Ann Sci For (1992) 49, 1-15

Original article

Frequency and evolution of *Melampsora larici-populina* Klebahn races in north-western France

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(Received 11 February 1991; accepted 7 September 1991)

Summary — Race populations in *M larici–populina* were studied for 4 years (nearly 7 000 identifications). Race E1 is ubiquitous in France (and probably in western Europe), E2 exists at least in the northern half of France, in Belgium and in the Netherlands and E3 is present in the east of France and very probably in the west and south-west as well. Races E2 and E3 occurred irregularly among years on larch, the alternate host. There was some link between race populations on larch and on poplar: when E2 and E3 were infrequent on poplar at the end of the growing season, they were practically undetectable on larch the following spring. On universal poplar clones (clones susceptible to all known races), E2 and E3 were in the minority but their counterselection was not evident. On differential clones (susceptible only to E2 or E3) the compatible race was in the majority, but at the end of the growing season infections by incompatible races (that do not infect poplar growing actively in the greenhouse) were detected. This phenomenon is discussed and several hypotheses are proposed. Specific resistance delays the epidemics in differential clones. Up to now, no race combining all the virulences has been found which is in agreement with the low theoretical frequency of such a race. E2 and E3 seem to remain stable and it is suggested that race populations reflect host populations.

races / population / *Melampsora larici–populina* / *Populus* / resistance

Résumé — Fréquence et évolution des populations de races de *Melampsora larici-populina* Klebahn dans le Nord-Est de la France. *Les populations raciales de* M larici-populina *ont été étudiées pendant 4 ans (près de 7 000 identifications), dans plusieurs pépinières de l'est de la France. La race E1 est ubiquiste en France (et très probablement en Europe occidentale), la race E2 existe au moins dans la moitié nord de la France, en Belgique, aux Pays-Bas, et la race E3 semble présente dans l'ouest et le sud-ouest de la France et en Italie, dans la vallée du Pô. Les races E2 et E3 ont une fréquence irrégulière d'une année à l'autre sur le mélèze, l'hôte alternant (tableau I). Il existe un certain lien entre les populations raciales du mélèze et celles des peupliers (tableaux I et II). En particulier lorsqu'une race est très peu fréquente sur peuplier, en fin de saison de végétation, elle sera très difficilement détectable sur le mélèze au printemps suivant. De même, la race majoritaire sur les peupliers le sera ensuite sur l'hôte alternant. Sur les clones universels de peuplier (c'est-à-dire sensibles à toutes les races européennes), ces deux races sont toujours minoritaires (tableaux II et III, fig 1), mais il n'est pas certain qu'elles y soient pour autant contre-sélectionnées. Sur les clones différentiels (ceux qui ne peuvent être infectés que par la race E2 ou la race E3), la race compatible est majoritaire (figs 2 et 3), mais à la fin de la saison de végétation, des infections par les races incompatibles (sur plantes en croissance active en serre) ont néanmoins été détectées et plu-*

sieurs hypothèses sont émises pour tenter de comprendre ce phénomène. La résistance spécifique se traduit par un retard de l'épidémie sur les clones différentiels (fig 4). Jusqu'à présent, aucune race combinant l'ensemble des virulences n'a pas été trouvée, mais ceci peut être dû à la très faible fréquence théorique qu'aurait une telle race. Alors que la fréquence des clones différentiels augmente dans notre pépinière, celle des races E2 et E3 semble suivre la même évolution, et l'infection de ces clones s'accroît d'année en année (fig 4). Il est donc suggéré que les populations raciales reflètent les résistances présentes dans les populations de peuplier.

races / populations* / Melampsora larici–populina / Populus / *résistance

INTRODUCTION

Rust fungi are well known for variability in their pathogenicity. This phenomenon is frequently described in agriculture (cereals, coffee tree) but less often in forestry where host populations are maintained as genetically diverse. Consequently populations of forest trees do not put any strong and uniform selection pressure on parasite populations. Poplar is an exception because of its easy vegetative propagation which results in clonal populations that exert a uniform pressure on the rust population. In addition, poplar clones offer a simple tool to explore rust variability. When new races appear, cultivars previously selected for immunity are often highly infected. New breeding programmes and new types of cultivar management must be developed taking into account race populations.

In 1949, Van Vloten in the Netherlands described 3 physiological races of *Melampsora larici–populina* Kleb, one having an albino variant. Since then, these races have not been investigated. In Belgium, poplar clones which were usually rust-free recently became infected by *M larici–populina*, and Steenackers (1982) suggested that a new race had appeared. Our laboratory experiments confirmed this hypothesis (Pinon and Bachacou, 1984; Pinon *et al*, 1987). Soon after, a third race was detected (Pinon and Peulon, 1989). In Australia, several races of *M medusae* Thuem and *M larici–populina* were described (Sharma and Heather, 1976; Chandrashekar and Heather, 1980) and in the United States, along the Mississippi River, several races of *M medusae* were also discovered by Prakash and Thielges (1987).

In the case of the European races of *M larici–populina*, some poplar clones are totally resistant in the laboratory while others are susceptible. These clear distinctions allowed us to develop simple tests in order to identify races. It therefore became possible to study race frequencies on clones in relation to various seasons, years and locations. Finally, we describe the structure and dynamics of race populations which untill now have not been studied on poplar or on larch, the alternate host. This study offers some epidemiological indications that are useful for breeding and host management.

MATERIALS AND METHODS

When a high number of clones were inoculated in the laboratory with the 3 races of *M larici–populina* known at the time in France (E1, E2 and E3) most of the clones appeared susceptible to the 3 races (universal clones), while others were infected only by E2 or E3 (differential clones). Such clonal reactions can be reproduced easily on fast-growing cuttings from the greenhouse. In the present paper, we used *Populus* x *euramaricana* cv *Robusta* (universal), Ogy (susceptible only to E2) and generally Can-

dicans (susceptible only to E3) as test clones for race identifications. On a few occasions Candicans was not available, and was replaced by NL 2842 or Carpaccio. To avoid natural infection these clones were grown in a greenhouse in 5-l containers. The substrate was composed of a mixture of sand and peat, in equal proportions, the pH being adjusted to approximately 5.5–6.0 with limestome and magnesium carbonate (150–200 g/m^3). This substrate was fertilized by Osmocote Plus or Nutricote (13/13/13/2) at 5 kg/m^3. Poplar shoots grew vigorously and their leaves reacted clearly to races after inoculation.

To identify the races, discs (12 mm in diameter) were cut in the leaves of the test clones and placed on water (abaxial face up) in dwell boxes. To identify the race to which each sore, collected on naturally infected poplar or larch, belonged, a spore suspension was prepared: 15 µl of water agar (10^{-4}) were deposited on the sore and spores were scraped off with a disposable micropipette. The spore suspension was then sucked off with a micropipette, and small drops were deposited on a disc of each test clone. Dwell boxes were left for incubation on the laboratory bench under continuous fluorescent light (50 µmol m^{-2} s^{-1}) or in an illuminated incubator when the ambient temperature exceeded 22 °C. Ten to 12 days later, infections (presence or absence of sporulated sores) on the discs inoculated with each isolate were recorded and the following data became available:

– rate of successful identifications, *ie* the percentage of isolates which had at least infected Robusta (susceptible to all known races);

– frequency of E1 (virulent to Robusta and avirulent to Ogy and Candicans), of E2 (virulent to Robusta and Ogy and avirulent to Candicans), and of E3 (virulent to Robusta and Candicans and avirulent to Ogy).

If the 3 test clones were infected by an isolate, then a race combining all virulences could be detected. Sizes of the specimen fluctuated according to the material available in the nurseries as shown in the tables and figures.

Among the clones whose race populations were surveyed, the following are cultivated at present in France: Luisa Avanzo, Blanc du Poitou, Cima, Fritzi Pauley, Robusta and Unal. Robusta is well represented in our nursery and is the most frequent in the European poplar stands. This justifies a special interest in the rust populations on this clone and their evolution both during the growing season and annually.

The reaction to *M larici–populina* of the clones cultivated in France has been described in another paper (Pinon, 1991).

Ninety-five percent confidence intervals were calculated when possible, *ie* when the percentage x the number of identified isolates was ≥ superior to 5; these intervals have been presented in the tables.

RESULTS

Geographical distribution of the races

The race E1 has been identified in the main poplar cultivation areas and is likely to be ubiquitous. E2 may have already been present in the INRA forest tree nursery at Orleans (Loiret) in 1975 because we identified *M larici–populina* at that time on cv Rap which was found to be susceptible only to E2. In 1983, a survey was conducted in northern France (Pinon, 1986). Our laboratory tests on the specimens collected during this survey showed that E2 was present in 9 nurseries in the Aisne, in the Oise, 1 in the Pas-de-Calais and 6 in the Nord department. Clones infected at least by E2 were the following: Columbia River, Fritzi Pauley, Heimburger, Hunnegem, I 214', Rap, Raspalje, Robusta, Sélys, Spijk, Trichobel and Unal. Rap was found to be infected with *M larici–populina* at Guéméné-Penfao (Loire-Atlantique) in 1988 and at Tiercé (Mainte-et-Loire) in 1989. The latter observations suggest that E2 is present in the lower Loire River valley. Many identifications were also conducted in the Lorraine (eastern France), which will be described in detail later. Therefore E2 exists throughout the northern half of France (the southern half still remains to be surveyed), in Belgium (Steenackers, 1982) and in the Netherlands (Pinon *et al,* 1987).

Race E3 was first described in the Lorraine (Pinon and Peulon, 1989) and is

probably present in the west and southwest of France since infections were found there on clones that are susceptible only to E3: Luisa Avanzo (Orleans; in 1989), Cima (Guéméné-Penfao, Loire-Atlantique; in 1987), Altichiero and Tiepolo (Bordeaux, Gironde; in 1988). Since clones which are differentially susceptible to E3 have been introduced into France only recently, it is impossible to determine how long this race has been present in the country. It may have existed in Europe for at least 10 years because we found (Pinon and Peulon, 1989) that it was identical to the NZ-2 race described in New Zealand by Latch and Wilkinson in 1980, a race of likely European origin.

Race populations on larch (the alternate host)

In spring, *M larici–populina* may alternate on larch, on which its yellow aecidia usually develop at the beginning of May in the Lorraine. It is of interest to determine the race populations on this host for 2 reasons. Firstly, infection on larch is the consequence of the infection which developed the previous year on poplar and is the origin of the poplar contamination at the beginning of the next growing season. Nevertheless, without larch, rust can survive as urediospores on overwintering poplar leaves on the ground (Chiba and Zinno, 1960; Pinon, 1980). Secondly, the sexual stage of rust occurs on larch and consequently recombination may occur on this host.

Between 1987 and 1990 we studied race populations on the naturally-infected larch trees in our nursery at Champenoux (Meurthe-et-Moselle). In 1987, E3 was not yet known and E1 frequency may have included E3 (table I).

In 1987 it was impossible to detect E2 on the different larch species (European, Japanese and their hybrid). The same was established for European larch in 1988. Nevertheless we carried out positive inoculation with E2 on young European larches in a growing chamber (14 h 30 photoperiod, 11°/4 °C thermoperiod and saturated humidity). In addition, poplar leaves of clones susceptible only to E2 and bearing teliospores were placed above the larch seedlings in our nursery and maintained wet. This induced some infection in May and the resulting aecidiospores were collected and analysed in the laboratory. We determined that infections were due to E2, so it was established that this race was able to contaminate larch both under controlled and natural conditions.

In 1989 natural contamination on larch was more frequent in our nursery and the 3 races were detected. In 1990 only scarce infections were observed and E3 was not found. Up to now, no race combining the different virulences has been recognized.

Race populations on Robusta (the universal host)

When inoculated separately in the laboratory with the 3 races, Robusta appeared to be equally susceptible to all of them. Nearly 2 700 race identifications have been carried out on samples collected on this clone in our nursery during the last 4 years (table II). A clear tendency appears: E1 is always predominant and the evolution of the 2 other races depends on the year. In 1987 E2 was quite abundant at the beginning of the growing season, but it decreased and finally disappeared in August. In 1989 it was again more frequent at the time of the first infections. Then its frequency decreased but it was detected until late in the season.

Table I. Race populations on larch in the east of France between 1987 and 1990.

Year	*Larch species (day/month)*	*No of identifications*	*Races (No)* E1	E2	E3
1987	European larch				
	22 May	19	19	0	*
	29 May	30	30	0	*
	4 June	20	20	0	*
	19 June	9	9	0	*
	Japanese larch				
	29 May	14	14	0	*
	Hybrid larch				
	12 June	14	14	0	*
1988	European larch				
	24 May	7	7	0	*
	25 May	6	6	0	*
	26 May	2	2	0	*
	30 May	3	3	0	*
	8 June	9	9	0	*
1989	European larch				
	6 May	66	53	8	5
	19 May	80	80	0	0
	European larch under poplar				
	6 May	26	25	1	0
1990	European larch				
	22 May	37	36	1	0

* No identification of race E3, as it was unknown in 1987 and 1988.

Conversely, in 1988 and 1990 E2 populations appeared stable, even though they were a minority.

E3 was scarce in the spring of 1988 and could no longer be detected at the end of August and the following year. In 1990 it was more frequent and persisted until the end of the season, and an increase was even recorded at that time. In order to determine whether the tendencies that we have described for the race populations on Robusta could be generalized, we surveyed populations on other clones and in other nurseries.

Race populations on other universal clones

Race populations were identified on *P trichocarpa* cv Fritzi Pauley in different nurseries in the Lorraine (table III). Here again, E1 was in the majority whatever the location or the year. In 1986 at least, E2

Table II. Race populations on cv Robusta between 1987 and 1990 in our nursery in the east of France.

Year	*Date of sampling (day/month)*	*No of identifications*	*Races (%)* E1	E2	E3
1987	22 June	191	73 ± 6.3	27	*
	7 July	73	100	0	*
	22 July	200	99.5	0.5	*
	4 Aug	100	100	0	*
	18 Aug	190	100	0	*
	31 Aug	192	100	0	*
	25 Sept	100	100	0	*
1988	23 June	56	95	2	3
	13 July	74	95	4	1
	29 Aug	143	97	3	0
1989	27 June	58	88 ± 8.3	12	0
	17 Aug	148	96 ± 3.2	4	0
	13 July	168	97 ± 2.6	3	0
	4 Oct	200	99.5	0.5	0
1990	2 July	197	88 ± 4.5	7	5
	3 Aug	199	89	1	10
	4 Sept	196	82 ± 5.4	10	8
	8 Oct	205	56.6	7.3	36.1

* No identification of E3 as it was unknown in 1987.

remained stable during the growing season.

On other universal clones E2 was in the minority in Champenoux in 1987 among 2 000 race identifications: on 12 clones E2 was undetectable and on those which were sufficiently infected to allow more than 100 identifications per clone, the frequency of E2 was similar to that previously described on Robusta. The survey conducted in 1990 on a smaller number of clones (1 000 identifications), again led to the conclusion that E1 was in the majority (fig 1). On Unal, the mid-September control showed a stagnation of E3 and a slight increase of E2.

Race populations on differential clones

Figures 2 and 3 present all the race identifications carried out on the differential clones, *ie* clones which are susceptible only to E2 or E3 after the inoculation tests in the laboratory. On each clone, the predominant race was the one that the clone had been described as susceptible to, which is logical. Nevertheless, around the time of cessation of growth in the nursery, we detected E1 or the race lacking in the virulence required to infect the clone considered. This surprising phenomenon (even if those "intruding" races are in the minority) will be discussed later.

Table III. Race populations on cv Fritzi Pauley in nurseries in the east of France.

Year	*Date (day/month)*	*Location (department)*		*Races (%)* E1	E2	E3
1986	27 Aug	Harmel	(88)	98.5	1.5	–
	29 Aug	Optel	(55)	98.9	1.1	–
	3 Sept	Kappel	(57)	98.7	1.3	–
1987	15 June	Champenoux	(54)	98	2	–
	28 July	"		98	2	–
1989	25 July	Champenoux	(54)	99	1	0

* No identification of race E3 as it was unknown in 1986 and 1987. Each sample included at least 100 identifications.

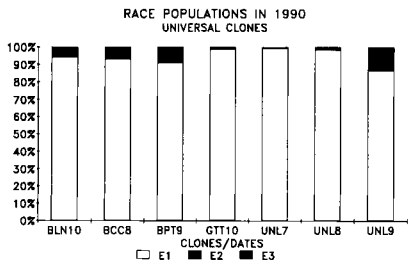


Fig 1. Race populations on universal clones in 1990 in our nursery. Clones and number of identifications in brackets: BLN = Bellini (104), BCC = Boccalari (141), BPT = Blanc du Poitou (102), GTT = Gattoni (104), UNL = Unal (160, 136 and 199). Dates: 7 = July, 8 = August, 9 = September, 10 = October.

Vertical resistance and delayed epidemics

According to Van der Plank (1974), clones with vertical resistance (differential clones) present a delayed epidemic as compared with the clones without this type of resistance (universal clones). This delay occurs when races pathogenic to differential clones are infrequent at the beginning of the growing season. In 1990 we detected the first natural infections in the nursery on clones whose reaction to the different races had previously been established in our laboratory. In fact, infection appeared earlier on the universal clones (table IV). As proposed by Van der Plank, we calculated the mean date for the beginning of the epidemics on the different types of clones. This was evaluated to be July 11 for the universal clones, July 24 for those only susceptible to E2 (*ie* a delay of 13 days) and August 4 for those susceptible to E3 (*ie* 24 days after the universal clones).

It also became possible to estimate the speed of infection of the race E2 using the formula suggested by Van der Plank:

$$x_0 = x_{ov}\ e^{rdt}$$

where x_0 is the number of sores (at the beginning of the growing season) of all the races together (here E1 + E2 because E3 was not detected on larch), x_{ov} the number of sores belonging to the virulent race (E2), r the speed of infection and dt the delay of the epidemic by the race E2. Since

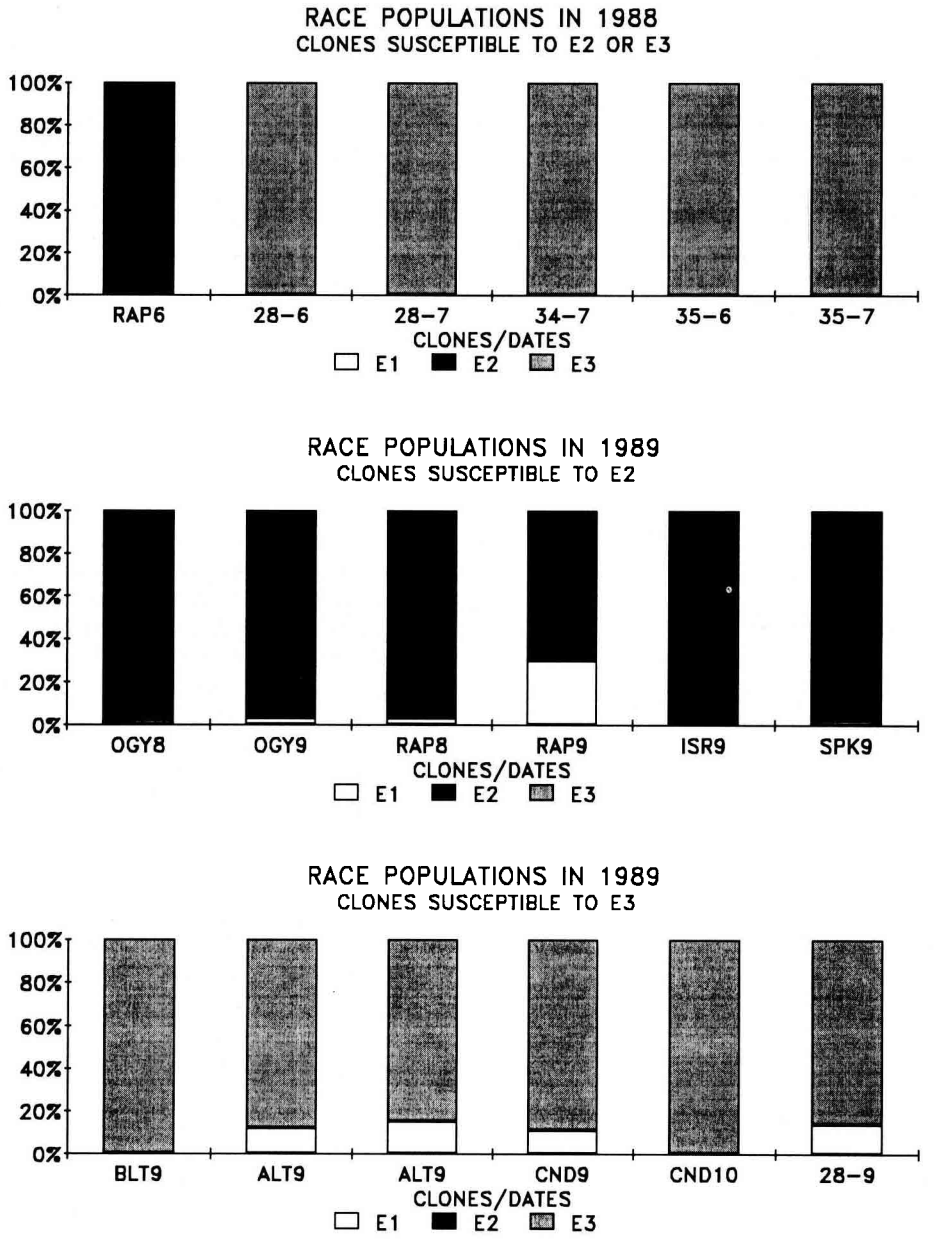
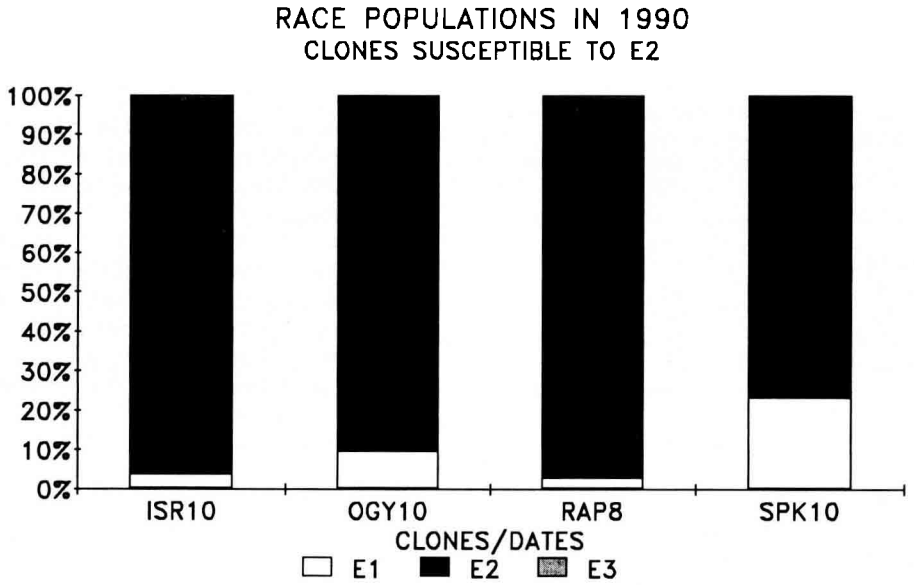


Fig 2. Race populations on differential clones in 1988 and 1989 in our nursery. Clones and number of identifications in brackets: RAP = Rap (17), 28 = NL 2842 (12), 34 = NL 3495 (11), 35 = NL 3512 (18 ad 6). OGY = Ogy (54 and 60), RAP = Rap (37 and 30), ISR = Isières (58), SPK = Spijk (55), BLT = Bellotto (46), ALT = Altichiero (57 and 60), CND = Candicans (53 and 204), 28 = NL 2842 (14). Dates: 6 = June, 7 = July, 8 = August, 9 = September.



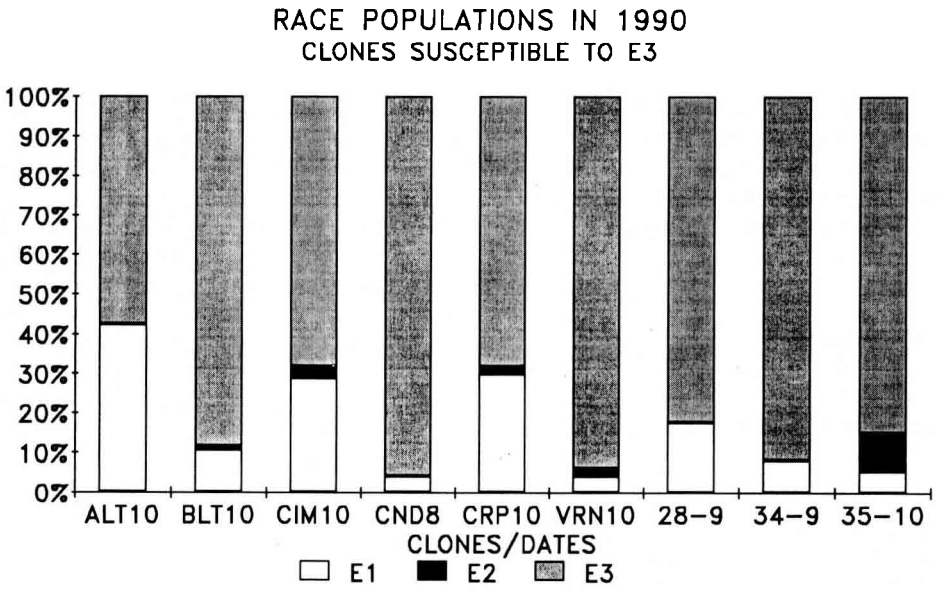


Fig 3. Race populations on differential clones in 1990 in our nursery. Clones and number of identifications in brackets: ISR = Isières (105), OGY = Ogy (104), RAP = Rap (159), SPK = Spijk (103). ALT = Altichiero (104), BLT = Bellotto (105), CIM = Cima (101), CND = Candicans (197), CRP = Carpaccio (104), VRN = Véronèse (104), 28 = NL 2842 (102), 34 = NL 3495 (100), 35 = NL 3512 (114). Dates: 8 August, 9 = September, 10 = October.

Table IV. Early detection of rust in our nursery in 1990.

Date of observation	*No of clones beginning infection*		
	Universal	*Susceptible to E2*	*Susceptible to E3*
June 19	10	0	1
July 18	19	2	4
July 30	4	2	3
September 3	0	0	2
October 10	0	0	1

the delay is 13 days for race E2 and taking its frequency into account, *r* equals 0,269. This value is very similar to the values described by Van der Plank for other diseases. If we state that *r* has the same value for race E3, and if we take into account the 24-day delay in the case of this race, the formula indicates that the frequency of this race on larch was 1/611. In other words, it would have been necessary to survey 611 sores to have a chance of finding one sore belonging to the race E3. Such a sample is far greater than the sample we could obtain, and so explains why we could not detect E3.

DISCUSSION

Race populations on larch

Most of the clones present in our nursery are universal, and among them Robusta is the most frequent, so it is logical to compare race populations on Robusta at the end of the growing season (*ie* when teliospores develop) and the populations on larch during the following spring.

The absence of E2 on larch in 1988 is in agreement with its scarcity (or absence) on Robusta in 1987 on which it was not detected from July until October. The presence of E2 on larch in 1989 is in relation with its detection on Robusta at the end of August 1988 (3%). The same relationship exists between the infection of Robusta on October 4 1989 (1% of the race E2) and the infection of larch the following spring (3% of the race E2). So it seems that the frequency of this race on poplar at the end of the growing season can predict its presence (or absence) on larch the following spring.

E3 was not detected on Robusta at the end of 1988. The following year, we found it on 1 group of larches but not on the other. This may indicate that larch is infected mainly from poplar leaves in the immediate vicinity and consequently is dependent on the race populations borne by these poplar leaves. In fact, it is generally accepted that the basidiospores emerging from poplar leaves are very fragile and able to infect larch only over a very short distance. In 1990 E3 was not found on larch; neither had it been found on Robusta the previous year. Finally there seems to be a link between the race populations of larch and those of the most frequent poplar clones. It is evident that the above-mentioned frequencies must be considered as indicative. They depend on the number of identifications performed, especially on larch whose infection was scarce for certain years which reduced the probability of detecting the infrequent races.

No detection of a race combining all the virulences

To try to explain why such a race was never detected, we must take into account the observed frequencies of E2 and E3 and

accept the hypothesis that there must be relationship between these 2 races and a recombining one.

Taking into account the frequency of E2 (12%) and of E3 (8%) on larch in 1989, we can calculate that the theoretical size of a sample in which one sore of the combined race might have existed is 104. This number is close to the number of identifications we carried out (105). In 1990 E3 was not found on larch, which prevented the detection of the combined race.

In the future, we intend to inoculate larch seedlings with poplar leaves bearing teliospores of E2 and E3 in an isolated chamber in order to determine whether a combination of their virulences can occur. If we are successful, we will look for such a race on naturally infected larches to ascertain its existence in the open.

It would have been necessary to test 1 667 sores on Robusta in June 1988 and 286 in July 1990 to have a chance of detecting this hypothetical combined race. In 1990 isolates were collected on clones with a relatively high frequency of E2 and E3. These isolates have been stored for further study with the aim of detecting a combined race.

Race frequencies on universal clones

The low frequency of the races E2 and E3 observed on the different universal clones may refer to Van der Plank's theory of counterselection of unnecessary genes of virulence. During the first years of detection of these races we noticed that they decreased in frequency during the growing season on Robusta. But this phenomenon was not confirmed until recently. We can compare the change in the race populations with that of the infection on the differential clones in our nursery during the past few years (fig 4). Infection on such clones increased after E2 and E3 became obvious and is now stable or perhaps still increasing. This tendency does not reflect a general climatic change (and consequently an evolution of the infections) since infections on Robusta remained quite stable during the same period.

Steenackers (personal communication) considers that some differential clones like Ogy and Isières were rust-free in his nursery before 1982, which may indicate that E2 was absent or very scarce at that time. So the populations that we have described here may be interpreted as reflecting a settlement stage of E2 and E3 followed by a stable phase with an eventual increase in relation to the recent introduction and propagation of some differential clones. Some observations relative to E3 on Robusta suggest a relationship between the poplar population (especially poplar differential clones) and race frequencies. The first estimation of E3 frequency in July 1990 revealed 5% of this race, while it was not detected a few weeks before on the larch trees. E3 contaminating Robusta early in the season probably originated from Candicans, whose first detectable infection was observed in the middle of June. In October 1990 E3 was unusually frequent (36%) on Robusta, but samples were collected next to clones known for their differential susceptibility to this race. If this interpretation is correct it will mean that the race populations are closely related to the host population (*ie* the frequency of the differential clones). To validate this hypothesis, it would be interesting to survey race populations in nurseries or stands including various proportions of universal and differential clones.

In the present study, the counterselection of unnecessary genes of virulence is not evident. For other diseases many exceptions to the theory of counterselection have been described. Grant and Archer (1983) indicated that such a decline of un-

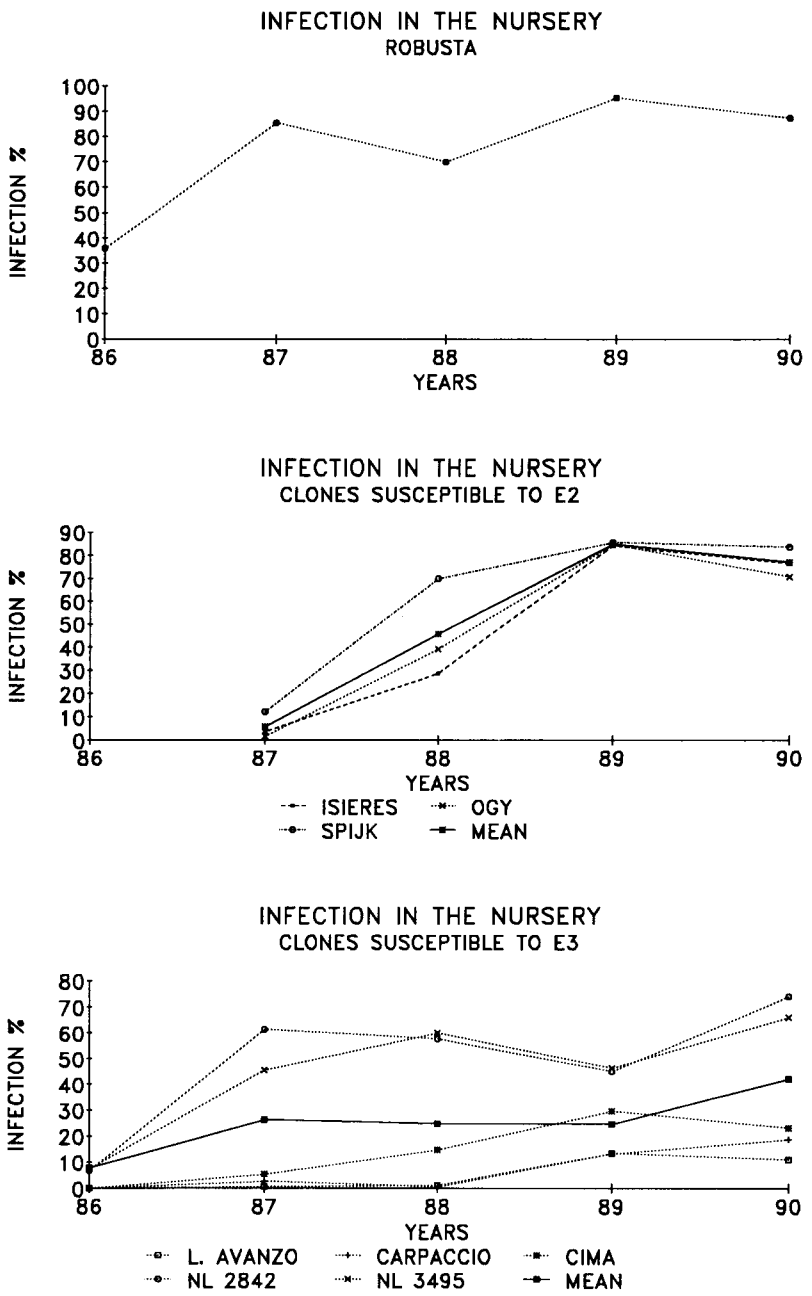


Fig 4. Changes in the infection of different clones in our nursery between 1986 and 1990.

necessary genes of virulence in *Puccinia graminis tritici* is more evident in the greenhouse than in the field. Leonard and Czochor (1980) gave evidence of one isolate cumulating several genes of virulence and presenting an increased competitivity. Also, in *Erysiphe graminis*, Bronson and Ellingboe (1986) proved that fitness and virulence genes were independent. Finally, to determine whether the counterselection of unnecessary genes of virulence operates in *M larici–populina*, it is necessary to manage epidemics in which the original race populations are controlled and to follow, cycle after cycle, the frequency of the virulent races on universal clones.

Epidemiological models often lack climatic and physiological parameters. Bronson and Ellingboe (1986) indicated that counterselection may be effective or not according to environmental conditions. Heather and Chandrashekar (1982) have shown that on poplar, the expression of resistance in the laboratory was dependent on temperature. In our study, it is evident that the climatic parameters changed continuously between spring and late autumn. So it would be valuable not only to manage artifical epidemics but also to simulate different climates.

Race populations on the differential clones

Inoculations of the differential clones in the laboratory led to very clear and reproducible expressions of virulence and resistance: a clone which resists one race is always free of rust when inoculated with this incompatible race. We have shown that under natural infection the compatible race is in the majority but is not exclusive. Races which are not pathogenic on differential clones in the laboratory induced small infections in the nursery late in season. This raises the question of the factors which can modify the expression of resistance to races. We have already noticed in the laboratory that incompatibility may be expressed in 2 ways: lack of symptoms (immunity) or necrotic flecks suggesting hypersensitivity (for example, Ogy inoculated with E1).

In the nursery, the infection of differential clones by incompatible races was detected mainly when poplar was coming to the end of or had finished its growth, while resistance to such races was the rule on fast-growing greenhouse cuttings. Does this mean that the physiology or phenology of poplar may modify its reaction to disease? It is also evident that the inoculum pressure is much higher at the end of the growing season, but its effect has not been studied. At the same time, the decrease in the temperature is noteworthy as resistance may depend on temperature, as shown for cereals by Dyck and Kerber (1985) and for poplar by Chandrashekar and Heather (1981). The last question is: can preliminary infection by the compatible race reduce resistance to further infection by an incompatible race?

CONCLUSION

This first description of race populations in *M larici–populina* indicates that E2 and E3 races are in the minority on the universal clones but that it is not evident that unnecessary genes of virulence are counterselected. It seems more likely that the frequency of these races may reflect the frequency of the differential clones susceptible to them. If the culture of the differential clones is increased, the population of the races virulent on those clones will probably increase and consequently these clones will become more heavily infected. This phenomenon is likely to be occurring

in Italy. When we demonstrated that E3 was able to infect Luisa Avanzo, this clone was still healthy in Italy. Now Luisa Avanzo suffers from heavy infections in the Pô River valley (Anselmi, personal communication). According to the host genotypes that will be cultured, rust populations may continue to evolve. Especially when differential clones are cultivated more often, rust populations that are still more or less wild will be replaced by host-selected populations. This is what happened with cereal rusts during the last 30 years leading to the boom and bust cycle. In forestry when a gene of resistance is defeated, plantations cannot be protected (because of the cost of the treatments) and it is impossible to move quickly towards new resistant genotypes. Present results underline the evolution of rust populations in connection with changes in the host population. Specific resistance, especially governed by a limited number of genes, is the most likely to be defeated. It means that tree-breeders must increase their knowledge of the genetic basis of the resistance thay they are selecting and must look for genotypes with a sufficient level of general resistance. Unfortunately such genotypes are the minority at the moment among the cultivated clones (Pinon, 1991) and heritability of such resistance is not well documented. Because the number of genes of resistance (and symetrically of genes of virulence) are unknown, we cannot forecast the number of races that may exist. So, we continue to explore rust variability including its molecular approach.

ACKNOWLEDGMENTS

The authors are grateful to F Collinet, G Costa, D Masson, M Perochon, V Peulon, N Schell, A Schipfer for technical assistance and to the EEC for financial support.

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