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Variation in traits associated with parasitism and saprotrophism in a fungal root-rot pathogen invading intensive pine plantations

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ABSTRACT

Armillaria ostoyae, the causal agent of root- and butt-rot in several forest trees, has a lifecycle consisting of alternating parasitic and saprotrophic stages. It causes high levels of mortality in the intensively managed monospecific plantations of maritime pine (*Pinus pinaster*) in south-western France (Landes forest). In this region, the pathogen was native to the forests, pre-dating the large plantations of the 19th century. The first objective of this study was to estimate the variation in aggressiveness on maritime pine, determined as rate of host mortality caused by the infection process. The second objective was to characterize relationships between aggressiveness and traits likely to be involved in parasitism (i.e. rhizomorph production and colonization of host tissues) and saprotrophism (ability to decompose wood). The *A. ostoyae* isolates studied caused high rates of mortality in maritime pine, with significant differences between isolates. However, there was no variation of aggressiveness between *A. ostoyae* isolates from ancient forested and from more recently afforested areas, and did not support the hypothesis of a higher aggressiveness linked to a recent range expansion and the intensification of silviculture in this area. Rhizomorph production and aggressiveness were significantly correlated. In addition, we did not detect any trade-off between components of parasitism and saprotrophism, suggesting no significant evolutionary constraint driving these traits.

1. Introduction

Emerging plant diseases, i.e. diseases that have been recently reported or are increasing in incidence or geographic range, are mainly due to the anthropogenic introductions of alien pathogens and recent climatic changes (Anderson et al., 2004). However, human activities, such as range expansion or domestication of the plant host or a change in the host environment, may also result in an increasing impact of indigenous pathogens, especially when these practices result in more dense and uniform host populations (Stukenbrock and McDonald, 2008). Indeed, farming or cultural practices were identified as major drivers of plant diseases caused by fungal pathogens (Anderson et al., 2004). In forests, increases in planted forest surfaces (FAO, 2013) are thus probably enhancing the risk of fungal disease emergence (Pautasso et al., 2005). Consistent with this hypothesis, root-rot diseases, such as *Armillaria ostoyae*, have been increasing in conifer plantations worldwide over the last few decades (Morrison and Mallett, 1996; Roux et al., 2005). In south-western France, in the Landes forest, which occupies an area of about one million hectares of monospecific plantations of maritime pine (*Pinus pinaster* Aiton), mortalities caused by *A. ostoyae* in this conifer species have been increasingly reported in recent decades (Aumonier, 2007). In this region, *A. ostoyae* was likely present in ancient forested areas, pre-dating the intensive plantation of maritime pine of the 19th century, and is expanding to the more recent afforested areas won on the ancient wetlands which are currently drained (Labbé et al., 2015). The emergence of *A. ostoyae* root-rot might thus be linked to two combined factors: first, an expansion of the range of its host, and second, a change in silvicultural practices.

Armillaria species have a lifecycle consisting of alternating parasitic and saprotrophic stages. They infect roots and kill host tissues to obtain resources, but they also colonize and use dead wood substrates, thereby contributing to carbon recycling

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(Baumgartner et al., 2011). Among the seven *Armillaria* species geographically distributed in Europe (*Armillaria borealis*, *Armillaria cepistipes*, *Armillaria ectypa*, *Armillaria gallica*, *Armillaria mellea*, *A. ostoyae* and *Armillaria tabescens*), *A. ostoyae* is the most damaging pathogen of conifers. The nutritional strategy of *A. ostoyae* probably depends on the host species and forest management: it may act as a primary parasite (i.e. attacking healthy and unstressed trees), a secondary pathogen (i.e. infecting stressed trees) or almost exclusively as a saprotroph (Guillaumin and Legrand, 2005). In hardwood forests, *A. ostoyae* is mostly saprotrophic, only rarely infecting living trees, whereas, in unmanaged coniferous forests, it often acts as a secondary parasite (Ferguson et al., 2003; Laflamme, 2010). By contrast, many studies have reported that this species acts as a primary parasite in managed conifer plantations (with thinning, short rotation periods and sometimes fertilization) (Morrison and Mallett, 1996; Lung-Escarmant and Guyon, 2004).

The infection process of *Armillaria* spp. can be broken down into different stages: the production of infectious organs (pre-entry events), contact and penetration (entry), and the establishment and colonization of host tissues. *A. ostoyae* infects hosts by developing subterranean rhizomorphs. These structures are aggregated hyphae obtaining their nutrients from a source of inoculum (a dead or living root). They expand in the soil, coming into contact with woody conifer roots, which they penetrate by a combination of enzymatic degradation and mechanical force (Zeller, 1926; Solla et al., 2002). A positive correlation between mortality caused on *Picea abies* by *A. ostoyae* isolates and rhizomorphs produced by these isolates during a controlled plant inoculation experiment has been observed (Prospero et al., 2004). Hyphae originating from infected roots or wood residuals can also infect roots directly, although this tree-to-tree infection process is less common (Shaw, 1980; Lung-Escarmant et al., 2003). In the Landes forest, a small number of rhizomorphs have been observed in pine disease foci (Guillaumin and Legrand, 2005). In this particular forest context, tree-to-tree parasite transmission by root contact remains the most likely infection process, and host density might be an important factor for *A. ostoyae* spread. Within host tissues, the fungus spreads in both directions, to the root tip and the root collar. Mycelial fans form under the root bark, and they break down the cambium and the secondary xylem by producing massive amounts of several enzymes, such as laccases and polygalacturonases, which decompose lignin and pectin, respectively (Robene-Soustrade et al., 1998). *A. ostoyae* breaks down wood much less effectively than other wood-decomposing fungi (Guillaumin and Lung-Escarmant, 1985; Robene-Soustrade, 1993; Prospero et al., 2004), but it can persist in the stumps and roots for more than 40 years possibly through the action of different enzymes (Rishbeth, 1972; Garrett, 1950). Biochemical and genomic studies have lead different authors to suggest that a trade-off might exist between saprotrophism and parasitism in some root-rot pathogens (Robene-Soustrade and Lung-Escarmant, 1997; Olson et al., 2012). Enzymes involved in the decomposition of wood are produced by *A. ostoyae* in different steps during its saprotrophic stage (Robene-Soustrade et al., 1998). Mn-peroxidase, involved in lignin breakdown, is produced early in the wood degradation process, whereas CM-cellulase and xylanase, responsible for breaking down cellulose and hemicellulose, respectively, are produced later in this process. This difference in enzymatic activities between the parasitic and saprotrophic stages may result in different genotypes having different capacities to exploit living or dead wood, and, therefore, in genotype selection according to life-cycle stage.

To be able to predict *Armillaria* spp. impacts on intensive monospecific forests and the evolutionary dynamics of these emerging pathogens, a better knowledge of their life-history traits linked both to parasitic and saprotrophic strategies are required. Theoretical studies predict that traits associated with dispersal and reproduction would be favored in an expansion front, where selective pressures differ to the ones prevailing in the original range, whereas traits linked to competitive ability decreased (Burton et al., 2010). Consistent with this prediction, selection for dispersal capacity has been demonstrated in invasive populations of the fungal pathogen *Seiridium cardinale*, the causal agent of cypress canker (Garbelotto et al., 2015). In the front of expansions, pathogens may also find a higher density of susceptible hosts than in their original range, as postulated by Phillips and Puschendorf (2013). Indeed, their results suggest an increase of mortality caused by *Batrachochytrium dendrobatidis*, a fungal pathogen of amphibians, in its invasive range. For plant pathogens, the ability to cause disease (or pathogenicity) is commonly described by a quantitative component (i.e. the aggressiveness) and a qualitative one (i.e. virulence; Lannou, 2012). Both components are clearly linked to pathogen fitness, since pathogens need to infect their host before being transmitted (Alizon and Michalakis, 2015). Changes in the qualitative component of pathogenicity associated with a range expansion have been demonstrated (Gladieux et al., 2015). By contrast, few studies have investigated whether changes in aggressiveness of plant pathogens were associated with their range expansion. For example in *S. cardinale*, Garbelotto et al. (2015) did not observe any increase of aggressiveness associated with pathogen expansion. When considering aggressiveness as a component of competitive ability of the pathogen, this result was in agreement with predictions made by Burton et al. (2010). Changes in silvicultural practices might also favor aggressiveness. In classical theoretical models, there is a trade-off between parasite transmission and virulence (Anderson and May, 1982; May and Anderson, 1983). These models were developed to predict the evolutionary trajectories of obligate pathogens, the horizontal transmission of which is dependent on host survival. For root-rot pathogens, such as *A. ostoyae*, which can survive and be transmitted even if the host dies, theoretical predictions should be treated with considerable caution. For example, if there is a trade-off between transmission and virulence, then, in environmental conditions facilitating pathogen transmission from tree-to-tree (e.g. high host density), the most virulent genotypes may be favored (Thrall and Burdon, 1999). Variability of phenotypic traits associated with parasitic and/or saprotrophic stages exist in *A. ostoyae* populations (Morrison and Pellow, 2002; Prospero et al., 2004), so we can hypothesize that a high host density may favor the most parasitic genotypes over the most competitive saprotrophic genotypes. However, trade-offs or other relationships between life history traits may also exist and have an influence on the evolutionary potential of pathogens (Morris et al., 2009). Overall, it is difficult to monitor accurately changes in pathogenicity, since this complex phenotype depends on different related traits subject to different selective pressures (Ghalambor et al., 2003). Investigations of potential evolutionary trends and relationships between traits involved in parasitism and saprotrophism are required in order to better understand the emergence of a fungal pathogen having a complex life cycle, like *A. ostoyae*, in an intensive silviculture forest, like the Landes forest.

In this study, we addressed the following questions: (1) What is the variation of aggressiveness in the *A. ostoyae* population in an intensive monospecific pine forest? (2) Can a spatial effect be detected at this scale, consistent with the observed colonization gradient? (3) How are different quantitative traits linked to parasitism and saprotrophism (i.e. rhizomorph production, lesion size, ability to decompose wood and mycelial growth *in vitro*) related to each other, and, particularly, to aggressiveness assessed by the rate of mortality?