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Variability among African populations of Rigidoporus lignosus and Phellinus noxius

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Abstract

Pathogenicity and physiological characteristics were studied for the two populations of isolates. Evidence of variations has been demonstrated for each fungus but no correlations could be established between the *in vivo* and *in vitro* parameters.

1 Introduction

In tropical climates, the white rots of *Hevea brasiliensis* (Wild. ex Adr. de Juss) Mull. Arg. caused by *Rigidoporus lignosus* (Kl.) Imazeki and *Phellinus noxius* (Corner) G. H. Cunn have been studied in Ivory Coast since 20 years (BOISSON 1968; GEIGER et al. 1976; NICOLE et al. 1982 a, b; TRAN VAN CANH 1982; NANDRIS et al. 1985).

In the context of the ORSTOM research program on these parasites, epidemiological surveys in rubber plantations have shown the existence of differences in the course of the infectious cycle, both among different foci and among trees in the same focus (NANDRIS et al. 1983 a). These differences can be attributed either to differences in host reaction capacities (GEIGER et al. 1983) or to variations in pathogenicity of the infecting strains. In order to decide between these two hypotheses, a pathogeny study was carried out in a greenhouse by artificially infecting young rubber trees with different isolates of each of the above parasites. In parallel, the possibility of a physiological heterogeneity in these strains was sought *in vitro* (GEIGER et al. 1984). The present paper describes first results acquired on pathogenicity variations and the attempt to correlate the parasitic behaviour of these strains with their saprophytic characteristics.

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2 Materials and methods

2.1 Artificial infections

R. lignosus and *R. noxius* strains were isolated from roots of infected trees of different species collected in different countries (Cameroon, Ivory Coast, Liberia) or in a same plantation (Ivory Coast). The inoculum and the experimental system of rubber trees inoculation were previously described by NANDRIS et al. (1983 b).

2.2 Evaluation of pathogenicity

The quantitative determination of the infestation level of each inoculated plant was evaluated on the basis of a scoring scale (NANDRIS et al. 1983 b). Some other criteria were also taken into account:

- growth of stem (measured weekly),
- the percentage of plants manifesting anatomic reactions (secondary rhizogenesis) which have been described elsewhere (NICOLE et al. 1983),
- the localization of necrosis into the tap root,
- enzymatic activities (laccases, hydrolases) of the fungus in the inoculum, assayed before, during and after the experiment as described by GEIGER (1975).

2.3 In vitro physiological variability of isolates

Effect of pH and temperature on the growth rate

The effects of pH (3 to 9) on fungal growth rate was determined by growing the different strains at 30 °C on malt medium (2 %).

The effect of temperature on fungal growth was determined by depositing cultures on malt agar and growing them in incubators maintained at 20, 25, 30 and 35 °C.

In both cases, differences among strains were evaluated on the basis of their growth after 3 days of culture (mean of two perpendicular thallus diameters).

Evaluation of wood degrading capacity

Test blocks were formed from pieces of rubber tree wood ($5 \times 1 \times 0.3$ cm in size) taken from healthy tap roots in plantations. After drying, the test blocks were placed in constriction tubes containing 20 ml of water. Each tube was inoculated with a mycelial implant. After 7 months of incubation, the infested blocks were used for two series of tests. Wood degradation, i. e. the loss in dry weight (DWL) and lignin content (LIG) were measured. In addition, fungal enzymatic activities in the wood were measured: beta-glucosidase (β-glu), alpha-galactosidase (α-gal), beta-galactosidase (β-gal), pectinase (pect.), cellulase (cell.) and catalase (cat.). Conventional techniques were used for these assays (GEIGER 1975).

2.4 Statistical interpretation of results

All data of each experiment were subjected to a principal component analysis (P.C.A.) and to a multiple regression test.

3 Results

3.1 Pathogenicity of R. lignosus isolates

At the end of the experiment (5 months after inoculation with strains collected in different plantations) although the totality of plants infected with each isolate was contaminated and penetrated – in the absence of qualitative differences among strains – the percentages of

Table 1

Incidence and severity on rubber seedlings of Rigidoporus lignosus isolates collected in different areas from various hosts

Isolates (stock no.)	Rating criteria									
	Severity Index (S.I.)	Standard error of S.I.	Growth of stem cm	Contamination %	Penetration %	Foliar symptoms %	Mortality %	Secondary rhizogenesis %	Amount of decay %	Location of infection site
52	8,9	0,22	41	100	100	0	95	0	91	Tap root
42 b	8,7	0,5	47	100	100	0	90	5	78	Tap root + collar
13	8,7	0,37	44	100	100	0	85	5	86	Tap root
1	8,4	0,56	47	100	100	0	75	10	72	Tap root + collar
38	8,3	0,62	48	100	100	5	70	0	85	Tap root
21	8,1	0,76	54	100	100	0	70	15	60	Tap root + collar
9	7,15	1,08	55	100	100	5	45	10	45	Tap root + collar
37 b	5,47	1,16	78	100	90	0	10	60	32	Tap root

Means of 20 inoculated plants.

plant mortality in fact varied between 10 and 95%, depending on the isolate. In addition, differences in infestations were also manifested by the various indicators characterizing parasitic attack (Table 1).

In order to further define this analysis, additional experimentation was carried out to test the pathogenic capacity of *R. lignosus* strains isolated in the same rubber plantation and mutually separated by a distance of several hundred meters. The data gathered for each isolate (Table 2) show, as before, that there is a considerable variability among the 7 isolates examined. This variability is most apparent at the level of the percentage of necrotic tissues

Table 2

Incidence and severity on rubber seedlings of Rigidoporus lignosus isolates collected in the same plantation

Isolates (stock no.)	Rating criteria									
	Severity Index (S.I.)	Standard error of S.I.	Length of stem cm	Contamination %	Penetration %	Foliar symptoms %	Mortality %	Secondary rhizogenesis %	Amount of decay %	Location of infection site
64 C	8,8	0,23	59	100	100	6,6	86,6	13	73	Tap root + collar
64 F	7,9	0,50	71	100	100	3,3	50	35	68	Tap root + collar
36	7,3	0,42	72	100	100	0	20	23	59	Tap root
64 E	7	0,93	84	100	93	0	36,6	32	55	Tap root + collar
64 D	6,8	0,48	83	100	100	0	16,6	30	45	Tap root
9	5,7	0,67	102	100	100	0	6,6	17	27	Tap root
64 A	5,3	0,54	97	100	100	0	0	43	17	Tap root

Means of 30 inoculated plants.

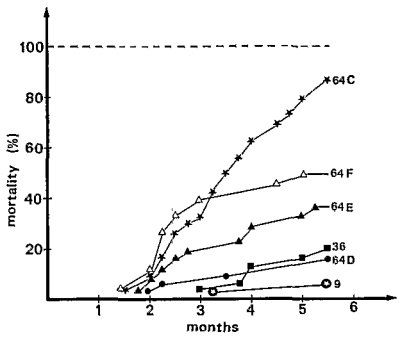

Fig. 1. Rates of mortality of plants inoculated with isolates of *Rigidoporus lignosus*, collected in a same plantation

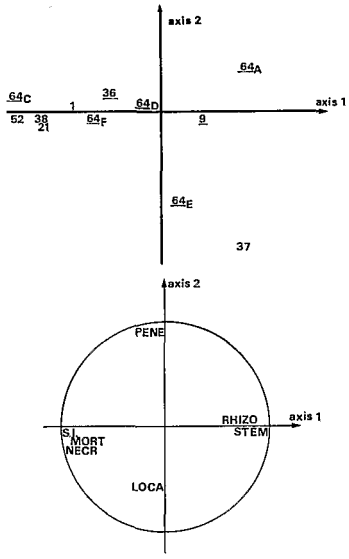

Fig. 2. Pathogenicity variation among *Rigidoporus lignosus* isolates collected in the same rubber estate: a. position of the strains on the plan 1–2 of the P.C.A. The most aggressive strains lie on axis 1 negative. b. position of the variables on the correlation circle. LOCA: location of the infection site; MORT: mortality; NECR: root necrosis; PENE: root penetration; RHIZO: reactional rhizogenesis; S.I.: severity index; STEM: length of stem

and of the number of dead plants. It was also noted that some strains preferentially attacked the lower part of the root system and then progressively worked upwards to the collar, while others simultaneously attacked the collar and the tap root.

Differences in aggressiveness among these strains were confirmed by the mortality rates (Fig. 1). Thus, attacks by the most pathogenic strains was brutal, with mortality occurring as soon as the sixth week. In the case of other isolates (No. 64 D, 9 and 64 A), on the other hand, the infestation remained quite moderate. The statistical positioning of the isolates on the basis of their characteristics is shown in Fig. 2 a, b, after PCA processing.

For the sake of comparison, reference strains are also included in this representation (axes 1 and 2) but they were not considered directly in the analysis of the 7 strains examined. It should be noted that the classification obtained for some strains on the basis of plant mortality level (Table 2) differs from that furnished by the P.C.A. for all the variables considered.

3.2 In vitro physiological variability of R. lignosus

In parallel to the demonstration of *in vivo* pathogenic variability, we carried out a study of the growth and physiological characters of these strains (GEIGER et al. 1984). It was found first that the isolates could not be discriminated on the basis of growth rates on agar medium as a function of pH and of temperature. The analysis of test blocks degradation parameters (loss of weight and lignin contents) and of enzymatic characteristics, showed consequent variations of data recorded for the isolates of each parasite. Nevertheless the existence of a correlation between enzyme activities and loss of weight was established.

3.3 Attempt to correlate pathogenicity and physiological characteristics of R. lignosus isolates

Table 3 contains the values characterizing the most representative variables (*a priori*) of the saprophytic behavior of the isolates, and also the S. I. of the attacks. The multiple regression test enabled us to demonstrate the absence of significant correlations between pathogenicity (S. I.) and enzymatic activities assayed *in vitro*. Indeed, the F values established (F = 1,25 and F = 1,19) are much lower than the F table at 5 % confidence level (F = 8,35).

Table 3

Comparison of in vitro and in vivo characteristics of R. lignosus

Isolates (Stock no.)	Severity Index (S. I)	Secondary Rhizogenesis (RHIZO) %	Speed of growth (S. GRO) cm / day	Optimum temperature °C	Optimum pH	Δ Weight Loss (DWL) %
52	8,9	0	2,0	30	7,6	21,2
42	8,7	5	1,7	30	7,0	19,5
13	8,7	5	1,8	30	7,6	17,8
1	8,4	10	2,0	30	7,6	17,2
38	8,3	0	1,4	30	7,6	19,7
21	8,1	15	2,0	30	7,6	18,7
9	7,1	10	1,5	30	> 9 (?)	35,7
37	5,5	60	1,8	30	> 9 (?)	16,0

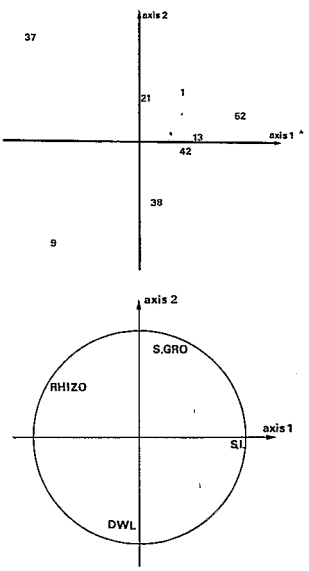

Fig. 3. Correlation between *in vitro* and *in vivo* characteristics of *Rigidoporus lignosus* isolates. a. position of the strains on the plan 1–2 of the P.C.A. The most aggressive strains lie on axis 1 positive. b. position of the variables on the correlation circle. DWL: difference in weight loss; RHIZO: secondary rhizogenesis; S. GROW: speed of isolate growth; S. I.: severity index

As a result of the lack of correlation between these two types of variation, it was of interest to determine the effect of the *in vitro* characteristics on the structure of the parasite population, previously determined on the basis of only pathogenic criteria (see Fig. 2).

Four active non-correlated variables were used for this new P.C.A.: the S.I., the percentage of plants reacting to the attack, the weight loss of the test blocks, and the growth rate of the isolates in culture at 30 °C and optimal pH. The comparisons of positions of individuals and of variables (Figs. 3 a, 3 b) stress the preponderant role of the S. I. on the configuration of the population. Although the statistical representation of this set of isolates remains globally comparable to that seen in Fig. 2 it is nonetheless noted that there is a better discrimination among the 5 most aggressive isolates.

3.4 Pathogenic and physiological variabilities of P. noxius isolates

Experimentation identical to that carried out with *R. lignosus* was performed with *P. noxius* isolates (Table 4). Although there are obvious quantitative differences in the parasitic and saprophytic behaviours of the two fungal species, the conclusions of the analysis performed with the *P. noxius* strains are qualitatively similar to those obtained with *R. lignosus*.

Table 4

Incidence and severity on rubber seedlings of Phellinus noxius isolates collected in different areas from various hosts

Isolates (stock no.)	Rating criteria (*in vivo*)									
	Severity Index (S.I.)	Standard error of S.I.	Length of stem cm	Contamination %	Penetration %	Foliar symptoms %	Mortality %	Secondary rhizogenesis %	Amount of decay %	Location of infection site
45	6,45	1,76	49	90	80	15	45	5	53	Tap root
32	6,2	1,3	59	95	95	5	35	25	24	Tap root
35	5,05	1,56	60	85	80	15	15	30	30	Tap root
2	4,54	1,9	62	65	65	5	20	25	16	Tap root
7	2,6	1,21	74	60	60	5	0	7	5	Tap root
39	1,5	1,3	74	35	30	0	5	10	5	Tap root
31	0,35	0,56	78	10	10	0	0	0	0	Tap root

Means of 20 inoculated seedlings.

4 Discussion

A comparable analysis has been carried out by LIYANAGE et al. (1977) with *R. lignosus* isolates collected in different Sri Lanka rubber plantations. Even though the general experimental design and the main conclusions are analogous in both studies, there are nevertheless certain differences.

First, the authors stated that the most aggressive strain differed from the others by the lack of rhizomorph differentiation. They also stated that the rhizomorph was initiated only when individual hyphae were incapable of penetrating host roots. These observations are in contradiction with results obtained in our laboratory concerning the parasitic behaviour of the African isolates of this fungus (BOISSON 1968). All the strains we studied in fact produce rhizomorphs, the contamination structure "par excellence". This shows the absence of a relationship between rhizomorphogenesis and pathogenicity in the model in question.

Secondly, LIYANAGE et al. (1977) observed that the same highly aggressive strain was obtained in a region of Sri Lanka which was devastated by root diseases. In the Ivory Coast, on the other hand, no direct relation between the origin of the strain and its pathogenic capacity could be established, since there are considerable differences in aggressiveness among strains obtained in the same area (Table 2). Similar variability has been reported in the case of a population of *Armillaria*, whose individuals were isolated in a distance of less than 100 meters from each other (REDFERN 1975).

The reproducibility of the results, reflected by the low variations of the S.I. of each isolate with time shows that experimental conditions of storage during the saprophytic stage and of greenhouse inoculations of the plants did not affect the pathogenic capacity of the strains (NANDRIS et al. 1985). The pathogenic variations observed among these isolates may thus be considered as being constitutive of each.

We may now question the basis of this variability. In this context, attempts to correlate pathogenicity with the capacity of different strains to degrade plant polymers *in vitro* have not furnished positive results. Furthermore, several authors have also reported the absence of a correlation between saprophytic (*in vitro*) and parasitic characteristics (RAABE 1967; JAMES and COBB 1982; WORRAL et al. 1983).

We may invoke two hypotheses to explain this result:

– pathogeny does not depend only on the degradation of root structures. This would be suggested by some recent observations of young rubber plants killed by *R. lignosus* (artificial

infection), whose tap root was only partially necrosed. We may thus reconsider the hypothesis of the participation of a fungal toxin in the infectious process already suggested by PERIES 1959;

– the saprophytic behavior of these fungi, i. e. their capacity to degrade inert substrates, would not characterize the actual mechanisms utilized by the parasite to colonize host tissues. Extrapolation of results acquired on parasite behavior *in vitro* would thus not be valid.

Other explanations have still been advanced to explain the variability existing among individuals (PRILLINGER and MOLITORIS 1979):

– a variation related primarily to the stage of development of each isolate,
– a variation depending on the ecological conditions prevaling in the region in which the isolate originated,
– a variation caused by genetic differences.

The results described in the present article, in particular the stability of the pathogeny of a given strain with time and the variability recorded in a limited perimeter tend to prove that the third of the above hypotheses is the best adapted to explain the observed phenomenon. Thus, as reported for the genus *Armillaria* (REDFERN 1975), the African populations of *Rigidoporus lignosus* and *Phellinus noxius* would each constitute a mosaic of "clones" with different pathogenic potential. This variability must be considered when practical means for combatting these root diseases are put into practice. Henceforth, resistant tree selection and fungicides screening will no longer involve a single strain for performing tests for extrapolating the results to all the strains comprising the parasite population of a given region.

Summary

Isolates of the root rot fungi *Rigidoporus lignosus* and *Phellinus noxius* were tested first *in vivo* for their pathogenicity and secondly, *in vitro*, for their physiological characteristics.

Evidence of pathotypes of *R. lignosus* was demonstrated by differences in mortality levels and in pathogenesis. Multivariate analyses of the data confirm the existence of a variability in pathogenicity within isolates originated either from various countries or from the same rubber plantation. This suggests that the fungal population could be considered as a mosaic of pathogenic clones. *In vitro* investigations concerning some physiological characteristics, demonstrate differences between each isolate, that could not be correlated with pathogenicity. Same experiments and similar results were gathered also for *Phellinus noxius* isolates. Effects of these variations on control method schedules are discussed.

Résumé

Variabilité d'isolats africains de Rigidoporus lignosus et Phellinus noxius

Ces études démontrent l'existence d'une variabilité au sein des souches de *R. lignosus*, qui est perceptible au niveau du taux de mortalité des plants infectés (de 10 à 95 %) et du déroulement des infections. Cette variabilité, confirmée par des analyses multivariées, s'exerce à la fois pour des souches de provenances diverses ainsi que pour des souches isolées dans une même plantation. Ceci suggère que la population parasitaire puisse être considérée comme une mosaïque de clones différant par leur pathogénie. Les recherches menées *in vitro* concernent leurs caractéristiques culturales, le dosage des activités des enzymes extracellulaires et la capacité à dégrader le bois. Il s'avère que les différences ainsi mises en évidence entre les souches, ne peuvent pas être corrélées à leur pouvoir pathogène. Des expérimentations identiques, menées avec des isolats de *Phellinus noxius*, ont apporté des résultats comparables. En conclusion, les conséquences de ces variations sur la mise en œuvre de méthodes de lutte contre ces parasites sont discutées.

Zusammenfassung

Variabilität zwischen afrikanischen Populationen von Rigidoporus lignosus und Phellinus noxius

Isolate der Wurzelfäuleerreger *Rigidoporus lignosus* and *Phellinus noxius* wurden zuerst *in vivo* auf ihre Pathogenität und dann *in vitro* auf ihre physiologischen Eigenschaften hin untersucht. Isolate unterschiedlicher Pathogenität wurden für *R. lignosus* auf Grund der Unterschiede in der hervorgerufenen

Mortalität und der Pathogenese nachgewiesen. Die statistische Auswertung der Daten bestätigt das Vorkommen einer Variabilität hinsichtlich der Pathogenität sowohl bei Isolaten aus verschiedenen Ländern wie auch aus derselben Gummibaumplantage. Dies legt die Annahme nahe, daß die Pilzpopulationen als ein Mosaik aus unterschiedlich pathogenen Klonen aufzufassen sind. *In vitro* gefundene Unterschiede bezüglich einiger physiologischer Eigenschaften waren nicht mit der Pathogenität korreliert. Die gleichen Untersuchungen wurden mit *P. noxius* durchgeführt und ergaben ähnliche Resultate. Die Bedeutung der Variabilität der Pilze für die Bekämpfung wird diskutiert.

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