J. Rubb. Res. Inst. Malaysia, **24(1)**, 15-25 (1974)

Stem Galls of Hevea brasiliensis

W.C. LIM[1]

Galls of up to 50 cm diameter were found on the trunks and branches of trees of RRIM 612 on an estate in Selangor. The galls were formed by proliferation of the outer parts of the branch or trunk, probably as a result of enhanced manufacture of growth substances. No pathogens were found associated with gall tissue. Galls can be easily removed and are of little economic cosequence.

Stem galls were first reported on *Hevea brasiliensis* in Malaysia in 1965 (RUBBER RESEARCH INSTITUTE OF MALAYA, 1965) in a 14-acre field of eight-year-old trees of clone RRIM 612 in Selangor. Galls were first noticed on the trunks and branches a few years after planting the clone and, at the time of examination in 1970, they ranged in size from 2 – 50 cm in diameter *(Figures 1 – 3)*, some almost circumscribing the trunk. Gall-bearing trees were distributed at random in the 14 acres, about 5% of trees being affected. Such gall-bearing trees were otherwise healthy and of normal girth. Adjacent trees of clones RRIM 603 and 605 were unaffected.

DISCUSSION

The surface texture of a gall depends on its age and rate of growth; the bark of rapidly-growing galls flakes from the surface *(Figure 1)*, whereas the surfaces of

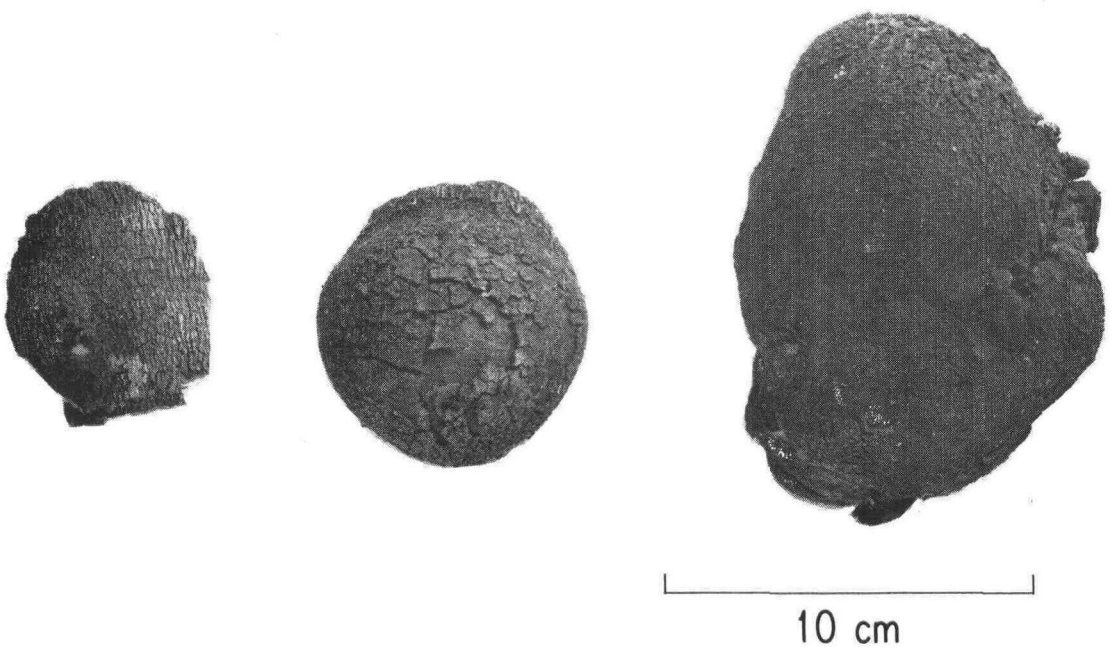

Figure 1. Three trunk galls from clone RRIM 612.

[1]Now at School of Biological Science, Universiti Sains Malaysia, Penang.

COMMUNICATION 575

Figure 2. Trunk gall decaying as a result of invasion by Ustulina zonata *at the junction between gall and trunk.*

slow growing galls are similar to normal bark *(Figure 3)*. Young, actively growing galls have a very light pinkish tinge, while mature galls are similar in colour to the trunk. Most of the affected trees had one gall, although up to six galls per tree were seen. In such cases galls were either separate or confluent *(Figure 4)*. Generally, the galls occured between the base of the trunk and the main fork, as well as on branches *(Figures 3* and *4)*, with the bigger galls mainly on the trunk and main forks. No galls were found on green twigs, leaves, flower stalks or roots.

At the sites of insertion of galls stress marks were seen; large galls (*i.e.* > 15 cm*), due to their growth and weight, had sizeable cracks at their junction with the trunk which may have served as entry points for pathogens such as *Ustulina zonata (Figure 2)*. Twenty-seven trees with large galls showed such infection, as well as a general decay, at the gall-trunk junctions. Small (> 8 cm^a) and medium (8 – 15 cm^a) galls showed no apparent effect on the tree apart from reducing the amount of tappable bark if they happened to occur below panel height.

*Horizontal diameter

EXPERIMENTAL

Galls were examined *in situ* and excised from the tree with a saw. All tissues infected by *Ustulina zonata* were removed, and the exposed wounds on the tree treated with a wound dressing. The largest gall exicsed weighed 90 kilograms.

Histology

Small pieces of gall and tissues from unaffected trees were fixed in formalin acetic alcohol (JOHANSEN, 1940), sectioned transversely and stained with safranin and fast green. Unstained sections were mounted in a dilute solution of iodine and potassium iodide to determine the distribution of starch granules. Longitudinal and transverse sections of bark specimens were prepared and stained with Sudan III to show the laticiferous tissues.

Isolation of Micro-organisms

Specimens were surface-sterilised with 95% alcohol and isolations of bacterial and fungal pathogens made from the inner bark and the wood. Organisms thus obtained were wound-inoculated into rubber seedlings and tomato plants to see if they could induce galls, and compared with a gall-producing isolate of *Agrobacterium tumefaciens*. Bark and wood shavings were also used to inoculate wounded stems of rubber seedlings and tomato plants.

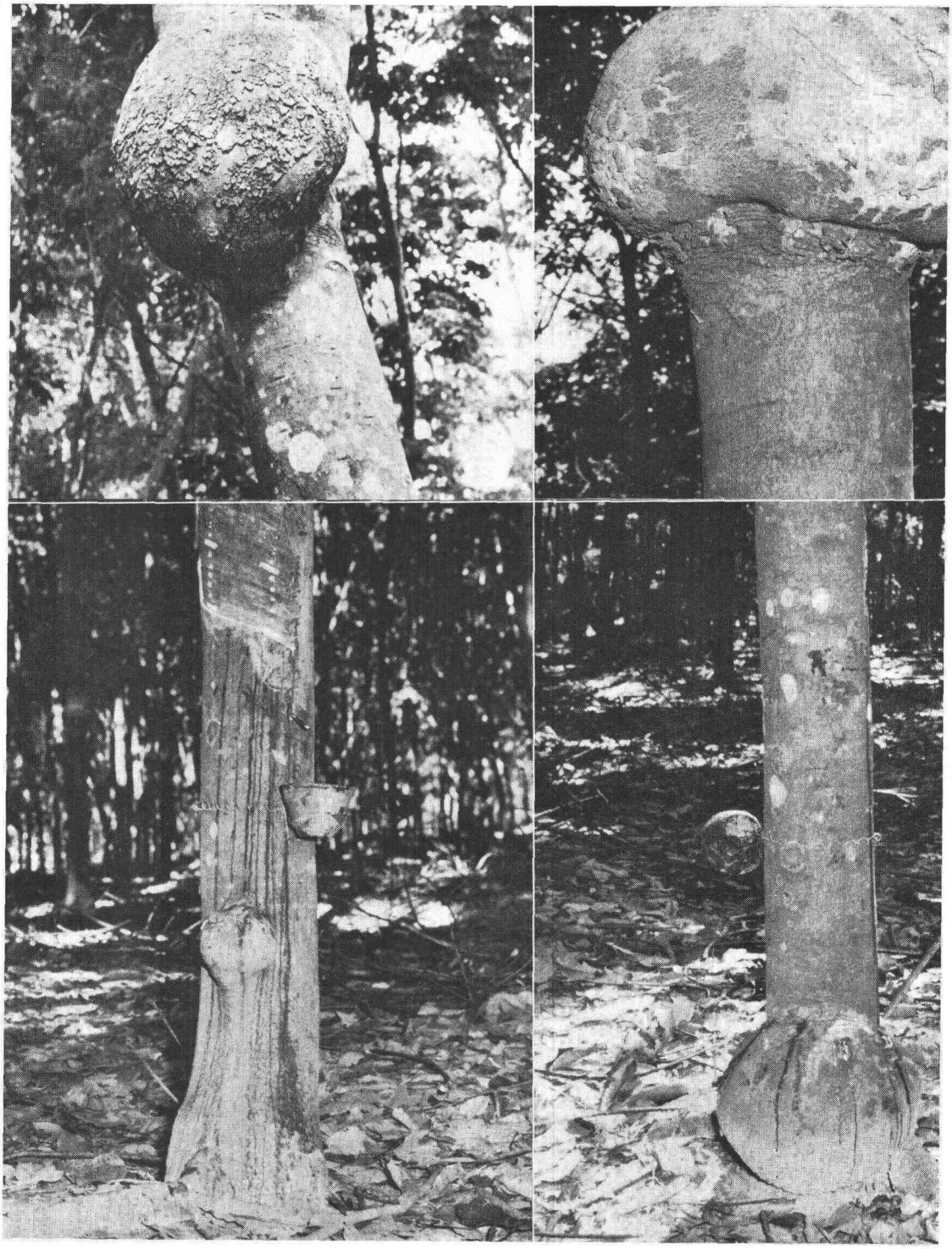

Figure 3. Trunk galls in several positions.

Figure 4. Multiple trunk galls.

To detect the presence of a virus, leaves from galled and healthy trees were held overnight at 4°C before extraction of sap, which was prepared in 2% phosphotungstic acid and distilled water using the epidermal strip technique (HITCHBORN AND HILLS, 1965). Sap was similarly extracted from the inner bark and wood of galls and branches. Partially purified sap from bark and wood shavings was prepared in phosphate-veronal buffer, pH 7.8 (CHOD AND POLAK, 1969). Sap preparations were spotted on carbon-colloidion coated grids, shadowed with platinum (except when negatively stained with phosphotungstic acid), and examined with an electron microscope.

Crude sap from leaves and wood shavings of galled and normal trees were extracted in phosphate-veronal buffer of pH 7.8 (POLAK AND KLIR, 1969). The saps were immediately inoculated to leaves of potted test plants previously dusted with carborundum (mesh 600) and subjected to a period of 24 h darkness before use (BAWDEN

AND ROBERTS, 1948). Carborundum was removed after inoculation by washing with tap water. Inoculated plants were kept under observation in a glasshouse for a few weeks. Test plants were *Hevea brasiliensis, Euphorbia hirta, Manihot esculenta* (varieties *Black twig, Puteh, Yellow twig, Medan* and *Green twig*), *Nicotiana tabacum, Lycopersicon esculentum, Cucumis sativus, Vigna sinensis* and *Gomphrena globosa.*

Extraction of Auxins

Shavings from the inner bark and outer wood of galls and normal branches were extracted with 50 ml ethanol at 4°C; 50 ml distilled water was added to the extract and the pH adjusted to 2.8 with dilute sulphuric acid. The acidified mixture was extracted thrice with 50 ml ether, the ether fractions combined and extracted thrice with 50 ml of 5 % sodium bicarbonate solution to obtain a neutral fraction. The combined aqueous portions were acidified to pH 2.5 with dilute sulphuric acid and again extracted thrice with 50 ml ether to obtain an acid fraction. Both fractions were subjected to ascending paper chromatography on Whatman No. 1 paper with isobutanol – methanol – water (80:5:15 by volume) as solvent. Branch and gall extract fractions were spotted on the same chromatogram for comparative purposes. Chromatograms were viewed under ultraviolet light and were either sprayed with ferric/perchloric or nitrite/nitric reagents (BOATMAN, 1960). Unsprayed chromatograms were assayed for growth substances using the oat coleoptile straight-growth test of BOATMAN (1960), with 3-indole propionic acid (IPA), indole-3-acetic acid (IAA), indole butyric acid (IBA) and indole-3-aldehyde (I3A) as standards. Blank chromatograms were incorporated. Coleoptile sections (0.3 cm) were cut under green light from four-day-old *Avena sativa* L. seedlings (variety *Victory*) grown in total darkness at 25 ± 2°C, and placed in incubation vials containing 1 ml of 2% sucrose solution in 10^{-2}M phosphate-pitrate buffer (pH 5), a piece of chromatographic paper with the test substance, and ten sections of coleoptile. Parallel tests were carried out on pieces cut from equivalent portions of the chromatogram of gall and branch extracts. Vials were incubated for 20 h in the dark with gentle agitation on an oscillating table. Harvested sections were placed on a glass slide in a photographic enlarger, magnified four times, and photographed. Changes in length were expressed as a percentage of the change in length of coleoptiles in contact with chromatographic sections spotted with branch extract (control), and plotted against the Rf on the chromatogram.

RESULTS

The trunks and branches where galls were removed healed satisfactorily, with no signs of regeneration of galls one year later.

Longitudinal sections show that galls are circular to oval in shape, with the site of insertion smaller than the diameter *(Figure 5)*. Galls are woody, without pith or other cavities.

The bark covering galls is thicker than that over surrounding tissues *(Table 1)*, but is normal except for the fact that the rings of laticiferous tissues are closer in the bark over the galls and, especially in larger galls, contain more latex vessels *(Figure 6)*. The cambium proliferates faster centripetally than centrifugally, with the result that galls consist mostly of xylem. The ground tissue of bark and wood of galls is similar in cell size to that of surrounding normal tissues, although the metaxylem vessels of large galls are smaller in diameter than those of the branch *(Table 2)*. Starch granules are confined to the medullary rays, which in a gall are distorted by the arrangements of the xylem tissues *(Figure 7)*.

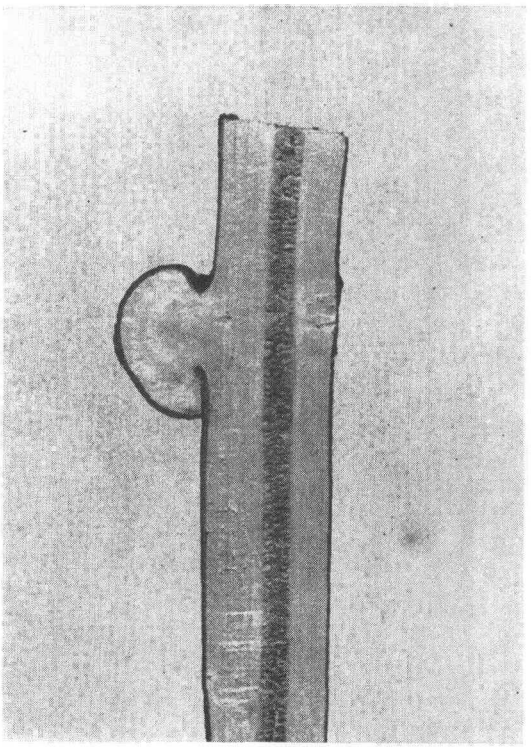

Figure 5. Longitudinal section of branch gall.

TABLE 1. THICKNESS OF BARK OVER GALLED AND NORMAL WOOD OF RRIM 612 (THREE TREES)

Item	Bark thickness (mm)[a]	
	Mean	Range
Small gall (< 8 cm)	4.5	3–6.5
Normal bark[b]	3.0	2–4
Medium gall (8–15 cm)	4.0	3–6
Normal bark	3.0	2–4
Large gall (> 15 cm)	5.0	4–6
Normal bark	4.5	3–5

[a]Mean of five readings.
[b]Bark over normal tissue about 5 cm away from the gall.

No bacteria or fungal mycelium were seen in sections of normal and galled tissuse, but four fungi were isolated from large galls and two actinomycetes from medium-sized galls. Bacteria were isolated from galls of all sizes. Inoculating these organisms, or shavings of galls, did not give rise to galls on rubber seedlings or tomato plants, although an isolate of *Agrobacterium tumefaciens* did so. The bark of such induced galls was rough and unlike that of natural galls.

TABLE 2. CROSS-SECTIONAL MEASUREMENTS[a] OF THE METAXYLEM OF A LARGE GALL AS COMPARED WITH THAT OF ADJACENT NORMAL TISSUES

Vessel	Size (μ)	
	Large gall	Normal wood (branch)
Single	105 x 54	186 x 131
Twin	179 x 71	287 x 159

[a]Average of twenty measurements.

Leaf and gall tissue sap did not show any virus particles when examined under the electron microscope; neither did leaf and gall tissue extracts produce any consistent symtoms of a virus on test plants.

When viewed under ultraviolet light, chromatograms of gall and branch extracts showed one major spot, the Rf value of which *(Table 3)* differed from those of IPA and IAA. When sprayed with ferric/perchloric or nitrite/nitric reagents, chromatograms of extracts were either without reaction or showed faint spots which coincided with those detected under ultraviolet light.

Using the more critical chromatographic bioassay methods described, it was found that extracts of small and medium-sized galls contained more growth hormones than large galls or normal branches *(Figure 8)*. Areas of principal activity of the chromatogram were around Rf 0.2 – 0.4 and Rf 0.7 in the acid fractions, and around Rf 0.3 – 0.4, Rf 0.7 and Rf 0.9 in the neutral fractions of

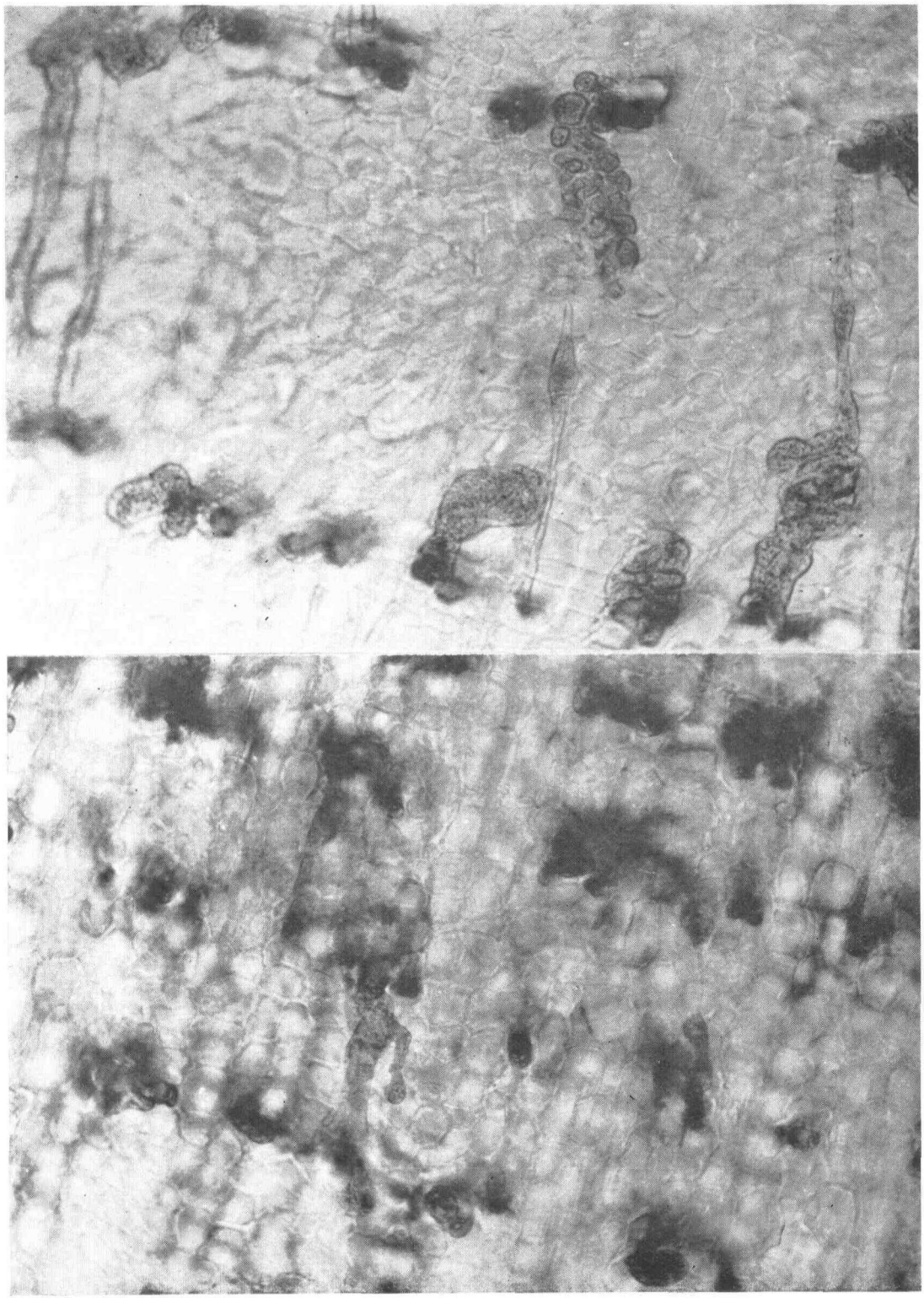

Figure 6. Transverse section of inner bark, stained with Sudan III to show the distribution of latex tissues. Top, normal bark; bottom, bark from large gall (× 860).

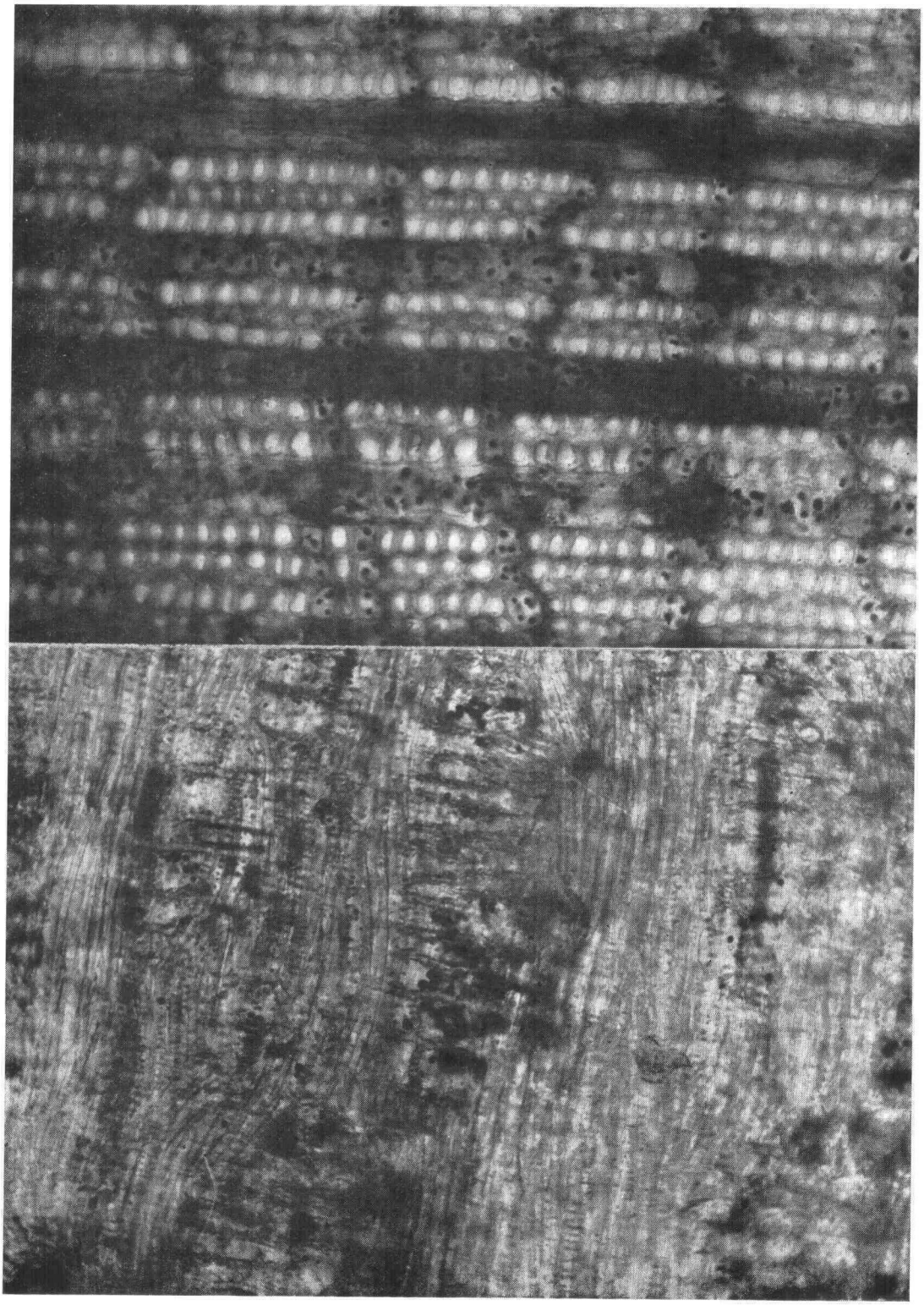

Figure 7. Transverse section of wood stained with iodine in potassium iodide solution. Top, normal branch; bottom, wood from gall (× 860).

TABLE 3. RF VALUES OF THE MAIN SPOTS SEEN BY ULTRAVIOLET LIGHT AFTER CHROMATOGRAPHY[a] OF GALL EXTRACTS

Spot	Rf
Small gall	0.7 – 0.9
Medium and large galls	0.9
Normal tissue	0.8 – 0.9
3-Indole propionic acid	0.3 – 0.4
Indole-3-acetic acid	0.6 – 0.7
Indole butyric acid	0.8 – 0.9
Indole-3-aldehyde	0.8 – 0.9

[a]Solvent mixture: isobutanol – methanol – water (80:5:15).

the gall extracts *(Figure 8)*. Rf values of 0.2 – 0.4 are probably due to the presence of IAA *(Table 3)*.

DISCUSSION

Plant galls are caused by fungi, bacteria, nematodes, mites and insects (MANI, 1964), but the even contour and regular internal morphology of *Hevea* galls do not indicate these organisms to be likely causes of the galls. The failure to induce virus symptoms on indicator plants when inoculated with extracts of leaves and gall tissues and the lack of virus particles in the sap indicate that galls are not likely to be caused by a virus.

Fungi, actionomycetes and bacteria were isolated from galls, but inoculation experiments failed to reproduce gall symptoms. This does not rule out fungi or bacteria as possible causes, but suggests that they are not likely to be causatively involved.

Bark burrs are common on the trunk and branches of RRIM 612. Similar burrs on trees have been attributed to a physiological disturbance (METCALFE AND CHALK, 1950). Very young galls are difficult to distinguish from burrs, and it is indeed possible that burrs may give rise to galls. Galls with bark similar to normal bark are normally caused by non-infectious agents, whereas those with bark markedly differing in character from the normal are caused by pathogens (BOYCE, 1961). Since the bark of *Hevea* galls is similar to that of normal stems, and there is no evidence of the presence of a causal organism, it is likely that galls are produced as a result of some unknown physiological stress.

TOLLENAAR (1966) reported that boron deficiency might cause swellings and galls in cacao, although this was not confirmed by SHAW AND BURNETT (1969). Leaves of galled trees of *Hevea* were found to be low in nitrogen, potassium, magnesium and boron *(Table 4)* in comparison with normal trees (TAN, 1970), but this does not reflect a direct relationship between galls and nutrient deficiency since only a few trees were sampled.

TABLE 4. LEAF ANALYSIS[a] OF LEAVES OF RRIM 612 TAKEN FROM THE EDGE OF THE CANOPY OF A GALL-BEARING TREE

N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Mn (p.p.m.)	B (p.p.m.)
2.56	0.16	1.38	0.35	0.16	112	12

[a]Expressed on oven-dry basis.

The enhanced growth capacity of crown gall tumour tissue, as compared with normal tissue, seems to depend on its ability to manufacture a growth hormone (MANI, 1964). This appears to be the case with galls on *Hevea*. Plant hormones have been found in latex (BOATMAN, 1960) and in leaves (BOLLE-JONES, 1954). IAA was found in leaves but not in latex. Auxins were found in galls of *Hevea*, and at least three active compounds, one of which is probably IAA, were detected.

Hevea galls of the type described are of little economic consequence and may easily be excised if necessary.

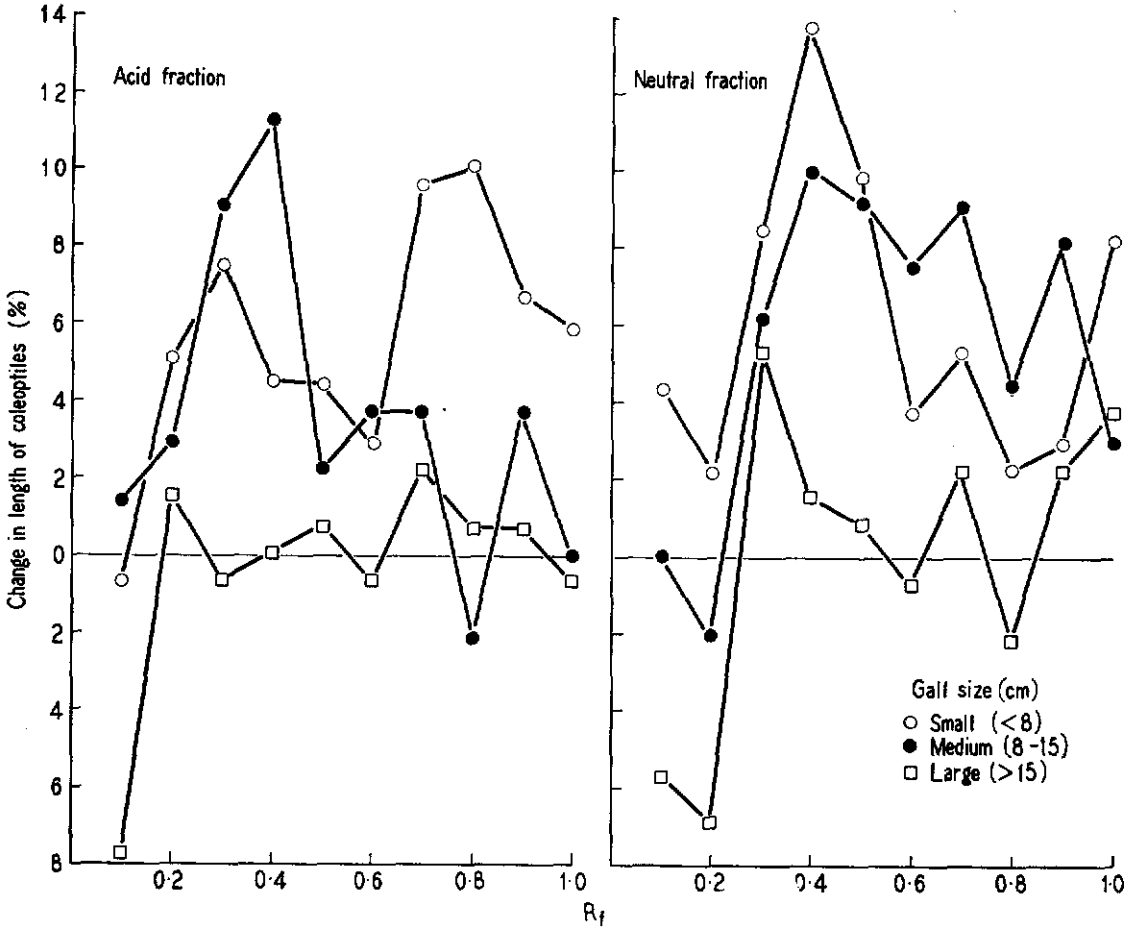

Figure 8. Percentage change in length of oat coleoptiles caused by extracts of different Rf values from chromatograms of gall tissue sap.

ACKNOWLEDGEMENT

Acknowledgement is made to the manager of the estate concerned for assistance; to Mr G.C. Iyer for statistical analysis; to Dr J.B. Gomez for electronm icroscope facilities; to Mr K.H. Tan of Soils Division and colleagues in the Analytical Chemistry Division for leaf analysis;and to Messrs Mohd Rosli bin Bachik and K.C. Wong for technical assistance.

Rubber Research Institute of Malaysia
Kuala Lumpur *December 1970*

REFERENCES

BAWDEN, F.C. AND ROBERTS, F.M. (1948) Photosynthesis and predisposition of plants to infection with certain viruses. *Ann. appl. Biol.*, **35**, 418.

BOATMAN, S.G. (1960) Detection and estimation of growth substances in *Hevea* latex. *J. Rubb. Res. Inst. Malaya*, **16(4)**, 166.

BOLLE-JONES, E.W. (1954) Occurrence of indole-3-acetic acid in laminae of *Hevea brasiliensis*.*Nature, Lond.*, **173**, 127.

BOYCE, J.S. (1961) *Forest Pathology*. New York: McGraw-Hill.

CHOD, J. AND POLAK, J. (1969) Purification and conservation of infective sugar beet mosaic virus. *Biol. Plant. (Praha)*, **11**, 324.

HITCHBORN, J.H. AND HILLS, G.J. (1965) Electron microscopic examination of wild cucumber mosaic virus in negatively stained crude preparations. *Virology*, **26**, 756.

JOHANSEN, D.P. (1940) *Plant Microtechnique*. New York: McGraw-Hill.

MANI, M.S. (1964) *Ecology of Plant Galls*. The Hague: W. Junk.

METCALFE, C.R. AND CHALK, L. (1950) *Anatomy of the Dicotyledons*. Oxford: Clarendon Press.

POLAK, J. AND KLIR, O. (1969) Mechanical transmission of beet yellows virus to *Chenopodium quinoa* Willd. and *Chenopodium foliosum* (Moench.) Asch. *Biol. Plant. (Praha)*, **11**, 366.

RUBBER RESEARCH INSTITUTE OF MALAYA (1965) *Plrs' Bull. Rubb. Res. Inst. Malaya No. 78*, 105.

SHAW, D.E. AND BURNETT, W.M. (1969) Galls of cacao in Papua and New Guinea. *Papua and New Guin. agric. J.*, **21**, 25.

TAN, K.H. (1970) Personal communication. Rubber Research Institute of Malaya.

TOLLENAAR, D. (1966) Boron deficiency in cacao, bananas and other crops on volcanic soils of Ecuador. *Neth. J. agric. Sci.*, **4**, 1138.