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Evaluation of the health status of *Picea abies* provenances growing on the IUFRO 1964/68 experimental plots

Abstract: Due to the deterioration in the health condition of Norway spruce on the IPTNS-IUFRO 1964/68 observation plots in Krynica (Poland), there was an urgent need to determine the cause of the decline in order to design preventive measures. For this purpose, a health inventory was carried out in the years 2002 and 2003. This included an assessment of the condition of crowns in 144 trees and a thorough examination of 29 felled trees. It was found that individual trees or groups of trees have declined in some blocks of the experiment. The tops of some trees were dying and the crowns were getting transparent. The discolouration (turning brown) of needles was quite common and even green needles were shed. Some of the roots showed symptoms of necrosis and died. Seven taxa of pathogens were isolated from the diseased roots and trunk parts of spruces, among them *Phytophthora citrophthora*, *Fusarium avenaceum*, *F. solani* and *Trichoderma*. The number of diseased trees was related to provenance. The provenances Babenhausen (Germany), Frantiskovy Lazne (Czech Republic), Traunstein 1/4 D, 6 A, B, 7 A (Germany), Wundsiedel-Weissenstadt (Germany), Mestwinowo (Poland), and Magland (France) proved to be more susceptible than others to fungal pathogen attack.

Additional key words: Norway spruce, fungal infection, spiral disease

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Introduction

A total of 1096 Norway spruce provenances were planted under the IUFRO 1964/68 experiment at the Experimental Forest Station in Krynica. Unexpectedly, in the year 1999 crown dieback was first observed, with characteristic symptoms progressing from initial dead branches at the top of infected trees to the infection reaching the lowest part of crowns within a number of years. The mechanism behind the steady decline of trees or even whole stands was explained by Manion (1981). The symptoms of spiral disease (or complex disease), such as the shedding of needles, dying of the tops of trees, and resin exudates on stems, have been well described (Barszcz and Małek 2006; Mańka 2006). Three main sets of driving forces behind the spiral disease have been identified, namely: (i) predisposing factors, (ii) inciting factors, and (iii) contributing factors. The predisposing factors act over long periods of time and comprise, for example, a prolonged drought period, a hot summer, or unfavourable forest site conditions for particular species. The inciting factors are biological or physical drivers that cause damage to trees, e.g. insect defoliators, other pathogens (among them *Phytophthora* spp.), or severe winter frost. The contributing factors include other organisms like secondary pests or root rot fungi which contribute to the complex disease and often are the direct cause of tree mortality. In contrast with the chain disease theory, the spiral disease theory suggests that all these factors act simultaneously. There is no need for their subsequent occurrence (as is the case with the chain disease) and there are many possible variants of the sequence of factors, e.g. drought can be followed by *Phytophthora* spp., then *Armillaria* spp. (roots and stem tissue), and insect borers attack.

The objectives of the present study were to assess the current health status of Norway spruce provenances on the IPTNS-IUFRO 1964/68 observation plots, to identify the threats posed by pathogenic organisms, especially *Phytophthora citrophthora*, and to determine the effect of the genetic factor (provenance) on the susceptibility of spruces to fungal infections.

Material and methods

The study was carried out in 11 blocks of the IUFRO 1964/68 International Provenance Test with Norway Spruce (area No. 19, Poland), situated at the Krynica Experimental Forest Station in the Wojkowa Forest Range (compartment 165; longitude 20°58', latitude 49°21', altitude 770–795 m; blocks 02, 06 and 10) and the Kopciowa Forest Range (compartment 14; longitude 21°01', latitude 49°28', altitude 705–745 m; blocks 01, 03, 04, 05, 07, 08, 09 and 11) . Each block comprised 100 provenances represented by 25 trees each, on average. The experimental design and the location of the blocks are described in an earlier publication (Bałut and Sabor 2001). The trees represented the provenances from most of the geographical regions distinguished by Krutzsch (1975).

In 2002 and 2003, 144 trees (Table 1) were assessed on the basis of the condition of crowns. The number of spruces to be assessed resulted from their health condition. Twenty nine trees were felled and examined in detail for the occurrence of disease symptoms on the needles, stem and root system. Attention was given to the discolouration and shedding of needles and the appearance of pathogenic organisms, rot or tissue lesions (dark exudates and necrosis beneath the bark). In addition, radial increments were observed on the cross-sections of trunks.

Table 1. IUFRO 1964/68 Inventory Provenance Test with Norway Spruce in Krynica. Number of trees under study by block

Block No.	Number of trees	
	examined for crown condition	felled and examined for disease symptoms
01	19	5
02	–	–
03	27	5
04	21	3
05	16	–
06	8	8
07	19	–
08	20	3
09	6	2
10	3	3
11	5	–
Total	144	29

To isolate potential pathogens, wood samples were taken from trees with disease symptoms. After the examination and isolation, tests on agar malt were performed for *Basidiomycetes*, while for *Oomycetes* the diseased parts of superficial roots and bark were examined in a laboratory. Following disinfection over a burner flame, fragments of tissue ca. 3–5 mm in diameter were placed on Difco potato-dextrose agar (PDA) in 90 mm Petri dishes (6 pieces per dish and 6 plates per tree). After 6 days of incubation at 25°C in the dark, colonies grown around the tissue fragments were transferred to PDA slats. Additionally, the diseased parts of trees were put into apples (3 fruits per tree) at three points, and when brown discolouration was seen, circles of fruit flesh with 3 mm diameter were transferred into PDA slants. Microorganisms were identified using the available monographs.

Results

As follows from the assessment of 144 spruce trees done in 2002 and 2003, individual trees or groups of trees declined in some blocks of the UFRO 1964/68 Inventory Provenance Test with Norway Spruce in Krynica. In places where trees had fallen to the ground, gaps were created in the stand. The tops of some trees were dying and the crowns were getting transparent. Frequent resin exudates, turning dark near the bases of trunks, were observed on the trees (Fig. 1). Some of the roots showed symptoms of necrosis and died. There were also other symptoms such as discolouration and shedding of the needles in the vegetation season, especially intensive at the tops of trees and spreading down. Observations of the annual radial increments on the cross-sections of spruces showed their dramatic decrease over the past 4 years.

In several cases *Armillaria* sp., a root pathogen, was found to cause substantial damage. White mycelium and rhizomorphs beneath the bark appeared in some trees (block 1 – tree No. 3, block 3 – tree No. 17, block 4 – trees No. 25 and 51; Fig. 2). A lot of dead rotting roots without apparent disease symptoms, and sometimes the white mycelium of non-identified fungus (probably belonging to a saprotrophic group of fungi) were observed. Out of 29 cut trees, 17 were dying, 11 were dead and 1 was blown over by the wind due to severe damage to the roots. In one case (block 3 – tree No. 14), several-meter-long inner rot appeared along

Fig. 1. Resin exudates turning dark near trunk bases (phot. T. Oszako)

Fig. 2. Mycelium of *Armillaria* on root (phot. T. Oszako)

the trunk, which may be attributed to *Heterobasidion* sp. since this pathogen was isolated from the wood samples (Fig. 3). The wood (xylem) and inner bark (phloem) representing other trees were considered as healthy.

Seven taxa of pathogens were isolated from the diseased roots and trunk parts of spruces (Table 2). *Phytophthora citrophthora* was isolated from 3 trees, while *Fusarium avenaceum* and *F. solani* were found on 4 and 2 trees, respectively. Species of *Trichoderma* were observed on 4 trees. Apple fruits performed well as a medium for the isolation of *P. citrophthora* from 2 trees.

There was a clear relationship between provenance and the number of diseased trees. Susceptibility to pathogens was exhibited by spruces from the following provenances:

Table 2. Fungi isolated from 6 dying trees of Norway spruce

Genus/Species	Number of infested trees (n = 6)	Number of isolates	
		from trunks	from roots
Alternaria alternaria Nees	3	3	1
Fusarium avenaceum (Fr.) Sacc.	4	2	7
Fusarium solani (Mart.) Sny. et Hans.	2	0	5
Mucor spp.	6	7	11
Penicillium spp.	4	8	1
Phytophthora citrophthora (Smith et Smith) Leonian.	3	6	10
Trichoderma spp.	4	4	7

a b

Fig. 3. Several-meter-long inner rot along trunk caused by *Heterobasidion* sp. (a), and fruit body of *Heterobasidion* on roots (b) (phot. T. Oszako)

- 04 75 Babenhausen from region 23 – Swabian-Bavarian Upland (Swabia) 2; Germany,
- 07 31 Frantiskovy Lazne from region 10 – Erzgebirge; Czech Republic,
- 08 04 Traunstein 1/4 D, 6 A, B, 7 A from region 26 – East Alps; Germany,
- 08 43 Wundsiedel-Weissenstadt from region 19 – Franconia, Upper Palatinate; Germany,
- 08 78 Mestwinowo from region 67 – East-Pomeranian Lakeland, Warmia, Masuria; Poland,
- 08 86 Magland from region 2 – West Alps; France.

Discussion

The results of the research correspond with other studies showing the threats faced at an older age by spruce plantations, especially those established on former agricultural land (Żółciak and Oszako 2002; PGL 2004; Barszcz and Małek 2006; Mańka 2006). The threats include infections with pathogens, among them *Phytophthora citrophthora*.

For the first time in Poland, *P. citrophthora* was reported in 2003 from an experimental forest stand in the Krynica Forest District in the Beskid Mts. (Oszako and Orlikowski 2004). There were several sites of infection taking the form of circular gaps in the stand. In the infection centres, trees had died at least 2 years prior to the observations reported here, whereas in the peripheral parts, trees remained alive but were in decline. *P. citrophthora* was isolated from the border of the necrotic zone in the wood beneath the bark of declining trees and from the rotted parts of roots. The diseased tissues contained also *Fusarium* spp. and *Penicillium* spp. whose role in damaging the fine roots may be important, too. The dry vegetation seasons of 2002 and 2003 appeared to have predisposed trees to infection with pathogens. Apart from *P. citrophthora* causing root rot, *Armillaria* sp. and *Heterobasidion* sp. probably contributed to the death of many trees, as well.

Studies conducted in the last decade have suggested that many previously unrecognised *Phytophthora* spp. may be associated with diseased trees (Belbahri et al. 2006). Most *Phytophthora* species are not native of the area where they cause serious problems. For example, *P. ramorum*, the cause of the sudden death of oak, was brought separately into North America and Europe. Up to now, at least 60–80 *Phytophthora* spp. have been described. Most of them are soil-borne pathogens that cause damping-off, root rots, collar and stem rots and foliar blights in different woody plant species (Erwin and Ribeiro 1996). As a genus, *Phytophthora* mainly act like parasites on plants, among them trees and tree seedlings. According to Tsao (1990), most of the crown diseases of woody plants can be attributed to *Phytophthora* spp., although in most studies no adequate techniques have been used to confirm the causal agents.

Phytophthora spp. and other oomycete microorganisms have long been considered fungi, but more recently, evolutionary phylogenetic analyses have led scientists to distinguish a new kingdom, *Chromista*, which for example includes also brown algae (Erwin and Ribeiro 1996; Baldauf et al. 2000). In plant tissues, *Phytophthora* spp. produce mainly diploid hyphae, oospores and chlamydospores. Although the oospores can survive in the organic matter of soil for extended periods, the asexual chlamydospores are the main resting structures of oomycetes. For this reason *P. citrophthora*, once introduced into an area with planted tree seedlings, could survive there for many years. The asexual, biflagellated zoospores of *Phytophthora* spp. that initiate plant infection are produced in sporangia under wet soil conditions. In such conditions, zoospores may attack and damage even up to 90% of fine roots. During subsequent dry seasons, chlamydospores form again in the soil, carrying the infection over to the next wet period.

In order to accurately identify and diagnose the *Phytophthora* disease, new DNA-based methods have been developed (Oszako et al. 2006). Occasionally, *Phytophthora* spp. may form interspecific hybrids, as is probably the case with the *P. alni*-complex. The *P. alni*-complex, which comprises particularly virulent *P. alni* ssp. *alni*, has altered riparian ecosystems throughout Europe (Brasier et al. 1999; Brasier and Kirk 2004).

Each year, new species of *Phytophthora* are being reported. Some of them, having a greater adaptability to the global climatic change, are likely to replace other, less well adapted species. To take an example, *P. polonica* found in Polish declining alder stands grows best at 30°C, a temperature which now is more and more common in Polish forests (Belbahri et al. 2006). Other newly described *Phytophthora* species include *P. nemorosa* and *P. ramorum* found in an extensive survey into the sudden death of oaks in California and Oregon. A similar survey done in the UK revealed the presence of *P. kernoviae* in the isolates from *Fagus sylvatica*, *Quercus robur* and *Liriodendron tulipifera* (Brasier et al. 2005). In Finland a new, homothallic *Phytophthora* sp. from rhododendron was shown to be highly pathogenic to many woody hosts including Norway spruce (Lilja et al. 2006).

The climatic change is associated with the increased frequency of weather extremes that may create favourable conditions for the development of some species (e.g. floods). For example, *P. inundata* was recently described as infecting *Salix* in riparian ecosystems (Brasier et al. 2003). The same species attacks *Aesculus*, *Olea* and *Prunus*, and might be highly pathogenic after floods or water logging (Brasier et al. 2003). Extensive studies on oak decline provided evidence about the presence of *P. quercina*, *P. psychrophila*, *P. europaea*, *P. uliginosa* and *P. pseudosyringae* in declining stands (Jung et al. 1999, 2002, 2003). *Phytophtho-*

ra pseudosyringae was also found in the necrotic fine roots and the stem lesions of *Fagus sylvatica* and *Alnus glutinosa* (Jung et al. 2003). *Phytophthora cambivora* and *P. citricola* were observed in declining beech stands in Poland (Oszako et. al 2006). *Phytophthora quercina* was most frequently isolated from a rhizosphere soil near declining oaks in Sweden (Jönsson et al. 2003). A correlation was established between the presence of this pathogen and the vitality of oak stands (Jönsson et al. 2005). Similarly, some spruce provenances on the Krynica plots proved to be more affected than others (however, further research is needed to produce statistically sound evidence).

The spruce decline phenomenon observed on the Krynica experimental plots might have been triggered by several factors. It is worth mentioning that the trees were planted at a dense spacing (2 × 2 m) on former agricultural land. Due to the presence of the mycelia of many pathogenic fungi (among them *Heterobasidion* sp.), such grounds create a risk to forest plantations. The risk is further aggravated if the neighbouring stands suffer from fungal diseases. No dieback symptoms were observed until 1999. In contrast to 1980 with a high rainfall, the year 1999 had low precipitation (drought). The shift in the weather conditions (from extremely wet to dry) may have caused serious damage to the fine roots of trees, resulting in an increased susceptibility of trees to the infection with root pathogens. Under such conditions, organisms from the genera *Armillaria, Heterobasidion* and *Phytophthora* can damage the roots and cause growth inhibition, severe dieback and, as a consequence, death of many trees.

Final remarks

Since the trees on the plots were planted rather densely, their root systems were intensively growing and vastly expanding with age, and very often created a dense network in the soil. The numerous root grafts formed among the diseased and healthy trees undoubtedly facilitated the spread of the disease. More and more trees got infected around the initial source of infection, and the dying ones created a permanently growing gap in the stand.

Our study focused on the Krynica stand of Norway spruce as an exemplary area of occurrence of *P. citrophthora* in Polish forests. Probably, some other *Phytophthora* spp. also occur in stands where spruce trees show trunk and root rot symptoms. It seems possible that the pathogen had spread from one host plant to the others already in nurseries.

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References

Baldauf S.L., Roger A.J., Wenk-Siefert L., Doolittle W.F. 2000. A kingdom-level phylogeny of eukaryotes based on combined protein data. Science 290: 972–977.

Bałut S., Sabor J. 2001. Inventory Provenance Test of Norway Spruce (*Picea abies* (L.) Karst.) IPTNS-IUFRO 1964/68 in Krynica. Part I. Description of the experimental area. Test material. IUFRO Working Party S 2.02.11 Norway Spruce Provenances and Breeding, Kraków.

Barszcz J., Małek S. 2006. The quality and viability of *Picea abies* (L.) Karst. seedlings in young crops laid out after a decay of stands at high mountain elevations in the Śląski Beskid Mountains and the western part of the Beskid Żywiecki Mountains. In: Current problems of forest protection in spruce stands under conversion. Forest Research Institute, Warsaw, pp. 21–36.

Belbahri L., Calmin G., Oszako T., Moralejlo J.A.G., Descals E., Lefort F. 2006. *Phytophthora polonica*: a new species isolated from a Polish declining alder forests. In: Alien Invasive Species and International Trade. Forest Research Institute, Sekocin Stary.

Brasier C.M., Kirk S. 2004. Production of gametangia by *Phytophthora ramorum* in vitro. Mycological Research 108: 823–827.

Brasier C.M., Cooke D.E.L., Duncan J.M. 1999. Origin of a new *Phytophthora* pathogen through interspecific hybridization. Proceedings of the National Academy of Science, USA 96: 5878–5883.

Brasier C.M., Sanchez-Hernandez E., Kirk S.A. 2003. *Phytophthora inundata* sp. nov., a part heterothallic pathogen of trees and shrubs in wet or flooded soils. Mycological Research 107: 477–484.

Brasier C.M., Beales P.A., Kirk S.A. Denman S., Rose J. 2005. *Phytophthora kernoviae* sp. nov., an invasive pathogen causing bleeding stem lesions on forest trees and foliar necrosis of ornamentals in the UK. Mycological Research 109: 853–859.

Erwin D.C., Ribeiro O.K. 1996. *Phytophthora* Diseases Worldwide. APS Press, St. Paul, Minnesota.

Jönsson U., Lundberg L., Sonesson K., Jung T. 2003. First record of soil-borne *Phytophthora* species in Swedish oak forests. Forest Pathology 33: 175–179.

Jönsson U., Jung T., Sonesson K., Rosengren U. 2005. Relationship between *Quercus robur* health, occurrence of *Phytophthora* species and site conditions in southern Sweden. Plant Pathology 54: 502–511.

Jung T., Cooke D.E.L., Blaschke H., Duncan J.M., Oâwald W.F. 1999. *Phytophthora quercina* sp. nov., causing root rot of European oaks. Mycological Research 103: 785–798.

Jung T., Hansen E.M., Winton L., Oβwald W.F., Delatour C. 2002. Three new species of *Phytophthora* from European oak forests. Mycological Research 106: 397–411.

Jung T., Nechwatal J., Cooke D.E.L., Hartmann G., Blaschke M., Oβwald W., Duncan J.M., Delatour C. 2003. *Phytophthora pseudosyringae* sp. nov., a new species causing root and collar rot of deciduous tree species in Europe. Mycological Research 107: 772–789.

Krutsch P. 1975. Die Pflanzschulenergebnisse eines inventierenden Fichtenherkunftsversuches (*Picea abies* (L.) Karst). Silvae Genetica 41(3): 143–150.

Lilja A., Kokkola M., Hantula J. 2006. *Phytophthora* species, a new threat to forest trees and seedlings. In: Possible Limitation of Decline Phenomena in Broadleaved Stands. Forest Research Institute, Warsaw.

Manion P.D. 1981. Tree Disease Concepts. Prentice-Hall Inc., Englewood Cliffs, N.J.

Mańka M. 2006. *Armillaria* root rot in Beskidy Mountains in research after World War II. In: Current Problems of Forest Protection in Spruce Stands under Conversion. Forest Research Institute, Warsaw, pp. 21–23.

Oszako T., Orlikowski L.B. 2004. The first noting of *Phytophthora citrophthora* on *Picea abies* in a forest stand. Phytopathologia Polonica 34: 81–85.

Oszako T., Belbahri L., Calmin G., Lefort F. 2006. Real time PCR diagnostic of *Phytophthora alni* and its three subspecies. In: Possible Limitation of Decline Phenomena in Broadleaved Stands. Forest Research Institute, Warsaw.

PGL. 2004. Raport o stanie lasów w Polsce 2003. Państwowe Gospodarstwo Leśne "Lasy Państwowe", Warszawa.

Tsao P.H. 1990. Why many *Phytophthora* root rots and crown rots of tree and horticultural crops remain undetected. EPPO Bulletin 20: 11–17.

Żółciak A., Oszako T., 2002. Ocena stanu zdrowotnego świerków z doświadczenia prowieniencyjnego świerka pospolitego IUFRO 1964/68. Ekspertyza IBL. Zakład Fitopatologii Leśnej, Sękocin.