| Title | Ecological and evolutionary implications of spatial heterogeneity during the off-season for a wild plant pathogen |
|---|---|
| Author(s) | Tack, Ayco Jerome Michel; Laine, Anna-Liisa |
| Citation | Tack , A J M & Laine , A-L 2013 , ' Ecological and evolutionary implications of spatial heterogeneity during the off-season for a wild plant pathogen ' New Phytologist , vol 202 , no. 1 , pp. 297-308 . , http://dx.doi.org/ 10.1111/nph.12646 |
| Date | 2013 |
| URL | http://hdl.handle.net/10138/42970 |
| Version | Peer reviewed |

1   **Title:** Ecological and evolutionary implications of spatial heterogeneity during the off-season for a

2   wild plant pathogen

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| Total word count (excluding summary, reference and legends): | 6494 | No. of figures: | 5 (Figs 2-5 in colour) |
|---|---|---|---|
| Summary: | 195 | No. of tables: | 1 |
| Introduction: | 1166 | No. of Supporting Information files: | 11 (Figs S1-S5; Tables S1-S4; Methods S1; Note S1) |
| Materials and Methods: | 2433 | | |
| Results: | 1126 | | |
| Discussion: | 1652 | | |
| Acknowledgements: | 110 | | |

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20   *Submitted as a regular research article to New Phytologist*

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23   **Abstract**

24   • While recent studies have elucidated many of the factors driving parasite dynamics during
25      the growing season, the ecological and evolutionary dynamics during the off-season (i.e. the
26      period between growing seasons) remain largely unexplored.

27   • We combine large-scale surveys and detailed experiments to investigate the overwintering
28      success of the specialist plant pathogen *Podosphaera plantaginis* on its patchily distributed
29      host plant *Plantago lanceolata* on the Åland Islands.

30   • Twelve years of epidemiological data establish the off-season as a crucial stage in pathogen
31      metapopulation dynamics, with approximately forty percent of the populations going extinct
32      during the off-season. At the end of the growing season, we observed environmentally-
33      mediated variation in the production of resting structures, with major consequences for
34      spring infection at spatial scales ranging from single individuals to populations within a
35      metapopulation. Reciprocal transplant experiments further demonstrated that pathogen
36      population of origin and overwintering site jointly shaped infection intensity in spring, with
37      a weak signal of parasite adaptation to the local off-season environment.

38   • We conclude that environmentally-mediated changes in the distribution and evolution of
39      parasites during the off-season are crucial for our understanding of host-parasite dynamics,
40      with applied implications for combating parasites and diseases in agriculture, wildlife and
41      human disease systems.

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**Introduction**

While the question of why parasite populations crash has puzzled theoreticians and empiricists alike

for nearly a century (Kermack & McKendrick, 1927), few studies have explored what happens

during and after cessation of growth and subsequent crash in size of parasite populations. Hence, we

now know that in seasonal environments several mechanisms ranging from changing environmental

conditions (Soper, 1929; Pascual & Dobson, 2005; Altizer *et al.*, 2006; Barrera *et al.*, 2012) and

acquired immunity (Soper, 1929) to the evolution of increased host resistance (Duffy & Sivars-

Becker, 2007) may all contribute to stabilization or decline of parasite populations, but we know

hardly anything about pathogen dynamics during the ensuing off-season. In many systems where

the host does not allow parasite growth or systemic (within-host) persistence during the off-season,

the epidemic decline is matched by the production of specialized resting structures for survival

(Combes, 2001; Agrios, 2005). Notably, the off-season may involve drought, heat and cold periods,

depending on the climatic region and host and pathogen life-history. By neglecting this off-season

we may ignore a major part of the parasite puzzle: What role does the off-season play in explaining

parasite population crashes and spatiotemporal disease dynamics? Do parasites exhibit genetic

variation for survival in different off-season environments, and therefore show evolutionary

responses to spatial and temporal heterogeneity during the off-season? Understanding this is non-

trivial as off-season survival is a key determinant of the demographic and genetic structure of the

epidemic in the next season.

The striking focus of the literature on the within-season growth of the parasite (often

characterized by multiple generations of asexual reproduction; e.g. Tibayrenc & Ayala, 2012) is

likely due to two reasons. First, parasitologists may have thought *a priori* that the off-season lacks

any mechanistic underpinning or ecologically and evolutionary interesting dynamics, and can hence

be satisfactorily ignored, or described by a single stochastic parameter. Second, the off-season is

notoriously hard to study, and even in well-studied pathosystems researchers may lack sufficient

insight in the production, maturation, storage and revival of resting structures to allow for detailed

experimentation. Possibly for these reasons, the majority of empirical and theoretical studies still

regard off-season survival of parasites as a stochastic process or black box. Illustratively, the index

of many ecological and evolutionary textbooks on host-parasite interactions entirely lacks an entry

for 'off-season survival' or 'overwintering' (e.g. Thomas *et al.*, 2009; Schmid-Hempel, 2011). In

contrast, the rapidly increasing research on the transmission phase of the parasite life-cycle has

revealed a wealth of information on spatiotemporal disease spread, and has elucidated a key role for

genotype-by-environment interactions (Laine, 2008; Wolinska & King, 2009; Hall & Ebert, 2012).

79          Survival during the off-season may - just like infection and epidemic spread during the

80    growing season - be affected by the interaction between host genotype, parasite genotype and the

81    environment (Fig. 1). However, while the impact of the abiotic and biotic environment on the off-

82    season survival of parasites has been recognized for a long time (Roelfs, 1982; Marçais *et al.*, 1996;

83    Bergot *et al.*, 2004), only recent studies have shown that parasite genotypes may differentially

84    survive in the absence of the host, possibly through a trade-off with host transmission stages

85    (Barrett *et al.*, 2011; Sommerhalder *et al.*, 2011). Further, to date we don't know whether parasite

86    genotypes vary in their ability to survive different off-season environments. Given that

87    spatiotemporal variation in environmental conditions is likely, it is crucial to assess whether

88    parasites may adapt to changing off-season environmental conditions or shift distributions.

89          Off-season survival of parasites may depend on several life-stages. First, parasite genotypes

90    may vary in the production of resting structures (Fig. 1, triangle B). This variation may also be

91    mediated by heterogeneity in the abiotic (e.g. temperature or humidity) or biotic (e.g. host

92    genotype) environment in the period before the off-season (e.g. Gadoury & Pearson, 1988; Legler *et*

93    *al.*, 2012; Asalf *et al.*, 2013). Second, the maturation and release of resting structures in spring may

94    depend on pathogen genotype, the host-genotype on which the resting structures were produced,

95    and environmental conditions during the off-season itself (Fig 1, triangle C; Gadoury & Pearson,

96    1988; Abang *et al.*, 2006; Marçais *et al.*, 2009; Cohen *et al.*, 2013). Infection in spring may then

97    depend on the spring conditions, pathogen genotype and receiving host genotype (Fig. 1, triangle

98    D). In order to generalize our understanding of the impact of genotype and environment in shaping

99    the spatial ecology and evolution of parasites during the off-season, we need to shift our focus to

100   these multiple steps that underlie successful survival between growing seasons.

101          In this article, we investigate the off-season dynamics of the specialist obligate plant

102   pathogen *Podosphaera plantaginis* on its perennial host *Plantago lanceolata*. In  the Åland Islands

103   in southwestern Finland, the pathogen population persists in a network of *c.* 4000 highly

104   fragmented host populations, which range in size from a few square meters to several hectares, with

105   a median size of 300 m$^2$ (Nieminen *et al.*, 2004; Laine & Hanski, 2006; Laine, 2008; Tack *et al.*,

106   2013b) Yearly autumnal surveys conducted in the period 2001-2012 indicate that this highly

107   dynamic pathogen metapopulation persists in the face of high population turnover with

108   approximately half of the pathogen populations going extinct from one year to the next (Laine &

109   Hanski, 2006). Seasonality is considered to be a key driver of the metapopulation dynamics with

110   extinctions taking place during the environmentally harsh off-season, and colonizations occurring

111   during the favourable growing season. Further modeling of the presence/absence pathogen data of

112   *P. plantaginis* has predicted that there is spatial variation in overwintering success, with major

4

113　consequences  for local densities and distribution of the pathogen during the following growing

114　season (Soubeyrand *et al.*, 2009). However, as these studies were based on snapshot data collected

115　every September, they may not accurately disentangle the extinctions during the off-season and the

116　growing season. Here, we use data on the presence and local abundance of disease after infection

117　has taken place in spring for years 2011 and 2012. Furthermore, we investigate whether parasite

118　genotype, environment and their interaction ─ which have been shown to have a major ecological

119　and evolutionary impact on this system during the growing season (Laine, 2007; Laine, 2008; Tack

120　*et al.*, 2013a) ─ play an equally important role during the off-season. More specifically, we i) study

121　extinction rate during the off-season using data from the end of one growing season and the

122　beginning of the next year's growing season, and assess whether environmental and spatial factors

123　affect the extinction rate and abundance at the onset of disease dynamics; ii) assess large-scale

124　spatial and temporal variation in the production of resting structures of the powdery mildew across

125　the metapopulation, and investigate whether environmental and spatial factors drive the observed

126　variation; iii) demonstrate that resting structures are capable of infecting plants in spring;  iv)

127　conduct field experiments to test whether overwintering environment and genetic variation affect

128　the ability of resting structures to infect plants in spring, and whether pathogens survive best in their

129　sympatric (local) off-season environment; and v) assess the impact of resting spore production on

130　small- and large scale patterns in overwintering success in the pathogen metapopulation.

131

132　**Materials and methods**

133　*Study system*

134　The powdery mildew *Podosphaera plantaginis* (Castagne; U. Braun & Takamatsu) is a fungal plant

135　pathogen specific to *Plantago lanceolata* L. Like all members of the powdery mildews

136　(Erysiphaceae), it is an obligate pathogen requiring living host tissue throughout its life cycle.

137　During the growing season, *P. plantaginis* grows on the surface of the plant, with only its feeding

138　roots (haustoria) penetrating the epidermis. Wind-dispersed spores (conidia) are produced on chains

139　growing vertically on the leaf surface. Over the winter, *P. plantaginis* survives (see section *Results*)

140　with specialized resting structures (i.e. chasmothecia, formerly cleistothecia; Braun *et al.*, 2002).

141　The resting structures of *P. plantaginis* are produced on the leaf surface (Fig. S1), and are relatively

142　difficult to dislodge from the leaf (cf. Gadoury *et al.*, 2010). The number of resting structures on an

143　infected leaf in autumn can vary strongly, ranging from none or few resting structures up to several

144　thousand (e.g. x-axis in Fig. 5a). During development the resting structures change from

145　inconspicuous white, yellow and green to conspicuous brown and black (Fig. S1b). Each resting

146　structure contains a single ascus, in which usually develop eight ascospores during successful

147    maturation (Fig. S1c). Maturation takes place from late autumn to spring. During spring, the resting

148    structures burst open and the ascospores can infect living plant material. Dormancy of resting

149    structures for more than a single season, as equivalent to seed banks for plants, has not been

150    reported and is regarded unlikely for powdery mildews (Spencer, 1978; Bélanger *et al.*, 2002). A

151    recent study has revealed that resting structures can be produced through haploid selfing in *P.*

152    *plantaginis*, in contrast to the heterothallic nature of the majority of powdery mildews (Tollenaere

153    & Laine, 2013).

154         The host plant *P. lanceolata* (ribwort plantain) is a monoecious, rosette-forming perennial

155    herb (Sagar & Harper, 1964). The pollen is wind-dispersed and *P. lanceolata* is an obligate

156    outcrosser (Ross, 1973). Seeds frequently drop close to the mother plant (Bos, 1992). In contrast to

157    the pathogen, the spatial distribution of plant populations is consistent from year to year (Nieminen

158    *et al.*, 2004; Ojanen *et al.*, 2013), possibly due to a combination of long-term seed bank and clonal

159    propagation. During the winter in Åland the plant dies back to the rootstock and the resting

160    structures remain attached to the dead leaves.

161         In the Åland archipelago in southwestern Finland, *P. lanceolata* populations are fragmented

162    into discrete patches. The locations of the *c. 4000* plant populations have been mapped since the

163    early 1990's in the context of the Glanville fritillary butterfly (*Melitaea cinxia*) metapopulation

164    project (Nieminen *et al.*, 2004; Ojanen *et al.*, 2013). The host plant mainly grows on dry meadows,

165    pastures, and comparable habitats, which occur mostly as well-defined, discrete habitat patches

166    (Nieminen *et al.*, 2004; Ojanen *et al.*, 2013). Since 2001, the incidence of *P. plantaginis* in this

167    meadow network has been recorded systematically in early September of each year since 2001

168    (Laine & Hanski, 2006). At this time the fungus is highly conspicuous and resting structures

169    become frequent (Fig. S1a). At the same time, several environmental and spatial variables have

170    been recorded for each patch, including host plant coverage, distance to shore, road presence, host

171    plant spatial connectivity, plant dryness and patch shadow (Nieminen *et al.*, 2004; Laine & Hanski,

172    2006; Ojanen *et al.*, 2013). Patch shadow was estimated in 2001 using a categorical scale: 1=no

173    shadow, the entire patch is directly exposed to daylight for the majority of the day; 2=partly

174    shadowed, a large part of the patch is shaded for part of the day; 3=shadowed, the entire patch is in

175    shadow for most of the day. Light, temperature, and humidity are considered the key abiotic

176    variables affecting disease establishment and development in powdery mildews, and some variation

177    in these conditions are expected to be captured by this variable. Our focal populations in the Åland

178    Islands are distributed across fourteen geographical districts, and we use this division to obtain

179    roughly equally-sized units to visualize the extent of large-scale variation in overwintering success.

180   For more details on methodology of the large-scale survey and the variables measured we

181 refer to Laine and Hanski (2006) and Ojanen et al (2013).

182

183 *(1) The key role of the off-season in pathogen population extinction*

184 While the long-term survey has indicated a high extinction rate of *P. plantaginis* from one year to

185 the next (grey bars in Fig. 2a), such annual surveys cannot unambiguously disentangle extinctions

186 during the off-season and the subsequent growing season. To accurately quantify the extinction rate

187 during the off-season, we re-visited in early July 2011 a random subset of 47 populations occupied

188 by the pathogen in the previous autumn. As this survey indicated that extinctions during the off-

189 season were frequent (see *Results*), we visited the majority of populations occupied in September

190 2011 (n=287 populations) again in July 2012 to further investigate the impact of spatial and

191 environmental factors on off-season extinction and July abundance (triangles B-D, Fig. 1; Table 1).

192 We chose to re-visit populations in early July, as this period is after the re-establishment of the

193 powdery mildew from resting structures but well before epidemic spread of the pathogen within

194 populations and across the metapopulation (R. Alanen, unpublished). In July 2011, when infection-

195 levels were low, we recorded for each visited population the presence / absence of the powdery

196 mildew in the plant patch; at the same time, we scored local abundance of the powdery mildew as

197 the total number of infected plants within the plant population (where the search was terminated

198 when a threshold number of ten infected plants was detected; given low infection numbers in 2011,

199 this only applied to 14.9% of the populations). In July 2012, when the overall pathogen abundance

200 was much higher than in the previous year, local abundance was measured following a categorical

201 scale, where 0=absence of mildew, 1=1-9 infected plants, 2=10-99 infected plants, 3=100-999

202 infected plants, and 4=1000 or more infected plants.

203   To investigate the temporal consistency of disease distribution within patches before and

204 after the off-season, we additionally recorded the location of up to five infected plants in 259 host

205 populations in September 2011. The next July, we surveyed the presence and absence of infection

206 in these marked locations (1-m$^2$ quadrats) as well as in an equal number of control quadrats at

207 random locations. Random quadrats were selected to match the range of host density found in the

208 marked quadrats to avoid a confounding effect of host density.

209   To analyse the impact of environmental and spatial factors on the spatial pattern of winter

210 extinction and July abundance, we fitted a Bayesian spatial model using the integrated nested

211 Laplace approximation (Cameletti *et al.*, 2012) as implemented in the package *INLA* (Rue *et al.*,

212 2009; Lindgren *et al.*, 2011) in *R* version 2.15.1 (R Core Team, 2012). The advantage of this

213 method is that it efficiently and accurately estimates both covariates and the spatial range of

214    autocorrelation (as based on Euclidean distance between populations). We included the

215    environmental variables distance to shore, plant dryness, patch shadow, habitat openness, July

216    rainfall, August rainfall and population age (i.e. how many years ago the pathogen population had

217    been established by colonization, with a maximum value of 5) and the spatial factors host plant

218    coverage, road presence and host plant spatial connectivity as explanatory covariates (Table 1). The

219    average rainfall in July and August was estimated separately for each population using detailed

220    radar-measured rainfall data. For further details on the statistical models, see Methods S1.

221

222    *(2) Spatial and temporal variation in the production of resting structures*

223    The last few decades have seen a rise in the number of studies that have shown that resting

224    structures play a major role in the overwintering of powdery mildews, although experimental

225    demonstrations in wild systems are still rare (Pearson & Gadoury, 1987; Braun, 1995; Mmbaga,

226    2000; Marçais *et al.*, 2009). To assess the extent and environmental causes of variation in the

227    production of resting structures (Fig. 1, triangle B; Table 1), we surveyed natural variation in the

228    production of resting spores across the metapopulation during the years 2010-2012 in the Åland

229    Islands. In September 2010 and 2011, we selected a random subset of pathogen-infected plant

230    populations for our survey (n=91 out of 172 populations in 2010; n=96 out of 268 populations in

231    2011), and nearly all populations were surveyed in September 2012 (n=46 populations). For each

232    infected plant population, we randomly selected approximately five infected plants (mean±SD:

233    5.10±1.57), or less when less than five infected plants were detected. For each plant we recorded

234    whether infection and full-grown resting structures were present on ten haphazardly selected leaves

235    (or less on small plants). Selecting five plants but scoring a large number of leaves allows for a

236    similar survey effort across years and patches in the face of high temporal and spatial variation in

237    the number of infected plants per population (cf. Fig. 3a).

238         To analyse the impact of environmental and spatial factors on the spatial pattern of resting

239    structure production, we fitted a Bayesian spatial model using the integrated nested Laplace

240    approximation as described in the previous section (using the same environmental and spatial

241    covariates; Table 1).

242

243    *(3) Experiment on overwintering and spring infection*

244    We first carried out a trial overwintering experiment to i) investigate whether resting structures are

245    able to infect plants in spring in this pathosystem, and ii) assess the impact of winter conditions

246    (both indoors and outdoors) on the survival of resting structures. This experiment is described in

247    detail in Notes S1.

248　　　　　Given successful infection in the trial experiment (Notes S1, Fig. S5), we set up a larger

249　　experiment in spring 2012 to pinpoint the impact of pathogen population of origin, overwintering

250　　site, and their interaction on the ability of resting structures to infect plants in spring (Fig. 1,

251　　triangles C and D; Table 1). In autumn 2011, we collected ten leaves bearing resting structures from

252　　five plants in five populations (pink circles in Fig. S2). These leaves were stored in polyester

253　　pollination bags (PBS International) in a fully reciprocal design within these same five populations.

254　　Within each population, we stored the infected leaves in two locations (henceforth referred to as

255　　'micro-sites') to probe the impact of micro-environmental variation within a single pathogen

256　　population. As a single leaf and population can contain multiple pathogen genotypes (Tollenaere *et*

257　　*al.*, 2012), we note that such an experiment cannot measure variation among individual pathogen

258　　genotypes for overwintering, but it may indicate whether there is genetic differentiation among

259　　pathogen populations (Tack *et al.*, 2012). After snow-melt in mid-April 2012, samples were

260　　recovered from the field (samples from a single microsite were lost). To assess whether leaves

261　　bearing resting structures were able to infect plants in spring, individual leaves were positioned

262　　above a randomly assigned plant individual using two vertical sticks and horizontal iron wire.

263　　Plants were individually caged using a polyester pollination bag (PBS International 10-1; 1-

264　　window; 255 x 510 mm), as previous work has shown that infection develops well in these bags and

265　　spores cannot leave or enter (Laine, 2011; Notes S1). To reduce the impact of the receiving host

266　　plant genotype on the infection process, we used plant offspring from eight crosses between plant

267　　genotypes that were highly susceptible to a large number of pathogen genotypes (H. Susi,

268　　unpublished). Plants were scored on 19 June for the presence of powdery mildew infection. We

269　　implemented a total of 240 cages with 21 control cages in a largely balanced design. None of the

270　　control cages became infected. The experiment was conducted outdoors at Kumpula Botanic

271　　Garden (Helsinki, Finland).

272　　　　　To analyse the impact of pathogen population of origin, site of overwintering and their

273　　interaction on spring infection, we used the framework of generalized linear mixed-effects models

274　　(Table 1; Littell *et al.*, 2006). All models were fitted with procedure GLIMMIX in SAS 9.3. For

275　　models with multiple interactions, we used the principle of backwards stepwise model

276　　simplification to arrive at a minimum adequate model, where variables were retained when p<0.1

277　　(Crawley, 2007). We used two different analyses to probe for the existence of local adaptation of

278　　pathogen resting structures to survival in their sympatric (local) off-season environment. See

279　　Methods S1 for a more detailed verbal description of the statistics and measures of local adaptation.

280

281　　*(4) The impact of resting structures on overwintering in the field at two spatial scales*

282 We next aimed to investigate how natural variation in the amount of resting structures produced

283 affects overwintering in the field at two spatial scales: individual plants and populations.

284     To assess the ability of resting structures to re-infect the plant on which they were produced

285 (i.e. autoinfection), we enclosed 59 individual plants with variable numbers of resting structures

286 inside polyester pollination bags at the end of the 2011 growing season. By placing them in these

287 bags, any infection visible on the plants in the following spring would result from the previous

288 infection on the plant rather than from external sources of inoculum. As overwintering success may

289 vary among plant populations, we selected 10 plants in each of six populations (Fig. S2; n=9 in one

290 population). We also enclosed an additional 7 uninfected control plants (n=3 and n=4 in two

291 populations, respectively). In autumn, we recorded for each enclosed plant the total number of

292 leaves with resting structures. Field cages were checked for infection in early July 2012. During this

293 re-survey, nine cages did not contain a plant individual (due to mortality or dormancy) and one cage

294 could not be recovered. None of the control cages became infected.

295     To assess the impact of resting structures on overwintering at the population level, we used

296 data on the subset of populations where we collected both the July presence and abundance of the

297 powdery mildew (see section *The key role of the off-season in pathogen population extinction*) and

298 the level of resting structures in the previous autumn (see section *Spatial and temporal variation in*

299 *the production of resting structures*). Data were available for 34 populations for the overwintering

300 period from September 2010 to July 2011 and 89 populations from September 2011 to July 2012.

301     To assess the impact of resting structures on overwintering success at the plant level,

302 infection (0/1) and disease intensity (number of infected leaves / total number of leaves) in spring

303 were modelled as a function of the number of leaves with resting structures in the previous autumn

304 (Table 1). The pathogen population was used as random factor to account for variation among

305 populations in overwintering success. To investigate the impact of resting structures on

306 overwintering at the population level, off-season survival and July abundance were modelled as a

307 function of the fraction of infected leaves with resting structures in the previous autumn (Table 1).

308

309 **Results**

310 *(1) The key role of the off-season in pathogen population extinction*

311 More than 30% of the pathogen populations went extinct during the winter of 2010/2011 and

312 2011/2012 (Fig. 2), thereby confirming that extinctions during the off-season play a major role in

313 the high turnover rate in the pathogen metapopulation. Nonetheless, the extinction rates were in

314 both years lower based on September-to-July surveys (*c.* 37%) than for the extinction rates based on

315 the September-to-September surveys (*c.* 63%). Hence, while the off-season clearly represented a

316    major period of extinctions (black bars), these data suggest that a significant number of extinctions

317    also take place during the growing season (difference between black and grey bars in Fig. 2).

318    Abundance estimates show that the abundance within powdery mildew populations crashes severely

319    during the winter in each of the twelve geographical districts (Fig. 3a). Moreover, these data

320    indicated that powdery mildew populations declined more severely in some districts than in others

321    (repeated-measures ANOVA: $F_{11,253}$=1.81 and $P$=0.05). While a decline in densities during the off-

322    season is not trivial given the large number of resting structures that can be produced even on a

323    single leaf in autumn (cf. x-axis in Fig. 5a), only five out of 89 populations had higher densities in

324    July than in the previous autumn and twenty populations remained at similar densities. This

325    suggests that a decline (or crash) during the off-season is not universal but may be regarded as a

326    general rule. At a finer spatial scale, disease disappeared during the off-season from 73.8% of the 1-

327    $m^2$ quadrats known to be infected during the previous autumn. Pathogen distribution within the

328    patch was clearly related to the distribution of the disease in the previous autumn, as disease was

329    more than twice as common in quadrats with known disease incidence in the previous autumn as

330    compared to random quadrats (Fig. 3b; ANOVA: $F_{1,1290}$=18.43 and $P$<0.001).

331            Our Bayesian model of the survey data in July 2012 revealed that pathogen extinction was

332    high in patches that were i) small (in terms of host coverage), ii) exhibited no dryness of plants in

333    the previous autumn, iii) received little rainfall in the previous July, and iv) were recently colonized

334    (Table S1). When populations survived during the off-season, the abundance in July was positively

335    affected by plant coverage, and negatively affected by distance to shore (Table S2). The mode for

336    the spatial range (reflecting the range of spatial autocorrelation) was 6.7 km and 7.9 km for

337    extinction and abundance, respectively (Fig. S3).

338

339    *(2) Spatial and temporal variation in the production of resting structures*

340    The production of resting structures was highly variable among populations (Fig. 4) and affected by

341    several environmental factors in each of the years 2010-2012, though the impact of individual

342    factors varied strongly among years (Table S3). In 2010, the formation of resting structures

343    increased with plant dryness and decreased with rainfall in August (Table S3). In 2011, August

344    rainfall likewise decreased the formation of resting structures, whereas July rainfall had the opposite

345    effect, indicating the complex impact of rainfall across the growing season. Population age

346    negatively affected the formation of resting structures in 2011, whereas it positively affected resting

347    structures in 2012. Habitat openness had a strong positive impact on the formation of resting

348    structures in both 2011 and 2012, while host spatial connectivity had a positive impact on the

349    formation of resting structures in 2011 only. Production of resting structures was spatially

350  correlated in each of the three years, with the mode for the spatial range varying from 2.0 km to 4.6

351  km in 2010 and 2012, respectively (Fig. S4). Notably, while the fraction of infected leaves with

352  resting structures covered the full range from zero to one in 2010 and 2012, there were no

353  populations with no or few resting structures in 2011 (Fig. 4). The production of resting structures

354  across populations was highly uncorrelated among years (all pairwise Pearson correlations $P > 0.3$).

355

356  *(3) Experiment on overwintering and spring infection*

357  The large overwintering experiment was aimed at disentangling the impact of pathogen population

358  of origin and large and small-scale environmental variation on the viability of resting structures and

359  subsequent infection of plants in spring. As expected, both the infection (0/1) and disease

360  prevalence (i.e. proportion of infected leaves) on caged plants in spring were positively correlated

361  with the number of resting structures on the overwintered leaf ($F_{1,161} = 4.71$, $P = 0.03$ and $F_{1,137} =$

362  26.13, $P < 0.001$, respectively; Fig. 5a; see table S4 for detailed statistical results). Furthermore, the

363  proportion of leaves infected was affected by the interaction between population of origin and the

364  location of overwintering ($X_1^2 = 15.43$, $P < 0.001$; Fig. 5b), suggesting that pathogen genotypes from

365  different populations vary in their ability to survive a range of off-season environments. Variation

366  among plant individuals within population of origin (e.g. due to within-population variation in

367  pathogen genotype) as well as the location of the bags within an overwintering location (i.e. micro-

368  environmental variation during the off-season) explained additional variation in the infection

369  intensity ($X_1^2 = 62.05$, $P < 0.001$ and $X_1^2 = 3.87$, $P = 0.05$, respectively). Finally, despite the selection

370  of generally susceptible plant genotypes as trap plants in the cages, we still detected variation in

371  infection level among plant genotypes ($X_1^2 = 19.90$, $P < 0.001$). We detected a trend for higher

372  disease infection in sympatric (back-transformed least-squares mean ± SE: 0.064 ± 0.032) as

373  compared to allopatric (back-transformed least-squares mean ± SE 0.043 ± 0. 021) combinations

374  using two alternative modeling approaches (Methods S1), suggesting some support for local

375  adaptation by the pathogen to its sympatric (local) overwintering environment ($F_{1,148} = 4.66$, $P =$

376  0.03 and $F_{1,133} = 3.68$, $P = 0.06$ for model 1 and 2, respectively).

377

378  *(4) The impact of resting structures on overwintering in the field at two spatial scales*

379  Finally, we investigated the impact of resting structures on overwintering at the level of individual

380  plants and populations. At the level of the individual plant, a large fraction of the plants that were

381  infected and enclosed in a cage during the off-season were infected during the following spring (i.e.

382  'auto-infection'; 41% or 20 out of 49 plants). The control plants (n = 7) all remained without

383  infection. There was a positive relationship between the number of leaves with resting structures in

384    the previous autumn and infection and disease intensity in spring ($F_{1,42}$ = 2.21, $P$ = 0.14 and $F_{1,42}$ =

385    33.23, $P$ < 0.001, respectively). There was significant variation among the pathogen populations in

386    the number of infections and the disease intensity ($X_1^2$ = 4.30, $P$ = 0.02 and $X_1^2$ = 50.16, $P$ < 0.001,

387    respectively).

388         The number of infected plants in a population in 2011 was positively correlated with the

389    fraction of infected leaves with resting structures in the previous autumn ($F_{1,32}$ = 4.07, $P$ = 0.05), but

390    no significant effect was detected for the presence / absence of infection ($F_{1,32}$ = 0.93, $P$ = 0.34).

391    The fraction of infected leaves with resting structures in September 2011 did not affect survival or

392    infection intensity in July 2012 ($F_{1,87}$ = 0.15, $P$ = 0.70 and $F_{1,87}$ = 0.06, $P$ = 0.80, respectively).

393

394    **Discussion**

395    Few previous studies have investigated the ecological and evolutionary dynamics of host and

396    parasites during the off-season. In this study, we combine observational and experimental studies to

397    demonstrate that i) the ephemeral nature of local pathogen populations is directly related to the off-

398    season, when forty percent of the populations go extinct and local population abundances strongly

399    decline across the metapopulation; ii) environmental and spatial factors strongly affect pathogen

400    overwintering and the production of resting structures; and iii) pathogen population of origin and

401    the off-season environment interact to jointly shape infection intensity in spring, with a weak signal

402    of pathogen adaptation to the local off-season environment. Overall, while an increasing number of

403    studies have explored how genotype and environment shape parasite transmission and evolution

404    during the growing season (triangle A in Fig. 1; e.g. Wolinska & King, 2009), the current study

405    emphasizes that equally fascinating, complex and unexplored ecological and evolutionary dynamics

406    play out across the off-season.

407

408    *The extended disease triangle*

409    McNew's disease triangle (1960) focuses on how the environment, plant genotype and pathogen

410    genotype jointly shape disease dynamics, with a clear focus on the growing season. Here, we extend

411    the disease triangle to the off-season.

412         We detected a strong impact of environmental and spatial factors on overwinter survival and

413    July abundance (triangles B – D, Fig. 1) and the production of resting structures (triangle B, Fig. 1).

414    However, the identity of the spatial and environmental drivers varied among response variables and

415    among years. Higher plant coverage (representing patch area) decreased extinction and increased

416    July abundance, thereby re-emphasizing the important role of patch area in classic metapopulation

417    dynamics (Hanski, 1994). Environmental factors like the percentage of dried plants and July rainfall

418    increased winter survival (probably by its direct effects on pathogen growth), whereas August

419    rainfall (-) and habitat openness (+) were the most consistent predictors of the presence of resting

420    structures. Population age increased the likelihood of both off-season survival and the production of

421    resting structures in 2012, probably because these patches provide a more optimal habitat to the

422    pathogen. Despite the detection of several environmental and spatial variables, the spatial scale of

423    autocorrelation (extending up to 8 km) suggests that our environmental and spatial variables fail to

424    capture all spatial variation. This may not be surprising, particularly given the fact that the

425    environmental variables included in the model were originally selected based on their potential

426    relevance for the epidemiology during the growing season. Importantly, the scale of autocorrelation

427    may guide us in the identification of environmental variables relevant for the off-season (e.g. snow

428    cover). While rarely considered, spatial patterns in the pathogen genetic and phenotypic distribution

429    may further explain patterns of spatial autocorrelation. For example, while the current study does

430    not directly address the role of plant or pathogen genotype in the production of resting structures,

431    other experimental work in this system shows that the production of resting structures by pathogens

432    strains is differentially affected by light (Tollenaere & Laine, 2013) and soil environment (A. J. M.

433    Tack, unpublished). Comparable studies in other systems reveal a key role for both host resistance

434    and environment on the production of resting structures of grape powdery mildew *Erysiphe necator*

435    (Gadoury & Pearson, 1988; Legler *et al.*, 2012) and the impact of pathogen genotype, temperature

436    and their interaction on the production of resting structures of strawberry powdery mildew

437    *Podosphaera aphanis* (Asalf *et al.*, 2013). Jointly, these observational and experimental studies

438    suggest that both the environment and genotype may explain variation in the production of resting

439    structures (triangle B, Fig. 1).

440        The maturation and viability of the resting structures may depend on pathogen genotype and

441    environment experienced during the off-season (triangle C, Fig. 1). Here, our experiment revealed a

442    relatively weak effect of pathogen population of origin or overwintering site *per se*: instead,

443    pathogens from different locations responded differently to the same off-season environment (i.e. a

444    GxE interaction). While the role of the off-season environment on the viability of resting spores is

445    well-known (Gadoury & Pearson, 1988; Cohen *et al.*, 2013), few studies have investigated the

446    effect of pathogen origin or GxE interactions. Finally, our experiment showed a significant impact

447    of receiving plant genotype on infection in spring (Fig. 1, disease triangle D). While not the focus of

448    our experiment, this warrants further study and suggests that plant genotype may be critical to

449    safeguard spring infection. Such a notion is in line with results from this (Laine, 2005; Laine, 2008;

450    Tack *et al.*, 2013b) and other (Thompson & Burdon, 1992; Laine *et al.*, 2011) disease systems

451    demonstrating that plant genotype  plays a key role in the infection process. Notably, the pattern of

452    high temporal consistency of disease distribution within plant populations between autumn and July

453    infection (Fig. 3B) may then be due to (a combination of) dispersal limitation, micro-environmental

454    variation and spatial variation in plant genotype (Tack *et al.*, 2013a).

455            While the fraction of leaves with resting structures in autumn 2010 explained disease

456    intensity in July 2011, we detected no relationship between levels of resting spores in September

457    2011 and infection in July 2012. The absence of a pattern for the latter year may be explained by the

458    absence of populations with no or few resting structures in September 2011 (Fig. 4). Interestingly,

459    this relatively high fraction of resting structures in nearly every pathogen population in September

460    2011 may also explain the large overall increase in the pathogen metapopulation from September

461    2011 to September 2012 (from 268 to 633 pathogen populations, respectively).

462

463    *The evolutionary implications of the extended disease triangle*

464    While the key aim of McNew's disease triangle was to understand and predict disease severity, the

465    inclusion of genetic factors and the potential for genotype-by-environment interactions within the

466    disease triangle provides a direct link between epidemiological patterns and evolutionary processes.

467    Previous results in this pathosystem have indicated the key role for genotype by environment

468    (temperature, nutrient levels) interactions on infection dynamics (Laine, 2007) and patterns of local

469    adaptation (Laine, 2008). We now have accumulating evidence that GxE interactions may also be

470    important during the production of resting structures (triangle B, Fig. 1; Tollenaere & Laine, 2013)

471    and during the ensuing off-season (triangle C, Fig. 1; Fig. 5). Such studies indicate the existence of

472    differentiation among pathogen genotypes and pathogen populations in the ability to produce

473    resting structures and subsequently survive a range of environmental conditions during the off-

474    season. At the same time, our results show that the ecological and evolutionary drivers of

475    interactions may vary in time, and the dynamics of an interaction can thus be a composite of

476    temporally distributed GxE (or more speculatively, GxGxE) interactions. The existence of temporal

477    variation in (Gx)GxE interactions has important implications for the coevolution within

478    metapopulations and across larger geographical scales for all forms of interactions between species

479    (Thompson, 2005).

480            Pathogen population differentiation and its interaction with the off-season environment

481    indicate that genetic variation exists for the pathogen to adapt to spatial variation in environmental

482    conditions within the metapopulation. Our reciprocal transplant experiment revealed a marginally

483    significant pattern of local adaptation (*P = 0.06*): while resting structures from four out of five

484    populations resulted in relatively high infection intensity in spring when stored in their local

485    population during the off-season, this pattern was reversed in population 609 (Fig. 5b). The absence

486 of a consistent pattern of local adaptation during the off-season may be explained by at least three

487 mechanisms. First, local adaptation may be swamped by high gene flow (Slatkin, 1987; Tack &

488 Roslin, 2010) or wiped out by frequent extinctions. A classic study on the mummy berry fungus

489 (*Monilinia vaccinii-corymbosi*) on blueberry illustrates this pattern: while spring germination of

490 overwintering structures was in some locations adapted to be synchronized with the phenology of

491 bud break in the host cultivar, such adaptation was absent when early and late cultivars were grown

492 in close proximity (Lehman & Oudemans, 2000). Second, there may be a trade-off between off-

493 season survival and within-season growth, thereby counteracting selection in separate stages of the

494 pathogen life cycle (cf. Carson, 1998; Martinez *et al.*, 2005; Abang *et al.*, 2006; Barrett *et al.*, 2011;

495 Sommerhalder *et al.*, 2011). Third, the magnitude of yearly variation in overwintering conditions

496 may (far) exceed heterogeneity in summer conditions: while resting structures may be protected

497 from degeneration by a thick and persistent snow cover in some years (cf. Pearson & Gadoury,

498 1987), the lack of a continuous snow cover in other years may expose the resting structures – at

499 least in some populations – to extreme weather and sub-zero temperatures. Such a mosaic pattern of

500 local adaptation has previously been reported in this study system for pathogen performance during

501 the growing season (Laine, 2005; Laine, 2008). The existence of genetic variation in our study is in

502 contrast with studies on the oak powdery mildew, which showed the absence of genetic variation in

503 the timing of spore release (and therefore local adaptation) in spring (Marçais *et al.*, 2009; Desprez-

504 Loustau *et al.*, 2010).

505

506 *Conclusion*

507 We demonstrate that parasite survival during the off-season is crucial to our understanding of

508 disease metapopulation dynamics and evolutionary responses of the pathogen to environmental

509 heterogeneity. We expect that ecological and evolutionary changes during the largely unexplored

510 off-season play a similar role in the dynamical behaviour of a wide range of other host-parasite

511 systems, irrespective of whether the parasite survives as resting structures, in low densities on the

512 few remaining or susceptible hosts, or saprophytically. Unravelling the ecological and evolutionary

513 drivers behind the off-season dynamics across host-parasite systems, both empirically as well as

514 theoretically, offers an exciting venue for future research and is needed to generate predictions

515 regarding disease dynamics from one season to the next. From an applied perspective, insights into

516 the off-season may be pivotal in combating diseases and parasites in agricultural, wildlife and

517 human disease systems (Bartlett, 1957; Altizer *et al.*, 2006; Rambaut *et al.*, 2008; Gunning &

518 Wearing, 2013; Jaspers *et al.*, 2013) and the design of environmentally friendly means of managing

519 diseases and parasites (Peterson *et al.*, 2005; Legler *et al.*, 2012; Cohen *et al.*, 2013).

520

532

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697

698

699 **Supporting information**

700

701 Additional supporting information may be found in the online version of this article.

702 **Fig. S1.** Resting structures of the powdery mildew *Podosphaera plantaginis*.

703

704 **Fig. S2.** Locations of overwintering cages and locations where resting structures were collected in

705 autumn and/or stored during the off-season.

706

707 **Fig. S3.** Posterior distribution of the spatial range estimate for a spatial Bayesian model for winter

708 extinction of the pathogen *Podosphaera plantaginis* from 2011 to 2012 and pathogen abundance in

709 July 2012.

710 **Fig. S4.** Posterior distribution of the spatial range estimate for the fraction of infected leaves with

711 resting structures for the pathogen *Podosphaera plantaginis* in its host populations in Åland,

712 southwestern Finland.

713

714 **Fig. S5.** Fraction of infected cage plants in the overwintering trial experiment.

715

716 **Table S1.** Factors that affect the extinction of the powdery mildew pathogen *Podosphaera*

717 *plantaginis* during the off-season in its host populations in Åland, southwestern Finland.

718

719 **Table S2.** Factors that affect the July abundance of the powdery mildew pathogen *Podosphaera*

720 *plantaginis* in infected host populations in Åland, southwestern Finland.

721

722 **Table S3.** Factors that affect the fraction of infected leaves with cleistothecia for pathogen

723 *Podosphaera plantaginis* in its host populations in Åland, southwestern Finland.

724

725 **Table S4.** The impact of population of origin and off-season storage location on the ability of

726 resting structures to infect individually caged plants in spring.

727

728 **Notes S1.** A trial experiment on overwintering survival using indoor and outdoor overwintering

729 sites.

730

731 **Methods S1.** A detailed description of the statistical methods.

732    **Table 1.** A summary of the experimental and observational materials used, the disease triangles

733    addressed, and the models fitted for analyses.

734

| Interaction targeted | Triangle(s) addressed (Fig 1) | Response(s) examined | Fixed effects (triangle) | Random effects (triangle) | Link[1] |
|---|---|---|---|---|---|
| (1) *The key role of the off-season in pathogen population extinction* | B - D | a) Extinction<br>b) July abundance | *Environmental and spatial covariates*[2] | | a) Logit<br>b) Identity |
| (2) *Spatial and temporal variation in the production of resting structures* | B | proportion of infected leaves with sexual resting structures[3] | *Environmental and spatial covariates*[2] | | Logit |
| (3) *Experiment on overwintering and spring infection* | B - D | a) Infection (0/1)<br>b) Proportion of infected leaves | *Number of resting structures* (B) | Pathogen population of origin (C) + Overwintering site (C) + Pathogen population of origin (C) × Overwintering site (C) + Micro-site (Overwintering site) (C) + Plant individual (Pathogen population of origin) (C) + Receiving plant genotype (D) | Logit |
| (4) *The impact of resting structures on overwintering in the field at two spatial scales* | B | Plant level:<br>a) Infection (0/1)<br>b) Proportion of infected leaves | *Number of leaves with resting structures* | Pathogen population | Logit |
| | B | Population level[4]:<br>a) Infection (0/1)<br>b) Number of infected plants | *Fraction of infected leaves with resting structures* | | a) Logit<br>b) Log |

[1] For continuous data, we assumed a normal distribution with an identity link; for count data, we assumed a Poisson distribution with a log link; for binomial data we assumed a binomial distribution with a logit link. Independent continuous variables are identified in italics

[2] Environmental and spatial covariates included are distance to shore, plant dryness, patch shadow, habitat openness, July rainfall, August rainfall, population age, host plant coverage, road presence and host plant spatial connectivity

[3] Separate models were constructed for 2010, 2011 and 2012

[4] Separate models were constructed for 2011 and 2012

735

736

737     **Legends**

738     **Figure 1.** The majority of studies – and the classic disease triangle of phytopathology (McNew,

739     1960; Scholthof, 2007) – focus on the infection and epidemic stage of the parasite (triangle A and

740     less frequently triangle D). As such, the disease triangle has emphasized for several decades how

741     plant genotype, pathogen genotype and environment jointly affect disease presence and intensity.

742     However, we lack crucial insight on how genotype and environment interact during alternative life-

743     stages of parasites: production of resting structures (triangle B), off-season survival (triangle C) and

744     infection of hosts at the start of the epidemic season (triangle D).

745

746     **Figure 2.** Extinction dynamics of the pathogen *Podosphaera plantaginis* in the *c.* 4000 host

747     populations of *Plantago lanceolata* on the Åland islands, southwestern Finland.

748     In panel **a**, the grey bars represent the extinction rate estimated from the large-scale survey

749     conducted each September since 2001 for the presence/absence of the pathogen *P. plantaginis*,

750     calculated as the fraction of populations occupied in September of year t-1 that were unoccupied in

751     September of year t. The black bars represent the extinction rate estimated from more recent bi-

752     annual surveys in September t-1 and July in year t. Panel **b** is based on a detailed survey in July

753     2012 of pathogen populations that were infected during the previous autumn, and shows the spatial

754     distribution of population survival in 2012 across the Åland Islands (Finland). Red triangles and

755     blue circles refer to extinct and persisting populations, respectively.

756

757     **Figure 3.** Patterns in off-season survival of the powdery mildew *Podosphaera plantaginis.* Panel **a**

758     shows the decrease in abundance during the winter for each of twelve geographical areas

759     ('districts') within the Åland Islands, where arrows point out the mean values. Note that the y-axis

760     follows a categorical scale, where 0=absence of mildew, 1=1-9 infected plants, 2=10-99 infected

761     plants, 3=100-999 infected plants, and 4=1000 or more infected plants. Panel **b** illustrates patterns

762     of July infection within pathogen populations. Plots (1 m$^2$) with known infection in the previous

763     autumn (2011) have a likelihood of infection in July (2012) of 26.2%. Randomly selected plots

764     have a much lower likelihood of infection (9.9%).
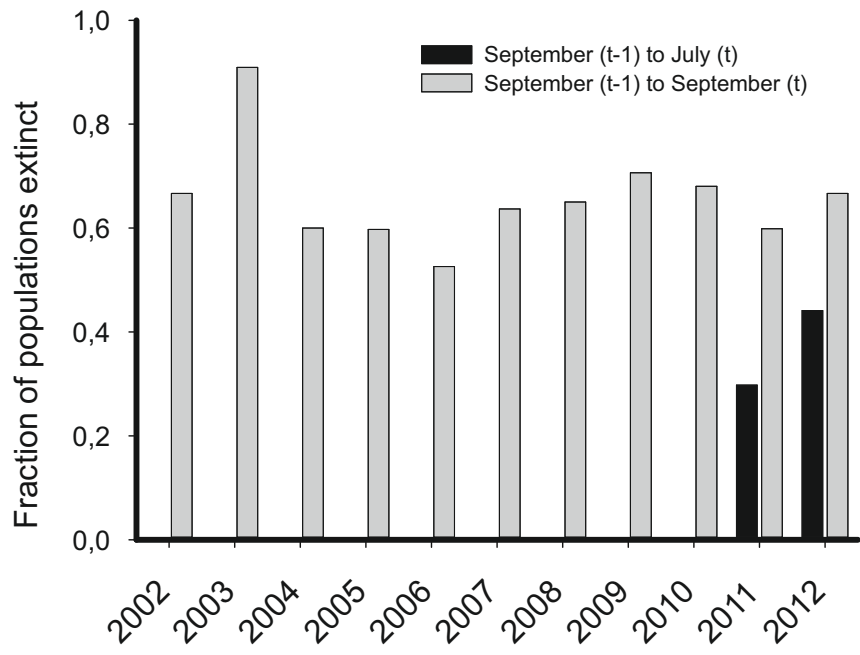
765

766     **Figure 4.** Spatial variation in the production of resting structures (i.e. fraction of infected leaves

767     with resting structures) by the powdery mildew *Podosphaera plantaginis* for each of three years.

768     The graphs on the right show the distribution of the fraction of infected leaves with resting

769     structures across populations. For each year, the fraction of infected leaves with resting structures is

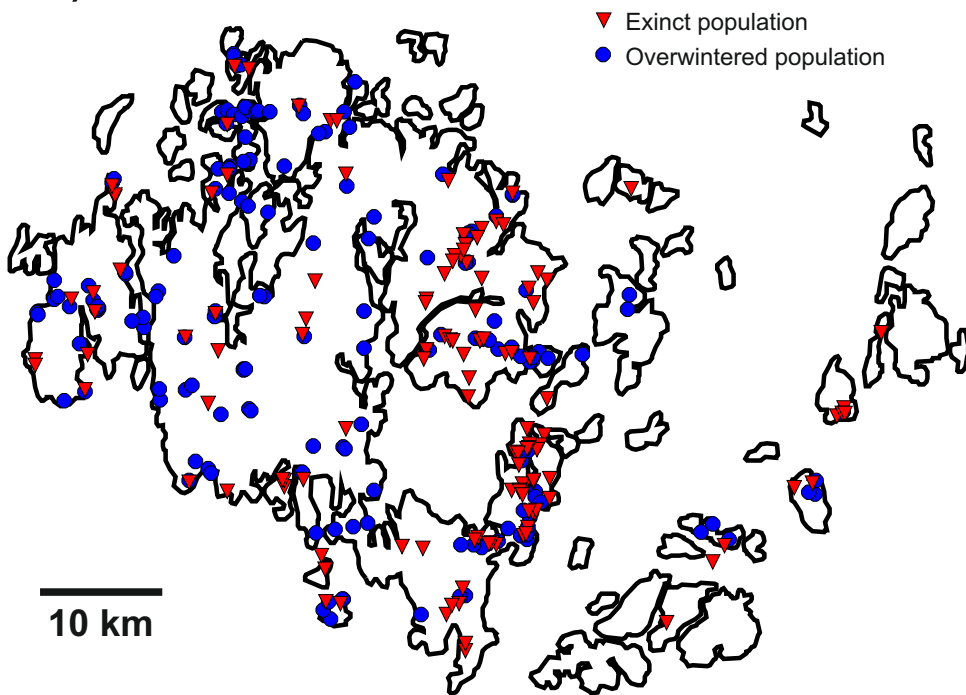770     highly variable among populations, but particularly so in 2010 and 2012.

771

772  **Figure 5.**  Some examples of factors that affect overwintering of the pathogen *Podosphaera*

773  *plantaginis* in a reciprocal experiment. Panel **a** shows the impact of the quantity of resting structures

774  on spring infection (a single data points falls to the right of the plotted range). Panel **b** depicts the

775  interaction between pathogen population of origin and storage location on infection intensity in

776  spring. The black line and associated grey shaded area in panel **a** shows the logistic regression line

777  and its 95% confidence interval, respectively. In panel **b** are plotted empirical means +/- SEs.

a)



b)

a)



b)

a)



b)