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Phytophthora species in oak forests of north-east France

Everett Hansen[a], Claude Delatour[b*]

[a] Botany and Plant Pathology, Oregon State University, Corvallis, OR 97331, USA
[b] Laboratoire de pathologie forestière, INRA Nancy, 54280 Champenoux, France

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Abstract – *Phytophthora* species were surveyed from the end of 1997 through July 1998 in oak forests in NE France. Healthy (Amance) or declining (Illwald) forests were compared. The *Phytophthora* population in both was diverse and locally abundant. At least eight species were present at Amance and six at Illwald. At Amance *Phytophthora* species had a localized distribution in water and low-lying soils. At Illwald distribution was more uniform apparently due to flooding events. Most often recovered were *P. citricola*, *P. gonapodyides* and *P. quercina. P. gonapodyides* was ubiquitous in water and colonized leaf debris. *P. quercina* was widely distributed in soil but not abundant, and was found in sites that did not otherwise appear to favor *Phytophthora.* No correlation was detected between presence of *Phytophthora* in soil and health of trees. Unusual combinations of environmental factors may be required for resident *Phytophthora* to have a detrimental impact on oaks. © 1999 Éditions scientifiques et médicales Elsevier SAS.

***Quercus* / *Phytophthora gonapodyides* / *Phytophthora quercina* / *Phytophthora* spp. / soil detection**

Résumé – Les *Phytophthora* des chênaies dans le nord-est de la France. Les *Phytophthora* ont été recherchés dans des chênaies du NE de la France entre fin 1997 et juillet 1998. Une forêt saine (Amance) et une dépérissante (Illwald) ont été comparées. La population de *Phytophthora* était variée et localement abondante. Au moins huit espèces étaient présentes à Amance et six à Illwald. A Amance, les *Phytophthora* étaient localisés dans l'eau et les bas fonds. A Illwald, la répartition était plus uniforme, apparemment à cause des inondations. Les espèces les plus fréquentes étaient *P. citricola*, *P. gonapodyides* et *P. quercina. P. gonapodyides* était ubiquiste dans l'eau et colonisait les débris de feuilles. *P. quercina* était largement répandu dans le sol mais peu abondant, il était présent dans des sites apparemment non particulièrement favorables aux *Phytophthora.* Aucune liaison n'a été trouvée entre la présence des *Phytophthora* dans le sol et l'état sanitaire des arbres. Des combinaisons inhabituelles entre facteurs du milieu seraient nécessaires pour que les *Phytophthora* résidents aient un effet défavorable aux chênes. © 1999 Éditions scientifiques et médicales Elsevier SAS.

***Quercus* / *Phytophthora gonapodyides* / *Phytophthora quercina* / *Phytophthora* spp. / détection dans le sol**

1. Introduction

Phytophthora is a genus of fungus-like microorganisms which belongs to a different kingdom (Chromista) than fungi (Mycetae), near algae. A typical feature of this group is motile spores (zoospores) which require free water to be produced and to move. Movement in infested soil is also important in many species. About 60 species of *Phytophthora*, mostly soil-borne, have been described; many undescribed species probably exist. Nearly all species are plant pathogens, mostly on roots.

With a few exceptions, little is known about the occurrence and behavior of *Phytophthora* species in

* Correspondence and reprints
delatour@nancy.inra.fr

temperate forests. While *P. cinnamomi* on numerous host plants, worldwide [23] and *P. lateralis* on *Chamaecyparis lawsoniana* in western North America [15] are relatively well known because of the destruction they have caused following introduction to new forest ecosystems, even they are poorly understood or unknown in their countries of origin. On forest trees in Europe, *P. cinnamomi* was first described as responsible for the ink disease and decline of sweet chestnut [13], then, of the red oak canker [1]. More recently, it was associated with severe dieback of evergreen oaks in Iberia [2]. *P. cinnamomi* is nevertheless limited in Europe owing to its susceptibility to low temperatures [20]; it has not been reported from continental Europe in forest conditions. It is not likely that it is involved in the severe oak decline episodes which develop periodically throughout central Europe [11].

Declining oaks exhibit non-specific general symptoms including progressive dieback of twigs and branches. Many variations in symptom development exist as well, such as occurrence of epicormic shoots, leaf clusters, reduced size and yellowing of leaves. Wilting of leaves, necrotic patches or stripes in bark, bleeding cankers, etc., are also mentioned occasionally. Decline occurs mainly on mature oaks, older than 100 years; usually, death of trees is only a possibility and in most cases they survive for a long time but in some exceptional circumstances oaks may die in large areas [11, 17].

Scattered observations confirm the presence of a variety of *Phytophthora* species on forest trees, usually associated with crown dieback or with root or root collar damage, but there have been few surveys for *Phytophthora* in "healthy" forests. Recent work in Germany described several *Phytophthora* species, especially the new species *P. quercina*, associated with decline and death of mature oak trees [17, 18]. Stimulated by that work, we began a survey of *Phytophthora* in deciduous forests of NE France. This work is in support of a larger European Union project termed "PATHOAK" exploring the interactions between root pathogens, environmental stress and oak decline.

Our work was concentrated on two areas, with scattered observations in other nearby forests. The Forêt d'Amance, on the Lorrain Plateau near Nancy in NE France, covers about 1 200 ha and is comprised primarily of *Quercus robur* and *Q. petraea*. Topography is gentle and most streams and drainage channels flow only during periods of heavy rain. Soils have a high clay content. Under the litter, pH ranges between 3.9 and 7.3 (mean 4.8) The area has been managed for forest products for hundreds of years, and periodic cuttings continue. Despite repeated disturbances from harvest and roads, insect defoliation, and war, Amance Forest is healthy. Portions of the forest are periodically defoliated by insects and individual trees are affected by *Collybia fusipes* and other pathogens. Overall, however, growth is good and symptoms of general decline are absent.

Illwald, or Forêt de l'Illwald, covers about 1 500 ha near Sélestat in Alsace, on the Rhine plain south of Strasbourg, France, and is comprised primarily of *Fraxinus excelsior, Alnus glutinosa* and *Quercus robur*. Topography is essentially flat, and in earlier times, much of the forest was inundated during flooding episodes on the Ill River. In recent decades, however, flooding has been less frequent and more localized. The alluvial soils are sandy (0.5–3 m deep) and overlay gravel. Under the litter, pH ranges between 5.2 and 7.0 (mean 6.0). Illwald has been continuously forested, and periodically harvested, for many centuries. Portions of the forest are considered to be in "decline", associated with episodes of drought, insect defoliation, and unknown causes.

2. Methods

Sampling for *Phytophthora* was carried out from November 1997 through July 1998, except where otherwise noted. *Phytophthora* was isolated from soil and water by baiting. Two types of baits were used extensively: *Chamaecyparis lawsoniana* (Lawson's cypress or Port Orford cedar) twigs [15]; and very young leaflets of *Quercus robur* [17]. Cedar baits 2–3 cm long were prepared from the green axis of cedar branch tips stripped of their lateral branchlets. Tender oak leaflets up to about 3 cm long were collected from seedlings kept in nearly continuous growth in the greenhouse by periodic cutting back to induce sprouting.

Soil samples were collected 1 m from the base of trees. Surface litter was scraped away and a portion of soil from about 5–20 cm deep was collected. Four collections from each tree, about 1 L in total, were mixed. About 200 mL of each soil sample were then flooded with deionized water to a depth of 2 cm (about 500 mL) and baited by floating cedar twigs and oak leaflets on the surface (about ten baits of each). Baiting was performed under standard laboratory conditions (about 18–20 °C, diffuse light). Baits were removed after 3 days and blotted dry; whole cedar baits and necrotic parts of oak leaflets were transferred to agar media. Two *Phytophthora* selective media were used frequently, CARPBHy (corn meal agar with 200 mg ampicilin, 10 mg rifampicin, 10 mg pimaricin, 15 mg benomyl, and 50 mg hymexazol per liter) and multivitamin juice (V-8 like) selective agar [17].

Streams and standing water in the forest were also sampled by baiting. Cedar and leaflet baits were held in nylon mesh bags in the stream or puddle for periods up to 1 week, then rinsed, blotted dry, and placed on selective media. Alternatively, submerged leaf litter was collected from the water in the field and returned to the lab, where it was flooded and baited as with the soil samples.

Plates were examined daily, and possible *Phytophthora* colonies were transferred to corn meal agar amended with β-sitosterol, potato dextrose agar, and multivitamin agar for identification. Isolates were grouped by growth pattern and morphology into species "types", then representative isolates were examined more critically for identification [12]; *Pythium* species and non-Oomycete species were discarded. Names were confirmed by analysis of ITS DNA sequences and comparison with published (Genbank) and unpublished (Jim Duncan et al., pers. comm.) *Phytophthora* sequence databases.

Soil and water samples from Amance Forest were referenced to a 200 m mapped grid that is used for many types of studies on this experimental forest. Fifteen sample sets came from a randomly selected subset of grid points known to fall in mature oak forest. Soil was collected from around the bases of 2–4 oak trees nearest to each reference point. If a stream, or body of standing water (puddle on soil compacted by harvesting machinery, drainage ditch or natural low spot in the forest, shell craters or trenches from WWI, etc.) was nearby, it was also sampled. Samples were collected without regard to tree health, but selected trees were rated for crown dieback. Trees were scored 0–4, for no dieback, scattered dead small branches in the outer crown, a few larger dead branches in upper crown, major portions of the upper crown dead, and tree severely damaged or recently dead, respectively. Two areas of low-lying forest at Amance (27 trees near grid point 31.15 and 12 trees near 16.31) were investigated more intensively. At these sites oaks as well as the other tree species present were sampled at several times.

At Illwald, three stands were selected for sampling because they were known to have mature oak trees, some with symptoms of decline. Two trees were selected in

Table I. *Phytophthora* species isolated three or more times from Forests in NE France.

Species	Morphology	Locations	Notes
P. gonapodyides	no oogonia, non-papillate often nested sporangia; colony margin regular and dense	Amance, Illwald, Forêt de Champenoux, Forêt de Haye, Forêt de Bezange	abundant in water and submerged leaves, also saturated soil
P. "type 3a"	like *P. gonapodyides*, but with chlamydospores	Forêt de Haye (stand 458)	from water in a beech stand
P. citricola	oogonia 30–35 μ, sometimes enveloped in hyphae; sporangia semi-papillate and often irregular	Amance, Illwald	recovered a few times from water, and abundantly from soil around trees of several species
P. quercina	oogonia present but often slow to form and irregular shapes; sporangia papillate and often irregular; very slow growth with distinctive branching	Amance, Illwald	present in soil collected around both healthy and "dieback" trees
P. megasperma	oogonia 40–50 μ; sporangia non-papillate	Amance	uncommon, recovered only three times from water at one location
P. "type 4"	large oogonia, non-papillate sporangia	Illwald	isolated repeatedly from around two adjacent trees
P. "type 6"	oogonia about 40 μ, with tapered base and thick oospore wall; sporangia non-papillate; fluffy colonies	Amance, Illwald	locally and perhaps seasonally abundant in soil around trees of several species
P. "type 8"	apparently heterothallic, with papillate or semi-papillate sporangia and a dense colony margin	Amance	from water at 11.13

Table II. *Phytophthora* from soil and streams at intensively sampled sites at Amance and Illwald.

Location	Sample	Number tested	Number positive	*Phytophthora* species*
Amance 16.31	*Quercus*	9	3	*P. citricola*, *P.* "type 6", *P.* "unknown"
	Carpinus	3	2	*P.* "type 6"
	stream	1	1	*P. gonapodyides, P. megasperma*
Amance 31.15	*Quercus* low ground	14	12	*P. citricola, P. gonapodyides, P. megasperma, P. quercina, P.* "type 6", *P.* "unknown"
	Quercus high ground	7	0	none
	Carpinus	3	2	*P. citricola, P.* "unknown"
	Fraxinus	3	3	*P. citricola*
	stream	1	1	*P. gonapodyides*
Illwald 183	*Quercus*	8	8	*P. citricola, P. gonapodyides, P.* "types 3, 4, 6", *P.* "unknown"
	Alnus	3	2	*P. citricola, P. megasperma, P.* "unknown"
	Fraxinus	3	2	*P. citricola*
	no tree	3	3	*P. citricola, P. gonapodyides, P.* "type 6", *P.* "unknown"
	water	1	1	*P.* "type 4"
Illwald 83	*Quercus*	5	5	*P. citricola, P. gonapodyides, P. quercina, P.* "type 4"
	stream	1	1	*P. gonapodyides*
Illwald 9	*Quercus*	2	2	*P. citricola, P. gonapodyides*
	stream	1	1	*P. gonapodyides, P.* "type 4"

* *P.* "unknown" indicates that *Phytophthora* was observed in the isolation plate, but could not be recovered in pure culture, usually because of mixture with *Pythium*, and thus was not identified.

stand 9, and six trees were sampled in stand 83 where decline was especially severe, including four trees sampled at two different times. Fifteen trees were tested in stand 183, including three trees sampled twice and trees of other species (*table II*). In each Illwald stand both "healthy" trees and trees with dieback were selected.

3. Results

Oak leaflet baits were effective with all *Phytophthora* species that we recovered, and proved to be the only bait tested that allowed consistent recovery of *P. quercina*. Cedar twig baits were also useful for recovering the common *Phytophthora* species in these forests except *P. quercina*. They had the advantage of year round ready availability from cedar trees that have been widely planted as ornamentals in France. Both CARPBHy and multivitamin selective agars supported isolation of all species of *Phytophthora*. We preferred CARPBHy, however, because it was transparent, and because *Pythium* species developed more slowly, allowing easier recovery of *Phytophthora* species.

A diverse and locally abundant *Phytophthora* community was present in both forests, including at least eight species (*table I*). *Phytophthora citricola* was readily recognized with practice by its colony pattern and rapid production of oogonia. *P. gonapodyides* was recognized by its regular colony margin and lack of oogonia. *P. quercina* was typically recognised by its slow growth, cottony colony with a loose margin, hyphae with dichotomous branching and zigzag growth. "*Phytophthora* type 3a" looked like *P. gonapodyides* but with chlamydospores, and ITS DNA sequences were similar to that species. Several homothallic isolates, including "*Phytophthora* type 4", also grouped in the ITS clade with *P. gonapodyides* and *P. megasperma* sensu stricto (the large oospore, BHR type) [14, 16]. "*Phytophthora* type 6" grew like *P. cambivora*, but was homothallic. ITS DNA sequences were different from described species, but aligned in the same clade as *P. cambivora*. The latter species was not found in these stands. "Type 6" represents a new species. "*Phytophthora* type 8" did not produce oogonia, and formed papillate or semi-papillate sporangia.

Phytophthora species were recovered from water and from leaves and soil in water (or low spots where water had been) throughout the forest, from soil in low-lying sites that are wet but not flooded through the winter, and from scattered upland sites. Only in a small forest nursery at Amance Forest was *Phytophthora* obviously associated with diseased trees (on Douglas-fir seedlings) and this unidentified heterothallic species was not found elsewhere in the forests (data not shown). Strip cankers, bleeding cankers, and other symptoms often associated with *Phytophthora* infection were not seen on the trees sampled in this study. *Phytophthora* was recovered from soil around all tree species sampled and regardless of dieback status of the tree. Non-specific symptoms of branch dieback or crown decline were evident on scattered trees. Some was associated with Collybia root rot, while in other cases dieback seemed to have resulted from past defoliation or drought events, with current foliage appearing healthy. Some trees were chlorotic, but this was usually associated with regrowth following early spring defoliation by insects.

At Illwald and at the low-lying intensively sampled sites at Amance *Phytophthora* species were present in most soil samples tested (*table II*). A total of 60 trees were sampled at these sites, and *Phytophthora* species were recovered from 41. Two or more species were isolated from soil around 12 of these trees. With the exception of *P. quercina*, *Phytophthora* species were seldom recovered from upland sites (see below). Repeated sampling of a few trees at each site gave similar results. Trees (and individual soil samples) that were negative in the first attempt were also negative when tested a second time, and vice versa. There was a tendency for more species to be recovered from individual trees with repeated sampling, however.

Intensively sampled Amance site 31.15 (*table II*) was a low-lying parcel of forest, drained by several shallow ditches. The area sampled measured about 50 m × 80 m, and included slightly higher ground (maximum elevation difference 1.4 m) on the north-west edge of the site. Water was standing in the ditches and low spots throughout the winter but tree bases were not flooded. Mature *Quercus* (about 50–90 cm dbh and 100–120 years old) predominated but *Fraxinus* and *Carpinus* were also present. Spring ephemeral herbaceous understory vegetation was present. The frequency of crown dieback was not noticeably different from adjacent upland areas. *Phytophthora* (and *Pythium*) was regularly and abundantly recovered from soil around trees in the low-lying portions of the site. *Phytophthora citricola* and "*Phytophthora* type 6" were abundant and *P. quercina* was present. Adjacent trees on slightly higher ground had no *Phytophthora*, and *Pythium* was very infrequent. *Phytophthora gonapodyides* was recovered abundantly from the water at all sample times, and occasionally from soil samples.

Intensively sampled Amance site 16.31 (*table II*) was similar to site 31.15, but trees were smaller (about 20 cm dbh). Soils were saturated or flooded at intervals through the winter. There was abundant grass around all trees, and *Pythium* isolations were frequent, making *Phytophthora* recovery more difficult. "*Phytophthora* type 6" was most abundant. *Phytophthora megasperma* was recovered several times from water at this site, but never from soil.

The Illwald stands (*table II*) comprised predominately large *Quercus* (about 80 cm dbh and 120 years old), with *Fraxinus, Alnus*, and *Carpinus* also present. Scattered dead trees were present, as well as trees showing evidence of old and current dieback. *Urtica* dominated the herbaceous layer at the Illwald sites. Stand 183 was sampled most intensively. Several *Phytophthora* species were present, but *P. citricola* dominated; it was recovered from soil around 10 of 12 trees in an area about 30 m by 65 m, and from all three samples collected at least 10 m away from any tree. *P. citricola* was also recovered from six of seven trees at the other Illwald sites.

3.1. *Phytophthora gonapodyides*

Phytophthora gonapodyides is apparently resident in most if not all of the streams on forest land in NE France, and in most places in forests where water accumulates after heavy rain. Both oak leaflet and *Chamaecyparis* baits were effective in recovering the fungus; most tests used *Chamaecyparis* baits alone. At Amance, 26 collections were made from 15 separate streams at different times of the year, and all were positive; 15 of 22 ephemeral puddles yielded *P. gonapodyides*. In a systematic sampling at 15 points on the Amance grid (*table III*), *P. gonapodyides* was readily baited directly from streams and standing water and from submerged leaf litter. It was also occasionally recovered from fallen leaves on the ground near streams, but never from the soil immediately beneath those leaf samples. At other locations at Amance, *P. gonapodyides* was recovered from soil samples collected near the water line in stream courses and where soil was saturated through the winter. At Illwald, *P. gonapodyides* was recovered from soil around seven of 19 trees in the three stands sampled.

Water only was sampled in three other forests in Lorraine, and *P. gonapodyides* was present in eight of ten streams or puddles sampled.

P. gonapodyides was seemingly abundant at all times of the year in the waters of Lorraine. At Amance, nearly every bait was colonized by this fungus in stream sampling conducted monthly from October 1997 to July 1998 in five streams, despite the fact that three of the streams were dry during portions of the sampling time. Even old leaf litter collected from dry streams yielded *P. gonapodyides* (data not shown). While other *Phytophthora* species were occasionally recovered from water, *P. gonapodyides* regularly comprised more than 90 % of the isolates.

P. gonapodyides colonized leaves as they fell from trees into water and persisted in those leaves. The fungus was readily recovered from bulk leaf litter samples collected from water at all times of the year. In one test in March 1998 it was isolated from five of 20 individual dead oak leaves collected from two pools at Amance site 31.15. Leaves were rinsed in tap water and flooded then baited individually. In another test in April 1998, dead, dry oak leaves picked from forest trees and green oak leaves from greenhouse trees were bagged and placed in water at Amance site 31.15 for 1 week and then baited, or baited directly (controls). *P. gonapodyides* was isolated from leaves in all bags at site 31.15, but not from any control bags. In May 1998, it was isolated from green fragments of young oak leaves fallen in water after being clipped by defoliating insects in the tree canopies.

3.2. *Phytophthora quercina*

P. quercina was isolated from one tree at Illwald, and several trees at Amance (*tables II* and *IV*). It is very slow growing and was difficult to isolate if *Pythium* and other *Phytophthora* species were abundant. It is likely that it is present around more trees in both forests, although not successfully isolated. Oak leaflets were the most efficient bait; *P. quercina* did not colonize *Chamaecyparis* baits.

Oak trees at five Amance sites that had been independently scored for dieback were sampled for *Phytophthora* in the surrounding soil (*table IV*). None of these sites were considered low lying or wet. *P. quercina* was present around one or more trees at each of the sites. It was abundant on the baits from samples from some trees where other *Phytophthora* or *Pythium* species were not present. There was no evident association between *P. quercina* and decline status of the trees.

Table III. Recovery of *Phytophthora gonapodyides* at Amance Forest by baiting from soil, leaf litter on soil, leaf litter in streams, and leaf litter in standing water, January 1998.

Grid point	Soil	Leaves on soil	Leaves in stream	Leaves in standing water
11.13	–	+	+	/
11.15	–	–	+	+
13.09	–	–	+	+
13.29	–	–	+	+
15.29	/	/	/	+
16.31	/	/	+	+
17.23	–	–	/	–
17.29	–	–	/	+
17.41	–	–	/	+
19.17	–	–	+	/
19.25	–	–	+	/
19.35	–	–	/	+
23.29	–	+	+	–
31.15	–	–	+	+
36.18	–	–	+	+

/: not sampled.

5. Discussion

A diverse and locally abundant *Phytophthora* population is present in the oak forests of NE France. These observations confirm and extend the recent reports of *Phytophthora* in forests in Germany and other European countries [17], and earlier, less systematic observations from England and elsewhere in Europe [3, 8, 9, 10]. In contrast to those works, our objective was not to establish etiology for a particular disease syndrome, but rather was intended to elucidate *Phytophthora* distribution and population structure in two forests with contrasting decline histories, without regard for symptomology of individual trees. While our sampling was inadequate to support firm conclusions, the observed similarities and differences between the forests do suggest directions for further research.

The diversity of *Phytophthora* species present is perhaps surprising. At least eight species are present at Amance Forest, and six at Illwald. Up to four species were regularly recovered from soil around individual trees. It is likely that more repeated sampling from other substrates, at different seasons and with different baits would reveal still more species. Presumably these *Phytophthora* species differ in pathogenicity or otherwise occupy different niches, or are active at different seasons [7]. Because *Phytophthora* may be in a soil sample as resting spores, mycelium in roots, or active sporangia and zoospores, careful and detailed sampling will be necessary to associate particular species with particu-

Table IV. *Phytophthora quercina* at Forêt d'Amance.

Site	Tree no.	Sampling date (1998)	Diam (cm)	Crown condition	*P. quercina*	*Phytophthora* spp.
17–29	DV (116)	June	38	1	+	–
17–29	DX (114)	June	24	1	+	–
17–29	DY (9)	June	30	1	–	*P. citricola*
17–29	DZ (115)	June	25	1	+	–
17–29	EA (10)	June	46	1	–	–
27–29	DW (20)	June	38	1	+	–
27–29	EB (21)	June	44	3	–	–
11–15	EN (118)	July	34	1	–	–
11–15	EO (117)	July	36	2	+	–
9–13	EP (63)	July	41	3	+	–
9–13	EQ (64)	July	41	1	–	?
11–13	ER(62.1)	July	61	0	–	–
11–13	ES (58)	July	53	2	–	–
11–13	ET (57)	July	69	2	–	–
11–13	EU (60)	July	70	1	+	–

Crown condition: 0 = healthy; 1 = scattered dead small branches in the outer crown; 2 = dead branches present in upper crown (< 50 %); 3 = dead branches present in upper crown (> 50 %); 4 = severely damaged or recently dead tree.

lar substrates or soil environmental conditions. Baiting methods can reveal presence, but allow only limited inferences about abundance or activity.

Jung et al. [17] distinguished seven *Phytophthora* species from declining oak stands in Germany and elsewhere in Europe. In their work, as in ours, *P. citricola* was most often identified. They also regularly encountered *P. gonapodyides* and *P. quercina*, as did we. Surprisingly, we did not recover *P. cactorum* or *P. cambivora*, regularly encountered in Germany and elsewhere in Europe. *Phytophthora cinnamomi* was not present in either species list, although it is well established in forests in southern France [19, 22]. Jung and colleagues [17] also listed *Phytophthora undulata*, but this species grows like a *Pythium* and recent molecular work reaffirms its affinities to that genus (Duncan and Cooke, unpublished data). It was identified twice in our survey, but was disregarded along with other *Pythium* species the rest of the time.

Phytophthora species appear to have a localized distribution in the forests of NE France. At Amance Forest they are present and seemingly abundant in streams and standing water, and in low lying, seasonally wet forest soils. On adjacent slightly elevated and better drained sites they are absent (except for *P. quercina*). At Amance, soil sampling at arbitrary grid points across the forest (*table III* and additional unreported data) revealed no *Phytophthora* species, presumably because the sample intensity was too low to pick up the relatively rare sites that favor *Phytophthora*. Sampling on a regional or national scale that is not directed toward likely *Phytophthora* habitats will probably be similarly inefficient, especially for the species that favor wet soils.

Illwald Forest apparently represents a very different *Phytophthora* distribution pattern. *Phytophthora* was present in soil around nearly every tree sampled in three different stands. Illwald is essentially flat, and the alluvial soils overlay gravel with a relatively shallow water table, especially in winter. The sampled stands appeared to be well drained, however. Perhaps the uniform distribution of *Phytophthora* at Illwald is the consequence of infrequent flood events.

Phytophthora species were not especially associated with symptomatic or declining trees in either forest. Amance Forest is considered by local foresters and pathologists to be generally healthy, although scattered trees show non-specific symptoms of dieback and decline. The frequency of symptomatic trees was no greater in the intensively sampled wet sites with their abundant *Phytophthora* populations than in the surrounding forest where *Phytophthora* was scattered or absent. Neither the incidence of *Phytophthora* nor its species composition differed between symptomatic and non-symptomatic trees at either of the intensively sampled sites (data not shown) or at the upland sites where *P. quercina* was recovered (*table IV*).

Illwald, by contrast, has a history of dieback episodes, usually associated with insect outbreaks and drought. Local areas, such as stand 83, are suffering severe decline and mortality perhaps representative of the "oak decline syndrome" although symptomatic trees and mortality are much more widely scattered in other parts of the forest, such as stand 183. *Phytophthora* was generally distributed at Illwald but even here there was no obvious difference in kind or amount of *Phytophthora* between stands with active decline and mortality and the other stands with only scattered evidence of old dieback episodes.

The *Phytophthora* species encountered on these sites are not uniquely associated with oaks. Limited sampling of soil around *Carpinus, Alnus,* and *Fraxinus* in these stands also yielded *Phytophthora* species. At Illwald, they were even recovered from the soil in gaps between trees. There are many other plant species growing on these sites, and it will take careful root examinations and isolations to identify the important hosts. Indeed, there is the possibility that some species maintain their populations saprophytically.

Phytophthora gonapodyides is nearly ubiquitous in streams and in pools (even ephemeral) of standing water. It colonizes both living and dead leaf debris as it falls into water, produces sporangia on these substrates (witness its ready recovery by baiting from flooded leaf litter). In most circumstances it is recovered only from water or from saturated soil immediately adjacent to streams or pools. At Amance Forest, for example, it was found in nearly every body of water, no matter how small, but almost never in the soil unless the spot was periodically flooded. At Illwald, however, it was recovered from well-drained soil around several different trees, perhaps suggesting flooding events. *P. gonapodyides* was first isolated and described from plant debris in water [21] and several subsequent authors have considered it a saprophyte [12]. It is also a plant pathogen, although generally considered a rather weak parasite [12]. Its pathogenicity has been demonstrated on several hosts, including oak, and it was isolated from fine roots and collar stripe cankers on oak in Germany [17]. Its ecological role remains unclear. It is an interesting species, often misidentified [5, 6].

Phytophthora quercina has received much attention because of its recent description and its association with declining oak trees in Germany [17, 18]. In our sampling, we only began to recover *P. quercina* after we started using oak leaflet baits and subculturing within 2–3 days of plating baits on selective media. At Amance Forest it was present in soil around some oak trees, on both wet and upland sites (*tables II* and *IV*). It may be more widely present, but our methods in earlier samples were not adequate for its detection. It was recovered from trees with and without symptoms of dieback, and on sites that did not otherwise appear to favor *Phytophthora*. Our evidence suggests that it is widely distributed but not abundant, and not a recent introduction to these forests.

Phytophthora is known as a genus of plant pathogens, and indeed pathogenicity to oak has been demonstrated [17] for most of the species identified here. The widespread occurrence of these pathogens in sites favorable for their growth perhaps should not be surprising, but their long time association with mature trees that seemingly remain healthy is interesting. Clearly it takes more than the spatial association of these pathogens with a susceptible host, even on wet sites, to result in significant damage to the trees [4]. It will take much more focused work than simple surveys of *Phytophthora* incidence to determine if any or all of the species encountered are causal agents of oak decline, as has been suggested by Jung and colleagues [17]. This is the case in southern France, where there was no obvious correlation between presence of *P. cinnamomi* in the soil and oak decline [22]. Future research should focus on unusual combinations of environmental factors, such as insect defoliation, drought, and temporary flooding that might under certain circumstances combine to prevent regeneration of fine roots killed by seasonal activity of *Phytophthora*, or predispose trees to accelerated invasion by the pathogen.

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