

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

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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. Breeding natural resistance in host plants to insect pests
10 has been used in many crop species to reduce damage as part of an integrated pest management
11 strategy. Here, we conducted a common garden experiment in a previously clearfelled forestry
12 plantation where *H. abietis* are known to occur. 672 saplings, grown from seed collected from
13 21 naturally occurring populations of *Pinus sylvestris* across Scotland were planted together in
14 a regular grid, as is common in plantation forestry, 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 between *P. sylvestris* population. In contrast we found that sapling populations did not
17 differ in their likelihood of being attacked by *H. abietis*. A latitudinal pattern was observed, with
18 saplings sourced from populations found further north being attacked more heavily than those
19 further south. From these results it is suggested that as part of an integrated pest management
20 strategy, planting of *P. sylvestris* saplings from more southerly populations may reduce pine
21 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
27 on the bark and buds of saplings (Figure 1) as a result of feeding may cause a reduction in growth
28 rate, stem deformation and an increased susceptibility to infection by airborne diseases of trees
29 (Leather et al., 1999). Heavy damage may lead to stem girdling and death of the terminal growing
30 bud resulting in a malformed trunk, limiting economic use as timber when fully grown (Alfaro,
31 1989; Gill, 1992). While *H. abietis* may inhabit adult coniferous trees in both natural and planted
32 coniferous forests, recently clearfelled and restocked coniferous plantation sites provide an enriched
33 habitat for breeding *H. abietis* and so pose more of a danger to planted saplings than those in
34 naturally regenerating stands (Willoughby et al., 2004; Ördlander and Nilsson, 1999). Adults lay
35 eggs within the stumps of clearfelled trees, which are rarely removed after clearfelling, with newly
36 emerged juvenile weevils feeding on young saplings (Willoughby et al., 2004). Planted coniferous
37 saplings are more susceptible to *H. abietis* damage than naturally regenerating saplings, probably
38 due to water stress as a result of damage to root systems during planting (Selander et al., 1990).



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.

39 A single adult weevil can damage several plants over the course of a season, with ~50% sapling
 40 mortality observed across affected plantation sites in the UK and Ireland (Heritage and Moore,
 41 2000). On commercial conifer plantations, *H. abietis* causes annual economic losses of €140 million
 42 *per annum* in Europe, of which €2.75 million (~£2.47 million) occurs in the UK (Evans et al., 2015).
 43 Currently, *H. abietis* is the most important pest of newly planted trees in Northern Europe (Evans
 44 et al., 2015). The potential for climate change to enhance the damage caused by *H. abietis*, by
 45 reducing life cycle length (Leather et al., 1999) and encouraging migration into previously weevil
 46 free areas (Inward et al., 2012; Barredo et al., 2015), especially in more northerly regions, has
 47 prompted discussion of the effectiveness of current *H. abietis* management practices and possible
 48 alternative methods (Kaprana et al., 2017; Mc Namara et al., 2018).

49 Management of *H. abietis* currently relies on a variety of chemical, biological and physical mea-
 50 sures, with integrated pest management schemes tending to yield greater success (Willoughby et al.,
 51 2004). Physical deterrents include piling debris produced by the clearfelling process over exposed
 52 stumps to discourage egg laying (Rahman et al., 2015), or stump removal to limit the availability of
 53 substrate for egg laying. The application of entomopathogenic nematodes after clearfelling has been
 54 shown to reduce the number of adult weevils in clearfelled sites (Dillon et al., 2006; Kaprana et al.,
 55 2017; Williams et al., 2013). The most common method of control is the addition of chemicals at the
 56 time of restocking, with *H. abietis* being the only insect pest against which routine chemical controls
 57 are applied in the UK and Ireland (Willoughby et al., 2004, 2017). The most common chemical ap-
 58 plication for *H. abietis* in the UK are synthetic pyrethroids of various formulation, which are sprayed
 59 directly onto saplings as a prophylactic treatment, acting as a strong deterrent for *H. abietis* feeding
 60 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
87 woodland, forestry and primary wood processing in the United Kingdom*, 2018). It is one of the
88 UK's three native coniferous tree species (Dines et al., 2005). There is increasing interest to plant
89 native tree species in an attempt to preserve native biodiversity and landscape heritage. *H. abietis*
90 is the most serious pest of UK *P. sylvestris* plantations, with infestations sometimes precluding
91 sustainable future planting completely due to sapling mortality on clearfell sites (Willoughby et al.,
92 2017).

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

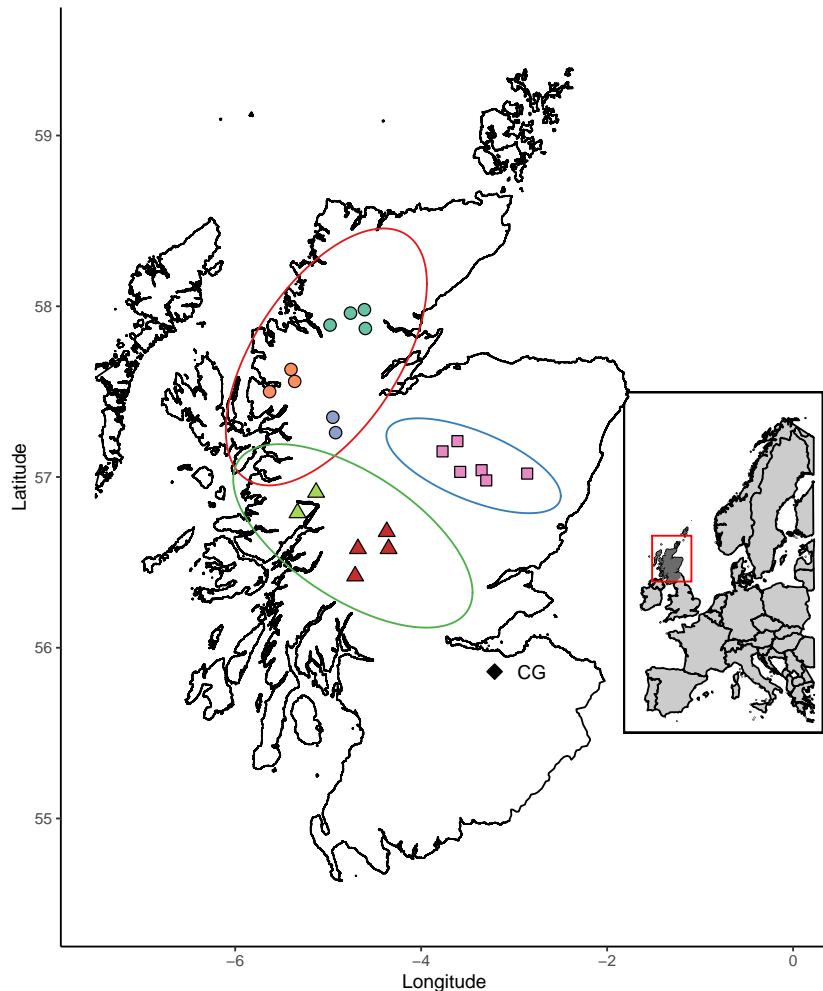


Figure 2: Map of seed collection sites within Scotland, from which seed populations were collected. Elliptic hulls and site point shapes define the three Regional zones. Points are coloured according to Geographic zone clusters, which are nested within Regions.

153 **Experimental design**

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

165 **Data collection**

166 The area of bark lesions caused by *H. abietis* was counted on each sapling stem in July 2015. This
167 is roughly between the two seasonal peaks of weevil feeding that are commonly observed in the UK,
168 which occur in the spring and late summer, coinciding with the end of adult hibernation and the
169 emergence of new adults from pupae, respectively (Nordenhem, 1989; Leather et al., 1999). Only
170 damage sustained by *H. abietis* during the current growing season was counted and could be clearly
171 separated from damage sustained in previous years by the lack of bark edge scarring and presence
172 of sap at the wound edge (Figure 1). Isolated lesions tended to be roughly circular with a diameter
173 of ~3 mm. Where a larger continuous lesion was found, as when a stem was girdled, the larger
174 lesion was photographed with a scale and the area estimated by tracing the lesion with ImageJ
175 version 1.50g7 (Schneider et al., 2012). Weevil damage is therefore expressed as the mm^2 area of
176 stem lesions per sapling.

177 **Statistical analysis**

178 To assess the effect of sapling genetic origin on damage by pine weevils, and to test our hypothesis
179 that two effects are responsible for *H. abietis* damage, we implemented a hurdle model framework
180 with generalised linear mixed models, using the *glmmTMB* package in R (Brooks et al., 2017).
181 First, a binomial logistic mixed effects regression assessed variation in the probability of a sapling
182 being initially damaged according to *P. sylvestris* parent population. The response variable of this
183 model was binomial, describing whether an individual sapling had experienced any damage by *H.*
184 *abietis*. Then a linear mixed effects model using data only saplings where damage had occurred,
185 assessed whether saplings varied in the total area of bark damaged by continued feeding by *H. abietis*
186 according to *P. sylvestris* parent population. The response variable of this model was the area of
187 weevil damaged bark visible on the sapling. Area of bark damaged was log transformed in order
188 to better meet model assumptions. In both analyses, parent tree was used as a random intercept
189 effect to account for pseudo-replication in sapling parent. The nested geographic nature of the seed
190 collection sites was also used as a random effect, with site nested within geographic zone (Figure ??).
191 All statistical analyses were performed in R version 3.4.2 (R Core Team, 2019). Model goodness-of-fit
192 was assessed for both model types by comparing models with equivalent random effects models and
193 null models using AIC_r (Akaike Information Criterion) and Log-likelihood estimates (Bolker et al.,
194 2008). During model comparison all models were fitted using Maximum Likelihood (ML) (Bolker
195 et al., 2008). To investigate which populations of *P. sylvestris* differed in their resistance to *H.*
196 *abietis* attack, the models were refitted using Restricted Maximum Likelihood (REML) and model

197 slope estimates were compared. Tukey's HSD multiple comparisons test assessed which populations
198 were significantly different from each other for both models, using the PACKAGE package ().

199 Spatial autocorrelation in area of bark damaged due to damaged saplings acting as olfactory beacons
200 to attract weevils was investigated using Generalised Least Squares (GLS) models of damaged bark area with spatial autocorrelation structures as a covariate. Multiple spatial autocorrelation
201 structures were tested and models fitted using ML were compared in their goodness-of-fit using AIC
202 (Akaike Information Criterion) values, Log-likelihood estimates and pseudo R-squared model values
203 calculated by the *MuMin* package (Bartoń, 2019). After model selection, the best generalised least
204 squares model was re-fitted using REML for model interpretation to assess the predictive effect of
205 spatial auto-correlation on weevil damage. Semi-variograms of the best fitting model residuals and
206 raw damaged area mm² data confirmed that spatial autocorrelation between saplings was negligible
207 within the common garden and so spatial autocorrelation structures were not included in other
208 models.

210 Results

211 Sapling damage

212 36.9% (248/672) of the saplings in the common garden were damaged by *H. abietis* feeding activity.
213 Figure 4 shows the number of saplings damaged divided into seed collection site populations. All
214 saplings were alive prior to data collection and sapling mortality was not recorded during the experiment.
215 All seed populations had at least eight affected saplings out of a total of 32. The population
216 with the highest number of damaged saplings was Loch Clair (LC), which had 18 damaged saplings.
217 The sapling with the highest mm² damaged area was from Cona Glen (CG) and had 325.8 mm²
218 of bark damaged. Rhidorroch (RD) had the highest cumulative damaged area with 1057.1 mm².
219 Variation in bark area damaged within seed populations was high (Figure 5), with some geographic
220 zones having similar levels of damage while others varied a widely within geographic zone.

221 Spatial autocorrelation

222 Multiple Generalised Least Squares (GLS) models of damaged sapling area fitted with different
223 correlation structures were compared against a null model with no correlation structure using AIC
224 values (Table 1). A Gaussian correlation structure fit the data best, but explained only a very low
225 percentage of the variation in sapling damaged area. Gaussian, Exponential and Rational quadratic
226 models had AIC values within 2 points of each other and explained only negligibly different amounts
227 of variation in damaged bar area, according to pseudo-R² model values, so these models can be
228 interpreted as fitting the data similarly well. All three models were better than a null model which
229 explained none of the variation in damaged bark area. A semivariogram of damaged bark area with
230 distance between saplings showed that there was no appreciable spatial auto-correlation (Figure 7).
231 This was supported by a visual inspection of a schematic map of damaged bark area per sapling in
232 the common garden (Figure 6). As a result, further modelling with mixed effects models did not
233 include a spatial auto-correlation covariate structure.

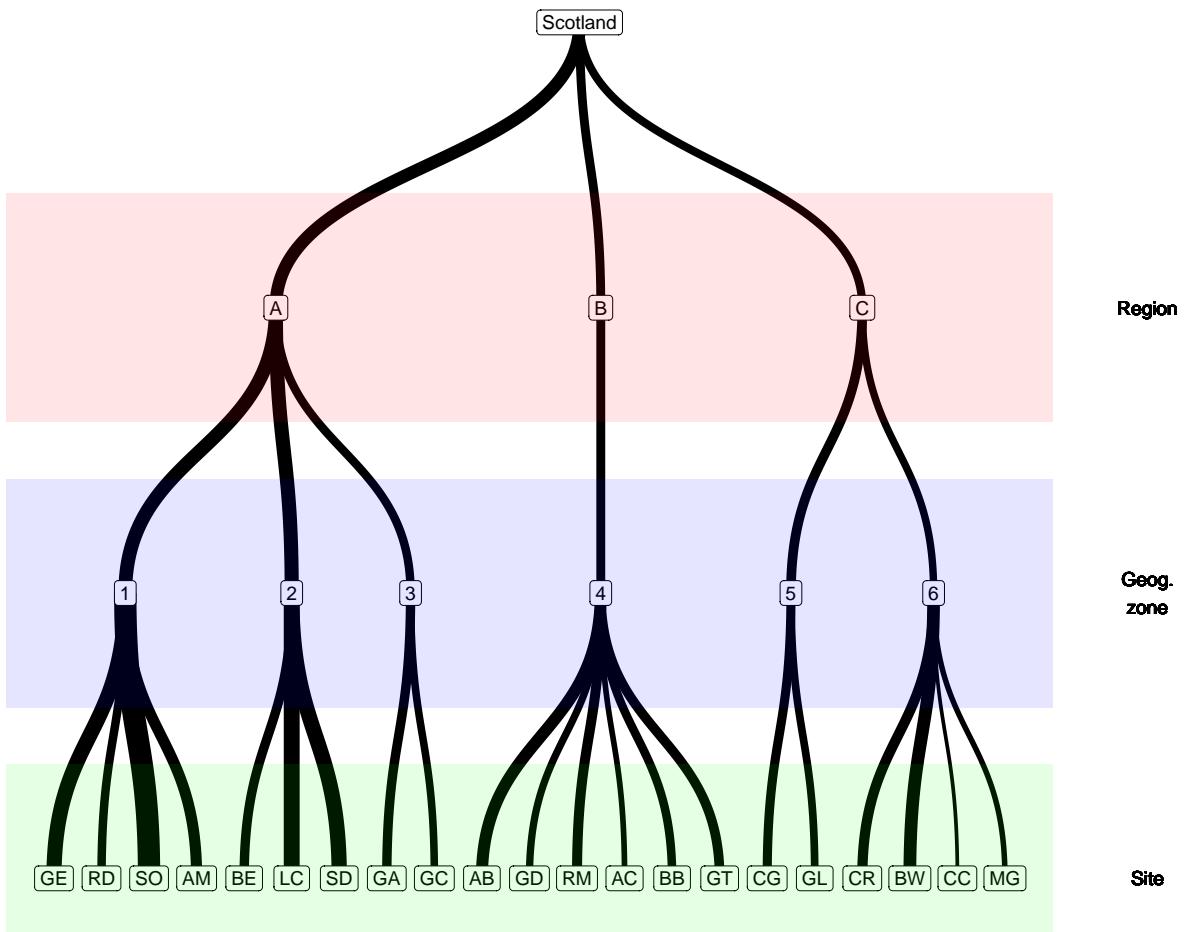


Figure 3: Dendrogram showing nested grouping of seed populations. Graph edge widths vary relatively according to the total bark area damaged on saplings collected from each site. Width edges are weighted according to the number of saplings at each grouping level to account for differences in number of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

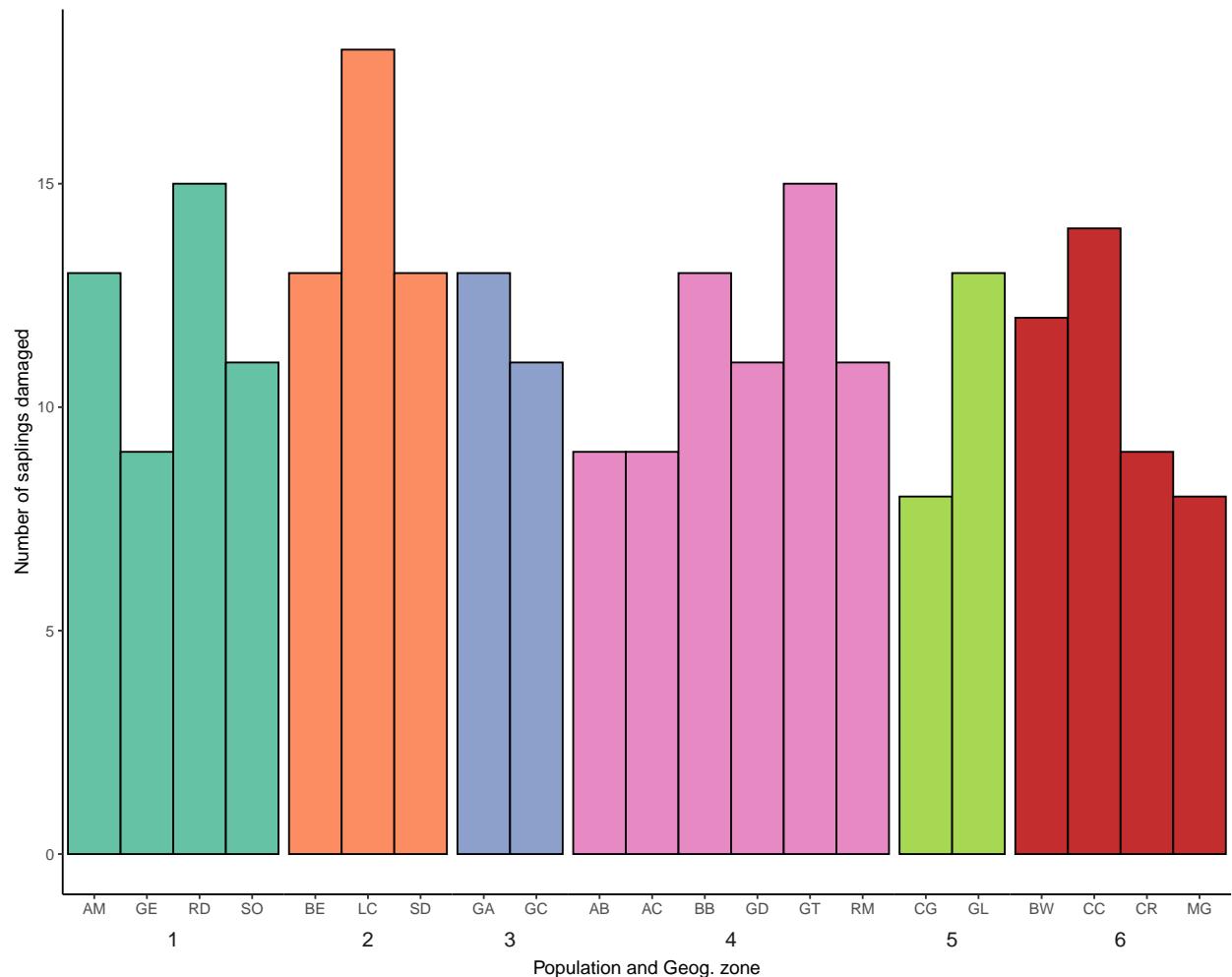


Figure 4: The number of saplings with visible damage by *H. abietis*, divided by seed population. Groups of bars denote Geographic zones.

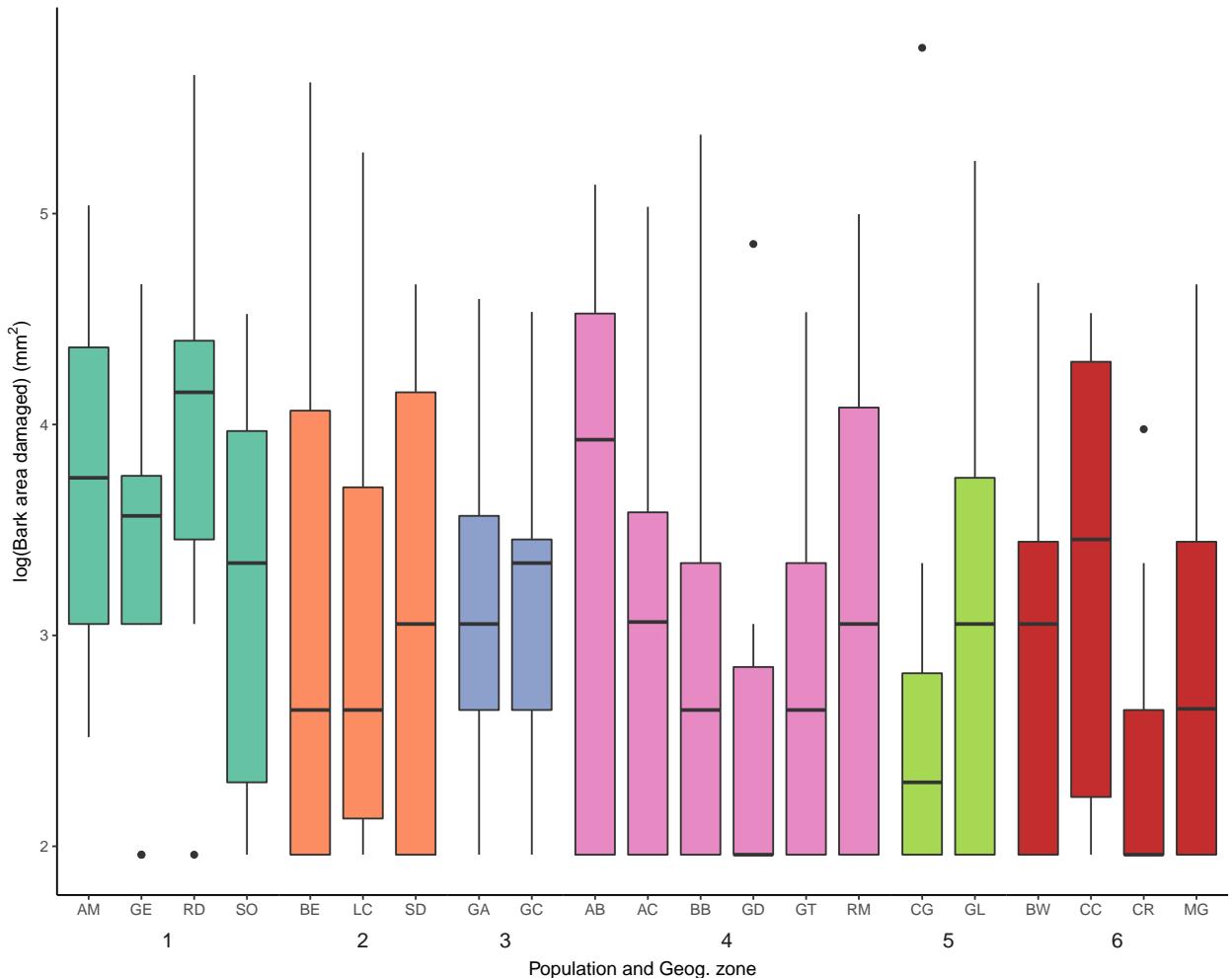


Figure 5: Variation in bark area damaged by *H. abietis*, divided by seed population. Coloured groups of bars denote Geographic zones. Thick bars denote the median value per population.

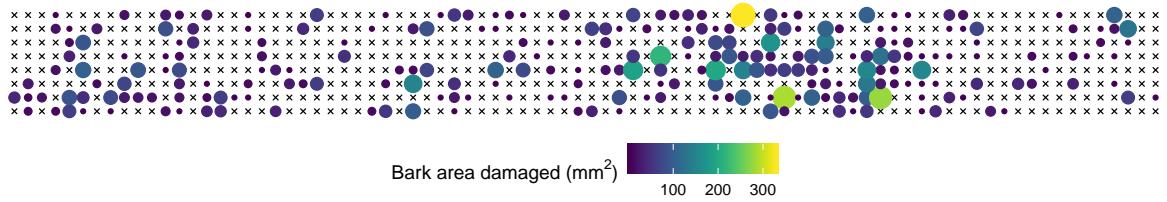


Figure 6: Schematic diagram of sapling relative position within the Common Garden, with sapling points coloured and sized according to the area of bark damaged. The distance between saplings is 3 m in both the X and Y directions.

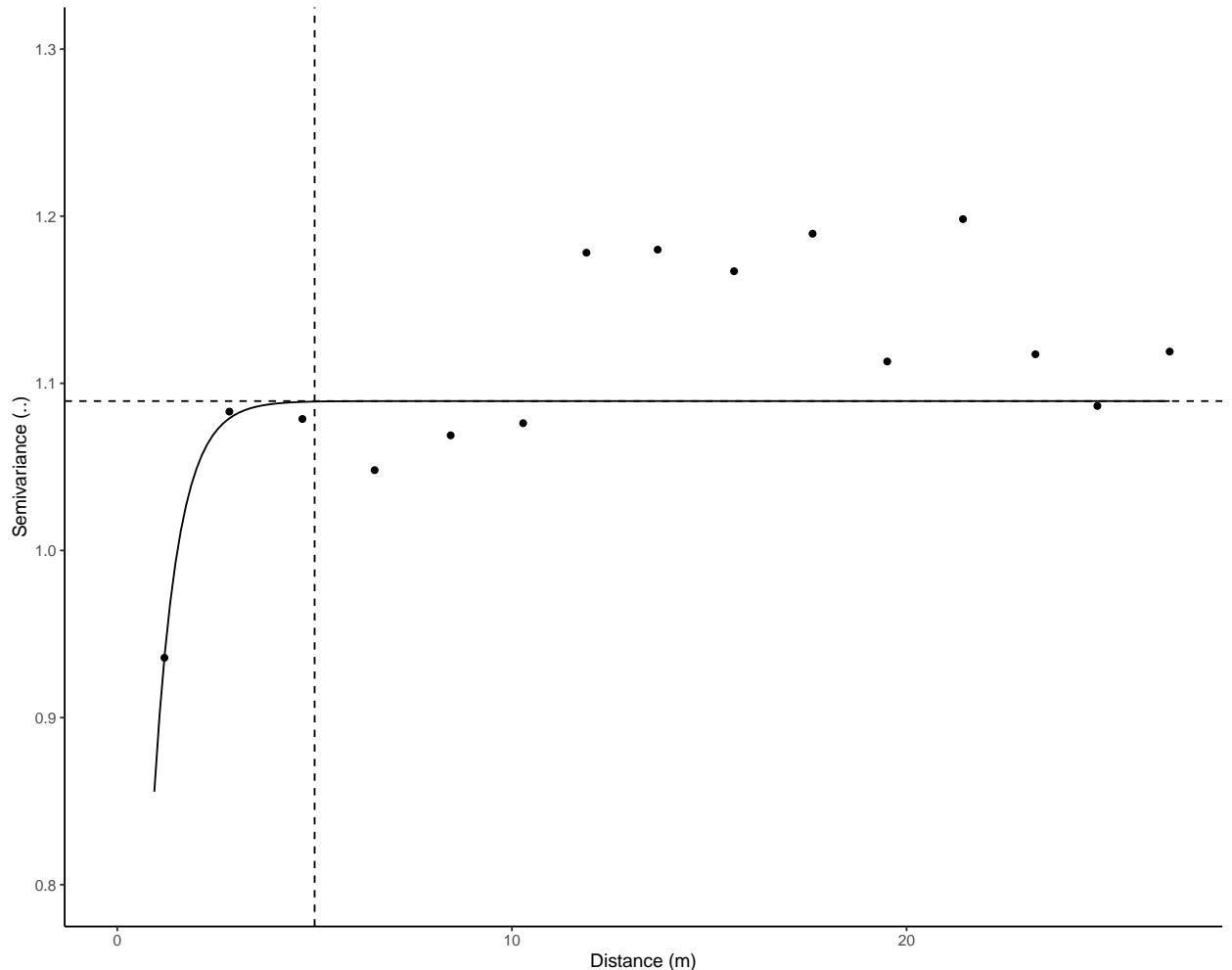


Figure 7: Semivariogram showing spatial autocorrelation of damaged bark area according to distance between saplings. Vertical dotted line denotes the nugget and the horizontal dotted line denotes the sill of the semivariogram.

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_m
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

234 The effect of seed population on sapling damage

235 The first part of the hurdle model process explored variation among seed populations in the probability of a sapling being damaged by *H. abietis*. The most parsimonious model was a null model, as
236 estimated by AIC values. Fixed effects models using Geographic Zone and Collection Site explained
237 little of the variance in likelihood of a sapling being damaged, while models using Parent as the
238 fixed effect explained ~95% of the variance (Table 2). Family models were the least parsimonious
239 however, with Δ AIC values of 147.73 and 145.73. The fixed effect of Family accounted for most of
240 the model variance for those models (~94%). Estimated marginal means of Geographic zones did
241 not vary meaningfully for these models.
242

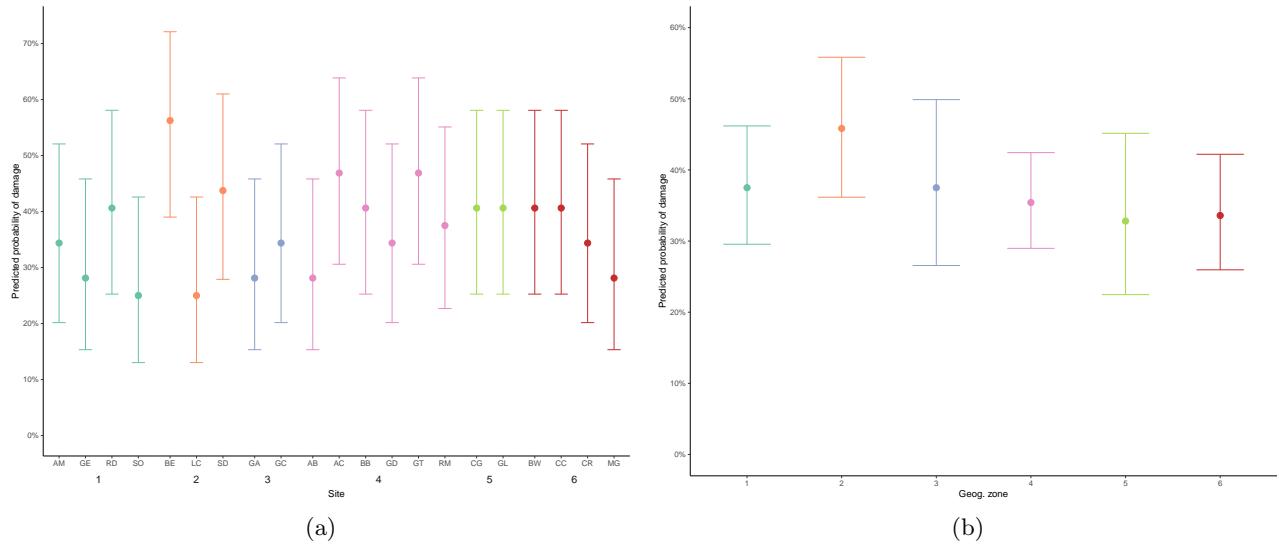


Figure 8: Predicted values with 95% confidence intervals for the probability of a sapling being damaged with seed collected from different (a) sites and (b) aggregated by geographic zone.

243 The fixed effect of Site was weak. In a model using seed population as a fixed effect and Parent as
244 a random intercept effect, seed population only accounted for 2.7% of the variation in the probability
245 that a sapling would be initially damaged by *H. abietis*. According to the best fitting model with
246 site as a fixed effect, saplings from Beinn Eighe (BE) had a greater chance of being initially damaged
247 than others (Figure 8a), however, these predicted values were not significantly different from other
248 sites according to a comparison of marginal means.

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

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

249 The second part of the hurdle model explored populations level variation in the area of sapling
250 bark damaged by *H. abietis*, for those saplings which were damaged. The best model included
251 Geographic zone as the fixed effect and Site as a random intercept effect(Table ??).

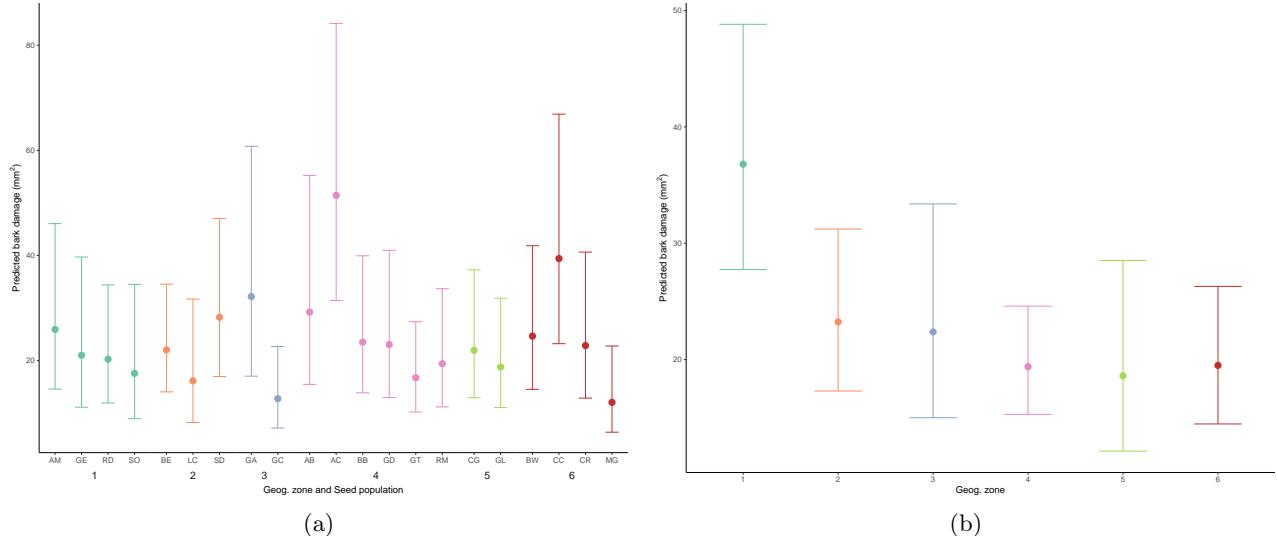


Figure 9: Predicted values of mm² for saplings with seed collected from different Geographic Zones.

252 Population level spatial patterns

253 There was a weak positive but significant effect of latitude on the total bark area damaged by
254 *H. abietis* ($Z = 3.249(1, 248)$, $p = <0.005$, $R^2 = 0.041$), when the nested random effects of seed
255 collection site and family were accounted for (Figure 13). In a similar model, there was no effect of
256 longitude on bark area damaged ($Z = -1.377(1, 248)$, $p = 0.168$, $R^2 = 0.009$).

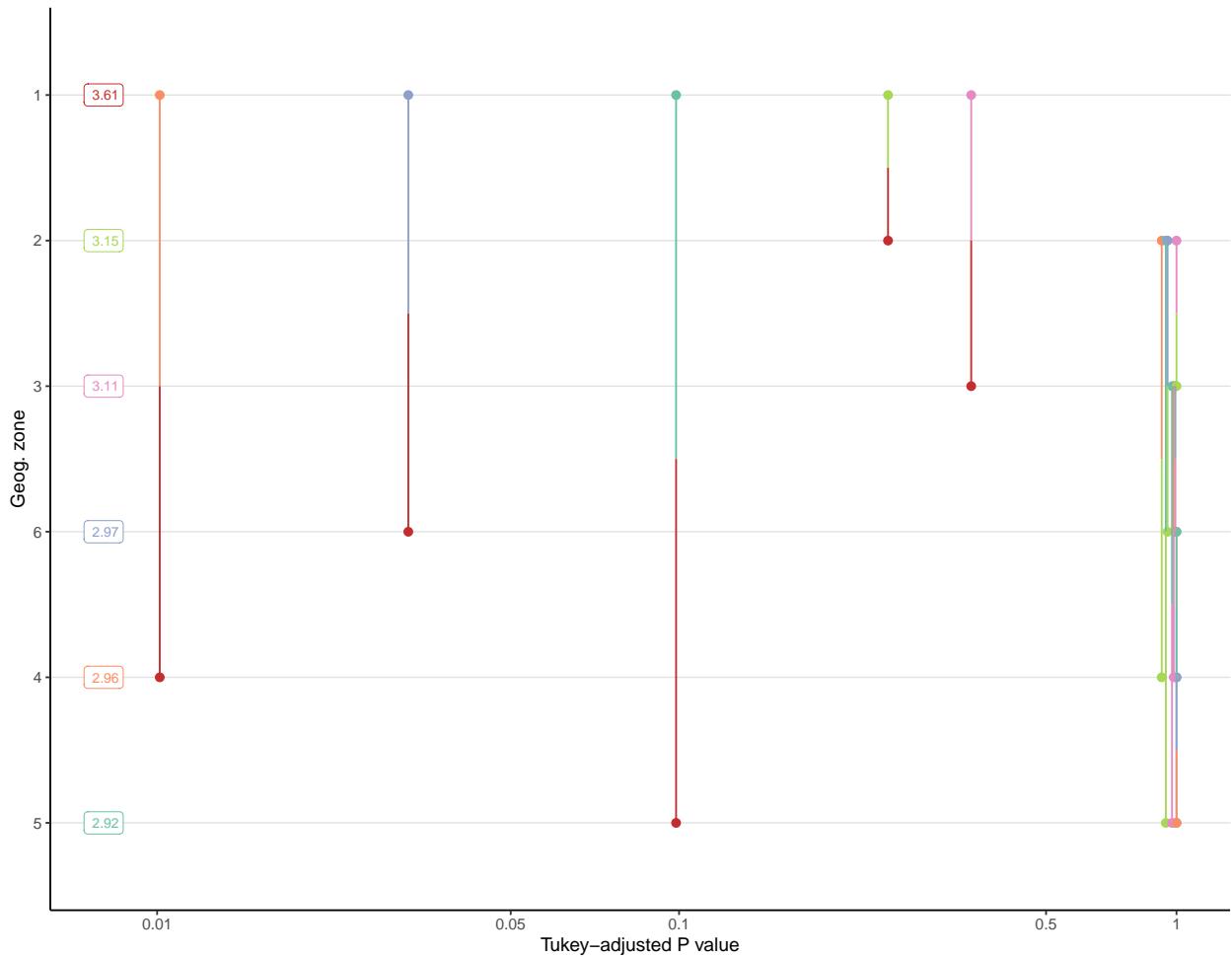


Figure 10: Pairwise p-values of a Tukey HSD test comparing geographic zones in the best model predicting variation in bark area damaged by *H. abietis*. Geographic zone 1 is significantly ($p < 0.05$) different from zones 4 and 6. P-values for all other comparisons outwith Geographic zone 1 are close to 1.

Table 3

Fixed eff.	Random eff.	AIC	logLik	R^2_m	R^2_c
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	0.033
NA	Site / Family	724.929	-358.464	0	0.026
Site	Geog. zone	736.127	-345.063	0.106	0.106
Site	Family	736.127	-345.063	0.106	0.106
Site	Geog. zone + Family	738.127	-345.063	0.106	0.106
Family	Geog. zone	810.131	-256.066	0.565	0.565
Family	Geog. zone / Site	812.131	-256.066	0.565	0.565
Geog. zone	Site / Family			0.056	0.056

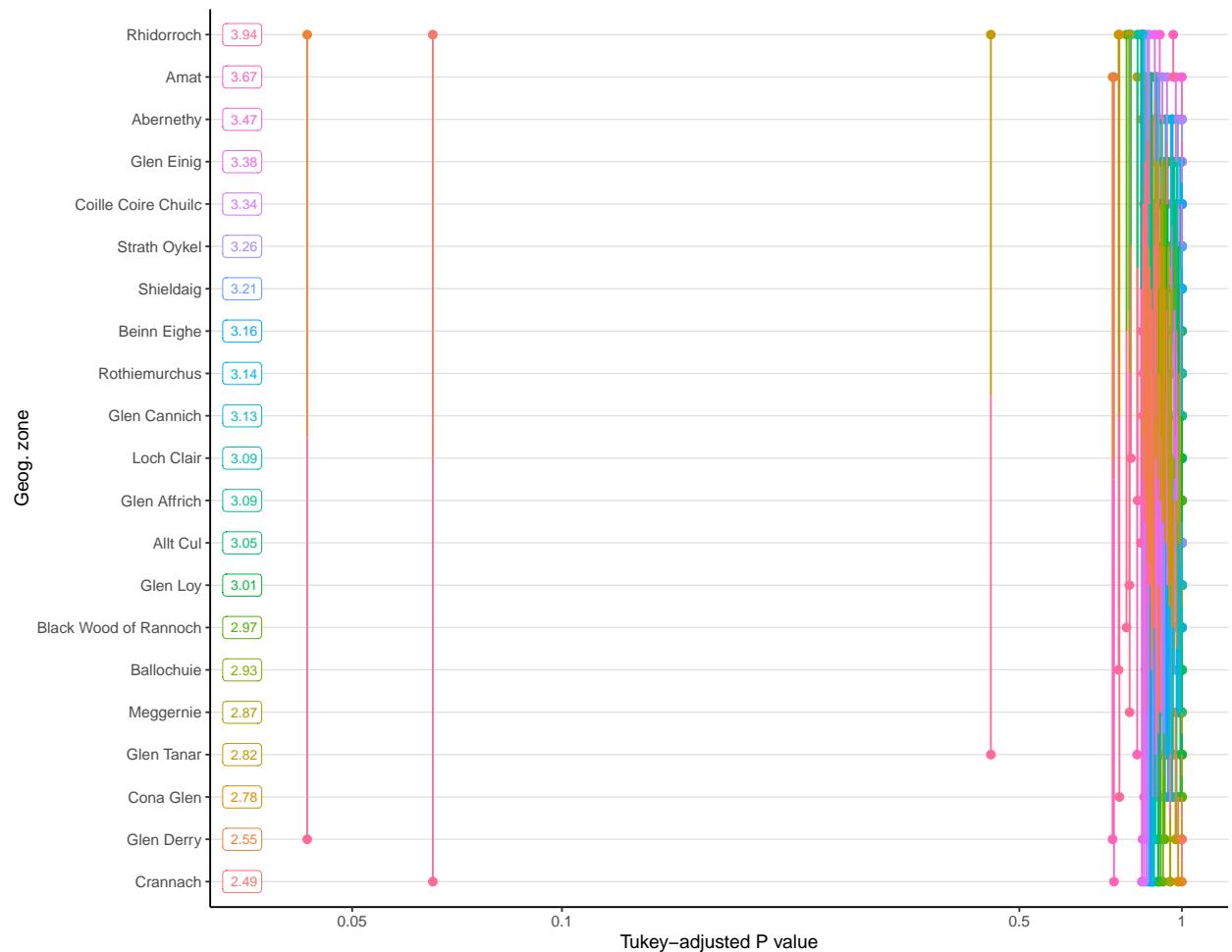


Figure 11: Pairwise p-values of a Tukey HSD test comparing geographic zones in the best model predicting variation in bark area damaged by *H. abietis*. Geographic zone 1 is significantly ($p<0.05$) different from zones 6 and 4. P-values for all other comparisons outwith Geographic zone 1 are close to 1.

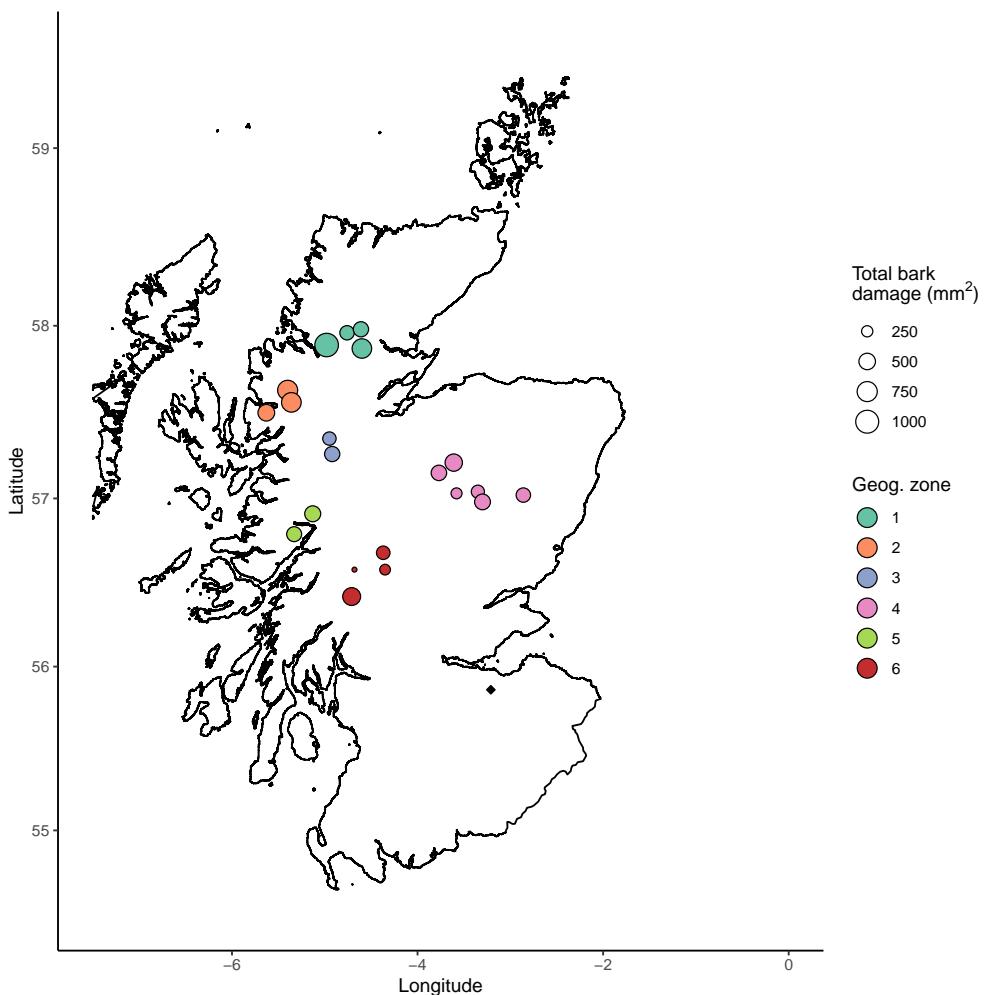


Figure 12: Map of study sites with bubbles coloured according to Geographic zone and relatively sized according to the total bark area damaged for all 32 saplings per site.

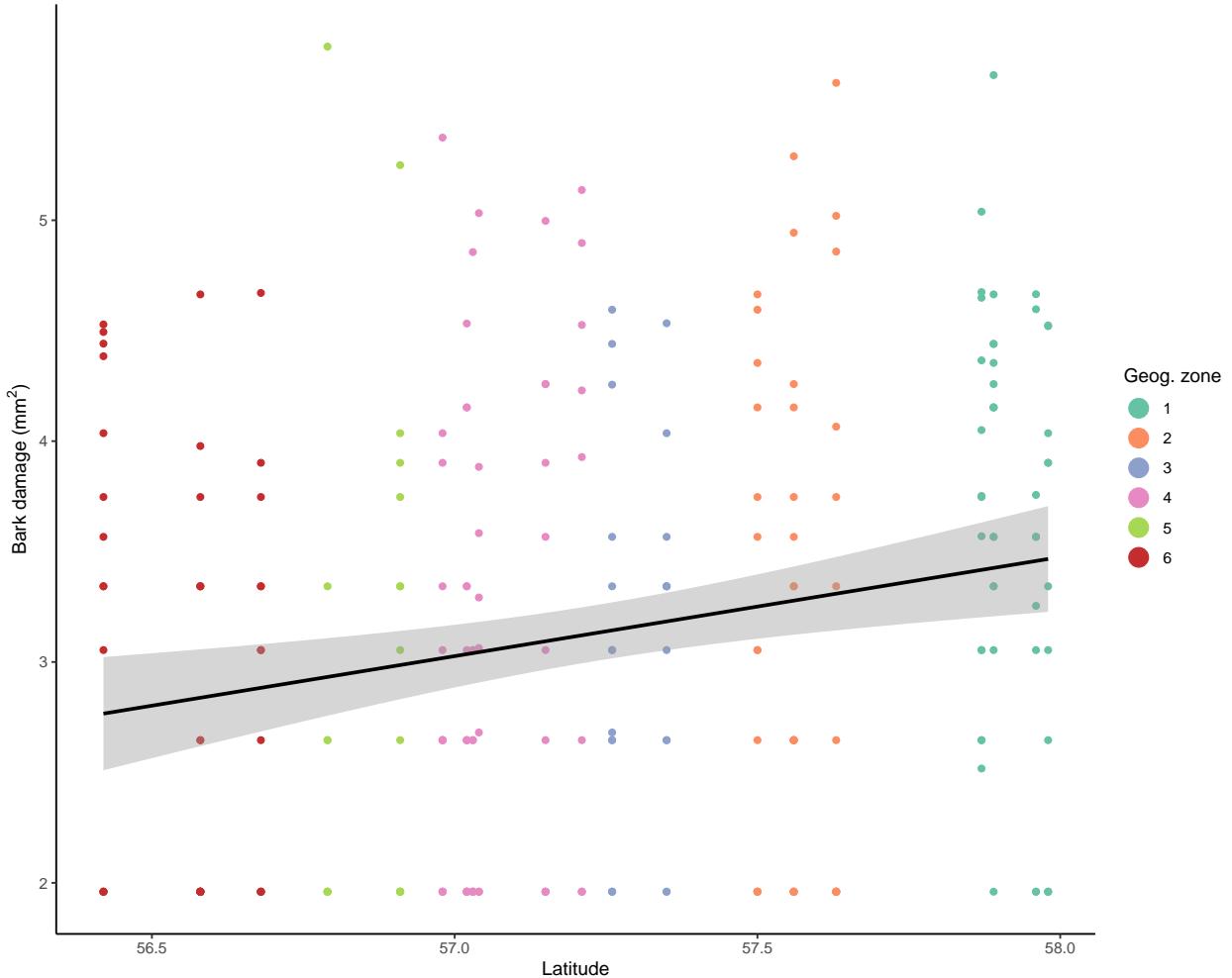


Figure 13: Relationship between bark area damaged and latitude of sapling population, for those saplings which were damaged. Each point is an individual sapling. Points are coloured by Geographic zone. The linear model fit (black line with grey 95% confidence interval) shows a weak positive trend.

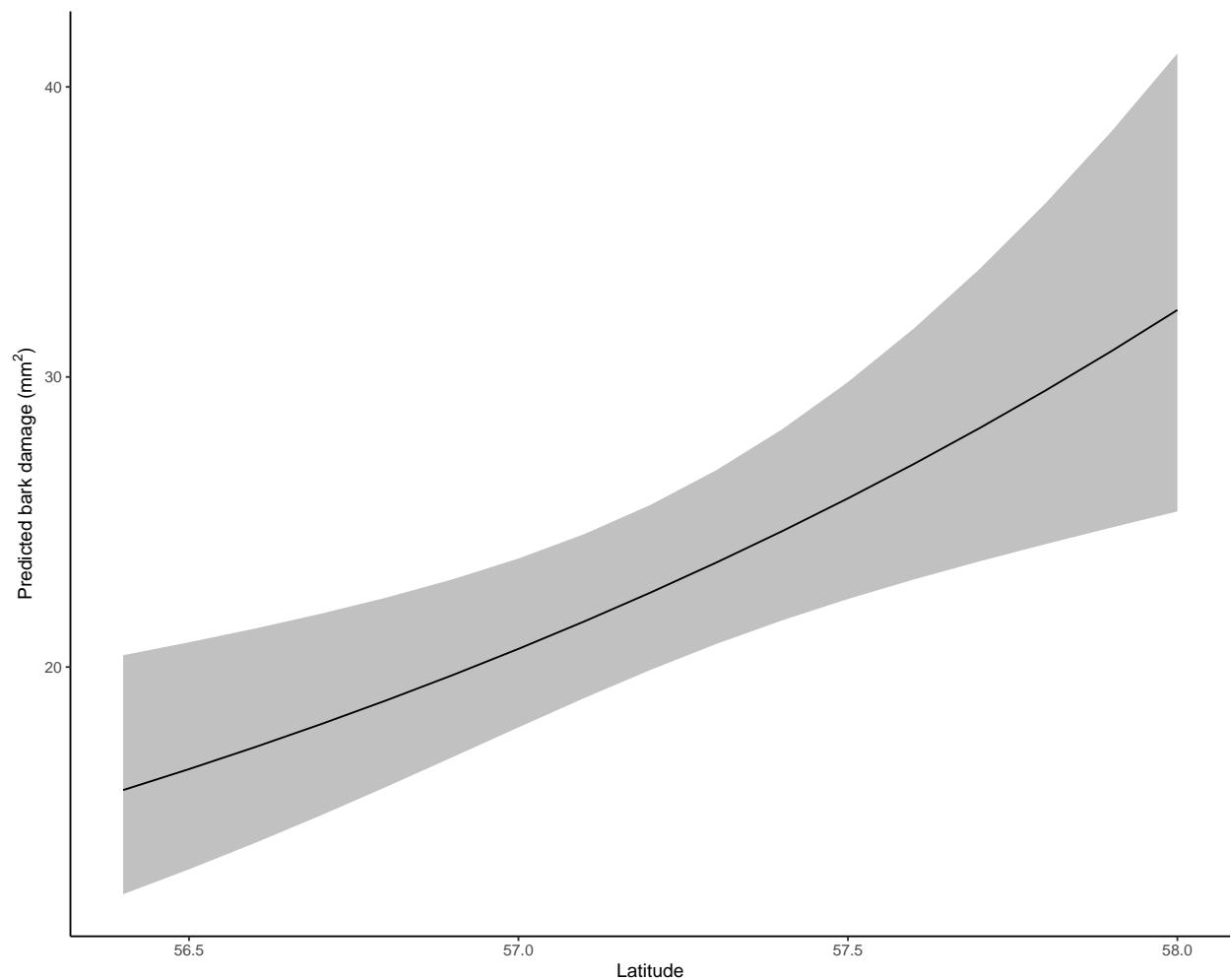


Figure 14: Predicted values with 95% confidence interval for the bark area damaged on a sapling when the seed was collected at different latitudes.

257 **Parent effects - variation within populations**

258 Variance in bark area damaged was high in all seed populations. The highest being 216.5 mm² in
259 Cona Glen (Figure 5).

260 **Discussion**

261 The model selection process determined that there was an effect of seed population on the area of
262 weevil damage found on damaged saplings, but could not account for variation in the probability
263 that a sapling became damaged initially. Linear models demonstrated that there was a general
264 latitudinal effect on sapling damaged area. Saplings with parents at higher latitudes typically
265 experienced higher levels of damage, but this had a much weaker effect than population itself.
266 Studies on the distribution and life cycle of *H. abietis* have shown that life cycle length is strongly
267 linked with mean temperature in the summer months, with higher temperatures leading to a short
268 life cycle and therefore higher numbers of pine weevils where infestations occur (Leather et al., 1999).
269 *H. abietis* abundance reduces with latitude in Scotland (Barredo et al., 2015). The latitudinal effect
270 may therefore be a result of adaptation to resist *H. abietis* damage. *P. sylvestris* leaves and bark
271 have resin canals which act to deter herbivores (). While it has not been explicitly tested for *H*
272 *abietis*, other studies involving **SOME INSECTS** have found a negative correlation between resin
273 canal density in leaves and feeding behaviour for *P. sylvestris*. found that *H. abietis* was discouraged
274 from eating **tree name** needles with higher resin canal concentration and cuticle thickness.

275 Within population variability was high. **This is expected given the high gene flow**. The effect
276 of population was higher however.

277 Other things that might have caused variation among seed populations **dunno**.

278 **Conclusion**

279 This study sought to test whether adaptive variation for resistance to the large pine weevil (*Hylobius*
280 *abietis*) existed in genetically distinct populations of scot's pine (*Pinus sylvestris*) in Caledonian
281 remnant forest patches in Scotland. A weak positive effect of latitude of seed collection site was
282 found in the damaged area of sapling bark, suggesting that more southerly populations may be less
283 attractive to *H. abietis* attack. It is suggested that further studies investigate bark morphological
284 variation and concentrations and variation in Volatile Organic Compounds (VOCs) emitted when
285 bark is damaged in young saplings cultivated from seed stock collected from these Caledonian
286 remnant forest patches to understand the underlying mechanism for this variation in attractiveness.

287 **References**

- 288 Alfaro, R. (1989), Stem defects in sitka spruce induced by sitka spruce weevil, *pissodes strobi* (peck),
289 *in* R. Alfaro and S. Glover, eds, 'Insects affecting reforestation: biology and damage', Forestry
290 Canada, Berlin, Germany, pp. 177–185.
- 291 Alfaro, R. I., Fady, B., Vendramin, G. G., Dawson, I. K., Fleming, R. A., Sáenz-Romero, C.,
292 Lindig-Cisneros, R. A., Murdock, T., Vinceti, B., Navarro, C. M., Skrøppa, Baldinelly, G., El-
293 Kassaby, Y. A. and Loo, J. (2014), 'The role of forest genetic resources in responding to biotic and

- 294 abiotic factors in the context of anthropogenic climate change', *Forest Ecology and Management*
295 **333**, 76–87.
- 296 Alfaro, R. I., King, J. N. and vanAkker, L. (2013), 'Delivering sitka spruce with resistance against
297 white pine weevil in british columbia, canada', *The Forestry Chronicle* **89**(2), 235–245.
- 298 Antwi, F. B. and Reddy, G. V. P. (2015), 'Toxicological effects of pyrethroids on non-target aquatic
299 insects', *Environmental Toxicology and Pharmacology* **40**, 915–923.
- 300 Barredo, J. I., Strona, G., de Rigo, D., Caudullo, G., Stanganelli, G. and San-Miguel-Ayanz, J.
301 (2015), 'Assessing the potential distribution of insect pests: case studies on large pine weevil
302 (*Hylobius abietis* l) and horse-chestnut leaf miner (*Cameraria ohridella*) under present and future
303 climate conditions in european forests', *EPPO Bulletin* **45**(2), 273–281.
- 304 Bartoń, K. (2019), *MuMIn: Multi-Model Inference*. R package version 1.43.6.
- 305 Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H.
306 and White, J. S. (2008), 'Generalized linear mixed models: a practical guide for ecology and
307 evolution', *Trends in Ecology and Evolution* **24**(3), 127–135.
- 308 Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug,
309 H. J., Maechler, M. and Bolker, B. M. (2017), 'glmmTMB balances speed and flexibility among
310 packages for zero-inflated generalized linear mixed modeling', *The R Journal* **9**(2), 378–400.
- 311 Byun-McKay, A., Godard, K., Toudefallah, M., Martin, D. M., Alfaro, R., King, J., Bohlmann,
312 J. and Plant, A. L. (2006), 'Wound-induced terpene synthase gene expression in sitka spruce
313 that exhibit resistance or susceptibility to attack by the white pine weevil', *Plant Physiology*
314 **140**, 1009–1021.
- 315 Carlisle, A. and Brown, A. H. F. (1968), 'Pinus sylvestris, l.', *Journal of Ecology* **56**(1), 269–307.
- 316 Dillon, A. B., Ward, D., Downes, M. J. and Griffin, C. T. (2006), 'Suppression of the large pine weevil
317 *Hylobius abietis* (l.) (coleoptera: Curculionidae) in pine stumps by entomopathogenic nematodes
318 with different foraging strategies', *Biological Control* **38**, 217–226.
- 319 Dines, T. D., Jones, R. A., Leach, S. J., McKean, D. R., Pearman, D. A., Preston, C. D., Rumsey,
320 F. J. and Taylor, I. (2005), 'The vascular plant red data list for great britain. species status 7'.
- 321 Donnelly, K., Cavers, S., Cottrell, J. E. and Ennos, R. A. (2016), 'Genetic variation for needle traits
322 in scots pine (*Pinus sylvestris* l.)', *Tree Genetics & Genomes* **12**(3), 40.
- 323 Donnelly, K., Cavers, S., Cottrell, J. E. and Ennos, R. A. (2018), 'Cryptic genetic variation and
324 adaptation to waterlogging in caledonian scots pine, *Pinus sylvestris* l.', *Ecology and Evolution*
325 **8**, 8665—8675.
- 326 Edwards, C. E. and Mason, W. L. (2006), 'Stand structure and dynamics of four native scots pine
327 (*Pinus sylvestris* l.) woodlands in northern scotland', *Forestry* **79**, 261–277.
- 328 Evans, H., McAllister, F., Saunders, T., Moore, R., Jenkins, T., Butt, T., Ansari, M., Griffin,
329 G. Williams, C., Teck, R. and Sweeney, J. (2015), 'The impact project guide to *Hylobius* man-
330 agement 2015'.
- 331 Eyles, A., Bonello, P., Ganley, R. and Mohammed, C. (2009), 'Induced resistance to pests and
332 pathogens in trees', *New Phytologist* **185**, 893–908.
- 333 *Forestry Statistics 2018: A compendium of statistics about woodland, forestry and primary wood*
334 *processing in the United Kingdom* (2018).
- 335 GBIF (2019), 'Gbif occurrence download'.

- 336 Gershenson, J. and Croteau, R. (1991), Terpenoids, in G. A. Rosenthal and M. Berenbaum, eds,
337 'Herbivores: Their interactions with secondary plant metabolites', Elsevier Science, Oxford,
338 pp. 165–219.
- 339 Gill, R. M. A. (1992), 'A review of damage by mammals in north temperate forests: 3. impact on
340 trees and forests', *Forestry* **65**(4), 363–388.
- 341 González-Díaz, P., Cavers, S., Iason, G. R., Booth, A., Russell, J. and Jump, A. S. (2018), 'Weak
342 isolation by distance and geographic diversity gradients persist in scottish relict pine forest',
343 *iForest* **11**, 449–458.
- 344 Heijari, J., Blande, J. D. and Holopainen, J. K. (2011), 'Feeding of large pine weevil on scots
345 pine stem triggers localised bark and systemic shoot emission of volatile organic compounds',
346 *Environmental and Experimental Botany* **71**, 390–398.
- 347 Heritage, S. and Moore, R. (2000), 'The assessment of site characteristics as part of a management
348 strategy to reduce damage by *Hylobius*'.
- 349 Inward, D. J. G., Wainhouse, D. and Peace, A. (2012), 'The effect of temperature on the development
350 and life cycle regulation of the pine weevil *Hylobius abietis* and the potential impacts of climate
351 change', *Agricultural and Forest Entomology* **14**, 348–357.
- 352 Kapranas, A., Malone, B., Quinn, S., O'Tuama, P., Peters, A. and Griffin, C. T. (2017), 'Optimizing
353 the application method of entomopathogenic nematode suspension for biological control of large
354 pine weevil *Hylobius abietis*', *BioControl* **62**, 659–667.
- 355 Keeling, C. I. and Bohlmann, J. (2006), 'Genes, enzymes and chemicals of terpenoid diversity in
356 the constitutive and induced defence of conifers against insects and pathogens', *New Phytologist*
357 **170**, 657–675.
- 358 King, J. N., Alfaro, R. I., Lopez, M. G. and van Akker, L. (2011), 'Resistance of sitka spruce
359 (*Picea sitchensis* (bong.) Carr.) to white pine weevil (*Pissodes strobi* Peck): characterizing the
360 bark defence mechanisms of resistant populations', *Forestry* **84**(1), 83–91.
- 361 Kiss, G. K. and Yanchuk, A. D. (1991), 'Preliminary evaluation of genetic variation of weevil
362 resistance in interior spruce in british columbia', *Canadian Journal of Forest Research* **21**(2), 230–
363 234.
- 364 Kivimäenpää, M., Magsarjav, N., Ghimire, R., Markkanen, J., Heijari, J., Vuorinen, M. and
365 Holopainen, J. K. (2012), 'Influence of tree provenance on biogenic voc emissions of scots pine
366 (*Pinus sylvestris*) stumps', *Atmospheric environment* **60**, 477–485.
- 367 Leather, S. R., Day, K. R. and Salisbury, A. N. (1999), 'The biology and ecology of the large
368 pine weevil, *Hylobius abietis* (coleoptera: Curculionidae): a problem of dispersal?', *Bulletin of
369 Entomological Research* **89**, 3–16.
- 370 Manlove, J. D., Styles, J. and Leather, S. R. (1997), 'Feeding of the adults of the large pine weevil,
371 *Hylobius abietis* (coleoptera: Curculionidae)', *European Journal of Entomology* **94**, 153–156.
- 372 Mason, W. L., Hampson, A. and Edwards, C. (2004), 'Managing the pinewoods of scotland'.
- 373 Mc Namara, L., Kapranas, A., Williams, C. D., O'Tuama, P., Kavanagh, K. and Griffin, C. T.
374 (2018), 'Efficacy of entomopathogenic fungi against large pine weevil *Hylobius abietis*, and their
375 additive effects when combined with entomopathogenic nematodes', *Journal of Pest Science*
376 **91**, 1407–1419.
- 377 Mian, L. S. and Mulla, M. S. (1992), 'Effects of pyrethroid insecticides on nontarget invertebrates
378 in aquatic ecosystems', *Journal of Agricultural Entomology* **9**(2), 73–98.

- 379 Nordenhem, H. (1989), 'Age, sexual development, and seasonal occurrence of the pine weevil *Hylobius abietis* (L.)', *Journal of Applied Entomology* **108**, 260–270.
- 380
- 381 Nordlander, G. (1987), 'A method for trapping *Hylobius abietis* (L.) with a standardized bait and its potential for forecasting seedling damage', *Scandinavian Journal of Forest Research* **2**, 199–213.
- 382
- 383 Nordlander, G. (1991), 'Host finding in the pine weevil *Hylobius abietis*: effects of conifer volatiles and added limonene', *Entomologia Experimentalis et Applicata* **59**, 229–237.
- 384
- 385 Nordlander, G., Eidmann, H. H., Jacobsson, U., Nordenhem, H. and Sjödin, K. (1986), 'Orientation of the pine weevil *Hylobius abietis* to underground sources of host volatiles', *Entomologia Experimentalis et Applicata* **41**, 91–100.
- 386
- 387
- 388 Ördlander, G. and Nilsson, U. (1999), 'Effect of reforestation methods on pine weevil (*Hylobius abietis*) damage and seedling survival', *Scandinavian Journal of Forest Research* **14**, 341–354.
- 389
- 390 Ördlander, G., Nilsson, U. and Nordlander, G. (1997), 'Pine weevil abundance on clear-cuttings of different ages: a 6-year study using pitfall traps', *Scandinavian Journal of Forest Research* **12**, 225–240.
- 391
- 392
- 393 Perry, A., Wachowiak, W., Brown, A. V., Ennos, R. A., Cottrell, J. E. and Cavers, S. (2016), 'Substantial heritable variation for susceptibility to *Dothistroma septosporum* within populations of native british scots pine (*Pinus sylvestris*)', *Plant Pathology* **65**, 987–996.
- 394
- 395
- 396 R Core Team (2019), *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna, Austria.
- 397
- 398 Rahman, A., Viiri, H., Pelkonen, P. and Khanam, T. (2015), 'Have stump piles any effect on the pine weevil (*Hylobius abietis* L.) incidence and seedling damage?', *Global Ecology and Conservation* **3**, 424–432.
- 399
- 400
- 401 Rose, D. (2002), Control of the Large Pine Weevil, *Hylobius abietis*, L., PhD thesis, Imperial College, London.
- 402
- 403 Rose, D., Leather, S. R. and Matthews, G. A. (2005), 'Recognition and avoidance of insecticide-treated scots pine (*Pinus sylvestris*) by *Hylobius abietis* (coleoptera: Curculionidae): implications for pest management strategies', *Agricultural and Forest Entomology* **7**, 187–191.
- 404
- 405
- 406 Salmela, M. J., Cavers, S., Cottrell, J. E., Iason, G. R. and Ennos, R. A. (2013), 'Spring phenology shows genetic variation among and within populations in seedlings of scots pine (*Pinus sylvestris* L.) in the scottish highlands', *Plant Ecology & Diversity* **6**(3-4), 523–536.
- 407
- 408
- 409 Schiebe, C., Hammerbacher, A., Birgersson, G., Witzell, J., Brodelius, P. E., Gershenson, J., Hansson, B. S., Krokene, P. and Schlyter, F. (2012), 'Inducibility of chemical defenses in norway spruce bark is correlated with unsuccessful mass attacks by the spruce bark beetle', *Oecologia* **170**, 183–198.
- 410
- 411
- 412
- 413 Schneider, C. A., Rasband, W. S. and Eliceiri, K. W. (2012), 'Nih image to imagej: 25 years of image analysis', *Nat Methods* **9**(7), 671–675.
- 414
- 415 Selander, J., Immonen, A. and Raukko, P. (1990), 'Resistance of naturally regenerated and nursery-raised scots pine seedlings to the large pine weevil', *Folia Forestalia* **766**, 1–199.
- 416
- 417 Telford, A., Cavers, S. and Cottrell, J. E. (2014), 'Can we protect forests by harnessing variation in resistance to pests and pathogens', *Forestry* **88**, 3–12.
- 418
- 419 Tilles, D. A., Sjödin, K., Nordlander, G. and Eidmann, H. H. (1986), 'Synergism between ethanol and conifer host volatiles as attractants for the pine weevil, *Hylobius abietis* (L.) (coleoptera: Curculionidae)', *Journal of Economic Entomology* **79**(4), 970–973.
- 420
- 421

- 422 Toivonen, R. and Viiri, H. (2006), 'Adult large pine weevils *hylobius abietis* feed on silver birch
423 *betula pendula* even in the presence of conifer seedlings', *Agricultural and Forest Entomology*
424 **8**, 121–128.
- 425 Trapp, S. and Croteau, R. (2001), 'Defensive resin biosynthesis in conifers', *Annual Review of Plant
426 Physiology and Plant Molecular Biology* **52**, 689–724.
- 427 Wallertz, K., Nordenhem, H. and Nordlander, G. (2014), 'Damage by the pine weevil *Hylobius abietis*
428 to seedlings of two native and five introduced tree species in sweden', *Silva Fennica* **48**(4), 1–14.
- 429 Williams, C. D., Dillon, A. B., Harvey, C. D., Hennessy, R., McNamara, L. and Griffin, C. T. (2013),
430 'Control of a major pest of forestry, *Hylobius abietis*, with entomopathogenic nematodes and fungi
431 using eradicate and prophylactic strategies', *Forest Ecology and Management* **305**, 212–222.
- 432 Willoughby, I., Evans, H., Gibbs, J., Pepper, H., Gregory, S., Dewar, J., Nisbet, T., Pratt, J.,
433 McKay, H., Siddons, R., Mayle, B., Heritage, S., Ferris, R. and Trout, R. (2004), 'Reducing
434 pesticide use in forestry'.
- 435 Willoughby, I., Moore, R. and Nisbet, T. (2017), 'Interim guidance on the integrated management
436 of *Hylobius abietis* in uk forestry'.