UNIVERSITY OF WEST-HUNGARY

SUMMARY OF PH.D. THESIS

OCCURENCE OF SOILBORNE AND WATERBORNE PHYTOPHTHORA SPECIES IN WEST-HUNGARY AND THEIR ROLE IN THE DECLINE OF BLACK WALNUT, COMMON ALDER AND WILD CHERRY TREES

Sárándi-Kovács Judit

Sopron

2015

University of West-Hungary
'Roth Gyula' Doctoral School of Forestry and Wildlife Management
Head of the Doctoral School: *Sándor Faragó DSc.*
Programme: *Biological basics of forest management (E2)*
Head of the programme: *Ferenc Lakatos CSc.*
Supervisors: *Ilona Szabó CSc., Ferenc Lakatos CSc.*

Introduction

Species belonging to the genus *Phytophthora* are devastating pathogens of cultivated plants or forest trees (JAKUCS 1999). They cause root or trunk rot, crown transparency, dwarf, discolouration and early fall of leaves. Approximately 106 officially described species belongs to the genus nowadays. The number of species may thrive in the near future, because there are a lot of recently discovered, undescribed taxa (ÉRSEK and RIBEIRO 2010). There are two other reasons explaining the quick growth of number of described species: the scientific interest turned to the natural ecosystems in the last decade resulting a number of new *Phytophthora* taxa; and the trade of nursery plants reaches higher and higher volumes, resulting new pathogen taxa and new plant-pathogen interactions (ÉRSEK and RIBEIRO 2010).

Phytophthora species cause the death of alder trees or the ink disease of sweet chestnut all over Europe. Its role was proven in the oak decline of the Mediterranean region. They play also a role in the decline or sudden death of walnut species. In some regions of Western-Europe they also assist in the oak and beech decline (HARTMANN and BLANK 1998, JUNG et al. 2000).

According to the investigations of the Institute of Silviculture and Forest Protection (University of West-Hungary, Sopron), *Phytophthora* species occure in common alder (*Alnus glutinosa*)l, sessile oak (*Quercus petraea*), turkey oak (*Q. cerris*) and eastern black walnut (*Juglans nigra*) stands. The highest diversity was detected in the rhizosphere soil of alder stands, with nine species: *P. alni*, *P. plurivora*, *P. gonapodyides*, *P. inundata*, *P. megasperma*, and four, that time undescribed taxa (SZABÓ et al. 2013). There are also data about the occurrence of ink disease in Hungary.

Heat stress, flooding, soil salinity, hardpans, drought and the simultaneous attack of other pathogens can cause predisposition to *Phytophthora* infection (ERWIN and RIBEIRO 1996).

The *Phytophthora* species, as other hemibiotrophic pathogens, cannot colonize dead tissues. They can exist only for short periods in the soil without their host plants. To compensate the weak survival ability, a small inoculum quantity is enough to cause epidemics in the presence of host plants and adequate site conditions. (ERWIN and RIBEIRO 1996)

Phytophthora inoculum can disperse via the movement or transportation of soil particles, plant tissues, via floods, irrigation water and the trade of infected but asymptomatic nursery stock (GHIMIRE et al. 2009, ERWIN and RIBEIRO 1996).

The integrated plant protection against *Phytophthora* diseases includes management methods decreasing predisposition and avoiding the introduction of pathogens, the use of phosphite and fungicides as necessary. Biological control methods are also availeable with the use of *Gliocladium virens*, *Trichoderma harzianum* or *Pythium oligandrum* (ERWIN and RIBEIRO 1996, JUHÁSOVÁ and BERNADOVIČOVÁ 2004). Ectomycorrhiza could also mean a potential control method.

One of the important ecological and economic problems is the fast growing number of invasive species. The second most invasive plant pathogen species belongs to the Oomycetes nowadays in Europe (SANTINI et al. 2012). Their number is three times higher than in the 1980's. The invasive species of the genus *Phytophthora* endanger the ecological and economic sustainability of the forests worldwide (HANSEN 2008b). That is the reason

why it is important to gain as many as possible information about forest Phytophthoras, their importance in forest protection and about the control methods.

Hypothesis of the research

1. The presence of *Phytophthora* species in the rhizosphere soil decreases the health condition of the forest stand.
2. Watercourses should have a diverse *Phytophthora* community.
3. *Phytophthora* species living in watercourses have more diverse genetic.
4. Predominantly the precipitation affects the occurrence of *Phytophthoras* and the diversity of the *Phytophthora* communities.
5. There are seasonal changes in the virulence of Phytophthora species causing diseases of woody species.
6. Certain *Phytophthora* species are more common in spring, others in fall.
7. Copper-containing fungicides can be used for the control of *Phytophthora* diseases.
8. Saplings with ectomycorrhized roots are more resistant against *Phytophthoras* and other soilborne pathogens.

Aims of the research

1. To know the soilborne *Phytophthora* communities of forest stands with different health conditions. To monitor the changes of health condition and the changes of *Phytophthora* communities in long ago declining forest stands.
2. To know the waterborne *Phytophthora* community living in the brook Rák and in the springs belonging to its watershed.
3. To analyse the factors affecting the changes in *Phytophthora* communities. Are there seasonal changes in the species composition of soilborne and waterborne *Phytophthora* communities? What are the factors affecting the occurrence of *Phytophthora* species in the soil or watercourses?
4. To analyse the effect of site conditions on the host plant-pathogen interactions. How can different site parameters affect the host-pathogen interaction in declining forest stands? Which site parameters are important at the beginning of new epidemies?
5. To characterize the collected *Phytophthora* strains with morphological, phylogenetical investigations, to characterize the genetic diversity of the most common *Phytophthora* species.
6. To prove the pathogenicity of the most important *Phytophthora* species of my collection against the saplings of forest tree species.
7. To test control methods with special emphasis of phosphite, copper containing fungicide and ectomycorrhizal fungi.

Materials and methods

Field surveys

Field surveys were carried out near Sopron, Sárvár and in the Hanság region. In the Hanság region, two sampling sites were assigned, each containing 20 labelled trees. *Phytophthora* infection was detected earlier by the researchers of theUniversity of West-Hungary in both forest stands. The aims of the research were to monitor the health condition

of the labelled trees, to asses the occurring *Phytophthora* species and to compare the resulting data with the older data. Field surveys were carried out in June and September 2011 and in June and September 2012. The health condition assessment was carried out based on the crown symptoms in the black walnut sampling site. Samples were taken every time from the rhizosphere soil of the labelled trees. Water samling was carried out in the irrigation channel and in the River Rábca near the sampling site. The health condition assessment was carried out based on crown and collar symptoms in the common alder sampling site. Samples were taken every time from the rhizosphere soil of the labelled trees. Water sampling was carried out in the irrigation channel. When collar symptoms with fresh exudates were observed, barks samples were also collected. The aims of the field surveys were to identify the causal agents of the decline in the Sárvár 5L and Sárvár 19G forest stands. There were no sampling sites in these stands. Trees with different severity of symptoms were chosen after the reconnaisence. Health condition assessment and sampling was carried out with the chosen trees. The health condition of ten black walnut trees was assessed based on the crown symptoms in the Sárvár 5L forest stand. Soil samples were taken from the rhizosphere of the trees. The health condition of six wild cherry (*Prunus avium*) trees was assessed based on the collar symptoms in the Sárvár 19G forest stand. Rhizosphere soil samples and symptomatic roots were collected. Water sampling was also carried out in the backwater of the River Rába, near the forest stand. The aims of the field survey near Sopron, in the catchment of the Brook Rák was to detect and identify the *Phytophthora* species occurring in the Sopron Hills, and to identify the parameters affecting their occurrence. Water sampling was carried out in 18 sampling points in April, May, July, September and October 2011, April, May, June, July, August, September and October 2012 in order to fulfil the aims.

Isolation methods, species identification and strain collection maintenance

Isolation from soil samples was carried out using the leaf baiting method with healthy cherry laurel and rhododendron leaves. The leaves were disinfected with 10% sodium-hypochlorite solution previously. 5 mm x 5 mm segments were cut from the growing edge of the lesions on the baits. The segments were placed onto *Phytophthora* selective agar plates. The collected plant tissues were soaked in daily changed distilled water for a couple of days until fenols dissolved. Small segments were cut from the edge of the living and dead tissues after disinfection with 10% sodium-hypochlorite solution. The segments were placed onto selective agar plates. The plates were incubated in dark, on 20°C. White colonies characteristic to the genus *Phytophthora* appeared in 2-4 days. Both morphological and molecular traits were used for species identification. Colonies for morphological characterization were grown on carrot agar (BRASIER 1969), 20°C, in dark. Filtered non-sterile soil extract (JEFFERS and ALDWINCKLE 1987) was used to induce sporangia formation. Growth rates were measured, colony morphology was identified and microscopic features were assessed.

Colonies used for molecular identification were grown on PDA plates covered with a sterile cellophane film on 20°C in dark. The ITS1-5.8S-ITS2 region of the ribosomal DNA was amplified using the ITS4-ITS6 primer pair (COOKE et al. 1997). The translation elongation factor 1-alpha (TEF1A) region of the nuclear DNA was also amplified with the EF1A-for – EF1A-rev primer pair (KROON et al. 2004) in cases of species belonging to the Clades 8-10 (BLAIR et al. 2008). PCR reactions were made with the REDExtract N-Amp Plant Kit (Thermo Scientific, 'direct protocol'), according to the manufacturer's

instructions. Sequencing was carried out in the EUROFIN Laboratory (Ebersberg, Germany). Sequence editing and aligning was made with the Sequence Scanner, GeneRunner and Clustal X softwares. BLAST searches against the NCBI GenBank and against the Phytophthora Database were carried out using the BLASTN software. A strain collection of 400 individuals were created from *Phytophthora* isolates for the further investigations. The strains are maintained on PDA on 7-10°C. Their condition is regularly controlled. They are subcultured two times per year.

Morphological examinations

The aim was to characterize every collected *Phytophthora* species. 10-10, or every collected isolates of the given species were used to do this. Colonies were grown as described by the morphological identification. Growth rates were measured, colony morphology was identified and microscopic features were assessed. Microscopic examinations were doe every 2nd day until 50-50 pieces of every characteristic organs were measured and photographed or until 1 month. Basic statistics were calculated from the collected data with the MS Office Excel 2010 Software.

Pathogenicity tests

Black walnut seedlings

The first infection was done in Spetember 2012. One *P. plurivora* (202a) and one *P. cactorum* (174/2) strain was used, both originationg from the Hanság black walnut sampling site. 14 days old colonies were used for the wound inoculation. The colonies were grown on PDA agar, 20°C, in dark.The saplings were 3.5 months old. They were grown in 10 l plastic containers. 8 saplings/ pathogen strain plus 8 control saplings were used. The inoculation was made on 8th September 2012. Saplings were watered as it was necessary but they were maintained under natural conditions. They overwintered in a frost-free, closed chamber. Evaluation of the experiment was made after 10 months of incubation. Length of the shoot (cm), diameter of the root collar (mm), length and width of the necrosis (mm) were measures and the health condition of roots and shoots was evaluated based on a 5-point scale.

The second infecion was done on 8th May 2013. 14 days old colonies were used for the wound inoculation. One *P. plurivora* (202a), one *P. cactorum* (174/2) and one *P. polonica* (141/2) strain was used, *P. plurivora* and P. *cactorum* originationg from the Hanság black walnut sampling site, *P. polonica* from the Sárvár black walnut sampling site. The colonies were grown on PDA agar, 20°C, in dark. The saplings were one year old. They were grown in nursery field. 15 saplings/ pathogen strain plus 15 control saplings were used. Saplings were watered as it was necessary but they were maintained under natural conditions. Evaluation of the experiment was made after 5 months of incubation. Length of the shoot (cm), diameter of the root collar (mm), length and width of the necrosis (mm) were measures and the health condition of roots and shoots was evaluated based on a 5-point scale.

Common alder seedlings

The pathogenicity of *P. alni*, *P. taxon raspberry*, *P. inundata*, *P.lacustris* and *P. gonapodyides* was examined. The strains used were collected in 2011 from the common alder sampling site (*P. alni*, *P. taxon raspberry*, *P. inundata*, *P.lacustris*) or from the brooks of the Sopron Hills (*P. gonapodyides*). 14 days old colonies were used for the tests. The

colonies were grown on PDA agar, 20°C, in dark. The saplings were one year old. They were replanted into 2.5l plastic containers in March 2013. The planting media proved to be free of Phytophthoras. 18+18 saplings/isolate plus 17 control saplings were used for the test. Infection was made on 26-27. April 2013 with wound inoculation (18 saplings / isolate) and with soil infection (18 saplings/isolate) methods. The saplings were watered as it was necessary but they were maintained under natural conditions until September 2013. After 5 months of incubation, the health condition of the root system and the health condition of the shoots was evaluated; length of the shoot (cm), diameter of the root collar (mm), length and width of the necrosis (mm), length and width of the root system were measured. The area of the necrosis in case of wound inoculated saplings was calculated based on the formula of the ellipsoid.

Wild cherry saplings

Pathogenicity test was combined with a test evaluating the effect of sandy loam soil type to the health condition of wild cherry saplings, in comparison with the effect of a loamy, control soil type. Both planting media proved to be free of Phytophthoras. Colonies of one *P. plurivora* (207/1) and one *P. polonica* (210/2) were used for the test. Both strains were collected in the Sárvár 19G stand, from the rhizosphere soil of dying wild cherry trees. 14 days old colonies were used for the tests. The colonies were grown on PDA agar, 20°C, in dark. The saplings were two years old. They were replanted into 2,5l plastic containers in March 2013. Infection was made on 26-27. April 2013, with wound inoculation and soil infection methods. 10 saplings were planted into sandy loam soil and infected with *P. polonica*, 10 saplings were planted into loamy soil and infected with *P. polonica*, 10 saplings were planted into sandy soil and infected with *P. plurivora*, 10 saplings were planted into loamy soil and infected with *P. plurivora*, 10 plus 10 saplings were noninfected controls. The saplings were watered as it was necessary but they were maintained under natural conditions until September 2013. After 13 weeks of incubation, the health condition of the root system and the health condition of the shoots was evaluated; length of the shoot (cm), diameter of the root collar (mm), length and width of the necrosis (mm), length and width of the root system were measured. The area of the necrosis was calculated based on the formula of the ellipsoid.

Sessile oak saplings

One *P. plurivora*, one *P. gonapodyides* and one *P. lacustris* strains, all collected from the brooks of the Sopron Hills were used for the tests. They were 14 days old, grown on PDA agar, 20°C, in dark. Saplings were 2 years old. Saplings for wound inoculation (7 saplings/strain plus 7 control saplings) were grown in nursery field. Saplings for soil infection (7 saplings/strain plus 7 control saplings) were replanted into 2.5l plastic containers. The planting media used proved to be free of Phytophthoras. The saplings were watered as it was necessary but they were maintained under natural conditions. until September 2013. After 5 months of incubation, the health condition of the root system and the health condition of the shoots was evaluated; length of the shoot (cm), diameter of the root collar (mm), length and width of the necrosis (mm), length and width of the root system were measured. The area of the necrosis in case of wound inoculated saplings was calculated based on the formula of the ellipsoid.

The test of control methods

The aims were to test the effect of ectomycorrhiza, phosphite and copper-oxychloride against the ink disease of sweet chestnut; and to test the effect of phosphite and copper-oxychloride on ectomycorrhiza. 40 ectomycorrhized sweet chestnut saplings (*Scleroderma spp.*) and 40 non-mycorrhized saplings were used. 10 days old colonies of a *P. cambivora* strain, grown onPDA, 20°C, in the dark were used for wound inoculation and soil infection experiments (done in May 2012). Phosphite and fungicide treatments (spraying) were done in June 2012 (Phosphite: Fosfitex FR 5% solution, 50 ml/sapling; copper-oxychloride 0.2% solution, 50ml/sapling). 10 non-mycorrhized saplings were infected through their planting media (5 also with wound inoculation, 5 without wound inoculation), without any spraying. 10 non-mycorrhized saplings were infected through their planting media (5 also with wound inoculation, 5 without wound inoculation), with phospite spraying. 10 non-mycorrhized saplings were infected through their planting media (5 also with wound inoculation, 5 without wound inoculation), with fungicide spraying. 10 non-mycorrhized saplings were maintained as controls. 10 mycorrhized saplings were infected through their planting media (5 also with wound inoculation, 5 without wound inoculation), without any spraying. 10 mycorrhized saplings were sprayed with phosphite. 10 mycorrhized saplings were maintained as controls. The saplings were watered as it was necessary but they were maintained under natural conditions until June 2013. After the incubation, the health condition of the root system and the health condition of the shoots was evaluated; length of the shoot (cm), diameter of the root collar (mm), length and width of the necrosis (mm), length and width of the root system were measured. The area of the necrosis in case of wound inoculated saplings was calculated based on the formula of the ellipsoid. The condition of mycorrhizae was checked under light microscope.

Data processing, statistical analysis

PAST 3.05 software was used for rarefaction analysis, for calculating diversity indices and diversity profiles and to do PCA. STATISTICA 11 software was used to compare health condition datasets and to evaluate the pathogenicity and control methods test. Basic statistics, parametric and non-parametric tests were conducted. MEGA 5.0.2 and Topali v2.5 softwares were used to create phylogenetic trees. MEGA 5.2.2 software was used to calculate within and between group distances and to evaluate the genetic diversity of the most common *Phytophthora* species.

Summary of the results

Phytophthora species occurring in the forest stands and their role in forest declines

Common alder sampling site

While collar symptoms with fresh exudates could be seen only occasionally, wilting symptoms were continuously present. The health condition of the trees was significantly wronger in 2012 than in June 2011, based on the crown symptoms (p= p=0,006145, p=0,002195, respectively). Nine *Phytophthora* taxa were isolated from the samples: *P. alni ssp. multiformis*, *P. lacustris*, *P. taxon raspberry*, *P. inundata*, *P. plurivora*, *P. polonica*, *P. sp. oaksoil*, *P. taxon hungarica* and *P. gonapodyides*. *P. lacustris* was present in the rhizosphere soil of the trees every sampling time, *P. taxon raspberry* and *P. inundata* could be isolated two times, the other species were detectable only at one sampling time.

Black walnut sampling sites

There were no collar symptoms present on the trees in the Hanság region. Wilting symptoms suggesting root disease could be seen continuously. The health condition of the trees was significantly better in June 2011 than in June and September 2012 (p=0,023000). *P. plurivora* was present in the rhizosphere soil of the trees at every sampling time. *P. cactorum* was detectable only in June 2011 and September 2012.

In the Sárvár 5L forest stand, severe wilting symptoms suggesting root disease were present on 50% of the trees. There were no collar symptoms in the stand. *Phytophthora* species were present in every rhizosphere soil samples. 2 *Pythium sp*., 2 *P. polonica*, 1 *P. cactorum* and 15 *P. plurivora* isolates were collected.

Sudden death of wild cherry trees near Sárvár

Approximately 50% of the wild cherry trees were dead in a 12 years old mixed deciduoud forest stand. Many of the living trees showed also dieback symptoms and severe collar symptoms with bark cracking and gummosis. *P. polonica* (5 isolates) could be isolated from root and rhizosphere soil samples, while *P. plurivora* (3 isolates) could be isolated from rhizosphere soil samples.

Baiting in the brooks and springs of the Sopron Hills

The 2 years of the monitoring proved to be enough to detect the *Phytophthora* species of the region, based on the rarefaction curve. 123 *Phytophthora* and 7 *Pythium* isolates were identified during the 2 years of the survey. Isolation was the most successful in July in both years. 50,41% of the collected *Phytophthora* isolates belongs to the *P. gonapodyides* species, 36,59% is *P. lacustris*, 12,2% *P. plurivora* and 0,81% is *P. pseudosyringae*. While *P. lacustris* was detectable from the water almost every time, *P. gonapodyides* was present in spring and early summer, *P. plurivora* in summer and fall. *P. pseudosyringae* could be isolated only one time in October 2011.

Factors affecting the changes of Phytophthora communities

Common alder sampling site

There was no appreciable seasonality in the changes of species composition, or in the diversity of sampling times. June 2011 and September 2012 were the two most diverse sampling times. June 2011 was the most diverse according to the Simpson-index. However, the Shannon-index and the diversity profiles don't show significant differences between the two diverse sampling times. The cluster analysis based on the Bray-Curtis index differentiates the years 2011 and 2012. It doesn't show any seasonality.

Black walnut stand in the Hanság region

There was no appreciable seasonality in the changes of species composition, or in the diversity of sampling times. June 2011 and September 2012 were the two most diverse sampling times. They were significantly more diverse than September 2011 or June 2012. The Cluster analysis based on the Bray-Curtis index differentiates September 2011 and June 2012. It doesn't show any seasonality.

Baiting in the brooks of the Sopron Hills

There may be seasonal changes in the species composition of waterborne Phytophthoras in the region. We can differentiate a spring-early summer group with the dominant presence of *P. gonapodyides* and a summer-fall group with the presence of P. *lacustris* and *P. plurivora*. Diversity profiles and diversity indices separate the most diverse

sampling times of July 2011 and September 2012 from the other sampling times. There are no seasonal changes based on the diversity profiles. However, the cluster analysis based on the Bray-Curtis index supports both previous assumptions. There is a group with the two most diverse sampling times and two other groups, one with the spring-early summer sampling times and an other with the summer-fall sampling times.

Meteorological and hydrological factors affecting the changes of Phytophthora communities

Based on the results of the PCA and the Spearman's correlation, we can say that the presence or absence of waterborne *Phytophthora* species is affected mainly by the quantity of precipitation of the given and previous month, less by the mean temperature of the previous month, by the soundings and by the water temperature in the Sopron Hills.

The presence or absence of *P. plurivora* and *P. cactorum* was affected by the above mentioned environmental factors in the Hanság region. The quantity of precipitation of the given and previous month, the number of frost and winter days and the number of hot days were mainly relevant based on the PCA. Temperature parameters, like the minimum temperature of the given month or the mean temperature of the previous month showed a weaker correlation with the presence of these species.

Effects of site conditions on the host-pathogen interactions

Hanság region

Climatic conditions got better between 2000-2005, and then continuously wrong between 2005-2012, based on the values of the modified Ellenberg-index. Climatic conditions were the worst in 2012 in the region. The groundwater table also continuously decreased. The maximum groundwater level in the hydrological year 2011-2012 was lower than the minimum levels of the previous years. These lead to a weaken immunity of forest trees, which could not tolerate the root loss caused by the Phytophthoras.

Sárvár

Climatic conditions did not decrease in the region of Sárvár. However, the decrease of the groundwater level was continuous in the last five years. This might lead to weaken immunity of the black walnut trees. In case of wld cherry trees, the poor soil conditions might swell the predisposition. Rainstorms with unordinary high quantity of precipitation, floods of the River Rába could also prosper the increase of *Phytophthora* inoculum. The weaken trees could not olerate the root loss.

Characterisation of the identified species

Morphology and Phylogeny

The detailed morphological examination, the phylogenetic trees (maximum likelihood-, maximum parsimony and Bayesian Inference trees) based on the ITS1-5.8S-ITS2 and TEF1A regions support the results of the BLAST searches. However, the identification of two isolates needs further molecular analysis. These isolates belong to the species *P. polonica* based on the BLAST searches with ITS and TEF1A sequences, and based on the morphology. However, their ITS sequences differ in 160 sites from all of the other *P. polonica* sequences.

Genetic diversity of the most common species

P. cactorum

There are two different alleles present in the Hanság black walnut sampling site. They differ in only one site on the 792 bp long region.

P. plurivora

There are four alleles based on four variable sites on the 762 bp long ITS region. The most common allele is the A1 allele. It occurs in both black walnut sampling sites and also in the brooks of the Sopron Hills. The A2 allele occurs only in the Sopron Hills. The A3 allele was the most common in the Sopron Hills, but it also occurred near Sárvár, in the rhizosphere soil of the dying wild cherry trees. A4 allele was the 2nd most common. It was present in the soil of both black walnut stands.

P. gonapodyides

There were five variable sites on the 820 bp long section of the ITS1-5.8S-ITS2 region. These differentiated seven alleles. A2 was the most common, A5 the 2nd most common. The other alleles are singletons. Every allele originated from the water of the brooks in the Sopron Hills.

P. lacustris

P. lacustris was the most diverse based on the ITS sequences. 11 different lleles appeared on the 821 bp long section of the ITS1-5.8S-ITS2 region. The most common alleles were A10 and A6. Both were present in the rhizosphere soil of the common alder sampling site and in the water of the brooks in the Soprn Hills. The other alleles are singletons.. P. lacustris proved to be equally diverse in the soil of the Hanság region and in brooks of the Sopron Hills. 3 alleles occurred in both sampling sites, 4 only in the Sopron Hills and 4 only in the Hanság.

P polonica

There were 10 variable sites on the 839 bp long region. These differentiate four alleles. Three of them were found in the rhizosphere soil of the dying wild cherries, the 4th in the rhizosphere soil of the common alder sampling site.

Results of the pathogenicity tests

Black walnut seedlings

Although the saplings didn't show any symptoms during the test period, all of the examined *Phytophthora* species were able to cause necrosis on the lower stem of the saplings. During the fall infection, *P. plurivora* proved to be more aggressive against black walnut saplings than *P. cactorum* did (p=0.049883). On the other hand, during the spring infection, *P. cactorum* proved to be more aggressive than *P. plurivora* (p=0.000444) or *P. polonica* (p=0.000444) did. There were no significant differences in the size of the necrosis caused by *P. plurivora* or *P. polonica*. There were no significant seasonal differences in the virulence of *P. plurivora*. *P. cactorum* was significantly more virulent in spring than in fall (p=0.000370).

Common alder saplings

By the soil infection test, two infected seedlings died during the test period. Both seedlings were infected with *P. alni ssp. multiformis*.

According to the results of the Kruskal-Wallis nonparametric ANOVA, the health condition of the roots of the seedlings was significantly different between the groups with different treatments (p=0.0000). The soil infestation test did not result significant differences in the health condition of the shoots during the test period (p=0.051300).

Based on the results of the pairwise comparisons with the Mann-Whitney U-test, the health condition of the root system was worse in every infected group than in the non-infected control group. The most significant damage was caused by the *P. alni* (p=0.0000). Similar, severe damage was caused by the *P. lacustris* (p=0.000100). The root system of the seedlings infected with *P. taxon raspberry* or *P. inundata* was also significantly weaker than the health condition of the roots of the control seedlings (p=0.001400 and p=0.017700). However, the root system of *P. gonapodyides*-infected seedlings was not significantly damaged in comparison with the root system of the control seedlings (p=0.149700). P. alni damaged significantly stronger the root system of the seedlings than *P. taxon raspberry* (p=0.029300), *P. inundata* (p=0.000200) or *P. gonapodyides* (p=0.000100) did. Damages caused by *P. inundata* were weaker than those caused by *P. lacustris* (p=0.0020), but they were not significantly different from the damages caused by *P. gonapodyides* (p=0.474500). *P. lacustris* caused significantly more severe damage to alder roots, than *P. gonapodyides* did (p=0.000600). The damages caused by *P. taxon raspberry* did not differ significantly from the damages caused by *P. inundata* (p=0.172900), *P. lacustris* (p=0.143400) or *P. gonapodyides* (p=0.066500).

By the stem inoculation experiment, four saplings died during the test period. They were inoculated with the *P. alni ssp. multiformis* isolate.

According to the results of the Kruskal-Wallis non-parametric ANOVA, the treatment groups differ significantly based on the health condition of the shoots of the seedlings (p=0.001100). There are also significant differences between the groups based on the health condition of the root system of the seedlings (p=0.000700). However, the planting media of these seedlings was not infested with Phytophthoras. The lesion sizes were also significantly different (p=0.000000) between the treatment groups.

Based on the pairwise comparisons with the Mann-Whitney U-test, the health condition of the shoots was significantly poorer in the *P. alni*-infected group, than in the non-infected control seedling (p=0.024400), *P. taxon raspberry*-infected (p=0.008100), *P. gonapodyides*-infected (p=0.0027) or *P. inundata*-infected (p=0.011400) groups. However, it did not differ significantly from the health condition of the *P. lacustris*-infected group (p=0.522800). The health condition of the shoots in the *P. lacustris*-infected group damaged significantly stronger than the health condition of the shoots in the *P. taxon raspberry* (p=0.038000), *P. gonapodyides* (p=0.013100) or *P. inundata* (p=0.048700)-infected groups. The health condition in the *P. lacustris*-infected group did not differ significantly in comparison with the *P. alni ssp. multiformis* -infected group.

Every *Phytophthora* species used for the stem inoculation proved to be a pathogen of alder seedlings (p=0.000100). The biggest necroses were caused by the *P. alni ssp. multiformis* strain. These necroses were significantly bigger than the necroses caused by *P. lacustris* (p=0.030900), *P. taxon raspberry* (p=0.003100), *P. gonapodyides* (p=0.003100) or *P. inundata* (p=0.000800). The necroses caused by *P. lacustris* was also significantly bigger than those caused by *P. taxon raspberry* (p=0.002700), *P. gonapodyides* (p=0.004000) or *P. inundata* (p=0.000100).

Wild cherry saplings

In case of the uninfected saplings, the root system remained rich and healthy and only callus formation was observed on the stem at the inoculation point. In contrast altogether six *P. polonica*-, and four *P. plurivora*- infested saplings died during the thirteen weeks of the examination. Sunken, dark necrotic lesions developed at the inoculation points and rare root system, dead fine roots and root tips, and extremely short main roots were observed. Significant differences were observed in case of the health condition of the shoots (p=0.002000), health condition of the roots (p=0.000000), in the width of the roots (p=0.000000) and the area of the necrosis (p=0.001). For the health condition of the roots both the effect of the soil type (p=0.015000) and the effect of the pathogen (p=0.000000) was significant. *P. polonica* seemed to be more virulent than *P. plurivora*. For the health condition of the shoots only the effect of the soil type was significant (p=0.000000). The soil type did not show significant differences in the wound inoculation experiment. *P. plurivora* and *P. polonica* caused significantly bigger necrosis than observed in the control groups. There was no significant difference in the virulence of the two species.

Sessile oak saplings

In case of the soil infection test, one, *P. plurivora*-infected sapling died during the test period. *P. lacustris* (p=0.044359) and *P. gonapodyides* (p=0.003923) reduced significantly the width of the root system, in comparison with the uninfected control saplings. *P. plurivora* also reduced the width of the root system, but the difference isn't significant in comparison with the uninfected control saplings. There were no significant differences int he virulence of the three examined *Phytophthora* species based on the root width datasets. Every examined *Phytophthora* species spoilt the health condition of the root system of the saplings significantly (*P. plurivora*-control: p=0.007992, P. *gonapodyides*-control: p=0.000666, *P. lacustris*-control: p=0.042624). There were no significant differences int he virulence of the three examined *Phytophthora* species based on the root condition datasets.

In case of the stem inoculations, two saplings died during the test period. Both were inoculated with *P. plurivora*. Every *Phytophthora* species caused significantly bigger necrosis than observed in case of the control saplings. *P. plurivora* caused the biggest necrosis (p=0.006061 in comparison with the controls). The necrosis caused by *P. lacustris* and *P. gonapodyides* were similar in size (p=0.006061 and p=0.0040400, respectively, in comparison with the controls). The necrosis caused by *P. plurivora* were significantly bigger than those caused by *P. gonapodyides* (p=0.040093). There were no significant differences between the sizes of necrosis caused by *P. lacustris* and *P. gonapodyides*. There were no significant differences between the treatment groups according to the shoot condition, length of shoots or diameter datasets.

Control methods

Phosphite treatment did not impair the state of mycorrhizae, bot copper-oxychloride treatment did. Mycorrhized saplings did not die during the test period, however, 10% of non-mycorrhized saplings did. The root system of mycorrhized saplings remained rich, healthy, asymptomatic, while in the non-mycorrhized groups we could find a lot of saplings with poor root system, plentiful dead fine roots. In the non-mycorrhized groups, phosphite treatment could save the saplings, while 30% of the infected, copper-oxichloride treated saplings died. The most advisable control method is the planting of mycorrhized saplings and the phosphite treatment.

Theses

1. Nine *Phytophthora* species were isolated from the rhizosphere soil of the common alder sampling site: *P. alni ssp. multiformis, P. lacustris, P. gonapodyides, P. inundata, P. taxon raspberry, P. sp. oaksoil, P. sp. hungarica, P. plurivora* and *P. polonica*.
2. Three *Phytophthora* species were isolated from the rhizosphere soil of black walnut stands: *P. cactorum, P. plurivora* and *P. polonica*. This is the first data of *P. polonica* in black walnut stands.
3. *P. polonica* was isolated from root and rhizosphere soil samples; *P. plurivora* was isolated from rhizosphere soil samples of dying wild cherry trees. Both species proved to be new pathogen of wild cherry trees.
4. The following Phytophthora species were isolated from the brooks of the Sopron Hills: *P. gonapodyides*, *P. lacustris*, *P. plurivora* and *P. pseudosyringae*.. Seasonal changes can be observed in the species composition of waterborne Phytophthoras near Sopron.. Their occurrence is affected by the mean temperature of the given and of the previous month, by the minimum and maximum temperature values of the given month and by the water temperature in the Sopron Hills (p=0,341577, Table 5.8). However, the isolation success is affected by the quantity of precipitation.
5. The presence or absence of *Phytophthora* species was affected by the quantity of precipitation of the given and previous month (p=-0,370230), the number of frost and winter days and the number of hot days, the mean temperature of the given month and the previous month (p=0,356348) and the ground water level of the given and of the previous month (p=-0,192925) significantly.
6. There are two new *P. plurivora*, four new *P. gonapodyides*, 10 new *P. lacustris* alleles based on the ITS sequences in Hungary in comparison with earlier reports.
7. The pathogenicity of *P. plurivora, P. cactorum, P. polonica* against black walnut saplings was proven This is the first data about P. polonica.
8. There are seasonal changes in the virulence of *P. cactorum* against black walnut saplings.
9. The pathogenicity of *P. plurivora* and *P. polonica* against wild cherry saplings was proven. This is the first data about *P. polonica.*
10. The effect of site condition onto *P. polonica*-wild cherry interaction was proven.
11. The pathogenicity of *P. plurivora, P. lacustris* and P. *gonapodyides* against sessile oak saplings was proven.
12. Copper-oxychloride can't save the sapings against ink disease, but it impairs the mycorrhizae, while phosphite doesn't.
13. The occurrence of *P. hydropathica* and *P. gallica* in the channel near the Hanság black walnut sampling site are the first Hungarian data of these two *Phytophthora* species.

Publication list

Research articles

KOVÁCS, J., LAKATOS, F., SZABÓ, I. (2013): Occurrence and Diversity of Soilborne Phytophthoras in a Declining Black Walnut Stand in Hungary. Acta Silvatica & Lignaria Hungarica 9 (2013):57-69.

SÁRÁNDI-KOVÁCS, J., LAKATOS, F., SZABÓ, I.: Post-epidemic Situation of a Previously *Phytophthora alni* Infected Common Alder Stand. Acta Sylvatica & Lignaria Hungarica (in press.)

SÁRÁNDI-KOVÁCS, J., NAGY, L., LAKATOS, F., SIPOS, Gy.: Sudden *Phytophthora* dieback of wild cherry trees in northwest-Hungary. Forest Pathology (submitted)

Conference proceedings and abstracts

KOVÁCS, J. (2011): The role of Phytophthora species in the health condition of forest trees. In: Lakatos, F., Polgár, A., Kerényi-Nagy, V. (Ed.): Scientific Conference of PhD Students– University of West-Hungary Faculty of Forestry – Proceedings of the Conference, PALATIA Kft, 2011: 177-180. ISBN:978-963-334-013-4. (Hungarian).

KOVÁCS, J. , LAKATOS, F. , EGYED, K., SZABÓ, I. (2011): *Phytophthora* infection in a sweet chestnut orchard in South-Transdanubia, Hungary. IUFRO 2011 WP 7. 02. 02. Meeting: Global change and forest diseases: new threats, new strategies. Cantabria, Spain, 23-28. May 2011. Abstracts book Ed. JAVIER-DIEZ, J., MARTINEZ-ÁLVAREZ, P., ROMERALO, C., Palencia, 2011: 101.

KOVÁCS, J., LAKATOS, F., SZABÓ, I. (2011) :*Phytophthora* species in the decline of black walnut (*Juglans nigra L.*) stands. In: COST Action FP0801 Management Committee and Working Groups Meeting, 21-22. November 2011 Budapest. Programme and Abstracts (2011): 36.

KOVÁCS, J., LAKATOS, F., SZABÓ, I. (2011): The Role of Phytophthora Species in the decline of black walnut trees. In.: Lakatos, F. and Szabó, Z. (Ed., 2011): Scientific Conference of the Faclty of Forestry- Abstracts and Proceedings of the Conference :61. (Hungarian).

KOVÁCS, J., LAKATOS, F., SZABÓ, I. (2012): Seasonal variation of inoculum density and species composition of soilborne Phytophthoras in an infected black walnut stand in Hungary. In.: 6th IUFRO Meeting Working Party 7.02.09 Phytophthora in Forest and Natural Ecosystems Meeting Abstracts Córdoba (Spain) 9th-14th Sept 2012:42.

KOVÁCS J., LAKATOS F., SZABÓ I. (2012): *Phytophthora* species in the decline of black walnut (*Juglans nigra L.*) stands In: Kőmíves, T., Haltrich, A., Molnár, J. (Ed.).: 58. Scientific Days of Plant Protection, RePRINT Kft. Budapest, ISBN 963 8131 071: 54. (Hungarian).

KOVACS, J., LAKATOS, F., SZABÓ, I. (2012): The Role of *Phytophthora* Species in the Decline of Black Walnut stands. International Scientific Conference on Sustainable Developement and Ecological Footprint, 26-27. March 2012, Sopron, Hungary, Proceedings of the conference (Ed.: Neményi, M., Heil, B., Kovács, A. J., Facskó, F.), University of West-Hungary Press, ISBN: 978-963-334-047-9, Sopron, 2012.

SÁRÁNDI-KOVÁCS, J., Lakatos, F., Szabó, I. (2014): Studies on *Phytophthora* species in a declining common alder stand In: Horváth, J., Haltrich, A., Molnár, J. (Ed.): Scientific Days of Plant Protection 2014. Hungarian Plant Protection Association, Budapest, ISBN 0231 2956. (Hunagrian).

Posters and presentations

KOVÁCS, J., LAKATOS, F., EGYED, K., SZABÓ, I. (2011): *Phytophthora* infection in a sweet chestnut orchard in South-Transdanubia, Hungary. IUFRO 2011 WP 7. 02. 02. Meeting: Global change and forest diseases: new threats, new strategies. Cantabria, Spain, 23-28. May 2011.

KOVÁCS, J., LAKATOS, F., SZABÓ, I (2011): *Phytophthora* species in the decline of black walnut (*Juglans nigra L.*) stands. In: COST Action FP0801 Management Committee and Working Groups Meeting, 21-22 November 2011 Budapest.

KOVÁCS, J., LAKATOS, F., SZABÓ, I. (2011): The Role of Phytophthora Species in the decline of black walnut trees. In.: Lakatos, F. and Szabó, Z. (Ed., 2011): Scientific Conference of the Faclty of Forestry, Sopron, 2011.

KOVACS, J., LAKATOS, F., SZABÓ I. (2012): Seasonal variation of inoculum density and species composition of soilborne Phytophthoras in an infected black walnut stand in Hungary. 6th IUFRO Meeting Working Party 7.02.09 Phytophthora in Forest and Natural Ecosystems, Córdoba (Spain), 9-14. September 2012.KOVACS, J., LAKATOS, F., SZABÓ, I. (2012): The Role of *Phytophthora* Species in the Decline of Black Walnut stands. International Scientific Conference on Sustainable Development and Ecological Footprint, 26-27. March 2012, Sopron, Hungary.

KOVÁCS, J. (2011): The role of Phytophthora species in the health condition of forest trees. Scientific Conference of PhD Students– University of West-Hungary Faculty of Forestry Sopron, 13rd April 2011.

KOVÁCS, J., LAKATOS, F., SZABÓ, I. (2012): The Role of Phytophthora Species in the Decline of Black Walnut Stands. 58. Scientific Day of Plant Protection, Budapest, 21st February 2012.

KOVACS, J., LAKATOS, F., SZABÓ I. (2012): Seasonal variation of inoculum density and species composition of soilborne Phytophthoras in an infected black walnut stand in Hungary. In.: 6th IUFRO Meeting Working Party 7.02.09 Phytophthora in Forest and Natural Ecosystems, Córdoba (Spain) 11. September 2012.

KOVACS, J. (2012): Studies about *Phytophthora* species of Hungarian forest ecosystems. Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Birmensdorf, Switzerland, 7th November 2012.

SÁRÁNDI-KOVÁCS, J., Lakatos, F., Szabó, I. (2014): Studies on *Phytophthora* species in a declining common alder stand 60. Scientific Day of Plant Protection, Budapest,18th February 2014.