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Pathogenic variation in poplar rust *Melampsora larici-populina* from England

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Abstract

Using a leaf disc method, 19 isolates of the poplar rust, *Melampsora larici-populina*, and one isolate of *M. populnea* from England were inoculated on to 25 poplar clones belonging to *Populus nigra* and *P. trichocarpa*, and hybrids between *P. deltoides* and *P. nigra*, *P. deltoides* and *P. trichocarpa*, *P. tacamahaca* and *P. trichocarpa*, and *P. alba* and *P. tremula*. Disease was scored based on the pustule area and inoculum density. In terms of whether sporulating uredinia formed, the 19 isolates showed seven different patterns to the tested poplar clones. The majority of the rust isolates infected *P. nigra* 'P3090' and 'Vereecken', *P. nigra* × *P. deltoides* 'Casale' and 'Tasman', *P. tacamahaca* × *trichocarpa* '36' and 'Balsam Spire', and *P. trichocarpa* 'Blom'. *Populus trichocarpa* × *P. deltoides* '69039/4' was infected by only three isolates collected from southern England. No visible symptoms appeared on *P. alba* × *P. tremula* 'Tower' and *P. trichcarpa* × *P. deltoides* × *P. deltoides* '76028/5' in inoculations with *M. larici-populina* isolates. *Populus alba* × *P. tremula* 'Tower' was infected only by *M. populnea*. When *M. larici-populina* isolates were tested using AFLP, no differences were found either between isolates from different geographical regions or between those having 'narrow' spectrum of virulence and those showing 'wide' spectrum of virulence on the tested clones. The results suggest that the UK rust populations possess virulences which were found in races E1, E2, E3 and E4 in continental Europe and that rust having virulence patterns similar to race E4 has occurred in UK poplar plantations since 1996.

Introduction

Poplars (*Populus* spp.) occur naturally in most parts of the northern hemisphere from subarctic to subtropical regions (Bean, 1976). Species belonging to *Populus* are grouped into five sections: Secs. *Leuce* (white poplars, grey poplars and aspens), *Tacamahaca* (balsam poplars), *Aegiros* (black poplars), *Leucoides* and *Turanga*. Poplars have long been cultivated in various regions of the world and those most widely planted for farmland sheltering, urban amenity, wood and biomass production are the species and hybrids of black and balsam poplars.

In Europe, poplar cultivation started to expand in 18th century, when North American poplars were introduced to hybridise with European poplars (Guinier, 1958). In the 20th century, several institutions in Europe worked on selection and breeding of poplars, especially sections *Aigiros* and *Tacamahaka*. For example, over the past 50 years, a poplar breeding programme based in Belgium has been concentrated on four species, *P. deltoides*, *P. nigra*, *P. trichocarpa* and, to a lesser degree, *P. maximowczii* (Steenackers, 1988). *Populus deltoides* and *P. nigra* belong to section *Aigiros*, whilst *P. trichocarpa* and *P. maximowiczii* to section *Tacamahaca*. *Populus deltoides* and *P. trichocarpa* are

native to North America, *P. nigra* to Europe and *P. maximowczii* to the Far East.

Rust caused by *Melampsora* spp. is one of the most serious diseases of poplars. Of several *Melampsora* species described on poplar, the most widespread and frequent is *M. larici-populina*. This rust occurs on poplars belonging to Sections *Tacamahaca*, *Aegiros* and *Leucoides*. It produces five spore stages during the life-cycle and alternates on larch (*Larix*). The rust is indigenous to Eurasia, but was spread to Australia in the 1970s (Van Kraayenoord et al., 1974) and, more recently, to North America (Newcombe and Chastagner, 1993). In Western Europe, native *P. nigra* is susceptible to *M. larici-populina* (Pinon, 1992). Introduced clones of *P. deltoides* of northern origin are generally more resistant to rust than those of southern origin (Steenackers, 1988; Pinon, 1992). *Populus trichocarpa* varies in rust resistance, while *P. maximowczii* is relatively resistant (Steenackers, 1988). In the Far East, *M. larici-populina* occurs on a wide range of poplar species and hybrids, and often causes severe damage (Pei and Shang, 1984).

In Europe, hybrids between *P. deltoides* and *P. nigra* or *P. trichocarpa* were selected in the mid 20th century for their immunity to rust (Steenackers, 1988). Cultivation of these so-called 'Unal' clones (e.g. *P. trichocarpa* × *P. deltoides* 'Unal', 'Boelare' and 'Beaupré') were successful for many years (Steenackers, 1991) and they are grown widely throughout north-western Europe. Breakdown of resistance to *M. larici-populina* in these clones was detected in the early 1980s with appearance of races E1 and E2 (Steenackers, 1982; Pinon et al., 1987). Race E3 was detected in 1986 in Italy, but not considered to have great impact on important commercial clones (Pinon and Peulon, 1989). In the autumn of 1994, rust infections were encountered on 'Boelare' and 'Beaupré', and on *P. deltoides* × *P. nigra* 'Primo' , 'Ghoy' and 'Gibecq' in continental Europe. Subsequent studies revealed that the outbreaks of rust on these clones were caused by a new race, E4 (Steenackers et al., 1994).

In the UK, several 'Unal' clones have been planted in plantations. Up to 1995, UK poplar plantations sustained slight to moderate rust infections (Longsdale and Tabush, 1998). In 1996, rust outbreaks occurred in many UK plantings, with short-rotation coppice (SRC) and some close-spaced single-stem plantations in south-west England being among the most seriously affected. In some cases, the rust resulted in stem dieback and even death of coppice stools. Since 1996, poplars in south-west England were severely infected by rust each year (M. Pei and T. Hunter, unpublished observations). It is likely that the poplars in the UK have become susceptible due to the appearance of new pathotypes of the rust. To date, however, little is known of pathogenic variation in *M. larici-populina* in the British Isles. The present study was conducted to determine pathogenic variation and genetic background of *M. larici-populina* from England and to compare the results with the existing information from continental Europe.

Materials and methods

Rust isolates

Rust-infected poplar leaves collected from Markington, North Yorkshire (northern England), Alice Holt, Surrey (southern England), South Molton, Devon, and Long Ashton, North Somerset (south-west England), during 1995–2000 (Table 1). Rust isolates were derived from single uredinial pustules. To obtain the single-pustule isolates, urediniospores from each collection were inoculated on to detached leaves of the host clone or, in the cases that host clones were not available, of 'substitute' clones. The 'substitute' clones were chosen from those found to be susceptible to the rust collections in preliminary inoculation tests. As a result, *P. trichocarpa* 'Blom' was used for isolation and bulking up of the isolates C, G, K, O and P. *Populus alba* × *P. tremula* 'Tower' was used for isolate T. The rust spores were stored at −20 °C.

Species identity of the isolates was determined by morphological examination of the rust-infected leaves collected from the field and confirmed by examining the telia produced by the isolates. To produce telia, each isolate was inoculated on to detached leaves of the host clone or the 'substitute' clone, which were placed on water-soaked filter paper in Petri dishes. The inoculated leaves were incubated for 2 weeks in a growth chamber at 16 °C with 16 h day^{-1} illumination at an intensity of 80 μE m^{-2} s^{-1} and for a further 6 weeks in an illuminated cold room at 4 °C.

Table 1. Melampsora isolates tested

Rust isolate	Host species	Clone	Site and date of collection	
A	*P. trichocarpa* × *P. deltoides*	'Beaupré'	Markington, N Yorkshire, N England	Sept. 1997
B	*P. trichocarpa* × *P. deltoides*	'Beaupré'	South Molton, Devon, SW England	Sept. 2000
C	*P.* × *berolinensis*		Alice Holt, Surrey, S England	Sept. 2000
D	*P. trichocarpa*	'Blom'	Long Ashton, N Somerset, SW England	Oct. 1998
E	*P. trichocarpa* × *P. deltoides*	'Boelare'	Long Ashton, N Somerset, SW England	Sept. 1996
F	*P. trichocarpa* × *P. deltoides*	'Boelare'	Markington, N Yorkshire, N England	Sept. 1997
G	*P. cathayana*		Alice Holt, Surrey, S England	Sept. 2000
H	*P. deltoides* × *P. trichocarpa*	'Donk'	Alice Holt, Surrey, S England	Sept. 2000
I	*P. deltoides* × *P. nigra*	'Ghoy'	Alice Holt, Surrey, S England	Sept. 2000
J	*P. deltoides* × *P. nigra*	'Ghoy'	South Molton, Devon, SW England	Sept. 2000
K	*P. laurifolia*		Alice Holt, Surrey, S England	Sept. 2000
L	*P. deltoides* × *P. nigra*	'Robusta'	Alice Holt, Surrey, S England	Sept. 2000
M	*P. deltoides* × *P. nigra*	'Robusta'	Markington, N Yorkshire, N England	Sept. 1995
N	*P. deltoides* × *P. nigra*	'Robusta'	Markington, N Yorkshire, N England	Sept. 1995
O	*P. simonii*		Alice Holt, Surrey, S England	Sept. 2000
P	*P. szechuanica*		Alice Holt, Surrey, S England	Sept. 2000
Q	*P. trichocarpa* × *P. trichocarpa*	'Trichobel'	South Molton, Devon, SW England	Sept. 2000
R	*P. nigra*	'Vereecken'	Markington, N Yorkshire, N England	Sept. 1996
S	*P. nigra*	'Vereecken'	Markington, N Yorkshire, N England	Sept. 1997
T	*P. grandidentata* × *P. alba*		Alice Holt, Surrey, S England	Sept. 2000

The isolates, except for isolate T, were inoculated on to detached leaves of the respective host clones or 'Blom' 2 weeks before the inoculation experiment to produce fresh urediniospores. Isolate T was inoculated on to 'Tower' 4 weeks before inoculation experiment because its incubation period proved be longer than other isolates in preliminary tests.

Poplar plants

Twenty-five poplar clones (Table 2) were grown from 1-year-old dormant cuttings in pots containing 'John Innes' Compost No. 3 in a glasshouse for 8 weeks. At the time of inoculation, plants from these cuttings were 60–90 cm tall and actively growing.

Inoculation experiment

Leaf discs, 1.6 cm diameter (2.01 cm^2 area), were cut from 5th to 10th leaves on actively growing poplar shoots. The discs were placed, abaxial surface uppermost, on blotting paper bridges soaked in tap water in 25 (5 × 5) compartments of 10 × 10 cm^2 Petri dishes. Five leaf discs, each from different leaves, were used for each poplar clone/rust isolate combination. A 60 mm diam. Petri dish containing 1.2% water agar was also placed in the spray target area. Rust spores were suspended in tap water containing 0.004% Tween 20 (1 drop in 100 ml) and spore suspensions were adjusted to 40,000 spores ml^{-1}. The spore suspensions were sprayed on to the target area (1 ml per 10 × 10 cm area) using a Humbrol air brush (Humbrol Ltd., England).

After inoculation, the leaf discs were incubated in a growth chamber at 16 °C with 16 h day^{-1} illumination (80 μE m^{-2} s^{-1}). The inoculum density (viable spores per leaf disc) for each rust isolate was determined by counting the number of germinating spores on the water agar in 10 microscopic fields (10 × 10 magnification, 2.4 mm^2 each field) 24 h after inoculation.

AFLP procedure

A two-tube method was used to extract genomic DNA from rust urediniospores (Pei and Ruiz, 2000). For each rust isolate, approximately 5 mg spores were used. The AFLP was performed as described by Pei and Ruiz (2000), which was modified from the two-step PCR protocol by Vos et al. (1995). In the first step (pre-amplification), *Eco*RI and *Mse*I primers, each having 1 bp extension, were used. In the second step, *Eco*RI and *Mse*I primers having 3 bp extensions were used in four combinations: E-AAA/M-CAA,

Table 2. Infection type scores of *M. larici-populina* isolates from England on 25 poplar clones tested

Clone	G	K	L	M	N	O	P	R	B	F	A	D	E	J	Q	S	C	H	I
alb × trem 'Tower'	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
d × n 'Dorskamp'	0	0	0	0	0	0	0	0	2	2	3	1	2	1	1	2	1	2	2
d × n 'Ghoy'	0	0	0	0	0	0	0	0	2	2	3	1	2	2	3	2	2	3	2
d × n 'Gilbecq'	0	0	0	0	0	0	0	0	2	3	3	2	2	3	2	2	2	2	3
d × n 'Primo'	0	0	0	0	0	0	0	0	1	3	2	2	2	3	2	2	2	2	3
d × tr 'Barn'	0	0	0	0	0	0	0	0	2	3	3	2	2	4	4	3	2	2	2
n 'P3090'	2	2	3	1	3	2	0	2	2	2	3	1	1	4	4	3	2	2	3
n 'Vereecken'	3	4	4	3	4	3	4	4	4	4	3	3	3	4	4	4	3	3	3
n × d 'Casale'	3	3	3	2	4	2	2	4	1	2	2	2	2	3	3	3	2	3	2
n × d 'Flevo'	0	0	0	0	0	0	0	0	1	2	3	1	2	3	3	3	2	2	3
n × d 'Spijk'	0	0	0	1	0	0	1	0	1	3	2	1	2	3	3	1	2	2	2
n × d 'Tasman'	3	3	2	2	4	2	3	3	2	3	3	1	3	2	4	4	2	3	3
ta × tr '36'	3	4	3	2	4	2	3	3	3	3	3	2	3	3	4	4	3	4	3
ta × tr 'Balsam Spire'	3	4	2	2	4	2	3	3	3	1	3	2	2	4	4	3	3	3	2
tr 'Blom'	2	2	3	1	4	2	3	3	2	2	2	2	3	4	4	4	2	3	3
tr 'Heimberger'	1	2	1	0[a]	3	1	1	1	2	1	1	1	1	1	2	2	2	2	1
tr 'Tichobel'	1	2	1	1	2	0	1	2	1	2	1	1	2	2	2	2	1	2	2
tr × d '69039/04'	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2
tr × d '71009/01'	0	0	0	0	0	0	0	0	0	3	2	2	2	3	4	3	2	4	3
tr × d 'Beaupré'	0	0	0	0	0	0	0	0	3	2	3	2	3	4	4	3	3	4	3
tr × d 'Boelaré'	0	0	0	0	0	0	0	0	3	3	3	2	4	4	4	2	2	3	3
tr × d 'Raspalje'	0[a]	1	1	1	2	0[a]	3	1	1	1	1	1	2	3	4	4	2	3	1
tr × d 'Unal'	0	0	1	1	1	0	1	0	1	1	0	0	0	1	1	0	1	1	0
tr × d × d '75028/3'	0	0	0	0	0	0	0	0	3	3	2	2	4	4	3	4	4	4	4
tr × d × d '76028/5'	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a] Only poorly developed, unruptured uredinia (flecks) appeared.

Table 3. Pathogenicity of E1-E4 of *M. larici-populina* from north-western Europe to some poplar clones (extracted from Pinon (1992) and Steeanckers (1994))

Host species/hybrid	Clone	E1	E2	E3	E4
P. deltoides × *P. nigra*	'Ghoy'	−	−	−	+
P. deltoides × *P. nigra*	'Gibecq'	−	−	−	+
P. trichocarpa × *P. deltoides*	'Beaupré'	−	−	−	+
P. trichocarpa × *P. deltoides*	'Boelare'	−	−	−	+
P. trichocarpa × *P. deltoides*	'69039/4'	−	−	−	−
P. trichocarpa × *P. deltoides*	'Unal'	+	+	+	
P. trichocarpa × *P. deltoides*	'Raspalje'	+	+	+	
P. nigra × *P. deltoides*	'Spijk'	+	+		
P. trichocarpa × *P. trichocarpa*	'Trichobel'		+		

E-AAA/M-CAC, E-AAA/M-CAG and E-AAA/M-CAT. The PCR products were denatured and run on a 6% polyacrylamide sequencing gel and autoradiographs were obtained using Hyperfilm MP (Amersham).

Disease assessment

Inoculated leaf discs were examined daily from 5 days after inoculation and the incubation period was recorded as days from inoculation until the appearance of ruptured uredinia on the leaf disc. The incubation period for each poplar clone/rust isolate combination was calculated by averaging the data from the replicates. Isolate T was assessed 5–12, 16, 20, 24 days after inoculation.

Fourteen days after inoculation, reactions on leaf discs were recorded using a digital camera (Olympus C-2500L). Image analysis software SigmaScan Pro 5.0 (SPSS Inc.) was used to assess the disease. For each leaf disc, the number of pustules were counted and the diameter of ruptured uredinia were measured manually using 'Trace Measurement Mode' function. If there were more than 10 pustules on a leaf disc, only 10 randomly selected pustules were measured to

obtain an estimate of average pustule diameter on the leaf disc.

Data analyses

Disease data. Previous studies suggested that inoculum densities can greatly influence disease severity in *M. larici-populina* (Giocelli et al., 1996; Pei et al., 2003). In this study, disease was scored based on pustule area and inoculum density data according to the method described by Pei and Hunter (in press) using the following steps.

1. For each clone/isolate combination, a slope factor was calculated by dividing average square root pustule area per leaf disc by square root inoculum density per leaf disc for the isolate.
2. For each isolate, the clone which produced the largest pustule area (the most susceptible reaction) was chosen and the maximum slope factor was calculated by dividing its average square root pustule area by square root inoculum density. An average maximum slope factor (AMSF) was calculated by averaging the maximum slope factors from all the isolates.
3. Disease was scored using 0–4 scales. An assumption was made that the AMSF represents the mid point for the scale 4, the most susceptible reaction. Disease scores were given as scale 0, slope factor = 0; scale 1, 0 < slope factor ≤ AMSF × 2/7; scale 2, AMSF × 2/7 < slope factor ≤ AMSF × 4/7; scale 3, AMSF × 4/7 < slope factor ≤ AMSF × 6/7; scale 4, slope factor > AMSF × 6/7.

Analyses of variance were performed for the number and size of uredinia on tested clones for each isolate and by grouping the poplar clones according to their species/hybrid origin. Principle coordinate analyses were conducted by coding the clone/isolate combinations which supported growth of sporulating uredinia (infection types 1–4) as '1' and those which did not support rust sporulation (infection type 0) as '0'. Nei and Li's (1979) similarity coefficient were used to construct the similarity matrix and principal coordinate analysis was performed using unweighted pair-group method using arithmetic means (UPGMA) (Sneath and Sokal, 1973).

AFLP data. The presence and absence of an unambiguous band was scored as 1 and 0, respectively. Similarities were computed between all pairs of isolates (except T and K, see results) using the Nei and Li's similarity coefficient. All AFLP markers, both mono- and polymorphic, were included in data analysis. A dendrogram was constructed using UPGMA (Sneath and Sokal, 1973). The robustness of the clusters was examined by applying the bootstrap method with 100 replicates and the dendrogram was plotted using TREECON software (Van de Peer and De Wachter, 1994). An analysis of molecular variance (AMOVA) (Excoffier et al., 1992) was conducted (a) by grouping the isolates according to the regions, i.e. northern England (Markington), southern England (Alice Holt) and south-west England (South Molton, Devon and Long Ashton), and (b) by grouping them according to their virulence patterns ('narrow' or 'wide' spectrum of virulence on the tested clones, see Results). The variance components and Φ-statistics were tested by nonparametric randomisation tests using 1000 repetitions.

Spearman's rank correlation was tested for correlation between the similarities derived from AFLP and that from by coding the clone/isolate combinations which supported growth of sporulating uredinia (infection types 1–4) as '1' and those which did not support rust sporulation (infection type 0) as '0'.

Calculations were done using GenStat 5 Release 4.2 (Genstat Committee, 2000).

Results

Inoculation experiment

All the rust isolates, except T, had unevenly ehinulate, relatively thin-walled urediniospores and epiphyllous telia. They were identified as *M. larici-populina*. Isolate T was identified as *M. populnea* (Pers.) P. Karst. on the basis that its urediniospores were relatively small, evenly ehinulate and its telia were hypophyllous (Wilson and Henderson, 1966).

Inoculum densities were estimated in a range of 134–624 viable spores per leaf disc. During the 14 days of incubation, all leaf discs were sustained well on the filter paper bridges soaked in tap water.

The incubation period was recorded from 9 days after inoculation and a further 2–3 day period was needed for most uredinia to appear. No visible symptoms appeared on *P. alba* × *P. tremula* 'Tower' and *P. trichcarpa* × *P. deltoides* × *P. deltoides* '76028/5' inoculated with *M. larici-populina* isolates when examined 14 days after inoculation. Isolate T only produced symptoms on 'Tower' after prolonged incubation (uredinia were not observed on 'Tower' until when it was examined 20 days after inoculation). Only poorly developed unruptured uredinia (flecks) appeared on *P. trichocarpa* × *P. deltoides* 'Raspalje' inoculated with isolates G and O, and on *P. trichocarpa* 'Heimberger' inoculated with isolate M, 14 days after inoculation. The majority of rust isolates produced uredinia on *P. nigra* 'P3090' and 'Vereecken', *P. nigra* × *P. deltoides* 'Casale' and 'Tasman', *P. tacamahaca* × *trichocarpa* '36' and 'Balsam Spire', and *P. trichocarpa* 'Blom' (Table 2). Isolate E, collected in Long Ashton in 1996, was virulent to the tested *P. trichocarpa* × *P. deltoides* clones, e.g. 'Beaupré' and 'Boelare'. *Populus trichocarpa* × *P. deltoides* '69039/4' was infected only by isolates I, C and H, all collected from Alice Holt in 2000. About half of the isolates caused severe infections (Infection types 3–4) on *P. trichocarpa* × *P. deltoides* × *P. deltoides* '75028/3'.

Among the replicates, the size of uredinia appeared to be more consistent than the number of uredinia. The infection efficiency was the highest in 'Vereecken'/N (0.09). ANOVA revealed that, for each *M. larici-populina* isolate, there were significant differences in the number and size of uredinia among the poplar clones which produced uredinia (data not shown). The average diameter of uredinia on *P. deltoides* × *P. nigra* clones was significantly smaller than that on the majority of other poplar species/hybrids.

In terms of whether sporulating uredinia developed on the tested clones, the 19 *M. larici-populina* isolates could be placed into seven groups (Figure 1). Among those placed in the same group, there were marked differences in the degree of virulence. For example, under the similar inoculum pressure, uredinia produced by isolate S were larger than those developed from isolate E, notably on *P. nigra* 'Vereecken'.

AFLP

Isolate T was excluded from analyses because it shared few common AFLP bands with other isolates. Isolate K failed to produce PCR products. For the remaining 18 isolates, a total of 103 AFLP markers were scored for the four primer combinations. Of those, 94 markers (87.4%) were polymorphic. Nei and Li's (1979) similarity coefficients among the isolates were calculated as in a range of 0.45–0.92 (Figure 2). Bootstrap values for most of the branches were lower than 50%. AMOVA analysis showed no evidence of differentiation

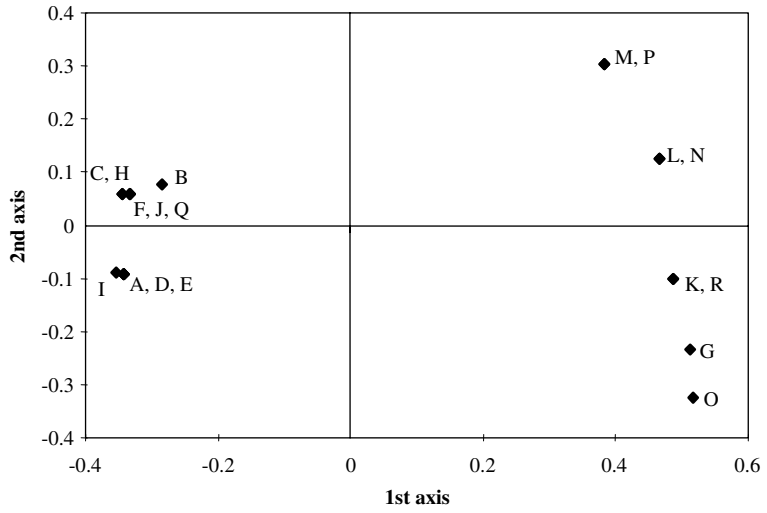

Figure 1. Principal coordinate analysis of *Melampsora larici-populina* isolates, based on whether sporulating uredinia formed.

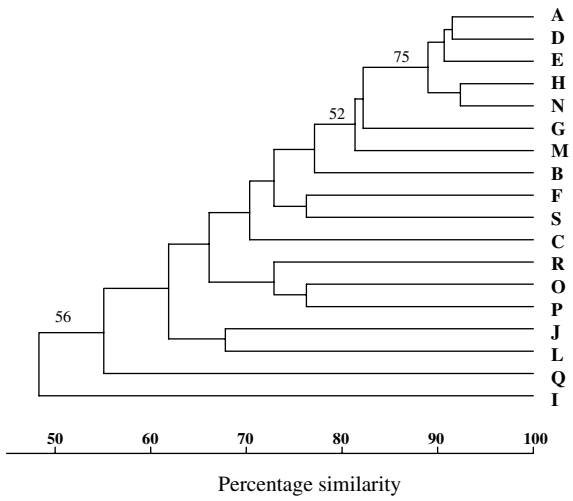

Figure 2. Dendrogram of *Melampsora larici-populina* isolates based on 103 AFLP loci. The dendrogram is constructed using Nei and Li's similarity coefficient and bootstrap values of 50% or greater (100 replicates) are given.

between the regions, 100% variation being attributed to among isolates within regions (data not shown). When AMOVA was performed by treating the isolates having 'narrow' spectrum of virulence, i.e. G, K, L, M, N, O, P and R (see Table 2) as one group and the remainder as another, all the variation was again attributed to among isolates within groups.

Spearman rank correlation test showed no significant correlation between the similarities derived from AFLP data and that from virulence/avirulence patterns ($P = 0.35$).

Discussion

This study revealed considerable variation in the pathogenicity within populations of *M. larici-populina* in England. In terms of whether sporulating uredinia formed, seven different patterns could be identified among the 19 isolates (Figure 1). Moreover, there were marked differences in the degree of virulence among the isolates showing similar patterns of pathogenicity.

The experimental procedures used were simple and effective. Previously, this method had been used for studies of quantitative relationships between inoculum and corresponding disease in the willow rust *M. larici-epitea* and *M. larici-populina* (Pei et al., 2002b, 2003). In this study, a total of 25×19 poplar clone/*M. larici-populina* isolate combinations were tested and the size and numbers of uredinia were examined. With such a large number of leaf discs to be assessed, it would have been extremely difficult to score the disease accurately using conventional methods by the time the experiment was terminated. The digital profiling method solved the problem by allowing the pustule numbers to be counted and the sizes to be measured later using image analysis software.

Inoculum densities were used to adjust the disease (pustule area) data between inoculations to obtain comparable scores. In rust fungi, the number of pustules produced can be greatly influenced by the number of spores applied and this was found with *M. larici-populina* (Pei et al., 2003). In inoculation experiments involving many isolates, each isolate has to be applied separately and, therefore, it is unlikely to achieve deposition of identical amount of spores on the leaf surface for each inoculation. Because of such practical difficulties, to date, the criteria for assessing interactions between hosts and pathogens have been ambiguous and the accuracy of disease scores relies largely on the experience and skills of the assessors. To address the problem, it has been proposed that inoculum densities should be taken into consideration when disease is scored in inoculation experiments (Pei and Hunter, in press). In the present study, such procedures were effective in obtaining disease scores comparable between inoculations. It needs to be pointed out that the AMSF should be representative of highly susceptible reactions. Otherwise, if the AMSF is estimated based on somewhat resistant reactions, the results may be biased toward exaggerating susceptibility.

In France, race surveys for *M. larici-populina* were conducted during 1987–1990 (Pinon, 1992) and virulence structure of the rust was investigated during 1992–1994 (Pinon and Frey, 1997). The results showed that, in the late 1980s, E1 was the most prevalent of the three races identified (E1, E2 and E3). Pinon and Frey (1997) also tested rust samples collected from two clones of *P. trichocarpa* × *P. deltoides* in Northern Ireland in 1992. Their results showed that the rust collections from Northern Ireland in 1992 did not possess virulence to *P. trichocarpa* × *P. deltoides* 'Unal'. In the autumn of 1994, rust infections occurred in Belgium on

'Beaupré', 'Boelare' and some other clones which had previously been highly resistant to *M. larici-populina* (Steenackers et al., 1994). When tested, several clones resistant to E1, E2 and E3 were shown to be susceptible to new race which was designated E4 (Steenackers et al., 1994).

In the UK, well-developed rust uredinia were observed on a few leaves of 'Beaupré' in the autumn 1994 (Lonsdale, unpublished observations) and severe outbreaks of rust on 'Beaupré' and 'Boelare' occurred in 1996 (Longsdale and Tabush, 1998). In the present study, isolate E collected at Long Ashton in 1996, was highly virulent (IT 3–4) to the tested *P. trichocarpa* × *P. deltoides* clones, e.g. 'Beaupré' and 'Boelare'. It appears that E4 crossed the English Channel in 1994 but did not become widespread in the UK during 1994–1995. Since 1996, however, the rust virulent to the 'Unal' clones has become epidemic in UK poplar plantations. Table 3 lists some of the results from inoculation tests by Pinon (1992) and Steenackers et al. (1994) involving the poplar clones which were included in our study. Because other poplar clones tested by Pinon (1992) and Steenackers et al. (1994) were not tested in our study, comparisons in virulence patterns cannot be made between the continental and UK rust collections. However, it is certain that the UK rust populations possess virulences which were found in E1–E4 rust.

In this study, *P. trichocarpa* × *P. deltoides* '69039/4' was infected with three isolates, all collected from Alice Holt. In Western Europe, the records between 1983 and 1994 (Steenackers, 1991; Steenackers et al., 1994) suggest that '69039/4' was highly resistant to rust. In the UK, '69039/4' was grown in SRC network trials which were established in 1995–1996 for biomass energy production. Disease surveys suggested that '69039/4' had been free of rust in these trials until 1998, when rust infections on this clone was detected in southwest England (David Lonsdale, unpublished results). Among the 24 clones of the species and hybrids of black and balsam poplars tested in this study, only one clone, *P. trichocarpa* × *P. deltoides* × *P. deltoides* '76028/5', showed complete resistance to all the isolates. However, the recent history of poplar rust outbreaks in Europe raises the question whether the resistance in '76028/5' will remain effective in the coming years.

AFLP profiles showed no differentiation between poplar rust isolates from different geographical regions or between those having different virulence patterns. In contrast, AFLP analysis of willow rust, *M. larici-epitea*, showed clear differentiation among the pathotypes tested (Pei et al., 2002a). Results from laboratory crossing experiments suggested that some of the willow rust pathotypes do not exchange genes freely (Pei et al., 1999). It is not known whether different pathotypes in *M. larici-populina* are reproductively isolated. Because only very small numbers of rust isolates were tested, the AFLP data cannot provide any clues as to whether different mating populations exist in *M. larici-populina*. It is well known that, like *M. larici-epitea*, *M. larici-populina* completes a full sexual life-cycle and alternates on larch. If different pathotypes are sexually compatible, as the alternate host European larch (*Larix decidua*) is widespread in the British Isles and the continental Europe (Bean, 1973), more complex virulence patterns could arise through exchange and recombination of genes for virulence.

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