Winter Conditions Correlate with *Phytophthora alni* Subspecies Distribution in Southern Sweden

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ABSTRACT

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During the last century, the number of forest pathogen invasions has increased substantially. Environmental variables can play a crucial role in determining the establishment of invasive species. The objective of the present work was to determine the correlation between winter climatic conditions and distribution of two subspecies of the invasive forest pathogen *Phytophthora alni*: *P. alni* subsp. *alni* and *uniformis* killing black alder (*Alnus glutinosa*) in southern Sweden. It is known from laboratory experiments that *P. alni* subsp. *alni* is more pathogenic than *P. alni* subsp. *uniformis*, and that *P. alni* subsp. *alni* is sensitive to low temperatures and long frost periods. By studying the distribution of these two subspecies at the northern limit of the host

species, we could investigate whether winter conditions can affect the geographical distribution of *P. alni* subsp. *alni* spreading northward. Sixteen major river systems of southern Sweden were systematically surveyed and isolations were performed from active cankers. The distribution of the two studied subspecies was highly correlated with winter temperature and duration of periods with heavy frost. While *P. alni* subsp. *uniformis* covered the whole range of temperatures of the host, *P. alni* subsp. *alni* was recovered in areas subjected to milder winter temperatures and shorter frost periods. Our observations suggest that winter conditions can play an important role in limiting *P. alni* subsp. *alni* establishment in cold locations, thus affecting the distribution of the different subspecies of *P. alni* in boreal regions.

Additional keywords: *Alnus* spp., invasiveness, *Phytophthora plurivora*.

During the last century, the number of forest pathogen invasions has drastically increased owing to an escalation of international trade bringing together evolutionarily distant host–pathogen combinations (Stenlid et al. 2011). Following introduction, invasive pathogens must be able to establish and spread in the new environment, and it has been suggested that temperature can pose a major barrier at the establishment phase for some pathogens (Sturrock et al. 2011). Future warmer climates could make forest pathogens overcome environmental thresholds that limited their activity earlier and, subsequently, increase their damage (Bergot et al. 2004; Brasier 1996; Dukes et al. 2009; Fabre et al. 2011; Ireland et al. 2013; Santini et al. 2013; Woods et al. 2005).

A significant example of the correlation between temperature and invasiveness is illustrated by the emergence of *Phytophthora alni* damage in Europe (Aguayo et al. 2014). *P. alni* is a tree pathogen that causes dieback and mortality in alder species (*Alnus* spp.). The symptoms associated with *P. alni* include excessive seed production and small, yellow, sparse foliage with premature leaf fall. Infected trees display tongue-shaped exuding cankers, often with reddish-brown phloem tissue starting from the root collar that sometimes is surrounded by black or rusty-colored exudates (Gibbs et al. 1999; Jung and Blaschke 2004; Schumacher et al. 2006) (Supplementary Fig. S1). *P. alni* was first reported in southern Britain during the early 1990s causing disease on alder (Brasier et al. 1995; Gibbs 1995). Since this first report, the pathogen has been observed across Europe and some parts of North America (Adams et al. 2010;

Gibbs et al. 2003; Jung and Blaschke 2004; Olsson 1999; Streito et al. 2002). Within the European continent, Scandinavian countries constitute the northern limit to the distribution of *P. alni*. Initial isozyme studies considered *P. alni* to be a hybrid complex and divided it into three groups (Olsson 1999). Finally, Brasier et al. (2004) described the pathogen as *Phytophthora alni* and divided it into three subspecies: *P. alni* subsp. *alni*, corresponding with the standard type; *P. alni* subsp. *uniformis*, coinciding with the Swedish variant; and *P. alni* subsp. *multiformis*, which included the Dutch, German, and U.K. variant hybrid types.

There are significant differences between *P. alni* subspecies regarding pathogenicity and cold tolerance. In pathogenicity tests, *P. alni* subsp. *alni* was more aggressive when infecting *Alnus glutinosa* compared with *P. alni* subsp. *uniformis* (Brasier and Kirk 2001; Santini et al. 2003). *P. alni* subsp. *alni* has also shown a low tolerance to long frost periods under laboratory conditions (Černý et al. 2012; Schumacher et al. 2006) and in natural environments (Aguayo et al. 2014; Černý and Strnadová 2012). In addition, *P. alni* subsp. *uniformis* has been found in locations with harsh winter conditions such as Alaska or Sweden, which could suggest a higher cold tolerance than *P. alni* subsp. *alni*.

In this work, we studied the distribution of *P. alni* infecting alder in Sweden. Both *P. alni* subsp. *uniformis* and *alni* have been present in Sweden since the 1990s, when they were first observed in the Sävå and Mölndalsån river systems (in 1996 and 1998, respectively) along the west coast near Gothenburg, and in an alder nursery at Ljungbyhed irrigated with water from the Rönne å river (in 1997) (Olsson 1999). Later surveys performed in the Rönne å river system in southern Sweden and in Klarälven (*A. incana*) in the city of Karlstad in 2006 and 2010 confirmed the presence of *P. alni* subsp. *alni* and *uniformis* in these parts of Sweden (C. H. B. Olsson, unpublished results). In 2013, we performed a comprehensive systematic sampling in South Sweden covering 16 rivers in

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order to (i) assess the current distribution of *P. alni* subspecies and (ii) investigate the correlation between the distribution of the different subspecies and the winter temperature and length of continuous frost periods. We found that *P. alni* subsp. *alni*, despite being more pathogenic, was only isolated in areas with mild winters, whereas *P. alni* subsp. *uniformis* was recovered all over the studied area. Our study showed that there is a strong correlation between the winter conditions and the distribution of *P. alni* subsp. *alni* and *uniformis* isolates.

MATERIALS AND METHODS

Field survey. We surveyed 16 major rivers along both the eastern and western coast of southern Sweden from September to November 2013 (Table 1; Fig. 1). In each river, 11 sampling plots were systematically established along the entire stream. In total, 176 plots were surveyed. In every sampling plot, all alder trees located at river banks or at river-connected swamps within a radius of approximately 100 m from the center of the plot were inspected for active *Phytophthora* cankers. Active cankers were typically observed as black tarry spots in exposed coarse roots or at the base of the stem. Lesions with unclear symptoms were confirmed to be caused by *Phytophthora* spp. by using a *Phytophthora* detection kit (*Phytophthora* spp. ALERT-LF; Neogen). From each lesion, the bark was removed to expose a brownish, flame-shaped active front, from where approximately 35 1-mm slices of phloem tissue were directly plated onto four plates of CMA-PARPHB selective medium (Jeffers and Martin 1986; Pérez-Sierra et al. 2010). The plates were kept cool (approximately 10°C) during the fieldwork period and, within 2 to 3 days, incubated in the laboratory at 20°C in the dark. Sampled trees were classified as small (<5 cm in diameter), medium (5 to 15 cm), or large (>15 cm). Additionally, for 10 trees in each plot whose root systems were in contact with the water, the percentage of damaged trees was estimated by noting the presence or absence of *Phytophthora* spp. infection symptoms such as defoliation and dieback. During the fieldwork period, six plots with alder trees displaying active lesions were observed in areas that did not belong to the established sampling plots. In those cases, *Phytophthora* spp. isolation was attempted and the plot was added

as an extra sampling plot in the stream. In order to keep the systematic impact assessment, the information from these extra plots was used only in the subspecies distribution analyses and not in the damage evaluation.

Isolation and identification procedures. Within 2 to 4 days following plating, the phloem tissue samples were examined for *Phytophthora*-like hyphae that were subsequently transferred onto PARPBH and incubated at 25°C for 4 days. Hyphal tips from putative pure cultures were transferred to V8 media and stored at 25°C. Within 7 to 10 days, the growth pattern and possible oogonia formed were examined for a tentative *P. alni* subspecies identification (Brasier et al. 2004). All *Phytophthora* isolates, including those not resembling *P. alni*, were typed by molecular identification. Together with the isolates obtained from this study, we also conducted molecular identification of 39 isolates collected in earlier surveys in Sweden (Table 1).

For molecular identification, isolates were grown on V8 agar for 1 week and DNA was extracted from mycelia harvested from the active growing front using NucleoSpin Plant II (Macherey-Nagel). Putative *P. alni* isolates were confirmed to be *P. alni* by using the sequence-characterized amplified region-based polymerase chain reaction (PCR) primers PA-F and PA-R from Ios et al. (2005). For subspecies identification, we performed two PCR rounds, the first with TRP-PAU-F/-R primers (positive signal for *P. alni* subsp. *alni* and *uniformis* isolates), and the second with RAS-PAM1-F/-R primers (positive signal for *P. alni* subsp. *alni* and *multiformis* isolates) (Ios et al. 2006). Cycling conditions were as in the respective papers (Ios et al. 2005, 2006). Unknown *Phytophthora* isolates were identified to species level based on the internal transcribed spacer (ITS) region. DNA was amplified using the ITS6f and ITS4 primers (Cooke and Duncan 1997; White et al. 1990) according to PCR conditions described by Samils et al. (2011) and sequenced by Macrogen. Blast searches in GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>) showed that the isolates corresponded to *P. plurivora*. The ITS sequences were subsequently compared with putative related isolates found in Jung and Burgess (2009) using ITS sequences obtained from GenBank. Sequence data for the ITS region was aligned by Muscle and a neighbor-joining tree was constructed in Mega software (version 6) using default settings.

TABLE 1. Distribution of *Phytophthora alni* isolates obtained in Sweden since 1996^a

Year	River code	River	Number of <i>Phytophthora</i> isolates					Number of plots with <i>Phytophthora</i> spp.					
			Paa	Pau	Pam	Plu	Total	Paa	Pau	Pam	Paa and Pau	Plu	Total
1996	1	Säveån	...	4	...	...	4	...	...	...	...	...	...
1998	2	Mölnålsån	1	...	...	...	1	...	...	...	...	...	...
2006	1	Säveån	1	...	...	...	1	...	...	...	...	...	...
		Klarälven	...	2	...	...	2	...	...	...	...	...	...
	2	Mölnålsån	9	...	1	...	10	...	...	...	...	...	...
	7	Roneå	4	...	1	...	5	...	...	...	...	...	...
2010	1	Säveån	12	...	...	...	12	...	...	...	...	...	...
	2	Mölnålsån	4	...	...	...	4	...	...	...	...	...	...
2013	1	Säveån	...	...	...	...	...	...	...	...	...	...	...
	2	Mölnålsån	7	...	...	...	7	3	...	...	...	...	3
	3	Örekilsälven	...	2	...	...	2	...	1	...	...	...	1
	4	Viskan	2	1	...	...	3	2	1	...	...	...	3
	5	Åtran	...	...	...	...	...	...	...	...	...	...	...
	6	Lagan	...	7	...	...	7	-	3	...	...	...	3
	7	Rönnea	8	2	...	2	12	1	1	...	1	2	5
	8	Kävlingeå	8	3	...	...	11	2	1	...	...	...	3
	9	Helgeå	6	2	...	...	8	2	1	...	...	...	3
	10	Ronnebyån	6	2	...	...	8	1	1	...	1	...	3
	11	Lyckebyån	2	11	...	...	13	1	3	...	...	...	4
	12	Alsteran	...	8	...	...	8	...	2	...	...	...	2
	13	Storån	...	8	...	2	10	...	4	...	...	2	6
	14	Nyköpingsån	...	11	...	2	13	...	7	...	...	1	8
	15	Gävleån	...	4	...	...	4	...	3	...	...	...	3
	16	Fyrisån	...	7	...	...	7	...	2	...	...	...	2

^a In the survey of 2013, all rivers but Örekilsälven and Viskan had a total of 11 sampling plots. Total number of sampling plots in 2013 = 176. Paa = *Phytophthora alni* subsp. *alni*, Pau = *P. alni* subsp. *uniformis*, Pam = *P. alni* subsp. *multiformis*, and Plu = *P. plurivora*.

Climatic data. Air temperature data were obtained from the Swedish Meteorological and Hydrological Institute website (luftweb.smhi.se). The database contains gridded daily temperature and precipitation data interpolated from weather station data with a resolution of 4 by 4 km. The monthly average temperature and the average of the maximum period of consecutive days with temperatures below -5°C for the last 20 years was calculated for each sampling plot.

Statistical analysis. We compared the average monthly temperature and the maximum number of consecutive days with temperatures below -5°C between sampling plots with presence of *P. alni* subsp. *alni* and *uniformis* and without any *Phytophthora* spp. by a general linear model. Means were compared by Tukey's honestly significant difference at $P < 0.05$. The same test was performed to compare the percentage of healthy trees between plots with the presence of *P. alni* subsp. *uniformis* and *P. alni* subsp. *alni*, those with *P. plurivora*, and those without apparent *Phytophthora* spp. damage. In order to test whether *P. alni* subsp. *uniformis* appeared more frequently in colder areas of the temperature distribution range of the host, we compared the median temperature of the coldest month of sampling plots with *P. alni* subsp. *uniformis* and all sampling plots together with the Mann-Whitney U non-parametric test. The association between the presence of *P. alni* subsp. *uniformis* and *alni* and February temperature was evaluated by logistic regression using *logit* as a link function and assuming a binomial distribution. All statistical tests were performed in Minitab (version 16.1.0; Minitab Inc.), except for the logistic regression that was performed in JMP Pro (version 11.0.0; SAS Institute Inc.).

RESULTS

Temperature-based *P. alni* distribution. We observed symptoms of *Phytophthora* spp. in all but two of the sampled rivers, with an overall incidence of 28% across sampling plots. In total, we obtained 113 isolates from 157 alder trees with cankers. Two subspecies (*P. alni* subsp. *uniformis* and *alni*) were found (68 isolates in 31 plots, and 39 isolates in 13 plots, respectively). In addition, six *P. plurivora* isolates were also recovered from five plots (in three rivers). Although *P. alni* subsp. *uniformis* was present across the distribution range of the host, *P. alni* subsp. *alni* was restricted to southern coastal areas, associated with milder winter conditions (Fig. 1). The monthly average temperature of locations where *P. alni* subsp. *alni* was observed was systematically over or very close to 0°C (Fig. 2). *P. alni* subsp. *alni* was isolated in plots with an average temperature of the coldest month (February) of -0.05°C (standard error [SE] = 0.31) and an average of maximum heavy frost duration of 4.45 days in length (SE = 0.52). *P. alni* subsp. *uniformis* was recovered in significantly colder sampling plots than *P. alni* subsp. *alni* (-1.75°C ; SE = 0.20), with average frost periods of 7.26 days (SE = 0.34) (Fig. 2 and 3A). February average temperature was highly correlated ($R^2 = 0.97$) with the maximum number of consecutive days with temperatures below -5°C (Fig. 3B). Although observations of *P. alni* subsp. *uniformis* were recorded throughout the entire range of the host, they tended to be shifted toward colder locations (Fig. 2) (median February temperature in *P. alni* subsp. *uniformis* plots = -2.0°C versus median all plots = -1.3°C ; $P = 0.036$). In contrast, *P. alni* subsp. *alni* observations were recorded in warmer locations (median February temperature in *P. alni* subsp. *alni* plots = 0.07°C versus median all plots = -1.3°C ; $P < 0.0001$). The probability of finding *P. alni* subsp. *uniformis* in a plot decreased as the average February temperature increased ($P = 0.027$). In contrast, the probability of finding *P. alni* subsp. *alni* increased as the average February temperature increased ($P < 0.0001$).

Impact assessment. The impact assessment showed that 31% of the alder stands had $\geq 50\%$ of the trees with symptoms of decline. In sampling plots with *P. alni* presence, the percentage of trees with a healthy crown was significantly lower than in plots without

symptoms of the pathogen (54 versus 77%, $P = 0.02$) (Fig. 4). No differences were found between *P. alni* subspecies. The majority of infected trees were *A. glutinosa* (93%), which is also the most common alder species in these river systems (as opposed to *A. incana*). The infected trees were more commonly found in sampling plots including river-connected swamps or ponds than plots restricted to river banks (57 isolates in 16 plots versus 56 isolates in 33 plots). The majority of *Phytophthora* colonies (86%) were isolated from trees of less than 15 cm in diameter.

Morphological and molecular identification. Microscopic morphological identification matched molecular identification based upon specific primers and ITS sequence analysis. *P. alni* subsp. *alni* isolates displayed uniform growth in V8 agar; sparse aerial mycelium; and abundant, ornamented, brown, and sometimes comma-shaped oogonia with amphigynous antheridia. In contrast, *P. alni* subsp. *uniformis* showed irregular and dense aerial mycelium; less abundant and mostly smooth-walled oogonia, rarely displaying protuberances; with amphigynous antheridia. The growth pattern of *P. plurivora* was radiate and slightly chrysanthemum shaped, with limited aerial mycelium. Oogonia were extremely abundant, with paragynous antheridia, and developed rapidly in V8 agar.

DISCUSSION

Role of temperature in *P. alni* subspecies distribution.

The geographical distribution of *P. alni* subspecies correlated with the monthly winter temperature and with the duration of heavy frost (consecutive days with temperature below -5°C). *P. alni* subsp. *alni* was isolated in the warmest locations of the studied area whereas *P. alni* subsp. *uniformis* was recovered within the whole temperature range of the host (Fig. 1). It could be hypothesized that the absence of *P. alni* subsp. *alni* in areas with average winter temperatures below -1°C was because the duration of heavy frost periods impaired *P. alni* subsp. *alni* survival in those regions (Fig. 3). Previous studies have shown that, even though *P. alni* subsp. *alni* was able to tolerate temperatures as low as -2.5°C , the survival rate decreases rapidly when temperature was between -5 and -10°C for several consecutive days (Černý and Strnadová 2012; Černý et al. 2012). In Sweden, locations with average February temperatures of -2.5°C , where *P. alni* subsp. *alni* is currently absent, can occasionally undergo frost periods twice as long as in other regions in Europe, where *P. alni* subsp. *alni* has been reported under similar temperature conditions (4 days in Czech Republic versus 8 days in Sweden) (Černý et al. 2012) (Fig. 3B). We suggest that the observed distribution of *P. alni* reflects the differences in cold and frost tolerance between *P. alni* subsp. *alni* and *uniformis*. Possibly, these differences are explained by their capacity to produce viable overwintering structures (i.e., *P. alni* subsp. *alni* generally produces fewer viable oospores than *P. alni* subsp. *uniformis*) (Delcán and Brasier 2001).

An alternative but less likely explanation for the limited distribution of *P. alni* subsp. *alni* in southern Sweden could be a more recent introduction of *P. alni* subsp. *alni* than *P. alni* subsp. *uniformis*. The observed distribution of *P. alni* subsp. *alni* would then be part of an ongoing northward expansion over areas previously occupied by *P. alni* subsp. *uniformis* (Štěpánková et al. 2013). However, there seems to be little evidence supporting that hypothesis. On the one hand, it is known from historical data that both *P. alni* subsp. *alni* and *uniformis* were present in Sweden since the 1990s, discarding a recent introduction of *P. alni* subsp. *alni*. On the other hand, because both subspecies are invasive in Sweden and they presumably spread by the same route of invasion, there seems to be little support for a faster dispersion of *P. alni* subsp. *uniformis* than of *P. alni* subsp. *alni*. Within similar time frames (2000 to 2013) but under less harsh winter conditions, other studies have shown that *P. alni* subsp. *alni* rapidly spread and dominated entire areas where *P. alni* subsp. *uniformis* was presumably established before (Štěpánková et al. 2013). Although perhaps *P. alni* subsp. *alni*

is still not covering its entire potential range, we argue that this replacement has not occurred in northern areas of Sweden because low winter temperatures and long periods of heavy frost occurring in these locations play a role in hampering such expansion.

The current distribution of the subspecies of *P. alni* could also be the consequence of different geographical introductions among subspecies. Initial *P. alni* introductions in Sweden have probably

occurred via infected nursery stock. Both *P. alni* subsp. *alni* and *uniformis* were first reported in Sweden in southwest nurseries and alder-planted areas during the 1990s (C. H. B. Olsson, unpublished results). During the present survey, both *P. alni* subspecies have been recovered from several riverbank alder plantations (Supplementary Fig. S1D). After *P. alni* establishment in rivers via alder plantations, further spread might have occurred via wild animal migrations or livestock and human movements (Adams et al. 2010;

Fig. 1. Distribution of *Phytophthora* spp. isolated from alder tissue. Shown is the average temperature of February (the coldest month of the year) for the last 20 years. The limit of -0.66°C in temperature corresponds to the limit value for the 95% confidence interval (average temperature of *P. alni* subsp. *alni* locations $-1.96 \times$ standard error). "None" indicates absence of the species. The number for each river corresponds to the river code in Table 1.

Webber and Rose 2008). Because *P. alni* subsp. *alni* is more often present in nursery stock than *P. alni* subsp. *uniformis* (Jung and Blaschke 2004; Schumacher et al. 2006), the abundance of *P. alni* subsp. *alni* in coastal areas might be related to higher planting activities as a consequence of a higher human density in these regions. However, *P. alni* subsp. *alni* only dominates coastal areas when these are located in the warmest areas of Sweden, whereas it appeared to be absent in more northern and colder coastal areas. To our knowledge, there seems to be no reason why *P. alni* subsp. *alni* would have been introduced more often in southern than in northern Sweden.

We found indications that *P. alni* subsp. *alni* might be replacing *P. alni* subsp. *uniformis* in climatically suitable areas. *P. alni* subsp. *uniformis* was observed across the entire range of temperatures of the host, although findings tended to be more common in the colder locations of the studied area. When looking at intrasite distribution, *P. alni* subsp. *uniformis* was present in only 2 of 14 locations where *P. alni* subsp. *alni* was observed. Previous observations in Sweden also support a local replacement. During the first *P. alni* surveys performed in the 1990s, both *P. alni* subsp. *alni* and *uniformis* were isolated. In Sävå, *P. alni* subsp. *uniformis* was first isolated in 1996 but, in 2006 and 2010, only *P. alni* subsp. *alni* was recovered from the same river system (Table 1). In contrast, *P. alni* subsp. *alni* has been systematically recovered in Mölndalsån (in 1998, 2006,

2010, and 2013), indicating that, where established, *P. alni* subsp. *alni* became prevalent in the long run. Most likely, *P. alni* subsp. *alni* is able to suppress *P. alni* subsp. *uniformis* in some areas due to its higher virulence (Brasier and Kirk 2001; Štěpánková et al. 2013). Replacement of well-established invasive or native pathogens with more virulent strains has been observed in other pathosystems such as *Ophiostoma novo-ulmi* replacing *O. ulmi* in Europe (Brasier and Buck 2001) or *Heterobasidion irregulare* replacing *H. annosum* s.s. in Italy (Garbelotto et al. 2010). To confirm the replacement, more isolations per site should be carried out, possibly complemented with isolation from the rhizosphere in order to confirm the absence of *P. alni* subsp. *uniformis* in areas where *P. alni* subsp. *alni* is present.

Impact assessment. In southern Sweden, *P. alni* was widespread in river systems, with 28% of sampling plots associated with severe decline crown symptoms. Similar observations have been reported in other Southern and Central European countries (Gibbs et al. 2003; Jung and Blaschke 2004; Streito et al. 2002). The results of the crown health assessment performed showed that the percentage of healthy crowns in the plots with *P. alni* was significantly lower than in the plots without this pathogen (Fig. 4).

Nevertheless, there was a large variation of alder health status in places where *Phytophthora* symptoms were observed. A reason for that could be that we observed *Phytophthora* spp. in sites where they had just established, and damages had not yet been manifested in the

Fig. 2. Association between temperature and *Phytophthora alni* subspecies distribution. **A**, Average daily temperature for every month for locations where *P. alni* subsp. *alni* and *uniformis* were isolated and locations without symptoms of *Phytophthora* infection; an asterisk (*) indicates significant differences between *P. alni* subsp. *alni* and *uniformis*. **B**, Average temperature of the coldest and warmest month of the year in plots with observed symptoms of *P. alni* subsp. *alni* and *uniformis*. The line inside the box indicates the median temperature.

Fig. 3. **A**, Association between maximum number of consecutive days with heavy frost and *Phytophthora alni* subspecies. **B**, Correlation between average February temperature and duration of heavy frost period for the last 20 years in southern Sweden.

dominant trees assessed. It has been previously observed that trees marked with active lesions still displayed a healthy-looking crown (Jung and Blaschke 2004; Thoirain et al. 2007). Occasionally, alder trees can also recover from decline (Aguayo et al. 2014). It can also be considered that many alder trees eventually die from the attack of secondary pathogens such as *Inonotus* or *Armillaria* spp. (Jung and Blaschke 2004). Consequently, high variation in health estimations can be related to the presence or absence of these secondary pathogens. Damage differences between *P. alni* subspecies were not observed, in contrast with previous observations of pathogenicity tests in seedlings (Brasier and Kirk 2001). A possible explanation could be that *P. alni* subsp. *alni* aggressiveness was decreased in Sweden by cold climate or short vegetative period, resulting in a similar aggressiveness of subspecies.

At the river scale, the rate of infected trees per plot was higher in flooded areas than in riverbank sites (3.6 and 1.7 isolates/plot, respectively). Some of the flooded areas were also highly disturbed sites in which cattle had been grazing. Swamps and ponds could be considered hot spots that might act as inoculum sources facilitating downstream spread of *Phytophthora* spp. (Jung and Blaschke 2004; Schumacher et al. 2006; Streito et al. 2002; Thoirain et al. 2007). The role of cattle movement as a vector for spreading *Phytophthora* spp. could be considered in future studies.

In five locations, *P. plurivora* was also isolated from alder bark tissue constituting additional reports of *P. plurivora* in alder trees in Sweden (Fig. 1). Earlier reports of *P. plurivora* on alder in Sweden correspond to the isolations made in Asslebyn (Bengtssons locality) in Sept 2012, although *P. plurivora* isolates had been previously obtained from surveys near Nyköping in 1999 (C. H. B. Olsson, unpublished results). *P. plurivora* is an invasive species infecting a wide range of host species, previously observed in other European countries also infecting alder (Haque et al. 2014; Jung and Blaschke 2004; Jung and Burgess 2009). Due to its potential to affect multiple woody species, the presence of *P. plurivora* in water streams could facilitate the spread to adjacent ecosystems, such as oak, beech, and Norway spruce forests (Jung and Burgess 2009; Rytönen et al. 2013).

The results of the present work showed that winter conditions could affect the distribution of the different subspecies of *P. alni* in southern Sweden, thus limiting further spread of *P. alni* subsp. *alni*. The high incidence and widespread damage caused by *P. alni* poses a threat to Swedish riverbank ecosystems and requires further impact monitoring and research on dispersal pathways. Climatic

suitability has been suggested as a major determinant for invasiveness in forest pathogens (Aguayo et al. 2014). The results of the present work support that idea, and suggest that low temperatures in Northern Europe could currently be preventing the establishment of invasive forest pathogens. Consequently, a future warmer climate could enable higher invasion rates.

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Fig. 4. Percentage of healthy trees in the sampling plots with presence and absence of *Phytophthora* spp.

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