PATHOTYPES OF *RIGIDOPORUS LIGNOSUS* AND *PHELLINUS NOXIUS* ISOLATED FROM DIFFERENT HOSTS IN WEST AFRICA

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INTRODUCTION

In West Africa, the fungi that cause root rot are the major economic problem affecting commercial monocultures of ligneous trees. *Rigidoporus lignosus* and *Phellinus noxius* cause significant damages to the plantations of rubber (*Hevea brasiliensis*) that are customarily established on newly cleared forest land.

In this ecological context, extrapolation of the recommended methods for combatting these parasites in South-East Asia has turned out to be largely ineffective. On the other hand, various preventive measures are now being developed in the Ivory Coast. They are based mainly on characterization of anti-fungal substances and a search for resistant or tolerant individual trees.

However, considering the diversity of soil and climatic conditions in which these root rot agents develop, and the large number of tree species that they attack, it was necessary to know if there is any variation in pathogenicity within populations of these fungi. In this case indeed, development of methods for combatting these fungi should take into account this heterogeneity in the aggressiveness of the parasites. To answer this question, the pathogenicity of various isolates of *R. lignosus* and *P. noxius* was tested experimentally against young artificially inoculated Hevea plants.

MATERIAL AND METHODS

The table 1 shows the characteristics of the various isolates of *R. lignosus* and *P. noxius* that were tested. Three countries of West Africa - the Ivory Coast, Liberia, and Cameroon were surveyed. Fungal strains were isolated from trees of the primary forest, from *Hevea brasiliensis* (rubber), *Tectona grandis* (teak), or *Cedrela odorata*.

Using a procedure developed in the laboratory for artificial inoculation in controlled conditions under greenhouse, young rubber plants were infected as follows : plant-growth tubs were filled with soil, whose humidity level was controlled with neutron probes. Sticks of Hevea wood, sterilized in Roux flasks and seeded with one of the fungi, constituted the inoculum. Because of the different degradative powers of these fungi, the incubation before inoculation lasted five months for *P. noxius* and eleven months for *R. lignosus*. For each one-month-old plant, five of these sticks were applied against the tap root, 20 cm deep in the soil. Each isolate was inoculated onto 30 plants in a single tub. The experiment lasted 5 months but 10 randomly chosen plants were harvested early, 2 months after inoculation.

In order to quantify the infection of each inoculated plant, the lengths of the stem and the root, the appearance of symptoms on the leaves, the date of death of the plant, the presence of rhizomorphs on the root, any reactive rhizogenesis, and the degree of colonization of the tissues (local penetrations and proportion of necrosis) were recorded. A plant health scale from 0 to 9 was established, incorporating these various criteria, so that each plant and each isolate could be given a score representing the severity of the attack (table 2).

PATHOTYPES OF *RIGIDOPORUS LIGNOSUS*

* Infectious processes appraisal (after 2 months)

From the sample at 2 months it is possible to appraise the initial stages of pathogenesis, namely rhizomorphogenesis, which conditions the contamination and initial penetrations.

The results obtained with each batch of 10 plants were collected in table 3. The strains are classified in order of decreasing phytosanitary scores (PS). For all the isolates, all the inoculated plants were contaminated, and all the tap roots, except those of certain plants with isolates 1 and 37, were penetrated by the fungal hyphae. For the first four isolates, nearly 50 % of the plants inoculated were dead or had leaf symptoms of impending death. The examination of their tap roots showed that on average they were 70 % necrosed from apex to collar. The pathogenicity of the four other isolates seems, on the other hand, to have been notably lower, especially that of strain 37, with which the mortality level was 0. Correspondingly, the reactional secondary rhizogeneses of the plants infected by this isolate were clearly perceptible. Finally this sampling clearly demonstrates that as early as two months there were real differences in pathogenicity between the strains tested. These differences were not dependent on the quality of the inocula, that is, on the initial capacity of each isolate to contaminate plants via rhizomorphs, since all the plants were contaminated a few weeks after inoculation.

Fungal attack affected plants' growth, since weekly measurements of the stem heights of the plants showed a delay in growth at two months that became more marked up to the end of the experiment (figure 1).

* Pathogenesis analysis (after 5 months)

These results collected after five months confirm the existence of differences in pathogenicity between the strains tested (table 4). For some isolates indeed, such as number 52, virtually all the plants succumbed to the fungal attack. Their tap roots were on average more than 90 % necrosed. On the other hand, the low level of plant mortality with other isolates, such as number 37, is attributable to merely partial necrosis (32 %) of the seedlings tap roots ; in this case, reactional anatomical structures had been often differentiated by the plants. Comparison of the mean phytosanitary scores of each isolate shows that six had scores of 8 or more, and so, are very pathogenic one.

The table 5 shows otherwise that with these six isolates the scatter in severity of damage among plant/parasite pairs was low. On the other hand, for isolates 9 and 37, which seemed less pathogenic, the scores were widely scattered. This heterogeneity might be due to the effectiveness of the plants' anatomical, cellular, and molecular reactions, whose existence has been demonstrated in the laboratory. Indeed, the establishment of these reactions seems to be mainly a function of the speed of progression of the fungus in the tissues. On this point, strain 37, the least aggressive, was characterized by a progression of necrosis in the root estimated at 0,8 mm per day ; this value is only half that for strain 52, the most aggressive. So, the plant might exploit this relative slowness by differentiating anatomical reactions that compensate for the destruction of part of the tap roots.

Biochemical analyses carried out in the laboratory showed otherwise that the various isolates all had qualitatively the same arsenal of extracellular enzymes. Therefore, and in view of the results shown in this table, one may hypothesize that the observed differences between isolates resulted either from variations in the levels of activity of the enzymes, or from delayed induction of the pool of enzymes, allowing so the host's reactions to get under way.

A complementary approach to the pathogenicity consists in observing the evolution of the mortality rate with time. These data (fig. 2) confirm the previous analysis, since for some strains the attack was early and severe, whereas for others it seemed to be more gradual. From month three onwards, one can distinguish a falling-off of the various curves, which remained parallel up to the end of the experiment, thus indicating some stagnation of the infection. It seems, at this time, that the reactional capacity of Hevea became really effective. Then, this balance of power determines whether the host survives.

These experiments have demonstrated that there is some variability in pathogenecity among the eight tested isolates. However principal-component statistical analysis was used to discriminate better among the various data got for each plant-isolate couple (figure 3). This kind of analysis with computer showed that the two first axes of statistical representation correspond respectively to 85 % and 12 % of the total variability. Plane 1-2 thus gives a good diagram of the structure of the population, but still

does not yield any new information. The most pathogenic isolates lie on axis 1 negative. As an indication, in this group of five strains, three were isolated from *Hevea*, one from *Tectona grandis*, one from a forest tree, in Cameroon and in various regions of the Ivory Coast...

PATHOTYPES OF *PHELLINUS NOXIUS*

A similar experiment, also comparing eight isolates, was carried out with *P. noxius*. In order to understand this analysis, it is important to bear in mind that the biology of this fungus is different from that of *R. lignosus*. *P. noxius* has no mycelial propagation structures comparable with rhizomorphs and so, contaminates the roots by means of a mycelial sleeve. The contamination is therefore a critical phase in the achievement of artificial infection.

* Infectious processes appraisal (after 2 months)

Indeed, and unlike *R. lignosus*, *P. noxius* contaminated plants very unequally after two months ; this affected the later development of the infection with regard to penetration and mortality (table 6). With all but three isolates (numbers 2, 32, and 45, which killed yet some of the inoculated plants) the infectious process was just initiated. This is corroborated by the absence of signs of anatomical reaction. At this stage the impact of the parasitism on the growth of the plants was still barely perceptible, but it increased significantly during the following months and conveyed the development of the infection into the roots tissues (figure 4).

* Pathogenesis analysis (after 5 months)

Overall 7 out of the 8 isolates tested demonstrated their parasitic ability after the 5 months of confrontation but the previously noticed differences became more marked (table 7). The variability in the parasitic behavior of each isolate can be illustrated by the following examples :

- strain 45 showed great ability both to contaminate and to colonize tap roots, right from the start of the experiment. The severity of its attack, shown by the rate of 65 % dead or dying plants, was inversely correlated with the ability of Hevea to establish secondary rhizogenesis.

- isolate 4 is similar to the preceding isolate in its abilities to contaminate and penetrate tap roots. Howevers it caused clearly lower mortality, while paradoxically the frequency of host reactions remained low. It can be assumed that there were real differences in aggressiveness between these two isolates, since in both cases the contamination rates were excellent. But, on the other hand, what conclusions can be drawn about strain 31, whose contamination rate was particularly low ?

Informations about the scatter of the results for this isolate are given in table 8. In fact it can be seen that only two of the twenty inoculated plants were contaminated. This homogeneity rules out any technical error at the time of inoculation and one may justifiably deduce that this strain has a low contamination capacity. On the other hand, the fact that the plants infected with the very aggressive isolates (45,32,35, 4 and 2) had phytosanitary scores that were scattered between 3 and 9, reveals a substantial intra-isolate variability. At first sight this could be attributable to the fact that contamination of the plants was not synchronous and the impact of the infection was thus to some extent delayed.

The overall development of infestation of the plants by each isolate between two and five months is shown in figure 5. Contrary to the results with *R. lignosus*, differences in behavior between isolates of *P. noxius* take the form of differences in slope between straight line segments. Thus, for example, isolate 35 made up for the delay at the time of inoculation, whereas strain 45 scarcely progressed between two and five months. The behavior of the other isolates was intermediate between these two extremes.

The kinetics of the mortality of the plants adds to the analysis. Two types of behavior can be distinguished in the figure 6:

- that of strains 39, 4 and 35 for which pathogenesis began slowly and remained low ;

- that of strains 45, 32 and 2, whose mortality kinetics were bimodal, with an initial ascending phase that was particularly marked for isolate 45 producing a mortality of 40 % in onemonth. This was followed by a second, plateau phase observable from the third month. To explain this phenemenon of stagnation of infection three possible hypotheses can be suggested : first, exhaustion of the fungus due to impoverishment of its trophic reserves in the sticks that carried the inoculum ; second, an abrupt arrest of the progression of the fungus in the tissues by host cellular and/or anatomical reactions ; third, delayed contamination of a fraction of the inoculated plants in the tub, whose level of infestation because of this delay scarcely reached that of the plants contaminated right from the beginning of the experiment.

Integration of all the data by principal-component analysis established a clear hierarchy of isolates (figure 7). This type of analysis made it possible to discriminate between isolates 45 and 32 even though their average phytosanitary scores were very similar. It is therefore reasonable to suppose that the modalities of infection are different for these two most aggressive isolates. Isolates 4, 35, and 2 then form one set distinct from isolates 7, 39 and 31.

Finally it is interesting that strains 31 and 32, which differ greatly in pathogenicity were nevertheless isolated from the same plantation.

CONCLUSION

First, the proposed experimental system showed differences in parasitic behavior that can serve as a basis for classifying isolates of each parasite. In fact there were considerable variations in pathogenicity within each of these populations. To complement this analysis of *in vivo* pathogenic variability, experiments are under way to quantify *in vitro* for each of these isolates the cultural characteristics, the enzymatic capacities, and the wood degradative properties.

Secondly, and fundamentally, these inter-isolate and intra-isolate variabilities in pathogenicity require that multiple isolates of any one parasite must be tested together when a control method is being developed. This is particularly true in case of screening of tree varieties or individuals for resistance.

	N° ISOLATE	COUNTRY CRIGIN	HOST	ISOLATION DATE
RIGIDOPORUS LIGNOSUS	1	South East IVORY COAST	*Hevea brasiliensis*	1978
	9	East IVORY COAST	Primary Forest	1978
	13	West IVORY COAST	Primary Forest	1978
	21	South East LIBERIA	*Hevea brasiliensis*	1979
	37	South East IVORY COAST	*Hevea brasiliensis*	1981
	38	CAMEROON	*Hevea brasiliensis*	1981
	42	South West IVORY COAST	*Hevea brasiliensis*	1981
	52	East IVORY COAST	*Tectona grandis*	1981
PHELLINUS NOXIUS	7	West IVORY COAST	Primary Forest	1978
	45	East IVORY COAST	*Cedrela odorata*	1981
	31	South West IVORY COAST	*Hevea brasiliensis*	1980
	4	South East IVORY COAST	*Hevea brasiliensis*	1978
	35	East IVORY COAST	*Hevea brasiliensis*	1981
	39	CAMEROON	*Hevea brasiliensis*	1981
	2	South West IVORY COAST	*Hevea brasiliensis*	1977
	32	South West IVORY COAST	*Cedrela odorata*	1980

TABLE 1 ORIGINS OF ISOLATES

RIGIDOPORUS LIGNOSUS		*PHELLINUS NOXIUS*	
NOTE		NOTE	
0	no mycelium on roots	0	no mycelium on roots
1	non aggregate hyphae	1	mycelium crust in formation
2	rhizomorphs	2	well - formed mycelium crust
3	rhizomorphs and ponctual penetration	3	mycelium crust and ponctual penetration
4	rhizomorphs and localized necrosis	4	mycelium crust and localized necrosis
5	rhizomorphs and partial root decay <20%	5	mycelium crust and partial root decay <20%
6	rhizomorphs and 20 to 50% root decay	6	mycelium crust and 20 to 50% root decay
7	rhizomorphs and root decay >50%	7	mycelium crust and root decay >50%
8	foliar symptoms	8	foliar symptoms
9	plant death	9	plant death

TABLE 2 PHYTOSANITARY SCALE

% CONTAMINATION : note 1 to 9

% PENETRATION : note 3 to 9

% MORTALITE : note 9

Pathogenicity variability in 8 Rigidoporus lignosus strains

Isolates	N°	PS	Contamination (%)	Penetration (%)	Foliar symptoms + Mortality (%)	Roots Necrosis ratio (%)	Secondary rhizogenesis (%)
Hevea IC South West	42	7,7	100	100	50	70	10
Forest IC South West	13	7,7	100	100	50	75	0
Teak IC East	52	7,5	100	100	40	72	10
Hevea CAM	38	7,4	100	100	40	70	10
Hevea LIB South East	21	6,2	100	100	20	33	10
Hevea IC South East	1	6,0	100	90	20	40	0
Forest IC East	9	5,7	100	100	20	31	10
Hevea IC South East	37	3,3	100	70	0	8	20

TABLE 3 INFECTIOUS PROCESSES DESCRIPTION *2 MONTHS* AFTER INOCULATION

Pathogenecity variability in 8 Rigidoporus lignosus strains

Isolates	N°	PS	Penetration (%)	Foliar symptoms + Mortality (%)	Root necrosis ratio (%)	Secondary rhizogenesis(%)
Teak IC East	52	8,9	100	95	91	0
Forest IC South West	13	8,7	100	85	86	5
Hevea IC South West	42	8,7	100	90	79	5
Hevea IC South East	1	8,4	100	75	72	10
Hevea CAM	38	8,3	100	75	85	0
Hevea LIB South East	21	8,1	100	70	61	15
Forest IC East	9	7,2	100	50	45	10
Hevea IC South East	37	5,5	90	10	32	60

TABLE 4 INFECTIOUS PROCESSES DESCRIPTION *5 MONTHS* AFTER INOCULATION

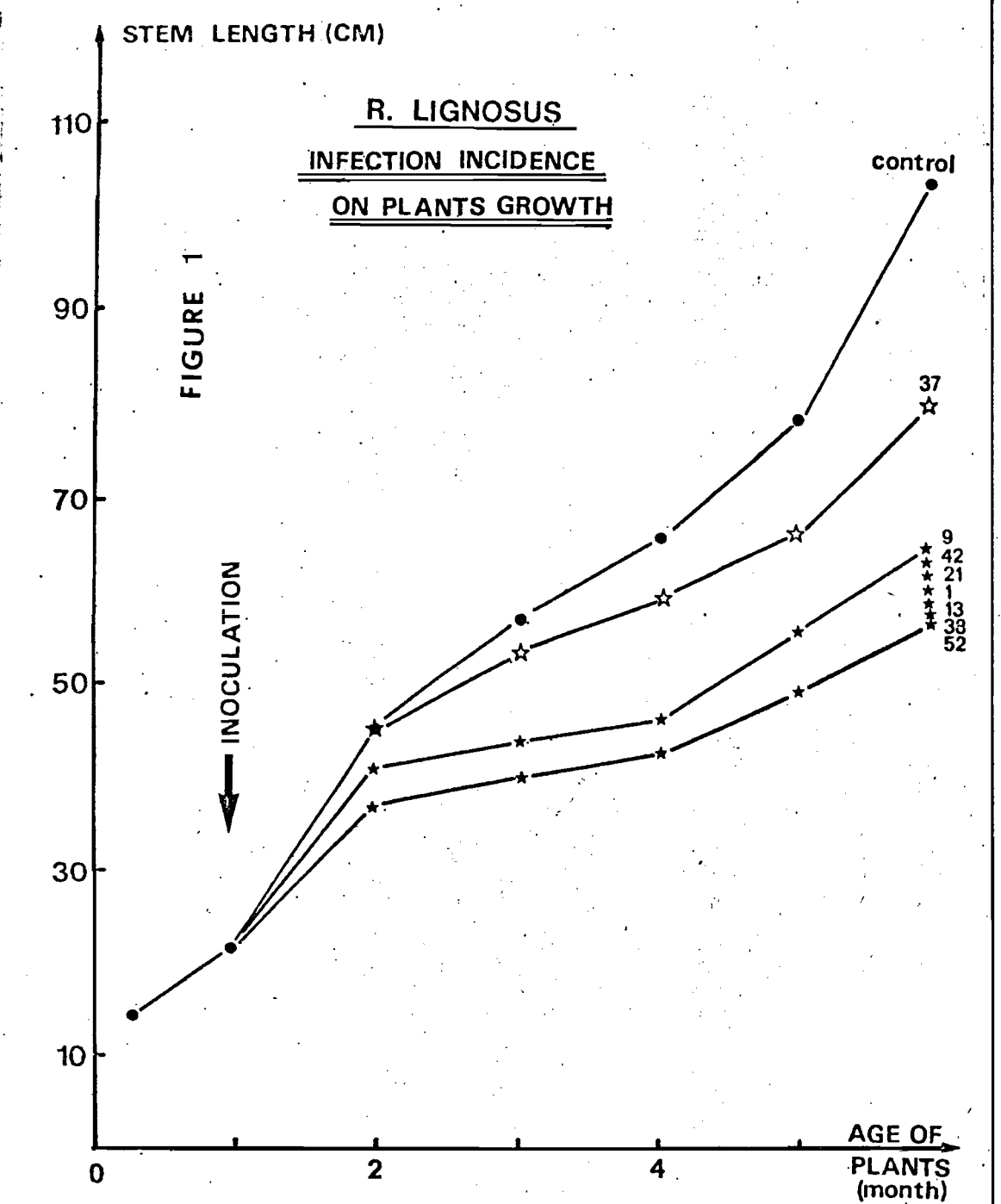

FIGURE 1

R. LIGNOSUS

INFECTION INCIDENCE ON PLANTS GROWTH

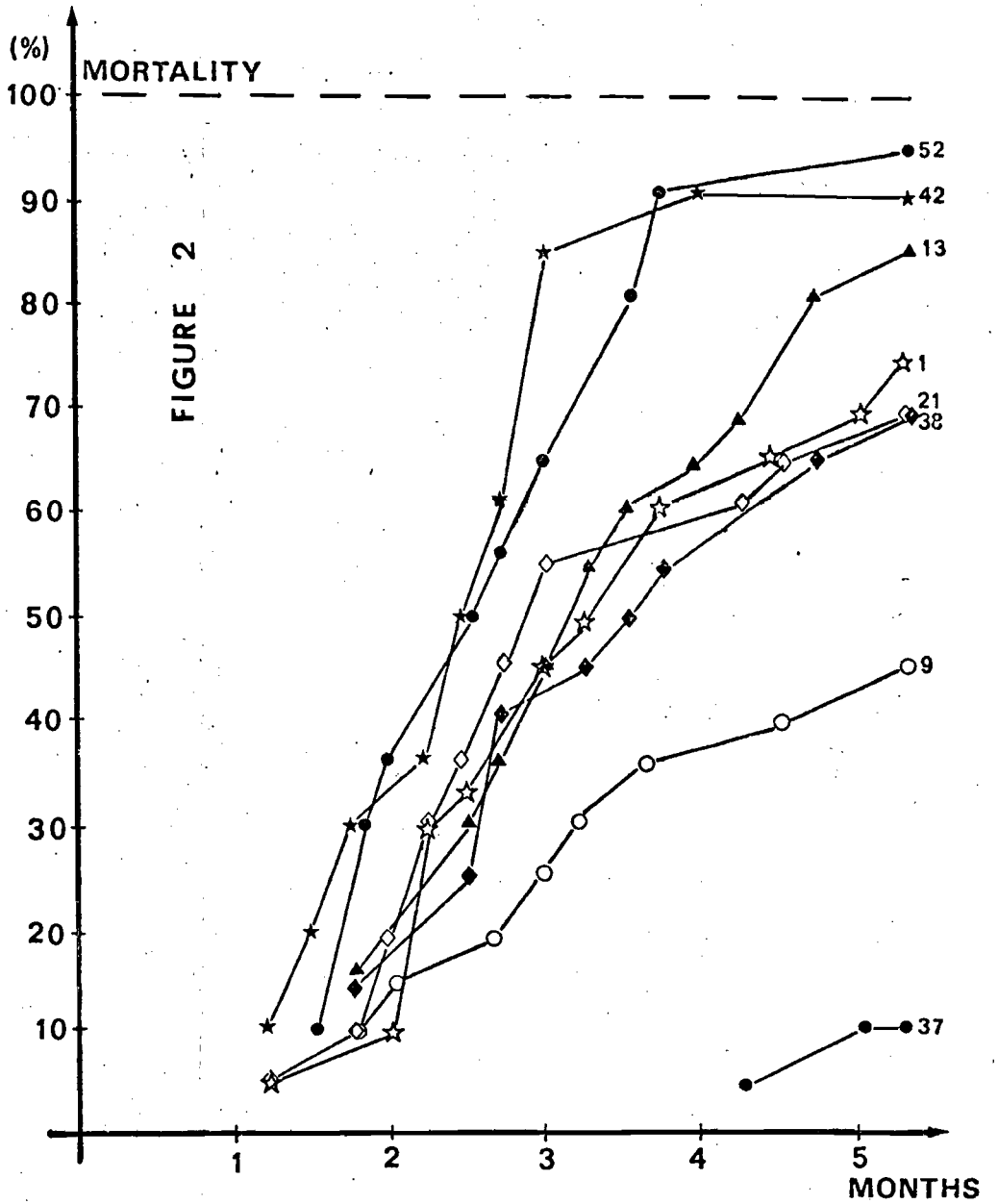

FIGURE 2

R. LIGNOSUS ISOLATES

KINETIC OF PLANTS MORTALITY

N°	Strains	0	1	2	3	4	5	6	7	8	9	PS
52	*Teak* IC East								5		95	8,9
42	*Hevea* IC South West						5		5		90	8,7
13	Forest IC South West								15		85	8,7
38	*Hevea* CAM						5	5	15	5	70	8,3
21	*Hevea* LIB South East						10	10	10		70	8,1
1	*Hevea* IC South East							10	15		75	8,4
9	Forest IC East			5		10		25	10	5	45	7,2
37	*Hevea* IC South East		15			5	20	35	15		10	5,5

TABLE 5 PHYTOSANITARY CLASSIFICATION FOR EACH HOST ISOLATE COMBINATION

Results in % of infected rubber trees for each phytosanitary class (0 to 9)

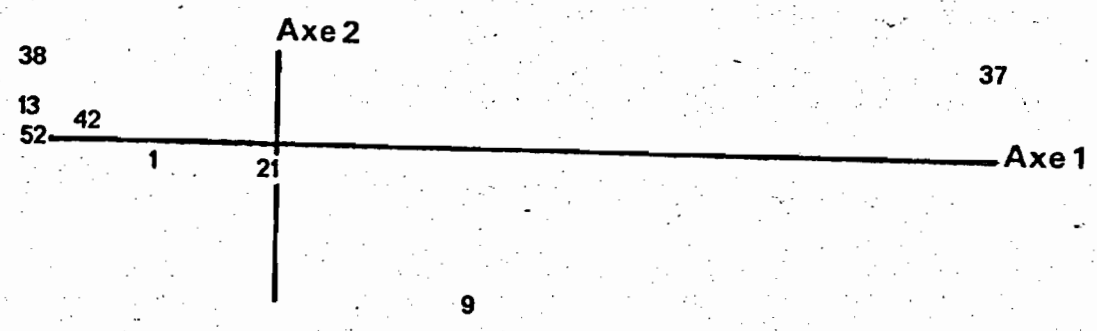

FIGURE 3 POSITION OF EACH ISOLATE ON THE PLAN 1 – 2 OF THE PRINCIPAL COMPONENT ANALYSIS , FIVE MONTHS AFTER INOCULATIONS WITH RIGIDOPORUS LIGNOSUS.

Pathogenicity variability in 8 Phellinus noxius strains

Isolates	N°	PS	Contamination (%)	Penetration (%)	Foliar symptoms + Mortality (%)	Necrosis (%)
Cedrela IC East	45	6,3	100	100	50	40
Cedrela IC South West	32	3,6	80	60	20	21
Hevea IC South West	2	3,4	50	50	20	28
Hevea IC South East	4	3,0	70	70	0	0
Hevea IC East	35	1,2	30	0	0	0
Forest IC South West	7	0,8	30	10	0	3
Hevea CAM	39	0,3	10	10	0	0
Hevea IC South West	31	0,2	20	0	0	0

TABLE 6 INFECTIOUS PROCESSES DESCRIPTION *2 MONTHS* AFTER INOCULATION

Pathogenicity variability in 8 Phellinus noxius strains

Isolates	N°	PS	Contamination (%)	Penetration (%)	Foliar symptoms + Mortality (%)	Necrosis (%)	Secondary rhizogenesis (%)
Cedrela IC East	45	6,5	90	80	65	53	5
Cedrela IC South West	32	6,2	95	95	40	24	25
Hevea IC East	35	5,1	85	80	30	30	30
Hevea IC South East	4	4,8	85	80	15	22	5
Hevea IC South West	2	4,5	65	65	25	16	25
Forest IC South West	7	2,6	60	60	5	7	5
Hevea CAM	39	1,5	35	30	5	5	10
Hevea IC South West	31	0,4	10	10	0	0	0

TABLE 7 INFECTIOUS PROCESSES DESCRIPTION *5 MONTHS* AFTER INOCULATION

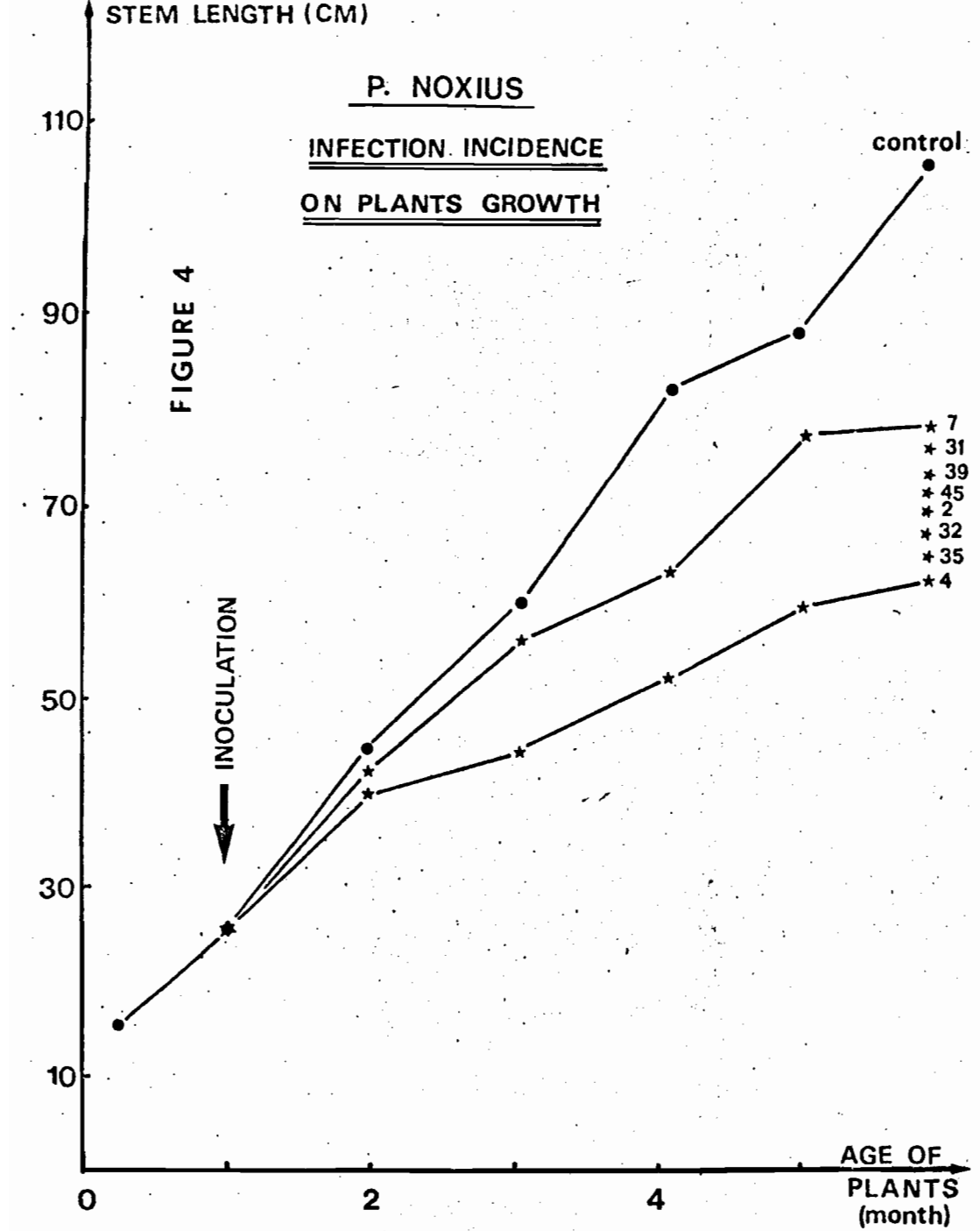

FIGURE 4

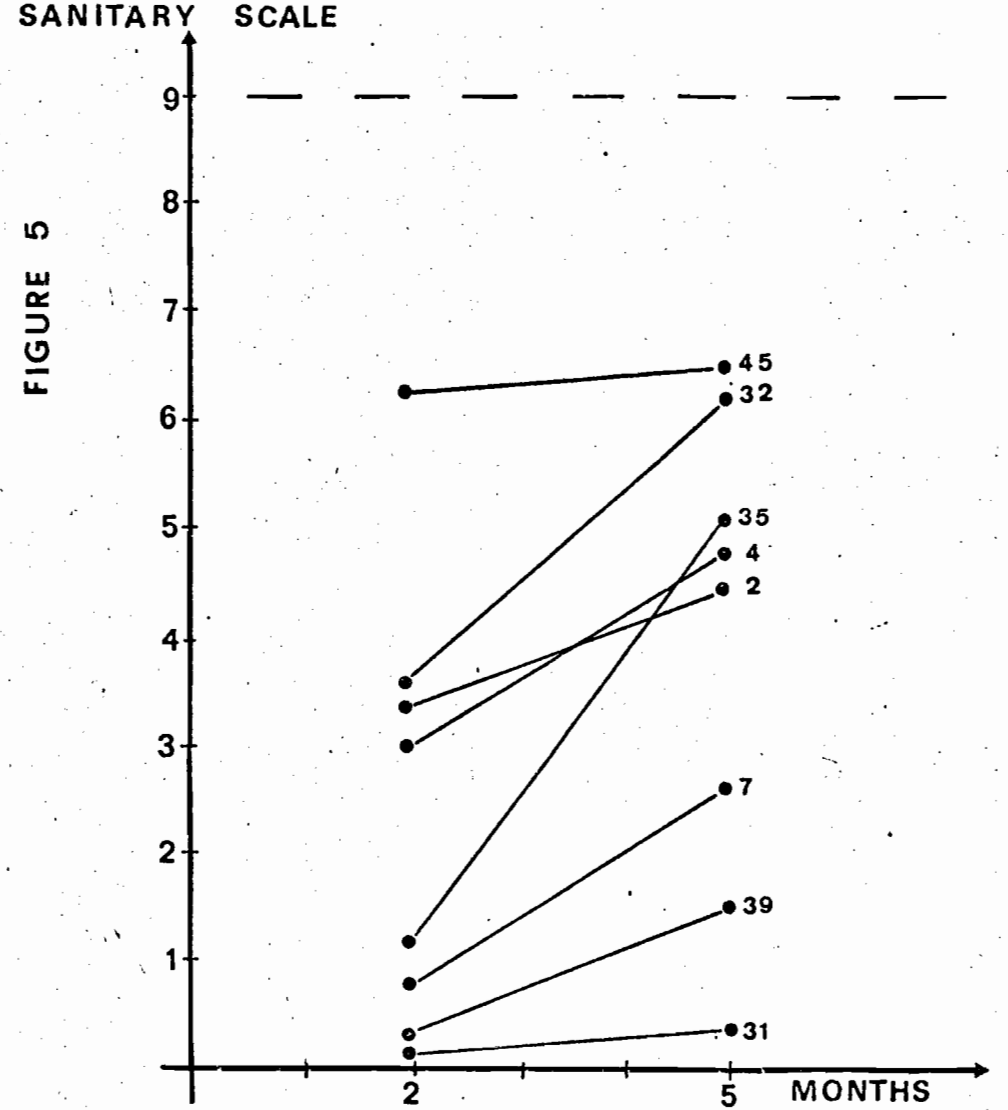

FIGURE 5

INFESTATION LEVELS COMPARISON

between P. NOXIUS Isolates

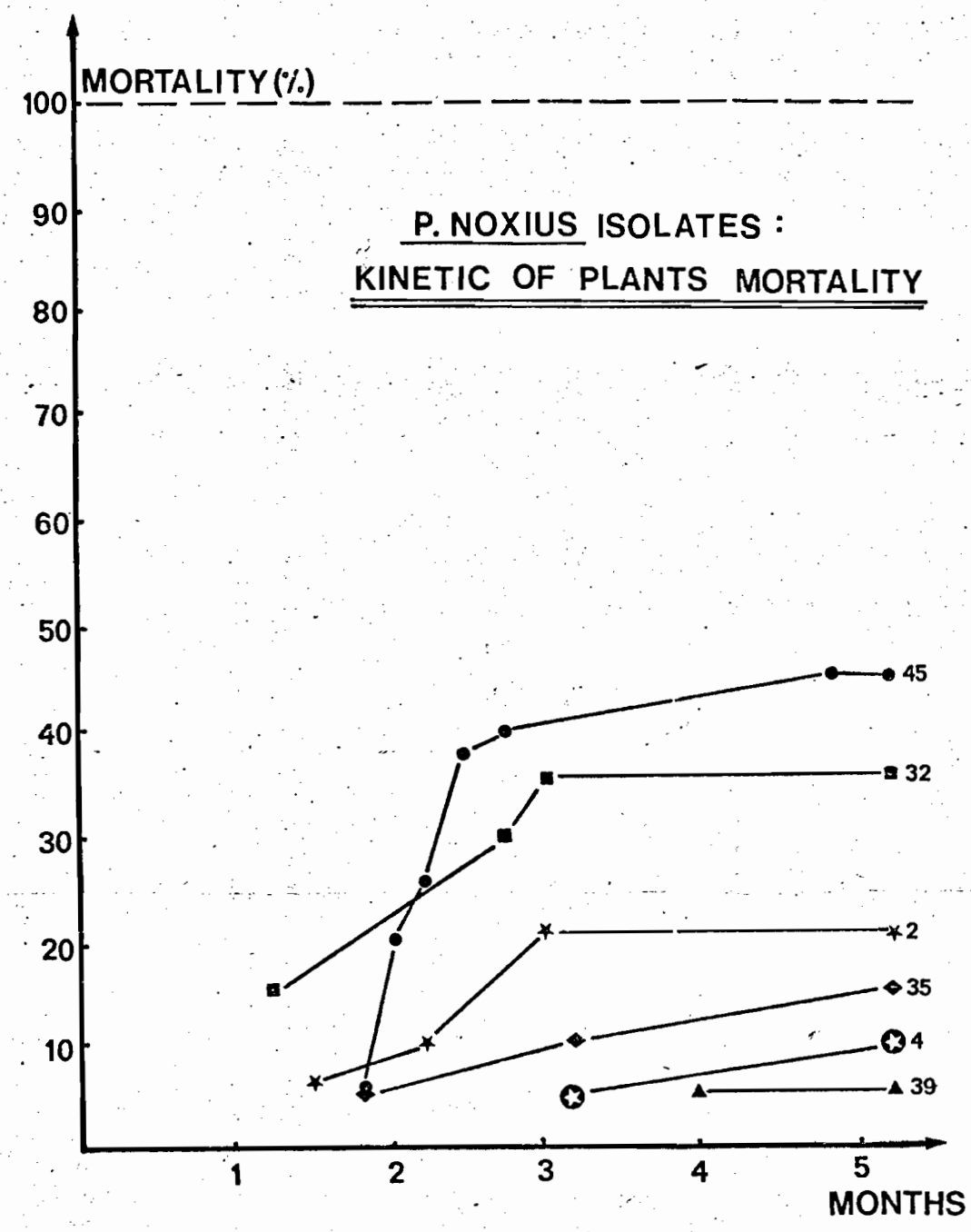
MORTALITY (%)
100
90
80
70
60
50
40
30
20
10
P. NOXIUS ISOLATES :
KINETIC OF PLANTS MORTALITY
45
32
2
35
4
39
1
2
3
4
5
MONTHS

FIGURE 6

Strains \ Phytosanitary scale		0	1	2	3	4	5	6	7	8	9
45	*Cedrela* IC East	10				5	10			20	45
32	*Cedrela* IC South West					20	30		5	5	35
35	*Hevea* IC East	15	5		5	10	20	15		15	15
4	*Hevea* IC South East	15	5			10	40	5	10	5	10
2	*Hevea* IC South West	35				5	10	10	15	5	20
7	Forest IC South West	40			10	35	10			5	
39	*Hevea* CAM	65	5			20	5				5
31	*Hevea* IC South West	90			5	5					

TABLE 8 PHYTOSANITARY CLASSIFICATION FOR EACH HOST ISOLATE COMBINATION

Results in % of infected rubber trees for each phytosanitary class (0 to 9)

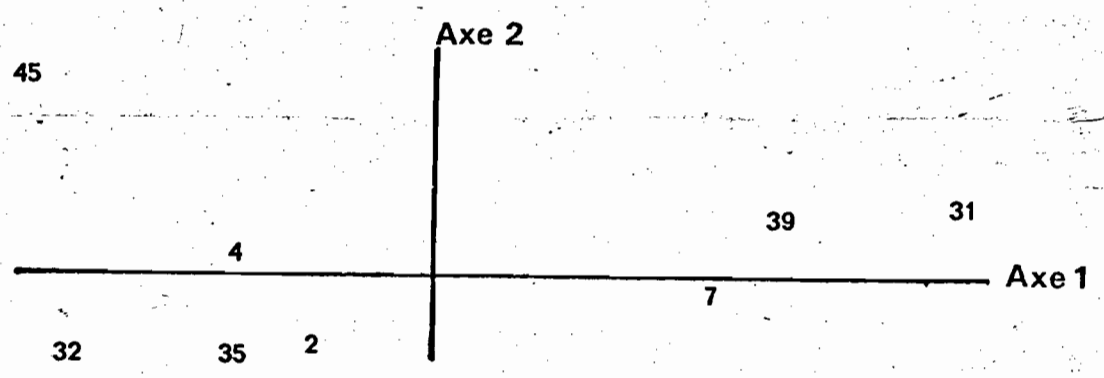

FIGURE 7 POSITION OF EACH ISOLATE ON THE PLAN 1 - 2 OF THE PRINCIPAL COMPONENT ANALYSIS, FIVE MONTHS AFTER INOCULATION WITH PHELLINUS NOXIUS.