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PHYTOPHTHORA SPP. - RISING THREAT FOR THE NATURAL AND AGRO ECOSYSTEMS IN BULGARIA

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Abstract

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Species from genus *Phytophthora* are known as pathogens mainly on agricultural plants in Bulgaria, causing diseases as potato blight disease, black shank on tobacco, blights on peppers and tomatoes, etc. They also cause problems in orchards and nurseries. Less data concerning these plant pathogens are available for forestry and in fact there was no comprehensive investigation of the *Phytophthora* biodiversity in Bulgarian ecosystems. The problem with the increasing number of incidents of severe disease outbreaks caused by introduced *Phytophthora* pathogens in different types of ecosystems is discussed in this review. Some of the *Phytophthora* species known as inhabitants in the European countries, but still not found in Bulgaria, are pointed and discussed as a potential invaders for the country and increased danger for the natural and agro ecosystems in the country. The awareness is focused on some factors that might influence this process, like human activity, global climate changes and natural interspesific hybridization.

Key words: Phytophthora, biodiversity, distribution, introduction

Introduction

Literally translated from Greek the name *Phytophthora* means "plant destroyer". *Phytophthora* is a genus of plant pathogens that completely correspond to its name, comprising approximately 100 species described in the literature by now (Kroon et al., 2012).

The number of the newly described species is growing rapidly, in response of the increased interest among scientists to this group of devastating plant pathogens. The best studied and probably the most popular species of all the members of this genus is *Phytophthora infestans*, the causing agent of the potato late blight. When first appeared on potato crop in Ireland in 1845 this new pathogen have completely destroyed the potato yield in two subsequent years, resulting in mass starvation, migration and the death of millions of people in Ireland at that time (Erwin and Ribeiro, 1996). *P. infestans* is the first microorganism proven to be responsible for disease, and the work on it initiated the science of plant pathology (Judelson and Blanco, 2005). Beside its importance as a crop plant pathogens *Phytophthora* spp. appears to be involved in oak decline phenomenon, which is of great concern in Europe since 1980's and also is associated with other prominent cases of trees mortality within natural forest ecosystems (Jung et al., 2002; Brasier and Jung, 2004; Jung et al., 2005; Oszako, 2007).

In this review we focus on the problem with the increasing number of incidents of severe disease outbreaks caused by introduced *Phytophthora* pathogens in different types of ecosystems. Particular concern is caused, when such cases lead to permanent disturbing of the natural balance in intact ecosystems. It is ongoing process, the consequences of which are hard to predict. We discuss some of the factors that might influence this process, like human activity, global changes in climate and natural interspesific hybridization.

Taxonomy

Microorganisms from the genus *Phytophthora* have been considered to be typical fungi for a very long time. Traditionally, *Phytophthora* have been included in *Oomycetes*, within *Fungi*, based on the superficial resemblance of the hyphae and apparent similarities in the sexual reproduction (Dick, 2001).

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Based on the evolutionary phylogeny it is clear now that *Oomycetes* are not fungi, and are closely related to diatoms and golden-brown algae (Lamour et al., 2007). Despite that, in broad sense species from genus *Phytophthora* are still considered as fungi, or fungus-like organisms, mainly due to the similarities in the growing pattern and ecological behavior.

According to the classification of Dick (2001), the genus *Phytophthora* belongs to Kingdom *Straminipila*, class *Peronosporomycetes* (*Oomycetes*), a diverse group of microorganisms, including saprotrophs, facultative and obligate parasites on plants, animals and crustaceans. Economically the most important members of the *Peronosporomycetes* are the phytoparasites, which include the obligate downy mildews (*Peronospora* spp., *Bremia* spp., *Plasmopara* spp., *Pseudoperonospora* spp, *Sclerospora* spp., etc.), white rusts (*Albugo* spp.), semi-biotrophs from *Phytophthora* spp. and the saprotriophs from genus *Pythium*.

In the revision of the classification of the *Eukaryotes* (Adl et al., 2005) which incorporates results from both ultrastructural research since 1980 and molecular phylogenetic studies, *Phytophthora* spp. fall in the super-group *Chromalveolata*. Distantly, the *Fungi* belong to the super-group *Opisthokonta*, together with *Animalia*.

The chromalveolates brings together the stramenopiles (oomycetes, diatoms, and brown algae) and the alveolates (apicomplexans, ciliates and dinoflagellates). Genome sequences of *Phytophthora ramorum* and *Phytophthora sojae* reveals similarity to photosynthetic organisms such as red algae or cyanobacteria. These and other analyses support the view that a photosynthetic ancestor founded the stramenopiles. In other words: *„It appears that devastating pathogens, such as Phytophthora, have evolved from benign phototrophic ancestors"* (Lamour et al., 2007). In addition, oomycetes appears to be more closely related to the apicomplexans, including parasites such as *Plasmodium* and *Toxoplasma*, than to *Fungi*. Oomycetes and apicomplexans share similar mechanisms of pathogenesis, and that might be a reflection of their common evolutionary history (Lamour et al., 2007).

Life cycle of *Phytophthora* in relation with the pathogenesis of the disease

The thallus of *Phytophthora*, as well as the most oomycetes is filamentous, composed of hyphae, and is diploid in its vegetative stage (Erwin and Ribeiro, 1996; Judelson and Blanco, 2005). Hyphae are developed in the convergent evolutionary process, and are analogous to hyphae of the *Eumycota* (Dick, 2001).

Oomycetes has several clearly defined developmental stages in their life cycle (Walker and Van West, 2007). Several types of spores have important role in the life and the disease cycles of *Phytophthora* species. In nature, the survival of *Phytophthora* spp. as a saprophyte is limited, and spores are required to allow movement to new host. The asexual spore, called sporangium is multinucleate spore, which is capable to convert its cytoplasm into multiple spores (zoospores) (Judelson and Blanco, 2005). The process of zoosporogenesis is considered one of the fastest developmental processes in any biological system (Walker and Van West, 2007). The zoospore is the primary infecting unit and its role is the transmission of the pathogen from host to host. It is a free-swimming spore, possessing two flagella of different length. Once released, they can swim for hours. Directional swimming of zoospores towards chemical, nutrient or electrical gradients are typical of zoospore taxis (Walker and Van West, 2007). After the motile period zoospores encyst, involving the loss of both flagella and the formation of a primary cell wall (Judelson and Blanco, 2005). The resulting cyst germinates by producing germ tubes and mycelia. After direct germination, hyphae ramify through living plant tissue, forming feeding relationship. The epidemic spread of the diseases, caused *Phytophthora* spp. to be often based on the rapid dispersal from host to host by zoospores that are released from sporangia.

Some of the species have sporangia, which are able to detach from the sporangiophores and when shed, are both airborn and water-born (Erwin and Ribeiro, 1996).

The motile zoospores extend the range of *Phytophthora* beyond the landing cites of air-born sporangia. The sporangia are metabolically active, but vulnerable to death, caused by the harsh environmental conditions as solar radiation and drought. By contrast more true fungi produce desiccated spores which can remain dormant for long periods of time, but which can consequently miss opportunities for host infection (Judelson and Blanco, 2005). The increase of inoculums of *Phytophthora* spp. is caused by the rapid production of sporangia and zoospores from infected plant tissues, when the environmental conditions, the most important of which is the presence of free water are favorable.

The evolutionary strategies of straminipilious fungi and eumycote fungi are similar in that they depend on large population with ephemeral generation times (Dick, 2001). All of the oomycetous microorganisms have short generation time and great reproductive capacity. For this reason, caused by *Phytophthora* spp. diseases are generally considered to be multicyclic (Erwin and Ribeiro, 1996).

Some of the *Phytophthora* species forms chlamydospores. These asexual spores have thicker walls and are capable of persisting in soil and plant tissue for long periods. They are of particular importance as survival propagules to some species (Erwin and Ribeiro, 1996).

The sexual spores (oospores) are the most persistent of all propagules produced by *Phytophthora*. As a product of sexual reproduction, the resulted progeny have been considered more variable than the parent cultures, and might be a source of new variants with increased virulence. The heterothallic species produce oospores mainly when the A1 and A2 (opposite) mating type grow together. Species that produce oospores in single cultures are designated homothallic (Erwin and Ribeiro, 1996).

The function of mating of the A1 and A2 types has been considered to be a true hybridization phenomenon, in which factors that condition parasitism and many other characters would recombine in the progeny. This is supported of the results of tests with nuclear genetic markers, showing that recombination of factors occurs (Erwin and Ribeiro, 1996).

Alien and native *Phytophthora* spp.

The introduction and spread of exotic and invasive species is one of the most important problems in conservation biology. Defining invasive species, not only the causing agents of emerging infective plant diseases should be included as invaders, but also fungi with unknown impact (saprotrophs and mycorrhizal fungi) and fungi with economic, but unknown ecological impact (pathogens of crop plants). It has been estimated that 65-85 % of plant pathogens worldwide are alien in the locations where they were recorded. Some of the most devastating alien species belong to the genus *Phytophthora* (Desprez-Loustau et al., 2007). According to the COP 6 Decision VI/23 (2002, Hague, Netherlands), of the Convention on Biological Diversity (2002), the definition for "invasive alien species" is species, or lower taxon, which is present outside its natural distribution and whose introduction and/or spread threaten biological diversity.

According to Brasier et al. (1999), the risk of emergence of new plant diseases is likely to increase as world trade in plants intensifies and more plants and their associated pathogens are introduced into new biogeographic environments. It raises the possibility closely related, but previously geographically isolated taxa, which lack strong reproductive barriers to come into contact. Many hybrids or introgressants are unlikely to be detected by the conventional methods, mainly morphologically based diagnostic methods used in international quarantine (Brasier et al., 1999).

A brief description of some very dangerous *Phytophthora* species distributed in the world is following. Most of them are still not recorded in Bulgaria, but found in Europe. They are considered as potential very hazardous invaders for the forests and orchards in the country.

Invasive *Phytophthora* pathogens with caduceus sporangia

As already mentioned, some *Phytophthora* species have sporangia which are able to detach from the sporangiophores. They are known as species possessing caduceus sporangia, and undoubtedly, this trait gives them epidemiological advantage (Erwin and Ribeiro, 1996). Some of the most notorious *Phytophthora* spp., causing agents of severe diseases as *P. infestans*, *P. capsici*, *P. ramorum*, *P. kernoviae* and *P. palmivora* has caducous sporangia. From those *P. infestans* and *P. capsici* are largely distributed in Bulgaria on agricultural crops as potato, tomato and pepper.

Since 1995 a new, never observed before disease on native California oak have been causing the death of tens of thousands trees along California and Oregon coast in USA. The disease was named Sudden Oak Death (SOD), or oak mortality syndrome. Since the time when the disease was first noted on tanoaks bordering a creek in California, until 1999 it turned into epidemic, resulting in tens of thousands dying native oaks (Garbelotto et al., 2001). The causing agent, a new *Phytophthora* sp. was isolated in 2000 and described in 2001 as *Phytophthora ramorum* sp. nov. This same pathogen has been detected several years earlier on ornamental plants across nurseries in Europe. For the first time it was found in 1993 on Rhododendron nursery stock in Germany and Netherlands. In 2003 nursery survey across European Union reveals more than 300 nurseries infected with *P. ramorum* only in UK, and infected nurseries in at least 11 other European countries. Soon after that, the pathogen was found spreading from nurseries to woodlands in Netherlands and UK, causing bleeding cankers and foliar lesions on *Quercus*, *Fagus* and other genera (Brasier and Jung, 2004). In 2010 *P. ramorum* continues to be the most intensively managed monitored and researched forest *Phytophthora* species in North America (Frankel and Hansen, 2011).

Another invasive species, *P. kernoviae* was discovered when a range of surveys have been initiated for *P. ramorum* in the nurseries and in the woodlands of UK. The pathogen has overlapping, but smaller range of host plant species compare to *P. ramorum*. It is found to cause large bleeding cankers on European beech (*Fagus sylvatica* L.) and is serious foliar pathogen on *Rhododendron* spp. (Brasier et al., 2005).

Phytophthora ramorum and *Phytophthora kernoviae* are both introduced, but the place of their origin is unknown. Both species have genetically uniform populations', which is explained with the bottleneck effect of introduction (Brasier, 2007).

There are revealed four distinct evolutionary lineages of *P. ramorum*. Lineage NA1 is found in nurseries and forests in

North America, lineage EU1 found both in Europe and North America. Third lineage NA2 was found only in nurseries in North America. The NA1 and NA2 isolates are both from mating type A2, as the isolates from EU 1 lineage are from A1 mating type (Grünwald et al., 2009). The fourth lineage EU2 recently was discovered on larch in Northern Ireland (Van Poucke et al., 2012).

Both *P. ramorum* and *P. kernoviae* has caducous sporangia and air-born pattern of dissemination, they can cause symptomless sporulation on leaves of *Rhododendron* and other hosts, and they are able to colonize the xylem tissue (Brasier, 2007). Major difference between these two species is that *P. ramorum* is out crossing, as well as *P. kernoviae* is self-crossing (Webber, 2008).

Another two new air-born species - *Phytophthora nemorosa* and *Phytophthora folorium* were discovered. *P. nemorosa* has remarkably similarity with *P. ramorum* in host range and symptomology and it was not possible to distinguish the diseases caused by these pathogens in the field**.** In contrast with *P. ramorum*, *P. nemorosa* is usually associated with single killed trees and has behavior suggesting an endemic pathogen (Hansen et al., 2003). The other species with caducous sporangia - *Phytophthora foliorum* is a pathogen on the azalea, causing leaf spot symptoms, and is not associated with significant mortality of azalea (Donahoo et al., 2006).

Invasive *Phytophthora* pathogens with non-caduceus sporangia

One of the most prominent cases of *Phytophthora* spp. with non-caduceus sporangia and soil-inhabiting lifestyle is *P. cinnamomi*.

P. cinnamomi is a pathogen with a cosmopolite distribution and over 3000 host species. It is typical root pathogen and can grow saprophytically in the soil for long periods (Hardam, 2005 and Erwin and Ribeiro, 1996).The pathogen is responsible for the widespread damage in natural ecosystems in Western Australia. It is believed that the pathogen have been introduced in contaminated nursery stock in 1920 and spread into the jarrah (*Eucalyptus marginata* Donn ex Sm.) forests, probably from a centre of origin in the Papua New Guinea-Celebes region (Brasier, 1999) It is likely that the pathogen has a significant role in the mortality and decline of evergreen oaks in the beginning of the 1990s in the Mediterranean region (Brasier, 1999). *P. cinnamomi* has been widely spread also on conifers and woody ornamentals in nurseries and gardens across Europe during the 1960s and 70s. *P. cinnamomi* have also been associated with the little leaf disease of *Pinus echinata* in the 1930s. Symptomatic trees have been first evident adjacent to roads and forest tracks. The disease have been present across about one third of the tree's range, and have forced changes in management on about 2 million hectares of forest land. Both *P. cinnamomi* and *P. cambivora* are responsible for the devastating epidemic on the native European chestnut (*Castanea sativa* Miller) in the beginning of the 20th century (Brasier, 1999).

Another exotic pathogen *Phytophthora lateralis*, introduced probably with infected nursery stock from France in the far 1923 is posing a serious threat to cedar (*Chamaecyparis lawsonia* (Murray) Parl) forests in the Northwest and in British Columbia, Canada. The disease has been so severe in some localities, leading to the abandoning of the Lawson cypress plantings (Erwin and Ribeiro, 1996). The pathogen colonizes trees rapidly, advancing upward from infection points on fine roots until it reaches the main stem. Aerial infection can occur if the weather is humid, although *P. lateralis* has persistent sporangia (Hansen, 1999).

A lethal *Phytophthora* disease threatens the alder population in Europe. It shows to be highly aggressive on alder trees (*Alnus* spp.), and is currently spreading in natural ecosystems across Europe (Ioos et al., 2006). It was observed first in southern United Kingdom in 1993 on *Alnus glutinosa* L., growing along rivers and in horticultural shelterbelts (Brasier et al., 2004; Streito and Gibbs, 1999). The majority of diseased trees are to be found on the banks of streams and rivers or on sites subject to flooding from adjacent watercourses. It has also been found in orchard shelterbelts and occasionally in new woodland plantings (Streito and Gibbs, 1999).

Three years later the occurrence of the Alder disease was conformed from France. Tarry spots associated with a continuous basal lesion and crown transparency symptoms are highly characteristic of the disease (Streito and Gibbs, 1999). In Poland, trees with damaged roots did not have a cankerous discharge on trunks, just bark splitting (Oszako, 2007).

Devastating alder *Phytophthora* appears to comprise a range of species hybrids. Brasier and colleagues describes it as a *Phytophthora alni* sp. nov. and its variants. The standard hybrid type is formally designated as *Phytophthora alni* subsp. *alni* (Brasier et al., 2004). It was shown to be near-tetraploid (Ioos et al., 2006), and the other unstable variant types, with chromosome numbers intermediate between diploid and tetraploid, were designated as *P. alni* subsp. *uniformis*; and P. *alni* subsp. *multiformis.* The results of pathogenicity experiments carried out over 4 year period (1995-1998) shows that the isolates of the standard alder *Phytophthora* from several European countries are potentially highly aggressive pathogens of alder bark and appears to be relatively specific to alder. The other natural variants were comparatively weak pathogens, with one exception (Brasier and Kirk, 2001).

The standard alder *Phytophthora* type is widely distributed across Britain, Netherlands, France, Germany and Austria (Brasier, 2000). To the moment it is reported also from Belgium, Czech Republic, Hungary, Ireland, Italy, Lithuania, Poland, Slovakia, Slovenia, and Sweden (Forestry Commission, http://www.forestry.gov.uk/fr/INFD-737J2S).

Recently *P. alni* subsp. *uniformis* was reported to be isolated from soil beneath *Alnus incana* subsp. *tenuifolia* exhibiting dieback at two sample locations in remote, unmanaged stands hundreds of miles apart on the Kenai Peninsula and near Denali National Park in Alaska (Adams et al., 2008).

Non caducous species causing aerial cankers

The *Phytophthora* species which sporangia remain attached to the sporangiophores have soil inhabiting lifestyle and are predominantly root-infecting parasites (Cooke et al., 2000).

In both southern England and Bavaria (Germany) it was found that the non-caducous soil and root inhabitants *P. cambivora*, *P. citricola* and *P. gonapodyides* can cause aerial bark lesions on beech (Jung et al., 2005). Also in UK *P. citricola*, which is a soil born species with non-caducous sporangia was found causing aerial bark lesions on European sycamore (*Acer pseudoplatanus* L.). It is still unknown how the inoculum of this soil borne pathogens becomes “aerial”. There were proposed two possible explanation for this unexpected pathogen behavior. One is the transport of inoculum via snails, which have been feeding on the exudates, and the other explanation might involve movement of inoculum via embolisms in infected xylem (Brasier and Jung, 2004).

Interspecific hybrization within the genera *Phytophthora*

In 1998 in The Netherlands from diseased ornamental plants (*Spathiphyllum* and *Primula*), grown on hydroponics system have been obtained three *Phytophthora* species, which have been proved to be interspecific hybrids between *P. cactorum* and *P. nicotianae*. Hydroponics systems facilitate physical encounters between different *Phytophthora* species, and it is possible natural hybridization processes to occur, as it is known that many different *Phytophthora* species are present in greenhouses. Since the introduction of hydroponic systems in greenhouses in the Netherlands, an increase of *Phytophthora* outbreaks has been recorded (Man in‘t Veld et al., 1998).

Brasier and colleagues proposed that the standard alder *Phytophthora* is a recently evolved hybrid involving *P. cambivora* and a *P. fragariae*-like species as parents (Brasier et al., 2004; Ioos et al., 2006). In order to examine this hypothesis, Ioos and colleagues investigate the occurrence and the allelic distribution of four nuclear and two mitochondrial genes, on a wide collection of *P. alni* and closely related species. They favor the hypothesis that *P. alni* subsp. *alni* may have arisen via hybridization of two taxa close to and *P. alni* subsp. *uniformis* and *P. alni* subsp. *multiformis,* if not themselves. Sexual, rather than somatic, hybridization is more likely to have occurred. The authors guess that *P. alni* subsp. *multiformis* and *P. alni* subsp. *uniformis*, or *P. alni* subsp. *multiformis*-like and *P. alni* subsp. *uniformis* -like species, might have existed for a long time near alder trees before the recent emergence of large-scale decay in the European alder population. The occurrence of these species in the past might not have been noticed because of the lack of conspicuous symptoms or declines of whole trees (Ioos et al., 2006).

In addition, the appearance of new hybrids in natural conditions, for instance *P. alni* and its subspecies is hardly to predict. The resulting new species might posses’ qualitatively new features-differences in virulence, host preferences and others.

Climate change and *Phytophthora*

Little is known about the influence of the climate change on the resident *Phytophthora* species, which appears not to be of great concern, as far as they do not cause significant diseases on their host plants. Much more attention is paid to the introduced species, and how their behavior will alter in answer of the global climate change.

The second assessment report of the Intergovernmental Panel on Climate Change (IPCC), edited in 1995 concludes that the climate has changed over the past century in response to increasing atmospheric CO_2 with the global mean surface air temperature increasing between 0.3 and 0.6°C. Climate model simulations suggest an increase in global mean temperature between 1 and 3.5°C by the year 2100. The Kyoto Conference in December 1997 led to a climate treaty ratified by more than 150 countries. It is expected climate change to influence the geographical distribution and growth of plant species around the world. The most likely impacts of climate change would be shifts in the geographical distribution of host and pathogen, changes in the physiology of host-pathogen interactions, and changes in crop loss. Another important impact may be through changes in the efficacy of control strategies. The mechanism of pathogen dispersal, suitability of the environment for dispersal, survival between seasons, and any change in host physiology and ecology in the new environment will largely determine how quickly pathogens become established in a new region. Warming and other changes could also make plants more vulnerable to damage from pathogens that are currently not important because of unfavorable climate (Coakley et al., 1999).

A series of computer models has been run to asses how predicted change in the mean annual temperature will alter the activity of *P. cinnamomi* in Europe. *P. cinnamomi* activity is constricted by both summer and winter temperatures. According to these models up to the year 2050, activity of *P. cinnamomi* is likely to increase significantly in the Mediterranean region and in maritime climates such as that of coastal western Britain, but not in central Europe (Brasier, 2000). There is a possibility the rise in the mean annual temperatures to lead to the increased activity of other forest *Phytophthora* spp. (Brasier, 2003). It is unknown what will be the influence of the possible climate changes on the endogenous species, and to what extend it might alter their ecological behavior.

The problem with the worldwide spread of *Phytophthora* by human activities

Movement of plants and plant products between biogeographically zones by human activities is now generally accepted to be the primary mode of introduction of exotic pathogens and pests (Brasier, 2008). There are many examples of introduced invasive species causing significant damage to both managed and natural ecosystems. The history of *Phytophthora infestans* appearance on potato crop in Ireland in 19th century is the most frequently cited example for the consequences of introduction of an invasive species on new place. In a review from 1997, Goodwin summarizes the literature on the population genetic of *Phytophthora* available to that moment. He hypothesized that the first global migration of *P. infestans* has occurred on three stages. First it has appeared in the eastern United States in 1843, probably from Mexico on infected tubers. The second step in the migration took place during 1844 or 1845, probably in a single shipment of potatoes, and subsequently in almost all potato growing areas worldwide (Goodwin, 1997). Brasier (2007) outlines the problems behind the increased sudden appearance of pathogens as *P. ramorum*, *P. kernoviae*, *P. alni* and *P. pinifolia.* It is the growing threat to forests and natural ecosystems from invasive forest pathogens. The author is making a conclusion that although the international system is well regulated in many countries, for example North America and the U.K., it still has certain fundamental flaws. One is that many invasive pathogens were unknown to science until they escaped from their natural range, becoming an invasive. Unknown threats cannot be formally regulated against because they do not meet the requirement of being "listable" as a named threat organism. *Phytophthora* pathogen listed on quarantine schedules as causing damage on a specific host in particular country might be a serious threat to entirely different hosts and ecosystems in another country. The absence of that specific host is therefore insufficient reason to consider the pathogen "non-threatening" (Brasier, 2007).

The appearance of *P. lateralis* on the wild growing Lawson cypress in the geologically and climatically distinctive Klamath ecological province in SW Oregon and NW California is another documentation of a system of unregulated international plant movements. The probable place of origin of *P. lateralis* might be Eastern Asia. It is supported by the tolerance of the Asian species to the pathogen (Hansen, 1999).

Another issue is that visually healthy plants may harbour a sporulating pathogen in the roots or the foliage, and a visual inspection alone may be insecure. Thus shipping stock bare-rooted is not a guarantee of plant biosecurity, even more it might provide a false sense of security.

The movement of inoculum on feet of people, animals and on wheeled vehicles is clearly a major potential pathway for local and even international spread of *P. ramorum* and *P. kernoviae*. At least two invasive *Phytophthora* species has been shown to spread on feet and machinery: *P. cinnamomi* in jarrah forests and native vegetation in Western Australia, and *P. lateralis* in Port-Orford-cedar areas in the Pacific Northwest (Brasier, 2007).

Pest Risk Analysis (PRA) is the fundamental process, which is prescribed by the World Trade Organization Sanitary and Phytosanitary Agreement (WTO) as well as the International Plant Protection Convention (IPPC). The WTO Sanitary and Phytosanitary Agreement require that a scientific assessment of the risk posed by a plant pest is undertaken before any phytosanitary measures are set. Such measures must be the minimum needed to reduce the risk without unnecessarily impacting on trade. The organism in question must be identified as being a unique taxonomic entity. Recently, pest risk analysts have had to consider the risks posed by previously unidentified pathogens new to science (Sansford, 2009).

Phytophthora spp. in Bulgaria

In Bulgaria, species from genus *Phytophthora* are known generally as pathogens on some annual agricultural crops as potato, tomato, pepper and tobacco. They also cause problems in orchards and nurseries. Some *Phytophthora* species are causing rotting of ornamental plants grown in greenhouses (Stancheva and Rosnev, 2005). *Phytophthora fragarie*, a species that is included in the quarantine list is considered not to present in Bulgaria. In her DSc. thesis, Nakova (2012) summarizes available information in the literature concerning the presence of *Phytophthora* species in the Bulgarian agro ecosystems, as well as in the semi-natural and natural ecosystems (Table 1).

Nakova (2010) reported disease symptoms that are appearing on 2-3-year old apple trees in the region of Bjaga village (Plovdiv) during the period 1998-1999, and also on 2-year old cherry rootstocks (Katunitza, Plovdiv) and 2-year old cherries (Trilistnik, Plovdiv). She also is informing about *Phythophthora* infections of root and crown of cherries registered in Kjustendil, Sliven, Jambol, Karnobat, Bourgas and Svishtov regions, and on apples from Plovdiv (Bjaga, Asenovgrad and Tzalapitza), Kjustendil, Pazardzik and Haskovo (Stalevo) regions. She isolated and identified as *Phytophthora cactorum*, *Phytophthora citrophthora* and *Phytophthora cryptogea*, based on morphological and molecular traits from infected plant tissue from apple in Plovdiv and Kjustendil regions. She concludes that the disease spread was 2-3 % in most gardens, only in an apple orchard in Bjaga (Plovdiv region) it was up to 8-10 %.

Phytophthora citricola and *Phytophthora citrophthora* have been reported from raspberry (*Rubus idaeus* L.) grown in a plantation of the experimental station of small fruits in Kostinbrod, Sofia region (Ilieva et al., 1995).

All mentioned above *Phytophthora* species described as invasive and alien species are potentially extreme hazard plant pathogens for both natural and agro ecosystems in Bulgaria. Recently an international project for searching the biodiversity and distribution of *Phytophthora* species in the country and most of the Balkan region was conducted (Project ERA 138/01). It was considered as more detailed investigation of *Phytophthora* species in the natural ecosystems accomplished recently. In the frame of this project no one of the alien species discussed in this review, was found in the country. Nevertheless of this fact, larger, continues and more comprehensive monitoring is needed to be carried out regularly in order to cover all the specific and potentially inhabitance for the *Phytophthora* pathogens.

Regulation of the movement of plants and plant products in Bulgaria

In Bulgaria, the state policy in the field of pest control is carried out by the Ministry of Agriculture and Food through the National Plant Protection Service (NPPS), its regional offices for Plant Protection (RSPP) and the Central Laboratory for Plant Quarantine (CLPQ). National plant protection interacts with the competent authorities of other countries - EU members and the European Commission. The Minister of Agriculture and Food on the proposal of National Service approves the lists of pests, plants, plant products and other goods subject to phytosanitary control (LPP, 2011). Plant protection low and Ordinance № 1 of 27 May 1998 on phytosanitary control is the legal basis of the state policy in plant protection. Only two species of *Phytophthora* are included in the quarantine lists: *P. ramorum* and *P. fragariae*, and are subject of phytosanitary control (LPP and Commission decision, 2002). If we compare this number with the number of known and the presumable number of unknown to science *Phytophthora* species it becomes clear that this is extremely insufficient. Intensifying the control and investigations of those plant pathogens is needed in the light of the discussed above impact of the climate change and human activities on the increased distribution and biodiversity of the *Phytophthora* spp.

Methods for diagnostic of *Phytophthora* spp.

Although *Phytophthora* spp. are aggressive pathogens, they are also seasonally active, delicate ephemeral organisms that are quickly replaced in host tissues by other fungi and by bacteria. It is therefore often difficult to isolate those pathogens for diagnosis, for taxonomic identification or for quarantine tests (Brasier, 1999). Some of the advantages and drawbacks of the available tools for detection and identification of *Phytophthora* species according to Cooke et al. (2007) are shown on Table 2.

In regard to prevent introduction of new invasive pathogens in new geographical regions, which impact on the biodiversity in a given country is difficult to predict, it is important to remember the history of the appearance of newly emerged pathogens worldwide. We need to know the likely ways of the movement of the microorganisms between different points of the globe, and with the help of the continuously developing tools for detection, and more strict legislation rules in the world movement of goods to restrict the devastating impact of the human progress on the world biodiversity. The case with invasive *Phytophthora* pathogens is one of the many other cases of introduction of new organ-

Law on the protection of plants. Reflected by the denomination on 05.07.1999. Prom. SG. 91 from 10 October 1997., amend. SG. issue 90 from 15 October 1999., amend. SG. issue 96 from 9 November 2001., suppl. SG. issue 18 from 5 March 2004., amend. SG. issue 26 from 28 March 2006., amend. SG. issue 30 from 11 April 2006., amend. issue SG. 31 from 14 April 2006., amend. SG. issue 96 from 28 November 2006., amend. SG. issue 13 from 8 February 2008., amend. SG. issue 36 of 4 April 2008., amend. SG. issue 43 of 29 April 2008., amend. SG. issue 82 from 16 October 2009., amend. SG. issue 8 from 25 January 2011., amend. SG. issue 28 from 5 April 2011(Bg).

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Table 1
***Phytophthora* species reported in Bulgaria (according Nakova, 2012)**

***Phytophthora* spp.**	**Hosts**	**Authors**
Phytophthora parasitica var *nicotianae*	*Nicotiana tabacum*	Atanasov, 1928; Vanev et al., 1993
	Lyccopersicon esculentum	Vanev *et al*., 1993,; Ilieva 1979, 1990
	Dianthus caryophyllus	Vanev *et al*., 1993,; Ilieva 1979, 1990
Phytophthora parasitica var *terestria*	*Lyccopersicon esculentum*	Kovachevski and Hristov, 1929
Phytophthora infestans	*Solanum tuberosum*	Vanev et al., 1993 (The report is from Atanasov, 1934)
	Lyccopersicon esculentum	Vanev *et al*., 1993 (The report is from Atanasov, 1934)
Phytophthora erythroseptica	*Lyccopersicon esculentum*	Vanev *et al*., 1993 (The report is from Atanasov and Kovachevski, 1929)
	Solanum tuberosum	Vanev *et al*., 1993 (The report is from Atanasov and Kovachevski, 1929)
Phytophthora erythroseptica var. *erythroseptica*	*Lyccopersicon esculentum*	Ilieva, 1990
Phytophthora cryptogea var. *richardiae*	*Calla aetiopica*	Hristova, 1941
Phytophthora cryptogea	*Lyccopersicon esculentum*	Ilieva 1979, 1990
	Capsicum annum	Ilieva, 1990; Ilieva *et al*., 1992
	Fragaria spp.	Ilieva, 1990; Ilieva *et al*., 1992
	Cucumis melo	Ilieva, 1990; Ilieva *et al*., 1992
	Nicotiana tabacum	Ilieva, 1990
	Leontopodium alpinum	Ilieva, 1990; Ilieva *et al*., 1992
	Beta vulgaris	Ilieva, 1990; Ilieva *et al*., 1992
Phytophthora cactorum (Lebert et Cohn)	*Calceolaria* sp.	Vanev *et al*., 1993
	Clarkia sp.	Vanev *et al*., 1993
	Fragaria elatior	Vanev *et al*., 1993
	Paeonia pelegrina	Vanev *et al*., 1993
	Panax quinquefolius	Bobev, 2003
	Fragaria sp.	Ilieva, 1995
Phytophthora capsici (Leonian)	*Capsicum annum*	Kovachevski, 1934; Elenkov and Bahriev, 1975; Elenkov and Hrelkova, 1977; Elenkov and Hristova, 1978; Ilieva, 1976, 1979, 1990; Vanev et. al, 1993; Georgieva, 1985
	Citrulus vulgaris	Vanev et al., 1993
	Dianthus caryophyllus	Ilieva, 1988;Vanev *et al*., 1993;
	Lycopesicum esculentum	Vanev *et al*., 1993, Vanev *et al*., 1993
	Melo sativus	Ilieva, 1990; Vanev *et al*., 1993
	Solanum melongena	Ilieva, 1990; Vanev *et al.,* 1993
Phytophthora citricola	*Fragaria elatior*	Vanev *et al*., 1993
	Fragaria sp.	Ilieva, 1995
	Rubus sp.	Ilieva, 1995
Phytophthora dreschleri	*Cucumis sativus*	Ilieva, 1985, 1990;Vanev *et al*., 1993
	Cucumis melo	Ilieva, 1985, 1990
Phytophthora fragariae	*Fragaria* spp.	Vanev *et al.,* 1993; Nakov, 1976
Phytophthora richardiae	*Zantedeschia aetiopica*	Vanev *et al*., 1993
Phytophthora megasperma	*Fragaria* sp.	Ilieva, 1995

Table 2
Tools for detection and identification of *Phytophthora* species according to Cooke et al. (2007)

Approach	Advantages	Drawbacks
Traditional detection methods		
Visual detection for signs of *Phytophthora* on infected plant parts and direct microscopic examination of diseased material		Limited to restricted number of host-pathogen combinations in which airborne spores are visible as a downy mass of sporulation (Cooke et al., 2007) In woody trees symptoms might not be detected even if the tree have lost 50% of its lateral roots (Erwin and Ribeiro, 1996).
Baiting with plant material and Isolation of the pathogen from infected plant tissues, water and soil, using general or selective agar media	Common and appropriate approach, cost effective, simple and familiar to many workers. In the case of river sampling the bait can be left in the substrate for relatively long periods and may thus recover low levels of *Phytophthora*.	The number of species detected might be strongly reduced due to the selective colonization and development on different types of baits. The oospore population might be not detected. Masking or inhibition of the relatively slowly growing *Phytophthora* by other competitive microorganisms. Assessments based solely on baiting are likely to underestimate the diversity.
Serological methods		
Use of antibodies (molecules produced by the mammalian immune systems to identify invading organisms or substances.	Monoclonal antibodies have been utilized for the production of *Phytophthora* species and genus specific commercial detection kits, using both membrane-based and ELISA formats. Some of these kits proved appropriate for the identification of important forest Phytophthoras.	Monoclonal antibodies are generally slow to produce, expensive to both produce and maintain, and occasionally cell lines may die or stop producing the required antibody.
Molecular methods		
Conventional PCR	The most important and sensitive technique presently available for the detection of plant pathogens. Microorganisms do not need to be cultured. Single target molecule can be detected in a complex mixture	The adoption of PCR for routine detection of plant pathogens is relatively slow, often due to technical limitations related to the post-amplification amplicon detection procedures.
Nested PCR	Improved sensitivity and specificity compared to conventional PCR	Increases the risk of false positives due to cross contamination and involves more time and effort.
Real-time PCR	Provides a more rapid means for screening water, plant and soil samples. Eliminates the requirement for post amplification processing steps thus saving time and labor, compared to conventional PCR. Reduces the risk of cross contamination. It is suitable for estimating plant pathogen biomass in host tissues or environmental samples. It is not affected by external factors such as other fungal species that could conceal the presence of the pathogen.	
Multiplex real time PCR	Enable detection multiple templates within a single reaction, particularly important for *Phytophthora* species in forest ecosystems, where they are frequently found in “clusters,, on the same site(tree) Increased sensitivity in comparison to other detection methods, including conventional PCR.	
DNA micro-and macro-arrays	Enables the simultaneous detection of many plant pathogens. Macro-arrays have proved appropriate for the multiplex detection of plant pathogens from complex environmental samples including those derived from soil and plants.	
Direct sequencing from environmental samples	Lack of cross-reaction with the ubiquitous *Pythium* species. Enables the detection of many *Phytophthora* species.	

isms from all existing taxa, which turns into causing agents of devastating epidemics.

Conclusion

In regard to prevent introduction of new invasive pathogens in new geographical regions, which impact on the biodiversity in a given country is difficult to predict, it is important to remember the history of the appearance of newly emerged pathogens worldwide. We need to know the likely ways of the movement of the microorganisms between different points of the globe, and with the help of the continuously developing tools for detection, and more strict legislation rules in the world movement of goods to restrict the devastating impact of the human progress on the world biodiversity. The case with invasive *Phytophthora* pathogens is one of the many other cases of introduction of new organisms from all existing taxa, which turns into causing agents of devastating epidemics. In Bulgaria as a country with different climate areas and with active plant materials trade and nursery stock exchange, and very high biodiversity of natural plant species and agricultural crops, it should be taken into account. Permanent control of *Phytophthora* should continue to be applied with increased number of investigated *Phytophthora* species.

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