Effect of Inoculum Size and Age of Trees on Root Disease Infection of Hevea Brasiliensis

K. P. JOHN

An experiment on the effect of size of inoculum of two major root parasites of Hevea *rubber—* Fomes lignosus *and* Ganoderma pseudoferreum*—on the age of trees is described. The ability of either parasite to infect and kill a tree is directly related to the size of the inoculum. With* F. lignosus, *this is also inversely related to the age of the trees; with* G. pseudoferreum, *however, the age difference is not apparent.*

Wide variations in the infectivity or rate of decay of inocula of different sizes have been found in inoculation experiments conducted in recent years (JOHN, 1958 and 1960). Hence, it was felt that an enquiry should also be made into the minimum size of a piece of infected wood necessary to induce a self-propagating infection. Experience has suggested that there might also be a relationship between the age of a tree and the size of an inoculum which can cause a successful infection.

DE JONG (1933) found that *Fomes lignosus* Klotzsch Bres. could infect young seedlings of *Hevea brasiliensis* Muell. Arg. with natural or artificial inocula 3 and 1.8 in^3 in size respectively. In early inoculation experiments with *F. lignosus* using natural inocula on rubber seedlings less than one year old, (RUBBER RESEARCH INSTITUTE OF MALAYA, 1933), the relationship between inoculum size and percentage of successful infections was found to be as follows:

Inoculum Size (in^3)	<0.25	0.25–0.50	0.5–1.0	1.0–5.0	>5.0
Successful infections(%)	0	12	22	69	100

However, the peak of root disease infection occurred in older trees—two to three years after planting in the case of *F. lignosus* and one or two years later for *G. pseudoferreum* (Wakef.) Over. & Steinm. Even after the peak incidence, root disease continues to occur in mature plantations up to any age. An experiment was therefore conducted to relate the size of inoculum and the age of tree, for an infection to be established by *F. lignosus* or *G. pseudoferreum,* the two principal root parasites of *Hevea* in Malaya.

MATERIALS AND METHODS

From plantings on the R.R.I.M. Experiment Station, ten budded trees aged approximately 3, 6, 13 and 23 years were inoculated, the 3 year-old trees on the tap root and the older ones on a lateral root. The inocula were sections of naturally infected root, of volume approximating to 5, 15, 45 or 135 cubic inches. The inocula were standardised by selecting those which were well penetrated yet still hard.

Trees in the four age groups were located in different parts of the Experiment Station, and on different soils. The 3-year-old trees were growing on flat ground, in a sandy loam area; the 6- and 13-year-old trees were also on flat and low lying ground but with an alluvial peaty sand; the 23-year-old trees were growing in colluvial sandy loam on the lower slope of a hill.

A preliminary examination was made one year after inoculation to record the initiation of rhizomorphs from the inocula. Thereafter the soil was not distributed for 2½ years, when the roots were exposed to score the degree of infection (0=no infection; 1=superficial rhizomorphs only; 2=penetration confined to inoculated roots; 3=collar penetration confined to bark but later healed; 4=collar penetration

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into wood; 5=dead or dying). During the 3½ years, however, trees which were dead or could be seen to be dying were counted at quarterly intervals.

RESULTS

The scored degrees of infection of all the experimental trees after 3½ years are presented in *Table 1* for *F. lignosus* and in *Table 2* for *G. pseudoferreum*. With both parasites, the extent of infection was directly related to the size of the inoculum; with *F. lignosus* the infection was inversely related to the age of the tree, whereas with *G. pseudoferreum* no age difference was apparent except that the 3-year-old trees succumbed to the largest inocula.

Tables 3 and *4* show the number of dead or dying trees at various intervals after inoculation. Though the largest inocula of both parasites killed an equal number of the youngest trees, the smaller inocula of *F. lignosus* killed more young trees than *G. pseudoferreum* inocula of similar size; there is also an indication that the latter may be a more effective parasite of old trees.

DISCUSSION AND CONCLUSION

Under most conditions, far more young trees are lost from *F. lignosus* than from *G. pseudoferreum* because rhizomorphs of the former grow much faster. However, both parasites killed 3-year-old trees in 6 months and 6-year-old trees in 12 months in the present experiment. The 13- and 23-year-old trees took a longer, and more variable, time to die.

The ability of either parasite to infect and kill a tree depends on the size of the inoculum and, in the case of *F. lignosus*, on the age of the tree. With *G. pseudoferreum* the age factor is not so apparent though the largest inocula killed as many of the youngest trees as *F. lignosus* did. Rhizomorphs were produced by all the 5 in^3 inocula of *F. lignosus* and by about half the *G. pseudoferreum* inocula of the same size, but no tree was killed because the inoculum potential was sufficient to sustain a self-propagating infection before the food base was lost.

GARRETT (1956) has defined inoculum potential as 'the energy of the growth of a fungal parasite available for infection of a host at the surface of the host organ to be infected'. He further stated, 'For every host parasite combination, under any given set of environmental conditions, there must be a limiting value for inoculum potential of parasite, below which establishment of a progressive infection will fail to occur. The energy and other nutrients necessary for supplying this inoculum potential are provided by the food base, which consists of mycelium in dead infected host tissue, or of resting propagule of the fungus (e.g. a sclerotium). These nutrients are conveyed from the food base through the mycelium of the fungus to the spices of growth, which form the spearhead of fungal invasions.'

The importance of food base for root infection was also appreciated by PETCH (1921, 1928) and GADD (1936). BANCROFT (1912) found that *F. lignosus* mycelium separated from its source of nourishment was incapable of infecting young rubber seedlings because it soon died, whereas among the 3-month-old rubber seedlings, 13 of the 18 inoculated with naturally infected roots were killed.

JOHN (1960) found that the root parasites would survive for a longer period in roots buried in bare soil than in those buried under rubber or cover plants, where conditions are more favourable for microbial activity. The feeding roots of rubber and cover plants also play an important role in the destruction of infected roots. Although all inoculated trees were in the clean-weeded planting rows, the nature and amount of cover in the interrows varied considerably between fields and their effect on the inocula is unknown. FOX (1965), who considered other factors which may play a part in the biological control of root disease, found that root parasites were attacked by antagonistic micro-organisms; roots infected with *G. pseudoferreum* were often invaded by *Trichoderma viride* (Pers) Fries. In discussing antibiosis and fungistatis of soil micro-organisms, JACKSON (1965) quotes the findings of RISHBETH (1951) concerning the antagonism of *T. viride* to *Fomes annosus* and its possible effect on the degree of infection by this root disease of pine trees in different soil types.

TABLE 1. EFFECT OF SIZE OF INOCULUM OF *F. LIGNOSUS* ON TREES OF DIFFERENT AGES. NUMBERS OF TREES, OUT OF TEN INOCULATED, SHOWING DEGREE OF INFECTION SCORED ON A SCALE OF 0—5.

Initial age of trees (years)	3						6						13						23					
Degree of infection score	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
Inoculum size (in^3)																								
5	–	8	–	–	2	–	–	5	5	–	–	–	–	9	1	–	–	–	–	9	1	–	–	–
15	–	6	–	–	1	3	–	–	8	–	2	–	–	2	3	5	–	–	–	9	1	–	–	–
45	–	–	–	–	1	9	–	2	6	–	1	1	–	1	2	7	–	–	–	7	2	–	1	–
135	–	–	–	–	1	9	–	–	2	–	1	7	–	–	1	4	3	2	–	1	6	1	1	1

TABLE 2. EFFECT OF SIZE OF INOCULUM OF *G. PSEUDOFERREUM* ON TREES OF DIFFERENT AGES. NUMBER OF TREES, OUT OF TEN INOCULATED, SHOWING DEGREE OF INFECTION SCORED ON A SCALE OF 0—5.

Initial age of trees (years)	3						6						13						23					
Degree of infection score	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5	0	1	2	3	4	5
Inoculum size (in^3)																								
5	7	3	–	–	–	–	5	5	–	–	–	–	3	5	2	–	–	–	5	5	–	–	–	–
15	2	7	–	1	–	–	1	5	1	3	–	–	2	5	2	1	–	–	–	5	4	–	–	1
45	–	7	–	–	1	2	–	3	4	3	–	–	1	–	1	8	–	–	1	1	4	2	2	–
135	–	–	–	–	1	9	1	2	–	4	1	2	–	–	–	8	2	–	–	1	1	2	3	3

TABLE 3. EFFECT OF SIZE OF INOCULUM OF *F. LIGNOSUS* ON THE AGE OF TREES. CUMULATIVE NUMBER OF TREES DEAD OR DYING AT DIFFERENT PERIODS, OUT OF 10 TREES INOCULATED.

Initial age of trees (years)	3					6					13					23				
Months after treatment	6	12	24	36	42	6	12	24	36	42	6	12	24	36	42	6	12	24	36	42
Inoculum size (in^3)																				
15	–	3	3	3	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
45	–	7	8	8	9	–	–	–	–	1	–	–	–	–	–	–	–	–	–	–
135	8	9	9	9	9	–	1	6	6	7	–	–	–	–	2	–	–	–	–	1

TABLE 4. EFFECT OF SIZE OF INOCULUM OF *G. PSEUDOFERREUM* ON THE AGE OF TREES. CUMULATIVE NUMBER OF TREES DEAD OR DYING AT DIFFERENT PERIODS, OUT OF 10 TREES INOCULATED.

Initial age of trees (years)	3					6					13					23				
Months after treatment	6	12	24	36	42	6	12	24	36	42	6	12	24	36	42	6	12	24	36	42
Inoculum size (in^3)																				
15	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
45	1	2	2	2	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
135	–	8	9	9	9	–	–	–	2	2	–	–	–	–	–	–	–	3	3	3

EVANS (1965) had shown quite clearly that the size of the infected root sample, the presence or absence of bark as well as the sealing of the cut ends with wax are significant as regards the survival of *F. annosus* in soil. This may apply to all root parasites. All inocula used in the present experiment had their bark intact, but it is a matter of conjecture as to what extent the results would have been influenced if their cut ends had been sealed.

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Pathological Division
Rubber Research Institute of Malaya
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