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Structure of *Melampsora larici-populina* populations on wild and cultivated poplar

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Abstract

Populations of *Melampsora larici-populina*, causal agent of poplar leaf rust, have been collected during 1992–1994 in three types of sites: natural stands of *Populus nigra* in the south-east of France; cultivated poplar stands in the northern half of France, including mainly interspecific and exotic hybrids of poplar; and two overseas populations collected from Northern Ireland and Western USA. The pathotype of each rust isolate was determined by inoculating a differential set of poplar clones. The number of virulences per isolate and the frequency of each virulence in each population were compared, according to the sites and to the clones from which the isolates were collected.

Several diversity indices were applied to the populations. The α and Simpson indices were retained since they described best the richness and the evenness, respectively, of the populations, and they were the least sensitive to sample size. The complexity of races was lower in the natural stands of *P. nigra* than in the cultivated poplar stands. Populations of *M. larici-populina* collected on *Populus* × *euramericana* 'Robusta' and *P. nigra* 'italica' (Lombardy poplar) presented a high richness and evenness, confirming the value of these clones for describing race populations. Populations from Western USA presented a very low diversity, which is in accordance with the recent introduction of the pathogen in North America. These results suggest that the race populations of *M. larici-populina* are mainly influenced by the structure of the host populations.

Introduction

Poplar cultivation in Europe has always been subject to phytosanitary hazards. This situation is due to the high number of pathogens that can attack poplars and to the artificiality of the system of cultivation (Pinon, 1984). Stands are even-aged and monoclonal and the number of cultivated clones is limited. Their genetic basis is narrow and most are hybrids sharing a parent, *Populus deltoides* Bartr., which did not co-evolve with European parasites. In addition, recent plantings for biomass production (mainly for pulp) have been at close spacing, which has led to the creation of a cool and humid microclimate and has therefore enhanced damage due to foliar rusts (Pinon and Schvester, 1985).

The main approach to disease control has been breeding for resistance (Villar et al., 1995; Lefèvre et al., 1994). By taking advantage of the natural diversity in the host population (among species, provenances, families and individuals), major advances were made in selection for resistance to bacterial canker caused by *Xanthomonas populi* (Ridé) Ridé and Ridé, and, to a lesser extent, to *Marssonina brunnea* (Ell. et Ev.) P. Magn. and to rusts, particularly poplar leaf rust caused by *Melampsora larici-populina* Kleb.

However, within a decade, a number of clones selected for complete resistance to *M. larici-populina* became infected after the development of virulent isolates of the fungus (Steenackers, 1982; Pinon and Bachacou, 1984; Pinon et al., 1987; Pinon and Lefèvre, 1994; Steenackers et al., 1994). Most of the clones in which resistance has been overcome by the parasite exhibit too low a level of general resistance to be useful, and the cultivation of some of these clones has been abandoned (i.e. *Populus trichocarpa* Torr. & Gray × *deltoides* 'Unal', 'Raspalje' or 'Hunnegem' and *P.* × *euramericana* (Dode) Guinier 'Luisa Avanzo'). During this same period no rust epidemic was evident on the indigenous black poplar, *P. nigra* L.

The first physiological races within *M. larici-populina* were described by van Vloten (1949). At present seven virulences are known (Pinon, 1995),

Table 1. Origins of the populations of *M. larici-populina*

Year	Country	Location	Species	Clone	Number of isolates
1992	France				
	East	Nancy	*P. nigra*	italica	100
			P. maximowiczii × *P. nigra*	Rochester	92
			P. trichocarpa	Fritzi Pauley	101
			P. trichocarpa	36-134	92
			P. × *euramericana*	Robusta	184
			P. × *euramericana*	Adige	29
			P. × *euramericana*	Stella	35
			P. × *euramericana*	Blanc du Poitou	30
			P. × *euramericana*	I 45-51	64
			P. trichocarpa × *P. deltoides*	Hunnegem	104
			P. trichocarpa × *P. deltoides*	Unal	105
			P. deltoides	Lux	21
		Rosières	*P.* × *euramericana*	Robusta	110
	Center	Orléans	*P.* × *euramericana*	Robusta	117
		Orléans	*P. nigra*	?	108
	North	Travecy	*P. nigra*	italica	108
	South-East	Asse	*P. nigra*		81
		Bresis	*P. nigra*		31
		Dèze	*P. nigra*		99
		Pont-du-Gard	*P. nigra*		24
		Vinon	*P. nigra*		91
	Ireland				
			P. trichocarpa × *P. deltoides*	n°1	77
			P. trichocarpa × *P. deltoides*	n°2	110
	USA				
	C	California	?	?	48
	G	California	*P.* × *euramericana*	I 488 (purified)	(30 sori)
	H	California	?	?	28
	I	California	*P. nigra*		19
	T	Washington	*P. trichocarpa*		48
	W	Washington	*P. trichocarpa*		45
1993	France				
	East	Nancy	*P.* × *euramericana*	Banc du Poitou	170
			P. × *euramericana*	Tiepolo	144
			P. × *euramericana*	Robusta	179
			P. × *euramericana*	I 45-51	151
			P. deltoides	64-18	100
			P. deltoides	64-21	101
			P. deltoides	64-23	104
			P. deltoides	64-28	98
			P. deltoides	64-29	98
			P. trichocarpa	19-73	93

Table 1. Continued

Year	Country	Location	Species	Clone	Number of isolates
			P. trichocarpa	19-77	94
			P. trichocarpa	36-77	94
			P. trichocarpa	36-100	96
			P. yunnanensis	?	180
	South-East	Dèze	*P. nigra*		92
		Gréoux	*P. nigra*		41
		Manosque	*P. nigra*		13
		Mézel	*P. nigra*		13
		Sisteron	*P. nigra*		8
		Vinon	*P. nigra*		20
1994	France				
	East	Nancy	*P.* × *euramericana*	Robusta	216
	South-East	Dèze	*P. nigra*		151

which theoretically can give rise to 128 different races. While the structure and the dynamics of rust race populations are well documented in agriculture (Groth and Roelfs, 1987; Kolmer, 1991; Andrivon and de Vallavieille-Pope, 1995), no comparable information has been available for poplar rust. The present study provides the first full description of the populations of *M. larici-populina*.

The main aim of this paper is to provide a description of the race populations of *M. larici-populina* according to the different host populations (wild *P. nigra*, selected clones), different clones (with or without race-specific resistance), and sites (same clone in different sites and a comparison between native and 'exotic' sites) in order to estimate the role of the host on the structure of the parasite populations. These populations were described and compared on the basis of the analysis of their diversity, as understood in ecological studies (Magurran, 1983). Four criteria were used to compare populations (frequencies of virulences, complexity of the isolates, richness and evenness of the populations). To compare populations for richness and evenness, several of the indices described by Magurran (1983) were tested in order to select the least sensitive to sample size. Finally, this study of race populations could provide useful information for the breeders to define their selection strategy (general versus race-specific resistance).

Material and methods

Populations surveyed

Isolates of *M. larici-populina* were collected from three types of poplar stands (Table 1). Stands of indigenous *P. nigra* were located as far as possible from areas of poplar cultivation, in order to get information from natural situations. The stands were distributed along different rivers in the south-east of France (Figure 1): the Durance river (Manosque, Sisteron), the Verdon river (Vinon, Gréoux), the Asse river (Pont d'Asse, Mézel), the Gard river (Pont du Gard, Dèze) and the Cèze river (Brésis). Most of the time it was necessary to collect sori from several trees in a stand to get sufficient samples of the fungus. Because of the natural origin of the trees, no information was available on their genetic diversity. A second set of isolates was collected on well-known clones in different nurseries. Some were experimental nurseries (Nancy, Orléans) characterised by the presence of many different clones, some with race-specific resistance to *M. larici-populina*, and also by the presence of the alternate host (*Larix decidua* Miller); others (Rosières and Travecy) were commercial nurseries cultivating only clones with general resistance or clones with race-specific resistance still efficient. Nancy and Rosières are both located in the east of France, while Travecy is in the north and Orléans in the centre (Figure 1). The last set of isolates was collected outside continental Europe (Northern Ireland and Pacific coast of the

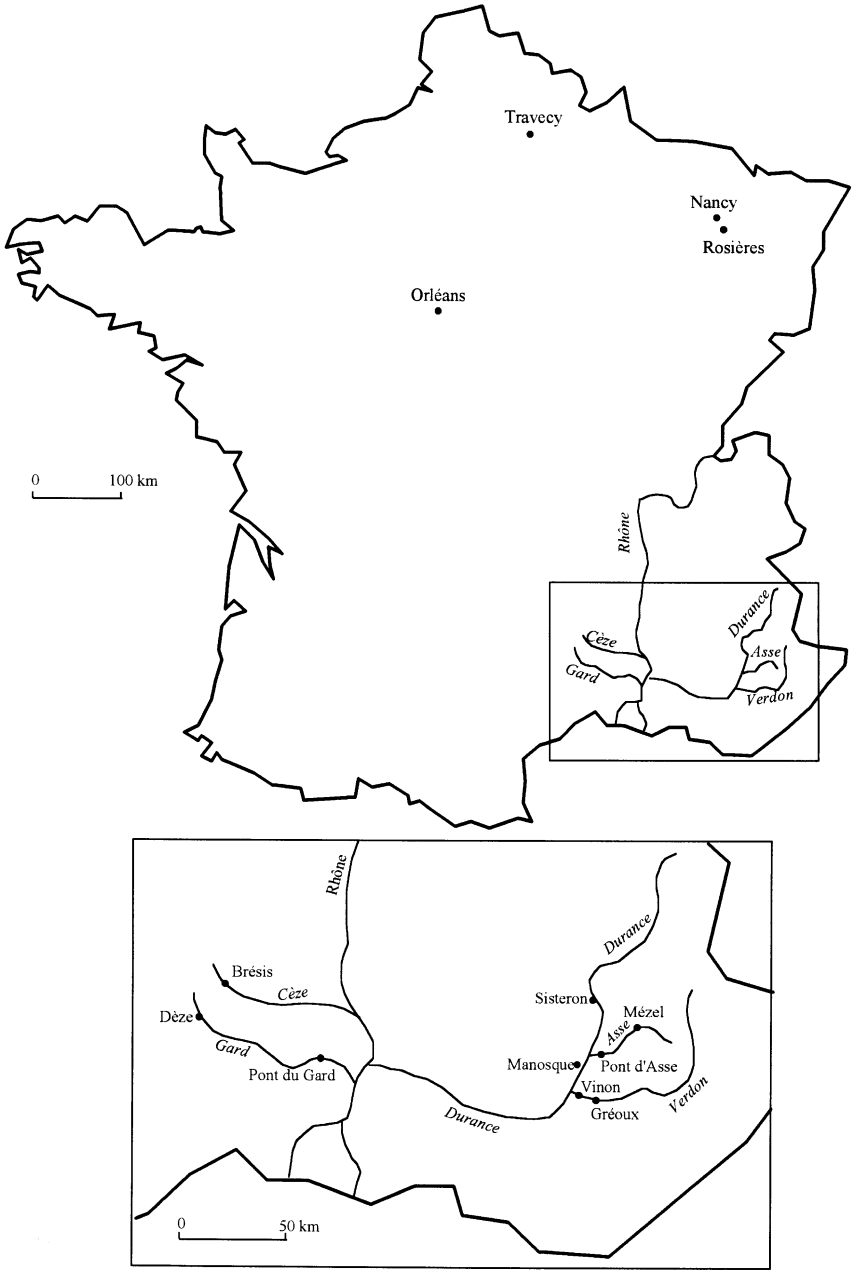

Figure 1. Location of the French poplar nurseries (Nancy, Orléans, Rosières, Travecy) and the wild poplar stands in the south-east of France, from which the populations were collected.

United States). There is almost no poplar cultivation in Northern Ireland and samples were collected from an experimental plot principally cropped with clones selected in Belgium. In the USA, *M. larici-populina* is a recent introduction (Newcombe and Chastagner, 1993). Samples were collected from a number of plantations in California and Washington States. In 1994 two populations were again surveyed, one from 'Robusta' in Nancy and one in the south-east of France (Dèze) from wild *P. nigra*.

Collection of specimens

Most of the collections were conducted in 1992 and 1993. Only leaves bearing a few sori were collected in order to minimise the chance of cross-contamination. Individual leaves were kept in aluminum foils at 10 °C until processed in the laboratory. Because *M. larici-populina* and *M. allii-populina* Kleb. are often found on the same leaf (especially on *P. nigra* in the south-east), the first step was to sort out *M. larici-populina.* This was achieved by inoculating spores from each sorus onto leaf disks of the two clones: *P.* × *euramericana* 'Robusta' (susceptible to both *Melampsora* species) and *P. trichocarpa* × *P. deltoides* 'Beaupré' (totally resistant, at that time, to *M. larici-populina* and susceptible to *M. allii-populina*). Isolates which could infect only 'Robusta' belonged to *M. larici-populina.*

Race identification

A 20 μl droplet of water agar (0.1 g.l^{-1}) was deposited over each sorus with a micropipette and spores were dispersed within the droplet with the micropipette. The resulting spore suspension was sucked and then applied as small droplets on leaf disks of a differential set of clones floating on water, as described by Pinon and Peulon (1989). The differential set consisted in two types of clones: 'Robusta' which allows the maintenance of all isolates, no specific resistance being known, and differential clones for the various virulences (V_x). These were: *P.* × *euramericana* 'Ogy' (V_1), *P. candicans* 'NNT' (V_2), *P.* × *euramericana* 'Brabantica' (V_3), *P. trichocarpa* × *P. deltoides* 'Unal' (V_4) and *P. trichocarpa* × *P. deltoides* 'Rap' (V_5). Because V_6 and V_7 have been discovered only recently (Pinon and Lefèvre, 1994; Steenackers et al., 1994) and their distribution is still narrow, the study was limited to the V_1-V_5 virulences. In 1992, no foliage from 'Rap' was available, hence only four virulences were studied. Isolates able to infect only 'Robusta' were considered to be V_0.

Analysis of the populations

All the isolates collected at one time on a clone (or on a group of trees in the case of wild *P. nigra*) at one site are considered a population. Each isolate is described by its range of virulence. The populations were described and compared by the mean of several variables: frequency of each virulence in the population, complexity of the isolates (number of virulences per isolate), richness in terms of the number of different races and relative abundance (dominance or evenness) of the races. When two or more populations had similar characteristics (frequency of the virulences, number of virulences per isolate), they were presented as an unique graph.

Indices of richness (α, Shannon, and Margalef indices) and of evenness (Shannon evenness and Simpson) were calculated as described by Magurran (1983):

- α (*alpha*) = N(1−x)/x after obtaining the x value from S/N = [(1−x)/x][−ln(1−x)] where S is the number of species (here races) and N the total number of individuals (here isolates),
- H′ (*Shannon diversity*) = $\Sigma p_i \ln p_i$ where p_i is the proportion of the *i*th race (n_i/N),
- D_{Mg} (*Margalef*) = (S−1)/lnN where S and N as defined above,
- D (*Simpson*) = $\Sigma[(n_i(n_i-1))/(N(N-1))]$ with n_i is the number of isolates of the *i*th race and N as defined above,
- E (*Shannon evenness*) = H′/lnS, with H′ and S as defined above.

The populations were compared using the 2I test (Arbonnier, 1966). This test is very similar to χ^2 (using the same table of probabilities and the same calculation of degrees of freedom) but has no constraint relative to the number of individuals per class.

Results and discussion

Frequencies of the virulences

In the 1992 samples, four virulences could be detected. Their frequencies varied with the sites and within a site with the clones. They were quite similar in four French populations: Nancy (10 clones), Rosières, Orléans (2 clones) and Travecy (Figure 2). Those populations came from clones without specific resistance. Very few isolates were of the type V_0, and the four virulences were detected in these populations. V_3 (29 to 43%) and V_4 (44 to 56%) were the most frequent virulences. The strong similarity between the populations of Nancy and Rosières may be due to their geographical proximity. In Nancy, two clones were easily distinguished by their race populations: V_2 was not found on 'I 45-51' and was less frequent on 'Blanc du Poitou' (4%) than on the other ten clones (14%). This difference is related with the fact that 'I 45-51' and 'Blanc du Poitou' are very

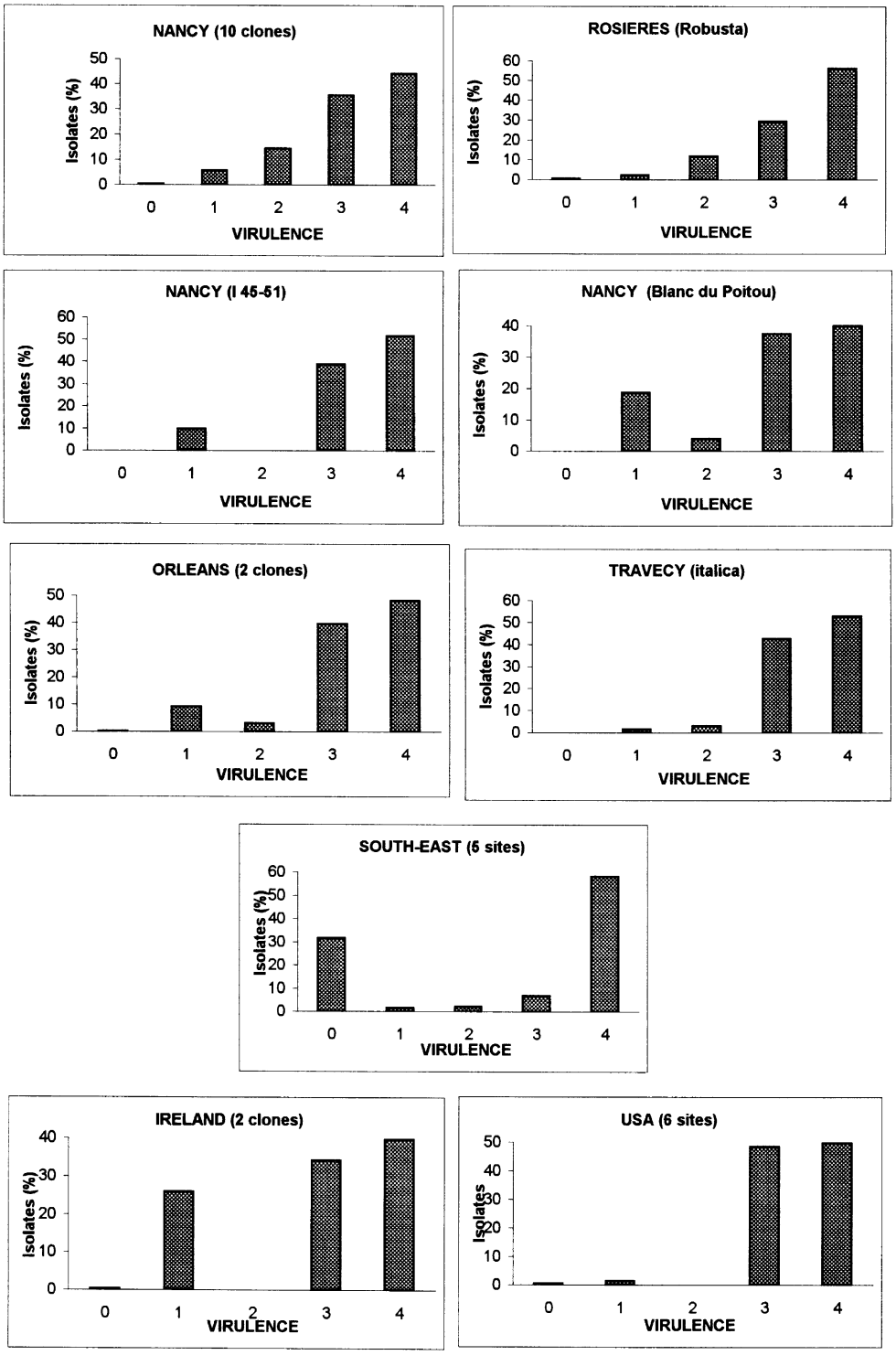

Figure 2. Frequencies of the virulences in 1992. Nancy (10 clones: 'italica', 'Rochester', 'Fritzi Pauley', '36-134', 'Robusta', 'Adige', 'Stella', 'Hunnegem', 'Unal', 'Lux').

resistant to V_2, contrary to the remaining ten clones. In the south-east, all virulences were also present, but the populations were very different from those described above. The frequencies of V_1 (1.4%), V_2 (2%) and V_3 (7%) were much lower than that of V_0 (32%). The high frequency of V_4 (58%) was the only common feature with the populations collected on cultivated poplars.

In Northern Ireland as well as in the United States, V_2 was not observed, indicating that this virulence has possibly not been introduced there. A clear difference existed between the two sites: V_1 was frequent in Ireland (26%) but rare in the United States (1.4%). This difference likely reflects the type of clones from which samples were taken.

A fifth virulence (V_5) was tested in 1993. In Nancy, V_5 had a frequency quite similar to that of V_1 (Figure 3), and the relative frequency of V_1 to V_4 in 1993 was identical to that in 1992. Clones 'I 45-51' and 'Blanc du Poitou' confirmed their relative resistance towards virulence V_2. The virulence distribution on those two clones did not differ ($2I = 1.9$, df = 4, $0.5 < P < 0.9$). Although V_2 (30%) was more frequent than V_3 (16%) on four *P. trichocarpa* clones ('19-77', '36-77', '36-100', '19-73'), the global virulence distribution on those clones was comparable to that on the other six clones ('Robusta', '64-21', '64-18', '64-23', '64-29', *P. yunnanensis*) in Nancy ($2I = 2.34$, df = 4, $0.5 < P < 0.9$). Despite the fact that virulence distributions on 'Tiepolo' and '64-28' were significantly different ($2I = 23.59$, df = 4, $P < 0.001$), those two clones shared an almost complete absence of V_1, in agreement with their resistance to this virulence. On *P. nigra* stands from south-eastern France, the virulence distribution was quite different from that in Nancy, with a lower frequency of V_1 (1%), V_2 (13%), V_3 (17%) and V_5 (5%) (Figure 3). Compared to 1992, 1993 populations showed mainly an increase in V_2 and a decrease in V_0 (1%). The contrast between the virulence distributions in Nancy and in the south-east (*P. nigra*) was again important in 1994 ($2I = 79.6$, df = 4, $P < 0.001$) (data not shown).

Overall, virulence V_4 appeared to be the most frequent virulence, which may indicate that it has been integrated for a long time in the pathogen populations in various locations or that the matched resistance gene is frequent in the host population. Although V_3 occurred less frequently, it has a similar distribution. Virulences V_1, V_2 and V_5 are less frequent and their development may be more recent. Those virulences are rare in the populations of the wild *P. nigra* stands, on which they are probably unnecessary. Although *P. nigra* might not favour the multiplication of virulences as a result of direct selection, it might do it as an hitchhiking effect with other selective traits (e.g. aggressiveness ...). At the same time there were clones in Orléans, but more evidently in Nancy, with race-specific-resistance, and these clones acted as selective clones for the multiplication of the relevant virulences. Even if these clones were not included in our study, they acted as a selection pressure on the inoculum. Consequently, the clones having only general resistance were infected partly with isolates exhibiting unnecessary virulences on these clones. Finally, the structure of the host population has a strong effect on the race populations, as previously suggested (Pinon, 1992). These results raise the question of a possible counter-selection of unnecessary virulences and of the genetic basis of the virulences. Further studies are needed to improve the understanding of the role of sexual reproduction in the biology of the fungus and its impact on population structures.

The absence of one virulence in the populations from Northern Ireland and from the North-Western United States (compared with continental Europe), is in accordance with a recent introduction in these sites. It is especially likely in the United States, were *M. larici-populina* was only recently discovered (Newcombe and Chastagner, 1993; Pinon et al., 1994).

Complexity of the isolates

In 1992 it was possible to detect up to four virulences per isolate. The data on the populations studied on the different clones were grouped per site in order to study the complexity of the populations. Figure 4 indicates that most of the isolates combined two virulences in the four locations in France (Nancy, Rosières, Orléans and Travecy). Isolates without virulence or cumulating the four virulences were unusual. The populations from the native stands of *P. nigra* were markedly different from the populations sampled on cultivated poplars: 34% of isolates from *P. nigra* had no virulence, 56% had one and 10% had two. No isolate was found with three or four virulences.

The Irish poplar host populations were represented by only two clones from Belgian origin belonging to the hybrid *P. trichocarpa* × *P. deltoides*, according to their leaf morphology. Most of the isolates cumulated several virulences (39 and 47% of the isolates had two and three virulences, respectively). This complexity of the isolates suggests that they required several virulences to infect those clones. In fact, such hybrids

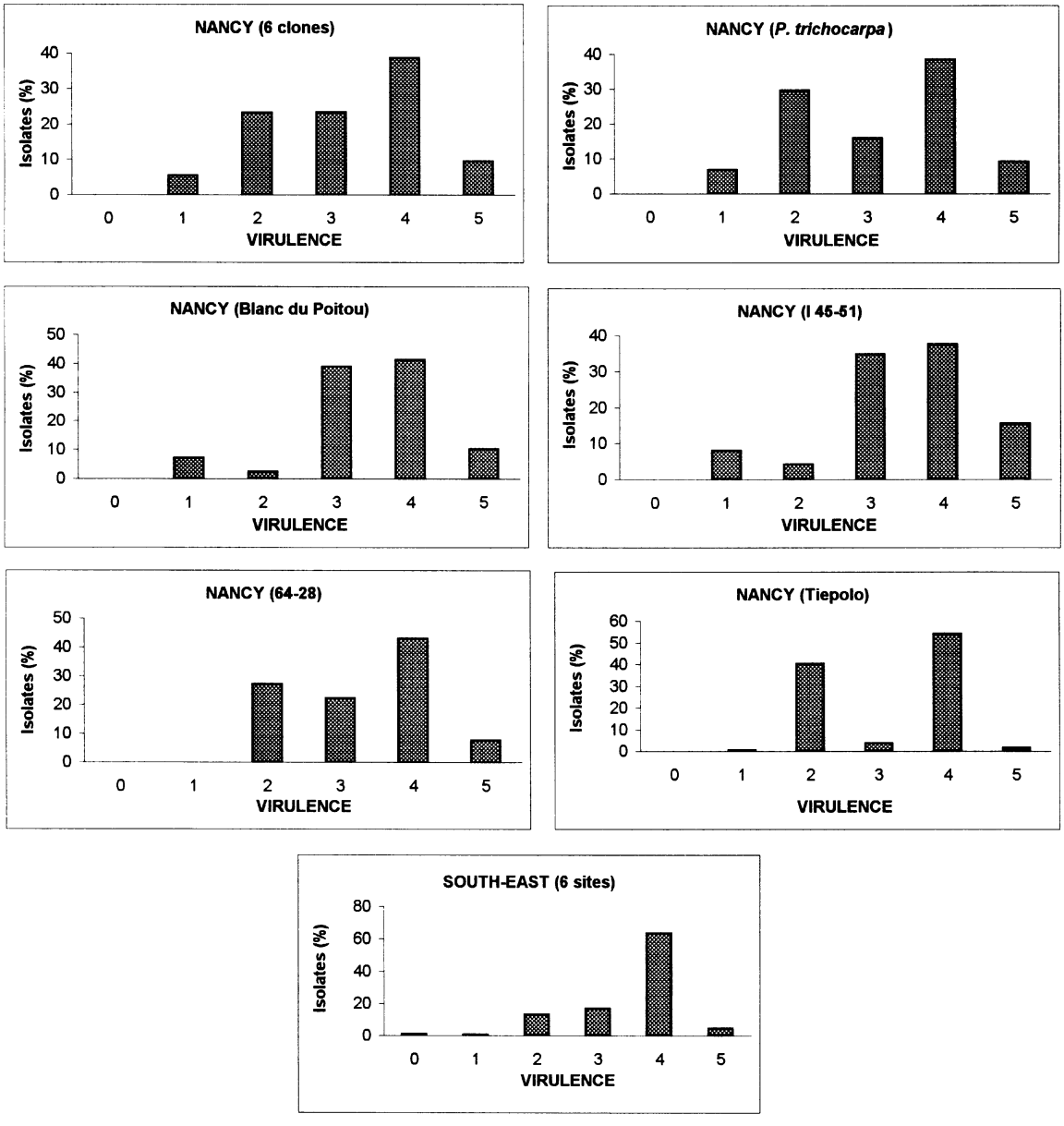

Figure 3. Frequencies of the virulences in 1993 in Nancy and in the south-east of France. Nancy (6 clones: 'Robusta', '64-18', '64-21', '64-23', '64-29', *P. yunnanensis*), Nancy (*P. trichocarpa*: '19-73', '19-77', '36-77', '36-100').

selected in Belgium frequently exhibit race-specific resistance. An alternative explanation to the necessity of three virulences to infect clones grown in Ireland is that the population of *M. larici-populina* there could be composed of a very low number of isolates, which happened to have three virulences for the most part. This would thus be a founder effect, not reflecting necessarily the selection status in Ireland but the composition of the population from which these isolates were derived. This hypothesis is supported by the low richness of the Irish populations (see below), and by the fact that poplar cultivation is not common in Ireland. The correct explanation of the population structure of *M. larici-populina* in Ireland (local selection or founder effect) would thus require additional information about the resistance genes present in the

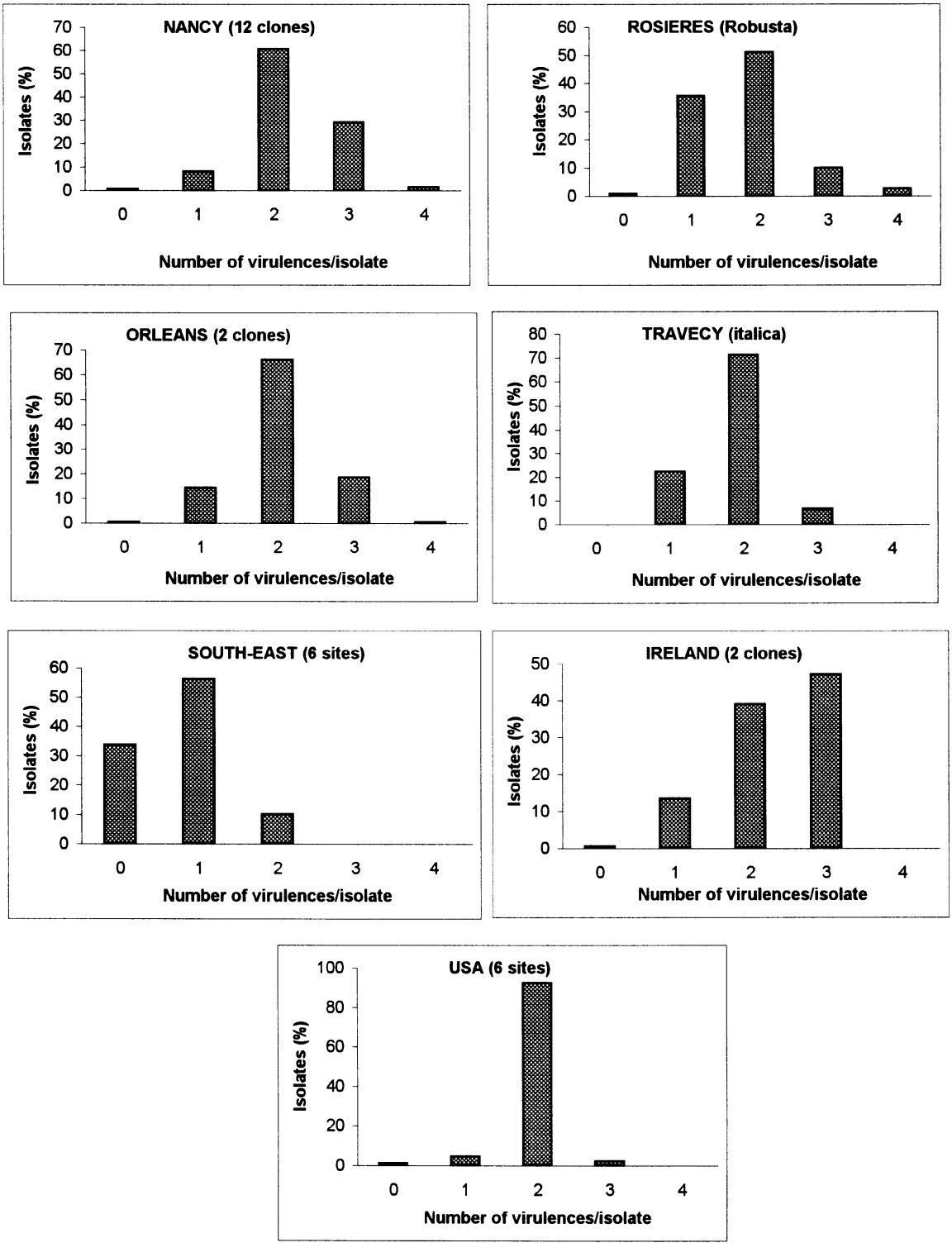

Figure 4. Populations of *M. larici-populina* in 1992. Numbers of virulences per isolate in seven regions.

clones sampled in Ireland. The American populations, like the French ones, presented generally two virulences (92% of the isolates).

Five virulences were surveyed in 1993 and 1994 (Figure 5). In Nancy, in 1993, 54% of the isolates had two virulences, 24% had three virulences, 13% had four virulences, while 9% had only one virulence. V_0 did not occur. During the same year, *M. larici-populina* was less abundant in the south-east of France than in 1992. Despite the fact that one more virulence was included in 1993 studies, Figure 5 confirms that isolates from *P. nigra* are significantly less complex than those from cultivated poplars, i.e. those from Nancy ($2I = 74.7$, $df = 4$, $P < 0.001$). The same trend was observed in 1994, comparing populations sampled on 'Robusta' in Nancy and on *P. nigra* in Dèze (Figure 5), since the two distributions were highly significantly different ($2I = 181.9$, $df = 4$, $P < 0.001$).

Clearly the populations in areas of poplar cultivation are different from those on the wild *P. nigra*. Isolates tend to accumulate more virulences in the former situation. This is in agreement with the culture of clones selected for race-specific resistance. This type of resistance has broken down within about 15 years due to the development of virulent isolates. Therefore, the development of races carrying multiple virulences seems to be driven by the structure of the cultivated population, which is increasingly composed of clones with complete resistance.

Choice of indices

Several indices of richness and of evenness were applied to our populations. For each population (29 in 1992 and 20 in 1993, Table 1) a correlation coefficient was calculated between the size of each population and the index value relative to the population (Table 2). Among the three richness indices, the Margalef and Shannon indices were repeatedly sensitive to sample size. The α index was not sensitive to population size in 1993 and less sensitive than the other indices in 1992 according to the probability thresholds.

To characterise evenness, the Simpson index proved insensitive to population size (Table 2), contrary to the Shannon evenness index. Groth and Roelfs (1987) also showed that the Shannon index was more sensitive to sample size than the Simpson index, in a pluriannual study of race populations of *Puccinia graminis* f. sp. *tritici*.

Therefore, each population has been characterised by the α and the Simpson indices on Figures 6 and 7. The highest values of α and Simpson indices indicate respectively a high richness in races and a high evenness of abundance of each race. Dotted lines divide in four parts each figure in order to highlight low versus high richness and evenness versus unevenness (i.e. dominance of some races).

Richness of the populations in races

The populations collected in 1992 (Figure 6) showed a wide range of richness (α index). Clearly, the populations from United States were characterised by a very low richness, in agreement with the hypothesis of a founder effect due to the recent introduction of the pathogen in the USA. The total absence of diversity in USA G is to be related to the fact that this population was provided as an isolate purified on *P.* × *euramericana* 'I 488'. Irish populations were also characterised by low values of the α index. This can be related to the fact that poplar is not very common there and only a limited number of clones is cultivated. In France, populations on the wild stands of *P. nigra* were slightly poorer than those from cultivation areas (e.g. Nancy), but there was an important diversity of situations within the wild stands of *P. nigra*. The Pont d'Asse population was very poor (similar to the American samples) while the Vinon population was quite rich. Nancy populations were generally rich, but important differences appeared according to the clones. For instance, populations from 'I 45-51' and 'Blanc du Poitou' were poor, which is in relation with their resistance to virulence V2. Similarly, virulence V_4 is necessary to infect 'Unal', which therefore cannot support all types of isolates. Clones 'Robusta' and 'italica' supported the richest populations, although there were differences between sites. These clones are useful models to study populations because they support the highest diversity of isolates. The comparison of the populations found on 'Robusta' in Nancy and Orléans, or on *P. nigra* 'italica' in Nancy and in Travecy suggests that the populations are richer in Nancy than in both other sites.

A range of diversity appeared in 1993 (Figure 7) among the populations on *P. nigra* in wild stands. The Dèze population was once more characterised by a low diversity. But the Vinon population appeared to be poorer than in 1992, which was most likely due to the small sample size. In Manosque, the race population was extremely rich. This particular result may be related with the location of this stand. It is the only stand which is relatively close to artificial plantations. The poorest population in Nancy was that on clone

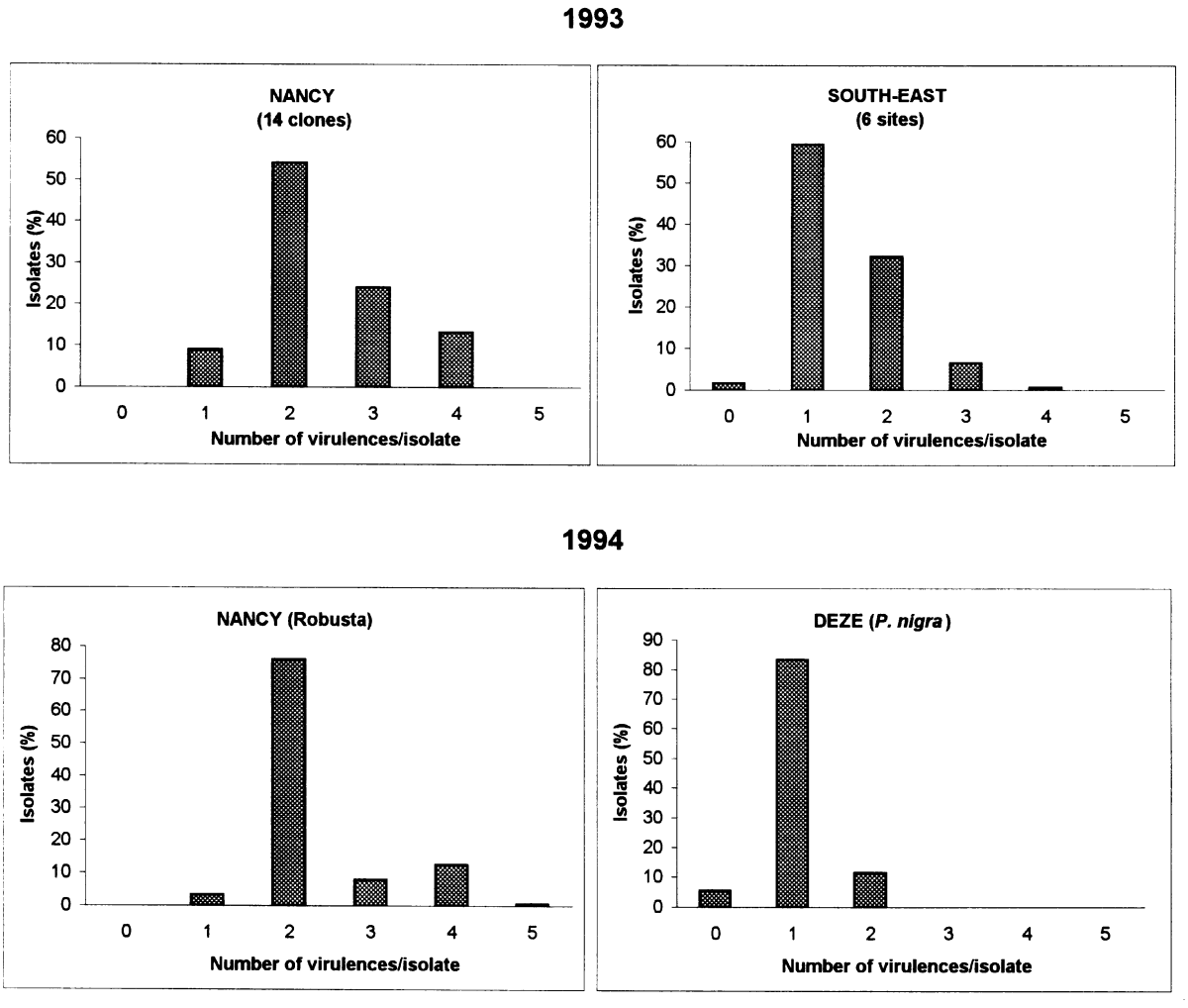

Figure 5. Populations of *M. larici-populina* in 1993 and 1994. Numbers of virulences per isolate in Nancy and in the south-east of France.

Table 2. Sensitivity of diversity indices to sample size

Year	Index		Correlation coefficient	Number of couples	Probability
	Name	Type			
1992	Alpha	Richness	0.46	29	0.05
	Margalef	"	0.54	29	0.01
	Shannon	"	0.47	29	0.01
	Simpson	Evenness	0.25	29	NS
	Shannon evenness	"	0.15	29	NS
1993	Alpha	Richness	−0.24	20	NS
	Margalef	"	0.40	20	0.05
	Shannon	"	0.41	20	0.05
	Simpson	Evenness	−0.10	20	NS
	Shannon evenness	"	−0.61	20	0.01

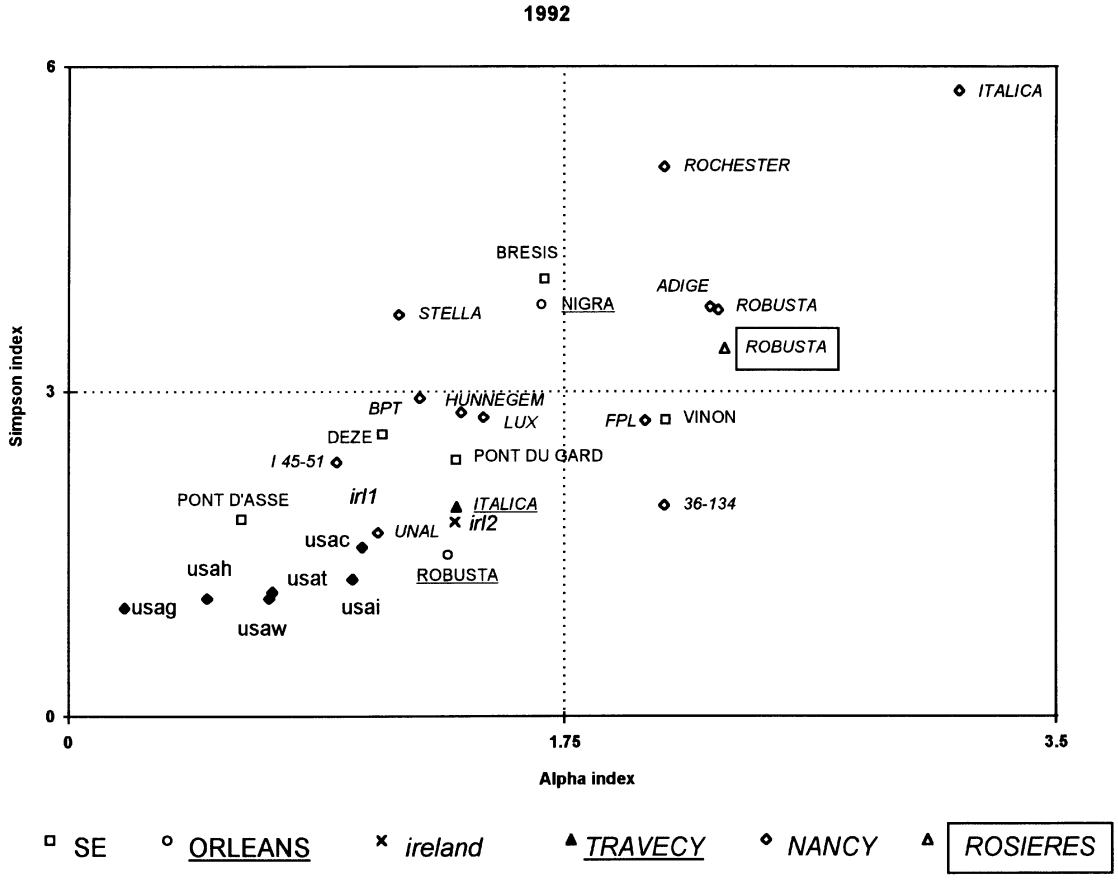

Figure 6. Distribution of the 1992 populations according to their richness (α index) and their evenness (Simpson index). SE = south-east of France, BPT = 'Blanc du Poitou', FPL = 'Fritzi-Pauley'.

'64-28', which is in accordance with the fact that this clone is quite resistant to virulence V_2. The higher values of the α index in 1993 compared to 1992 were due to the increased range of studied virulences (V_0 to V_4 in 1992 and V_0 to V_5 in 1993), which resulted in the identification of more races.

Evenness of the races

The distributions of the points representing the various populations clearly suggest a relationship between richness and evenness (Figures 6 and 7). However, Groth and Roelfs (1987) suggested that more than one index could be applied to better describe populations of plant pathogens. In 1992 and in 1993 there was a significant correlation between the α and the Simpson indices (respectively r = 0.79, df = 27, $P < 0.001$ and 0.70, df = 18, $P < 0.001$). In particular, the American populations (Figure 6) were characterised by an important dominance of one of the few races (low value of Simpson index). On the contrary, a high evenness was observed in Nancy on *P. nigra* 'italica' which confirms the value of this clone for describing race populations. Only a limited number of populations exhibited a looser relationship between the two indices. In Nancy, the population on 'Stella' was relatively poor but dominance was not evident, whereas the population on '36-134' was rich but with a clear dominance. This may suggest the existence of a quantitative interaction between this clone and some virulences, or the effect of small foci of inoculum nearby clones with race-specific resistance in the Nancy nursery.

In 1993, populations on clones '64-18' and '64-21' (with the same parentage) were relatively poor but with high evenness (Figure 7). The population on '36-77' was rich but marked by some dominance as it was the case in 1992 for '36-134'. Those two clones are genetically close (half-sib). The population from *P.*

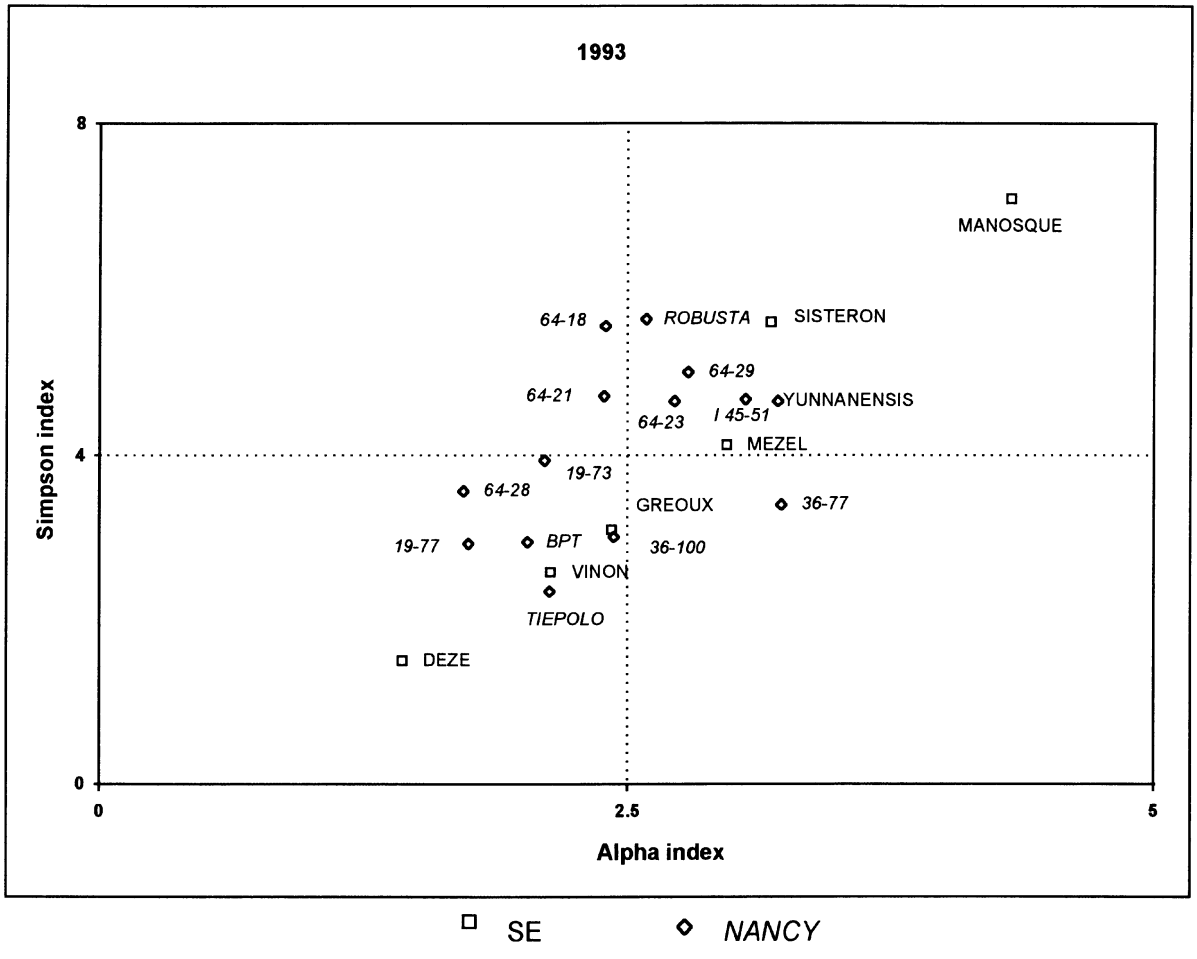

Figure 7. Distribution of the 1993 populations according to their richness (α index) and their evenness (Simpson index). SE = south-east of France, BPT = 'Blanc du Poitou'.

nigra in Dèze and 'Blanc du Poitou' in Nancy, which exhibited a strong dominance in 1992, confirmed this tendency in 1993. The high evenness in the populations from 'Robusta' was evident during both years. Despite the small sample size in Vinon in 1993 (most of the sori belonged to *M. allii-populina)*, there was a good agreement between the observations of 1992 and 1993: the decreased richness in 1993 resulted in increased dominance.

Finally, populations from the USA and from Northern Ireland were poorer and consequently affected by more dominance than the populations from continental Europe. In Nancy, the populations were surveyed on many clones under an overall richness. When populations were surveyed on different clones in the same location (Nancy), it appeared that differences were merely depending on the level of resistance of the clones towards the different virulences.

Conclusions

The populations of *M. larici-populina* surveyed were collected in three types of sites which appeared to be clearly distinct: a) natural stands of *P. nigra* (coevolution with the fungus and no selection pressure resulting from human activity), b) cultivated stands (including artificial hybrids with exotic parents) and c) overseas populations (especially the recent introduction of *M. larici-populina* in the USA).

The four criteria used for the analysis of the populations are useful to understand the structure of these

populations. Nevertheless, there is a link between richness and evenness (or dominance) as is often the case in ecological studies (Dupouey pers. com.; Andrivon and de Vallavieille-Pope, 1995). Presently, nothing is known about the relations between races (competition) and on the possible metabolic costs for the fungus that may result from the accumulation of unnecessary virulence genes. Analysis of data sets from different years showed constant trends, such as the virulence frequencies on some cultivated clones in relation with their behaviour towards these virulences, the higher complexity of the populations in Nancy than in south-eastern France and the low richness in some sites sampled during three consecutive years (e.g. Dèze). Finally it appears that the structure of populations is mainly driven by the resistance genes in the host population within or between sites.

In areas of poplar cultivation, more complex races were identified. Most of the populations described here were collected on clones without race-specific resistance planted in the vicinity of clones with race-specific resistance. These latter clones favour the multiplication of races with the virulence(s) necessary to break the race-specific resistance and, consequently, the richness of the populations is increasing. Likely, the presence of Larch in the nursery allows recombination and finally complex races may appear. The general trend is an increase of richness of the populations including more complex races, and exceptions are most probably due to clones with partial resistance.

Populations from wild *P. nigra* are characterised by a low number of virulences per isolate (simple races) and a low frequency of virulences that recently developed. This situation may raise the question of a counter-selection of unnecessary virulence genes (there is no race-specific resistance known in *P. nigra*, at least for V_1, V_2 and V_5). Experiments must be later engaged to test this hypothesis. In addition, most of these populations evolve in isolation from major virulence sources (i.e. cultivated stands). Nevertheless, these populations are diverse: they cover the full range of richness and evenness (or dominance) described in this study. To interpret this variability between stands, it will be necessary to study the genetic structure of the host population especially by cloning trees and testing their response to the different races of *M. larici-populina* in the laboratory.

In the native range of *Melampsora larici-populina*, race populations depend mainly on the host populations. This suggests that breeding and subsequent cultivation of selected hybrids carrying race-specific resistances strongly influence the parasite populations. A good knowledge of these populations in breeder nurseries is also a critical point for testing clones. In the present case, the experimental nursery in Nancy appears more suitable because of the high richness of its populations. It is also necessary to detect clones which better 'trap' all kinds of races. This is the case of *P. nigra* 'italica'.

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