The effects of flooding and *Phytophthora alni* infection on black alder

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**ABSTRACT**: The influences of long-term flooding and *Phytophthora alni* subsp. *alni* infection on the growth and development of 4-year-old *Alnus glutinosa* (black alder) saplings were investigated. The black alder saplings were divided into four groups and then subjected to combinations of both factors – flooded and inoculated with pathogen, flooded non-inoculated, non-flooded inoculated, and control. The biomass of the living roots and actinorrhiza, increase in stem length, length of leaves, rate of chlorotic foliage, amount of foliage biomass and length of stem necrosis were assessed after seven weeks. Both factors, flooding and *P. alni* infection significantly affected the black alder. In addition, a significant effect of interaction was observed. The inoculated flooded group had a substantially lower biomass weight of living roots, actinorrhiza and leaves than the other groups. The necroses caused by the pathogen in the flooded group were more extensive than those in the non-flooded one. These findings demonstrate that the simultaneous incidence of stress caused by flooding and *P. alni* infection is highly dangerous for black alder.

**Keywords**: alder decline; *Alnus glutinosa*; flooding; *Phytophthora alni*

In August 2002, the west part of the Czech Republic was afflicted with flooding that exerted stress on hundreds of kilometres of riparian alder stands in several catchments, especially in western, middle and southern Bohemia. The flooding or total water saturation of soil lasted for several weeks or months in many of the affected areas. In the years following the floods, the extended alder population appeared to decline in many of the affected riparian stands, which had been healthy prior to the floods (STRNADOVÁ et al. 2006). Therefore, it was likely that this decline was connected to the flooding that occurred in 2002 (VYHLÍDKOVÁ et al. 2005) because the increased water level and flooding could damage the alders and induce morphological changes as seen by McVEAN (1956). The dangerous pathogen of alders *Phytophthora alni* has been spreading rapidly in alder stands, particularly in the western part of the Czech Republic in recent years, and has leading to significant losses in highly affected stands (ČERNÝ et al. 2008). We felt it important to distinguish which of these factors was the real cause of the decline. Extensive field studies have taken place over the last few years (2003–2009) in the Czech Republic. While they are still in progress, one preliminary study on this topic has been published so far (STRNADOVÁ et al. 2006). This study showed that both factors could contribute to the alder decline in the investigated stands because the incidence of *P. alni* symptoms as well as an increased water level and extent of flooded area in August 2002 (STRNADOVÁ et al. 2006) were significantly correlated with the damage to alder stands.

Ground water table fluctuation and long-term waterlogging could be primary abiotic causes of damage to several tree species (KOZŁOWSKI 1997). Flooding...
alpers the soil structure, depletes oxygen and leads to the accumulation of carbon dioxide. This, in turn, induces anaerobic conditions, which inhibit growth and lead to the decay of the root system (Kozlowski 1997). The changes in several tree species in response to flooding were summarized by Kozlowski (1997), in black alders by McVean (1956) and in speckled alders by Ohmann et al. (1990). Black alders subjected to flooding produced a considerable amount of adventitious roots and hyprophotomed lenticels, and created new roots near the soil surface. The flooding led to a decrease in the number of nodules in deeper layers, the death of deep roots, stunting growth, the death of some branches and, in some cases, the death of alder seedlings or trees (McVean 1956).

The mechanism by which flooding induced the current decline in black alders in the Czech Republic was described by Vyhlídková et al. (2005), who stated that the alder decline along the Lužnice River (southern Bohemia) was induced by the mechanical effects of flooding, depletion of soil oxygen and invasion by microorganisms of the weakened alders (polymicrobial decline). The preliminary outcomes of a multidimensional analysis showed that floods could play an important role in the decline of alders along the Lomnice River in southern Bohemia (Strnadová et al. 2006).

The pathogen that had a key role in the decline of the black alder, *Phytophthora alni*, was first isolated in northwest Bohemia in the Czech Republic in 2001. Since then, the pathogen has been isolated from about 60 alder stands and continues to spread rapidly, particularly in the western part of the Czech Republic (Cerný et al. 2008). The disease has also been found in several river systems, some of which are connected to watercourses in eastern Bavaria (Jung, Blaschke 2004) and northern Austria (Cech 2001).

During flooding, *P. alni* zoospores spread from naturally infected bark and infect other trees (Streit et al. 2002). It is known that natural infestations of alder trees by *P. alni* occur during floods (Jung, Blaschke 2004), and greater disease incidences have been described in areas that hold flood water for a long period of time (Gibbs et al. 1999, 2003; Streito et al. 2002; Jung, Blaschke 2004; Schumacher et al. 2006; Thoirain et al. 2007). Schumacher et al. (2006) found that flooding during the growing season causes the highest risk of infection. The anaerobic conditions in flooded soil could inhibit growth and lead to decay of the root system (Kozlowski 1997).

This study was conducted to determine whether *P. alni* had a greater affect on the alders that were stressed by flooding and to describe some of changes that occur in alders after being subjected to flooding, *P. alni* infection and a combination of both factors.

**MATERIAL AND METHODS**

**Infection experiment**

Four-year-old black alder plants (*Alnus glutinosa*) were used for the inoculation experiment. Eighty plants with well-developed actinorrhiza were potted in 18 × 18 × 18 cm plastic containers that were filled with sterile peat substrate (pH 5). Several months later, when the plants took root readily, they were randomly divided into four groups of 20 plants each. The first group of plants (the first treatment) was artificially infected with *P. alni* subsp. *alni* and then flooded up to the soil surface with filtered pond water without *Phytophthora* infection; this stable water level was maintained for the duration of the experiment. The second group (the second treatment) was flooded in the same manner as the first group but was not inoculated (non-inoculated). The third group (the third treatment) was inoculated but not flooded (non-flooded). The fourth group (the fourth treatment) was a control (non-flooded, non-inoculated). The experiment was conducted for seven weeks in May and June 2005 in a greenhouse. The temperature was maintained at 20–30°C in day/night temperature regime, and the air humidity was varied from 40 to 60%. The plants were controlled and watered with filtered pond water as needed to prevent the substrate from drying. The used pond water contained a relatively low oxygen concentration (< 4 mg.l⁻¹) to simulate the situation in flooded stands. It was filtered through the sand filter during the experiment. The catchment area of the tributary to the pond was free of disease caused by *P. alni*.

*Phytophthora alni* subsp. *alni* isolated from the bleeding canker of a black alder tree growing in a stand highly affected by the disease (Velký Pěčín, district Jindřichův Hradec, southern Bohemia, geographical coordinates 49°6'38"N and 15°26'49"E) was used for the inoculation. The microscopic and cultural characteristics of the isolate used here were identical to those of *P. alni* subsp. *alni* (Brasier et al. 2004). In addition, its colonies were uniform on carrot agar and V8 juice agar (Erwin, Ribeiro 1996) without any chimaeric zones. The optimal growth temperature was 24°C, and it produced oogonia that were moderately ornamented. The abortion of oogonia reached 50–70%. A comparison of the rDNA sequence of the ITS region of the isolate with
Table 1. The effect of long-term flooding and Phytophthora alni infection on the development of black alder saplings

<table>
<thead>
<tr>
<th>Treatment per valid N</th>
<th>Length of necrosis (cm)</th>
<th>Root biomass (g)</th>
<th>Actinorrhiza biomass (g)</th>
<th>Height growth (cm)</th>
<th>Leaf length (cm)</th>
<th>Leaf biomass (g)</th>
<th>Chlorotic foliage</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-P-/20</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.65 ± 0.78&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.61 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>40.65 ± 2.55&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.12 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.50 ± 0.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F-P+/19</td>
<td>10.62 ± 1.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.41 ± 0.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.55 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.32 ± 2.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.17 ± 0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.68 ± 1.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.89 ± 0.21&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>F+P-/20</td>
<td>0.00 ± 0.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.36 ± 0.66&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.55 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>22.75 ± 2.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.89 ± 0.18&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.46 ± 0.70&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.75 ± 0.22&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>F+P+/20</td>
<td>17.86 ± 2.52&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.85 ± 0.60&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.09 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>21.15 ± 2.34&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.51 ± 0.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.60 ± 1.11&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.25 ± 0.19&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

F-P- treatment: non-flooded and non-inoculated plants (control group); F-P+ treatment: non-flooded, inoculated plants; F+P- treatment: flooded, non-inoculated plants; F+P+ treatment: flooded and inoculated plants. Values (mean and standard error) followed by the same letter are not significantly different (P > 0.05). The degree of chlorotic foliage (8<sup>th</sup> column) rating on a scale 0–4 according to percentage (0 = 0–10%, 1 = 11–25%, 2 = 26–50%, 3 = 51–75%, 4 = 75–100%). All results are presented as means ± standard errors.
analyzed with use of a unilateral t-test. The rate of chlorotic foliage was analyzed using a Kruskal-Wallis test followed by Tukey’s post-hoc test for unequal n (Spjtvoll-Stoline test).

RESULTS AND DISCUSSION

Confirmation of flooding and P. alni infection effects on black alder

The analysis of covariance confirmed that the both factors (flooding and P. alni infection) and their combination significantly influenced (P < 0.05) the characteristics of alder plants that were evaluated in this study. The assumptions of normality and homogeneity were fulfilled (P > 0.05).

Flooding had a significant effect (P < 0.05) on root and actinorrhiza biomass, height growth and foliar length and biomass. Flooding had the most important effect on root biomass (F = 36.00, P < 0.001).

The Phytophthora alni infection had a significant effect (P < 0.05) on root and actinorrhiza biomass and foliar length and biomass. Infection had the most prominent effect on actinorrhiza biomass (F = 32.76, P < 0.001).

The interaction of both factors (flooding and P. alni) significantly affected (P < 0.05) root and actinorrhiza biomass, height growth and foliar biomass, the most prominent effect was identified in reduced root biomass (F = 35.08, P < 0.001).

The effect of covariate (plant height) was identified (P < 0.05) in root and actinorrhiza biomass, stem increase and foliar biomass.

General differences in morphology among treatments

The plants subjected to flooding, artificial P. alni infection and the combination showed many morphological changes when compared to the control group. These differences include yellowing, the presence of small, sparse foliage in the crown, height growth, secondary stem base thickening, hypertrophy of lenticels on the stems, development of adventitious roots and necrosis development. In addition, the distribution and amount of root and actinorrhiza biomass differed between treatment plants and control plants.

The plants in the flooded treatment were characterized by the presence of yellowing, small and sparse foliage. The collars and basal portions of the stems were thickened, apparently as a result of the formation of a higher proportion of aerenchyma tissues. The lenticels on the collars, bases of stems and roots growing on the surface of the substrate were hypertrophied. A greater amount of adventitious roots on the collars were produced, and the root biomass was developed primarily near the soil surface. The actinorrhizal nodules were often found on the roots near the soil surface or on the collars. These symptoms resemble those that have been generally described for trees subjected to flooding (McVean 1956; Kozlowski 1997).

The infected treatment showed symptoms characteristic of bleeding cankers and black alder decline, including the presence of small, yellowing and sparse foliage and bleeding cankers on the stems and collars (Jung, Blaschke 2004). The rot of roots growing near the soil surface caused by the pathogen was noted in several cases. Adventitious roots developed on collars of many inoculated plants. All plants infected with P. alni showed symptoms characteristic of bleeding cankers and black alder decline, with the exception of one plant that did not develop any cankers. In that plant, bacterial colonization was observed at the inoculation point. It is possible that bacterial antagonism prevented infection by P. alni. The stem necroses varied in length considerably.

The symptoms of the combined treatment included factors found in both the flooded treatment and the infected treatment. These symptoms include the presence of yellowing, small and sparse foliage. Secondary thickening of the stem was observed on some plants, at least partially, in non-infested areas. The lenticels on the collars, bases of stems and roots growing on the soil surface of many plants were hypertrophied. Adventitious roots developed on the plants, although they were often killed by the pathogen invading from the necroses of the main stems. The biomass of the roots and actinorrhizal nodules was predominantly localized near the soil surface as a response to flooding. These surface roots, however, can be probably more easily colonized and killed by P. alni than the deeper ones, which is in agreement with observation of Jung and Blaschke (2004).

Differences among treatments in detail

When the development of P. alni infection in the flooded and non-flooded condition was compared, the length of the stem necroses in the flooded treatment was found to be 17.9 cm after seven weeks, which was significantly longer (P < 0.05) than that of the non-flooded treatment (10.6 cm). The length of necroses varied greatly in both treatments, however (Table 1). This variation is similar to those of other studies conducted on black alder saplings and excised logs (Brasier, Kirk 2001; Lonsdale 2003;
causes more significant

ences observed among these three treatments were

treatment was not significantly different from that of the com-
all cases). The stem length of the flooded treatment
stress factors and by their combination ().
consequence of extending stem necroses.
surface roots are colonized and killed by
root system dies as a result of hypoxia and that the
oomycetous infection on deep roots after a few days
environment for the development of a substantial
was subjected to high acidity and anaerobic reduc-
large roots and extends toward the root collar; the
infected and regenerated alders the infection usu-
al ready starts at the collar or at the surface of exposed
large roots and extends toward the root collar; the
distal part of root system remains healthy. Moreover,
in our experiment, flooded inoculated treatment
was subjected to high acidity and anaerobic reduc-
conditions that probably created an unsuitable
vironment for the development of a substantial
omycetous infection on deep roots after a few days
(ERWIN, RIBEIRO 1996; SCHUMACHER et al. 2006).
These results can indicate that the lower part of the
root system dies as a result of hypoxia and that the
surface roots are colonized and killed by P. ALNI as a
consequence of extending stem necroses.
The stem length was reduced by 25 to 50% by both
stress factors and by their combination (P < 0.001 in
all cases). The stem length of the flooded treatment
was not significantly different from that of the com-
bined one (Table 1).
The length of the leaves was significantly reduced
in the inoculated treatment (P < 0.05), flooded
-treatment (P < 0.01) and the combined treatment
(P < 0.001) when compared to the control. The differ-
ces observed among these three treatments were
not statistically significant (Table 1).
The foliar biomass of the combined treatment was
reduced by approximately 50% when compared to
the other treatments (P < 0.001). The differences in
the foliar biomass observed among the other treat-
ments were not statistically significant (Table 1).
The use of the Kruskal-Wallis test revealed a
significant difference in the rate of chlorotic foli-
age among treatments. The post-hoc comparisons
revealed that the control group differed signifi-
cantly from the inoculated (P < 0.01), flooded and
combined (both P < 0.001) treatments. The rate of
chlorotic foliage was highest in the combined
treatment (3.25 on a scale of 0 to 4); however, there
was no significant difference observed between this
treatment and the flooded one (Table 1). The flood-
ing and subsequent hypoxia leads to the yellowing
of foliage (KOZLOWSKI 1997; GÜNTHARDT-GOERG,
VOLLENWEIDER 2007). These significant differences
in the rate of chlorotized foliage in the flooded treat-
ments compared to the non-flooded ones indicate a
role for hypoxia.

CONCLUSIONS

The majority of the assessed criteria in the ex-
periment, including the amount of biomass of all
investigated plant parts, was significantly reduced
in the combined (flooded inoculated) treatment.
These results are consistent with P. ALNI being more
effective in the flooded treatment than in the non-
flooded one. The plants affected by both factors were
underdeveloped and declined quickly; some were
dying by the end of the experiment.
The most important outcome of this study is the
confirmation that P. ALNI causes more significant
damage to alders that are stressed by flooding than
to unstressed plants. Flooding clearly induces a de-
crease in host resistance (reduced uptake of nitrogen
and other nutrients, investment to rebuilding of the
root system, etc.) and accelerates the development
of the disease caused by P. ALNI.

From an ecological point of view, alder stands with
periodical or summer flooding and/or with a high
water table can have a higher incidence of disease, as
well as a more severe course of epidemics and higher
losses of trees. This situation very probably occurred
in great extent in the Vltava River catchment after
the summer floods in 2002. The subsequent substan-
tial stress persisted several months and contributed
to the sudden onset of phytophthora alder decline in
large affected areas in the Vltava River catchment.

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References


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