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Additive genetic variation in *Pinus radiata* bark chemistry and the chemical traits associated with variation in mammalian bark stripping

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Secondary metabolites are suggested as a major mechanism explaining genetic variation in herbivory levels in *Pinus radiata*. The potential to incorporate these chemical traits into breeding/deployment programmes partly depends on the presence of additive genetic variation for the relevant chemical traits. In this study, near-infrared spectroscopy was used to quantify the constitutive and induced levels of 54 compounds in the bark of trees from 74 *P. radiata* full-sib families. The trees sampled for chemistry were protected from browsing and induced levels were obtained by subjecting half of the trees to artificial bark stripping. The treatment effect on bark chemistry was assessed along with narrow-sense heritability, the significance of non-additive genetic effects and the additive genetic correlations of compounds with bark stripping by mammalian herbivores that was observed in unprotected replicates of the field trial. The results indicated: (i) significant additive genetic variation, with low-moderate narrow-sense heritability estimates for most compounds; (ii) while significant induced effects were detected for some chemicals, no significant genetic variation in inducibility was detected; and (iii) sugars, fatty acids and a diterpenoid positively genetically correlated while a sesquiterpenoid negatively genetically correlated with bark stripping by the mammalian herbivore, the Bennett's wallaby (*Macropus rufogriseus*). At the onset of browsing, a trade-off with height was detected for selecting higher amounts of this sesquiterpenoid. However, overall, results showed potential to incorporate chemical traits into breeding/deployment programmes. The quantitative genetic analyses of the near infrared predicted chemical traits produced associations with mammalian bark stripping that mostly conform with those obtained using standard wet chemistry.

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INTRODUCTION

Herbivory by mammalian herbivores can have significant deleterious impacts on plants and their protection against such damage can enhance productivity in both natural and managed systems (Endress et al. 2016; O'Reilly-Wapstra et al. 2012). In managed forest systems, naturally occurring plant defences offer a durable and less costly means of tree protection against mammalian herbivores in comparison to other management strategies such as fencing and animal control (Iason et al. 2011; Vourc'h et al. 2002). Natural defence against herbivory is achieved by constitutive and inducible physical and chemical traits that act directly or indirectly on herbivore feeding (Franceschi et al. 2005; Hudgins et al. 2004) and understanding their genetic architecture is of interest to the field of evolutionary ecology as well as plant breeding (Johnson 2011). In *Pinus* species and other conifers, such physical traits include bark thickness and texture, constitutive and traumatic resin ducts and specialized phloem parenchyma cells (Franceschi et al. 2005; Hudgins et al. 2004; Nantongo et al. 2020). The chemical traits include secondary metabolites mainly terpenoids and phenolics, where higher amounts are linked to increased resistance of the needles and the bark to mammalian and insect herbivores (Chiu et al. 2017; Iason et al. 2011; Zhang and States 1991). A few studies have also directly or indirectly associated primary compounds (metabolites involved in basic life functions) with herbivory responses (Gershenzon 1994; Tauzin and Giardina 2014; Tiffin 2000). These chemical and physical traits, which are often present in basal levels in plants, can increase or reduce following actual or artificial herbivory (Miller et al. 2005; Nantongo et al. 2021b; Raffa and Smalley 1995; Sampedro et al. 2011). In *Pinus* species, both constitutive and induced traits are under genetic control (Baradat and Yazdani 1988; Iason et al. 2011; Ott et al. 2011; Westbrook et al. 2015; Zhang et al. 2016) and are potentially amenable to natural and artificial selection. However, for different traits, there is variation in the extent to which phenotypic selection on parents will have an impact on their progeny. Assuming no genetic constraints, this impact will depend on the amount of additive genetic variation in the trait under selection, as determined by the phenotypic variance and narrow-sense heritability of the trait. Other factors being constant, traits with low heritability will genetically respond to selection more slowly than traits with higher heritability (Falconer and Mackay 1996).

While the presence of additive genetic variation is an important requirement for a genetic response in traits under selection,

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current theories on the evolution of plant resistance predict the existence of evolutionary trade-offs (negative genetic correlations) between resistance and fitness traits in the absence of herbivory, or between individual traits that can constrain selection in breeding programmes (Huot et al. 2014). Terpenes and phenolics are carbon-based and their production requires carbon resources, resulting in potential conflicts among the compounds or with other plant functions such as growth (Deslauriers et al. 2015; Sampedro et al. 2011; Villari et al. 2014). When there is a genetic basis to these trade-offs, improving resistance through selection and breeding could negatively impact growth or other defence traits, and vice versa. Mixed evidence for the existence of trade-offs in *Pinus* species has been documented (Sampedro et al. 2010; Villari et al. 2014) but generally trade-offs are not expected in environments that are resource-rich as predicted by the resource availability hypothesis (Coley et al. 1985; Sampedro et al. 2011) and growth–differentiation balance hypothesis (Lorio 1986). Similarly, where multiple traits are required for effective defence, limited trade-offs may occur among such traits (Carmona and Fornoni 2013).

Pinus radiata (radiata pine) is a tree native to California and is the main plantation softwood in both Australia and New Zealand where it has been subject to breeding to improve productivity and wood quality (Burdon et al. 2008; Burdon and Brown 2018). However, in many plantations in Australia, trees are subject to bark stripping by mammalian (mainly marsupial) herbivores, which can markedly reduce the genetic gain achieved in breeding programmes (Page et al. 2013). In Tasmania, bark stripping on young trees (1–6 yrs), especially by the Bennett's wallaby (*Macropus rufogriseus*) is the most important pest problem, affecting up to 40% of the Tasmanian plantations (Miller et al. 2014). Genetic variation in herbivory by both mammals and insect herbivores has been documented in *Pinus radiata* (Moreira et al. 2013b; Nantongo et al. 2020). However, the associated defence mechanisms are not well established. A few studies have documented the involvement of physical structures such as thick bark, rough bark and obstructive branches on the stem in deterring herbivores (Miller et al. 2014; Nantongo et al. 2020). Other studies have focussed on chemical defences and have found some relationships between the chemical defences and insect herbivory on *P.* radiata (Moreira et al. 2013a; Moreira et al. 2013b; Sampedro et al. 2011) but narrow-sense heritabilities have been estimated for only a few compound groups (Moreira et al. 2012; Moreira et al. 2013b). In the case of mammalian bark stripping, it has been suggested that increased sugar levels in the bark of *P. radiata* may contribute to susceptibility (Page et al. 2013). Studies also indicate induced responses of *P. radiata* to both actual and artificial herbivory as well as stress elicitors, mostly by increasing terpenes and phenolics and decreasing sugars (Moreira et al. 2013a; Reglinski et al. 2019). However, there is still limited support for the role of induced chemistry in deterring herbivores or its variation between families (Moreira et al. 2013a). The presence of trade-offs between growth and chemistry, and between different chemical traits has been demonstrated in *P. radiata* mainly at the phenotypic level (Gould et al. 2008; Reglinski et al. 2019). However, the existence of a genetic basis for the trade-offs for individual compounds has not been investigated.

The present study focuses on genetic variation in primary and secondary metabolites in the bark of *Pinus radiata* using a field trial of full-sib families, parts of which were protected from browsing whereas the remainder was open to uncontrolled mammalian browsing. Using this system we aimed to: (1) determine the extent to which variation in *P. radiata* bark chemistry is under additive and non-additive genetic control, (2) test whether there are genetic differences in the inducibility of bark chemicals following artificial bark stripping; (3) identify compounds that genetically correlate with uncontrolled mammalian bark stripping; and (4) identify potential trade-offs by determining the genetic correlations among key chemical compounds and their correlations with growth.

MATERIALS AND METHODS

P. radiata progeny trial

The genetic field trial used for this study was established at Wilmot in northern Tasmania (−41.454271°N, 146. 106801°E, 580 m ASL), Australia in 2015 using genetic material sourced from the New Zealand Radiata Pine Breeding Company (RPBC) and is described by Nantongo et al. (2020). The genetic material comprised 74 full-sib (cross-pollinated; CP) families generated from 55 unique parents and 54 grandparents which were planted in the field in a randomised incomplete block design of 26 replicates, and three incomplete blocks per replicate. Each family was represented as a single-tree plot within each replicate. The field trial was fenced to prevent bark stripping by native mammals. The dominant native herbivore on site was the Bennett's wallaby (*Macropus rufogriseus* subspecies *rufogriseus*). The density of the Bennett's wallaby within the general area was estimated at 32 animals/km^2 (DPIPWE 2018). In 2017 (when plants were 25 months of age), the gates of the trial were opened during winter (autumn and winter are peak bark stripping periods) for about two months to allow animal access and bark stripping. Six of the 26 replicates had been further protected using internal fencing to totally exclude the herbivores and allow chemistry to be assayed in the absence of uncontrolled browsing (see chemistry experiment described below). The 6 protected replicates were disconnected and randomly spread through the trial and thus were individually fenced. The remaining 20 replicates were freely accessible to browsing and were used to assess the genetic variation in susceptibility to bark stripping.

Experiment 1: assessment of bark stripping

The details of the bark stripping assessment were described in earlier studies (Nantongo et al. 2020). In brief, at 2 years of age, after ~2 months of exposure to mammalian bark stripping, the amount of bark removed from each tree was scored in the 20 unprotected replicates ($n = 1372$ plants due to some losses) on a 0–5 scale (0 = no damage, 1 = <25%, 2 = 25–50%, 3 = 50–75%, 4 = >75%, 5 = 100% damage [completely ring barked]). At the same time the height of trees from all 26 replicates was measured. By this time, bark stripping had not differentially influenced the height of the different families as depicted from the non-significant tree*protection interaction term (described later in the methods). However, bark stripping possibly caused a reduction in the overall height of the browsed trees given that the average height of the trees at the time of bark stripping assessment in the 20 unprotected replicates was 147.4 ± 0.90 cm compared to 163.7 ± 1.54 cm for trees in the 6 protected replicates. Browse scores were converted to class mid-point values for data analyses, except for scores 0 and 100, following Nantongo et al. (2020).

Experiment 2: chemistry experimental design and chemical analysis

Three weeks after the bark stripping assessment was conducted, an experiment was initiated to assess the constitutive and induced chemical differences among all 74 families using trees in the 6 protected replicates ($n = 390$ plants due to some losses). Half of the plants were subject to artificial bark stripping (treated trees; $n = 195$) at time zero (T0) and half were untreated ($n = 195$) and used as controls (more details of the sampling are presented in Nantongo et al. (2021b). Each family was represented by a maximum of 3 treated and 3 untreated seedlings (maximum $n = 6$). The treatment was applied to alternate trees in each row and thus was randomised with respect to pedigree. It involved removing a vertical strip of 15 cm of bark from the north side of the stem, starting 2 cm above the ground, and covering 30% of the stem circumference (Fig. 1a). The dimensions were selected based on the most common browsing level observed in Experiment 1. Figure 1b shows seedlings in the field that have been damaged by the mammals. Three weeks after the treatment was applied, bark samples were collected from both the control and the treated trees. This bark sample was collected from all the trees ~1 cm above the stripped part on the treated trees as illustrated in Fig. 1a. On the control trees, a bark sample of similar size was collected from a similar height as the one from the treated plants (Fig. 1a). Samples were kept in a cool box until transportation to the laboratory for near-infrared spectroscopy (NIRS) scanning of fresh samples. After scanning, each sample was divided into 2 parts; one part was stored in a −20 °C freezer until wet

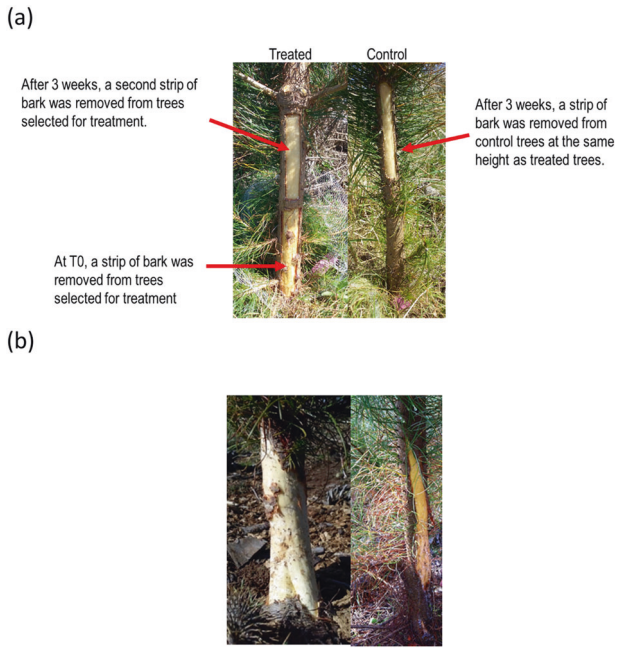

Fig. 1 Artificial and natural bark stripping on young *Pinus radiata* trees. a Fifteen month-old *P. radiata* trees showing the bark stripping treatment (lower left) and how bark was sampled for chemical analysis (upper strip removed) from treated (left) and untreated control (right) plants. After 3 weeks a strip of bark for chemical analysis was collected 1 cm above the treated area of the treatment tree and at similar height for the control tree (see text for details), **b** two seedlings that have been bark stripped by marsupials in the field.

chemical extraction (Maier et al. 2010) and the other was freeze-dried and ground using a Cyclotec 1093 sample mill (FOSS, Denmark) and stored in glass vials for NIRS scanning of dried-ground samples.

Near infrared reflectance spectroscopy models and wet chemical analysis

Assessment of chemistry is conventionally performed using wet chemistry. However, the need for large sample sizes for genetic analysis puts a constraint on the use of wet chemistry given the associated cost and labour (Siesler and Ozaki 2002). The ability of near infrared reflectance spectroscopy (NIRS) to accurately predict the amount of many primary and secondary compounds in *P. radiata* bark has been shown (Nantongo et al. 2021a). This approach enables fast, low cost and large-scale chemotyping and was adopted in the present genetic study.

To predict the chemistry of *P. radiata* using NIRS, bark samples from all trees in the 6 protected replicates ($n = 390$) were scanned when fresh and when freeze-dried and ground using a Bruker MPA Fourier-transform NIR spectrometer (Bruker, Optics, Ettlingen, Germany) in the diffuse reflectance mode (12,000–3800 cm^{-1}) according to the methods in Nantongo et al. (2021a). The fresh bark samples were divided into two; the part closer to the original strip (proximal) and one further from the strip (distal). At similar positions, spectra were also taken on the bark collected from control trees. For both the proximal and distal samples, spectra were collected from the inner and outer sides of the bark sample. In brief, at each position the fresh bark samples were scanned using an optic fibre reflective probe at five different points and the spectra averaged. The dried-ground samples were scanned through the bottom of the glass vials. Each absorbance spectrum was collected at 8 cm^{-1} using the OPUS (ver. 7.2; Bruker Optik GmbH, Germany) software and reflectance (R) data was stored as log (1/R). All quantitative analyses were performed using the Unscrambler® X software (CAMO software version 10.2, CAMO AS, Trondheim, Norway).

Principal component analysis (PCA) was carried out on the spectra of all fresh bark samples, to enable selection of a subset (a third of the total number of samples) of samples for wet chemical analysis (see below) for model calibration purposes. 150 samples representative of the spectral variation present in the entire sample set were selected and these were used to develop the prediction models using partial least squares regression (PLSR). The aim of the PLSR analysis method is to create models for an accurate prediction of the attributes of unknown samples. PLSR models were based on either cross- or external validation and these were developed for each compound using each of the 5 bark NIRS scans (outer distal, outer proximal, inner distal, inner proximal and dried-ground). For leave-out-one cross-validation models, all the 150 samples were used. For external validation models, 100 samples were used as the training data set for model construction and the 50 extra samples as a validation data set against which the model was tested before predicting the unknown samples ($n = 240$). The factors that were automatically selected by the models were retained. In most cases, spectral data were transformed by pre-treatments before the calibration process (Nantongo et al. 2021a; Rinnan et al. 2009). Spectral pretreatment was applied to reduce the spectral noise and to remove or minimize the influence of irrelevant information in order to develop more simple and robust models (Nantongo et al. 2021a; Rinnan et al. 2009). The performance of the PLS models was evaluated according to the root mean square error (see below) and the coefficient of determination (R^2) of the plot between the predicted values and the reference values. The better model of either the cross-validated or the externally validated model was used to predict the chemistry of the unknown samples. The metrics for the final predictive models for each compound or group of compounds can be found in Supplementary Table 1.

Wet chemical extractions that targeted terpenes, phenolics and sugars were performed separately for the bark from each tree using three extraction solvents: dichloromethane (DCM; for the volatile terpenes and phenolics), acetone (for the diterpenoids and fatty acids) and hot water (for the sugars), according to the methods documented in Nantongo et al. (2021c). The DCM extracts were analysed by gas chromatography-mass spectrometry (GC-MS) whereas the acetone extracts and the hot-water extracted sugars were analysed by ultra-high-performance liquid chromatography-mass spectrometry (UHPLC-MS). The preliminary identification of the compounds was based on the comparison of the retention time and mass spectra with the National Institute of Standards and Technology mass spectra library (NIST 2014). However, to verify the retention times for final identification of diterpenoid resin acids by UPLC-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 UPLC-MS. The internal standards n-heptadecane, nonadecanoic acid and specific sugars were used respectively for DCM, acetone and sugar extracts. The components were expressed as equivalents to the respective internal standard, except for the sugars that were measured in absolute amounts. Some compound peaks that could not be resolved on chromatograms were reported as groups of compounds as shown in Supplementary Table 1. Some terpenes and sugars could not be identified as this was a huge undertaking that was out of the scope of this work. A unique number was given to each compound for ease of identification in the tables. Some samples were extracted in triplicate for estimation of lab-error to enable calculation of the NIRS predictive error relative to the lab error (PRL). The compounds that were included in the final data analysis were selected based on 2 criteria. First, was the ratio of the range of the original data to the RMSE (ratio error range - RER). A minimum RER of 6.00 has been suggested as sufficient for detecting differences between classes of samples and for initial screening (Malley et al. 2004). Second, among those that did not meet this criteria, further selection was based on PRL and in this case, a PRL < 3.00 was selected based on suggestions that prediction errors within approximately twice the standard wet chemistry precision are sufficient for application (Yang et al. 2017). Graphical plots and linear regression between NIRS model metrics (RER, PRL and R^2) and genetic parameters (e.g. h^2, see below) were used to explore any bias caused by the NIRS predictive method.

Estimation of additive genetic variation in chemical traits

For all chemical traits successfully modelled with NIRS, the presence of additive genetic variation was tested based on variance components generated by fitting univariate linear mixed models in ASReml v4.1 (Gilmour et al. 2015). The general linear mixed model was fitted

$$\boldsymbol{y} = \boldsymbol{X\beta} + \boldsymbol{Zu} + \boldsymbol{e}, \quad (1)$$

where, $\boldsymbol{y}$ is a vector of the amounts of individual compounds, $\boldsymbol{\beta}$ is a vector of fixed effects (i.e. treatment-inducibility), $\boldsymbol{u}$ is the vector of random

effects (see below), $\boldsymbol{X}$ and $\boldsymbol{Z}$ are design matrices associated with the fixed and random effects, and $\boldsymbol{e}$ is a vector of random residuals. The random effects included replicates, blocks within replicates, tree (additive genetic effect—estimated using the relationship matrix derived from the recorded three-generation pedigree file for trial trees and their ancestors), specific combining ability (fitted by including the full-sib family identity in the model) and the tree × treatment interaction (differential inducibility).

The significance of the fixed treatment effect (inducibility) was tested using the Wald-F statistics (Gilmour et al. 2015). The significance of the random terms was sequentially tested in univariate models using likelihood ratio tests (LRT) starting with family (specific combining ability-SCA), differential inducibility (tree × treatment) and then the additive genetic variation. This testing involved comparing full models with respective reduced models using one-sided likelihood ratio tests with one degree of freedom (Gilmour et al. 2015). Bonferroni's correction was applied to the p-values associated with SCA, inducibility, differential inducibility and additive genetic variation to reduce the chances of obtaining false-positive results (type I errors) when multiple tests are performed (McDonald 2009). The Bonferroni correction was applied within each compound group (monoterpenoids, diterpenoids, sesquiterpenoids, volatile phenolics, sugars, fatty acids or unknowns). With this adjustment, *P*-values were considered significant at 0.05/n, where n is the number of statistical tests (McDonald 2009) (Table 1).

From univariate analyses, individual narrow-sense heritability (h^2) was estimated as the additive genetic variance (σ_a^2) divided by the sum of the additive genetic variance and the error variance (σ_e^2) as below:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2} \quad (2)$$

The associated standard errors were estimated through the "delta method" using ASReml (Gilmour et al. 2015) based on Taylor expansion (Lynch and Walsh 1998). The variance components used for this heritability calculation were derived from re-fitting the above model (Eq. 1) excluding the family and the interaction terms since they were not significant for any compound after Bonferroni correction (see 'Results').

Genetic correlations between chemistry and bark stripping

To determine the relationship between genetic variation in specific chemical compounds and amount of mammalian bark stripping, genetic correlations were estimated in trivariate models. The trees where bark stripping and chemistry was assessed were not the same individuals but belong to the same set of families in different parts of the trial (protected and unprotected, respectively). Therefore, the chemical data from trees that were scored for bark stripping data were treated as missing values in the models and vice versa. Height was the only trait that was assessed in all 26 replicates and therefore acted as the bridging trait between the 20 unprotected replicates where bark stripping was scored and the 6 protected replicates where chemistry was assessed. In each model, year 2 height, spatially adjusted year 2 bark stripping (Nantongo et al. 2020) and one chemical compound were fitted as response variables. The family and the tree × treatment interaction random terms were not fitted at this stage, just the additive tree term. The terms "protected" and "treatment" were fitted as fixed effects in the model. The fixed term "protected" was fitted for height to distinguish the 20 plots that were not protected (from which bark stripping was estimated) from the 6 protected plots (from which chemistry was estimated). This term was not fitted for the chemical traits as they were only assessed from the protected treatment nor for bark stripping which was only assessed in the unprotected replicates. The treatment term was fitted only for the chemical compound in the model as the height was assessed prior the treatment being imposed. The design terms (replicates and blocks) and additive genetic term were retained as random terms in the trivariate models. The spatial variation within incomplete blocks was particularly noticeable for mammalian bark stripping, where more damage occurred on the edge of blocks (Nantongo et al. 2020). Accordingly, spatially adjusted bark stripping scores from Nantongo et al. (2020) were used in the present study, which were derived after fitting design terms and an AR1 model to the residuals. In the case of the chemical traits and height spatial effects were accounted for within the model by fitting replicate and incomplete block terms. The unstructured variance-covariance matrix was fitted for the tree term and a diagonal matrix for the replicates and blocks within replicates.

The genetic correlation r_g between two traits measured was estimated as:

$$r_g = \frac{cov_a(x,y)}{\sqrt{\sigma_{ax}^2 \cdot \sigma_{ay}^2}}, \quad (3)$$

where $cov_a(x, y)$ is the additive genetic covariance between traits x and y, σ^2_{ax} is the additive genetic variance components for trait x, and σ^2_{ay} is the additive genetic variance components for trait y. The associated standard errors were estimated through the "delta method" (Gilmour et al. 2015). To test if the additive genetic correlation was different from zero, the likelihood from the full trivariate mixed model which allowed for additive genetic covariance among the three traits was compared to that from the model in which the covariance between bark stripping and the chemical compound was fixed to zero. This was done using a two-tailed LRT with one degree of freedom. No adjustment was applied to the *p*-values of the genetic correlations for compounds that were associated with bark stripping in Nantongo et al. (2021b) as there were clear a priori reasons for specifically testing these compounds. However, for interpreting significance of any new correlations, Bonferroni's correction was applied within compound groups as indicated above. A network diagram for the genetic correlations was generated in R using igraph.

Genetic correlations among chemical compounds and height

For the chemical compounds that had significant additive genetic correlations with bark stripping, the genetic correlation between chemical traits and height were estimated to test for genetic-based trade-offs with growth. This was done using the trivariate models described above. Before this model was fitted and genetic correlation tests undertaken, a random tree*protected interaction term was fitted in the univariate model (model 1) and its significance tested using a one-tailed LRT. This aimed to test if additive genetic effects on the height differentially responded to protection by the time of assessment. However, there was no evidence for a significant tree*protected interaction effect on height (results not shown) at the time of measurement (Nantongo et al. 2021b). Therefore, the models fitted for the LRT for the genetic correlations were undertaken without this term and compared the full trivariate model estimating all additive covariances to that from the model where the covariation between height and the chemical compound was fixed to zero.

Bivariate models were used to test the genetic correlations among all compounds that had a significant genetic correlation with bark stripping. Bivariate models included the treatment as a fixed term which was fitted for both compounds. The tree and design terms were included as random terms. The unstructured variance-covariance structure was fitted for all the random terms, including the error. Bonferroni's correction to the correlations was not applied at this stage. Pearson's phenotypic correlations among these chemical traits were also estimated in ASReml from bivariate models above (Gilmour et al. 2015) and the test that the phenotypic correlations were different from zero was done using the cor. test function of R v 3.6.1.

RESULTS

Predictions of chemical traits

Near infrared spectroscopy models were developed for all 65 compounds quantified in the bark by wet chemical analysis (Table 1; Supplementary Table 1). Better calibration models with higher R^2 were mostly developed with the spectra collected from the dried-ground bark compared to the rest of the scan positions with few exceptions (Supplementary Table 1). Therefore, the chemical predictions presented were derived using NIRS models developed with spectra collected from the dried-ground bark.

Based on dried-ground samples, the predictive accuracy of NIRS models, determined by the RER, PRL and R^2 varied considerably between compounds (Fig. 2, Supplementary Table 1). Of the primary compounds, the models developed for sugars, glucose [55] (RER = 11.12, PRL = 1.76, $R^2 = 0.79$) and fructose [54] (RER = 10.55, PRL = 1.63, $R^2 = 0.77$) showed the highest predictive power (Supplementary Table 1, superscripts in the text follow compound numbers in this Table and are identifiers for quick identification of the compound in the various tables). Of the secondary

Table 1. Mean and standard deviation (SD) of bark chemical compounds, the significance of fixed and random terms, narrow-sense heritability (h^2) and genetic correlations from the univariate models fitted.

ID	Group	mean ± SD	fixed effect (treatment-inducibility)		LRT *p*-values for random terms			narrow-sense heritability (h^2) ± se	genetic correlation with bark stripping (r_g) ± se	*P*-value	genetic correlation with height (r_g) ± se
			% inducibility	Wald *p*-value	differential inducibility (additive*treatment)	SCA	additive genetic variation				
	monoterpenoids										
1	α-pinene	782.61 ± 419.98	−4.3	0.666	0.021	0.040	**<0.001**	0.26 ± 0.10	−0.20 ± 0.29	0.498	0.02 ± 0.33
2	α-terpineol	29.49 ± 18.71	−2.6	0.737	0.063	0.070	0.045	0.09 ± 0.07	−0.17 ± 0.42	1.000	−0.56 ± 0.45
3	β-phellandrene	80.19 ± 39.76	−13.8	**<0.001**	0.258	0.021	**<0.001**	0.50 ± 0.13	0.10 ± 0.24	0.488	−0.02 ± 0.29
4	β-pinene	1840.94 ± 996.04	−0.4	0.913	0.103	0.007	**<0.001**	0.33 ± 0.11	−0.01 ± 0.28	1.000	−0.04 ± 0.32
5	camphene	7.81 ± 3.84	10.7	0.036	0.038	0.024	**<0.001**	0.30 ± 0.10	−0.12 ± 0.29	0.671	−0.00 ± 0.33
6	citronellal	42.35 ± 49.75	3.1	0.710	0.054	0.160	**<0.001**	0.19 ± 0.09	−0.14 ± 0.32	0.313	−0.32 ± 0.36
7	citronellic acid	18.72 ± 8.97	2.6	0.608	0.028	0.034	**<0.001**	0.34 ± 0.12	0.08 ± 0.27	1.000	0.03 ± 0.31
8	citronellol	49.01 ± 33.91	−5.7	0.287	0.058	0.054	**<0.001**	0.22 ± 0.10	−0.13 ± 0.29	1.000	−0.31 ± 0.32
9	γ-terpinene	5.87 ± 3.63	5.3	0.430	0.376	0.026	**0.001**	0.20 ± 0.09	0.29 ± 0.28	1.000	0.04 ± 0.35
10	limonene	54.30 ± 18.25	−1.2	0.562	0.189	0.008	**<0.001**	0.43 ± 0.13	0.34 ± 0.24	0.084	0.15 ± 0.29
12	sabinene	149.82 ± 91.47	2.5	0.715	0.344	0.139	**<0.001**	0.17 ± 0.09	0.16 ± 0.31	0.403	−0.01 ± 0.37
15	terpinene-4-ol	26.88 ± 16.59	−1.8	0.831	0.500	0.067	**<0.001**	0.28 ± 0.10	0.32 ± 0.27	0.260	−0.28 ± 0.32
16	unknown m/z 150	3.52 ± 1.85	0.0	0.931	0.212	0.000	0.010	0.13 ± 0.08	0.22 ± 0.34	1.000	−0.02 ± 0.40
	sesquiterpenoids										
17	bicyclogermacrene	1.93 ± 0.75	5.3	0.494	0.231	0.500	0.022	0.23 ± 0.09	0.09 ± 0.30	0.888	−0.14 ± 0.34
18	trans-farnesol	18.16 ± 15.36	−18.3	**0.008**	0.018	0.018	**0.004**	0.15 ± 0.09	0.08 ± 0.35	0.752	−0.37 ± 0.39
19	unknown sesquiterpenoid alcohol	4.37 ± 1.68	9.5	**0.003**	0.444	0.500	**0.001**	0.33 ± 0.12	−0.69 ± 0.22	**0.008**	−0.85 ± 0.22
	GC-MS diterpenoids										
20	agathadiol	550.26 ± 506.94	18.9	0.054	0.123	0.186	0.003	0.22 ± 0.10	−0.03 ± 0.31	0.752	0.00 ± 0.36
21	agatholal	340.57 ± 213.96	8.9	0.160	0.324	0.500	**0.000**	0.22 ± 0.10	−0.12 ± 0.29	0.708	0.18 ± 0.33
22	copalol	34.83 ± 18.34	4.3	0.390	0.028	0.336	**<0.001**	0.29 ± 0.10	−0.09 ± 0.28	0.767	−0.20 ± 0.32
23	levopimaral	13.34 ± 7.42	−5.6	0.196	0.354	0.389	**<0.001**	0.31 ± 0.11	0.08 ± 0.27	0.762	0.04 ± 0.31
24	methyl dehydroabietate	14.04 ± 6.15	−14.0	**<0.001**	0.292	0.058	**<0.001**	0.37 ± 0.12	0.22 ± 0.25	0.298	−0.00 ± 0.30
25	unknown diterpene-3	184.25 ± 116.46	−21.1	**<0.001**	0.189	0.005	**0.000**	0.18 ± 0.09	0.30 ± 0.29	0.247	0.03 ± 0.35
26	unknown m/z 109 A	17.44 ± 10.98	−6.3	0.204	0.086	0.006	**<0.001**	0.37 ± 0.12	0.07 ± 0.27	0.675	−0.11 ± 0.31
27	unknown m/z 109 B	21.38 ± 6.12	−2.3	0.166	0.500	0.139	**0.001**	0.20 ± 0.10	0.52 ± 0.24	**0.030**	0.77 ± 0.26
28	unknown m/z 239	6.63 ± 2.33	−5.8	0.021	0.240	0.095	0.107	0.13 ± 0.09	0.35 ± 0.30	0.237	0.70 ± 0.30
29	unknown m/z 272	7.79 ± 2.91	−13.1	**<0.001**	0.254	0.071	**0.002**	0.18 ± 0.09	0.34 ± 0.28	0.237	0.30 ± 0.34
	LC-MS diterpenoids										
30	dehydroabietic acid	24704.5 ± 5156.17	0.5	0.593	0.202	0.500	0.500	0.12 ± 0.07	−0.27 ± 0.37	0.462	0.39 ± 0.39
31	$C_{20}H_{30}O_2$ resin acids	26090.98 ± 6003.77	−5.0	0.020	0.075	0.034	0.022	0.21 ± 0.10	−0.35 ± 0.31	0.273	−0.43 ± 0.35
33	unknown m/z 316	13772.93 ± 5570.38	4.1	0.257	0.224	0.259	**0.001**	0.26 ± 0.11	−0.29 ± 0.29	0.337	−0.05 ± 0.34
34	unknown $C_{20}H_{30}O_3$	25954.20 ± 9277.74	−4.8	0.129	0.161	0.054	**0.001**	0.21 ± 0.10	0.15 ± 0.30	0.624	−0.03 ± 0.35
35	unknown $C_{20}H_{32}O_3A$	692.45 ± 303.45	5.1	0.240	0.011	0.133	**<0.001**	0.22 ± 0.09	−0.04 ± 0.30	0.888	−0.13 ± 0.34

Table 1 continued

ID	Group	mean ± SD	fixed effect (treatment-inducibility)		LRT *p*-values for random terms			narrow-sense heritability (h^2) ± se	genetic correlation with bark stripping (r_g) ± se	*P*-value	genetic correlation with height (r_g) ± se
			% inducibility	Wald *p*-value	differential inducibility (additive*treatment)	SCA	additive genetic variation				
36	unknown $C_{20}H_{32}O_3B$	20689.06 ± 10286.11	−11.1	**<0.001**	0.090	0.079	**<0.001**	0.36 ± 0.13	0.37 ± 0.24	0.077	0.56 ± 0.25
37	unknown $C_{20}H_{30}O_4$	60605.73 ± 18178.65	−5.5	0.037	0.107	0.095	**0.000**	0.27 ± 0.10	0.05 ± 0.28	0.841	0.07 ± 0.32
38	unknown $C_{20}H_{30}O_5$	11354.00 ± 4595.08	−0.0	0.959	0.115	0.107	**<0.001**	0.28 ± 0.11	0.07 ± 0.28	0.806	0.13 ± 0.32
39	unknown $C_{20}H_{30}O_6A$	223.74 ± 132.64	10.7	0.095	0.291	0.035	**<0.001**	0.33 ± 0.11	0.18 ± 0.27	0.517	0.01 ± 0.31
40	unknown $C_{20}H_{30}O_6C$	6297.58 ± 1894.94	2.6	0.412	0.110	0.009	**<0.001**	0.36 ± 0.11	0.32 ± 0.25	0.209	0.16 ± 0.30
41	unknown $C_{20}H_{30}O_6D$	3583.38 ± 1316.9	−6.6	0.008	0.500	0.078	**<0.001**	0.42 ± 0.13	0.61 ± 0.19	**0.006**	0.45 ± 0.26
	phenolics										
42	anethole/estragole	1.63 ± 0.65	−16.7	**<0.001**	0.145	0.208	**0.005**	0.21 ± 0.10	0.00 ± 0.58	0.752	0.43 ± 1.00
44	coniferyl alcohol	4.63 ± 1.23	6.7	**0.006**	0.274	0.254	0.037	0.21 ± 0.10	0.34 ± 0.27	0.237	0.39 ± 0.33
48	phenylethanol	5.13 ± 4.5	−5.7	0.218	0.500	0.036	0.431	0.01 ± 0.05	0.52 ± 0.56	1.000	0.15 ± 0.61
51	thymol	7.92 ± 5.66	−8.2	**<0.001**	0.500	0.154	0.103	0.10 ± 0.08	0.30 ± 0.33	1.000	−0.40 ± 0.37
52	trans-ferulic acid	58.25 ± 27.78	39.6	**<0.001**	0.205	0.500	**0.000**	0.20 ± 0.10	0.48 ± 0.27	0.051	0.32 ± 0.35
53	vanillin	4.56 ± 0.76	6.8	**<0.001**	0.500	0.500	**<0.001**	0.25 ± 0.10	−0.28 ± 0.30	1.000	0.15 ± 0.34
	sugars										
54	fructose	13303.09 ± 3607.02	−22.2	**<0.001**	0.500	0.500	**0.001**	0.31 ± 0.11	0.55 ± 0.23	**0.018**	0.06 ± 0.31
55	glucose	15298.65 ± 4309.32	−18.5	**<0.001**	0.376	0.500	**0.001**	0.29 ± 0.11	0.80 ± 0.20	**0.002**	0.62 ± 0.24
56	inositol	10935.47 ± 3551.37	−23.6	**<0.001**	0.354	0.500	0.060	0.15 ± 0.08	−0.14 ± 0.33	0.671	−0.01 ± 0.38
58	unknown monosaccharide	521.33 ± 236.64	24.1	**<0.001**	0.208	0.084	**0.003**	0.28 ± 0.11	−0.47 ± 0.28	0.066	−0.48 ± 0.32
	fatty acids										
59	linoleic acid	16914.05 ± 3747.38	−9.0	**<0.001**	0.500	0.500	**<0.001**	0.51 ± 0.14	0.68 ± 0.16	**0.001**	0.69 ± 0.22
60	linolenic acid	7689.81 ± 1454.91	1.7	0.430	0.500	0.015	**<0.001**	0.44 ± 0.13	0.65 ± 0.19	**0.004**	0.50 ± 0.26
61	palmitic acid	16656.04 ± 2440.36	4.0	**<0.001**	0.500	0.402	**0.004**	0.37 ± 0.12	0.08 ± 0.27	0.752	0.07 ± 0.31
	unknowns										
62	unknown m/z 104	2.64 ± 1.36	−16.7	**<0.001**	0.336	0.020	**<0.001**	0.43 ± 0.13	0.42 ± 0.22	**0.042**	0.27 ± 0.28
63	unknown Mol Wt 274	1115.47 ± 398.55	−9.0	**0.004**	0.100	0.052	**<0.001**	0.32 ± 0.11	0.03 ± 0.28	0.888	0.05 ± 0.32
64	unknown Mol Wt 406 A	530.63 ± 167.07	8.8	**0.001**	0.500	0.500	0.500	0.07 ± 0.06	0.77 ± 0.56	0.067	0.77 ± 0.66
65	unknown Mol Wt 406 B	5984.92 ± 2308.79	8.0	0.021	0.346	0.500	**<0.001**	0.19 ± 0.09	−0.46 ± 0.27	0.058	−0.68 ± 0.27

For the various terms, the unadjusted probabilities are shown and bolded when significant following Bonferroni adjustment ($p < 0.05$). The change that occurred in the different compounds following treatment (inducibility) is shown, where negative values (−) indicate reduction in the treated samples relative to the control and positive values indicate increase in the compounds relative to the control. The unadjusted *p*-values for the likelihood ratio test (LRT) associated with the significance of the random terms - differential inducibility (tree × treatment), family (specific combining ability, SCA) and tree (additive genetic variation) - are shown. The narrow-sense heritability and standard error (se) and the genetic correlation plus standard error (se) of the individual chemical compound with bark stripping and height are also included. The unadjusted *p*-value for the LRT test from zero are indicated for the genetic correlations (se) of the chemical compounds with bark stripping. For the correlation with height, only compounds that significantly correlated with bark stripping after Bonferroni adjustment were tested and unadjusted p- values are indicated in Table 2. The GC-MS components (monoterpenoids, sesquiterpenoids, GC-MS diterpenoids and phenolics) are expressed as micrograms of heptadecane equivalents (HE) per gram of dry weight of the sample (µg HE/g dw) and the LC-MS analytes (LC-MS diterpenoids and fatty acids) are expressed as micrograms of nonadecanoic acid equivalents (NE) per gram of dry weight of the sample (µg NE/g dw). Sugars are expressed in µg/g dw. Unknown indicates a compound that could not be identified. All compounds were given a unique identifier (ID) for ease of identification following Supplementary Table 1.

compounds, the highest prediction was achieved for unknown diterpenoids; unknown $C_{20}H_{32}O_3A$[35] (RER = 12.52, PRL = 4.50, $R^2 = 0.83$), unknown $C_{20}H_{30}O_3$[34] (RER = 14.79, PRL = 3.72, $R^2 = 0.83$), unknown m/z 316[33] (RER = 11.87, PRL = 2.97, $R^2 = 0.72$) and unknown $C_{20}H_{30}O_5$[38] (RER = 12.24, PRL = 4.59, $R^2 = 0.71$) as well as monoterpenoids; α-pinene[1] (RER = 7.63, PRL = 0.81, $R^2 = 0.73$) and β-pinene[4] (RER = 10.30, PRL = 1.01, $R^2 = 0.73$) (Fig. 2). Additionally, there are several other compounds that had $R^2 > 0.50$ and these included one sugar (inositol[56]), two fatty acids (linoleic acid[59] and linolenic acid[60]) and secondary compounds that included two monoterpenes (camphene[5], citronellal[6]), and ten diterpenoids (agathadiol[20], agatholal[21], copalol[22], levopimaral[23], dehydroabietic acid[30] and several unknown diterpenoids[26] [37] [38] [39] [40]) as well as other unknown compounds[63] [64] (Supplementary Table 1). The following results focussed on the 54 compounds that were retained from the 65 listed in Supplementary Table 1 after applying the selection criteria defined by the PRL and RER (see 'Materials and methods') and these 54 are listed in Table 1.

Inducibility of chemical traits

Twenty-seven out of the 54 compounds responded to the bark stripping treatment by significantly increasing or reducing their amounts, with 21 (39%) retaining their significance after Bonferroni adjustment (Table 1). The strongest increment in the amount of compounds was detected for the phenolic compound trans-ferulic acid[52], which increased by 39.6% ($p < 0.001$). In contrast, the bark sugars reduced following treatment, where inositol[56], fructose[54] and glucose[55] reduced by 23.6, 22.2, and 18.5%, respectively (Table 1). Only 6 out of 54 (11%) compounds, comprising three monoterpenoids[1,5,7], a sesquiterpenoid[18] and two diterpenoids[22, 35], showed significant ($p < 0.05$) genetic differences in inducibility as indicated by the unadjusted p-values of the additive by treatment interaction term (Table 1). However, these interactions were not significant after Bonferroni correction. There is thus little evidence to suggest the presence of genetic variation in chemical inducibility and this term was not included in the genetic models used to estimate heritabilities.

Family (SCA) variation

Based on unadjusted probabilities, 30% of the compounds showed significant ($P < 0.05$) non-additive genetic variation (i.e. SCA variation), including several monoterpenoids[1,3,4,5,7,9,10], a sesquiterpenoid[17], diterpenoids[24,25,30,38,39], a phenolic compound[48], a fatty acid[60] and an unknown compound[62]. However, after Bonferroni adjustment, the SCA variation was not significant for any of the compounds (Table 1), so the SCA term was also excluded from the models used to estimate heritability.

Genetic variation in *P. radiata* chemistry

Using univariate models minus the SCA term and the random tree × treatment interaction, significant (adjusted) levels of additive genetic variation were evident for most of the selected chemical compounds, with narrow-sense heritability estimates ranging between 0.01 and 0.51(Fig. 3, Table 1), with standard errors between 0.05 and 0.13 (Supplementary Fig. 3). Only 12 compounds including two monoterpenoids[2,16], a sesquiterpenoid[17], four diterpenoids[20,28,30,31], three phenolic compounds[44,48,51], a sugar[56] and an unknown compound[64] did not show significant additive genetic variation.

Of the secondary compound groups, considering only compounds with significant additive genetic variation, the average heritability of monoterpenoids ($h^2 = 0.29 \pm 0.10$), diterpenoids ($h^2 = 0.28 \pm 0.11$) and sesquiterpenoids ($h^2 = 0.24 \pm 0.10$) appeared to be consistently higher than the phenolics ($h^2 = 0.22 \pm 0.10$). The heritability for the terpenoids was similar to that of sugars ($h^2 = 0.29 \pm 0.11$) but lower than fatty acids. The fatty acids had the highest average heritability ($h^2 = 0.44 \pm 0.13$). There was no relationship between the univariate narrow-sense heritability estimate and the NIRS predictive accuracy for the 54 selected compounds as indicated by (i) the ratio of NIRS root mean square error (RMSE) relative to the laboratory error—PRL, (iii) the range error ratio—RER, and (iii) the NIRS coefficient of determination (R^2) (Supplementary Fig. 1). In addition, there was no significant relationship between heritability and mean amount of the compounds (Supplementary Fig. 2)

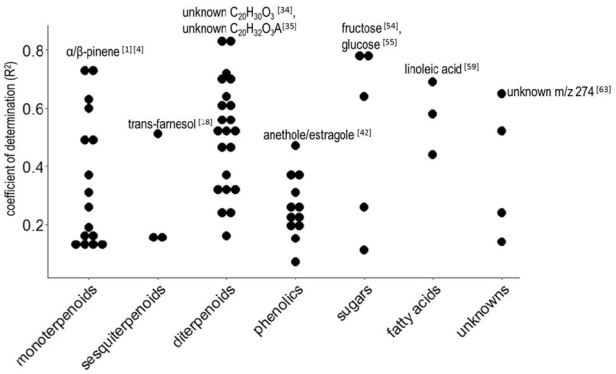

Fig. 2 Dot plot of the distribution of the coefficient of determination (R^2) for the NIRS PLS models for the 65 chemical compounds identified in the bark. Each dot represents one R^2 estimate for a specific compound and these have been grouped by major compound groups. The figure also shows the compound that exhibited the highest R^2 estimate in each major compound group. Unknown indicates a compound that could not be identified. Numbers in parentheses refer to the location of the compound in Supplementary Table 1.

Traits genetically associated with bark stripping

A greater number of positive than negative genetic correlations between compounds and bark stripping were detected suggesting that preference may be a stronger driver of genetic variation in bark stripping than defence (Table 1; Fig. 4). Significant unadjusted positive genetic correlations were detected between bark stripping and the sugars - glucose[55] ($r_g = 0.80 \pm 0.20$, $p < 0.01$) and fructose[54] ($r_g = 0.55 \pm 0.23$, $p < 0.05$); fatty acids - linoleic acid[59] ($r_g = 0.68 \pm 0.16$, $p < 0.01$) and linolenic acid[60] ($r_g = 0.65 \pm 0.19$, $p < 0.01$), as well as two diterpenoids - unknown m/z 109 B[27] ($r_g = 0.52 \pm 0.24$, $p < 0.01$) and unknown $C_{20}H_{30}O_6$ D[41] ($r_g = 0.61 \pm 0.19$, $p < 0.01$). One compound of unknown group, unknown m/z 104[62] also positively correlated with bark stripping ($r_g = 0.42 \pm 0.22$, $p < 0.01$). The only significant negative genetic correlation observed was between bark stripping and an unknown sesquiterpenoid alcohol[19] ($r_g = -0.69$, $p < 0.05$; Table 2). No adjustment was made on the p-values for these genetic correlations as they were based on an a priori hypothesis (see 'Materials and methods').

Genetic correlations among compounds and with height

The genetic correlation between bark stripping and height was positive, but non-significant ($r_g = 0.40 \pm 0.29$, $p = 0.11$). However, several of the chemical compounds correlated with bark stripping were genetically correlated with height (Table 2; Fig. 4). A significant negative genetic correlation was detected between the unknown sesquiterpene alcohol[19] and height ($r_g = -0.85 \pm 0.22$, $p < 0.01$), suggesting that selecting for higher amounts of this compound will reduce growth in the absence of herbivores. Similarly, a positive correlation of the sugars[55] and fatty acids[59,60] with height (Table 2) indicates that selecting for reduced sugar levels may result in reduced early growth. The strong positive genetic correlation between sugars and height suggests that fast-growing trees possibly have more sugar in the

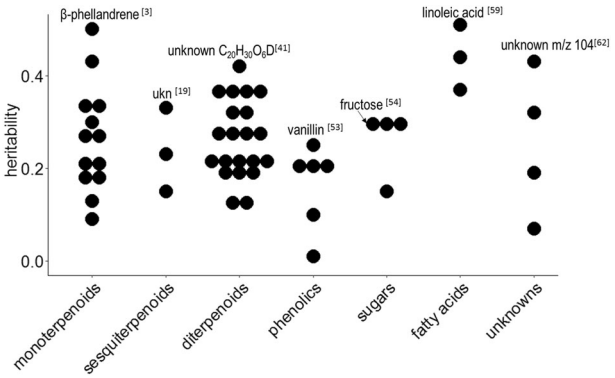

Fig. 3 Dot plot of the distribution of estimated narrow-sense heritabilities for selected chemical compounds in the bark. 54 chemical compounds that had RER > 6 or PRL < 3 (see 'Materials and methods') were included in the plot. Each dot represents a narrow-sense heritability estimate. The figure also shows the compound that exhibited the highest heritability estimate in each group, where ukn is an unknown sesquiterpenoid alcohol. Unknown indicates a compound that could not be identified. Numbers in parentheses refer to the ID code of the compound in Supplementary Table 1.

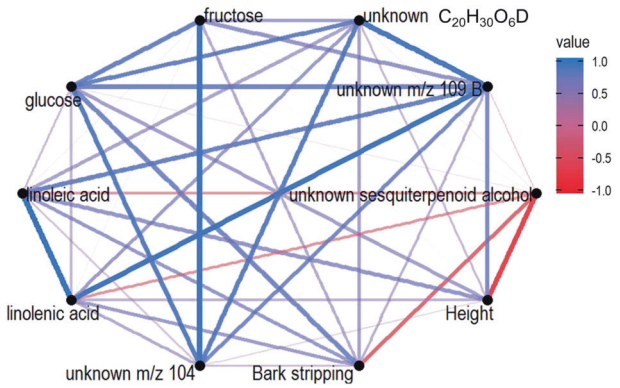

Fig. 4 A network diagram showing the genetic correlations between different traits that significantly correlated with bark stripping. Blue indicates a positive relationship and red indicates a negative relationship as indicated in Table 2. A thicker line indicates a stronger correlation.

bark sample than slow growing trees and conversely slow growing trees have less sugar and more sesquiterpenes.

Among the compounds that significantly correlated with bark stripping, genetic correlations indicated that selecting for higher amounts of the unknown sesquiterpenoid alcohol[19] to reduce bark stripping will slightly reduce the amount of the fatty acid - linoleic acid[59] (Table 2), which could contribute to its positive association with bark stripping noted above. There was no evidence for a genetic correlation between the unknown sesquiterpenoid alcohol[19] and the rest of the compounds, including the sugars. The positive genetic correlations also indicate that selecting for low sugars to reduce bark stripping will shift the fatty acids and the unknown diterpenoids in the same direction, offering possibilities for multi-trait selection. The highest positive correlations were detected between the unknown compound[62] and the sugars; glucose ($r_g = 0.96$, $p < 0.001$)[55] and fructose ($r_g = 0.90$, $p < 0.001$)[54].

Phenotypic correlations between compounds showed similar trends as the genetic correlations (Table 2). However, some significant correlations were detected at the phenotypic level that were not detected at the genetic level. Where genetic correlations were significant, the corresponding phenotypic correlations were smaller, except for the phenotypic correlations of the unknown sesquiterpenoid alcohol with glucose, fructose and the unknown diterpenoid (unknown $C_{20}H_{30}O_6D$[41]), which were higher than genetic correlations.

DISCUSSION

The results of this study showed that: (i) most of the primary and secondary chemical compounds of *Pinus radiata* bark are under additive genetic control, with non-additive effects of little significance; (ii) there are at most only weak additive genetic based differences for inducibility; (iii) sugars (glucose and fructose) and fatty acids (linoleic and linolenic acids) genetically, positively correlate with bark stripping while an unknown sesquiterpenoid alcohol negatively correlated with bark stripping; and (iv) the unknown sesquiterpenoid alcohol negatively correlated with height while the remainder of the compounds positively or did not correlate with height. Genetic differences in the constitutive and induced variation in secondary and primary metabolites have been noted in earlier studies in *P. radiata* and other pine species using quantitative (Sampedro et al. 2010; Zhang et al. 2016) and molecular (Lamara et al. 2018; Vázquez-González et al. 2019) genetic studies. Of the secondary compounds, terpenes and phenolics (including condensed tannins) have been the focal defence compounds, where genetic variation in these compound groups has been repeatedly documented in the bark of conifers (Sampedro et al. 2011). However, other secondary compounds such as alkaloids have also been found to be under genetic control in the needles of conifers (Gerson et al. 2009). In *P. radiata*, the presence of genetic variation has been previously detected for total terpenes and total phenolics (Moreira et al. 2012; Moreira et al. 2013b) in the bark but not individual compounds. Genetic variation in several individual cortical monoterpenoids have also been reported in undomesticated populations (Burdon et al. 1992a; Burdon et al. 1992b). However, the present study is the first to estimate narrow-sense heritability and genetic correlations for numerous individual primary and secondary compounds in *P. radiata* bark. This was only practical due to the development of NIRS models for these chemical traits, a possibility signalled in an earlier study of green-house grown plants (Nantongo et al. 2021a), and here shown possible for a wide range of chemical compounds with field-grown plants and using the freeze-dried, ground bark samples on which the genetic analyses focused.

Most of the chemical traits exhibited significant additive genetic variation with low to moderate narrow-sense heritability estimates. Numerous factors affect the heritability of a trait, and these may be context and population specific. The low heritabilities of some primary and secondary chemical traits could be a result of the erosion of genetic variation through drift or indirect selection during domestication, a possibility given that the species has undergone breeding for up to four generations (Dungey et al. 2009). It could also be a base population effect reflecting the erosion of additive genetic variation through natural selection which could occur if the trait is closely related to fitness (Kruuk et al. 2000; Mousseau and Roff 1987). Both primary and secondary metabolites are particularly important as chemical cues in plant-herbivore interactions. Primary metabolites like sugars indicate the physiological status and nutritional value of the plants while secondary metabolites may indicate toxicity and defence status. Fatty acids exhibited the highest average heritability of the compound groups studied. The importance of fatty acids in the bark of *P. radiata* is not well studied. However, the storage and structural functions of fatty acids, as well as their direct pathogen defence properties have been documented in the needles of *P. radiata* (Franich et al. 1983). Indeed, some of the most studied signalling molecules like jasmonic acid belong to a group of compounds formed by the oxygenation of fatty acids (Kachroo and Kachroo 2009), emphasizing the role of fatty acids in stress responses. In this study, the negative genetic correlation of the

Table 2. Genetic (below) and phenotypic (above) correlations and the standard error of the compounds that significantly correlated with bark stripping.

ID	Traits	Bark stripping	Height	unknown sesquiterpenoid alcohol	unknown m/z 109 B	unknown $C_{20}H_{30}O_6$ D	fructose	glucose	linoleic acid	linolenic acid	unknown m/z 104
	Height	0.40 (0.29)		0.24 (0.04)***	−0.30 (0.05)***	−0.13 (0.04)**	−0.12 (0.00)*	0.36 (0.08)	−0.09 (0.00)	0.00 (0.00)	−0.23 (0.06)**
19	unknown sesquiterpenoid alcohol	−0.69 (0.22)*	−0.85 (0.22)**		−0.27 (0.06)	−0.28 (0.12)**	−0.37 (0.05)***	−0.44 (0.05)***	−0.18 (0.19)***	−0.17 (0.05)**	−0.10 (0.11)
27	unknown m/z 109 B	0.52 (0.24)*	0.77 (0.26)**	−0.29 (0.29)		0.59 (0.06)	0.15 (0.09)	0.21 (0.09)	0.34 (0.10)	0.14 (0.08)	0.70 (0.04)***
41	unknown $C_{20}H_{30}O_6D$	0.61 (0.19)**	0.45 (0.26)	−0.15 (0.26)	0.93 (0.06)***		0.28 (0.40)	0.41 (0.09)***	0.48 (0.09)***	0.35 (0.09)***	0.84 (0.02)***
54	fructose	0.55 (0.23)*	0.06 (0.31)	−0.06 (0.27)	0.69 (0.23)*	0.64 (0.18)*		0.85 (0.03)***	0.07 (0.05)	0.05 (0.05)	0.50 (0.06)***
55	glucose	0.80 (0.20)**	0.62 (0.24)**	−0.19 (0.27)	0.82 (0.20)**	0.85 (0.14)**	0.84 (0.09)***		0.07 (0.15)*	0.18 (0.12)***	0.41 (0.07)***
59	linoleic acid	0.68 (0.16)**	0.69 (0.22)***	−0.51 (0.19)*	0.84 (0.12)***	0.59 (0.17)*	0.14 (025)	0.44 (0.23)		0.58 (0.06)***	0.30 (0.09)***
60	linolenic acid	0.65 (0.19)**	0.50 (0.26)**	−0.52 (0.21)	0.95 (0.13)***	0.65 (0.16)**	0.12 (026)	0.47 (0.23)	0.97 (0.06)***		0.19 (0.08)**
62	unknown m/z 104	0.42 (0.22)*	0.27 (0.28)	0.20 (0.26)	0.79 (0.11)***	0.88 (0.06)***	0.96 (0.08)***	0.90 (0.11)***	0.37 (0.20)	0.55 (0.19)*	

The phenotypic correlation between bark stripping with the compounds was not estimated since the chemistry was estimated in separate trial replicates from where bark stripping was estimated. Genetic correlation between traits were estimated using the trivariate models that included a chemical trait, height and bark stripping (see 'Materials and methods'). Unadjusted *p*-values are indicated, $^{**}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$. Unknown indicates a compound that could not be identified. All compounds were given a unique identifier (ID) for ease of identification following Supplementary Table 1.

fatty acid linoleic acid[59] and the unknown sesquiterpenoid alcohol[19] suggests that fatty acids may be partly linked to the formation of sesquiterpenoids (Pott et al. 2019). Several fatty acids were also positively associated with bark stripping.

Within the secondary compound groups, the terpenoids appeared to have higher narrow-sense heritability compared to phenolics (Moreira et al. 2013b). The average narrow-sense heritability estimates of the sugars was similar to that of the terpenoids. The heritability values of the secondary compounds were lower or in range of what has been established for the same compounds in the bark of other conifers (Sampedro et al. 2010; Zhang et al. 2016). For sugars in other *P. radiata* populations, low genetic variation for bark and wood has been detected (Cranswick et al. 1987; Donaldson et al. 1997). However, in other conifer species, observations have been mixed. For example, while no genetic variation was observed for sugars in the bark of juvenile *Pinus pinaster* (Sampedro et al. 2011), high heritability of glucose levels was observed in the wood of *Pseudotsuga menziesii* (Ukrainetz et al. 2008). These counter examples may also suggest population, tissue or species-specific differences in additive genetic variation for sugars. However, it is noted that differences in heritability can also arise from differences in the residual variance due to unaccounted environmental (e.g. phenotypic plasticity), and non-additive genetic variance or a combination of all these effects, rather than from different levels of additive genetic variation (Price and Schluter 1991; Visscher et al. 2008). Although the SCA variation, a major component of the non-additive genetic variation, was not significant after accounting for multiple testing for the traits in this study, possibly better accounting for spatial heterogeneity in the genetic models (Nantongo et al. 2020) may improve the genetic estimates in the chemical traits that exhibited low heritability. It has also been noted for secondary compounds that the relative amount of additive genetic variation may be related to the amount of compound harboured by the plant, where compounds that occur in higher amounts have been found to have higher heritability estimates than those in lower amounts (Haviola et al. 2006). For *P. radiata* bark, the amount of monoterpenoids often dominate the other terpenoid components (Nantongo et al. 2021c), which could explain their high heritability estimates compared to other secondary metabolites. However, over all chemicals studied no significant effect of mean levels on heritability estimates was observed. The low heritability of the phenolics could arise from their poor predictability by NIRS, but this is also an unlikely explanation as the present study showed no link between NIRS accuracy and heritability. Regardless, while heritability estimates for different secondary compounds may be variable between studies (Sampedro et al. 2010; Zhang et al. 2016), the low to moderate narrow-sense heritability values from different studies indicate that the secondary compounds in the bark of *P. radiata* and other conifers have sufficient additive genetic variation to be potentially responsive to natural or artificial selection.

Induced changes in the amounts of primary and secondary compounds were observed in response to artificial bark stripping. This is consistent with previous studies (Moreira et al. 2012; Nantongo et al. 2021c; Sampedro et al. 2011) that show a reduction in the amounts of sugars and an increase in some of the secondary compounds after chemical and biotic stress treatments. However, genetic differences in inducibility as a result of artificial bark stripping did not appear to be evident for individual compounds in the present study despite an earlier greenhouse study that showed the amounts of some individual terpenes, phenolics and sugars reduced or increased differentially between susceptible and resistant families (Nantongo et al. 2021b). While several terpenoids showed genetic variation in the induced response to bark stripping, the effect was weak and not significant after statistical correction for multiple testing. In contrast, Moreira et al. (2013b) and Sampedro et al. (2011) found high genetic

variation in inducibility of stem resins in *P. radiata* and diterpenoids in *P. pinaster*, respectively. The presence of genetic variation in inducibility suggests that this trait can be selected for. However, overall, the results for the populations studied suggest that selection for reduced susceptibility of *P. radiata* to bark stripping is more feasible based on the constitutive than the induced chemistry, although correlations between bark stripping and induced chemistry need further exploration.

The sugars fructose[54] and glucose[55] positively genetically correlated with bark stripping, which clearly demonstrates an additive genetic basis to this association which was previously noted by Nantongo et al. (2021b). The initial association was based on the comparison of the wet chemistry of families identified as more and less susceptible to bark stripping. In other conifers, this association between mammalian herbivory and sugars has been mainly detected at the phenotypic level (Kimball et al. 1998). Of the secondary compounds, an unknown sesquiterpenoid alcohol[19] negatively correlated with bark stripping and this is consistent with the earlier study of Nantongo et al. (2021b). In contrast, the sesquiterpenoid bicylogermacrene that was the major compound that differentiated the less from the more susceptible families in Nantongo et al. (2021b) did not exhibit significant additive genetic variation (after Bonferroni adjustment) and did not significantly correlate with bark stripping at the additive genetical level. This difference could possibly be in part due to non-linear genetic associations with bark stripping. Non-linear genetic relationships, where the range of a trait varies drastically from one extreme to another have been detected in *Arabidopsis thaliana* (Vasseur et al. 2019) and this may have affected the genetic correlations for bicyclogermacrene that were estimated based on linear models. The only terpenoid that significantly genetically correlated with bark stripping was the one unknown diterpenoid that had a positive correlation. This diterpenoid was also highlighted by Nantongo et al. (2021b) where its amount was higher in the susceptible compared to the resistant families (although this was non-significant). This positive association contrasts with the documented role of diterpenoids in reducing herbivory in conifers (Franceschi et al. 2005). However, it may be in part due to the capability of the marsupials to ingest and metabolise a range of terpenes that would be toxic to many other herbivore species (Boyle 1999; El-Merhibi et al. 2007). Consistent with the wet chemistry results of Nantongo et al. (2021b), the genetic correlations provided no evidence for the involvement of the monoterpenoids in determining susceptibility of *P. radiata* to bark stripping.

Although a significant negative correlation between the height and the unknown sesquiterpenoid alcohol that could signify presence of defence-growth trade-off was detected, further evidence for presence of this trade-off may be required since the unknown sesquiterpenoid alcohol occurs in very low quantities and it is unlikely be solely responsible for the observed reduced plant growth. A similar trend was still observed by Nantongo et al. (2021b). However, trade-offs in expression of defences and growth have also been detected in conifers based on molecular studies (Porth et al. 2011). Similarly, the positive correlation between sugars and height suggests that if susceptibility to bark stripping is mainly driven by the sugars, positive selection for early growth in the absence of bark stripping will increase the vulnerability of the population to bark stripping. Positive correlations of herbivory of the bark with plant height are common in conifers (Porth et al. 2011; Zas et al. 2017) and may be explained by fast-growing trees potentially investing less in secondary compounds, especially in the presence of resource constraints (Ferrenberg et al. 2015; Moreira et al. 2015). In the present case, genetic correlations suggest that fast-growing trees invested less in specific bark terpenoids and more in sugars. In terms of the correlation between early-age height and bark stripping, the positive correlation (but non-significant) in the trivariate models in this study was consistent with the results from bivariate models presented in Nantongo et al. (2020) and the non-parametric comparison of susceptible and resistant families presented in Nantongo et al. (2021b) which were both statistically significant. However, in the linear models that included three response variables - height, bark stripping and chemical compounds as response variables, bark stripping was not significantly correlated with height. While this may be due to higher statistical power of the previous bivariate models, it may also imply that height is an associational rather than independent predictor of susceptibility, especially given its positive correlation with the levels of glucose in the bark.

The genetic correlations among individual compounds were mostly positive or non-significant, except for the negative correlation between the unknown sesquiterpenoid alcohol[19] and the fatty acid, linoleic acid[59]. Positive genetic correlations may suggest common biochemical pathways which is common for traits that interact together to perform a given function (Conner and Via 1993), and suggest the potential for multi-trait selection. In the current system, susceptibility is potentially achieved by multiple interdependent primary and secondary compounds (Nantongo et al. 2021b). On the other hand, negative genetic correlations, such as those between the unknown sesquiterpenoid alcohol and fatty acids are predicted when the traits arise through intermediary metabolites such as acetyl-CoA that is implicated both in primary (e.g. fatty acid biosynthesis) and secondary metabolism (e.g. isoprenoid precursor biosynthesis) (Pott et al. 2019).

Phenotypic correlations also indicate similar trends, where positive phenotypic correlations indicate simultaneous resource investment in multiple traits and negative correlations suggest trade-offs (Moreira et al. 2015). For example, phenotypic correlations indicate that a higher amount of the unknown sesquiterpenoid alcohol[19] will come at the expense of fatty acids, consistent with a trade-off. This observation may explain the strong reduction in fatty acids detected following stress treatments in Nantongo et al. (2021c), consistent with suggestions that fatty acids can be precursors to the formation of secondary compounds (Kachroo and Kachroo 2009). Negative correlation between sugars and secondary compounds have been hypothesized in most stress studies, where after stress sugars should reduce to favour the formation of secondary compounds (Herms and Mattson 1992; Lombardero et al. 2000; Moreira et al. 2015; Sampedro et al. 2011). In this present study, however, fatty acids rather than the sugars appear to be more negatively associated with secondary compounds.

CONCLUSION

The use of NIRS offers opportunities for large scale, non-invasive, low-cost, and environmentally safe chemical phenotyping to back genetic studies. While, there is still a need for calibration improvement for most of the compounds, which may be achieved by increasing sample size, the current NIRS models have allowed the detection of genetic variation for a large number of chemical traits. Significant additive genetic variation was evident for most primary and secondary compounds in the bark of *P. radiata*, with low-moderate heritability estimates and little evidence of non-additive genetic variation. The chemical associations detected with bark stripping are consistent with those found using standard wet chemistry procedures. While increased consititutive levels of sugars (particularly glucose) and fatty acids in the bark are genetically associated with susceptibility, an unknown sesquiterpenoid alcohol was genetically associated with reduced bark stripping. The unknown sesquiterpenoid alcohol was genetically, negatively correlated with height, whereas glucose and fructose as well as the fatty acids genetically positively correlated with height, suggesting that positive selection for early-age height (up to 6 years) in the absence of bark stripping would shift the chemistry of the plants towards increased susceptibility. Whether or not these traits affect performance subsequent to browsing requires further investigation. Overall, there is the possibility that selection

for the unknown sesquiterpenoid in current breeding/deployment programmes of *P. radiata* could be used to reduce bark stripping by the marsupials. However, better knowledge of the extent to which their heritability, genetic correlations and trade-offs change in different environments (G × E) is needed, particularly correlations involving growth.

DATA AVAILABILITY

All data is available within the article or its supplementary materials.

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AUTHOR CONTRIBUTIONS

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 & editing: JSN, BMP, NWD, HF, TR and JOW; Funding and resource allocation: BMP and JOW; Project supervision: BMP and JOW.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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