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Phytophthora Root Rot in Declining Forest Trees

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Summary

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Studies by light and scanning-electron microscopy in combination with specific isolation techniques revealed the presence of soil-borne *Phytophthora* - species in declining oak, beech and other forest trees. The sequence of histopathological reactions in infected roots and distinct fungal structures found in necrotic tissues are documented. Specific laboratory procedures used for the detection and identification of *Phytophthora* are presented. Pathogenicity of the isolated *Phytophthora* - species is shown by common tests of soil infestation and stem inoculation. The possible role of predisposing and contributing factors leading to this type of decline is discussed with emphasis on climate change and excess nitrogen.

Introduction

In the last fifteen years forest decline in Europe has caused great concern to foresters and silviculturists. Affected trees display several aboveground symptoms e.g. bark necrosis, deteriorations of the branching system, a higher transparancy of the crown, yellowing of leaves and finally crown dieback, and distinct disorders in their root systems expressed as a conspicuous lack of functional mycorrhizal associations, dieback of fine roots and extensive root rot (BLASCHKE 1994, VINCENT 1987, 1991). More recently there has been an alarming intensification of dieback of pedunculate oak in Central and Eastern Europe (SIWECKI & LIESE 1991, LUISI & al. 1993).

Because of symptomatology resembling important *Phytophthora* diseases of chestnuts in Europe (DAY 1938, GRENTE 1961, REICHARD & BOLAY 1986) and

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USA (CRANDALL & al 1945), oaks in Spain (BRASIER & al. 1993) and California (MIRCETICH & al. 1977), beech and other forest trees in Britain (DAY 1938, BRASIER & STROUTS 1976) and eucalypt in Australia (SHEARER & TIPPET 1989) we have tested the hypothesis that soil-borne *Phytophthora* - species could be involved in the decline phenomenon.

This paper reports the identity and pathogenicity of different species of *Phytophthora* isolated from affected trees and illustrates the histopathological changes in primary and secondary root tissues after infection by the pathogens.

Materials and Methods

Comparative root studies were conducted at 25 locations in Bavaria, Northern Germany, Switzerland, Hungary, Italy and Slovenia during replicate inventories (1993-1995) on solitary trees and in mixed stands of *Quercus robur, Q. petraea, Q. cerris, Q. pubescens, Q. ilex, Fagus sylvatica, Castanea sativa, Acer platanoides, Betula pendula, Robinia pseudoacacia,* and *Aesculus hippocastanum*. 10 apparently healthy and 80 declining *Q. robur* trees (age 15-150 years) and single affected trees of the other species were examined. Root and soil samples were taken by soil coring and by careful hand excavation of severed coarse roots with attached fine root systems.

After visual examination of washed roots under the dissection microscope, laboratory routines were used for histopathological studies of diseased root tissues including staining of fungal structures (ALT & SCHMIEDLE 1980) and examination by light and scanning electron microscopy.

Isolations from necrotic fine roots and coarse root segments and from the watersoaked margins of advancing collar cankers were carried out by plating small pieces of diseased tissue directly onto a modified selective PVPNH agar (TSAO 1983) or by using a modified apple trap (RIBEIRO 1978). Specific baits like apples and pears (TSAO 1983, RIBEIRO 1978) and young leaves of *Q. robur* floated over flooded soil (JUNG & al. in prep.) were used for the isolation of *Phytophthora* and *Pythium* species from soil samples containing diseased roots.

Species were identified by comparing colony growth patterns and morphological features of sporangia, oogonia, chlamydospores and hyphae with known isolates and descriptions by WATERHOUSE 1970, KRÖBER 1985, STAMPS & al. 1990. Formation of sporangia was stimulated by flooding agar discs from the growing edge of the culture in nonsterile soil leachate (filtrate of a 25% soil suspension). Sexual reproduction of heterothallic isolates was reached by cros-sing them with tester strains of *P. cinnamomi* Rands, *P. cambivora* (Petri) Buisman and *P. drechsleri* Tucker of known compatibility group.

The pathogenicity of *Phytophthora* and *Pythium* isolates was determined by common methods of soil infestation (HAMM & HANSEN 1982, MATHERON & MIRCETICH 1985) using *Q. robur* seedlings and stem inoculation (ROBIN 1992) on 5 year-old *Q. robur* and *F. sylvatica* trees.

Toxigenicity of the isolated *Phytophthora* species was determined by a wilting test with young *Q. robur* cuttings which were transferred for 72 hours into test tubes containing diluted (10%) sterile (not autoclaved) culture filtrates of the isolates.

Results

In *Q. robur* symptoms of root infection in the field resembled those described earlier by investigators of oak decline (BLASCHKE 1994, EICHHORN 1992, VINCENT 1991, GALOUX & DUTRECQ 1990, NÄVEKE & MEYER 1990), ink disease of chestnut (GRENTE 1961, CRANDALL & al. 1945, DAY 1938) littleleaf disease of pines (JACKSON 1945) and dieback of eucalypts (SHEARER & TIPPETT 1989). The

most characteristic symptom was the massive destruction of rootlets by a *Phytophthora* species. There were marked differences between the branching frequency of root segments of healthy and declining trees. For example on healthy oaks the mean number of lateral roots (diameter <2mm) on coarse root segments (diameter 0.5-5cm) ranged from 43.5- 51.2 per m, whereas on coarse roots of declining oaks less than 3.8 per m were present (SALOMON 1995). In affected trees a progression of symptoms was observed from cessation of root growth to discoloration and abnormal shedding of fine and small woody roots, and further to tissue necrosis and callussing canker on coarse roots. Discrete blackened patches on the bark indicating extensive phloem necrosis were found along the coarse roots. In most cases cortical necrosis was associated with the development of stripe cankers on infected woody roots. Some small callussing lesions originating from the killing of unlignified roots remained limited in size. However on coarse roots and buttress roots callussing wounds in the secondary phloem had also impaired vascular tissue thus leading to the formation of tyloses in xylem vessels and cankers girdling parts of the woody root system.

Comparing the anatomy of healthy roots with cellular and histological changes induced by *Phytophthora* species in every tree species examined we found a typical discoloration of phellem cells at the site of invasion of coralloid hyphae.

The formation of papillae within the primary root tissue and the formation of lignitubers in the inner periderm layer was evident in affected fine roots. Oospores were observed in longitudinal sections of necrotic rootlets.

In most of the examined declining trees *Phytophthora* species were not only detected in deteriorated rootlets and in callussing wounds on coarse roots but also in stripe cankers which were mainly found on the stem base.

Results of isolation tests are presented in Tab. 1.

Preliminary results of ongoing pathogenicity tests with some of the isolates indicate the potential of *Phyto-phthora* species to cause symptoms observed in the field:

In the soil infestation test with seedlings of *Q. robur P. citricola*, *P. cactorum*, *P. gonapodyides*, *P. undulata* and *Pythium* Group P are able to induce intercostal yellowing and emergence of resting buds on the lower part of the stem and to cause necrosis on fine roots and dieback of unsuberized long roots. In addition *P. gonapodyides*, *P. cactorum*, *P. undulata* and *Pythium* Group P caused necrosis on some coarse roots and at the root collar. Oospores and hyphae could be observed in thin sections of infected roots and the pathogens were reisolated from the necrotic tissues. *P. cambivora*, *P. megasperma* and *Phytophthora* sp. 1 and 2 have not been included in the first test series. All control plants remained healthy.

Results of the stem inoculation test are presented in Tab. 2.

Tab. 1. Recovery of *Phytophthora* and *Pythium* species from declining forest trees

Phytophthora (*P.*)/ *Pythium* (*Py.*) species	Host tree	No. of locations	Sample type	Isolation method[1]
P. citricola	*Quercus robur*	20	soil, fine roots	1, 2, 3, 4, 5
	Fagus sylvatica	4	soil	5
	Acer platanoides	1	soil	5
	Aesculus hippocastanum	1	soil	5
	Robinia pseudoacacia	1	soil	5
P. cactorum	*Quercus robur*	3	soil	3, 5
P. cambivora	*Fagus sylvatica*	1	soil,roots,collar	1, 5
	Quercus robur	1	soil	5
	Castanea sativa	1	stem	1
P. gonapodyides	*Quercus robur*	2	soil,roots,collar	1, 3, 5
	Betula pendula	1	soil	3
P. megasperma	*Quercus robur*	1	soil	5
P. undulata	*Quercus robur*	1	soil	3, 4, 5
	Fagus sylvatica	2	soil	5
Phytophthora sp.1[2]	*Quercus* spp.[3]	7	soil, fine roots	1, 5
Phytophthora sp.2[4]	*Quercus robur*	1	soil	5
Py. anandrum	*Quercus spp.*[5]	3	soil, fine roots	1, 3, 5
Pythium Group P	*Quercus robur*	2	soil, fine roots	1, 3, 5
Pythium spp.	*Quercus* spp.[3]	25	soil, fine roots	1, 3, 4, 5

[1] 1 = direct plating, 2 = apple trap, 3 = apple baiting, 4 = pear baiting, 5 = baiting with oak leaflets

[2] = homothallic *Phytophthora* species with affinity to *P. cactorum*, but having non caduceous often intercalary or laterally attached sporangia and chlamydospores and hyphal swellings with radiating hyphae. Vegetative hyphae often branching in a dichasium.

[3] = *Q. robur, Q. petraea, Q. cerris, Q. pubescens, Q. ilex*

[4] = homothallic *Phytophthora* species with amphigynous antheridia and semipapillate sporangia. Low optimum temperature (17°C). Vegetative hyphae often branching in a dichasium.

[5] = *Q. robur* and *Q. petraea*

After 3 months control inoculations on *Q. robur* and *F. sylvatica* remained healthy and the wounds had been completely closed. On *Q. robur* all 6 *Phytophthora* species tested and *Pythium* Group P caused sunken necrotic lesions sometimes associated with a dark bleeding.With a mean lesion length of 40.2 mm *P. undulata* caused the greatest damage whereas *Phytophthora* sp.1 led to smaller lesions (length 16.5 mm) than *Pythium* Group P (length 21.5 mm). There had been marked differences in the mean lesion length caused by different isolates of the same *Phytophthora* species as well as in the lesion length caused by the same isolate on different *Q. robur* trees indicating differences in aggressiveness of the isolates and differences in the susceptibility of the trees.

On *F. sylvatica* the 3 *Phytophthora* species tested caused greater damage than on *Q. robur*. With a mean lesion length of 112 mm and 2 of 3 inoculated trees dying *P. cambivora* was the most aggressiv pathogen on beech.

The toxigenicity test revealed the production of a not yet identified phytotoxin by *P. gonapodyides* and *Pythium* Group P leading to a marked wilting of *Q. robur* cuttings after 72 hours.

Tab. 2. Results of the stem inoculation test and the toxigenicity test with *Phytophthora* and *Pythium* species on *Q. robur* and *F. sylvatica* (- = not tested)

Phytophthora (*P.*)/ *Pythium* species	No. of isolates tested		Stem inoculation test[1] lesion length mean (range) in mm		Toxigenicity test[2] wilting index mean (range)
	oak	beech	oak	beech	
P. citricola	6	1	26.3 (14.0-50.0)	56.6 (41.0-73.0)	1.0 (0.75-1.5)
P. cactorum	2	-	33.2 (27.0-45.0)	-	1.0 (0.75-1.25)
P. cambivora	2	1	34.7 (20.0-61.0)	112.0 (101.0-123.0)	-
P. gonapodyides	4	1	26.9 (13.0-65.0)	32.3 (27.0-38.0)	2.33 (1.25-3.33)
P. undulata	2	-	40.2 (15.0-52.0)	-	0.75 (0.5-1.0)
Phytophthora sp.1	2	-	16.5 (10.0-23.0)	-	-
Pythium Group P	2	-	21.5 (18.0-42.0)	-	1.9
Control			0	0	0.5
Sterile water			-		0

[1]= after 3 months
[2] = after 72 hours; 0 = no wilting, 4 = all cuttings completely wilted

Discussion

Our results of microscopic, cultural and infection studies of fine root necrosis are consistent with descriptions of root and collar rot diseases found in other woody perennials caused by *Phytophthora* spp.. *Phytophthora* species were isolated from oak, beech, birch, maple, chestnut, horse chestnut and black locust trees and appeared to be the main cause of the progressive destruction of rootlets. These fungi also infect bark of coarse roots and collar and are also able to induce tyloses in large xylem vessels thus reducing their conductivity for water and nutrients. Both fine root decay and plugging of vessels are probably the main cause for the aboveground symptoms of water deficiency and malnutrition. First results of biotests with culture filtrates of some isolates show that the known production of phytotoxins by *Phytophthora* species (WOOD & al. 1972) can also play a role in symptom expression.

The question arises why soil borne fungi of the natural mycoflora of Central Europe in recent years have led to such a devastive decline of forest trees. The following hypothesis can be presented:

1. Excess nitrogen has led to the observed reduction of mycorrhiza (MEYER 1987, BLASCHKE & JUNG unpublished data) which is known as effective mechanical and biochemical barrier against infection by *Phytophthora* species (ZAK 1964).

2. The frequent occurrence of mild-humide periods during wintertime and springtime of the last decade (RAPP & SCHÖNWIESE 1995) has favoured the infection of nonmycorrhizal roots by zoospores thus causing an increasing population of *Phytophthora* and a progressive destruction of fine root systems from year to year. It could be shown in the laboratory that most of the *Phytophthora* -

isolates are able to form sporangia and to release zoospores in non sterile soil extract water at temperatures between 2 to 8 ° C.

The role of *Phytophthora* species in other parts of Europe suggests that the possibility of root rot of forest trees remains to be elucidated with respect to predisposing climatic factors and nitrogen impact of forest soils trig-gering fine root turnover and the onset of the root rot complex.

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