Variation in aggressiveness plasticity between northern and southern European populations of *Hymenoscyphus fraxineus*, an invasive fungal pathogen of ash

Authors

C. Bécans[1], C. Robin[1], A. Martelli[2], C. Lepoittevin[1], A. Aubert[1], J.P. Soularue[1]*

[1] Univ. Bordeaux, INRAe, UMR1202 BioGeCo, 69 Route d'Arcachon, 33612 Cestas Cedex, France

[2] UCA - Université Clermont Auvergne (49, bd François-Mitterrand / CS 60032 / 63001 Clermont-Ferrand Cedex 1 – France)

* corresponding author: jean-paul.soularue@inrae.fr

Abstract

Hymenoscyphus fraxineus is an invasive fungal pathogen responsible for the ash dieback epidemic, which continues to cause severe mortality of common ash (*Fraxinus excelsior* L.) across Europe. Following its likely introduction in northeastern Europe, the pathogen rapidly colonized most regions where common ash is present. As it spread southward, *H. fraxineus* encountered warmer climates and a greater occurrence of *Fraxinus ornus* L., a species largely resistant to the disease. Despite this environmental heterogeneity, which probably imposed adaptive challenges to *H. fraxineus* at the epidemic front, ash dieback continues to expand in southern Europe. Aggressiveness is a key life-history trait that is expected to evolve during epidemics and to exhibit plasticity in response to environmental variation. We investigated whether the plasticity of aggressiveness in response to temperature and ash species has evolved in *H. fraxineus* during its propagation towards southern Europe. Using a synchronic approach based on leaf inoculations, we characterized individual reaction norms for aggressiveness in a long-established Lithuanian population and a recently established Italian population of *H. fraxineus*. The Italian *H. fraxineus* population is exposed to warmer summers than the Lithuanian population, while *F. ornus* is present in Italy but absent in

Lithuania. We observed no difference in the aggressiveness expressed on *F. excelsior* under moderate temperature between the two *H. fraxineus* populations. However, the ability of Italian isolates to cause severe leaf symptoms was less negatively affected by increasing temperature and host species change than that of Lithuanian isolates, suggesting local adaptation of *H. fraxineus* during its spread toward southern Europe. Our findings highlight the importance of considering the evolution of adaptive traits and their plasticity in fungal pathogens when anticipating disease risk. They also suggest that ash trees in southern Europe may be slightly more vulnerable to ash dieback than previously anticipated.

Keywords : ash dieback, *Hymenoscyphus fraxineus*, invasive fungal pathogen, plasticity evolution, *Fraxinus excelsior*, *Fraxinus ornus*, thermal adaptation, host adaptation, virulence evolution, aggressiveness evolution

Introduction

The emergence of forest diseases caused by the accidental introduction of non-native pathogens has intensified markedly in recent decades (e.g., Desprez-Loustau et al., 2010; Roy et al., 2014; Santini et al., 2013). These diseases inflict severe biodiversity losses on forest ecosystems and threaten the wide array of ecosystem services provided by natural and exploited forests (Freer-Smith & Webber, 2017; Guégan et al., 2020; Keesing et al., 2010). Effective disease management strategies rely on accurate assessments of disease risk, which in turn require clear insights into the key drivers of forest epidemics. However, our understanding of these drivers remains limited, notably because forest disease management has historically overlooked the evolutionary dynamics shaping interactions between pathogens and their biotic and abiotic environments (Desprez-Loustau et al., 2016).

Phenotypic plasticity refers to environmentally induced variations in phenotype (Sommer, 2020), typically represented using reaction norms (Ghalambor et al., 2007). In variable environments, plastic trait expression can give rise to polyphenism, a form of phenotypic diversity that may combine with genetic polymorphism (Mayr, 1963; West-Eberhard, 2003),

and may sustain population adaptation to novel environmental conditions (Gomulkiewicz & Stinchcombe, 2022; Hoffmann & Sgrò, 2011; Lande, 2009; Merilä & Hendry, 2014; Vinton et al., 2022). The role of plasticity is especially critical in organisms such as some forest pathogens which have limited capacity to track favorable environments during the lifetime of an established genotype (Chevin et al., 2013; de la Mata & Zas, 2023; Forsman, 2015). Importantly, when phenotypic plasticity is heritable, the shape and diversity of reaction norms may evolve according to various patterns. Theoretical models predict the evolution of adaptive reaction norms conferring high fitness to genotypes across environmental variation provided sufficient initial genetic variation associated with reaction norm shapes (Via & Lande, 1985) and substantial gene flow (Scheiner et al., 2012) exist. In the case of linear reaction norms, such evolution is expected to result in steep reaction norms under divergent selection (*i.e.*, when optimal phenotypes differ between environments) but flat reaction norms under uniform stabilizing selection across environments (*i.e.*, a single optimal phenotype across environments). This latter outcome, also called phenotypic buffering, is likely to arise for traits tightly linked to fitness, such as mycelial growth or the ability to infect hosts, and is considered a special case of plasticity (Bradshaw, 1965; Eriksson et al., 2023; Reusch, 2014). Alternatively, evolved reaction norms may be maladaptive when the environmental variation is highly stochastic (Ghalambor et al., 2007; Leonard & Lancaster, 2020), because of genetic drift following founder events (Price et al., 2003), when genetic variation associated with adaptive plasticity is lacking (DeWitt et al., 1998), or in the presence of plasticity costs (Murren et al., 2015; Scheiner, 2013). Understanding the ecological success of introduced forest pathogens, and anticipating their future epidemiological dynamics, requires insights into the evolution of plasticity in their life history traits. Yet, empirical knowledge in this area remains limited (but see Garbelotto et al. 2015), which is problemactic for forest disease management given the accelerating effects of global changes on the establishment and range expansion of invasive pathogens (George et al., 2025). A critical first step is therefore to empirically assess the diversity of individual reaction norms for adaptive traits in forest tree pathogens across plausible environmental variation.

Aggressiveness refers to the extent of damage a pathogen causes to its host (Pariaud et al., 2009). This quantitative trait is typically assessed at the tree level through measurements such as lesion size (e.g., Kosawang et al., 2020; Lygis et al., 2017) or symptom severity (e.g., Orton et al., 2019). Aggressiveness, also sometimes called virulence (Shaner et al., 1992), is a key life-history trait in plant pathogens as it influences their capacity to reproduce, spread, and persist within host populations (May & Anderson, 1983). It is expected to evolve during the course of epidemics along distinct trajectories shaped by the pathogen life history, host availability, the spatio-temporal distribution of host resistances, and the time since the pathogen's establishment (Cressler et al., 2016; Dutt et al., 2022; Tollenaere et al., 2016). Several models predict that the competition among genotypes for host resource is likely to increase the aggressiveness level in a pathogen population, especially when a single host can be infected by multiple genotypes of the pathogen species, or by multiple pathogen species (Bashey, 2015; Tack et al., 2012). Nonetheless, the deleterious effects of exacerbated pathogenicity on pathogen transmission may drive the evolution of aggressiveness toward an intermediate level that enables pathogen genotypes to establish and develop in the presence of other genotypes (of the same or different species), while also maximizing transmission to healthy hosts (the so-called virulence–transmission trade-off; Alizon et al., 2009; Mideo et al., 2008). Under the assumption of uniform resistance within the host population, this trade-off suggests that aggressiveness is more likely shaped by stabilizing selection toward an overall intermediate optimal value, rather than by divergent selection among geographical sites or by directional selection favoring ever-increasing aggressiveness. In addition, aggressiveness is expected to vary plastically with both temperature conditions and host resistance (e.g. Laine 2008, Castiblanco et al. 2020, Fidanoğlu et al. 2023, see also the reviews by Pariaud et al. 2009 and Cressler et al. 2016). Although it strongly determines epidemic severity, the evolution of the relationship between environmental conditions and the expression of aggressiveness expression remains largely unstudied in forest pathogens.

Ash dieback is an emblematic example of a forest emerging disease. It is caused by *Hymenoscyphus fraxineus*, an ascomycetous fungal pathogen native to Asia, which infects ash tree leaves during summer through wind-dispersed ascospores. These infections cause leaf wilting and necrotic lesions that can extend to the petioles and rachises, leading to premature leaf abscission (Lione et al., 2024). Lesions can also spread into and girdle the twigs and stems, damaging the cambium and inducing cankers on the bark (Gross, Holdenrieder, et al., 2014). The combined effects of leaf abscission and branch desiccation gradually lead to crown thinning and dieback, ultimately resulting in overall tree decline (Lione et al., 2024). In some regions, ash mortality rates have reached up to 85% (Coker et al., 2019). The severity of ash dieback tends to be highest under moderate summer and autumn temperatures, rather than during particularly warm periods (Grosdidier, Ioos, & Marçais, 2018a; Marçais et al., 2023). Three ash species are predominantly found in Europe: the common ash (*Fraxinus excelsior* L.), the most widespread, and the narrow-leaved ash (*Fraxinus angustifolia* Vahl) and the manna ash (*Fraxinus ornus* L.), both largely restricted to southern regions. The disease particularly affects *F. excelsior* and *F. angustifolia*, whereas *F. ornus* is less susceptible (Adamčíková et al., 2018; Gross & Sieber, 2016; Schwanda & Kirisits, 2016).

The European epidemic of ash dieback likely results from a single introduction event, followed by the propagation of descendants of two genetically divergent strains of *H. fraxineus* (Gross, Hosoya, et al., 2014; Mcmullan et al., 2018). The disease was first reported in Poland in 1992 (Przybył, 2002; Timmermann et al., 2011) and progressively reached most of the European ash distribution range (McKinney et al., 2014). Nowadays, the ash dieback epidemic has reached an endemic state in central Europe (Lygis et al., 2017; Marçais et al., 2022), but it continues to expand at the periphery of the continent, notably in southern Europe, like in Spain (Stroheker et al., 2021) and Italy (Migliorini et al., 2022). This successful invasion may appear paradoxical (Estoup et al., 2016), given the severe initial bottleneck experienced by the fungus (Mcmullan et al., 2018) and the

decreasing biotic (host species) and abiotic (temperature) environmental favourability along the latitudinal gradient it colonized. Indeed, as the fungus spread southward, both temperature (Fick & Hijmans, 2017) and the occurrence of resistant *F. ornus* increased, potentially exposing *H. fraxineus* to adaptive challenges. This context makes it plausible the plasticity of *H. fraxineus*' life-history traits and its evolution contributed to the development of the ash dieback epidemic.

To our knowledge, the evolution of aggressiveness following *H. fraxineus* introduction into Europe has been addressed only once, by Lygis et al. (2017), who reported no difference in aggressiveness between Lithuanian and Swiss populations, the latter located at the epidemic front at the time. However, this study did not consider the plasticity of aggressiveness and was conducted during an earlier stage of the epidemic's development. In *H. fraxineus*, plastic variation in aggressiveness has so far been investigated only in relation to host species, but the evolution of plasticity in aggressiveness during the range expansion of *H. fraxineus* has not yet been investigated. Besides, how aggressiveness in *H. fraxineus* varies plastically with temperature remains unstudied, although temperature is a key determinant of ectothermic fungal distributions (Gerken et al., 2015; McLean et al., 2005). In *H. fraxineus*, prior research has already documented thermal plasticity of *in vitro* mycelial growth (Hauptman et al., 2013; Kowalski & Bartnik, 2012) and viability (Hauptman et al., 2013), with optimal *in vitro* growth typically observed between 22 °C and 24 °C. More recently, Bécans et al. (2025) investigated the evolution of thermal plasticity of the same traits in five European *H. fraxineus* populations sampled along a latitudinal gradient. They found that the northernmost population (Lithuania) showed reduced viability at high temperatures, whereas the southernmost population (northern Italy), located near the epidemic front, displayed significantly greater growth than other populations under elevated temperatures. As mycelial growth and survival may be related to aggressiveness expression (Blenis et al., 1984; Kowalski & Bartnik, 2012 but see Kosawang et al., 2020), these findings

suggest that the thermal plasticity of aggressiveness may be evolving during the ongoing range expansion of *H. fraxineus*, and ultimately promoting further disease spread.

In this study, we aimed to characterize the plasticity of aggressiveness at the individual level in two distinct populations of *H. fraxineus*: one from Lithuania, where the pathogen is long-established and located near the historical center of ash dieback emergence in Europe, and another from northern Italy, representing a recently established peripheral population near the southern edge of the current epidemic front. Assuming that the southward expansion of ash dieback from its north-eastern centre of origin was continuous (Mcmullan et al., 2018), the Lithuanian population is composed of genotypes recurrently exposed to the seasonal temperature regimes characteristic of northern Europe and without prior contact with *F. ornus* (Caudullo & De Rigo, 2016). In contrast, the ancestors of the Italian genotypes colonised a latitudinal gradient and were subjected both to summer heatwaves characteristic of southern Europe (Fick & Hijmans, 2017) and to a host community comprising *F. excelsior* and *F. ornus* (Caudullo & De Rigo, 2016).

We characterized aggressiveness reaction norms through replicated plant inoculation experiments and specifically tested the following hypotheses:

1. There is no significant differences in baseline aggressiveness between Lithuanian and Italian isolates on *F. excelsior* under moderate temperature conditions.

2. The aggressiveness of *H. fraxineus* overall decreases under warm temperature conditions or on *F. ornus*, compared to moderate temperatures and *F. excelsior*.

3. The aggressiveness of Italian isolates is less decreased by temperature increase and host species change than that of Lithuanian isolates, indicating a higher level of phenotypic buffering. These differences in plasticity likely result in contrasting trait expression under non-optimal environmental conditions between the two populations.

Materials and methods

190 *H. fraxineus populations*

Black infected rachises of *F. excelsior* were sampled between July 8 and the end of July 2020 at two sites: one in Lithuania and the other in Italy. These sites differ in their distance from Estonia, where the first evidence of *H. fraxineus* presence in Europe was found (Agan et al., 2023). These sites also differ in their altitudinal and climatic characteristics: mean
195 annual temperature and mean summer temperature were higher at the Italian site (844 m elevation) than at the Lithuanian one (62 m), while summer temperature variability was greatest in Lithuania (Table 1). Moreover, *F. ornus* coexists with the highly susceptible *F. excelsior* in northern Italy, but is absent from Lithuania (Caudullo & De Rigo, 2016).

200 Upon collection, the rachises were stored at 4 °C, and isolations were performed between September 17 and 19, 2020, following the protocol of Kowalski & Bartnik (2012), with two modifications: ash leaf malt extract agar (AMEA, Kirisits et al., 2013) was used as the growth medium, and rachis surfaces were sterilized using 96% ethanol. The isolation yielded a batch of 15 isolates from each site. The two *H. fraxineus* populations analyzed in this study
205 comprised the same isolates as those examined by Becans et al. (2025). Sample size justification followed a precision-based approach (Lakens, 2022). We aimed to estimate population-level effects using random-intercept and random-slope mixed models repeatedly fitted to bootstrapped datasets, which allowed us to obtain confidence intervals reflecting estimation precision and formed the basis of our inferences (see details below). Fungal
210 isolates displaying the morphological characteristics of *H. fraxineus* were maintained on AMEA. Based on whole-genome sequencing (data not shown), we confirmed that all selected isolates belonged to *H. fraxineus* species (using the NCBI database and BLAST (https://blast.ncbi.nlm.nih.gov), and that they were genetically distinct from one another. *H. fraxineus* isolates were stored at 4°C in the dark in a cold room and then incubated on a new
215 AMEA plate three weeks before inoculation experimentations for inoculum production, at

22°C under controlled day/night cycles (16 hours light/8 hours dark). In what follows, the terms ‘isolate’ and ‘individual’ are used interchangeably.

Plant material

220 Three hundred ash seedlings of *F. excelsior* and 100 ash seedlings of *F. ornus* from the Plantfor nursery (Uchacq et Parentis, 40090, France), showing no visible symptoms of ash dieback, were potted in October 2022 in 4L containers and maintained outside until inoculation. On average, *F. excelsior* seedlings were 33.60 cm tall, while *F. ornus* seedlings measured 53.08 cm at the time of inoculation. Stem diameters, measured 10 cm above the
225 soil surface, ranged from 3 to 6 mm for *F. excelsior* and from 5 to 8 mm for *F. ornus*. These two ash species have been selected based on their distinct susceptibility to the disease; hereafter *F. ornus* seedlings were considered resistant, while *F. excelsior* were considered susceptible (Schwanda & Kirisits 2016, Nielsen *et al.* 2017).

230 *Aggressiveness assessment of* H. fraxineus *isolates through inoculation of ash trees*

There is considerable variation in susceptibility to ash dieback among common ash trees, which has been associated with differences in iridoid glycoside content (Sollars et al., 2017). Because the 30 isolates could not be inoculated on the same ash genotype, variation in host
235 susceptibility to *Hymenoscyphus fraxineus* may have biased our assessment of population-level differences in pathogen aggressiveness and its plasticity. Previous work has shown that analyses of phenolic bark extracts using Fourier-transform infrared spectrometry, combined with modeling of the resulting spectral data, can successfully discriminate among ash trees according to their susceptibility to *H. fraxineus* (Villari et al., 2018). To limit
240 intraspecific variation in resistance among the *F. excelsior* (susceptible) and *F. ornus*

(resistant) seedlings used in this study, we implemented the following strategy. We first analyzed the near-infrared spectra of the seedlings using an approach adapted from Villari et al. (2018), relying on leaf-level near-infrared spectrometry and principal component analysis of the spectral data. Two distinct groups corresponding to the two ash species were identified, although three *F. ornus* individuals grouped within the much larger *F. excelsior* cluster (Figure S1). These individuals, together with those located at the margins of the cluster, were excluded from the inoculation experiments. The retained susceptible group comprised 46 *F. excelsior* seedlings, while the resistant group included 23 *F. ornus* seedlings. Second, to further limit the effect or residual variations in host resistance on the expression of aggressiveness, three distinct trees were inoculated with each isolate under each environmental condition. Five inoculations were done on each plant: two distinct Italian isolates, two distinct Lithuanian isolates and one control inoculation with a mycelium-free AMEA plug. Each tree was inoculated with a unique combination of isolates. Foliar inoculations were carried out from July 19 to 21, 2023, following the protocol by Orton et al. (2019), except that we used standardized AMEA plugs colonized by *H. fraxineus* mycelium instead of mycelium agar mix. A 5 mm AMEA plug of three-week-old mycelium was placed on the epidermal tissues of a rachis between the second and third pair of leaflets, at a site that had been preliminary wounded with a standardized 5 mm incision. The inoculum was maintained in place and prevented from drying by wrapping the inoculation site with parafilm, which was removed seven days after inoculation.

Following the approach of Bécans et al. (2025), we characterized the thermal reaction norm of aggressiveness for all isolates on *F. excelsior* seedlings by exposing inoculated plants to two temperature conditions: 22°C (reference) and 26°C (treatment). The plants were maintained in separate climate chambers set at 22°C or 26°C, with controlled day/night cycles (16 h light / 8 h dark) and a constant relative humidity of 70%. In addition, we characterized the reaction norm of aggressiveness as a function of host species for all isolates, using *F. excelsior* as the reference host and *F. ornus* as the treatment host. The inoculated *F. ornus* seedlings were incubated in the same climate chambers at 22°C.

Overall, a total of 345 foliar inoculations were performed on 69 ash trees. Following
270 inoculation, isolates were anonymized to prevent bias in the monitoring of the experiments. We specifically assessed the aggressiveness using three proxies: (*i*) lesion length, a continuous variable representing the extent of the necrosis (in cm), (*ii*) symptom occurrence, a binary variable indicating whether any symptoms were visible on the inoculated leaf (0: no symptoms, 1: symptoms present),
275 (*iii*) symptom severity, a binary variable recorded only for symptomatic leaves, indicating whether the symptoms became severe (0: no severe wilting, 1: severe wilting or leaf shedding). We monitored lesion length and leaf symptoms weekly, starting two weeks after inoculation and continuing until the ninth week. We stopped measuring lesions at week seven because they could no longer be reliably distinguished after leaf fall.

280

Statistical analysis

The variations of lesion length, symptom occurrence and symptom severity as a function of temperature or host species were analyzed using generalized and linear mixed models in R software (v4.3.3, R Core Team 2024). The Tidyverse package (v2.0.0, Wickham et al., 2019)
285 was used for data handling, the lme4 package (v1.1-35.4, Bates et al., 2015) for model fitting and the stats package (v4.4.1, R Core Team 2024) for model prediction. The conditional R^2 (MuMIn R package, v1.48.4, Bartón & MK, 2023) and visual comparisons of model predictions against the data were used to assess the goodness of fit of our models.

For each monitoring day, we estimated the linear reaction norms of each isolate for lesion
290 length, symptom occurrence, and symptom severity. Each reaction norm is characterized by an intercept and slope (Hendry, 2016). The intercept represents the trait value expressed in the reference environmental conditions, *i.e.*, on *F. excelsior* at 22°C. The slope reflects the extent of plasticity, defined as the change in trait value in response to a treatment condition,

i.e., temperature increase (26°C) or species change (*F. ornus*). As advocated by Bolker et al. (2009) and Arnold et al. (2019), the best-fitting model specified the environmental condition (*i.e.*, 'temperature' or 'host species') as a fixed effect, while the interactions between 'population' and the environment, and the 'isolate' and the environment were specified as random. The 'isolate' effect was nested within 'population' to account for the hierarchical structure of the random effects. Note that adding "tree" as a fixed effect did not improve model fit according to likelihood ratio test, indicating no significant tree-level influence on isolate aggressiveness in our experiment. The model retained can be written as following:

$$Y_{ijk} = \beta_0 + b_{0j} + \gamma_{0ij} + \left(\beta_1 + b_{1j} + \gamma_{1ij}\right) e_{ijk} + \epsilon_{ijk} \quad (1)$$

In this model, Y_{ijk} corresponds to either the lesion length (in cm), symptom occurrence (probability) or symptom severity (probability) in replicate k, for isolate i within population j. When modeling symptom occurrence or symptom severity, the response variable followed a Bernoulli distribution, and $\log(\pi_{ijk} / 1 - \pi_{ijk})$ replaced Y_{ijk} in equation 1, with π_{ijk} representing the probability that replicate k of isolate i from population j induces a symptom or a severe symptom, depending on the aggressiveness proxy investigated. The parameter β_0 denotes the fixed intercept, *i.e.*, the expected value of the response variable when the environmental covariate e_{ijk} (*e.g.*, temperature or host species) is set to its reference level. The fixed slope β_1 captures the average change in the response per unit change in the environmental variable (*e.g.*, per °C or when host species changes). The terms b_{0j} and b_{1j} represent the random intercept and slope for population j, corresponding to deviations from the overall intercept and slope, respectively. Similarly, γ_{0ij} and γ_{1ij} are the isolate-level random effects (intercept and slope), nested within populations, capturing isolate-specific deviations from the corresponding population-level trends.

To test Hypothesis 1, we evaluated whether populations differed in the three aggressiveness proxies on *F. excelsior* under reference conditions (*F. excelsior*, 22°C), which represent the intercept of the reaction norm for each trait. For this purpose, we repeatedly fitted model 1 to bootstrapped datasets (boot package (v1.3-30; Davison & Hinkley, 1997). For each isolate, day, and trait, we averaged estimated trait value under reference conditions over 1,000 bootstrap iterations, then averaged these isolate-level estimates within each population and computed 95% confidence interval of the resulting means. We estimated the confidence intervals using a *t*-distribution, which has heavier tails than the Gaussian distribution and therefore reduces the risk of underestimating standard deviations in small datasets. We applied a stringent criterion across all tests: differences among populations were considered statistically significant when the confidence intervals of the estimates did not cross, that is, when their ranges were non-overlapping or just contiguous. We used a similar approach to test Hypothesis 2, which was considered supported when either (*i*) the mean slope of the estimated reaction norms was significantly negative, or (*ii*) the estimated difference in trait expression between the reference and treatment environments was significant. Hypothesis 3 addressed between-population differences in the isolates reaction norms. It was tested from the same bootstrap procedure and the same rationale as Hypothesis 1. Tables 2, S1, S2 and S3 summarize the results of our estimations and details on model performance at each monitoring day.

Results

The Lithuanian and Italian H. fraxineus *populations express similar aggressiveness in the reference environment (hypothesis 1)*

Eleven Italian and 13 Lithuanian *H. fraxineus* isolates populations induced lesions and symptoms following *F. excelsior* inoculation at 22 °C (Figure S2). No lesions or symptoms developed after control inoculations. None of the rachis inoculations we performed extended in lesions on the trunks of the inoculated ash seedlings during the experiment.

The lesion length, symptom occurrence, and symptom severity estimated under reference conditions were highly variable depending on the isolate used for the inoculation (Figure 1 thin points at 22°C in panels a, c, and e, Figures S3 and S4 panels a, d, g). This variability remained similar between the two *H. fraxineus* populations throughout the monitoring period (see the overlapping confidence intervals in panels a, d, g of Figures S3 and S4). Lesion length showed moderate to strong positive correlations with both symptom occurrence and symptom severity (Table S4).

A non significant increase in the estimated mean lesion length was observed during the first six weeks of the experiment in both populations, as indicated by overlapping confidence intervals across days in each population (Figure 2a). On day 42 after inoculation, the mean estimated lesion length reached 3.43 cm (confidence interval (CI): 1.86; 5.02)) in the Lithuanian population, a value close to the 3.81 cm (CI: 1.99, 6.63) estimated for the Italian population. After day 42 the lesion lengths decreased sharply, as a consequence of leaf abscission occurring at this stage of the experiment (see the decrease in lesion measurements between days 42 and 49 in Figure 2a), which removed the largest lesions from the dataset.

Under reference conditions, there was no difference in the ability of the two *H. fraxineus* populations to cause symptoms, which overall remained largely stable throughout the monitoring period (Figure 2d). At 63 days post-inoculation, the estimated probability of symptom occurrence averaged 0.71 (CI: 0.48; 0.93) in rachises inoculated with Italian isolates and 0.82 (CI: 0.64; 1.00) in rachises inoculated with Lithuanian isolates. The

probability of symptoms becoming severe increased over time following inoculation with isolates from both *H. fraxineus* populations, reaching 0.92 (CI: 0.76; 1.08) and 0.69 (CI: 0.49; 0.88) in rachises inoculated with the Italian and Lithuanian isolates, respectively, at 63 days post-inoculation (Figure 2g).

370

Temperature increase and F. ornus *reduce aggressiveness in Lithuanian and Italian* H. fraxineus *populations (hypothesis 2)*

Symptom occurrence in inoculated rachises was consistent across the two temperature
375 levels considered throughout the monitoring period. Hence, the mean slopes of the estimated thermal reaction norms for symptom induction remained consistently non-significant in both *H. fraxineus* populations (Figure 2f). Likewise, the probability of symptom induction at 22 and at 26 °C remained similar (Figure 2 panel d *vs* e).

In contrast, the lesion length and probability of severe symptom were significantly decreased
380 by temperature increase on several observation days (Figure 2c, i). From day 14 to day 42, the estimated slope of the thermal reaction norms for lesion length ranged from from –0.43 cm/°C to –1.48 cm/°C (Figure 2c). The probability of severe symptom was also mostly negatively impacted by temperature increase in the Lithuanian population, reaching a maximum decrease of –0.32/°C on day 21, whereas it was significantly negative only on day
385 28 in the Italian population (–0.27/°C) (Figure 2i). These significant negative levels of thermal plasticity resulted in a lower probability of severe symptom induction at 26°C than at 22°C on days 21 and 28, in Lithuanian isolates only. In all other cases, no significant differences in trait expression was detected between the reference environment (22°C) and the treatment environments (26°C) (compare panels a *vs* b, d *vs* e, and g *vs* h in Figure 2).

390 The change in ash species had few effects on the ability of the *H. fraxineus* isolates to induce symptoms. The estimated reaction norms mostly showed non significant slopes, except in Lithuanian isolates (-0.15) on days 49, 56, and 63 (Figure 3f). The probability of symptom occurrence was also similar on the two ash species, regardless of the *H. fraxineus* population used for inoculation (compare panels d *vs* e in Figure 3). By contrast, we
395 observed a pronounced negative effect of host shift on the ability of both *H. fraxineus* populations to cause lesions and severe symptoms. The estimated reaction norms showed predominantly negative slope (Figure 3c, l) ranging from –2.28 to –0.67 cm/°C for lesion length and from –0.37 to –0.05/°C for the probability of severe symptoms induction. In line with these negative plastic responses, both *H. fraxineus* populations generated shorter
400 lesions and showed a reduced ability of severe symptom induction on *F. ornus* than on *F. excelsior* (compare panels a *vs* b and g vs h in Figure 3).

The response of H. fraxineus *populations' aggressiveness to increasing temperature differed (hypothesis 3)*

405 The thermal plasticity of lesion length and probability of symptom induction did not differ between the *H. fraxineus* populations throughout the experiment (Figure 2c). For instance, at 21 days post-inoculation, the estimated reaction norms slopes were -1.01 cm/°C (CI: -1.65; -0.38) for the Italian population and -0.98 cm/°C (CI: -1.42; -0.55) for the Lithuanian population. Likewise, the lesion length and probability of symptom production estimated at
410 26 °C did not differ between populations (Figure 2b and 2c).

Interestingly, the probability of severe symptom induction was less negatively affected by temperature increase in Italian than in Lithuanian isolates on days 21, 28 and 35 (Figure 2i). In particular, on days 21 and 35 post-inoculation, a non significant slope was estimated for

the Italian mean reaction norm (0.04 (CI: -0.12; 0.20) on day 21, -0.08 (CI: -0.17; 001) on day 35), whereas the Lithuanian reaction exhibited a clearly negative slope (-0.32 (CI: -0.44, -0.19) on day 21, -0.27 (CI: -0.36, -0.18) on day 35). From day 42 onward, slope estimates no longer differed significantly between populations, although it remained negative in the Lithuanian population but non-significant in the Italian population. In addition, the probability of symptoms becoming severe at 26°C was significantly higher on days 42 and 49 in seedlings inoculated with Italian isolates than in those inoculated with Lithuanian isolates (Figure 2h). For example, at 42 days post-inoculation, the estimated probability of symptoms becoming severe was 0.79 (CI: 0.72; 0.85) in seedlings inoculated with Italian isolates, compared to 0.51 (CI: 0.37; 0.65) in those inoculated with Lithuanian isolates.

The aggressiveness of the Lithuanian and Italian H. fraxineus *populations differed on* F. ornus *(hypothesis 3)*

The estimated reaction norms for the three aggressiveness components as a function of host species showed similar mean negative slopes in the two *H. fraxineus* populations (Figure 3 panels c, f, i). For instance, on day 21 after inoculation, the slopes of the reaction norms for the probability of symptom and severe symptom induction were estimated at -0.11 (CI: -0.28; 0.06) and -0.41 (CI: -0.59; -0.24) for the Italian population, and 0.10 (CI: -0.24; 0.04) and -0.54 (CI: -0.70; -0.37) for the Lithuanian population, respectively. Interestingly, despite these similar plasticity levels, the estimated probability of severe symptom induction was higher on day 63 in Italian (0.67 (CI: 0.54; 0.81)) than in Lithuanian isolates (0.41 (CI: 0.27; 0.54)) (Figure 3h).

Discussion

We characterized reaction norms for aggressiveness in a Lithuanian and an Italian population of *H. fraxineus*, an invasive fungal pathogen responsible for the ash dieback epidemics currently developing in Europe. The Lithuanian population is located near the site of the initial emergence of the disease in Europe, whereas the Italian population is more recently established and close to the southern edge of the epidemic front. Using leaf inoculations, we assessed variation in the plasticity of three aggressiveness proxies: lesion length, symptom induction, and severe symptom induction. The environmental variables were temperature and ash species. Under reference conditions (*F. excelsior*, 22 °C), we detected no difference in aggressiveness between the two *H. fraxineus* populations, and thus no differentiation in the intercepts of their estimated reaction norms. The aggressiveness of the Lithuanian and Italian isolates was generally reduced at high temperature (26 °C) and on *F. ornus*, relative to reference conditions. The negative effect of host species shift was mostly comparable across populations for all three traits, except on the last day of the experiment when the of foliar symptoms induced by Italian were more severe than those induced by Lithuanian isolates. In addition, the negative effect of increasing temperature on foliar symptom severity was less pronounced in Italian than in Lithuanian isolates at different time points of the experiment, as indicated by (*i*) the shallower slopes of their estimated reaction norms, (*ii*) their higher ability to induce severe symptoms at 26°C, or (*iii*) both.

Increased leaf symptom severity may lead to higher mortality rates in infected ashes

Among the three proxies of aggressiveness we assessed, only the occurrence of severe foliar symptoms (pronounced wilting or premature abscission) allowed us to discriminate between the two *H. fraxineus* populations, even though lesion length was substantially correlated with both symptom induction and severe symptom induction. From a

methodological point of view, this result supports the use of multiple, closely related proxies at biologically relevant scale when quantifying variation in aggressiveness (Bock et al. 2010), as recommended for *H. fraxineus* by Orton et al. (2019).

Ash dieback severity has often been linked to the capacity of *H. fraxineus* to extend infections from leaves into woody tissues, but this ability has also been regarded as an epidemiological dead end for the fungus because stem infections do not contribute to apothecia formation and onward transmission (Gross et al. 2014; Marçais et al. 2023). Conversely, accelerated leaf abscission induced by foliar infections may facilitate a more rapid transition into the saprotrophic phase in the litter, thereby reducing exposure to the high canopy temperatures typical of sunny periods of southern Europe, and potentially improving strain viability (Hauptman et al. 2011; Becans et al. 2025).

Severe and recurrent defoliation caused by *H. fraxineus* weakens infected ashes (Combes et al. 2024), increasing their susceptibility to secondary fungal pathogens that further accelerate decline (Chandelier et al. 2016; Benigno et al. 2023; Peters et al. 2023; Spiegel et al. 2025). Necrotic leaf lesions can also impair photosynthetic efficiency and reduce carbohydrate storage, which in turn further constrains photosynthesis, radial growth and hydraulic function, thereby increasing the likelihood of mortality in infected trees (Klesse et al. 2020). Consequently, the induction of strong foliar symptoms under high temperatures or on *F. ornus* by the Italian isolates suggests that the evolution of phenotypic plasticity could provide *H. fraxineus* with the potential to inflict damages in southern Europe comparable to those already documented in central Europe, despite high summer temperature conditions, and *F. ornus* presence.

Abundance of naïve hosts may have prevented differentiation in aggressiveness on F. excelsior *at 22 °C between the studied* H. fraxineus *populations*

Lygis et al. (2017) investigated the evolution of *H. fraxineus* aggressiveness in Europe and reported high variability among isolates (also reported by Orton et al. (2019) and Kosawang et al. (2020)), but no difference in aggressiveness between a long-established Lithuanian population and a recently established Swiss population, the latter located at the epidemic front at the time. Although our analysis was based on a relatively small number of isolates, the absence of differentiation in aggressiveness between the Italian and Lithuanian *H. fraxineus* populations under reference conditions (*F. excelsior*, 22 °C) tends to support their findings. Under the aggressiveness–transmission trade-off hypothesis (Anderson & May, 1982), aggressiveness (also commonly referred to as virulence in the literature) is predicted to evolve during the course of epidemics according to complex, and sometimes antagonistic patterns (Cressler et al., 2016; Pariaud et al., 2009). A widely used conceptual framework posits that high aggressiveness may be favored during the early epidemic stages, when host availability relative to inoculum pressure is high, followed by a decline in aggressiveness as the pathogen populations approach endemic equilibrium ((Berngruber et al., 2013; Griette et al., 2015; Nørgaard et al., 2019). Although this prediction has received empirical support in various pathosystems (e.g., Berngruber et al., 2013; Fenner & Ratcliffe, 1965; Fraser et al., 2014), it does not align with the findings of Lygis et al. (2017) or with ours.

The strong bottleneck undergone by *H. fraxineus* during its introduction in Europe, likely coincided with a decrease in its aggressiveness against *F. excelsior*, in comparison with isolates from the native range of the fungus in Japan (Gross & Sieber, 2016; Mcmullan et al., 2018). It is plausible that the large availability of naïve hosts in the introduction area rapidly selected *H. fraxineus* isolates (Buschbom 2022) with an optimal, limited level of aggressiveness. During its subsequent spread toward southern Europe, the average aggressiveness of the fungus did not change, even though resistant *F. ornus* and susceptible *F. excelsior* coexisted. Such coexistence could have favored increased aggressiveness on susceptible hosts if resistance in *F. ornus* had imposed a directional selective pressure on pathogen aggressiveness (Gandon & Michalakis, 2000; Soularue et

515 al., 2025). Nonetheless, the weak initial genetic variation in the founding *H. fraxineus* population, coupled with repeated founder effects and strong genetic drift, may have further hindered the fixation of alleles enabling the evolution of aggressiveness in such situation. Moreover, the number of *H. fraxineus* genotypes infecting *F. ornus* trees likely remained too low (Kirisits 2017) to influence the evolution of aggressiveness at a regional scale in Italy,
520 particularly given the extensive gene flow connecting European *H. fraxineus* populations over large distances (Burokiene et al., 2015; Grosdidier, Ioos, Husson, et al., 2018b; Gross, Hosoya, et al., 2014), and the recent establishment of *H. fraxineus* in this area (Luchi et al. 2016).

Interestingly, the higher ability of Italian isolates to induce severe symptoms on *F. ornus* on
525 the last day of the experiment may represent an early sign of *H. fraxineus* adaptation to this host in northern Italy. Such *H. fraxineus* adaptation to *F. ornus* should be further investigated in future experiments. These experiments should allow investigation of the reaction norms for aggressiveness in relation to ash species, to determine whether (*i*) *H. fraxineus* adaptation to *F. ornus* is confirmed and (*ii*) this adaptation is associated with increased
530 aggressiveness on *F. excelsior*. If confirmed, such result would mean that the co-existence of these two ash species exerts divergent selection on *H. fraxineus* aggressiveness in southern Europe, potentially leading to more severe ash dieback damage in this region.

Transient differentiation in thermal reaction norm of aggressiveness suggests thermal
535 *adaptation in* H. fraxineus *at the southern epidemic front*

This is the second report of differentiation for a life-history trait among European *H. fraxineus* populations. Bécans et al. (2025) found that Italian isolates had a twice higher *in vitro* growth rate at 26 °C than Lithuanian isolates, as well as greater viability at temperatures above 30 °C. Hence, our results align with these observations. Taken together, the findings of the two
540 studies suggest that *in vitro* viability and mycelial growth are correlated with pathogen aggressiveness, as previously proposed by Blenis et al. (1984) and reported for *H. fraxineus*

by Orton et al. (2019) based on leaf inoculations, although Kosawang et al. (2020) found no relationship between *in vitro* growth and the lesion length caused on ash seedling stems by Danish *H. fraxineus* genotypes.

The Italian isolates and their close ancestors have largely been exposed to warm summer conditions, coinciding with the onset of new leaf infections (Landolt et al., 2016), than the Lithuanian isolates. This suggests that the lowest thermal plasticity of their aggressiveness could result from an adaptive response of the pathogen, that has led to the selection of reaction norms buffering aggressiveness under warm conditions (Eriksson et al., 2023). Considered together, our results and those of Becans et al. (2025) strengthen the hypothesis of thermal adaptation in *H. fraxineus* subsequent to the severe initial bottleneck experienced by the European *H. fraxineus* population (Gross, Hosoya, et al., 2014; Mcmullan et al., 2018), and potentially strong asymmetric gene flow from core populations to newly founded ones. Similar patterns of differentiation consistent with thermal adaptation have been reported in plant pathogens in recent years (e.g., Robin et al., 2017). Nonetheless, determining the extent to which the differences between the Italian and Lithuanian reaction norms observed here arise from selection rather than genetic drift will require genomic analyses to quantify neutral divergence and identify trait-associated loci through genome-wide association studies.

Conclusion

Our study suggests that epidemic severity and development in relation to temperature may be slightly higher than anticipated under the warm summer conditions of southern Europe, or despite the presence of *F. ornus*. These findings highlight the importance of precisely accounting for the evolution of pathogens response to environmental variation when assessing disease risk. Future experiments including more extensive sampling within each site would allow the characterization of additional individual reaction norms for each *H. fraxineus* population, thereby increasing statistical power. Future research should also investigate the evolution of plasticity in aggressiveness components across a broader

environmental range incorporating other environmental factors, such as humidity, that may interact with temperature and host resistance (Sturrock et al., 2011).

Acknowledgments

Clémence Becans received a doctoral scholarship from the French Ministry for Research and Higher Education. We thank Patrick Léger, Aurélien Kohler, Estelle Ducourtioux and Noémie Chaland for their assistance during the experimental work. We thank the Phenobois facility (*Phenobois, INRAE, 2018. Wood and Tree Physicochemical Phenotyping Facility for Genetic Resources, https://doi.org/10.15454/1.5572410490640864E12*) for its contribution to NIR spectroscopy measurements.

Conflict of interest

The authors declare no conflict of interest.

Bibliography

Adamčíková, K., Pažitný, J., & Pastirčáková, K. (2018). Individual resistance of Fraxinus angustifolia and F. excelsior clones to Hymenoscyphus fraxineus. *Journal of Plant Protection Research*. https://doi.org/10.24425/122937.

Agan, A., Tedersoo, L., Hanso, M., & Drenkhan, R. (2023). Traces of *Hymenoscyphus fraxineus* in Northeastern Europe Extend Further Back in History than Expected. *Plant Disease*, *107*(2), 344-349.https://doi.org/10.1094/PDIS-04-22-0807-RE.

Alizon, S., Hurford, A., Mideo, N., & Van Baalen, M. (2009). Virulence evolution and the trade-off hypothesis : History, current state of affairs and the future. *Journal of evolutionary biology*, *22*(2), 245-259.

Anderson, R. M., & May, R. M. (1982). Coevolution of hosts and parasites. *Parasitology*, *85*(2), 411-426. https://doi.org/10.1017/S0031182000055360.

Arnold, P. A., Kruuk, L. E. B., & Nicotra, A. B. (2019). How to analyse plant phenotypic plasticity in response to a changing climate. *New Phytologist*, *222*(3), 1235-1241. https://doi.org/10.1111/nph.15656.

Bartón, K., & MK, B. (2023). Package 'MuMin'https://cran. R-project. Org/web/packages. *MuMIn/index.html*.

Bashey, F. (2015). Within-host competitive interactions as a mechanism for the maintenance of parasite diversity. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1675), 20140301.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using **lme4**. *Journal of Statistical Software*, *67*(1). https://doi.org/10.18637/jss.v067.i01.

Bécans, C., Robin, C., Budde, K. B., Ghelardini, L., Gross, A., Lygis, V., Nielsen, L. R., Saint-Jean, G., & Soularue, J. (2025). Evolution of Thermal Plasticity in *Hymenoscyphus fraxineus* During Ash Dieback Expansion in Europe. *Ecology and Evolution*, *15*(6), e71513. https://doi.org/10.1002/ece3.71513.

Benigno, A., Bregant, C., Aglietti, C., Rossetto, G., Tolio, B., Moricca, S., & Linaldeddu, B. T. (2023). Pathogenic fungi and oomycetes causing dieback on Fraxinus species in the Mediterranean climate change hotspot region. *Frontiers in forests and global change*, *6*, 1253022.

Berngruber, T. W., Froissart, R., Choisy, M., & Gandon, S. (2013). Evolution of virulence in emerging epidemics. *PLoS pathogens*, *9*(3), e1003209.

Blenis, P. V., Patton, R. F., & Spear, R. N. (1984). Effect of temperature on the ability of Gremmeniella abietina to survive and to colonize host tissue. *European journal of forest pathology*, *14*(3), 153-164.

Bock, C. H., Chiang, K. S., & Del Ponte, E. M. (2022). Plant disease severity estimated visually: a century of research, best practices, and opportunities for improving methods and practices to maximize accuracy. *Tropical Plant Pathology*, *47*(1), 25-42.

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J.-S. S. (2009). Generalized linear mixed models : A practical guide for ecology and evolution. *Trends in Ecology & Evolution*, *24*(3), 127-135. https://doi.org/10.1016/j.tree.2008.10.008.

Bradshaw, A. D. (1965). Evolutionary Significance of Phenotypic Plasticity in Plants. In *Advances in Genetics* (Vol. 13, p. 115-155). Elsevier. https://doi.org/10.1016/S0065-2660(08)60048-6.

Buschbom, J. (2022). Genomic patterns and the evolutionary origin of an invasive fungal pathogen (Hymenoscyphus fraxineus) in Europe. *Basic and Applied Ecology*, *59*, 4-16.

Burokiene, D., Prospero, S., Jung, E., Marciulyniene, D., Moosbrugger, K., Norkute, G., Rigling, D., Lygis, V., & Schoebel, C. N. (2015). Genetic population structure of the invasive ash dieback pathogen Hymenoscyphus fraxineus in its expanding range. *Biological Invasions*, *17*(9), 2743-2756. https://doi.org/10.1007/s10530-015-0911-6.

Castiblanco, V., Castillo, H. E., & Miedaner, T. (2020). Be flexible and adapt easily—The great role of plasticity relative to genetic variation for aggressiveness of Fusarium culmorum isolates. Journal of Phytopathology, 168(3), 162-174.

Caudullo, G., & De Rigo, D. (2016). Fraxinus ornus in Europe : Distribution, habitat, usage and threats. *The European Atlas of Forest Tree Species: Modelling, Data and Information on Forest Tree Species*, 100-101.

Chandelier, A., Gerarts, F., San Martin, G., Herman, M., & Delahaye, L. (2016). Temporal evolution of collar lesions associated with ash dieback and the occurrence of Armillaria in Belgian forests. *Forest Pathology*, *46*(4), 289-297.

Chevin, L., Collins, S., & Lefèvre, F. (2013). Phenotypic plasticity and evolutionary demographic responses to climate change : Taking theory out to the field. *Functional Ecology*, *27*(4), 967-979. https://doi.org/10.1111/j.1365-2435.2012.02043.x.

Coker, T. L. R., Rozsypálek, J., Edwards, A., Harwood, T. P., Butfoy, L., & Buggs, R. J. A. (2019). Estimating mortality rates of European ash (*Fraxinus excelsior*) under the ash dieback (*Hymenoscyphus fraxineus*) epidemic. *PLANTS, PEOPLE, PLANET*, *1*(1), 48-58. https://doi.org/10.1002/ppp3.11.

Cressler, C. E., McLEOD, D. V., Rozins, C., Van Den Hoogen, J., & Day, T. (2016). The adaptive evolution of virulence : A review of theoretical predictions and empirical tests. *Parasitology*, *143*(7), 915-930. https://doi.org/10.1017/S003118201500092X.

Davison, A. C., & Hinkley, D. V. (1997). *Bootstrap Methods and their Application* (1re éd.). Cambridge University Press. https://doi.org/10.1017/CBO9780511802843.

de la Mata, R., & Zas, R. (2023). Plasticity in growth is genetically variable and highly conserved across spatial scales in a Mediterranean pine. *New Phytologist*, *240*(2), 542-554.

Desprez-Loustau, M.-L., Aguayo, J., Dutech, C., Hayden, K. J., Husson, C., Jakushkin, B., Marçais, B., Piou, D., Robin, C., & Vacher, C. (2016). An evolutionary ecology perspective to address forest pathology challenges of today and tomorrow. *Annals of Forest Science*, *73*(1), 45-67. https://doi.org/10.1007/s13595-015-0487-4.

Desprez-Loustau, M.-L., Courtecuisse, R., Robin, C., Husson, C., Moreau, P.-A., Blancard, D., Selosse, M.-A., Lung-Escarmant, B., Piou, D., & Sache, I. (2010). Species diversity and drivers of spread of alien fungi (sensu lato) in Europe with a particular focus on France. *Biological Invasions*, *12*(1), 157-172.

DeWitt, T. J., Sih, A., & Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution*, *13*(2), 77-81. https://doi.org/10.1016/S0169-5347(97)01274-3.

Dutt, A., Andrivon, D., & Le May, C. (2022). Multi-infections, competitive interactions, and pathogen coexistence. *Plant Pathology*, *71*(1), 5-22.

Eriksson, M., Kinnby, A., De Wit, P., & Rafajlović, M. (2023). Adaptive, maladaptive, neutral, or absent plasticity : Hidden caveats of reaction norms. *Evolutionary Applications*, *16*(2), 486-503.

Estoup, A., Ravigné, V., Hufbauer, R., Vitalis, R., Gautier, M., & Facon, B. (2016). Is there a genetic paradox of biological invasion? *Annual review of ecology, evolution, and systematics*, *47*(1), 51-72.

Fenner, F., & Ratcliffe, F. N. (1965). *Myxomatosis.*

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2 : New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, *37*(12), 4302-4315. https://doi.org/10.1002/joc.5086.

Fidanoğlu, B. T., Mestav, B., & Özkılınç, H. (2023). A case study of the effect of temperature on aggressiveness in the Monilinia-peach pathosystem. European Journal of Plant Pathology, 167(1), 1-10.

Forsman, A. (2015). Rethinking phenotypic plasticity and its consequences for individuals, populations and species. *Heredity*, *115*(4), 276-284. https://doi.org/10.1038/hdy.2014.92.

Fraser, C., Lythgoe, K., Leventhal, G. E., Shirreff, G., Hollingsworth, T. D., Alizon, S., & Bonhoeffer, S. (2014). Virulence and pathogenesis of HIV-1 infection : An evolutionary perspective. *Science*, *343*(6177), 1243727.

Freer-Smith, P. H., & Webber, J. F. (2017). Tree pests and diseases : The threat to biodiversity and the delivery of ecosystem services. *Biodiversity and Conservation*, *26*(13), 3167-3181.

Gandon, S., & Michalakis, Y. (2000). Evolution of parasite virulence against qualitative or quantitative host resistance. Proceedings of the Royal Society of London. Series B: Biological Sciences, 267(1447), 985-990.

Garbelotto, M., Rocca, G. D., Osmundson, T., di Lonardo, V., & Danti, R. (2015). An increase in transmission-related traits and in phenotypic plasticity is documented during a fungal invasion. *Ecosphere*, *6*(10), 1-16. https://doi.org/10.1890/ES14-00426.1.

George, M. E., Gaitor, T. T., Cluck, D. B., Henao-Martínez, A. F., Sells, N. R., & Chastain, D. B. (2025). The impact of climate change on the epidemiology of fungal infections : Implications for diagnosis, treatment, and public health strategies. *Therapeutic Advances in Infectious Disease*, *12*, 20499361251313840. https://doi.org/10.1177/20499361251313841.

Gerken, A. R., Eller, O. C., Hahn, D. A., & Morgan, T. J. (2015). Constraints, independence, and evolution of thermal plasticity : Probing genetic architecture of long-and short-term thermal acclimation. *Proceedings of the National Academy of Sciences*, *112*(14), 4399-4404.

Ghalambor, C. K., McKAY, J. K., Carroll, S. P., & Reznick, D. N. (2007). Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology*, *21*(3), 394-407. https://doi.org/10.1111/j.1365-2435.2007.01283.x.

Gomulkiewicz, R., & Stinchcombe, J. R. (2022). Phenotypic plasticity made simple, but not too simple. *American Journal of Botany*, *109*(10), 1519-1524. https://doi.org/10.1002/ajb2.16068.

Griette, Q., Raoul, G., & Gandon, S. (2015). Virulence evolution at the front line of spreading epidemics : VIRULENCE EVOLUTION AT THE FRONT LINE. *Evolution*, *69*(11), 2810-2819. https://doi.org/10.1111/evo.12781.

Grosdidier, M., Ioos, R., & Marçais, B. (2018a). Do higher summer temperatures restrict the dissemination of *Hymenoscyphus fraxineus* in France? *Forest Pathology*, *48*(4), e12426. https://doi.org/10.1111/efp.12426.

Grosdidier, M., Ioos, R., Husson, C., Caël, O., Scordia, T., & Marçais, B. (2018b). Tracking the invasion : Dispersal of Hymenoscyphus fraxineus airborne inoculum at different scales. *FEMS Microbiology Ecology*, *94*(5). https://doi.org/10.1093/femsec/fiy049.

Gross, A., Holdenrieder, O., Pautasso, M., Queloz, V., & Sieber, T. N. (2014). *Hymenoscyphus pseudoalbidus* , the causal agent of European ash dieback. *Molecular Plant Pathology*, *15*(1), 5-21. https://doi.org/10.1111/mpp.12073.

Gross, A., Hosoya, T., & Queloz, V. (2014). Population structure of the invasive forest pathogen *Hymenoscyphus pseudoalbidus*. *Molecular Ecology*, *23*(12), 2943-2960. https://doi.org/10.1111/mec.12792.

Gross, A., & Sieber, T. N. (2016). Virulence of *Hymenoscyphus albidus* and native and introduced *Hymenoscyphus fraxineus* on *Fraxinus excelsior* and *Fraxinus pennsylvanica*. *Plant Pathology*, *65*(4), 655-663. https://doi.org/10.1111/ppa.12450.

Guégan, J.-F., Ayouba, A., Cappelle, J., & De Thoisy, B. (2020). Forests and emerging infectious diseases: Unleashing the beast within. *Environmental Research Letters*, *15*(8), 083007.

Hauptman, T., Piškur, B., de Groot, M., Ogris, N., Ferlan, M., & Jurc, D. (2013). Temperature effect on *Chalara fraxinea* : Heat treatment of saplings as a possible disease control method. *Forest Pathology*, *43*(5), 360-370. https://doi.org/10.1111/efp.12038.

Hendry, A. P. (2016). Key Questions on the Role of Phenotypic Plasticity in Eco-Evolutionary Dynamics. *Journal of Heredity*, *107*(1), 25-41. https://doi.org/10.1093/jhered/esv060.

Hoffmann, A. A., & Sgrò, C. M. (2011). Climate change and evolutionary adaptation. *Nature*, *470*(7335), 479-485. https://doi.org/10.1038/nature09670.

Keesing, F., Belden, L. K., Daszak, P., Dobson, A., Harvell, C. D., Holt, R. D., Hudson, P., Jolles, A., Jones, K. E., & Mitchell, C. E. (2010). Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature*, *468*(7324), 647-652.

Kirisits, T., Dämpfle, L., & Kräutler, K. (2013). *Hymenoscyphus albidus* is not associated with an anamorphic stage and displays slower growth than *H ymenoscyphus pseudoalbidus* on agar media. *Forest Pathology*, *43*(5), 386-389. https://doi.org/10.1111/efp.12042.

Kirisits, T. (2017). Further Observations on the Association of Hymenoscyphus fraxineus with Fraxinus ornus. Baltic Forestry, 20, 60-67.

Klesse, S., von Arx, G., Gossner, M. M., Hug, C., Rigling, A., & Queloz, V. (2021). Amplifying feedback loop between growth and wood anatomical characteristics of Fraxinus excelsior explains size-related susceptibility to ash dieback. *Tree Physiology*, *41*(5), 683-696.

Kosawang, C., McKinney, L. V., Nielsen, L. R., & Kjær, E. D. (2020). Variation in aggressiveness of *Hymenoscyphus fraxineus* genotypes amid the ash dieback epidemic. *Plant Pathology*, *69*(4), 677-684. https://doi.org/10.1111/ppa.13158.

Kowalski, T., & Bartnik, C. (2012). Morphologial variation in colonies of Chalara fraxinea isolated from ash (Fraxinus excelsior L.) stems with symptoms of dieback and effects of temperature on colony growth and structure. *Acta Agrobotanica*, *63*(1), 99-106. https://doi.org/10.5586/aa.2010.012.

Laine, A. (2008). Temperature-mediated patterns of local adaptation in a natural plant–pathogen metapopulation. *Ecology Letters*, *11*(4), 327-337. https://doi.org/10.1111/j.1461-0248.2007.01146.x.

Lakens, D. (2022). Sample size justification. *Collabra: psychology*, *8*(1), 33267.

Lande, R. (2009). Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of Evolutionary Biology*, *22*(7), 1435-1446. https://doi.org/10.1111/j.1420-9101.2009.01754.x.

Landolt, J., Gross, A., Holdenrieder, O., & Pautasso, M. (2016). Ash dieback due to *Hymenoscyphus fraxineus* : What can be learnt from evolutionary ecology? *Plant Pathology*, *65*(7), 1056-1070. https://doi.org/10.1111/ppa.12539.

Laubray, S., Buée, M., & Marçais, B. (2024). *Hymenoscyphus fraxineus* persistence in the ash litter. *Plant Pathology*, ppa.13948. https://doi.org/10.1111/ppa.13948.

Leonard, A. M., & Lancaster, L. T. (2020). Maladaptive plasticity facilitates evolution of thermal tolerance during an experimental range shift. *BMC Evolutionary Biology*, *20*(1), 47. https://doi.org/10.1186/s12862-020-1589-7.

Lione, G., Ongaro, S., Prencipe, S., Giraudo, M., & Gonthier, P. (2024). Drivers of *Hymenoscyphus fraxineus* Infections in the Inner-Alpine Valleys of Northwestern Italy. *Forests*, *15*(4), 732. https://doi.org/10.3390/f15040732.

Luchi, N., Ghelardini, L., Santini, A., Migliorini, D., & Capretti, P. (2016). First record of ash dieback caused by *Hymenoscyphus fraxineus* on *Fraxinus excelsior* in the Apennines (Tuscany, Italy). Plant Disease, 100(2), 535.

Lygis, V., Prospero, S., Burokiene, D., Schoebel, C. N., Marciulyniene, D., Norkute, G., & Rigling, D. (2017). Virulence of the invasive ash pathogen *Hymenoscyphus fraxineus* in old and recently established populations. *Plant Pathology*, *66*(5), 783-791. https://doi.org/10.1111/ppa.12635.

Marçais, B., Giraudel, A., & Husson, C. (2023). Ability of the ash dieback pathogen to reproduce and to induce damage on its host are controlled by different environmental parameters. *PLOS Pathogens*, *19*(4), e1010558. https://doi.org/10.1371/journal.ppat.1010558.

Marçais, B., Kosawang, C., Laubray, S., Kjær, E., & Kirisits, T. (2022). Ash dieback. In *Forest Microbiology* (p. 215-237). Elsevier. https://doi.org/10.1016/B978-0-323-85042-1.00022-7.

May, R. M., & Anderson, R. M. (1983). Epidemiology and genetics in the coevolution of parasites and hosts. *Proceedings of the Royal society of London. Series B. Biological sciences*, *219*(1216), 281-313.

Mayr, E. (1963). *Animal species and evolution*. Harvard University Press.

McKinney, L. V., Nielsen, L. R., Collinge, D. B., Thomsen, I. M., Hansen, J. K., & Kjær, E. D. (2014). The ash dieback crisis: Genetic variation in resistance can prove a long-term solution. *Plant Pathology*, *63*(3), 485-499. https://doi.org/10.1111/ppa.12196.

McLean, M. A., Angilletta, M. J., & Williams, K. S. (2005). If you can't stand the heat, stay out of the city : Thermal reaction norms of chitinolytic fungi in an urban heat island. *Journal of Thermal Biology*, *30*(5), 384-391. https://doi.org/10.1016/j.jtherbio.2005.03.002.

Mcmullan, M., Rafiqi, M., Kaithakottil, G., Clavijo, B. J., Bilham, L., Orton, E., Percival-Alwyn, L., Ward, B. J., Edwards, A., Saunders, D. G. O., Accinelli, G. G., Wright, J., Verweij, W., Koutsovoulos, G., Yoshida, K., Hosoya, T., Williamson, L., Jennings, P., Ioos, R., … Clark, M. D. (2018). The ash dieback invasion of Europe was founded by two genetically divergent individuals. *Nature Ecology & Evolution*, *2*(6), 1000. https://doi.org/10.1038/s41559-018-0548-9.

Merilä, J., & Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity : The problem and the evidence. *Evolutionary applications*, *7*(1), 1-14.

Mideo, N., Alizon, S., & Day, T. (2008). Linking within-and between-host dynamics in the evolutionary epidemiology of infectious diseases. *Trends in ecology & evolution*, *23*(9), 511-517.

Migliorini, D., Luchi, N., Nigrone, E., Pecori, F., Pepori, A. L., & Santini, A. (2022). Expansion of Ash Dieback towards the scattered Fraxinus excelsior range of the Italian peninsula. *Biological Invasions*, *24*(5), 1359-1373. https://doi.org/10.1007/s10530-021-02716-z.

Miñana-Posada, S., Lorrain, C., McDonald, B. A., & Feurtey, A. (2025). Thermal adaptation in worldwide collections of a major fungal pathogen. *Molecular Plant-Microbe Interactions*, *38*(2), 252-264.

Nørgaard, L. S., Phillips, B. L., & Hall, M. D. (2019). Infection in patchy populations: Contrasting pathogen invasion success and dispersal at varying times since host colonization. *Evolution Letters*, *3*(5), 555-566. https://doi.org/10.1002/evl3.141.

Orton, E. S., Clarke, M., Brasier, C. M., Webber, J. F., & Brown, J. K. M. (2019). A versatile method for assessing pathogenicity of *Hymenoscyphus fraxineus* to ash foliage. *Forest Pathology*, *49*(2), e12484. https://doi.org/10.1111/efp.12484.

Pariaud, B., Ravigné, V., Halkett, F., Goyeau, H., Carlier, J., & Lannou, C. (2009). Aggressiveness and its role in the adaptation of plant pathogens. *Plant Pathology*, *58*(3), 409-424. https://doi.org/10.1111/j.1365-3059.2009.02039.x.

Peters, S., Fuchs, S., Bien, S., Bußkamp, J., Langer, G. J., & Langer, E. J. (2023). Fungi associated with stem collar necroses of Fraxinus excelsior affected by ash dieback. *Mycological Progress*, *22*(7), 52.

Price, T. D., Qvarnström, A., & Irwin, D. E. (2003). The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1523), 1433-1440. https://doi.org/10.1098/rspb.2003.2372.

Przybył, K. (2002). Fungi associated with necrotic apical parts of *Fraxineus excelsior* shoots. *Forest Pathology*, *32*(6), 387-394. https://doi.org/10.1046/j.1439-0329.2002.00301.x.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reusch, T. B. (2014). Climate change in the oceans : Evolutionary versus phenotypically plastic responses of marine animals and plants. *Evolutionary applications*, *7*(1), 104-122.

Robin, C., Andanson, A., Saint-Jean, G., Fabreguettes, O., & Dutech, C. (2017). What was old is new again: thermal adaptation within clonal lineages during range expansion in a fungal pathogen. Molecular Ecology, 26(7), 1952-1963.

Roy, B. A., Alexander, H. M., Davidson, J., Campbell, F. T., Burdon, J. J., Sniezko, R., & Brasier, C. (2014). Increasing forest loss worldwide from invasive pests requires new trade regulations. *Frontiers in Ecology and the Environment*, *12*(8), 457-465.

Santini, A., Ghelardini, L., De Pace, C., Desprez-Loustau, M. L., Capretti, P., Chandelier, A., Cech, T., Chira, D., Diamandis, S., Gaitniekis, T., Hantula, J., Holdenrieder, O., Jankovsky, L., Jung, T., Jurc, D., Kirisits, T., Kunca, A., Lygis, V., Malecka, M., … Stenlid, J. (2013). Biogeographical patterns and determinants of invasion by forest pathogens in Europe. *New Phytologist*, *197*(1), 238-250. https://doi.org/10.1111/j.1469-8137.2012.04364.x.

Scheiner, S. M. (2013). The genetics of phenotypic plasticity. XII. Temporal and spatial heterogeneity. *Ecology and evolution*, *3*(13), 4596-4609.

Scheiner, S. M., Barfield, M., & Holt, R. D. (2012). The genetics of phenotypic plasticity. Joint evolution of plasticity and dispersal rate. *Ecology and Evolution*, *2*(8), 2027-2039. https://doi.org/10.1002/ece3.327.

Schwanda, K., & Kirisits, T. (2016). Pathogenicity of *Hymenoscyphus fraxineus* towards leaves of three European ash species : *Fraxinus excelsior*, *F. angustifolia* and *F. ornus*. *Plant Pathology*, *65*(7), 1071-1083. https://doi.org/10.1111/ppa.12499.

Shaner, G., Stromberg, E. L., Lacy, G. H., Barker, K. R., & Pirone, T. P. (1992). Nomenclature and concepts of pathogenicity and virulence. *Annual Review of Phytopathology*, *30*(1), 47-66.

Sollars, E. S. A., Harper, A. L., Kelly, L. J., Sambles, C. M., Ramirez-Gonzalez, R. H., Swarbreck, D., Kaithakottil, G., Cooper, E. D., Uauy, C., Havlickova, L., Worswick, G., Studholme, D. J., Zohren, J., Salmon, D. L., Clavijo, B. J., Li, Y., He, Z., Fellgett, A., McKinney, L. V., … Buggs, R. J. A. (2017). Genome sequence and genetic diversity of European ash trees. *Nature*, *541*(7636), 212-216. https://doi.org/10.1038/nature20786.

Sommer, R. J. (2020). Phenotypic plasticity : From theory and genetics to current and future challenges. *Genetics*, *215*(1), 1-13.

Soularue, J. P., Halkett, F., Saubin, M., Denni, S., Demené, A., Dutech, C., & Robin, C. (2025). Quantitative Resistance Deployment Can Strengthen Epidemics in Perennial Plants by Selecting Maladapted Pathogen Strains. Evolutionary Applications, 18(7), e70123.

Spiegel, P., Hintze, T., Kopp, A., Sahli, M., Detter, A., Queloz, V., ... & Heinzelmann, R. (2025). Synergistic negative effects of ash dieback and Armillaria root rot on health and stability of mature ash trees. *Forest Ecology and Management*, *580*, 122476.

Stroheker, S., Queloz, V., & Nemesio-Gorriz, M. (2021). First report of *Hymenoscyphus fraxineus* causing ash dieback in Spain. *New Disease Reports*, *44*(2), e12054. https://doi.org/10.1002/ndr2.12054.

Sturrock, R. N., Frankel, S. J., Brown, A. V., Hennon, P. E., Kliejunas, J. T., Lewis, K. J., Worrall, J. J., & Woods, A. J. (2011). Climate change and forest diseases. *Plant pathology*, *60*(1), 133-149.

Tack, A. J. M., Thrall, P. H., Barrett, L. G., Burdon, J. J., & Laine, A. -L. (2012). Variation in infectivity and aggressiveness in space and time in wild host–pathogen systems : Causes and consequences. *Journal of Evolutionary Biology*, *25*(10), 1918-1936. https://doi.org/10.1111/j.1420-9101.2012.02588.x.

Timmermann, V., Børja, I., Hietala, A. M., Kirisits, T., & Solheim, H. (2011). Ash dieback : Pathogen spread and diurnal patterns of ascospore dispersal, with special emphasis on Norway*. *EPPO Bulletin*, *41*(1), 14-20. https://doi.org/10.1111/j.1365-2338.2010.02429.x.

Tollenaere, C., Susi, H., & Laine, A.-L. (2016). Evolutionary and Epidemiological Implications of Multiple Infection in Plants. *Trends in Plant Science*, *21*(1), 80-90. https://doi.org/10.1016/j.tplants.2015.10.014.

Via, S., & Lande, R. (1985). Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution*, *39*(3), 505-522.

Villari, C., Dowkiw, A., Enderle, R., Ghasemkhani, M., Kirisits, T., Kjær, E. D., Marčiulynienė, D., McKinney, L. V., Metzler, B., Muñoz, F., Nielsen, L. R., Pliūra, A., Stener, L.-G., Suchockas, V., Rodriguez-Saona, L., Bonello, P., & Cleary, M. (2018). Advanced spectroscopy-based phenotyping offers a potential solution to the ash dieback epidemic. *Scientific Reports*, *8*(1), 17448. https://doi.org/10.1038/s41598-018-35770-0.

Vinton, A. C., Gascoigne, S. J. L., Sepil, I., & Salguero-Gómez, R. (2022). Plasticity's role in adaptive evolution depends on environmental change components. *Trends in Ecology & Evolution*, *37*(12), 1067-1078. https://doi.org/10.1016/j.tree.2022.08.008.

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. Oxford University Press.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T., Miller, E., Bache, S., Müller, K., Ooms, J., Robinson, D., Seidel, D., Spinu, V., … Yutani, H. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, *4*(43), 1686. https://doi.org/10.21105/joss.01686.

610

Table 1: Characteristics of sampling sites of the two studied *Hymenoscyphus fraxineus* populations. Temperature conditions were estimated by Wordclim for 2010-2019 (v2, Fick & Hijmans, 2017). For each sampling site, values correspond to the spatial mean across all raster grid cells located within a 20-km radius around the focal coordinates. MAT: mean annual temperature, Mean ST: mean summer temperature, Var ST: variance of summer temperature at each sampling site. The black cursor indicates Estonia, where the first evidence of *H. fraxineus* presence in Europe was found (Agan et al., 2023). The blue star represents the Lithuanian site, and the red star represents the Italian site.

	Lithuanian site	Italian site
Coordinates	54.8284 N; 24.1013 E	44.0514 N; 10.8430 E
Elevation	62 m	844 m
MAT	6.56	10.05
Mean ST	15.3	17.3
Var ST	5.24	3.86
Presence of *Fraxinus ornus*	No	Yes
Year of first ash dieback report	1996	2016

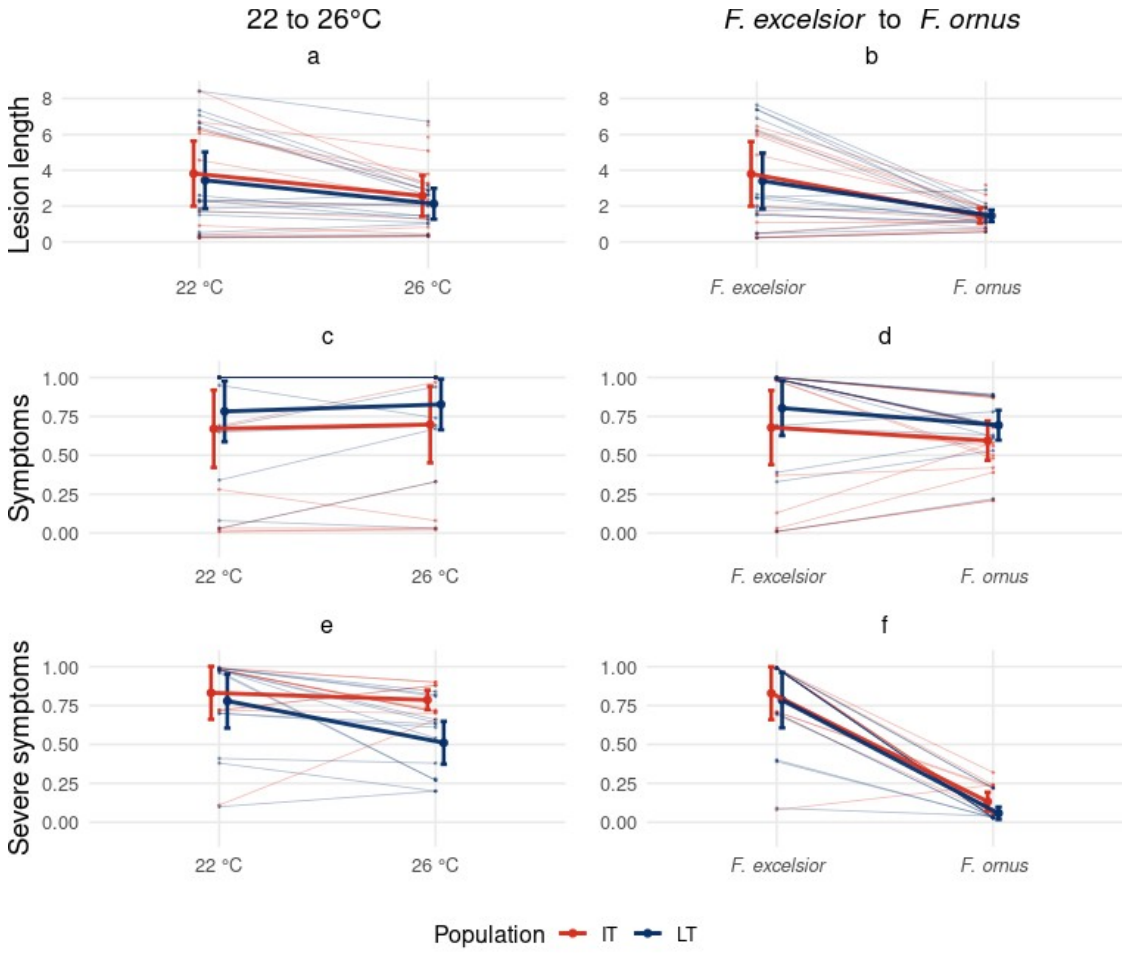

Figure 1. Reaction norm estimates for lesion length (cm) (a, b), symptom occurrence (c, d), and symptom severity (e, f) for two *Hymenoscyphus fraxineus* populations in response to temperature (left column) and host species (right column), on day 42 after inoculation. Thin lines represent individual reaction norms, estimated from three replicates per isolate and modality. Thick lines represent the mean reaction norm for each population. Large dots represent the mean estimate value for each population. Error bars indicate 95% confidence intervals of the mean.

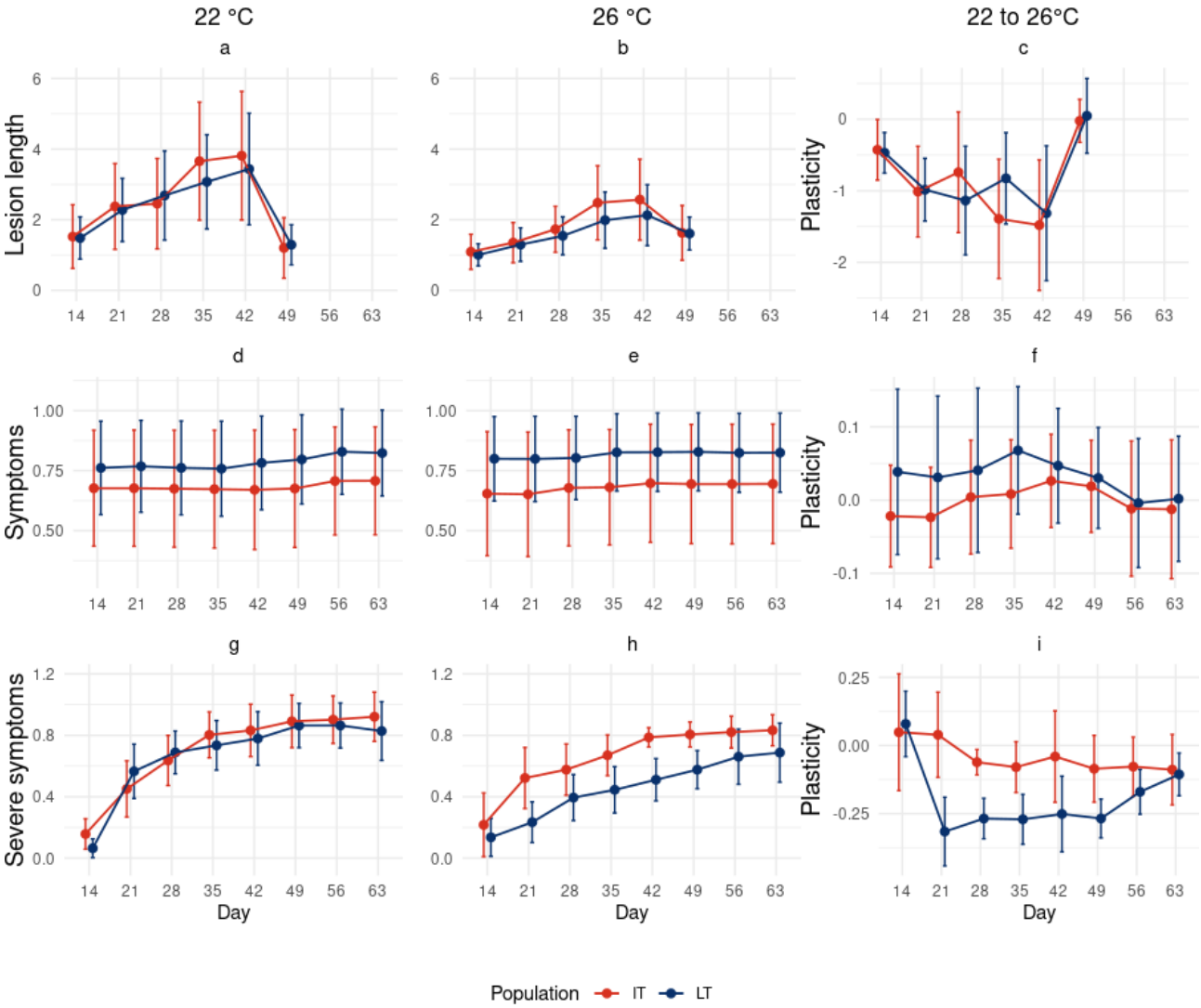

Figure 2. Estimated trait values and plasticity in relation to temperature for lesion length (cm) (a, b, c), symptom probability (d, e, f), and severe symptom probability (g, h, i), from day 14 to day 63 post-inoculation, in two *Hymenoscyphus fraxineus* populations (red: Italy, blue: Lithuania). The first column (a, d, g) shows the temporal variation of estimated trait values under the reference condition (22°C), these values correspond to the intercept estimates of the reaction norms. The second column (b, e, h) shows the temporal variation of estimated trait values under the treatment condition (26°C), and the third column (c, f, i) shows the temporal variation of the estimated slopes of the reaction norms. Each point represents the mean of isolate-level estimates, based on 15 fungal isolates per population, with three replicates per isolate and condition. Error bars indicate 95% confidence intervals.

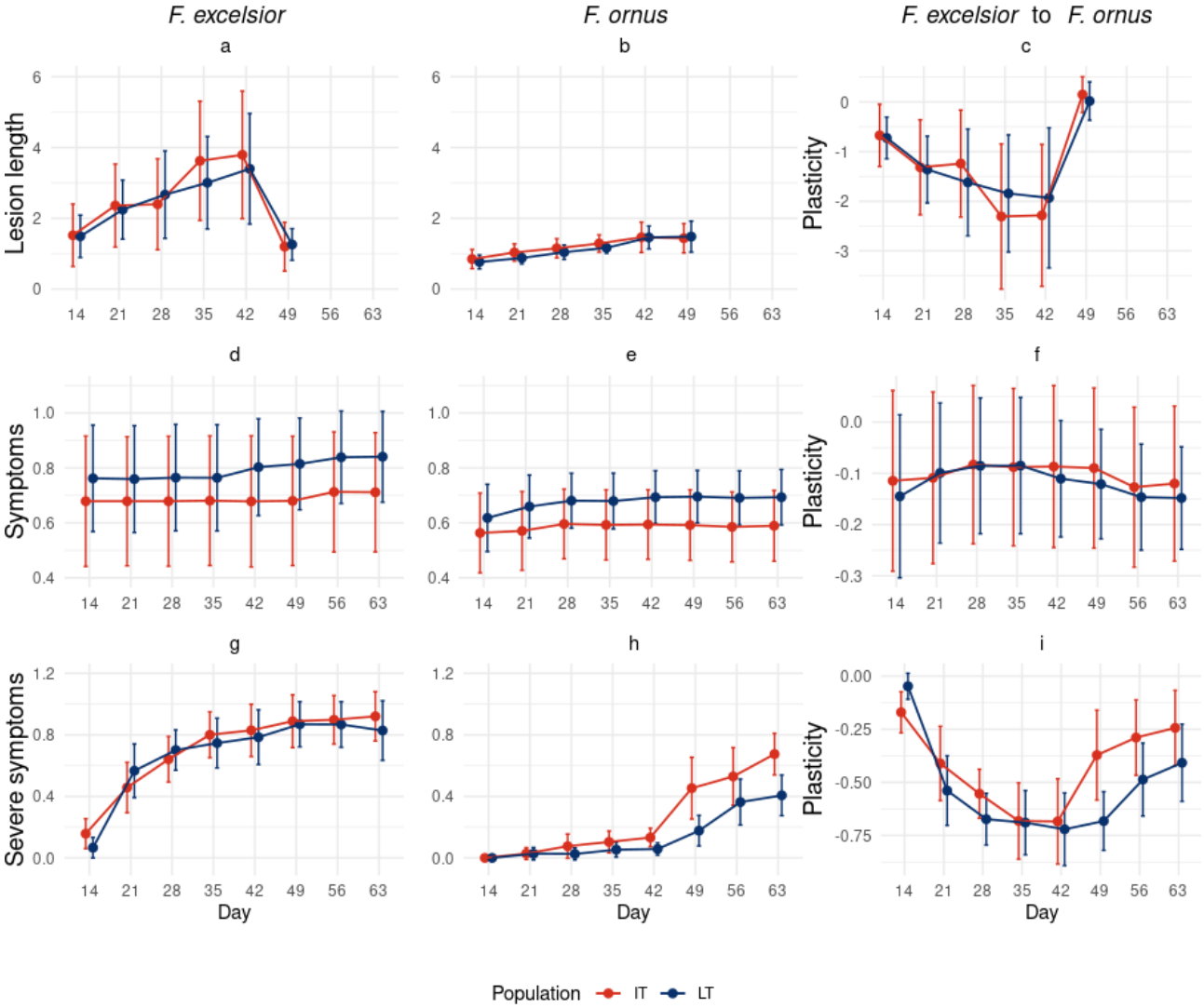

Figure 3. Estimated trait values and plasticity in relation to ash species for lesion length (cm) (a, b, c), symptom probability (d, e, f), and severe symptom probability (g, h, i), from day 14 to day 63 post-inoculation, in two *Hymenoscyphus fraxineus* populations (red: Italy, blue: Lithuania). The first column (a, d, g) shows the temporal evolution of estimated trait values under the reference condition (*Fraxinus excelsior*), these values correspond to the intercept estimates of the reaction norms. The second column (b, e, h) shows the evolution of estimated trait values under the treatment condition (*Fraxinus ornus*), and the third column (c, f, i) shows the evolution of the estimated slopes of the reaction norms. Each point represents the mean of isolate-level estimates, based on 15 fungal isolates per population, with three replicates per isolate and condition. Error bars indicate 95% confidence intervals.