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Editorial

Recent Advances in the Monitoring, Assessment and Management of Forest Pathogens and Pests

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Abstract: Tree pathogens and pests are fundamental components of forest ecosystems. By killing and decomposing susceptible trees, they regulate the cycle of nutrients and energy flow, thus shaping the structure and composition of forest stands. However, ecosystems can be seriously disrupted when the population density of these parasites increases beyond their tolerance level. Ascertaining the origin of pathogen and pest outbreaks, recognizing their causal agents in a precise and unequivocal way, while understanding their reproductive and dispersive dynamics are all crucial for the implementation of effective control measures. The studies collected in this special issue cover a wide range of topics in the field of forest pathology and entomology. Investigations range from molecular diagnosis of pathogens and pests to their monitoring and quantification in the field, from measurements of their proliferation rate to the analysis of their genetic variability, from the assessment of the role of plant diversity and ecosystem heterogeneity on pathogen and pest impacts to disease and pest management. Specific case studies show how applied research conducted with innovative methods is key to solving taxonomic issues that were, until now, controversial. The variety of experimental approaches and the range of scientific issues addressed document the trends and topicality of modern forest health protection science.

Keywords: fungi; insects; invasive species; diagnosis; surveillance; disease and pest management

This special issue was launched in view of the United Nations' International Year of Plant Health in 2020, with the purpose of demonstrating how forest health protection from pathogens and pests must necessarily go through certain fundamental and unavoidable steps: recognizing the "enemy"; measuring/quantifying it; developing suitable strategies to combat it. These stages are stepping stones: better understanding of the parasite's life-history strategies and impacts helps us to progress towards the final goal of disease and pest management.

A first, unavoidable issue, especially when dealing with alien parasites which, as such, are also little known, is to identify them accurately and unambiguously [1]. Accurate and timely diagnosis is furthermore of paramount importance for the subsequent implementation of effective disease and pest management, since it allows us to have a better understanding of the parasite's potential impact on woodland ecosystems. Several studies address this issue with both traditional and molecular approaches. For example, useful guidelines were provided by Mercado et al. [2] for the accurate determination of *Dendroctonus* species attacking *Pinus contorta* Douglas ex Loudon in subalpine forest in the southern Rocky Mountains. Simplified insect identifications based on morphological characters, their attack pattern, and signs have improved classification of these beetles which are normally difficult to distinguish from congeneric species.

Advances in molecular-based diagnostics of infectious diseases are greatly enhancing our ability to identify and characterize damaging agents, allowing a more accurate testing for pathogen identification [3]. Current significant research efforts are aimed at identifying new markers for highly specific diagnosis. In a study targeting the destructive and highly

polyphagous pathogen *Phytophthora cinnamomi*, for instance, a novel biomarker unique to this oomycete, the RxLR effector gene PHYCI_587572, was identified through a comparative genomics approach. Furthermore, a recombinase polymerase amplification-lateral flow dipstick assay targeting the PHYCI_587572 biomarker has proven able to detect, with high specificity, *P. cinnamomi* isolates of diverse sources and different geographic origins, with no cross reactions from 37 other oomycete and fungal species, among which, the sister taxon *P. parvispora* [4].

An additional recent breakthrough is the loop-mediated isothermal amplification (LAMP) technique, initially developed to diagnose animal/human diseases [5] but which is also being applied to phytosanitary issues and to solving taxonomic uncertainties. A LAMP assay was developed for unambiguously identifying *Dothistroma septosporum* (G. Dorog.) M. Morelet, one of the agents of *Dothistroma* needle blight (DNB), an emergent pathogen harmful to both natural and plantation pine forests. The assay proved highly sensitive, detecting as little as 1 pg of fungus DNA; it was also highly specific, discriminating this pathogen from the related *Dothistroma pini* Hulbary and *Lecanosticta acicola* (Thüm.) Syd. [6]. The LAMP method was also employed to solve the question of the identity of the pathogen responsible for the first outbreak of DNB in Italy [7]. This first outbreak of DNB there, about half a century ago (mid-1970s), had been ascribed to *D. pini* on the basis of micromorphological identification alone [8]. The re-checking of this old, never-confirmed report by using pathogen testing with the LAMP method, revealed the causal agent of the early outbreak of the last century to have been, instead, *D. septosporum*, probably introduced into the area by plantations of exotic *Pinus radiata* D. Don.

Once a damaging agent has been accurately identified, the next step is to broaden the knowledge about this species as much as possible [9]. In fact, pest control efforts need to be based on extensive scientific knowledge of this organism's key traits and its population dynamics. The citrus long-horned beetle *Anoplophora chinensis* (Forster) and the gypsy moth *Lymantria dispar* L., for example, are both invasive, destructive pests which can damage many tree species in orchard, urban, and forested habitats. Hence, information on temperature requirements of the citrus long-horned beetle, and on the key traits affecting the gypsy moth's dispersal capability, are provided in order to develop phenological models for management or eradication efforts [10,11]. Specifically, Keena et al. [10] evaluated adult survival, reproduction, and the egg hatching of *A. chinensis* at eight constant temperatures under laboratory conditions; these can be used to predict the timing of stages. Srivastava [11], in addition, provided a review of the current literature on the variations in flight capability and flight distance of gypsy moth populations for each subspecies, as well as shifts in other traits of concern. Rapid tools for assessing key traits in non-native pest populations, as well as knowledge about population dynamics in post-invasion sites, furnish further fundamental information to adequately deal with new introductions into novel habitats. For example, Robinett et al. [12], demonstrated that white ash trees are persisting in several forested areas in the heart of the *Agrilus planipennis* Fairmaire invasion site in Michigan, more than a decade after invasion, indicating that these beetle populations remain below the sites' carrying capacity.

Research efforts in recent years, greatly boosted by modern molecular and bioinformatic methods, have set their sights on surveying the fungal and pseudofungal biodiversity in protected areas. Protected areas are reservoirs of biodiversity that are an essential tool for its conservation, thus they demand particular attention. These areas, being relatively undisturbed ecosystems, are especially useful for studying the ecology and dynamics of populations of plant pathogens among diverse vegetation types. A survey on the diversity and distribution of *Phytophthora* species in aquatic, riparian and terrestrial habitats in a protected area in Eastern Sicily (Italy), for instance, has revealed the presence of 11 species of *Phytophthora* and, interestingly, a correlation between the occurrence of some oomycete taxa and certain vegetation type groups [13]. Another study investigated the impact and spread of the emergent charcoal canker agent *Biscogniauxia mediterranea* (De Not.) Kuntze in a protected, lowland residual forest (Castelfidardo Forest, central Italy) [14]. The impact of

the charcoal canker pathogen in this sub-Mediterranean deciduous forest with mesophilic traits, dominated by oak species, ended up devastating the stand in recent decades. Moreover, it turned out to be related to environmental stresses, such as precipitation deficit and extended drought; further factors predisposing oaks to decay were an absence of silvicultural management, a high competition among physiologically mature trees, and the geographic isolation of this residual forest.

Understanding the factors governing the spread and virulence of pathogens that cause substantial ecological and economic damage continues to remain central to forest pathology studies. Variation in the landscape, in land use (e.g., forest lands vs. former agricultural lands) and, of course, in the main ecological parameters, strongly influences tree susceptibility and the dispersal patterns of forest pathogens [15]. In this regard, Cheng et al. [16] found that in Japanese red pine (*Pinus densiflora* Sieb. et Zucc.) forests, the impact of Diplodia tip blight caused by *Sphaeropsis sapinea* (Fr.) Dyko and B. Sutton, a serious pathogen for several conifer species from the *Abies*, *Cedrus*, *Juniperus*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga* and *Thuja* genera, varied according to stand types and vertical structure layers. Diplodia tip blight disease, for example, was higher in *P. densiflora*–conifer mixed forest than in the *P. densiflora*–hardwood mixed forest or in the purely *P. densiflora* forest. Furthermore, a higher plant diversity in the understory resulted in a more intense competition between young Japanese red pines and shrub–herb plants, resulting in poor resistance of this pine species to Diplodia tip blight [16].

The paper by Klavina et al. [17] provided evidence that Norway spruce (*Picea abies* (L.) Karst.) plantations established in former non-forest lands become highly susceptible to the root rot pathogen *Heterobasidion parviporum* Niemelä and Korhonen after thinning, and that in plantations growing in former pastures and meadows, the pathogen, probably favored by the scarcity of a competing soil microbial community, is able to expand with large territorial clones. For this reason, stump treatment with biological or chemical control agents during thinning operations is fundamental to preventing the transfer of *Heterobasidion* infection to the next generation of trees through root contacts. Stumps are, on the other hand, a primary source of infection for *Heterobasidion annosum* s.l., since they permit pathogen spread to healthy trees by vegetative mycelium via root anastomoses. Airborne *Heterobasidion* sp. spore deposits on the stumps of susceptible species can be estimated by means of wood discs [18]. Brūna et al. [19] have utilized wood discs to investigate the susceptibility of seven conifer species (*Larix sibirica* Ledeb., *P. abies*, *Picea sitchensis* (Bong.), *P. contorta*, *Pinus strobus* L., *Pinus sylvestris* L. and *Pseudotsuga menziesii* (Mirb.)) to artificial inoculation with conidia of *H. annosum* sensu stricto (s.s.) and *H. parviporum* under controlled conditions or to natural airborne infection. These authors observed that the rate of infection of wood discs sprayed with conidial suspensions under controlled conditions was comparable to that obtained from exposing the same species' discs to natural airborne inoculum in spruce stands infested either by *H. annosum* or *H. parviporum*. The importance of such local spore sources in the range of dispersal of the two forest pathogens was confirmed by the fact that the infection rate of wood discs decreased as the distance from the inoculum sources (fruiting bodies) increased.

Proper management of forest pathogens and pests must take into consideration not only the pest itself, but also the other living components of the ecosystem, as well as their multitrophic interactions. The role of insects as vectors of pathogenic fungi is well established [20,21], nonetheless, many aspects of insect/fungus interactions still need to be clarified. A great quantity of *Heterobasidion* spp. fruit bodies, for example, develop on large, infected *P. abies* logs when they are left in stands after forest operations. These high-spore-density fruiting bodies are a considerable risk; therefore, several studies recommend removing large, infected logs from stands. All the same, these logs are important as a habitat for many fungi and invertebrates, including rare and endangered species. Legzdina et al. [22], for instance, found a rich community of invertebrates in *Heterobasidion* fruit bodies, decayed logs and adjacent soil; however, as these authors stated, the nature of such associations requires further investigation. Moreover, Vissa et al. [23] examined

differences in the diversity of symbiotic mite communities associated with *Dendroctonus ponderosae* Hopkins, an economically damaging North American bark beetle. Specifically, they observed that symbiotic mite biodiversity varied geographically according to environmental differences; they suggest that this information may be useful for further steps towards identifying key species interactions in forest ecosystems.

Eco-sustainable control strategies must be favored and improved to minimize the impact of pest control measures on forest ecosystems. The use of these strategies requires more expertise, being more difficult to apply in comparison to traditional measures based on pesticides. Additional studies are needed to better understand how to improve their efficacy, while reducing undesirable effects. Bracalini et al. [24], for example, have investigated the negative effects of *Ips sexdentatus* Böerner mass trapping, especially non-target catches among the target's natural enemies. They tested slot traps modified with mesh screens and escape windows to improve their selectiveness, demonstrating how trap modifications may mitigate the problem, especially for beetles larger than the target. This expedient should be adopted together with precise mass-trapping scheduling, in order to avoid seasons in which the target adults are less active than the main predator adults. Since biodiversity conservation is an integral component of sustainable forest management, considering the impact of all management strategies on the entire biological community is essential. In this regard, Hartshorn [25] assessed the impact of different types of forest management (e.g., clear cutting, burning, and chemical control of invasive species) on leaf litter arthropods, which are some of the most abundant and diverse communities in forests, and which provide several ecosystem services, from decomposition and mineralization to pollination and predation.

Microbial insecticides may be employed in forest ecosystems, where the use of pesticides is not an option. These biological agents are safe for both their users and woodland dwellers, due to their high selectivity. Among these environmentally friendly insecticides, *Bacillus thuringiensis*-based products are the most used all over the world [26]. *Bacillus thuringiensis* var. *kurstaki* (Btk), combined with mating disruption, are reported to be effective in controlling *Thaumetopoea pityocampa* Denis et Schiffermüller [27], a harmful insect for forest, urban and peri-urban pines, that needs to be kept under control because its larvae also cause public health problems for humans and pets. Finally, the use of Btk can also be integrated with other biological products, as proposed by Ruiu et al. [28]. These authors suggested applying Btk in combination with the agent of natural gypsy moth epizootics, the species-specific multicapsid nucleopolyhedrovirus (LdMNPV), to contain the lepidopteran defoliator *L. dispar* in multi-year integrated programs. In other instances, various fungal species have shown a marked antagonism towards important phytopathogenic fungi. The fungus *Phlebiopsis gigantea* (Fr.) Jülich, for example, is a biocontrol agent that has been very successful in the biocontrol of the root rot of conifer trees caused by *Heterobasidion annosum* (Fr.) Bref. Wit et al. [29] utilized elongation factor 1-alpha (EF1α) partial DNA sequencing and in silico data to infer the relationships between representative isolates of *P. gigantea* and selected Basidiomycota species. The results obtained indicated that the EF1α region is a valid marker for selecting the most competitive isolates of *P. gigantea* to be possibly employed for limiting damage by forest tree root pathogens.

This special issue is furthermore enhanced by two reviews that deal with two important themes: the emerging problem of the invasion of forest ecosystems by alien parasites and phytoplasma diseases of forest trees. The former is topical and very popular; the latter, little investigated, if not totally disregarded. The first review, by Panzavolta et al. [30], analyzed the main entry pathways of non-native pathogens and pests, as well as the factors affecting their establishment and spread into forest ecosystems and uncontaminated territories. It spotlights the importance of early diagnosis, monitoring and eradicating harmful organisms, noting the role of research and the valuable contribution of volunteers in early detection (citizen science). It also highlights how government policies must be enacted to effectively counter this international problem. In the second article, Marcone et al. [31] reviewed the major phytoplasma taxa that cause diseases to forest tree species. These dam-

aging, wall-less, obligate bacteria, which harm thousands of plant species worldwide, have been poorly investigated compared to both phytoplasmas infecting fruit trees and other groups of plant pathogens. Deeper research into the vectors, plant host range, strain virulence, pathogenicity and host tolerance/resistance would all be crucial for appropriately managing phytoplasma diseases.

The topics covered in the 21 papers that make up this special issue indicate the current trends in research on forest health protection. The variety of techniques used, spatial scales, methodological approaches, and topics covered are a measure of the versatility and importance of today's forest pathology and entomology research.

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