Inoculation Experiment with Fomes lignosus, Klotzsch

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An experiment is described in which the roots of Hevea brasiliensis trees five or twentyfive years old were inoculated with white root disease. The inocula were pieces of root naturally infected with Fomes lignosus. By physically preventing external growth of the fungus on the roots, the rate of spread of infection was virtually halted. Incidental observations have been made on the penetration of F. lignosus and on host reaction.

FOMES LIGNOSUS IS THE COMMONEST CAUSE of losses from root disease in *Hevea brasiliensis* during the first five years in the life of the plantation (SANDERSON 1930), (NAPPER 1932a). It can infect and kill trees of all ages. Infection of a healthy tree takes place when a root comes into contact with a diseased root in the soil. The rhizomorphs of the fungus spread inwards from the point of contact to the collar, then outwards along other lateral roots. Neighbouring trees are in turn infected and the process is repeated if control measures are not taken. The external growth of the fungus proceeds ahead of the region where the root is penetrated and killed (NAPPER 1932b). The rate of spread of the rhizomorph thus normally determines the rate of spread of the disease. It was not known to what extent the disease could spread internally in an infected root, and it is this that has been investigated, by physically suppressing the external growth.

EXPERIMENTAL METHOD AND MATERIALS

The experiment to be described was carried out at the R.R.I. Experiment Station, Sungei Buloh, Selangor. The root systems of fourteen five-year-old trees and ten twentyfive-year-old trees were exposed to permit the selection of twenty lateral roots of each group. These roots were chosen as being reasonably straight, at a depth of not more than nine inches, and ranging in size from three quarters of an inch to two inches in diameter. Any branch roots or roots in contact with them were removed.

The young trees were growing in a well drained site on peaty sand that had been cleared from swamp in secondary jungle six years ago. Of the twenty roots inoculated in this area five were near a drain edge where the soil is a coarse sand with but little peat in it. A sparse growth of natural cover plants was present in the tree inter-rows but the selected roots were in the clean-weeded planting rows. The old trees were growing in a low lying site between two small ravines where the soil is a sandy loam, and five of their selected roots were also near a drain edge where the soil is a coarse sand with little organic matter in it.

All the roots were inoculated with *F.lignosus* by burying in contact with them sections of naturally infected wood, well penetrated, but still firm. The inocula, all obtained from the same source, varied in volume from 12 to 61 cubic inches. They were placed anywhere between 3 and 7½ feet away from the collar.

One month after inoculation an inspection hole was dug one foot away from the inoculum to expose about 7 inches of the proximal part of each root. For the duration

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of the experiment these holes were kept covered with roofing tiles with a layer of earth over them to prevent the desiccation of rhizomorphs growing along the root in the hole, and to maintain the temperature close to that of the surrounding soil.

All the inspection holes were opened at weekly intervals and, with a stiff brush, the mycelium was removed from the exposed section of one half of the roots of each age group. These roots are hereinafter referred to as the treated roots. The remaining ten roots of each age group were left untouched except for the weekly opening of the holes to note the progress of the disease and to give the same degree of exposure as was received by the treated roots. These roots are hereinafter referred to as the control roots.

RESULTS

All the forty roots were dug out and examined a year after the start of the experiment.

In eight roots of the young trees and twelve roots of the old trees there was complete death either at the point of inoculation or for some further length on either side of it. Partial death at the point of inoculation was found on eight young roots and five of the old. In two roots each of the young and old trees there was only restricted penetration through wounds. There was no penetration at all in two of the young and one of the old roots, despite the fact that there had been vigorous superficial growth of the fungus over them in the early part of the experiment.

The results presented in Tables 1 and 2 show that preventing surface growth on the treated roots effectively checked internal extension of the disease for the duration of the experiment. In no case where the external growth was removed had the pathogen penetrated the root as far as the other side of the inspection hole although in the early months of the experiment the entire root surface within the holes became covered by superficial growth between the weekly treatments. The maximum penetration within those parts of the treated roots in the inspection holes was 4 inches, with an average of 1.74 inches in the young trees and 0.86 inch in the old trees. This greatly contrasts with the control roots, where the rhizomorphs were allowed to grow. In these, death was found in the young trees to extend beyond the inspection holes up to 19 inches with an average of 3.65 inches; in the old trees the maximum was 66 inches and the average 22.92 inches. The difference is illustrated by comparing *Figures* 2 and 3 (treated) with *Figures 4* and 5 (control).

OBSERVATIONS

When the inspection holes were dug, one month after inoculation, mycelium was already present on about half the roots. After a further month it was present on all except five roots of the old trees. In most cases the growth became very profuse, covering the entire surface of the roots. On the control roots much of this investing mycelium disappeared after twelve to sixteen weeks, leaving well defined rhizomorphs that had developed from it. The mycelium, at first white, became yellow after two to four weeks; the rhizomorphs, within three to six weeks, were orange or rust coloured. This colouration, when encountered in the field, is often attributed to the adsorption of pigments from the soil, especially in lateritic soil, but in this case such an explanation is difficult to accept since the mycelium within the inspection holes was not in contact with the soil. Although diffusion or translocation of pigments might have occurred from the soil at the edge of the pit, it is more likely that the pigment was produced by the fungus as it aged. Moreover, pigment is often found in old cultures.

TABLE I. ROOT DISEASE EXPERIMENT. DETAILS OF ROOTS, INOCULA AND RESULTS OF INOCULATION AFTER ONE YEAR, WHEN THE SURFACE GROWTH OF *Fomes lignosus* WAS SCRAPED OFF

5-year-old trees

Root	Root diameter at point of inoculation (inches)	Inoculum volume (cubic inches)	Length of penetration from outer end of hole (inches)	Length of penetration from inner end of the hole (inches)
1	1.75	48	4.0	0.0
2	2.0	31	0.0	0.0
3	1.5	48	0.0	0.0
4	1.25	42	0.0	0.0
5	1.0	59	1.0	0.0
6	1.0	39	2.9	0.0
7	1.0	31	3.0	0.0
8	0.75	18	0.0	0.0
9	0.75	22	4.0	0.0
10	0.75	18	2.5	0.0
Mean	1.18	35.6	1.74	0.0

25-year-old trees

Root	Root diameter at point of inoculation (inches)	Inoculum volume (cubic inches)	Length of penetration from outer end of hole (inches)	Length of penetration from inner end of the hole (inches)
1	2.0	61	0.0	0.0
2	2.0	53	0.8	0.0
3	1.5	31	2.5	0.0
4	1.5	21	0.0	0.0
5	2.5	59	0.0	0.0
6	1.0	49	0.0	0.0
7	1.0	39	1.8	0.0
8	1.0	29	2.0	0.0
9	0.75	18	0.0	0.0
10	0.75	46	1.5	0.0
Mean	1.40	40.6	0.86	0.0

TABLE 2. ROOT DISEASE EXPERIMENT. DETAILS OF ROOTS, INOCULA AND RESULTS OF INOCULATION AFTER ONE YEAR, WHEN THE SURFACE GROWTH OF *Fomes lignosus* WAS NOT SCRAPED OFF

Root	Root diameter at point of inoculation (inches)	Inoculum (volume) (cubic inches)	Length of superficial mycelium from inoculum: Inwards (inches)	Outwards (inches)	Length of penetration from inoculum: Inwards (inches)	Outwards (inches)	Length of penetration from outer end of hole (inches)	Length of penetration from inner end of hole (inches)
5-year-old trees								
1	2.0	53	66	54	27	54	16.0	7.5
2	1.0	21	54	12	12	0	3.0	0.0
3	1.5	24	42	10	13	0	8.5	2.5
4	2.0	39	50	6	23	0	22.5	19.0
5	1.5	18	36	18	5	5	0.0	0.0
6	1.25	49	48	7†	24	7†	1.5	0.0
7	1.0	24	54	18	6	0	2.5	0.0
8	0.75	12	56	14	15	0	14.5	7.5
9	0.75	24	*Mycel. disappeared*		0	0	0.0	0.0
10	1.0	32	48	24	11	8	1.25	0.0
Mean	1.28	29.6	45.4	16.3	13.6	7.4	6.98	3.65
25-year-old trees								
1	1.5	29	24	3	9	0	0.0	0.0
2	2.0	31	56	33	51	14	41.0	33.0
3	2.0	40	30	10	16	0	10.7	3.7
4	1.25	58	102	60	87	45	72.0	66.0
5	1.25	46	40	9	26†	0	11.5	4.5
6	1.0	46	60	60	50	48	38.0	30.0
7	0.75	18	12*	10*	4	0	0.0	0.0
8	0.75	44	84	36†	50	36	40.0	33.0
9	0.75	39	54	12†	48	12	41.0	30.0
10	1.0	18	54	12†	46	12	36.0	29.0
Mean	1.23	36.9	51.6	24.5	38.7	16.7	29.02	22.92

* = traces of rhizomorphs only present on roots
† = roots disintegrated beyond the distance shown

A record of the amount of growth found in the inspection holes of the treated roots is presented as a combined weekly average for young and old trees in *Figure 1*. Eighteen weeks after inoculation the rate of growth decreased rapidly, doubtless due to the exhaustion of food material in the original inoculum.

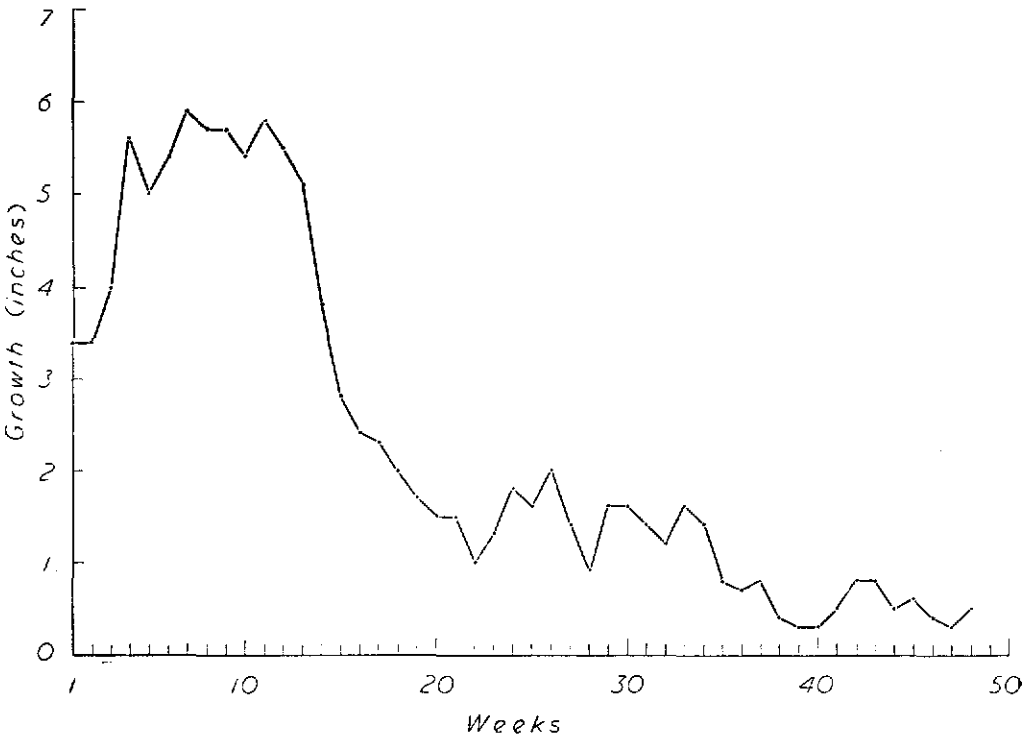

Figure 1. The average weekly growth of Fomes lignosus in the inspection holes.

Four and a half months after inoculation, that is about three months after the rhizomorphs had covered the root surface within the inspection holes, the bark of four untreated roots of the old trees and that of one of the young trees had been killed by the fungus within the holes. After six months these figures had doubled.

At the termination of the experiment (at the end of one year) all the inocula were found to be partially or completely disintegrated. In no case could the pathogen be isolated from them. Although a few retained their form, the inside was found to be either reduced to a soft black rot or converted into a mass of humus which, in many cases, was permeated by rubber feeding roots (*Figures 6* and *7*). Feeding roots had also penetrated dead sections of some of the inoculated roots (*Figures 8* and 9). The condition of the inocula and their lack of viability is in marked contrast to previous experimental results. In an earlier experiment on the length of viability of the three major root pathogens (*F.lignosus*, *F.noxius* and *Ganoderma pseudoferreum*), sections of infected roots were buried in a loose sandy loam in an area free from cover plants which was kept clean weeded. In root sections of a size similar to those used in the present experiment, *F.lignosus* was found viable for up to two years (ALTSON 1953a, 1953b). There is no doubt that cover plants hasten the rate of decay and in this instance the feeding roots of the rubber appear also to have aided the process.

The inocula used in this experiment were all of sufficient volume to be able to initiate an infection. The smallest one used, of 12 cubic inches, sufficed to cause partial death of a lateral root and penetration at the collar. The few failures may have been due to variability of the inocula or to some undetermined soil effect. In a previous inoculation experiment with young seedlings in a nursery ALTSON (1953c) reported that inocula of 5 cubic inches or more were necessary to assure initiating an infection. With progressively smaller inocula, the proportion of successful inoculations fall off. The smallest size that induced an infection was ¼ cubic inch. However, in his experiment the inocula were placed in contact with the tap root of young seedlings a little below the collar — an area known to be especially susceptible to infection. DE JONG (1933) reported infection of young seedlings with natural inocula of 3 cubic inches and with artificial inocula of 1.8 cubic inches.

When the control roots were examined at the termination of the experiment it was noticed that both the external rhizomorph growth and penetration of the wood was more extensive inwards from the inoculum than outwards towards the distal portion of the root. Growth and penetration inwards was approximately twice as great as outwards from the inoculum.

The mean penetration inwards from the inoculum in the control roots was 38.7 inches for the old trees and 13.6 inches for the young trees. The two figures differ significantly ($P<0.02$). This result might be attributable to different degrees of resistance, or to different amounts of food reserves within the roots. If the old roots contained greater quantities of reserve food materials and thus might be expected to give greater impetus to fungal growth, it would be reasonable to expect a comparable increase in the amount of external growth on the older roots. However, although the average external growth was greater on the old roots—51.6 inches against 45.4 inches—the difference was found to be not significant. There was no significant difference between the volumes of inocula used for the two groups. It does not seem likely that this difference in penetration could be attributed to soil factors, thus it would appear to result from the young roots having a greater degree of resistance. This view is substantiated by the greater degree of barrier formation observed in the roots of young trees.

A contrary opinion was expressed by NAPPER (1932a), who believed that as the tree grows older it becomes more resistant to attack. The apparent contradiction might be resolved by supposing that, whilst the tree is growing rapidly and vigorously—up to, say, its twelth year—its resistance increases, and thereafter decreases.

Although both NAPPER (1932a) and DE JONG (1933) thought that the fungus did not penetrate through wounds, there is clear evidence from the present experiment that it does. Examples are shown in *Figures 10* to *13*, the presence of the fungus having been confirmed in all cases by its isolation in pure culture. The wounds had been caused when the root systems were first exposed in preparation for the experiment. Lenticels, also, were more readily invaded than the intact bark (*Figures 14* to *17*), while infected branch rootlets in addition facilitated entry of the fungus (*Figure 18*). In all cases the identity of the fungus was confirmed by isolating it from the lesions.

The cracking and fissuring of the bark at the collar might be regarded as a sort of self-wounding. The greater susceptibility of such bark to infection by *F.lignosus* than other parts of the root system is confirmed by this experiment. In four of the young trees it was found that there was extensive death of the bark and some penetration of the wood at the collar, although the lateral roots along which the rhizomorphs had grown were free of all traces of penetration for two to three feet proximal to the collar (*Figure 19*).

Casual field observations confirm the statement of DE JONG (1933) that the rot caused by *F.lignosus* will sometimes stop without treatment. Detailed examination of the roots from this experiment show that host resistance may be effected in three ways.

Primary resistance may come from the cork cambium, which reacts to invasion by proliferating to form patches of thick corky bark which tend to crack longitudinally and to flake off (*Figure 20*). This proliferation is also noticeable when infection occurs at the lenticels and is accompanied by proliferation of parenchyma within the lenticels (*Figure 17*, p). If the fungus penetrates to the wood, wound barriers may be formed. These however, are unlikely to be successful where a vigorous inoculum is promoting the infection, each attempt at wound barrier formation being defeated by further penetration behind it from the advancing external rhizomorphs. When, however, the initial inoculum potential is too low for the infection to reach a self propagating level, then the wound barriers may succeed in completely sealing off the lesion. *Figure 21* shows evidence of a wound barrier having formed and been broken through, and the pathogen then having been halted successfuly by a second barrier. Other examples of barrier formation are shown in *Figures 10* to *13*. The final stage of the host reaction is callus formation commencing from the edges of the lesion (*Figures 12* and *22* to *25*). From some of the partly healed infections the pathogen could still be isolated, but in others it had died out and been replaced by *Diplodia* and other saprophytes.

Evidence from the present experiment supports the view that the fungus penetrates into the wood of the infected root through medullary rays (DE JONG 1933). In most cases of partial penetration the infection had stopped at, or just short of, the centre of the root. This was sometimes so even when a root was dead along one side for many feet, the other side (with no rhizomorph) remaining healthy.

DISCUSSION

The most striking finding of this experiment is that, by preventing external growth of *F.lignosus*, the internal spread of infection is virtually halted. This observation greatly encourages the belief that chemical treatments may come to play an important part in control measures. If the collar and proximal part of the lateral roots can be kept free of rhizomorphs by chemical means, the host reaction may be able to cut off the disease, which will decay to nothing.

The experiment has incidentally provided evidence of wounds and lenticels being more readily invaded than is the intact bark. It has also indicated the changes in activity of the fungus in the pieces of naturally infected wood used as inocula. Defensive mechanisms of the host are also indicated.

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In the following *Figures* the white lines on the entire roots, and the black lines on sectioned roots, denote the position of the inspection hole.

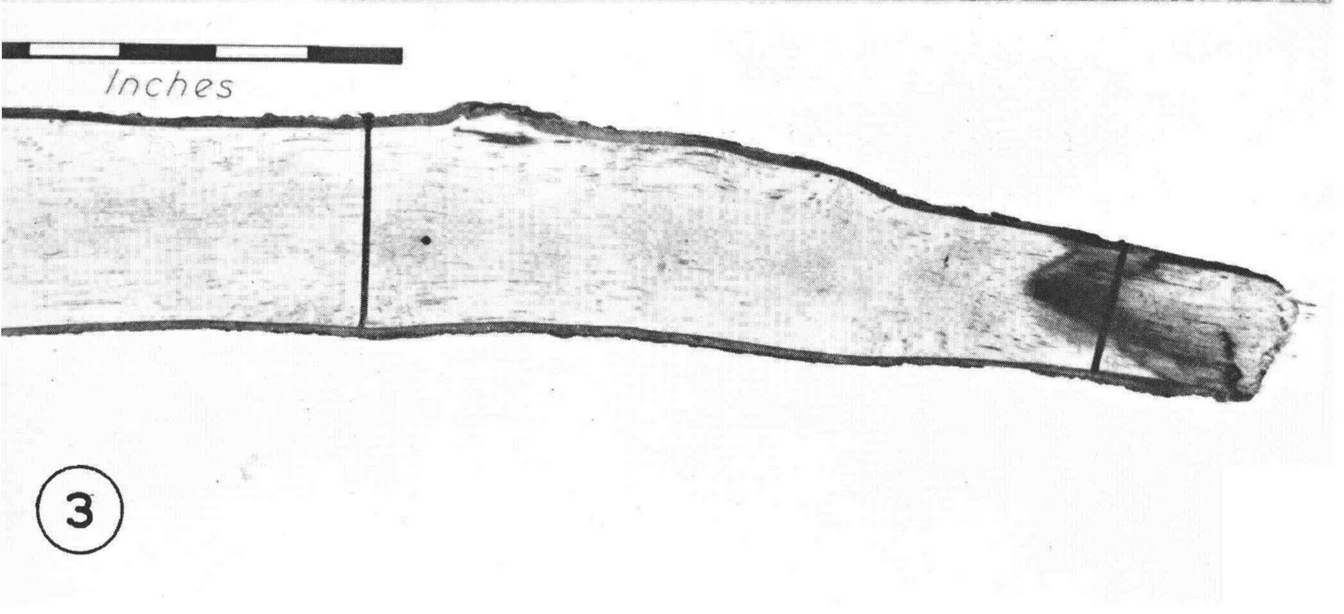
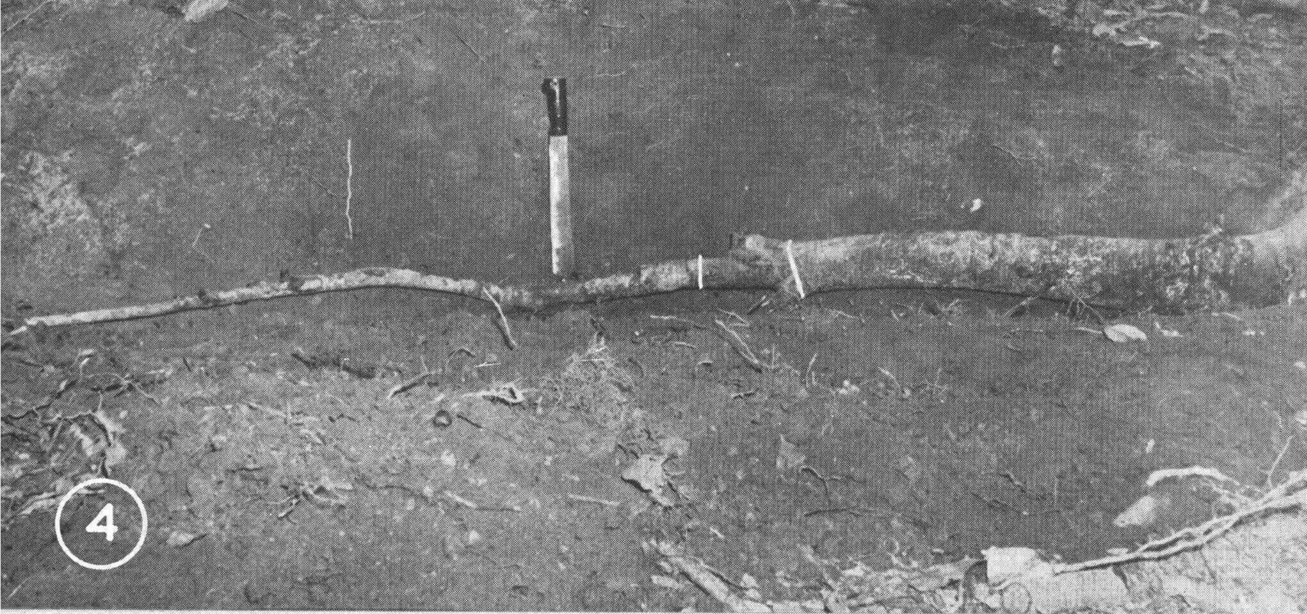


Figure 2. A treated root of an old tree, dead up to the distal end of the hole and breaking into pieces at the site of the inoculum (marked with a chisel), the rot being so far advanced.

Figure 3. The same root in section showing the limit of the rot, which ends about an inch within the inspection hole at the distal end.



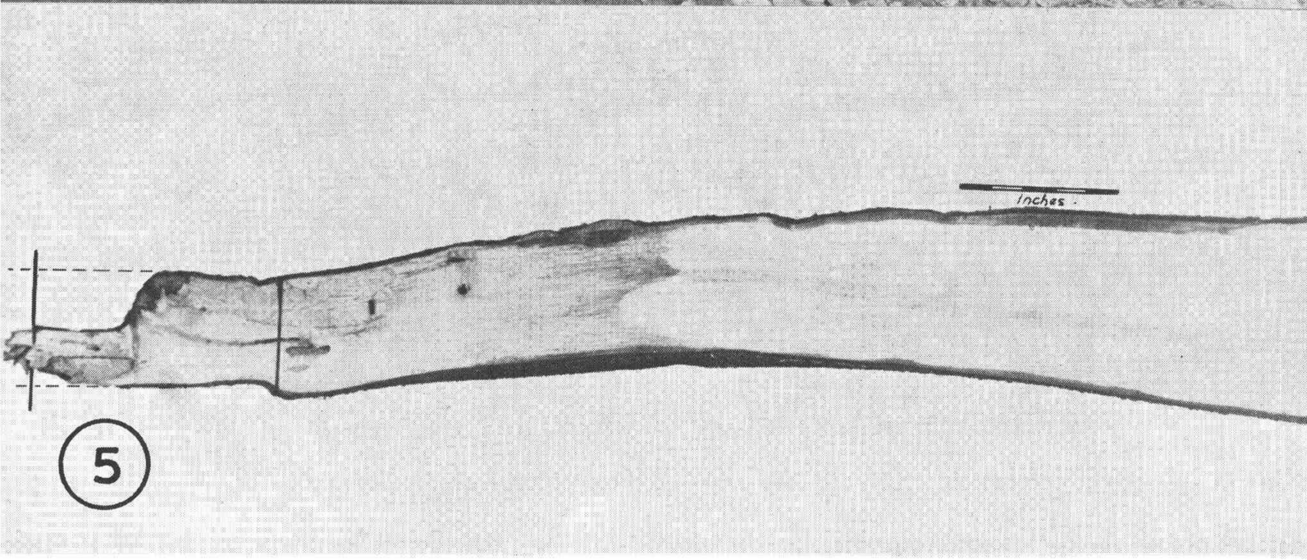
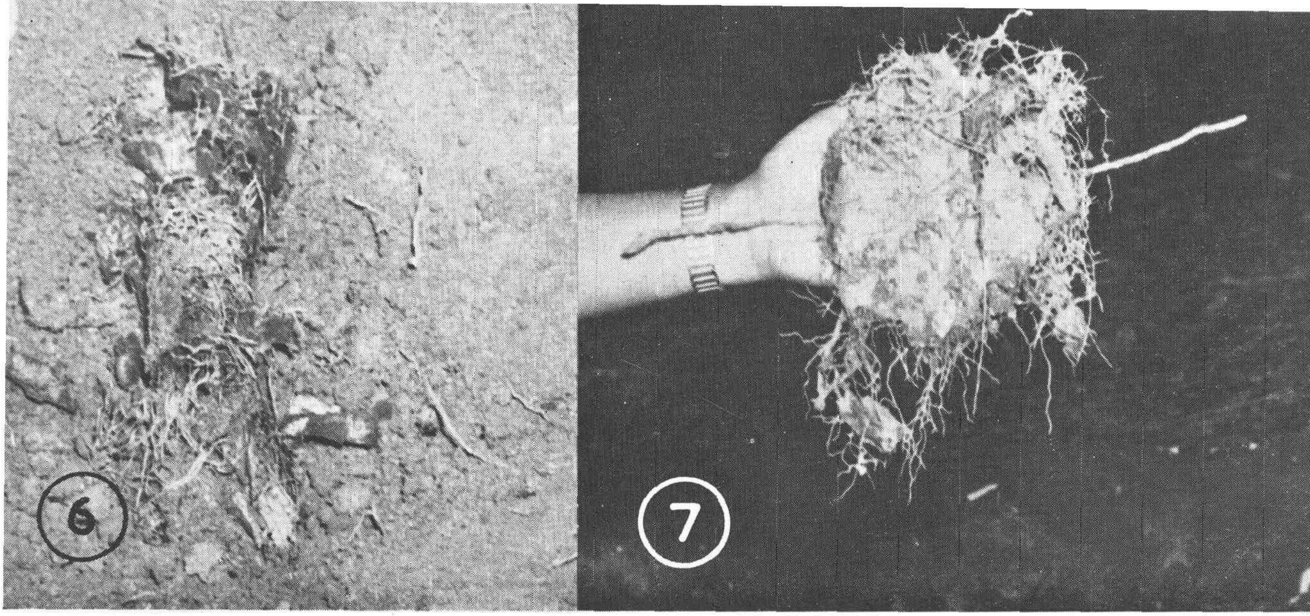
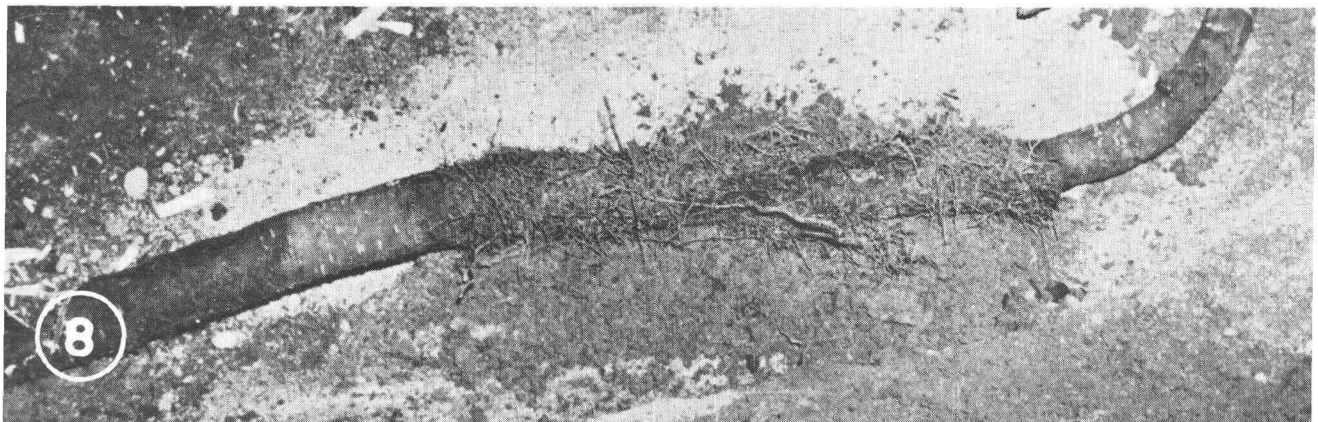


Figure 4. A control root of an old tree showing the position of the inspection hole and of the inoculum (marked with a parang). Rhizomorphs have grown right up to the collar of the tree.

Figure 5. The same root in section showing complete death of the root inside the inspection hole and for a further distance towards the tree, with death of the outer bark extended considerably further still.



Figures 6 & 7. Inocula which are almost rotted away and penetrated by feeding roots of rubber.



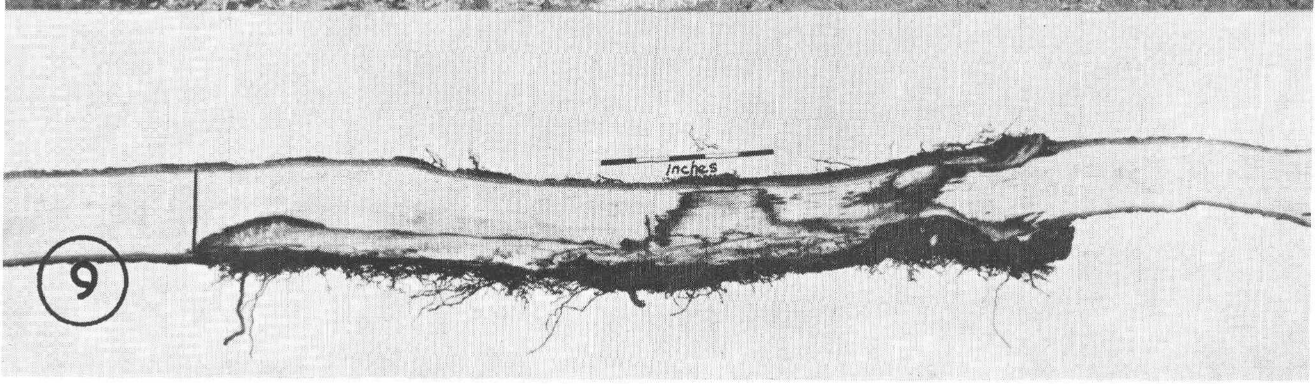


Figure 8. A root of an old tree, the dead part penetrated by numerous feeding roots.

Figure 9. The same root in section showing the rot, with feeding roots on the surface and healthy parts on either side.

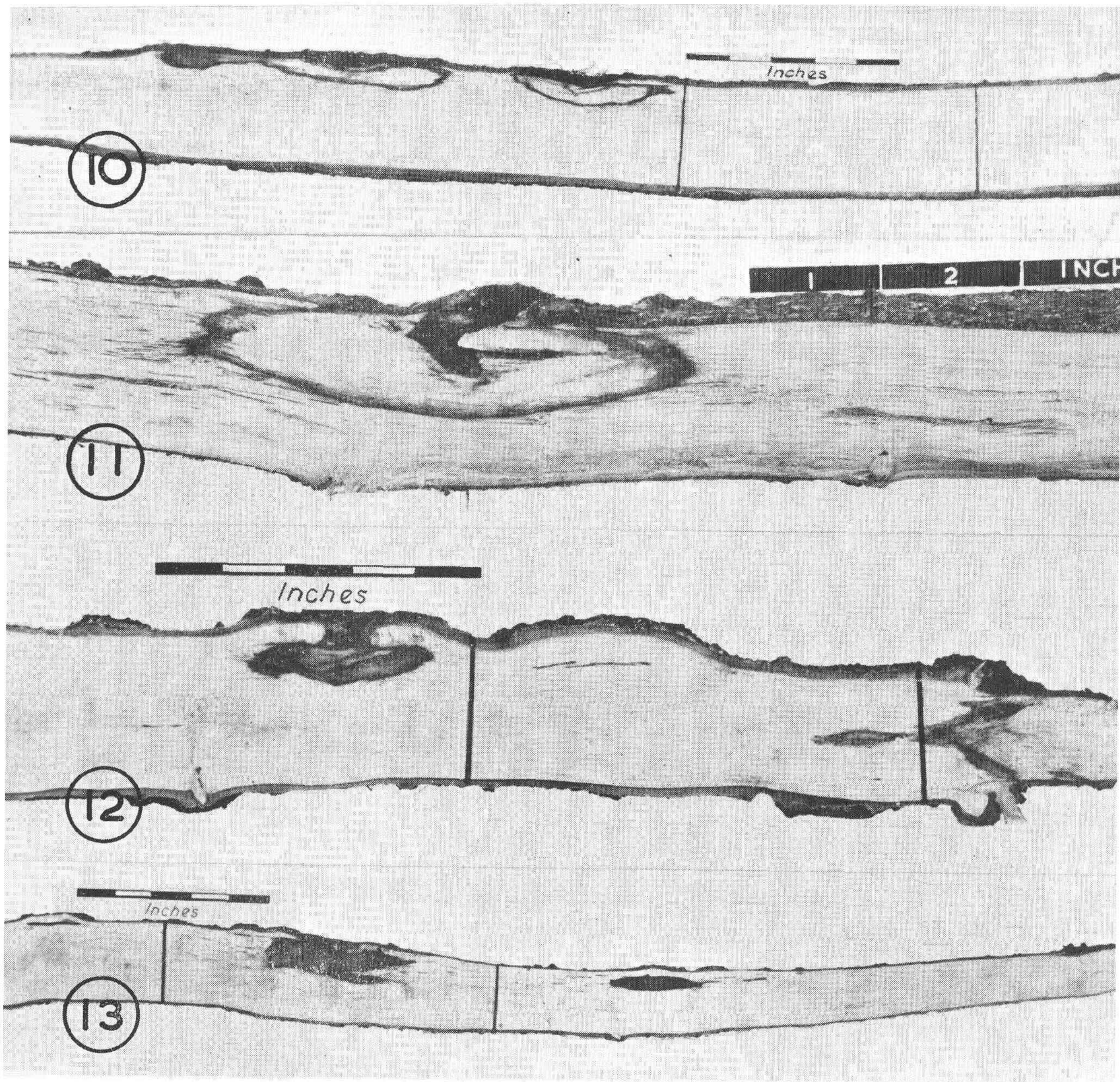


Figure 10. Section of a root of a young tree, showing two sites of wound penetration and barrier formation.

Figure 11. Section of a root of a young tree showing penetration through a deep wound, limited by formation of a barrier.

Figure 12. Section of a root of a young tree showing wound penetration where the wound is being closed by callus formation.

Figure 13. Section of a root of a young tree showing two sites of wound penetration. The one on the right is completely healed and covered by callus, whilst that on the left is partially healed and not yet covered.

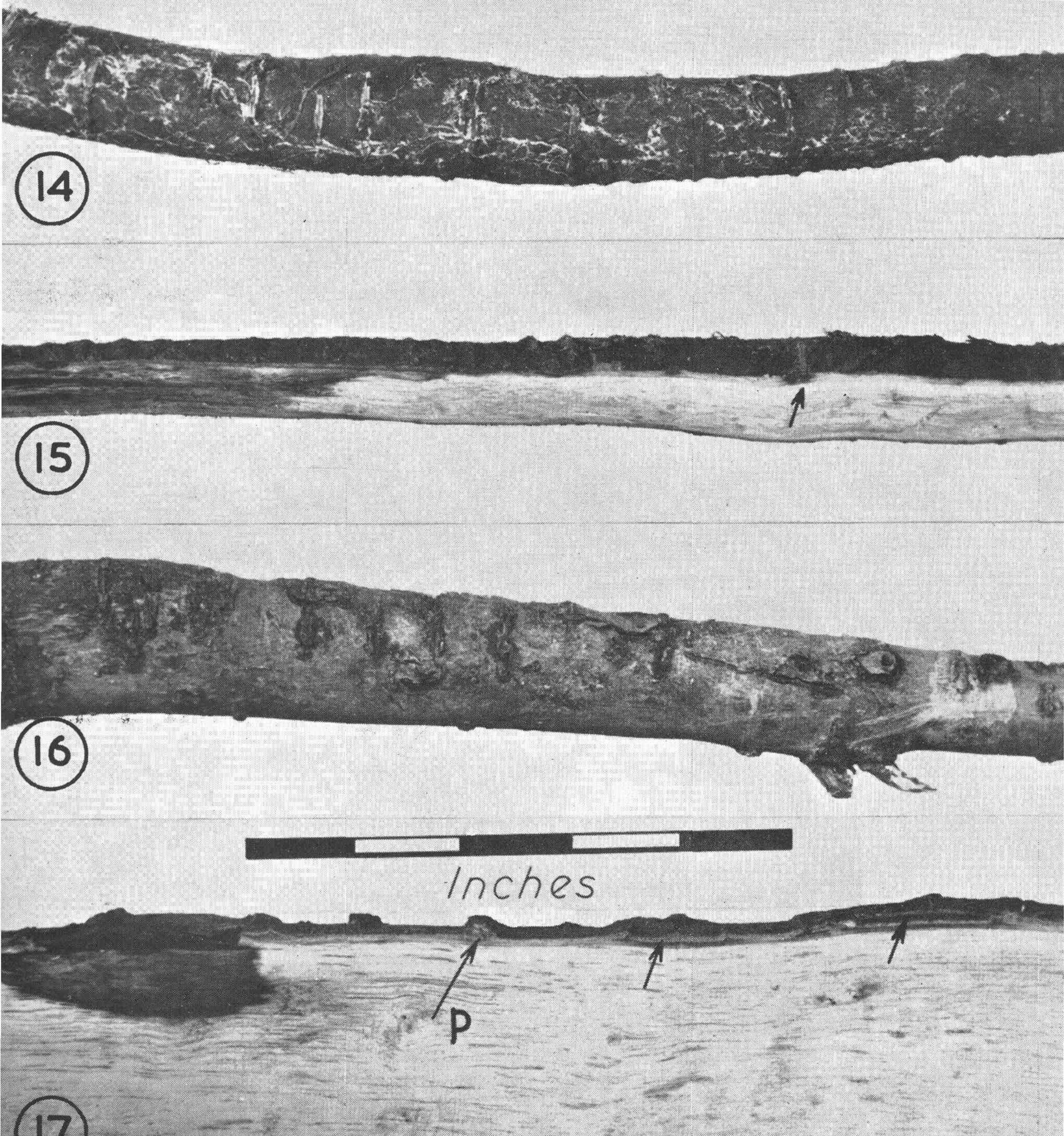
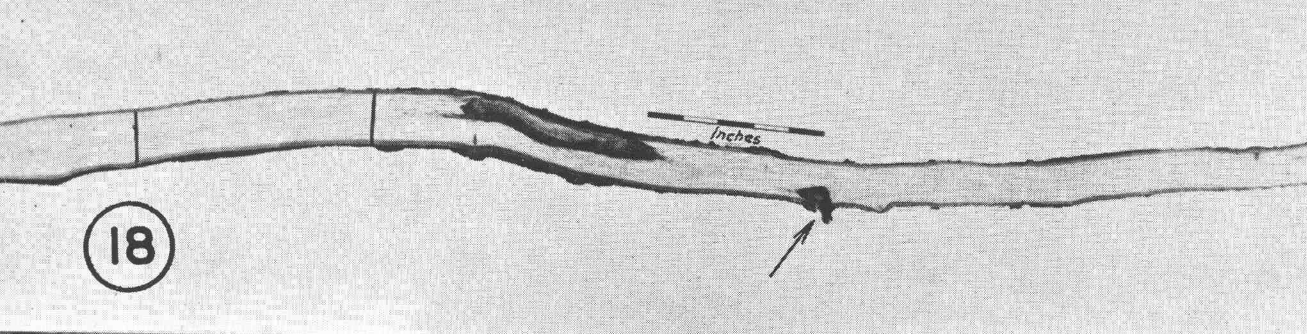


Figure 14. A control root of an old tree showing enlarged lenticels and rhizomorphs running along them.

Figure 15. The same root in section showing dead bark (seen black) beneath the lenticels (indicated by the arrow).

Figure 16. An infected root of a young tree showing enlarged lenticels.

Figure 17. The same root in section showing thick layers of dead bark (seen black) beneath the lenticels, with healthy bark beneath. The letter p shows the prolifera-tion of parenchyma within the lenticels.



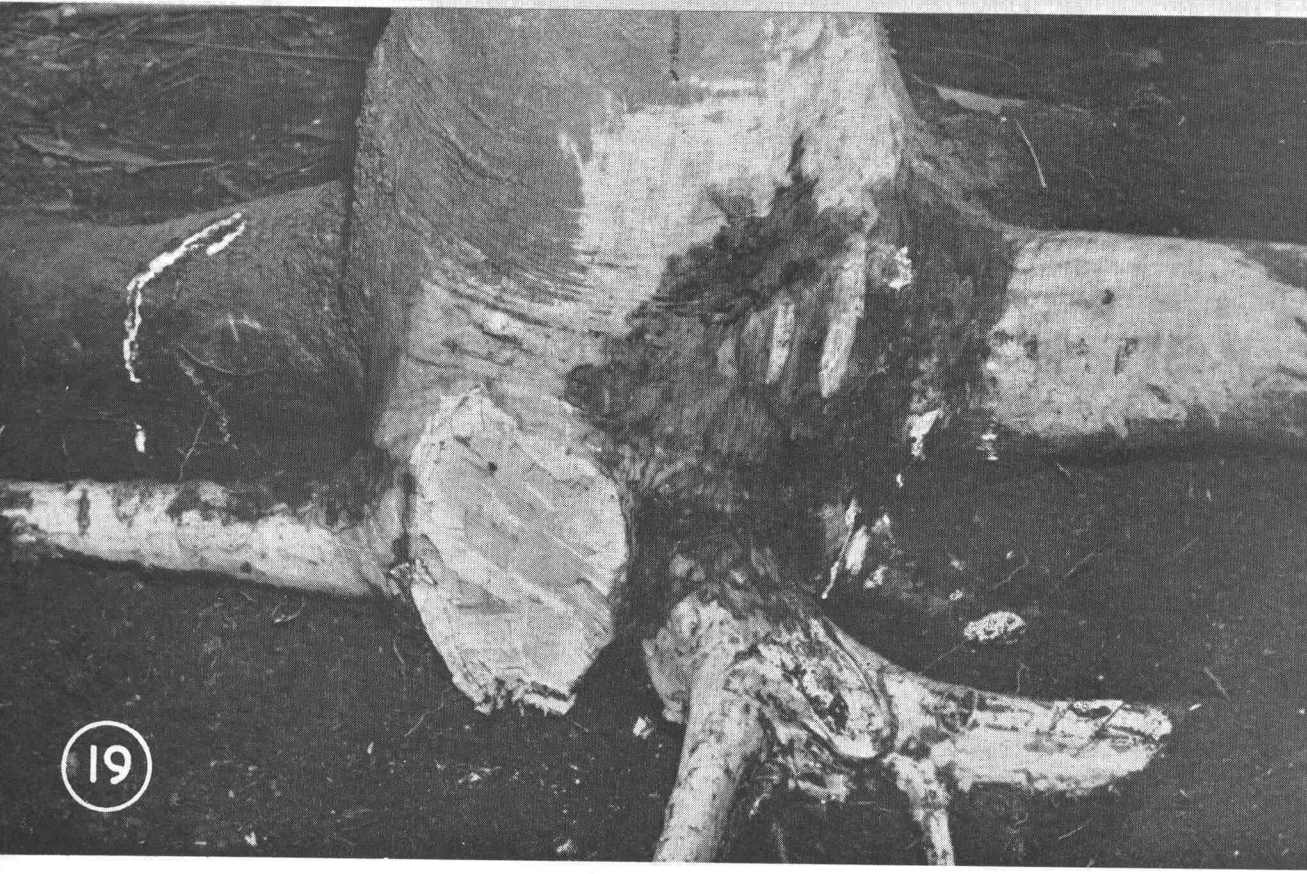
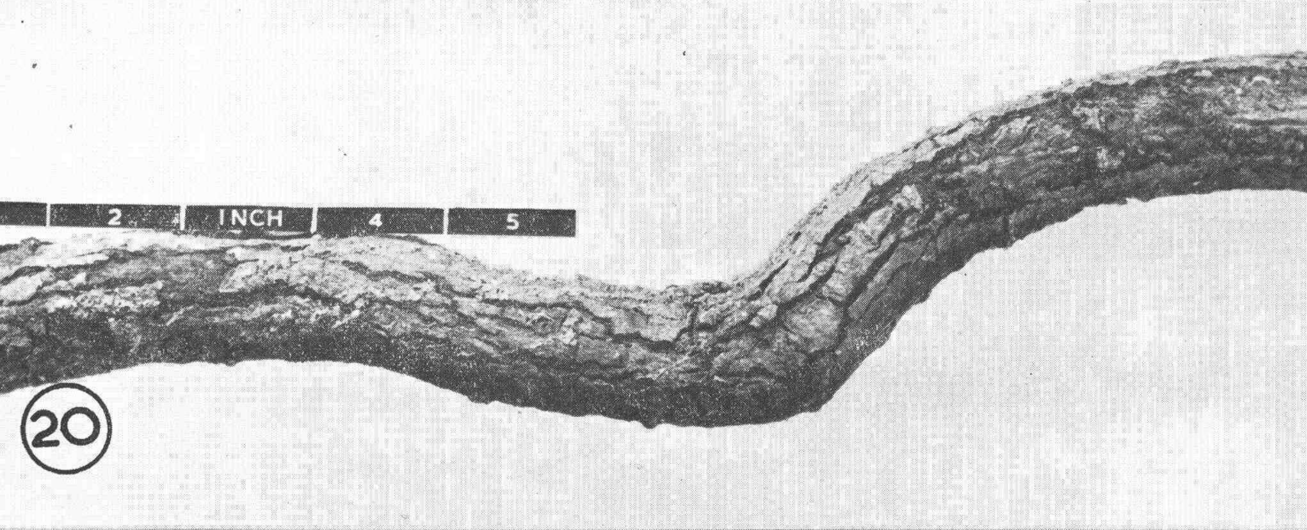


Figure 18. Section of a root of a young tree showing penetration through a small infected branch root (indicated by the arrow).

Figure 19. The bole of an untreated young tree scraped to show areas of collar penetration and death (seen black) with healthy areas (seen white) around. The inoculated root, which was not penetrated for some length near the collar, has been cut off.



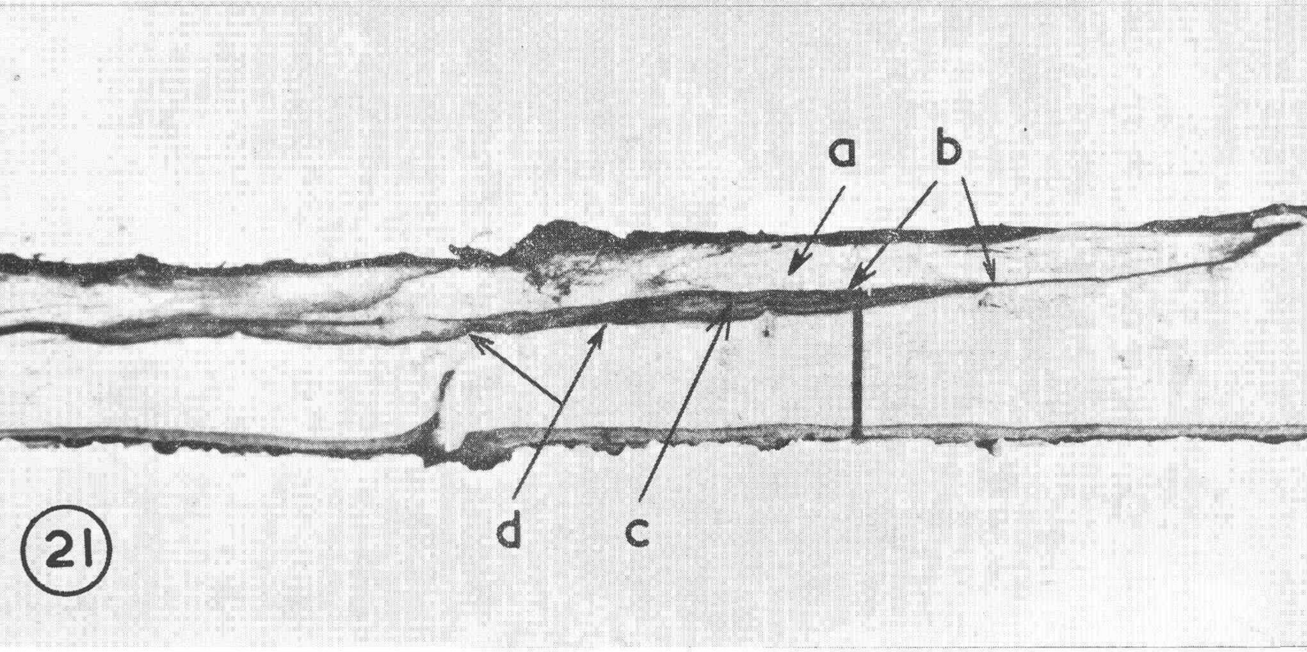


Figure 20. Section of a root of a young tree showing proliferation and death of the outer bark, which is being sloughed off.

Figure 21. Section of a root of a young tree showing how penetration of the fungus has been limited by barrier formation. The white part at the top *a* shows an advanced soft rot which was initially checked by a brown barrier *b*, the black part below shows a hard rot, of recent origin *c*, the fungus having broken through the original barrier, whilst a second barrier is now forming *d*.

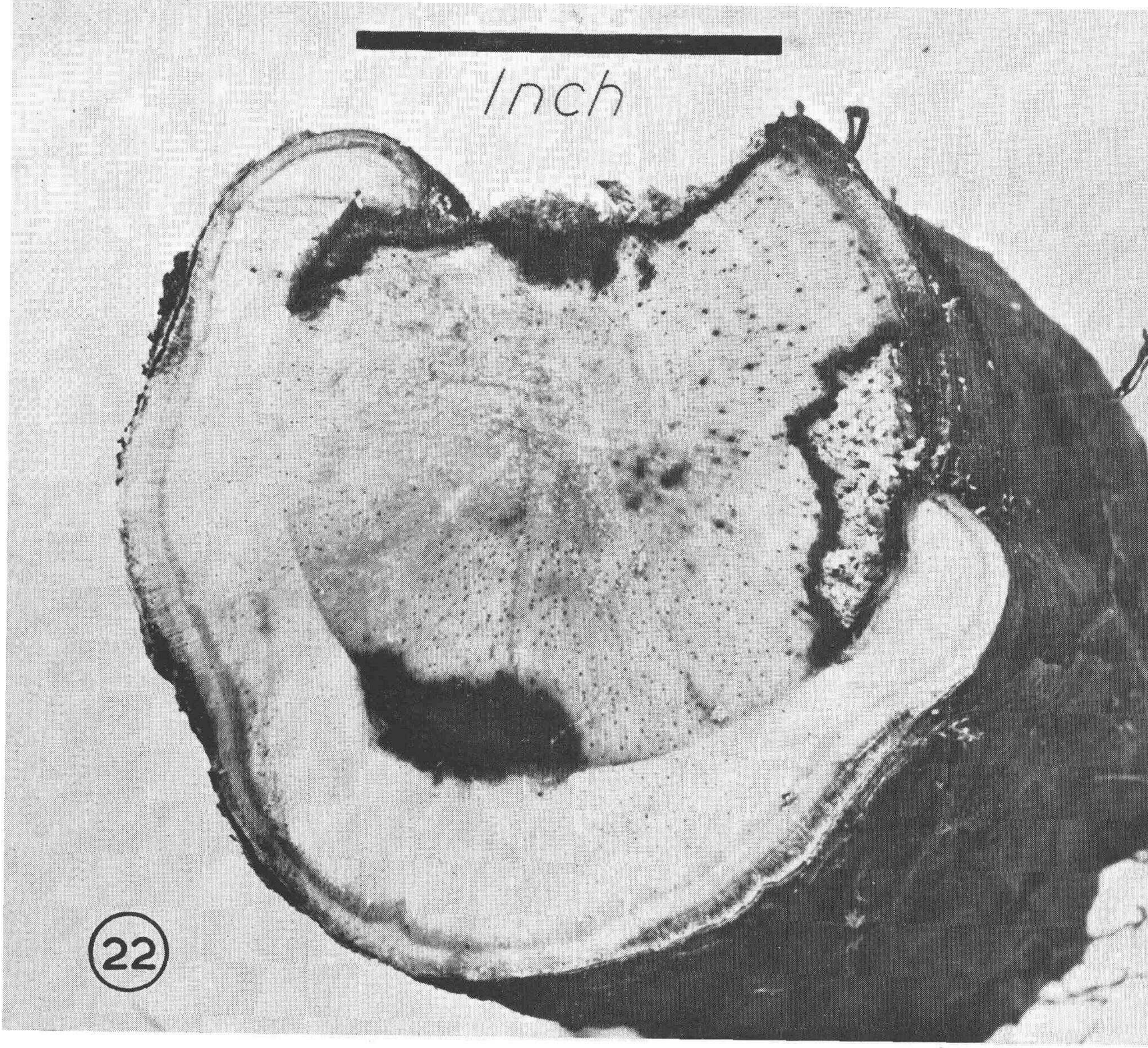


Figure 22. The cross section of a root of a young tree showing extensive infection and regeneration by callus growth.

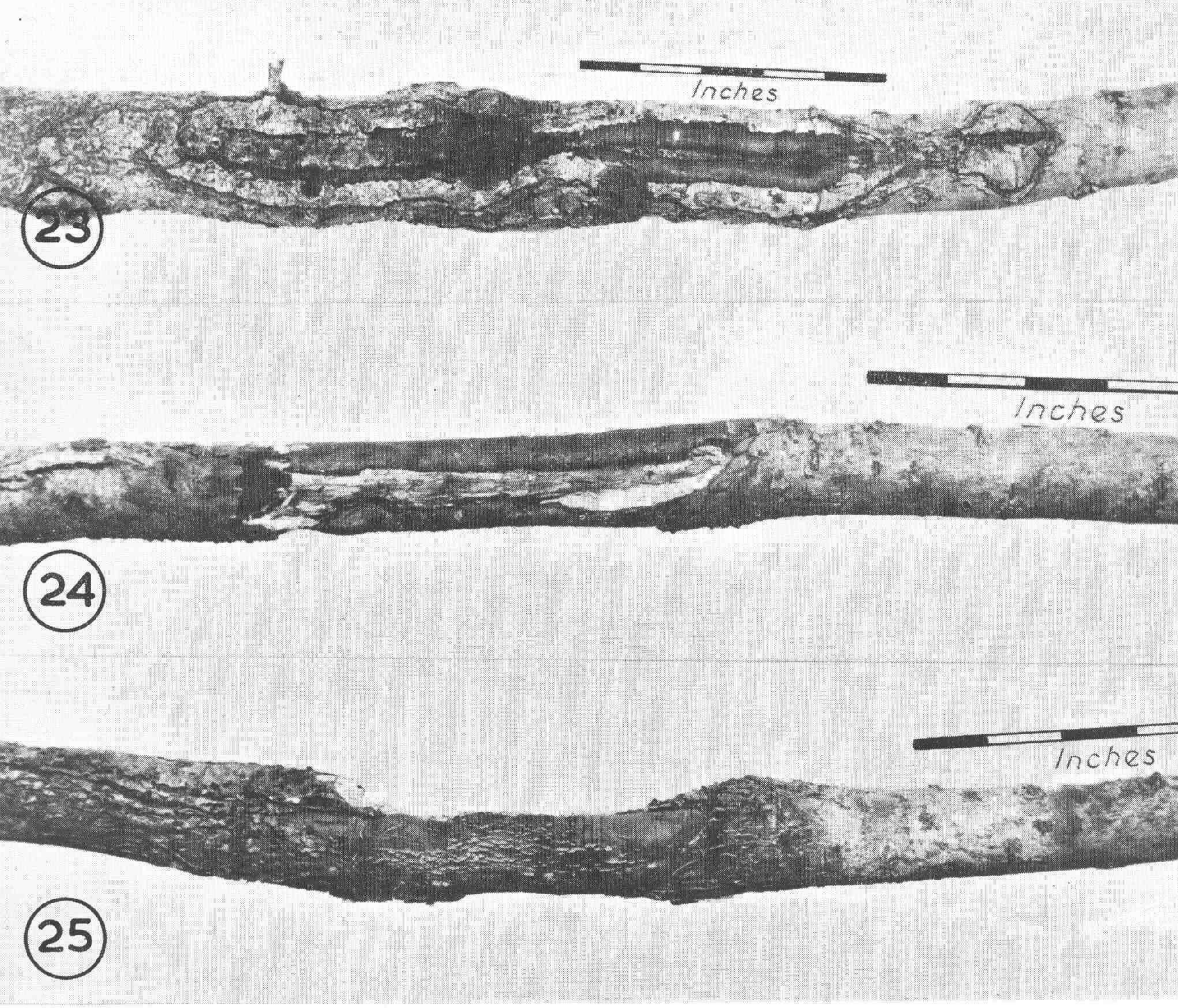


Figure 23. A root of a young tree showing healing of a large lesion.

Figure 24. A root of a young tree showing healing of a part, where extensive penetration had originally killed more than three quarters of the cross section of the root.

Figure 25. The other side of the same root shown in Figure 24.