Never-Ending Presence of *Phytophthora* Species in Italian Nurseries

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Plant trade coupled with climate change has led to the increased spread of well-known and new *Phytophthora* species, a group of fungus-like organisms placed in the Kingdom Chromista. Their presence in plant nurseries is of particular concern because they are responsible for many plant diseases, with high environmental, economic and social impacts. This text offers a brief overview of the current status of *Phytophthora* species in European plant nurseries. Focus was placed on Italian sites. Despite the increasing awareness of the risk of *Phytophthora* spread and the management strategies applied for controlling it, the complexity of the *Phytophthora* community in the horticulture industry is increasing over time. Since the survey carried out by Jung *et al*., new *Phytophthora* taxa and *Phytophthora*-host associations were identified. *Phytophthora hydropathica, P. crassamura, P. pseudocryptogea* and *P. meadii* were reported for the first time in European plant nurseries, while *P. pistaciae*, *P. mediterranea* and *P. heterospora* were isolated from Italian ornamental nurseries. Knowledge of *Phytophthora* diversity in plant nurseries and the potential damage caused by them will help to contribute to the development of early detection methods and sustainable management strategies to control *Phytophthora* spread in the future.

Phytophthora Italy nursery Europe plant trade biosecurity biological invasions plant disease plant pathogens

1. Introduction

Phytophthora species are Oomycetes, classified within the Stramenopile lineage; they consist of soilborne and airborne species and require water to complete their life cycles. They produce infectious propagules, including zoospores, chlamydospores and oospores, that can be spread short or long distances. These structures enable long-term survival (oospores) and short-term survival (chlamydospores) facilitating the adaptation of *Phytophthora* taxa to different environments [1][2][3][4]. Some *Phytophthora* species are aggressive pathogens that can cause damping-off, root and collar rot, wilting and blight on over 1000 plant species. While some taxa, such as *P. infestans* Mont. de Bary, have a limited range of hosts, species such as *P. cinnamomi* Rands, can infect more than 109 plant species [1].

2. *Phytophthora* Species Diversity in Plant Nurseries

Numerous *Phytophthora* species have been documented in commercial plant nurseries worldwide, causing significant economic losses [1][5][6][7][8]. For instance, a total of 28 and 15 *Phytophthora* taxa, have been found in

Oregon and California ornamental nurseries, respectively [9][10]. Some of the *Phytophthora* taxa found in plant nurseries are of regulatory concern. Among those, *P. ramorum* Werres, de Cock and Man in 't Veld has received the most notoriety, but also other regulated taxa can be present. The first European detection of *P. lateralis* Tucker and Milbrath (EPPO List) is dated 1999 in French nurseries [11]. Of over 36 *Phytophthora* species identified in Pennsylvania nurseries and greenhouses, three*, P. parvispora* Scanu and Denman; *P. chrysanthemi* Naher, Watanabe, Chikuo and Kageyama; and *P. sojae* Kaufm. and Gerd., were listed in the U.S.- regulated Plant Pest Risk [12][13].

2.1. The Case of *Phytophthora* Species in Italian Plant Nurseries

The survey conducted by Jung *et al*. [14] described a total of 36 different *Phytophthora* taxa in Italian plant nurseries, forest and landscape plantings. Those taxa were not exclusive to Italy, confirming nurseries as a potential basin of plant pathogens [14][15]. *Phytophthora cinnamomi, P. cambivora* Petri Buisman and *P. cryptogea* Pethybr. and Laff. occurred mainly in oak stands, while *P. palmivora* E.J. Butler was isolated from all nurseries stands of *Olea europaea* L. Over more than 100 *Phytophthora*-host associations, 33 new hosts were reported exclusively in Italy, including *Agave attenuata* Salm-Dyck., *Coronilla valentina* L. and *Solanum melongena* L. *Phytophthora ramorum, P. fragrariae* Hickman and *P. lateralis* were the only three *Phytophthora* species recorded in Italian plant nurseries and plantings also included on the EPPO quarantine list. Interestingly, *P. ramorum* was first isolated in Italy in 2002, on *R. yakushimanum* Ken Janeck in a Piedmont nursery [16]. Later, in 2013 the pathogen was detected by pyrosequencing analysis in chestnut stands and by culture-based methods on *Viburnum tinus* L. in Pistoia, where currently it is considered eradicated [17][18]. Nevertheless, its record in Italian plant nurseries confirmed that it was not halted despite strict quarantine regulations.

A literature survey was carried out using databases available for academic research, such as Scopus [19] and Web of Science [20], using "*Phytophthora* and Italy" as keywords. The dataset was then restricted to nurseries. Results highlighted the presence of 43 *Phytophthora* species associated with horticultural, forest and ornamental plant species. The richness of *Phytophthora* taxa in Italy could be associated with the geographic characteristics of the country and its extensive trading traditions. Italy is a long peninsula with mostly mountainous hinterland and is surrounded on every side except the north by the Mediterranean Sea. Thus, its climate is highly diverse. The lack of information of the exact geographic locations of the nurseries reported in the dataset analyzed has prevented us from highlighting a possible link between *Phytophthora* diversity and climate conditions. Nevertheless, the diversity of the *Phytophthora* community decreased from Southern to Northern Italy (Figure 1). Sardinia, Tuscany and Piedmont were the regions with the highest *Phytophthora* spp. richness in the three Italian zones (Southern, Central and Northern Italy, respectively) (Figure 1). *Phytophthora ramorum, P. cinnamomi, P. nicotianae* Breda de Haan and *P. niederhauserii* Abad were present throughout the peninsula. However, the structures of *Phytophthora* communities varied in the three zones also in accordance with the distribution of hosts and efforts to characterize occurrence, with most of the survey conducted after 2000. The rising outbreaks of *Phytophthora* species in forests and natural ecosystems in Europe in the late 1990s probably stimulated the scientific community to investigate with systematic surveys the presence of pathogens in nurseries.

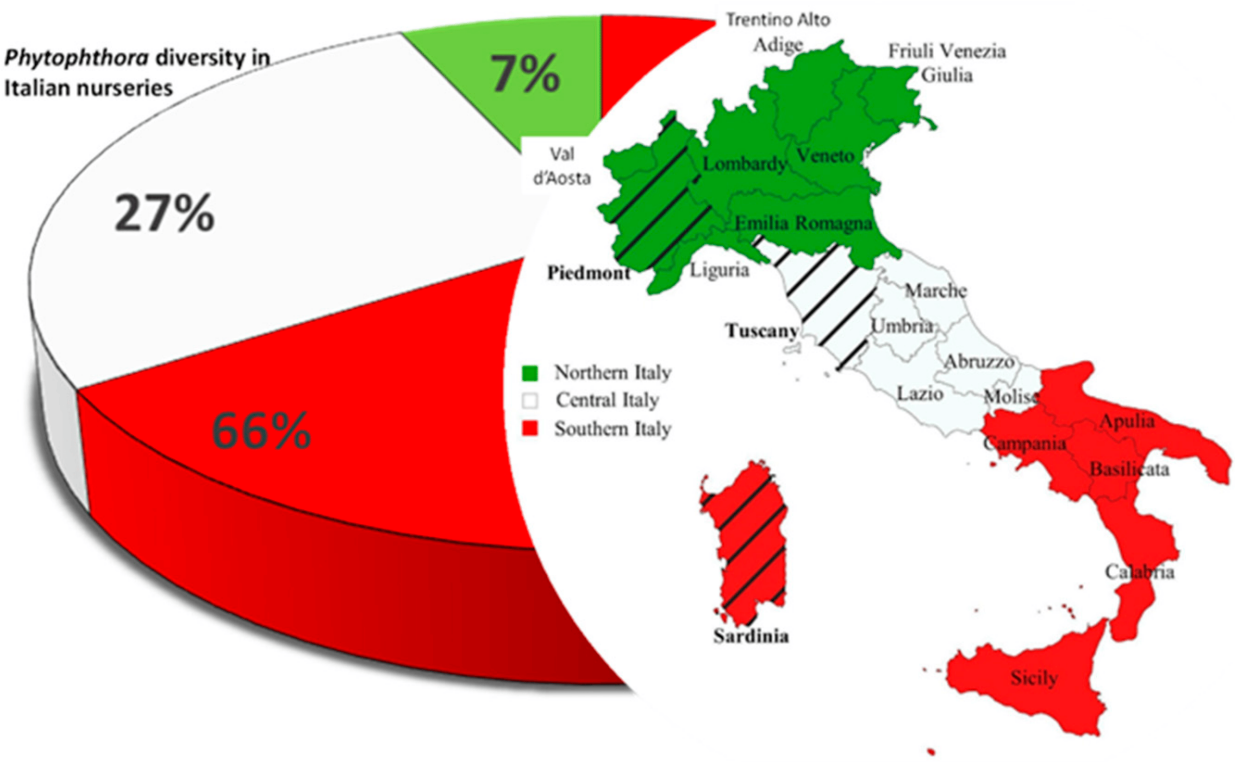

Figure 1. Distribution map of *Phytophthora* spp. in Italian plant nurseries. Different colors represent the three Italian zones: red = Southern Italy; white = Central Italy; green = Northern Italy. The regions within each zone with the highest *Phytophthora* diversity are indicated by stripes.

The previous survey by Jung *et al*. [14] focused on data collected from 1992 to 2013. Since then, three new *Phytophthora* species, *P. pistaciae* Mirabolfathy; *P. mediterranea* Bregant, Mulas and Linaldeddu; and *P. heterospora* Scanu, Cacciola, Linald. and T. Jung, were described in Italy. These were the first reports in Europe (Figure 2).

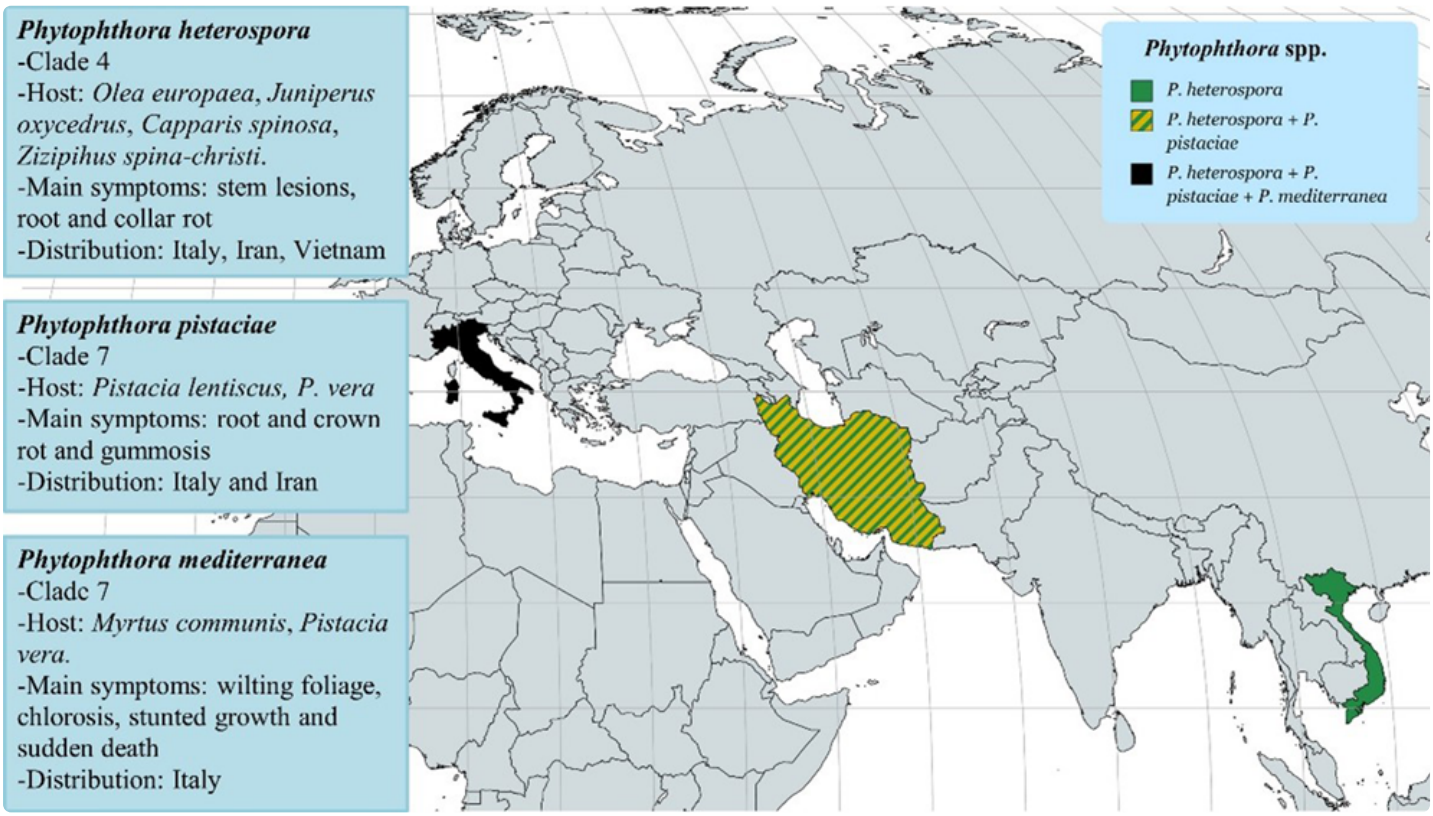

Figure 2. Worldwide geographical distribution of *P. pistaciae, P. mediterranea* and *P. heterospora*.

Phytophthora mediterranea was isolated from declining potted myrtle seedlings (*Myrtus communis* L.) in Italy [21]. It was previously observed on pistachio (*Pistacia vera* L.) in California [22]. Although phylogenetically, *P. mediterranea* is closely related to *P. cinnamomi*, the two species can be easily distinguished on the basis of some morphological differences, such as size of the sporangia, colony growth pattern and cardinal temperature values. Several Mediterranean maquis species are highly susceptible to this newly recognized pathogen [21]. *Phytophthora pistaciae* causes leaf reddening, wilted shoots, root and collar rot on nursery plants of *P. lentiscus* L. in Italy [23]. It is considered the most aggressive pathogen of *P. vera* in Iran [24][25]. *Phytophthora heterospora* has been isolated from stem lesions and root and collar rot of *Olea europaea* (2010, Italy), *Ziziphus spina-christi* L. Desf (2011, Fars Province, Iran), *Juniperus oxycedrus* L., *Capparis spinosa* L. (2013–2014, Italy) and *Durio zibethinus* L. (2013, Mekong River delta, Vietnam) [26]. *Phytophthora heterospora* and *P. palmivora* have many similar morphological characteristics in terms of colony morphology, sporangia, chlamydospores, and gametangia shape and size. However, *Phytophthora heterospora* produces pseudoconidia, a unique asexual dissemination structure of *Phytophthora* species. This feature was previously described on isolates obtained from *Theobroma cacao* L. in the Ivory Coast and named *P. palmivora* var. *heterocystica* Babacauh [27]. Unfortunately, it is unclear whether *P. heterospora* and *P. palmivora* var. *heterocystica* represent the same taxon. Pathways of *P. heterospora* and *P. pistaceae* introduction are unknown. It is worth noting that Italy has a long history of trade in goods with Iran, where both *P. heterospora* and *P. pistaceae* are present. It is the second-largest importer of shelled pistachios in Europe (after Germany), with a value of USD 193 million in 2020 [28]. Since 2013, the list of *Phytophthora*-host combinations was reviewed with novel associations including *P. psychrophila* Jung and Hansen/*Ilex aquifolium*, *P. pseudosyringae*/*I. aquifolium*, *P. pseudocryptogea* Safaiefarahani, Mostowfizadeh, Hardy and Burgess/*Laurus*

nobilis L., *P. megasperma* Dreschsler/*L. nobilis*, *P. citrophthora* R.E. Smith and E.H. Smith Leonian /*L. nobilis*, *P. bilorbang* Aghighi and Burgess/*Phyllirea latifolia* L. and *P. palmivora*/*P. latifolia* [21]. *Phytophthora* x *pelgrandis* Gerlach, Nirenberg and Gräfenhan, previously observed in potted plants in the Netherlands, Hungary and Germany, and *Phytophthora hydropathica* Hong and Gallegly, previously never detected in European nurseries, were also reported on *Lavandula* spp., *Buxus sempervirens* L., *C. lawsoniana* and *Viburnum tinus* L. in Italy [29][30][31]. During 2012–2014, *P. pseudosyringae* was first detected using ITS DNA metabarcoding [32][33] and only in 2021, was isolated from potted plants of *Ilex aquifolium* L. in Sardinia (Italy) [21]. In all probability, this is only the beginning of the story; the final picture of the occurrence of *Phytophthora* species in plant nurseries will probably never be complete, as additional species are being discovered every year. Researchers believe that the diversity of *Phytophthora* species presently is well underestimated. Although a total number of 200 *Phytophthora* taxa have been described [26][34], another 200–400 species may remain to be discovered in environments not yet surveyed [35] or not yet formally identified [32][33].

3. How to Tackle the Spread of *Phytophthora* Species

Before the COVID-19 pandemic, the value of horticulture production in Italy exceeded EUR 2.7 billion. Regarding plant nurseries, the figures also included cut flowers and flowering plants. During the periods of lockdown, all seasonal products were irremediably lost due to the impossibility of watering, for a short period, and the demand for ceremonies and anniversaries. Fortunately, matters are gradually improving for this sector in both the domestic and foreign markets. The return to gardening practices has led to an appreciable increase in sales. Export, however, is the driving force for the sector, with a value of about USD 28,765,318.00 [36], with demand coming mainly from Northern European countries (Holland, Germany and France). Italian imports of plants and live plant materials (import values USD 86,437,699.00; data 2020) comes mainly from The Netherlands (71%), Germany, Spain and Poland [36]. In globalized trade, plants and plant products are continuously on the move. Marketing has switched from conventional to web-commerce sites, exacerbating potential phytosanitary risks as delivery often bypasses traditional screening by NPPOs [37][38][39]. Not least, the distribution of pests is clearly altered by climate change. The presence of *P. cinnamomi* in alpine areas is emblematic. Its quick spreading in new geographic areas was reported in forests [40][41][42] as well as in German nurseries, where generally it is rare due to its sensitivity to frost [43]. In this scenario, the future of *Phytophthora* spp. occurrence is dangerously uncertain. Addressing the risks of *Phytophthora* spread is a highly complex task. Despite good intentions to control pest introduction and spread, we must be aware of the weakness and the lack of harmonization of phytosanitary regulations and processes [39][44][45][46][47][48][49][50]. The recently adopted new Plant Health Regulation (EU) 2016/2031, enhancing more effective measures for the protection of the Union territory and its plants, ensures safer trade, as well as proposing mitigation measures for the impacts of climate change on the health of crops and forests. The application of the new law cannot tackle the issue alone. It is essential to develop pest risk assessments that underpin policy and decision-making to assess the risks of introduction, spread and the environmental impact posed by invasive alien species (IAS). However, during the introduction steps, pathogens could be particularly hard to identify. They can express a pathogenic lifestyle only following introduction into new areas and in association with new hosts. Several guidelines and protocols for risk assessments have already been drawn up, but an effort to harmonize them and

enhance communication and information exchanges with other countries is suggested [51][52][53]. The development of new rapid, reliable, accurate and cost-effective detection methods is also widely desirable to prevent spread of *Phytophthora* spp. Apart from molecular approaches, such as environmental DNA metabarcoding, aerobiology or the use of sentinel plants, represent a challenging but helpful research line for bio-surveillance of IAS [54][55][56]. Once in a nursery, the spread of *Phytophthora* is difficult to stop. Several guidelines were published to help to maintain a nursery system that excludes *Phytophthora* pathogens [57][58]. The application of those protocols, however, could be hampered by practical issues. They could require technical practices, such as testing irrigation water for the presence of pathogens, which represent additional costs for professional growers. In this context, it is important to inform professionals in the sector of the risk and consequences of plant diseases that are often hidden by chemical treatments. It is increasingly recognized that surveillance activities should be developed for early detection both in the areas of interest and in the exporting regions outside the EU. Thus, field workers and inspectors at borders should continuously update their knowledge or skills to recognize symptoms of plant diseases. In recent years, several molecular methods have been developed for early detection of *Phytophthora*; however, they often require expertise not generally present in plant nurseries, meaning that growers need to pay for external services. It is a matter of fact that plant nurseries are generally small-sized enterprises, about 1.3 ha/nursery in Italy, that could hardly bear the costs of biosecurity strategies, despite the necessity. External financial aid, for example from EU plant health organizations, could support bio-surveillance practices. Among the strategies suggested for *Phytophthora* disease management, the biological protection approach results in one of the most eco-sustainable control methods by inhibiting plant pathogens, improving plant immunity and/or stimulating microorganisms beneficial to the plants. Gaining a better understanding of the interaction of biological control agents with the environment and the development of new eco-friendly products, such as nanoparticles as carriers of plant extracts or other chemicals [59][60][61][62], will be important for the improvement of environmentally sustainable management protocols. Given the global nature of *Phytophthora* disease problems, bio-surveillance should be introduced encompassing global cooperation in monitoring, detection, studying and managing the pathogen. Encouragement of better collaborations among research centers, growers and national and international organizations will optimize efforts for protecting plants. Moreover, a reciprocal exchange dialogue is required with the public and industry to work in synergy in order to fully share common control strategies, increase awareness of the risks in plant trade and the importance of protecting and maintaining local biodiversity.

4. Conclusions

The plant nursery industry is a reservoir for *Phytophthora* species, whose spread will be exacerbated by the effects of the ever-increasing global plant trade, climate change, the introduction of highly susceptible or asymptomatic hosts and the emergence of new threats, or a combination of these issues. These factors will have a decisive influence on the geographic distribution of pathogens, their virulence and host range into the future. It is, therefore, not surprising that in the future, new combinations of host-pathogens or new *Phytophthora* hybrids will occur. The growing number of publications and citations for *Phytophthora* species could be interpreted as an increasing awareness of their environmental, economic and social impacts. Nonetheless, there remains a lack of information about the occurrence of *Phytophthora* spp. in nurseries, illustrating the need to develop simple, efficient early

detection methods and management strategies. More efforts should be addressed to highlight the risk posed by new introductions of *Phytophthora* species as a matter of urgency by government agencies, international health organizations, managers, plant nurseries and citizens. In this scenario, nurseries will play a crucial role. By enforcing appropriate biosecurity practices and early detection, they can reduce their economic losses and limit pest spread into forests and urban areas. The study highlighting the rapid increase in the number of *Phytophthora* species in European plant nurseries will contribute to raising awareness of managers and scientists on the importance of implementing appropriate biosecurity measures to minimize the ecological and economic threat posed to the forest and food chains as well as natural ecosystems and urban areas.

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