

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

4 John L. Godlee

5 August 7, 2019

6 **Abstract**

7 Damage to coniferous tree plantation crops from the large pine *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* are known to occur. 672 saplings,
13 grown from seed collected from 21 naturally occurring populations of *Pinus sylvestris* across
14 Scotland 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
16 bark lesions between *P. sylvestris* populations. In contrast we found that sapling populations
17 did not differ in their likelihood of being attacked by *H. abietis*. A weak latitudinal pattern
18 was observed, with saplings sourced from populations found further north being attacked more
19 heavily than those 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
26 bark and buds of coniferous saplings, consuming sugar rich phloem tissue (Nordlander, 1991).
27 Lesions on the bark and buds of saplings (Figure 1) as a result of feeding may cause a reduction
28 in growth rate, stem deformation and an increased susceptibility to infection by airborne diseases
29 of trees (Leather et al., 1999). Heavy damage may lead to stem girdling and death of the terminal
30 growing bud resulting in a malformed trunk, limiting economic use as timber when fully grown
31 (Alfaro, 1989; Gill, 1992). While *H. abietis* may inhabit adult coniferous trees in both natural and
32 planted coniferous forests, recently clearfelled and restocked coniferous plantation sites provide an
33 enriched habitat for breeding *H. abietis* and so pose more of a danger to planted saplings than those
34 in naturally regenerating stands (Willoughby et al., 2004; Ördlander and Nilsson, 1999). Adults
35 lay eggs within the stumps of clearfelled trees, which are rarely removed after clearfelling, with
36 newly emerged juvenile weevils feeding on young saplings until adulthood (Willoughby et al., 2004).
37 Planted coniferous saplings are more susceptible to *H. abietis* damage than naturally regenerating
38 saplings, probably due to water stress as a result of damage to root systems during planting (Selander

et al., 1990). A single adult weevil can damage several plants over the course of a season, with ~50% sapling mortality observed across affected plantation sites in the UK and Ireland (Heritage and Moore, 2000). On commercial conifer plantations, *H. abietis* causes annual economic losses of €140 million *per annum* in Europe, of which €2.75 million (~£2.47 million) occurs in the UK (Evans et al., 2015). Currently, *H. abietis* is the most damaging insect pest of newly planted trees in Northern Europe (Evans et al., 2015). The potential for climate change to enhance the damage caused by *H. abietis*, by reducing life cycle length (Leather et al., 1999) and encouraging migration into previously weevil free areas (Inward et al., 2012; Barredo et al., 2015), especially in more northerly regions, has prompted discussion of the effectiveness of current *H. abietis* management practices and possible alternative methods (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.

Management of *H. abietis* currently relies on a variety of chemical, biological and physical measures, with integrated pest management schemes tending to yield greater success (Willoughby et al., 2004). Physical deterrents include piling debris produced by the clearfelling process over exposed stumps to discourage egg laying (Rahman et al., 2015), or stump removal to limit the availability of substrate for egg laying. The application of entomopathogenic nematodes after clearfelling has been shown to reduce the number of adult weevils in clearfelled sites (Dillon et al., 2006; Kapranas et al., 2017; Williams et al., 2013). 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 Ireland (Willoughby et al., 2004, 2017). The most common chemical application for *H. abietis* in the UK are synthetic pyrethroids of various formulation, which are sprayed directly onto saplings as a prophylactic treatment, acting as a strong deterrent for *H. abietis* feeding on treated bark (Rose et al., 2005). There are concerns however about run-off from spraying events

61 entering watercourses, where it is highly toxic to aquatic organisms (Willoughby et al., 2017; Mian
62 and Mulla, 1992; Antwi and Reddy, 2015). There are also concerns about the health of forestry
63 workers who apply the sprays (Rose, 2002). Additionally, the application of pyrethroid sprays can
64 cost ~£80 per hectare of planted land, and requires additional top-up sprays in subsequent years if
65 the problem persists during the sapling stage (Willoughby et al., 2017).

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

82 While *H. abietis* is a generalist of a number of coniferous tree species (Wallertz et al., 2014;
83 Toivonen and Viiri, 2006), they are common pests in scots pine (*Pinus sylvestris* L. Pinaceae)
84 plantations (Manlove et al., 1997). An increasing percentage of coniferous plantation forestry in
85 the UK is *P. sylvestris*. It currently constitutes ~17% of the UK's commercial coniferous plantation
86 forestry by area and ~15% by biomass (*Forestry Statistics 2018: A compendium of statistics about
woodland, forestry and primary wood processing in the United Kingdom*, 2018). It is one of the UK's
87 three native coniferous tree species (Dines et al., 2005). There is increasing interest to plant native
88 tree species in an attempt to preserve native biodiversity and landscape heritage (). *H. abietis*
89 is the most serious pest of UK *P. sylvestris* plantations, with infestations sometimes precluding
90 sustainable future planting completely due to sapling mortality on clearfell sites (Willoughby et al.,
91 2017).

92 Selective breeding and identification of *P. sylvestris* varieties that are resistant to *H. abietis*
93 attack may provide a low cost method to reduce damage to saplings. Resistant varieties could
94 form part of an integrated pest management scheme (Telford et al., 2014) and planting of multiple
95 varieties in a single forest patch could act as good insurance against potential future attacks in a
96 rapidly changing pest landscape due to climate change (Alfaro et al., 2014). Indeed, selecting for
97 and inducing natural resistance to *H. abietis* and other bark boring insects is being heavily explored
98 with other coniferous tree species such as *Picea abies* (Norway spruce) (Eyles et al., 2009; Schiebe
99 et al., 2012), *Picea sitchensis* (Sitka spruce) (King et al., 2011), and *Picea glauca* (white spruce)
100 (Kiss and Yanchuk, 1991), but *P. sylvestris* has not received the same attention. Byun-McKay
101 et al. (2006) found that *P. sitchensis* populations varied in their expression of genes responsible for
102 the production of bark oleoresin ducts when saplings were damaged, which act as a defence against
103 stem boring insects. Similarly, Alfaro et al. (2013) developed varieties of *P. sitchensis* resistant to
104 the white pine weevil (*Pissodes strobi* Peck Coleoptera: Curculionidae). They concluded that resin
105 canals and sclereid cells in the bark as well as terpene production and variation in tree phenology
106 were heritable characteristics which confer resistance to attack by *P. strobi*.

108 Natural populations *P. sylvestris* are restricted to enclaves in the north of Scotland. Remnant
109 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 pine remnants, adaptive variation in attractiveness to *H. abietis* as a food source would exist between populations of *P. sylvestris*. 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 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 and 30. Previous studies have shown cryptic genetic variation between the Caledonian remnant forest sites from which seeds used in this study are sourced (Donnelly et al., 2018), which supports the assertion that despite strong cross-pollination effects between the populations, some degree of genetic isolation occurs. Variation in isolatedness between sites follows a predictable longitudinal gradient, with sites on the western extreme of the Caledonian pine range being more isolated due to the prevailing easterly wind direction limiting pollen dispersion to the west (González-Díaz et al., 2018).

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

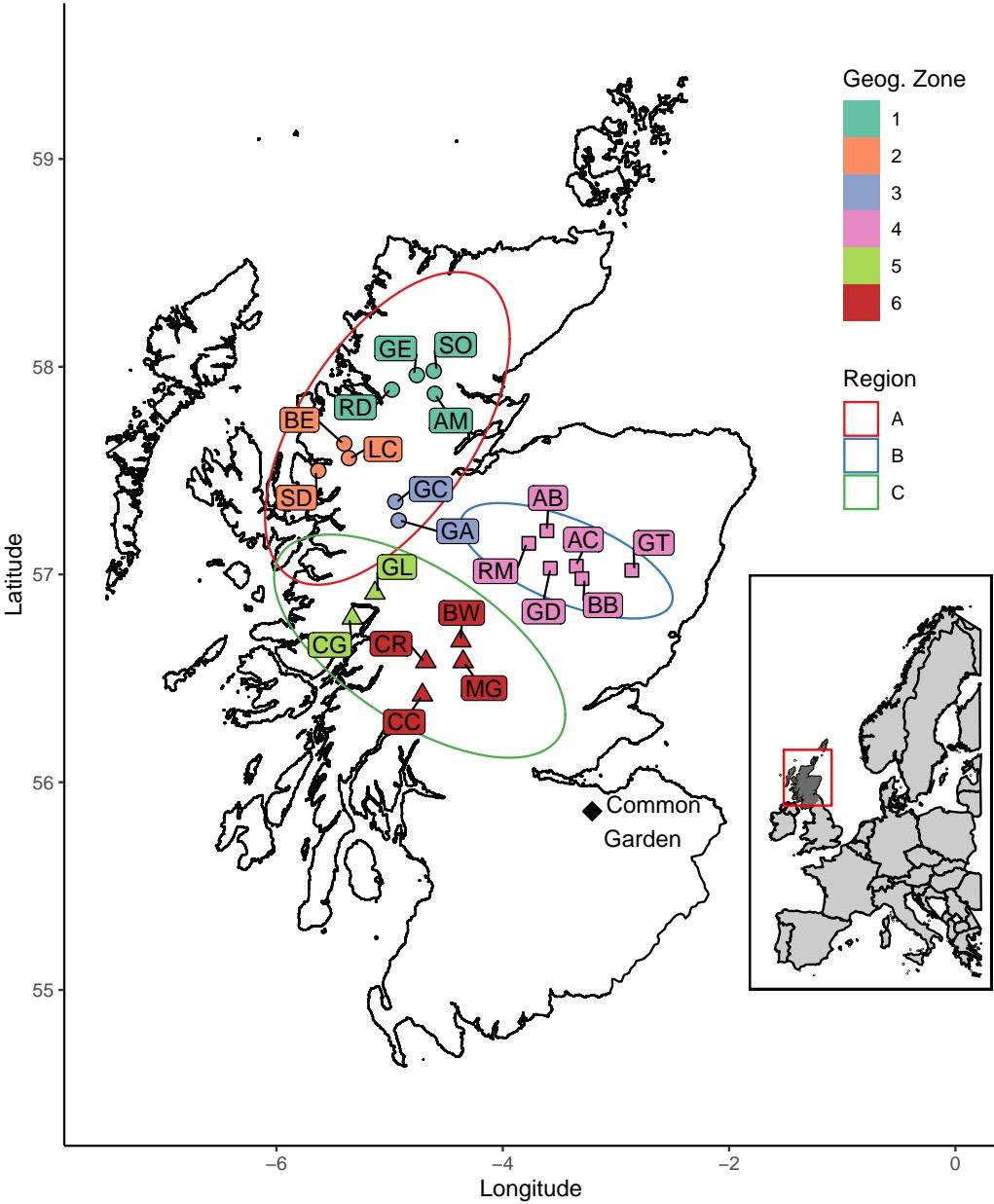


Figure 2: Map of seed collection sites within Scotland, from which seed populations were collected. Elliptic hulls and site point shapes define the three Regions. Points are coloured according to Geographic Zone clusters, which are nested within Regions. Point labels are Site codes used as a shorthand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

154 Experimental design

155 The common garden was located in Southern Scotland ($N 55.86^\circ$, $E -3.21^\circ$) in a patch of recently
 156 clear-felled sitka spruce (*Picea sitchensis*) plantation, surrounded by existing adult *P. sitchensis*
 157 plantation on all sides. This mimicks the conditions found in commercial plantation forestry sites
 158 that will be replanted, which often have adjacent existing plantation. A mown grass border of 10 m
 159 on all sides separated the newly planted *P. sylvestris* from the surrounding *P. sitchensis* plantation,
 160 to avoid competitive edge effects. All *P. sitchensis* surrounding the common garden was planted at
 161 the same time in 2005, making it 10 years old when the common garden was established. Saplings
 162 were randomly assigned to grid points within 4 adjacent blocks with a distance of 3 m between
 163 each sapling. This resulted in a total grid size of 84 x 8 saplings, a total of 672 saplings. *H. abietis*

164 infestation occurred naturally across the site, with adult weevils likely travelling from the adult *P.*
165 *sitchensis* plantation around the common garden.

166 Data collection

167 The area of bark lesions caused by *H. abietis* was measured on the main growing stem of each
168 sapling in June 2015. This is roughly between the two seasonal peaks of weevil feeding that are
169 commonly observed in the UK, which occur in the spring and late summer, coinciding with the
170 end of adult hibernation and the emergence of new adults from pupae, respectively (Nordenhem,
171 1989; Leather et al., 1999). Only damage sustained by *H. abietis* during the current growing season
172 was counted and could be clearly separated from damage sustained in previous years by the lack
173 of bark edge scarring and presence of sap at the wound edge (Figure 1). Isolated lesions tended
174 to be roughly circular with a diameter of ~3 mm. Where a larger continuous lesion was found, as
175 when a stem was girdled, the larger lesion was photographed with a scale and the area estimated by
176 tracing the lesion with ImageJ version 1.50g7 (Schneider et al., 2012). Weevil damage is therefore
177 expressed as the mm² area of stem lesions per sapling.

178 Statistical analysis

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

184 We used a linear mixed effects model, using only saplings where damage had occurred, to as-
185 sess whether saplings varied in the total area of bark damaged by continued feeding by *H. abietis*
186 according to *P. sylvestris* Site. The response variable of area of bark damaged was log transformed
187 in order to better meet model assumptions. In both analyses, a combination of fixed and random
188 intercept effects were modelled to obtain the optimal model structure and to compare the relative
189 effect sizes of Geographic Zone, Site and Parent tree. Parent tree was used as a random intercept
190 effect in all analyses to account for pseudo-replication in sapling Parent. The geographically nested
191 nature of the seed collection Sites within Geographic Zones was also used as a random effect in
192 the appropriate models (Figure 3). Model goodness-of-fit was assessed for both model types by
193 comparing models with equivalent random effects models and null models using AIC_r (Akaike In-
194 formation Criterion) and Log-likelihood estimates (Bolker et al., 2008). During model comparison
195 all models were fitted using Maximum Likelihood (ML) (Bolker et al., 2008). To investigate which
196 populations of *P. sylvestris* differed in their resistance to *H. abietis* attack, the models were refitted
197 using Restricted Maximum Likelihood (REML) and model slope estimates were compared. Tukey's
198 HSD multiple comparisons tests of marginal means assessed which populations were significantly
199 different from each other for both models, using the *emmeans* package (L., 2019). All statistical
200 analyses were performed in R version 3.4.2 (R Core Team, 2019).

201 A post-hoc linear mixed effects model investigated the effect of latitude of seed collection Site
202 on the area of damaged bark, with nested random intercept effects of Parent within Site. Predicted
203 values of this model were generated and used to assess the effect of latitude on damaged bark area.

204 Spatial autocorrelation may have been present within the Common Garden, with some damaged
205 saplings acting as olfactory beacons to attract more *H. abietis* to the area. This potential effect was
206 investigated using Generalised Least Squares (GLS) models of damaged bark area with spatial

autocorrelation structures as a covariate. Multiple spatial autocorrelation structures were tested and models fitted using ML were compared in their goodness-of-fit using AIC (Akaike Information Criterion) values, Log-likelihood estimates and pseudo R-squared model values calculated by the *MuMin* package (Bartoń, 2019). After model selection, the best generalised least squares model was re-fitted using REML for model interpretation to assess the predictive effect of spatial autocorrelation on weevil damage. Along with the GLS model effect size, semi-variograms of the raw damaged area mm² data confirmed that spatial autocorrelation between saplings was negligible within the Common Garden and so spatial autocorrelation structures were not included in other models.

Results

Sapling damage

36.9% (248/672) of the saplings in the common garden were damaged by *H. abietis* feeding activity. Figure 4 shows the number of saplings damaged divided into their origin seed collection Sites. All saplings were alive prior to data collection and sapling mortality was not recorded during the experiment. All seed populations had at least eight affected saplings out of a total of 32. The population with the highest number of damaged saplings was Loch Clair (LC), which had 18 damaged saplings. The sapling with the highest mm² damaged area was from Cona Glen (CG) and had 325.8 mm² of bark damaged. Rhidorroch (RD) had the highest cumulative damaged area with 1057.1 mm². Variation in bark area damaged within seed populations was high (Figure 5), with some geographic zones having similar levels of damage while others varied a widely within geographic zone.

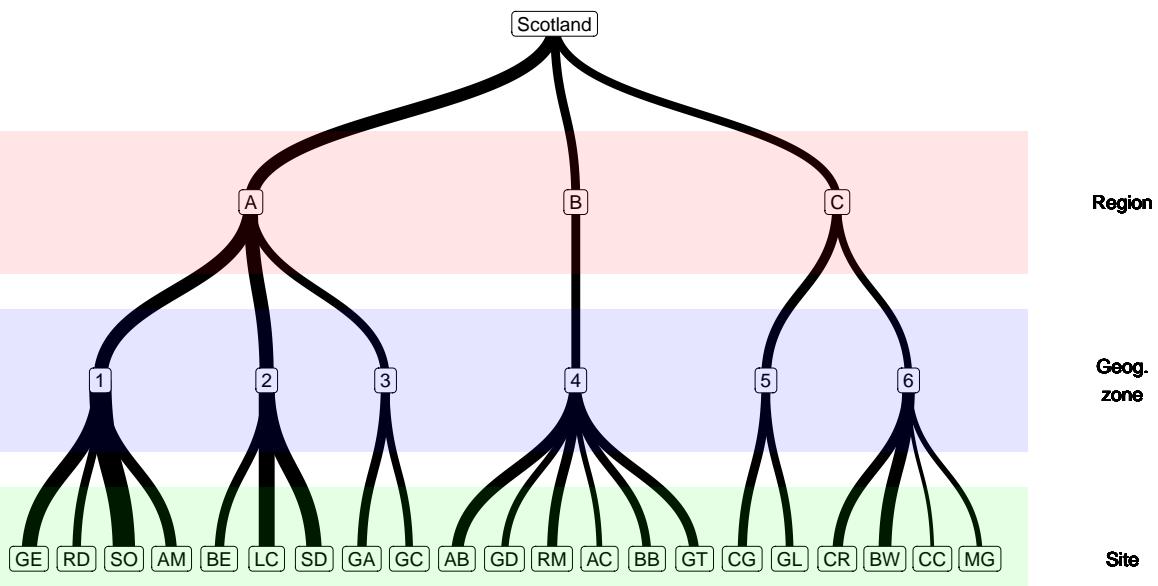


Figure 3: Dendrogram showing nested grouping of seed populations. Graph edge widths vary relatively according to the total bark area damaged on saplings collected from each Site. Width edges are weighted according to the number of saplings at each grouping level to account for differences in number of Sites per Geographic Zone and Region. This means edge widths should not be compared across vertical node levels.

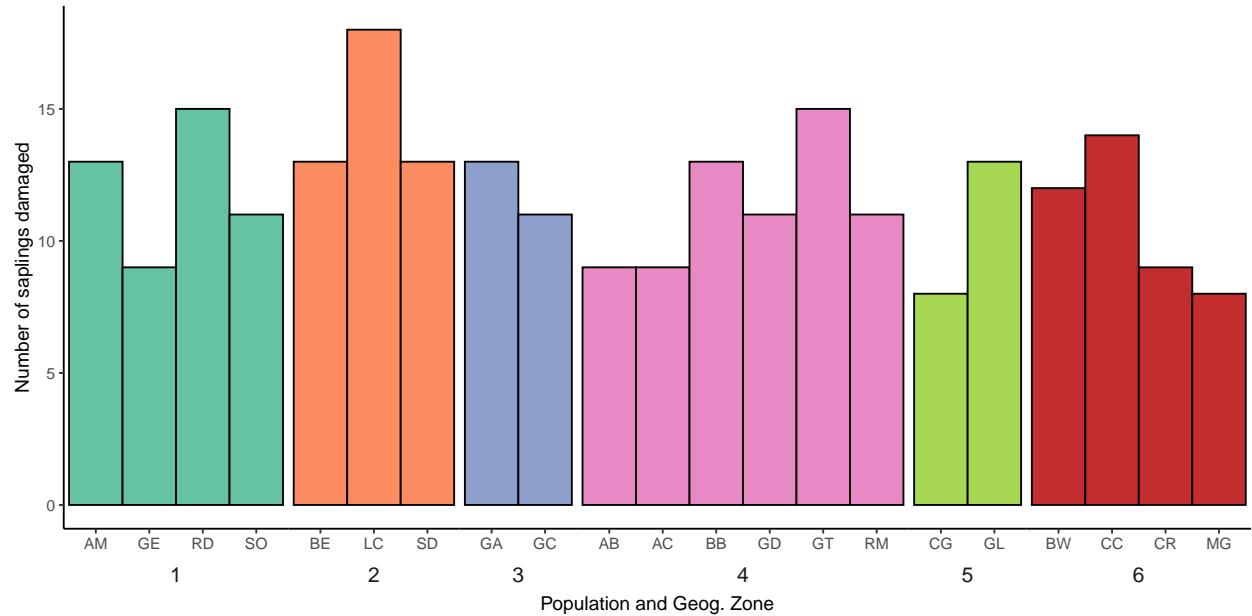


Figure 4: The number of saplings with visible damage by *H. abietis*, divided by Site. Groups of bars denote Geographic Zones.

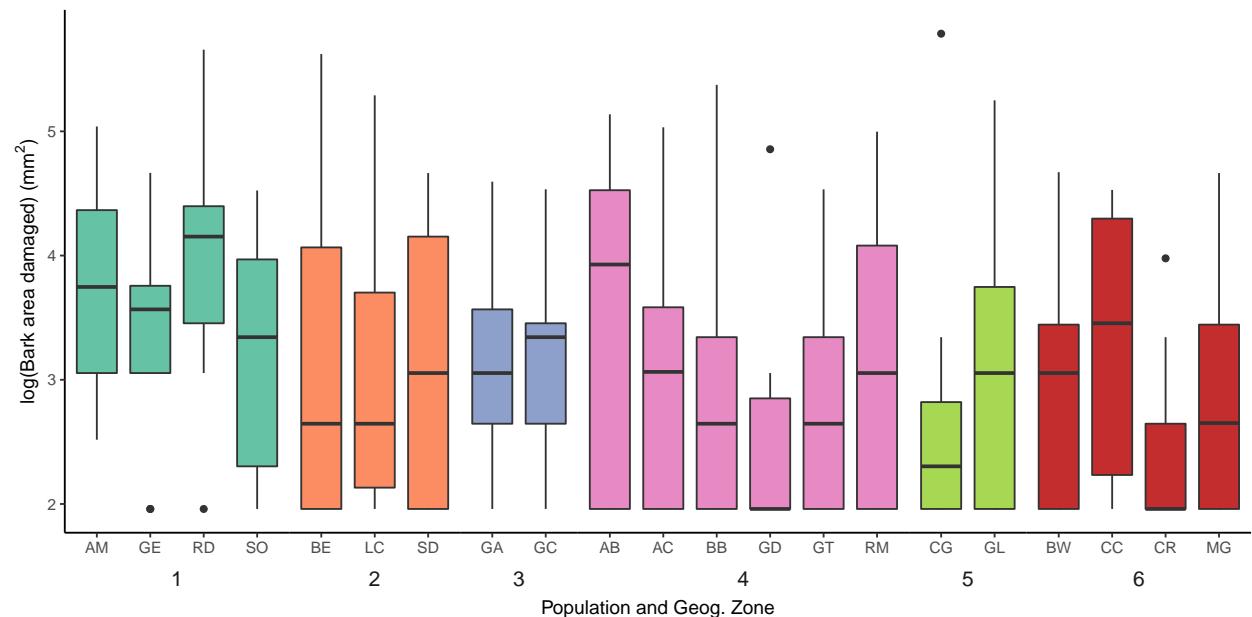


Figure 5: Variation in bark area damaged by *H. abietis*, divided by Site. Coloured groups of bars denote Geographic Zones. Thick bars denote the median value per population.

227 Spatial auto-correlation

228 Multiple Generalised Least Squares (GLS) models of damaged sapling area fitted with different
 229 correlation structures were compared against a null model with no correlation structure using AIC
 230 values (Table 1). A Gaussian correlation structure fit the data best, but explained only a very low
 231 percentage of the variation in sapling damaged area. Gaussian, Exponential and Rational quadratic
 232 models had AIC values within 2 points of each other and explained only negligibly different amounts
 233 of variation in damaged bar area, according to pseudo-R² model values, so these models can be

234 interpreted as fitting the data similarly well. All three models were better than a null model which
 235 explained none of the variation in damaged bark area. A semivariogram of damaged bark area
 236 with distance between saplings showed that there was no appreciable spatial auto-correlation, with
 237 all adjacent sapling distances occurring after the nugget of the semivariogram (Figure 7). This
 238 was supported by a visual inspection of a schematic map of damaged bark area per sapling in the
 239 common garden (Figure 6). As a result, further modelling with mixed effects models did not include
 240 a spatial auto-correlation covariate structure.

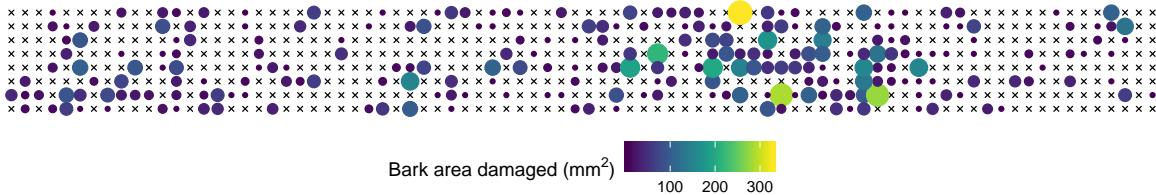


Figure 6: Schematic diagram of sapling relative position within the Common Garden, with sapling points coloured and sized according to the area of bark damaged. The distance between saplings is 3 m in both the X and Y directions.

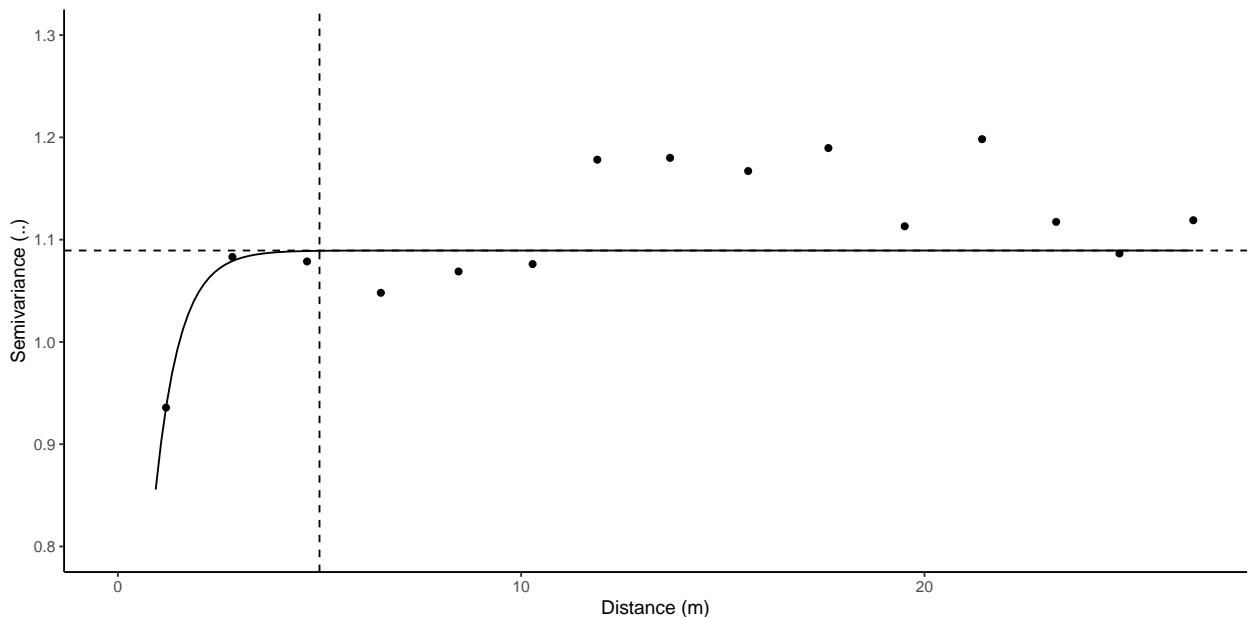


Figure 7: Semivariogram showing spatial autocorrelation of damaged bark area according to distance between saplings. Vertical dotted line denotes the nugget and the horizontal dotted line denotes the sill of the semivariogram.

Table 1: Model comparison of Generalised Least Squares models predicting damaged sapling bark area using different spatial autocorrelation structures. Models are ordered by increasing AIC value.

Cor. Struct.	AIC	logLik	R^2_c
Gaussian	719.573	-355.786	0.033
Exponential	720.306	-356.153	0.031
Rational quadratic	720.496	-356.248	0.028
Null	725.404	-360.702	0
Spherical	728.224	-360.112	0.004
Linear	728.224	-360.112	0.004

241 The effect of seed population on sapling damage

242 Binomial model

243 The first part of the hurdle model process explored variation among seed populations in the prob-
 244 ability of a sapling being damaged by *H. abietis*. The most parsimonious model was a null model,
 245 as estimated by AIC values. Fixed effects models using Geographic Zone and Site explained little
 246 of the variance in likelihood of a sapling being damaged, while models using Parent as the fixed
 247 effect explained ~95% (R^2_m) of the variance (Table 2). Parent models were the least parsimonious
 248 however, with ΔAIC values of 147.73 and 145.73. The fixed effect of Parent accounted for most
 249 of the model variance for those models ($R^2_c = \sim 94\%$), however a pairwise comparison of marginal
 250 means for each family revealed that none differed significantly from each other, indicating the model
 251 is likely overfitted due to the large number of Parent groups. We compared marginal means of the
 252 fixed effect groups for the best fitting models using Geographic Zone or Site as fixed effects and
 253 found that these groups did not vary significantly in a pairwise comparison (Figure 9a,b).

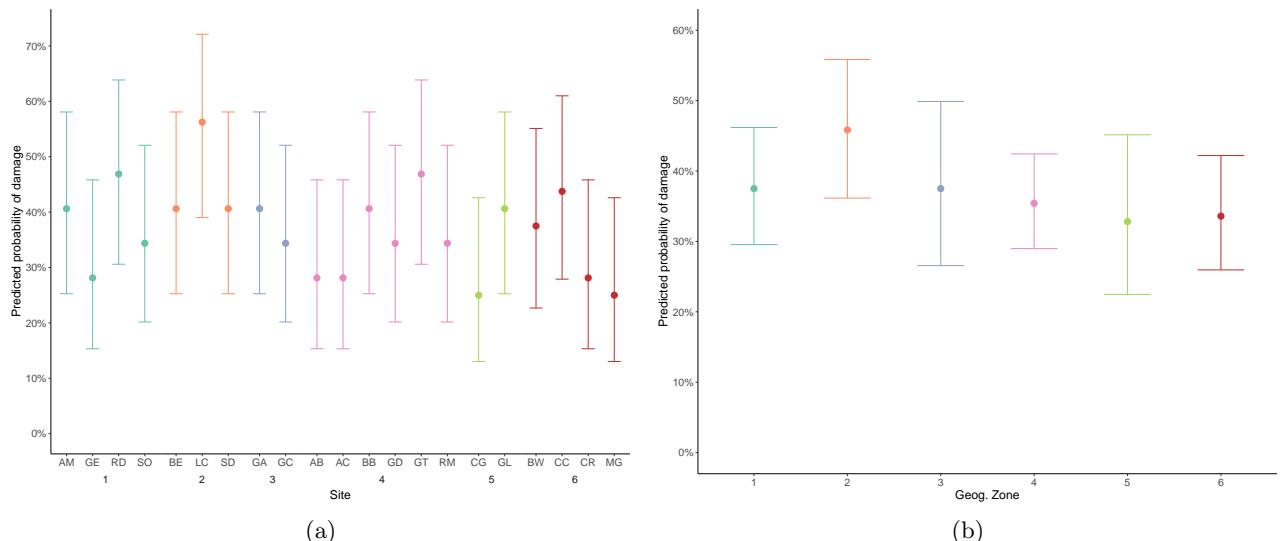


Figure 8: Predicted values with 95% confidence intervals for the probability of a sapling being damaged with seed collected from different (a) Sites and (b) aggregated by Geographic Zone.

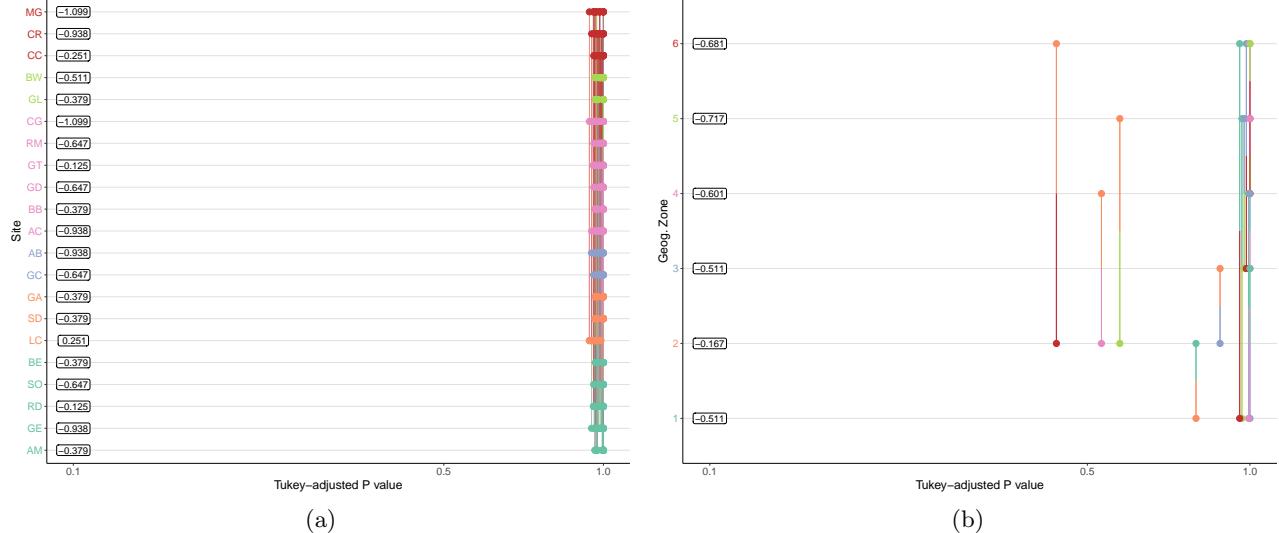


Figure 9: P-values for Tukey HSD pairwise comparisons of estimated marginal means between (a) Site and (b) Geographic Zone).

Table 2: Model comparison of logistic generalised linear mixed effects models predicting the likelihood of a sapling being attacked by *H. abietis*. Models are sorted according to increasing AIC.

Fixed eff.	Random eff.	AIC	logLik	R ² _c	R ² _m
NA	NA	886.953	-442.476	0	0
NA	Parent	888.953	-442.476	0	0
NA	Site	888.953	-442.476	0	0
NA	Site / Parent	890.953	-442.476	0	0
NA	Geog. Zone / Site / Parent	892.953	-442.476	0	0
Geog. Zone	Parent	894.462	-440.231	0.006	0.008
Geog. Zone	Site / Parent	896.462	-440.231	0.006	0.008
Site	Parent	910.721	-433.361	0.027	0.035
Site	Geog. Zone	910.721	-433.361	0.027	0.035
Site	Geog. Zone + Parent	912.721	-433.361	0.027	0.035
Parent	Geog. Zone	1034.683	-348.341	0.940	0.953
Parent	Geog. Zone / Site	1036.683	-348.341	0.941	0.954

254 The fixed effect of Site was weak as a predictor of likelihood of *H. abietis* damage. In a model
 255 using seed population as a fixed effect and Parent as a random intercept effect, seed population
 256 only accounted for 2.7% (R^2_m) of the variation in the probability that a sapling would be initially
 257 damaged by *H. abietis*. According to the best fitting model with Site as a fixed effect, saplings from
 258 Beinn Eighe (BE) had a greater chance of being initially damaged than others (Figure 8a), however,
 259 these predicted values were not significantly different from other Sites according to a comparison of
 260 marginal means (Figure 9a).

261 Non-zero damage model

262 The second part of the hurdle model explored variation in the area of sapling bark damaged by *H.*
 263 *abietis*, for those saplings which were initially damaged. The most parsimonious model according
 264 to AIC included the fixed effect of Geographic Zone and the random effect of Site to account for

265 pseudo-replication in seed origin. This model explained 5.6% (R^2_m) of the variance in sapling bark
 266 area damaged. This model was of better quality than a null model and multiple random effects
 267 models. As with the logistic models, models with Parent as a fixed effect explained more variance
 268 in damaged bark area, but a pairwise comparison of estimated marginal means showed that no
 269 families differed significantly from each other, indicating that the model is over-fitted. The Parent
 270 BE4, collected from Beinn Eighne contrasted weakly with a number of families from more southerly
 271 Sites, but none significantly ($P < 0.05$) (Figure 12).

272 In a pairwise comparison of estimated marginal means of Geographic Zones for the best-fitting
 273 model, Geographic Zone 1 differed significantly from Zones 4 and 6, and weakly with Zone 5. At
 274 the Site level, Rhiddoroch (RD) differed from Glen Derry and Crannach, both populations in the
 275 southern part of the study region. Geographic Zone one had a higher predicted damaged bark area
 276 according to the best fitting model (Figure 10b). AC and CC had a higher predicted damaged bark
 277 area than other Sites.

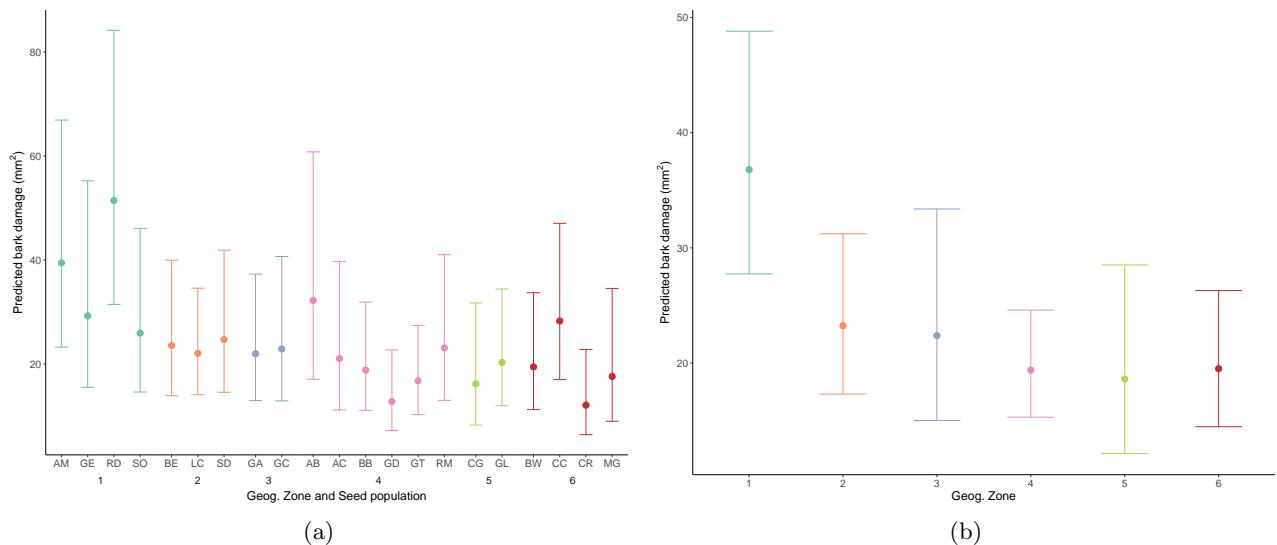


Figure 10: Predicted values of mm^2 for saplings with seed collected from different Geographic Zones.

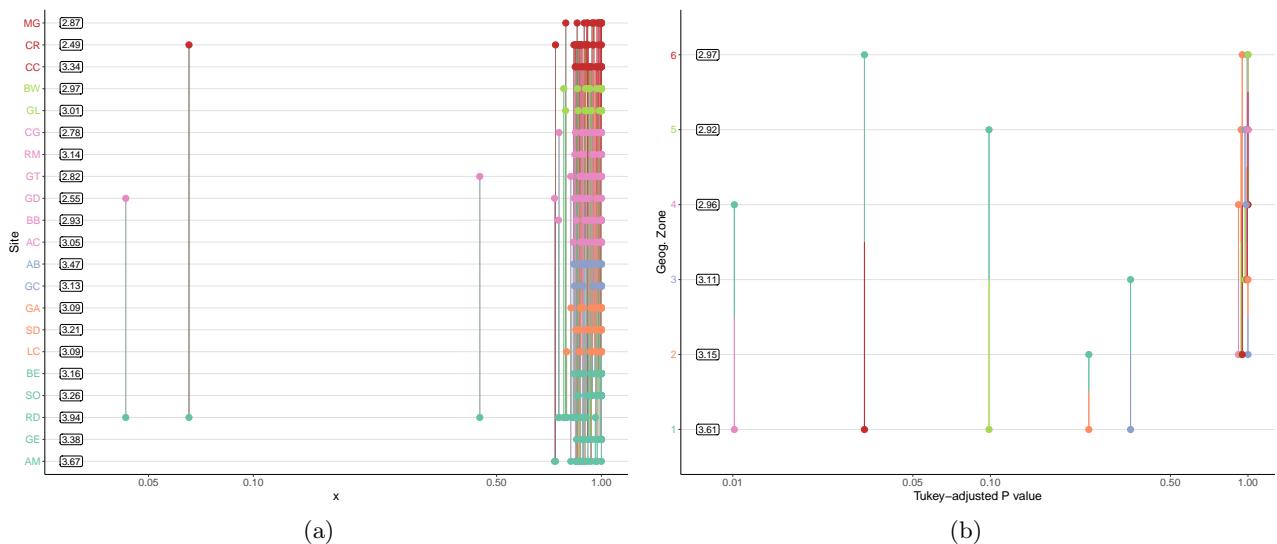


Figure 11: P-values for Tukey HSD pairwise comparisons of estimated marginal means between (a) Site and (b) Geographic Zone).

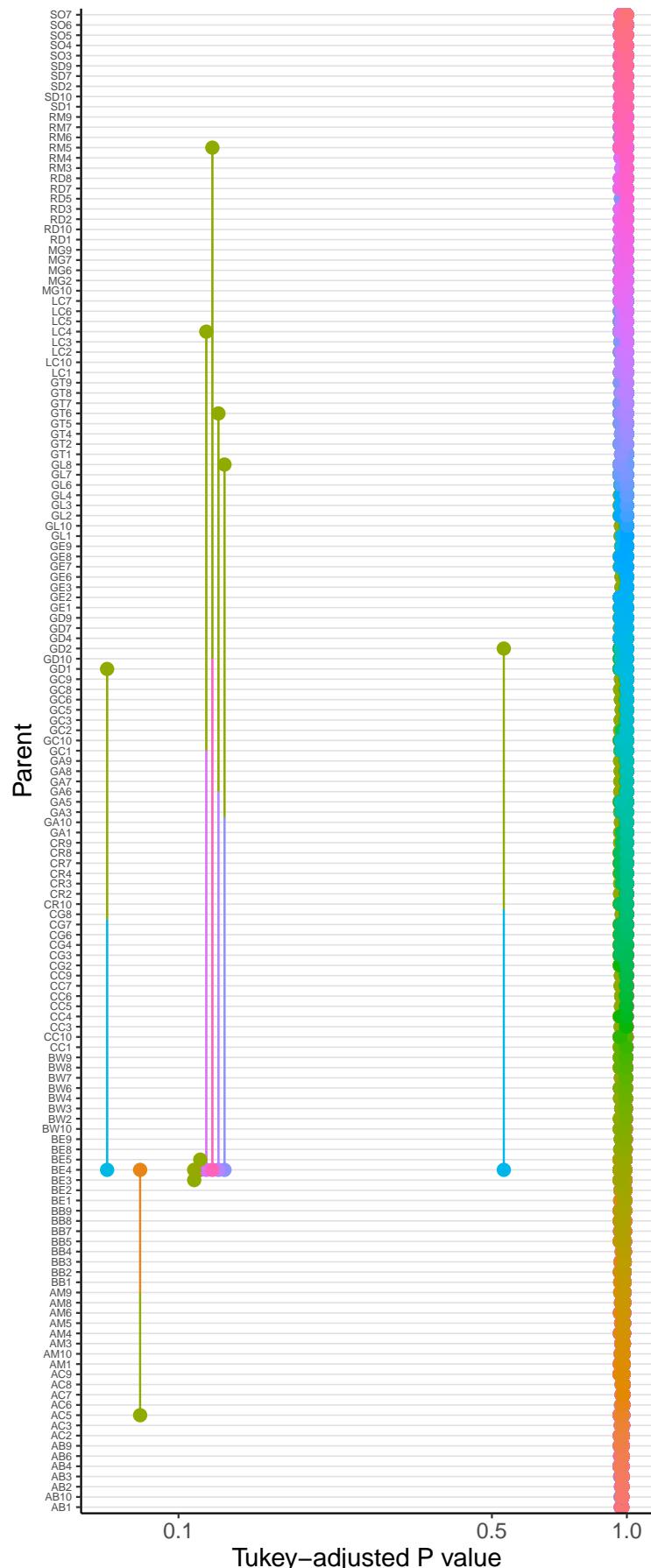


Figure 12: P-values for Tukey HSD pairwise comparisons of estimated marginal means between sapling Parent groups.

Table 3: Model comparison of general linear mixed effects models predicting the damaged bark area of a sapling, for those saplings which have been initially damaged. Models are sorted according to increasing AIC.

Fixed eff.	Random eff.	AIC	logLik	R ² _c	R ² _m
Geog. Zone	Site	719.471	-351.735	0.056	0.056
NA	NA	721.787	-358.893	0	0
NA	Geog. Zone / Site	722.193	-357.096	0.033	0
NA	Site / Parent	724.929	-358.464	0.026	0
Site	Geog. Zone	736.127	-345.063	0.106	0.106
Site	Parent	736.127	-345.063	0.106	0.106
Site	Geog. Zone + Parent	738.127	-345.063	0.106	0.106
Parent	Geog. Zone	810.131	-256.066	0.565	0.565
Parent	Geog. Zone / Site	812.131	-256.066	0.565	0.565
Geog. Zone	Site / Parent			0.056	0.056

278 Population level spatial patterns

279 There was a weak but significant positive effect of latitude on the total bark area damaged by *H.*
 280 *abietis* ($Z = 3.249(1, 248)$, $p = <0.005$, $R^2_m = 0.041$), when the nested random effects of seed
 281 collection Site and family were accounted for (Figure 14). In a similar model, there was no effect of
 282 longitude on bark area damaged ($Z = -1.377(1, 248)$, $p = 0.168$, $R^2_m = 0.009$).

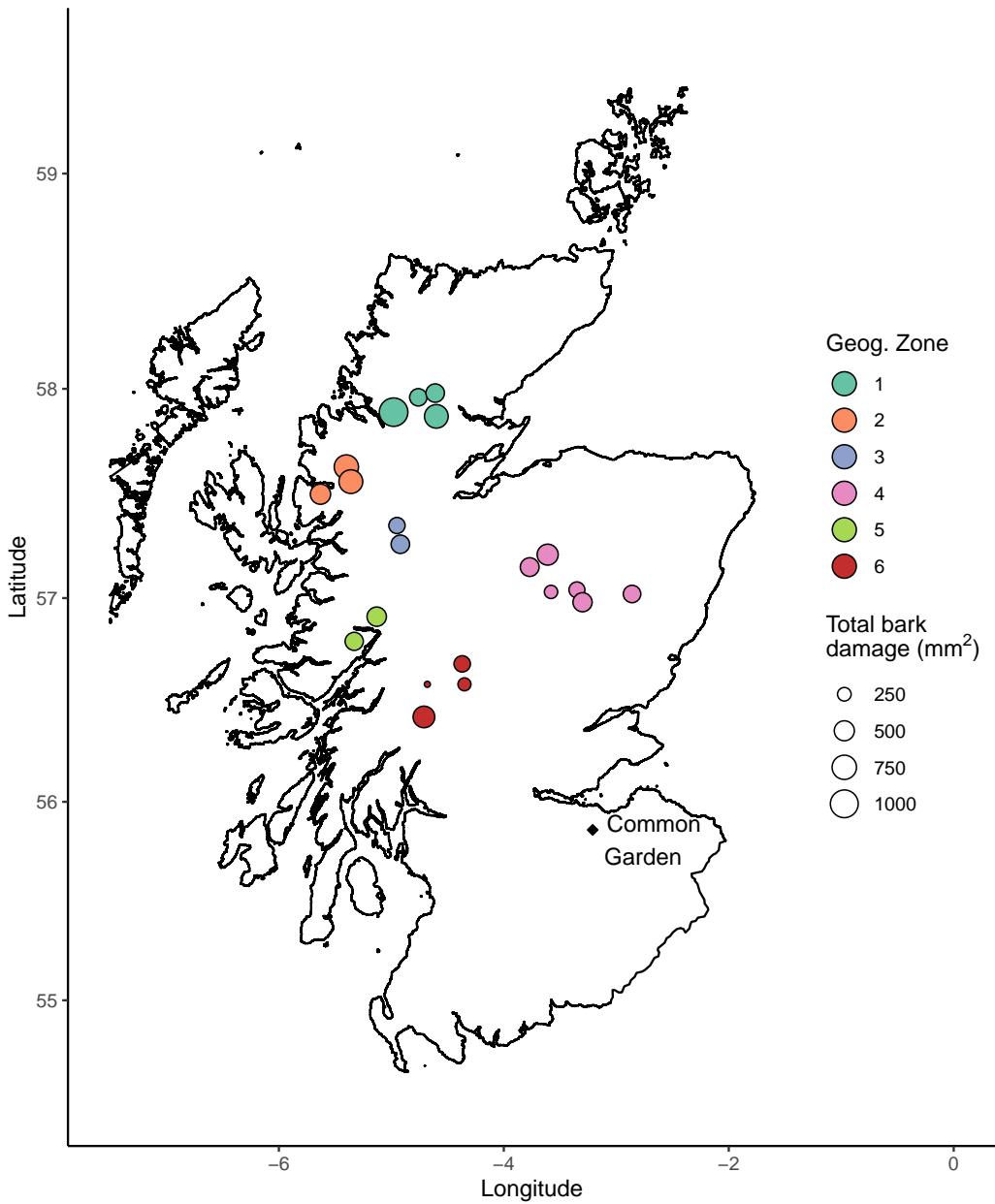


Figure 13: Map of study Sites with bubbles coloured according to Geographic Zone and relatively sized according to the total bark area damaged for all 32 saplings per Site.

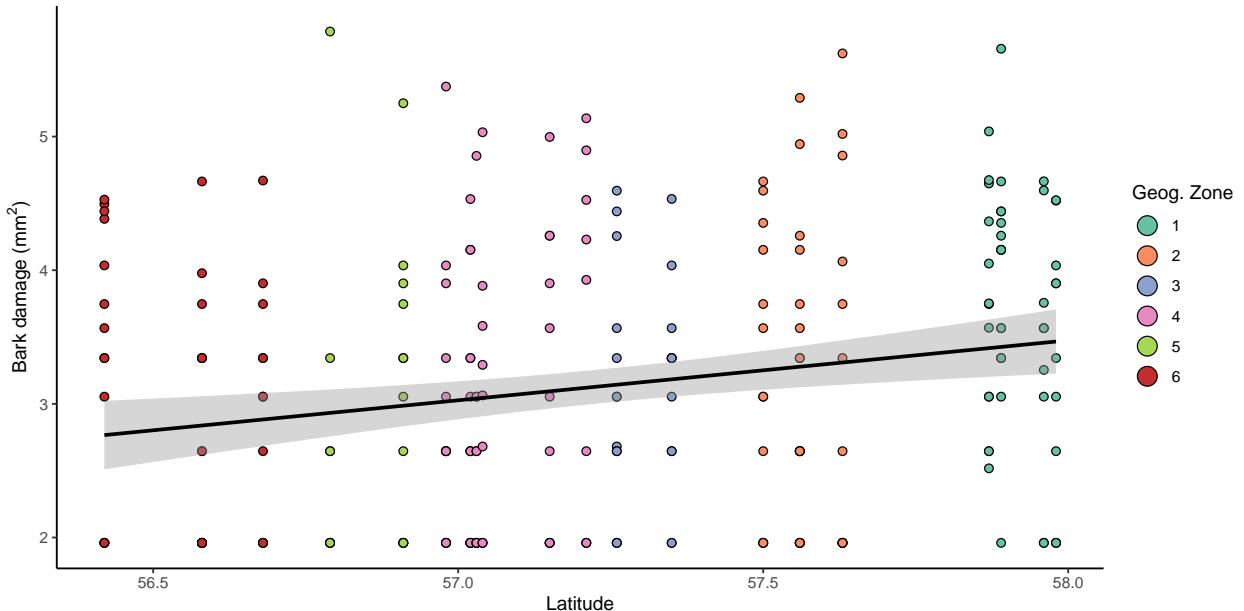


Figure 14: Relationship between bark area damaged and latitude of sapling population, for those saplings which were damaged. Each point is an individual sapling. Points are coloured by Geographic Zone. The linear model fit (black line with grey 95% confidence interval) shows a weak positive trend.

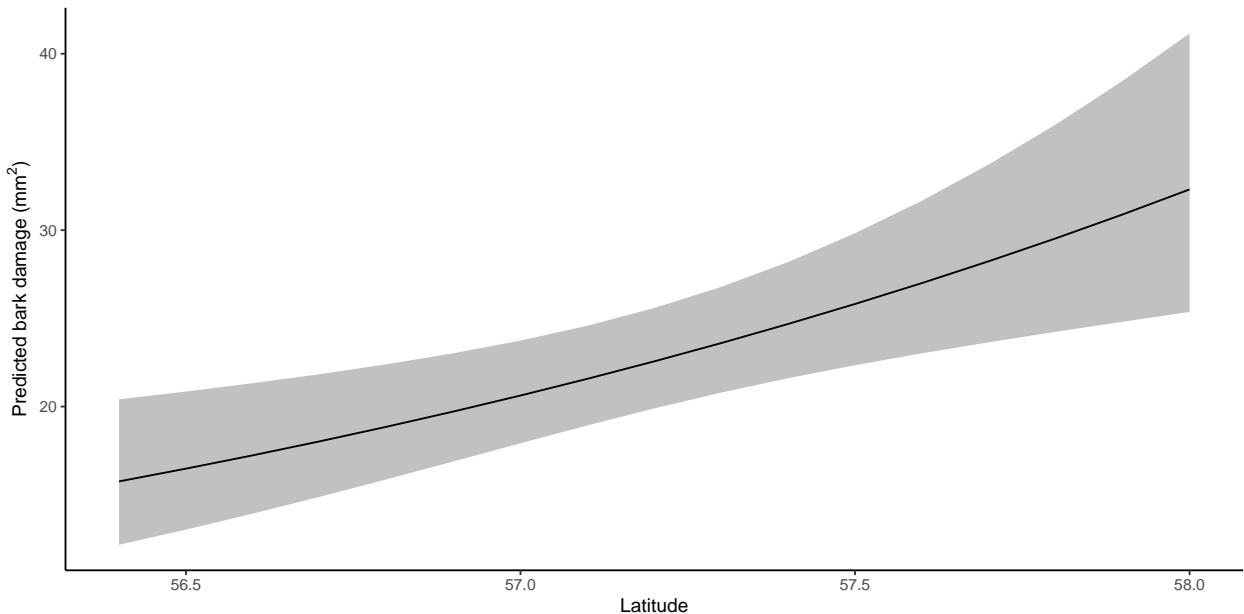


Figure 15: Predicted values with 95% confidence interval for the bark area damaged on a sapling with seed collected at different latitudes.

283 Discussion

284 The model selection process determined that there was an effect of Geographic Zone on the area
 285 of weevil damage found on damaged saplings and a possible weak effect of Site, but could not
 286 account for variation in the probability that a sapling became damaged initially. Linear Mixed
 287 Effects models demonstrated that there was a general latitudinal effect on sapling damaged area.

288 Saplings with Parents at higher latitudes typically experienced higher levels of damage, but this
289 had a much weaker effect than population or Geographic Zone itself. Within Site variability among
290 Parents was high and diluted the effects of Geographic Zone and Site. This is expected given the
291 high gene flow between populations (Donnelly et al., 2018). Nevertheless, some clear variation was
292 observed between groups.

293 In this study we identified weak but significant differences between *P. sylvestris* populations in
294 their susceptibility to *H. abietis* attack. It appears that a weak latitudinal pattern may be driving
295 these differences. It may be that historical exposure to *H. abietis* in more southerly populations
296 has driven adaptation to develop defensive structures to deter bark feeding insects. Studies on the
297 distribution and life cycle of *H. abietis* have shown that life cycle length is strongly linked with
298 mean temperature in the summer months, with higher temperatures leading to a short life cycle
299 and therefore higher numbers of pine weevils where infestations occur (Leather et al., 1999). *H.*
300 *abietis* abundance reduces with latitude in Scotland (Barredo et al., 2015). Historically, *H. abietis*
301 populations at high latitudes and in the west of Scotland have been low (Leather et al., 1999),
302 due to lower temperatures (Wainhouse et al., 2014). The latitudinal effect may therefore be a
303 result of adaptation to resist *H. abietis* damage. Additionally, phenological variation in latitudinal
304 populations may lead to VOC concentrations varying between saplings at the same time of year
305 in the common garden, making some saplings more desirable than others (Guenther, 1997). The
306 data collection for this study took place in June, approximately between the two seasonal peaks
307 of *H. abietis* activity. Other studies have shown that the growing season of *P. sylvestris* from
308 higher latitudes starts later in the year (Salmela et al., 2013), leading to a lower concentration of
309 VOC stored in bark resin when our study was conducted, potentially making these saplings more
310 attractive than those from southerly populations to *H. abietis*.

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

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

328 Conclusion

329 This study sought to test whether adaptive variation for resistance to the large pine weevil (*Hylobius*
330 *abietis*) existed in genetically distinct populations of scots pine (*Pinus sylvestris*) in Caledonian
331 remnant forest patches in Scotland. A weak positive effect of latitude of seed collection Site was
332 found in the damaged area of sapling bark, suggesting that more southerly populations may be
333 less attractive to *H. abietis* attack. No relationship was found between Site and probability of a

334 sapling being initially damaged. Variation exists between Geographic Zones and between Sites with
335 regards to resistance to continued *H. abietis* attack. The conclusions of this study suggest that under
336 warming temperatures as a result of anthropogenic climate change, there is a conservation concern
337 for less-resistant Caledonian remnant forest patches as *H. abietis* populations may increase, leading
338 to higher mortality of saplings, leading to changes in ecosystem structure. It is suggested that further
339 studies should investigate bark morphological and physiological variation amongst these natural
340 populations of *P. sylvestris*, with particular attention to variation in Volatile Organic Compounds
341 (VOCs) emitted when bark is damaged as a defensive response in young saplings, to understand
342 the underlying mechanism driving differences between these genetically distinct populations.

343 References

- 344 Alfaro, R. (1989), Stem defects in sitka spruce induced by sitka spruce weevil, *pissodes strobi* (peck),
345 in R. Alfaro and S. Glover, eds, 'Insects affecting reforestation: biology and damage', Forestry
346 Canada, Berlin, Germany, pp. 177–185.
- 347 Alfaro, R. I., Fady, B., Vendramin, G. G., Dawson, I. K., Fleming, R. A., Sáenz-Romero, C.,
348 Lindig-Cisneros, R. A., Murdock, T., Vinceti, B., Navarro, C. M., Skrøppa, Baldinelly, G., El-
349 Kassaby, Y. A. and Loo, J. (2014), 'The role of forest genetic resources in responding to biotic and
350 abiotic factors in the context of anthropogenic climate change', *Forest Ecology and Management*
351 **333**, 76–87.
- 352 Alfaro, R. I., King, J. N. and vanAkker, L. (2013), 'Delivering sitka spruce with resistance against
353 white pine weevil in british columbia, canada', *The Forestry Chronicle* **89**(2), 235–245.
- 354 Antwi, F. B. and Reddy, G. V. P. (2015), 'Toxicological effects of pyrethroids on non-target aquatic
355 insects', *Environmental Toxicology and Pharmacology* **40**, 915–923.
- 356 Barredo, J. I., Strona, G., de Rigo, D., Caudullo, G., Stanganelli, G. and San-Miguel-Ayanz, J.
357 (2015), 'Assessing the potential distribution of insect pests: case studies on large pine weevil
358 (*Hylobius abietis* l) and horse-chestnut leaf miner (*Cameraria ohridella*) under present and future
359 climate conditions in european forests', *EPPO Bulletin* **45**(2), 273–281.
- 360 Bartoń, K. (2019), *MuMIn: Multi-Model Inference*. R package version 1.43.6.
- 361 Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H.
362 and White, J. S. (2008), 'Generalized linear mixed models: a practical guide for ecology and
363 evolution', *Trends in Ecology and Evolution* **24**(3), 127–135.
- 364 Boucher, D., Lavallée, R. and Mauffette, Y. (2001), 'Biological performance of the white pine weevil
365 in relation to the anatomy of the resin canal system of four different host species', *Canadian
366 Journal of Forest Research* **31**, 2035–2041.
- 367 Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug,
368 H. J., Maechler, M. and Bolker, B. M. (2017), 'glmmTMB balances speed and flexibility among
369 packages for zero-inflated generalized linear mixed modeling', *The R Journal* **9**(2), 378–400.
- 370 Byun-McKay, A., Godard, K., Toudefallah, M., Martin, D. M., Alfaro, R., King, J., Bohlmann,
371 J. and Plant, A. L. (2006), 'Wound-induced terpene synthase gene expression in sitka spruce
372 that exhibit resistance or susceptibility to attack by the white pine weevil', *Plant Physiology*
373 **140**, 1009–1021.
- 374 Carlisle, A. and Brown, A. H. F. (1968), 'Pinus sylvestris, l.', *Journal of Ecology* **56**(1), 269–307.

- 375 Dillon, A. B., Ward, D., Downes, M. J. and Griffin, C. T. (2006), 'Suppression of the large pine weevil
376 *Hylobius abietis* (l.) (coleoptera: Curculionidae) in pine stumps by entomopathogenic nematodes
377 with different foraging strategies', *Biological Control* **38**, 217–226.
- 378 Dines, T. D., Jones, R. A., Leach, S. J., McKean, D. R., Pearman, D. A., Preston, C. D., Rumsey,
379 F. J. and Taylor, I. (2005), 'The vascular plant red data list for great britain. species status 7'.
- 380 Donnelly, K., Cavers, S., Cottrell, J. E. and Ennos, R. A. (2016), 'Genetic variation for needle traits
381 in scots pine (*Pinus sylvestris* l.)', *Tree Genetics & Genomes* **12**(3), 40.
- 382 Donnelly, K., Cavers, S., Cottrell, J. E. and Ennos, R. A. (2018), 'Cryptic genetic variation and
383 adaptation to waterlogging in caledonian scots pine, *Pinus sylvestris* l.', *Ecology and Evolution*
384 **8**, 8665—8675.
- 385 Edwards, C. E. and Mason, W. L. (2006), 'Stand structure and dynamics of four native scots pine
386 (*Pinus sylvestris* l.) woodlands in northern scotland', *Forestry* **79**, 261–277.
- 387 Evans, H., McAllister, F., Saunders, T., Moore, R., Jenkins, T., Butt, T., Ansari, M., Griffin,
388 G. Williams, C., Teek, R. and Sweeney, J. (2015), 'The impact project guide to *Hylobius* man-
389 agement 2015'.
- 390 Eyles, A., Bonello, P., Ganley, R. and Mohammed, C. (2009), 'Induced resistance to pests and
391 pathogens in trees', *New Phytologist* **185**, 893–908.
- 392 Farrell, B. D., Dussourd, D. E. and Mitter, C. (1991), 'Escalation of plant defense: do latex and
393 resin canals spur plant diversification?', *The American Naturalist* **138**(4), 881–900.
- 394 *Forestry Statistics 2018: A compendium of statistics about woodland, forestry and primary wood*
395 *processing in the United Kingdom* (2018).
- 396 GBIF (2019), 'Gbif occurrence download'.
- 397 Gershenzon, J. and Croteau, R. (1991), Terpenoids, in G. A. Rosenthal and M. Berenbaum, eds,
398 'Herbivores: Their interactions with secondary plant metabolites', Elsevier Science, Oxford,
399 pp. 165–219.
- 400 Gill, R. M. A. (1992), 'A review of damage by mammals in north temperate forests: 3. impact on
401 trees and forests', *Forestry* **65**(4), 363–388.
- 402 González-Díaz, P., Cavers, S., Iason, G. R., Booth, A., Russell, J. and Jump, A. S. (2018), 'Weak
403 isolation by distance and geographic diversity gradients persist in scottish relict pine forest',
404 *iForest* **11**, 449–458.
- 405 Guenther, A. (1997), 'Seasonal and spatial variations in natural volatile organic compound emis-
406 sions', *Ecological Applications* **7**(1), 34–45.
- 407 Heijari, J., Blande, J. D. and Holopainen, J. K. (2011), 'Feeding of large pine weevil on scots
408 pine stem triggers localised bark and systemic shoot emission of volatile organic compounds',
409 *Environmental and Experimental Botany* **71**, 390–398.
- 410 Heritage, S. and Moore, R. (2000), 'The assessment of site characteristics as part of a management
411 strategy to reduce damage by *Hylobius*'.
- 412 Inward, D. J. G., Wainhouse, D. and Peace, A. (2012), 'The effect of temperature on the development
413 and life cycle regualtion of the pine weevil *Hylobius abietis* and the potential impacts of climate
414 change', *Agricultural and Forest Entomology* **14**, 348–357.
- 415 Kapranas, A., Malone, B., Quinn, S., O'Tuama, P., Peters, A. and Griffin, C. T. (2017), 'Optimizing
416 the application method of entomopathogenic nematode suspension for biological control of large
417 pine weevil *Hylobius abietis*', *BioControl* **62**, 659–667.

- 418 Keeling, C. I. and Bohlmann, J. (2006), 'Genes, enzymes and chemicals of terpenoid diversity in
419 the constitutive and induced defence of conifers against insects and pathogens', *New Phytologist*
420 **170**, 657–675.
- 421 King, J. N., Alfaro, R. I., Lopez, M. G. and van Akker, L. (2011), 'Resistance of sitka spruce
422 (*Picea sitchensis* (bong.) Carr.) to white pine weevil (*Pissodes strobi* Peck): characterizing the
423 bark defence mechanisms of resistant populations', *Forestry* **84**(1), 83–91.
- 424 Kiss, G. K. and Yanchuk, A. D. (1991), 'Preliminary evaluation of genetic variation of weevil
425 resistance in interior spruce in British Columbia', *Canadian Journal of Forest Research* **21**(2), 230–
426 234.
- 427 Kivimäenpää, M., Magsarjav, N., Ghimire, R., Markkanen, J., Heijari, J., Vuorinen, M. and
428 Holopainen, J. K. (2012), 'Influence of tree provenance on biogenic VOC emissions of Scots pine
429 (*Pinus sylvestris*) stumps', *Atmospheric Environment* **60**, 477–485.
- 430 L., R. (2019), *emmeans: Estimated Marginal Means, aka Least-Squares Means*. R package version
431 1.3.5.1.
- 432 Leather, S. R., Day, K. R. and Salisbury, A. N. (1999), 'The biology and ecology of the large
433 pine weevil, *Hylobius abietis* (Coleoptera: Curculionidae): a problem of dispersal?', *Bulletin of
434 Entomological Research* **89**, 3–16.
- 435 Manlove, J. D., Styles, J. and Leather, S. R. (1997), 'Feeding of the adults of the large pine weevil,
436 *Hylobius abietis* (Coleoptera: Curculionidae)', *European Journal of Entomology* **94**, 153–156.
- 437 Mason, W. L., Hampson, A. and Edwards, C. (2004), 'Managing the pinewoods of Scotland'.
- 438 Mc Namara, L., Kapranas, A., Williams, C. D., O'Tuama, P., Kavanagh, K. and Griffin, C. T.
439 (2018), 'Efficacy of entomopathogenic fungi against large pine weevil *Hylobius abietis*, and their
440 additive effects when combined with entomopathogenic nematodes', *Journal of Pest Science*
441 **91**, 1407–1419.
- 442 Mian, L. S. and Mulla, M. S. (1992), 'Effects of pyrethroid insecticides on nontarget invertebrates
443 in aquatic ecosystems', *Journal of Agricultural Entomology* **9**(2), 73–98.
- 444 Nordenhem, H. (1989), 'Age, sexual development, and seasonal occurrence of the pine weevil *Hylo-
445 bius abietis* (L.)', *Journal of Applied Entomology* **108**, 260–270.
- 446 Nordlander, G. (1987), 'A method for trapping *Hylobius abietis* (L.) with a standardized bait and its
447 potential for forecasting seedling damage', *Scandinavian Journal of Forest Research* **2**, 199–213.
- 448 Nordlander, G. (1991), 'Host finding in the pine weevil *Hylobius abietis*: effects of conifer volatiles
449 and added limonene', *Entomologia Experimentalis et Applicata* **59**, 229–237.
- 450 Nordlander, G., Eidmann, H. H., Jacobsson, U., Nordenhem, H. and Sjödin, K. (1986), 'Orientation
451 of the pine weevil *Hylobius abietis* to underground sources of host volatiles', *Entomologia
452 Experimentalis et Applicata* **41**, 91–100.
- 453 Ördlander, G. and Nilsson, U. (1999), 'Effect of reforestation methods on pine weevil (*Hylobius
454 abietis*) damage and seedling survival', *Scandinavian Journal of Forest Research* **14**, 341–354.
- 455 Ördlander, G., Nilsson, U. and Nordlander, G. (1997), 'Pine weevil abundance on clear-cuttings
456 of different ages: a 6-year study using pitfall traps', *Scandinavian Journal of Forest Research*
457 **12**, 225–240.
- 458 Perry, A., Wachowiak, W., Brown, A. V., Ennos, R. A., Cottrell, J. E. and Cavers, S. (2016),
459 'Substantial heritable variation for susceptibility to *Dothistroma septosporum* within populations
460 of native British Scots pine (*Pinus sylvestris*)', *Plant Pathology* **65**, 987–996.

- 461 R Core Team (2019), *R: A Language and Environment for Statistical Computing*, R Foundation for
462 Statistical Computing, Vienna, Austria.
- 463 Rahman, A., Viiri, H., Pelkonen, P. and Khanam, T. (2015), ‘Have stump piles any effect on the
464 pine weevil (*Hylobius abietis* l.) incidence and seedling damage?’, *Global Ecology and Conservation*
465 **3**, 424–432.
- 466 Rose, D. (2002), Control of the Large Pine Weevil, *Hylobius abietis*, L., PhD thesis, Imperial College,
467 London.
- 468 Rose, D., Leather, S. R. and Matthews, G. A. (2005), ‘Recognition and avoidance of insecticide-
469 treated scots pine (*Pinus sylvestris*) by *Hylobius abietis* (coleoptera: Curculionidae): implications
470 for pest management strategies’, *Agricultural and Forest Entomology* **7**, 187–191.
- 471 Salmela, M. J., Cavers, S., Cottrell, J. E., Iason, G. R. and Ennos, R. A. (2013), ‘Spring phenology
472 shows genetic variation among and within populations in seedlings of scots pine (*Pinus sylvestris*
473 l.) in the scottish highlands’, *Plant Ecology & Diversity* **6**(3-4), 523–536.
- 474 Schiebe, C., Hammerbacher, A., Birgersson, G., Witzell, J., Brodelius, P. E., Gershenson, J., Hans-
475 son, B. S., Krokene, P. and Schlyter, F. (2012), ‘Inducibility of chemical defenses in norway
476 spruce bark is correlated with unsuccessful mass attacks by the spruce bark beetle’, *Oecologia*
477 **170**, 183–198.
- 478 Schneider, C. A., Rasband, W. S. and Eliceiri, K. W. (2012), ‘Nih image to imagej: 25 years of
479 image analysis’, *Nat Methods* **9**(7), 671–675.
- 480 Selander, J., Immonen, A. and Raukko, P. (1990), ‘Resistance of naturally regenerated and nursery-
481 raised scots pine seedlings to the large pine weevil’, *Folia Forestalia* **766**, 1–199.
- 482 Telford, A., Cavers, S. and Cottrell, J. E. (2014), ‘Can we protect forests by harnessing variation in
483 resistance to pests and pathogens’, *Forestry* **88**, 3–12.
- 484 Tilles, D. A., Sjödin, K., Nordlander, G. and Eidmann, H. H. (1986), ‘Synergism between ethanol
485 and conifer host volatiles as attractants for the pine weevil, *Hylobius abietis* (l.) (coleoptera:
486 Curculionidae)’, *Journal of Economic Entomology* **79**(4), 970–973.
- 487 Toivonen, R. and Viiri, H. (2006), ‘Adult large pine weevils hylobius abietis feed on silver birch
488 betula pendula even in the presence of conifer seedlings’, *Agricultural and Forest Entomology*
489 **8**, 121–128.
- 490 Trapp, S. and Croteau, R. (2001), ‘Defensive resin biosynthesis in conifers’, *Annual Review of Plant
491 Physiology and Plant Molecular Biology* **52**, 689–724.
- 492 Wainhouse, D., Inward, D. J. G. and Morgan, G. (2014), ‘Modelling geographical variation in
493 voltnism of *Hylobius abietis* under climate change and implications for management’, *Agricultural
494 and Forest Entomology* **16**, 136–146.
- 495 Wallertz, K., Nordenhem, H. and Nordlander, G. (2014), ‘Damage by the pine weevil *Hylobius abietis*
496 to seedlings of two native and five introduced tree species in sweden’, *Silva Fennica* **48**(4), 1–14.
- 497 Williams, C. D., Dillon, A. B., Harvey, C. D., Hennessy, R., McNamara, L. and Griffin, C. T. (2013),
498 ‘Control of a major pest of forestry, *Hylobius abietis*, with entomopathogenic nematodes and fungi
499 using eradicant and prophylactic strategies’, *Forest Ecology and Management* **305**, 212–222.
- 500 Willoughby, I., Evans, H., Gibbs, J., Pepper, H., Gregory, S., Dewar, J., Nisbet, T., Pratt, J.,
501 McKay, H., Siddons, R., Mayle, B., Heritage, S., Ferris, R. and Trout, R. (2004), ‘Reducing
502 pesticide use in forestry’.
- 503 Willoughby, I., Moore, R. and Nisbet, T. (2017), ‘Interim guidance on the integrated management
504 of *Hylobius abietis* in uk forestry’.