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STUDIES ON THE VARIABILITY AND PATHOGENICITY OF *RIGIDOPORUS LIGNOSUS*

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SUMMARY

White Root Disease in Hevea, *caused by the fungus* Rigidoporus lignosus (Fomes lignosus) *occurs widely in Sri Lanka. In recent years, the severity of the disease has increased alarmingly. One of the factors that could be attributed to the increased incidence of the disease is the virulence of the pathogen. This paper reports the results of laboratory experiments on the effects of pH, temperature, light and dark, and relative humidity on a number of isolates, obtained from sites located in different rubber growing areas.*

The fungus is able to grow over a wide range of temperature with maximum growth being recorded at 30° C. All the isolates grew better when kept in continuous darkness except one isolate which grew equally well under both light and dark conditions. There was wide variability in the pH requirements for the growth of the fungus.

The virulence of the different isolates was examined using healthy rubber roots under laboratory conditions and rubber seedlings in pot culture. The results show that there are differences in virulence and pathogenicity.

INTRODUCTION

White Root disease caused by the fungus *Rigidoporus lignosus* (*Fomes lignosus*) is by far the most destructive root disease of *Hevea* and causes death of plants in immature and mature clearings. The disease is widespread in all rubber growing areas. In the past, Fox (1961), Peries *et al.* (1963) ; (1965) reported effective and economical methods of controlling the disease. This effected a considerable check on the spread of the disease. However, in recent years the severity of the disease has increased alarmingly, in certain localities, and rendered many mature clearings uneconomic. The increased incidence of the disease can be attributed to the variability of the pathogen as reported for Armillaria root rot disease in tea and forest plantation in Nayasaland by Gibbson & Corbett (1964). Laboratory and pot experiments were therefore initiated to compare the pathogenic relationships of several isolates of the fungus.

EXPERIMENTAL

Eleven isolates of *R. lignosus* were obtained from diseased material collected from seven rubber growing districts, listed in Table 1. The stock cultures were maintained on Difco Malt Agar (DMA) slopes under sterile paraffin oil and subcultured when required. The following general method was employed to study the effect of pH, temperature, light and dark and relative humidity on the growth of the fungus. Discs, 6 mm diameter, were taken from the pheriphery of a seven day old culture of *Rigidoporus*, grown in 9 cm diameter petri dishes, each containing 20 ml of 2% DMA. The inoculum discs were placed in the centre of 9 cm diameter petri dishes containing 20 ml DMA. The plates were incubated at room temperature (28°C $\pm$ 1°) for all studies except temperature, where dishes were incubated under regulated temperatures. Four replicate dishes were inoculated in each treatment and the radial growth of the fungus along two diameters at right angles to each other, was measured daily. Analysis of variance on the growth of the fungus after 3 days was carried out to compare the behaviour of the isolates.

TABLE 1 : ORIGIN OF *Rigidoporus lignosus* ISOLATES

Isolate No.	Source	District	Estate collected
R. lignosus			
A	Rubber	Kalutara	Dartonfield Group, Agalawatta
B	Rubber	Kalutara	Dartonfield Group, Agalawatta
C	Rubber	Matara	Urumutta Estate, Akuressa
D	Rubber	Galle	Stokesland Group, Udugama
E	Rubber	Galle	Bentota Group, Elpitiya
F	Rubber	Ratnapura	Peenkanda Group, Uda Karawita
G	Rubber	Kelany Valley	Mahaoya Group, Dehiowita
H	Rubber	Kelany Valley	Woodend Estate, Dehiowita
J	Rubber	Kurunegala	Muwankanda Estate, Mawathagama
K	Rubber	Matale	Wariyapola Group, Matale
L	Cacao	Matale	Wariyapola Group, Matale.

Effect of pH

Growth was tested at pH values ranging from 3 to 11. The medium was brought to the required pH after autoclaving with 0·1N hydrochloric acid or 1N sodium hydroxide. The lowest value tested, pH 3, was found to be lethal for growth of all isolates and when the inoculum plugs were transferred onto fresh malt agar no growth was recorded. The response to pH between the isolates was significant and their general response is shown in Fig. 1.

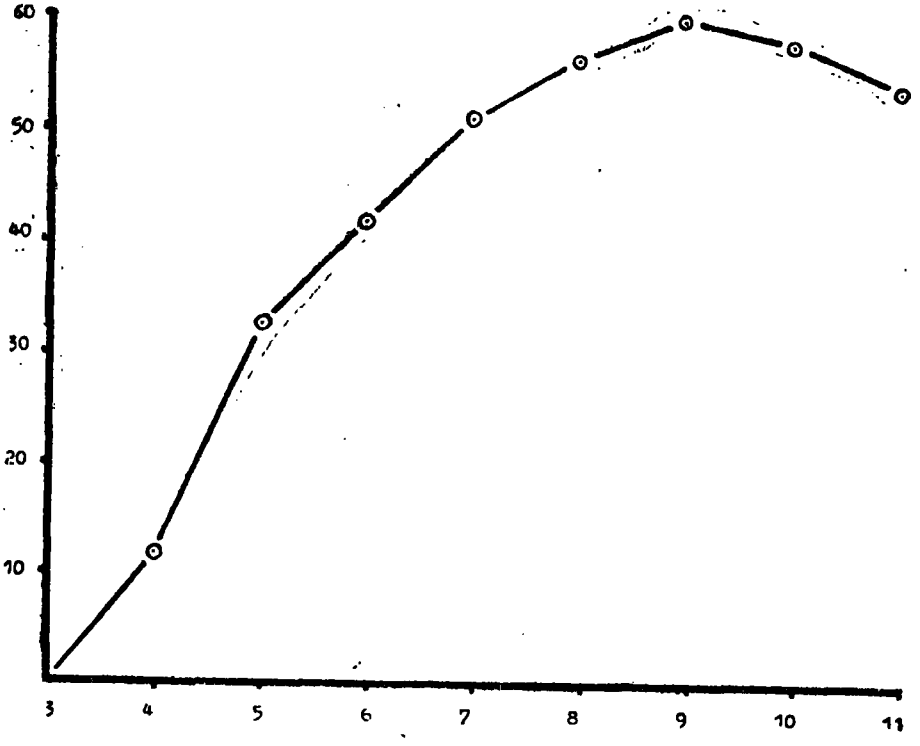

Fig. 1. Mean radial growth of the fungus on 2% malt agar at various pH levels, 3 days after inoculation.

The best growth was found at pH 9. Both the linear and quadratic effects of pH on fungal growth were significant. This indicated an increase in growth with an increase in pH. However, at higher pH values a diminishing pattern of growth

was noted. The linear into linear component of interaction between isolates and pH was significant. This showed that growth of various isolates at low pH is different but the tendency for the growth to fall away with increase in pH is alike in all isolates. Thus, separate regression equations to each isolate were fitted and grouped on the basis of the linear regression coefficient (Table 2). The regression coefficients indicate the pattern of tolerance to low pH by the isolates.

TABLE 2 : THE PATTERN OF TOLERANCE TO pH BY ELEVEN ISOLATES OF *Rigidoporus*, BASED ON LINEAR REGRESSION COEFFICIENT

	Isolates										
	H	K	G	E	C	J	B	L	D	A	F
Linear regression coefficient	49·1	30·8	30·7	28·6	27·9	27·8	26·3	26·0	24·5	23·1	22·7

Isolate H withstood low pH levels better than all the other isolates, whereas isolate F was the most sensitive to low pH levels. Rhizomorph production was evident on all isolates at low and high pH levels except isolate H where it produced no rhizomorphs.

Effect of temperature

The growth of all the isolates was tested at 15°, 20°, 25°, 30°, 35° and 40°C. It was found that all isolates grew poorly at 15°C and the fungus was killed when the temperature was 40°C. The growth of isolates in response to temperature is shown in Fig. 2.

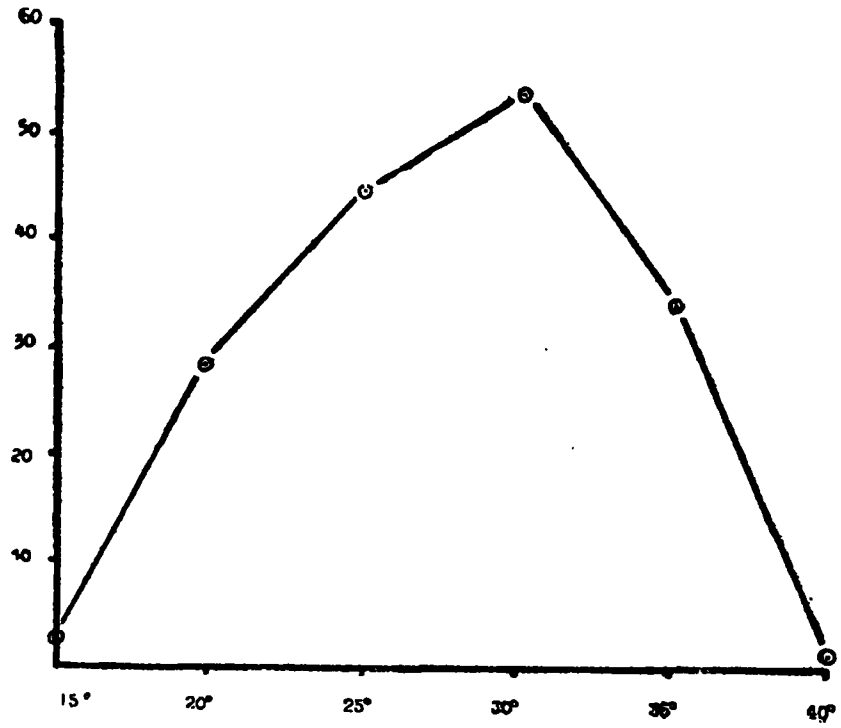

Fig. 2. Mean radial growth of the fungus on 2% malt agar at different temperatures, 3 days after inoculation.

TABLE 3 : CALCULATED TEMPERATURE OPTIMUM AND LINEAR GROWTH (MM) OF *Rigidoporus lignosus* ISOLATES, 3 DAYS AFTER INOCULATION

Isolate	Optimum temperature C°	linear growth (mm)
A	27·4	41·5
B	27·5	48·4
C	27·9	51·3
D	27·7	44·5
E	27·5	51·2
F	27·8	37·8
G	27·6	52·2
H	27·2	76·5
J	27·6	48·0
K	27·8	52·6
L	27·9	50·2

regression equation $y = a + bx - cx^2$

The interaction of the isolates at lower temperature, was not significant whereas at higher temperatures it was found to be significant. This indicated that isolates are sensitive to higher temperatures. The growth rate at optimum temperatures for all isolates were calculated (Table 3). Although temperature optima of isolates were found to be identical, growth rates at the optimum temperature differed between isolates. The highest growth rate was recorded for isolate H and the lowest for isolate F. Production of rhizomorphs was evident in all isolates at 30° and 35°C. except isolate H where rhizomorphs were not produced.

Effect of light and dark

Culture dishes, kept on a laboratory bench, were exposed to direct florescent light (110 cm below 300 lux). The effect of darkness was assessed by wrapping the dishes in black polythene. The isolates differed significantly in their response to dark and light, and isolates grew significantly better when kept continuously in the dark than when exposed to continuous light, except isolate H which grew well under both conditions (Table 4). This observation is confirmed by the significant negative correlation coefficient ($r = -0 \cdot 653$).

Effect of relative humidity (RH)

The effect of 100%, 75% and 50% RH was tested. The growth was not affected by differences in humidities.

TABLE 4 : EFEECT OF EXPOSURE TO CONTINUOUS LIGHT AND DARK CONDITIONS ON THE GROWTH OF ELEVEN ISOLATES OF *R. lignosus*, 3 DAYS AFTER INOCULATION.

Isolate	Mean colony diameter (mm)		Difference	Significance
	dark	light		
H	68·7	66·4	2·3	NS
J	34·2	29·8	4·4	*
E	39·2	32·7	6·5	*
D	38·6	34·1	4·5	*
C	38·4	30·9	7·5	*
A	37·8	28·7	9·1	*
B	36·4	26·4	10·0	*
L	32·6	21·6	11·0	*
K	32·2	20·6	11·6	*
G	40·4	28·4	12·0	*
F	29·2	15·8	13·4	*
Mean	38·8	30·4		

LSD = 0·041 at 5% level
NS = not significant

Pathogenicity of the fungus on Hevea *roots.*

The ability of the fungus to grow in healthy autoclaved rubber wood was tested, to assess the difference that exist between the isolates. Rubber roots, about 1 cm diameter, were carefully excised from a 15 yr old PB 86 clearing, avoiding damage to the roots. The roots were washed free of soil, cut into 6 cm long pieces and autoclaved in steam at 121°C for 15 minutes. 'Fomac 2' which contained 20% penta chloro nitrobenzene was aseptically applied on the outer surface of root pieces leaving a 5 mm area free of 'Fomac 2'. This treatment prevented the external growth of the fungus but permitted it to grow internally in the wood tissues (Fox, 1966) Fig. 3. The untreated end of the wood piece was then implanted into a 7 day old culture of *R. lignosus* grown on 20 ml of 2% DMA in boiling tubes, and incubated at room temperature. Each replicate comprised roots from a single tree and each treatment was replicated ten times. On the 3rd day after inoculation, root lengths were removed, split longitudinally and incubated overnight under moist conditions. The relative position of the fungus in the root tissues was recognised by the appearance of white hyphae (Fig. 4). An analysis of variance showed the isolates to be significantly different from each other and were compared in the multiple range test, at the 5% confidence level. (Table 5).

TABLE 5 : MEAN LINEAR GROWTH (MM) OF ELEVEN ISOLATES OF *R. lignosus* ON HEALTHY AUTOCLAVED *Hevea* ROOTS OF CLONE PB 86, 3 DAYS AFTER INOCULATION

Isolate										
H	A	E	G	B	C	J	D	K	L	F
51·5	21·8	20·0	19·2	15·2	15·0	13·9	13·5	12·6	12·2	4·9

Any two means not underscored by the same line are significantly different, any two means underscored by the same line are not significantly different, at 5% level of confidence by the multiple range test.

Isolate H had a greater ability to colonise wood than the others, Isolate F was slow growing and had the least ability to colonise wood. Furthermore, the growth rate in autoclaved wood pieces compared favourably with that on 2% DMA.

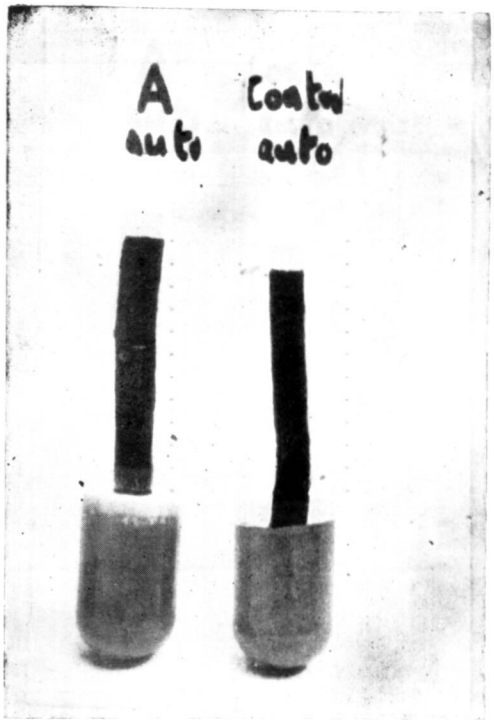

Fig. 3. Treated wood pieces show prevented external growth of the fungus.

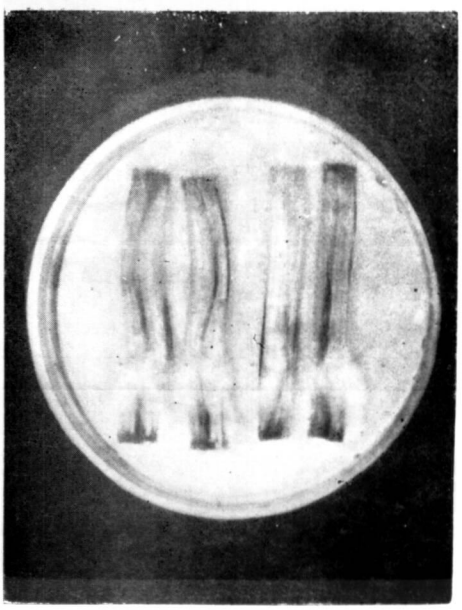

Fig. 4. Root Tissues showing the white hypae of *R. Lignosus*.

The same experiment was repeated using fresh healthy roots without autoclaving. However, the root pieces were surface sterilized using Cholorox solution, containing 2% sodium hypochlorite. Unlike in autoclaved roots, the linear growth of the fungus was recognised as a white to light gray rot. Incipient wound barriers were also recognised at the edges of lesions. This suggests that the progress of the fungus had been arrested by means of inherent resistant factors. The differences in growth between isolates, 7 and 14 days after inoculation are significant and the isolates were compared in the multiple range test. (Table 6).

TABLE 6 : MEAN LINEAR GROWTH (MM) OF ELEVEN ISOLATES OF *R. lignosus* ON HEALTHY *Hevea* ROOTS OF CLONE PB 86, 7 AND 14 DAYS AFTER INOCULATION

7 days after inoculation

H	B	A	C	E	G	D	K	L	J	F
31·8	8·3	7·6	7·5	7·4	7·2	6·3	6·2	6·1	5·2	3·2

14 days after inoculation

H	C	E	K	G	J	L	D	B	A	F
55·3	24·5	23·8	23·4	21·1	20·9	20·2	20·0	18·9	18·7	8·4

Any two means not underscored by the same line are significantly different, any two means undescored by the same line are not significantly different at 5% level of confidence by the multiple range test.

The order of the virulence of the isolates in colonising healthy root pieces was essentially the same for 7 and 14 days. All isolates were pathogenic, however isolate H was significantly more virulent while isolate F was significantly less virulent than all other isolates.

Pathogenicity of the fungus on rubber seedlings

The pathogenicity of the isolates was also examined using the rubber seedlings technique developed by Liyanage (unpublished). Seedlings of clones PB 86, Tjir 1, and RRIC 52 were used. Fifteen germinated seeds of each variety were placed in a cement pot (30 cm diameter) where 200 × 1 cm^3 artificially infected rubber stem wood pieces were buried 8 cm below the level of the soil. Each treatment was replicated three times. The mortality rate of seedlings due to infection by the fungus was observed for a period of 4 months and the results are summarised in Table 7.

TABLE 7 : PERCENTAGE SEEDLINGS DEAD DUE TO INFECTION, 4 MONTHS AFTER INOCULATION WITH ELEVEN ISOLATE OF *R. lignosus*

	Isolate										
Variety	A	B	C	D	E	F	G	H	J	K	L
PB 86	0	44	0	2·2	2·2	6·6	6·6	26·4	0	0	0
Tjir 1	0	0	0	6·6	2·2	0	4·4	15·4	2·2	0	2·2
RRIC 52	0	0	0	0	0	0	0	2·2	0	0	0

45 seedlings in each treatment.

Isolate H caused more deaths, of seedlings than any other isolate on all 3 clones tested, PB 86 being the most susceptible clone tested. Seedlings of clone RRIC 52 showed a high degree of resistance to all isolates, except H. The pattern of infection confirmed that isolate H was the most virulant one tested.

DISCUSSION

Robert *et al.* (1962), Gibbson *et al.* (1914) and Rabbe (1966; 1967) have recorded the variability in pathogenicity and morphology of the root parasites *Fomes annosus* and *Armillaria mella* occurring in forests. The present investigation is the first record of the presence of variations in the pathogenicity, growth behaviour and morphology of the isolate of *R. lignosus*. Inoculation tests carried out on living rubber roots and seedlings showed that there were differences in the pathogenicity of the isolates. When non-autoclaved healthy root pieces were used the spread of the pathogen slowed down, clearly demonstrating the inherent resistance of living tissue to infection, as noted by Fox (1966). Further, this experiment showed the rate of spread of established infections within living roots. The growth of the fungus on autoclaved healthy rubber root pieces was comparable to that on malt agar. Inoculation tests on rubber seedlings revealed differences in pathogenicity among *Rigidoporus* isolates.

The growth rate of the various isolates at low pH levels differed from one another and significantly slower than at higher pH levels. Fungal growth was not recorded when the pH dropped below 3. Peries (1965) advocated the amendment of soils in planting holes with sulphur to reduce the incidence of the disease in the early years after replanting and reported that sulphur increased the acidity of the soil. Satchuthananthavale (1970) recorded that *R. lignosus* did not grow in soils amended with sulphur. These results confirm that highly acidic conditions are unfavourable for the growth of *R. lignosus*. Sulphur helps to increase the acidity, and this may partly account for the apparent reduction in the incidence of the disease in the replantings, where sulphur is used. Highly alkaline conditions too are inimical to the fungus. Therefore, liming the soil to increase the alkalinity would be another method of controlling this disease. Petch (1921) recommended the application of lime to soils in infected areas. However, large amounts of lime would be required to increase the alkalinity to a level that would be antagonistic to the fungus and hence the use of such a method would be uneconomical. Further, the optimum soil pH for the growth of *Hevea* is 4 – 5, so that highly alkaline soil conditions would be detremental to the growth of the crop.

The distribution of the fungus appeared to be determined by its temperature requirements. The disease has not been reported in Moneragala, which is a dry rubber growing district. This study showed that at extreme temperatures either the fungus grew slowly or was killed. The ability of the fungus to survive for long periods and cause greater damage in wet areas could be mainly due to a moisture effect, but a concomitant reduction in the soil temperatures may also have helped the fungus to be active. Thus the low incidence of White Root disease in the dry district, Moneragala, can be attributed to the low moisture status and high soil temperature conditions.

These studies have shown that the fungus grows best in continuous darkness, so that it is highly adapted to a subterranean habitat. Therefore, greater damage could be expected to roots that are deeply submerged than those exposed above the surface of soil, due to soil erosion.

When plants affected with White Root disease are treated it is customary in some estates to leave the root system exposed for a few days before refilling with fresh soil ; as it is believed that this helps to kill the fungus. The combined effect

of direct exposure to light and high temperatures would be responsible for this effect. The light and high temperature can dessicate the fungus in infected wood. However, it must be emphasized that such treatment is only complementary and not a substitute for complete eradication of the source of infection.

There were distinct differences in the morphology of the different isolates. These variations could be largely dependent on the conditions under which they were grown. As reported by Fox (1970) there was a tendency for most isolates to produce rhizomorphs when grown under sub-optimal conditions such as high temperature and high and low pH. The most significant feature was that the most virulent isolate did not form any rhizomorphs even under adverse conditions. This suggests that the rhizomorphs are formed only when the individual hyphae are incapable of penetrating the host tissue. Garret (1951) also suggested such a mechanism in the development of rhizomorphs amongst root inhabiting fungi. Therefore, rhizomorph formation can be viewed as a mechanism to overcome unfavourable conditions. The aggregation of hyphae would allow the fungus to penetrate the host and establish an infection.

The most virulent isolate detected in this study was collected from an area which was devastated by the disease, so much so that this area was uprooted for replanting six years before the scheduled date. The isolate grew equally well under continuous light or darkness, tolerated both low and high pH levels and withstood extremes of temperature. This suggests that it is highly adapted to withstand changes in environmental conditions. Furthermore, it was observed that it could grow over the root pieces which were painted with ' Fomac 2 ' indicating that this fungicide is not effective against this strain (Fig. 5). The possibility that such strains occur in other areas cannot be excluded. Such strains are likely to cause greater

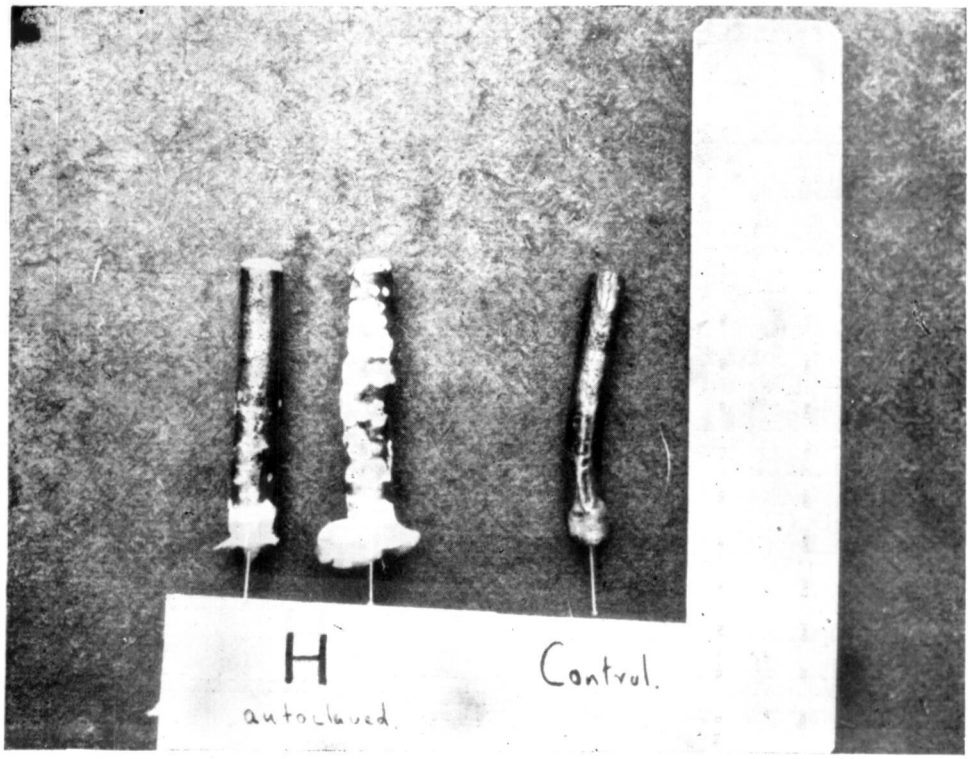

Fig. 5. Root pieces showing fungal growth,

damage if the disease is not detected early and proper remedial measures taken. It is therefore strongly recommended that regular foliar inspections be carried out and the spread of the disease stopped as advocated by the Institute.

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