for linoleic acid [67] levels in the bark and needles ($r_s = -0.53$, $p < 0.05$) and unknown disaccharide B [65] in the needles and roots ($r_s = -0.88$, $p < 0.01$) (Table 1).

Within plant parts, Spearman rank correlations (r_s) focussed on correlations among the levels of total compound classes (Table 2). In the bark, the amount of total monoterpenoids positively correlated with total volatile phenolics ($r_s = 0.92$, $p < 0.001$) and with total GC–MS diterpenoids ($r_s = 0.97$, $p < 0.001$). Total sesquiterpenoids in the bark also positively correlated with total phenolics and resin acids. In the needles, high positive correlation was detected between total monoterpenoids and sesquiterpenoids ($r_s = 0.90$, $p < 0.001$) and the GC–MS diterpenoids ($r_s = 0.83$, $p < 0.001$). In the roots, the only significant correlation detected was between total monoterpenoids and GC–MS diterpenoids ($r_s = 0.62$, $p < 0.01$).

Induced responses

Total compound classes

To understand how the total compound classes responded to treatment, pair-wise Kruskal–Wallis tests comparing the MJ and strip treatments to the controls at each point in time were undertaken (Supplementary Table 1). These revealed non-significant changes in all secondary compound classes in the needles, bark and roots. (Supplementary Table 1). However, contrary to the lack of significant change in

Table 2 Spearman's rank correlations of the constitutive (T0) amounts of total compound classes within the bark, needles and roots

	Plant part	Total monoterpenoids	Total GC-MS diterpenoids	Total sesquiterpenoids	Total volatile phenolics	Total sugars
Total GC− MS diterpenoids	Bark	0.97***				
	Needles	0.83***				
	Roots	0.62**				
Total sesquiterpenoids	Bark	0.62**	0.55*			
	Needles	0.90***	0.77			
	Roots	NA	NA			
Total volatile phenolics	Bark	0.92***	0.89***	0.55*		
	Needles	0.19	0.15	0.25		
	Roots	− 0.07	− 0.05	NA		
Total sugars	Bark	− 0.11	− 0.11	− 0.26	− 0.10	
	Needles	− 0.19	− 0.01	− 0.09	− 0.03	
	Roots	0.30	0.32		0.18	
$C_{20}H_{30}O_2$resin acids	Bark	0.32	0.28	0.52*	0.30	− 0.41
	Needles	0.43	0.46	0.45	0.08	0.17
	Roots	-0.21	− 0.27	NA	-0.10	-0.07

The unadjusted p values that the correlation is different from zero are indicated as $*p<0.05$, $**p<0.01$ and $***p<0.001$. No sesquiterpenoids were detected in the roots

secondary compounds after treatment, the amount of total sugars was significantly reduced after treatment in the needles ($p<0.001$; *Dn, Bf*) and bark ($p<0.001$; *Dn, Bf*), but not in the roots. The reduction occurred only in the MJ-treated plants at T7 and T14 Supplementary Table 1. The time progression of induced changes in total compound classes appeared to differ between classes, plant parts and treatments (Fig. 4).

Individual compounds

Similar to the non-significant changes in total compound classes, the individual secondary compounds responded non-significantly to treatment (Supplementary Table 1), but stronger changes were detected in individual sugars. In the bark of MJ-treated plants, fructose [60] and glucose [61] reduced significantly at T7–T14 (Supplementary Table 1). Glucose [61] and the unknown disaccharide [65] also reduced significantly at T28. In the needles, fructose [60] and glucose [61] reduced in the MJ-treated plants at T7 and T14, as did inositol [62] at T14 and T21 ($p<0.01$; *Dn, Bf*). The unknown disaccharide [65] also reduced in the needles of MJ-treated plants from T7–T21. The responses of individual sugars in the roots or strip-treated plants were not evident (Supplementary Table 1).

As observed with the total compound groups, the induced responses and time progression of the individual compounds also varied between individual compounds, plant parts and treatments. For individual compounds that were identified in at least two plant parts, this is illustrated in Supplementary Figure S3. Secondary compounds for example generally increased in the bark but reduced in the needles and the roots following MJ treatment (Supplementary Figure S3). In contrast, following the strip treatment, an overall reduction in secondary compounds was observed in all the plant parts with a few exceptions. Overall, MJ caused greater responses than bark stripping in the bark and needles (Supplementary Table 1; Supplementary Figure S3).

Spearman's rank correlations after treatment

Between plant parts To detect the influence of treatments on the correlations of the levels of compounds between plant parts, Spearman's rank correlations after treatment were calculated and are shown in Table 1. There were more significant correlations for total compound classes and individual compounds between plant parts in the induced than constitutive chemistry. Induced positive correlations were for example detected between the total sesquiterpenoids of the needles and the bark at T7 and the $C_{20}H_{30}O_2$ resin acids [39] of the bark and the roots at T7 and T28 (Table 1). For individual compounds, the plant parts mostly exhibited positive correlations after treatment except for the negative correlation of the unknown diterpenoid $C_{20}H_{30}O_4$ [48], unknown diterpenoid $C_{20}H_{30}O_5$ [49], ethyl 4-ethoxybenzoate [54], palmitic acid [69] at different time points (Table 1).

Within plant parts Within plant parts, several correlations that were not detected at T0 became evident at T7 (Table 3 versus Table 2), such as a significant negative correlation

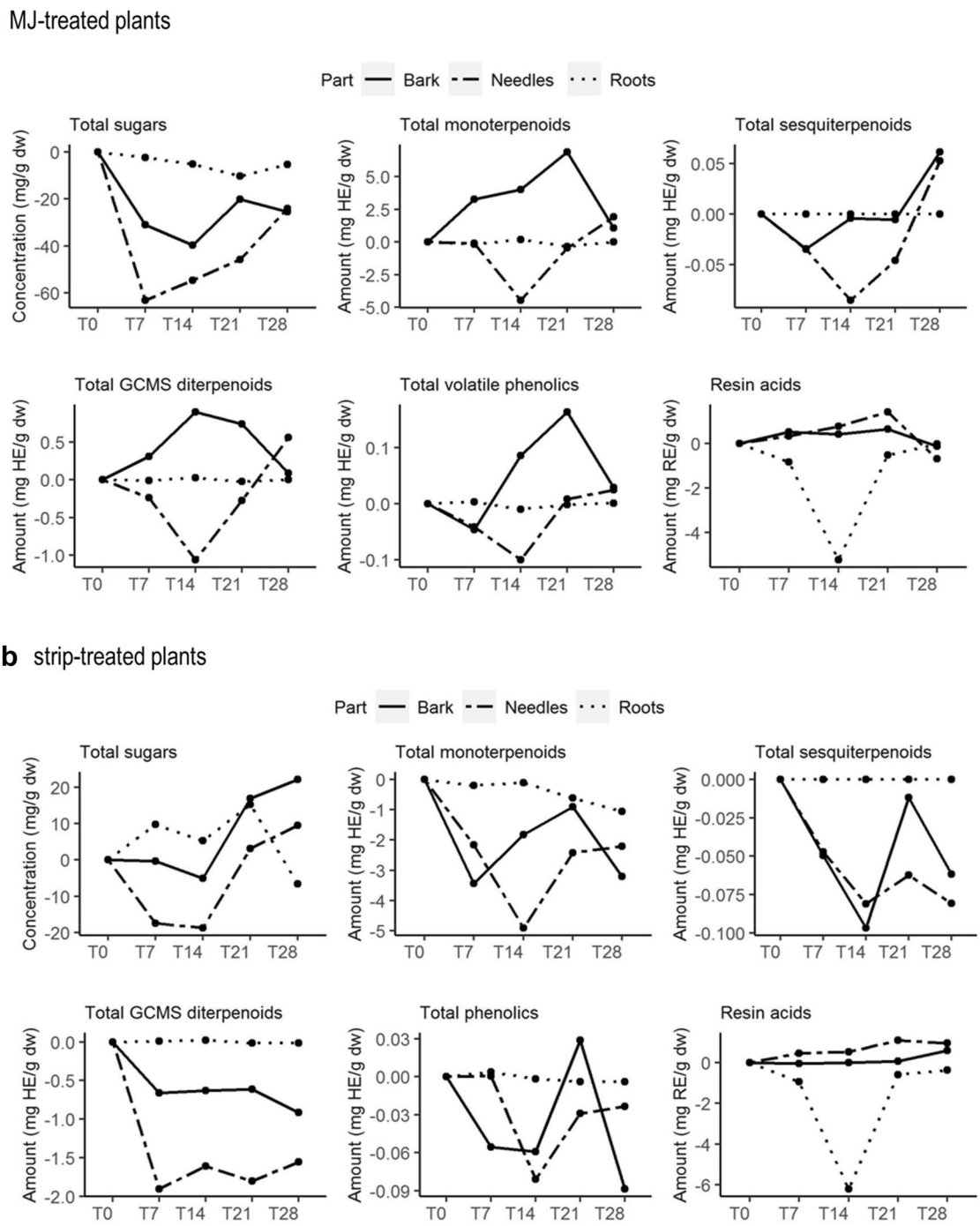

Fig. 4 Average progressive change in the amounts of total compound classes in **a** methyl jasmonate (MJ) and **b** bark strip-treated plants relative to the controls in the bark, needles and roots of *Pinus radiata* plants. At each time point, induced changes were detected by comparing the mean values for the MJ and control treatments (mean of treatment–mean of control) for a specific time. The comparisons were undertaken at each sampling time: T0—before treatment applications, then, T7, T14, T21 and T28, which are, respectively 7, 14, 21 and 28 days after treatment application. The terpenoids and phenolics are expressed as mg heptadecane equivalents (HE)/g dw, while the resin acids are expressed as mg rutin equivalents (RE)/g dw, as no absolute quantitation was carried out on these analytes. The absolute amounts of sugars are quantified in mg/g dw. Note the different scales for **a** and **b**

between total monoterpenoids and total volatile phenolics in the needles of MJ-treated plants (Table 3). In contrast, some correlations detected at T0 were not significant after treatment such as, the significant correlation between the constitutive monoterpenoids and diterpenoids in the roots. More

Table 3 Spearman's rank correlations of compound classes within plant parts at T7 within the bark, needles and roots in a) MJ-treated and b) strip-treated plants.

	Plant part	Total monoterpenoids	Total GC-MS diterpenoids	Total sesquiterpenoids	Total volatile phenolics	Total sugars
(a) MJ-treated plants						
Total GC-MS diterpenoids	Bark	0.94**				
	Needles	0.94**				
	Roots	− 0.14				
Total sesquiterpenoids	Bark	0.54	0.60			
	Needles	0.83*	0.89*			
	Roots	NA	NA			
Total volatile phenolics	Bark	0.43	0.37	− 0.26		
	Needles	− 0.83*	− 0.77	− 0.54		
	Roots	0.09	0.31	NA		
Total sugars	Bark	0.71	0.60	0.09	0.43a	
	Needles	− 0.71	− 0.60	− 0.43	0.89*	
	Roots	− 0.54	− 0.09	NA	− 0.66	
$C_{20}H_{30}O_2$ resin acids	Bark	− 0.09	0.03	0.71	− 0.37	− 0.60
	Needles	0.54	0.60	0.83	− 0.37	− 0.14
	Roots	0.83*	− 0.31	.NA	− 0.20	− 0.31
(b) Strip-treated plants						
Total GC-MS diterpenoids	Bark	0.09				
	Needles	0.77				
	Roots	0.14				
Total sesquiterpenoids	Bark	0.81	0.32			
	Needles	0.71	0.94			
	Roots	NA	NA			
Total volatile phenolics	Bark	−0.31	−0.09	0.14		
	Needles	0.03	− 0.14	− 0.31		
	Roots	0.61	0.49			
Total sugars	Bark	− 0.31	0.77	− 0.03	− 0.09	
	Needles	0.60	0.37	0.26	− 0.31	
	Roots	0.27	0.11	.NA	0.54	
$C_{20}H_{30}O_2$ resin acids	Bark	0.49	0.03	0.09	− 0.49	0.71
	Needles	0.83*	0.09	0.46	− 0.54	− 0.14
	Roots	0.83*	− 0.14	.NA	0.64	− 0.26

p values were set as $p < 0.05$*, $p < 0.01$** and $p < 0.001$***. The values were not Bonferroni adjusted

significant correlations were detected with MJ-induced chemistry than strip-induced chemistry (Table 3a, b).

Discussion

This study showed that based on detectable peaks: (i) two-year old *Pinus radiata* harbours a diversity of primary and secondary compounds that occur in one or other plant part and that the chemical profile between bark, needles and roots is different; (ii) the chemical compounds in the roots responded to above-ground treatment with methyl jasmonate and bark stripping but the progression of the changes over time differed for the chemical compounds, plant parts and treatments; and that (iii) mostly positive correlations between the amounts of compounds occur within and between plant parts. To date, comparatively few studies have examined plant-wide variation in chemistry across plant parts and this is the first study to examine the secondary compound composition of roots in *P. radiata* and how it varies with reference to other plant parts. Results have shown that *P. radiata* roots have high numbers and amounts of both primary and secondary metabolites, with unique compounds also being recorded. However, the number of compounds detected in the roots was less than that detected in the bark and the needles. The allocation patterns of the amount of compounds in the roots compared with the bark and needles also varied depending on the class of chemical compounds, for example, with the sugars, fructose dominated in the roots while glucose dominated in the bark and

the needles. All identified compounds have been previously reported in conifers (Salem et al. 2014; Zhang et al. 2016), and most in *P. radiata* (Cool and Zavarin 1992). However, in this work many unidentified diterpenoids were also important in differentiating the needles, bark and roots and require further investigation.

Needles, bark and roots varied in the constitutive allocation of compounds

This is the first study to explore the chemistry of the three plant parts of *P. radiata* simultaneously. The temporal and spatial variation in the amount of secondary metabolites in a plant is thought to be shaped by organisms that interact with the plant, the nature of the interactions but also by neutral processes (Franceschi et al. 2005; López-Goldar et al. 2019; McKey 1979). According to the optimal defence theory, secondary metabolites that act as defences will be concentrated in parts or tissues that are at higher risk of herbivory and/or tissues that are more valuable (McKey 1974). In this study, the higher number of terpenoid compounds in the bark may imply that the bark is more protected since it is nutrient rich and sought by many herbivores (Felicijan et al. 2015; Franceschi et al. 2005). The bark is also the first barrier of protection (Franceschi et al. 2005). In addition, bark damage may have a stronger impact on plant fitness as it increases the risk of secondary infection to the wood (Franceschi et al. 2005; Welch et al. 1988) and this can reduce the mechanical, hydraulic, and physiological integrity of a tree. Additionally, severe bark stripping can lead to complete ring-barking of the tree and tree death. Studies also show that *P. radiata* can easily compensate for relatively high levels of defoliation (Eyles et al. 2011; Lombardero et al. 2016), which may explain why the number and amount of secondary compounds was lower in the needles compared to the bark. Similar patterns in phenolic allocation in the bark and the needles have been detected in other *Pinus* species (Apetrei et al. 2011). The lower amounts of mono- and di-terpenoids and volatile phenolics in the roots and the absence of sesquiterpenoids altogether, implies that this plant part is not attacked by pests and pathogens to the same degree as the above-ground parts or may rely on other defense mechanisms such as physical defences. However, currently, there are no comprehensive studies of above-ground versus below-ground herbivory in most conifers (Dettlaff et al. 2018). It is also possible that the ongoing artificial selection pressure in *Pinus radiata* could directionally alter the chemistry in the different plant parts, but also chemical composition may vary with age. However, in *Populus tremuloides* Michx., root tannins were also lower than those of the leaves, suggesting that low allocation to roots may be common in trees (Dettlaff et al. 2018). However, defence allocation is also predicted to change during ontogeny (Barton and Koricheva 2010) (and our preliminary investigation—see Materials and methods—of the differences in bark chemistry between young and older trees suggests this might be the case in this species). Although the compounds that have been well described in terms of their involvement in defence activities in conifers, such as α/β-pinene, camphene and limonene, were found in all three plant parts, several monoterpenoids, diterpenoids and volatile phenolics were below detection levels in the needles and the roots. An overlap of some compounds between plant parts may be beneficial where some pathogens and herbivores are non-specific in plant part preferences (Mead 2013). However, it should be noted that secondary metabolites play other roles in plants (Wink 2008) and these may also influence their distribution in the plants.

The constitutive amount of total and individual sugars and fatty acids was found to be highest in the needles, which is consistent with other studies in *P. radiata* (Cranswick et al. 1987) and other conifer species (Dobbelstein et al. 2019; Piper et al. 2017). Generally, the distribution of non-structural carbon compounds including glucose and fructose between photosynthesizing needles and non-photosynthesizing plant parts has been viewed as a passive sink–source process resulting from imbalances between carbon supply and demand (Wiley and Helliker 2012). The lack of detection of sucrose may be related to the season of sampling since sugars and other chemical compounds are very prone to seasonal changes (Cranswick et al. 1987; Dobbelstein et al. 2019). However, Cranswick et al. (1987) also showed that sucrose was relatively low compared to glucose or fructose in *P. radiata* needles compared to the bark and the roots.

Secondary metabolites in conifers, appearing as non-volatile or volatile compounds are diverse and include terpenoids, alkaloids, tannins and phenolic compounds (Holopainen et al. 2018). Maintaining metabolite diversity may pose possible conflicts in resource allocation in the plant that manifest as negative correlations between pairs of traits that share a resource (Kant et al. 2015; Saeki et al. 2014). In the constitutive amounts, the results, however, showed limited phenotypic trade-offs in resource investment between and within plant parts for individual primary and secondary metabolites, or between secondary compounds, consistent with other studies (Deslauriers et al. 2015; Moreira et al. 2013b; Sampedro et al. 2011; Villari et al. 2014). For constitutive secondary metabolites, it has been suggested that trade-offs are not expected where resources are sufficient, or in presence of shared regulatory processes (Sampedro 2014; Sampedro et al. 2011), and when reduction in herbivory is achieved by multiple defence traits (Pearse et al. 2018). Trade-offs are disrupted in crops that have undergone translocation and/or breeding that reduces pest and pathogen pressures and subsequent investment in secondary compounds (Kempel et al. 2011), which could explain the reduced trade-offs in *P. radiata*.

Roots, bark and needles were differentially responsive to MJ and stripping

The results of this study support an earlier study that signalled induced chemical responses in the roots of *P. radiata* to above-ground stressors (Nantongo et al. 2021). Overall, different responses were detected between compound groups, plant parts, and treatments at each time period. Stronger responses were detected in primary compounds compared to secondary compounds, with a very strong overall reduction of sugars especially in the bark and the needles. The consistent reduction of glucose, fructose, sucrose and fatty acids at various times of measurement following the treatments suggests their significant involvement in induced stress responses. The reconfiguration of sugars following herbivory and similar stress treatments (Roth et al. 2018) could result from diminished photosynthesis, which would decrease the overall pool of energy reserves as a result of damage to photosynthetic machinery, loss of photosynthetic tissue, and/or disruption of the vasculature affecting water and sugar transport, and/or from a diversion of resources for defence activities including repair of damaged tissues (Gershenzon 1994; Gould et al. 2008; Huot et al. 2014; Ralph et al. 2006; Roth et al. 2018; Schwachtje and Baldwin 2008). While the reduction in photosynthesis has been observed in *P. radiata* after methyl jasmonate treatment (Gould et al. 2008), this has been explained by the following premises that: (1) resistance traits are costly and frequently up-regulated after attack—the cost is reflected as trade-offs that manifest as negative correlations among chemical traits or between chemicals and growth, reproduction or storage. In the present study, however, there is no strong evidence that defence is costly, contrasting with other studies in conifers (Goodsman et al. 2013; Raffa et al. 2017; Roth et al. 2018), which could also be related to the short time of assessment; (2) resources are translocated to areas inaccessible to herbivores to support the physiological adjustments for subsequent recovery—an aspect of tolerance (Moreira et al. 2012b; Sampedro et al. 2011), which was not strongly supported by the present study because no sugars showed significant increase in the roots after treatment; (3) sugars function as signals in defence pathways, where sugar signals can reportedly be generated either by carbohydrate concentration and ratios relative to other metabolites, such as C:N or by flux through sugar-specific sensors and/or transporters (Eveland and Jackson 2012). This was not tested in the study; and (4) induced changes in primary metabolism could themselves be defensive was also not tested, but whether sugars have a direct defensive role needs to be tested in *P. radiata*. The direct and indirect role of sugars in defence has been implicated in both insect (Clancy 1992) and pathogen–plant (Morkunas and Ratajczak 2014; Schwachtje and Baldwin 2008; Zhou et al. 2015) systems.

The reduction in sugars contradicts observations with *P. pinaster* that did not show any response in bark sugars following methyl jasmonate treatment (Sampedro et al. 2011) and in *Fagus sylvatica* that showed an increase in bark glucose and fructose after bark stripping (Saint-Andrieux et al. 2009). Sometimes, responses of sugars will depend on the amount of bark removed from the plant, for example girdling experiments in non-conifer trees show an accumulation in leaves and bark above the girdle and a strong decline in soluble sugar and starch concentrations in organs below the girdle (roots) (Li et al. 2003). The strong reduction of fatty acids in the needles and the bark following treatments in the present and similar studies (López-Goldar et al. 2018) is consistent with an indirect role in plant defences, because fatty acids can be precursors to the formation of secondary compounds (Kachroo and Kachroo 2009). Additionally, direct defence properties of specific fatty acids against the fungus *Dothistroma pini* in the needles of *P. radiata* as well as in *Pinus banksiana* Lamb. against beetles have been implicated (Franich et al. 1983; Ishangulyyeva et al. 2016).

For secondary compound groups and individual compounds, no or weak responses of terpenoid compounds in the bark, needles or roots following both bark stripping or methyl jasmonate treatments were detected. Within *Pinus radiata*, most studies have reported significant increases in total or individual terpenes and phenolics following herbivory (Gould et al. 2009; López-Goldar et al. 2020; Lundborg et al. 2019; Moreira et al. 2012a; Zas et al. 2014), with a few exceptions (Lombardero et al. 2013), which may reflect the timing of assessment or the nature of the treatments to which the plants are subjected. Mechanical treatments may also differ in their responses from actual herbivory (Miller et al. 2005).

After treatment, this study detected more significant correlations of compounds within and between plant parts compared to the chemistry before treatment. The negative correlations are usually attributed to resource limitations especially in this case where the traits involved rely on a common pool of resources (Sampedro et al. 2011). Whether the observed trade-offs are genetic or can translate into reduced growth remains to be tested. Gould et al. (2008) also showed that in *P. radiata* the costs of expressing defences may be short lived and easily compensated for and are expressed only under nutrient deficiency (Sampedro et al. 2011). Hence, further understanding the genetic basis of these trade-offs may provide a foundation for the development of breeding strategies. However, the induced responses of methyl jasmonate were more visible in the bark than in the needles or roots, which was contrary to the theory of trade-offs between constitutive and induced chemical defences that has been documented in pines (Moreira et al. 2014; Villari et al. 2014). The extent to which the observations may be representative of the natural populations may need further

testing to support extrapolation of the results, including an investigation of ontogenetic changes in chemistry. Although the genetic diversity of Australian and the original natural populations may not differ substantially (Moran and Bell 1987), the chemistry may change in different environments and due to selection during domestication.

Conclusion

This study has shown that plant parts of 2-year old *P. radiata* harbour unique assemblages of compounds. The results confirm earlier studies that show differential allocation of compounds in different plant parts. The allocation of compounds to the roots differs from that of the needles and bark. The results showed strong induced changes in sugars and fatty acids that has not been documented in most conifers and limited induction of mono-, sesqui- and di-terpenoids and volatile phenolic compounds. The importance of sugars and fatty acids in conifer defences needs a more comprehensive investigation. This detailed assessment of *Pinus radiata* chemistry in the needles, bark and roots, including the compounds that respond to simulated biotic stress will potentially facilitate the identification of related chemical defence traits. However, incorporating the effect of genetics and ontogenetic variation in chemistry for target herbivore species will further our understanding of *Pinus radiata* defences, and their potential in the management of browsing herbivores.

Author contribution statement

Experimental design: JSN, BMP, NWD, HF, TR, and JOW; sample collection and management; JSN and HF; data collection of all laboratory work: JSN, NWD, and HF; data curation and analysis: JSN, BMP, NWD, HF, TR, and JOW; writing, review and editing: JSN, BMP, NWD, HF, TR, and JOW; funding and resource allocation: BMP and JOW; project supervision: BMP and JOW.

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Declarations

Conflict of interest The authors declare no conflict of interest.

Code availability Not applicable.

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