Review of the most important pathogens in Serbian forest nurseries

Nenad Keča

Faculty of Forestry, University of Belgrade, Kneza Višeslava 1, Belgrade, Serbia

✉ nenad.keca@sfb.bg.ac.rs

ARTICLE INFO

Citation:
Keča N (2016) Review of the most important pathogens in Serbian forest nurseries. Reforesta 1: 164-177.DOI: http://dx.doi.org/10.21750/REFOR.1.09.9

Editor: Vladan Ivetić, Serbia
Received: 2016-02-07
Accepted: 2016-05-06
Published: 2016-05-25

CrossMark

This article is Chapter 8 of establishing issue of Reforesta Journal, edited in form of Thematic Proceedings, by Vladan Ivetić and Steven Grossnickle.

Abstract

Successful reforestation depends on the quality and health of seedlings. Targeted production in forest nurseries should produce plants that will be able to survive unfavorable environmental and weather conditions in Serbia. Pathogens can reduce the vitality of seedlings and decrease survival after outplanting. The most common pathogens identified on seeds of both conifer and broadleaved seedlings are presented. The most frequently used fungicides for the control of pathogens in Serbian forest nurseries, as well as the best time of their application, are reviewed.

Keywords

Seed, Seedling, Nursery production, Pathogens, Fungicides

Contents

1 Introduction

Serbia, having 29.1% (2.252.000 ha) of its area covered in forest, is one of the mild-forested countries of Europe (Banković et al. 2009). The forestry sector is currently under pressure to improve the situation and increase reforestation efforts. An action plan for Serbian forestry has predicted an increase of forest coverage to 35% by 2050. The reforestation of such a large area will pose many challenges to the forestry professionals; among them, seedling health will certainly be one the most challenging.

Forest ecosystems throughout Serbia consist of 49 autochthonous woody species. Broadleaf species (40) dominate over coniferous species (9) (NFI 2009). Despite the great diversity of trees, only a few species dominate in Serbian nursery production.

In Serbia there are 55 registered forest nurseries. The average volume of production in the past 10 years is around 12 million, of which about 2/3 are coniferous and 1/3 deciduous seedlings. Different types of planting materials are represented ranging from bare root and Nisula rolls, to container trees (Ivetić and Vilotić 2014). The ages of produced material vary from 1+0, for hardwood and container seedlings, to 2+3, for spruce. Mostly produced seedlings are bare roots, while containerized seedlings participate in a total production of approximately 20%. Nursery infrastructures are differently equipped with a range of nurseries for producing seedlings with bare roots in traditional seedbeds, through Dunemann beds, to two nurseries equipped with the most modern lines for automatic planting and filling Hiko type containers (V. Ivetić, pers. comm.). Dominant species produced in Serbia are Austrian pine (*Pinus nigra* Arn.), Scots pine (*Pinus sylvestris* L.), and Norway spruce (*Picea abies* L.). The production of oak (*Quercus* spp.) seedlings has considerably increased in recent years (Directorate of Forests, unpublished).

Natural regeneration dominates Serbian forestry practice; this maintains genetic diversity and enables new stands to be more vigorous to the challenges of coming climate change (Spittlehouse and Stewart 2003; Millar et al. 2007). Despite the domination of natural regeneration, some sites are difficult or impossible to regenerate without seeding or planting seedlings. One species that is regularly planted on harsh sites is Austrian pine; most reforestations done in Serbia are performed using this species (Ranković 2009).

The production of large numbers of trees, as is the case with seedlings, is threatened by of a number of abiotic and biotic factors (Karadžić and Anđelić 2001). A great number of plants of the same age could be easily attacked and destroyed by various pathogens. Without appropriate pest control, damage could occur, not only during the process of seed storing and manipulation, but also during development of the root system, stem, and crown. Disease detection and application of appropriate control measures play vital roles in the production of adequate planting material (Lazarević et al. 2012a, 2012b).

This paper presents some of the most important pathogens that cause destruction of plant seeds and parts of the plant during development in forest nurseries and plantations. According to the National Forest Strategy, only seedlings of adequate origin and properties, which meet all requirements and are not diseased, may be used for afforestation (Forestry development strategy for the Republic of Serbia 2006; Lazarević et al. 2012b). This review presents current knowledge concerning the identification and control of diseases in Serbia.

2 Main groups of pathogens in forest nurseries

Intensive production of seedlings depends on a constant supply of seeds. To ensure the quality of descendant seedlings, seeds are collected in established seed orchards and selected stands (Ivetić and Vilotić 2014). Collection and manipulation of seeds present many opportunities for contamination by various microorganisms (viruses, bacteria, phytoplasma, fungi, etc.) (Salisbury 1953). They can cause decay and destroy seed nutritional reserves or act as saprotrophs that cause no damage to the seed or seedling (Peno 1971).

The first step to assess the presence of microorganisms consists in routine seed tests for germination and purity. Sometimes a seed may appear to be healthy, even if it is abundantly infected with pathogens (Rees and Phillips 1986). Laboratory tests will not detect some pathogens that can cause damage in the field, on the germ tube, or to seedlings. A ten-year study of mycobiota on four coniferous and the five most important broadleaved hosts is presented in Table 1.

Among studied samples, the most frequently observed species were from the *Penicillium*, *Aspergillus*, and *Fusarium* genera and *Botrytis cinerea*. Some species from the genus *Fusarium* can cause damping-off on young seedlings not only during the first few weeks after germination, but also later, on seedlings up to the age of one year. The most-frequently observed species are not host specific and can destroy the seed of many tree species (Sutherland et al. 2002).

2.1 Seed-borne diseases

Aspergillus species are well known contaminants of various substrata and are common seed contaminants. A considerable number of species (*A. clavatus*, *A. fumigatus, A. nidulans*, *A. niger*, etc.) can be found on seeds, especially if seeds have been stored in humid conditions and have reduced germination capacity (Malone and Muskett 1964). Production of various chemical substances, such as alcohols, enzymes, antibiotics, and toxins, can cause diseases in plants, animals, and man (Bennett 2010). The species that could be linked to seed damage is *A. niger*, which was also reported to have caused systemic infection on ornamental plants (Samson et al. 2001).

Penicillium species are known to contaminate bulbs, rhizomes, and fruit, but are generally considered as saprophytes (Bennett 2010). Sometimes the secondary spore contamination of seeds is quite large, but it is generally less than 1%, particularly in laboratory conditions in which germ tubes were infected with representatives of this genus. *Trichothecium roseum* Link ex Fr. is a saprophyte of various substrates, but in some conditions it could act as a weak parasite (Malone and Muskett 1964). Browning of the fruit (a nut-like drupe) and cotyledons of *Tilia* species during seed health tests was observed. The cause of this phenomenon, however, was not thoroughly studied.

Phomopsis spp. was associated with acorns of *Quercus* spp. and other tree species without any further information on pathogenic behavior (Kehr and Schroeder 1996). Both pathogenic *P. quercella* and saprobic *P. glandicola* were observed on *Q. robur and Q. petraea* acorns. In this study, *Phomopsis* was found to abundantly spoilage non-germinating ash seeds (*Fraxinus* spp.), but we could not confirm that the species was the cause of seed death. The *Rhizopus* genus has many species that are regularly found on seeds. Samples from coniferous hosts originating in the western regions of Serbia were severely contaminated with *Rhizopus* spp.

Graphium, similar to *Acremonium* and *Aureobasidium,* was rarely observed and was without obvious influence on the cotyledons and the seed germ tube development. Some *Acremonium* species (e.g. *A. implicatum)* are entophytic and are transmitted in the wild by seeds of *Lolium* spp. (Ewis et al. 1997).

Many molds, including *Mucor mucedo* de Bary and Woron., are considered as saprophytes that are present in the laboratory; contamination of seed lots is inevitable during routine tests.

Observed bacteria contamination was significant for some examined samples, but the destruction of seeds and cotyledons could not be correlated to the bacterial infection.

Table 1. Nursery pathogens observed on seeds and seedlings in Serbia

Pathogen type	Pathogen	Tree host species	References	Author observations	
				Observed (%)	Significance*
Seeds					
Viruses	Elm mottle virus (EMoV) Cherry leaf roll virus (CLRV) Plum pox mosaic virus Tobacco mosaic virus	*Ulmus* spp. Different hosts *Prunus* spp. Different hosts	Mink 1993	n.a.	??
Bacteria	Unidentified			0-41	??
Phytoplasmas	Unindentified			n.a.	??
Fungi	*Botrytis cinerea*	*Quercus* spp.		2-28	+++
	Graphium sp.			7-35	++
	Penicillium spp.			3-6	??
	Penicillium spp.	*Fagus sylvatica*		28	++
	Aspergillus spp.			23	++
	Botrytis cinerea			15	+++
	Fusarium sp.			31	+++
	Rhizoctonia sp.			15	++
	Penicillium spp.	*Robinia*		17	++
	Aspergillus spp.	*pseudoacacia*		13	++
	Fusarium spp.			13	+++
	Penicillium spp.	*Fraxinus* spp.		22	++
	Phomopsis sp.			33	+
	Aspergillus spp.			6	++
	Fusarium sp.			8	+++
	Aspergillus spp.	*Pinus* spp.		3-12	++
	Penicillium spp.			11-25	++
	Botrytis cinerea			0-34	+++
	Epicoccum sp.			0-10	-
	Fusarium spp.			3-8	+++
	Aureobasidium sp.			1-4	??
	Penicillium sp.	*Picea* spp.		5-19	++
	Aspergillus sp.			0-21	++
	Fusarium sp.			2-10	+++
	Botrytis cinerea			5-7	+++
	Rhizopus sp.			0-31	++
	Aspergillus sp.	*Abies* spp.		2-14	++
	Cladosporium sp.			5-27	+
	Fusarium sp.			1-7	+++
	Penicillium sp.			1-9	++
	Penicillium sp.	*Picea omorika*	Cvjetković 2011	2-35	++
	Rhizopus sp.			5-51	??
	Botrytis cinerea			3-25	+++
	Fusarium sp.			3-8	+++
	Acremonium spp.			1-8	??
	Aureobasidium sp.			1-3	??

Table 1. Nursery pathogens observed on seeds and seedlings in Serbia, cont.

Pathogen type	Pathogen	Tree host species	References	Author observations	
				Observed (%)	Significance*
Seedlings of deciduous species					
Viruses	Poplar mosaic virus (PopMV)	*Populus* spp.	Nienhaus 1985, Büttner et al. 2013	n.a.	??
	Tabacco mosaic virus	*Quercus* spp.			
	Tabacco necrosis virus	*Quercus* spp.			
	Elm mottle virus (EMoV)	*Ulmus* spp.			
	Cherry leaf roll virus (CLRV)	*Ulmus* spp., *Fagus* spp.			
	Bean yellow mosaic virus	*Fagus* spp.			
	Plum pox mosaic virus	*Prunus* spp.			
Bacteria	*Agrobacterium tumefaciens*	Different hosts		n.a.	++
Phytoplasmas	Mycoplasma-like organisms	*Ulmus* spp.		n.a.	??
Fungi	*Cryptodiaporthe populea* (Sacc.) Butin	*Populus* spp.	Keča et al. 2015		+++
	Drepanopeziza punctiformis Gremmen	*Populus* spp.			+++
	Erysiphe alphitoides Griffon and Maublanc	*Quercus* spp.	Karadžić and Milijašević 2005		+++
	Melampsora allii-populina	*Populus* spp.	Keča et al. 2015		+++
	M. larici-populina	*Populus* spp.			+++
Seedlings of coniferous species					
Viruses	Tomato mosaic virus	*Picea* spp.	Nienhaus 1985, Büttner et al. 2013	n.a.	??
	Scots pine mosaic virus	*Pinus* spp.			
	Tobacco necrotic virus	*Pinus* spp.			
	Not identified	*Abies* spp., *Picea* spp., *Pinus* spp.			
Bacteria	*Agrobacterium tumefaciens*	Different hosts		n.a.	++
Phytoplasmas	Mycoplasma-like organisms	*Larch* spp.		n.a.	++
Fungi	*Botrytis cinerea*	Different hosts		n.a.	++
	Chrysomyxa abietis (Wallr.) Unger	*Picea* spp.			++
	Chrysomyxa pirolata Wint.	*Picea* spp.			+
	Diplodia sapinea	*Pinus* spp.			++
	Fusarium spp.	*Pinus* spp.			+++
	Herpotrichia juniperi (Duby) Petrak	*Picea* spp.			+
	Lophodermium seditiosum	*Pinus* spp.			+++
	L. pinastri	*Pinus* spp.			++
	Mycosphaerella pini Rostr. apud Munk	*Pinus* spp.			+++
	Phacidium infestans P.Karst.	*Pinus* spp.			+
	Pythium spp.	Different hosts			+++
	Phytophthora spp.	Different hosts			+++
	Thanatephorus cucumeris (A.B.Frank) Donk	Different hosts			++

* (-) typical saprophytes; (+) decaying fungi; + weak pathogen; ++ facultative parasites, they cause problems only exceptionally; +++ strong pathogens that could be the practical problem; ?? unknown trophic behavior (Keča et al. 2015).

2.2 Conifer tree diseases

Conifers are the dominant species produced in forest nurseries in Serbia. Generally, conifers are susceptible to the attack of pathogens in all phases of development, from germination, in the seedling phase, but also during growth in the second and third year. A wide range of diseases can cause problems on root systems, stem and needles. The most often observed in nurseries are presented.

Chrysomyxa pirolata Wint. is a pathogen that can frequently be found on spruce cones and cause their destruction. It was earlier reported that *C. pirolata* decreases the quality of Norway spruce seeds (Lilja et al. 2010). According to Karadžić (2007) up to 90% of seeds from infected cones show a decrease in weight and vitality.

Fusarium is a genus with a great number of plant-pathogenic species, ranging from 53 (Lević 2008) up to 1,000. These cause diseases in several agriculturally important crops, including cereals, and could also be harmful to humans and animals (Booth 1971). Different *Fusarium* species can be found on seeds (*F. proliferatum* (Matsush.) Nirenberg ex Gerlach and Nirenberg, *F. semitectum* Berkeley and Ravenel, *F. subglutinans* (Wollen et Reink) Nelson, Toussoun & Marasas (Cvjetković 2011), but most post-emergence damping off in pine production was related to *F. oxysporum* or to *F. oxysporum* subsp. *pini* and *F. avenaceum* (Fr.) Sacc. (Lilja et al. 1995). Damage is usually present if soil humidity is too high or after late sowing, when temperatures reach 22–26°C (Karadžić and Anđelić 2001), which is optimal for development of fusariosis (Sutherland and Davis 1991). Soil pH (6–7) and humidity are important for *Fusarium* incidence; fertilization can accelerate the infection process. Less damage is observed in sandy soil as well as rich soil with antagonistic organisms.

Rhizoctonia species damage is more prevalent in natural regeneration than in nurseries. Inadequately treated seeds, especially those of beech or seedbeds, can exhibit substantial losses due to *Rhizoctonia solani* Kühn, while damage is rarely observed in container production. Among resistant conifers are *Juniperus*, *Cupressus, Thuja*, and *Chamaecyparis*, while *Quercus*, *Ulmus* and *Robinia* are considered as resistant hardwood species (Tomić et al. 2011).

Grey mold can be found as a saprophyte in almost all nurseries in its teleomorphic (*Botryotinia fuckeliana* (de Bary) Whetzel) and asexual stages (*Botrytis cinerea*). The fungus is usually present on dead plant parts or lower branches (Lilja et al. 2010). The first symptoms of its presence involve needle chlorosis, with later developments of infection, greyish mycelium and the presence of branched conidiophores that appear on necrotic tissues (Lilja et al. 2010). Damage from this parasite is connected with other abiotic damage such as frost, herbicides, low light intensity, extended drought, or water saturation (Zhang and Sutton 1994). In the Balkan region, seedling decline is also usually connected with cold and wet weather (Karadžić 2010). Under these conditions, even healthy plants could be infected (Karadžić and Anđelić 2001). It was observed that two-year-old seedlings could die in less than three weeks under those conditions. Weedy nurseries with dense seedbeds that maintain high relative humidity favor pathogen infections. Forest nurseries that are closer to the plantations on natural forests are exposed to the inoculum produced in forest litter. Spores germinate between -2 and 32°C (Karadžić and Anđelić 2001) with the optimal temperature for infection being 7-25°C (Petäistö 2006; Karadžić and Anđelić 2001). Spores germinate and infections occur within three hours if water is present on the plants surface. Keeping the canopy well aerated and as dry as possible can reduce infection (Mittal et al. 1987). Irrigation should occur during the morning hours to enable the faster drying of needles; also reduction of nitrogen could prevent incidences of grey mold. Spruces (*Picea* spp.) are sensitive to *Botrytis cinerea* attack, but the pathogen can also be found on larch (*Larix deciduas* Mill.), Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), sequoia (*Sequoiadendron giganteum* (Lindl.) J.Buchh.), and cypress (*Cupressus* spp.) seedlings (Williamson et al. 2007). It was observed that Serbian spruce (*Picea omorika* (Pančić) Purk) could suffer very severe damage if not grown under adequate conditions. *Botrytis* spp. may develop tolerance to fungicides that are used repeatedly, so fungicides should be used in rotation during the growing season.

Phytophthora and *Pythium* species are well-known pathogens of seedlings and young trees in nurseries and plantations. During the 20th century, the most damage in nurseries was attributed to *P. cactorum* (syn. *P. omnivora*) (Karadžić and Anđelić 2001). Recent results show that at least 15 species are present in Serbian forest ecosystems, plantations, and nurseries (Milenković 2015). *Phytophthora* can cause pre-emergence decay of the germ tube, damping-off, and root rot in 1–2 year old seedlings. Because of motile reproductive structures, the presence of free water in poorly drained soils increases the chance of damage from both species. Because resting oospores in dead plant parts also play an important role in disease control, reduction of inoculum potential is important.

Spruce needle rust (*Chrysomyxa abietis* (Wallr.) Unger) is a pathogen native to Europe and Northern Asia. This rust does not require an alternative host. Hyphae penetrate through the stomata and cause chlorosis after several weeks (Hansen 1997; Uotila et al. 2000). Basidiospores are released during wet periods in May and June. Needles fall off after the basidiospores are released. *Picea abies* (L.) Karst, *Picea sitchensis* (Bong.) Carrière, *Picea rubens* Sarg., *Picea pungens* Engelm., and *Picea engelmannii* Parry ex Engelm. could be hosts for *C. abietis* (Uotila et al. 2000). Diseased needles are observed in Serbian nurseries mostly on *Picea pungens.* Control should be applied to severely infected plants from the end of April until June; older infected trees in the vicinity of nurseries should be cut down (Karadžić and Milijašević 2003).

Needle cast diseases are caused by *Lophodermium* species, the most important pathogen of pines in nursery production (Lazarev 2006). Evidently, no resistant pine species exist, but the damage to Scots pine is the most important (Lilja et al. 2010). *Lophodermium seditiosum* Minter, Staley and Millar attack needles from current vegetation, while *L. pinastri* (Schrad.) Chevall. infects needles from the previous year. Damage can be also seen on Austrian and Aleppo (*Pinus halepensis* Miller) pines. The critical period for needle infections occurs from mid-August to the end of September. The first symptoms can be observed 2–3 months upon being infected (November–December) and depend on environmental conditions. Infected needles first turn red, then brown, and fall off the following spring (Lazarev 1981). Higher precipitation during autumn favors ascosporic infection (Lilja et al. 2010). If planting is done in the early autumn, seedlings can appear healthy, but disease development could worsen their condition during winter and kill them by the following spring. Early identification of latent infections is crucial because infected seedlings do not survive planting stress (Lilja 1986). Seedling protection is necessary for the production of healthy material. Annual application of fungicides has become a standard process in most European countries (Lilja et al. 2010; Lazarev 2006). Two treatments, at the end of August and in mid-September, are sufficient if copper-based fungicides are used. According to the Rule Book on Health Inspection of Planting Material in Nurseries, up to 10% of seedlings could have needle cast infection. An infection of less than 5% of a plant's needles is tolerated (Official Gazette of R. Serbia).

Dothistroma needle blight (DNB) (*Mycosphaerella pini* Rostr. apud Munk) is a problem in pine plantations, but could also be present on seedlings in nurseries, especially if older trees surround them. The species has a worldwide distribution and attacks more than 50 pine species, including larch; Norway (*Picea abies* (L.) H. Karst), Serbian (*Picea omorika* (Pančić) Purk.), Sitka spruce (*Picea sitchensis* (Bong.) Carr.); and Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Karadžić 2004). Fungus is often observed as anamorphic with acervuli forming inside the red bands on needles.

Teleomorph is rare and perithecia are produced on 2–3 year-old needles. Serbia is among a few countries where teleomorph is found. Infections occur during May and June; red -band symptoms can be seen from late September and become very clear in November and December (Karadžić 2004). Austrian pine is very susceptible to the attack of Dothistroma needle blight, while other economically important pine species show moderate or low susceptibility (Karadžić 2004). The situations in Central and Northern Europe are somewhat different because the most of the DNB damage is observed on Scots pine. Control in nurseries is necessary and consists of applications of copper-based fungicides during the critical period of infections (May–June).

Snow blights are usually observed in nurseries established on higher altitudes where snow covers seedlings at an earlier time. Symptoms become visible after the snow melts in spring. *Phacidium infestans* P.Karst. and *Herpotrichia juniperi* (Duby) Petrak attack needles and develop mycelium that grow under the snow. A whitish or blackish mycelium covers needles and prevents them from falling off. It was observed in Finland that *P. infestans* could attack container seedlings of Norway spruce (Lilja et al. 2010). In the Balkan region, snow blights are quite often observed on Norway and Serbian spruce, Scots pine, and rarely on European fir (*Abies alba* Mill.), Junipers (*Juniperus* spp.), and *Thuja* spp. (Karadžić and Anđelić 2001). Infections occur in October and November before the first snow of the season falls. Ascospores are released at temperatures close to 0°C (Kurkela 1995). Further development of the disease depends on the height of snow. *P. infestans* is a quarantine disease and should be prevented from migrating to new regions (Karadžić 2010). Control in nurseries and plantations are possible by spraying fungicides as late as possible (Lilja et al. 2010; Karadžić and Anđelić 2001).

In nurseries, *Diplodia sapinea* (Fr.) Fuckel (syn. *Sphaeropsis sapinea*) is generally considered as a wound parasite, infecting tissue that has already begun to wilt due to other causes, such as drought or wrench, during dry weather (Milijašević 2003). Diseases seldom progress to cause the death of the seedling, but the destruction of leading shoots renders the plant inadequate for planting. Nurseries should be established in the vicinity of pine plantations. Effective control could be achieved by spraying with fungicides from mid-April until mid-June (Milijašević 2002).

2.3 Broadleaved tree diseases

It is a common opinion that pathogens cause less damage in broadleaved nurseries in comparison to conifers. However, some diseases are observed quite regularly and can cause significant loss in number and reduce seedling quality from year to year.

Erysiphe alphitoides Griffon and Maublanc (syn. *Microsphaera alphitoides* Griffon and Maublanc) is a serious disease of oak species (*Quercus* spp.). The disease appeared as an epidemic at the beginning of the 20th century in France, from where it spread throughout Europe. Damage could be observed in nurseries, plantations, and natural stands. *Erysiphe* species attack young leaves and soft shoots, covering them with whitish mycelium. Powdery mildew can have a damaging impact on natural oak regeneration and a significant role in the decline of mature trees (Marcais and Desprez-Loustau 2014). Affected seedlings have reduced height increments of as much as 30% (Karadžić and Anđelić 2001). Ascospores are released during April and May from chasmothecia and overwinter on fallen oak leaves. Conidia are produced on infected leaves from mycelium during the vegetation period, while young flag shoots become infected by mycelium that overwinter in buds. After the first growing season, well-formed oak plants 2–3 years of age, tolerate the presence of powdery mildew which is no longer a limiting factor for their development in natural regeneration (Pap et al. 2012). Systemic fungicides, applied from June to the end of August are efficient and necessary in the control of oak mildew (Glavaš 2011; Karadžić and Anđelić 2001).

Cryptodiaporthe populea (Sacc.) Butin (anamorph *Dothichiza populea*) causes canker on poplar trees, spreading widely in nurseries and hybrid poplar plantations (Keča 2008; Ostry et al. 2014). Damage is most often observed on the representatives of section *Aigeiros.* On older trees in natural stands and plantations, infections usually occur on branches with formation of cankers; on cuttings in nurseries, the main stem becomes infected. The main inoculum consists of conidia that are produced, in picnidia, on the edges of a necrosis and spread within drops of water (Marinković 1965). Infections proceed through lenticels, buds, injuries, and the base of the shoot. Non-suberized leaf scars from early defoliation, caused by rust or *Marssonina* species, could serve as an entrance for *Dothichiza* (Tomović 1990). Most infections occur in late autumn, late winter, and early spring before vegetation starts. Control should be based on disease prevention by keeping plants in optimal growth conditions. Nurseries should be established on soils with a good texture and structure for poplar growth. Irrigation, tillage, use of herbicides to remove weed competition, and protection from leaf diseases, will avoid predisposition to *Dothichiza* and therefore are efficient in disease control. Fungicides are necessary for the production of healthy nursery material.

Poplar rust forms orangeurediniospores on the under or upper side of leaves in July. *Melampsora allii-populina* and *M. larici-populina* produce uredinial and telial stages on the *Poplar* spp. leaves, while the aecial stage has been observed on *Allium* leaves and *Larix* spp. needles (Keča 2008). Aecidiospores transfer infection to poplars during summer when damage has been observed on leaves. Infection is followed by the production of pustules and necrotic lesions at infected sites. Differences in susceptibility among cultivars have been reported (Keča 2003b). Susceptible clones loose leaves quite early (in late August). The development of a disease is, beside differences in clone susceptibility, greatly influenced by weather conditions. It was shown that *P.* x *euramericana* cv. "*Pannonia*" is more resistant to attack than other clones grown in Serbia. Disease control can be obtained by strict hygiene in nurseries (Lilja et al. 2010). The application of fungicides labeled for control of *Melampsora* on poplar from June until mid-July reduce infection levels on leaves (Ostry et al. 2014).

Marssonina leaf spot (*Drepanopeziza punctiformis* Gremmen) is one the most important foliage diseases of *Populus* species (Beare et al. 1999; Keča 2003a, 2003b; Keča et al. 2015). After infection of ascospores, small (up to about 1 mm in diameter) circular-to-irregular dark-brown spots develop on leaves during spring. Necroses spread on leaves and cause premature defoliation, weakening, and dieback of infected trees. Fast-growing hybrid poplars that were planted worldwide after the Second World War are now ready for the production of technical wood and woody biomass (Keča et al. 2012; FAO 2011). *Marssonina* and other leaf spot causing pathogens (*Septoria*, *Phyllosticta*, etc.) considerably reduce the growth and productivity of hybrid poplars and cause significant economic loss (Zhu et al. 2012; Keča 2008). The most promising control is to select and grow poplar varieties resistant or tolerant to the disease (Zhu et al. 2012; Han et al. 2000). Application of fungicides during the summer months is necessary in nurseries in order to reduce leaf infections and prevent early defoliation (Keča 2003b).

Further development of marker-assisted and biotechnological approaches in breeding and selection of resistant poplar clones are currently limited, but will play an important role in the future.

Table 2. Chemical protection periods and fungicides used in the forest nurseries in Serbia.

Pathogen	Month								Fungicide
	IV	V	VI	VII	VIII	IX	X	XI	
Botrytis cinerea	■	■	■	**If necessary** ■	■	■			Chlorothalonil Dicloran Ferbam Thiophanate-methyl
Cryptodiaporthe populea						■	■	■	Hexaconazole Carbendazime Chlorthalonil
Diplodia sapinea	■	■	■						Azoxystrobin Copper Hydroxide Propiconazole Thiophanate- methyl
Erysiphe alphitoides				■	■				Kresoxim-methyl *Carbendazime+Flusilazol* Epoxiconazole+Thiophanat e-mehtyl *Azoxystrobin*
Fusarium spp.	■	■	■						Thiophanate-methyl Iprodione Maneb
Herpotrichia juniperi						■	■	■	Copper hydroxide Ditiocarbamates
Lophodermium seditiosum					■	■			Azoxystrobin Copper Sulfate Chlorothalonil Mankozeb Triadimefon
L. pinastri	■	■							
Marssonina spp.			■	■	■				Chlorothalonil Copper hydroxide Oxychloride sulfate
Melampsora spp.				■	■				tebuconazole, cyproconazole propiconazole
Mycosphaerella pini =Dothistroma septosporum		■	■						Copper Oxychloride Copper Hydroxide
Chrysomyxa abietis	■	■							Chlorothalonil Myclobutanil
Phacidium infestans						■	■	■	Copper hydroxide Ditiocarbamates
Phytophthora spp. and *Pythium* spp.	■	■	■						Fosetyl-Al Metalaxyl Propamocarb

3 Disease prevention and control measures

The application of control measures is in Serbian nurseries usually connected with the occurrence of the pathogens or damages. A treatments' effectiveness is dependent on correlation between timing application measures, and the host and pathogen phenology. There are still no developed plans for protection against the most important diseases, so procedures depend only on the personal involvement of nursery personnel/managers responsible for the production. Chemical control measures options for the most important forest nursery pathogens, and the best time period for application are presented in Table 2.

The application of chemicals for diseases control is quite questionable in forest nursery production in Serbia. Available fungicides are registered mainly for agricultural crops. Wholesalers do not find interest to register for the use in forestry because this market is very small.

4 Concluding remarks

Intensive production, as in nurseries, presents a constant risk of damage caused by pests and diseases. Factors such as high seedling density, irrigation, fertilization, and herbicides, encourage the development of pathogenic fungi. Hygiene in nurseries and application of available measures in seed management, disinfection of soil, and treatments by pre-emergence and post-emergence pesticides prevent the survival of pathogens and the development of diseases. Application of pesticides at a critical period for infections maximizes their efficiency and minimizes environmental contamination.

Educating nursery employees about pathogen life cycles and ecology is necessary for symptom recognition and the application of appropriate protective measures. Modern technologies can help identify diseased seedlings and thereby facilitate their exclusion for their use in reforestation. Recent changes in climate require the use of vigorous and healthy plants that will be able to survive environmental extremes.

5 References

Anonymous (2006) Forestry development strategy for the Republic of Serbia. The Republic of Serbia - Ministry of Agriculture, Forestry and Water Management Directorate of Forests, pp 30.

Banković S, Medarević M, Pantić D, Petrović N, Šljukić B, Obradović S (2009) The growing stock of the Republic of Serbia - state and problems. Bulletin of the Faculty of Forestry 100:7-30.

Beare JA, Archer SA, Bell JNB (1999) *Marssonina* leafspot disease of poplar under elevated ozone: pre-fumigated host and in vitro studies. Environ Poll 105:409-417.

Bennett JW (2010) An Overview of the Genus *Aspergillus*. *Aspergillus*: Molecular Biology and Genomics. Caister Academic Press, Haverhill.

Booth C (1971) The genus *Fusarium*. CAB International, Wallingford.

Büttner C, von Bargen S, Bandte M, Mühlbach H-P (2013) Forest Diseases Caused by Viruses. In: Infectious Forest Diseases ed. by P. Gothier and G. Nicolotti, CAB International, Wallingford.

Cvejtković B (2011) Influence of fungi on dynamic of Serbian spruce (*Picea omorika* Panč./ Purkyně) seed germination and possibility of storage. Master of Science Thesis.University of Belgrade.

Ewis GC, Ravel C, Naffaa W, Astier C, Charmet G (1997) Occurrence of *Acremonium endophytes* in wild populations of *Lolium* spp. in European countries and a relationship between level of infection and climate in France. Annals of Applied Biology 130: 227-238. doi: 10.1111/j.1744-7348.1997.tb06828.x

FAO (2011) State of the World's Forests. FAO Rome.

Glavaš B (2011) Protection of oak stands form Powdery Mildew (*Microsphaera alphitoides* Greff. et Maubl.). Cro J For Res 31(1):205-210.

Han ZM, Yin TM, Li CD, Huang MR, Wu RL, (2000) Host effect on genetic variation of *Marssonina brunnea* pathogenic to poplars. Theor Appl Genet 100:614-620.

Hansen E (1997) Needle and broom rusts. In: Hansen EM, Lewis KJ (eds) Compendium of confer diseases. 1st edn. APS Press, St. Paul Minnesota, pp 51-53.

Ivetić V, Vilotić D (2014)The role of plantation forestry in sustainable development. Bulletin of the Faculty of Forestry:157-180.

Karadžić D. (2004) The distribution, hosts, epidemiology, impact and control of fungus *Mycosphaerella pini* E. Rostrup apud Munk. in Serbia. Bulletin of the Faculty of Forestry 90:7-35. doi:10.2298/GSF0490007K

Karadžić D (2007) *Chrysomyxa pirolata* G. Wint in Rabenh. - New parasitic fungus on the cones and seeds of spruce. Bulletin of the Faculty of Forestry 96:45-53.

Karadžić D, Milijašević T (2003) The most frequent "rusts" on trees and shrubs in SERBIA. Bulletin of the Faculty of Forestry 88:77-101. (in Serbian with English summary)

Karadžić D, Milijašević T (2005) The most frequent powdery mildews on forest woody species and their impact. Bulletin of the Faculty of Forestry 91:9-29. (in Serbian with English summary)

Karadžić; D (2010) Forest Pathology. University of Belgrade-Faculty of Forestry, Belgrade

Karadžić D, Anđelić M (2001) Diseases in forest nurseries. University of Belgrade-Faculty of Forestry, Belgrade.

Keča Lj, Keča N, Pantić D (2012) Net Present Value and Internal Rate of Return as Indicators for Assessment of Cost-efficiency of Poplar Plantations: a Serbian case study. International Forestry Review 14(2):145-157.

Keča N (2003b) Possibility of parasite (*Marssonina brunnea* (Ell.&Ev.)P. Magnus and *Melampsora* spp.) control on poplar foliage with some fungicides. Bulletin of the Faculty of Forestry 88:103-120. doi:10.2298/GSF0388103K

Keča N (2003a) Test of resistance of Populus x euramericana cv. Pannónia to the attack of Discosporium populeum (Sacc.) Sutton in the conditions of Gornje Potisje. Bulletin of the Faculty of Forestry 87:139-149.

Keča N (2008) Diseases in poplar plantations. Bulletin of the Faculty of Forestry 97:7-31. doi: 10.2298/GSF0897007K

Keča N, Milenković I, Keča Lj (2015) Mycological complex of poplars in Serbia. J For Sci 61(4):169-174. doi: 10.17221/13/2014-JFS

Kehr RD, Schroeder, T (1996) Long-term storage of oak seeds-new methods and mycological aspects. In: Unknown (ed) Proceedings of the tree seed pathology meeting, Opocno, Czech Republic, October, pp 50-61.

Kurkela T (1995) Short-term variation in ascospore release by *Phacidium infestans* on needles of *Pinus sylvestris*. European Journal of Forest Pathology 25:274-281.

Lazarev V (2006) Diversity of *Lophodermium* species on pines. Bulletin of the Faculty of Forestry 89:9-40. (in Serbian with English summary)

Lazarev V (1981) Bioecological characteristics of *Lophodermium* spp. on Scots pine in the nurseries of Bosnia. Current research on conifer needle diseases, IUFRO Working Party on needle diseases, Sarajevo, pp 59-67.

Lazarević J, Keča N, Vilotić D (2012b) Mycorrhization and use of super absorbent polymers in targeted production of planting material, International Conference: Role of research in sustainable development of agriculture and rural areas, Podgorica 24-27 May 2012, pp 74.

Lazarević J, Keča N, Martinović A (2012a) Mycorrhization of containerized *Pinus nigra* seedlings with Suillus granulates under open field conditions.Forest systems 21(3):498-507.

Lević J (2008) Vrste roda *Fusarium*. u oblasti poljoprivrede, veterinarske i humane medicine. Cicero, Beograd.

Lilja A, Hallaksela AM, Heinonen R (1995) Fungi colonizing Scots pine cone scales and seeds and their pathogenicity. European Journal of Forest Pathology 25:38-46.

Lilja A, Poteri M, Petäistö R-L, Rikala R, Kurkela T, Kasanen R (2010) Fungal diseases in forest nurseries in Finland. Silva Fennica 44(3):525-545.

Lilja S (1986) Diseases and pest problems on Pinus sylvestris nurseries in Finland. Bulletin OEPP/EPPO Bulletin 16:561-564.

Malone JP, Musket AE (1964) See-borne fungi. The International Seed Testing Association, Wageningen.

Marçais B, Desprez-Loustau M-L (2014) European oak powdery mildew: impact on trees, effects of environmental factors, and potential effects of climate change. Annals of Forest Science 71:633-642.

Marinković P (1965) New studies on the biology of the pathogenic fungus *Dothichiza populea* Sacc. et Briard with special reference to the possibility of its control. Dissertation,.Bulletin of the Faculty of Forestry 30:1-65.

Milenković I (2015) Diversity of species form the *Phytophthora* genus and their role in the decline of broadleaved forest trees in Serbia. Dissertation, University of Belgrade-Faculty of Forestry, Belgrade.

Milijašević T (2002) Bioecology of the pathogenic fungus *Sphaeropsis sapinea* Dyko & Sutton - agents of Pinus species decline. Bulletin of the Faculty of Forestry 86:7-29. doi:10.2298/GSF0286007M (in Serbian with English summary)

Milijašević T (2003) Life cycle of the pathogenic fungus *Sphaeropsis sapinea* Dyko & Sutton. Bulletin of the Faculty of Forestry 88:141-156. doi:10.2298/GSF0388141M

Millar CI, Stephenson NL, Stephens SL (2007) Climate change and forests of the future: managing in the face of uncertainty. Ecological Applications 17(8):2145-2151.

Mink GI (1993) Pollen- and Seed-Transmitted Viruses and Viroids. Ann. Rev. Phytopathol. 31:375-402.

Mittal RK, Singh P, Wang BSP (1987) Botrytis: a hazard to reforestation. A literature review. European Journal of Forest Pathology 17:369-384.

Nienhaus F (1985) Infectious diseases in forest trees caused by viruses, mycoplasma-like organisms and primitive bac-teria. Experientia 41:597-603.

Ostry M, Ramstedt M, Newcombe G, Steenackers M (2014) Diseases of Poplars and Willows. In: Isebrands JG, Richardson J (eds) Poplars and Willows: Trees for Society and the Environment, FAO, Rome, pp 443-458.

Pap P, Ranković B, Maširević S (2012) Significance and need of powdery mildew control (*Microsphaera alphitoides* Griff. et Maubl.) in the process of regeneration of the pedunculate oak (*Quercus robur* L.) stands in the Ravni Srem area. Periodicum Biologorum 114(1):91-102.

Peno M (1971) The biological method of control against the pathogenic *Fusarium* species on Austrian pine seedlings. Institut de recherché Forestieres el l'Industie du bios. No. 30. Belgrade, pp 1-99.

Petäistö R-L (2006) Botrytis cinerea and Norway spruce seedlings in cold storage. Baltic Forestry 12:24-33.

Ranković N (2009) Afforestation in Serbia in the period 1961-2007. with special reference to Austrian pine and Scots pine. Bulletin of the Faculty of Forestry 99:115-134. doi:10.2298/GSF0999115R

Rees AA, Phillips DH (1986) Detection, Presence and Control of Seed-Borne Pests and Diseases of Trees with special reference to seeds of tropical and sub-tropical pines. Danida Forest Seed Centre. Technical Note no. 28.

Salisbury PJ (1953) Some aspects of conifer seed microflora. Bimonthly Progress Report 9:3-4

Zhu S, Cao Y-Z, Jiang C, Tan B-Y, Wang Z, Feng S, Zhang L, Su X-H, Brejova B, Vinar T, Xu M, Wang M-X, Zhang S-G, Huang M-R, Wu R., Zhou Y (2012) Sequencing the genome of *Marssonina brunnea* reveals fungus-poplar co-evolution. BMC Genomics 13:382-391.

Samson RA, Houbraken J, Summerbell RC, Flannigan B, Miller JD (2001) Common and important species of fungi and actinomycetes in indoor environments. Microogranisms in Home and Indoor Work Environments. CRC, pp 287-292.

Spittlehouse DL, Stewart RB (2003) Adaptation to climate change in forest management. BC Journal of Ecosystems and Management. 4 (1):1-11.

Sutherland JR, Davis C (1991) Diseases and insects in forest nurseries in Canada. In: Sutherland JR. Glover SG (eds). Proceedings of the first meeting of IUFRO Working Party S2.07-09. Diseases and insects in forest nurseries. Victoria, Brittish Columbia, Canada. August 23-30,1990. Forestry Canada, Pacific and Yokon Region, Pacific Forestry Centre. BC-X-331, pp 25-32.

Sutterland JR, Diekmann M, Berjak P (2002) Forest tree seed health for germplasm conservation. IPGRI Technical Bulletin, Rome 6:1-85.

Tomić Z, Rakonjac Lj, Isajev V (2011) The selection of species for reforestation and amelioration in Central Serbia. Institute of Forestry, Belgrade.

Tomović ZM (1990) Variability and heredity of susceptibility of black poplars to bark canker (*Dothichiza populea* Sacc. et Br.). Radovi - Institut za Topolarstvo 22:1-105.

Uotila A, Wang T, Wang L (2000) Some ecological aspects of *Chysomyxa abietis* (Wallr.) Unger epidemiology. Metsanduslikud Uurimused 34: 17-23

Williamson B, Tudzynski B, van Kan JA (2007) Botrytis cinerea: the cause of grey mould disease. Mol. Plant Pathol 8(5):561-580.

Zhang PG, Sutton JC (1994) High temperature, darkness, and drought predispose black spruce seedlings to gray mold. Canadian Journal of Botany 72:135-142.