JOURNAL OF PLANT PROTECTION RESEARCH
Vol. 43, No. 1 (2003)

FIRST RECORD OF ALDER *PHYTOPHTHORA* IN POLAND

Leszek B. Orlikowski[1], *Tomasz Oszako*[2], *Grażyna Szkuta*[3]

[1]Research Institute of Pomology and Floriculture
Pomologiczna 18, 96-100 Skierniewice, Poland
e-mail: lorlikow@insad.pl
[2]Forest Research Institute
Bitwy Warszawskiej 1920 r. 3, 00-973 Warsaw, Poland
e-mail: T.Oszako@iblrs.waw.pl
[3]Main Inspectorate of Plant Protection and Seed Service, Central Laboratory
Żwirki i Wigury 73, 87-100 Toruń, Poland
e-mail: gszkuta@wp.pl

Accepted: February 17, 2003

Abstract: Common alder (*Alnus glutinosa*) decline has been observed in most of European countries since 1993. In Poland decline of alder trees has been observed during the last 6 years. Alder *Phytophthora* was recorded, however, only from one sampling area in the middle of the country. Species of *Armillaria*, *Fusarium*, *Mucor*, *Penicillium* and *Trichoderma* were also isolated from diseased trees. Inoculation of alder stem parts, leaves and seedlings with *Phytophthora* isolates resulted in the development and spread of necrosis. Studies will be continued in the nearest years.

Key words: common alder, isolation, *Phytophthora*, fungi, distribution, pathogenicity

INTRODUCTION

Alnus glutinosa (L.) Gaertn. known as Common alder is an important species component of some forest stands but mainly component of the riparian ecosystem. The species has a great conservation value, providing food and protection for many organisms and in association with genus *Frankia* is strongly nitrogen fixing (Streito and Gibbs 1999). Alder root system helps to stabilise the river banks. A severe decline and death of alder have become common in Europe. Streito et al. (2002) reported that in 1988 *Phytophthora cambivora* (Petri) Buisman was isolated from diseased *A. corgata* in southern France. Three years later alder mortality was observed in natural woodland on the banks of lakes in south-eastern France but the causal agent of the disease was not determined. The tree mortality was noticed also in north-eastern France in 1993 but alder *Phytophthora* was isolated in 1998 (Streito et al. 2002). In 1993 *Phytophthora* sp. was recorded on alder at several sites in Great

Britain (Gibbs 1995). Examination of isolates by Brasier et al. (1995) has indicated that they resembled *P. cambivora*. Development of colonies, however, with an appressed-felty appearance and sparse or no aerial mycelium or similar colonies with a little uniformly woollly aerial overgrowth, sometimes rather irregular in outline development of cultures caused that Brasier et al. (1995) described the causal agent as alder *Phytophthora*. Data presented by de Gruyter & Man in't Veld (2000) indicated on the first record of alder *Phytophthora* in the Netherlands in 1992 and since 1995 in Germany, Austria, Sweden, Belgium, Ireland, Hungary and Italy. Losses caused by alder *Phytophthora* varied from a few percent till more than 50% (Cech 1999; Streito et al. 2002). In opinion of Jung et al. (2000) alder *Phytophthora* introduced to a river system, spreads downstream infecting collar or bare roots of riparian trees via lenticells and adventitious roots. The authors observed the disease on young alder plantations on former agricultural land. Alder *Phytophthora* was isolated also from rootstocks in tree nurseries (Jung et al. 2000).

A severe decline of Common alder has been observed in Poland during the last 6 years. Dead and declining trees 5–90 years old, distributed along rivers, streams, lakes or water ponds but also on seasonally flooded forest stands were often found side by side. The spread of disease and mortality of trees varied greatly from site to site. Similar symptoms like those described by Gibbs (1995) and Streito et al. (2002) are often observed on affected alders. Usually leaves were abnormally small, pale-green or yellow-green and sparse. On some trees on one side of trunk dark-brown necrosis spread from the base up to 50–130 cm. Sometimes tarry spots and wet exudations were seen on the lower part of stems. Beneath the bark reddish to brown discoloration in the cambium zone was visible after fresh exposure. On some trees, even 5–15 years old, advanced root rot was observed. Often new water sprouts grown at the stems of diseased or dead trees.

In 2002 we attempted to isolate specific primary alder pathogens, including *Phytophthora* spp. and to determine their possible involvement in the alder mortality.

MATERIALS AND METHODS

Field survey. A total of 86 samples were collected from 14 sample points in different part of middle and eastern Poland within decline area from April till November, 2002. Each sample was taken from the base of alder with visible infection symptoms on trunk and/or leaves. Diseased tissue parts were taken from the border of bark and cambium with discoloration symptoms, placed into plastic bags and transferred to the laboratory.

Isolation of fungi. Pieces of diseased tissues (about 5 mm diameter) after disinfection over a burner flame, were placed on potato-dextrose agar (PDA) in 90 mm Petri dishes (6 pieces/dish and 5 plates for each sample) and incubated in the dark at 24°C. Plates were checked every day for the presence of fungi colonies. Small fragments of colonies, growing around of tissue pieces were transferred on PDA slants. Additionally, green apples were used as alternative medium. Immediately after taken of samples from trees or in the laboratory, pieces of bark were inserted into fruit in 2–3 holes, covered with adhesive tape and incubated in plastic bags at room temperature. When first, brown lesions were observed, after surface disinfec-

tion over burner flame, pieces of discolorated fruits were transferred into PDA slants. After segregation of isolates obtained representative cultures were cleaned and identified to genera and /or to species. Procedure described by Orlikowski et al. (2002) was used for identification of *Phytophthora*. A taxonomical identification and isozyme analysis of isolates of alder *Phytophthora* were carried out according to Brasier et al. (1995), Erwin and Ribeiro (1996), de Gruyter and Man in't Veld (2000), Mant in't Veld (1995), Mant in't Veld et al. (1998; 2002), Oudemans et al. (1991), Stamps et al. (1990).

Colonisation of alder stems and leaves by *Phytophthora* isolates. Isolates PH/0102 from alder isolated from tree in Poland, PD/20010932 of alder *Phytophthora* from the Netherlands and PH/0028 of *P. cambivora* from striped maple (*Acer pennsylvanicum* L.) were used. Stock cultures were maintained on PDA at 24°C in the dark. In laboratory trials 3 mm diam mycelial plugs, taken from the edges of colonies, were applied on leaf petioles and base of stems of common alder. Plant parts were placed on the moist blotting paper covered with plastic net in the polystyrene boxes and covered with foil (Orlikowski et al. 2001). Length of necrosis on tested organs was measured after 3, 5 and 7-day-incubation at 19–22°C. In greenhouse trials 3 mm diam mycelial disks, taken from the edge of 7-day-old cultures of alder *Phytophthora* PH/0102, PD/20010932 and PH/0028 of *P. cambivora* were used for stem inoculation trial. Four-month-old alder sedlings were used for inoculation. Mycelial disks were put under the cut part of bark (about 15 mm long and 3 mm wide) 2–3 cm above the substratum and covered with tape. After 5 and 8-day-incubation of plants on greenhouse bench at 18–22°C length of necrosis was measured.

Experimental design was completely randomised with 4 replications and 5 plant parts or seedlings (greenhouse trials) in each rep. Trials were repeated at least twice.

RESULTS AND DISCUSSION

Isolation and identification of fungi. Many fungal colonies were isolated from diseased trees but isolates identified as alder *Phytophthora* were found only in one sample point in woodland in the middle part of Poland (Tab. 1). *Phytophthora* was isolated from the base of trees with black spots on the bark up to 1 m above the soil and/or small pale-green leaves. Isolates were obtained only from 15-years-old trees (Tab. 1). Alder *Phytophthora* colonies were already seen on PDA 2 days after transferring of bark fragments on Petri dishes. Later, colonies were overgrown by other fungi but especially by *Mucor* spp., *Fusarium culmorum* and *Trichoderma* spp. (Tab. 1). From diseased bark parts *Alternaria alternata, F. oxysporum, F. solani* and *Penicillium* spp. were also isolated (Tab. 1). Alder *Phytophthora* was not isolated from seeded green apples. Isolates of *Phytophthora* were tested on PDA, V8 juice agar (Erwin and Ribeiro 1996), CMA, cherry decoction agar (Gams et al. 1987), and carrot agar (Erwin and Ribeiro 1996). Typical, not numerous oogonia were only formed on carrot agar and resembled those produced by *P. cambivora*. The nonpapillate zoosporangia were produced both in sterilised 1% soil extract and sterile water. They were similar to those formed by *P. cambivora*. Results of isozyme analysis indicated that Polish isolate of alder *Phytophthora* belong to the standard polyploid hybrid-group II (Germany, UK) according to data of de Gruyter and Man in't Veld (2000).

Table 1. Fungi isolated from diseased Common alder; number of trees colonised by fungi (a) and number of isolates obtained (b)

Fungal species	Observation period					
	2002.04.25 (6 trees 90 years old)		2002.04.25 (5 trees 15 years old)		2002.06.05 (7 trees 15 years old)	
Alternaria alternata Nees.	6	11	5	11	2	6
Fusarium culmorum (W.G.Sm.) Sacc.	4	10	–	–	–	–
Fusarium oxysporum Schlecht.	2	2	–	–	1	2
Fusarium solani (Mart.) Sny. et Hans.	–	–	2	4	2	4
Mucor hiemalis Wehmer	6	12	–	–	–	–
Mucor spp.	2	4	3	6	7	15
Penicillium spp.	5	12	3	7	3	11
Phytophthora sp.	–	–	4	15	4	8
Trichoderma spp.	4	15	5	18	–	–
Brown, nonsporulating fungi	4	14	–	–	–	–

Colonisation of alder parts or seedlings by fungi. Inoculation of leaves or stem parts by *Fusarium* spp. did not cause any disease symptoms so further trials were only done with *Phytophthora* spp. Inoculation of stem parts of alder with 2 alder *Phytophthora* isolates resulted in the fast spread of necrosis (Tab. 2). Browning and decaying of alder parts spread much faster when stems both 10 and 20 mm diameter were inoculated with isolate PH/0102 (Tab. 2). In the next trial alder shoots used for inoculation with *Phytophthora* isolates from alder and striped maple were taken from the base and top of stems (Tab. 3). After 3-day-incubation there were no

Table 2. Development of necrosis on alder stems inoculated with alder *Phytophthora*; length of necrosis in mm. Inoculation: 2002.09.06

Isolates	Source of isolates	Diam. of stems in mm	Days after inoculation	
			3	5
PH/0102	Poland	10	42.0 a	58.3 a
		20	35.0 a	56.6 a
PD/2001 0932	Holland	10	22.5 a	37.0 a
		20	29.3 a	49.5 a

Note: means in columns followed by the same letter do not differ at 5% of significance (Duncan's multiple range test)

Table 3. Spread of necrosis on alder shoots inoculated with *Phytophthora* spp.; length of necrosis in mm. Inoculation: 2002.08.12

Source of isolates	Stems	Days after inoculation		
		3	5	7
PH/0102 (alder)	Base	14.1 a	36.6 b	61.3 d
	Top	15.3 a	31.0 b	96.5 f
PH/0028(maple)	Base	22.5 a	50.0 c	73.0 e
	Top	22.0 a	38.0 b	101.5 f

Note: see table 2

significant differences in the spread of necrosis. After the next 2 and 4 days, however, necrosis spread significantly faster on stem parts inoculated with isolate PH/0028 from striped maple (Tab. 3). Inoculation of leaf base with both *Phytophthora* isolates resulted in the spread of necrosis on petioles and leaf blades (Tab. 4). The development of necrosis on leaves, however, was not so fast like on stem parts (Tabs. 3, 4). In greenhouse trial, inoculation of stem with 3 *Phytophthora* isolates resulted in the development of disease symptoms already after 2 days. Three days later brown or dark-brown spots reached 26–31 mm (Tab. 5). Eight days after inoculation the significantly longer bark canker was observed on seedlings inoculated with alder *Phytophthora* isolates than PH/0028 from striped maple. The necrosis spread about 0.7 mm/hr (Tab. 5).

Prior to this study *Phytophthora* species have not been recognized as pathogens of Common alder in Poland although *P. cambivora* was found on striped maple (Orlikowski et al. 2002). Our survey showed that among sampled plots alder *Phytophthora* was recorded only in one place but isolation of the pathogen was positive in 2 sampling periods. This indicates that potato-dextrose agar was suitable medium for the isolation of *Phytophthora*. In studies of Streito and Gibbs (1999) satisfactory isolation of *Phytophthora* from diseased bark parts of alder was obtained when corn meal agar or selective medium were used. In opinion of de Gruyter and Man in't Veld (2000), however, cherry decoction medium proved to be the most suitable.

The most striking results obtained from the mycological analysis of declined trees is that the pathogen was not recovered from alders growing along river banks but in woodland ecosystem without possibility of flooding with moving water but seasonally flooded by water from snow or/and rain. A transmission of the pathogen via infected, young plants from nursery, observed by Jung et al. (2000) in Bavaria, was not possible as well because natural regeneration of alders in natural woodland ecosystem. This confirms Gibbs (1995) and Jung et al. (2000) statements that alder

Table 4. Spread of necrosis on alder leaf blades inoculated with *Phytophthora* spp.; diam. of necrosis in mm. Inoculation: 2002.08.12

Source of isolates	Days after inoculation		
	3	5	7
PH/0102 (alder)	12.0 a	20.3 ab	41.8 c
PH/0028 (maple)	13.0 a	24.3 b	36.9 c

Note: see table 2

Table 5. Development of stem rot on alder seedlings inoculated with *Phytophthora* spp.; length of necrosis in mm; greenhouse trial. Inoculation: 2002.09.10

Isolates	Source of isolates	Days after inoculation	
		5	8
PH/0102	Alder (Poland)	31.0 b	37.0 b
PD/2001 0932	Alder (Holland)	26.0 a	35.8 b
PH/0028	Maple (Poland)	26.0 a	21.0 a

Note: see table 2

Phytophthora transmission by moving water is not the only one way of the pathogen dissemination.

Cech (1999) concluded that alder decline is a consequence of phloem destruction at the stem base by alder *Phytophthora*. In our study occurrence of *Armillaria* and *Fusarium* species under diseased bark was also observed. In Streito et al. (2002) studies besides mentioned fungi *Cryptosporiopsis*, *Phoma* and *Cylindrocarpon* species were recovered from declined alder trees. It is possible that in the most majority of surveyed plots, besides of unfavourable climatic conditions (Cech 1999; Streito et al. 2002) those fungi may be the secondary agents of alder mortality.

Studies concerning distribution of alder decline, detection of *Phytophthora* sp., its harmfulness and biology will be continued in the next years. It is already known that alder mortality affects the appearance of landscape and there would be a wide range of biological repercussions (Streito and Gibbs 1999).

ACNOWLEDGEMENTS

We would like to thank H. de Gruyter for his valuable support during taxonomical studies of alder *Phytophthora* and W. Man in't Veld (both from Plant Protection Service in the Netherlands) for their guidance with isozyme methods and confirmation of our analysis. Additionally, we are grateful to the Mycology Section of Plant Prot. Service for kindly providing of *Phytophthora* reference isolates used in our taxonomical and isozyme studies. Thanks are expressed to Z. Sierota for reviewing the manuscript and his valuable remarks.

REFERENCES

Brasier C.M., Rose J., Gibbs J.N. 1995. An unusual *Phytophthora* associated with widespread alder mortality in Britain. Plant Pathology 44: 999–1007.

Cech Th.L. 1999. Alder decline in Austria. Disease? Environment Interactions in Forest Decline. Proc. of Workshop of the Working Party. Vienna, 1998.03.16–21: 16–21.

De Gruyter J., Man in't Veld W.A. 2000: *Phytophthora* disease of alder. Diagnostic Activities, Plant Protection Service, Annual Report, Wageningen: 85–94.

Erwin D.C., Ribeiro O.K. 1996. *Phytophthora* disease worldwide. APS Press, St Paul, Minnesota: 258–261.

Gams W., van der Aa H.A., van der Plaats-Niterink A.J., Samson R.A., Staplers J. A. 1987. CBA Course of Mycology, CBS Institute, Baarn.

Gibbs J.N. 1995. *Phytophthora* root disease of alder in Britain. Bull. OEPP/EPPO 25: 661–664.

Jung T., Schlenzig A., Blaschke M., Adolf B., Osswald W. 2000. Droht Bayerns Erlen eine Epidemie? LWF aktuell 24: 22–25.

Man in't Veld W.A. 1995. *Phytophthora* taxonomy – progress in isozyme analysis. Diagnostic Centre of Plant Protection Service, Wageningen. Ann. Report 1995: 90–97.

Man in't Veld W.A., Veenbaas-Rijks W.J., Iljeva E., de Cock A.W.A.M., Bonants P.J.M., Pieters R. 1998. Natural hybrids of *Phytophthora nicotianae* and *Phytophthora cactorum* demonstrated by isozyme analysis and random amplified polymorphic DNA. Phytopathology 88 (9): 923–929.

Man in't Veld W.A., de Cock A.W.A.M., Ilieva E., Levesque C.A. 2002. Gen flow analysis of *Phytophthora* porri reveals a new species *Phytophthora brassicae* sp. nov. Eur. J. Plant Pathol., 108: 51–62.

Orlikowski L.B., Jaworska-Marosz A., Szkuta G. 2002. Maple stem rot induced by *Phytophthora cambivora*. Phytopathol. Pol., 24: 17–26.

Orlikowski L.B., Skrzypczak Cz., Szkuta G. 2001. Occurrence of *Phytophthora* root and foot rot of dieffenbachia, peperomia and radermachera in Polish greenhouses. Phytopathol. Pol., 21: 109–117.

Oudemans P., Coffey M.D. 1991. Isozyme comparison within and among worldwide sources of three morphologically distinct species of *Phytophthora*. Mycol. Res., 95: 19–30.

Streito J.C., Gibbs J.N. 1999. Alder *Phytophthora* in France and the United Kingdom: symptoms, isolation methods, distribution and damage. First Int. Meeting on Phytophthoras in Forest and Wildland Ecosystem. *Phytophthora* Diseases of Forest Trees, Grant Pass, Oregon: 37–39.

Streito J.C., Legrand Ph., Tabary F., Jarnouen de Villartay G. 2002. *Phytophthora* disease of alder (*Alnus glutinosa*) in France: investigation between 1995 an 1999. Forest Path., 32: 179–191.

Stamps D.J., Waterhouse G.M., Newhook F.J., Hall G.S. 1990. Revised tabular key to the species of *Phytophthora*. Mycol. Paper 162, IMI , Kew, 28 pp.

POLISH SUMMARY

PIERWSZE DONIESIENIE O *PHYTOPHTHORA* SP. NA OLSZY W POLSCE: WYSTĘPOWANIE I CHOROBOTWÓRCZOŚĆ

W minionych 6 latach na olszach rosnących w lasach, wzdłuż brzegów rzek i strumieni, nad jeziorami i innymi zbiornikami wodnymi obserwowano symptomy zahamowania wzrostu, przejaśnienia liści, brązowienia i brunatnienia kory u nasady pnia, ciemne plamy z czarną wydzieliną cieczy oraz zamieranie drzew. Obserwacjami objęto 14 miejsc w Polsce środkowej i wschodniej, w których przynajmniej jednorazowo pobierano do badań mykologicznych próby porażonych tkanek z podstawy pnia drzew w wieku od 5 do 90 lat. Tylko w jednym miejscu centralnej Polski stwierdzono występowanie fytoftorozy, a przyczynę choroby Brasier i współ. (1995) określili jako „alder *Phytophthora*". Zakażenie części łodyg i liści oraz siewek olszy przez izolaty *Phytophthora* spp. powodowało ich brązowienie, brunatnienie i zamieranie. U nasady pędów siewek olszy, zakażonych przez „alder *Phytophthora*", nekroza rozwijała się z szybkością 0,7 mm na godzinę.