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Low persistence of *Phytophthora ramorum* (Werres, De Cock, and Man in 't Veld) in western France after implementation of eradication measures

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Abstract

Key message Presence of *Phytophthora ramorum* (Werres, De Cock, and Man in 't Veld**)** in western France was studied after the detection of this invasive pathogen in 2017 in *Larix kaempferi* (Lamb.) and eradication of the affected stands. *P. ramorum* was seldom detected in the area of the outbreak in the year following eradication. However, we confirm that *P. ramorum* can multiply to epidemic level on chestnuts (*Castanea sativa* Mill.) in the absence of larch (*Larix* spp.). This represents the major risk in France.

Context *Phytophthora ramorum* is an invasive oomycete that causes significant damage in the USA and Europe. Although the pathogen has been present in nurseries in France since 2002, the first outbreaks in forest stands were identified in 2017 in plantations of *Larix kaempferi* in two forests in western France (Saint-Cadou and Hanvec). In order to limit the development of the epidemic, neighboring larch stands were clear-cut.

Aim This study investigated the presence of *P. ramorum* in the affected area after the eradication treatment.

Methods Larch stands located within a 18-km radius of the reported outbreaks were investigated. We also monitored the native woody hosts present in infected clear-cut larch stands and in the vicinity of seven ornamental nurseries that had been infected by *P. ramorum* on several occasions in the past.

Results Overall, a very limited presence of *P. ramorum* was detected in 2018–2021. Two new stands of infected *L. kaempferi* were found close to the main initial outbreak, in Saint-Cadou and Saint-Rivoal. The pathogen was only detected on rhododendrons and chestnut trees *(Castanea sativa Mill.)* in the vicinity of the outbreaks. In the Saint-Cadou state Forest, an outbreak of the disease developed in 2019–2021 on chestnut trees even though all the mature larch trees had been felled. *P. ramorum* was also detected near two of the formerly infected ornamental nurseries, on *Castanea sativa* and on rhododendrons.

Conclusion While larches and rhododendrons are uncommon in the forests of north-western France, chestnut trees are present in 21–25% of the forest and therefore represent the major risk for the survival of *P. ramorum* in the region.

Keywords *Castanea sativa*, *Larix kaempferi*, Epidemiology, Invasive forest pathogen

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1 Introduction

Invasive pathogens are a major threat to forest ecosystems and can affect timber production and biodiversity (Ghelardini et al. 2016). Introductions of invasive forest pathogens in Europe have been increasing rapidly in recent decades due to the intensification of international trade (Santini et al. 2013; Sikes et al. 2018). Invasive pathogens are responsible for a large fraction of forest disease cases reported by the French forest health network, with an increase from around 20% in 1990 to around 50% after 2010 (Desprez-Loustau et al. 2016). The management of invasive pathogens requires knowledge of the pathways of introduction and adequate surveillance to rapidly detect possible outbreaks before they spread (Paap et al. 2020; Parnell et al. 2017). The ability to eradicate a disease in a natural environment mainly depends on the size of the outbreak and the speed of the eradication process (Branco et al 2023; Vainio et al. 2019). Surveillance is particularly difficult for generalist pathogens, not only because of the large number of hosts that need to be surveyed but also because disease symptoms may be inconspicuous in many hosts, allowing the pathogen to persist undetected; cryptic infection is a major difficulty in the surveillance of quarantine organisms (Parnell et al. 2017).

Phytophthora ramorum is a good example of an invasive pathogen causing severe damage in forests. In the early 2000s, this oomycete was reported to cause Sudden Oak Death in coastal California and Oregon, USA (Rizzo et al. 2002; Goheen et al. 2002). In Europe, *P. ramorum* was first detected on woody ornamentals, mainly rhododendrons (Werres et al. 2001; Sansford et al. 2009; Harris et al 2018), before it was reported to cause a severe epidemic on larch in the UK (Brasier and Webber 2010). The pathogen was probably first introduced in forest ecosystems via infected ornamental shrubs (Mascheretti et al. 2008; Grünwald et al. 2016; Harris et al 2018). *P. ramorum* can infect many woody species (Davidson et al. 2005, 2008; Hansen et al. 2005). However, a limited number of hosts are usually identified as important sources of inoculum, such as California bay laurel (*Umbellularia californica* (Hook. and Arn.) Nutt.) and tanoak (*Notholithocarpus densiflorus* (Hook. and Arn.) Rehder) in California (Davidson et al. 2005, 2008; Garbelotto et al. 2017), tanoak in Oregon (Hansen et al. 2019) and larch (mainly *Larix kaempferi* Lamb., but also *L. decidua* Mill and *L.* × *eurolepis* Henry) in the UK and Ireland (Harris and Webber 2016; O'Hanlon et al. 2018). In affected stands and their surroundings, the pathogen may be able to persist at low levels by limited infection of a wide range of hosts, but also in soil and litter. This may be critical to understand, particularly if eradication is attempted (Harris 2014; O'Hanlon et al. 2018; Hansen et al. 2019). *P. ramorum* spreads locally by splash dispersal (Davidson et al. 2005, 2008). At a larger scale, the pathogen can spread up to several kilometers, presumably by the movement of airborne sporangia (Perterson et al. 2015). However, in the early stage of the epidemic in Oregon, it was estimated that 80% of the effective dispersal occurred within 300 m of the inoculum source (Hansen et al. 2008). Transportation and planting of infected plants (e.g., nursery stock) remain the primary long-distance dispersal mechanism (Mascheretti et al. 2008).

The detection of *P. ramorum* in France has been summarized in Desprez-Loustau et al. (2018). The pathogen was reported in ornamental nurseries and trade in 2002, shortly after its description by Werres et al. (2001). The number of outbreaks in ornamental nurseries was significant from 2002 to 2008 and then decreased in the following years, although the surveillance efforts remained intensive (about 2100 sites surveyed throughout the period, Fig. 1A). Outbreaks in nurseries mainly affected rhododendrons (68%) and *Viburnum tinus L.* (28%) and were concentrated in western France, in Bretagne and Pays-de-Loire (Fig. 1B). However, the pathogen was reported in ornamental plants retailers throughout the country. In forests, surveillance was carried out by the DSF, the French forest health survey system (Département de la Santé des Forêts). Prior to 2017, mainly *Rhododendron ponticum L.*, larch (*L. kaempferi* and *L. decidua*), oak (*Quercus petraea* L. and *Q. robur* L.) and *Vaccinium myrtillus L.* were surveyed (58, 21, 7, and 7% of the reports respectively) and most of the surveillance efforts were located in western France where approximately 50 stands were surveyed per year (Fig. 1C). *P. ramorum* was detected on rhododendrons at four sites in Bretagne and Normandie in 2007 (twice), 2008, and 2014 (Fig. 1D). Infected *L. kaempferi* were then reported in 2017 in Bretagne in two sites, in the state forest of Saint-Cadou (EPSG4326: − 3.99701, 48.37289) and in the "bois du Gars" at Hanvec (EPSG4326: − 4.21251 48.33629). The outbreak in Saint-Cadou was large with about 13 ha of larch stands severely affected by *P. ramorum,* whereas it was less severe and more limited in Hanvec (< 1 ha). Isolates recovered in infected larch stands forests belong to the EU1 clonal lineage (Schenck et al. 2018). Following the discovery of these two outbreaks, eradication measures were implemented. The report of *P. ramorum* in forest stands also triggered an increased surveillance effort with approximatrly 150 forest stands surveyed annually by the DSF throughout France between 2018 and 2021 (97% larch and 1.5% chestnut).

The aim of this study was to better document the distribution of *P. ramorum* in Bretagne on known susceptible hosts (larch and native woody species) after eradication measures had been applied in *L. kaempferi* plantations.

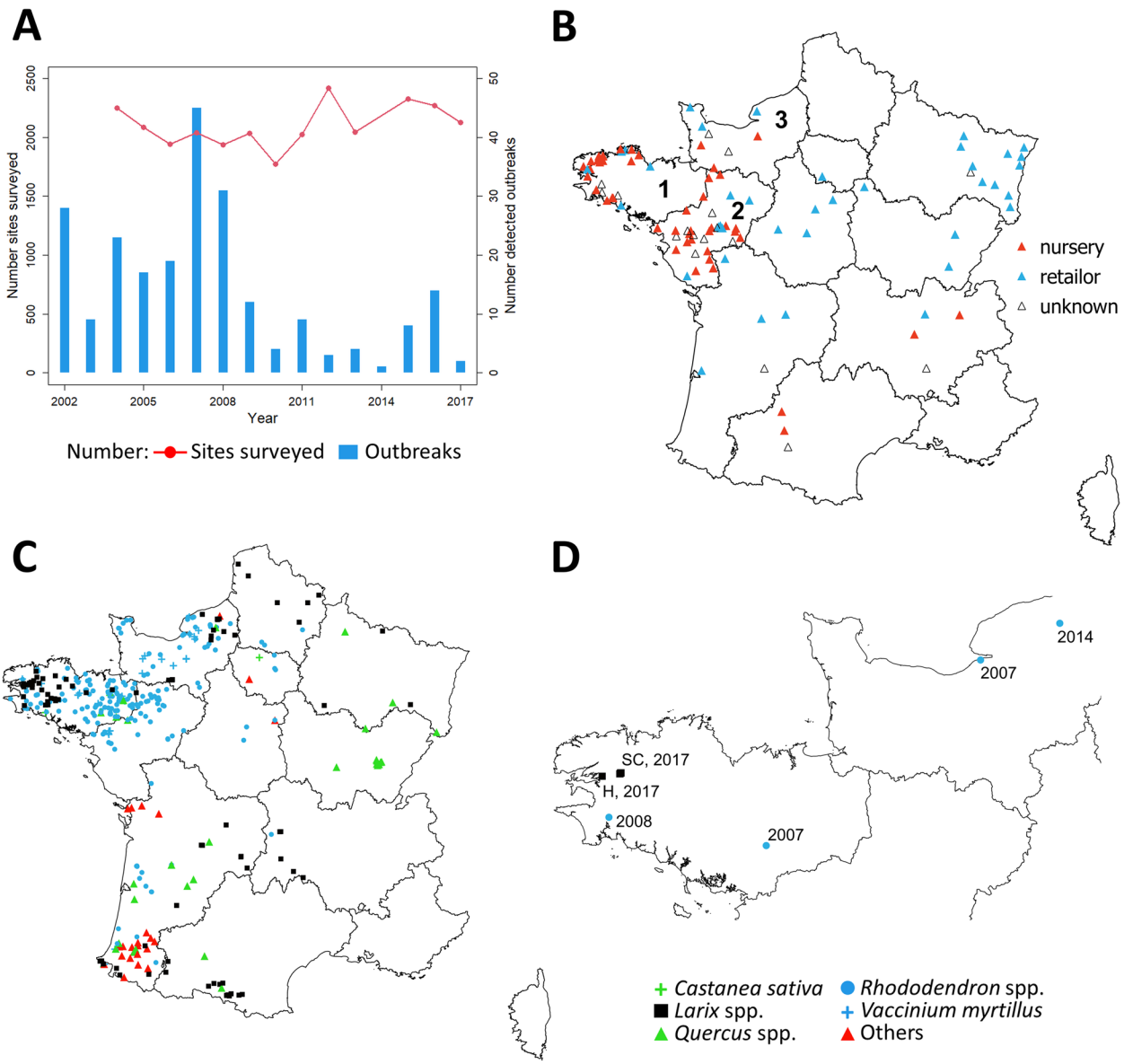

Fig. 1 Surveillance of *P. ramorum* in France between 2004 and 2017. **A** Evolution of the report and of the surveillance effort, **B** Location of *P. ramorum* reports by the plant protection service in ornamental nurseries and retailors (unknown are either nurseries or retailors), 1. Bretagne, 2, Pays de Loire, 3, Normandie, **C** Location of the forest stands surveyed by the DSF (forest health surveillance system), **D** Location of *P. ramorum* reports in forests of Bretagne and Normandie (the year of the report is indicated, SC, Saint-Cadou state forest, H, Bois du Gars in Hanvec) (Desprez-Loustau et al. 2018)

To this end, we surveyed the neighborhood of reported outbreaks both in the forest and in the vicinity of selected ornamental nurseries. We also analyzed DSF data on diseases reported in larch plantations to determine whether *P. ramorum* may be transmitted to the natural environment by hosts other than rhododendrons. Finally, we examined the distribution of the hosts of *P. ramorum* in Bretagne using the data from the forest inventory and of the Conservatoire Botanique of Brest.

2 Material and methods

2.1 Survey in larch stands (pre-eradication treatment)

A survey was conducted in 2017–21 focusing on the area where *P. ramorum* was observed on larch in 2017 (Hanvec and Saint-Cadou state forest). This area in western Bretagne is referred to as "Finistère" (see Fig. 2). In November 2017, rain traps were placed in five Japanese larch plots including two plots infected by *P. ramorum* in Hanvec and Saint-Cadou prior to tree felling for

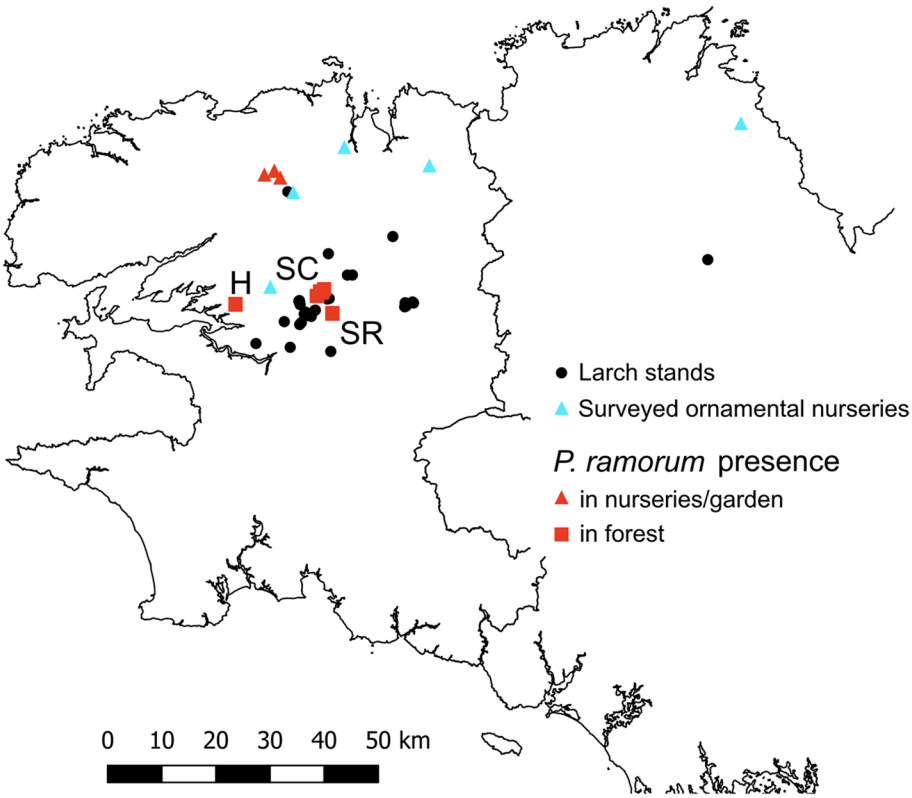

Fig. 2 Japanese larch stands and nurseries surrounding surveyed in 2017–2021 in Finistère. H, Bois du gars in Hanvec, SC, Saint-Cadou, SR, Saint Rivoal. Finistère is the administrative departement delineated on the map at the west of Bretagne where the *P. ramorum* outbreaks are located

eradication. The rain traps consisted of 250 ml buckets filled with 100 ml tap water in which three fresh *Rhododendron* leaves (*R. caucasicum*×*ponticum* 'Cunningham's White') were placed floating on the surface. The traps were covered with a net to prevent coarse debris from falling in. The rhododendron leaves were left for a single 2 weeks period and then collected for laboratory analysis. In December 2017, freshly fallen larch needles were sampled in six different larch stands of the Saint-Cadou state forest, including four stands known to be infected by *P. ramorum* and two others that showed no crown symptoms. The samples were analyzed by plating about 20 larch needles on *Phytophthora* selective media (Legeay et al. 2020) in order to recover *P. ramorum*.

In 2018, the survey was extended. Aerial photographs (20 cm pixels Orthophotos from the IGN, 2015, http://professionnels.ign.fr) were used to locate all possible larch stands up to 18 km from the Saint-Cadou state forest and Hanvec. All putative larch stands in the area were visited to assess the presence of *P. ramorum* (Fig. 2). Several methods of detection were used. We established plots on 25 m transects along a planting line in each of the stands. The stands were surveyed for trees with possible symptoms of *P. ramorum*, ranging from brown to dead branches in the upper crown to canopy dieback. Each transect began with a symptomatic tree. Five rain traps were placed along the line at 5 m intervals. Rain traps consisted in 15 l plastic buckets filled with 2 l of tap water and buried to half height for stability. Three fresh *Rhododendron* leaves ('Cunningham's White') were placed floating on the tap water. Each bucket was covered with a net to avoid falling coarse debris. The *Rhododendron* leaves were left for a single 2-week period, then removed and returned to the laboratory for analysis. One to five litter samples were collected along each transect, selecting locations under trees with *P. ramorum* symptoms. Litter samples were collected over an area of approximately 0.5 m^2, targeting the freshest larch needles. Finally, crown symptoms on trees along the 25 m transects were scored as follows: 0, healthy crown, 1, presence of one or several small branches in the upper crown that are either dead or have an abnormal reddish color, 2, dead tree-top/dieback, 3, dead tree. Twenty-two larch plots were sampled, with both rain traps and litter samples, in April–May 2018 and ten in autumn 2018 (including eight sampled in spring). In autumn, the rain traps were set in October and the litter was sampled in December.

In autumn 2018, we also used a different survey method to better target symptomatic trees, as *P. ramorum* was detected in very few plots. Twenty-five larch plots were sampled in different stands, and only litter samples were collected (including 4 plots that had already been sampled with the first survey method). The stands were first surveyed in September to locate and mark trees with *P. ramorum* symptoms (1–5 per plot). Litter was then sampled in December at the base of symptomatic trees, as described above. Thus, in the autumn of 2018, litter samples were collected in 31 different plots (twice for 4 plots), and rain traps were set up in 10 of these plots. The new survey procedure was repeated in December 2019 for nine larch stands and in February 2021 for five larch stands.

2.2 Survey in the vicinity of outbreaks (post-eradication treatment)

Specific surveys were set up near eradication sites in two different types of environments (i) near the infected Japanese larch stands that were clear-cut in 2018–2019 (in Saint-Cadou, Hanvec and the new outbreak in Saint-Rivoal), (ii) around ornamental nurseries where *P. ramorum* had been detected in Bretagne over the last decade. In this area, *P. ramorum* was detected in about 50 ornamental nurseries since 2002, on *Rhododendron* spp. and *Viburnum* spp. (Fig. 1B). We selected seven nurseries, six where *P. ramorum* was detected in at least 3–4 different years with one detection in the last 5 years and one located close to the larch stands infected by the pathogen (about 7 km away). In addition, a garden with a extensive *P. ramorum* infection on a large *Rhododendron* sp. hedge was surveyed in May 2021. Samples collected during these surveys are listed in Table 2 in Appendix.

The selected sites were inspected for *P. ramorum* symptoms by teams of three observers in September 2019, September 2020, February 2021, and May 2021 (5 days of survey per visit). This pathogen can infect a wide range of woody hosts in these environments (Sansford et al., 2009; Desprez-Loustau et al. 2018). We focused on trees that represent a high risk such as chestnut (*Castanea sativa*) and oak (*Quercus ilex* L. and *Q. rubra L.*). These species, especially *C. sativa* and *Q. ilex*, combine a significant susceptibility with the ability to multiply the pathogen (foliar hosts) (Desprez-Loustau et al. 2018). Several other woody hosts that are abundant in the area and showed some leaf/shoot symptoms were also surveyed (*Vaccinium myrtillus, Calluna vulgaris Hull., Viburnum tinus, Camellia* spp., *Fraxinus excelsior* L., *Ilex aquifolium* L., *Lonicera periclymenum* L., *Sambucus nigra* L., *Rubus* spp.). The presence of symptoms was assessed in formerly infected larch stands and on possible host plants within a 50-m radius. In the infected larch stands located in the Saint-Cadou state forest, the surveys were extended to two areas. First, we surveyed the edge of the main dirt road crossing the forest where many chestnut trees had been planted several decades earlier. Second, the presence of chestnuts showing *P. ramorum* symptoms was surveyed in hedges within 500–800 m from the forest. Around the selected nurseries, we surveyed hedges and small woods that were located 50 to 100 m from the nursery, but not the nurseries themselves. When *P. ramorum* symptoms were observed, such as shoot mortality or foliar symptoms (necrotic spots to extensive lesions on leaves), samples were taken for confirmation by laboratory analysis. An attempt to quantify the survey effort was made by counting the number of individuals for each woody host surveyed (not possible for *Rubus* spp., *V. myrtillus* or *C. vulgaris).*

An outbreak of *P. ramorum* was identified on chestnuts located along the main road crossing the Saint-Cadou forest (Fig. 3B). At this location, five rain traps were placed under the mature chestnuts in October 2018 according to the methodology used in the larch stands (15 l buckets along the line at 5 m intervals). We also tried to better characterize the frequency of symptoms, by counting both the number of infected individuals and the total number of observed individuals, and by occasionally counting the number of infected suckers on infected individuals. Then, in February 2021, three chestnut trees were felled to assess whether *P. ramorum* had colonized the upper crown. Many of the trees in the plot were in advanced decline and trees with some live crown were selected. Selected trees either showed some symptoms of *P. ramorum*, usually dead suckers starting at the lower bole (Fig. 4) or were within 1–2 m of trees showing these symptoms. The presence of dead shoots with possible *P. ramorum* symptoms was then quantified for each in the lower (1–3 m from the ground), middle (5–7 m from the ground) and upper part of the trunk (> 10 m from the ground) by counting the number of symptomatic shoots in several groups of 10 shoots each.

2.3 Laboratory analysis for detection of *P. ramorum*

The *Rhododendron* leaves recovered from the rain traps were blotted dry and brought back to the laboratory in sealed bags. Presence of necrotic spots on each leaf was recorded. Isolation was attempted from 5 to 10 necrotic spots per trap, when available. Leaf fragments with the margin healthy/necrotic tissues were transferred to V8 juice agar selective medium (Legeay et al. 2020) which were incubated at 19 °C in the dark for 7 days. Isolates recovered were morphotyped and those suspected to be *P. ramorum were* DNA extracted using the DNeasy Plant mini-kit (Qiagen) and identified with real-time PCR (specific primer pairs Pram-C62-F/R/P, Lamarche et al. 2015).

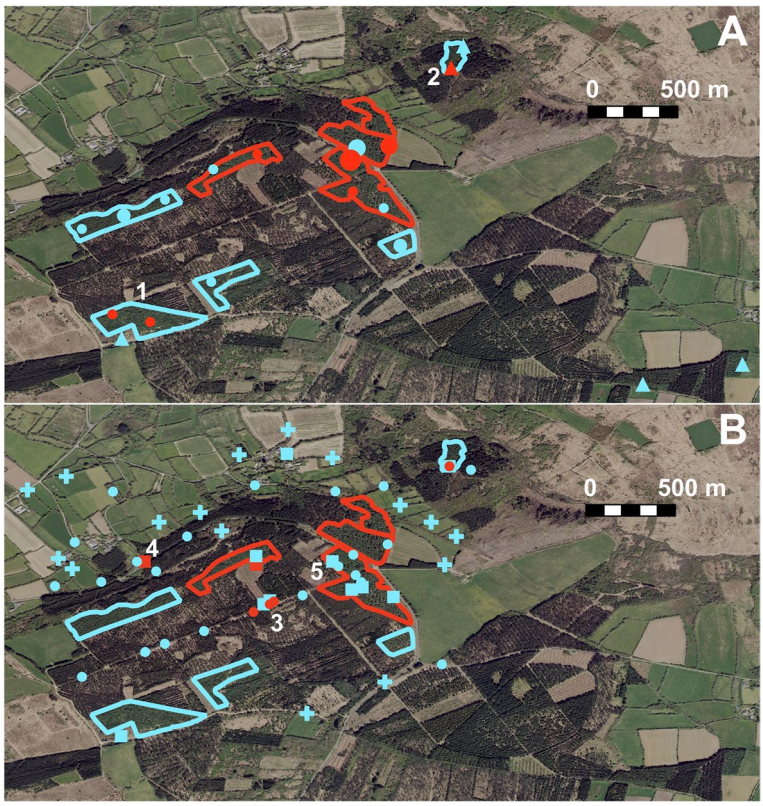

Fig. 3 Survey in the Saint-Cadou state forest area. **A** Pre-eradication survey. Plots with crown assessment (circle, size of the circle proportional to the proportion of tree with a crown assessment > 1), plots with no crown rating (triangle). *P. ramorum* detected (red) or not detected (blue). **B** Post-eradication survey. No suitable host present (plus), chestnuts present (circles), other hosts such as larch saplings, *Rhododendron* sp., *Vaccinium myrtillus* or *Calluna vulgaris* (squares). Larch stands clear-cut in 2018 or 2021 are indicated by red and blue lines. The stands outlined in red are the stands that were assumed to represent the inoculum sources in 2017s–2018 to assess the distance of *P. ramorum* dispersal. 1, Detection of *P. ramorum* in autumn 2017 in stands with crown in good condition; 2, Privately owned larch stand clear-cut in April–May 2021 where *P. ramorum* was first detected in 2019 and; 3, Plot where rain traps were placed under chestnuts in October 2018 and where trees were felled for examination of *P. ramorum* presence in the crown (declining mature chestnuts, and detection of *P. cinnamomi* on a dying seedlings); 4, wild rhododendrons patch near planted rhododendrons; 5, Multiple healthy looking larch and chestnut saplings within the larch stands that were clear-cut during the eradication treatment

Fig. 4 Symptoms of *P. ramorum* on *Castanea sativa*. **A**, **B** Typical necrotic lesions on shoots, (notice the black color of dead shoots). **C** Dead shoots are frequently observed on vigorous regrowth starting either from stumps or at the lower bole of heavily declining individuals

Litter samples were stored in the laboratory at − 20 °C until processing for detection of *P. ramorum.* The litter samples where ground twice for 30 s with a lab grinder (Kinematica MB550) in a 125 ml boro-silicate bowl. The grinding device was washed and disinfected between each sample by soaking in a 20% hydroxide peroxide solution for 20 min and then rinsed twice in distilled water. DNA from three subsamples of about 500 mg was extracted with the Fast DNA™ spin kit for soil (MPBio). Following grinding, samples were placed in Lysing Matrix E tubes provided with the kit and stored at − 20 °C until further processing. To improve DNA extraction, the lysis step was adapted to remove potential PCR inhibitors present in the samples. For this purpose, 15 mg PVPP was added to the sodium phosphate/MT buffer mixture (1100 µl volume) for each sub-sample of ground litter contained in the Lysing Matrix E tube. The subsamples were then shaken twice in a FastPrep mill (MPBio) for 40 s at 6.0 $m.s^{-1}$ to disrupt the tissue and release the content of the cells, and centrifuged for 10 min at 14,000×*g*. The next steps followed the instructions of the kit manufacturer. The supernatant from each litter subsample was transferred to a 2-ml tube with 250 µl of PPS and then homogenized and centrifuged for 5 min at 14,000×*g* to precipitate the proteins. To fix the DNA, the supernatant was transferred to 15 ml tubes containing 1 ml of binding matrix solution and placed in a rotator for 2 min at 14,000×*g*. The samples rested for 3 min to precipitate the binding matrix and 500 µl of supernatant was removed. The remaining solution was resuspended and transferred to a spin filter column for a 1-min centrifugation at 14,000×*g*. The filtered solution was removed and this filter step was repeated with the binding matrix solution remaining (after resuspending it). The DNA bound to the matrix in the spin filter column was washed with the addition of 500 µl of SEWS-M solution containing alcohol. After homogenization with a pipette, the tube was centrifuged at 14,000×*g* for 1 min. After complete filtration and removal of the filtrate, the DNA binding matrix was dried by centrifugation for 2 min at 14,000 g and placed in a collection tube and left for 5 min at room temperature to complete the drying. The final elution step was performed with the addition of 100 µl of DES solution and the solution was centrifuged a final time at 14,000×*g* for 1 min. The elution filtrate containing the extracted DNA was diluted with H_2O (1/50) and stored at − 20 °C until analysis.

The presence of *P. ramorum* in the litter DNA extracts was detected by real-time PCR (qPCR) (Lamarche et al. 2015). The reaction mixture for qPCR was 1×Brillant II master mix,0.02 µM reference dye supplied by Agilent Technologies, 0.01 U.µl-of UDG (New England BioLabs), 0.02 µM of primer pairs Pram-C62-F/R, 0.01 µM of probe Pram-C62-P 5 and 2 µl of DNA template, molecular grade water was added to 15 µL. The amplification reaction was run in a Quantstudio 6 thermocycler (Applied Biosystem) and was initiated by first pre-cycling steps at 37 °C for 10 min to activate the UDG and at 95° for 15 min to the initial denaturation by 50 cycles of denaturation at 95 °C for 15 s and hybridization /elongation at 62.5 °C for 90 s. The fluorescence was measured at the end of each hybridization/elongation step. A sample of plasmidic DNA containing the target region diluted at 1.10^{-9} $ng.\mu l^{-1}$ was included in each run and served as a limit of detection positive control (LOD). The DNA samples yielding a mean cycle threshold value (Ct value) inferior to the LOD mean Ct value was considerate as a positive detection.

Isolation of the pathogen was attempted from the collected symptomatic leaves and shoots. The samples were washed under water, surface sterilized (1 min in sodium hypochlorite at 3.75% active chlorine), and rinsed three times in sterile water. Pieces of the margin between necrotic and healthy tissues were plated on *Phytophthora*-selective V8 medium (Legeay et al. 2020). Mycelia growing out of the plated pieces were transferred to fresh V8 medium plates and later identified by qPCR test (Lamarche et al. 2015). During the isolation process, some of the selected tissue pieces were placed in Eppendorf tube for later analysis by DNA extraction and PCR. Extraction was performed using the Qiagen DNeasy plant minikit according to manufacturer instructions. The detection of *P. ramorum* DNA was performed by end-point PCR (Ioos et al. 2006). The end-point PCR method was used because preliminary attempts showed that the qPCR test of Lamarche et al. (2015) was unreliable for chestnut stems infected by *P. ramorum*.

2.4 Analysis of dispersal pattern in the Saint-Cadou area

To characterize the dispersal pattern of *P. ramorum* in the Saint-Cadou area, we hypothesized that the sources of inoculum were the larch stands severely affected by the pathogen that had been documented in 2017 (stands outlined in red in Fig. 3). The distance to these plot of all plots surveyed during 2017–2021, either in *L. kaempferi* stands or in *C. sativa*/rhododendrons was computed using GIS software. The number of surveyed and affected trees at each surveyed plot was computed. Trees were counted as presumably infected if they showed *P. ramorum* symptoms and the pathogen had been successfully detected on the plot. The presence and impact of *P. ramorum* were analyzed with a zero-inflated binomial model using the glmmTMB package of R. The proportion of affected plants at a surveyed location was examined in relation to the distance to the putative inoculum source. Distance to inoculum sources was introduced in both

the Bernoulli response (probability of *P. ramorum* presence) and in the binomial response (proportion of trees affected when *P. ramorum* was present).

2.5 Presence of *P. ramorum* in 1-year-old larch plantations

Each year, as part of the DSF's standard monitoring, forest stands planted during the previous winter are surveyed for plantation success. Any symptoms of tree disease in the surveyed plantations are reported. The stands investigated are representative of the tree species planted locally. We selected the larch stands surveyed in 2015–2021, which represents 33–52 stands per year (294 *L. decidua* and 12 *L.*×*eurolepis*, Fig. 5). Stands are surveyed twice, once in spring and once in autumn. Prior to 2019, no particular emphasis was placed on the presence of *P. ramorum*; however, any type of visible symptoms are required to be reported. After 2019, the surveyors were asked to report whether *P. ramorum* symptoms were present or absent in the larch stands and to send samples for analysis if they were observed.

2.6 Frequency of *P. ramorum* hosts in Bretagne

To investigate the frequency of potential hosts of *P. ramorum* in the study area, we used data from the French forest inventory (https://inventaire-forestier.ign.fr/dataifn/). The abundance of several potential woody hosts in Finistère, Bretagne outside of Finistère and France were determined within the 108,741 plots surveyed between 2005 and 2021 (750 plots in Finistère and 3116 in Bretagne). As some of the species, particularly rhododendrons, are common in non-forest situations and are poorly covered by the forest inventory data, we used data from the botanical conservatory of Brest (https://www.cbnbrest.fr/observatoire-plantes/cartes-de-repartition/ecalluna) on the presence of *R. ponticum* in the natural environment at the scale of the village (commune). This represents rhododendron populations established in natural environments, outside parks/gardens for Bretagne and Normandie. We then compared, at the scale of the commune, the presence of *P. ramorum* reported by the DSF in a forest with the presence of *R. ponticum* in the natural environment, using Fisher's exact test.

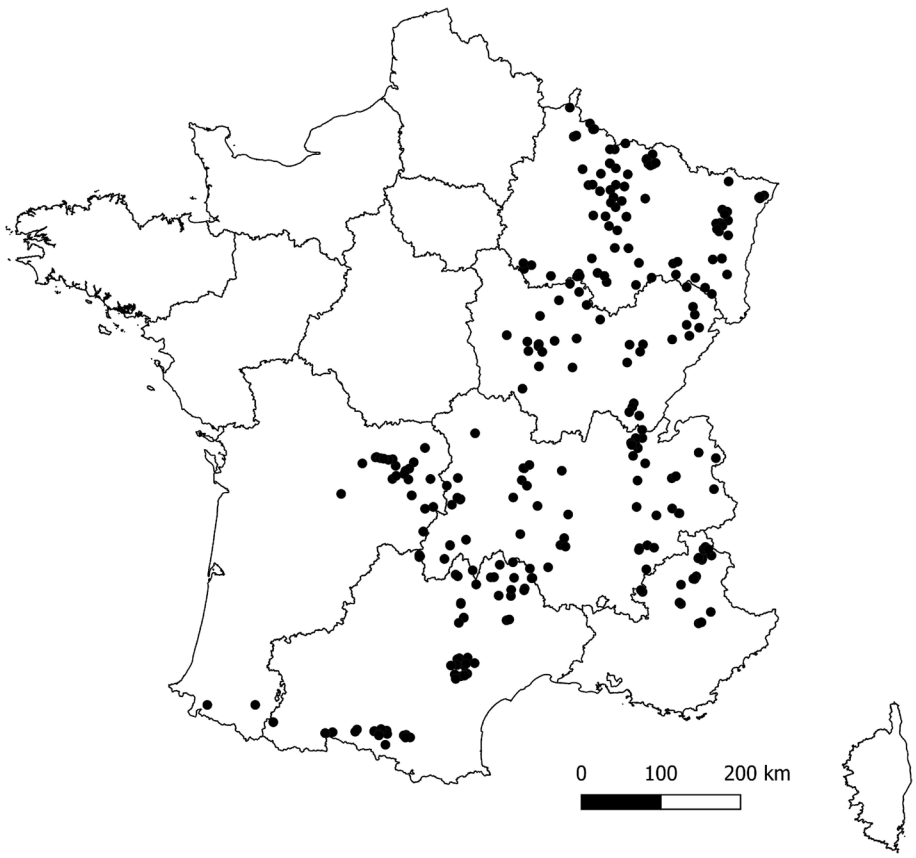

Fig. 5 One-year-old larch plantations surveyed by the DSF (Forest Health Survey System, 2015–2022). *P. ramorum* was never observed

3 Results

3.1 Survey in larch stands (pre-eradication treatment)

Fourty-nine Japanese larch plots were visited in Finistère between 2017 and 2021 (Fig. 2). However, the survey encountered difficulties: due to both the scarcity of *P. ramorum* detection and the speed of the eradication treatment, very limited replicates were available to compare the different detection methods. These results are not reported. Most of the rhododendron trap leaves collected in November had more than 10 lesions. However, *P. ramorum* was detected in the rain trap in only one out of the five stands; the five rain traps were positive. In December 2017, *P. ramorum was* recovered from litter sampled in six plots, including the plot with the positive rain trap in November. The six larch stands with detections of *P. ramorum* are all located in the Saint-Cadou state forest.

Detection of *P. ramorum* was scarce in 2018. In the spring, the presence of lesions on the rhododendron leaves from the rain traps was very limited (lesions in only 10 of the 109 traps). *P. ramorum* was detected in only two traps, both located in a larch plot in Saint-Rivoal at about 4.5 km east of the *P. ramorum* outbreak in the Saint-Cadou state forest (Fig. 2). No samples were collected from the Hanvec plot, as the stand had been clear-cut in winter 2017–2018. Detection from litter was also very limited, with *P. ramorum* detected in only 1 out of the 81 litter samples analyzed in spring 2018 (in the Saint-Cadou outbreak, 1–5 litter samples from 22 stands). Moderate crown symptoms were common in the surveyed plots with the presence of a few reddish or dead small branches in the upper crown (from 8 to 90% of observed trees depending on the plot). More severe symptoms such as death of the treetop to severe dieback were present only in the plots of Saint-Cadou and Saint-Rivoal where *P. ramorum* was detected, with a frequency of 30–50% of the observed trees. Some of the plots of the Saint-Cadou state forest where *P. ramorum* was detected in the litter in December 2017 still appeared very healthy, with only limited presence of moderate crown symptoms (8% of the trees with a crown status of 1 and none with a crown status > 1, Fig. 3A).

Detection of *P. ramorum* was even more limited in autumn 2018 with no detection in the 60 rain traps set up (12 larch stands) and in the 81 litter samples analyzed (33 larch stands). Necrotic spots were observed in only 10 of the 60 rain traps; but the pathogen was not isolated from them. No samples were collected in the Saint-Cadou forest, as all larch stands had been clear-cut in June 2018. *P. ramorum* was not detected in the Saint-Rivoal larch stands which were positive in April–May 2018. With the exception of Saint-Rivoal, the larch plots surveyed in autumn showed good crown condition with a maximum of 5–7% of trees having a crown rating above 1.

In autumn 2019, the detection of *P. ramorum* was slightly more common. The pathogen was detected in three out of 22 litter samples. The three positive samples were all from a privately-owned Japanese larch stand very close to the Saint-Cadou state forest (approximately 600 m from the *P. ramorum* outbreak eradicated in spring 2018, Fig. 3A). No samples were collected from the Saint-Rivoal plots which were clear-cut in winter 2018–2019. In February 2021, 2 out of the 10 litter samples analyzed were positive for *P. ramorum*. Both samples came from the privately owned Japanese larch stand that was previously positive in September 2019. This stand was clear-cut in April–May 2021.

3.2 Survey in the vicinity of outbreaks (post-eradication treatment)

A high diversity of potential woody hosts for *P. ramorum* was observed around the surveyed nurseries (Table 1). Many of them (*Viburnum tinus, Rhododendron* spp., *Camellia* spp.) were planted as ornamentals shrubs. Occasionally some host trees were also planted (*Q. ilex* and *Q. rubra*). The observations are summarized in Table 1. *P. ramorum* was detected around two nurseries located in the same village about 15 km north of the Saint-Cadou state forest (Fig. 2). In one case, the infected individuals were *Rhododendron* sp., probably transplanted from the nursery; one infected individual was observed in 2019 and another in 2020. In the second case, the infected individual was a chestnut from a hedge adjacent to the nursery. In both cases, *P. ramorum* could be isolated from the infected shoots. However, the infection was very localized (1–2 individuals) and not epidemiologically significant for the surrounding areas. The infected garden surveyed was located in the same village as the two nurseries where *P. ramorum* was detected. *P. ramorum* was observed only on the *Rhododendron* sp. hedge; about 10 mature *Q. rubra* in close contact with the infected hedge did not show symptoms that we could attribute to *P. ramorum*.

In the formerly infected larch plantation and the surrounding area, we observed mostly *C. sativa*, *V. myrtillus, C. vulgaris, Rhododendron* spp., and larch saplings (Table 1). In the Hanvec stand, *C. sativa* saplings were the only available hosts observed and were abundant with about 200 saplings present. Only one symptom (a dead shoot) was observed on the plot; *P. ramorum* was not isolated and the PCR test was negative. *P. ramorum* hosts were also present on the stands of Saint-Rivoal, with few remaining mature larches and several patches of *C. vulgaris*. However, *P. ramorum* was not detected in the Saint-Rivoal stands in 2019, 2020, and 2021.

Table 1 Results of 2019–2021 surveys on woody hosts within or in the neighborhood of former *P. ramorum* outbreaks (post-eradication survey)

Type	Species	Number of individuals observed	Number of individuals with symptoms	Number of samples	Number of *P. ramorum* detections
Forest settings	***Castanea sativa***	**> 1060**	**48**	**45**	**7**
	Ilex aquifolium	> 80	1	1	0
	Larix kaempferi	104	5	5	0
	***Rhododendron* spp.**	**> 100**	**7**	**6**	**2**
	Others: *Acer pseudoplatanus, Rubus* spp., *Taxus baccata*	14	3	3	0
	Total	**> 1600**	**33**	60	9
Nurseries surrounding, Garden	*Camellia* spp.	56	0	0	0
	Castanea sativa	**> 390**	**11**	**11**	**1**
	Fraxinus excelsior	46	6	6	0
	Ilex aquifolium	45	1	1	0
	Larix spp.	3	0	0	0
	Lonicera periclymenum	42	1	1	0
	Quercus ilex	17	1	1	0
	Quercus rubra	48	3	3	0
	***Rhododendron* spp.**	**> 230**	**19**	**15**	**6**
	Sambucus nigra	77	4	4	0
	Viburnum tinus	49	2	2	0
	Others (*Acer pseudoplatanus, Arbutus unedo, Calluna vulgaris, Euonymus europaeus, Ligustrum vulgare, Magnolia soulangeiana, Quercus palustris, Salix caprea, Syringa vulgaris)*	29	2	2	0
	Total	**> 1170**	**10**	**47**	**7**

By contrast, in the Saint-Cadou state forest, *P. ramorum* hosts were abundant with numerous Japanese larch and chestnut saplings that either appeared in the years following the clear-cut or pre-existed in a neighboring Scots pine stand (see Fig. 3B). Large patches of both *V. myrtillus* and *C. vulgaris* were also present in the larch stands that had been clear-cut in June 2018. However, no symptoms of *P. ramorum* were observed in 2019, 2020, and 2021 in the area directly under the formerly infected larch.

A large patch of wild *Rhododendron* sp. of about 100–200 m^2 was present at the border of the state forest, close to a group of houses where a few *Rhododendron* sp. individuals had been planted (Fig. 3B). In February 2021, dead shoots were observed on two individuals out of more than a hundred surveyed. *P. ramorum* was recovered by isolation from these dead shoots. We re-surveyed the patch in May 2021 but did not observe any additional symptomatic individuals even though the individuals infected a few months earlier had not been removed. The *Rhododendron* sp. patch was located at 500–600 m from the *P. ramorum*-infected larch stands observed in 2017–2018.

Finally, mature chestnut trees had been planted along the main dirt road through the forest several decades before the *P. ramorum* epidemic (Fig. 3B). Many of these chestnuts were in advanced decline and discussions with the forest managers indicated that the decline was not new with recurrent salvage logging resulting in many stumps, often with stools growing from them. The cause of the decline appeared to be complex. Large basal cankers were commonly observed on the declining chestnuts and isolation from the root systems of a nearby dying seedling yielded *P. cinnamomi*. No *P. ramorum* was detected in the rain traps set up in the area in October 2018 (Fig. 3B, absence of necrotic spots). An increasing trend in disease severity was observed in later years. In 2019, symptoms of *P. ramorum* were very infrequent on the plot. One infected sucker was observed at the base of a tree and tested positive for *Phytophthora* by Elisa test (lateral flow device), but we did not detect *P. ramorum* from this sample either by isolation or PCR. However, *P. ramorum* was isolated from similar symptoms on the same tree 1 year later. In fact, in September 2020, *P. ramorum* symptoms were more frequent on a section

approximately 250 m long (Fig. 3B). The observed symptoms were either dying suckers at the base of severely declining trees or dead stool on stumps. A count of infected individuals showed that 30–50% of suckers at the base of trees or on stumps showed necrosis typical of *P. ramorum* (Fig. 4). Lastly, in February 2021, 13 ± 3.6% of the chestnuts in this section showed dead shoots typical of *P. ramorum* and the pathogen was isolated from the 6 samples collected in the area in 2020–21. The crown of the three felled chestnuts was carefully observed for the presence of *P. ramorum* symptoms. No infected shoots were found in the upper crowns (number of shoots observed was 73, 122, and 86). On one of the trees, symptoms typical of infected shoots were observed at 5–6 m from the ground (10 shoots with symptoms out of the 32 observed); *P. ramorum* was recovered from one of the shoots. The other two felled trees showed typical dead shoots on the trunk within 1–2 m from the ground. In order to eradicate the infestation, the chestnut trees on the infected section of the dirt road were felled in the summer of 2021 and all stumps were ground to prevent re-sprouting.

We also surveyed hedges within 500–800 m of the Saint-Cadou state forest. Chestnut was scarce in this area, present in small groups of less than 10 individuals in most locations (Fig. 3B). We did not observe typical symptoms of *P. ramorum* in any of the hedges, and the pathogen was not detected by PCR or isolation in any of the five samples collected.

3.3 Analysis of dispersal pattern in the Saint-Cadou area

We attempted to estimate the dispersal pattern of *P. ramorum* in the Saint-Cadou area, with the hypothesis that the sources of inoculum were the larch stands severely affected in 2017 (Fig. 3). The median dispersal distance was 350 m (interquartile interval of 300–460 m) with dispersal events up to 1000 m (Fig. 6). The relationship between the frequency of *P. ramorum* detection and the distance to putative inoculum sources was not significant (zero-inflated model part, p value = 0.564). In contrast, the proportion of symptomatic hosts when *P. ramorum* was detected on the plot was significantly related to the distance to putative inoculum sources (p value < 0.001). This lack of a significant relationship between pathogen presence and distance to inoculum sources can be explained by the absence of *P. ramorum* symptoms on the chestnut and larch saplings present in 2019–2021 in the stands where larch had been eradicated in 2018.

3.4 Presence of *P. ramorum* in 1-year-old larch plantations

In total, 286 1-year-old larch plantations were visited by the DSF between 2015 and 2021 (274 *L. decidua* and 12

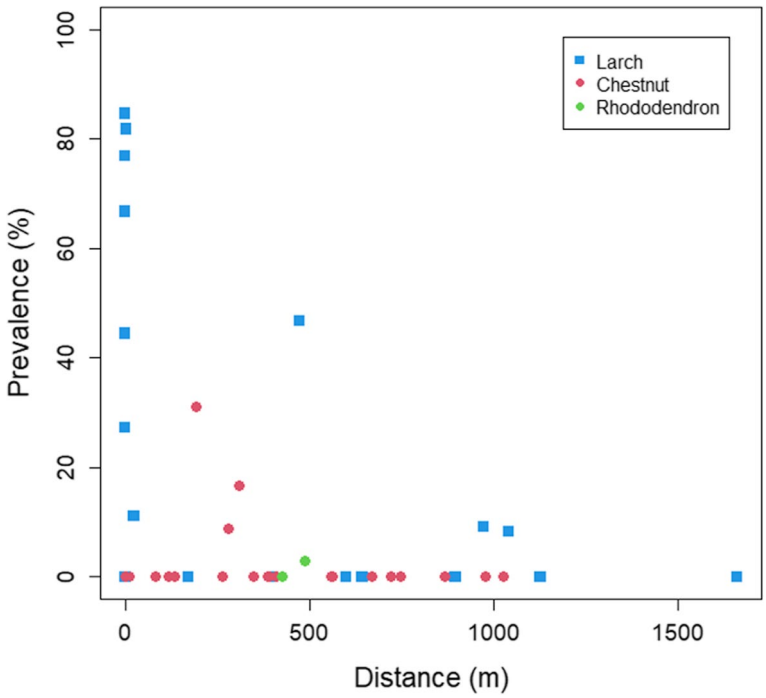

Fig. 6 *Phytophthora ramorum* dispersal pattern in the Saint-Cadou area. Prevalence is the proportion of plants with *P. ramorum* symptoms (trees or shrubs) at each surveyed plot

L. × *eurolepis)*. The surveyed stands were mainly located in eastern France and in the Massif Central, where most larch is planted in France (Fig. 5). No 1-year-old plantations were surveyed in Bretagne. No symptoms of *P. ramorum* were reported in any of the stands surveyed.

3.5 Frequency of *P. ramorum* hosts in Bretagne

The main susceptible species of *P. ramorum* in forest stands, both in Finistère and in Bretagne excluding Finistère (see Figs. 1B and 2 for localization), is chestnut present in over 20% of the stands (20.6% in Finistère and 24.3% in Bretagne). However, chestnut is particularly common along the southern Bretagne coast and is less common in the area where the *P. ramorum* outbreaks occurred (Fig. 2). Common susceptible species include *V. myrtillus* and *C. vulgaris* present in 7–12% of the forests stands in Finistère and in Bretagne. Larch, holm oak, and red oak are infrequent, present in less than 1% of the forest stands in both Finistère and Bretagne (0.7 and 0.4% respectively). Holm oaks are mainly found along the coast. Finally, while Finistère represents the French region with the largest presence of *R. ponticum* in forests, the species is present in only 1.7% of the surveyed stands (0.5% in Bretagne). Overall, in France, *R. ponticum* is present in 0.05% of the stands surveyed by the forest inventory teams.

However, the species is much more frequently reported by the Brest Botanical Conservatoire. It is reported as a wild species in the natural environment in 30% of the villages of Finistère, but it is less frequently in Bretagne and in Normandie (5.7 and 1.5% of the villages respectively). The occurrence of DSF reports on the presence of *P. ramorum* in the forest is significantly correlated with the presence of *R. ponticum* in the natural environment in the village (Fisher exact test p value of 0.02).

4 Discussion

The evolution of the presence of P. *ramorum* in western France matches the pattern reported in the United Kingdom and Ireland (Brasier and Webber 2010; McCracken et al. 2015; O'Hanlon et al. 2018; Webber et al. 2017), arriving first in ornamental nurseries, then in rhododendrons in the natural environments, Japanese larch stands and finally on chestnut trees. However, the presence of *P. ramorum* in forest situations in France was much less frequent than in the UK, with very few outbreaks detected during the surveys. The pathogen persisted after eradication in the larger outbreak, in the Saint-Cadou state forest, with spread to nearby chestnut and larch trees.

We showed that *P. ramorum* is still infrequent in larch stands of Finistère. The pathogen was detected in autumn 2017 in several stands throughout the Saint-Cadou state forest, even in stands with few crown symptoms. However, in the spring of 2018, detection was scarce. The time of sampling was probably the main reason for this pattern. In autumn, we sampled at needle shedding and the presence of viable *P. ramorum* inoculum was abundant on the sampled larch needles. This high frequency of *P. ramorum* at the time of needle shedding has been reported by Webber et al. (2010). Conversely, in 2018, we sampled in early May due to the planned clearcutting and this period was likely sub-optimal for the pathogen recovery. The poor recovery in the Saint-Rivoal stands in the 2018 autumn is more surprising as the period in late October and December should have been favorable. The dieback in Saint-Rivoal was severe and the presence of *P. ramorum* was expected to be high. We assume that other environmental factors such as high tree density and lack of management contributed to the severity of the dieback in Saint-Rivoal.

C. sativa represents a high risk for the development of *P. ramorum* in the area as it is often found in forest stands and hedgerows. The ability of chestnut to support a *P. ramorum* outbreak was demonstrated in the UK. Denman et al. (2006) and Harris and Webber (2016) showed that chestnut enables the pathogen multiplication (so-called sporulating host) while Webber et al. (2017) reported outbreaks on chestnut trees in the absence of other sporulating hosts such as *Larix spp.* or rhododendron in the vicinity. Our observations in the Saint-Cadou forest support this view. We observed the onset of an outbreak on chestnut trees, despite the removal of all larches by clear-cutting 2–3 years earlier. Chestnut trees did not appear to be directly infected in their crowns. Crown-to-crown spread of *P. ramorum* has been described in western North America on *N. densiflorus* and in the UK on *Larix spp.* (Webber et al. 2010; Peterson et al. 2015) and we expected the chestnuts at Saint-Cadou to be infected in this way as they were located approximately 300–400 m from heavily infected larch stands. However, the infection was localized close to the ground and *P. ramorum* was absent from the upper crowns of the three felled trees. Symptoms of *P. ramorum* were very rare in 2019 and we assumed that the outbreak started from a limited source of inoculum remaining after the eradication process, either from a few chestnut or larch saplings or from understory susceptible shrubs. Indeed, *V. myrtillus* and *C. vulgaris* were abundant in the stands and, although we did not observe symptoms of *P. ramorum*, this source cannot be ruled out as they have been reported

to support inoculum production (Sansford et al. 2009; Harris and Webber 2016). All symptoms observed on chestnuts corresponded to dead, formerly vigorous sprouts from the lower boles of mature trees or from stumps (Fig. 4). No symptoms were observed on the numerous chestnut saplings that appeared post-eradication in the clear-cut infected larch stands. This is surprising since the presence and survival of *P. ramorum* have been reported in the litter of infected stands several years post-eradication (Fichtner et al. 2007, 2009; Harris 2014; Goheen et al. 2017). Thus, it was expected that the saplings of susceptible species that appeared in the stands would be infected by splashing from the soil (Goheen et al. 2008; Harris 2014; Hansen et al. 2019). However, it has been reported that despite the survival of *P. ramorum* in the soil, little infection of the susceptible vegetation is observed during post-eradication monitoring (Goheen et al. 2017; McCracken et al. 2015; O'Hanlon et al. 2018); the opening of stands apparently creates conditions unfavorable to *P. ramorum.*

The *P. ramorum* eradication programs conducted in Oregon, the UK, and Ireland concluded that while eradication failed at the regional level, *P. ramorum* symptoms were no longer observed in many of the treated sites (McCracken et al. 2015; Goheen et al. 2017; O'Hanlon et al. 2018; Hansen et al. 2019). Undetected foci provided a sufficient amount of inoculum to keep the epidemic active often because the eradication was conducted too late. Our observations are consistent with these conclusions. In two of the forests with eradication treatment (Hanvec and Saint-Rivoal, respectively < 1 and 8 ha), we did not detect symptoms of *P. ramorum* in the 2–3 years following the clearcut, while in the larger outbreak, in Saint-Cadou (13 ha), the pathogen persisted at a low level in nearby chestnut and larch trees. We cannot claim successful eradication in the treated stands with the survey effort undertaken: with about 230 chestnut saplings observed, we can only say that the proportion of infected chestnuts in clearcut larch stands of Saint-Cadou was less than 1.3% (Parnell et al. 2017). Also, *P. ramorum* may have survived to a limited extent in the soil and litter for several years after eradication as shown elsewhere (Turner et al. 2006; Goheen et al. 2008, 2017; Harris 2014; O'Hanlon et al. 2018). However as discussed above, residual litter or soil inoculum may not result in significant infection of the remaining susceptible hosts (Hansen et al. 2019). The *P. ramorum* outbreaks in Saint Cadou, Hanvec, and Saint Rivoal appear to remain under control with only a few new foci reported. The elimination of most of the inoculum production during the eradication process contributed to disease control. In particular, all larch was removed, leaving no sporulating trees; while infected sporulating shrubs may have persisted, they have been shown to disperse the pathogen to a smaller range of 10–20 m because of their smaller height (Clarke et al. 2021). The dispersal observed in Saint-Cadou was within the range reported in Oregon at the onset of the epidemic (Hansen et al. 2008). The result must be interpreted with caution as inoculum sources are not fully known: asymptomatic infection can occur on larch (Harris and Webber 2016). Similarly, in Oregon, although the program ultimately failed to eradicate *P. ramorum* from the forest, it was shown to have significantly slowed down the epidemic (Peterson et al. 2015; Goheen et al. 2017; Hansen et al. 2019). The main specificity of the Finistère case may be the limited availability of suitable hosts. Larch is infrequent. With 27 ha of monospecific larch plantation, the Saint-Cadou state forest was the forest with the largest area of monospecific larch plantation in Finistère (average area of monospecific larch plantation per forest of 6.1 ha, interquartile interval of [2.1, 7.7], source https://geoservices.ign.fr/bdforet). Chestnut, although present in 21% of Finistère forest stands was not abundant in the Saint Cadou, Saint Rivoal, and Hanvec stands. Within the pathogen's dispersal range (i.e., 500 m from the infected stands), we did not observe many chestnuts in the hedgerows and forest in the Saint-Cadou area, which greatly facilitated the control of the disease. Since larch was not a critical forest component, it was easier to decide to remove all *Larix* in the three affected forests. In Saint-Cadou, healthy larch stands located at more than 500 m from any recorded infected stands were clear-cut. This situation contrasts with Oregon where N. *densiflorus* are abundant in the area affected by *P. ramorum* (Peterson et al. 2015) or with UK where *L. kaempferi* represent large areas (about 5–6% of the forest, Harris 2014; McCracken et al. 2015). Parnell et al. (2010) found that host density and aggregation strongly influence the outcome of eradication programs, affecting both the optimal size of the culling area and the duration of the epidemic. Another important feature is the rainfall pattern. *P. ramorum* inoculum production and dispersal are strongly enhanced by wet conditions and the eradication program in Oregon was abandoned after 1 year of exceptional spring rainfall (Peterson et al. 2015). Saint Cadou, Saint Rivoal, and Hanvec are located in an area with high rainfall (an average of about 1200 mm per year). The study period was characterized by average rainfall in the area.

New outbreaks could be expected after a year with a very wet vegetation period. In conclusion, we assume that large clear-cuts of larch stands were an efficient strategy to control the disease in Finistère. It created unfavorable conditions for the survival of *P. ramorum* in the treated stands, and it limited the spread of the pathogen due to the limited number of larches in the vicinity. Eradication remains a controversial measure, especially under forest conditions. Branco et al. (2023) evaluated the success of eradication implemented in Europe for forest pests either insects or microorganisms, from 1945 to 2022. While the success was high in confined environments and when the outbreak was smaller than 1 ha (about 80%), it became far less effective in the natural environment, and when the outbreak area was larger than 10 ha (less than 50% success rate). The outbreaks of *P. ramorum* on larch in Finistère, with an area of 1–13 ha, are in the range of area that makes eradication difficult.

As reported elsewhere (Mascheretti et al. 2008; Grünwald et al. 2016), the most likely pathway for the transfer of *P. ramorum* to forest stands is the planting of infected ornamental plants. We documented the presence of *P. ramorum* on a chestnut hedge adjacent to a formerly contaminated ornamental nursery. Although the outbreak was limited to only a single infected tree, it may have been locally significant, partly explaining why *P. ramorum* was detected in the nursery for several years. In the Saint-Cadou state forest, the largest forest outbreak, infected wild rhododendrons, were present up to 500 m from the infected larch stands and in close proximity to planted rhododendrons. Finally, we observed a correlation between the presence of rhododendron populations in the natural environment in a village and the detection of *P. ramorum* in the forest by the DSF. On the other hand, we could not document any transfer of the pathogen during the planting of infected larch seedlings from forest nurseries (no observation of *P. ramorum* in 1-year-old plantations).

The likelihood of transfer of *P. ramorum* from infected ornamental plants to forest stands in other parts of France is of considerable interest. Most of the ornamental nurseries where *P. ramorum* was detected are located in Bretagne and Pays de Loire. Nevertheless, the pathogen has been detected in retailers throughout the country (Fig. 1B), suggesting that the risk is widespread. However, the risk of transfer also depends on the proximity between planted infected ornamentals and susceptible wild woody species, either trees or shrubs. Wild rhododendron populations appear to be important in bridging the gap between planted-infected ornamentals and forests and are very rare in France. Wild rhododendrons occur mainly in the Finistère, the area where the Saint Cadou, Saint Rivoal, and Hanvec forests are located. While larch is not frequent in Bretagne and should no longer be planted there, this is not the case in other regions of France, as hybrid larch between *L. kaempferi* and *L. decidua* (*L.* × *eurolepis*) is increasingly being planted in the mountains of eastern and central France, in areas with a climate favorable to *P. ramorum* (Desprez-Loustau et al. 2018). Hybrid larch has been assessed as susceptible by Harris and Webber (2016) and is not considered a replacement for *L. kaempferi* in the UK. These hybrid larch stands are at high risk and will require very careful monitoring in the future. While chestnut is the main risk identified in France, it must be noted that the susceptibility of a large fraction of the woody hosts present in France to *P. ramorum* remains unknown (Desprez-Loustau et al. 2018). The observation of severe outbreaks of larch in the UK in 2010 was unexpected (Brasier and Webber 2010), and additional host jumps may occur as the pathogen encounters other possible hosts in novel ecosystems.

5 Conclusion

Overall, a very limited presence of *P. ramorum* was detected in the forests of Finistère during the survey. While the pathogen persisted at the main outbreak site of Saint-Cadou after the eradication treatment, the outbreak is still under control. Ongoing surveillance and the scarcity of suitable hosts on the site should limit the risk in Saint-Cadou. The main risk in Bretagne is probably the existence of undetected outbreaks on planted ornamentals that might transfer the pathogens to nearby hedges or forests. During the time frame of this study, two outbreaks of Rhododendrons, in one case recently planted, were detected. The EU regulation on *P. ramorum* has recently changed and only non-EU isolates of the pathogen remain quarantine pests; forest outbreaks caused by isolates already present in the EU thus no longer need to be eradicated from a regulatory perspective. This may however not change the management of *P. ramorum* in France: French authorities have recommended eradication treatment whatever the origin of the isolates (EU or not EU) and we can hope that this policy will be maintained.

Appendix

Table 2 Schedule of the pre and post-eradication surveys with the number of samples

Name	Year	Month	eradication	Species sampled	*N* plots	*N* sample			N sample with *P ramorum*			N plots with
						Litter	Rain trap	Plants	Litter	Rain trap	plants	*P. ramorum* detection
Commana	2018	10	Pre	*L. kaempferi*	1	1	5	–	0	0	–	0
	2018	12	Pre	*L. kaempferi*	2	6	–	–	0	–	–	0
	2019	11	Pre	*L. kaempferi*	1	3	–	–	0	–	–	0
	2021	02	Pre	*L. kaempferi*	1	1	–	–	0	–	–	0
Glujau Astach	2018	10	Pre	*L. kaempferi*	4	9	–	–	0	–	–	0
Hanvec	2017	11	Pre	*L. kaempferi, Vaccinium myrtillus*	1	0	5	1	–	0	0	0
	2021	05	Post	*C. sativa*	2	–	–	2	–	–	0	0
Huelgoat	2018	10	Pre	*L. kaempferi*	5	4	25	–	0	0	–	0
Kervel	2018	12	Pre	*L. kaempferi*	1	2	–	–	0	–	–	0
	2019	11	Pre	*L. kaempferi*	1	1	–	–	0	–	–	0
Landivisiau	2018	12	Pre	*L. kaempferi*	1	1	–	–	0			0
Lanrodec	2017	11	Pre	*L. kaempferi*	1	0	5	5	-	0	0	0
Loperec	2018	12	Pre	*L. kaempferi*	1	1	5	–	0	0	–	0
	2019	11	Pre	*L. kaempferi*	1	1	–	–	0	–	–	0
Menez Meur	2017	11	Pre	*L. kaempferi*	1	–	5	–	–	0	–	0
	2018	05	Pre	*L. kaempferi*	4	8	–	–	0	–	–	0
	2018	12	Pre	*L. kaempferi*	5	9	–	–	0	–	–	0
Nursery 1	2019	09	Post	*Arbutus unedo, Camellia spp, C. sativa, Fraxinus excelsior, Larix spp, Ligustrim vulgare, Quercus ilex, Q.rubra, Sambuccus nigra*	–	–	–	5	–	–	0	0
	2020	09	Post		–	–	–	6	–	–	0	0
Nursery 2	2019	09	Post	*C.sativa, Euonymus europaeus, F. excelsior, Ilex aquifolium, L. periclymenum, Prunus lustanica,V. tinus, Rhododendron, S. nigra*	–	–	–	4	–	–	0	0
	2020	09	Post		–	–	–	2			0	0

Name	Year	Month	eradication	Species sampled	*N* plots	*N* sample			N sample with *P ramorum*			N plots with
						Litter	Rain trap	Plants	Litter	Rain trap	plants	*P. ramorum* detection
Nursery 3	2019	09	Post	*Camelia spp, C.sativa, F. excelsior, Ilex aquifolium, L. periclymenum, Magnolia soulangeiana, Quercus rubra, Rhododendron, Salix caprea, S. nigra, Viburnum tinus*	–	–	–	4	–	–	**1**	**1**
	2020	09	Post		–	–	–	1	–	–	**1**	**1**
Nursery 4	2019	09	Post	*Acer pseudoplatanus, Camelia spp, C.sativa, F. excelsior, Ilex aquifolium, L. periclymenum, Rhododendron, S. nigra, Syringa vulgaris, V. tinus*	–	–	–	2	–	–	0	0
		09			–	–	–	1	–	–	0	0
Nursery 5	2019	09	Post	*Camelia spp, C.sativa, F. excelsior, Ilex aquifolium, L. periclymenum, V. myrtillus, Rhododendron, V. tinus*	–	–	–	3	–	–	0	0
	2020	09			–	–	–	6	–	–	0	0
Nursery 6	2020	09	Post	*A. unedo, Calluna vulgaris, Camellia spp, C. sativa, Rhododendron, S. nigra*	–	–	–	1	–	–	**1**	**1**
	2021	02	Post		–	–	–	2	–	–	0	0
	2021	05	Post	*C. sativa*	–	–	–	3	–	–	0	0
Nursery 7	2021	05	Post	*C.sativa, Rhododendron*	–	–	–	3	–	–	0	0
Plougar garden	2021	02	Pre	*Rhododendron, Q. rubra*	1	–	–	4	–	–	**2**	**1**
Penn Ar Guer	2018	12	Pre	*L. kaempferi*	2	6	–	–	0	–	–	0
	2019	11	Pre	*L. kaempferi*	1	1	–	–	0	–	–	0
Pleyben	2018	10	Pre	*L. kaempferi*	1	1	5	–	0	0	–	0
	2018	12	Pre	*L. kaempferi*	1	2		–	0	–	–	0
Pont de Buis	2018	10	Pre	*L. kaempferi*	1	–	5	–	–	0	–	0
Pleyber Christ	2018	12	Pre	*L. kaempferi*	1	3	–	–	0	–	–	0
Roudouderch	2018	12	Pre	*L. kaempferi*	2	7	–	–	0	–	–	0
	2019	11	Pre	*L. kaempferi*	2	2	–	–	0	–	–	0
	2021	02	Pre	*L. kaempferi*	1	1	–	–	0	–	–	0

Name	Year	Month	eradication	Species sampled	*N* plots	*N* sample			N sample with *P ramorum*			N plots with
						Litter	Rain trap	Plants	Litter	Rain trap	plants	*P. ramorum* detection
Saint Cadoux	2017	11	Pre	*L. kaempferi, V. myrtillus*	1	–	5	3	**-**	**5**	**2**	**1**
	2017	12	Pre	*L. kaempferi*	6	6	–	–	**6**	–	–	**6**
	2018	05	Pre	*L. kaempferi*	14	53	69	2	**1**	**0**	**2**	**1**
	2018	10	Pre	*C. sativa*	1	–	5	–	–	0	–	0
	2018	12	Post	*L. kaempferi*	3	5		–	0		–	0
	2019	09	Post	*A. pseudo-platanus, C.sativa, L. kaempferi, Ilex aquifolium, V. myrtillus*	4	–	–	10	–	–	0	0
	2019	09	Post	*C.sativa, L. kaempferi, C. vulgaris, V. myrtillus*	4	–	–	7	–	–	**4**	**1**
	2019	11	Pre	*L. kaempferi*	2	13	–	–	**3**	–	–	**1**
	2021	02	Post	*C.sativa, L. kaempferi, Rhododen-dron*	7	5	–	18	2	–	4	**2**
	2021	05	Post	*C.sativa, Rho-dodendron, Rubus spp.*	15	–	–	19	–	–	0	0
Saint Rivoal	2018	05	Pre	*L. kaempferi*	4	20	20	–	0	**4**	–	**2**
	2018	10	Pre	*L. kaempferi*	2	2	20	–	0	0	–	0
	2018	12	Post	*L. kaempferi*	4	20	–	–	0	–	–	0
	2019	09	Post	*Ilex aquifo-lium, Taxus baccata V. myrtillus*	4	–	–	3	–	–	00	0
	2019	11	Post	*L. kaempferi*	1	1	–	–	0	–	–	0
	2020	09	Post	*V. myrtillus*	–	–	–	1		–	0	0
	2021	02	Post	*L. kaempferi*	1	3	–	–	0	–	–	0
Saint Sau-veur	2018	12	Pre	*L. kaempferi*	1	2	–	–	0	–	–	0

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Authors' contributions
RI, SL, and BM designed the study. AB, SL, and BM gathered, analyzed, and interpreted the data and were major contributors in writing the manuscript. CH provided the DSF data. CH and RI participated in writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References

Branco S, Douma JC, Brockerhoff EG et al (2023) Review of eradication attempts against non-native pest and pathogens of woody plants in Europe. Neobiota 84:281–317. https://doi.org/10.3897/neobiota.84.95687
Brasier C, Webber J (2010) Sudden larch death. Nature 466:824–825. https://doi.org/10.1038/466824a
Clarke M, Guitan-Castrillon JM, Herrero ML et al (2021) Report of a pest risk management for *P. kernoviae* and *P. ramorum*. European and Mediterranean Plant Protection Organization (EPPO). https://pra.eppo.int/pra/5c4a233e-b6d2-4b83-99f2-572b50287636. Accessed 26 Oct 2023
Davidson JM, Wickland AC, Patterson HA et al (2005) Transmission of *Phytophthora ramorum* in mixed-evergreen forest in California. Phytopathology 95:587–596. https://doi.org/10.1094/PHYTO-95-0587
Davidson JM, Patterson HA, Rizzo DM (2008) Sources of Inoculum for *Phytophthora ramorum* in a Redwood Forest. Phytopathology 98:860–866. https://doi.org/10.1094/PHYTO-98-8-0860
Denman S, Kirk S, Whybrow A et al (2006) *Phytophthora kernoviae* and *P. ramorum*: host susceptibility and sporulation potential on foliage of susceptible trees. EPPO Bull 36:373–376. https://doi.org/10.1111/j.1365-2338.2006.01014.x
Desprez-Loustau M-L, Aguayo J, Dutech C et al (2016) An evolutionary ecology perspective to address forest pathology challenges of today and tomorrow. Ann for Sci 73:45–67. https://doi.org/10.1007/s13595-015-0487-4
Desprez-Loustau ML, Robin C, loos R, et al (2018) Espèces hôtes dans le cadre de la lutte contre *Phytophthora ramorum*. ANSES collective expertise report 2017-SA-0259. https://www.anses.fr/fr/system/files/SANTVEG2017SA0259Ra.pdf. Accessed 15 Feb 2023
Fichtner EJ, Lynch SC, Rizzo DM (2007) Detection, distribution, sporulation, and survival of *Phytophthora ramorum* in a California Redwood-Tanoak Forest Soil. Phytopathology 97:1366–1375. https://doi.org/10.1094/PHYTO-97-10-1366
Fichtner EJ, Lynch SC, Rizzo DM (2009) Survival, dispersal, and potential soil-mediated suppression of *Phytophthora ramorum* in a California Redwood-Tanoak Forest. Phytopathology 99:608–619. https://doi.org/10.1094/PHYTO-99-5-0608
Garbelotto M, Schmidt D, Swain S et al (2017) The ecology of infection between a transmissive and a dead-end host provides clues for the treatment of a plant disease. Ecosphere 8:e01815. https://doi.org/10.1002/ecs2.1815
Ghelardini L, Pepori AL, Luchi N et al (2016) Drivers of emerging fungal diseases of forest trees. For Ecol Manage 381:235–246. https://doi.org/10.1016/j.foreco.2016.09.032
Goheen EM, Hansen EM, Kanaskie A, McWilliams MG, Osterbauer N, Sutton W (2002) Sudden oak death caused by *Phytophthora ramorum* in Oregon. Plant Dis 86:441–441. https://doi.org/10.1094/PDIS.2002.86.4.441C
Goheen E, Kanaskie A, Navarro S, Hansen E (2017) Sudden oak death management in Oregon tanoak forests. For Phytophthoras 7:45–53. https://doi.org/10.5399/osu/fp.7.1.4030
Goheen EM, Hansen EM, Kanaskie A et al (2008) Vegetation response following Phytophthora ramorum eradication treatments in southwest Oregon forests. In: Frankel JS, Kliejunas TJ, Palmieri MK (tech. Coords.) Proceedings of the sudden oak death third science symposium. Gen. Tech. Rep. PSW-GTR-214. U.S. Department of Agriculture, Forest Service, Pacific Southwest Research Station, Albany, p 301. https://www.fs.usda.gov/research/treesearch/29915. Accessed 11 Jan 2024
Grünwald NJ, Larsen MM, Kamvar ZN et al (2016) First report of the EU1 clonal lineage of *Phytophthora ramorum* on tanoak in an Oregon forest. Plant Dis 100:1024–1024. https://doi.org/10.1094/PDIS-10-15-1169-PDN
Hansen EM, Parke JL, Sutton W (2005) Susceptibility of Oregon forest trees and shrubs to *Phytophthora ramorum*: a comparison of artificial inoculation and natural infection. Plant Dis 89:63–70. https://doi.org/10.1094/PD-89-0063
Hansen EM, Kanaskie A, Prospero S et al (2008) Epidemiology of *Phytophthora ramorum* in Oregon tanoak forests. Can J For Res 38:1133–1143. https://doi.org/10.1139/X07-217
Hansen E, Reeser P, Sutton W, Kanaskie A et al (2019) Efficacy of local eradication treatments against the sudden oak death epidemic in Oregon tanoak forests. Forest Pathol 49:e12530. https://doi.org/10.1111/efp.12530
Harris AR, Webber JF (2016) Sporulation potential, symptom expression and detection of *Phytophthora ramorum* on larch needles and other foliar hosts. Plant Pathol 65:1441–1451. https://doi.org/10.1111/ppa.12538
Harris AR, Mullett MS, Webber JF (2018) Changes in the population structure and sporulation behaviour of Phytophthora ramorum associated with the epidemic on Larix (larch) in Britain. Biol Invasions 20:2313–2328. https://doi.org/10.1007/s10530-018-1702-7
Harris AR (2014) The epidemiology of *Phytophthora ramorum* associated with Larix in the UK. Ph.D. https://doi.org/10.25560/24862
loos R, Laugustin L, Schenck N et al (2006) Usefulness of single copy genes containing introns in *Phytophthora* for the development of detection tools for the regulated species *P. ramorum* and *P. fragariae*. Eur J Plant Pathol 116:171–176. https://doi.org/10.1007/s10658-006-9051-2
Lamarche J, Potvin A, Pelletier G et al (2015) Molecular detection of 10 of the most unwanted alien forest pathogens in Canada using real-time PCR. PLoS One 10:e0134265. https://doi.org/10.1371/journal.pone.0134265
Legeay J, Husson C, Boudier B et al (2020) Surprising low diversity of the plant pathogen *Phytophthora* in Amazonian forests. Environ Microbiol 22:5019–5032. https://doi.org/10.1111/1462-2920.15099
Mascheretti S, Croucher PJP, Vettraino A et al (2008) Reconstruction of the sudden oak death epidemic in California through microsatellite analysis of the pathogen *Phytophthora ramorum*. Mol Ecol 17:2755–2768. https://doi.org/10.1111/j.1365-294X.2008.03773.x
McCracken AR, Finlay J, Wilson M et al (2015) Introduction and spread of *Phytophthora ramorum* in Northern Ireland, UK. In: Sutton W, Reeser P, Hansen EM (eds) Proceedings of the 7th Meeting of the IUFRO Working Party 7-02-09 phytophthora in forests & natural ecosystems. pp152–162. http://forestphytophthoras.org/sites/default/files/proceedings/IUFRO_Proceedings_2014.pdf. Accessed 15 Feb 2023
O'Hanlon R, Choiseul J, Brennan JM, Grogan H (2018) Assessment of the eradication measures applied to *Phytophthora ramorum* in Irish *Larix kaempferi* forests. Forest Pathol 48:e12389. https://doi.org/10.1111/efp.12389
Paap T, Wingfield MJ, Burgess TI et al (2020) Harmonising the fields of invasion science and forest pathology. NeoBiota 62:301–332. https://doi.org/10.3897/neobiota.62.52991
Parnell S, Gottwald TR, Gilligan CA, Cunniffe NJ, van den Bosch F (2010) The effect of landscape pattern on the optimal eradication zone of an invading epidemic. Phytopathology 100:638–644. https://doi.org/10.1094/PHYTO-100-7-0638
Parnell S, van den Bosch F, Gottwald T, Gilligan CA (2017) Surveillance to inform control of emerging plant diseases: an epidemiological perspective. Annu Rev Phytopathol 55:591–610. https://doi.org/10.1146/annurev-phyto-080516-035334
Peterson EK, Hansen EM, Kanaskie A (2015) Temporal epidemiology of sudden oak death in Oregon. Phytopathology 105:937–946. https://doi.org/10.1094/PHYTO-12-14-0348-FI
Rizzo DM, Garbelotto M, Davidson JM et al (2002) *Phytophthora ramorum* as the cause of extensive mortality of *Quercus* spp. and *Lithocarpus densiflorus* in California. Plant Dis 86:205–214. https://doi.org/10.1094/PDIS.2002.86.3.205
Sansford CE, Inman AJ, Baker R et al (2009) Risk analysis for Phytophthora ramorum, a newly recognised pathogen threat to Europe and the cause of Sudden Oak Death in the USA (RAPRA). EU 6th Framework Project RAPRA, Deliverable Report 28. Retrieved the 29/01/2024 from https://hal.science/hal-01245979v1/document
Santini A, Ghelardini L, De Pace C et al (2013) Biogeographical patterns and determinants of invasion by forest pathogens in Europe. New Phytol 197:238–250. https://doi.org/10.1111/j.1469-8137.2012.04364.x

Schenck N, Saurat C, Guinet C et al (2018) First report of *Phytophthora ramorum* causing Japanese Larch dieback in France. Plant Dis 102:2045. https://doi.org/10.1094/PDIS-02-18-0288-PDN

Sikes BA, Bufford JL, Hulme PE et al (2018) Import volumes and biosecurity interventions shape the arrival rate of fungal pathogens. PLoS Biol 16:e2006025. https://doi.org/10.1371/journal.pbio.2006025

Turner J, Jennings P, Humphries G Lockley D (2006) Epidemiology of natural outbreaks of *Phytophthora ramorum*. Project report PH0195. Forest Research, York. https://sciencesearch.defra.gov.uk/ProjectDetails?ProjectID=12514. Accessed 15 Feb 2023

Vainio EJ, Bezos D, Bragança H et al (2019) Sampling and detection strategies for the pine pitch canker (PPC) disease pathogen *Fusarium circinatum* in Europe. Forests 10:723. https://doi.org/10.3390/f10090723

Webber JF, Turner J, Jennings P (2010) Report on research undertaken between October 2009 to March 2010, on *Phytophthora ramorum* incited dieback on larch (Larix kaempferi). https://www.semanticscholar.org/paper/Project-Title-%3A-Report-on-research-undertaken-2009/459a4038ff5d33afdaf59cf479640c4b8cdc4aee. Accessed 15 Feb 2023

Webber J, Harris A, Brasier C (2017) Shifting disease dynamic of *Phytophthora ramorum* causing localized epidemics on sweet chestnut (*Castanea sativa*). In: Phytophthora in forests and natural ecosystems, proceedings of the 8th Meeting of the International Union of Forest Research Organizations (IUFRO). Working party S07-02-09. https://forestphytophthoras.org/proceedings. Accessed 15 Feb 2023

Werres S, Marwitz R, Man In't veld WA et al (2001) *Phytophthora ramorum* sp. nov., a new pathogen on Rhododendron and Viburnum. Mycol Res 105:1155–1165. https://doi.org/10.1016/S0953-7562(08)61986-3

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