

1 Geographically and genetically distinct populations of scots pine
2 (*Pinus sylvestris*) differ in resistance to damage by the large pine
3 weevil (*Hylobius abietis*): a common garden translocation study

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6 **Abstract**

7 Damage to coniferous tree plantation crops from the large pine weevil *Hylobius abietis* causes
8 economic losses of €140 million in Europe *per annum*. Current mitigation strategies are labour
9 intensive and only partially effective. Identifying and breeding natural resistance in host plant
10 cultivars to insect pests has been used in many crop species to reduce damage as part of an
11 integrated pest management strategy. Here, we conducted a common garden experiment in a
12 previously clearfelled forestry plantation where *H. abietis* is known to occur. 672 saplings, grown
13 from seed collected from 21 naturally occurring populations of *Pinus sylvestris* across Scotland
14 were planted together to assess resistance to attack by *H. abietis*.

15 On those saplings which were attacked, we found significant variation in the total area of bark
16 lesions among *P. sylvestris* populations. In contrast we found that sapling populations did not
17 differ statistically in their likelihood of being attacked by *H. abietis*. A weak latitudinal pattern
18 was observed, with saplings sourced from populations further north being attacked more heavily
19 than those from further south. From these results it is suggested that as part of an integrated
20 pest management strategy, planting of *P. sylvestris* saplings from more southerly seed-stock may
21 reduce pine weevil attack in affected areas.

22 **Introduction**

23 The large pine weevil (*Hylobius abietis* L. Coleoptera: Curculionidae) is a common pest of newly
24 planted coniferous tree plantations in Europe, causing damage to plantation saplings up to around
25 five years old (Ördlander et al., 1997). Adult weevils emerge from tree stumps and feed on the bark
26 and buds of coniferous saplings, consuming sugar rich phloem tissue (Nordlander, 1991). Lesions on
27 the bark and buds of saplings as a result of feeding (Figure 1) may cause a reduction in growth rate,
28 stem deformation and an increased susceptibility to infection by airborne diseases of trees (Leather
29 et al., 1999). Heavy damage may lead to stem girdling and death of the terminal growing bud
30 resulting in a malformed trunk, limiting economic use as timber when fully grown (Alfaro, 1989;
31 Gill, 1992).

32 While *H. abietis* may inhabit adult coniferous trees in both natural and planted coniferous
33 forests, recently clearfelled and restocked coniferous plantation sites provide an enriched habitat
34 for breeding *H. abietis* and so pose more of a danger to planted saplings than those in naturally
35 regenerating stands (Willoughby et al., 2004; Ördlander and Nilsson, 1999). Adults lay eggs within
36 the stumps of clearfelled trees, which are rarely removed after clearfelling, with newly emerged

37 juvenile weevils feeding on young saplings until adulthood (Willoughby et al., 2004). Planted
 38 coniferous saplings are more susceptible to *H. abietis* damage than naturally regenerating saplings,
 39 probably due to water stress as a result of damage to root systems during planting, which leads
 40 to saplings not producing defensive chemical compounds (Selander et al., 1990). A single adult
 41 weevil can damage several plants over the course of a season, with ~50% sapling mortality observed
 42 across affected plantation sites in the UK and Ireland (Heritage and Moore, 2000). On commercial
 43 conifer plantations, *H. abietis* causes annual economic losses of €140 million *per annum* in Europe,
 44 of which €2.75 million (~£2.5 million) occurs in the UK (Evans et al., 2015). Currently, *H. abietis*
 45 is the most damaging insect pest of newly planted trees in Northern Europe (Evans et al., 2015).
 46 The potential for climate change to enhance the damage caused by *H. abietis*, by reducing life cycle
 47 length (Leather et al., 1999) and encouraging migration into previously weevil free areas (Inward
 48 et al., 2012; Barredo et al., 2015), especially in more northerly regions, has prompted discussion
 49 of the effectiveness of current *H. abietis* management practices and possible alternative methods
 50 (Kapranas et al., 2017; Mc Namara et al., 2018).



Figure 1: Damage caused by *Hylobius abietis*, destroying phloem tissue and causing scarring of the stem. (a) shows light damage with individual circular lesions, while (b) shows heavier damage with exposure of wood beneath the bark. Images taken from (a) Milan Zubrik, Forest Research Institute - Slovakia, Bugwood.org, and (b) Petr Kapitola, Central Institute for Supervising and Testing in Agriculture, Bugwood.org.

51 Current management of *H. abietis* through a variety of chemical, biological and physical mea-
 52 sures, can be costly and are not completely effective. Integrated pest management schemes tend to
 53 yield greater success, but increase costs further (Willoughby et al., 2004). Physical deterrents in-
 54 clude piling debris produced by the clearfelling process over exposed stumps to discourage egg laying
 55 (Rahman et al., 2015), or stump removal to limit the availability of substrate for egg laying. The
 56 application of entomopathogenic nematodes after clearfelling has been shown to reduce the number
 57 of adult weevils in clearfelled sites (Dillon et al., 2006; Kapranas et al., 2017; Williams et al., 2013).

58 The most common method of control is the addition of chemicals at the time of restocking, with *H. abietis* being the only insect pest against which routine chemical controls are applied in the UK and
59 Ireland (Willoughby et al., 2004, 2017). The most common chemical application for *H. abietis* in
60 the UK are synthetic pyrethroids of various formulation, which are sprayed directly onto saplings as
61 a prophylactic treatment, acting as a strong deterrent for *H. abietis* feeding on treated bark (Rose
62 et al., 2005). There are concerns however about run-off from spraying events entering watercourses,
63 where it is highly toxic to aquatic organisms (Willoughby et al., 2017; Mian and Mulla, 1992; Antwi
64 and Reddy, 2015). There are also concerns about the health of forestry workers who apply the
65 sprays (Rose, 2002). Additionally, the application of pyrethroid sprays can cost ~£80 per hectare of
66 planted land, and requires additional top-up sprays in subsequent years if the problem persists dur-
67 ing the sapling stage (Willoughby et al., 2017). With attitudes and regulations regarding chemical
68 insecticides changing and with advancements in genetics and breeding of crop species it has been
69 postured that *H. abietis* resistant tree cultivars could be developed and/or selected for to reduce
70 costs of active pest management.

72 A particular selectable trait for *H. abietis* tree cultivars may be that of Volatile Organic Com-
73 pound (VOC) production in bark, which may differ according to provenance of naturally occurring
74 tree populations. *H. abietis* adults rely on olfaction to search for coniferous hosts, responding to
75 VOCs, dominated by α -pinene and other monoterpenes released by the host plant (Nordlander
76 et al., 1986; Nordlander, 1987). At the local scale, when adult *H. abietis* are searching for feeding
77 material while on the ground, after their flight muscles regress, VOCs released by open wounds on
78 the bark caused by previous pine weevil feeders may attract more individuals (Nordlander, 1987;
79 Tilles et al., 1986), worsening the damage caused to the sapling. A positive feedback mechanism
80 may therefore exist, whereby damaged saplings are more likely to be further damaged, acting as
81 beacons for other *H. abietis* individuals in the area. Conifer saplings may also use VOCs as a de-
82 fensive strategy however, to deter insect pests (Gershenson and Croteau, 1991; Trapp and Croteau,
83 2001). Conifer saplings may differ in the concentration of VOCs produced both prior to damage
84 and after bark has been damaged by feeding (Kivimäenpää et al., 2012; Keeling and Bohlmann,
85 2006), and in their chemical composition (Heijari et al., 2011) potentially causing variation in the
86 likelihood of a sapling becoming damaged by *H. abietis*. Other defensive strategies employed by
87 coniferous tree species against insect herbivores include higher concentrations of sclereid cells in the
88 bark and resin canals in the needles, making the plant material less palatable to herbivores, thus
89 deterring continued feeding (Donnelly et al., 2016; King et al., 2011).

90 While *H. abietis* is a generalist of a number of coniferous tree species (Wallertz et al., 2014;
91 Toivonen and Viiri, 2006), they are common pests in scots pine (*Pinus sylvestris* L. Pinaceae) plan-
92 tations (Manlove et al., 1997). An increasing percentage of coniferous plantation forestry in the
93 UK is *P. sylvestris*. It currently constitutes ~17% of the UK's commercial coniferous plantation
94 forestry by area and ~15% by biomass (Forest Research, 2018). It is one of the UK's three native
95 coniferous tree species (Dines et al., 2005). There is increasing interest to plant native tree species
96 in an attempt to preserve native biodiversity and landscape heritage (Priestley and Sutherland,
97 2016). *H. abietis* is the most serious pest of UK *P. sylvestris* plantations, with infestations some-
98 times precluding sustainable future planting completely due to sapling mortality on clearfell sites
99 (Willoughby et al., 2017).

100 Selective breeding and identification of *P. sylvestris* varieties that are resistant to *H. abietis*
101 attack may provide a low cost method to reduce damage to saplings. Resistant varieties could
102 form part of an integrated pest management scheme (Telford et al., 2014) and planting of multiple
103 varieties in a single forest patch could act as good insurance against potential future attacks in
104 a rapidly changing pest landscape due to climate change (Alfaro et al., 2014). MacAllister et al.
105 (2019) found in an experimental study on *P. sylvestris* seedlings, that drought conditions prevent
106 the production of defensive compounds such as tyrosine which decrease insect larval performance
107 (Lokvam et al., 2006). As northern Scotland particularly is predicted to experience more drought

as climate change progresses (Gosling, 2014), this presents the possibility that insect pests may become more damaging as tree host defenses are degraded. Indeed, selecting for and inducing natural resistance to *H. abietis* and other bark boring insects is being heavily explored with other coniferous tree species such as *Picea abies* (Norway spruce) (Eyles et al., 2009; Schiebe et al., 2012), *Picea sitchensis* (Sitka spruce) (King et al., 2011), and *Picea glauca* (white spruce) (Kiss and Yanchuk, 1991), but *P. sylvestris* has not received the same attention. Byun-McKay et al. (2006) found that *P. sitchensis* populations varied in their expression of genes responsible for the production of bark oleoresin ducts when saplings were damaged, which act as a defence against stem boring insects. Similarly, Alfaro et al. (2013) developed varieties of *P. sitchensis* resistant to the white pine weevil (*Pissodes strobi* Peck Coleoptera: Curculionidae). They concluded that resin canals and sclereid cells in the bark as well as terpene production and variation in tree phenology were heritable characteristics which confer resistance to attack by *P. strobi*.

Natural populations *P. sylvestris* are restricted to enclaves in Scotland. Remnant Caledonian pine populations in Scotland, where *P. sylvestris* is the dominant species (Edwards and Mason, 2006) are comprised of 84 fragmented woodland stands dominated by *P. sylvestris*, over a total area of 17,882 hectares (Mason et al., 2004), which maintain adaptive genetic variation. Previous studies have shown that these populations vary in their ability to tolerate pathogens (Perry et al., 2016) and environmental extremes (Salmela et al., 2013). This study contributes further by assessing the tolerance of natural *P. sylvestris* populations to *H. abietis* attack, with the hope of informing future selection of pine weevil resistant *P. sylvestris* cultivars for plantation forestry, and identifying potential future conservation concerns for naturally occurring *P. sylvestris* in Caledonian remnant forests.

We conducted a common garden experiment in a recently clearfelled plantation already affected by *H. abietis* with *P. sylvestris* saplings in southern Scotland to assess sapling resistance to damage from the large pine weevil *H. abietis*. We compared germinated seedstock collected in naturally occurring *P. sylvestris* populations in remnant Caledonian pine forest patches across Scotland (Figure 2). We hypothesised that due to limited gene flow between Caledonian forest remnants, adaptive variation in attractiveness to *H. abietis* as a food source would exist between populations of *P. sylvestris*. Given previous research on *H. abietis* feeding behaviour we suggest that this adaptive variation may exist in bark VOC production and chemical composition. We tested this using biochemical fingerprints extracted from each sapling in the study. We hypothesised that two effects contribute to the extent of damage which a sapling is subject to, based on the previous work discussed above regarding *H. abietis* host searching behaviour: the probability of *H. abietis* initially choosing to feed on a sapling and damaging its bark (a), and the intensity of continued feeding by *H. abietis* (b).

Materials & Methods

Study sites and species

Scots pine (*Pinus sylvestris*) is the most widely distributed pine species in the world. Its range spans Eurasia from the arctic circle in Scandinavia to the dry northern mediterranean in high altitude areas of Spain and Turkey and from Scotland to the eastern edge of Siberia (GBIF, 2019; Carlisle and Brown, 1968). Scotland represents the western limit of its Eurasian distribution, where it is the dominant canopy tree species of the Caledonian pine forest. *P. sylvestris* grows well under conditions of low grazing, shade and competition.

P. sylvestris is wind pollinated, with monoecious flowering beginning between the ages of 15

152 and 30. Previous studies have shown cryptic genetic variation between the Caledonian remnant
153 forest sites from which seeds used in this study are sourced (Donnelly et al., 2018), which supports
154 the assertion that despite strong cross-pollination effects among populations, some degree of genetic
155 isolation occurs. Variation in isolation between sites follows a predictable longitudinal gradient, with
156 sites on the western extreme of the Caledonian pine range being more isolated due to the prevailing
157 westerly wind direction, which limits pollen dispersion to westerly populations (González-Díaz et al.,
158 2018).

159 Seed populations of *P. sylvestris* were collected from 21 sites where genetic variation has already
160 been identified across Scotland in March 2007 (Figure 2). At each site four open-pollinated trees
161 were located at least 100 m apart. From each of these trees at least 20 cones with seeds were
162 collected. To minimise seedling mortality, seeds were germinated and grown in a glasshouse for
163 3 years before four randomly selected surviving seedlings per Parent tree were transplanted to a
164 common garden. This resulted in 168 distinct maternal lines. All seed was collected from old
165 adult trees, in an attempt to avoid sampling trees descended from nearby plantation forestry as this
166 study focussed only on natural populations. Sites were situated within the historical range of the
167 Caledonian pine forest. Seed collection sites were chosen by accessibility in six geographic clusters.
168 Each cluster was located to ensure geographical isolation from others.

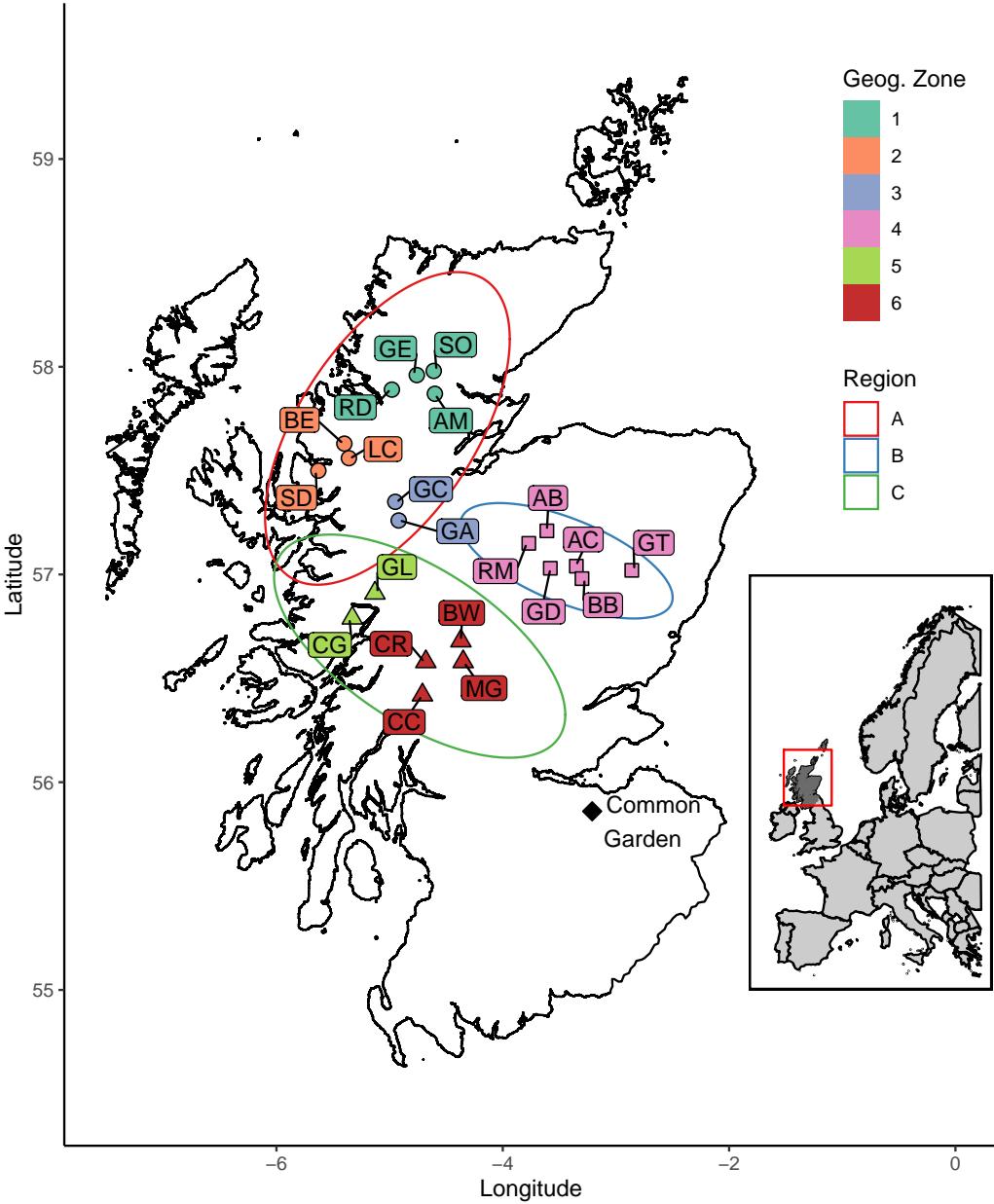


Figure 2: Map of seed collection sites within Scotland, from which seed populations were collected. Elliptic hulls and site point shapes define the three Regions. Points are coloured according to Geographic Zone clusters, which are nested within Regions. Point labels are Site codes used as a shorthand for the sites throughout this report.

169 Experimental design

170 The common garden was located in Southern Scotland ($N 55.86^\circ$, $E -3.21^\circ$) in a patch of recently
 171 clear-felled sitka spruce (*Picea sitchensis*) plantation, surrounded by existing adult *P. sitchensis*
 172 plantation on all sides. This mimicks the conditions found in commercial plantation forestry sites
 173 that will be replanted, which often have adjacent existing plantation. A mown grass border of 10 m
 174 on all sides separated the newly planted *P. sylvestris* from the surrounding *P. sitchensis* plantation,
 175 to avoid competitive edge effects. All *P. sitchensis* surrounding the common garden was planted at
 176 the same time in 2005, making it 10 years old when the common garden was established. Saplings
 177 were randomly assigned to grid points 3 m between each sapling. This resulted in a total grid size
 178 of 84 x 8 saplings, and a total of 672 saplings. *H. abietis* infestation occurred naturally across the

site, with adult weevils likely travelling from the adult *P. sitchensis* plantation around the common garden.

181 Data collection

182 The area of bark lesions caused by *H. abietis* was measured on the main growing stem of each
183 sapling in June 2015. This is roughly between the two seasonal peaks of weevil feeding that are
184 commonly observed in the UK, which occur in the spring and late summer, coinciding with the
185 end of adult hibernation and the emergence of new adults from pupae, respectively (Nordenhem,
186 1989; Leather et al., 1999). Only damage sustained by *H. abietis* during the current growing season
187 was counted and could be clearly separated from damage sustained in previous years by the lack
188 of bark edge scarring and presence of sap at the wound edge (Figure 1). Isolated lesions tended
189 to be roughly circular with a diameter of ~3 mm. Where a larger continuous lesion was found, as
190 when a stem was girdled, the larger lesion was photographed with a scale and the area estimated by
191 tracing the lesion with ImageJ version 1.50g7 (Schneider et al., 2012). Weevil damage is therefore
192 expressed as the total mm² area of all stem lesions per sapling.

193 Statistical analysis

194 To assess the effect of *P. sylvestris* sapling genetic origin on damage by pine weevils (*H. abietis*), and
195 to test our hypothesis that two effects are responsible for *H. abietis* damage, we implemented a two-
196 part framework using generalised linear mixed models, using the *glmmTMB* package in R (Brooks
197 et al., 2017). First, a binomial logistic mixed effects model assessed variation in the probability of
198 a sapling being initially damaged according to *P. sylvestris* Site.

199 We used a linear mixed effects model, using only saplings where damage had occurred, to as-
200 sess whether saplings varied in the total area of bark damaged by continued feeding by *H. abietis*
201 according to *P. sylvestris* Site. The response variable of area of bark damaged was log transformed
202 in order to better meet model assumptions. In both analyses, a combination of fixed and random
203 intercept effects were modelled to obtain the optimal model structure and to compare the relative
204 effect sizes of Geographic Zone, Site and maternal line. Maternal line was used as a random inter-
205 cept effect in all analyses to account for pseudo-replication in sapling Parent. The geographically
206 nested nature of the seed collection Sites within Geographic Zones was also used as a random effect
207 in the appropriate models (Figure 5). Model goodness-of-fit was assessed for both model types
208 by comparing models with equivalent random effects models and null models using AIC_r (Akaike
209 Information Criterion) and Log-likelihood estimates (Bolker et al., 2008). During model comparison
210 all models were fitted using Maximum Likelihood (ML) (Bolker et al., 2008). To investigate which
211 populations of *P. sylvestris* differed in their resistance to *H. abietis* attack, the models were refitted
212 using Restricted Maximum Likelihood (REML) and model slope estimates were compared. Tukey's
213 HSD multiple comparisons tests of marginal means assessed which populations were significantly
214 different from each other for both models, using the *emmeans* package (L., 2019). All statistical
215 analyses were performed in R version 3.4.2 (R Core Team, 2019).

216 A post-hoc linear mixed effects model investigated the effect of latitude of seed collection Site
217 on the area of damaged bark, with nested random intercept effects of maternal line within Site.
218 Predicted values of this model were generated and used to assess the effect of latitude on damaged
219 bark area.

220 Spatial autocorrelation may have been present within the Common Garden, with some damaged
221 saplings acting as olfactory beacons to attract more *H. abietis* to the area. This potential effect was

222 investigated using semi-variograms of model residuals for the best-fitting generalised linear mixed
 223 effects models. Semi-variograms of the damaged area mm^2 suggested that spatial autocorrelation
 224 between saplings was negligible within the Common Garden and so spatial autocorrelation structures
 225 were not included in generalised linear mixed effects models (Figure 3).

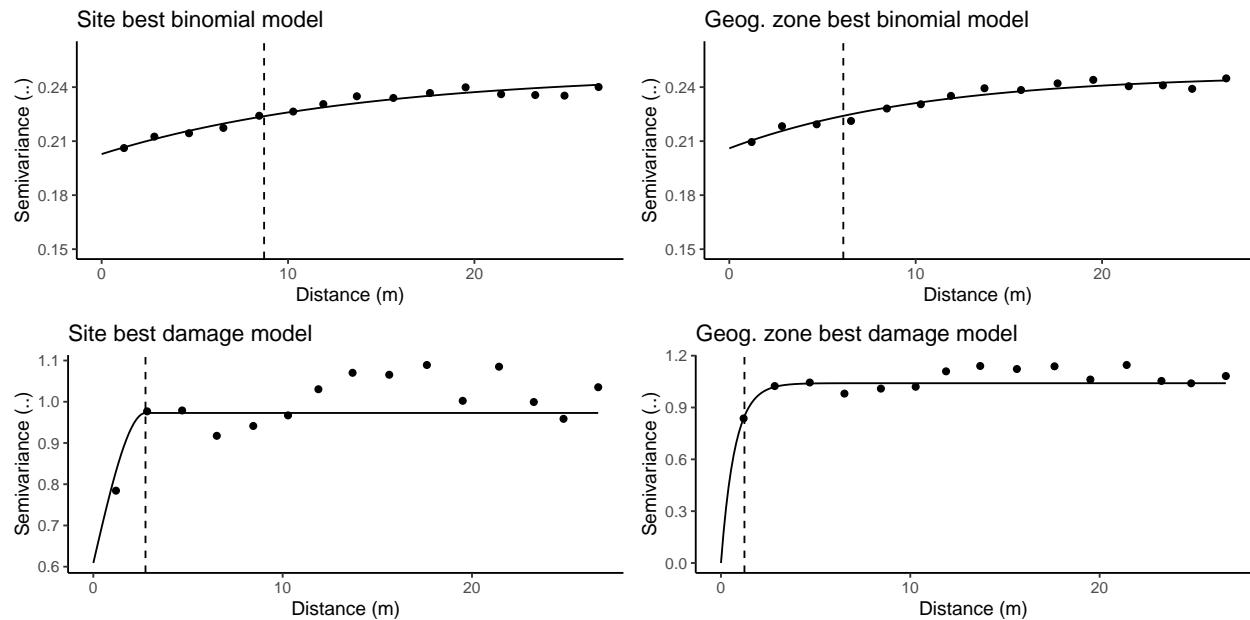


Figure 3: Semivariograms showing spatial autocorrelation of damaged bark area according to distance between saplings. Horizontal dotted line denotes the sill of the semivariogram. Saplings further apart than this distance are predicted to not affect the degree of bark damage, or the probability of being damaged by *H. abietis*, respectively.

226 To test whether damage by weevils was a result of variation in VOC production and composition
 227 in the bark, we used Principal Component Regression (PCR). First we ran a Principal Components
 228 Analysis (PCA) using all 20 identified terpene compounds. The first three axes of the PCA explained
 229 50% of the variation in terpene composition across saplings. We used the first three axes of the
 230 PCA in a simple multiple regression with bark area damaged as a measure of

231 Results

232 Sapling damage

233 36.9% (248/672) of the saplings in the common garden were damaged by *H. abietis* feeding activity.
 234 Figure 4 shows the number of saplings damaged divided into their origin seed collection Sites. All
 235 saplings were alive prior to data collection and sapling mortality was not recorded during the exper-
 236 iment. All seed populations had at least eight affected saplings out of a total of 32. The population
 237 with the highest number of damaged saplings was Loch Clair (LC), which had 18 damaged saplings.
 238 The sapling with the highest mm^2 damaged area was from Cona Glen (CG) and had 325.8 mm^2
 239 of bark damaged. Rhidorroch (RD) had the highest cumulative damaged area with 1057.1 mm^2 .
 240 Variance in bark area damaged within seed populations was high (Figure 5). Some geographic zones
 241 showed comparable levels of damage, while others varied widely within geographic zone.

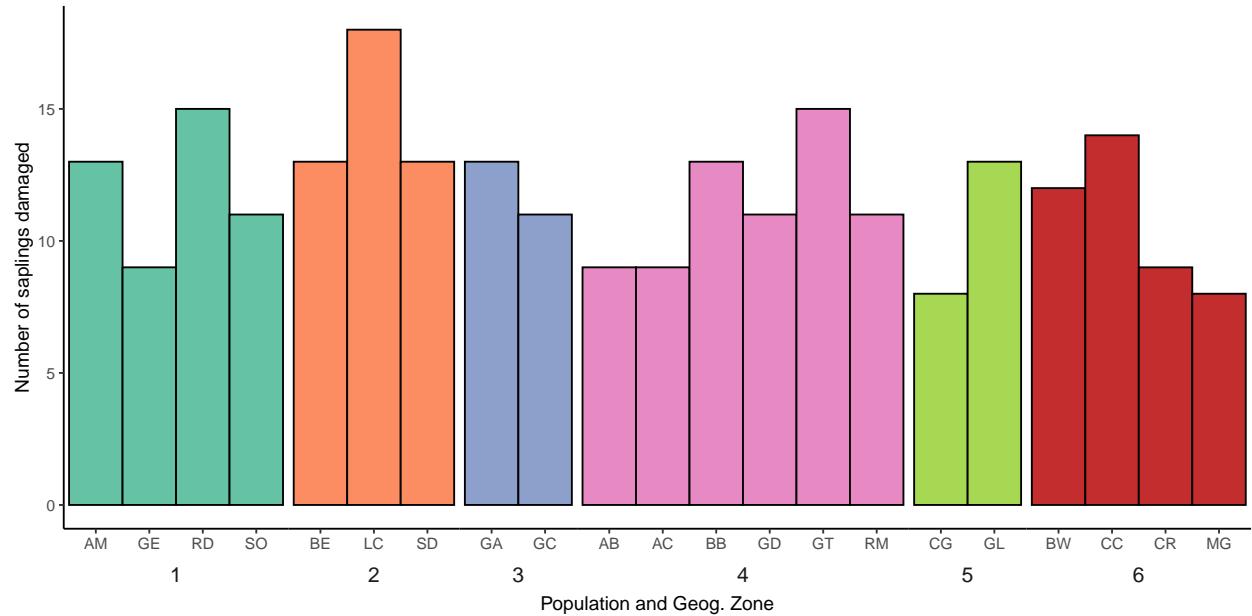


Figure 4: The number of saplings with visible damage by *H. abietis*, divided by Site. Groups of bars denote Geographic Zones, ordered from left to right by decreasing mean latitude

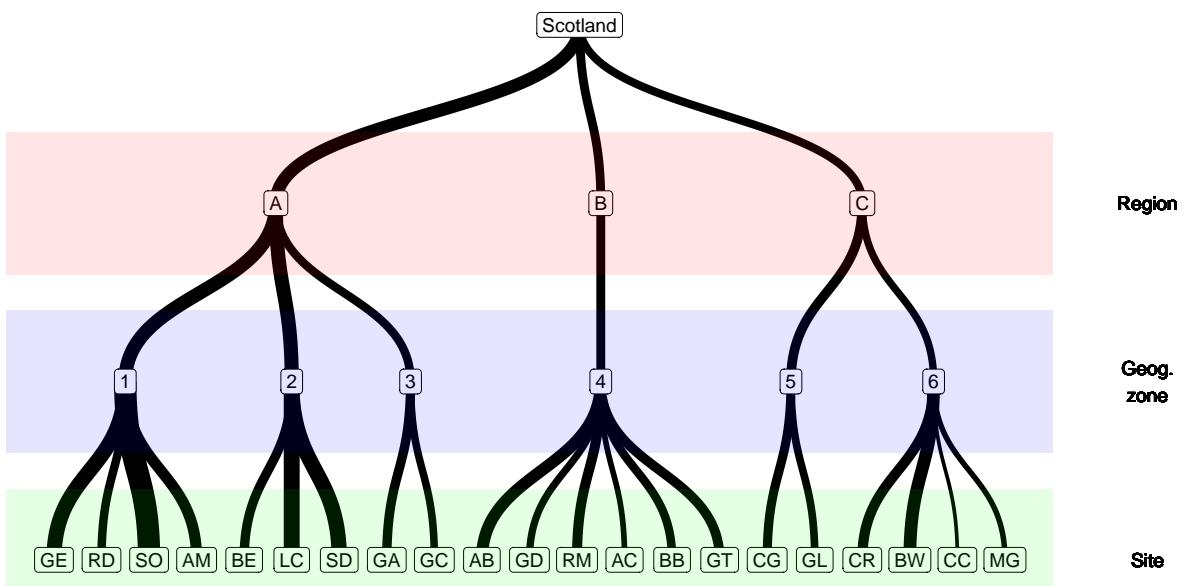


Figure 5: Dendrogram showing nested grouping of seed populations. Graph edge widths vary according to the total bark area damaged on saplings collected from each Site. Geographic zones are ordered from left to right by decreasing mean latitude. Width edges are weighted according to the number of saplings at each grouping level to account for differences in number of Sites per Geographic Zone and Region. This means edge widths should not be compared across vertical node levels.

242 The effect of seed population on sapling damage

243 Binomial model

244 The first part of the modelling process explored variation among seed populations in the probability
 245 of a sapling being damaged by *H. abietis*. The most parsimonious model was a null model, as
 246 estimated by AIC values. Fixed effects models using Geographic Zone and Site explained little of
 247 the variance in likelihood of a sapling being damaged, while models using maternal line as the fixed
 248 effect explained ~95% (R^2_m) of the variance (Table 1). We compared marginal means of the fixed
 249 effect groups for the best fitting models using Geographic Zone or Site as fixed effects and found
 250 that these groups did not vary significantly in a pairwise comparison using Tukey's HSD test.

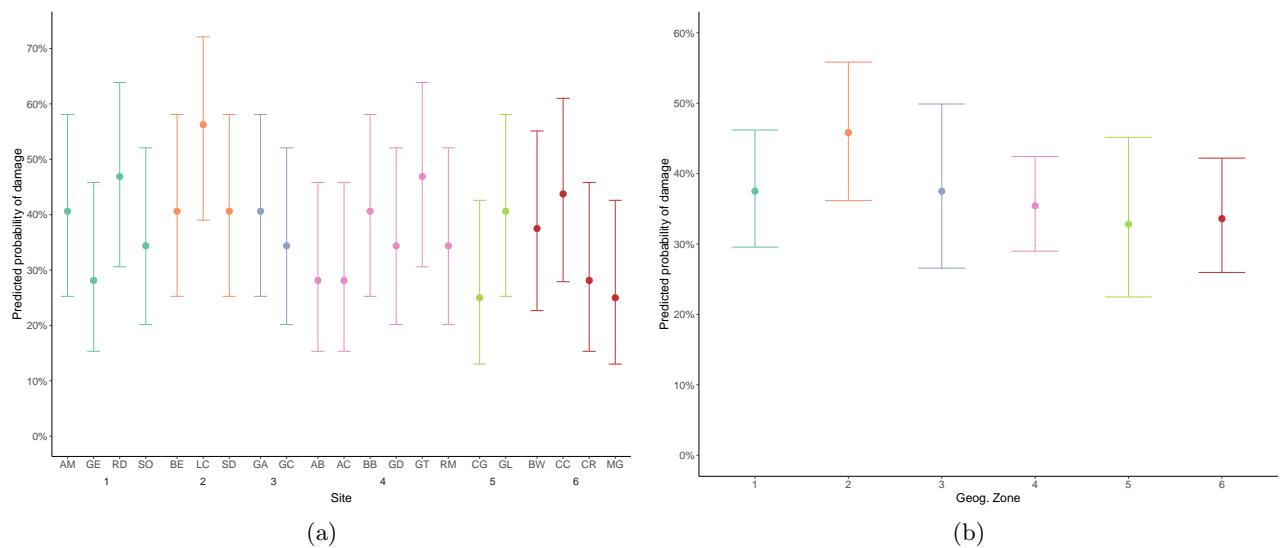


Figure 6: Predicted values with 95% confidence intervals for the probability of a sapling being damaged with seed collected from different (a) Sites and (b) aggregated by Geographic Zone.

Table 1

Fixed eff.	Random eff.	AIC	logLik	R^2_c	R^2_m
NA	NA	887.000	-442.500	0	0
NA	Parent	889.000	-442.500	0	0
NA	Site	889.000	-442.500	0	0
NA	Site / Parent	891.000	-442.500	0	0
NA	Geog. Zone / Site / Parent	893.000	-442.500	0	0
Geog. Zone	Parent	894.500	-440.200	0.006	0.008
Geog. Zone	Site / Parent	896.500	-440.200	0.006	0.008
Site	Parent	910.700	-433.400	0.027	0.035
Site	Geog. Zone	910.700	-433.400	0.027	0.035
Site	Geog. Zone + Parent	912.700	-433.400	0.027	0.035

251 The fixed effect of Site was weak as a predictor of likelihood of *H. abietis* damage. In a model
 252 using seed population as a fixed effect and maternal line as a random intercept effect, seed population
 253 only accounted for 2.7% (R^2_m) of the variation in the probability that a sapling would be initially
 254 damaged by *H. abietis*. According to the best fitting model with Site as a fixed effect, saplings from
 255 Beinn Eighe (BE) had a greater chance of being initially damaged than others (Figure 6a), however,

256 these predicted values were not significantly different from other Sites according to a Tukey's HSD
 257 comparison of marginal means.

258 Non-zero damage model

259 The second part of the hurdle model explored variation in the area of sapling bark damaged by *H.*
 260 *abietis*, for those saplings which were initially damaged. The most parsimonious model according
 261 to AIC included the fixed effect of Geographic Zone and the random effect of Site to account for
 262 pseudo-replication in seed origin. This model explained 5.6% (R^2_m) of the variance in sapling bark
 263 area damaged. This model was of better quality than a null model and multiple random effects
 264 models. The maternal line BE4, collected from Beinn Eighe contrasted weakly with a number of
 265 families from more southerly Sites, but none significantly at $P < 0.05$ when parent was used as a
 266 fixed effect in a mixed effects model predicting bark area damaged.

267 In a pairwise comparison of estimated marginal means of Geographic Zones for the best-fitting
 268 model, Geographic Zone 1 differed significantly from Zones 4 and 6, and weakly with Zone 5. At
 269 the Site level, Rhiddoroch (RD) differed from Glen Derry and Crannach, both populations in the
 270 southern part of the study region. Geographic Zone one had a higher predicted damaged bark area
 271 according to the best fitting model (Figure 7b). Sites AC and CC had a higher predicted damaged
 272 bark area than other Sites.

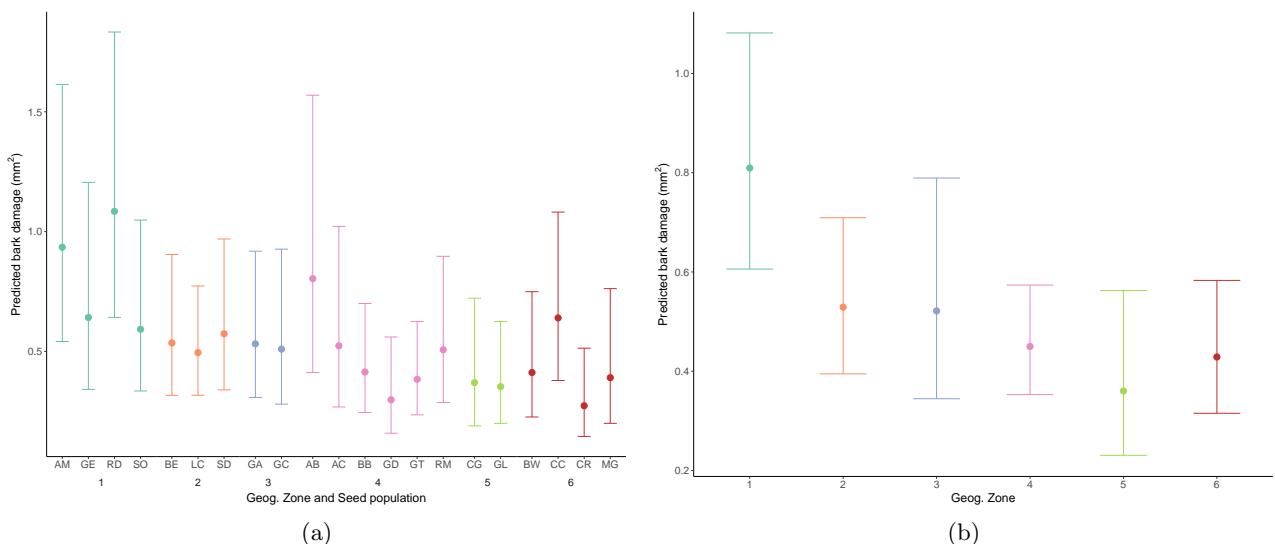


Figure 7: Predicted values of mm^2 for saplings with seed collected from different Geographic Zones.

273 Site level spatial patterns

274 There was a weak but significant positive effect of latitude on the total bark area damaged by *H.*
 275 *abietis* ($Z = 3.249(1, 248)$, $p = <0.005$, $R^2_m = 0.041$), when the nested random effects of seed
 276 collection Site and family were accounted for (Figure 8). In a similar model, there was no effect of
 277 longitude on bark area damaged ($Z = -1.377(1, 248)$, $p = 0.168$, $R^2_m = 0.009$).

Table 2

Fixed eff.	Random eff.	AIC	logLik	R^2_c	R^2_m
Geog. Zone	Site	676.200	-330.100	0.058	0.058
NA	NA	678.200	-337.100	0	0
Geog. Zone	Site / Parent	678.200	-330.100	0.058	0.058
NA	Geog. Zone / Site	679.200	-335.600	0.034	0
NA	Site / Parent	681.800	-336.900	0.020	0
Site	Parent	693.500	-323.800	0.108	0.108
Site	Geog. Zone	693.500	-323.800	0.108	0.108
Site	Geog. Zone + Parent	695.500	-323.800	0.108	0.108

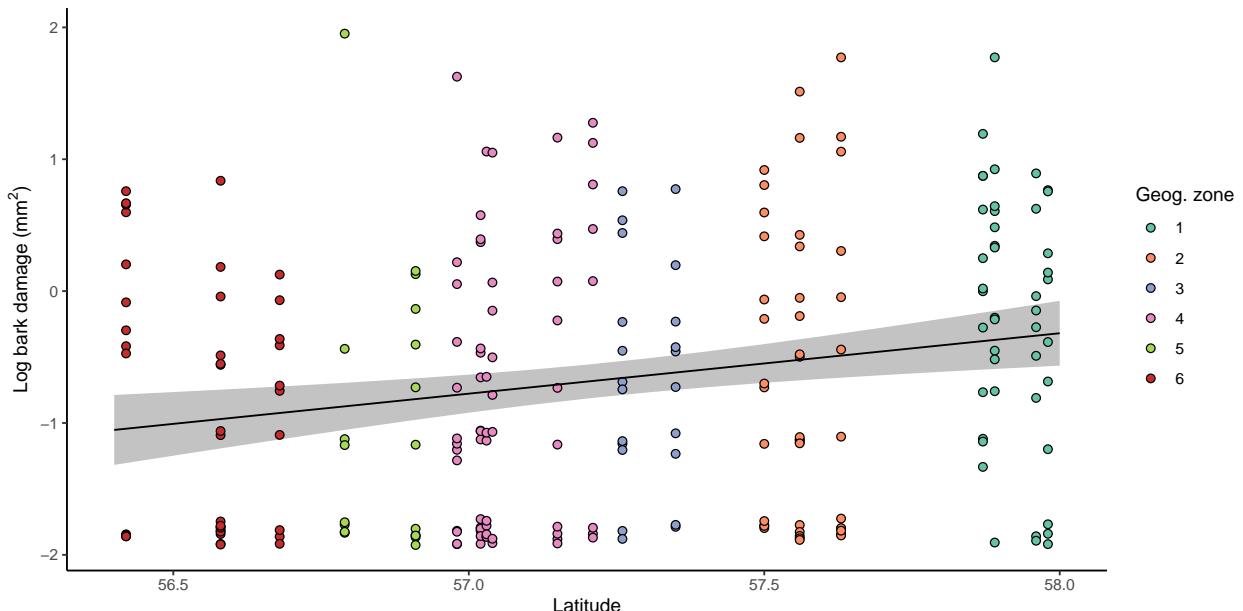


Figure 8: Predicted values with 95% confidence interval for the bark area damaged on a sapling with seed collected at different latitudes.

278 Discussion

279 We found a statistically clear, but weak, latitudinal gradient in the level of damage from *H. abietis* to
 280 *P. sylvestris* saplings in a common garden experiment. Saplings from more northerly populations
 281 experienced greater damage than those from more southerly populations. The model selection
 282 process determined that there was an effect of Geographic Zone on the area of weevil damage found
 283 on damaged saplings and a possible weak effect of Site, but could not account for variation in the
 284 probability that a sapling became damaged initially. Saplings with maternal lines found at higher
 285 latitudes typically experienced higher levels of damage, but this had a much weaker effect than
 286 population or Geographic Zone itself. Within Site variability among maternal lines was high and
 287 diluted the effects of Geographic Zone and Site. This is expected given the high gene flow between
 288 populations (Donnelly et al., 2018). Nevertheless, some clear variation was observed between groups.

289 In this study we identified weak but significant differences between *P. sylvestris* populations in
 290 their susceptibility to *H. abietis* attack. It appears that a weak latitudinal pattern may be driving

these differences. It may be that historical exposure to *H. abietis* in more southerly populations has driven adaptation to develop defensive structures to deter bark feeding insects. Studies on the distribution and life cycle of *H. abietis* have shown that life cycle length is strongly linked with mean temperature in the summer months, with higher temperatures leading to a short life cycle and therefore higher numbers of pine weevils where infestations occur (Leather et al., 1999). *H. abietis* abundance reduces with latitude in Scotland (Barredo et al., 2015). Historically, *H. abietis* populations at high latitudes and in the west of Scotland have been low (Leather et al., 1999), due to lower temperatures (Wainhouse et al., 2014). The latitudinal effect may therefore be a result of adaptation to resist *H. abietis* damage. Additionally, phenological variation in latitudinal populations may lead to VOC concentrations varying between saplings at the same time of year in the common garden, making some saplings more desirable than others (Guenther, 1997). The data collection for this study took place in June, approximately between the two seasonal peaks of *H. abietis* activity. Other studies have shown that the growing season of *P. sylvestris* from higher latitudes starts later in the year (Salmela et al., 2013), leading to a lower concentration of VOC stored in bark resin when our study was conducted, potentially making these saplings more attractive than those from southerly populations to *H. abietis*. Yazdani et al. (1985) found that *P. sylvestris* individuals in Sweden varied in the composition of monoterpenes found in oleoresin, with northern populations containing lower limonene, lower β -pinene and higher $\Delta 3$ -carene. Nordlander (1990) found that *H. abietis* limonene inhibits the efficiency of α -pinene, a chemical which is known to attract *H. abietis*, meaning more northerly *P. sylvestris* populations may be more attractive to *H. abietis*.

This study found no correlation between longitude of source population and damage by *H. abietis*. *P. sylvestris* needles and bark possess resin canals which act to deter herbivores. While it has not been explicitly tested for *H. abietis*, other studies involving similar bark feeding insects have found a negative correlation between resin canal density and feeding behaviour on coniferous tree species. Boucher et al. (2001) found that the white pine weevil *Pissodes strobi* was discouraged from eating the needles of four different pine species with higher resin canal concentration and cuticle thickness. Donnelly et al. (2016) found that for a subset of the same *P. sylvestris* Sites studied here, that resin canal density in needles varied with longitude and between Sites, but did not test latitudinal variation. They suggested that resin canal density may be linked to water stress, as it plays a role in water regulation (Farrell et al., 1991). A lack of longitudinal effect in this study may suggest that *H. abietis* response to

As climate change increases average temperatures at high latitudes, there is the possibility that *H. abietis* and other bark feeding insect herbivores will become more present at high latitudes (Inward et al., 2012). This study shows that there is a potential risk to both naturally occurring and planted forests with seed stock gathered from high latitudes as these varieties appear more susceptible to *H. abietis* attack. We suggest that future work seeks to identify variation in VOC concentration and composition among *P. sylvestris* populations to identify at risk populations to allow the creation of prioritised conservation management strategies as climate change progresses.

Conclusion

This study sought to test whether adaptive variation for resistance to the large pine weevil (*Hylobius abietis*) existed in genetically distinct populations of scots pine (*Pinus sylvestris*) in Caledonian remnant forest patches in Scotland. A weak positive effect of latitude of seed collection Site was found in the damaged area of sapling bark, suggesting that more southerly populations may be less attractive to *H. abietis* attack. No relationship was found between Site and probability of a sapling being initially damaged. Variation exists between Geographic Zones and between Sites with regards to resistance to continued *H. abietis* attack. The conclusions of this study suggest that under

warming temperatures as a result of anthropogenic climate change, there is a conservation concern for less-resistant Caledonian remnant forest patches as *H. abietis* populations may increase, leading to higher mortality of saplings, leading to changes in ecosystem structure. We suggest that further studies should investigate bark morphological and physiological variation amongst these natural populations of *P. sylvestris*, with particular attention to variation in Volatile Organic Compounds (VOCs) emitted when bark is damaged as a defensive response in young saplings, to understand the underlying mechanism driving differences between these genetically distinct populations.

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