This is the peer reviewed version of the following article:
Puidet, B., Mabon, R., Guibert, M., Kiiker, R., Loit, K., Le, V. H., Eikemo, H., Dewaegeneire, P., Saubeau, G., Chatot, C., Aurousseau, F., Cooke, D. E. L., Lees, A. K., Abuley, I. K., Hansen, J. G., Corbière, R., Leclerc, M., Najdabbasi, N., & Andrivon, D. (2023). Investigating phenotypic traits as potential drivers of the emergence of EU_37_A2, an invasive new lineage of Phytophthora infestans in Western Europe. *Plant Pathology*, 72(4), 797–806. Portico.,
which has been published in final form at https://doi.org/10.1111/ppa.13700.

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Investigating phenotypic traits as potential drivers of the emergence of EU_37_A2, an invasive new lineage of *Phytophthora infestans* in Western Europe

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26

27 ## Abstract

28 Since the mid 2010s, *Phytophthora infestans* clones that have been dominant in Western Europe
29 since the beginning of the 21st century, for example, EU_13_A2, EU_6_A1 and EU_1_A1, are
30 being replaced by several other emerging clones, including EU_37_A2. The objective of this
31 study was to determine whether the main drivers for the success of EU_37_A2 in Western
32 Europe are associated with decreased fungicide sensitivity, increased virulence and/or
33 aggressiveness. Axenic *P. infestans* cultures were sampled in the 2016 and 2017 growing
34 seasons from potato crops in France and the United Kingdom. Among these, four genotypes
35 were identified: EU_37_A2, EU_13_A2, EU_1_A1 and EU_6_A1. Although a wide range of
36 fluazinam sensitivity was found amongst individual isolates, clonal lines EU_13_A2 and
37 EU_37_A2 showed decreased sensitivity to fluazinam. EU_37_A2 overcame the R5 differential
38 cultivar more often than isolates of EU_1_A1 or EU_6_A1. However, this does not explain the
39 competitive advantage of EU_37_A2 over the virulent EU_13_A2. The fittest genotype, as

measured by aggressiveness under controlled conditions, was EU_6_A1, followed by EU_37_A2, EU_13_A2 and then EU_1_A1. EU_37_A2 isolates also showed a shorter latent period than either EU_6_A1 or EU_13_A2, which could favour its long-term persistence. Overall, the data suggest that the emergence of EU_37_A2 in Western Europe was driven by its resistance to a then major fungicide and shorter generation time. This conclusion is further supported by the fact that EU_37_A2 emergence was slowed by the progressive reduction in the use of fluazinam as a single active ingredient in the years following its initial detection.

Keywords

Potato late blight, fungicide sensitivity, fluazinam, generation time, fittest genotype, aggressiveness

1 Introduction

Potato late blight has been the cause of significant economic losses globally for over a century. In the European Union alone, yearly losses due to late blight are estimated at about €900 million, including production losses and the cost of protection methods (Haverkort et al., 2008), making this disease a persisting challenge to control and manage sustainably.

Potato late blight, caused by the pathogen, *Phytophthora infestans*, is mainly controlled through fungicide applications, which make up about 10% of the total production costs and add a significant burden to the environment (Haverkort et al., 2008, 2009). *P. infestans* is a heterothallic oomycete, of which both mating types (A1 and A2) are widely distributed, but with spatially different proportions of each across Europe. Although the simultaneous occurrence of

61 both mating types in many regions should favour sexual reproduction and high population
62 diversity, *P. infestans* populations in Western Europe are mostly clonal (Cooke et al., 2012,
63 Mariette et al., 2016b)

64 One of the most successful and long-lasting clonal lineages of *P. infestans* in Europe has
65 been EU_13_A2, which was first detected in the Netherlands and the United Kingdom in 2004 to
66 2005 (Cooke et al., 2012; Li et al., 2012) and persists to this day. Its wide geographical
67 distribution and longevity in many populations have shown that it is a fit genotype (Chowdappa
68 et al., 2013, 2015;, Cooke et al., 2012; Li et al., 2013b). Furthermore, this genotype has also
69 proven to be phenylamide-resistant (Cooke et al., 2012, 2013), which could be one of the reasons
70 for its global success.

71 Whatever the fitness of individual lineages, most clonal populations undergo massive
72 changes in their genetic structures over relatively short periods (Mariette et al., 2016b). Such
73 selective sweeps raise the question of the drivers behind the emergence of new clones and about
74 ways of predicting which and when new clones will displace existing ones. According to the
75 most recent monitoring results from the EuroBlight network (EuroBlight, 2022), the three
76 dominating clones from the past 15 years (EU_1_A1, EU_6_A1 and EU_13_A2) are currently
77 being replaced by several emerging clones, notably EU_41_A2, EU_36_A2 and EU_37_A2.
78 EU_41_A2 spreads mainly in northern Europe and is more virulent than sexually reproducing
79 populations (Puidet et al., 2022). EU_36_A2 was first identified in 2014 in the Netherlands and
80 has shown a greater aggressiveness than several other major clones (EuroBlight, 2022). Finally,
81 EU_37_A2 was first observed in the Netherlands in 2013 (Schepers et al., 2018) and started
82 spreading rapidly in 2015, reaching England, France, Germany, Poland and Belgium (Figure S1,
83 2021). In 2018, reduced efficacy of the active ingredient fluazinam against *P. infestans* genotype

84 EU_33_A2 was reported in the Netherlands (Schepers et al. 2018). In addition to the field testing
85 on EU_33_A2, in-vitro sensitivity tests were also carried out on different genotypes. The results
86 showed significantly higher minimum inhibitory concentration values for EU_33_A2 and
87 EU_37_A2 than for isolates from genotypes EU_13_A2 and EU_6_A1. Fluazinam acts via an
88 uncoupling of oxidative phosphorylation that affects energy production in the pathogen.
89 Although development of resistance to fluazinam was considered extremely unlikely, its long-
90 term and widespread use throughout the growing season is considered to have created a strong
91 selection pressure and driven the emergence of these new lineages (Schepers et al., 2018).
92 Despite the reports of insensitivity to fluazinam (Schepers et al., 2018), other phenotypic data of
93 this genotype remain sparse and large scale studies are lacking. Such changes in the dominant
94 genotypes of *P. infestans* populations might lead to ineffective protection of the potato crop
95 against the pathogen (Fry et al., 2015) and suggests the need to adjust control methods according
96 to the main pathogenicity and epidemic features of invasive genotypes. Ideally, anticipating such
97 invasions would also allow for optimal deployment of active ingredients and resistance genes
98 (Kessel et al., 2018). Therefore, the objective of this study was to determine whether the main
99 drivers for the success of EU_37_A2 compared to EU_1_A1, EU_6_A1 and EU_13_A2 in
100 Western Europe lie in its phenotypic characteristics. We hypothesised that the drivers for the
101 success of genotype EU_37_A2 were primarily associated with a decrease in sensitivity to
102 fluazinam—a rare trait in *P. infestans* populations at the time and a major selective force given
103 its widespread use in Europe in the mid 2010s. However, and as shown by a similar analysis of
104 another emerging clone, EU_41_A2, in northern Europe, invasive ability is often driven by
105 multiple rather than single traits (Puidet et al., 2022). Therefore, we also compared pathogenicity

traits between EU_37_A2 and contemporary lineages to determine whether they could also be integral to the emergence of this genotype.

2 Materials and methods

2.1 Isolate collection and genotyping

Isolates of *P. infestans* were collected during the 2016 (*n* = 71) and 2017 (*n* = 66) growing seasons from potato crops in France (*n* = 82) and the United Kingdom (*n* = 55) from a mix of conventional fields and gardens. Up to four leaves, each with a single lesion of *P. infestans* were collected from each sampled field throughout the season. Leaflets were collected randomly across the field, each from a different plant. Half of each lesion was used to produce an axenic culture (Puidet et al., 2022), whereas the other half was used for simple-sequence repeat (SSR) genotyping.

The media for isolation, experiments and storage differed among laboratories (V8 agar, rye B agar, pea agar or a 50/50 mixture of pea and rye B agar; Puidet et al., 2022). To avoid contamination, isolates obtained from the leaf lesions were first grown on media amended with antibiotics (10 µg/ml pimaricin, 30 µg/ml rifamycin and 150 µg/ml ampicillin). Purified axenic isolates were subsequently kept on agar media without antibiotics at 15–18℃ in the dark and were transferred to fresh plates every 4 to 7 weeks until phenotypic characterization. Isolates were grown on cv. Bintje potato leaves prior to phenotypic characterization to restore their natural aggressiveness. All phenotypic assays were conducted within a year after sampling.

126 Genotyping was carried out using the EuroBlight 12-plex SSR marker set as described by
127 Li et al. (2013a). Genotypes were assigned to clonal lineages by matching their SSR profiles to
128 those of the reference isolates in the EuroBlight database (EuroBlight, 2022).

129 **2.2 Fungicide sensitivity**

130 Fungicide sensitivity of the collected isolates was determined for four formulated active
131 ingredients: fluazinam (Shirlan 500 SC, a.i. 500 g/L; Syngenta), mandipropamid (Revus 250 SC,
132 a.i. 250 g/L; Syngenta), cyazofamid (Ranman Top, a.i. 160 g/L; Belchim Crop Protection) and
133 propamocarb (Previcur N, a.i. 605 g/L; Bayer Crop Science). Fluazinam and mandipropamid
134 were tested in Estonia (the Chair of Plant Health, Estonian University of Life Sciences, Estonia),
135 and cyazofamid and propamocarb were tested in Norway (the Division of Biotechnology and
136 Plant Health, Norwegian Institute of Bioeconomy Research, Norway). According to the
137 Fungicide Resistance Action Committee Code List in 2022 (FRAC, 2022a), the resistance risk of
138 the active ingredients tested is considered to be low (group 29: fluazinam), low to medium
139 (group 40: mandipropamid and group 28: propamocarb) or unknown, but assumed to be medium
140 to high (group 21: cyazofamid). For each product, sensitivity was assessed using different doses
141 and a distilled water control (Table 1). The range of concentrations was increased in 2017 to
142 match the product manufacturer's field dose recommendations while covering the baseline
143 sensitivity, as was previously done with fluazinam in 2016.

144 The experiment was conducted on detached leaflets collected from 6- to 8-week-old
145 plants of the late blight susceptible potato cv. Bintje as described by Puidet et al. (2022). In brief,
146 six fully developed cv. Bintje leaflets were tested for each isolate and concentration. The leaflets
147 were dipped for a few minutes into the product suspension, and then incubated in closed trays

148 with moist filter paper for 24 h at 18°C with a 16 h photoperiod. Twenty-four hours after
149 treatment, the abaxial sides of the leaflets were each inoculated with a 10-µl droplet of
150 sporangial suspension at a concentration of c.3 × 10^4 sporangia/ml, and further incubated under
151 the conditions described above. They were assessed visually 7 days after inoculation. The test
152 was considered successful, and the data subsequently analysed, if at least 10 out of 12
153 inoculation points formed sporulating lesions larger than the initial droplet size in the untreated
154 control set. The test was repeated only for the isolates that failed the first test. The results were
155 discarded for the isolates that failed the experiment twice.

156 **2.3 Virulence profiles**

157 Virulence profiles were determined for all collected isolates using Black's differential set of 11
158 potato genotypes (Malcolmson & Black, 1966; Zhu et al., 2015), grown in the greenhouse for 6–
159 8 weeks. The isolates were randomly distributed between three different laboratories (Estonian
160 University of Life Sciences, Tartu, Estonia; FN3PT-Inov3PT, Achicourt, France and Germicopa
161 Breeding, Châteauneuf du Faou, France), where the profiling was conducted according to
162 Andrivon et al. (2011). Each laboratory tested five identical reference isolates, which were
163 selected to verify the procedure. In brief, sporangial suspensions of c.3 × 10^4 sporangia/ml were
164 prepared from individual isolates of *P. infestans*. Two leaflets from each potato genotype were
165 inoculated with two 20-µl suspension droplets per leaflet. The test was evaluated after 7 days of
166 incubation at 18°C with a 16 h photoperiod in closed trays lined with moist filter paper. The test
167 results were analysed if at least three of the four inoculation spots on the leaflets of the late blight
168 susceptible cv. Bintje developed sporulating lesions larger than the inoculation droplet. The
169 resistance of a given differential was considered to be overcome by an isolate if sporulation was
170 present on at least two of the four inoculation points on the leaflets of this differential.

171 **2.4 Characterization of aggressiveness**

172 For each isolate, components of aggressiveness were assessed in one laboratory (INRAE, Le
173 Rheu, France) under the same conditions in two consecutive years. Suspensions of c.3 × 10^4
174 sporangia/ml were used to inoculate 10 cv. Bintje leaflets with a single 20 µl droplet per leaflet,
175 deposited on the abaxial side. The inoculated leaflets were incubated in inverted Petri dishes
176 (two leaflets per dish) containing 10 g/L water agar, which acted as humidity chambers, for 5
177 days at 18°C during the16 h photoperiod and at 15°C during the 8 h dark period (Mariette et al.,
178 2016b). Subsequently, each lesion was assumed to be an elliptic shape and was measured with an
179 electronic caliper from two different diameters: one along the midrib and one perpendicular to it.
180 The lesion area was calculated as described by Mariette et al. (2016b). Immediately after
181 measuring the lesion diameters, sporangia were washed from leaflets with 5 ml of sterile water,
182 and the suspensions were stored in glass tubes at −20°C until the sporangia were counted with a
183 particle counter (Occhio S.A. Flowcell FC200S+), as described by Kröner et al. (2017). The
184 latent period was determined as the time between inoculation and observation of the first
185 sporangia using a stereomicroscope. The inoculated leaflets were checked for sporangia daily.
186 Finally, the lesion growth rate was calculated by dividing the lesion area by the time of the lesion
187 growth without the latent period, and a fitness index, proposed by Montarry et al. (2010), was
188 calculated from the data for each isolate.

189 **2.5 Statistical analysis**

190 The isolates which had at least 10 successful infections out of 12 inoculations in the fungicide
191 sensitivity experiment, and had the same number of successful infections for all tested doses,
192 were automatically regarded as resistant, and their half-maximal inhibitory concentration (IC_{50})

193 values were not calculated (IC_{50} > highest concentration). For the rest of the isolates, fungicide
194 sensitivity was analysed separately for each isolate considering a likelihood function based on a
195 n~Binomial[N, p] where n is the number of successful infections, N the total number of
196 inoculated leaflets, and p the probability that an inoculation creates a successful infection. Using
197 a logit link function, a generalised linear model (logistic GLM) was obtained and used to assess
198 the effect of the dose on the probability of successful infection (Dunn & Smyth, 2018). The fitted
199 logistic models were then used to obtain the IC_{50}, i.e. the concentration at which $p = 0.5$ (Figure
200 S2). The effects of genotype, country, year, country × year interaction and genotype × year
201 interaction on IC_{50} were tested using a type II analysis of variance (ANOVA). Afterwards, post
202 hoc analyses using Tukey's honestly significant differences (Tukey's HSD) were performed to
203 compare treatments ($\alpha = 0.05$).

204 Virulence profiling results were analysed as binary data (distribution: binomial) using
205 GLM (Type III SS) followed by Tukey's unequal N HSD post hoc test ($\alpha = 0.05$).

206 The effects of the genotype and the country on individual aggressiveness traits (i.e., latent
207 period, spore density, lesion growth rate and fitness index) were analysed with ANOVAs
208 followed by post hoc analyses with Tukey tests.

209 All statistical analyses of fungicide sensitivity and aggressiveness were performed using
210 R (R Core Team v. 3.6.2, 2019; Faraway, 2016) while virulence data were processed using
211 Statistica v. 12.0.

212

213 3 Results

214 **3.1 Isolate genotypic structure**

215 Genotyping the 137 isolates with the 12-plex SSR set of markers revealed that 29 isolates (17
216 from France and 12 from the United Kingdom) matched the genetic fingerprint of clonal lineage
217 EU_37_A2 (Table 2). The markers assigned 16, 44 and 48 isolates to EU_1_A1, EU_6_A1 and
218 EU_13_A2, respectively.

219 **3.2 Fungicide sensitivity**

220 Fungicide sensitivity data were obtained for 116 *P. infestans* isolates in the tests with fluazinam
221 and mandipropamid and 123 isolates with cyazofamid and propamocarb. Ninety percent of the
222 tested EU_37_A2 isolates in 2016 and 27% in 2017 gave complete successful infections on all
223 fluazinam rates tested, and were rated as resistant to this active ingredient (Table 3). Ten
224 EU_13_A2 isolates and one EU_6_A1 isolate collected in 2016 were also resistant to fluazinam
225 (36% and 5%, respectively). Three isolates, of genotypes EU_1_A1, EU_6_A1 and EU_13_A2,
226 collected in 2016 were resistant to the active ingredient propamocarb (Table 3).

227 IC_{50} values were calculated for the remaining isolates. The analysis of variance on the
228 IC_{50} values showed a significant effect of sampling/experiment year for all active ingredients.
229 There was also a significant effect of country for propamocarb sensitivity (Table 4). The most
230 influential variable was the year, which explained 8.8% to 21.3% of the total variance. The
231 genotype, country of origin, and the interactions of country × year and genotype × year had less
232 influence, explaining up to 7.1% of the variance.

233 The mean IC_{50} values for fluazinam and mandipropamid were significantly higher in
234 2016 than in 2017, while those for propamocarb and cyazofamid were significantly lower (Table

235 5). The mean IC_{50} value for fluazinam in 2016 was close to the field dose suggested by the
236 manufacturer (1000 mg/L). By contrast, IC_{50} values for all other tested products were well below
237 the suggested dose in both years. The IC_{50} mean for propamocarb was significantly higher for the
238 samples collected from France than from the United Kingdom (Table 5). However, there were no
239 significant differences between the countries for other active ingredients.

240 **3.3 Virulence profiles**

241 Altogether, 122 isolates (15 isolates for EU_1_A1; 43 for EU_6_A1; 38 for EU_13_A2 and 26
242 for EU_37_A2) were successfully tested for virulence against a range of known R genes. From
243 92% to 100% of the isolates overcame the resistance of differentials *R1*, *R3*, *R4*, *R7*, *R10* and
244 *R11*. However, none of the tested EU_6_A1 and EU_37_A2 isolates overcame *R9*, whereas 13%
245 of EU_1_A1 and 45% of EU_13_A2 isolates showed virulence on this differential. Interestingly,
246 the resistance of differential *R5* was significantly more often overcome by EU_13_A2 and
247 EU_37_A2 than by any other lineage, while *R2* was overcome only by EU_13_A2 ($p < 0.001$)
248 (Table 6). Differential *R6* was overcome significantly more often by EU_13_A2 than either
249 EU_6_A1 or EU_37_A2 ($p < 0.001$).

250 All lineages showed a diversity of virulence profiles, including the recently emerging
251 EU_37_A2 (10 profiles for 26 tested isolates) (Table S1). This level of diversity in EU_37_A2 is
252 comparable to those in the older lineages EU_1_A1 (8 profiles for 15 isolates), EU_6_A1 (16
253 profiles for 43 isolates), and EU_13_A2 (15 profiles for 38 isolates). The most prevalent
254 virulence profile for genotype EU_13_A2 (13 isolates) included virulence to all known R genes,
255 whereas the most prevalent for EU_37_A2 (9 isolates) showed virulence to eight of the R genes.
256 For genotypes EU_1_A1 and EU_6_A1, the predominant virulence profiles (5 and 15 isolates,

257 respectively) were less complex. On average, EU_1_A1, EU_6_A1 and EU_37_A2 isolates
258 overcame fewer R genes (mean = 7.1 for EU_1_A1 and EU_6_A1, and 7.6 for EU_37_A2) than
259 EU_13_A2 isolates (mean = 9.3).

260 **3.4 Characterization of aggressiveness**

261 Aggressiveness traits were measured successfully for 116 isolates. There were significant
262 differences within and between genotypes for all the variables tested (Table 7). The latent period
263 was shorter in EU_37_A2 isolates than in genotypes EU_6_A1 and EU_13_A2. Spore density
264 was lowest for EU_1_A1. The lesion growth rate of EU_37_A2 was significantly higher than
265 that of EU_13_A2, but significantly lower than that of EU_1_A1 and EU_6_A1. The fitness
266 index was similar for EU_37_A2 and EU_13_A2. However, the smallest index value was
267 calculated for EU_1_A1 and the greatest for EU_6_A1. The latent period was significantly
268 shorter and the fitness index was higher for isolates collected from France than from UK (Table
269 7).

270

271 4 Discussion

272 Several clonal lineages of *P. infestans*, for example, EU_13_A2, EU_6_A1 and EU_1_A1, have
273 proven successful in establishing and persisting in Western Europe. However, in recent years,
274 these lineages have declined and are now challenged by new emerging genotypes.

275 One of these newcomers is EU_37_A2, a genotype first observed in the Netherlands in
276 2013 (Schepers et al., 2018) and which has since rapidly expanded over most potato-growing
277 regions from the UK to Poland (Figure S1).

278 An initial characterization of this genotype showed a high proportion of isolates with a
279 much lower sensitivity than that of other genotypes to the active ingredient fluazinam (Schepers
280 et al., 2018). Because fluazinam was, until recently, one of the key fungicides used in late blight
281 protection programmes, this characteristic could be an important factor for the epidemic success
282 of EU_37_A2. However, this trait might not be sufficient to account for its rapid expansion in
283 2015–2017, as another lineage less sensitive to fluazinam, EU_33_A2, had previously been
284 detected but failed to establish itself on a wide scale (Schepers et al., 2018) and is now only
285 sporadically detected within European surveys (EuroBlight, 2022). Therefore, the present study
286 was undertaken to analyse whether EU_37_A2 might possess other phenotypic traits that
287 contribute to its higher fitness relative to historical lineages of the pathogen still prevalent within
288 west European populations.

289 The majority of the isolates which showed resistance to fluazinam belonged to the
290 genotype EU_37_A2, with more than half of the isolates not responding to the highest tested
291 dose, *i.e.* the field dose suggested by the manufacturer. However, the mean estimated fluazinam
292 IC_{50} value for the EU_37_A2 isolates collected in 2016 was markedly higher than that tested in
293 2017, which could reflect a change in response or some unexplained variation in the assay
294 between the two years. Such an experimental artefact due to changes in methodology seems
295 unlikely as the testing protocol for fluazinam sensitivity remained identical in both testing years.
296 We propose that more monitoring and fungicide sensitivity testing should be undertaken to
297 investigate the cause of this change. A similar fluctuation in fluazinam sensitivity was also seen
298 in the study of EU_41_A2 and the sexually reproducing *P. infestans* population from northern
299 Europe in the same years (Puidet et al., 2022). Such a change may reflect a reduction in the use
300 of this active ingredient in the first years of EU_37_A2 expansion, and thus reduced selection

301 pressure for the highest levels of resistance. This hypothesis would need confirmation, both by
302 an in-depth analysis of local selection pressure and fungicide use and through experimental
303 evolution experiments with plants submitted to different regimes of fluazinam sprays. The
304 resistance to fluazinam found amongst isolates of EU_13_A2 and a few isolates of EU_6_A1
305 was unexpected. There were no reports of blight management failure with fluazinam when
306 EU_13_A2 predominated and Scheper et al. (2018) indicated effective control in laboratory
307 assays and in field plots infected with the EU_13_A2 genotype. It is known that fluazinam is
308 highly effective against the zoospore phase of *P. infestans* and the fact that these assays were
309 conducted at 18°C, higher than the optimal temperature for zoospore germination, may have
310 influenced the assay sensitivity. A re-evaluation using zoospore motility assays is recommended.

311 Virulence tests with the set of 11 differential *R* genes derived from *Solanum demissum*
312 showed that EU_37_A2 isolates, like other major west European lineages, displayed a range of
313 virulence patterns. While EU_37_A2 overcame the *R5* differential more often than did EU_1_A1
314 or EU_6_A1, it is doubtful that this virulence constitutes a strong selective advantage in Western
315 Europe as *R5* is not one of the genes known to be present in major European potato cultivars.
316 Furthermore, the virulence spectrum of EU_37_A2 is relatively narrower than that of
317 EU_13_A2, notorious for its ability to overcome a wide range of race-specific resistance genes
318 (Cooke et al., 2012; Stellingwerf et al., 2018). Therefore, the ability to overcome *R5* cannot
319 alone explain the competitive advantage of EU_37_A2 over the multivirulent EU_13_A2.

320 EU_37_A2 isolates showed a significantly shorter mean latent period than isolates of the
321 genotypes EU_6_A1 and EU_13_A2, as well as a greater lesion growth rate than EU_13_A2.
322 The shorter latent period might provide EU_37_A2 with an opportunity to complete more
323 epidemic cycles within a season, leading to it spreading faster than other genotypes. In addition,

324 the faster lesion growth rate could result in more severe symptoms, and hence greater crop yield
325 or quality losses. These differences are also somewhat reflected in the cumulative fitness index,
326 heavily influenced by spore density and lesion growth rate. It is important to remember that the
327 aggressiveness measurements were taken under optimal, controlled conditions and not under the
328 fluctuating ones found in the field. Additionally, this study did not include other important traits
329 such as between-season survival of inoculum, infection efficiency, zoospore motility, zoospore
330 survival or temperature adaptation (Chepsergon et al., 2020; Fall et al., 2015; Mariette et al.,
331 2016a; Pasco et al., 2016; Savory et al., 2014). Therefore, the fitness index should be regarded as
332 an estimate of the genotype epidemic potential, rather than a direct measure of its actual
333 performance under field conditions.

334 In conclusion, genotypes EU_37_A2, EU_13_A2, EU_6_A1 and EU_1_A1 coexist in
335 large areas of potato cropping regions in Western Europe. The fast, but transient emergence of
336 EU_37_A2 shows that this genotype had an initial selective advantage over other sympatric
337 lineages. Part of this advantage was possibly due to its shorter generation time, allowing for
338 more numerous multiplication cycles during a single epidemic season, but mainly due to its
339 better performance in crops sprayed with fluazinam, which would probably disappear wherever
340 fluazinam is not used intensively (see Fungicide Resistance Action Committee (FRAC)
341 recommendations on fungicide resistance management [FRAC, 2022b]). This active ingredient
342 has been largely and rapidly abandonned in areas where EU_37_A2 was widespread, which
343 might explain why EU_37_A2 frequency tended to decrease in 2020 and 2021 (Figure S1;
344 EuroBlight, 2022). The transient emergence dynamics of EU_37_A2 in Western Europe
345 therefore illustrates the complex competitive mechanisms working in *P. infestans*
346 metapopulations. It provides a useful example of how understanding the drivers for emergence,

as well as the responses to changing section pressures, can be exploited to design sustainable control strategies over large spatial and temporal scales.

Acknowledgements

We acknowledge the BrACySol Biological Resource Centre (INRAE Ploudaniel, France) for providing the differential set and Germicopa Breeding for distributing cv. Bintje seed tubers used in this study. The study visit of BP to the French National Research Institute for Agriculture, Food and Environment was supported by the European Social Fund's Doctoral Studies and Internationalisation Programme DoRa, carried out by the Foundation Archimedes. We thank Marika Mänd, Eve Runno-Paurson, Seyed Mahyar Mirmajlessi and Liina Soonvald from the Estonian University of Life Sciences, Ragnhild Nærstad from the Syngenta Group, Albert Schirring and Daniela Portz from Bayer AG and Jadwiga Śliwka from the Plant Breeding and Acclimatization Institute – National Research Institute, Poland for useful input in the preparation of the project, planning of the experiments, or technical support. We also thank members of the EuroBlight network for useful discussions on early presentations of these data. This study was supported by the Estonian Ministry of Rural Affairs (grant 8T150054PKTK), Estonian Ministry of Education and Research (project IUT36-2) and the European Union, European Regional Development Fund (Estonian University of Life Sciences ASTRA project ”Value-chain based bio-economy“); INRAE and The French Office for Biodiversity; the Ministry of Environment and Food of Denmark (Miljø- og Fødevareministeriet project MST-667-00174); The Research Council of Norway (project number 259826); and the Scottish Government's Rural and Environment Science and Analytical Services Division (RESAS), as contributions to the project

IPMBlight 2.0 selected in response to the call of ERANET C-IPM (Coordination of Integrated Pest Management in Europe). The authors have no conflicts of interest to disclose.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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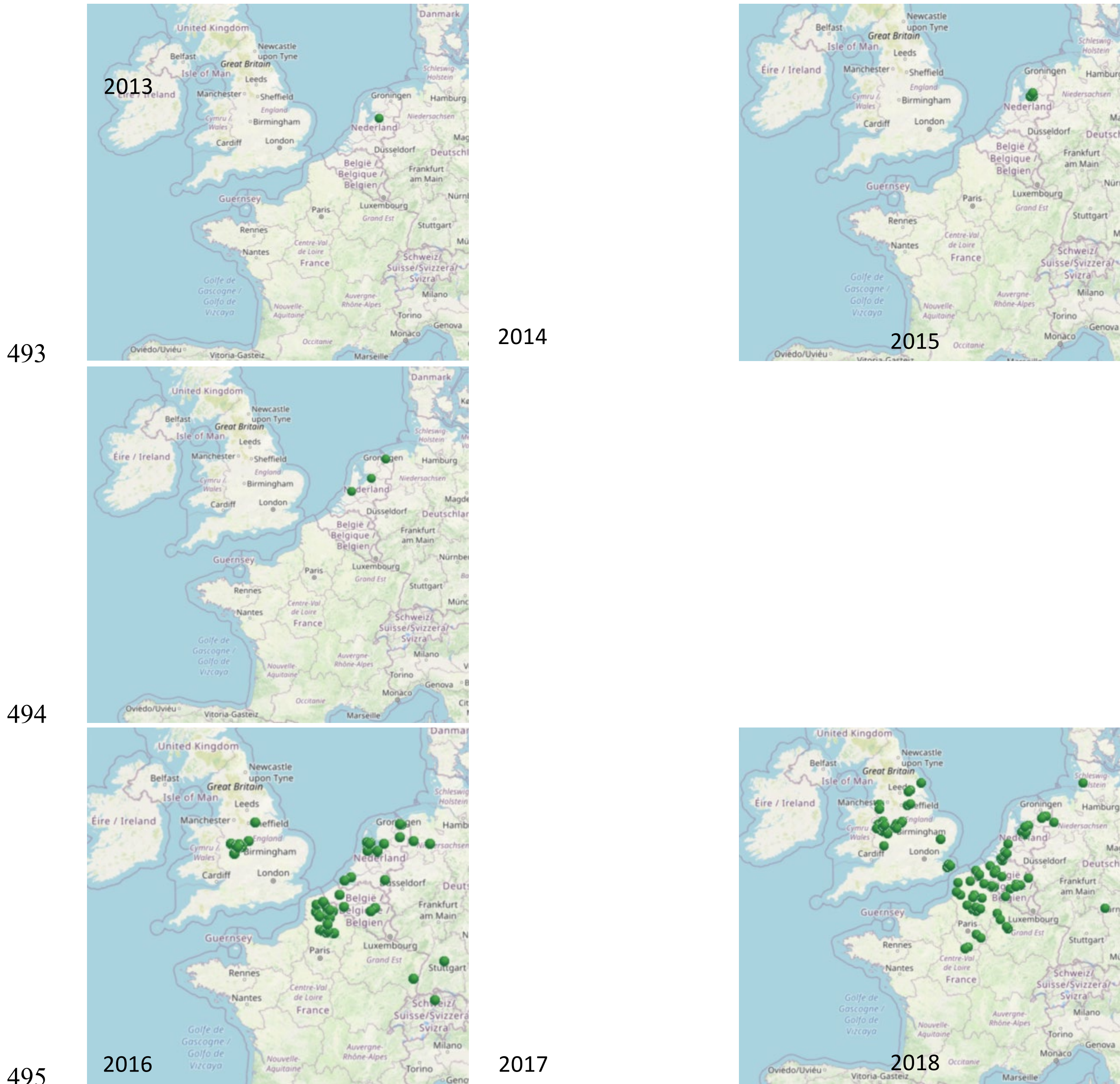
475 Supporting information legends

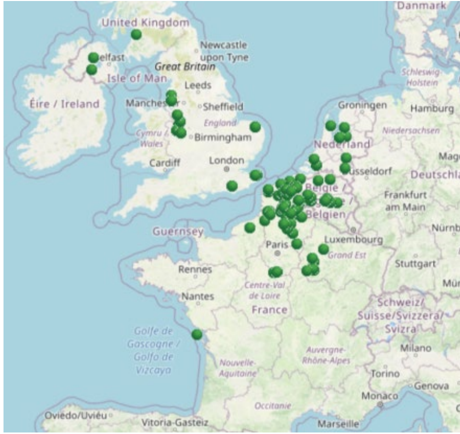
476 **Figure S1** Distribution of *Phytophthora infestans* isolates belonging to genotype EU_37_A2
477 sampled from Europe in 2013–2021. The genotype maps are retrieved from Euroblight Pathogen
478 monitoring site (https://agro.au.dk/forskning/internationale-platforme/euroblight/pathogen-
479 monitoring/genotype-map/; accessed on 2 June 2022).

480 **Figure S2** Dose–response analysis for *Phytophthora infestans* isolates sampled in 2016 and
481 2017 and tested on four commercially available active ingredients: fluazinam (Shirlan 500 SC,
482 a.i. 500 g/L; Syngenta), mandipropamid (Revus 250 SC, a.i. 250 g/L; Syngenta), cyazofamid
483 (Ranman Top, a.i. 160 g/L; Belchim Crop Protection) and propamocarb (Previcur N, a.i. 605
484 g/L; Bayer Crop Science). The IC_{50} values (mg/L) were obtained through fitted logistic models
485 based on the proportion of successful infections on each tested product dose.

486 **Table S1** Occurrence by genotype (EU_1_A1, *n* = 15; EU_6_A1, *n* = 43; EU_13_A2, *n* = 38;
487 EU_37_A2, *n* = 26) of *Phytophthora infestans* virulence profiles on Black's differential set of 11
488 potato genotypes.

FIGURE S1. Distribution of *Phytophthora infestans* isolates belonging to genotype EU_37_A2 sampled from Europe in 2013–2021. The genotype maps are retrieved from Euroblight Pathogen monitoring site (https://agro.au.dk/forskning/internationale-platforme/euroblight/pathogen-monitoring/genotype-map/) accessed on 02.06.2022).





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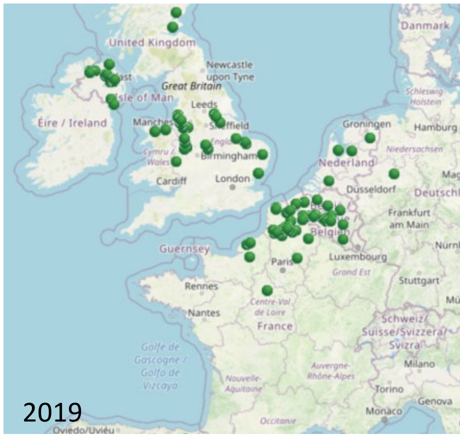

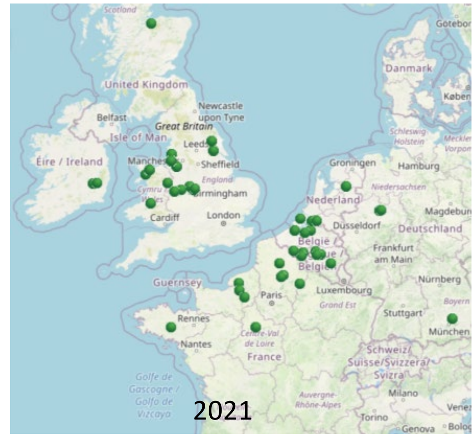

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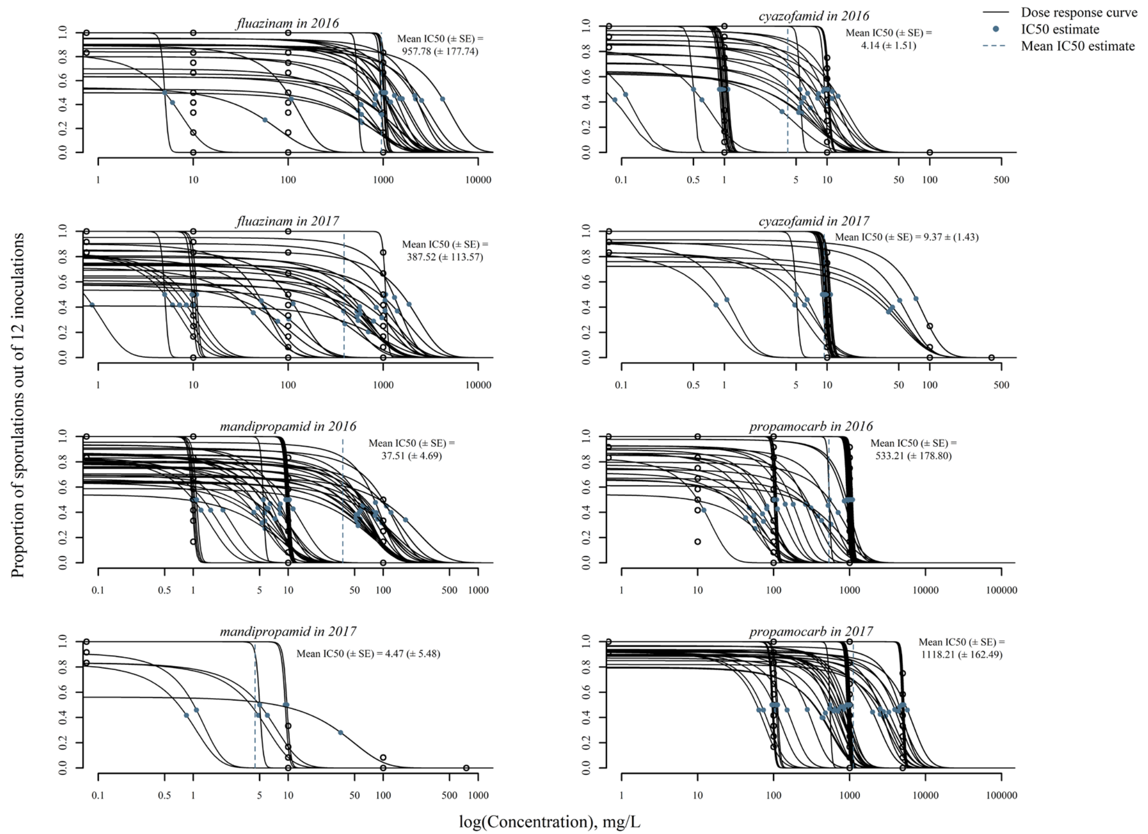
FIGURE S2. Dose response analysis for *Phytophthora infestans* isolates sampled in 2016 and 2017 and tested on four commercially available active ingredients: fluazinam (Shirlan 500 SC, a.i. 500 g/L, Syngenta), mandipropamid (Revus 250 SC, a.i. 250 g/L, Syngenta), cyazofamid (Ranman Top, a.i. 160 g/L, Belchim Crop Protection) and propamocarb (Previcur N, a.i. 605 g/L, Bayer Crop Science). The IC_{50} values (mg/L) were obtained through fitted logistic models based on the proportion of successful infections on each tested product dose.



508 **TABLE 1.** Concentration range (mg/L) used for testing fungicide sensitivity for *Phytophthora*
509 *infestans* isolates sampled in 2016 and 2017 from France and the United Kingdom on different
510 active ingredients. The field dose rate suggested by the manufacturer is the highest
511 concentration tested for each active ingredient in 2017.

Year	Active ingredient	Mobility	Concentration (mg/L)			
			I	II	III	IV
2016	Fluazinam	Contact	0	10	100	1000
	Mandipropamid	Contact/translaminar	0	1	10	100
	Cyazofamid	Contact	0	1	10	100
	Propamocarb	Systemic	0	10	100	1000
2017	Fluazinam	Contact	0	10	100	1000
	Mandipropamid	Contact/translaminar	0	10	100	750
	Cyazofamid	Contact	0	10	100	400
	Propamocarb	Systemic	0	100	1000	5000

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514 **TABLE 2.** Number of *Phytophthora infestans* isolates obtained from France and the United
515 Kingdom by sampling/experiment year and genotype.

Genotype	Country and year				Total
	France		The United Kingdom		
	2016	2017	2016	2017	
EU_1_A1	9	7	0	0	16
EU_6_A1	10	12	12	10	44
EU_13_A2	15	12	15	6	48
EU_37_A2	8	9	2	10	29
Total	42	40	29	26	137

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518 **TABLE 3.** Number of *Phytophthora infestans* isolates sampled in 2016 and 2017 from France and
519 the United Kingdom grouped by genotype and year in each sensitivity range group according to
520 the calculated IC_{50} values obtained from the fungicide sensitivity tests (fluazinam and
521 mandipropamid: n = 116; cyazofamid and propamocarb: n = 123), where a ≤ the smallest tested
522 concentration, b ≤ the highest tested concentration, c > the highest tested concentration to the
523 active ingredient (Table 1) and d = isolates, which gave complete successful infections on all the
524 tested concentrations, and were rated as resistant to the active ingredient).

Active ingredient	Genotype	Year	Number of isolates in the sensitivity range			
			a	b	c	d
Fluazinam	1_A1	2016	0	4	5	0
		2017	2	3	0	0
	6_A1	2016	2	7	12	1
		2017	6	12	2	0
	13_A2	2016	0	7	11	10
		2017	4	5	2	0
	37_A2	2016	0	1	0	9
		2017	2	4	2	3
Mandipropamid	1_A1	2016	2	6	1	0
		2017	0	5	0	0
	6_A1	2016	1	21	0	0
		2017	0	20	0	0
	13_A2	2016	3	23	2	0
		2017	0	11	0	0
	37_A2	2016	1	7	2	0
		2017	0	11	0	0
	1_A1	2016	3	5	0	0

Active ingredient	Genotype	Year	Number of isolates in the sensitivity range			
			a	b	c	d
		2017	0	7	0	0
Cyazofamid	6_A1	2016	10	11	0	0
		2017	0	20	0	0
	13_A2	2016	12	9	0	0
		2017	0	18	0	0
	37_A2	2016	3	6	0	0
		2017	0	19	0	0
Propamocarb	1_A1	2016	1	6	0	1
		2017	2	5	0	0
	6_A1	2016	5	15	0	1
		2017	2	16	2	0
	13_A2	2016	3	17	0	1
		2017	4	13	1	0
	37_A2	2016	1	8	0	0
		2017	1	18	0	0

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527 **TABLE 4.** Results of variance analysis on IC_{50} estimates of *Phytophthora infestans* isolates by ac-
528 tive ingredient (fluazinam: EU_1_A1, n = 14; EU_6_A1, n = 41; EU_13_A2 n = 29; EU_37_A2, n =
529 9; mandipropamid: EU_1_A1, n = 14; EU_6_A1, n = 42; EU_13_A2 n = 39; EU_37_A2, n = 21;
530 cyazofamid: EU_1_A1, n = 15; EU_6_A1, n = 41; EU_13_A2 n = 39; EU_37_A2, n = 28; and pro-
531 pamocarb: EU_1_A1, n = 14; EU_6_A1, n = 40; EU_13_A2, n = 38; EU_37_A2, n = 28).

Active ingredient	Effect	df[x]	F[y]	Pr(>F)[z]	Explained variance (%)
Fluazinam	Genotype	3	2.121	0.104	5.3
	Country	1	0.545	0.462	<1
	Year	1	24.728	<0.001***	21.3
	Country × year	1	3.270	0.074	1.6
	Genotype × year	3	1.844	0.146	4.4
Mandipropamid	Genotype	3	0.447	0.720	1.2
	Country	1	0.014	0.908	<1
	Year	1	25.081	<0.001***	18.8
	Country × year	1	0.070	0.792	<1
	Genotype × year	3	0.256	0.857	<1
Propamocarb	Genotype	3	0.357	0.785	1.0
	Country	1	9.574	<0.01**	7.1
	Year	1	13.286	<0.001***	9.5
	Country × year	1	2.353	0.128	1.4
	Genotype × year	3	0.201	0.896	<1
Cyazofamid	Genotype	3	0.326	0.807	<1
	Country	1	0.492	0.484	<1
	Year	1	11.246	<0.01**	8.8
	Country × year	1	0.616	0.434	<1
	Genotype × year	3	0.300	0.826	<1

532 [x] Degrees of freedom.
533 [y] F statistic.
534 [z] Calculated probability: *** Pr(>F) < 0.001; ** Pr(>F) < 0.01; * Pr(>F) < 0.05.
535

536 **TABLE 5.** Comparison of IC_{50} estimates (mg/L) of *Phytophthora infestans* isolates between A)
537 years and B) countries. Values are listed as least-square mean ± standard error. Different letters
538 within the same line indicate statistical difference between the groups within the tested active
539 ingredient using linear-mixed effects models following Tukey post hoc tests at $\alpha = 0.05$.

540 A) Mean IC_{50} estimates compared by years (fluazinam: 2016, n = 49; 2017, n = 44;
541 mandipropamid: 2016, n = 69; 2017, n = 47; propamocarb: 2016, n = 56; 2017, n = 64; and
542 cyazofamid: 2016, n = 59; 2017, n = 64).

Active ingredient	**Year**	
	2016	**2017**
Fluazinam	957.78 (±177.74) a	387.52 (±113.57) b
Mandipropamid	37.51 (±4.69) a	4.47 (±5.48) b
Propamocarb	533.21 (±178.80) a	1118.29 (±162.49) b
Cyazofamid	4.14 (±1.51) a	9.37 (±1.43) b

543 B) Mean IC_{50} estimates compared by countries (fluazinam: France, n = 55; the United
544 Kingdom, n = 38; mandipropamid: France, n = 67; the United Kingdom, n = 49;
545 propamocarb: France, n = 75; the United Kingdom, n = 45; and cyazofamid: France, n =
546 76; the United Kingdom, n = 47).

Active ingredient	**Country**	
	France	**The United Kingdom**
Fluazinam	620.27 (±122.33) a	725.03 (±135.10) a
Mandipropamid	21.21 (±4.23) a	20.77 (±5.59) a
Propamocarb	1155.72 (±131.28) a	495.78 (±191.87) b
Cyazofamid	7.38 (±1.14) a	6.12 (±1.65) a

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550 **Table 6** Percentage of isolates of *Phytophthora infestans* genotypes (EU_1_A1: *n* = 15;
551 EU_6_A1: *n* = 43; EU_13_A2: *n* = 38; EU_37_A2: *n* = 26) that overcame resistance of potato
552 differentials (*R1*–*R11*)

	Genotype						
Differential	**EU_1_A1**	**EU_6_A1**	**EU_13_A2**	**EU_37_A2**	***df***	***F***	***p***
R1	100.0 a	93.0 a	94.7 a	92.3 a	3	0.54	0.656
R2	20.0 a	11.6 a	81.6 b	11.5 a	3	29.76	<0.001***
R3	93.3 a	90.7 a	94.7 a	92.3 a	3	0.18	0.908
R4[a]	100.0 a	100.0 a	100.0 a	100.0 a	–	–	–
R5	40.0 a	16.3 a	76.3 b	96.2 b	3	30.98	<0.001***
R6	46.7 ab	27.9 a	84.2 b	23.1 a	3	12.65	<0.001***
R7	100.0 a	100.0 a	97.4 a	100.0 a	3	1.02	0.388
R8	6.7 a	72.1 b	68.4 b	57.7 b	3	9.05	<0.001***
R9	13.3 a	0.0 a	44.7 b	0.0 a	3	15.30	<0.001***
R10	93.3 a	97.7 a	94.7 a	100.0 a	3	0.63	0.599
R11	100.0 a	97.7 a	92.1 a	100.0 a	3	2.11	0.103

553 [a]Data not variable.
554 Results were analysed as binary data (distribution: binomial) using GLM (Type III SS) followed
555 by Tukey's unequal N HSD post hoc test ($\alpha = 0.05$). Different letters indicate statistically
556 significant differences. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.
557

TABLE 7. Comparison of genotypes (A) and countries (B) for each aggressiveness trait through post-hoc analyses of ANOVA models and Tukey tests at α = 0.05. Means with different letters in one row are statistically significantly different.

A) Average (± standard error) results for different aggressiveness traits by genotype (EU_1_A1, n = 6; EU_6_A1, n = 43; EU_13_A2, n = 41; EU_37_A2, n = 26).

Variable	Genotype		
	EU_1_A1	**EU_6_A1**	**EU_13_A2**
Latent period (days)	3.24 (± 0.07) ab	3.44 (± 0.03) c	3.39 (± 0.03) bc
Spore density (sporangium/mm^2)	78.54 (± 10.02) a	119.90 (± 3.64) b	120.17 (± 3.74) b
Lesion growth rate (mm^2/day)	577.00 (± 36.19) a	544.66 (± 13.13) a	360.42 (± 13.51) b
Fitness index	47584.98 (± 5336.95) a	74830.21 (± 1936.40) b	62612.73 (± 1992.79

564 B) Average (± standard error) results for different aggressiveness traits by country (France,
565 n = 69; the United Kingdom, n = 47).

Variable	**Country**	
	France	**The United Kingdom**
Latent period (days)	3.29 (± 0.02) a	3.36 (±0.03) b
Spore density (sporangium/mm^2)	110.16 (± 3.31) a	105.61 (± 4.35) a
Lesion growth rate (mm^2/day)	478.77 (± 11.94) a	477.53 (± 15.71) a
Fitness index	65262.27 (± 1760.97) a	60386.60 (± 2316.61) b

566