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Stranger in a strange land: genetic variation of native insect resistance biomarkers in UK Sitka spruce (*Picea sitchensis* [Bong.] Carr.)

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Reforestation in the British Isles (UK and Ireland) has been dominated with the use of an exotic conifer tree species, Sitka spruce (*Picea sitchensis* [Bong.] Carr.). Sitka breeding in the UK was developed from a single provenance, the Haida Gwaii Islands (Canada), which is both well suited to the British climate and highly susceptible to the white pine weevil (*Pissodes strobi* L.) in its native range. We examined variation and heritability of insect resistance related traits and assessed potential trade-offs with tree growth in 50 full-sibling families and 13 clonally replicated genotypes growing in the UK. We measured bark levels of three terpenes (dehydroabietic acid, (+)-3-carene and terpinolene) shown to confer resistance to the white pine weevil in Sitka spruce's native range, on the principle that these defence compounds may also contribute to pest resistance in the UK. We compared our results with published findings from the native range and also used individuals from a Haida Gwaii seed lot grown in the UK for comparison of terpene levels. Dehydroabietic acid content in the UK breeding population was similar to populations from resistant native populations, but (+)-3-carene and terpinolene levels were relatively low. Narrow sense heritability for dehydroabietic acid, (+)-3-carene and terpinolene was estimated as 0.20, 0.93 and 0.98, respectively from the full-sib data, and this evidence of genetic variance was supported by estimates of broad sense heritability from the smaller clonal study. Terpene content was found to be positively correlated to growth traits. The heritability estimates and genetic correlations indicate that selective breeding should be effective in raising levels in the UK breeding population of the three candidate terpenes implicated in weevil resistance. However, low levels observed indicate that other provenances from the native range may produce greater short-term improvements for two of the terpenes.

Introduction

In natural populations of forest trees challenged by pests, naturally-occurring resistance will determine their ability to adapt and survive through natural selection (Telford *et al.*, 2015). On the other hand, in planted forests, naturally-occurring resistance provides a basis for developing forest reproductive materials that are resilient to pests through artificial selection, i.e. resistance breeding (Sniezko and Koch, 2017). The effectiveness of natural and artificial selection depends on the variability of resistance related traits (or phenotypic variation) within species or populations, the portion of the observed variance that is genetically heritable due to additive genetic control and the selection intensity asserted by nature or the breeder (Telford *et al.*, 2015; Woodcock *et al.*, 2018). Trait heritability is the proportion of phenotypic variance explained by genetic variance; it is expressed as a ratio with values between 0 and 1 and indicates the degree of genetic control over a trait. Additive genetic variance encompasses variation in breeding values across individuals and represents the opportunity for change by natural or artificial selection. High heritability indicates greater potential for change in a trait through a breeding program (White *et al.*, 2007). The genetics of natural resistance to damaging insect pests is poorly understood in the majority of forest tree species and this knowledge gap may be crucial for the health of introduced or exotic species (Yanchuk and Allard, 2009).

In commercial forestry both seed sourcing and establishment of a breeding population will influence phenotypic variation and additive genetic variance. Using a narrow genetic base composed of a relatively small number of trees is expected to reduce overall genetic variation (Nei *et al.*, 1975); however, the effect on phenotypic variation is less certain and additive genetic variance may actually increase following population size reduction (Goodnight, 1988; Taft and Roff, 2012). These potential effects are of

particular relevance in populations of introduced species that do not receive recurrent gene flow from the native range, such as exotic tree species used in productive planted forests. In such cases, additional germplasm from the native range may need to be integrated into the breeding program to provide sufficient diversity to attain new selection goals such as pest resistance.

Forestry has a long history of using introduced species. Poplars (*Populus* sp.) are commonly moved between countries in the northern hemisphere, eucalypts (*Eucalytpus* sp.) are transferred to sub-tropical and tropical regions worldwide and radiata pine (*Pinus radiata* D. Don), a native to California and Mexico, is widely planted in the southern hemisphere. Many European forestry programs use exotic conifer species from the Pacific Northwest of North America, such as Sitka spruce (*Picea sitchensis* [Bong.] Carr.) and Douglas fir (*Pseudotsuga menziesii* Mirb.). Seed for breeding populations of these introduced species is sourced from native provenances that are typically selected based on superior growth and comparable climatic conditions. The evolutionary consequences of forestry practices are now being investigated in introduced forestry species. European populations of Douglas fir have lower genetic diversity and are significantly different in terms of a range of adaptive traits compared with native populations (Eckhart *et al.*, 2017). Despite its importance, the variation in insect resistance related traits has not yet been well studied in breeding populations of exotic species. Furthermore, insect resistance related traits can potentially involve trade-offs such as reduced tree growth (Zas *et al.*, 2005), with impacts on both market value and carbon sequestration targets (Stephenson *et al.*, 2014). Where traits are negatively correlated genetically, selection for either trait in a breeding program would reduce the other trait through an adverse selection response (White *et al.*, 2007), so increased insect resistance could come at a cost of reduced growth for example. Although a negative relationship between traits like growth and insect attack may not reflect an adverse genetic correlation, but could instead be due to another factor such as insect behaviour (King *et al.*, 1997). With the increased demand for tree plantings to both supply industry and to offset carbon emissions (Bastin *et al.*, 2019), understanding existing variation in insect resistance related traits and the potential trade-offs with growth may be key to updating forest tree breeding practices to meet market and carbon targets under climate change.

Heritable variation in insect resistance related traits in Sitka spruce populations in Europe is of high economic importance as the species is the main timber producing species in the UK and Ireland (Lee *et al.*, 2013). Traditionally, outbreaks of insect pests, such as the large pine weevil (*Hylobius abietis* L.) and the great European spruce bark beetle (*Dendroctonus micans* Kugelann; Tuffen and Grogan, 2019), have been managed by the application of pesticides. However, these pesticides pose a risk to all insects and blanket restrictions are being put in place on the application of certain types of pesticides (e.g. Neonicotinoids, EU regulation Nos: 2018/783-785; Fipronil, EU regulation No: 781/2013) to protect bees (Bonmatin *et al.*, 2015). Therefore, alternative means of insect control, such as resistance breeding, are becoming increasingly important and will be crucial for the future of planted forests.

Putative resistance and resistance related traits associated with native and introduced exotic pest species in the UK are still being investigated in Sitka spruce. However, insect resistance related traits have already been characterized in the Sitka spruce native range to the native white pine weevil (*Pissodes strobi* L.; Robert *et al.*, 2010; Whitehill *et al.*, 2016). Across the Sitka spruce native range (Figure 1), high levels of resistance to *P. strobi* are geographically restricted to two regions in British Columbia, Canada: East Vancouver Island (Big Qualicum, BQ) and the lower Fraser Valley (Haney, FV), potentially due to introgression with white spruce (*Picea glauca* [Moench] Voss; King *et al.*, 2004). UK breeding populations are unlikely to have been sourced from these two regions as source provenances were mainly selected based on growth rate, frost hardiness and branching habit (Lee *et al.*, 2013). Although records are incomplete, the UK population is believed to originate mainly from a single provenance on Haida Gwaii (formerly known as Queen Charlotte Island; Lee *et al.*, 2013). Irish populations additionally use stock from Oregon and Washington State (US), which have low resistance to *P. strobi* (King *et al.*, 2004). The origin of the UK population is of particular interest because Haida Gwaii serves as the representative susceptible (low resistance) population in most *P. strobi* resistance studies (e.g. King *et al.*, 2011). Compared with BQ and FV, Haida Gwaii provenances have significantly lower densities of stone cells (Whitehill *et al.*, 2016) and significantly lower content of the three terpenes identified as important predictors for *P. strobi* resistance in BQ and FV: dehydroabietic acid, (+)-3-carene and terpinolene (Robert *et al.*, 2010). The relatively narrow geographic base of the UK breeding population (1800 trees), combined with the selection of 360 plus trees in the UK (Lee and Connolly, 2010) could potentially affect the quantity and variation of those same terpenes in the UK breeding population. Our study makes the assumption that the terpenes which are known to provide resistance to *P. strobi* in Sitka spruce in its native range should play a role in resistance to insect pests elsewhere, including the UK. Therefore, we sought to quantify these terpenes in the UK population and compare these results with the natural variation in the native range of Sitka spruce available in the literature.

The objective of this study is to examine phenotypic variation and additive genetic variation of terpenes associated with native insect resistance within an introduced population of Sitka spruce in the UK. Specifically, we (1) measured growth of 50 full-sibling families in the UK breeding population and open-pollinated trees sourced from Haida Gwaii grown on the same site as a control; (2) measured dehydroabietic acid, (+)-3-carene and terpinolene on samples from the same 50 full-sibling families and additionally on 13 clonally replicated genotypes within the UK breeding population; (3) estimated both narrow and broad sense heritability across traits to gain insight into the type of genetic control (additive and non-additive, for details see methods) of these traits; and (4) assessed trade-offs between growth and terpene content where appropriate. We compared UK breeding population terpene content with unselected, open-pollinated trees sourced from Haida Gwaii and the resistant individuals from FV population in the native range (Robert *et al.*, 2010). We hypothesized that the terpene content of the UK breeding population and the UK grown Haida Gwaii would be lower when compared with those of resistant populations within the native range and that there would be low variation across families within the breeding population, resulting in low estimates of heritability.

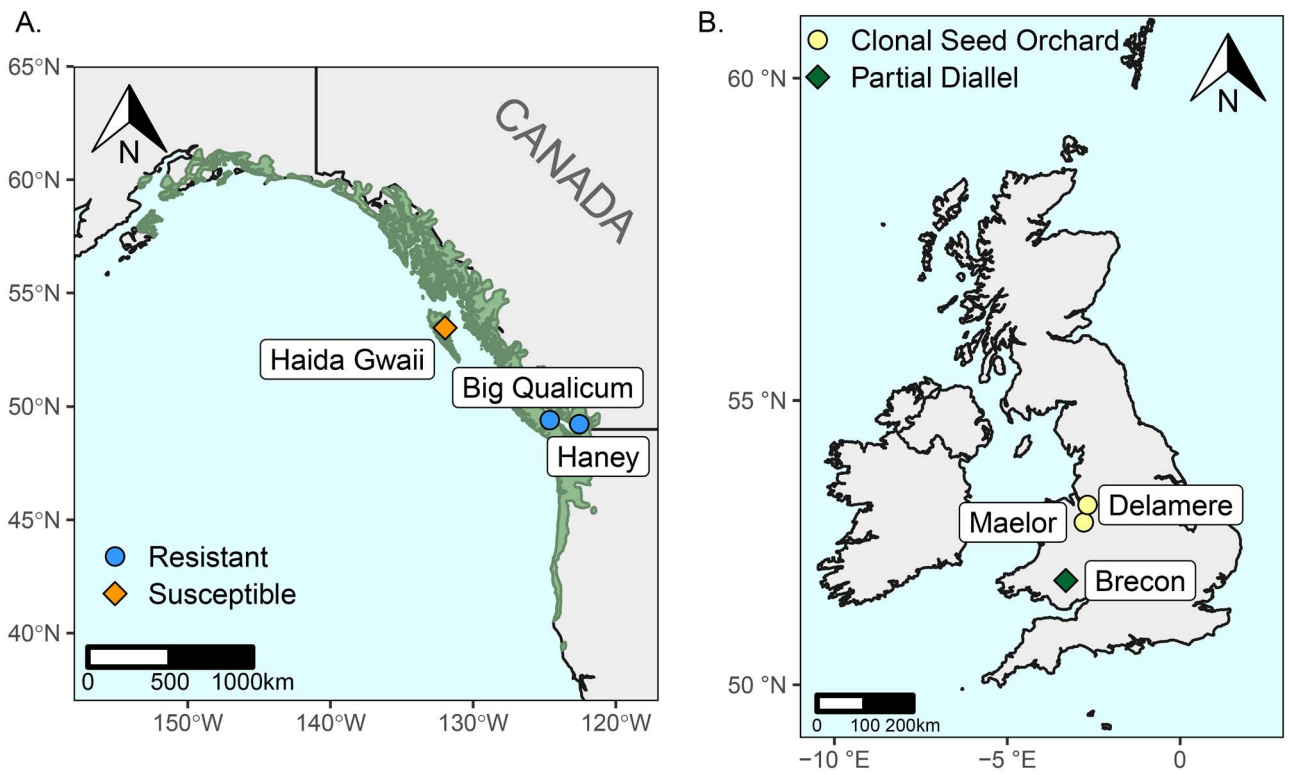

Figure 1 (A) The native range of Sitka spruce (green/dark grey; Little, 1971) across the Pacific Northwest of North America with the two regions showing high resistance to the white pine weevil (*Pissodes strobi*), Big Qualicum (BQ) and Haney (FV), (blue circle) and the highly susceptible Haida Gwaii provenance believed to be the source of the UK breeding population (orange diamond). (B) Samples for terpene and heritability analyses were collected from a partial diallel experiment (green diamond) and two clonal seed orchards (yellow circle) in the UK. This figure appears in colour in the online version of *Forestry*.

Methods

Sample collection and growth data

Samples of bark including the cambial layer and growth measurements were collected for 205 trees across 50 full-sib families in a progeny trial located at Brecon, UK to estimate narrow sense heritability of the three terpenes and genetic correlations with growth. Bark samples were collected from a smaller set of 42 trees in clonal seed-production orchards managed by Maelor Forest Nurseries Ltd in Whitchurch, UK and the Forestry Commission in Delamere Forest, UK (Figure 1) to estimate broad sense heritability of the three terpenes. Clonal samples were not measured for growth traits as these trees are part of a grafted seed orchard that is controlled for height.

The full-sib progeny trial was planted in 1996 with two-year-old trees using a partial-diallel mating design based on 59 parents assessed to have superior growth and stem form in the second breeding cycle of the UK breeding program. Trees were planted according to an incomplete block within replicates design (Alpha design) (Patterson and Williams, 1976). Each complete replicate contained one individual from each of 50 full-sib families based on crosses between pairs of parents assessed to have superior growth and one tree grown from open pollinated, unrelated and unimproved seed collected from Haida Gwaii Open Pollinated (HGOP). In July–August 2017, three to six trees from each full-sib family and four trees from HGOP were randomly sampled from each of 37 replicate blocks.

In August 2018, 42 ramets were sampled from 13 clonal genotypes planted in a randomized block design at 2 clonal seed orchards. Three ramets from eight clonal genotypes of 5–7-year-old grafts were sampled from three blocks at Maelor (24 total) and two ramets from nine clonal genotypes of 10–15-year-old grafts were sampled from two blocks at Delamere (18 total), with four common genotypes between sites. No damage from herbivorous insects was observed on the sampled trees at any of the three sites.

Immediately following felling of sample trees in both full-sib and clonal experiments, bark samples were collected from the top ~2 cm of the topmost branch within the node of the previous year's growth. Bark was removed from the segment, placed in a 1.5-mL conical centrifuge tube and immediately frozen on-site in a slurry of dry ice and ethanol (−70°C to −75°C). Sample tubes were placed into a −80°C freezer located near the sampling site within 8 h of harvest and stored for up to 6 weeks after which they were placed in dry ice for transfer to the University of Oxford for storage at −80°C prior to subsequent terpene analysis.

Growth measurements were taken on the same 205 trees at the Brecon site and an additional 479 trees from the same 50 full-sib families growing in the same trial. Diameter at breast height

(DBH) was measured at 1.3 m from the stem base using girth tape and tree height was measured on the ground after felling from the stem base to the tip of the apical meristem. The full set of growth measurements from 684 trees was used to calculate narrow sense heritability and genetic correlations of growth traits, while the 205 trees measured for terpene compounds were used to calculate narrow sense heritability and genetic correlations among terpenes as well as phenotypic correlations with growth traits.

Terpene extraction and analysis

Terpene compounds were extracted and analysed by gas chromatography mass spectrometry (GC/MS) to measure monoterpenes (+)-3-carene and terpinolene and diterpenoid dehydroabietic acid following protocols from Lewinsohn *et al.* (1993) and Martin *et al.* (2002) with the following adaptations. Approximately 0.2-g dry matter (DM) of bark tissue were extracted overnight in 1.5-mL *tert*-butyl methyl ether (anhydrous, 99.8 per cent MTBE, Sigma-Aldrich, Gillingham, Dorset, UK) containing 100 µg mL^{-1} (R)-(+)-Limonene (Sigma-Aldrich) and 200 µg mL^{-1} Tricosanoic acid (Sigma-Aldrich) as internal standards for monoterpenes and dehydroabietic acid, respectively. The extract was separated into two fractions: 0.5 mL for monoterpene analysis, and 0.5 mL to derivatise for diterpenoid analysis. The monoterpene fraction was washed with 0.3-mL aqueous 0.1 M $(NH_4)_2CO_3$ (pH 8.0). The diterpenoid fraction was derivatised using 200-µL methanol (Fisher Scientific, Loughborough, UK) with 200 µL (trimethylsilyl) diazomethane (2.0 M in diethyl ether, Sigma-Aldrich) at room temperature for 20 min, and evaporated under compressed nitrogen gas. The dried extracts were re-suspended in 1-mL anhydrous high-performance liquid chromatography (HPLC) grade, inhibitor-free ethyl ether (Honeywell, Bucharest, Romania).

Terpenoid compounds were identified using Agilent 7890B Series GC system coupled with mass spectrometry system (Agilent 5977B Series MSD, Santa Clara, California, US) using HP-5 capillary columns (Intuvo 19091J-413, 0.32 mm × 0.25 µm × 30 m, Agilent J&W), by comparing compound retention time to retention time of authentic standards. Monoterpenes were separated using the following GC program: 40°C initial temperature increased by 3°C min^{-1}–110°C, then increased at 10°C min^{-1}–180°C, then increased by 15°C min^{-1}–260°C and held for 15 min. Injector temperature was set at 250°C and the initial flow rate was 1.5 mL H_e min^{-1}. Diterpenoid compounds were separated using: 150°C initial temperature held for 1 min then increased by 1.5°C min^{-1}–220°C, then increased at 20°C min^{-1}–240°C, then held for 15 min. Injector temperature was set at 250°C, and the initial flow rate was 1.2 mL H_e min^{-1}.

Terpene concentrations were calculated by integrating the GC generated peaks with the GC/MSD ChemStation Software (Agilent) and comparing the peak areas to those of the internal standards. The bark terpenoid content (µg terpenoid g^{-1} DM) was back calculated using the extract concentration and the calculated DM weight of each sample.

Statistical analysis

Kernel density plots were created for both sample sets using the density statistic in ggplot2 (Wickham, 2016) in R statistical package v3.6.1 (R Core Team, 2019). Average values of terpene content for full-sib families were compared with average values reported in Robert *et al.* (2010) for susceptible and resistant populations in the Haney region (located in FV) of the native range using one standard error. Direct comparison to the native range would require common garden experiments with samples run using the same equipment and protocol. However, we follow the same protocol for terpene extraction as Robert *et al.* (2010) and the average values with standard errors are the only available data for the native range.

Narrow (h^2) and broad (H^2) sense heritability were estimated across full-sib and clonal samples, respectively. Narrow sense heritability is the ratio of additive genetic variance (V_A) to phenotypic variance (V_P) whereas broad sense heritability is the ratio of total genetic variance (V_G; i.e. additive and non-additive genetic variance) to phenotypic variance (Falconer and Mackay, 1996). Additive genetic control means that alleles associated with traits are independent and their effects are independent, indicting greater genetic gain can be expected from sexual crosses. Non-additive genetic control would mean that alleles and loci interact so that specific combinations of alleles (genotypes) are important for trait values (Falconer and Mackay, 1996). High non-additive genetic variance indicates that clonal forestry would deliver greater genetic gain (White *et al.*, 2007). Our study does not use the same genotypes or sites to measure the two types of heritability, which prevents us from calculating the narrow sense to broad sense heritability ratio and to make definitive conclusions about the type of genetic control. However, the two datasets and associated measures complement one another and provide different pieces of information on genetic control in the studied terpenes.

Terpene and growth trait measurements were transformed to the scale of natural logarithms to reduce the heterogeneity of variance observable in the data. Linear mixed models were fitted to terpene content and tree growth data using the ASReml-R4 package (Butler *et al.*, 2017) in R. Firstly, univariate linear models were fitted to the three terpenes to estimate heritability:

$$\mathbf{y} = \mathbf{1}\, m + \mathbf{Xg} + \mathbf{Wb} + \mathbf{Z}_1\mathbf{u} + \mathbf{Z}_2\mathbf{v} + e \quad (1).$$

where **y** is the vector of transformed terpene content; *m* a fitted mean with **1** a vector of 1's; **g** an effect of genetic group (i.e. the mean difference between the HGOP and improved trees) with design matrix **X**; **b** a vector of block effects, assumed random with design matrix **W**; **u** a vector of breeding values, assumed random with design matrix $\mathbf{Z}_1$; **v** a vector of non-additive full-sib family effects, assumed random with design matrix $\mathbf{Z}_2$ and **e** a vector of random residual effects. The breeding values were assumed to have a multivariate normal distribution, or MVN, (0, V_A **A**), where **A** is the numerator relationship matrix which incorporated the half- and full-sib family structure of the improved trees. All other random effects were also assumed to be MVN distributed (0, V**I**), with variance parameters V_B, V_D and V_E for blocks, family and residual effects, respectively, and **I** being the identity matrix of corresponding size. The narrow sense heritability was calculated as:

$$h^2 = V_A/(V_A + V_B + V_D + V_E) \quad (2).$$

and 95 per cent support intervals were calculated from the REML likelihood profiles obtained by fixing h^2 over a range of

Table 1 Phenotypic variances (V_P), the estimate of narrow sense (h^2) heritability and its 95% support interval and the fraction of V_P attributable to blocks (b^2) for the natural logarithms of content (μg g^{-1} DM) of (+)-3-carene, terpinolene and dehydroabietic acid across 205 trees from 50 full-sib families.

Variable	V_P	h^2	h^2 Support	b^2
(+)-3-carene	6.38 (0.36)	0.98 (0.06)	0.86–1.00	0.007 (0.024)
terpinolene	0.42 (0.03)	0.93 (0.06)	0.66–1.00	0.061 (0.038)
dehydroabietic acid	0.14 (0.02)	0.20 (0.13)	0.00–1.00	0.121 (0.061)
DBH	16.19 (1.05)	0.31 (0.09)	0.18–0.52	0.004 (0.013)
tree height	1.86 (0.12)	0.26 (0.08)	0.15–0.49	0.089 (0.030)

Note: Standard errors are given in parentheses.

values when fitting the model. Site effect replaced the genetic group effect and a vector of random clonal effects **c** replaced both **u** and **v** for analyses of clonal samples, with clones assumed to be distributed MVN(0, V_G**I**). The block term was also removed in clonal models because it was bound to 0 for all variance components. A genotype–environment (GxE) interaction was not considered for the clonal samples as only four clones were replicated across the sites and splitting samples by site would reduce the sample size, limiting the power of the analysis. The broad sense heritability was calculated as:

$H^2 = V_G/(V_G + V_E)$ (3).

Simple phenotypic correlations were calculated using Pearson's correlation in R. Genetic correlations, the correlation of breeding or clonal values, were estimated among terpenes and between terpenes and growth. Genetic correlations approximate the extent of pleiotropy, i.e. the same genes affecting more than one trait. This in turn gives an indication of the possible outcomes of selection on the two traits (Falconer and Mackay, 1996). Strong correlations indicate that artificial or natural selection for the first trait will cause a favorable or unfavourable change in the second trait depending on the direction of the correlation (White *et al.*, 2007). Estimates of genetic correlations (r_A) were obtained among the three terpenes and separately for each of the terpenes with DBH and tree height from trivariate versions of the above models, but with the following modifications. For the full-sib families, family effects, **v**, were excluded as estimates of V_D were found to be small and non-significant, and the vector of breeding values, **u**, was replaced by a vector of parent effects, assumed to be random and distributed MVN(0, $\mathbf{G}\otimes\mathbf{I}$) where **G** is three by three matrix of genetic variances and covariances among the three terpene traits. Likelihood ratio tests were used to test the hypotheses H_0: $r_A = 0$ against alternative H_1: $r_A \neq 0$. When null hypotheses were on the boundary of the parameter space, the critical value for the likelihood ratio test was adjusted following Self and Liang (1987).

Results

Terpene biomarkers

The three terpenes were present in every sample although their content varied across individuals and groups (full-sib families and clonal genets), with (+)-3-carene showing the greatest phenotypic variation (Tables 1 and 2). On average, dehydroabietic acid (1454 ± 527 μg g^{-1} DM) had the highest content across all individuals followed by (+)-3-carene (419 ± 505 μg g^{-1} DM) and terpinolene (60 ± 36 μg g^{-1} DM; Figure 2 and Supplementary Figure S1). In the open-pollinated unselected HGOP, dehydroabietic acid content was also the highest (1015.71 ± 229 μg g^{-1} DM) followed by terpinolene (39.2 ± 35 μg g^{-1} DM) and (+)-3-carene (3.5 ± 6 μg g^{-1} DM; Figure 3 and Supplementary Figure S1). Dehydroabietic acid had the greatest range (355–3363 μg g^{-1} DM) in both sample sets, although differences in (+)-3-carene (0.11–2157 μg g^{-1} DM) were more striking, showing a presence/trace pattern with very low content approaching zero in some samples and high content in others.

These patterns are further shown in the kernel density plots (Figure 4). For (+)-3-carene, two peaks are observed at low and high values across both sample sets. Dehydroabietic acid had a distribution approaching normal that was slightly skewed positive, while (+)-3-carene and terpinolene were positively skewed with higher densities at lower values (Figure 4).

Full-sib families had higher combined average content of all three terpenes than HGOP, although only (+)-3-carene was significantly greater ($P < 0.01$) (Supplementary Table S1). The majority (38) of the families had dehydroabietic acid content within one standard error of resistant FV populations, but three families were within one standard error of susceptible populations. One family was within one standard error of (+)-3-carene content in resistant FV populations and 24 families were within one standard error of (+)-3-carene in susceptible populations. All families had lower terpinolene than susceptible or resistant FV populations (Figure 3).

Heritability and correlations

The results of the univariate models across full-sib family samples showed strong evidence of additive genetic variation for both (+)-3-carene ($h^2 = 0.98$, SE = 0.06) and terpinolene ($h^2 = 0.93$, SE = 0.06), both with 95 per cent support intervals with lower bounds above 0.5 and upper bounds that include $h^2 = 1$ (Table 1). For dehydroabietic acid, a formal test of H_0: $h^2 = 0$ against the alternative H_1: $h^2 > 0$ did detect additive genetic variation ($P < 0.05$); however the evidence was much weaker and the 95 per cent support interval was uninformative. There was no evidence of non-additive genetic variance with the estimate of $V_D = 0$ for all three terpenes. Heritabilities of DBH and tree-height were estimated to be 0.31 (SE = 0.09) and 0.26 (SE = 0.08), respectively (Table 1). There was no evidence ($P > 0.05$) for non-additive variation for growth traits among families and variation between blocks was evident for tree height alone ($P < 0.01$). Broad sense heritability estimates from clonal trials for

Table 2 Phenotypic variances (V_P) and the estimate of broad sense (H^2) heritability and its 95% support interval for the natural logarithms of content (μg g^{-1} DM) of (+)-3-carene, terpinolene and dehydroabietic acid across 42 clonal samples from 13 genets.

Variable	V_P	H^2	H^2 Support
(+)-3-carene	8.55 (0.52)	0.77 (0.05)	0.52–0.92
terpinolene	0.27 (0.23)	0.67 (0.06)	0.40–0.87
dehydroabietic acid	0.08 (0.01)	0.60 (0.06)	0.30–0.84

Note: Standard errors are given in parentheses.

(+)-3-carene and terpinolene were 0.77 (SE = 0.05) and 0.67 (SE = 0.06), respectively. There was greater evidence for additive genetic variance in dehydroabietic acid across clonal samples (H^2 = 0.60, SE = 0.06) based on the informative 95 per cent support interval that had a lower bound above 0.3 (Table 2).

All three terpenes had significant positive phenotypic correlations to one another across both sample sets and to growth traits across the 205 full-sib family samples ($P < 0.05$), with the exception of dehydroabietic acid and terpinolene which were not significantly correlated across clonal samples (Table 3). Despite the small size of the terpene dataset, the strong genetic signals for (+)-3-carene and terpinolene allowed some inference on genetic correlations, but results involving dehydroabietic acid across full-sib samples are not presented due to its much weaker genetic signal. The estimate of r_A for (+)-3-carene and terpinolene was 0.91 (SE = 0.04) and the formal test of H_0: $r_A = 0$ was rejected ($P < 0.001$), indicating a positive genetic correlation. This correlation was also significantly different from zero across clonal samples, whereas correlations with dehydroabietic acid were positive but did not significantly differ from zero (Table 3). The estimated genetic correlations of (+)-3-carene with DBH and tree height were slightly positive at 0.23 (SE = 0.20) and 0.09 (SE = 0.22), respectively although neither were significantly different from zero ($P > 0.05$). The genetic correlations of terpinolene with DBH and tree height were also positive at 0.30 (SE = 0.21) and 0.21 (SE = 0.22), respectively, but again not significantly different from zero ($P > 0.05$; Table 3).

Discussion

The selection of native provenances for breeding populations of introduced species could potentially and inadvertently influence forest health outcomes through loss of heritable phenotypic variation in insect and pathogen resistance related traits. Here, we have studied constitutive terpenes as they offer an easily measurable and straightforward method of quantifying potential changes in insect resistance related traits as identified in Canada. In the absence of trials in the same environment, investigating insect attack rate (putative resistance) *in situ* would be confounded by differences in both pest and environment relative to the native range. Our study fills knowledge gaps on the variation and heritability of terpenes associated with resistance in the native range in a European Sitka spruce population selected mainly for growth-related traits. Although the UK breeding population was reportedly sourced from a single provenance known to be highly susceptible to a native pest, we found that variation was greater than expected for the three studied terpenes. However, this variation was restricted to the lower portion of the variation observed across the native range. Our data show that (+)-3-carene and terpinolene had high heritability estimates and all three terpenes were positively correlated to growth, the primary selection trait.

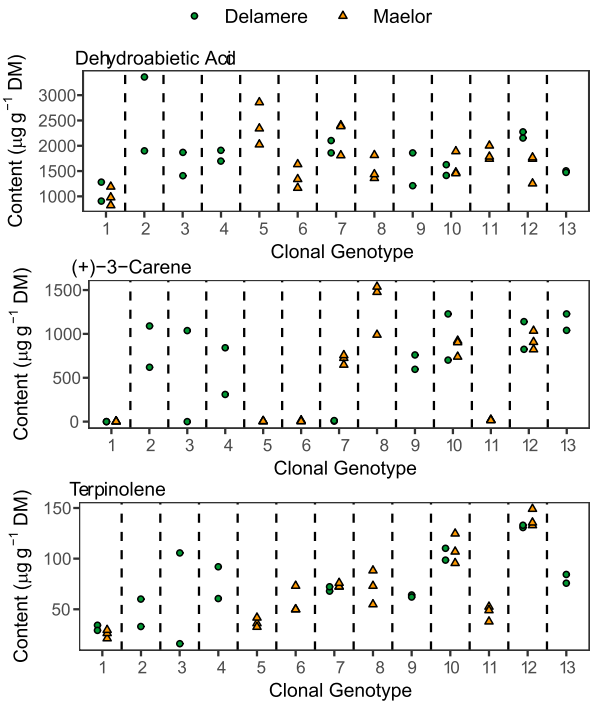

Figure 2 Terpene content (μg g^{-1} DM) for dehydroabietic acid (top), (+)-3-carene (middle) and terpinolene (bottom) for ramets of 13 clonally replicated genotypes sampled from Delamere Forest (green circle) and Maelor Forest Nurseries Ltd. (orange triangle). This figure appears in colour in the online version of *Forestry*.

Levels of terpene biomarkers

We found greater variation than expected based on the singular source of the breeding population in the three terpenes across full-sib families and clonal genotypes. However, only (+)-3-carene had high levels of phenotypic variation, most likely due to the trace/presence pattern, and all three terpenes had relatively low average values (Figure 3).

We compared the terpene data obtained in the 50 full-sib families from the UK breeding population that have been selected for growth and stem form to unselected individuals (HGOP) from the same source grown together on the same site. The overall mean of the full-sib family average terpene contents was greater than the HGOP average, suggesting that the artificial selection for growth has not had a negative effect on these resistance biomarkers. However, some individual family averages were lower or within one standard error of HGOP for each of the terpenes, indicating the biomarker levels may still be similar to those of the source population.

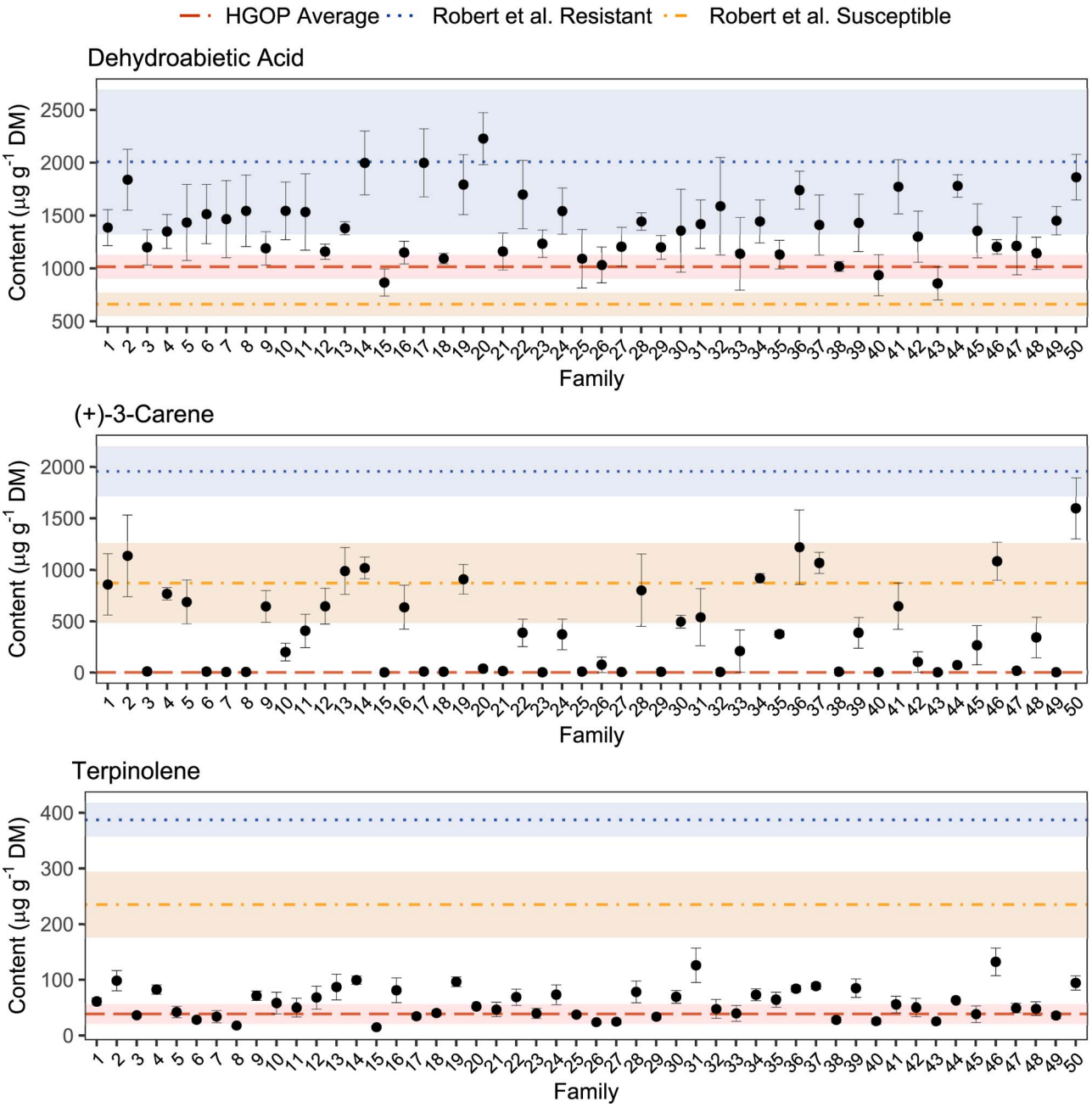

Figure 3 Average terpene content (µg g^{-1} DM) with standard errors within 50 full-sib families for dehydroabietic acid (top), (+)-3-carene (middle) and terpinolene (bottom). Dashed lines and shaded regions represent averages and standard errors of the respective terpenes in resistant (blue dotted) and susceptible (orange dot-dash) FV populations reported in Robert *et al.* (2010) and the open pollinated family (HGOP) sourced from the susceptible Haida Gwaii provenance (red dashed). This figure appears in colour in the online version of *Forestry*.

Next, we compared our results from the present study with published data on terpene levels in Sitka spruce grown in its native range (Robert *et al.*, 2010). The full-sib family averages for dehydroabietic acid in our study were similar to those of resistant native FV populations, whereas (+)-3-carene averages were more similar to susceptible populations and terpinolene averages were entirely below those of native FV populations. The differences between average values for the UK and native grown samples may be due to environmental or silvicultural differences, but the reportedly narrow geographic base of the UK breeding population is likely also playing some role in the reduced averages in the full-sib families. Although dehydroabietic acid content in our samples was comparable to the native range, its phenotypic variation was the lowest among the studied terpenes. Our results

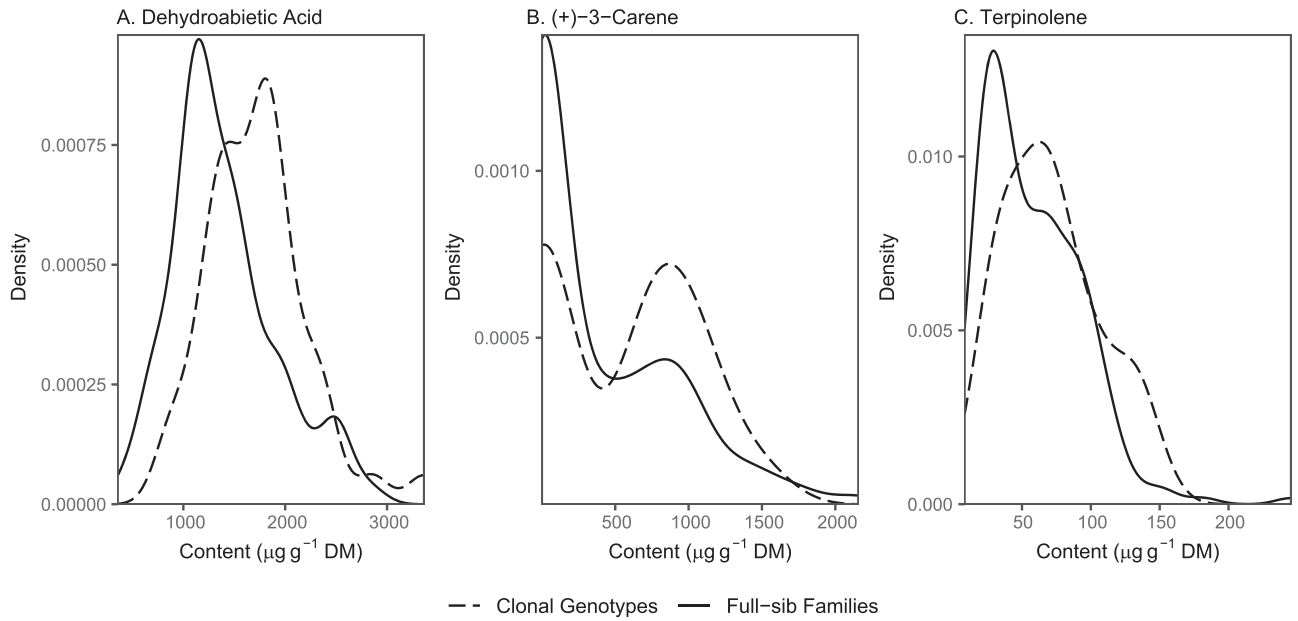

Figure 4 Kernel density plots of dehydroabietic acid (A), (+)-3-carene (B) and Terpinolene (C) content (μg g^{-1} DM) for full-sib family samples (solid line) and clonal genotype samples (dashed line).

Table 3 Pearson's correlations of phenotypic values with associated *P* values and genetic correlations with associated standard errors among terpenes and growth traits across clonal and partial diallel samples.

		Phenotypic correlations		Genetic correlations	
Variable 1	Variable 2	Estimate	*P* value	Estimate	Standard error
Clonal samples					
(+)-3-carene	terpinolene	**0.80**	1.387E−10	**0.75**	0.15
(+)-3-carene	dehydroabietic acid	**0.32**	3.60E−02	0.18	0.33
terpinolene	dehydroabietic acid	0.24	1.19E−01	0.13	0.34
Partial diallel samples					
(+)-3-carene	terpinolene	**0.73**	4.27E−36	**0.91**	0.04
(+)-3-carene	dehydroabietic acid	**0.21**	2.92E−03	**–**	–
terpinolene	dehydroabietic acid	**0.31**	8.26E−06	**–**	–
DBH	(+)-3-carene	**0.23**	1.14E−03	0.23	0.20
DBH	terpinolene	**0.24**	5.68E−04	0.09	0.22
DBH	dehydroabietic acid	0.03	6.57E−01	–	–
tree height	(+)-3-carene	**0.21**	3.14E−03	0.30	0.21
tree height	terpinolene	**0.25**	5.50E−04	0.21	0.22
tree height	dehydroabietic acid	**0.15**	4.39E−02	–	–
DBH	tree height	**0.58**	1.57E−18	**0.87**	0.08

Note: Significant phenotypic correlations ($P < 0.05$) and genetic correlations significantly different from zero are in bold.

indicate that the assumed narrow geographic base has likely affected both the variation and quantity of the three studied terpenes in the UK breeding population.

Heritability and trade-offs

We obtained high narrow and broad sense heritability estimates for (+)-3-carene and terpinolene. Individual terpene heritability has not been estimated for Sitka spruce in its native range, but putative resistance to *P. strobi* has been studied and found to be highly heritable ($h^2 = 0.9$; Moreira *et al.*, 2012). Furthermore, resin production (Liu *et al.*, 2013; Lai *et al.*, 2017), resin chemical components (Ericsson *et al.*, 2001) and individual terpene compounds (Sampedro *et al.*, 2010) have high heritability in other conifer species. The high heritability of the two compounds suggests (+)-3-carene and terpinolene are under high genetic

control. However, no conclusions can be drawn as to whether the high heritability estimates can be attributed to control by a major gene, as has been speculated to control resistance in Sitka spruce (King *et al*., 2004). The trace/presence pattern in (+)-3-carene is likely to be inflating the variation among families and genotypes, and therefore potentially driving up the heritability estimate. This pattern of very low and high concentrations of 3-carene across individual trees has been observed in other studies of Sitka spruce (Hall *et al*., 2011) and in other conifers, resulting in similarly high estimates of heritability of this terpene (Baradat and Yazdani, 1988; Hanover, 1966). In future work, increasing the sample size within families could determine the degree of variation across individuals and help further resolve if this oscillating phenotypic variation is due to additive genetic variance or to another source like phenotypic plasticity or environmental variation.

We also found high broad sense heritability of dehydroabietic acid in clonal samples. In contrast, the evidence for additive genetic variance across full-sib families (narrow sense heritability) is weaker for dehydroabietic acid than the other terpenes, potentially indicating dehydroabietic acid is under non-additive control.

The notably stronger genetic signal and pairwise correlation of the monoterpenes compared with the corresponding results for dehydroabietic acid have a plausible metabolic underpinning as the two monoterpenes are the two most abundant products of the same enzyme (Fäldt *et al*., 2003), whereas dehydroabietic acid is synthesized by a different enzyme (Hamberger *et al*., 2011; Whitehill and Bohlmann, 2019). This result is reflected across plant species, in which closely related compounds tend to be positively correlated while more divergent compounds generally have no significant correlation (Koricheva *et al*., 2004). The positive correlations among terpenes suggest an absence of resource trade-offs in the synthesis of these different terpenes and indicate selection for a terpene will not negatively affect other terpenes.

The positive phenotypic and genetic correlations between terpenes and growth traits indicate there is no evidence of a substantial trade-off between the studied terpenes and growth. This agrees with studies in other conifers that have found resin production to be highly and positively correlated to stem diameter and total height (Liu *et al*., 2013; Lai *et al*., 2017). Correlation between the studied terpenes and growth has not been studied in the native range, but the slight positive correlation between *P. strobi* attack rate and growth indicates a small to moderate negative trade-off between resistance and growth (King *et al*., 2004). However, as this result is related to attack rate generally rather than specific biomarkers, it could be driven by other resistance traits such as the number of resin canals or stone cells (King *et al*., 2011), differences in provenance or environment, or another factor like insect behaviour (King *et al*. 1997). Our results indicate that artificial selection for growth traits has not resulted in a decrease in the content of the measured terpenes, which is supported by the higher combined average terpene content in full-sib families obtained through selective cross-breeding compared with HGOP. Furthermore, our results imply that the formation of the breeding population from a narrow geographic base and two cycles of selection for growth have not negatively affected additive genetic variance in the three terpenes.

Implications for planted forests

Practical and effective pest management can be costly, difficult to develop and evidence is accumulating that the pesticides used in traditional management are environmentally detrimental (Bonmatin *et al*., 2015). Breeding trees for resistance traits in order to sustain wood supply and carbon sequestration is a viable option to reduce the economic and environmental costs of pest management. In the case of Sitka spruce, resistance breeding has been successfully developed within the native range with seed orchards generated from BQ and FV parents providing *P. strobi* resistant stock (Alfaro *et al*., 2013). However, the suite of traits needed for resistance to European pests in the introduced range is poorly understood. For resistance breeding to be effectively used against pests that attack Sitka spruce growing in Europe, further research is needed to assess natural resistance and identify biomarkers (Woodcock *et al*., 2018). A reasonable starting point would be to determine the degree of overlap with traits linked to resistance to *P. strobi* such as those studied here, as these traits have been well studied in the native range and are among the few resistance traits identified in Sitka spruce.

The high narrow sense heritability of two of the studied terpenes suggest genetic gain could be achieved through sexual crosses. This and the positive genetic correlations to growth indicate high potential for resistance breeding if further research links the same terpenes to insect resistance in the UK. However, due to the single geographic origin of the UK breeding population, the variation in the terpenes did not capture the full variation observed in the native range and was restricted to relatively low concentrations. Additional germplasm sampled more broadly from the native range may subsequently be needed to provide a sufficient base for more rapid and effective resistance breeding. This strategy is increasingly seen in agricultural crops (Warburton *et al*., 2006; Migicovsky and Myles, 2017). Our study emphasizes the need to include sufficient variation in resistance related traits when selecting provenances to increase the resilience of introduced forest tree populations to pest and pathogen outbreaks.

Conclusion

We analysed three terpenes that are known to confer resistance to *P. strobi*, a weevil in Canada, and found both low levels and low phenotypic variation in samples from 50 full-sib families and 13 clonal genotypes growing in the UK. This result was unsurprising considering that the UK breeding population was reportedly sourced from a single geographic area (Haida Gwaii) known for its high susceptibility to *P. strobi* and indicates that incorporating germplasm from elsewhere in the native range would offer opportunities for increasing relative levels and variability of the studied terpenes. High heritability estimates in two of the terpenes suggest that the makeup of the breeding population did not negatively affect heritability of traits linked to insect resistance in the native range. Terpene content was positively correlated to growth traits, signifying that selection for these terpenes is unlikely to result in reduced growth. These results indicate that there is significant scope for artificial and natural selection, and we propose that a

future research priority is to test the ability of these studied terpenes to provide Sitka spruce with resistance to pests in Europe.

Supplementary data

Supplementary data are available at *Forestry* online.

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Conflict of interest statement

None declared.

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Data availability

Data available on request.

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