

GALLS OF CACAO IN PAPUA AND NEW GUINEA

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ABSTRACT.

Fan and knob galls of cacao, previously recorded in Central America, are now recorded in Papua and New Guinea. Neither of these galls, the causes of which have not been determined, are of economic importance in the Territory at present.

Studies carried out on knob gall include microscopic examination of the tissues, isolations in nutrient agar of fungi present in the galls, inoculations with gall tissue (which did not result in galls), excision of galls in the field and the production of new galls from some old excision scars. The presence of knob galls could not be correlated with any nutritive state of the trees. No significant difference was found in auxin activity or in gibberellin levels in galled and normal samples and no virus-like particles were found in negatively stained-cell extracts in the electron microscope.

INTRODUCTION.

Of the various galls of cacao recorded overseas (Hutchins and Siller, 1961) the only ones so far identified in Papua and New Guinea are fan gall and knob gall. Occasionally trees with prolific flowering are located, but so far these have been attributed to causes other than flowery gall by the Cacao Agronomist, Mr. J. B. O'Donohue (unpublished data) who, on the senior author's recommendation, kept such trees under observation in one of the cacao-growing areas of Papua. No records of disc or green point gall have been made to date. Information on fan and knob galls as they occur in the Territory, and the work carried out on the latter, is given below.

FAN GALL.

Fan gall was described from Central America (Hutchins and Siller, 1961) as a stem-like outgrowth up to several inches in length, sometimes branched, with very short internodes and bract-like leaves; some of the outgrowths bore flowers. Groups of such stems had a fan-like appearance, hence the name.

In the Territory a condition resembling fan gall has been noted in New Britain, in the Markham Valley (New Guinea mainland) and

in the Northern District of Papua. An early stage is shown in *Plate I* and a later stage, where a cluster of such stems are forming a fan, is shown in *Plate II*. Often the accumulation of dead flowers from the small branches forms a mass of debris around the fan gall.

One tree was examined by the senior author several times a year for three years. When first noted it had apparently normal flowering on the trunk and all the branches except one. On the exceptional branch fan galls were present, not only on the thicker wood, but on thinner wood which would not usually carry flowers (*Plate I*). Apparently normal pods were being borne on other branches. Since that time the tree has continued to bear well and at the time of the last visit carried 17 pods. The incidence of fan gall on this tree seems to have fluctuated over the period of three years. It is growing on deep volcanic sand in an area where the cacao does not have symptoms of soil deficiency.

No cause has been ascribed to the condition overseas, nor has any claim been made that it is infectious.

The authors would be interested to learn of the occurrence of fan galls on Territory plantations other than those on which fans have already been noted and collections studied.

As mentioned previously, fan gall has been previously recorded on Central American cacao,

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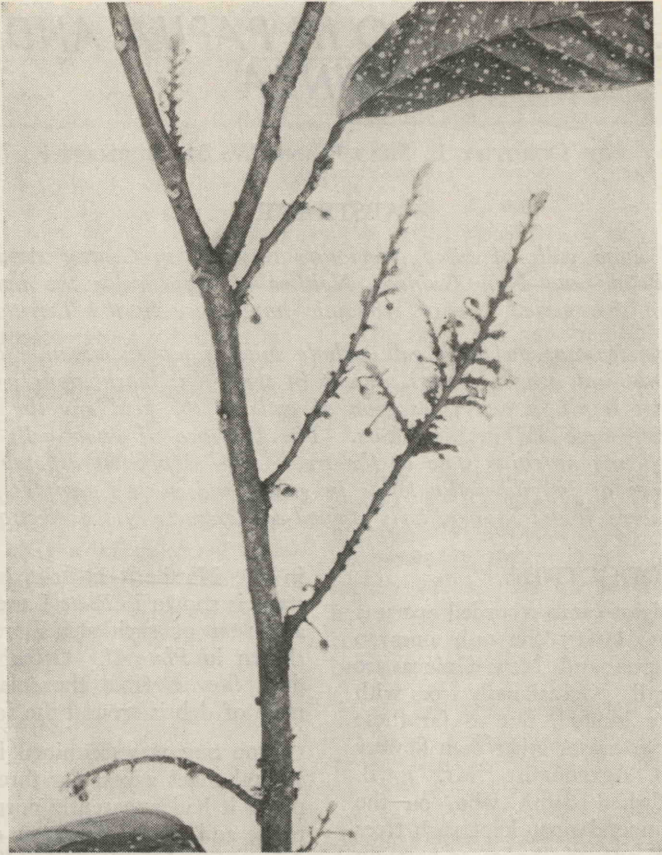


Plate I.—An early stage of fan gall of cacao. Note the flowers on the small shoots and the shortened internodes.

and now on cacao in Papua and New Guinea. It may occur in the other cacao-growing areas of the world but has not been recorded.

Trees and Collections Examined.

As well as the fan galls examined on trees in the field in New Britain, the Markham Valley on the New Guinea mainland and the Popondetta area of Papua, and later collections from these trees studied in the laboratory over a period of four years, other collections examined were as follows : TPNG 4187, C. Levy, New Britain ; 24.VI.64 ; TPNG 4188, P. G. Hicks, New Britain, 25.VI.64 ; TPNG 4238, J. Millar, Markham Valley, New Guinea, 17.VIII.64 ; TPNG 4260 and 4261, N. Robinson, Northern District, Papua, 28.VIII.64 ; TPNG 4478, A. E.

Charles, Northern District, Papua, 28.II.65 ; TPNG 5493, J. B. O'Donohue, Northern District, Papua, 24.VI.65.

KNOB GALL.

Galls similar to knob gall described overseas have been recorded in the Territory in New Britain, New Ireland and on an island off the New Guinea mainland.

Knob galls were described by Hutchins and Siller (1961) as hard, woody, smooth-surfaced swellings, which may occur in the flower cushion, but which bear no flowers. The galls were said to be widely distributed* but were

* Presumably throughout the American cacao-growing areas ; no records in other countries are known to the authors.

relatively unimportant. It was stated by the above authors that an affected tree seldom showed more than 10 to 15 galls, usually less, over its entire fruiting area. Hutchins (1964) reported that knob gall developed in tissues after excision of flowery galls. Tollenaar (1966) reported that in Ecuador deficiency of boron in cacao led to vast swellings along the main trunk and often to the formation of knob galls.

Habit and morphology of knob galls in the Territory.

In the Territory knob galls have been found occurring on the main trunk, especially immediately below the jorquette (*Plate III*), on the primary branches, especially just above the jorquette, and occasionally higher up the tree. At the New Guinea island location galls often

occurred in rows, on one or both sides of the fan branches (*Plate IV*, A, B and C) reflecting the $\frac{1}{2}$ phyllotaxy of these branches. At other times only one gall was found on a branch, and sometimes only one gall on the tree. In New Ireland galls were frequently found just below the forks of branches as shown in *Plate XI*, in each case examined with a slight inclination downwards. Most galls examined have been up to $1\frac{1}{2}$ in. in diameter (4 cm.), although some, especially in New Ireland, measured up to 6 in. long, 5 in. wide and 4 in. in radial cross section (approximately 15.2 by 12.7 by 10.2 cm.) and had bases as wide as the gall at the site of insertion onto the branch.

Galls were found on branches on which some cushions were flowering (*Plate IV*, A and B) and on trees which were bearing pods.



Plate II.—Fan gall on cacao. Note the accumulation of dead flowers from the small branches forming a mass of debris around the gall.



Plate III.—Tree with very large galls on trunk.

The galls were hard, woody, smooth-surfaced swellings, agreeing well with the description given by Hutchins and Siller (1961). Occasionally a tree was found with enlarged lenticels and in these cases the lenticels on the gall were also enlarged (Plate IV, D). The swellings were mainly hemi-spherical, that is, with a base usually or nearly as wide as the gall. Some were slightly pointed, mainly on the uppermost side, especially when the galls were on upright trunks or branches; some galls were slightly asymmetrical. A few galls illustrating these points are shown in Plate V.

Dissections of Galls.

Notes on the dissection of individual galls are as follows :

A gall just over 1 in. (2.8 cm.) long, 1½ in. (4 cm.) wide and 1 in. (2.5 cm.) in radial section is shown in Plate VI, A. The same gall is shown in profile in Plate VI, B; the wood still attached to the specimen when received was approximately 1 cm. thick. In Plate VI, C, a portion of the reverse of the block is shown; the position of the gall is marked by the vascular trace at a depth of 1 cm. in the wood.

In Plate VII, A, the same gall is shown with the bark cut away, revealing the bulbous outthrust of wood protruding from the normal circumference of the branch. In Plate VII, B, the bulbous outthrust has been pushed through the bark, leaving a hole, as clearly shown in the illustration. Although the longest axis of the gall was the horizontal, the longer axis of the opening in the bark through which the gall protruded was the vertical axis. The peeled excised gall, attached to the paper by a pin in Plate VII, B, can be seen to have grain forming concentric circles around the horizontal axis of the gall tangential to the trunk. A third centre of grain also occurred near the top, slightly off centre. Strips of tissue resembling thick irregular, slightly shredded strands of stiff cotton could easily be stripped off the gall at this stage.

In Plate VII, C, a cut across the gall near one of the ends clearly shows the concentric circles of tissue.

Microscopic examination revealed that :—

1. No pith or lysigenous cavities were present in the wood of the gall;
2. The tissue was composed of secondary xylem, i.e., ray parenchyma, fibres and xylem vessels, forming the grain lines discernible macroscopically with the bark peeled away; starch grains were few in number and solitary druses of calcium oxalate were noted only occasionally;
3. At the two horizontal extensions of the gall, the secondary xylem elements formed concentric circles, as shown in Plate VIII; and
4. Where the grain from the two ends met the grain encircling the third centre, the elements were mainly continuous but with a change of direction, the angle of change often being quite acute.



Plate IV.—A, B. Galls *in situ* on branches. C. Profile view of some galls. D. Gall with enlarged lenticels on the surface.

In *Plate IX, A*, a portion of trunk is shown with a longitudinal radial cut made through a gall so young as to be hardly discernible above the bark. In *Plate IX, B*, the two halves are opened out showing the young gall in profile and particularly showing the horizontal line of the trace at right angles to the bark leading into

the interior of the branch—only this portion of the wood was still attached to the specimen when it was received at the laboratory.

The wood supporting one of the galls originally shown in *Plate IV, C*, was exposed by transverse section and as is shown in *Plate X, A*, the gall is directly opposite to the sector formed of

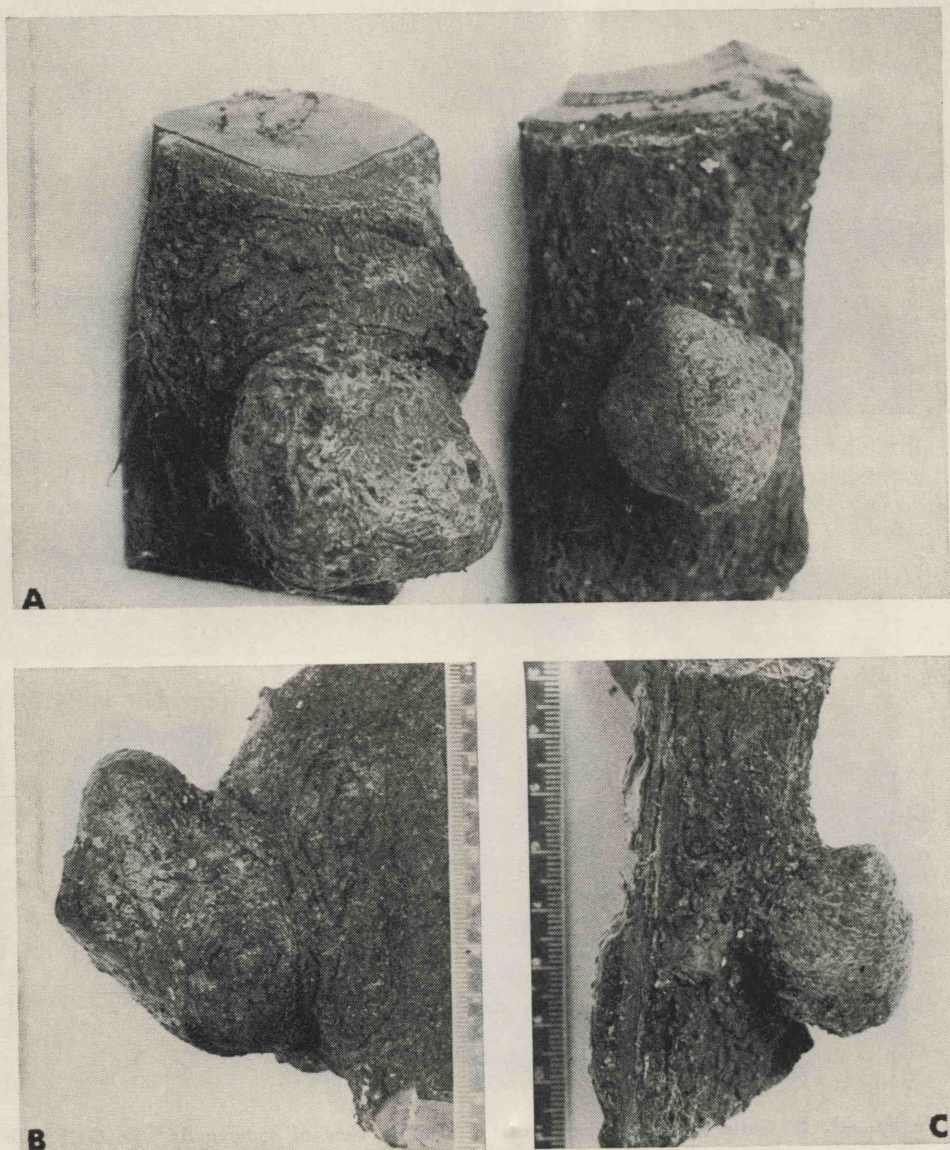


Plate V.—A. Two galls on cacao trunks. B, C. Two galls in profile. (Scale in cm.).

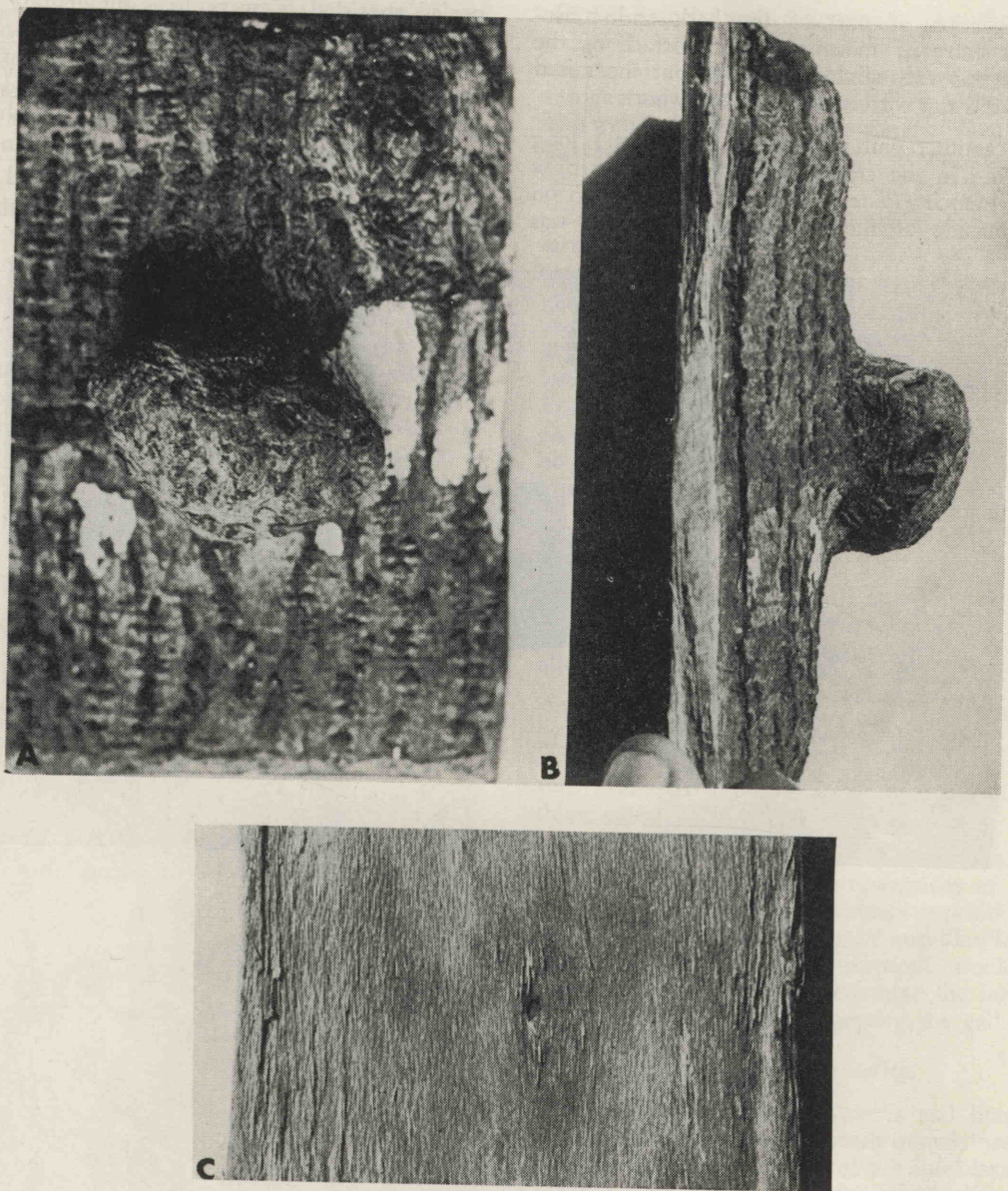


Plate VI.—A. Gall on cacao trunk. B. Same gall in profile. C. Reverse of A, with the position of the gall indicated by the 'core' in the wood.

gap parenchyma commencing at the edge of the eccentric pith; this is shown microscopically in Plate X, B.

In one large gall, 6 in. long, 5 in. wide and 4 in. in radial cross section (15.2 by 12.7 by

10.2 cm.) whorls of grain were visible on the outside of the wood when the bark was peeled off. The pattern was particularly evident because some of the parenchyma between the xylem and fibres had disintegrated due to

deterioration of part of the bark and consequent entry of moisture. The surface of the wood was very slightly knobbly, but the raised areas did not correspond with the whorls.

In another gall nearly 3 in. (7.5 cm.) long growing in the crease below the junction of a large branch (*Plate XI*) the pattern of grain on the outside of the wood when the bark was

peeled consisted of wavy lines integrading and some intermixing with whorls. Nine crests were counted along 1 in. of surface, the distance between crests and troughs being about $\frac{1}{8}$ in. Sections at and parallel to the surface are shown microscopically in *Plate XII*. It will be noted that the arrangement of continuous angled and whorled elements on the surface of this gall was

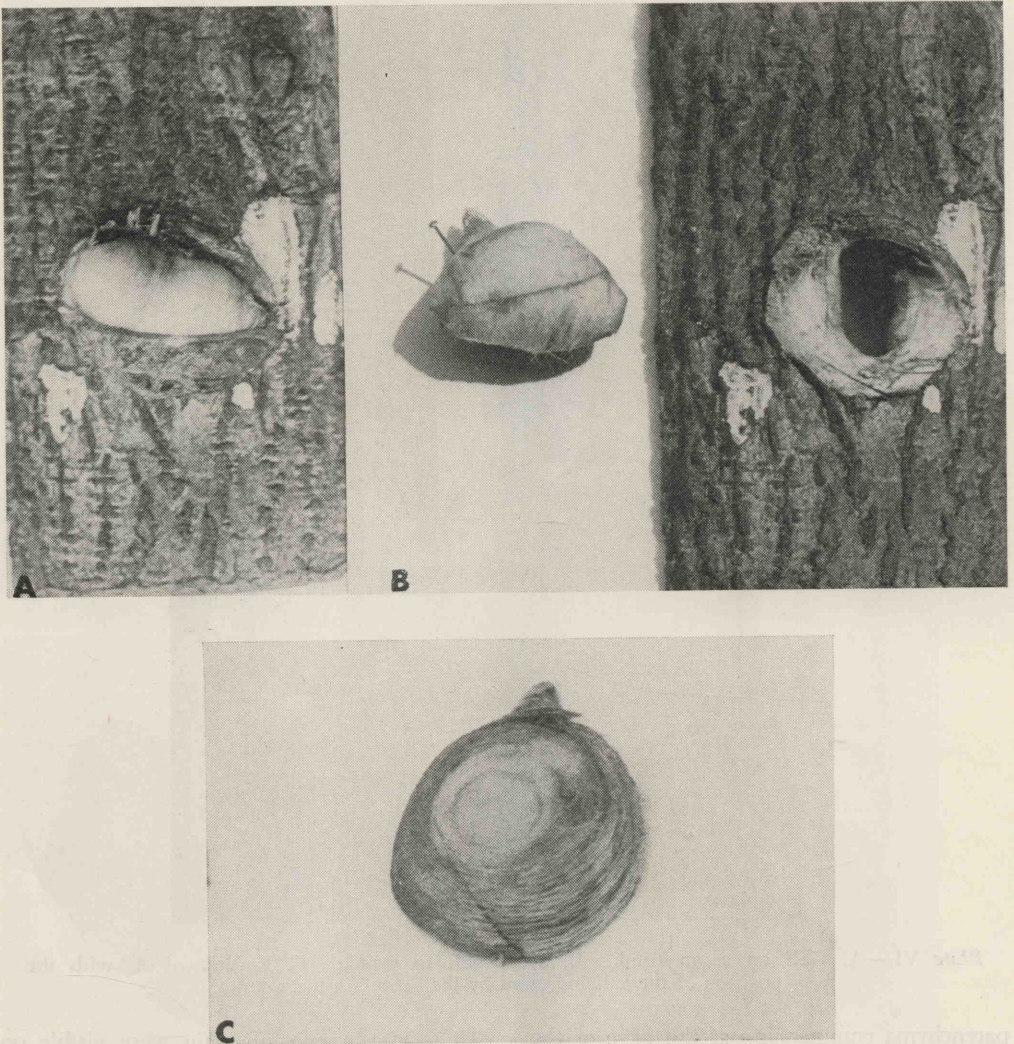


Plate VII.—A. Same gall as in *Plate VI*; bark peeled from gall. B. The peeled gall excised from the underlying wood and removed from the hole in the bark. (The straight line is a scalpel cut.) C. The concentrically-zoned wood of the gall revealed after the removal of the end. Note the grain running around the peeled gall.

of a type similar to the continuous angled and whorled elements from the interior of the gall shown microscopically in *Plate VIII*, which had straight grain on the surface except at the ends and one other centre.

The pattern of wavy lines and whorls was also impressed on the inside of the bark. Sections through the cambium zone of the gall did not reveal anything which could be recognized as abnormal when examined microscopically. Sections through the bark, however, revealed that the triangularity of the fibre bundles of the cortex and the reverse triangularity of the parenchyma capping the rays were greatly distorted. The bark of this gall could be fairly easily pulled apart by the fingers, mainly into irregular layers, a week or so after harvest. This ease of disintegration of the tissues was no doubt due to the disorganization of the elements of the cortex—the bark of the normal branch was still quite firm at that stage. During the dissection of the wood bearing the gall, no vascular trace or parenchymatous gap arising from the centre

of the branch was recognized. As the gall occurred in the crease below the junction of the branch, in a region of complex tissue, the trace and gap may have been missed during dissection. The wood of this gall, unlike some of the others, was particularly compact even weeks after harvest.

In all except one of the dissections of galls carried out to date, the position of the gall has corresponded with a right-angled parenchymatous gap in the wood supporting the gall; the vascular traces were no doubt present but were difficult to identify. Brooks and Guard (1951-1952) stated that the flowering cushion is derived from the solitary axillary bud of a leaf; the parenchymatous gap and trace opposite a gall is no doubt the trace and gap complex of the original leaf and axillary bud.

The linear orientation of many of the galls on fan branches, especially on cacao from the New Guinea island location, also supports the contention that vascular traces and parenchymatous gaps subtending galls were probably originally those of the leaf and axillary bud at that site.

On some occasions it was difficult to determine by external visual examination whether galls, especially those on trunks, occurred at cushion sites; internal dissection would be necessary in these cases.

In some cases galls seemed to occur near but not at cushions. While the eccentricity of some of the cacao stems and perhaps repositioning caused by haphazard excising by unskilled labour may account for these, dissections would be necessary in each case to determine the internal condition of the wood supporting the gall.

Possible Associated Abnormality.

In sections of wood of trunks and branches bearing galls it was occasionally noted that collapse of the ray parenchyma of the outer layers of the secondary xylem occurred in wood adjacent to the galls. When such wood was examined even with a hand lens about one week after collection, minute wedges of holes were present. This collapse of the outer ray parenchyma may have only taken place with differential drying in the immediate vicinity of the gall of the excised wood block and may never be manifested in the growing tree.

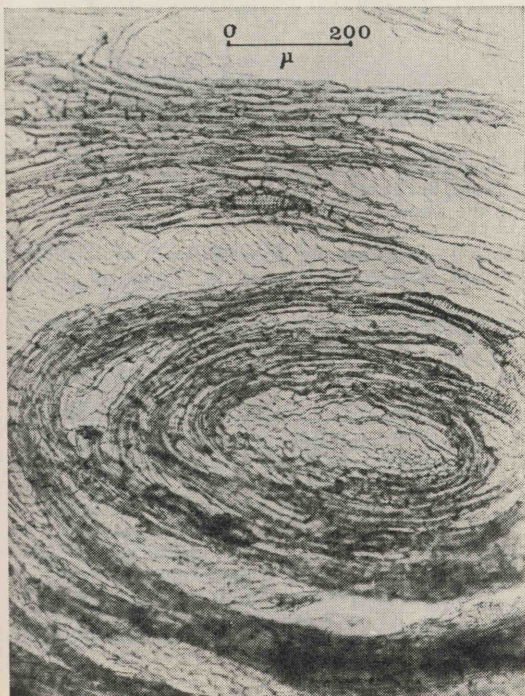


Plate VIII.—Section through one end of a gall showing whorled secondary xylem.

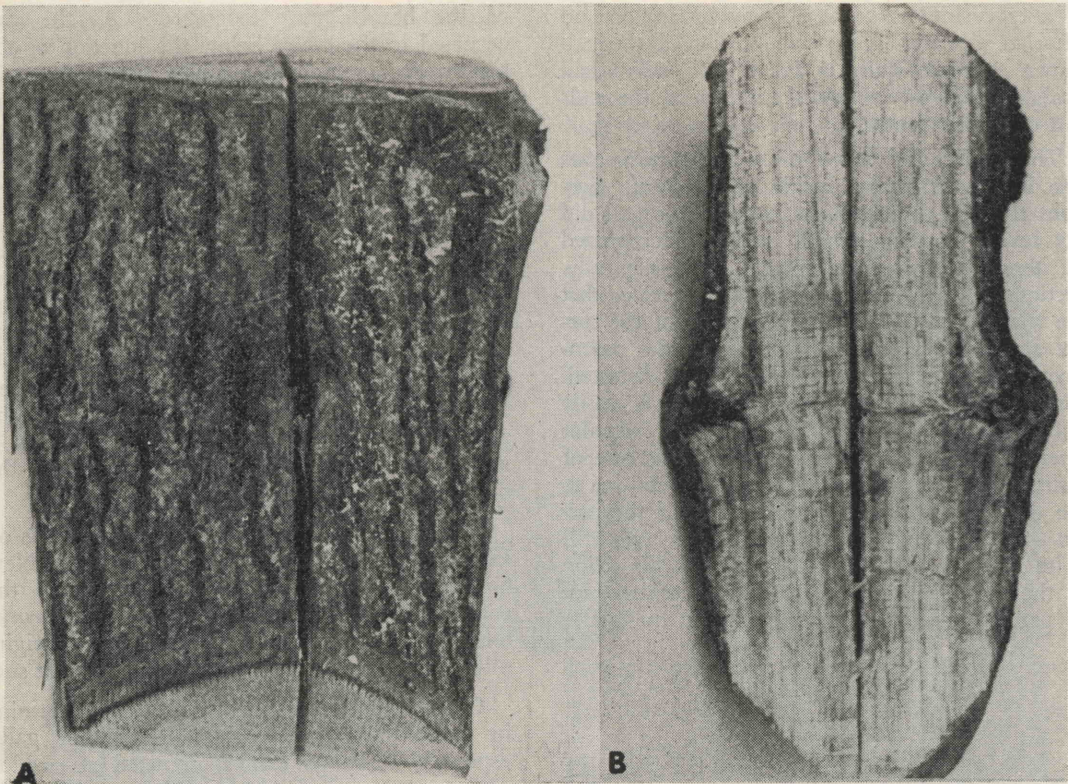


Plate IX.—A. Portion of cacao trunk with a radial cut through a young gall. B. Same block opened out to show young gall in half profile. Note horizontal black line running from centre of gall deep into the wood.

Galls and underlying wood with vertical traumatic lysigenous canals.

Some of the wood of trees with galls were found to have vertical traumatic lysigenous canals (Plate XIII, A and B). The wood of seven galls chosen at random by a field officer in New Ireland was examined to determine whether the canals always occurred in wood of the trunk or branch on which galls were found. The results are shown in Table 1.

From the Table it will be seen that some galls arose from wood in which no v.t.l. canals occurred. Since Shaw (1968) examined other specimens of wood with v.t.l. canals from which no galls arose, it appears as if the occurrence of galls and v.t.l. canals is coincidental.

Table 1.—Presence or Absence (—) of Traumatic Vertical Lysigenous Canals in Trunk or Branch Bearing Galls.

Size of gall. (tangential axis). cm.	Examination of wood.
14	One ring* of canals deep in wood.
7	Several rings of canals, possibly relating to previous injury (Plate XIII, A).
4	Two rings of canals (Plate XIII, B). (At the time this illustration was taken, the bark had been peeled and the gall excised from the wood; the position of the gall, however, is still evident.)
0.5	One ring of canals on one side only.
2.5	—
1.5	—
1.5	—

* One ring visible macroscopically—each ring may be composed of several rows of lysigenous canals.

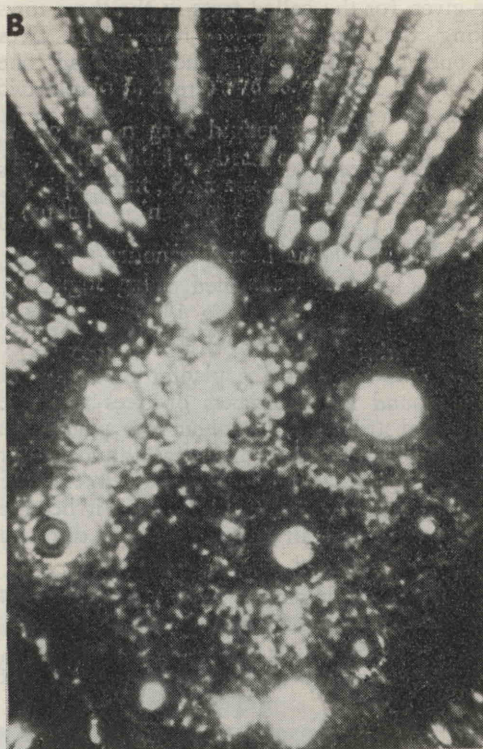
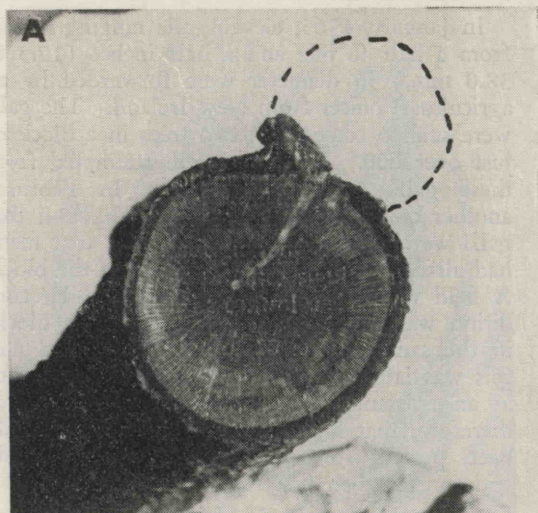


Plate X.—A. Transverse cut showing parenchymatous sector in the wood subtending gall which was previously removed. B. Transverse section through same stem showing pith and the beginning of the parenchymatous sector issuing north of the pith, 33x.

History of the occurrences.

1. New Britain.

One gall was received from a plantation in a relatively isolated area of New Britain in August, 1963. It was a typical knob gall. The planter was advised to destroy the tree, and, despite subsequent inquiry, no further notification of galls has been received from this plantation.

2. Island off the New Guinea mainland.

In November, 1966, a plantation owner and the agricultural officer at the above locality advised that one tree with galls had been located. One gall forwarded to Port Moresby, however, was very old, and had obviously been present for some time. As a precautionary measure, the planter was advised to destroy the tree and to report any further occurrence immediately.

In early January, 1967, another tree with galls was found and destroyed. The planter was requested to provide information on the position of the second tree in relation to the first.

In early February he advised that the second tree was about 150 yards (137 m.) from the first. He also reported that during his inspections he had found another area of 21 trees with galls and a third area of two trees with galls. The larger area was about one mile (1.6 km.) from the first tree found on the plantation, with the smaller area about 250 yards (228.5 m.) further away. The trees were mainly eight years old, with a small proportion five to ten years old.

The relative positions of the affected trees in the larger area are shown in *Figure 1*, the planter's assessment of the number of knobs per tree also being shown. No positions, however,

were given for the galled trees in the three rows flanking the most affected row. Two trees had one old gall each; one tree had one gall only on a branch; other trees had from two galls to more, and three trees were said to be 'covered'. The concentration of galled trees around two trees with one old gall each is suggestive of distribution around a centre. The area shown in *Figure 1* is at sea-level about 250 yards (228.5 m.) from the shore.

Some of the galls examined in the laboratory at Port Moresby were up to $1\frac{1}{2}$ in. (nearly 4 cm.) in diameter, and two were confluent.

By middle February the planter reported that he had found large numbers of trees with the gall well distributed throughout the plantation. This was confirmed by the agricultural officer who stated that 'many dozens' of trees were found with the condition, and that "it became apparent that the gall was much more common than at first thought".

It is stressed, therefore, that it is not known for how long galls had been occurring on trees on the plantation before the report of November, 1966, or how many trees were affected at that

time. It seems certain that the condition had been occurring for quite some time before it was reported.

3. New Ireland.

In January, 1965, eleven galls ranging in size from a half to one and a half inches (12.5 to 38.0 mm.) in diameter were forwarded by an agricultural officer from New Ireland. The galls were said to come from two trees in a block of just over 600. Isolations were attempted from these galls, as reported later. In February another officer checked the area and found that galls were present on some trees but that many had already been cut off other trees in the block. A field worker also claimed that a similar condition was occurring on cacao on other blocks in the same general area of New Ireland and this was later confirmed in a survey carried out by an assistant pathologist. It must be stressed, therefore, that the condition must have already been present for some time and was already widespread when first reported.

Although there is no claim in the literature that knob gall is infectious, arrangements were made in early March, 1965, for all trees in the

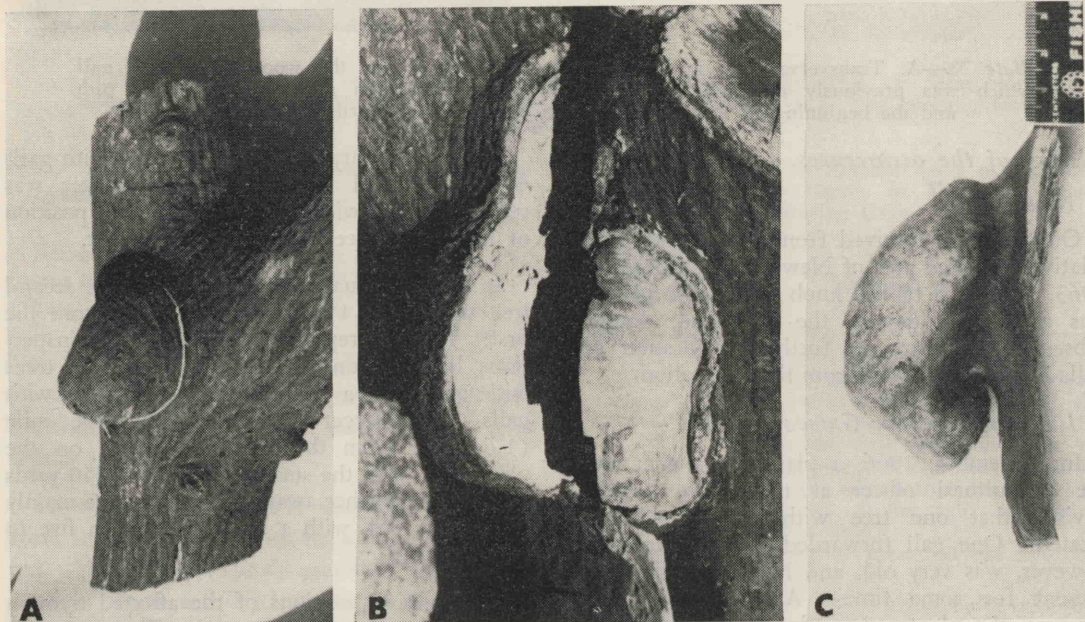


Plate XI.—A. Gall below junction of branch, $\frac{1}{4}x$. B. Gall split longitudinally and bark peeled from one half, $\frac{1}{4}x$. C. Gall in profile, bark removed, $\frac{1}{2}x$.



Plate XII.—Sections at and parallel to the surface of fall shown in Plate XI.

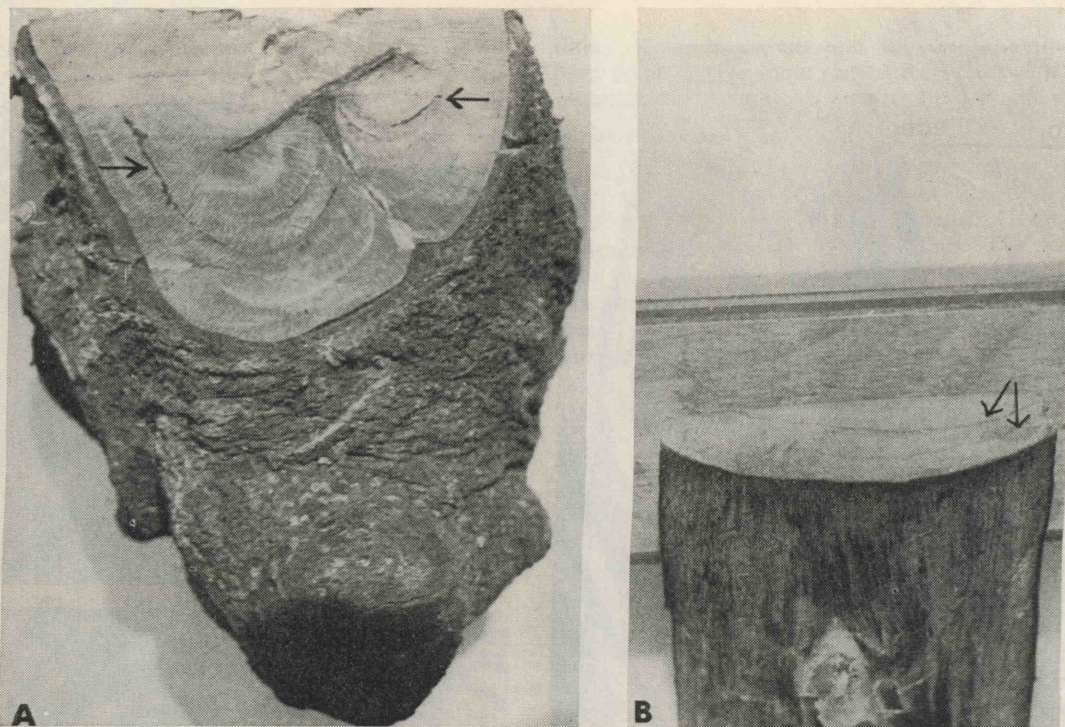


Plate XIII.—A. Cross section of portion of trunk above a large gall, showing deep-seated probable wound and partial rings of vertical traumatic lysigenous canals, indicated by arrows. B. Wood block with bark peeled and gall excised, showing two rings of vertical traumatic lysigenous canals, indicated by arrows.

block to be inspected, and any trees with galls were marked on the trunk with paint. Trees with galls were counted and marked by the local staff during the following year to try and determine whether any spread was occurring. However, continual difficulty was experienced with the labourers who persisted in cutting off galls during the regular removal of water shoots, despite instructions to the contrary; reliable results were, therefore, not obtained.

Occurrence of galls on cacao at two readings six months apart.

In May, 1966, each tree in the block of 600 in New Ireland was examined by the senior author who noted the presence or absence of galls, scars, and galls from scars. Similar readings were taken approximately six months later. The position and type of tree is shown in Figure 2, and the numbers of trees in each class are shown in Tables 2 and 3.

When reading the Tables and Figure, it should be kept in mind that before and during this six month period the trees, which were about 15 ft. high and interplanted with *Leucaena leucocephala* (Lam.) de Wit to provide shade and which were growing on shallow soil derived from coral, were subject to normal plantation procedure. This involved harvesting of mature pods by cutting with unsterilized knives and the removal of water shoots using unsterilized knives; also, despite instructions to the contrary, the labourers still apparently cut off galls from time to time, also with unsterilized knives.

From Table 2 it will be noted that at the first inspection 235 trees had galls but scars were plainly evident on 75 other trees, making 310 trees which had had or then had galls.

At the inspection six months later 181 trees had galls, with a cutting mark evident on one other tree, making 182 trees which obviously had galls or had had galls.

Table 2.—Numbers of Normal and Galled Cacao Trees at Two Readings Six Months apart.

Condition of tree.	May, 1966.		November, 1966	
	No.	Per cent.	No.	Per cent.
Normal	258	43.0	382	63.7
Galled	160	26.7	152	25.3
Gall scars	75	12.5	1	0.2
Galls and gall scars	39	6.5	0	0.0
Galls, some at least from scars	36	6.0	29	4.8
Missing	32	5.3	36	6.0
	600	100.0	600	100.0

These figures show that 128 more trees had or had had galls at the first reading than was evident six months later. Table 3 shows the detailed analysis of the tree readings. It seems that many of the galls had been removed, and scars had healed sufficiently to be unnoticeable, especially those at the higher levels which were more difficult to see in the deep shade under

the cacao and *Leucaena* trees. In some cases at least, however, the removal of galls did not prevent a new gall from arising from the old scar.

During the readings every care was taken to try and distinguish cuts caused by the removal of water shoots and those caused by the removal of galls, which mainly occurred higher up the trunk above and below the jorquette. While some errors may have occurred, scars scored as having arisen from galls comprised only 8.3 per cent. of the total and even if all the scars had arisen from water shoot scars the general position is not affected. It will also be noted that out of the 258 normal trees at the first readings, 227 or 88.7 per cent. were still normal at the end of six months. This was despite the continual harvesting of pods and removal of water shoots with unsterilized knives, and the probable removal of galls themselves from other trees during this period, also with unsterilized knives. Only 5 per cent. of new trees acquired galls during the six months' period.

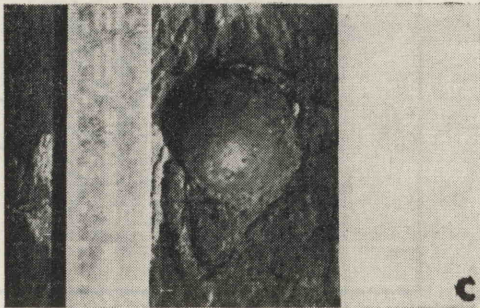
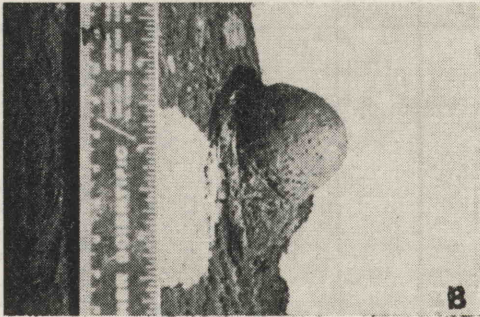


Plate XIV.—A. Profile view of knob gall growing in centre of scar of an excised gall. The tip of the pencil indicates the edge of the callus tissue. B and C. Profile and front view of another gall rising from scar; note callus tissue.

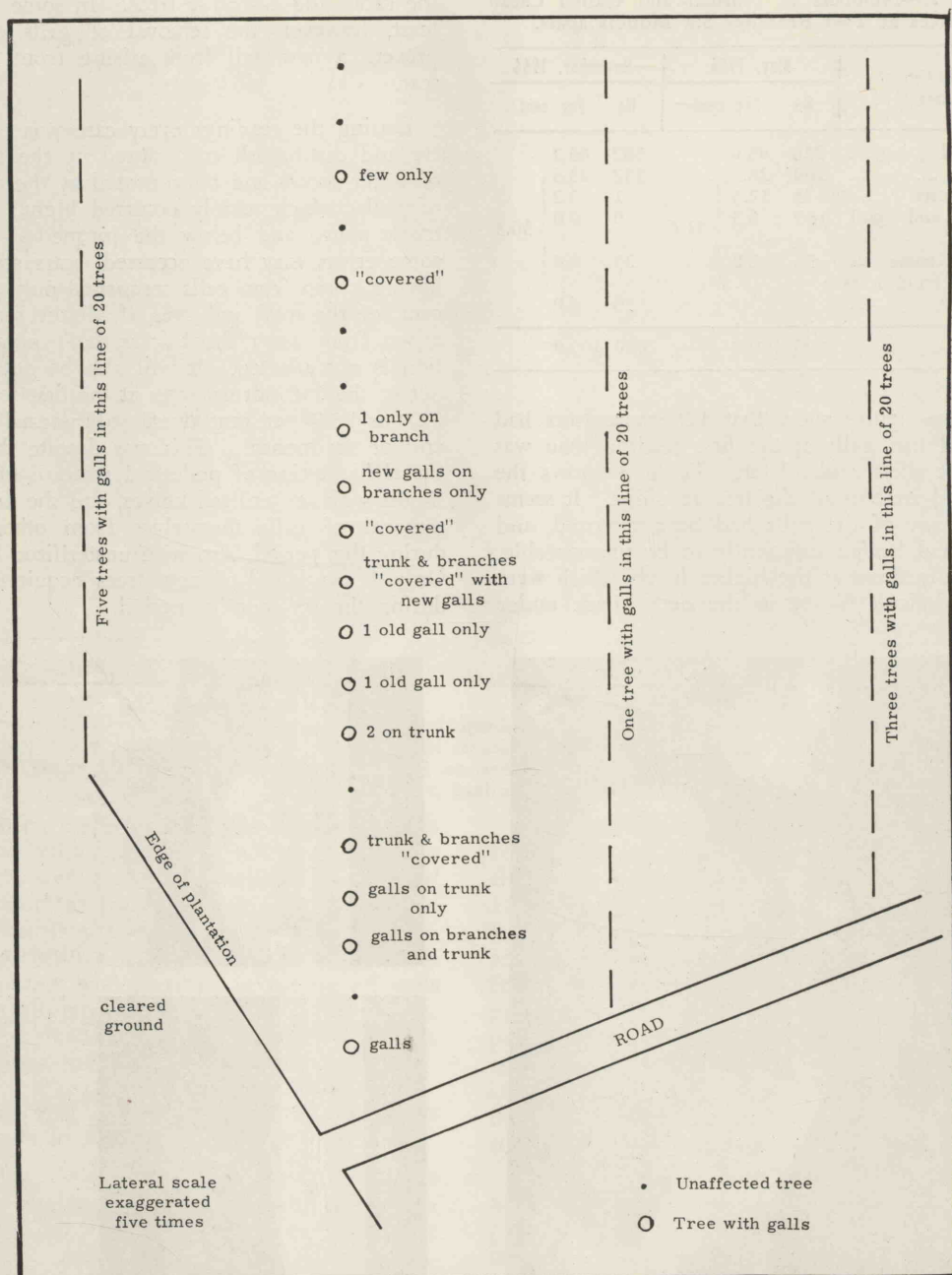


Figure 1.—Cacao trees with galls, New Guinea island. Distance between trees on vertical scale about 12 feet.

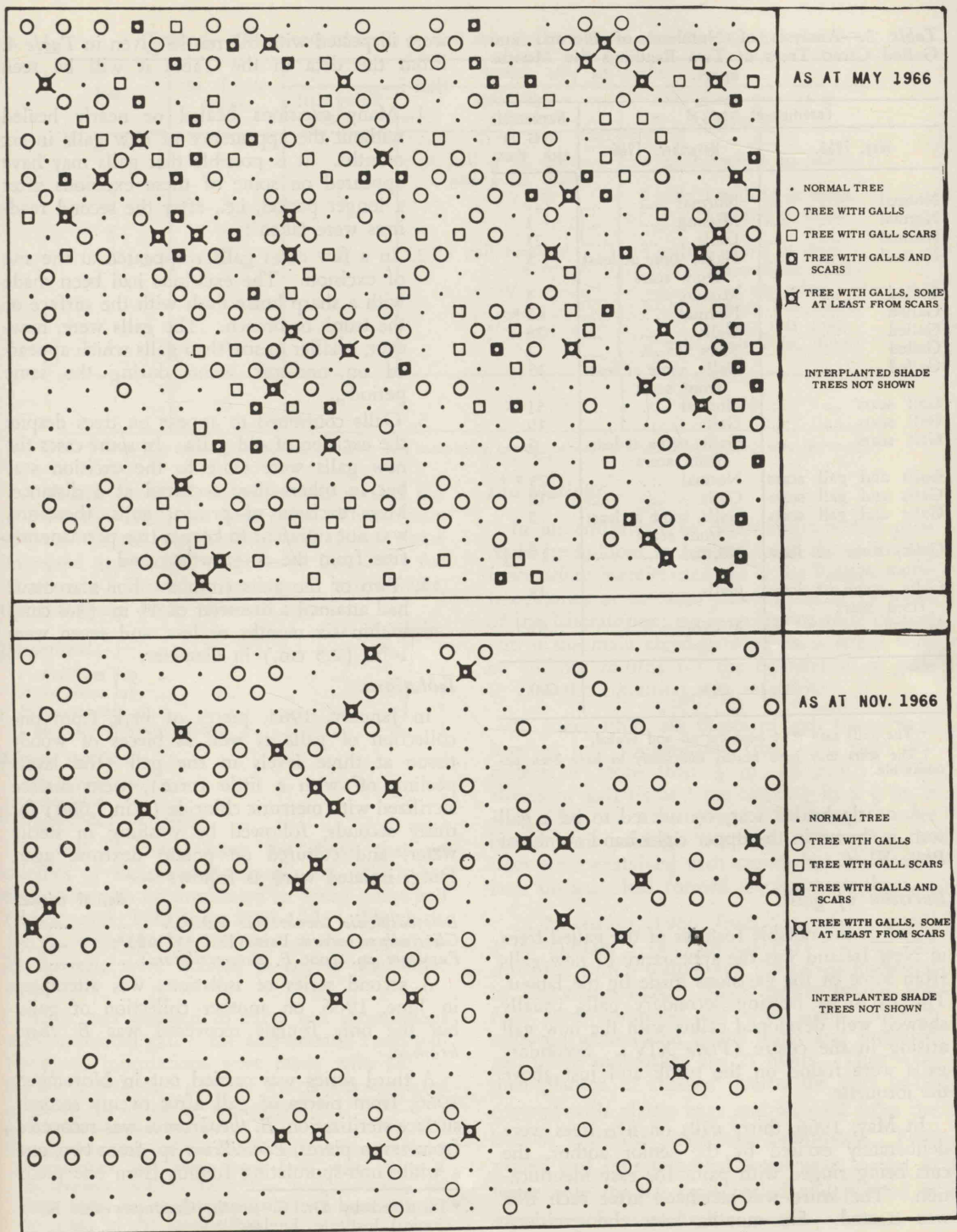


Figure 2.—Galls and scars on cacao trees at two readings six months apart.

Table 3.—Analysis of Numbers of Normal and Galled Cacao Trees at Two Readings Six Months apart.

Condition of trees at		Number of trees in each class.
May, 1966.	November, 1966	
Normal	Normal	227
Normal	Missing	1
Normal	Galls	25
Normal	Galls, some at least from scars	5
Galled	Missing	3
Galled	Normal	65 *
Galled	Galls	78
Galled	Scars	1
Galled	Galls, some at least from scars	13
Gall scars	Normal	51
Gall scars	Galls	19
Gall scars	Galls, some at least from scars	5
Galls and gall scars	Normal	23 * †
Galls and gall scars	Galls	12
Galls and gall scars	Galls, some at least from scars	3
Galls, some at least from scars	Normal	15 * †
Galls, some at least from scars	Galls	18
Missing	Missing	32
		600

* The galls may have been cut off and healed.
† The scars may have healed sufficiently to have been unnoticeable.

A nearly healed scar, considered to be a gall scar, is shown in the upper right hand corner of Plate VI, C.

Excision of galls.

One of the notable features of the galled trees in New Ireland was the appearance of new galls from some of the excisions made by the labour. The excisions bearing secondary galls usually showed well developed callus with the new gall arising in the centre (Plate XIV). Secondary galls were found on the trunk and just above the joquette.

In May, 1966, thirty galls on five trees were deliberately excised by the senior author, the cuts being ringed with paint for easy identification. The knife was sterilized after each tree was treated. Six months later the excisions

were inspected with the results given in Table 4. From the data in the Table it will be seen that :—

- 1. Many excisions healed or nearly healed without the appearance of new galls in six months. It is possible that galls may have appeared on some of these excisions after a longer period, i.e., after the second readings were taken ;
- 2. In a few cases galls reappeared at the site of excision. The excisions had been made with a sharp knife flush with the surface of the trunk or branch. The galls were, however, smaller in size than galls which appeared on non-scar tissue during the same period ;
- 3. Galls continued to appear on trees despite the excision of old galls. In some cases the new galls were close to the excision scar but in others they occurred at a distance. Mere excision of present galls, therefore, was not sufficient to keep a tree permanently free from the outgrowths ; and
- 4. Two of the galls from the non-scar tissue had attained a diameter of 1½ in. (3.8 cm.) within six months or less, and seven were 1 in. (2.5 cm.) in diameter.

Isolations.

In January, 1965, pieces of bark from one collection of galls as well as pieces of woody tissue at three levels in the gall (the layers peeling off with a little force), were surface sterilized with mercuric chloride (1 in 1,000) for thirty seconds, followed by washing in sterile water, and cultured on potato dextrose agar. Fungi isolated were as follows :—

	No. of colonies.
<i>Botryodiplodia theobromae</i> Pat.	18
<i>Gliocladium roseum</i> Bain (IMI 112160)*	4
<i>Fusarium</i> sp. (not <i>F. decemcellulare</i>)	4

A second series of isolations was attempted in June, 1966, on another collection of galls, but the only fungus recovered was *B. theobromae*.

A third series was carried out in November, 1966, from pieces of gall after twenty seconds surface sterilization. *B. theobromae* was recovered from seven pieces, *Penicillium* sp. from two, and a white non-sporulating fungus from one piece.

* Identified by Dr. C. Booth, Commonwealth Mycological Institute, England.

Table 4.—Occurrence of Galls on and near Excision Scars after Six Months.

Tree.	No. of galls excised.	Readings after six months.		
		Scars without galls.	Scars with galls.	No. of new galls not on excision scars.
1	9	7	1 gall $\frac{1}{2}$ in. diam. 1 gall $\frac{1}{4}$ in. diam.	4 galls each 1 in. diam.
2	3	3		2 galls, 1 with $1\frac{1}{2}$ in. diam. 1 with $\frac{1}{2}$ in. diam.
3	6	6 *	1 gall forming	5 galls, 3 with $\frac{1}{2}$ in. diam. 1 with $1\frac{1}{2}$ in. diam. at scar edge 1 with 1 in. diam. near scar edge
4	6	5		
5	6	6		2 galls each 1 in. diam.
	30	27	3	13

* Very big edges on callus tissue, some cuts completely healed, others with unhealed opening into the wood.

A fourth series of isolations was carried out in February, 1967, on three galls. From tissue without surface sterilization and from tissue immersed in mercuric chloride (1 in 1,000) for 20 seconds, followed by washing in sterile water, the following were obtained :—

No. of colonies.

Unsterilized—

<i>Penicillium</i> sp.				14
<i>Aspergillus</i> sp.				1
<i>Pestalotiopsis</i> sp.				1
Unidentified white colony				1

Surface sterilized—

<i>Penicillium</i> sp.				8
<i>Aspergillus</i> sp.				2
Unidentified (4 types)				5

It will be noted from the above that no colonies of *Fusarium decemcellulare*, which has been shown to be implicated in green point gall (Brunt and Wharton, 1961-1962; Hutchins, 1965), were obtained in any of the isolations. No bacteria were isolated in any of the above tests.

As it was decided to carry out inoculations with macerated gall tissue and extracts from gall tissue, no inoculations were made with any of the above isolates,* none of which was suspected of being the cause of the galls.

* In another study of fungi isolated from cacao, many inoculations into cacao have been made with *B. theobromae* isolated from this host, although not from galls, but to date no galls have been recorded from any of the inoculations.

Inoculations.

In an endeavour to determine whether galls could be induced in healthy trees, the following inoculations were carried out at the Port Moresby laboratories or in cacao planted within 12 miles of the laboratories; no inoculations were carried out in the main cacao-growing areas either with or without records for the occurrence of galls in case the condition was infectious.

In June, 1966, six pieces of gall tissue were inserted into young cacao trees with stem diameters of more than $\frac{1}{4}$ in. (6 mm.). The slivers of gall tissue were about $\frac{1}{4}$ in x $\frac{1}{8}$ in. x $\frac{1}{8}$ in. (6 x 3 x 3 mm.) and after insertion were bound with adhesive bandage. Thereafter the trees were examined each month but 12 months later no galls had formed anywhere on them.

In November, 1966, fresh tissue from a gall $\frac{3}{4}$ in. (nearly 2 cm.) in diameter was cut and shredded with a scalpel and the pieces immersed in de-ionized water. Eight newly peeled and four seeds unpeeled but with some mucilage removed were added to the water and shredded gall tissue and soaked for 21 hours. They were then planted in crushed quartz, the pots being covered with plastic bags and watered from the bottom until the seeds had germinated. Four peeled and four unpeeled seeds were soaked in water only for the same period and planted in separate pots as controls.

The emerging seedlings were examined weekly but six months later no galls had formed on the cotyledons or on the stems or petioles.

In February, 1967, a third series of inoculations was made on trees about seven years old near Port Moresby as follows :—

Gall extract inoculum—Four cuts about three inches (7.6 cm.) long by approximately one half inch (1.3 cm.) deep were made on the limbs about two feet (0.6 m.) above the jorquette in each of four trees, the knife being run through a gall still attached to a portion of the branch before each cut. Eight of the cuts were covered with adhesive bandage for five weeks until the first readings were taken ; eight cuts were left uncovered ;

Controls—As above, but the knife was drawn through healthy cacao tissue instead of through gall tissue prior to inoculation ;

Gall tissue inoculum—Pieces of gall tissue about $\frac{1}{2}$ in. (1.3 cm.) square and one sixteenth inch (1.5 mm.) deep were inserted at four sites in each of four trees, eight of the insertions being covered with adhesive bandage for five weeks ; eight insertions were left uncovered ; and

Controls—As above, but healthy cacao tissue was inserted instead of gall tissue.

No galls or malformed tissue was observed on or near the inoculation sites on any of the trees during the succeeding four months.

In February, 1967, 48 seeds were obtained from two healthy pods from trees in a non-gall area, and treated as follows :—

12 seeds. Peeled ; soaked in macerated gall tissue in water for 24 hours ; planted in soil in pots at the laboratory ;

12 seeds. Peeled ; each seed punctured with a needle three times on each side ; soaked in macerated gall tissue in water for 24 hours ; planted in soil in pots as above ;

12 seeds. Peeled ; soaked in water without gall tissue for 24 hours ; planted as above (controls) ; and

12 seeds. Peeled ; punctured with a needle as above ; soaked in water only for 24 hours ; planted as above (controls).

Four months later no galls or malformations had occurred on any part of the seedlings grown from the above seeds.

In April, 1967, a further series of inoculations was carried out on two-month-old seedlings as follows :—

Inoculum of macerated gall tissue suspension—

4. plants. Inoculum introduced per hypodermic syringe at three different positions into each seedling.

4. plants. Inoculum introduced per hypodermic syringe into base of seedlings.

4. plants. Inoculum introduced per hypodermic syringe at tip of seedlings.

Inoculum of gall tissue—

8 plants. Inoculum consisting of piece of gall tissue approximately one sixteenth inch (1.5 mm.) square by one quarter inch (3 mm.) thick inserted into each stem.

Inoculum of macerated gall tissue—

4 plants. Soil around seedlings watered with a suspension of macerated gall tissue.

Controls—

12. plants. Inoculated with water only per syringe.

8 plants. Inoculated with slivers of healthy cacao wood.

4. plants. Seedlings watered with tap water only.

All the injections and insertions were covered with adhesive bandage for at least four weeks, but some bandages were not removed at all.

Seven months later no galls or malformations were present on any of the above seedlings.

From the four series of inoculations carried out no galls were obtained in any of the test material. Further inoculations using different methods will be required before it can be stated firmly that the condition cannot be transferred by inoculation.

Sowing of seed from a tree with galls.

Seeds from two pods from a tree with galls in New Ireland and seeds from two pods from a tree in a non-gall area were dipped separately in 95 per cent alcohol for five seconds, washed in de-ionized water and sown in soil in pots in

the Port Moresby laboratory. Seven months after sowing no galls had appeared on the 16 seedlings raised from the pods from galled trees or on the 20 seedlings raised from seeds from normal trees.

It seems unlikely to the authors that a genetical factor is involved causing the production of knob galls on some trees but not on others.

Reported association overseas of knob gall with boron deficiency.

Tollenaar (1964) reported that in Ecuador deficiency of boron led to vast swellings along the main trunk and often to the formation of knob galls in cacao.

The Senior Chemist of the Department of Agriculture, Stock and Fisheries, Mr. P. J. Southern, kindly made available to the authors the results (unpublished) of analyses carried out on cacao leaves from the gall area in New Ireland and from non-gall cacao in many other parts of the Territory. The figures are shown in Table 5.

Table 5.—Range and Average p.p.m. of Boron in Cacao Leaf Samples from Gall and Non-Gall Areas in New Guinea.

Location.	No. of Samples.	Boron in p.p.m.	
		Range.	Average.
Gall area, New Ireland	15	32-44	38
Gall area, New Guinea island	1		45
Non-gall areas, various sites throughout the Territory	103		39

As is evident from the Table, both the range and average boron in p.p.m. of cacao leaves from the two gall areas are similar to those from the non-gall areas in the Territory.

Tollenaar considered that boron deficiency could be induced on soils with high calcium. While the gall area in New Ireland is on soil containing high amounts of available calcium, other non-gall areas in the Territory with similar leaf boron content are on soils with a similar or higher calcium status. Also, no other symptoms such as those described by Maskell *et al.* (1953), Lockard *et al.* (1959) or by Loue (1961-1962) for boron deficiency in cacao seedlings under

controlled conditions were present on the galled cacao, nor were there any of the other symptoms described by Tollenaar for cacao with boron deficiency in the field. It would seem, therefore, that the knob galls in this Territory cannot be attributed to deficiency of boron.

Mr. Southern summarized the position regarding the general nutrient status of the soils as follows :—

"One of the areas where galls occur is situated on fertile volcanic soil and soil and cacao leaf analysis do not indicate likely mineral deficiencies or toxicities. The other area is limestone derived soil of lower fertility but which still has a relatively high nutrient availability, as judged by both soil and leaf analysis. Many other cacao growing areas where galls are not occurring are chemically poorer. There is thus no evidence of mineral deficiency or toxicity associated with the production of galls in this Territory and in particular little possibility of an association with boron deficiency, as reported by Tollenaar (1964) in Ecuador."

Electron microscope check for virus particles.

In April, 1967, Dr. Adrian Gibbs of the Australian National University, Canberra, kindly examined in the electron microscope sap extracts from the sources listed below, the material having been mounted on grids and negatively stained with potassium phosphotungstate in Port Moresby using the methods described by Hitchborn and Hills (1965) :—

Unaffected cacao—

Strips of tissue from the underside of leaves ; and

Cambium zone and bark of stems.

Cacao with knob gall—

Strips of leaves from the underside of leaves ;

Cambium zone and bark of gall ;

Stem of young shoot from tree with galls ; and

Water shoot of three eighth in. (0.9 cm.) diameter arising from gall.

Gibbs (personal communication) found no virus-like particles in the preparations, though extracts of *Crotalaria anagyroides* leaves showing

mosaic symptoms, mounted in the same way and at the same time, contained filamentous virus-like particles. These results suggest that there is little likelihood of a virus with elongated particles being present in the trees with galls. A virus with isometric particles whose concentration is lower than about 10^{11} per ml. might be present, however, because small isometric virus particles are not easily distinguished from sound plant sap constituents in the electron microscope, unless they are abundant.

Check on giberellin and auxin content.

In 1967 arrangements were made with one of the English laboratories of Imperial Chemical Industries Ltd. to undertake bio-essays on cacao gall material in order to determine whether gibberellins or auxins occurred in gall tissue in excess of that found in normal cacao tissue. Samples were obtained from both New Ireland and from the island of the New Guinea mainland and were sent to England by air. Each consignment consisted of the following :—

1. Galls of various sizes freshly cut from branches or trunks ;
2. Portion of branch from a tree with galls ; and
3. Portion of branch from a tree never known to have had galls.

Dr. D. Broadbent of the Biochemical Research Department of Imperial Chemical Industries Ltd. reported (personal communication) that the method used by Mr. G. W. Elson for the assay of growth substances, and the results, were as follows :—

" Samples of sawdust produced from the wood were weighed and extracted by stirring with 70 per cent. methanol for 12 hours at 2 degrees C. Each sample was extracted twice. The two extracts were bulked and the methanol removed by vacuum distillation at 35-40 degrees C. The aqueous residue was divided into two equal fractions A and B.

Fraction A.—adjusted to pH2 with dilute hydrochloric acid and extracted three times with equal volumes of diethyl ether. The solvent extract was dried over anhydrous sodium sulphate, evaporated to dryness and assayed for indole auxin.

The dried extract was taken up in ethanol/ether/water (50 : 40 : 10 v/v/v) and strip loaded on to Whatman No. 1 chromatography paper. The chromatograms were developed with water/isopropanol (95 : 5 v/v) at 2 degrees C. in the dark. The strips were cut at 0.1 R.F. intervals and each segment eluted with 5 ml. of a solution containing 0.05 m. glucose and 0.0025 m. potassium dihydrogen phosphate at M4.8. Ten 5 mm. oat coleoptile segments were placed in each dish. The segments were measured after four hours incubation at 25 degrees C. in the dark.

Oat coleoptiles were grown for 96 hours at 25 degrees C. in continuous red light. Coleoptiles 25 mm. to 35 mm. in length were selected, the 3 mm. apical segment removed and the next 5 mm. segment used in the assay.

Each extract was made up from 8 gm. of material, as below :—

Extract 1 : from separated galls ;

Extract 2 : from branch of tree with galls ; and

Extract 3 : from branch of tree without galls.

There was some activity in each extract but only in Extract 3 was there much activity ; the overall pattern was very similar for all three extracts. Further tests would be desirable on more samples.

Fraction B.—adjusted to pH2 with dilute hydrochloric acid and extracted three times with one third volumes of ethyl acetate. The aqueous phase was discarded and the solvent phase extracted three times with equal volumes of 1 per cent. sodium bicarbonate. The solvent phase was discarded and the aqueous phase adjusted to pH2 and extracted three times with equal volumes of ethyl acetate. The ethyl acetate extract was dried over anhydrous sodium sulphate and the solvent removed by vacuum distillation. The residue was assayed for gibberellins. All extracts were stored at 20 degrees C. between operations.

Gibberellin assay.—The residue was dissolved in ethyl acetate and replicate aliquots of this solution equivalent to 20 gm. of the fresh weight were pipetted into assay vials containing the aqueous assay substrate. The ethyl acetate was removed under vacuum and the assay was carried out using barley endosperm assay (Jones and Varner, 1967).

The total gibberellins content of all three samples was equal to 10^{-3} ug/kg fresh weight. There was no significant difference between galls, gall-infected- or gall-free wood."

Economic importance.

To date knob gall has not been of economic importance in the Territory. If many galls occur on flower cushions, however, yields may be affected. It was because of this possible contingency that observations and experimental work were commenced on the condition several years ago.

Collections examined.

Apart from the knob galls studied in the field by the senior author and those studied in the Laboratory, other collections examined were as follows : TPNG 3985, R. Kelly, New Britain, 9.VIII.63 ; TPNG 4434, J. Cox, New Ireland, 26.I.65 ; TPNG 5324, G. R. Forbes, New Guinea island, 2.II.67, TPNG 5427, R. Burnett, New Ireland, 13.IV.67 ; TPNG 6161, D. Brown, New Ireland, 10.IX.68 ; TPNG 6401, J. Owen-Turner, New Ireland, 7.II.69 ; TPNG 6408, J. Owen-Turner, New Ireland, 13.II.69.

DISCUSSION.

Galls on plants can be caused by nematodes, mites and insects (Mani 1964), but dissection of knob galls in the Territory has not revealed any direct evidence of the above, although this itself cannot, of course, rule out these organisms as possible causes of knob gall.

The failure to transmit the condition from macerated gall tissue after inoculation into peeled cacao beans, seedlings and trees, and the lack of virus particles in sap extract preparations in the electron microscope examined by Dr. Gibbs would seem to indicate little likelihood of a viral cause, unless a virus with isometric particles in low concentration which is not easily transmitted by the methods used is present.

Certain bacteria and fungi can cause galls in cacao, for example *Agrobacterium tumefaciens* (Brunt and Wharton, 1961) and *Fusarium decemcellulare* (Brunt and Wharton, 1961, 1962 Hutchins, 1965). Although no bacteria or *F. decemcellulare* have been isolated in the tests carried out to date, and although no transmission was obtained in the inoculation experiments, neither bacteria nor fungi can be ruled out as possible causal organisms without further tests.

The galls occur in one area which is minerally relatively rich and in another area which, although on poorer soil derived from coralline rock, nevertheless is not chemically poorer than other soils of a similar composition in the Territory which are growing cacao without galls. It would seem therefore, that a mineral deficiency or toxicity is not involved. There is especially no evidence that deficiency of boron, which Tolenaar (1964) found associated with knob gall in Ecuador, is causing this condition in the Territory.

Cushions with flowers or pods were found adjacent to or interspersed with galls. If the cause is a nutritional or physiological one, considerable selectivity of action must be operating at these sites on the same tree.

Hutchins (1964) reported that knob gall developed in tissues after excision of flowery galls. No prolific flowering such as occurs with flowery gall has been reported from the New Guinea island, and no flowery galls occurred at the site in New Ireland where the senior author carried out the tree inspections and gall excisions, although a few trees did occur with profuse flowering.

It has been demonstrated in this paper that excision of all knob galls on a tree does not prevent the tree from continuing to produce new galls both on or near the scars or on other parts of the tree. Some excision scars healed apparently completely in the period of six months during which they were under observation.

It seems unlikely to the authors that a genetic factor is involved causing the production of knob galls on some trees but not on others, although normal seedlings raised from seed from a tree with galls are probably too young yet for this negative evidence to be conclusive.

The present studies have been handicapped because of the distance of the condition from the Port Moresby laboratories (approximately 400 miles) and the resulting inability to keep close surveillance on experiments in the field. Care had to be taken that no material with knob gall was taken to the other main-cacao-growing areas at present free from the condition, in case an infectious agent was present.

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