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

Hayley R. Tumas1,†, Ziad Soufi1,†, John A. Woolliams2, J. Paul McLean3, Steve Lee4, Joan E. Cottrell5, Joanna J. Ilska4, Gustavo Lopez1 and John MacKay1

1Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK
2The Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Easter Bush Campus, Midlothian EH25 9RG, UK
3Forest Research, Northern Research Station, Roslin, Midlothian EH25 9SY, UK

*Corresponding author Tel: +44 01865 275088; Fax: +44 1865 275074; E-mail: hayley.tumas@plants.ox.ac.uk
†Authors contributed equally to this research.

Received 4 August 2020

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 (Eucalyptus 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 (Hylaeus abietis L.) and the great European spruce bark beetle (Dendroctonus micans Kugelmann; 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 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 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 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 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 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.
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
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 monoterpane analysis, and 0.5 mL to derivatise for diterpenoid analysis. The monoterpane fraction was washed with 0.3-mL aqueous 0.1 M (NH\(_4\))\(_2\)CO\(_3\) (pH 8.0). The diterpenoid analysis. The monoterpene fraction was washed with 0.3-mL aqueous 0.1 M (NH\(_4\))\(_2\)CO\(_3\) (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 × 30 m, Agilent J&W), by comparing compound retention time to 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 Honey region (located in FV1) 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 (Falocner 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 (Falocner 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:

\[
y = \mathbf{1} \mathbf{m} + \mathbf{X} \mathbf{g} + \mathbf{W} \mathbf{b} + \mathbf{Z}_1 \mathbf{u} + \mathbf{Z}_2 \mathbf{v} + \mathbf{e}
\]

where \(y\) is the vector of transformed terpene content; \(\mathbf{m}\) a fitted mean with \(\mathbf{1}\) a vector of 1’s; \(\mathbf{g}\) an effect of genetic group (i.e. the mean difference between the HGOP and improved trees) with design matrix \(\mathbf{X}\); \(\mathbf{b}\) a vector of block effects, assumed random with design matrix \(\mathbf{W}\); \(\mathbf{u}\) a vector of breeding values, assumed random with design matrix \(\mathbf{Z}_1\); \(\mathbf{v}\) a vector of non-additive full-sib family effects, assumed random with design matrix \(\mathbf{Z}_2\); and \(\mathbf{e}\) 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 \(V_I\) for blocks, family and residual effects, respectively, and \(I\) being the identity matrix of corresponding size. The narrow sense heritability was calculated as:

\[
h^2 = V_A/(V_A + V_B + V_D + V_I)
\]

and 95 per cent support intervals were calculated from the REML likelihood profiles obtained by fixing \(h^2\) over a range of
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, $V_c$). 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:

$$H^2 = V_c/(V_G + V_c)$$

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 one trait will cause a favorable or unfavorable 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 $V_G$ 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, $G \otimes I$) 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 ($1454 \pm 527 \mu g$ g$^{-1}$ DM) had the highest content across all individuals followed by (+)-3-carene ($419 \pm 505 \mu g$ g$^{-1}$ DM) and terpinolene ($60 \pm 36 \mu g$ g$^{-1}$ DM; Figure 2 and Supplementary Figure S1). In the open-pollinated unselected HGOP, dehydroabietic acid content was also the highest ($1015.71 \pm 229 \mu g$ g$^{-1}$ DM) followed by terpinolene ($39.2 \pm 35 \mu g$ g$^{-1}$ DM) and (+)-3-carene ($3.5 \pm 6 \mu g$ g$^{-1}$ DM; Figure 3 and Supplementary Figure S1). Dehydroabietic acid had the greatest range ($355–3363 \mu g$ g$^{-1}$ DM) in both sample sets, although differences in (+)-3-carene (0.11–2157 \mu g 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 $V_Q = 0$ 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 1

| 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.
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          | $V_p$ (SE) | $H^2$ (SE) | $H^2$ 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 $r_g$ for (+)-3-carene and terpinolene was 0.91 (SE = 0.04) and the formal test of $H_0$: $r_g = 0$ 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 heritage 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.

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.
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 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.
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.

| Clonal samples | Phenotypic correlations | Genetic correlations |
|----------------|-------------------------|---------------------|
| Variable 1     | Variable 2              | Estimate | \(P\) value | Estimate | Standard error |
| (+)-3-carene   | terpinolene             | 0.80      | 1.38E-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 \((h^2 = 0.9; \text{Moreira et al.}, 2012)\). Furthermore, resin production \((\text{Liu et al., 2013}; \text{Lai et al., 2017})\), resin chemical components \((\text{Ericsson et al., 2001})\) and individual terpene compounds \((\text{Sampedro et al., 2010})\) 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 progenances 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.

Acknowledgements
We thank Imam Sayyed (Maelor Forest Nurseries); Alan Harrison and Sam Donnelly (Forestry Commission); Andy Price and David Gil-Moreno (Forest Research); and Gabriele Rizzuto and Glyndwr Jones (University of Oxford) for their assistance with sample collection. Natural Resources Wales provided in-kind support with the provision of materials and forest operations. Authors are grateful for the comments from two anonymous reviewers that improved this manuscript.

Conflict of interest statement
None declared.

Funding
This work was supported by the Biotechnology and Biological Sciences Research Council (BB/P2020488/1) as part of the Sitka Spruce project. Additional support in sampling the trees was provided by Natural Resources Wales and Scottish Forestry.

Data availability
Data available on request.

References
Alfaro, R.I., King, J.N. and VanAkker, L. 2013 Delivering Sitka spruce with resistance against white pine weevil in British Columbia. Canada. For. Chron. 89, 235–245. doi: 10.5558/tfc2013-024.
Baradat, P. and Yazdani, R. 1988 Genetic expression for monoterpenes in clones of Pinus sylvestris grown on different sites. Scand. J. For. Res. 3, 25–36. doi: 10.1080/02827588809382492.
Basin, J., Finegold, Y., Garcia, C., Mallicone, D., Rezende, M., Routh, D. et al. 2019 The global tree restoration potential. Science 365, 76–79. doi: 10.1126/science.aaax0848.
Bonmatin, J., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D.P., Krupke, C. et al. 2015 Environmental fate and exposure; neonicotinoids and iprofinil. Environ. Sci. Pollut. Res. 22, 35–67. doi: 10.1007/s11356-014-3332-7.
Butler, D.G., Cullis, B.R., Gilmour, A.R., Gogel, B.G. and Thompson, R. 2017 ASReml-R Reference Manual Version 4. VSN International Ltd, Hemel Hempstead, UK.
Eckhart, T., Walcher, S., Hasenauer, H. and van Lou, M. 2017 Genetic diversity and adaptive traits of European versus American Douglas-fir seedlings. Eur. J. For. Res. 136, 811–825. doi: 10.1007/s10342-017-1072-7.
Ericsson, T., Fries, A. and Gref, R. 2001 Genetic correlations of heartwood extractives in Pinus sylvestris progeny tests. For. Genet. 8, 73–79.
Falconer, D.S. and Mackay, T.F.C. 1996 Introduction to Quantitative Genetics. 4th edn. Longmans Green, Harlow, England, UK.
Falldt, J., Martin, D., Miller, B., Rawat, S. and Bohlmann, J. 2003 Traumatic resin defense in Norway spruce (Picea abies): Methyl jasmonate-induced terpene synthase gene expression, and cDNA cloning and functional characterization of (+)-3-carene synthase. Plant Mol. Biol. 51, 119–133. doi: 10.1023/A:1020714403780.
Goodnight, C.J. 1988 Epistasis and the effect of founder events on the additive genetic variance. Evolution 42, 441–454. doi: 10.1111/j.1558-5646.1988.tb04151.x.
Hamberger, B., Ohnishi, T., Hamberger, B., Seguin, A. and Bohlmann, J. 2011 Evolution of diterpene metabolism: Sitka spruce CYP720B4 catalyzes multiple oxidations in resin acid biosynthesis of conifer defense against insects. Plant Physiol. 157, 1677–1695. doi: 10.1104/pp.111.185843.
Hall, D.E., Robert, J.A., Keeling, C.I., Domanski, D., Quesada, A.L., Jancsik, S. et al. 2011 An integrated genomic, proteomic and biochemical analysis of (+)-3-carene biosynthesis in Sitka spruce (Picea sitchensis) genotypes that are resistant or susceptible to white pine weevil. Plant J. 65, 936–948. doi: 10.1111/j.1365-313X.2010.04478.x.
Hanover, J.W. 1966 Genetics of terpenes. 1. Gene control of monoterpenes level in Pinus monticola Doug. For. Sci. 17, 73–84.
King, J.N., Yanchuk, A.D., Kiss, G.K. and Alfaro, R.I. 1997 Genetic and phenotypic relationships between weevil (Pissodes strobi) resistance and height growth in spruce populations of British Columbia. Can. J. For. Res. 27, 732–739. doi: 10.1139/x97-009.
King, J.N., Alfaro, R.I. and Cartwright, C. 2004 Genetic resistance of Sitka spruce (Picea sitchensis) populations to the white pine weevil (Pissodes strobi); Distribution of resistance. Forestry. 77, 269–278. doi: 10.1093/forestry/77.4.269.
King, J.N., Alfaro, R.I., Lopez, M.G. and Van Akker, L. 2011 Bark defence mechanisms of resistant populations. Forestry. 84, 83–91. doi: 10.1093/forestry/cpq047.
Koricheva, J., Nykänen, H. and Gianoli, E. 2004 Meta-analysis of trade-offs among plant antiherbivore defenses are plants Jacks-of-All-Trades, Masters of All. Nat. 163, 64–75. doi: 10.1038/sj.nat.1610409.
Lai, M., Dong, L., Yi, M., Sun, S., Zhang, Y., Fu, L. et al. 2017 Genetic variation, heritability and genotype × environment interactions of resin yield, growth traits and morphologic traits for Pinus elliottii at three progeny trials. Forests. 8, 409. doi: 10.3390/f8110409.
Lee, S.J. and Connolly, T. 2010 Finalizing the selection of parents for the Sitka spruce (Picea sitchensis (Bong.) Carr) breeding population in Britain using mixed model analysis. Forestry. 83, 423–431. doi: 10.1093/forestry/cpq024.
Lee, S., Thompson, D. and Hansen, J.K. 2013 Sitka spruce (Picea sitchensis (Bong.) Carr) in Forest tree breeding in Europe: current state-of-the-art and perspectives, Managing Forest Ecosystems, vol 25. L. E. Páques (ed). Springer, Dordrecht, NL, pp. 177–227. doi: 10.1007/978-94-007-6146-9_4.
Lewinsohn, E., Savage, T.J., Gijzen, M. and Creutze, R. 1993 Simultaneous analysis of monoterpenes and diterpenoids of conifer oleoresin. Phytochem. Anal. 4, 220–225. doi: 10.1002/pca.2800040506.
Little, E. 1971 Atlas of United States trees. Volume 1. Conifers and Important Hardwoods. Miscellaneous Publication 1146. U.S. Department of Agriculture, Forest Service, Washington, D.C., US, 9 pp. doi: 10.5962/bhl.title.130546.
Liu, Q., Zhou, Z., Fan, H. and Liu, Y. 2013 Genetic variation and correlation among resin yield, growth, and morphologic traits of Pinus massoniana. Silvae Genetica. 62, 1–2. doi: 10.1515/sgf-2013-0005.
Martin, D., Tholl, D., Gershenzon, J. and Bohlmann, J. 2002 Methyl Jasmonate induces traumatic resin ducts, terpenoid resin biosynthesis, and terpenoid accumulation in developing xylem of Norway spruce stems. Plant Physiol. 129, 1003–1018. doi: 10.1104/pp.011001.
Moreira, X., Alfaro, R.I. and King, J.N. 2012 Constitutive defenses and damage in Sitka spruce progeny obtained from crosses between white pine weevil resistant and susceptible parents. *Forestry*. 85, 87–97. doi: 10.1093/forestry/cpr060.

Nei, M., Maruyama, T. and Chakraborty, R. 1975 The bottleneck effect and genetic variability in populations. *Evolution*. 29, 1–10. doi: 10.2307/2407137.

Patterson, H.D. and Williams, E.R. 1976 Some theoretical results on general block designs. In Proceedings of the 5th British Combinatorial Conference Congressus Numeratium XV. Utilitas Mathematica, Winnipeg, CAN, pp. 489–496.

R Core Team 2019 *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Robert, J.A., Madilao, L.L., White, R., Yanchuk, A., King, J. and Bohlmann, J. 2010 Terpenoid metabolite profiling in Sitka spruce identifies association of dehydroabietic acid, (+)-3-carene, and terpinolene with resistance against white pine weevil. *Botany*. 88, 810–820. doi: 10.1139/B10-049.

Sampedro, L., Moreira, X., Llusia, J., Peñuelas, J. and Zas, R. 2010 Genetics, phosphorus availability, and herbivore-derived induction as sources of phenotypic variation of leaf volatile terpenes in a pine species. *J. Exp. Bot.* 61, 4437–4447. doi: 10.1038/jxb/erq246.

Self, S.G. and Liang, K.Y. 1987 Asymptotic properties of maximum likelihood estimators and likelihood ratio tests under nonstandard conditions. *J. Am. Stat. Assoc.* 82, 605–610. doi: 10.2307/2289471.

Sniezko, R.A. and Koch, J. 2017 Breeding trees resistance to insects and diseases: Putting theory into application. *Biol. Invasions*. 19, 3377–3500. doi: 10.1007/s10530-017-1482-5.

Stephenson, N.L., Das, A.J., Condit, R., Russo, S.E., Baker, P.J., Beckman, N.G. et al. 2014 Rate of tree carbon accumulation increases continuously with tree size. *Nature*. 507, 90–93. doi: 10.1038/nature12914.

Taft, H.R. and Roff, D.A. 2012 Do bottlenecks increase additive genetic variance? *Conserv. Genet*. 13, 333–342. doi: 10.1007/s10592-011-0285-y.

Telford, A., Cavers, S., Ennos, R.A. and Cottrell, J.E. 2015 Can we protect forests by harnessing variation in resistance to pests and pathogens? *Forestry*. 88, 3–12. doi: 10.1093/forestry/cpy012.

Tuffen, M.G. and Grogan, H.M. 2019 Current, emerging and potential pest threats to Sitka spruce plantations and the role of pest risk analysis in preventing new pest introductions to Ireland. *Forestry*. 92, 26–41. doi: 10.1093/forestry/cpy036.

White, T., Adams, W. and Neale, D. 2007 *Forest Genetics*. 1st edn. CABI, p. 896 pp.

Whitehill, J.C.A. and Bohlmann, J. 2019 A molecular and genomic reference system for conifer defence against insects. *Plant, Cell Environ*. 42, 2844–2859. doi: 10.1111/pce.13571.

Woodcock, P., Cottrell, J.E., Buggs, R.J.A. and Quine, C.P. 2018 Mitigating pest and pathogen impacts using resistant trees: A framework and overview to inform development and deployment in Europe and north American. *Forestry*. 91, 1–16. doi: 10.1093/forestry/cpx031.

Zas, R., Sampedro, L., Prada, E. and Fernandez-Lopez, J. 2005 Genetic variation of *Pinus pinaster* ait. Seedlings in susceptibility to the pine weevil *Hylobius abietis* L. *Ann. For. Sci*. 62, 681–688. doi: 10.1051/forest.