Molecular Evidence of Distinct Introductions of the European Race of *Gremmeniella abietina* into North America

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ABSTRACT

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The presence of the European (EU) race of *Gremmeniella abietina* var. *abietina*, the causal agent of Scleroderris canker of conifers, was first reported in North America in 1975 in the northeastern United States and subsequently in southern Quebec and Newfoundland during the late 1970s, where it quickly became established. We analyzed DNA profiles in samples from a historic collection of *G. abietina* var. *abietina* that included some of the first isolates of the EU race reported in the United States to test hypotheses concerning the *G. abietina* var. *abietina* epidemic in North America. Genetic diversity was partitioned by an analysis of molecular variance with haplotype frequencies and distances. Genetic differentiation was high between populations in continental North America and Newfoundland (between region differentiation, $\Phi_{ct} = 0.665$, $P < 0.001$). This result was not consistent with the hypothesis of a single introduction of the pathogen into the northeastern United States followed

by secondary spread into northeastern Canada. In contrast, small levels of genetic differentiation were observed among continental North American populations ($\Phi_{ct} = 0.047$, $P = 0.079$), suggesting gene flow among these populations. A single haplotype of *G. abietina* var. *abietina* dominated the continental populations (80% of the isolates) but was absent from Newfoundland and Europe. Five haplotypes were found in the Newfoundland population, all of which were either absent or very rare on the continent. Populations from continental North America clustered together and were distinct from a second cluster composed of European and Newfoundland populations. A phylogenetic analysis of the haplotypes indicated that some of the rare haplotypes may have derived from somatic mutations, whereas others probably occurred as the result of new introductions. The results are consistent with a scenario of distinct primary introductions of this pathogen into Newfoundland and continental eastern North America followed by secondary asexual propagation.

Additional keywords: random amplified polymorphic DNA.

Gremmeniella abietina (Lagerberg) Morelet var. *abietina* Petri et al., the causal agent of Scleroderris canker, is a very damaging pathogen of conifers in North America, Europe, and Asia (16,30,37). In North America, a North American (NA) race that is believed to be indigenous and a race that was introduced earlier during this century, presumably from Europe (EU), are present (4). These two races have overlapping morphological characteristics (28), but they are fairly divergent based on their ecological, etiological, and molecular characteristics (1,3,7,9,10, 17,25). Subsequent analyses within the EU race also revealed genetic differentiation within the race (8,11), and genetic profiles in North America often differ from the profiles in Europe (8).

In North America, Scleroderris canker (presumably caused by the NA race) was first reported in Michigan and Ontario pine plantations during the 1950s (22,27). Subsequently, research established that the EU race caused mortality of large trees, whereas the NA race was restricted to infection on the portion of the tree crown that remains under snow cover (21). The EU race was first reported in New York State and then in Vermont, Maine, and New Hampshire (32,33). The first report in Canada was during the late 1970s from a plantation located near the U.S.-Quebec border (13). The pathogen rapidly spread to Quebec and is now prevalent throughout the red pine plantations in the province (15). In Newfoundland and New Brunswick, the disease was found in 1979, only a year after it was reported in Quebec (19,31). The chronology of these new reports is consistent with the scenario of

an introduction from Europe to the eastern United States followed by secondary spread to northeastern pine stands, but the precise routes and means of dissemination are impossible to establish based on available data.

The natural mode of dissemination of the EU race of *G. abietina* var. *abietina* in North America is thought to be water-splashed conidia. Apothecia, which produce airborne ascospores, have not been observed in North America, although apothecia for the EU race are commonly found in Finland (34,36). It is possible that natural spread through conidia only results in local spread. In western Quebec, the pathogen is prevalent in red pine plantations, but it is absent on the Ontario side of the Ottawa River. Strong circumstantial evidence exists that infected nursery material and Christmas trees are an additional source of inoculum and may be involved in local and long-distance spread of the fungus (20,32). This pathogen can survive for long periods in an endophytic state (14,29,37), and therefore, it is possible that apparently healthy seedlings are outplanted even after phytosanitary seedling inspections.

If the EU race of *G. abietina* var. *abietina* was introduced into northeastern North America followed by secondary asexual spread the genetic structure of the population would be expected to be uniform among regions with low overall levels of genetic diversity. If several introductions took place but were followed by extensive gene flow, larger levels of genetic diversity combined with low interregional genetic diversity would be expected. In contrast, if several genetically different introductions occurred followed by local secondary spread but no interregional gene flow a discontinuous population structure would be expected, with extensive genetic diversity among regions. The objective of this study was to analyze the genetic structure of this exotic pathogen,

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using molecular approaches to test the null hypothesis of a single introduction of the EU race into North America followed by secondary asexual spread.

MATERIALS AND METHODS

Isolates. Two hundred twenty isolates of *G. abietina* var. *abietina* race EU were sampled from seven regions in Quebec over a period of 20 years and preserved in liquid nitrogen (Fig. 1). Isolates were obtained either from a single spore or from a single pycnidium. The sampling was not particularly structured, because the objective of the initial survey was to record disease incidence in Canada. Nevertheless, the sampling represents a cross-section of the Scleroderris canker epidemics in North America. Confounding effects of time of collection and geographic origin are treated in the analysis.

In addition, 15 samples were obtained from several European countries. Only isolates belonging to the European biotype (8) were used in the current study, because the Scandinavian and Alpine biotypes have not been reported in North America. Twenty isolates representing the beginning of the *G. abietina* var. *abietina* epidemics also were obtained. However, only 10 of those isolates previously identified as race EU or intermediate (putative hybrids), using serology (4), were identified as race EU when rDNA-internal transcribed spacer and random amplified polymorphic DNA (RAPD) markers were used (1,7) and were retained for this study.

All isolates were grown for 1 month on dialysis membranes placed in petri dishes containing an agar medium (25 ml of Campbell's V8 juice, 15 g of Bacto agar, 7.5 g of malt extract, and 475 ml of water) dispensed at 20 ml per petri dish. Alternatively, the strains were inoculated in a V8 liquid medium and grown in the dark for 4 weeks at 18°C. Mycelia were harvested by filtration, lyophilized, and stored at -20°C until use.

DNA extraction. Approximately 10 mg of lyophilized mycelium was mixed with an equal amount of diatomaceous earth (Sigma Chemical Co., St. Louis) and ground with a mortar and pestle. Six hundred microliters of extraction buffer (1.4 M NaCl, 100 mM Tris-HCl [pH 9.5], 20 mM EDTA, 0.25% 2-mercaptoethanol, 2% cetyltrimethylammoniumbromide, and 1% PEG

6000) was added to the macerated mycelium and incubated at 65°C for 30 min. The mixture was emulsified by adding an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1), vortexing, and centrifuging for 5 min at 12,000 × g. The resulting pellet was washed with 70% cold ethanol, dried, and resuspended in 50 µl of 10 mM Tris-HCl and 1 mM EDTA (pH 8). All DNA was diluted 1:50 (≈25 ng/µl), which yielded the most reproducible RAPD patterns.

DNA amplification. Race identification was performed for each of the 245 isolates according to methods previously described (1,7). RAPD analyses were performed as described previously (7,8), except 2.5 mM MgCl₂ was used for primers OPA12 and C08 and 3.5 mM MgCl₂ was used for primer OPD05. Additional primers were screened for polymorphism within race EU. Primer kits (kits C, D, and F; Operon Technology, Alameda, CA) were tested with seven isolates originating from diverse geographic sources. Most primers were monomorphic even when including isolates from North America and Europe. Only two additional primers, OPC08 and OPD5, were polymorphic and were included in this study. Random amplified microsatellite (RAMS) reactions were performed as described previously, with primer DBH(CGA)₅ (degenerate 5' anchor: D = A, G, or T; B = C, G, or T; and H = A, C, or T) (10), except *Taq* Expand (Boehringer Mannheim, Laval, Quebec) polymerase mixture was used. The ≈600-bp polymerase chain reaction (PCR) product scored for primer CGA contained repeated motifs, creating length differences (data not shown).

Amplifications were carried out in a MJ-Research (Watertown, MA) thermal cycler programmed for a denaturation step of 94°C for 2 min, followed by 1 cycle of 40°C (61°C for RAMS primers) for 4 min, 72°C for 2 min, and 45 cycles of 94°C for 1 min, 40°C for 1 min, and 72°C for 2 min. The reactions ended with a 7 min extension at 72°C.

All isolates were assayed with the four selected primers, and PCR products were separated by electrophoresis on 1.5% agarose gels in Tris-acetate-EDTA buffer at 3 V/cm for 2 h or on 1.5% agarose gels and 1.5% synergel (Diversified Biotech Inc., Boston) in 0.5× Tris-phosphate-EDTA buffer at 3 V/cm for 4 h. Gels were stained in ethidium bromide and digitized under UV light with the Canberra IS1000 system (Alpha Innotech Corp., San Leandro, CA).

Fig. 1. Map of geographic regions sampled for *Gremmeniella abietina* var. *abietina* in North America.

Fragment lengths were calculated using available software. Amplifications of DNA templates and primer combinations were repeated regularly to ensure the reproducibility of the amplification profiles. All major diagnostic DNA fragments used in our analysis were reproducible between runs.

Data analysis. A matrix of isolates and markers was recorded, and haplotypic frequency was computed. Because the data were collected from various geographic regions over a number of years, frequency data were calculated by year as well as by region to

detect chronological trends. Data were pooled by region because no important chronological trends were noted in the preliminary analysis. Because of the perennial nature of this canker pathogen, large year-to-year variation, such as that observed for agricultural crop pathogens, would not be expected.

Because of the apparent clonal nature of the *G. abietina* var. *abietina* populations, deviations from random mating and strong linkages were observed between markers (R. C. Hamelin, *unpublished data*). Therefore, analyses of gene diversity based on marker frequencies were not conducted due to potential violations in the assumptions of independence between markers. Instead, an analysis of molecular variance (AMOVA) was carried out with a matrix of Euclidean distances and Nei and Li's (26) genetic distance between all pairs of haplotypes. The AMOVA was based on the measure of mean squares, using haplotypic frequencies and distances, and was appropriate for haplotypes because it was designed for nonrecombining mtDNA haplotypic data (5). A measure of genetic differentiation was obtained by *F*-statistic analogs (Φ statistics), which represent the proportion of the total variance attributable to sampling among subunits. Nonparametric tests of Φ_{st} , genetic differentiation among populations, and Φ_{ct} , genetic differentiation among geographic origin (continental North America, Europe, and Newfoundland), as well as variance within population, were conducted by 100 random permutations of the data set. Genetic distances between all pairwise population comparisons were calculated based on Φ_{st} . A dendrogram based on these distances was constructed by unweighted pair group arithmetic mean with PHYLIP software (J. Felsenstein, Department of Genetics, University of Washington, Seattle).

The relationship among haplotypes and correlation with geographic origin also were investigated by a phylogenetic analysis of the haplotypes, by parsimony analysis using PAUP 3.0 software (D. L. Swofford, distributed by the Illinois Natural History Survey, Champaign). The results of this analysis are presented as a network between haplotypes, represented as spheres connected by arms whose length corresponds to genetic distance. The intensity of fill in the spheres represents the haplotypic frequencies.

Fig. 2. Amplification products of the European race of *Gremmeniella abietina* var. *abietina*, using 5'-anchored microsatellite primer DBH9(CGA)₅. Lanes 1 and 18: 100-bp ladder; lanes 2 through 6: continental North America; lanes 7 through 12: Europe; and lanes 13 through 17: Newfoundland. Electrophoresis was run on 1.5% agarose plus 1% synergel.

TABLE 1. Binary coding of the DNA profiles of 27 haplotypes of the European race of *Gremmeniella abietina* var. *abietina* in North America

Haplotype	DNA profile ^a
1	0010100010110000000000
2	0011100001110000000000
3	0011100010000100001100
4	0011100010010000000000
5	0011100010110000000000
6	0011100010110000100100
7	0011101010110000100010
8	0011101010110000100100
9	0011110010110000000000
10	0011110010110001000000
11	0011111010110001100100
12	0100100100010000001000
13	0101000010001010001010
14	0101100100010000001000
15	0111100001010000001000
16	0111100001010000101100
17	0111100010010000000000
18	0111100010010000001000
19	0111100100010000001000
20	0111101001010000101100
21	1011110010010001100100
22	1011110010110001000000
23	1011110010110001100100
24	0111100010010000000001
25	1111110001010001001000
26	0011100001000000001000
27	0111110001010000001000

^a Sequential order of DNA markers (primer size in base pairs of scored fragments): A12, 500; A12, 800; A12, 1,100; A12, 1,350; CGA, 150; CGA, 300; CGA, 500; CGA, 550; CGA, 580; CGA, 620; D5, 350; D5, 800; D5, 750; D5, 500; C8, 250; C8, 350; C8, 700; C8, 1,200; C8, 1,300; C8, 1,400; C8, 1,500; and C8, 1,250.

RESULTS

Twenty-seven haplotypes were found among the two hundred forty-five strains of *G. abietina* var. *abietina* from North America and Europe, based on twenty-two DNA markers (Fig. 2 and Table 1). Haplotypes were sorted according to their binary code and arbitrarily given an identification number. A single haplotype (#5) dominated the *G. abietina* var. *abietina* populations in Quebec and the United States, independent of the year or region of sampling (Tables 2 and 3). Haplotype #5 comprised between 53 and 86% of the isolates sampled in each of seven geographic regions in Quebec and the eastern United States. When strains were pooled by year, between 47 and 89% of the samples belonged to haplotype #5 (Table 2). In 1985, a survey was conducted over a large

TABLE 2. Frequency of haplotypes of the European race of *Gremmeniella abietina* var. *abietina* sampled in Quebec, pooled by year of survey

Year ^a	N	Haplotypes ^b				
		1–4	5	6–15	15	16–27
1985	61	<0.06	0.62	<0.08	0.10	<0.05
1986	19	<0.06	0.47	<0.08	0.16	<0.05
1990	28	<0.06	0.89	<0.08	0.07	<0.05
1991	18	<0.06	0.89	<0.08	0.11	<0.05
1995	18	<0.06	0.83	<0.08	0.00	<0.05

^a All regions were pooled within a year; years 1984, 1988, 1992, and 1996 are not presented because sample size was less than 10. Frequencies are presented for each haplotype.

^b Haplotypes are based on 22 random amplified polymorphic DNA and random amplified microsatellite markers (defined in Table 1).

portion of the range of distribution of red pine in Quebec, and 62% of the samples belonged to haplotype #5 (Table 2).

However, haplotype #5 was completely absent from Newfoundland and Europe (Table 3). The *G. abietina* var. *abietina* population from Newfoundland had only five haplotypes, which were either rare or absent from Quebec and the eastern United States. For example, haplotypes #14 and #19 comprised 15 and 19% of the isolates from Newfoundland, respectively, but were absent from Quebec and the United States (Table 3).

An additional 25 rare haplotypes were found in Quebec. These haplotypes generally were not present in Newfoundland or Europe (Table 3). This observation should be viewed with caution, because a smaller sample size was obtained from Newfoundland and Europe than from Quebec, and the probability of obtaining rare haplotypes is proportional to sample size. Nevertheless, rare haplotypes also were found in Europe and Newfoundland that were absent from Quebec and the eastern United States (e.g., #24, #26, and #27).

A single haplotype (#15) was common to continental North America, Europe, and Newfoundland (Table 3) but occurred at different frequencies. It represented 58% of the Newfoundland samples, 27% of the European samples, and only 8% of the continental North American samples (Table 3). Two additional haplotypes were common to Newfoundland and Europe but were absent from Quebec and the eastern United States, one of which (#19) was equally frequent in both sampling regions (Table 3).

Some of the rare haplotypes in continental North America were present in several geographic regions (e.g., #6 and #8), whereas

others were restricted to a single region (e.g., #1, #2, and #3; data not shown). Direct comparisons between populations in different areas have to be made carefully because of differences in sample size between regions and the potential chronological gradient in sampling. Nevertheless, rare haplotypes were more frequent in the central Mauricie region (9 haplotypes; $n = 34$) than further east in the Gaspé Peninsula (4 haplotypes; $n = 20$).

Large values of genetic differentiation were obtained by AMOVA when all regions were considered (Table 4). Approximately 62% of the total genetic variability can be explained by the sampling of different geographic regions. Pairwise comparisons indicated that most of the genetic diversity among regions was attributable to differences between continental North America and Newfoundland ($\Phi_{ct} = 0.665$, $P < 0.001$), with smaller levels of genetic diversity between Newfoundland and Europe ($\Phi_{ct} = 0.201$, $P < 0.001$) and between continental North America and Europe ($\Phi_{ct} = 0.538$, $P < 0.001$). However, genetic differentiation among regions within continental North America was small ($\Phi_{ct} = 0.047$) and not significant ($P = 0.079$; Table 4).

Analysis of genetic distance also indicated that all continental North American populations were closely related and genetically distant from those from Newfoundland and Europe. Populations from Newfoundland and Europe were, however, more closely related than those from continental North America and Europe (Fig. 3).

Phylogenetic analysis of the haplotypes indicated that the occurrence and frequency of haplotypes were correlated to geographic origin (Fig. 4). A larger intersection of haplotypes could

TABLE 3. Frequency of *Gremmeniella abietina* var. *abietina* haplotypes by region

Region	N	Haplotype ^a							
		4	5	8	14	15	17	18	19
Continental North America									
Gaspé	22	0.00	0.86	0.00	0.00	0.00	0.00	0.00	0.00
Beauce	10	0.00	0.80	0.00	0.00	0.20	0.00	0.00	0.00
Mauricie	34	0.00	0.53	0.06	0.00	0.15	0.00	0.00	0.00
Eastern Townships	14	0.00	0.75	0.08	0.00	0.08	0.00	0.00	0.00
Abitibi	12	0.17	0.67	0.00	0.00	0.17	0.00	0.00	0.00
Montreal	20	0.00	0.70	0.10	0.00	0.10	0.05	0.00	0.00
Outaouais	80	0.00	0.78	0.04	0.00	0.05	0.00	0.00	0.00
United States	12	0.09	0.82	0.00	0.00	0.09	0.00	0.00	0.00
Newfoundland	26	0.00	0.00	0.00	0.15	0.58	0.00	0.00	0.19
Europe	15	0.00	0.00	0.00	0.00	0.27	0.07	0.40	0.13

^a Haplotypes are based on 22 random amplified polymorphic DNA and random amplified microsatellite markers (defined in Table 1). Haplotypes 1 through 3, 6, 7, 9 through 13, 16, and 20 through 27 had low frequencies and were mostly found in single regions.

TABLE 4. Analysis of molecular variance in populations of *Gremmeniella abietina* var. *abietina* based on haplotypic frequencies and distances

Source	df	Variance	Percentage of total variance (%)	Φ statistic	P value ^a
Among groups (continental North America-Newfoundland-Europe)	2	0.109	61.75	0.617	<0.001
Among populations within group	7	0.003	1.82	0.048	0.003
Within populations	233	0.064	36.43		<0.001
Among groups (continental North America vs. Newfoundland)	1	0.133	66.48	0.665	<0.001
Among populations within group	7	0.003	1.62	0.048	<0.001
Within populations	219	0.064	31.90		<0.001
Among groups (continental North America vs. Europe)	1	0.080	53.80	0.538	<0.001
Among populations within group	7	0.003	2.13	0.046	<0.001
Within populations	208	0.065	44.06		<0.001
Among groups (Newfoundland vs. Europe)	1	0.015	20.07	0.201	<0.001
Within populations	39	0.061	79.93		<0.001
Among groups (among continental North America)	7	0.003	4.68	0.047	0.079
Within populations	194	0.065	95.32		<0.001

^a Probability of a larger value obtained by chance, determined by 100 randomizations of the data set.

be seen between Europe and Newfoundland than between Europe and Newfoundland and continental North America. Some of the rare haplotypes found in Quebec probably were derived from distinct introductions, because there was large phylogenetic divergence among the most common haplotypes. For example, 11 RAPD markers differed between haplotypes #15 and #13, and 7 markers differed between haplotypes #21 and #5 (Fig. 4 and Table 1).

Other haplotypes probably derived from other haplotypes through mutations or somatic recombination. For example, single RAPD markers differed between haplotypes #5 and #4 and #9 (Fig. 4). Both haplotypes #4 and #9 were found in distant regions, in Abitibi and the Gaspé Peninsula, where variability in the pathogen population was low.

DISCUSSION

The discovery of the EU race of Scleroderris canker in New York State in 1975 was followed by intensive surveys to identify infection foci. The disease was almost simultaneously reported in three other states as well as in three Canadian provinces (13, 19, 31). The first report of the disease in Canada was in southern Quebec near the U.S.-Quebec border (13). During the following years, the disease was reported further north, west, and east and now occurs from Ontario and the Lake States to Newfoundland.

The chronology of these events suggests the pathogen was introduced from the northeastern United States followed by spread into other states and provinces. However, the results presented in this study are not consistent with this hypothesis. The uneven distribution of *G. abietina* var. *abietina* haplotypes in continental North America and Newfoundland and apparent discontinuity in the epidemic strongly suggest there were at least two distinct introductions of this fungus into North America: one on the continent and another in Newfoundland, with little or no gene flow between the two founding populations. On the other hand, popu-

lations in continental North America are clearly part of a single epidemiological unit, as indicated by the low level of gene diversity and low genetic distance among regions on the continent.

In Scandinavia, the population structure of *G. abietina* var. *abietina* belonging to the biotype found in North America resembles the population structure observed in continental North America. The same banding patterns occur in several geographically isolated populations, and genetic differentiation among populations is moderate ($F_{st} = 0.097$) and comparable to that observed among continental North American populations in our study (11). Nevertheless, genetic diversity within *G. abietina* var. *abietina* may be higher in Scandinavia, because 37 M13 banding patterns were found among 71 isolates compared to 20 RAPD haplotypes among 204 isolates from continental North America. Although this comparison may not be valid because different markers were used, we also found 6 profiles among 15 isolates from Europe, a proportion similar to that reported for M13 (11). The presence of a sexual cycle in Scandinavia and its absence from North America could explain these differences (35).

Similar high levels of gene diversity between distant geographic regions have been reported for other pathogenic ascomycetes. G_{st} values of 0.180 were reported among *Rhynchosporium secalis* populations from Australia, Europe, and Asia (6), and a G_{st} value of 0.37 was reported among *Cryphonectria parasitica* populations from North America, Europe, and Japan (24). Genetic differentiation is not always proportional to geographic distance, however. A large G_{st} value was reported for *C. parasitica* between populations that were only 16 km apart. This high level of genetic differentiation can be explained best by a restriction of gene flow between these local populations (23). Gene flow in *G. abietina* var. *abietina* appears to be restricted between Newfoundland and continental North America but apparently is extensive among regions on the continent. Even though the geographic distance between some continental populations (e.g., ~700 km between

Fig. 3. Unweighted pair group arithmetic mean dendrogram, based on Φ_{st} genetic distances between *Gremmeniella abietina* var. *abietina* populations.

Gaspé and Abitibi) is as large as that between some continental populations and Newfoundland (e.g., ≈600 km between Gaspé and Newfoundland), the genetic distance is far larger between any continental North American and Newfoundland population than between continental North American populations (Fig. 4).

Two hypotheses can be proposed to explain the dominance of a single haplotype of *G. abietina* var. *abietina* in continental North America. First, it is possible that this haplotype is better adapted or more aggressive than other rare haplotypes. If adaptation is an important factor in the population structure of this pathogen, however, the same haplotype would not be expected to dominate all of the eco-climatic zones represented in this survey. Also, it would be expected that the Newfoundland and Gaspé populations would share the same adapted haplotype, because maritime climates prevail in these regions.

A second hypothesis is that a founder effect followed by loss of mating type and genetic drift resulted in an unbalanced population structure with a few dominant haplotypes (2). Founder effects have been proposed to explain low diversity in some populations of *C. parasitica* (18). The founding population could have been composed mostly of haplotypes #5 and #15, and the rare haplotypes were either introduced later or were found at a much lower frequency. This hypothesis is supported by the dominance of haplotype #5 (82%) in the isolates representing the earliest records of the EU race of *G. abietina* var. *abietina* in North America.

The hypothesis of a founder effect in this pathogen is particularly plausible if the frequent haplotypes were present in a few large nurseries and were disseminated on nursery stock. Dissemination of this pathogen has been suspected to occur via infected symptomless seedlings that escaped inspections prior to outplanting (20,32). The endophytic nature of this pathogen predisposes its dissemination on nursery material (14,29,37).

Phylogenetic analysis of the haplotypes provided information about the possible processes underlying this epidemic. Some of the rare haplotypes in Quebec were genetically distant from all other haplotypes and clearly not derived from a common source. It is possible that these rare haplotypes also represented distinct introductions onto the continent, but unlike the haplotypes present in Newfoundland, subsequent gene flow probably distributed these haplotypes between populations on the continent.

Fig. 4. Phylogenetic relationship between 27 haplotypes of the European race of *Gremmeniella abietina* var. *abietina*.

Other haplotypes were phylogenetically closely related and may have evolved from mutations or somatic or sexual recombination. However, apothecia have never been observed for the EU race in North America, and it is believed that sexual reproduction does not take place. Mutations have been proposed to explain the occurrence of wheat leaf rust with very similar DNA profiles but distinct virulence (12) and *C. parasitica* lines differing only by one or two fingerprint fragments (18).

Genetic distance, gene diversity, and haplotype phylogeny analyses all indicated that the Newfoundland population was more closely related to the European population than was its continental North American counterpart. The complete absence of the most common haplotype in North America from our European samples indicates that either it is a rare haplotype in Europe that we failed to detect, or it originated elsewhere. The true center of origin of the EU race is still unknown, although northern Europe has been considered a likely source (8).

The current study highlights the importance of understanding global epidemics of exotic pathogens. Several countries are regulating *G. abietina* var. *abietina* through quarantines and seedling inspection programs. Our findings indicate that Newfoundland and continental North America can be considered distinct epidemiological units. Also, European populations of *G. abietina* var. *abietina* are genetically very different from continental North American populations of the *G. abietina* var. *abietina* EU race. A larger sampling of Europe and Asia would be necessary to fully elucidate the center of origin of this pathogen.

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