

Scotland's Rural College

## Planting exotic relatives has increased the threat posed by *Dothistroma septosporum* to the Caledonian pine populations of Scotland

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*Published in:*  
Evolutionary Applications

*DOI:*  
[10.1111/eva.12562](https://doi.org/10.1111/eva.12562)

Print publication: 27/02/2018

*Document Version*  
Peer reviewed version

[Link to publication](#)

### *Citation for pulished version (APA):*

Piotrowska, MJ., Riddell, C., Hoebe, PN., & Ennos, RA. (2018). Planting exotic relatives has increased the threat posed by *Dothistroma septosporum* to the Caledonian pine populations of Scotland. *Evolutionary Applications*, 11(3), 350 - 363. <https://doi.org/10.1111/eva.12562>

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1           Planting exotic relatives has increased the threat posed by *Dothistroma*  
2                           *septosporum* to the Caledonian pine populations of Scotland

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21  
22 **Running Title:** Multiple origins of *Dothistroma septosporum*

23

**24 Abstract**

25 To manage emerging forest diseases and prevent their occurrence in the future, it is essential  
26 to determine the origin(s) of the pathogens involved and identify the management practices  
27 that have ultimately caused disease problems. One such practice is the widespread planting of  
28 exotic tree species within the range of related native taxa. This can lead to emerging forest  
29 disease both by facilitating introduction of exotic pathogens, and by providing susceptible  
30 hosts on which epidemics of native pathogens can develop. We used microsatellite markers to  
31 determine the origins of the pathogen *Dothistroma septosporum* responsible for the current  
32 outbreak of Dothistroma needle blight (DNB) on native Caledonian Scots pine (*Pinus*  
33 *sylvestris*) populations in Scotland, and evaluated the role played by widespread planting of  
34 two exotic pine species in the development of the disease outbreak. We distinguished three  
35 races of *D. septosporum* in Scotland, one of low genetic diversity associated with introduced  
36 lodgepole pine (*Pinus contorta*), one of high diversity probably derived from the DNB  
37 epidemic on introduced Corsican pine (*Pinus nigra* subsp. *laricio*) in England, and a third of  
38 intermediate diversity apparently endemic on Caledonian Scots pine. These races differed for  
39 both growth rate and exudate production in culture. Planting of exotic pine stands in the UK  
40 appears to have facilitated the introduction of two exotic races of *D. septosporum* into  
41 Scotland which now pose a threat to native Caledonian pines both directly and through  
42 potential hybridisation and introgression with the endemic race. Our results indicate that both  
43 removal of exotic species from the vicinity of Caledonian pine populations, and restriction of  
44 movement of planting material are required to minimise the impact of the current DNB  
45 outbreak. They also demonstrate that planting exotic species that are related to native species  
46 reduces rather than enhances the resilience of forests to pathogens.

- 47 **Keywords:** *Dothistroma septosporum*, microsatellite, tree disease, genetic structure, pine,  
48 needle blight, emerging disease

## 49 **Introduction**

50 Over recent decades a dramatic rise in the incidence of tree disease epidemics has occurred  
51 on a global scale (Stenlid *et al.* 2011; Wingfield *et al.* 2015). Unregulated global trade in live  
52 plants, which facilitates the introduction of exotic pathogens, is largely responsible for this  
53 phenomenon (Brasier 2008; Santini *et al.* 2013). However there are a variety of other forestry  
54 practices that may be contributing significantly to our current tree disease problems (Ennos  
55 2015). One that deserves particular attention is the practice of planting exotic species in areas  
56 occupied by closely related native tree taxa (Burgess & Wingfield 2017). In these situations  
57 disease outbreaks can arise in two ways.

58

59 The first involves transfer of endemic pathogen species or races from the native to the related  
60 exotic tree (Gilbert & Webb 2007; Gilbert *et al.* 2012). The exotic species may prove  
61 susceptible to these native pathogens due to lack of previous co-evolution (Ennos, 2015). The  
62 natural resistance of the exotic may also be compromised because it is poorly adapted to the  
63 novel environment into which it has been planted (Read 1968; Karlman *et al.* 1984). High  
64 density planting in monoculture and reduced genetic diversity of the exotic host may further  
65 exacerbate disease problems. An epidemic of the native pathogen may therefore build up on  
66 the exotic plantation species. The pathogen pressure generated by this epidemic may be  
67 severe enough to produce damage on the (previously resistant) native tree species (Ennos  
68 2001).

69

70 The second route to epidemic disease involves the inadvertent introduction, along with the  
71 exotic tree species, of one of its co-evolved pathogens. The native species may suffer serious

72 damage because it has no history of co-evolution with the introduced pathogen (Anagnostakis  
73 1987). Disease problems can also arise on the exotic plantation species if the novel  
74 environmental conditions that it encounters either favour the introduced pathogen directly  
75 (Gibson, 1972), or impose stress on the exotic species and increase its susceptibility to  
76 disease (Schoenweiss 1975, 1981). Introduced pathogens may also hybridise with closely  
77 related native pathogens to generate genotypes that are more virulent than either parent  
78 (Brasier 2000; Brasier *et al.* 2004; Stukenbrock, 2016).

79

80 Well documented examples of disease outbreaks associated with planting of exotic relatives  
81 of native species include the white pine blister rust *Cronartium ribicola* (Lasch.) Dietr.  
82 epidemic on *Pinus strobus* L. in Europe that followed planting of this species within the  
83 native range of European five needled pines at the end of the nineteenth century (Hummer  
84 2000), and the epidemic of *Gremmeniella abietina* (Lagerberg) Morelet on *Pinus contorta*  
85 Douglas ex Loudon when this species was introduced into Sweden in the 1990's alongside  
86 native *Pinus sylvestris* L. (Karlman *et al.* 1994). More recently the ash dieback epidemic in  
87 Europe caused by *Hymenoscyphus fraxineus* has been linked with planting of Asian *Fraxinus*  
88 *mandschurica* Rupr. within the native range of European ash *Fraxinus excelsior* L. (Gross *et*  
89 *al.* 2014).

90

91 Given the diversity of ways in which planting of exotic relatives can give rise to tree disease  
92 epidemics, detailed forensic studies of such situations are needed to establish the origin(s) of  
93 the pathogens responsible and devise appropriate control measures. If the pathogen involved  
94 is native, removal of the exotic species may be sufficient to eliminate the disease threat.  
95 However if the pathogen is introduced, the prospects for the native species may be poor,

96 involving death of many trees and recovery only after a prolonged period during which there  
97 is evolution of enhanced host resistance (Gomulkiewicz & Holt 1995). Where both exotic and  
98 native pathogens are responsible the outcome is less predictable and will depend on the extent  
99 of genetic interactions between the pathogen sources (Brasier *et al.* 2001, 2004). Here we use  
100 microsatellite markers to analyse the origin(s) of the pathogen *Dothistroma septosporum*  
101 (Dorog.) Morelet responsible for a recent outbreak of Dothistroma needle blight (DNB) on  
102 pine in Scotland (Brown *et al.* 2012). We highlight the role of two exotic pine species in  
103 facilitating the DNB outbreak, and assess the threat that *D. septosporum* now poses to native  
104 pine populations.

105

106 DNB caused by the ascomycete *D. septosporum*, is currently the most important foliar  
107 disease of pine worldwide, affecting 82 host pine species across six continents (Drenkhan *et*  
108 *al.* 2016). Needle infection by rain-splashed conidia (Gibson 1972) or wind-borne ascospores  
109 (Funk & Parker 1966) leads to defoliation, reduction in growth and, in severe cases, death of  
110 trees (Brown & Webber 2008). *D. septosporum* is believed to be endemic on indigenous pine  
111 populations in the northern hemisphere (Welsh *et al.* 2009; Drenkhan *et al.* 2013). From here  
112 it has spread and caused severe damage to exotic pine plantations in the southern hemisphere  
113 (Barnes *et al.* 2014). Most recently DNB has emerged as a problem in plantations in the  
114 northern hemisphere; in North America on *P. contorta* (Roach *et al.* 2015); in mainland  
115 Europe on *Pinus nigra* (Poir.) Maire (Fabre *et al.* 2012; Tomsovsky *et al.* 2013; Boron *et al.*  
116 2016; Drenkhan *et al.* 2016); and in Britain on *P. sylvestris*, *P. nigra* and *P. contorta* (Brown  
117 & Webber 2008).

118

119 In Britain the native host for *D. septosporum*, Scots pine *P. sylvestris*, comprises two distinct  
120 populations. Caledonian pines represent the remnants of native *P. sylvestris* populations that  
121 recolonised Scotland after the last ice age. They are confined to the Scottish Highlands, are  
122 highly fragmented, and their distribution has been reduced to less than 1% of its former area  
123 (Steven & Carlisle 1959; Forestry Commission Scotland, 1998). Nevertheless they retain  
124 high genetic diversity (Kinloch *et al.* 1986; Wakowiak *et al.* 2011) and are of enormous  
125 conservation value because they support one of the few intact, semi-natural forest ecosystems  
126 remaining in Britain (McVean & Ratcliffe 1962; Mason *et al.* 2004). Outside the Caledonian  
127 pinewoods *P. sylvestris* stock derived from Forestry Commission seed orchards is used to  
128 establish plantations, and the species is extensively naturalised throughout British woodlands,  
129 representing 17% of total conifer area (Forestry Commission 2015).

130

131 Two exotic pine species closely related to Scots pine have been introduced to Britain and  
132 grown in large scale plantations for the last 60-100 years. Corsican pine *P. nigra* subsp.  
133 *laricio* accounts for 13% of conifer stands in England, and has been successfully introduced  
134 at a small number of coastal sites in Scotland (Forestry Commission 2015). Lodgepole pine  
135 *P. contorta* of two subspecies (*contorta* and *latifolia*) is grown principally in Scotland where  
136 it makes up 10% of the conifer area (Lines 1987; Forestry Commission 2015). Plantations of  
137 lodgepole pine often occur adjacent to, or even within Caledonian pine stands.

138

139 DNB was first found in Britain in the 1950s in southern English nurseries on four exotic  
140 species; Corsican pine, lodgepole pine, *Pinus ponderosa* Douglas ex C. Lawson and *Pinus*  
141 *bungeana* Zucc. ex Endl. (Murray & Batko 1962). No infection or damage to Scots pine was  
142 reported. Over the next 40 years the presence of *D. septosporum* was recorded sporadically in



143 southern England and southern Wales (Brown & Webber 2008), and in the 1980s on Scots  
144 pine in northern Scotland (British Mycological Society 2014) but was not associated with  
145 significant damage. However from 2000 onwards serious epidemics of DNB broke out in  
146 England on plantations of Corsican pine, with some infection of adjacent Scots pine. The  
147 very high level of damage led to a moratorium on plantings of Corsican pine in 2006 (Brown  
148 & Webber 2008). Subsequently DNB has been reported in Scotland on Corsican, lodgepole  
149 and plantation Scots pine, and on all three species in forest nurseries. Serious conservation  
150 concerns were raised in 2011 when *D. septosporum* was discovered in Caledonian pine  
151 populations where it had not previously been recorded (Brown *et al.* 2012).

152

153 To clarify the origins of the *D. septosporum* population in Scotland, assess the role of the  
154 exotic Corsican and lodgepole pine species in its appearance, and inform management plans  
155 for its control, particularly in the Caledonian pinewoods, we initiated a detailed analysis of  
156 the genetic structure of the pathogen across its hosts within Scotland. Recent work by Mullett  
157 *et al.* (2017), using microsatellite marker analysis of a large sample of *D. septosporum* from  
158 across the whole of Britain, has demonstrated that individuals can be assigned to one of three  
159 major genetic groups (see Figure 2 in Mullett *et al.*, 2017). These comprise: a genetic group  
160 with low diversity, present only in Scotland and found predominantly on lodgepole pine  
161 (DAPC cluster 1 of Mullett *et al.*, 2017) hereafter referred to as the lodgepole pine race, LPR;  
162 a genetic group with a markedly southern distribution found largely on Corsican pine (DAPC  
163 clusters 3-7 and 10-12 of Mullett *et al.*, 2017) designated here the southern race, SR; and a  
164 Britain wide but predominantly northern genetic grouping loosely associated with Scots pine  
165 (DAPC clusters 2, 8 and 9 of Mullett *et al.*, 2017), named here the native pine race, NPR;  
166 LPR shows genetic similarities with samples from lodgepole pine in Canada, while SR

167 clusters genetically with samples from northern France where it is found mainly on Corsican  
168 pine (Mullett *et al.*, 2017).

169

170 The aim of the present study was firstly to determine the involvement of the three genetic  
171 groupings of *D. septosporum* identified by Mullett *et al.* (2017) in the current outbreak of  
172 DNB in the native Caledonian pinewoods. We also sought to understand the degree to which  
173 the LPR, SR and NPR races are associated with different pine hosts in Scotland, and to  
174 determine the geographic distribution of these races. To do this we designed a sampling  
175 scheme that explicitly included samples from Caledonian pinewood populations and in which  
176 we took population samples from adjacent stands of different hosts so that the effects of host  
177 species and geographic location on the frequencies of the races could be determined  
178 independently.

179

180 Four different categories of host population were recognised in the sampling; Caledonian  
181 Scots pine populations; Scots pine plantations; lodgepole pine plantations; and Corsican pine  
182 plantations. Replicate sites throughout Scotland containing adjacent stands of these different  
183 host population types were identified, and from these sites population samples of *D.*  
184 *septosporum* were isolated. Clustering based on microsatellite data was used to assign  
185 individuals to races, assess the distribution of races with respect to host type within each site,  
186 and to ascertain the geographic pattern of races among sites. Further samples were obtained  
187 from isolated Caledonian pinewood sites and from infected pine nurseries to measure the  
188 proportions of *D. septosporum* races present in these situations. Analysis of mating type loci  
189 and multilocus microsatellite genotypes was used to infer the reproductive systems of the  
190 three races. In addition the races were compared in culture to establish whether they differed

191 significantly for important phenotypic characters. We then developed a scenario, based on  
192 our results, to account for the current distribution of *D. septosporum* races in Scotland,  
193 highlighting the role played by exotic plantations of Corsican and lodgepole pine, and  
194 assessed the likely impact of *D. septosporum* on Caledonian pine populations.

195

## 196 **Materials and Methods**

### 197 *Sampling*

198 We performed targeted sampling of *Dothistroma septosporum* (Dorog.) Morelet outbreaks  
199 identified in disease surveys in naturally regenerated and planted forest stands (Table 1,  
200 Figure 2). Classes of sample site and associated sampling strategies were:

- 201 i. Mixed plantations of Scots (*P. sylvestris*) and Corsican pine (*P. nigra* subsp.  
202 *laricio*). Three sites were sampled in 2015 (Culbin Forest (n=39), Torrs Warren  
203 (n=33), and Tentsmuir (n=30)). At each site roughly equal numbers of isolations  
204 were made from the two host species.
- 205 ii. Caledonian Scots pine sites with adjacent lodgepole pine (*P. contorta*) stands.  
206 Three sites were sampled in 2014 (Glen Einig (n=29), Glen Garry (n=35),  
207 Inshriach Forest (n=32)) and two in both 2014 and 2015 (Glen Affric (n=21),  
208 Dundreggan (n=39)). At each site we made isolations from roughly equal numbers  
209 of the two hosts. In addition we collected two lodgepole pine isolates from  
210 Strathpeffer in 2015 (n=2).
- 211 iii. Caledonian Scots pine sites isolated from exotic pine plantations.  
212 Two sites were sampled in 2015, Glen Tanar (n=15), and Beinn Eighe (n=23).
- 213 iv. Forest nursery sites with reported disease outbreaks.  
214 Samples were isolated by Forest Research (Alice Holt) during annual forest  
215 nursery DNB surveys between 2011 and 2015. Nursery samples were classified  
216 into three categories; southern Scotland (n=24), northern Scotland (n=13) and  
217 northern England (n=3). All other information relating to the samples and their  
218 location remains confidential.

219 At each site, we sampled needles bearing conidiomata from 15-40 individuals of the relevant  
220 tree species (current year or second year growth needles). At Caledonian pine sites infected  
221 needles originated mostly from naturally regenerated saplings, though in some cases mature  
222 trees were sampled. A single genotype of *D. septosporum* was obtained from each tree.

223 Single spore cultures were isolated following the procedure described by Mullet *et al.* (2015),  
224 with modifications described in Piotrowska *et al.* (2016). Cultures were stored in three ways  
225 as described by Mullett & Barnes (2012); as agar cubes at 4 °C, water storage at 4 °C and 15  
226 % glycerol stocks at -80 °C. In addition to our Scottish collection, Forest Research at Alice  
227 Holt provided a single isolate of *D. septosporum* from each of three North American  
228 populations of lodgepole pine ( Nass Valley, Brown Bear (1 and 7) and Kispiox, Buckley  
229 Canyon)) (Table 1).

230

### 231 *DNA extraction and genotyping*

#### 232 *i. DNA extraction*

233 Fungal mycelium was collected from cultures on agar plates into 2 ml cryovial tubes, freeze-  
234 dried overnight (Alpha 1-4 LDplus, Christ, Osterode am Harz, Germany) and tissue-lysed  
235 (Tissue Lyser LT; Qiagen, Hilden, Germany) prior to DNA extraction (~ 20 mg of  
236 lyophilized tissue). DNA extraction was performed using DNeasy Plant Mini Kit (Qiagen),  
237 following the manufacturer's guidelines. DNA for genotyping was re-suspended in sterile  
238 distilled water (SDW) and stored at -20 °C for further use.

#### 239 *ii. Mating type assay*

240 Mating type variants for *D. septosporum* were determined using species-specific primer  
241 combinations developed by Groenewald *et al.* (2007). The amplification reactions were

242 carried out using GoTaq Green Master Mix (Promega, Madison, USA). Each reaction  
243 comprised 1x Promega Master Mix, 200 nM of each forward (F) and reverse (R) primers,  
244 12.5 ng of DNA and SDW up to 25  $\mu$ l. The thermocycler (GeneAmp PCR System 9700  
245 thermocycler, Applied Biosystems, Foster City, CA) conditions included initial denaturation  
246 at 94 °C for 5 minutes, followed by 36 cycles of denaturation at 95 °C for 20 seconds,  
247 annealing at 60 °C for 30 seconds and extension at 72 °C for 40 seconds, and a final  
248 extension at 72 °C for 5 minutes. To determine mating type variants, samples were run on 1.2  
249 % agarose gels, and band sizes corresponding to mt-1 and mt-2 were scored manually against  
250 the Quick-Load Purple 100 bp DNA Ladder (New England BioLabs, Ipswich, USA). Both  
251 positive and negative controls for each mating type were run on every PCR plate.

### 252 *iii. Microsatellite scoring*

253 To investigate the population structure of *D. septosporum*, we scored 11 microsatellite loci,  
254 using primers developed by Barnes *et al.* (2008). Economic fluorescence labelling (Schuelke  
255 2000) was used in all genotyping assays; F primers were tailed at the 5' end with M13  
256 universal primer and M13 primer was labelled with 6-Carboxyfluorescein (6FAM) dye at the  
257 5' end. We grouped microsatellite primers into three multiplex combinations: (i) MixI:  
258 Doth\_E, Doth\_F, Doth\_I, Doth\_K, M13\_FAM; (ii) MixII: Doth\_J, Doth\_M, Doth\_DS1,  
259 Doth\_DS2, M13\_FAM; (iii) MixIII: Doth\_G, Doth\_L, Doth\_O, M13\_FAM. The  
260 amplification reactions were performed using Multiplex PCR Kit (Qiagen) with the following  
261 thermocycler conditions (GeneAmp PCR System 9700 thermocycler): initial denaturation at  
262 95 °C for 15 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds,  
263 annealing at 60 °C for 90 seconds, extension at 72 °C for 60 seconds, and final extension at  
264 60 °C for 30 minutes. For MixI and MixII PCR components comprised 1x Master Mix, 0.2  
265  $\mu$ M of each R and M13 primer, 0.05  $\mu$ M of each F primer, 12.5 ng of DNA template and

266 RNase free water (Qiagen) up to a final volume of 25  $\mu$ l. For MixIII the following  
267 modifications of primers' concentrations were used: M13 at 0.2  $\mu$ M, primer Doth\_L at 0.2  
268  $\mu$ M of R and 0.05  $\mu$ M of F, primers Doth\_G and Doth\_O at 0.1  $\mu$ M of R and 0.025  $\mu$ M of F.  
269 Genotyping reactions were run on the ABI 3730 sequencer (Applied Biosystems) at  
270 Edinburgh Genomics (UK) using size standards GS500LIZ (Life Technologies, Thermo  
271 Fisher Scientific, Wilmington, USA). Allele sizes were scored in Peak Scanner Software (v  
272 2.0, Applied Biosystems) and binned manually for population genetic analysis.

273

## 274 *Population genetic analysis of genotype data*

### 275 *i. Genotypic clustering*

276 In order to infer the number of genetic clusters within Scottish populations of *D.*  
277 *septosporum*, we performed analysis in R studio (v 1.0.136) using the adegenet package (v  
278 2.0.1, Jombart 2008; Jombart & Ahmed 2011). The isolates were assigned to genetic groups  
279 using the multivariate Discriminant Analysis of Principal Components method (DAPC,  
280 Jombart *et al.*, 2010). This method of clustering was chosen because it makes no assumptions  
281 about the mating system of the organisms concerned. The optimal number of clusters was  
282 inferred using the *find.cluster* function by computing both BIC (Bayesian Information  
283 Criterion) and WSS (within sum of squares) statistics for increasing number of clusters.

284

### 285 *ii. Genetic diversity and divergence among and within genetic clusters*

286 All the input files for genetic analysis were prepared in CREATE software (v 1.37, Coombs  
287 *et al.* 2008). Percentage of polymorphic loci, number of alleles, number of unique alleles  
288 possessed by each cluster, genetic diversity over all loci ( $H_t$ ), as well as genetic divergence

289 ( $\theta_{st}$ ) between *D. septosporum* clusters and among populations within these clusters, were  
290 calculated using the FSTAT programme (v 2.9.3.2, Goudet 1995, 2002).  $H_t$  was calculated  
291 according to Nei's (1987) unweighted estimator. Overall genetic differentiation between the  
292 clusters, among populations within the clusters, as well as pairwise differentiation between  
293 populations was measured using Weir & Cockerham's (1984) estimator of  $\theta_{st}$ . Significance of  
294  $\theta_{st}$  was tested with multiple bootstrapping over loci.

295

### 296 *iii. Multilocus structure of races*

297 For each cluster the number of multilocus genotypes was found using the program MLGsim  
298 (Stenberg *et al.* 2003). The program was also used to estimate which multilocus genotypes  
299 represented multiple times have a low probability ( $P < 0.05$ ) of being the product of sexual  
300 reproduction. These genotypes were then treated as clonal replicates to generate a clone-  
301 corrected data set. Allele frequencies used in the MLGsim simulation were those estimated in  
302 the complete dataset (prior to removal of clonal genotypes).

303

304 Analysis of multilocus structuring of the races was conducted in three ways. In the first  
305 analysis, the program Multilocus 1.3 Beta (Agapow & Burt 2001) was used to estimate the  
306 index of association among loci ( $I_A$ ) and mean correlation among loci ( $r_D$ ), with significance  
307 estimated using 1000 randomisations. In the second analysis, the proportion of locus pairs  
308 showing significant association (Weir 1996) was determined in FSTAT with allele  
309 permutations. In the third analysis the extent of genetic differentiation between the two  
310 populations of opposite mating type within a race ( $\theta_{mt}$ ) was computed using FSTAT. If sexual  
311 reproduction is prevalent, there will be no significant genetic differentiation between



312 populations of opposite mating type (Ennos & Hu *in prep.*). Analyses were conducted both  
313 on the original and on the clone-corrected data sets. Additionally, the extent of genetic  
314 differentiation ( $\theta_{mt}$ ) between the clone-corrected mt-1 population in the SR race and the  
315 clone-corrected mt-1 population in the NPR race was calculated in FSTAT.

316

### 317 *Analysis of growth rate and exudate production*

318 To investigate possible genetically determined phenotypic differences between *D.*  
319 *septosporum* clusters identified with genetic markers and between populations within these  
320 clusters, we examined the rate of fungal colony growth and exudate production *in vitro*. We  
321 randomly selected five individuals from three populations within each of the three *D.*  
322 *septosporum* races identified above. Initial cultures were grown on Dothistroma sporulating  
323 medium (DSM), (Bradshaw *et al.* 2000) at 20 °C at 12 h dark/12 h light mode (Gallenkamp,  
324 INF 780C, Weiss Technik Konigswinter, Germany). Mycelial plugs of 8 mm diameter were  
325 excised from the colony and sub-cultured onto fresh DSM media. Samples were incubated for  
326 a further 8 weeks, in 24 hour darkness. In total we used 6 growth cabinets (3- MIR-254  
327 incubator; Sanyo, Osaka, Japan and 3- Gallenkamp, INF 780C), 3 temperatures (10 °C, 17.5  
328 °C, 22.5 °C) and 2 technical replicates for each temperature, resulting in a total number of  
329 n=270 observations. Growth of isolates was measured as increase in the colony radius (mm)  
330 from week 0 to week 8.

331

332 In culture *D. septosporum* produces the exudate dothistromin which is known to be a  
333 virulence factor in DNB (Kabir *et al.*, 2015). Exudate production of isolates was scored at  
334 week 8 according to the degree to which it discoloured the growth medium using a four point

335 scale: no exudate=0, low=1, medium=2, high=3 (Figure S1). Statistical analysis of growth  
336 rate and exudate production was performed in Minitab v. 17 (Minitab Inc., State College,  
337 PA). Data were analysed in a split plot ANOVA framework with individual incubators as  
338 plots. The analysis was used to infer the significance of the following factors: race (fixed),  
339 temperature (fixed) and their interaction; population (nested within race, random effect) and  
340 isolate (nested within isolates and populations).

## 341 **Results**

### 342 *Microsatellite genotyping and genotypic clustering*

343 A total of n=338 isolates were successfully scored for both mating type and the 11 SSR loci  
344 (Table 1). All of the SSR loci were polymorphic, but total number of alleles per locus varied  
345 from 3 at locus O to 37 at the hypervariable M locus (Table S1). Clustering of the multilocus  
346 genotypes using DAPC analysis in the adegenet package inferred three discrete genetic  
347 clusters within the total *D. septosporum* population in Scotland (Figure 1).

348

### 349 *Host and geographic distributions of genetic clusters*

350 The highly genetically divergent cluster of *D. septosporum* isolates revealed by the DAPC  
351 analysis was isolated only from lodgepole pine (Table 1, Figure 2). This was true even at sites  
352 where adjacent stands of Caledonian Scots pine had been sampled and in nursery collections  
353 where isolates had also been made from both Scots and Corsican pine. This cluster will  
354 hereafter be referred to as the lodgepole pine race of *D. septosporum* (LPR). We found LPR  
355 in all sites where isolations were made from lodgepole pine with the exception of the  
356 northern Caledonian pinewood site at Glen Einig. In some locations, such as Glen Affric and  
357 Glen Garry, LPR was the predominant race on lodgepole pine, whereas in others, such as  
358 Dundreggan, it comprised a small proportion of lodgepole pine infections (Table 1, Figure 2).

359

360 In contrast to LPR, the remaining two genetic clusters distinguished by the DAPC analysis  
361 were found on all three host pine species. However they were very distinct in their  
362 geographic distributions. The first of these clusters (n= 92) predominated in samples from  
363 more southern locations, and its frequency declined towards the north of Scotland (Table 1,

364 Figure 2). In recognition of its distribution, this genetic cluster will be referred to as the  
365 southern race (SR) of *D. septosporum*. In the combined nursery samples from southern  
366 Scotland and northern England, 26 of the 27 isolates were from SR, while only 3 of 13  
367 isolates from northern Scottish nurseries belonged to this race. In the mixed Scots and  
368 Corsican pine plantations the frequency of SR declined from south to north; from 88% in  
369 Torrs Warren through 47% in Tentsmuir to 26% in Culbin Forest. SR was not isolated from  
370 Caledonian Scots pine trees except in the most easterly population at Glen Tanar where it was  
371 present at a frequency of 33%. In more westerly and northerly Caledonian pine sites, SR was  
372 either absent or present at a very low frequency (total 4 isolates) on lodgepole rather than  
373 Scots pine. Where it occurred at intermediate frequency in mixed plantations of Scots and  
374 Corsican pine e.g. at Tentsmuir, SR was significantly associated with Corsican pine ( $\chi^2=6.47$ ,  
375 d.f. =1,  $P<0.05$ ).

376

377 The third genetic cluster recognised by the DAPC analysis (n=209) was strongly associated  
378 with Caledonian Scots pine (Table 1, Figure 2). Of the 130 isolations made from Caledonian  
379 pine trees, 125 belonged to this cluster, which will henceforth be referred to as the native pine  
380 race (NPR). As well as being present on Caledonian Scots pine, NPR was also isolated from  
381 adjacent stands of lodgepole pine and was the main race present in northern Scottish nursery  
382 samples. In mixed stands of Scots and Corsican pine NPR predominated in the north at  
383 Culbin Forest, but its frequency declined to the south (Figure 2). Where present at  
384 intermediate frequency at Tentsmuir it was preferentially found on Scots pine ( $\chi^2=6.47$ , d.f.  
385 =1,  $P<0.05$ ).

386

387 *Genetic diversity and divergence among and within D. septosporum races*

388 LPR was the least diverse of the three *D. septosporum* races (Table 2). It was characterised  
389 by very low allelic richness  $A=1.55 \pm 0.16$ , low gene diversity  $H_T=0.041$  and relatively low  
390 percentage of polymorphic loci, 55%. It had the smallest total number of alleles  $n=17$ ,  
391 although 7 of these were private alleles distributed over 6 loci. Three of these alleles (E-243,  
392 G-195, I-319) were shared with the three individuals sampled from North American  
393 populations (Table 1).

394

395 In contrast, SR was characterised by the highest level of genetic variation (Table 2). All of its  
396 loci were polymorphic, with 68 alleles in total, 21 of which were unique and present across 7  
397 loci. SR displayed both high allelic richness  $A=5.50 \pm 1.25$  and high gene diversity  
398  $H_T=0.550$ . Allelic richness of SR was significantly higher in the population occupying  
399 plantations ( $A=3.75$ ) than on Caledonian pine ( $A=2.64$ ) ( $P=0.015$ ).

400

401 The NPR race was intermediate in genetic diversity between LPR and SR (Table 2). 91% of  
402 loci were polymorphic and total number of alleles ( $n=68$ ) and allelic richness  $A=4.23 \pm 1.60$   
403 were high. However gene diversity  $H_T=0.182$  was much lower than in SR because allelic  
404 diversity was present predominantly at a single hypervariable locus (M). NPR showed the  
405 highest number of unique alleles ( $n=22$ ) with 16 of these found at the hypervariable locus M  
406 (Table S1). Although mean allelic richness was greater on Caledonian pine ( $A=4.45$ ) than on  
407 plantation pines ( $A=3.91$ ) this difference was not significant ( $P=0.348$ ).

408

409 The three *D. septosporum* races were strongly genetically differentiated from each other with  
410 overall  $\theta_{st}=0.558$ ,  $P<0.01$ . LPR diverged the most from the other two races, showing pairwise

411  $\theta_{st}=0.8037$  ( $P<0.05$ ) with NPR and  $\theta_{st}=0.5119$  ( $P<0.05$ ) with SR. The other two races, NPR  
412 and SR, were genetically closer, with a moderate but still significant level of divergence  
413  $\theta_{st}=0.3997$  ( $P<0.05$ ).

414

415 Within the LPR race, there was no significant genetic differentiation ( $\theta_{st}=0.075$ ,  $P>0.05$ )  
416 among the major populations scored (Inshriach Forest, Glen Garry, Dundreggan, Glen  
417 Affric). For SR there was low but significant genetic differentiation ( $\theta_{st}=0.082$ ,  $P<0.01$ )  
418 among the plantation and nursery populations (Torrs Warren, Tentsmuir, Culbin Forest,  
419 Southern Scotland nursery) (Table S2). SR also showed significant genetic differentiation  
420 between Caledonian pine and plantation populations ( $\theta_{st}=0.101$ ,  $P<0.01$ ). In NPR there was  
421 low but significant genetic divergence among Caledonian pine populations ( $\theta_{st}=0.071$ ,  
422  $P<0.01$ ) but much higher genetic differentiation among plantation populations  $\theta_{st}=0.318$   
423 ( $P<0.01$ ) (Table S3). Mean pairwise genetic differentiation between Caledonian pine  
424 populations and the northernmost Culbin Forest plantation population was low ( $\theta_{st}=0.040$ ),  
425 while mean pairwise differentiation between Caledonian pine populations and the two  
426 plantation populations located further south was much higher (Tentsmuir  $\theta_{st}=0.147$ , Torrs  
427 Warren  $\theta_{st}=0.656$ ) (Table S3).

428

#### 429 *Multilocus structure and mating type variation of races*

430 LPR comprised only 5 multilocus genotypes (MLGs), among which one was a clonal MLG  
431 (Table 3). All of the individuals within this race were of one mating type variant, mt-2. There  
432 was high and significant linkage disequilibrium among loci, in both the original ( $I_A=0.842$ ,  
433  $r_D=0.176$ ,  $P=0.003$ ) and clone-corrected datasets ( $I_A=0.576$ ,  $r_D=0.124$ ,  $P=0.015$ ), as expected  
434 if LPR reproduces asexually.

435

436 SR was characterised by the highest number of MLGs  $n=58$ , 11 of which were clonal MLGs  
437 (Table 3). Mating type variants, mt-1 and mt-2, were present in a 1:1 ratio ( $\chi^2=0.043$ , d.f.=1,  
438  $P=0.835$ ), which suggests the possibility of sexual reproduction within SR populations. There  
439 was however significant linkage disequilibrium among loci present in the original ( $I_A=0.663$ ,  
440  $r_D=0.067$ ,  $P<0.001$ ) and clone-corrected datasets ( $I_A=0.322$ ,  $r_D=0.033$ ,  $P<0.001$ ). We also  
441 found a significant amount of genetic divergence between the two mating type populations  
442 (original  $\theta_{mt}=0.107$ ,  $P<0.01$ ; clone-corrected  $\theta_{mt}=0.022$ ,  $P<0.05$ ), rejecting the hypothesis of  
443 random mating within SR populations. The results are compatible with SR being  
444 predominantly asexual with low levels of sexual reproduction.

445

446 A moderate number of MLGs  $n=51$  were present in the NPR population with a relatively  
447 small number of clonal MLGs ( $n=5$ ) (Table 3). Within NPR multilocus genotypes were often  
448 distinguished by allelic differences at a single hypervariable locus. Linkage disequilibrium  
449 was high and significant in both the original ( $I_A=2.037$ ,  $r_D=0.245$ ,  $P<0.001$ ) and clone-  
450 corrected datasets ( $I_A=2.203$ ,  $r_D=0.261$ ,  $P<0.001$ ). Although both mating type variants (mt-1  
451 and mt-2) were present in NPR populations, the frequency of individuals bearing the mt-1  
452 allele was limited to only 3% of the population. Genetic differentiation was high and  
453 significant between the two mating type populations within NPR (original  $\theta_{mt}=0.378$ ,  $P<0.01$ ;  
454 clone-corrected  $\theta_{mt}=0.396$ ,  $P<0.01$ ). The mating system of NPR is therefore likely to be  
455 predominantly asexual. Genotypes possessing the mt-1 variant were noticeably more  
456 genetically variable than those possessing mt-2 (mt-1  $H_t=0.507$ , mt-2  $H_t=0.153$ ,  $P=0.002$ ).

457

458 *Growth rate and exudate production of races at different temperatures*

459 We found significant differences in mycelial growth rate among the races ( $F_{2,6} = 20.40$ ,  
460  $P=0.002$ ), and significant interaction between race and temperature for this character ( $F_{4,12} =$   
461  $13.31$ ,  $P<0.001$ ) (Table S4) (Figure 3a). This interaction remained significant even when the  
462 LPR isolates were removed from the analysis ( $F_{2,56} = 5.93$ ,  $P=0.005$ ). The three *D.*  
463 *septosporum* races showed different temperature optima for growth. LPR exhibited the  
464 slowest growth at all three temperatures tested, NPR was the fastest growing race at 10 °C  
465 and 22.5 °C, while SR exhibited the fastest growth at 17.5 °C.

466

467 There were also significant differences in exudate production among races ( $F_{2,6}=11.94$ ,  
468  $P=0.008$ ) and a significant race x temperature interaction ( $F_{4,12} = 5.61$ ,  $P=0.009$ ) (Table S4).  
469 LPR produced the least exudate at all three temperatures, NPR produced the most exudate at  
470 10 °C, and SR at the remaining two temperature points, 17.5 °C and 22.5 °C (Figure 3b).

471



**472 Discussion**

473 Our molecular analysis of the *D. septosporum* population in Scotland was able to assign  
474 individuals to the three major genetic groups previously recognised by Mullett *et al.* (2017) in  
475 their Britain wide study. In agreement with Mullett *et al.* (2017) we found that the first of  
476 these groups, LPR, possesses very low genetic variability and is completely asexually  
477 reproducing. In addition our analysis of mixed sites provided no evidence of LPR on trees  
478 other than lodgepole pine, implying a high degree of host adaptation. In contrast we found the  
479 second race SR to be highly genetically variable with the potential for sexual reproduction,  
480 again in line with the results of Mullett *et al.* (2017). Our results further show that within  
481 Scotland SR is mainly southern in distribution, occurs on all three pine species, but is  
482 preferentially found on Corsican rather than Scots pine where adjacent stands of these two  
483 hosts occur, implying a modest degree of host specialisation. Finally we have shown that  
484 NPR, the third major genetic grouping distinguished by Mullett *et al.* (2017), is the  
485 predominant race on Caledonian pine, and preferentially infects Scots pine when present in  
486 mixture with Corsican pine. In our purely Scottish sample, NPR possesses intermediate levels  
487 of variability and reproduces largely asexually.

488

489 Besides confirming and documenting the host and geographic distributions within Scotland  
490 of the genetic groups recognised by Mullett *et al.* (2017), we have also shown that LPR, SR  
491 and NPR are highly significantly different both in their growth rate response to temperature  
492 and their level of production of the exudate dothistromin, implicated as a virulence factor in  
493 DNB (Kabir *et al.*, 2015). This provides strong evidence that these groupings represent  
494 important biological, ecological and evolutionary units within *D. septosporum*, and that it is  
495 therefore appropriate to regard them as distinct races within the species.

496

497 The very low genetic and clonal variability of LPR strongly suggests that it derives from  
498 recent introduction of a limited number of isolates. However despite low variability, LPR  
499 harbours 7 alleles that are absent from SR and NPR, three of which were detected in the  
500 limited sample of North American isolates that were genotyped. These observations support  
501 the hypothesis of Mullett *et al.* (2017) that LPR has been introduced into Scotland on needle  
502 debris accompanying lodgepole pine seed imports from North America. Although seed  
503 importation is generally considered a low biosecurity risk, there are at least two other  
504 examples where it has led to transfer of important pine pathogens between North America  
505 and Europe (*Lecanosticta acicola* (Janousek *et al.* 2015); *Gremmeniella abietina* (Hamelin *et*  
506 *al.* 1998)).

507

508 SR is the most genetically variable of the three races implying a large effective population  
509 size as expected in an epidemic population. Equality of mating type frequencies and low  
510 (though significant) correlation among markers together with limited differentiation across  
511 mating types suggests that SR may practice a low frequency of sexual reproduction.  
512 Therefore some long distance dispersal by ascospores could occur in this race. Given the  
513 preference of SR for Corsican pine, a predominantly southern distribution, and near absence  
514 from Caledonian pine, SR is likely to have originated via recent dispersal from the epidemic  
515 of DNB on Corsican pine in England. Transfer of SR to northern plantations in Scotland via  
516 infected nursery stock is suggested firstly by the prevalence of SR on nursery material from  
517 south Scotland. In addition the genetic composition of the SR population found on southern  
518 nursery samples is very similar to that at Tentsmuir and Culbin Forest (low genetic

519 differentiation), a result that would be expected if infected nursery stock from southern  
520 nurseries was the source of SR at these northern sites.

521

522 The third and most enigmatic of the *D. septosporum* races, NPR, is strongly associated with  
523 Caledonian pine in our study (125 out of 130 isolates derived from Caledonian pine). NPR  
524 was also isolated from lodgepole pine adjacent to Caledonian pine stands and was  
525 predominant in northern Scottish nurseries. In plantations the frequency of NPR declined  
526 from north to south and data from Mullett *et al.* (2017) indicates that the equivalent ‘northern  
527 Scottish group’ is infrequent south of the Scottish border. A plausible hypothesis to account  
528 for this host and geographic distribution is that NPR is a northern race of *D. septosporum*  
529 endemic on Caledonian pine, that has recently spread to adjacent lodgepole pine stands,  
530 plantations of Corsican pine in northern Scotland, and pine nurseries in the same geographic  
531 area.

532

533 Genetic support for the hypothesis that NPR represents an endemic race is equivocal.  
534 Although allelic richness is relatively high (due to hypervariability at a single locus) overall  
535 gene diversity is low. The ratio of mating types in NPR is very highly skewed and there is  
536 strong allelic correlation among markers and genetic differentiation between mating types, all  
537 suggesting that NPR reproduces asexually. Neither low genetic diversity nor asexual  
538 reproduction are generally considered to be attributes of endemic populations. On the other  
539 hand NPR possesses 22 alleles that are not found in the most closely related race SR. Such  
540 race specific alleles can only have accumulated by mutation if the races have been isolated  
541 for a considerable number of generations.

542

543 To reconcile these results we hypothesise that *D. septosporum* arrived in Scotland with pine  
544 populations colonising from continental Europe after the last glaciation (Sinclair *et al.*, 1998).  
545 Following isolation of the Caledonian pine populations from their continental counterparts  
546 about 8Kypb, we propose that the *D. septosporum* population went through a population  
547 bottleneck, leading to loss of genetic variation. We further propose that the population was  
548 selected for reproductive assurance under conditions at the range edge that were unfavourable  
549 for sexual reproduction, leading to evolution of a predominantly asexual breeding system.  
550 Where environmental factors hinder outcrossing sexual reproduction, mating system  
551 transitions to uniparental reproduction are known to have taken place in a wide range of  
552 plants and animals (Avisé 2015; Holsinger 2000) and are equally likely to have occurred in  
553 fungi (Taylor *et al.* 2015).

554

555 If *D. septosporum* has been endemic in Caledonian pine populations it appears curious that its  
556 presence was not noted before 2011 (Brown *et al.*, 2012). One explanation may be that the  
557 proposed endemic race NPR, which apparently causes little damage to needles, has been  
558 overlooked, with attention being focussed on more damaging needle pathogens such as  
559 *Lophodermium seditiosum* (Minter & Millar, 1980). Another explanation may be that  
560 populations have recently increased in size due to more favourable environmental conditions  
561 for *D. septosporum*. This would be consistent with a rise in annual temperature of 0.75°C,  
562 and an increase of 23% in annual rainfall in Scotland since 1970 (Met Office, 2017).  
563 Circumstantial evidence that *D. septosporum* has had a long term endemic presence in the  
564 Caledonian pine populations comes from studies of geographic variation in their DNB  
565 susceptibility. Pine populations from areas of high rainfall, where conditions are most

566 favourable to *D. septosporum*, show significantly lower susceptibility than those in low  
567 rainfall areas, the pattern expected under long term co-evolution (Perry *et al.* 2016b).

568

569 Drawing together the information outlined above and that from Mullett *et al.* (2017), we can  
570 put forward a tentative scenario to account for the current situation in Scotland. Prior to  
571 planting of exotic conifers in Britain, we suggest that NPR was present both as a co-evolved  
572 endemic pathogen causing minimal damage in Caledonian pinewoods and on other Scots pine  
573 populations in Scotland. From the 1930s Corsican pine was widely planted in England and  
574 more locally in Scotland. In the 1950s the SR race was introduced into southern England  
575 probably from France (Mullett *et al.* 2017), possibly aided by transfer of diseased nursery  
576 stock. In the 1990s SR spread to genetically susceptible high density Corsican pine stands in  
577 East Anglia and expanded massively to create a DNB epidemic made particularly serious by  
578 a succession of unusually wet and warm summers (Brown & Webber 2008). High disease  
579 pressure may have facilitated limited adaptation to adjacent Scots pine, which was previously  
580 immune to attack (Murray & Batko 1962). From these footholds SR spread northwards  
581 through England and into Scotland in the 2000s via the stepping stones of vulnerable  
582 Corsican pine and to a lesser extent Scots pine plantations. SR has now established an outlier  
583 population in the most easterly Caledonian pine site (Glen Tanar) via natural dispersal, and in  
584 more northerly Caledonian pine sites by movement of infected lodgepole pine. The derived  
585 nature of SR in Caledonian pine sites is supported by its lower genetic diversity compared to  
586 that found on plantation pines.

587

588 Concurrent with northward movement of SR, the major Corsican pine plantation in northern  
589 Scotland (Culbin Forest) appears to have become infected by the endemic NPR race of *D.*

590 *septosporum* dispersed from Caledonian pine populations present in the region, with  
591 subsequent spread to plantations further south. This direction of transfer is supported by the  
592 low differentiation between the NPR population on Caledonian pine and that at Culbin  
593 Forest, and increasing differentiation between Caledonian pine populations and plantation  
594 populations to the south. Transfer via asexual spores may have been augmented by planting  
595 of stock infected with NPR from northern Scottish nurseries. Meanwhile lodgepole pine  
596 plantations established in the vicinity of Caledonian pinewoods have become infected by  
597 NPR, but this has done little damage. However inadvertent introduction of the lodgepole pine  
598 adapted LPR race from North America has led to devastating DNB outbreaks mainly on the  
599 *latifolia* subspecies of lodgepole pine (Brown & Webber 2008).

600

601 Our scenario implies that the planting of related exotic species has significantly altered the  
602 biodiversity threat posed by *D. septosporum* to Caledonian pine. The presence of susceptible  
603 Corsican pine throughout Britain has facilitated the introduction of race SR from continental  
604 Europe, while planted lodgepole pine has brought with it a second exotic race of *D.*  
605 *septosporum* LPR from North America. The two exotic races are now sympatric with race  
606 NPR which prior to their arrival was not causing any significant damage. What are the risks  
607 posed by these novel introductions?

608

609 Analysis of the outcome of natural inoculations by the SR race present at Torrs Warren  
610 showed high genetic variation for susceptibility both within and among populations of  
611 Caledonian pine (Perry *et al.* 2016b). Thus although SR is likely to cause some damage to  
612 Caledonian pine populations, this could be mitigated if populations are able to naturally  
613 regenerate, allowing evolution of greater resistance to SR by natural selection (Cavers &

614 Cottrell 2015). Our isolation results provide no evidence that LPR has established on  
615 Caledonian pine. Therefore if lodgepole pine is removed from the vicinity of native  
616 Caledonian pine, the impact of LPR may be minimal. However Mullet *et al.* (2017) did  
617 record rare presence of the ‘lodgepole population’ on Scots pine, so the possibility of host  
618 switching onto Caledonian pine cannot be discounted.

619

620 Our conclusion that introduction of SR and LPR into Caledonian pine populations will have  
621 limited impact are conditional on a lack of genetic interaction among the three races of *D.*  
622 *septosporum* now present. In this respect we note that introduction of SR brings with it a high  
623 frequency of isolates carrying the mt-1 allele which is absent or at very low frequency in the  
624 other two races. If the races are sexually compatible, this opens up the possibility for  
625 hybridisation between them and rapid evolution of either a new hybrid race (Brasier *et al.*  
626 2001, 2004), or introgression of important genetic attributes among the races (Paoletti *et al.*  
627 2006). In either circumstance, the virulence of the races may be increased, causing more  
628 serious damage to the Caledonian pine populations (Stukenbrock 2016).

629

630 Tentative evidence for hybridisation between NPR and SR comes from an analysis of the  
631 small number of NPR isolates from Caledonian pine that carry the mt-1 allele. Genetic  
632 differentiation of these isolates from mt-2 isolates of the NPR mating type is much larger ( $\theta_{st}$   
633 = 0.369,  $P < 0.01$ ) than from mt-1 isolates in the SR race ( $\theta_{st} = 0.048$ , n.s.). A possible  
634 explanation is that the mt-1 mating types within NPR are actually hybrids between the SR  
635 and NPR races. Analysis of the genomic constitution of these isolates, now underway, will  
636 provide further evidence for or against this hypothesis (Stukenbrock 2016).

637

638 Possible steps to reduce spread of SR into Caledonian pine include a moratorium on  
639 movement of nursery stock of either Scots or lodgepole pine into Caledonian pinewood sites,  
640 and removal of plantations of either Scots, lodgepole or Corsican pine from areas within the  
641 range of sexual spore dispersal. If the SR race nevertheless becomes established within  
642 Caledonian pine populations, control of DNB would be best achieved by promoting  
643 management of the pinewoods for natural regeneration (Cavers & Cottrell 2015) to maximise  
644 the opportunity for greater resistance to evolve (Perry *et al.* 2016a, b).

645

646 Overall our results demonstrate the power of adopting a forensic forest pathology approach in  
647 which population genetic analysis of molecular markers is used to unravel the origins and  
648 subsequent evolution of emerging forest pathogens. The approach has already provided  
649 previously inaccessible information on the introduction pathways and subsequent behaviour  
650 of forest pathogens in genera such as *Heterobasidion* (Garbelotto *et al.* 2013), *Cryphonectria*  
651 (Dulech *et al.* 2010) and *Hymenoschyphus* (Gross *et al.* 2014). Crucially this approach can  
652 identify previously cryptic but evolutionarily important units within recognised  
653 morphological taxa which may possess very different ecological attributes (Perez *et al.* 2012).  
654 Understanding these ecological differences may be key to explaining the epidemiology of the  
655 associated disease.

656

657 From a wider forest policy viewpoint our analysis of DNB in Scotland provides a clear  
658 illustration of the dangers of establishing plantations of exotic tree taxa that are related to and  
659 share pathogens with native tree species (Gilbert & Webb 2007; Gilbert *et al.* 2012; Burgess



660 & Wingfield 2017). Establishment of exotic Corsican and lodgepole pine has led to two  
661 separate and economically damaging epidemics of DNB on exotic plantations, caused by two  
662 new races of DNB that accompanied their exotic hosts. In addition the presence of these  
663 exotic pathogen races has increased the biosecurity threat to the iconic Caledonian pine  
664 populations. Our results are highly relevant to recent debate over the merits of introducing  
665 exotic species to increase the diversity and resilience of ecosystems (Schlaepfer *et al.* 2011;  
666 Vitule *et al.* 2012). Our overall conclusion, based on the outcome of a large scale though  
667 unplanned historical experiment, is that planting exotic trees related to native species is likely  
668 to decrease rather than increase the resilience of forest ecosystems to disease.

**669 Acknowledgements**

670 This project was funded jointly by a grant from BBSRC, Defra, ESRC, the Forestry  
671 Commission, NERC and the Scottish Government, under the Tree Health and Plant  
672 Biosecurity Initiative. We would like to thank Dr. Katherine Tubby, Richard Baden and Dr.  
673 Martin Mullett from Forest Research in Alice Holt for kindly supplying the nursery and  
674 North American isolates of *D. septosporum*.

675

**676 Author Contributions**

677 MJP, CR, PNH and RAE were all involved in the design and performance of the research  
678 together with data collection, analysis and interpretation. MJP and RAE wrote the manuscript  
679 with significant input from CR and PNH.

680

**681 Data Accessibility**

682 Data for this study are available at: (to be completed after acceptance of the manuscript)

683

684

685

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879 Table 1. Sampling sites, their locations and number of trees from which *Dothistroma septosporum* isolations  
 880 were made. Number of isolates of each genetic group identified by Mullett *et al.* (2017) and designated  
 881 lodgepole pine race (LPR), southern race (SR) and native pine race (NPR) are shown in the format  
 882 (LPR:SR:NPR).

| <i>(i) Mixed plantations of Scots pines and Corsican pines</i>              |                       |                      |                   |                         |                         |
|-----------------------------------------------------------------------------|-----------------------|----------------------|-------------------|-------------------------|-------------------------|
| Site                                                                        | Year of collection    | Total no of isolates | No of Scots pines | No of Corsican pines    | Lat/Long                |
| Culbin Forest                                                               | 2015                  | 39                   | 20<br>(0:3:17)    | 19<br>(0:7:12)          | 57.632986<br>-3.6792442 |
| Torrs Warren                                                                | 2015                  | 33                   | 18<br>(0:17:1)    | 15<br>(0:12:3)          | 54.865287<br>-4.8919655 |
| Tentsmuir                                                                   | 2015                  | 30                   | 16<br>(0:4:12)    | 14<br>(0:10:4)          | 56.413737<br>-2.8109709 |
| <i>(ii) Caledonian Scots pine sites with adjacent lodgepole pine stands</i> |                       |                      |                   |                         |                         |
| Site                                                                        | Year of collection    | Total no of isolates | No of Scots pines | No of lodgepole pines   | Lat/Long                |
| Glen Einig                                                                  | 2014                  | 29                   | 23<br>(0:0:23)    | 6<br>(0:0:6)            | 57.957361<br>-4.7397091 |
| Glen Garry                                                                  | 2014                  | 35                   | 20<br>(0:0:20)    | 15<br>(11:0:4)          | 57.051089<br>-4.9790647 |
| Inshriach Forest                                                            | 2014                  | 32                   | 13<br>(0:0:13)    | 19<br>(10:2:7)          | 57.097124<br>-3.9313490 |
| Glen Affric                                                                 | 2014<br>2015          | 21                   | 7<br>(0:0:7)      | 14<br>(13:0:1)          | 57.292428<br>-4.8939597 |
| Dundreggan                                                                  | 2014<br>2015          | 39                   | 29<br>(0:0:29)    | 10<br>(2:2:6)           | 57.196282<br>-4.7359641 |
| Strathpeffer                                                                | 2015                  | 2                    | 0                 | 2<br>(0:1:1)            | 57.621310<br>-4.5325429 |
| <i>(iii) Isolated Caledonian Scots pine forests</i>                         |                       |                      |                   |                         |                         |
| Site                                                                        | Year of collection    | No of Scots pines    |                   | Lat/Long                |                         |
| Glen Tanar                                                                  | 2015                  | 15<br>(0:5:10)       |                   | 57.043444<br>-2.8552516 |                         |
| Beinn Eighe                                                                 | 2015                  | 23<br>(0:0:23)       |                   | 57.629890<br>-5.3512581 |                         |
| <i>(iv) Forest nursery sites</i>                                            |                       |                      |                   |                         |                         |
| Site                                                                        | Year of collection    | Total no of isolates |                   |                         |                         |
| Southern Scotland                                                           | 2011-2015             | 24<br>(0:23:1)       |                   |                         |                         |
| Northern Scotland                                                           | 2011-2015             | 13<br>(1:3:9)        |                   |                         |                         |
| Northern England                                                            | 2011-2015             | 3<br>(0:3:0)         |                   |                         |                         |
| <i>(v) Northern American samples</i>                                        |                       |                      |                   |                         |                         |
| Site                                                                        | No of lodgepole pines |                      |                   |                         |                         |
| Nass Valley, Brown Bear, British Columbia, Canada                           | 2                     |                      |                   |                         |                         |

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 Kispiox, Buckley Canyon, British  
 Columbia, Canada
 

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884 Table 2. Genetic diversity measured at 11 microsatellite loci within three *Dothistroma*  
 885 *septosporum* races in Scotland.

| Race                     | LPR (n=37)      | SR (n=92)        | NPR (n=209)      |
|--------------------------|-----------------|------------------|------------------|
| Gene diversity $H_t$     | 0.041           | 0.550            | 0.182            |
| % Polymorphic loci       | 55%             | 100%             | 91%              |
| Total number of alleles  | 17              | 68               | 68               |
| Allelic richness $A$     | 1.55 +/- 0.16   | 5.50 +/- 1.25    | 4.23 +/- 1.60    |
| Number of unique alleles | 7 (over 6 loci) | 21 (over 7 loci) | 22 (over 4 loci) |

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888 Table 3. Multilocus structure of *D. septosporum* races in Scotland.

| Race                               | LPR                  | LPRcc <sup>a</sup> | SR       | SRcc <sup>a</sup> | NPR      | NPRcc <sup>a</sup> |
|------------------------------------|----------------------|--------------------|----------|-------------------|----------|--------------------|
| mt-1:mt-2                          | 0:37                 | 0:36               | 45:47    | 26:33             | 7:202    | 7:195              |
| Total no<br>MLGs <sup>b</sup>      |                      | 5                  |          | 58                |          | 51                 |
| No clonal<br>MLGs <sup>b</sup>     |                      | 1                  |          | 11                |          | 5                  |
| $I_A^c$                            | 0.842** <sup>d</sup> | 0.575*             | 0.663*** | 0.323***          | 2.037*** | 2.203***           |
| $r_D^e$                            | 0.176**              | 0.124*             | 0.067*** | 0.033***          | 0.245*** | 0.261***           |
| %<br>pairwise<br>l.d. <sup>f</sup> | 0                    | 0                  | 46       | 9                 | 38       | 27                 |
| $\Theta_{mt}^g$                    | -                    | -                  | 0.107**  | 0.022*            | 0.378*** | 0.396**            |

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890 <sup>a</sup> clone-corrected data set891 <sup>b</sup> MLG-multilocus genotype892 <sup>c</sup>  $I_A$ - index of association among loci893 <sup>d</sup> Significance of deviations from expectations under purely sexual reproduction are indicated894 (\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ )895 <sup>e</sup>  $r_D$ - mean correlation among loci896 <sup>f</sup> l.d.- linkage disequilibrium897 <sup>g</sup>  $\Theta_{mt}$ - genetic differentiation between mating types within races

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908 Figure 1. Plot of scores on first two axes from DAPC analysis (Jombart *et al.*, 2010), based  
909 on variation at 11 microsatellite loci, for *Dothistroma septosporum* isolates from Scotland.  
910 Races LPR (red), SR (orange) and NPR (blue) are indicated.

911

912 Figure 2. Distribution of *Dothistroma septosporum* races (LPR (red), SR (orange) and NPR  
913 (blue)) **a**). on Scots (S) and lodgepole (L) pine at Caledonian pine sites, illustrating data from  
914 Table 1, sections (ii) and (iii); **b**). on Scots (S) and Corsican (C) pine in mixed plantations  
915 and on pines in northern and southern Scottish nurseries (N), illustrating data from Table1,  
916 sections (i) and (iv).

917

918 Figure 3. Variation in **a**). growth rate and **b**). exudate production at three temperatures in  
919 three races (LPR, SR and NPR) of *Dothistroma septosporum* from Scotland.

920 **Supplementary Materials for Piotrowska *et al.***

921 Table S1. The range of allele sizes, number of alleles and allelic richness at 11 microsatellite  
 922 loci (Barnes *et al.*, 2008) in Scottish populations of *Dothistroma septosporum*.

| Locus | Allele range (bp) <sup>a</sup> | Number of alleles across all three races | Allelic richness in LPR <sup>b</sup> | Allelic richness in SR <sup>b</sup> | Allelic richness in NPR <sup>b</sup> |
|-------|--------------------------------|------------------------------------------|--------------------------------------|-------------------------------------|--------------------------------------|
| E     | 231-278                        | 6                                        | 2.000                                | 4.98                                | 1.907                                |
| F     | 192-208                        | 4                                        | 1.000                                | 2.000                               | 2.686                                |
| G     | 195-205                        | 4                                        | 1.000                                | 3.000                               | 2.230                                |
| I     | 319-328                        | 4                                        | 2.000                                | 2.957                               | 2.680                                |
| J     | 203-223                        | 7                                        | 2.000                                | 5.518                               | 2.773                                |
| K     | 355-438                        | 8                                        | 1.000                                | 7.283                               | 2.762                                |
| L     | 339-459                        | 14                                       | 2.000                                | 8.215                               | 5.401                                |
| M     | 233-579                        | 37                                       | 2.000                                | 16.38                               | 19.85                                |
| O     | 215-224                        | 3                                        | 1.000                                | 1.998                               | 1.000                                |
| DS1   | 165-175                        | 5                                        | 2.000                                | 2.985                               | 3.731                                |
| DS2   | 388-418                        | 6                                        | 1.000                                | 5.120                               | 1.543                                |

923 <sup>a</sup> all allele sizes include M13 tail length

924 <sup>b</sup> based on min. sample size of 37 individuals.

925 Table S2. Pairwise genetic differentiation ( $\theta_{st}$ ) between SR populations of *Dothistroma*  
926 *septosporum*.

|                         | Torrs Warren | Tentsmuir | Culbin Forest | Southern Scottish<br>Nursery |
|-------------------------|--------------|-----------|---------------|------------------------------|
| Caledonian<br>pinewoods | 0.1466       | 0.1134    | 0.0954        | 0.1293                       |
| Torrs Warren            |              | 0.0613    | 0.0722        | 0.1091                       |
| Tentsmuir               |              |           | -0.0055       | -0.0085                      |
| Culbin Forest           |              |           |               | 0.0559                       |

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930 Table S3. Pairwise genetic differentiation ( $\theta_{st}$ ) between NPR populations of *Dothistroma*  
 931 *septosporum*.

|                     | Inshriach<br>Forest | Glen<br>Garry | Dundreggan | Glen<br>Affric | Beinn<br>Eighe | Glen<br>Tanar | Torrs<br>Warren | Tentsmuir | Culbin<br>Forest | Northern<br>Scottish<br>Nursery |
|---------------------|---------------------|---------------|------------|----------------|----------------|---------------|-----------------|-----------|------------------|---------------------------------|
| Glen Einig          | 0.023               | 0.074         | 0.053      | 0.017          | 0.079          | 0.035         | 0.708           | 0.139     | 0.018            | 0.011                           |
| Inshriach<br>Forest |                     | 0.087         | 0.067      | 0.038          | 0.060          | -<br>0.017    | 0.623           | 0.115     | 0.028            | 0.009                           |
| Glen Garry          |                     |               | 0.126      | 0.056          | 0.129          | 0.070         | 0.585           | 0.141     | 0.084            | 0.051                           |
| Dundreggan          |                     |               |            | 0.161          | 0.127          | 0.112         | 0.801           | 0.218     | 0.025            | 0.035                           |
| Glen Affric         |                     |               |            |                | 0.064          | 0.020         | 0.638           | 0.127     | 0.025            | 0.027                           |
| Beinn<br>Eighe      |                     |               |            |                |                | 0.108         | 0.681           | 0.191     | 0.055            | 0.055                           |
| Glen Tanar          |                     |               |            |                |                |               | 0.554           | 0.099     | 0.044            | 0.005                           |
| Torrs<br>Warren     |                     |               |            |                |                |               |                 | 0.598     | 0.709            | 0.603                           |
| Tentsmuir           |                     |               |            |                |                |               |                 |           | 0.158            | 0.077                           |
| Culbin<br>Forest    |                     |               |            |                |                |               |                 |           |                  | -0.015                          |

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934 Table S4. Analysis of variance of growth rate and exudate production for three races of  
 935 *Dothistroma septosporum* grown at three temperatures. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

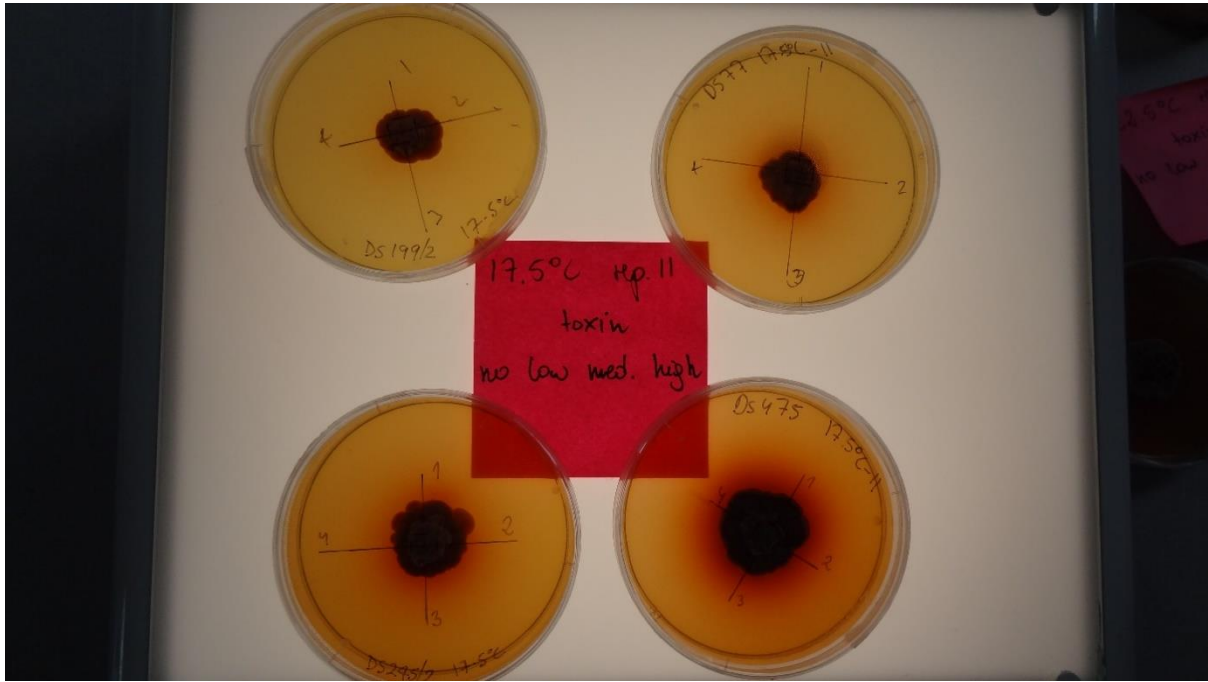
936

| 937 | <b><u>Source of Variation</u></b> | <b><u>df</u></b> | <b><u>MS Growth rate</u></b> | <b><u>MS Exudate production</u></b> |
|-----|-----------------------------------|------------------|------------------------------|-------------------------------------|
| 938 | Temperature                       | 2                | 487.83*                      | 15.03**                             |
| 939 | Incubator (Temp)                  | 3                | 30.83***                     | 0.48                                |
| 940 | Race                              | 2                | 475.07**                     | 9.73**                              |
| 941 | Population (Race)                 | 6                | 23.30                        | 0.82                                |
| 942 | Isolate (Race Pop)                | 36               | 20.13***                     | 1.32***                             |
| 943 | Temp x Race                       | 4                | 111.09***                    | 2.73**                              |
| 944 | Temp x Pop (Race)                 | 12               | 8.35*                        | 0.49                                |
| 945 | Temp x Iso (Race Pop)             | 72               | 4.37**                       | 0.46*                               |
| 946 | Error                             | 127              | 2.52                         | 0.30                                |

947

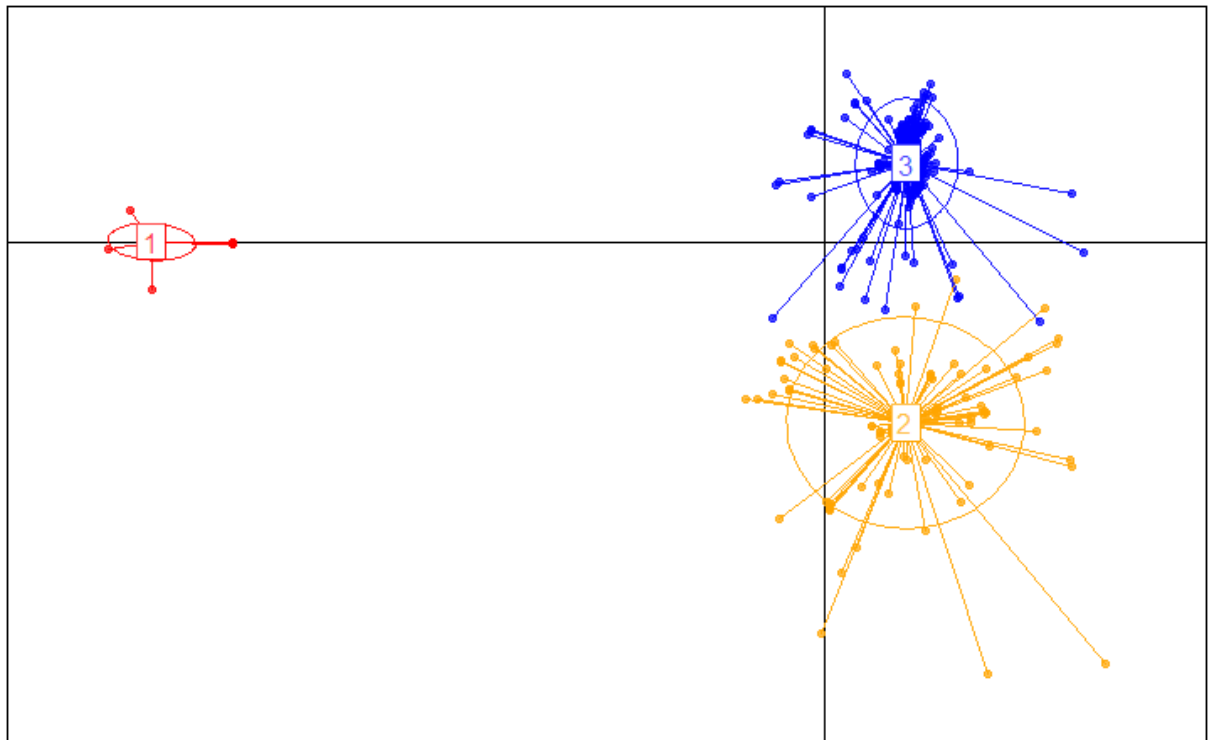


948 Figure S1. Exudate production scoring scale for *Dothistroma septosporum*



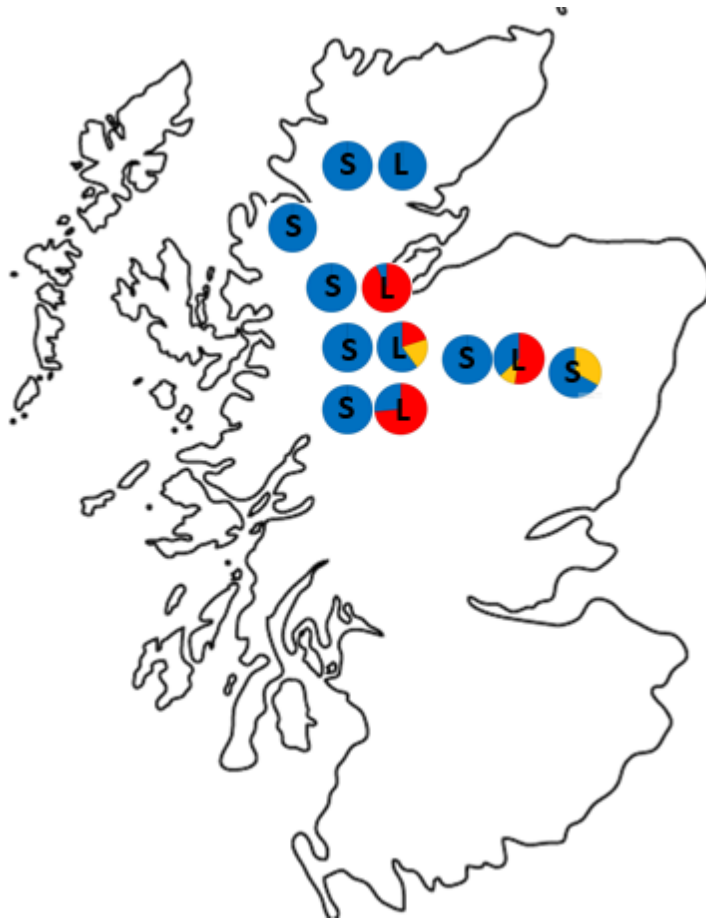
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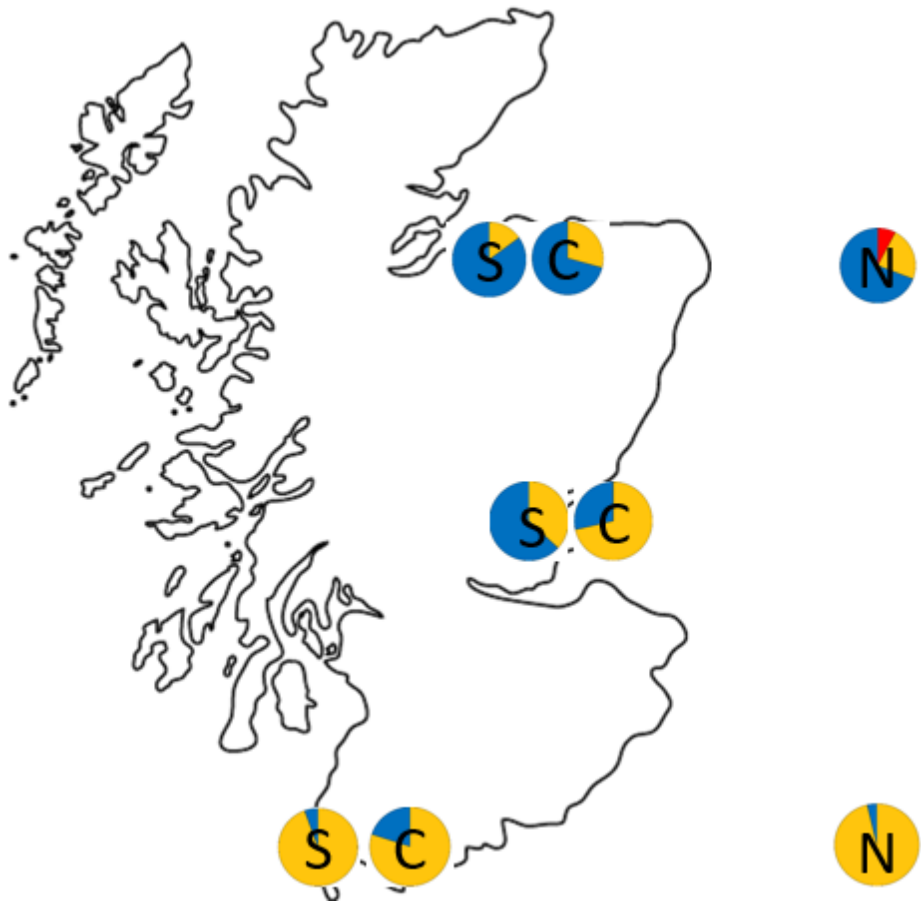
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952 Figure 1



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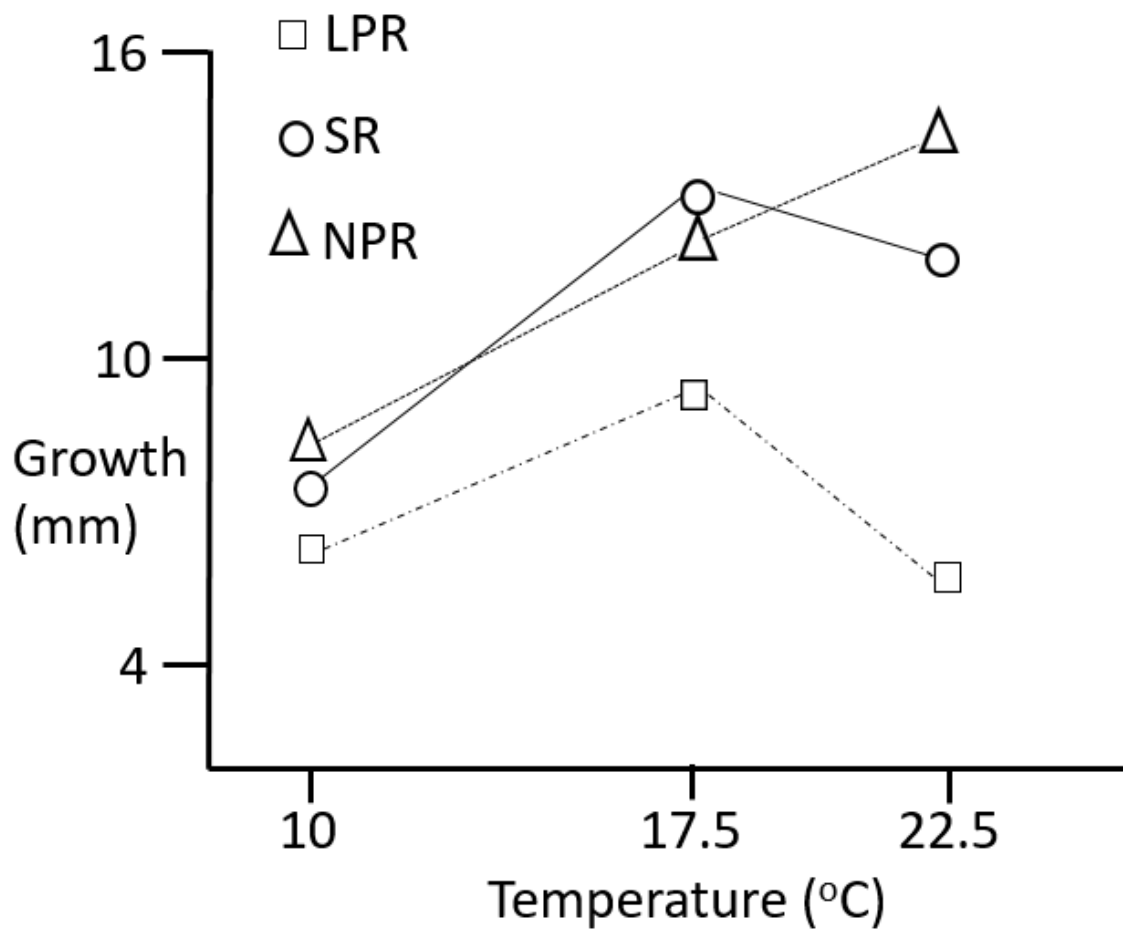
954 Figure 2a



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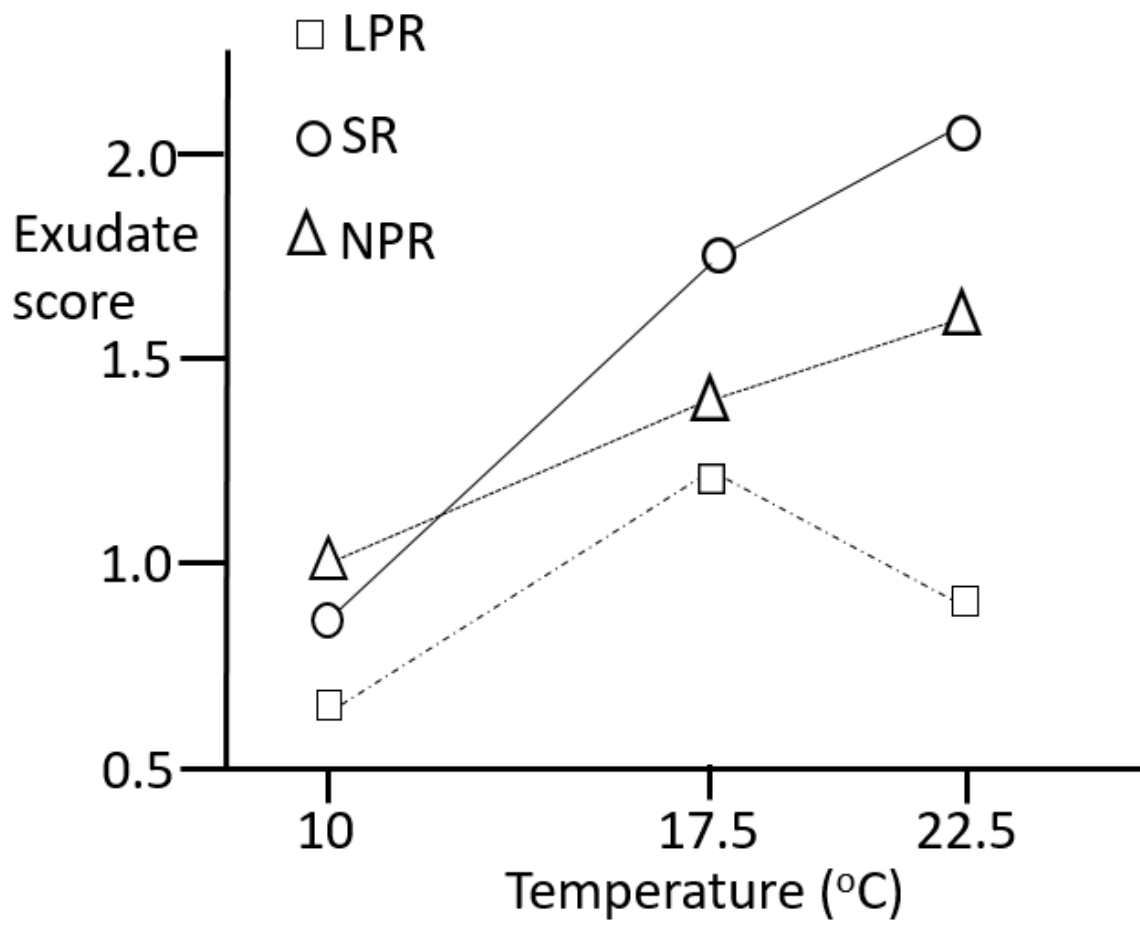
956 Figure 2b

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959 Figure 3a



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961 Figure 3b

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