Phytophthora Alder Decline: Disease Symptoms, Causal Agent and Its Distribution in the Czech Republic

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Abstract

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Phytophthora decline of riparian alder populations has recently become an important problem in many European countries, including the Czech Republic. The causal agent, *Phytophthora alni*, has spread quickly in the Czech Republic. Hundreds of kilometres of riparian alder stands, especially in the western part of the country, have been severely affected to date. Diseased trees show symptoms characteristic of *Phytophthora* root and collar rot; these include small, sparse and yellowing foliage, crown dieback, presence of exudates on the bark and necroses of collar and root tissues. Infected trees usually die within a few years, or they become irreversibly damaged, and their function in bank reinforcement declines. The ecological and mechanical functioning of severely affected alder stands may be seriously disrupted.

Keywords: *Phytophthora alni*; *Alnus glutinosa*; *Alnus incana*; common alder; grey alder; alder decline; bleeding canker; Czech Republic

Unknown before 1993, lethal root and collar rot of common alder (*Alnus glutinosa*) was first recorded in southern Britain. This disease, also known as *Phytophthora* alder decline or bleeding canker, occurred mainly along riverbanks and locally in orchards, shelterbelts and woodland plantations (Gibbs 1995). In subsequent years, the disease was also found in grey alder (*A. incana*) and Italian alder (*A. cordata*) and in Germany, France, Ireland, the Netherlands, Sweden, Poland, Hungary, Italy, and other European countries (Jung & Blaschke 2006; Érsek & Nagy 2008). The cause of the disease was identified as a novel *Phytophthora*, initially referred to as "alder-*Phytophthora*", a species hybrid (Brasier *et al*. 1995, 1999). Later it was described as *Phytophthora alni* Brasier et S. A. Kirk comprising a range of phenotypically diverse allopolyploid genotypes (Brasier *et al*. 2004). Therefore *P. alni* was split into three subspecies, *P. alni* ssp. *alni*, *P. alni* ssp. *uniformis*, and *P. alni* ssp. *multiformis*. In the Czech Republic, the pathogen was first isolated from damaged common alder trees in western Bohemia in 2001 (Černý *et al*. 2003). Six years later, the pathogen was identified in about 60 alder stands, mostly in the western part of the country (Cerny *et al.* 2008). To date, the pathogen has caused considerable losses of alder trees along hundreds of kilometres of riverbanks and has spread beyond control.

MATERIAL AND METHODS

Information about the distribution of phytophthora alder decline in the Czech Republic has been collected since 2000 in the course of our field sur-

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veys. Also, data were acquired via cooperation with many agencies concerned with the management, protection and preservation of riparian, forest and other alder stands, including the Vltava, Ohře and Labe River Authorities, State Phytosanitary Administration, Agency for Nature Conservation and Landscape Protection of the Czech Republic, Agricultural Water Management Authority, Forestry and Game Management Research Institute, Forests of the Czech Republic, Czech Union for Nature Conservation, etc. The declining alder stands were surveyed for symptoms of bleeding canker comprising the presence of exudates in bark cracks and characteristic lesions on stems and collars. When these symptoms were observed, active orange-brown to honey or red coloured lesions of the subcortical tissues were identified. Bark from the apical part of these lesions was removed to uncover the subcortical tissues. Samples (100–200 cm^2) of tissues (including cambium) were stripped out of the wood using a wood chisel and placed into sterile polyethylene bags. Several symptomatic trees were sampled in each locality. The samples were kept in a dark, cool place or box and processed immediately.

In the laboratory, the tissue samples were carefully cleaned off the bark remnants and thoroughly washed in running tap water. Next, several dozen segments (ca 5 × 5 × 5 mm) of each sample were cut, repeatedly washed in sterile water and 95% ethanol, blotted on sterile filter paper or pulp. Samples were then plated onto selective PARPNH V8 agar consisting of 100 ml V8 juice (Campbell Grocery Products Ltd., Norfolk, England), 3 g $CaCO_3$, 200 mg ampicillin, 10 mg rifampicin, 25 mg pentachloronitrobenzene (PCNB), 50 mg nystatin and 50 mg hymexazol per litre of deionised water. Ten segments were cultivated per agar plate; the plates were incubated in the dark, at 20°C. After three to five days, the plates were examined for the presence of *Phytophthora*-like hyphae and colonies. Single hyphae from the margins of growing colonies were transferred onto V8-juice agar (V8A) plates. For oogonium production carrot agar (CA) consisting of 15 g agar and 50 g carrot per litre of deionised water was plated and used. Sporangium formation was induced by incubating infested agar segments in soil extract or filtered pond water (Erwin & Ribeiro 1996). Cultural characteristics were observed on V8A and CA plates after seven to ten days of cultivation in the dark at 20°C. The acquired isolates have been preserved in tubes, on oat meal agar (Erwin & Ribeiro 1996) under mineral oil at ca. 12°C.

RESULTS AND DISCUSSION

Symptoms of disease. The symptoms characteristic of *Phytophthora* bleeding canker and decline observed in the afore-mentioned alder stands are consistent with those observed in other countries (e.g. Gibbs *et al.* 1999; Szabó *et al.* 2000; Streito *et al.* 2002; Nagy *et al.* 2003; Jung & Blaschke 2004).

The pathogen usually infects the host through the roots and collar and causes root rot and collar and stem necroses (Figure 1A). Rotting of adventitious roots growing near the soil surface beneath the necrotised areas is often seen. Tarry or rusty spots of exudates on the surface of the bark or in its cracks and fissures (Figure 1B) are characteristic. Necroses of subcortical tissues are orange or honey to red coloured and flame- or tongue-shaped (Figure 1C) and sometimes reach several metres in length. During the period of maximum pathogen activity, i.e. summer and early autumn, lesions on the cambium may increase in length by ca 3–7 mm in one day. The annual growth of stem necroses often reaches dozens of centimetres. Roots and collars of affected trees are usually damaged to a great extent (Figure 1D); the role of these trees in bank reinforcement quickly declines.

Diseased trees characteristically have abnormally small, yellowing and sparse foliage, early and often excessive fruiting with small cones and crown dieback (Figure 1E). The crowns in advanced stages of the disease are substantially withered.

The focal pattern of decline is characteristic of later stages of this disease (Figure 1F). If the infected alder stand was previously stressed by floods, this pattern is not clearly visible. Young trees and saplings often die during one season. Severely affected mature trees usually die within a few years, or they become irreversibly damaged. Major damage or disruption to infected alder stands has occurred in many cases in the Czech Republic (Figure 1G).

Morphological changes in the shape of bank slopes and streambeds (especially in small watercourses with high fluctuation of flow rate) can occur as a consequence of disrupted or non-functioning root systems. Among others, this can lead to the development of lateral pools and changes in flow rate (Figure 1H).

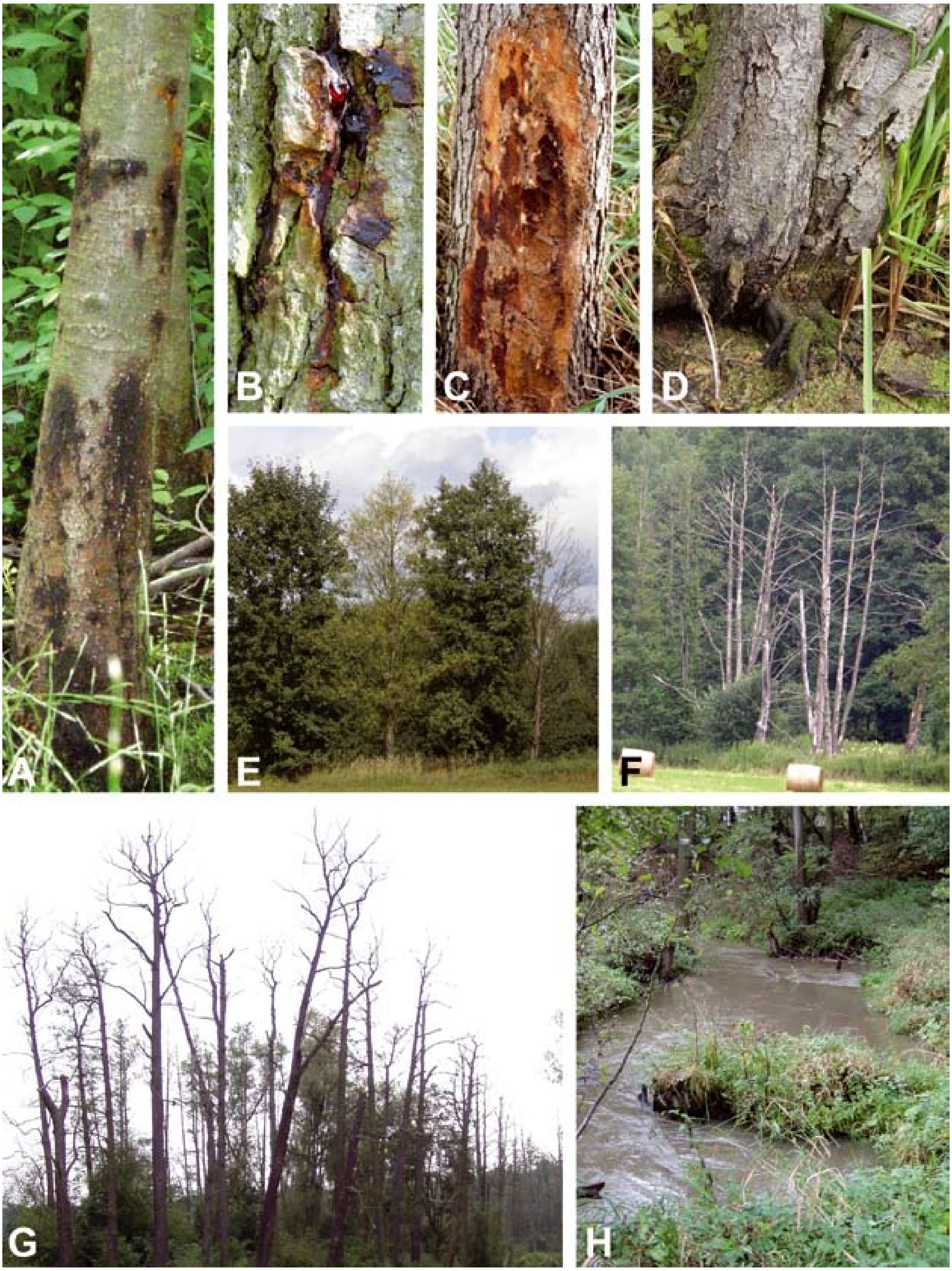

Figure 1. *Phytophthora* decline of common alder. (A) stem of young alder with characteristic symptoms of disease (bleeding canker); (B) tarry and rusty exudate in bark cracks, detail; (C) tongue-shaped red necrosis of subcortical tissues on stem base; (D) rotten roots and collars of severely affected trees; (E) yellowing and crown dieback in diseased young trees; (F) focal pattern of disease; (G) disruption of severely affected carr; (H) morphological changes in banks following the disruption of bankside alder stand

Pathogen description. In morphological studies ten selected isolates were examined. In a previous study, DNA sequences of the ITS region of two of these isolates (GenBank accession numbers EF194776 and EF194777) were compared with sequences available from the GenBank (Cerny *et al*. 2008). One isolate has been deposited in the Culture Collection of Fungi, Prague, under No. 3682. Both approaches confirmed that the corresponding isolates were identical with *P. alni*. Colonies growing on CA and V8A are usually uniform and appressed with sparse aerial mycelium (Figures 2A and 2B). Radial growth is 7–9 mm per day at 20°C on CA. The optimal growth temperature is 23–25°C. Strains are homothallic with usually two-celled (22–31×12–20 µm) amphigynous antheridia, producing abundant terminal, spherical oogonia (28–55 µm in diameter) often with moderately ornamented walls (Figure 2C). Many oospores are aborted (Figure 2D); the rate of abortion often reaches 20–60%. Sporangiophores are simple or sympodial with terminal sporangia proliferating internally, often nested, ellipsoid or ovoid in shape, measuring 38–65 × 25–41 µm (length:width ratio 1.4–1.6) and have a minute papilla or they are non-papillate (Figure 2E). On the whole, these morphological features correspond to those of *P. alni* subsp. *alni* (previously known as the standard hybrid type).

More than 130 isolates from throughout the area of the Czech Republic known to be inhabited by the pathogen have been deposited in the culture collection of Silva Tarouca Research Institute for Landscape and Ornamental Gardening and have been included in a detailed morphological study (unpublished data). Based on preliminary examination, most of these isolates belong to *P. alni* subsp. *alni*, which generally corresponds to the situation in Europe (Brasier *et al*. 2004). However, further studies, including molecular ones, are required for obtaining detailed data on the subspecies composition of *P. alni* populations in the country.

Pathogen and disease distribution in the Czech Republic. *Phytophthora alni*, the pathogen that plays a key role in the decline of common and grey alders, was first isolated in northwestern Bohemia in the Czech Republic in 2001 (Černý *et al*. 2003).

However, the decline of alder trees was previously reported in southern Bohemia during the 1980s and 1990s (Jančařík 1993; Struková *et al*. 1996), but its cause was not satisfactorily determined. Circumstantial evidence indicates a possibility that this earlier decline was connected with *P. alni*. First, when we developed efficient isolation techniques in 2005, we found that the entire Lužnice River basin was strongly affected by *P. alni* and that tens of thousands alder trees were apparently affected or killed by this pathogen. Second, mature trees that survived the previously reported decline in the studied sites (Jančařík 1993; Struková *et al*. 1996) showed the same type of flame-shaped lesions characteristic of *P. alni* disease, and these lesions originated about ten to fifteen years ago. The foliage and crowns of the trees resembled those of trees affected by *P. alni*. Of course, the remaining trees and those subsequently planted (now about fifteen years old) are currently affected by the pathogen as well. This situation can be found in several localities in southern Bohemia; the most typical is a bankside alder stand along the Nežárka River at Kruplov, near Jarošov nad Nežárkou (Jindřichův Hradec district, southern Bohemia; coordinates 49°10'59.01''N and 15°3'59.2'').

Alder decline possibly linked with *P. alni* infection was reported before 2000 from several regions in the Czech Republic by workers from three local management agencies (Ohře, Labe and Vltava River Authorities). Our first field investigation, carried out in 2001, confirmed the presence of the disease and characteristic symptoms of bleeding canker in alder stands along the Lužnice, Nežárka (southern Bohemia), Berounka, Jizera (central Bohemia), Radbuza, Úslava, Ohře (western Bohemia), Ploučnice (northern Bohemia) and Tichá Orlice and Labe (eastern Bohemia) Rivers. Since bleeding canker of alders has been known from central Europe since the mid-1980s in Austria and Bavaria (Cech 1997; Jung & Blaschke 2004) and the presence of *P. alni* has been known in adjacent regions in Germany and Austria since the 1990s (Hartmann 1995; Cech 1997), it can be assumed that the disease was present in several areas of the Czech Republic before 2000. This conclusion is very likely, although direct evidence is missing.

In 2006, the pathogen was known to be present in about 60 sites and to be spreading rapidly, particularly in the western part of the Czech Republic (Cerny *et al*. 2008). The loss of trees in affected areas has increased enormously: tens of thousands of alder trees have disappeared to date in the area of the Upper Vltava River Authority alone. At present, the pathogen has been confirmed in about 150 locations throughout the Czech Republic, and

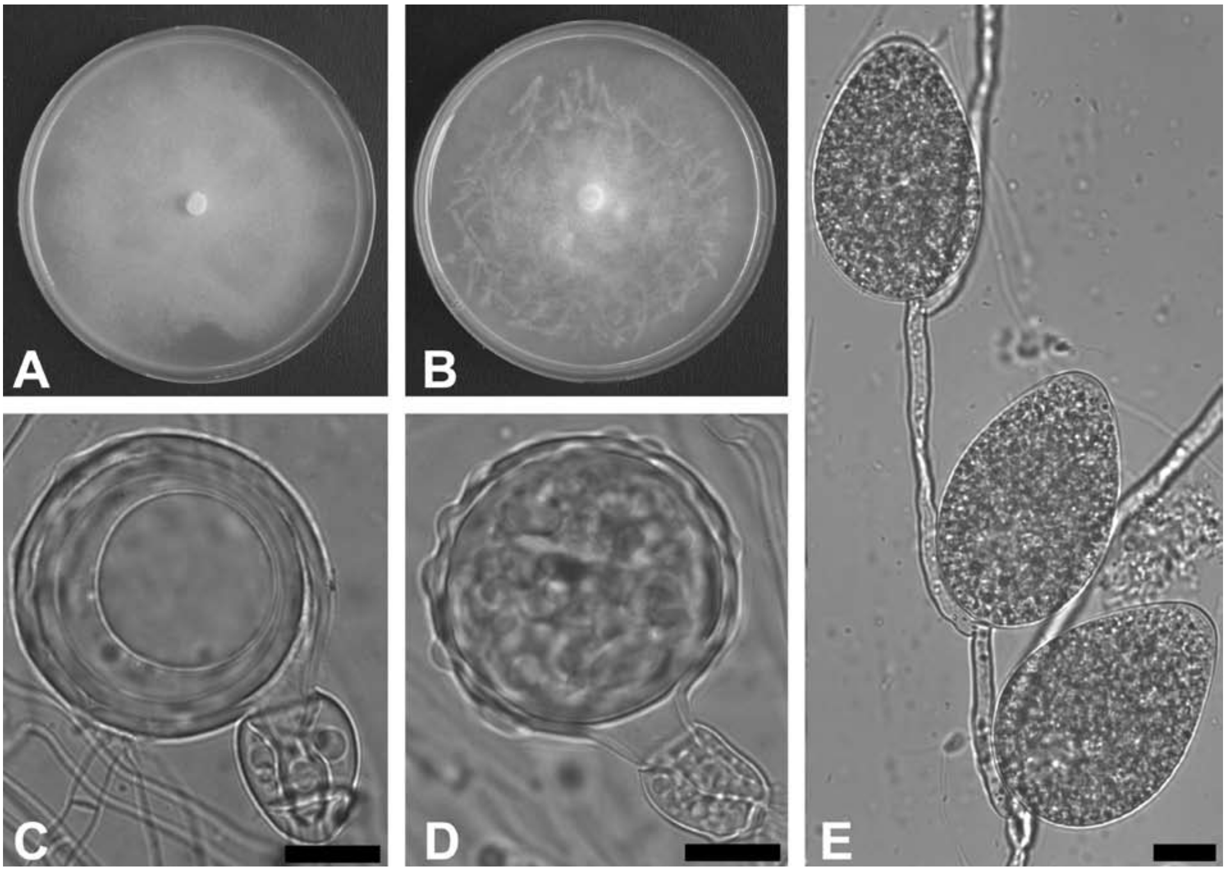

Figure 2. *Phytophthora alni*. (A) colony on V8-juice agar plate after one week in the dark at 20°C; (B) colony on carrot agar plate after one week in the dark at 20°C; (C) two-celled amphigynous antheridium and oogonium with mature oospore; (D) two-celled antheridium with basal septum and oogonium with aborted oospore (note the characteristically ornamented oogonial wall); (F) sympodial sporangiophore with ovoid sporangia

characteristic alder decline has been found in more than 300 sites. Along several watercourses (e.g. the Sázava and some its tributaries, the Lužnice and its tributaries, the Otava and some of its tributaries, the Berounka and its tributaries, and the Ohře, Ploučnice, Jizera, Doubrava, Jihlava and Moravská Dyje Rivers), bankside alder stands are affected nearly continuously along the length of dozen kilometres, and the pathogen probably occurs in thousands of kilometres of river banks in the Czech Republic at present. The disease has also been found in river systems that are connected with watercourses in eastern Bavaria (Jung & Blaschke 2004) and northern Austria (Cech 2001).

The exact geographic distribution of the pathogen in the Czech Republic remains partially unknown, and it should be noted that only the western part of the area has been relatively well surveyed to date, primarily in the Vltava River basin (especially in southern, western and central Bohemia). Major damage to alder stands has been observed along rivers including the Sázava, Želivka, Hejlovka, Vlašimská Blanice, Mastník, Smutná, Židova Strouha, Vltava, Žirovnice, Nežárka, Lužnice, Dračice, Zlatá Stoka, Stropnice, Malše, Blanice, Volyňka, Otava, Lomnice, Skalice, Úhlava, Úslava, Radbuza, Zubřina, Mže, Berounka, Střela and Litavka. The pathogen frequently occurs in alder stands along smaller watercourses and ponds as well.

To date, we have less information about the distribution of the pathogen in other river basins. However, it can be certainly stated that the disease is frequent in several watercourses in the Ohře River basin (e.g. the Ohře, Teplá, Blšanka, Bílina, Liboc, Svatava, Ploučnice and Svitávka Rivers and the Lobezský, Chodovský and Panenský potok brooks), in the Labe River basin (e.g. the Labe, Jizera, Cidlina, Divoká Orlice and Doubrava Rivers) and locally in the Morava River basin (e.g. the Jihlava, Moravská Dyje, Třebůvka, Oslava, Svratka and Svitava Rivers). We have not received any reports of the disease in the Odra River basin yet. Extensive decline of alder trees has not been detected in the eastern part of the country (Moravia) yet, but the pathogen and declining alder stands have been frequently found there along some watercourses, especially in the Jihlava River basin. It should be emphasized that the pathogen is rapidly spreading to the east.

The distribution of the pathogen and of the associated disease in the Czech Republic is mainly connected with the distribution of common and grey alders in bankside stands, mixed ash-and-alder stands along small and medium watercourses, alder stands in periodically flooded alluvial plains, alder carrs, etc. The disease has been found in forest plantations only in two cases. We have not found the pathogen in forest nurseries yet. The distribution of this disease partially resembles the situation in Great Britain, where the pathogen and disease are restricted to riverside banks for the most part (Gibbs *et al.* 1999, 2003). In Bavaria, on the other hand, the disease regularly occurs in forest alder stands as well as in riparian sites (Jung & Blaschke 2004). This important difference may be caused, at least in part, by different modes of the pathogen spread in these territories. In Great Britain, the primary mode of the pathogen dispersal is along watercourses (Gibbs *et al.* 1999) whereas in Bavaria the pathogen has also been introduced into many stands, especially forest plantations, by infected nursery stock (Jung & Blaschke 2004). The preliminary outcomes of our small-scale investigation of the pathogen spread in the Česká Sibiř area support the role of ponds with more or less intensive fish farming as primary inoculum sources in the landscape, but more field data are still needed. Among 23 primary sources of inoculum in the landscape studied (we have screened ca. 250 km^2 to date), 22 are ponds with more or less intensive fish farming, and only one case has been identified as a young grey alder carr. However, it is probably only a matter of time until the pathogen is introduced into forest nurseries, probably by irrigation water, and then by infected saplings into forests and other stands like in Bavaria (Jung & Blaschke 2004).

The worsening situation in the Spreewald biosphere reserve (Schumacher *et al.* 2006) indicates that the Czech alder carr ecosystem may also be endangered by the pathogen invasion, particularly in the Třeboňsko biosphere reserve (southern Bohemia), where the pathogen regularly occurs.

CONCLUSIONS

Phytophthora alder decline has become one of the most devastating epidemics in any common tree species since Dutch elm disease emerged in the Czech Republic. The pathogen has become a common component of the alder stand ecosystem throughout much of the Czech Republic. To date, we have detected the pathogen and the disease in about 150 and 300 sites, respectively, throughout the Czech Republic. The severity of the disease and its impact on riparian alder stands in the western part of the Czech Republic is comparable to the situation in Great Britain, northeastern France and Bavaria. The impact and rapid uncontrolled spread of the pathogen should provoke the concern of river and forest authorities, local administrations and other agencies dealing with nature conservation.

Recorded occurrences of the pathogen and associated disease in the Czech Republic are clearly restricted to bankside alder stands, alder carrs, mixed ash-and-alder stands along small and medium watercourses, alder stands in periodically flooded alluvial plains, etc. The occurrence of the disease in forest plantations seems to be very exceptional and no disease has been found in forest nurseries yet. Watercourses are probably the primary routes of infection, but fish farming ponds as such may also be important. The introduction of the pathogen into forests by infected alder saplings from nurseries has not been detected in the country yet, but this mode of introduction may take place in near future.

Determination of the full distribution of the pathogen, its role in the riverside alder stand ecosystem, its modes of introduction, and other characteristics will require more field data. Further investigation of alder decline is ongoing.

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