European Journal of Plant Pathology **106:** 771–779, 2000.

Heterogeneous nature of a 'new' pathotype of *Melampsora* rust on *Salix* revealed by AFLP

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Accepted 7 September 2000

Key words: pathotype, Shannon index, multiple sources

Abstract

An outbreak of rust on *Salix* × *mollissima* (*S. triandra* × *S. viminalis*) 'Q83', an important biomass willow, was first observed at several locations in the UK in 1992. Rust collections obtained from 'Q83' in 1992 at Long Ashton (south west England), Markington (Northern England) and Loughgall (Northern Ireland), were tested for pathogenicity and examined using amplified fragment length polymorphism (AFLP). All collections showed the same pathogenicity patterns on the eight willow differentials and were assigned to f.sp. *larici-epitea typica* of *Melampsora epitea*. A total of 304 AFLP markers was scored for 54 rust isolates, 20 from Long Ashton, 20 from Markington and 14 from Loughgall. Cluster analysis placed the isolates into three distinct groups according to the collection sites. Within each site, Markington isolates were least variable, Nei & Li's similarity coefficients averaging 0.996. Average similarities within isolates from Long Ashton and Loughgall were 0.899 and 0.883, respectively. Average per-locus diversity within site (H'_j), calculated using Shannon information index, was 0.014 in Markington, 0.24 in Long Ashton and 0.23 in Loughgall population. Most diversity (69.1%) was partitioned between populations. An analysis of molecular variance (AMOVA) attributed 85.8% of variance to between populations and 14.2% to the individuals within populations. The results suggest that, in 1992, this previously unknown pathotype was not spread from a common source but from separate sources. The AFLP analysis and early records on the host range of *M. epitea* indicate that the rust virulent to *S.* × *mollissima* may have existed in nature before 1992.

Introduction

The rise and spread of new virulent types in plant pathogens, especially in highly specialised biotrophs such as rust fungi, can drastically breakdown host resistance and seriously affect crop productivity. For most rusts, rapid increases in population size are achieved through repeated cycling of asexual urediniospores. The urediniospores are thick-walled and known to travel long distances. The longest documented single movement of wheat leaf rust, *Puccinia graminis* Pers., in North America was 680 km (Roelfs, 1984). To date, however, almost all the information on the spread of rusts is based on the combination of field observations, pathogenicity of rust, weather and spore-trapping data. Using these conventional methods, difficulties arise in determining whether the new type has single or multiple origin in cases where the new type occurs simultaneously or over a short period of time at different locations. So far, convincing evidence on the origin of new virulent types in rust pathogens has been lacking.

Rust caused by *Melampsora* spp. is the most widespread and frequent disease of willows (*Salix*) in short-rotation coppice plantations for renewable energy (biomass willows) in the UK and western Europe. When severe, rust can defoliate willows prematurely, predispose them to other secondary pathogens and lead to serious yield losses and even rootstock death (Parker et al., 1993; Hunter et al., 1996). The

most widespread species of *Melampsora* on biomass willows is the larch-alternating *M. epitea* Thüm. (*M. larici-epitea* Kleb.) (Pei et al., 1993). Within *M. epitea*, several pathotypes (a pathotype is defined as one or a group of pathogen genotypes showing the same virulence/avirulence patterns on a set of host differentials) have been identified and assigned to f.sp. *larici-epitea typica*, f.sp. *larici-retusae*, and f.sp. *larici-daphnoides* (Pei et al., 1996).

Salix × *mollissima* (*triandra* × *viminalis*) 'Q83' is one of the most important biomass willow clones currently grown in SRC plantations in Europe. It had been observed to be highly resistant to rust until 1992 (Hunter and Pei, unpublished results). Surveys of diseases and pests in willow plantations, which were undertaken at many sites in Europe and Canada during 1987–91 (Hunter et al., 1996), confirmed that 'Q83' had been one of the most resistant clones at all investigated sites. In the autumn of 1992, severe rust infections were detected on Q83', for the first time, simultaneously at Long Ashton (south west England), Markington (Northern England) and Loughgall (Northern Ireland). These three sites were among the first established biomass plantations in the UK and many willow clones, including 'Q83', have been grown since 1980s. Since 1992, infections on 'Q83' have occurred at all three sites every year (Hunter and Pei, unpublished results).

In a previous study (Pei et al., 1996), a rust isolate (M7) collected from 'Q83' at Long Ashton in 1992 was designated as pathotype LET4 of f.sp. *larici-epitea typica* of *M. epitea*. *Melampsora epitea* produces five spore stages during the life-cycle and alternates on larch (*Larix* spp.). During the growing season, the rust spreads in willow plantations as urediniospores, which are capable of producing the next generation in 6–7 days (Pei et al., 1996). The rust develops teliospores in the autumn and overwinters on fallen willow leaves. In spring, teliospores germinate to produce basidiospores that infect larch. Fertilization between spermagonia on larch needles results in formation of aeciospores which infect willow. The basidiospores and spermatia are monokaryotic, having a single haploid nucleus, while aeciospores, urediniospores and early phases of teliospores are dikaryotic, containing two nuclei.

Recently, we applied amplified fragment length polymorphism (AFLP) to willow rust (Pei and Ruiz, 2000). AFLP is a PCR-based DNA fingerprinting technique and has proven to be extremely efficient in genotyping and examining genetic variation. Rusts thrive only on living tissues of plants and bulking up material for conventional restriction fragment length polymorphisms (RFLPs), which usually need more than several hundred milligrams of sample, is often difficult and time-consuming. AFLP requires only a small amount of sample and, therefore, has a practical advantage over RFLP in studies of rust fungi.

This study used the AFLP technique to determine whether the rust that was first detected on 'Q83' in the UK in 1992 was spread from a common source (a single genotype or a pool of genotypes) or multiple sources in the growing season of 1992. If the field populations were spread from a single genotype, they would have the same clonal lineage and would show the same or almost identical DNA fingerprints and, if they were from the same pool of genotypes, the fingerprints would show little differentiation between the populations. Otherwise, if they were from different sources in the season of 1992, the DNA profiles would reveal the extent of variation within and between the populations.

Materials and methods

Rust collections and willow differentials

The rust samples on 'Q83' were collected from Long Ashton, Markington and Loughgall in September 1992 (Figure 1). Some 200 leaves bearing active uredinial pustules were collected at random from one-year-old stems on a diagonal transect from a plot, which had a configuration of 5×5 (=25) stools at 1×1 m spacing, at Long Ashton and Markington. Over 50 rust-infected leaves were randomly collected from a 'Q83' plot having the same plantation design at Loughgall. The urediniospores were collected into 2 ml polyethylene vials using a cyclone spore collector, dried at 4 °C for one week and stored at −15 °C until use. Eight willow clones, *S.* × *calodendron* Wimm., *S. burjatica* Nassarov 'Korso' and 'Germany', *S. disperma* Roxb. ex Don. 'LA068/01', *S.* × *mollissima* Hoffm. ex Elwert 'Q83', *S.* × *stipularis* Sm., *S. viminalis* L. 'Mullatin' and *S. viminalis* 'Bowles Hybrid', were grown from 25 cm dormant cuttings in pots containing John Innes Compost No. 3 in an unheated glasshouse. By the time of inoculation, the plants were 60–80 cm tall and actively growing.

Pathogenicity test

Rust collections were tested for pathogenicity to examine their virulence/avirulence pattern on the eight

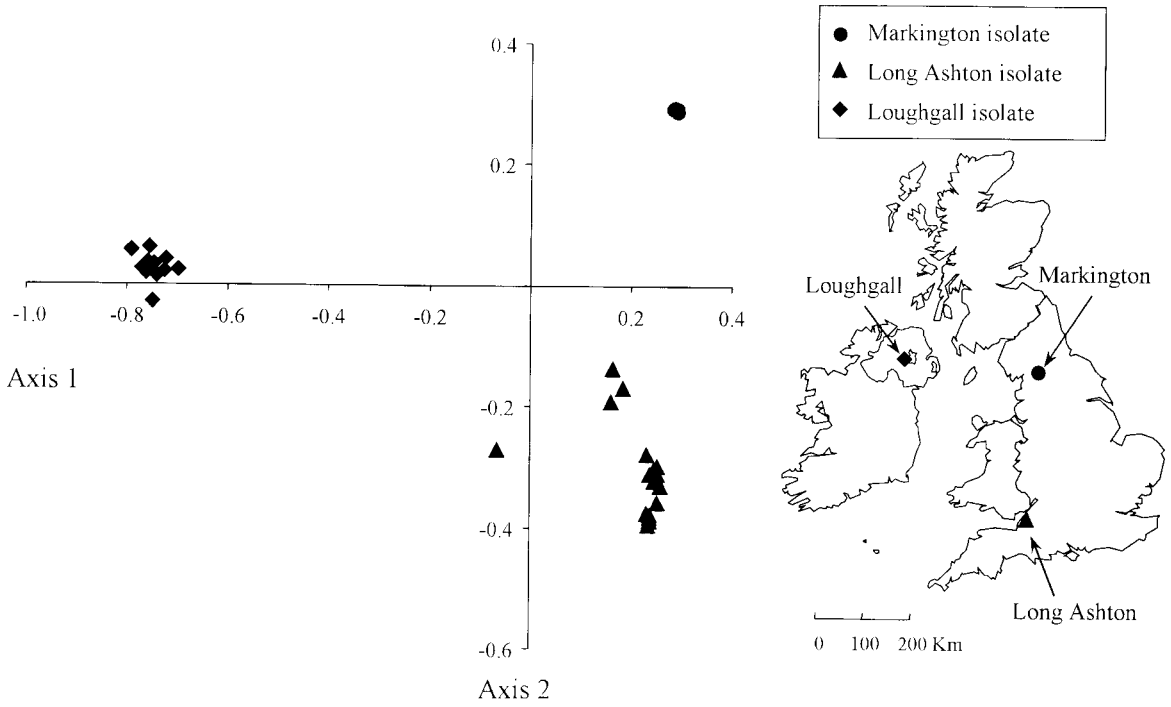

Figure 1. Principal coordinate analysis of *M. epitea* isolates collected from *S.* × *mollissima* 'Q83' at Markington (●), Long Ashton (▲) and Loughgall (◆) in 1992, based on 305 AFLP loci.

willow differentials. Willow leaves between the 5th and 15th leaf counted from the first unfurled (judged as furled edge less than 1/3) or the first expanded (leaf length longer than 2/3 of the leaves on middle of the shoots) were used. The test was done in two steps. In the first step, whole willow leaves of the eight willow differentials were placed, abaxial surface uppermost, on tap-water-soaked filter paper in 14 cm diameter Petri dishes. The urediniospores in the vials were mixed well with camel hair brushes and approx. 3 mg viable urediniospores (viability was checked on 0.5% water agar at 20 °C for 24 h) were suspended in 10 ml distilled water containing 0.004% Tween 20. The spore suspension was sprayed evenly over the detached leaves using an air brush (Humbrol). Four replicates, each having a leaf from each differential, were inoculated with each rust collection. In the second step, spores from 20 single pustules produced on 'Q83' from each collection in the first step were separately inoculated on to three replicates of 1.1 cm diameter leaf discs of *S.* × *mollissima* 'Q83' and *S.* × *stipularis* using a camel hair brush. Inoculated leaves and leaf discs were incubated at 18 °C with 16 h/day illumination at an intensity of 80 μE m^{-2} s^{-1} and examined 11–12 days after inoculation.

AFLP procedure

Fifty-four single-pustule isolates, 20 from Long Ashton, 20 from Markington and 14 from Loughgall, obtained in the second step pathogenicity test, were used for AFLP analysis. The urediniospores of the isolates were multiplied (1–2 generations) on detached leaves of 'Q83' in 14 cm diameter Petri dishes containing tap-water soaked filter paper. The spores were collected using cyclone spore collectors, dried at 4 °C for 1–2 week and stored at −15 °C before use.

A two-tube method, described by Pei and Ruiz (2000), was used to extract genomic DNA from rust urediniospores. For each isolate, approximately 10 mg spores were used. The AFLP procedure was modified from the two-PCR protocol by Vos et al. (1995) as described by Pei and Ruiz (2000). In the first step (pre-amplification), both E-A and M-C primers, each having one bp extension, were used.

In the second amplification, *Eco*RI and *Mse*I primers having three bp extensions were used: E-AAA and M-CAA, E-AAA and M-CAC, and E-AAA and M-CAT. The PCR products were denatured and run on a 6% polyacrylamide DNA sequencing gel and autoradiographs were obtained using Hyperfilm MP (Amersham). The second PCR amplification was repeated once.

Data analysis

The presence and absence of an unambiguous band was scored as 1 and 0 respectively. Similarities were computed between all pairs of isolates using the formula given by Nei and Li (1979): $s = 2xy/x + y$, where xy is the number of bands shared by the pair and $x + y$ is the total bands of the pair. All AFLP markers, both mono- and polymorphic, were included in data analysis. Principal coordinate analysis was performed and a dendrogram was constructed based on unweighted pair-group method using arithmetic means (UPGMA) (Sneath and Sokal, 1973). Computations were done using Genstat Release 5.

Phenotypic diversity was examined using Shannon information index, a method widely used in ecological studies to examine species richness and relative abundance (Kent and Coker, 1994). Each AFLP marker was treated as a putative locus with two alleles, 1 for presence, and 0 for absence, of a band. The diversity was analysed using the data (a) from all 54 isolates and (b) from 42 isolates, i.e. an equal sample (14 isolates) from each site. In the latter case, the 14 isolates from Markington and Long Ashton were randomly chosen from the 20 tested. The diversity was calculated for each single locus following the method of Monagahan and Halloran (1996) and Bussell (1999) as

$$H'_j = \sum p_i \, \mathrm{Log}_2 \, p_i,$$

where H'_j is the estimate of diversity within a population, p_i the frequency of either the presence ($i = 1$) or absence ($i = 2$) of an AFLP band. The average diversity over populations was calculated as

$$H'_{\mathrm{pop}} = \frac{1}{n} \sum H'_j,$$

where n is the number of populations. The species diversity (refers to the diversity of rust from 'Q83') was calculated for each locus as

$$H'_{\mathrm{sp}} = \sum p_s \, \mathrm{Log}_2 p_s,$$

where p_s is the frequency of either the presence ($s = 1$) or absence ($s = 2$) of the band in the whole sample (54(a) or 42(b) isolates). For each locus, diversity component within populations was calculated as $H'_{\mathrm{pop}}/H'_{\mathrm{sp}}$ and the component between populations as $(H'_{\mathrm{sp}} - H'_{\mathrm{pop}})/H'_{\mathrm{sp}}$.

All markers, including the monomorphic, were included in the calculation. Monomorphic markers were given zero values to allow the extent of monomorphism to be reflected in the values of per-locus diversity. The calculations were done using Microsoft Excel 97.

An analysis of molecular variance (AMOVA) (Excoffier et al., 1992) was also conducted to examine further the variation within and between populations. An Euclidean distance matrix was constructed between all pairs of multilocus phenotypes (a multilocus phenotype is referred to as the isolate(s) showing the same AFLP profiles over all AFLP loci examined). All markers were included in construction of distance matrix. AMOVA was performed using ARLEQUIN software provided by L. Excoffier. The variance components and Φ statistics were tested by nonparametric randomisation tests using 1000 repetitions.

Results

Pathogenicity test

All three rust collections showed the same virulence/avirulence patterns on the eight willow clones in the detached leaf inoculation: incompatible reactions (no symptoms, necrosis or negligible development (<0.2 mm diameter) of uredinia) on *S. disperma*, *S. burjatica* 'Korso', *S. burjatica* 'Germany', *S.* × *calodendron*, *S. viminalis* 'Bowles Hybrid' and 'Mullatin' and compatible reactions (well developed uredinia) on *S.* × *mollissima* 'Q83' and *S.* × *stipularis*. In the second step pathogenicity test, all 60 isolates (20 from each collection) produced well-developed uredinia on both 'Q83' and *S.* × *stipularis* but not on the others. Thus, all the rust collections could be assigned to the previously described LET4 of *M. epitea* (Pei et al., 1996).

AFLP analysis

A total of 305 AFLP bands, 100 for E-AAA and M-CAA, 104 for E-AAA and M-CAT, and 101 for E-AAA and M-CAC, were scored on AFLP profiles of

54 isolates. Of these, 17 were monomorphic. Thirteen bands were unique to all Markington isolates, one to all Long Ashton isolates and 17 to all Loughgall isolates.

Within each site, the collection from Markington was least variable, Nei & Li's coefficients being 0.976–1.0 (Table 1). Fourteen Markington isolates produced identical bands. The widest range of within-site variation was found among Long Ashton isolates (minimum similarity coefficient 0.677). All the isolates from Long Ashton and Loughgall, with the exception of two from Long Ashton, generated different AFLP profiles. Principle coordinate analysis and the dendrogram placed the isolates into three distinct groups according to collection sites (Figures 1 and 2). The isolates from Long Ashton were placed into 4 subclusters. Similarities were greater between isolates from Long Ashton and Markington compared with those between Loughgall and Long Ashton, and Loughgall and Markington.

Table 1. Summary of Nei & Li's similarity coefficients of *Melampsora* isolates within and between three sites in the UK

Site	Markington	Long Ashton	Loughgall
Markington			
Range	0.976–1.000		
Average	0.996		
Long Ashton			
Range	0.623–0.804	0.677–1.000	
Average	0.764	0.899	
Loughgall			
Range	0.328–0.385	0.300–0.540	0.829–0.941
Average	0.358	0.363	0.883

In analysis of phenotypic diversity, the average per-locus diversity indices were calculated for each primer combination (Table 2). The diversity was much lower in the Markington population ($H_j' = 0.014$) compared with that in Long Ashton and Loughgall populations ($H_j' = 0.242$ and 0.233 respectively). The estimated

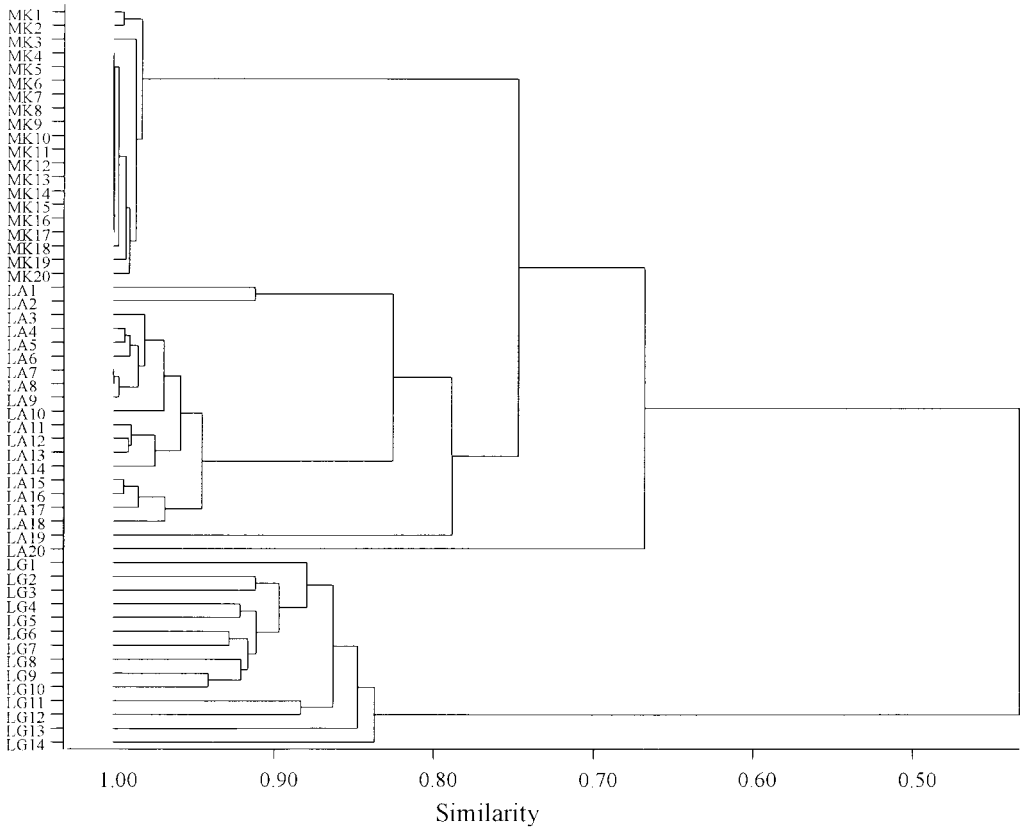

Figure 2. Dendrogram of *M. epitea* isolates collected from *S.* × *mollissima* 'Q83' at Markington (MK1…MK20), Long Ashton (LA1…LA20) and Loughgall (LG1…LG14) in 1992, based on 305 AFLP loci.

Table 2. Partitioning of average per-locus AFLP diversity within and between three populations of *M. epitea*

Primer combination	$H'_{\text{Markington}}$	$H'_{\text{Long Ashton}}$	H'_{pop}	H'_{sp}	$H'_{\text{pop}}/H'_{\text{sp}}$	$(H'_{\text{sp}} - H'_{\text{pop}})/H'_{\text{sp}}$	
(a) Including all isolates (54 in total)							
M-CAG	0.012	0.204	0.210	0.142	0.728	0.295	0.705
M-CAT	0.006	0.223	0.238	0.156	0.775	0.270	0.730
M-CAC	0.024	0.300	0.251	0.191	0.720	0.362	0.638
Average	0.014	0.242	0.233	0.163	0.741	0.309	0.691
(b) Including 14 isolates from each site (42 in total)							
M-CAG	0.008	0.215	0.210	0.144	0.863	0.253	0.747
M-CAT	0.004	0.234	0.238	0.159	0.961	0.200	0.800
M-CAC	0.008	0.323	0.251	0.194	0.863	0.319	0.681
Average	0.006	0.257	0.233	0.166	0.896	0.257	0.743

Table 3. AMOVA within and between three populations of the 'new' pathotype of *M. epitea*

Source of variation	d.f.	Variance components	Percentage of variation	*P*
Between population	2	0.20031	85.82	
Within population	52	0.03310	14.18	<0.00001

mean diversity within the species (H'_{sp}) was 0.741, much higher than that within populations ($H'_{\text{pop}} = 0.163$). Most AFLP variation (69.1%) was partitioned between populations. The analysis with the same sample size (14 isolates) from each site yielded similar results.

In AMOVA analysis, data from 40 different multilocus phenotypes, seven from Markington, 19 from Long Ashton and 14 from Loughgall, were used to construct an Euclidian distance matrix. The AMOVA analysis attributed 14.18% of variance to the individuals within populations and 85.82% between the three geographical populations. Differentiation between populations was highly significant ($P < 0.00001$) (Table 3).

Discussion

Pathogenicity testing and AFLP analysis were used to examine the *Melampsora* rust which caused breakdown of resistance in *S.* × *mollissima* 'Q83' in 1992. The pathogenicity test showed that the rust collections from three sites in the British Isles shared the same pathogenicity pattern on the eight tested willow differentials. Rust spores are airborne and can be dispersed over long distances by air currents. A number of rust species, including *Melampsora larici-populina* Kleb. and *M. medusae* Thüm. on poplars and *M. coleosporioides* Diet. on willow, are thought to have spread from Australia to New Zealand by trans-Tasmanian air currents (Latch, 1980). In this study, the three locations were 320–450 km apart from each other and the possibility that the rust spreads from one location to another cannot be excluded. Based on the occurrence of the rust in the same season, the same virulence/avirulence patterns on host differentials and the geographical scale, it is tempting to conclude that the new virulent type may have spread from a common source in the growing season of 1992. However, AFLP analysis revealed that the three geographical populations were distinct in their genetic background.

AFLP profiles of the Markington isolates were remarkably similar, 14 isolates being identified as the same clone. The genetic variation among the members of a clonal lineage mainly arises by mutation. No information on the mutation rate of rust fungi is available but it is expected to be generally low (10^{-8}–10^{-9} in most living organisms (Li, 1997)). Recently, Pei and Ruiz (2000) examined AFLP profiles from both sexually and asexually reproducing forms of willow *Melampsora*. The isolates of these two forms were collected from different geographical locations, including Long Ashton, Markington and Loughgall, over three years. Within the asexual form, Nei & Li's similarity coefficients were found to exceed 0.978. The isolates from 'Q83' used in this study were collected in the same plot in the same growing season and would have had little chance to differentiate in the process of asexual (uredinial) reproduction. It is likely that Markington isolates may have been derived from different recombinants, one of which became predominant.

The host willow *S.* × *mollissima* 'Q83' is a hybrid between *S. triandra* and *S. viminalis*, which are both widespread in Europe, including the British Isles

(Meikle, 1984). *Salix triandra* belongs to subgenus *Salix* and *S. viminalis* to subgenus *Vetrix*. Usually, willow species belonging to these two subgenera do not hybridise in nature. As an exception, natural hybrids between *S. triandra* and *S. viminalis* do occur (Meikle, 1975). *Salix triandra, S. viminalis* and *S.* × *mollissima*, are among the most important of basket willows (Stott, 1992). Basket-making is an age-old practice, its history dating back to the times of Egyptians and Romans (Bobart, 1936). Hence, *S.* × *mollissima* occurs not only as a result of natural hybridisation but also through introduction or escape of basket-willows. According to Meikle (1975), in the UK, *S.* × *mollissima* is common in river-banks and roadsides, and in moist thickets and osier-beds, where it is often planted. In continental Europe, *S.* × *mollissima* is widely, but locally, distributed in Germany, France, Belgium and Sweden (Meikle, 1984). It appears that there is no lack of host in nature for rust types virulent to 'Q83' to evolve, become established and spread.

Almost all the isolates from Long Ashton and Loughgall belonged to different genotypes. This suggests that they are likely to be sexually recombining populations. Previously, Pei et al. (1996) proved that the isolate M7 collected from 'Q83' at Long Ashton in 1992 readily completes a full *M. epitea* life-cycle . At Long Ashton, the first infections of rust on 'Q83' usually occur in June, some two months after bud burst (Hunter and Pei, unpublished results). This suggests that the rust is unlikely to overwinter in the plantation but develops as a result of ingress from outside sources. As the alternate host, European larch, is widespread in the UK (Bean, 1973), it is possible that there may be some locations where the willow host and European larch grow together or nearby. If virulent pathotypes are available, such locations would allow the rust to become established and to generate recombined inoculum each year.

The rust isolates used for this study were obtained from a single plot from each location and, therefore, the present results may not represent the genetic variation of the rust on 'Q83' in each geographical region. For the regional variation in *M. epitea* f.sp. *larici-epitea typica*, AFLP data from the leaf-infecting form of *Melampsora* on *S. viminalis* and its hybrids (Pei and Ruiz, 2000) may provide some clue. Like the rust on 'Q83', the leaf rust on *S. viminalis* has European larch as alternate host and completes a full sexual life-cycle. When the data comprising 215 AFLP loci were examined, Nei & Li's similarity coefficients were >0.73 (average 0.84) among the 11 leaf rust genotypes collected from *S. viminalis* and its hybrids in south west England during 1991–1993 (unpublished).

All the rust collections from 'Q83' in 1992 were non-virulent to *S. viminalis* 'Bowles Hybrid' and 'Mullatin'. During 1992–1995, field rust samples were collected annually from the three sites and tested for pathogenicity (Pei et al., 1999a). It was found that a pathotype virulent to both 'Q83' and *S. viminalis* also occurred at Long Ashton and Loughgall. In our laboratory experiments (Pei et al., 1999b), we have obtained rust hybrids by crossing isolate LA16 from Long Ashton (coded as Q8-1) with an isolate of a different pathotype of f.sp. *larici-epitea typica*, which is virulent to *S. viminalis*. The evidence indicates that the rust which occurred on 'Q83' is capable of hybridising with other pathotypes of f.sp. *larici-epitea typica*.

The rust on 'Q83' is now widespread in the UK, occurring at all the three study sites each year since 1992 (Hunter and Pei, unpublished results). In Sweden, rust collected from 'Q83' was also identified as LET4 (Fritz et al., 1996). Whether the rust on 'Q83' arose in 1992 from the local non-virulent populations at these sites is not certain. The present results suggest that the rust on 'Q83' from Markington may have come from a relatively newly established source, while that from Long Ashton and Loughgall was from well-established sources. In the late 19th century, Klebahn (1899) conducted inoculation experiments using *Melampsora* rust from *S. hippophaëfolia* Thuill. (= *S.* × *mollissima* var. *hippophaifolia* (Thuill) Wimm.) collected from Hamburg, Germany. The rust readily infected the host willow, producing uredinia in 8 days, but only formed poorly developed uredinia on *S. viminalis* after a prolonged period, 16 days after inoculation. He also proved that the rust from *S. hippophaëfolia* alternates on larch. In 'The British Rust Fungi', Wilson and Henderson (1966) included *S. triandra* × *viminalis* in the host list of larch-alternating *M. epitea* but the source of information is not clear. These records suggest that the rust capable of infecting *S.* × *mollissima* may have been in existence for a long time in continental Europe. However, its occurrence may have been rare or localised since no obvious rust infections on 'Q83' were detected in biomass willow plantations in western Europe during 1987–91, when extensive disease and pest surveys were carried out annually (Hunter et al., 1996).

In the present study, the between-population diversity component estimated using Shannon's index $((H'_{sp}-H'_{pop})/H'_{sp})$ and that using AMOVA were 69%

and 86% respectively. In plants, RAPD-based estimates of the between-population diversity component ranged from 15% to 38% in outbreeding species (seven examined) and 87.5% in the predominantly inbreeding species *Isotoma petraea* F. Muell (Bussell, 1999). Where RAPD diversity has been analysed using AMOVA, modified *F*-statistics and other methods, the value was usually $<20\%$ for outbreeding species and $>50\%$ for inbreeding species (Bussell, 1999). Crossing experiments using the alternate host, larch, suggested that *M. epitea* is heterothallic and sexual compatibility is controlled by a pair of alleles at a locus (Pei et al., 1999b). In terms comparable to the breeding behaviour in plants and animals, the breeding system in rust fungi involves both inbreeding and outcrossing. Where large numbers of willow and larch hosts occur together and rust populations are well established (effective population size is large), outcrossing would predominate. By contrast, where rust populations are newly established and few host plants are available, inbreeding would become more likely. In this study, the between-population diversity values appear to reflect fragmented co-existence of willow and larch hosts in nature.

Spread of rust pathogens has long been an important issue in plant disease epidemiology and various approaches have been suggested to investigate such events. For example, to identify whether a rust has spread over long distances, Zadoks and Bouwman (1984) listed 10 criteria, which include characteristics of host, rust and weather in both 'source' and 'target' areas, wind directions and spore content of the air from source to target, spore trapping data in the target area and matching of pathotypes in source and target areas. In practice, these criteria are never fully met and, even if they were met fully, no definitive conclusions could be drawn because two isolates from different sources may share the same phenotypic characteristics. Our results suggest that the AFLP method provides an efficient way of studying the spread of rust pathogens. Because AFLP can generate virtually unlimited numbers of DNA bands, it would be particularly useful in examining differentiation among pathogen populations having the same clonal lineage.

Acknowledgements

We thank Drs G. M. Arnold and A. Karp for their valuable comments on the manuscript. This research was funded by the Department of Trade and Industry (DTI) through the Energy Technology Support Unit (ETSU), as well as by the Ministry of Agriculture, Fisheries and Food (MAFF). IACR receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom.

References

Bean WJ (1973) Trees and Shrubs Hardy in the British Isles, Vol. II, 8th edn. John Murray Ltd., London, UK

Bobart HH (1936) Basketwork through the ages. Oxford University Press: Oxford, UK

Bussell JD (1999) The distribution of random amplified polymorphic DNA (RAPD) diversity amongst populations of *Isotoma petraea* (Lobeliaceae). Molecular Ecology 8: 775–789

Excoffier L, Smouse PE, and Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 179–191

Fritz RS, Johansson L, Åstrom B, Ramstedt M and Häggström H (1996) Susceptibility of pure and hybrid willows to isolates of *Melampsora epitea* rust. European Journal of Plant Pathology 102: 875–881

Hunter T, Royle DJ and Arnold GM (1996) Variation in the occurrence of rust (*Melampsora* spp.) and other diseases and pests, in short-rotation coppice plantations of *Salix* in the British Isles. Annals of Applied Biology 129: 1–12

Kent M and Coker P (1994) Vegetation Description and Analysis: A Practical Approach. John Wiley and Sons Ltd., Chichester, UK

Klebahn H (1899) Kulturversuche mit heteröcischen Rostpilzen. Zeitschrift für Pflanzenkrankheiten 9: 88–99

Latch BJ (1980) Weeping willow rust in New Zealand. New Zealand Journal of Agricultural Research 23: 535–538

Li WH (1997) Molecular Evolution. Sinauer Associates, Inc., Sunderland, Massachusetts, USA

Meikle RD (1975) *Salix* L. In: Stace CA (ed) Hybridization and the Flora of the British Isles (pp 305–338). Academic Press, London, UK

Meikle RD (1984) Willows and Poplars of Great Britain and Ireland. BSBI Handbook 4. Botanical Society of British Isles, London, UK

Monagahan BG and Halloran GM (1996) RAPD variation within and between natural populations of morama (*Tylosema esculentum* (Burchell) Schreiber) in southern Africa. South African Journal of Botany 62: 287–291

Nei M and Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences of the USA 76: 5269–5273

Parker SR, Royle DJ and Hunter T (1993) Impact of *Melampsora* rust on yield of biomass willows. P. 117. In: Abstracts of the 6th International Congress of Plant Pathology, Montreal, Canada, 28 July–6 August 1993, National Research Council Canada, Ottawa, Canada

Pei MH, Royle DJ and Hunter T (1993) Identity and host alternation of some willow rusts (*Melampsora* spp.) in England. Mycological Research 97: 845–851

Pei MH, Royle DJ and Hunter T (1996) Pathogenic specialization of *Melampsora epitea* var. *epitea* on *Salix*. Plant Pathology 45: 679–690

Pei MH, Hunter T and Ruiz C (1999a) Occurrence of *Melampsora* rusts in biomass willow plantations for renewable energy in the United Kingdom. Biomass and Bioenergy 17: 153–163

Pei MH, Royle DJ and Hunter T (1999b) Hybridisation in larch-alternating *Melampsora epitea* (*M. larici-epitea*). Mycological Research 103: 1440–1446

Pei MH and Ruiz C (2000) AFLP evidence of distinct patterns of life-cycle in two forms of *Melampsora* rust on *Salix viminalis*. Mycological Research 104: 937–942

Roelfs AP (1984) Epidemiology in North America. In: Roelfs AP and Bushnell WR (eds) The Cereal Rusts Vol II, Disease Distribution, Epidemiology and Control (pp 403–434) Academic Press, Orlando, USA

Sneath PHA and Sokal RR (1973) Numerical Taxonomy W.H. Freeman and Company, San Francisco, USA

Stott KG (1992) Nomenclature of the promising biomass coppice willows, *Salix* × *sericans* Tuasch ex Kern., *Salix dasyclados* Wimm. and *Salix* 'Aquatica Gigantea'. Botanical Journal of Scotland 46: 137–144

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, and Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acid Research 23: 4407–4414

Wilson M and Henderson DM (1966) The British Rust Fungi Cambridge University Press, Cambridge, UK

Zadoks JC and Bouman JJ (1984) Epidemiology in Europe. In: Roelfs AP and Bushnell WR (eds) The Cereal Rusts Vol II, Disease Distribution, Epidemiology and Control (pp 329–369). Academic Press, Orlando, USA