

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

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6 **Abstract**

7 Damage to coniferous tree plantation crops from the large pine weevil *Hylobius abietis* causes
8 economic losses of €140 million in Europe *per annum*. Current mitigation strategies are labour
9 intensive and only partially effective. Identifying and breeding natural resistance in host plant
10 cultivars to insect pests has been used in many crop species to reduce damage as part of an
11 integrated pest management strategy. Here, we conducted a common garden experiment in a
12 previously clearfelled forestry plantation where *H. abietis* 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 (?). Adult weevils emerge from tree stumps and feed on the bark and buds of
26 coniferous saplings, consuming sugar rich phloem tissue (?). Lesions on the bark and buds of
27 saplings (Figure ??) as a result of feeding may cause a reduction in growth rate, stem deformation
28 and an increased susceptibility to infection by airborne diseases of trees (?). Heavy damage may
29 lead to stem girdling and death of the terminal growing bud resulting in a malformed trunk, limiting
30 economic use as timber when fully grown (??). While *H. abietis* may inhabit adult coniferous trees in
31 both natural and planted coniferous forests, recently clearfelled and restocked coniferous plantation
32 sites provide an enriched habitat for breeding *H. abietis* and so pose more of a danger to planted
33 saplings than those in naturally regenerating stands (??). Adults lay eggs within the stumps of
34 clearfelled trees, which are rarely removed after clearfelling, with newly emerged juvenile weevils
35 feeding on young saplings until adulthood (?). Planted coniferous saplings are more susceptible to
36 *H. abietis* damage than naturally regenerating saplings, probably due to water stress as a result of
37 damage to root systems during planting (?). A single adult weevil can damage several plants over
38 the course of a season, with ~50% sapling mortality observed across affected plantation sites in the

39 UK and Ireland (?). On commercial conifer plantations, *H. abietis* causes annual economic losses of
40 €140 million *per annum* in Europe, of which €2.75 million (~£2.47 million) occurs in the UK (?).
41 Currently, *H. abietis* is the most damaging insect pest of newly planted trees in Northern Europe
42 (?). The potential for climate change to enhance the damage caused by *H. abietis*, by reducing life
43 cycle length (?) and encouraging migration into previously weevil free areas (??), especially in more
44 northerly regions, has prompted discussion of the effectiveness of current *H. abietis* management
45 practices and possible alternative methods (??).



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.

46 Management of *H. abietis* currently relies on a variety of chemical, biological and physical mea-
47 sures, with integrated pest management schemes tending to yield greater success (?). Physical
48 deterrents include piling debris produced by the clearfelling process over exposed stumps to dis-
49 courage egg laying (?), or stump removal to limit the availability of substrate for egg laying. The
50 application of entomopathogenic nematodes after clearfelling has been shown to reduce the number
51 of adult weevils in clearfelled sites (???). The most common method of control is the addition
52 of chemicals at the time of restocking, with *H. abietis* being the only insect pest against which
53 routine chemical controls are applied in the UK and Ireland (??). The most common chemical
54 application for *H. abietis* in the UK are synthetic pyrethroids of various formulation, which are
55 sprayed directly onto saplings as a prophylactic treatment, acting as a strong deterrent for *H. abi-*
56 *etis* feeding on treated bark (?). There are concerns however about run-off from spraying events
57 entering watercourses, where it is highly toxic to aquatic organisms (???). There are also concerns
58 about the health of forestry workers who apply the sprays (?). Additionally, the application of
59 pyrethroid sprays can cost ~£80 per hectare of planted land, and requires additional top-up sprays
60 in subsequent years if the problem persists during the sapling stage (?).

61 *H. abietis* adults rely on olfaction to search for coniferous hosts, responding to Volatile Organic
62 Compounds (VOCs), dominated by α -pinene and other monoterpenes released by the host plant
63 (??). At the local scale, when adult *H. abietis* are searching for feeding material while on the
64 ground, after their flight muscles regress, VOCs released by open wounds on the bark caused by
65 previous pine weevil feeders may attract more individuals (??), worsening the damage caused to the
66 sapling. A positive feedback mechanism may therefore exist, whereby damaged saplings are more
67 likely to be further damaged, acting as beacons for other *H. abietis* individuals. Conifer saplings
68 may also use VOCs as a defensive strategy however, to deter insect pests (??). Conifer saplings
69 may differ in the concentration of VOCs produced both prior to damage and after bark has been
70 damaged by feeding (??), and in their chemical composition (?) potentially causing variation in
71 the likelihood of a sapling becoming damaged by *H. abietis*. Other defensive strategies employed by
72 coniferous tree species against insect herbivores include higher concentrations of sclereid cells in the
73 bark and resin canals in the needles, making the plant material less palatable to herbivores, thus
74 deterring continued feeding (??).

75 While *H. abietis* is a generalist of a number of coniferous tree species (??), they are common
76 pests in scots pine (*Pinus sylvestris* L. Pinaceae) plantations (?). An increasing percentage of
77 coniferous plantation forestry in the UK is *P. sylvestris*. It currently constitutes ~17% of the UK's
78 commercial coniferous plantation forestry by area and ~15% by biomass (?). It is one of the UK's
79 three native coniferous tree species (?). There is increasing interest to plant native tree species in an
80 attempt to preserve native biodiversity and landscape heritage (). *H. abietis* is the most serious pest
81 of UK *P. sylvestris* plantations, with infestations sometimes precluding sustainable future planting
82 completely due to sapling mortality on clearfell sites (?).

83 Selective breeding and identification of *P. sylvestris* varieties that are resistant to *H. abietis*
84 attack may provide a low cost method to reduce damage to saplings. Resistant varieties could form
85 part of an integrated pest management scheme (?) and planting of multiple varieties in a single
86 forest patch could act as good insurance against potential future attacks in a rapidly changing
87 pest landscape due to climate change (?). Indeed, selecting for and inducing natural resistance
88 to *H. abietis* and other bark boring insects is being heavily explored with other coniferous tree
89 species such as *Picea abies* (Norway spruce) (??), *Picea sitchensis* (Sitka spruce) (?), and *Picea*
90 *glauca* (white spruce) (?), but *P. sylvestris* has not received the same attention. ? found that
91 *P. sitchensis* populations varied in their expression of genes responsible for the production of bark
92 oleoresin ducts when saplings were damaged, which act as a defence against stem boring insects.
93 Similarly, ? developed varieties of *P. sitchensis* resistant to the white pine weevil (*Pissodes strobi*
94 Peck Coleoptera: Curculionidae). They concluded that resin canals and sclereid cells in the bark
95 as well as terpene production and variation in tree phenology were heritable characteristics which
96 confer resistance to attack by *P. strobi*.

97 Natural populations *P. sylvestris* are restricted to enclaves in the north of Scotland. Remnant
98 Caledonian pine populations in Scotland, where *P. sylvestris* is the dominant species (?) are com-
99 prised of 84 fragmented woodland stands dominated by *P. sylvestris*, over a total area of 17,882
100 hectares (?), which maintain adaptive genetic variation. Previous studies have shown that these
101 populations vary in their ability to tolerate pathogens (?) and environmental extremes (?). This
102 study contributes further by assessing the tolerance of natural *P. sylvestris* populations to *H. abietis*
103 attack, with the hope of informing future selection of pine weevil resistant *P. sylvestris* cultivars for
104 plantation forestry, and identifying potential future conservation concerns for naturally occurring
105 *P. sylvestris* in Caledonian remnant forests.

106 We conducted a common garden experiment in a recently clearfelled plantation already affected
107 by *H. abietis* with *P. sylvestris* saplings in southern Scotland to assess sapling resistance to damage
108 from the large pine weevil *H. abietis*. We compared germinated seedstock collected in naturally oc-
109 curring *P. sylvestris* populations in remnant Caledonian pine forest patches across Scotland (Figure

110 ??). We hypothesised that due to limited gene flow between Caledonian pine remnants, adaptive
111 variation in attractiveness to *H. abietis* as a food source would exist between populations of *P.*
112 *sylvestris*. We hypothesised that two effects contribute to the extent of damage which a sapling
113 is subject to, based on the previous work discussed above regarding *H. abietis* host searching be-
114 haviour: the probability of *H. abietis* initially choosing to feed on a sapling and damaging its bark
115 (a), and the intensity of continued feeding by *H. abietis* (b).

116 Materials & Methods

117 Study sites and species

118 Scots pine (*Pinus sylvestris*) is the most widely distributed pine species in the world. It's range
119 spans Eurasia from the arctic circle in Scandinavia to the dry northern mediterranean in Spain and
120 Turkey and from Scotland to the eastern edge of Siberia (??). Scotland represents the western limit
121 of its Eurasian distribution, where it is the dominant canopy tree species of the Caledonian pine
122 forest. *P. sylvestris* grows well under conditions of low grazing, shade and competition.

123 *P. sylvestris* is wind pollinated, with monoecious flowering beginning between the ages of 15 and
124 30. Previous studies have shown cryptic genetic variation between the Caledonian remnant forest
125 sites from which seeds used in this study are sourced (?), which supports the assertion that despite
126 strong cross-pollination effects between the populations, some degree of genetic isolation occurs.
127 Variation in isolatedness between sites follows a predictable longitudinal gradient, with sites on the
128 western extreme of the Caledonian pine range being more isolated due to the prevailing easterly
129 wind direction limiting pollen dispersion to the west (?).

130 Seed populations of *P. sylvestris* were collected from 21 sites where genetic variation has already
131 been identified across Scotland in March 2007 (Figure ??). At each site four open-pollinated trees
132 were located at least 100 m apart. From each of these trees at least 20 cones with seeds were
133 collected. To minimise seedling mortality, seeds were germinated and grown in a glasshouse for
134 3 years before four randomly selected surviving seedlings per Parent tree were transplanted to a
135 common garden. This resulted in 168 distinct maternal lines. All seed was collected from old
136 adult trees, in an attempt to avoid sampling trees descended from nearby plantation forestry as this
137 study focussed only on natural populations. Sites were situated within the historical range of the
138 Caledonian pine forest. Seed collection sites were chosen by accessibility in six geographic clusters.
139 Each cluster was located to ensure geographical isolation from others.

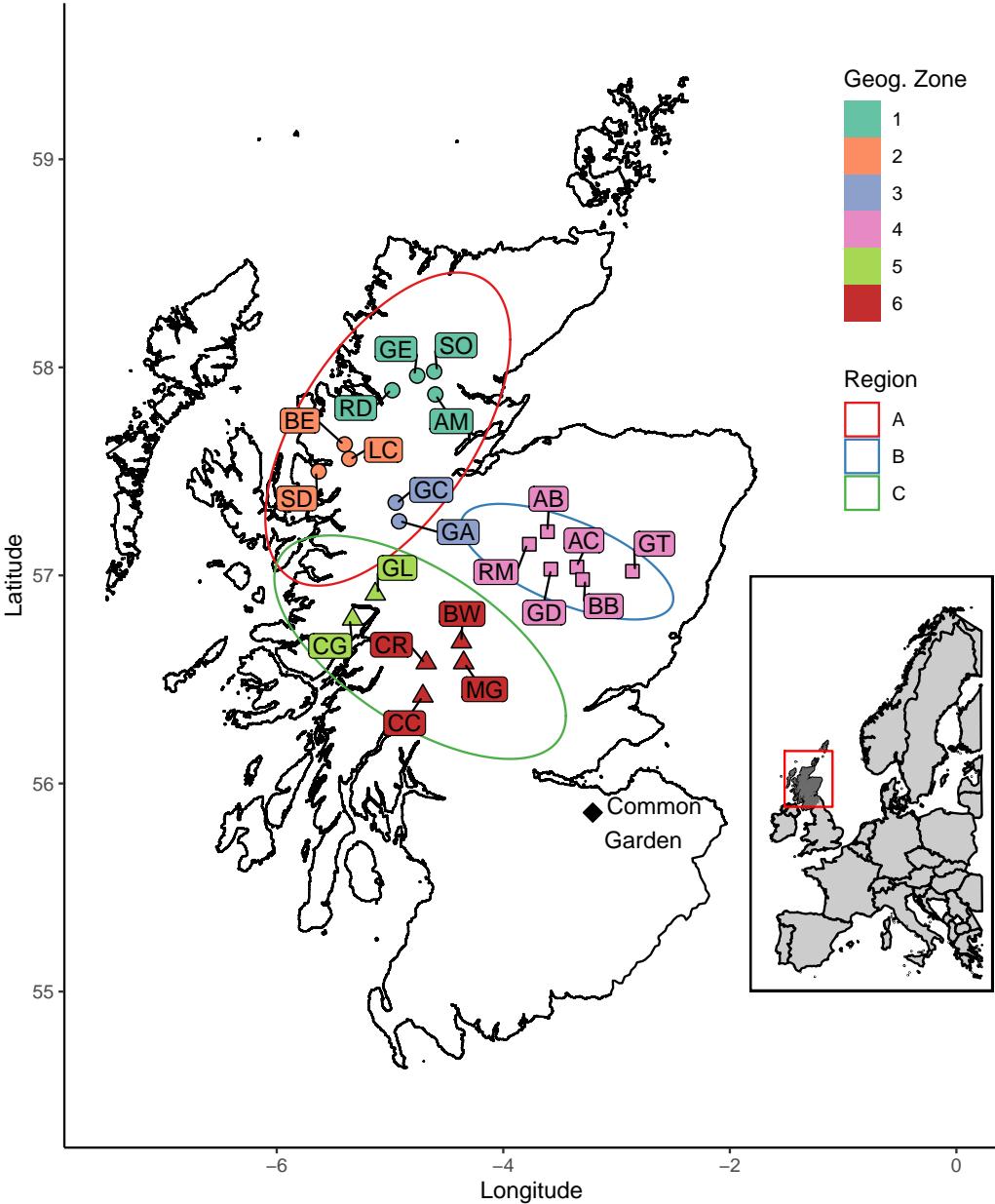


Figure 2: Map of seed collection sites within Scotland, from which seed populations were collected. Elliptic hulls and site point shapes define the three Regions. Points are coloured according to Geographic Zone clusters, which are nested within Regions. Point labels are Site codes used as a shorthand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

140 Experimental design

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

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

152 Data collection

153 The area of bark lesions caused by *H. abietis* was measured on the main growing stem of each
154 sapling in June 2015. This is roughly between the two seasonal peaks of weevil feeding that are
155 commonly observed in the UK, which occur in the spring and late summer, coinciding with the
156 end of adult hibernation and the emergence of new adults from pupae, respectively (??). Only
157 damage sustained by *H. abietis* during the current growing season was counted and could be clearly
158 separated from damage sustained in previous years by the lack of bark edge scarring and presence of
159 sap at the wound edge (Figure ??). Isolated lesions tended to be roughly circular with a diameter of
160 ~3 mm. Where a larger continuous lesion was found, as when a stem was girdled, the larger lesion
161 was photographed with a scale and the area estimated by tracing the lesion with ImageJ version
162 1.50g7 (?). Weevil damage is therefore expressed as the mm² area of stem lesions per sapling.

163 Statistical analysis

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

169 We used a linear mixed effects model, using only saplings where damage had occurred, to
170 assess whether saplings varied in the total area of bark damaged by continued feeding by *H. abietis*
171 according to *P. sylvestris* Site. The response variable of area of bark damaged was log transformed
172 in order to better meet model assumptions. In both analyses, a combination of fixed and random
173 intercept effects were modelled to obtain the optimal model structure and to compare the relative
174 effect sizes of Geographic Zone, Site and Parent tree. Parent tree was used as a random intercept
175 effect in all analyses to account for pseudo-replication in sapling Parent. The geographically nested
176 nature of the seed collection Sites within Geographic Zones was also used as a random effect in
177 the appropriate models (Figure ??). Model goodness-of-fit was assessed for both model types
178 by comparing models with equivalent random effects models and null models using AIC_r (Akaike
179 Information Criterion) and Log-likelihood estimates (?). During model comparison all models
180 were fitted using Maximum Likelihood (ML) (?). To investigate which populations of *P. sylvestris*
181 differed in their resistance to *H. abietis* attack, the models were refitted using Restricted Maximum
182 Likelihood (REML) and model slope estimates were compared. Tukey's HSD multiple comparisons
183 tests of marginal means assessed which populations were significantly different from each other for
184 both models, using the *emmeans* package (?). All statistical analyses were performed in R version
185 3.4.2 (?).

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

189 Spatial autocorrelation may have been present within the Common Garden, with some damaged
190 saplings acting as olfactory beacons to attract more *H. abietis* to the area. This potential effect was
191 investigated using Generalised Least Squares (GLS) models of damaged bark area with spatial
192 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 (?). After model selection, the best generalised least squares model was re-fitted using REML for model interpretation to assess the predictive effect of spatial auto-correlation 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 ?? 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 ??), with some geographic zones having similar levels of damage while others varied a widely within geographic zone.

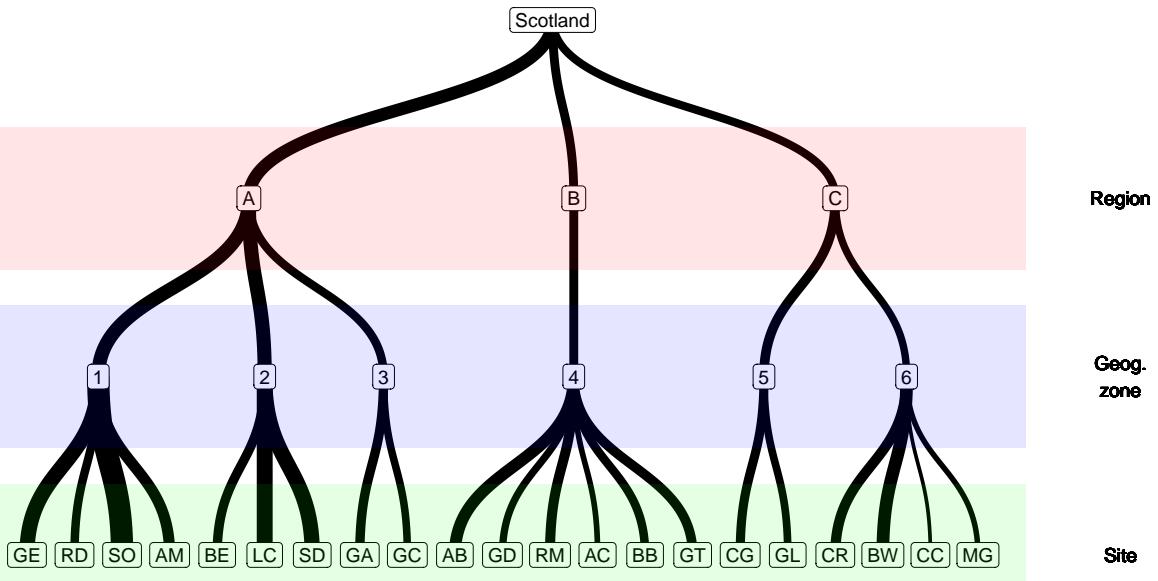


Figure 3: Dendrogram showing nested grouping of seed populations. Graph edge widths vary relatively according to the total bark area damaged on saplings collected from each Site. Width edges are weighted according to the number of saplings at each grouping level to account for differences in number of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

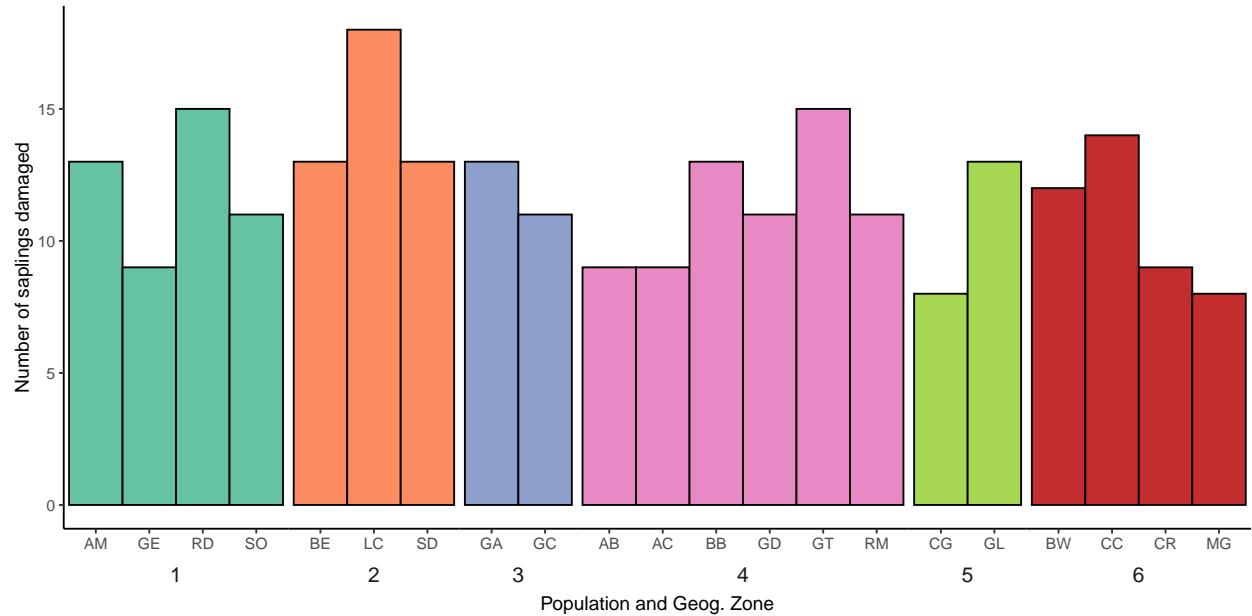


Figure 4: The number of saplings with visible damage by *H. abietis*, divided by Site. Groups of bars denote Geographic Zones.

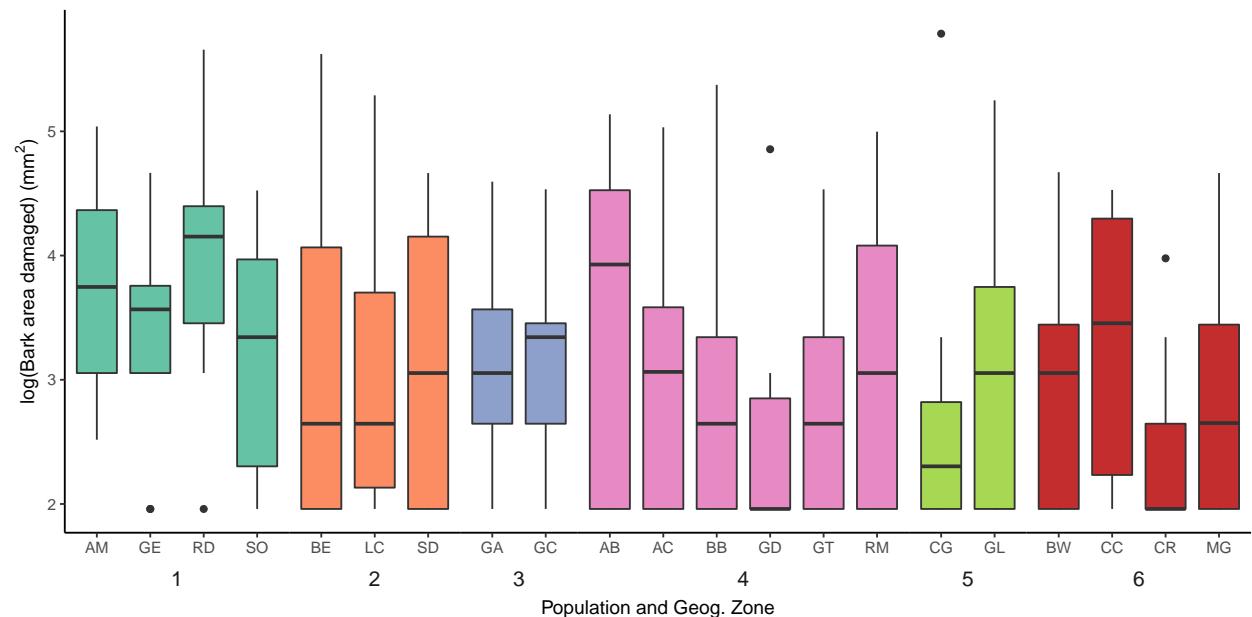


Figure 5: Variation in bark area damaged by *H. abietis*, divided by Site. Coloured groups of bars denote Geographic Zones. Thick bars denote the median value per population.

212 Spatial auto-correlation

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

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

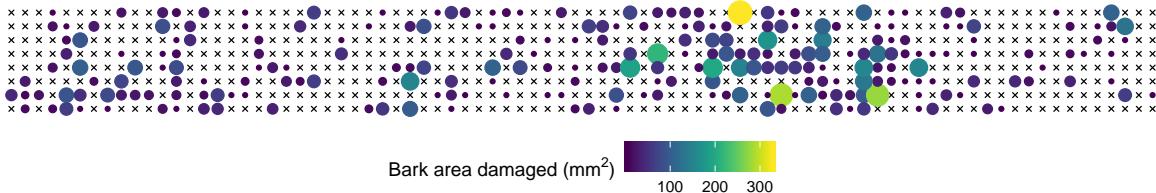


Figure 6: Schematic diagram of sapling relative position within the Common Garden, with sapling points coloured and sized according to the area of bark damaged. The distance between saplings is 3 m in both the X and Y directions.

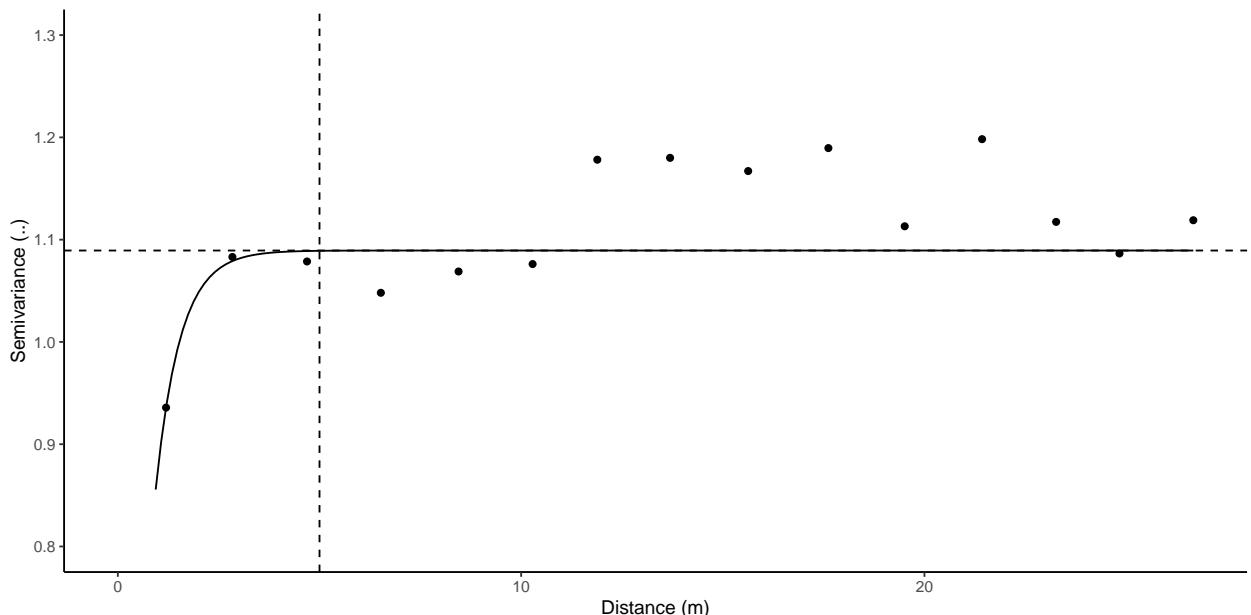


Figure 7: Semivariogram showing spatial autocorrelation of damaged bark area according to distance between saplings. Vertical dotted line denotes the nugget and the horizontal dotted line denotes the sill of the semivariogram.

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

226 The effect of seed population on sapling damage

227 Binomial model

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

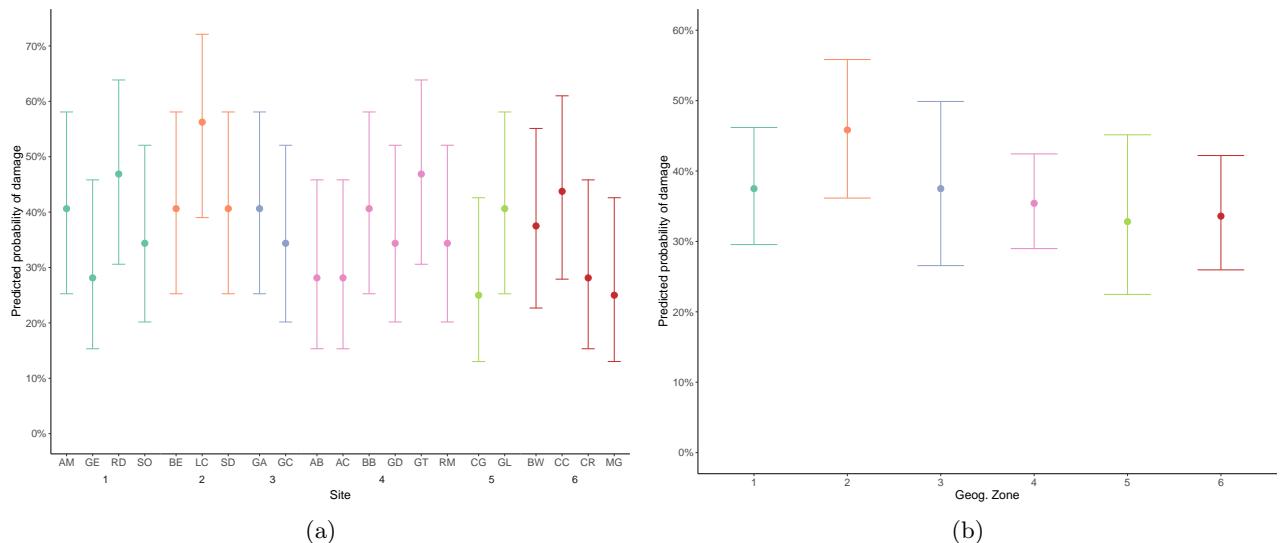


Figure 8: Predicted values with 95% confidence intervals for the probability of a sapling being damaged with seed collected from different (a) Sites and (b) aggregated by Geographic Zone.

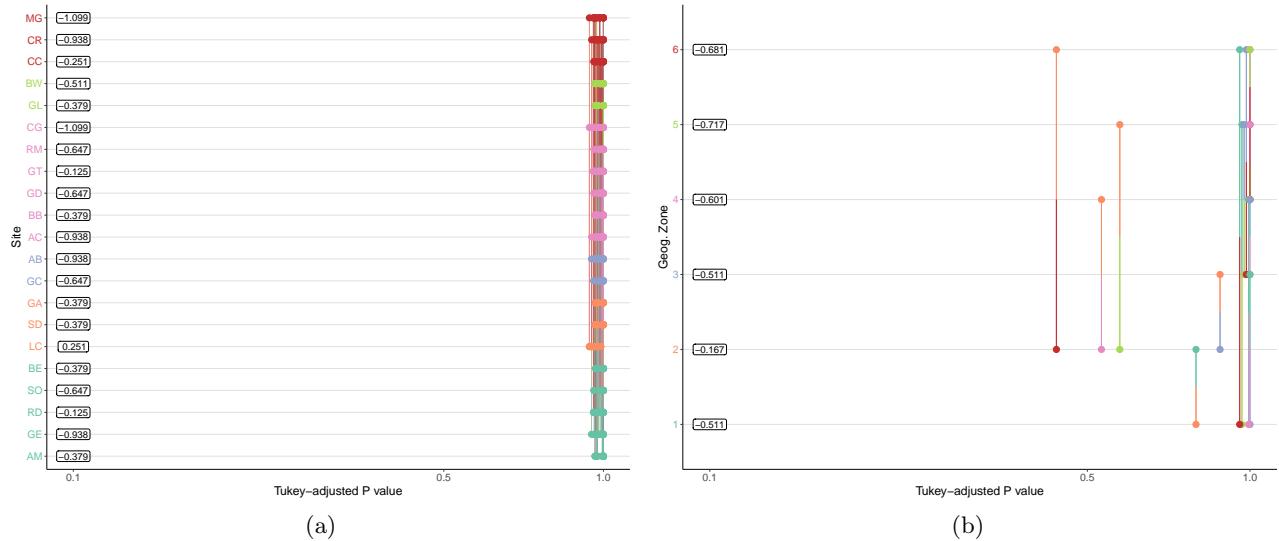


Figure 9: P-values for Tukey HSD pairwise comparisons of estimated marginal means between (a) Site and (b) Geographic Zone).

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

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

246 Non-zero damage model

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

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

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

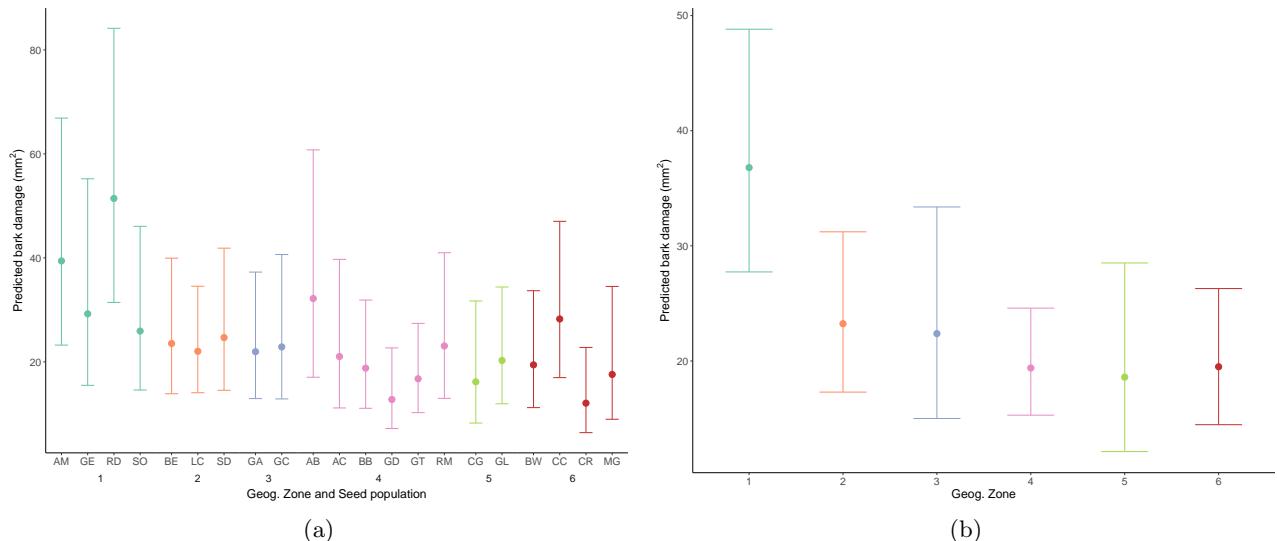


Figure 10: Predicted values of mm^2 for saplings with seed collected from different Geographic Zones.

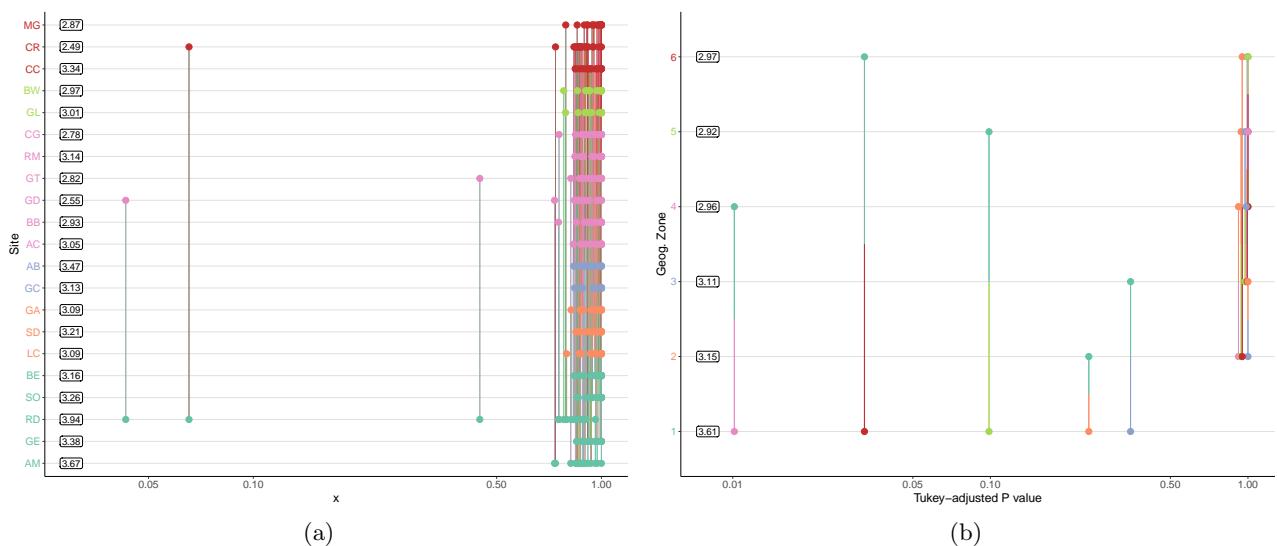


Figure 11: P-values for Tukey HSD pairwise comparisons of estimated marginal means between (a) Site and (b) Geographic Zone).

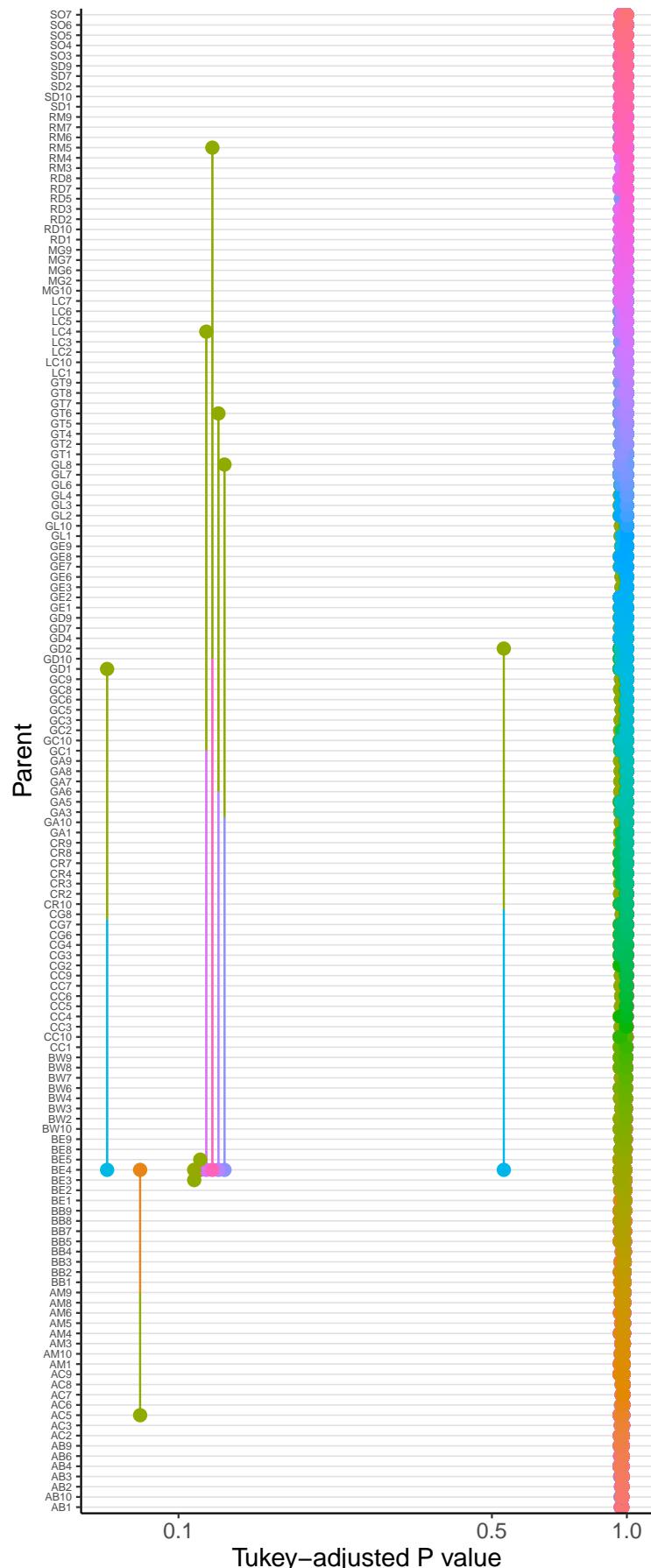


Figure 12: P-values for Tukey HSD pairwise comparisons of estimated marginal means between sapling Parent groups.

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

263 Population level spatial patterns

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

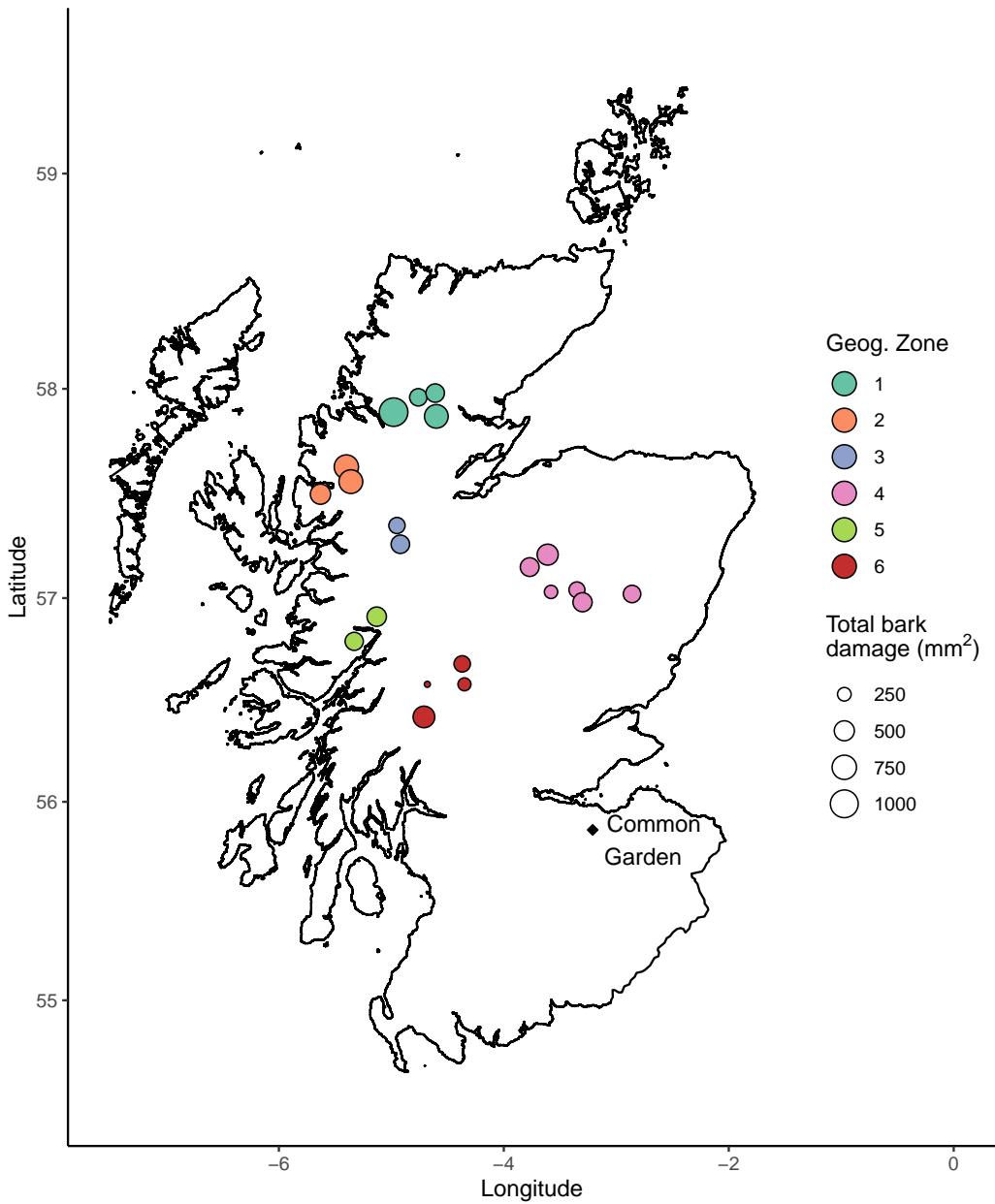


Figure 13: Map of study Sites with bubbles coloured according to Geographic Zone and relatively sized according to the total bark area damaged for all 32 saplings per Site.

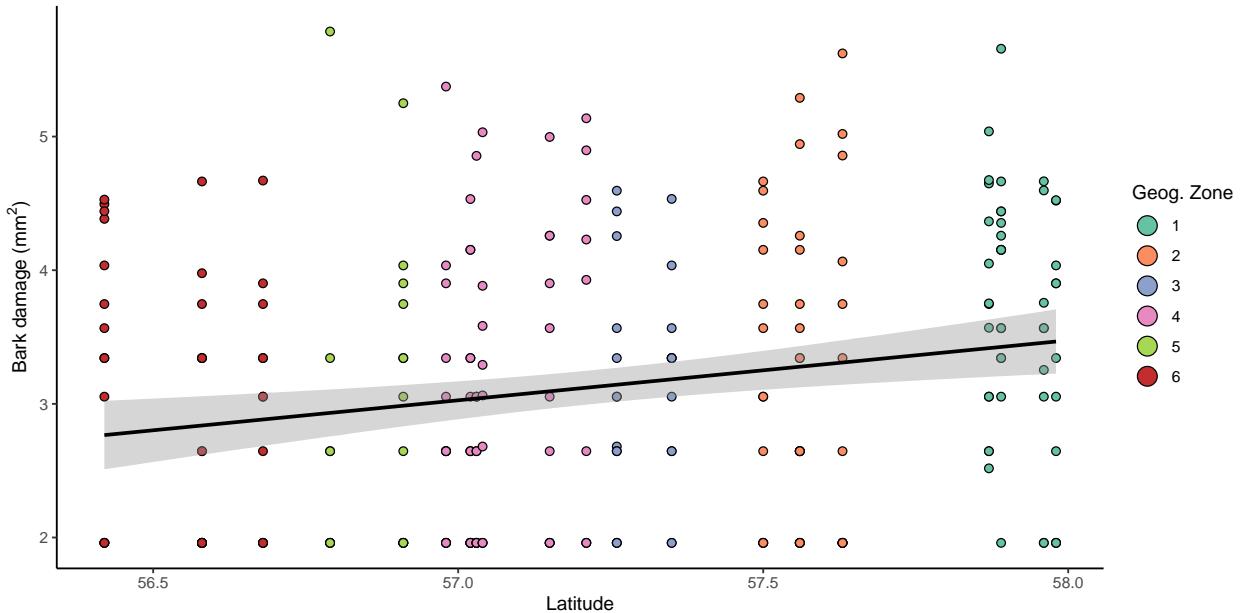


Figure 14: Relationship between bark area damaged and latitude of sapling population, for those saplings which were damaged. Each point is an individual sapling. Points are coloured by Geographic Zone. The linear model fit (black line with grey 95% confidence interval) shows a weak positive trend.

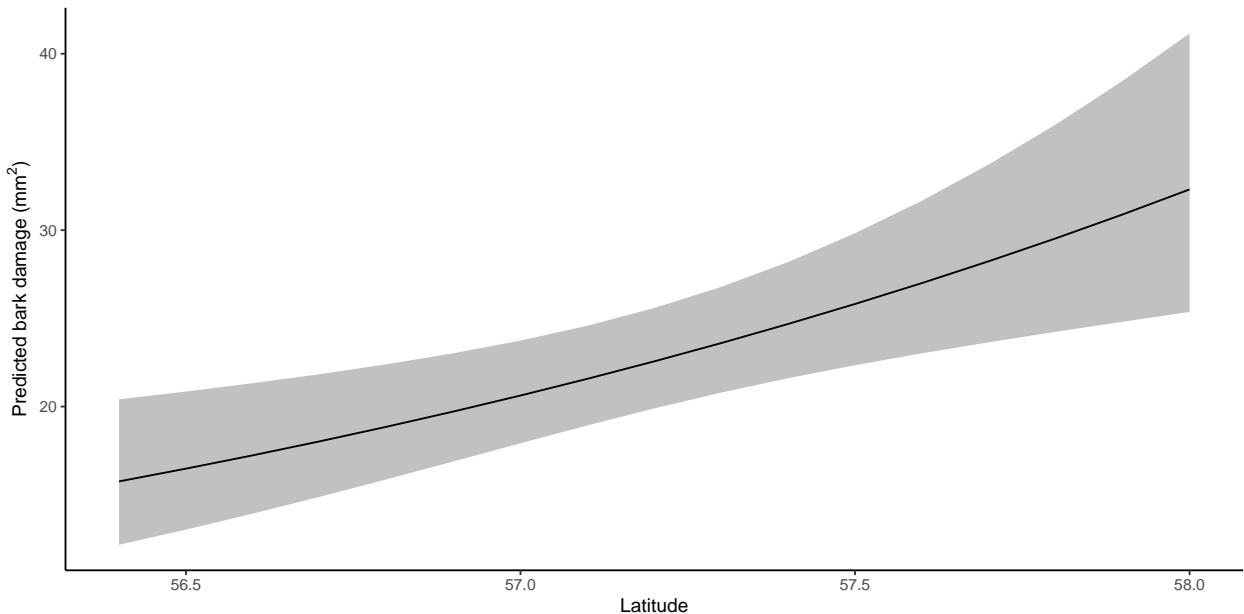


Figure 15: Predicted values with 95% confidence interval for the bark area damaged on a sapling with seed collected at different latitudes.

268 Discussion

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

273 with Parents at higher latitudes typically experienced higher levels of damage, but this had a much
274 weaker effect than population or Geographic Zone itself. Within Site variability among Parents was
275 high and diluted the effects of Geographic Zone and Site. This is expected given the high gene flow
276 between populations (?). Nevertheless, some clear variation was observed between groups.

277 In this study we identified weak but significant differences between *P. sylvestris* populations in
278 their susceptibility to *H. abietis* attack. It appears that a weak latitudinal pattern may be driving
279 these differences. It may be that historical exposure to *H. abietis* in more southerly populations
280 has driven adaptation to develop defensive structures to deter bark feeding insects. Studies on the
281 distribution and life cycle of *H. abietis* have shown that life cycle length is strongly linked with
282 mean temperature in the summer months, with higher temperatures leading to a short life cycle
283 and therefore higher numbers of pine weevils where infestations occur (?). *H. abietis* abundance
284 reduces with latitude in Scotland (?). Historically, *H. abietis* populations at high latitudes and in
285 the west of Scotland have been low (?), due to lower temperatures (?). The latitudinal effect may
286 therefore be a result of adaptation to resist *H. abietis* damage. Additionally, phenological variation
287 in latitudinal populations may lead to VOC concentrations varying between saplings at the same
288 time of year in the common garden, making some saplings more desirable than others (?). The
289 data collection for this study took place in June, approximately between the two seasonal peaks of
290 *H. abietis* activity. Other studies have shown that the growing season of *P. sylvestris* from higher
291 latitudes starts later in the year (?), leading to a lower concentration of VOC stored in bark resin
292 when our study was conducted, potentially making these saplings more attractive than those from
293 southerly populations to *H. abietis*.

294 *P. sylvestris* needles and bark have resin canals which act to deter herbivores. While it has not
295 been explicitly tested for *H. abietis*, other studies involving similar bark feeding insects have found
296 a negative correlation between resin canal density and feeding behaviour on coniferous tree species.
297 ? found that the white pine weevil *Pissodes strobi* was discouraged from eating the needles of four
298 different pine species with higher resin canal concentration and cuticle thickness. ? found that for
299 a subset of the same seed population Sites studied here, that resin canal density in needles varied
300 with longitude and between Sites, but did not test latitudinal variation. They suggested that resin
301 canal density may be linked to water stress, as it plays a role in water regulation (?). Interestingly,
302 this study found no complementary correlation between longitude and damage by *H. abietis*.

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

310 Conclusion

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

319 for less-resistant Caledonian remnant forest patches as *H. abietis* populations may increase, leading
320 to higher mortality of saplings, leading to changes in ecosystem structure. It is suggested that further
321 studies should investigate bark morphological and physiological variation amongst these natural
322 populations of *P. sylvestris*, with particular attention to variation in Volatile Organic Compounds
323 (VOCs) emitted when bark is damaged as a defensive response in young saplings, to understand
324 the underlying mechanism driving differences between these genetically distinct populations.

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