

Phytopathologia Mediterranea (2016) 55, 1, 20–40

DOI: 10.14601/Phytopathol_Mediterr-16423

REVIEW

***Phytophthora nicotianae* diseases worldwide: new knowledge of a long-recognised pathogen**

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Summary. *Phytophthora nicotianae* was first isolated from tobacco at the end of the 19th century. This organism is now considered as one of the most devastating oomycete plant pathogens, with a recognized host range of more than 255 species over five continents and a wide diversity of climates. The economic losses caused by *P. nicotianae* are difficult to estimate, because of the diversity of its hosts and ecological niches. For these reasons, this pathogen represents a continuous challenge to plant disease management programmes, which frequently rely solely on the use of chemicals. *Phytophthora nicotianae* is better adapted than its competitors to abiotic stresses, especially to climate warming. As a result, its importance is increasing. This review illustrates, with some examples, how *P. nicotianae* currently impacts plant economies worldwide, and how it may constitute more severe threats to agriculture and natural ecosystems in the context of global climate change.

Key words: global change, host range, re-emerging pathogen, oomycete.

Introduction

The development of modern agriculture has been heavily influenced by oomycete plant pathogens with three major epidemics spread over Europe in the middle 19th century. Potato, grapevine and citrus production were destroyed, respectively, by *Phytophthora infestans* (the Irish famine pathogen), *Plasmopara viticola* and a complex of *P. citrophthora* and *P. nicotianae* (Erwin and Ribeiro, 1996). These disasters led to the emergence of plant pathology as a formal science, and empirical observations contributed to

the search for resistant plants (Laviola *et al.*, 1990) and the elaboration of chemical preparations directed against pathogens, such as the Bordeaux mixture (Rivière *et al.*, 2011). Breeding for resistant cultivars and chemical control by fungicides became the cornerstones of nearly all crop protection strategies for more than a century.

Agriculture and agronomic research faced emerging challenges at the dawn of the 21st century. On one hand, genomics have provided invaluable tools for understanding the biology, pathogenicity and evolution of pathogens. On the other, major global changes (climate, international trade, human displacements, income growth in developing countries) currently modify agriculture and create unprecedented ecological situations. Many studies detailed

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effects of these changes on plant pests and pathogens, especially climatic changes (Gregory *et al.*, 2009). The present paper aims to illustrate how one of the earliest *Phytophthora* species to be described, *P. nicotianae*, benefits from current global change and provides new worldwide risks for agriculture, environment and food security.

***Phytophthora nicotianae*, a broad host range pathogen**

The genus *Phytophthora* was considered to comprise approx. 60 species until the end of the last century. This number has doubled in the last 15 years, reaching approx. 120 described species (Martin *et al.*, 2014). This blossoming is due to changes in species concepts, the refinement of identification tools and the subsequent resolution of species complexes, the development of large-scale surveys of natural ecosystems and new habitats, reports on new diseases, and the identification of new species arising from interspecific hybridization events (Hansen *et al.*, 2012). The expansion of the genus is likely to continue, and it has been proposed to contain 200–600 species (Brasier, 2009), so that the approx. 4,400 host-pathogen associations with *Phytophthora* spp. identified worldwide to date are also expected to evolve, with the probable emergence of new associations (Scott *et al.*, 2013).

Some *Phytophthora* species are more important than others, in terms of distribution, host range and impacts on agronomic productivity. *Phytophthora nicotianae* (Breda de Haan) stands out among *Phytophthora* spp. because it is responsible for heavy losses on a particularly high number of host plants. Since its initial description on tobacco in 1896 (Erwin and Ribeiro, 1996), it has been reported on 255 plant genera in 90 families, under this name or as *P. parasitica* Dastur (Cline *et al.*, 2008). The binomial species name *Phytophthora nicotianae* was coined first and has priority, but this name implicitly refers to tobacco, one of its numerous hosts, and therefore may be considered restrictive. Conversely, the name *P. parasitica* more accurately reflects the wide host range of this pathogen. So the two denominations are synonyms and are still used interchangeably despite a long debate, awaiting an eventual formal decision from the International Code of Botanical Nomenclature (Erwin and Ribeiro, 1996).

Phytophthora nicotianae has been isolated in multiple ecological niches over the five continents (Meng

et al., 2014). Its range and habitat extend from field crops such as solanaceous plants and numerous vegetables (Prigigallo *et al.*, 2015), to medicinal herbs, watersheds (Hulvey *et al.*, 2010), forest trees (Beever *et al.*, 2009), natural ecosystems, including mountain ecosystems (Vannini *et al.*, 2009), as well as recycling irrigation water systems (Hong and Moorman, 2005). *Phytophthora nicotianae* is one of the most common species in nurseries of potted ornamental and fruit tree species, the trade of which probably represents one of the most efficient dissemination pathways of *Phytophthora* species (Moralejo *et al.*, 2009; Olson and Benson, 2011). This versatility explains why annual economic impacts of *P. nicotianae* worldwide are impossible to estimate, keeping in mind that the real economical impacts must integrate productivity loss, the costs of disease management, and the eventual penalties for growing alternate crops of less value during rotation periods. Among the most notable diseases attributed to this pathogen are brown rot, foot rot, and black shank of tobacco (Gallup *et al.*, 2006). This disease is observed in almost all tobacco growing areas worldwide, and losses may reach 100%. *Phytophthora nicotianae* is also known to cause gummosis and root rot of citrus species (Erwin and Ribeiro, 1996).

A variable lifestyle favouring pathogenicity

Phytophthora nicotianae, generally considered as a root pathogen, possesses a wide host range of herbaceous and woody plants, and causes crown rots, and may attack aerial parts, including stems, leaves and fruits (Figure 1). Like several *Phytophthora* species, *P. nicotianae* is a hemibiotrophic pathogen. It establishes intimate contact with living host cells during the initial stages of infection (biotrophy), before it induces host cell death and grows in dead tissues (necrotrophy). This implies that the pathogen develops distinct, if not apparently contradictory, pathogenic strategies throughout infection cycles, from the suppression of plant basal defenses to the ultimate death of the host.

Phytophthora nicotianae may persist and develop on a broad range of plants and plant organs, but it may also survive outside of hosts. Multinucleate sporangia are produced on specialised hyphal structures (sporangiophores). Depending upon the availability of water and appropriate temperature, sporangia

germinate directly, or produce uninucleate, wall-less zoospores, which each possess two flagella allowing them to swim and reach host tissues through several attraction mechanisms, including chemotaxis and electrotaxis (Walker and van West, 2007). Zoospores are very efficient agents of dissemination as they are easily propagated in soil water, irrigation water and hydroponic solutions (Stanghellini and Rasmussen, 1994). Following host recognition, zoospores de-

velop a cell wall and form cysts, which germinate to develop germ tubes which penetrate plant tissues (Ludowici *et al.*, 2013).

Phytophthora is also able to reproduce sexually, forming thick-walled oospores that result from the fusion of male and female gametangia (Hemmes, 1983). Genetic variation is likely to be generated through this reproduction mode, which could lead to evolution of novel pathogenicity and virulence

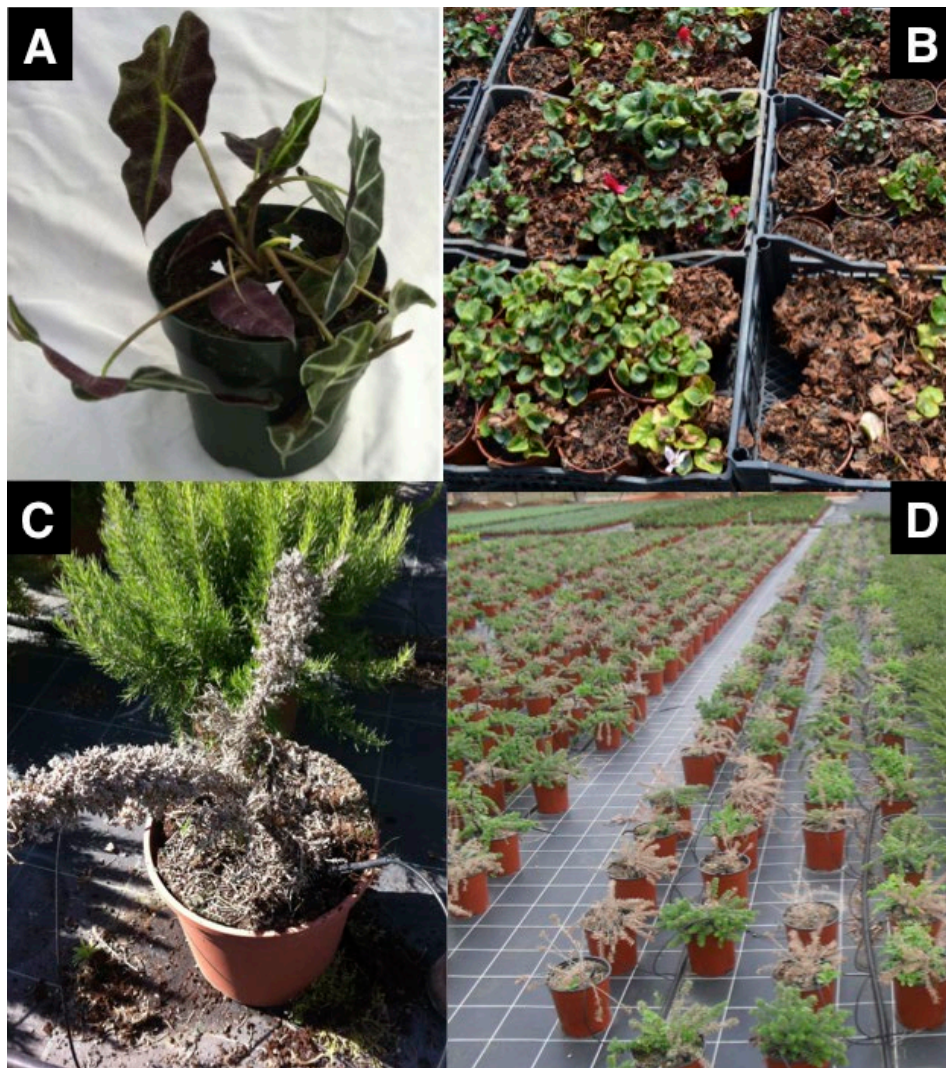


Figure 1. Symptoms associated with *Phytophthora nicotianae* infection on various hosts. (A) *P. nicotianae*-infected *Alocasia amazonica* recovered from an ornamental nursery in Florida. The pathogen initially infects near the collar region leading to the rapid collapse of leaves (indicated by arrows). (B) Disease caused by *P. nicotianae* on cyclamen. (C) Infected rosemary (*Rosmarinus officinalis*), and (D) *Phytophthora* symptoms on *Grevillea lanigera*. Both *P. nicotianae* and *P. cinnamomi* were recovered from these diseased plantlets.

factors. In addition, oospores persistent in soil for years, playing important roles in the epidemiology of *Phytophthora* diseases (Weste, 1983). Oospores generally form sporangia which release zoospores into soil water (Erwin and Ribeiro, 1996). Alternatively, oospores directly produce germ tubes that infect host plants.

Phytophthora nicotianae is a heterothallic species, requiring the co-occurrence of A1 and A2 (opposite) mating types for oospore production. The relative frequency of A1 and A2 isolates is often biased in nature (Andres *et al.*, 2003; Cohen *et al.*, 2003; Taylor *et al.*, 2008; Nerkar *et al.*, 2012), suggesting that the role of oospores in the aetiology of diseases caused by *P. nicotianae* may be less important than generally considered, and that the threat of emergence of new virulences generated by sexual reproduction may be overestimated.

Phytophthora nicotianae also produces chlamydospores, under unfavorable conditions of temperature and moisture (Erwin and Ribeiro, 1996). These thick-walled, asexual structures are responsible for long-term survival in soil and plant tissues for months to years. Like oospores, chlamydospores are assumed to be dormant in low temperature conditions, and are induced to germinate as the temperature increases. Oospores and chlamydospores allow *P. nicotianae* to overwinter in rhizospheres of host plants. Chlamydospores also constitute a dissemination source of inoculum, as they may be dispersed by irrigation water, rainsplash and soil movement (Thomson and Allen, 1976). Like oospores, they survive in gastrointestinal tracts and faeces of various animals, such as birds, termites or snails, and may be further dispersed by these vectors (Weste, 1983; Alvarez *et al.*, 2009a).

The diversity of propagules is arguably more important, considering that most of our knowledge on *Phytophthora* life cycles comes from studies performed in laboratory conditions, which cannot reproduce all environmental conditions encountered by the pathogen in nature. Complex organs or structures such as stromata, also occurring in true fungi, or atypical chlamydospores, have been reported in several *Phytophthora* species, including *P. nicotianae* (Lutz and Menge, 1991; Moralejo *et al.*, 2006; Crone *et al.*, 2013). The physiology of *Phytophthora* in natural conditions undoubtedly constitutes an important field of investigation to provide increased understanding of the epidemiology of the diseases it causes.

Population structure and host specificity

Despite the particularly wide host range of *P. nicotianae*, there are considerable differences in aggressiveness towards particular plants among natural populations, and there is much evidence of host specialization among isolates collected on various plants (Bonnet *et al.*, 1978; Colas *et al.*, 1998; Taylor *et al.*, 2012; Biasi *et al.*, 2016; Kamoun *et al.*, 2015). The boundaries of the species have long been a matter of debate, with emphasis on the status of tobacco isolates previously described as *P. parasitica* var. *nicotianae*, awaiting their possible elevation to a valid species (Erwin and Ribeiro, 1996). Nevertheless, the population structure of *P. nicotianae* remains poorly defined. Restriction Fragment Length Polymorphism (RFLP) analyses performed on mitochondrial DNA (Lacourt *et al.*, 1994) and nuclear DNA (Colas *et al.*, 1998) generally failed to draw genetic substructures on the basis of host plants. Yet, isolates associated with black shank of tobacco and recovered outside Australia may be distinguished from isolates collected on other hosts (Colas *et al.*, 1998). In agreement with pathogenicity tests, recent analyses conducted with mitochondrial and nuclear single nucleotide polymorphisms (SNPs) revealed a specific association between host of origin and genetic grouping (Mammella *et al.*, 2011; Mammella *et al.*, 2013). This was particularly true for citrus isolates collected from various world locations that clustered in the same mitochondrial group and shared at least one nuclear allele (Figure 2). A similar association was also observed for isolates recovered from *Nicotiana* and *Solanum* spp. These findings were partially confirmed using microsatellite markers (Biasi *et al.*, 2016). This association was less evident for isolates collected in nurseries (Biasi *et al.*, 2016), which also present greater levels of heterozygosity, and relative equilibria between A1 and A2 mating types. In contrast, clonal reproduction is hypothesized in tobacco fields and in citrus groves (Mammella *et al.*, 2013; Biasi *et al.*, 2016), consistent with the prevalence of single mating types in various surveys, with the identification of A1 on citrus and A2 on tobacco (Cohen *et al.*, 2003; Lamour *et al.*, 2003; Parkunan *et al.*, 2010). Similarly, the A2 mating type was found to be dominant in potato fields (see Taylor *et al.*, 2012 and below).

A significant geographical genetic structure was observed only for isolates from tobacco (sourced from Australia and United States) and from *Citrus maxima* (sourced from Vietnam), while a typical panmictic distribution characterized the majority of isolates including those from other *Citrus* species (Mammella *et al.*, 2013; Biasi *et al.*, 2016). Such structure may have originated in the different propagation and cultivation systems for these crops. Isolates obtained from potted ornamental and citrus (except pommelo) were likely to be disseminated worldwide with infected plant material (mainly potted plants). Conversely, tobacco is propagated by seeds that do not contribute to the spread of the pathogen. In addition, plantlets are very rarely transplanted in areas distant from those in which they have been produced. As regards to *C. maxima*, this species is a native plant of Vietnam, and plant material was not introduced from other countries, suggesting a specific co-evolution of host and pathogen.

The absence of geographic structures for most *P. nicotianae* populations and the concurrent existence of significant structures in relation to hosts or origins could be indicative of extensive migration via plant material or host adaptation. Although currently available data are limited, *P. nicotianae* isolates may have been spread worldwide with infected plant material and, afterwards, lineages may have progressively diverged. Additional molecular analyses and specific pathogenicity tests are necessary to determine whether recently determined molecular groups can be taken as evidence of physiological races, pathotypes or even subspecies. To this aim, an international initiative was instigated aiming to enable a comparative analysis of genes that determine host range in this cosmopolitan species [*Phytophthora parasitica* INRA-310 Sequencing Project, Broad Institute of Harvard and MIT (<http://www.broad-institute.org/>)]. This initiative aimed to create a high quality draft sequence of a *P. nicotianae* strain exhibiting a particularly broad host range (Attard *et al.*, 2008), and the identification of SNPs from additional isolates of diverse host range and geographical origins. This project is currently providing information and tools for increased understanding of the genetic structure and the evolution of *P. nicotianae* populations worldwide, in order to facilitate disease management. Furthermore, the availability of genomic assemblies of 14 different isolates of *P. nicotianae* enabled the selection and validation of highly polymor-

phic microsatellite markers that represent a valuable new tool for the characterization of *P. nicotianae* populations (Biasi *et al.*, 2015).

***Phytophthora nicotianae*: a renewed plague in a changing world**

World agriculture has undergone major changes as a conjunction of several factors, including globalization and intensification of international trade, new farming practices and climate change (Popkin, 1999). Such changes have immediate impacts on plant diseases, with the spreading of pathogens and the modification of their niches. Meanwhile, *P. nicotianae* is gaining importance in agriculture and plant health worldwide, a fact that may be considered to be resulting from global change. In some cases, this may rather reflect the natural evolution of pathogen populations. Nevertheless, it becomes apparent that anthropogenic activities favour the current development of *P. nicotianae*. As an example, the generalized westernization of diets worldwide provokes a shift from local staples towards temperate fruits and vegetables (Pingali, 2007), most of these crops being hosts for *P. nicotianae*. The rise of *P. nicotianae* worldwide is documented by a number of facts, such as its association with other pathogens resulting in enhanced damaging symptoms, the emergence of diseases on new hosts or in new geographical locations, the replacement of primary pathogens by *P. nicotianae*, as well as cases of interspecific hybridization. In addition, there are direct correlations between outbreaks of *P. nicotianae* diseases and changes in environmental conditions. These topics constitute the following paragraphs of this paper.

Associations of *Phytophthora nicotianae* with other pathogens

As a general soilborne pathogen, *P. nicotianae* affects root system development of plants and increases their vulnerability to harsh environmental conditions. The pathogen may then act as a predisposing factor and may be associated with diseases caused by other more devastating pathogens, as in the case for walnut decline observed in Europe (Belisario *et al.*, 2003) and California (Matheron and Mircetich, 1985). On the other hand, the association of *P. nicotianae* with other pathogens may cause unprecedented

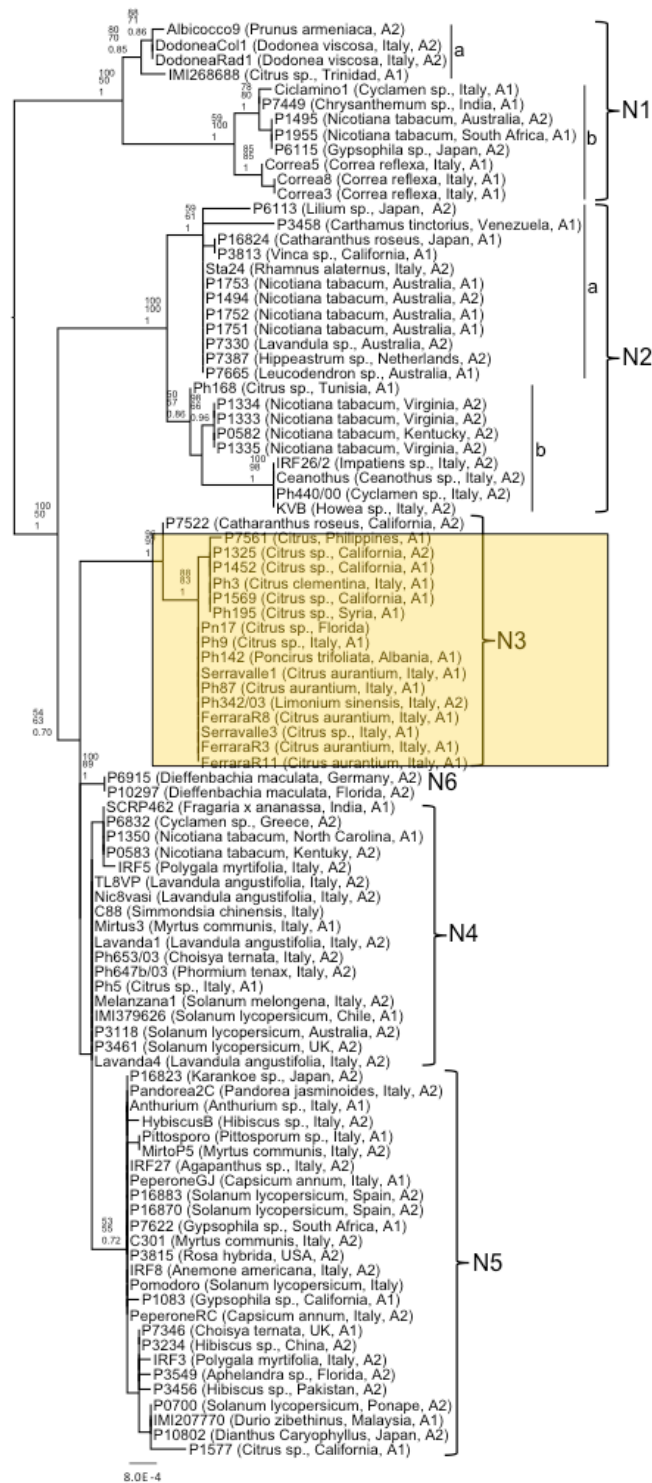


Figure 2. Phylogenetic relationships among isolates of *Phytophthora nicotianae* based on the combined dataset of sequences of the four mitochondrial regions (trnG-rns, rns-cox2, cox2+spacer, atp1-nad5), using maximum parsimony, maximum likelihood, and Bayesian methods. Adapted from Mammella *et al.*, (2013). Citrus isolates form a distinct clade, illustrated by the yellow rectangle.

diseases escaping traditional management strategies, exemplified by the case of “new” citrus diseases.

Phytophthora gummosis

Citrus constitutes the greatest value fruit crop in terms of international trade. Yet, this crop is highly vulnerable to disease outbreaks, including those caused by *Phytophthora*. Up to ten species have been described on citrus, three of which cause the most severe damage worldwide: *P. nicotianae*, *P. citrophthora* and *P. palmivora* (Erwin and Ribeiro, 1996). *Phytophthora nicotianae* is arguably the main *Phytophthora* pathogen on citrus, as it causes root and foot rot, gummosis, and may also attack the host canopies, essentially through water splash (Cacciola and Magnano di San Lio, 2008).

In warm climates like Florida or Southern Asia, *P. nicotianae* may be associated with *P. palmivora* (Graham *et al.*, 1998; Drenth and Guest, 2004). In Mediterranean climates in the northern hemisphere, it is frequently associated with *P. citrophthora*, which is predominant, particularly causing branch cankers (Cohen *et al.*, 2003; Alvarez *et al.*, 2011). Seasonal fluctuations are observed, and incidence of *P. citrophthora* is more important under cold conditions (Spring), while root infections due to *P. nicotianae* are more severe in summer and early autumn (Alvarez *et al.*, 2009b; Dirac and Menge 2003). Optimal growth temperatures for *P. nicotianae* are greater than for *P. citrophthora*, and this difference was frequently suggested to explain observed seasonal and geographical variations in relative distribution and incidence of these species (Timmer *et al.*, 1989). However, other factors are likely contribute to this phenomenon (Dirac and Menge, 2003). Both species may be found together in the same orchards, although it is difficult to draw conclusions on the possible influence of season or temperature on their relative rates of recovery (Cohen *et al.*, 2003; Meitz-Hopkins *et al.*, 2013). *Phytophthora nicotianae* has been found as the dominant species in Brazil, Egypt (Ahmed *et al.*, 2012), South Africa (Meitz-Hopkins *et al.*, 2013) and Tunisia (Kuhn and Panabières, unpublished results). *Phytophthora nicotianae* citrus diseases have been well-controlled with fungicides, resistant rootstocks, and sanitary practices (Vernière *et al.*, 2004), until recent observations of associations with other pathogens.

The *Phytophthora-Diaprepes* (PD) complex

Diaprepes abbreviatus is a polyphagous root weevil that was introduced into Florida from the Caribbean Basin, then dispersed in several citrus areas through contaminated nursery stock (Graham *et al.*, 2003). Larvae feed on roots of all rootstocks usually grown for citrus production. Eventually, roots are girdled by the weevil, causing the death of the trees. In addition, the injured roots become predisposed to *Phytophthora* infections (Graham *et al.*, 2003). This is particularly true for trees on rootstocks such as sour orange and “Cleopatra mandarin”, which are susceptible to *P. nicotianae*. The development of the resulting *Phytophthora-Diaprepes* (PD) complex is then difficult to control, and strategies for controlling *Diaprepes* must integrate *Phytophthora* control (Graham *et al.*, 2003).

The Huanglongbing (HLB) syndrome

Other pathogen complexes involving *P. nicotianae* may be more challenging than PD worldwide, such as the association with the Huanglongbing (HLB). This disease, otherwise designated as citrus greening or yellow dragon disease (the English translation of “huang long bing”), is one of the oldest citrus diseases, as it has been known in India and East Asia since 1919 (Bové, 2006). However, HLB remained largely neglected for decades, mainly because of its restricted geographic distribution to Asia and Africa. The simultaneous discovery of HLB symptoms, and the identification of its causal agents in the mid-2000s in Brazil and in Florida, which are the two most important citrus growing areas in the world, led to renewed interest in this disease (Bové, 2006). Since the first observations in the western hemisphere, HLB has been found in several regions in the United States, as well as South and Central America. The disease affects most of the main citrus growing areas worldwide, with the exception of the Mediterranean Basin, Australia and Japan (Wang and Trivedi, 2013).

HLB is transmitted by psyllid vectors, but also by grafting. It is caused by three unculturable, Gram negative bacteria belonging to the genus *Candidatus Liberibacter*, designated as *Candidatus Liberibacter africanus* (CaLaf), *Ca. L. asiaticus* (CaLas) and *Ca. L. americanus* (CaLam, Gottwald, 2010). The disease causes vascular decline, reduces fruit size and other fruit quality parameters, and ultimately kills affected trees (Graham *et al.*, 2013). No sources of HLB resist-

ance in citrus scions or rootstocks are known and no efficient management programme is currently available to control HLB, so crop losses may reach 100% locally (Graham and Feichtenberger, 2015). Furthermore, the bacteria infect all parts of host trees, including roots, causing symptoms that are not early detectable, substantially affecting the root health, with a potential impact on the fate of interactions with other soilborne pathogens, such as *P. nicotianae*. As reported some years before in the case of the PD, prior infection of citrus roots with CaLas accelerates *Phytophthora* infection. The CaLas-induced predisposition to *P. nicotianae* apparently results from greater attraction of zoospores to roots, and/or a breakdown of the defense system (Graham *et al.*, 2011). The HLB agent-*Phytophthora* interaction was shown to promote higher levels of root damage than each pathogen alone (Graham *et al.*, 2013). In addition, a survey conducted from 2008 to 2011 revealed a trend for increasing soil populations of *Phytophthora* in HLB prevalent areas, irrespective of grove location, rootstock and management practices (Graham *et al.*, 2012).

Initial HLB control strategies focused on initiatives to control psyllid vectors, and the removal of infected trees (Graham *et al.*, 2012). To avoid tree removal, and to keep trees infected with HLB alive as long as possible, the citrus industry developed enhanced nutritional programmes to sustain tree health (Graham *et al.*, 2012). The finding that *P. nicotianae* is a very significant component in the HLB syndrome requires revisiting of citrus management programmes, as there are not one, but at least two pathogens, to control, with distinct epidemiological histories. Moreover, the current management variables developed independently for each pathogen (rootstock selection, biological control, nature and pH of soils) have to be compatible for integrated management of the HLB-*Phytophthora* complex. As previously observed for DP, adding chemicals directed against *Phytophthora* in HLB control programmes brought an unexpected rise in cultural costs (Duncan *et al.*, 2014). In addition, phosphite-based chemicals that are generally used to induce host resistance to *Phytophthora* may be inefficient on roots, whose resistance has been already compromised by HLB infection (Graham *et al.*, 2011). Therefore, the use of chemicals acting directly on *P. nicotianae*, such as mefenoxam-based fungicides, is required to reduce disease symptoms. Such a management strategy has two drawbacks: it is expensive and it may become

ineffective in mid- to long-term perspectives, with the emergence of pesticide-resistant populations. Metalaxyl resistance was shown to be common in *P. nicotianae* populations collected from citrus areas in Florida (Timmer and Graham, 1998). Isolates showing some level of insensitivity to the fungicide were found, even in groves where the history of metalaxyl was unknown, suggesting that either resistant or insensitive strains spontaneously occur among natural populations of *P. nicotianae*, or that these strains have their origin in citrus nurseries where metalaxyl is extensively used, and were further disseminated into groves, limiting the efficacy of chemical treatments.

The associations between *P. nicotianae* and the Diaprepes root weevil and HLB have led to the emergence of new pathosystems and make development of integrated management programmes essential. However, one of the main threats that currently impact citrus production and that appears as an emerging disease results from the conjunction of two of the longest recognized pathogens that have been reported on this crop. Beyond any historical consideration, particular attention has to be taken on locations still free of HLB, in particular the Mediterranean Basin which is an important citrus production area, and which is currently undergoing significant environmental and climatic changes.

***Phytophthora nicotianae* as an emerging pathogen: the case of potato**

Phytophthora nicotianae has been sporadically reported to cause foliar blight and tuber rot of potato over the past 75 years, but was generally considered of minor importance (Taylor *et al.*, 2015). Tuber rot caused by *P. nicotianae* is very similar in symptomatology to the tuber disease referred to as pink rot, the principle cause of which is *P. erythroseptica*. Recent, repeated recoveries of *P. nicotianae* from pink rot-symptomatic tubers from the US states of Nebraska, Florida, Missouri, Texas, and Michigan, and from foliage with late blight-like lesions in Nebraska, Missouri and Texas, led to recognition of *P. nicotianae* as an important component of the tuber rot and foliar disease complex in a number of States (Taylor *et al.*, 2008; Taylor *et al.*, 2012, Figure 3). This suggests that *P. nicotianae* may be an emerging pathogen, in the current context of global change.

The optimal temperature for growth and infection of *P. nicotianae* isolates affecting potato is between 33

and 36°C, whereas *P. erythroseptica* is completely inhibited at these temperatures (Grisham *et al.*, 1983; Taylor *et al.*, 2008). *Phytophthora nicotianae* has been regarded as a potato pathogen confined to southern, warmer potato production areas. Global warming may therefore favour *P. nicotianae* diseases on potato. However, cases of tuber rot and foliar blight due to *P. nicotianae* were also detected in Nebraska and Michigan, above the 41st parallel, indicating that this pathogen is geographically becoming more widespread, and its impact on potato production may be increasingly widespread.

Recent research has developed substantial knowledge of the aetiology of this disease (Taylor *et al.*, 2008; Taylor *et al.*, 2012; Taylor *et al.*, 2015). All *P. nicotianae* isolates, regardless of geographic and tissue origin, were able to infect potato tubers and leaves (Taylor *et al.*, 2008). However, *P. nicotianae* was less aggressive on tubers than *P. erythroseptica* and less aggressive on foliage compared to *P. infestans* (Taylor *et al.*, 2008). Unlike *P. infestans*, the pathogen is incapable of sporulation and secondary inoculum development, a factor likely to affect aetiology and epidemiology of the foliar disease phase.

Unlike on other hosts, the A2 mating type of *P. nicotianae* largely predominates among populations of potato isolates regardless of location (Taylor *et al.*, 2008; Taylor *et al.*, 2012). As rare exceptions, both mating types have been recovered in a single field in Nebraska and a single field in North Texas. A1 isolates have only been recovered from foliage, although they are capable of infecting tubers and cause rots (Taylor *et al.*, 2008; Taylor *et al.*, 2012). Despite the prevalence of mefenoxam resistance in isolates of *P. nicotianae* recovered from other plant species (Timmer and Graham 1998; Hu *et al.*, 2008), all of the more than 100 isolates recovered from potato have been found to be sensitive to this fungicide (Taylor *et al.*, 2008; Taylor *et al.*, 2012). *Phytophthora nicotianae* isolates recovered from potato are significantly more aggressive on this plant compared to *P. nicotianae* isolates recovered from other hosts (Taylor *et al.*, 2012), suggesting potential host specialization.

The inability of *P. nicotianae* to sporulate on potato foliage posed questions of disease aetiology (Taylor *et al.*, 2008). Large areas of potato fields could be affected with foliar blight with stems becoming girdled and plants becoming completely defoliated (Taylor, *et al.*, 2008). These areas are usually clustered around irrigation wheel tracks of centre pivot irriga-

tors and in low areas of fields. Recently studies have demonstrated that soil infestation accompanied by water splash dissemination, either from irrigation water or rain, are the likely means by which infections of this potato pathogen are initiated (Taylor *et al.*, 2015). Grower recommendations for disease management may therefore be organized around water management practices, early applications of foliar fungicides, usually mefenoxam-based, and strategies otherwise developed to control pink rot and other *Phytophthora* diseases. These include planting into well-drained soils and avoiding excessive irrigation (Taylor *et al.*, 2015). This well-documented work demonstrates that all cases of *P. nicotianae* on potato reported over decades have to be carefully considered, and that monitoring programmes of potato diseases would integrate *P. nicotianae* as an important component of the *Phytophthora*-induced diseases on potato. In addition, the situation of potato diseases may participate to a more general phenomenon of the gaining incidence of *P. nicotianae* worldwide.

Physiological advantages of *Phytophthora nicotianae* over competitors/primary pathogens

Plants are generally susceptible to a range of pathogens, and overlapping host ranges are frequently observed with *Phytophthora*. However, the relative incidence and disease severities greatly vary from one species to another, depending on a range of variables, such as pathogenic strategies (including biotrophy vs. necrotrophy, airborne vs. soilborne) and environmental parameters including geographical location, soil status or cultural practices. On the basis of prevalence, incidence and severity of disease symptoms, *P. nicotianae* may be locally and/or temporarily a secondary pathogen behind other pathogens that generally display more restricted host ranges, like *P. infestans* on tomato, *P. capsici* on pepper, *P. citrophthora* or *P. palmivora* on citrus, *P. cactorum* on fruit trees (Erwin and Ribeiro, 1996), and *P. erythroseptica* on potato (Taylor *et al.*, 2008). Nevertheless, *P. nicotianae* displays several characteristics that may allow it to out-compete these other *Phytophthora* species.

Optimum temperature

Prior to the emergence of molecular techniques, traditional criteria for *Phytophthora* species identifi-

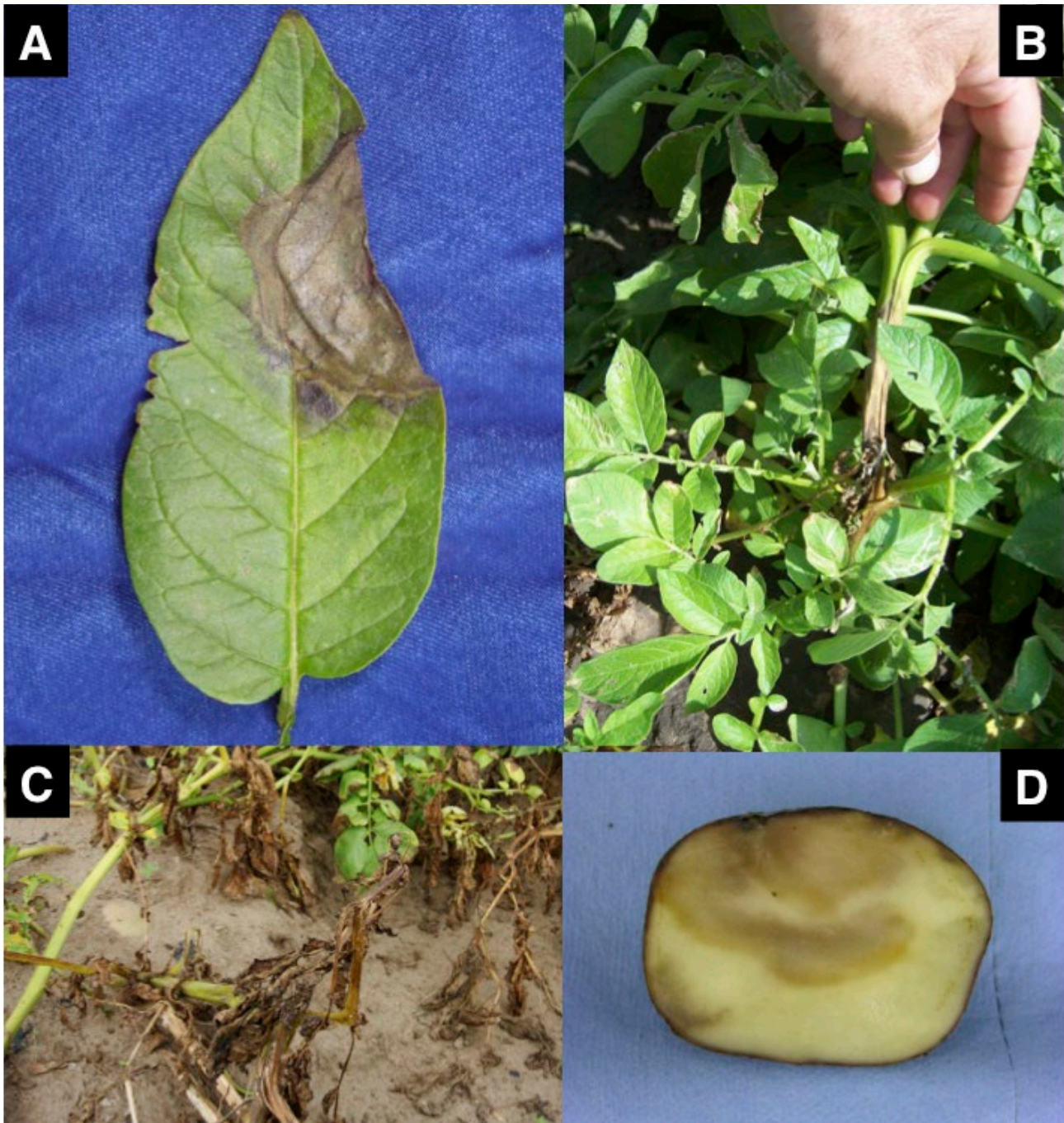


Figure 3. Symptoms associated with *Phytophthora nicotianae* infection on potato. (A) Leaflet field infection; (B) stem infection; (C) foliar infection; (D) tuber infection.

cation included morphological description of reproduction structures, the sexual status (homothallism vs. heterothallism) and optimum growth tempera-

tures (Erwin and Ribeiro, 1996). Optimum temperature may vary within a species, so that a range of optimum temperatures for growth is generally defined.

Alternatively, the optimum growth temperature is designated relative to a given host (Erwin and Ribeiro, 1996). Optimum temperatures for *P. nicotianae* are generally higher, by a few degrees, than for its main competitors (Erwin and Ribeiro, 1996). *Phytophthora nicotianae* may thus out-compete all these pathogens under warm conditions. Its prevalence may occur during warm seasons, or in warm production areas, as has been increasingly observed throughout the world. Current global warming may then constitute a strong catalyst for re-appraisal of the importance of *P. nicotianae* in a range of crop production systems, and this pathogen may replace primary pathogens that are less adapted to new environmental conditions.

Epidemiological advantages

Numerous studies highlight the importance of water management in the spreading of *Phytophthora* diseases, among which are those caused by *P. nicotianae*. Propagules of *P. nicotianae* such as zoospores and chlamydospores are frequently recovered from irrigation water and are easily disseminated in non-infested areas (Stanghellini and Rasmussen, 1994; Bush *et al.*, 2003). Compared to other *Phytophthora* species, *P. nicotianae* releases zoospores following very short periods of flooding, and these propagules remain motile for up to 20 h (Thomson and Allen, 1976). This allows *P. nicotianae* to colonize substrates or reach potential hosts more rapidly than many competitors. In addition, zoospores may form viable structures, such as cysts, appressorium-like structures, microsporangia, and hyphal fragments produced from cysts, that have been shown to survive in irrigation water up to 60 d (Thomson and Allen, 1976). Zoospores are also disseminated by splashing caused by irrigation or rainfall and reach the aerial parts of host plants. The rapid stimulation of zoospore release by short periods of contact with water and their subsequent dispersal by water splash may contribute to the success of plant infection.

Chlamydospores constitute an important means of long-term survival in the absence of host plants. Survival for up to 6 years has been reported for *P. nicotianae* chlamydospores (Gallup *et al.*, 2006). This confers *P. nicotianae* an ecological advantage over potential competitors such as *P. capsici* or *P. infestans*, which do not produce chlamydospores (Allagui *et al.*, 1995; Andres *et al.*, 2003; Judelson and Blanco, 2005).

These basic characteristics (higher optimum temperature, longevity and dispersal capacity of asexual structures) offer *P. nicotianae* a substantial advantage, which may partially explain the accumulating cases of prevalence of this pathogen, sometimes on new hosts and in a range of geographical regions.

Reports of *Phytophthora nicotianae* prevalence worldwide

In India and Southern Asia, *P. nicotianae* is one of the main pathogens among *Phytophthora* spp., and its hosts include, besides citrus and tobacco, betel, black pepper, brinjal (eggplant), coconut, durian, guava, orchids, periwinkle, pineapple, roselle, rubber and vanilla (Guha Roy and Grünwald, 2014). In other areas, the pathogen is recovered from new hosts otherwise infected by other *Phytophthora* species. *Phytophthora nicotianae* has been reported recently in Cuba causing root rot of avocado, while *P. cinnamomi* was thought to be the sole pathogen on this crop (Machado *et al.*, 2013). In South Africa, it is progressively replacing *P. cinnamomi* in eucalypt plantations (Nagel *et al.*, 2013). In southern States of India, including Kerala, it now affects coconut (Anandaraj, 2012) and in Eastern India, black pepper (Guha Roy *et al.*, 2009). Until the last decade, coconut was only reported to be attacked by *P. palmivora*, and black pepper by *P. capsici* (Chowdappa *et al.*, 2003).

Phytophthora cactorum has been frequently identified as a causal agent of apple tree dieback worldwide (Erwin and Ribeiro, 1996), and was prominent in Tunisian apple orchards until recently (Boughalleb *et al.*, 2006). However, current surveys conducted in that country reveal the association of *P. nicotianae* along with various *Pythium* spp. with the disease, while *P. cactorum* was no longer detected (Souli *et al.*, 2014). *Phytophthora nicotianae* was reported to be highly aggressive on a range of apple cultivars (Souli *et al.*, 2014).

Phytophthora nicotianae has also been identified as a particularly aggressive pathogen on hot (chill) pepper in Tunisia, while *P. capsici*, the primary pathogen in numerous pepper-growing areas, has not been detected in that country and probably not in Algeria (Allagui *et al.*, 1995). Climatic conditions encountered in North Africa could explain this particular association, as *P. capsici* is likely to be more adapted to humid regions whereas *P. nicotianae* prefers irrigated, warm and arid areas like the coastal zone of Tunisia. Tunisian isolates collected on pepper appear

to be specialized towards this host, and were proposed to constitute a new *forma specialis*, *P. nicotianae* f. sp. *tunisiana* (Allagui and Lepoivre 2000; Trabelsi *et al.*, 2007). The hypothesis of genetic isolation of these strains is reinforced by the observation that Tunisian *P. nicotianae* isolates collected on pepper has A1 mating types, and on apple has A2 types (Kuhn and Panabières, unpublished results). The aerial parts of the plants is generally not infected, and inoculation assays conducted on a range of artificial conditions failed to produce symptoms on leaves or stems (Allagui and Lepoivre, 1996). This suggested that Tunisian pepper isolates developed specific pathogenicity determinants to attack pepper roots. The origin of these strains is unknown, but they are clearly different from those collected on pepper in Northern Spain, which are essentially A2 isolates (Andres *et al.*, 2003), ruling out the hypothesis of a single lineage spreading over the various pepper-growing areas of the Mediterranean Basin.

Nursery propagation and pathogen dissemination

The nursery trade, with particular emphasis on potted ornamentals, is particularly exposed to *Phytophthora* infections, as a consequence of its globalization, intensive cultivation techniques and frequent turnover of new varieties and/or species. Furthermore, ornamentals are especially susceptible to *Phytophthora*, probably because they represent artificial ecosystems grown under conditions that increase their vulnerability. Numerous studies have revealed many *Phytophthora* species in nurseries and garden centers, of which *P. nicotianae* was frequently predominant (Schwingle *et al.*, 2007; Moralejo *et al.*, 2009; Ahmed *et al.*, 2012; Leonberger *et al.*, 2013; Bienapfl and Balci, 2014). In addition, *P. nicotianae* is frequently associated with asymptomatic plants, or may be baited from soilless potting media (Bienapfl and Balci, 2014), so that its importance in ornamental production is probably underestimated. Increased genetic differentiation observed among *P. nicotianae* isolates recovered from nurseries suggests an important role of this production system in the evolution of *P. nicotianae* by increasing the likelihood of genetic recombination (Mammella *et al.*, 2013). Many different plant species are commonly grown together in nurseries, favouring the co-occurrence of *P. nicotianae* isolates with opposite mating types,

and even distinct *Phytophthora* species. Nurseries may then be considered as cauldrons of “primordial pathogenic soups” containing emerging populations whose pathogenic potential is unknown. In addition, this industry is shaped by aesthetical prerogative that require extensive use of fungicides or fungistatic products. These compounds may temporarily mask symptoms but may not hamper the subsequent spread of pathogens, or may promote the dissemination of pesticide-resistant strains (Brasier, 2005). Moreover, massive, non selective destruction of stock is sometimes the sole alternative to disease outbreaks in nurseries. To avoid important financial losses and destruction of healthy stock, only visible diseased plants are destroyed, so that the health status of nurseries with regard to *Phytophthora* diseases may be over-estimated. Furthermore, considering that the raison d’être of nursery plants is mainly to move them into horticulture, agriculture, forests and other areas such as citizen environments, the main consequence of probable gene flow within nursery isolates is the dissemination of new genotypes in a wide range of environments. *Phytophthora nicotianae* can therefore be considered an ideal species to study diffusion pathways of soilborne pathogens since its polyphagy and relative abundance in nurseries of potted ornamentals and fruit tree species may promote its rapid dissemination on a global scale.

In a recent study conducted to assess *Phytophthora* diversity in Italian nurseries *P. nicotianae* was by far the most abundant species, and was detected on several new hosts unreported to date (Prigigallo *et al.*, 2015). A high number of ITS1-based sequence types (STs) were characterized, most of them being shared with isolates of worldwide origin and from diverse hosts. These observations suggest multiple introductions and extensive dissemination of *P. nicotianae* in ornamental production with infected plants. These results, contrasting the clonal structure detected in field cultures, pinpoint the complexity of *P. nicotianae* dynamics at the level of natural populations.

Based on the fact that nursery production and international trade is rising exponentially, scenarios concerning the consequences on agriculture and environment are pessimistic. The increasing movement of plants is expected to be accompanied by invasion of new niches by *P. nicotianae*, with the possible contact with other pathogens, including resident *P. nicotianae* populations with distinct evolutionary histories. Inappropriate practices in nursery production

may jeopardize a large range of agrarian cultures in many areas in the near future, making disease control more challenging. In addition, nurseries and hydroponics were suspected to be at the origin of a new challenge in horticulture, with the emergence of *Phytophthora* interspecific hybrids.

The ultimate survival strategy: reaching new hosts through interspecific hybridizations

The first cases of interspecific hybridizations involving *Phytophthora* were reported on ornamentals grown in hydroponic greenhouse systems (Man In 't Veld *et al.*, 1998). The parents were unambiguously identified as *P. nicotianae* and the homothallic *P. cactorum* (Bonants *et al.*, 2000). Such hybrids were later recovered on loquat trees in Taiwan and Peru, then on various hosts in Europe (Faedda *et al.*, 2013; Szigethy *et al.*, 2013). Molecular analyses indicate that the hybrids resulted from independent hybridization events (Chern *et al.*, 1998; Hurtado-Gonzales *et al.*, 2009). They are formally described now as *Phytophthora* × *pelgrandis* (Nirenberg *et al.*, 2009). As a rule, biparental inheritance of nuclear genes has been observed, whereas mitochondrial DNA is inherited from *P. nicotianae* (Man In 't Veld *et al.*, 1998; Bonants *et al.*, 2000), indicating that hybrids originated from sexual hybridization.

Interspecific hybridizations have been identified between related *Phytophthora* species belonging to various phylogenetic clades (Brasier *et al.*, 1999; Ioos *et al.*, 2006; Goss *et al.*, 2011; Man In 't Veld *et al.*, 2012; Bertier *et al.*, 2013; Nagel *et al.*, 2013), providing evidence that this phenomenon is common in the genus (Erzek and Man In 't Veld, 2013). They were first associated with horticultural crops in nurseries, suggesting that cultural practices (hydroponics, concentration of different hosts) would favour hybrid development (Man In 't Veld *et al.*, 2007; Man In 't Veld *et al.*, 2012). Due to prevalence of *P. nicotianae* in these cultures, the pathogen would be particularly suitable for interspecific hybridization. Some hybrids are important pathogens, like *Phytophthora* × *alni* which results from sequential hybridization events, and is responsible for alder decline in Europe (Husson *et al.*, 2015). As for other hybrids, *Phytophthora* × *alni* is believed to have occurred in nurseries, before being disseminated in natural ecosystems (Brasier *et al.*, 2004). Other hybrids have been isolated during

surveys of rivers, streams and natural waterways (Nagel *et al.*, 2013; Burgess, 2015). Their host ranges and pathogenicity are not documented to date, but they were recovered in high frequencies, indicating that human activity is not the sole factor bringing related species together and affecting generation of interspecific hybrids (Burgess, 2015).

The impacts of hybrids on agriculture, forestry and natural environments is still difficult to predict, but they constitute serious threats. The accumulating cases of hybridizations implying various described species presumably result from improved knowledge of the *Phytophthora* genus, and from increased surveys, now that hybrids can reasonably be expected to occur in most ecosystems (Erzek and Man In 't Veld, 2013; Burgess, 2015). They may also illustrate the current pace of *Phytophthora* evolution in nature, which has probably accelerated through human activities. Whether all species can hybridize is unknown, but some of them are more disposed than others. Among these candidates, *P. nicotianae* is particularly important, because of its prevalence in crops that are subject to extensive trade and international dissemination.

Phytophthora nicotianae and global climate change

Climate change is proposed as one of the biggest threats to agriculture worldwide (Gautam *et al.*, 2013). It is clear that human activity influences the global climate system more than before, which in return drives the adaptation of farming practices in a never-ending adaptive process. The exact impacts of global change on agriculture cannot be predicted accurately, because of the diversity of local situations. The consequences of climate change will be more important in countries heavily dependent on agriculture, which are mainly less developed countries, already facing other major challenges such as demographic expansion, and food security. Immediate consequences are water and arable land availability, leading to the clearing of forests for agriculture and livestock and influencing global carbon balance (Chakraborty *et al.*, 2000). Climate change is thus interrelated to other major issues, which together will impact both on crop production and the incidence of plant diseases. The effects of such changes on plant diseases are dependent on the nature of the hosts and the lifestyles of pathogens. Consequently positive,

negative or neutral consequences can be anticipated, according to the crops (Chakraborty *et al.*, 2000).

It is generally recognized that the most significant effects of climate change will result in increased emissions of carbon dioxide (CO₂), a general rise in temperature, and large changes in precipitation (Coakley *et al.*, 1999). The imbrication of these different factors generates complex interactions, and modifies the physiology both of plants and pathogens. The impacts of warming on plant diseases, and especially those caused by *P. nicotianae* are likely to be multiple.

First, we have discussed above how *P. nicotianae* is better adapted to a predicted mean temperature increase of few °C than its competitors. Warming may also cause shifts of agroclimatic zones to new locations. We can expect that pathogens, including *P. nicotianae*, will migrate with their hosts, possibly entering in contact with natural plant populations and expanding their host ranges. A direct consequence of host range expansion is that relying on crop rotation to control *P. nicotianae* diseases will become more problematic, as it will be difficult to identify non-host crops of economic value. Warming also implies that agronomic activities will require increased large scale irrigation practices, exacerbating the risks of diseases due to survival and migration of *P. nicotianae* propagules, as confirmed by current observations conducted on pepper production in Tunisia. North Africa is becoming warmer and drier, but agronomic activities are increasing to meet increasing food demand for increasing human populations. About 95% of available water in Tunisia is collected and distributed. Daily temperatures sometimes exceed 44°C. Despite these extreme conditions, severe damage caused by *P. nicotianae* has been observed in irrigated pepper fields. Droughts are also predicted to alternate with heavy rains due to climate warming. This will cause soil waterlogging, which will also favour dissemination of soilborne pathogens, among them *P. nicotianae*.

Warming also directly impacts plants and pathogens. An increase in temperature has been shown to induce susceptibility of soybean lines carrying resistance genes to *P. sojae* (Gijzen *et al.*, 1996), and tobacco to *P. nicotianae* (Sanden and Moore, 1978). Roots of hydroponically grown *Chrysanthemum* plants were more severely attacked by *P. cryptogea* following exposure to high temperatures (McDonald, 1991). On the other hand, recent *P. infestans* clonal lineages appear to have a fitness advantage at

warmer temperatures than other lineages, with direct influence on disease severity (Seidl Johnson *et al.*, 2015). Increasing temperature may also constitute severe abiotic stress both to plants and pathogens. We evaluated changes in the *P. nicotianae* transcriptome following a set of abiotic stresses, including heat shock (Panabières and LeBerre, unpublished). A wide range of genes were highly induced, encoding typical stress-induced proteins, as well as cytoplasmic effectors of the RxLR class, that are assumed to enable pathogens to colonize their hosts and cause disease (Panabieres *et al.*, 2009). Whether climate warming can modify the effector repertoire of *P. nicotianae* in field conditions is unknown, but several reports have demonstrated the involvement of stress-activated genes in *Phytophthora* infectivity (van West *et al.*, 1999; Kim and Judelson, 2003; Le Berre *et al.*, 2008; Li *et al.*, 2010; Kebdani *et al.*, 2010; Attard *et al.*, 2014; Gao *et al.*, 2015).

Evaluating other effects of climate changes is challenging, because such changes will differ in different geographical and agricultural areas. In the context of the present case study, we forecast several adaptive responses of *P. nicotianae*, such as overwintering, increased competitive properties over other pathogens, and expansion of geographical distribution, as has been predicted for other pathogens (Garrett *et al.*, 2006). The current disease management strategies have to be addressed, with a focus on the efficiency of disease control chemicals and the sustainability of resistant varieties.

Current challenges and future perspectives

Phytophthora nicotianae diseases were reported 120 years ago. Long-established methods, mainly relying on extensive use of fungicides, gave effective control of these diseases during most of the 20th century, but these are no longer adapted to the current situation. Most control strategies were developed in accordance with intensive crop production systems in industrialized countries to ensure adequate global food production, and consequences on human health and environment were poorly anticipated (Rivière *et al.*, 2011). Productivism is not now considered as the main issue, and ecological and social concerns feed the current debate for a redefinition of agriculture and sustainable plant disease control strategies. The systematic use of chemicals

is questioned in most countries of the industrialized world, because of the risk of adaptation of pathogen populations and the awareness of adverse effects on ecosystems and on human health and livestock. Therefore, current policies state that fewer pesticides should be used, and a substantial number of chemicals are planned to be banned for disease management in the near future. Consequently, efforts to manage *P. nicotianae* diseases have to be renewed, but solutions developed on major crops in the USA and Europe cannot be made at the expense of the rest of the world. Regulations or phytosanitary policies must take into account economic, social and cultural factors and differences.

Environmental care currently observed in industrialized areas occurs in countries where access to food and other plant products is not a central issue. On the other hand, less developed countries more dependent on agriculture, especially on local crops, are more exposed to plant diseases, due to fewer resources than developed countries. In addition, solanaceous crops, citrus and ornamentals are thriving industries in developing economies. Climate warming will facilitate the development of these crops, all of which are common hosts of *P. nicotianae*, which is thus likely to develop in these countries. Furthermore, the *P. nicotianae* host range is not formally delineated and still expanding. It includes a many plants that are poorly documented, or of limited interest for scientists or policy makers (Guha Roy and Grünwald, 2014). Nevertheless, these plants and crops are largely used at local scales for food production or as cash crops, and they may collectively have important economic impacts.

Without the rapid development of efficient, alternative disease management methods, it will be difficult to reduce the use of chemicals to limit *P. nicotianae* diseases elsewhere. To provide efficient solutions that would be applied to a range of crops and be affordable for developing countries, a reappraisal of our knowledge on *P. nicotianae* is required, and several research areas have to be strengthened.

Firstly, new knowledge is required of the diversity and dynamics of *P. nicotianae* populations. To date, population analyses performed with different tools have given convergent results, indicating host-genotype associations in some cases, and a global lack of geographical structure (Mammella *et al.*, 2013; Biasi *et al.*, 2016). Additional studies are required to confirm this trend. They have to be performed at large

scales and include additional crops, in order to better understand the bases of host-genotype associations, and identify eventual cases of speciation. As stated above, the boundaries of *P. nicotianae* were a matter of debate in regard to tobacco isolates (Erwin and Ribeiro, 1996). The genetic isolation of populations recovered from citrus further indicates that this debate is relevant, and that the species definition of *P. nicotianae* needs to be reconsidered. The population dynamics of *P. nicotianae* should also be studied at a worldwide level, in order to track eventual changes in populations. This should focus in areas where *P. nicotianae* appears to over-compete other species, and in regions where occurrence of the pathogen is recent. Collaborative efforts developed over many years to decipher the origin, structure and dynamics of *P. infestans* populations worldwide provided valuable results with major implications in management of late blight of potato (Fry *et al.*, 2015 and references therein). Although the epidemiology of diseases caused by *P. nicotianae* is likely to be complex, mainly due to the pathogen's large host range and the observed balance between sexual and clonal reproduction strategies, applying lessons learned from the *P. infestans* example would help to provide a more precise snapshot of the *P. nicotianae* situation worldwide.

Sequence-driven analyses constitute a second research area whose expected results will have implications for *P. nicotianae* management. The comparison of genomes from 14 isolates collected from various hosts and geographical areas not only allowed identifying SNPs and development of microsatellite markers, but also provided preliminary information on the variability of the repertoire of effectors and other virulence factors (The *Phytophthora parasitica* genome initiative, unpublished results). This analysis needs to be supported by additional sequence data and transcriptome profiling. Interactions between effectors and their host targets play pivotal roles in plant resistance, as all avirulence genes identified in *Phytophthora* so far encode RxLR effectors (Anderson *et al.*, 2015). Thus, effectors are currently used in breeding programmes for probing plant germplasm to detect major resistance (R) genes (Vleeshouwers and Oliver, 2014). It has also been suggested that evolution of targets among plants would impede recognition by effectors and subsequent manipulation of host functions, contributing to nonhost resistance (Schulze-Lefert and Panstruga, 2011). Considering

the hundreds of hosts and the hundreds of effectors present in the *P. nicotianae* genome, their variability observed among strains and their evolutionary potential, studies on the interactions between effectors and their targets have probably only just begun. This field of investigation is likely to provide understanding of the mechanisms that determine the host range of *P. nicotianae*, and will provide a basis for the design of new control programmes.

A third research area concerns methods for pathogen detection. This is particularly important when considering recent *Phytophthora* outbreaks, frequently due to association of well-characterized species and novel taxa (Prigigallo *et al.*, 2015). Due to its soil-borne niche, and its ability to infect a large number of hosts, including possible asymptomatic plants, *P. nicotianae* has probably been underestimated, so that improved detection and identification methods are necessary (Li *et al.*, 2013). PCR-based techniques and nested approaches have proven their efficiency and reliability for detection of *P. nicotianae*, even in pre-symptomatic infections (Ippolito *et al.*, 2002; Ippolito *et al.*, 2004; Meng and Wang, 2010). These methods are appropriate for high-throughput detection and quantification purposes, being ideal for evaluating inoculum threshold levels and study of epidemiology, biology and ecology of the pathogen (Sanzani *et al.*, 2014). An emerging reliable detection strategy relies on culture-independent metabarcoding analyses (Scibetta *et al.*, 2012; Sapkota and Nicolaisen, 2015). The combination of genus-specific primers and next generation sequencing approaches offers several advantages for assessment of the molecular ecology of *Phytophthora*. This approach provides accurate appraisal of the microbial diversity in environmental samples (soil and roots) and may lead to the identification of undescribed pathogen species (Prigigallo *et al.*, 2015; Prigigallo *et al.*, 2016). In the case of known species, like *P. nicotianae*, this method provides greater sampling depth, and thus increases sensitivity, compared to more conventional approaches (Prigigallo *et al.*, 2015; Prigigallo *et al.*, 2016). Again, the development of these techniques has just begun, and they will benefit from innovations in sequencing techniques, leading to greater knowledge of the diversity of microbial communities.

This emphasizes the need to invest in a fourth research area, a re-evaluation of the relationships between *P. nicotianae* and its biotic environment. For a better understanding of the factors that govern sur-

vival and fitness of *P. nicotianae* in a range of niches, the pathogen should be considered as one of the components of the microbial communities in contact with plant populations (Galiana *et al.*, 2011). Extensive research activities on *Phytophthora* diseases have been and are currently conducted at molecular levels, and they aimed at deciphering plant-pathogen dialogue occurring in laboratory conditions. These studies have revolutionized plant pathology, and their implications in pest management programmes must not be underestimated. However, the impacts of the microbial communities sharing *Phytophthora* habitats on the outcome of plant diseases have not been demonstrated. Metagenomics approaches will assist this field of investigation, and the study of molecular microbe-microbe interactions is a promising field of research. The description of *P. nicotianae* biofilm formation on the surface of wounded tobacco leaves revealed original relationships between zoospores (Galiana *et al.*, 2008). It showed that *P. nicotianae* biofilm constituted a heterogeneous micro-environment that could eventually serve as a niche for other microorganisms. Subsequent screening of a rhizosphere microbiome associated to *P. nicotianae* led to the identification of eukaryotic microbes that either inhibited *P. nicotianae* germination, or favoured its dissemination by forcing zoospores to leave the biofilm (Galiana *et al.*, 2011). These observations underline the importance of extending knowledge on the multiple interactions that *P. nicotianae* may establish with its more general biotic environment and not only with host plants, and how these interactions may impact disease epidemics. From an applied viewpoint, these results suggest that the development of new agro-ecosystems may efficiently contribute to the emergence of new control strategies (Rivière *et al.*, 2011).

Conclusions

Within the list of the “top 10 oomycete pathogens” recently established, *P. nicotianae* was ranked 8th on the basis of scientific and economic importance (Kamoun *et al.*, 2015). The present paper aims to convey that this pathogen was misplaced and is expected to gain increased ranking in the near future, due to the conjunction of human activities (globalization, climate change) and its intrinsic characteristics and adaptive potential. *Phytophthora nicotianae* causes global problems, and the definition of adapted

control strategies require global cross-disciplinary approaches. These will include close coordination among plant pathologists, climatologists, ecologists, economists, social scientists and growers.

Acknowledgements

F. Panabières and M. L. Kuhn thank Agnès Attard, Eric Galiana, Mathieu Gourgues, Harald Keller and Michel Ponchet for fruitful discussions. Research in the G.S. Ali laboratory is supported by the Institute of Food and Agricultural Sciences at the University of Florida. R. J. D. Dalio receives support from the National Council for Scientific and Technological Development CNPq/CsF/INCT Brazil, grant number 313139/2013-0. L. Schena is supported by grant FIRB 2010-RBFR10PZ4N from the Italian Ministry of Education, University and Research (MIUR).

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Accepted for publication: January 9, 2016

Published online: May 14, 2016