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Port Moresby

TERRITORY OF PAPUA AND NEW GUINEA

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[*Continued overleaf*]

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ERRATA.

ABSTRACTS (second page, unnumbered): "Investigation into the causes . . . ." should read "Investigation into the cause . . . ."

p. 173: Footnote at bottom of second column should be at bottom of first column.

p. 173: Footnote at bottom of first column should be at bottom of second column.

p. 175: Table 5 (*a*), fourth line under "Treatment", "possiblity" should read "possibility".

p. 176: Column 2, line 7 should read: Table 6 (*a*) and (*b*).

ABSTRACTS.

A LIGHTNING STRIKE OF COCONUTS IN NEW GUINEA.

DOROTHY E. SHAW. *Papua and New Guinea agric. J.* 19 (4) : 147-150.

The effects are recorded of a lightning strike on coconuts in a plantation on an island off the New Guinea coast. There was a delayed expression of symptoms (mainly frond break and frond droop), the most severe damage being apparent on 19 palms (including the struck palm) four months after the strike. Six palms died within 100 ft. of the struck palm but others within this radius were either apparently unaffected or showed only mild to severe symptoms. The recovery period of the 12 affected palms which did not die coincided in the main with a four-month drought which may have prevented the development of secondary rots. The site was under observation for 31 months.

A NOTE ON THE NON-TRANSMISSION OF CENTROSEMA MOSAIC VIRUS THROUGH SEED.

DOROTHY E. SHAW. *Papua and New Guinea agric. J.* 19 (4) : 151.

No symptoms of *Centrosema* mosaic virus were recorded on 1,252 plants of *Centrosema pubescens* raised from seed derived from *C. pubescens* plants with mosaic symptoms in three tests carried out in Port Moresby. New leaves produced on *C. pubescens* transplants infected with *Centrosema* mosaic virus grown in the same vicinity as the test seedlings had characteristic symptoms under the same conditions.

WELLMAN'S LEAF AND FRUIT SPOT OF ARABICA COFFEE IN NEW GUINEA.

DOROTHY E. SHAW. *Papua and New Guinea agric. J.* 19 (4) : 152-166.

A leaf and fruit spot of Arabica coffee, variety 'Bourbon', in New Guinea is similar to Wellman's blister spot of coffee in Costa Rica, attributed to a virus, and to Bitancourt's oily spot in Brazil. The leaf symptoms appear distinct from those of ring spot of coffee in the Philippines.

In experiments, spots occurred on leaves produced on tips of affected bushes kept under protective insecticide cover, indicating the probable systemic nature of the condition. Spots occurred on the cotyledons of volunteer seedlings in the field at 5,000 ft. altitude but not on the subsequent leaves of such seedlings transplanted to the glasshouse at sea level. In other experiments at sea level, spots were only rarely observed on new leaves grown after transplanting of older affected seedlings and a mature bush or on the new leaves of cuttings taken from affected plants. Nor were spots seen on leaves grown on healthy scions grafted onto affected bushes in the Highlands, except for four spots on three leaves of two grafts.

The condition was not transmitted through 477 seeds from affected bushes, the seedlings having been kept under observation for from 18 to 24 months.

In five areas totalling 629 trees with five per cent. of trees initially affected, only two additional trees (0.3 per cent. of the total) were observed to become newly infected during observation for one year.

[*Continued overleaf*]

No particles which could be ascribed to a virus were observed in preparations of sap extracts examined in the electron microscope.

As at least three common names are being used for conditions with similar symptoms in America, in New Guinea the condition is being designated as 'Wellman's leaf and fruit spot'.

INVESTIGATION INTO THE CAUSES OF LEAF TUMOURS OF TEA SEEDLINGS.

DOROTHY E. SHAW and W. M. BURNETT. *Papua and New Guinea agric. J.* 19 (4) : 167-192.

Tumours occurred on the early true leaves of tea seedlings derived from seed from two localities in New Guinea, and from Ceylon, India, Malawi and Australia, grown in a glasshouse and a laboratory at Port Moresby and in the field in New Guinea, New Britain and Australia. Leaves produced later on these plants were unaffected. Recently a report of leaf tumours of tea seedlings in nurseries in Malawi has also been received.

Experiments reported herein seem to eliminate the following as possible causes of the condition : micro-organisms, mites, insecticide, copper fungicide, hormone herbicide, charcoal packing, sawdust used in pregermination beds, timber used in pregermination boxes, the water supply and the local soil.

Slightly fewer seedlings with tumours occurred with whole or partial removal of the seed coat before germination than when seed remained intact, and slightly fewer with soil than with inert crushed imported quartz. No malformed leaves occurred on 66 'seedlings' developed from embryos excised from their cotyledons and grown to the two to six leaf stage on nutrient agar. Ten times more seedlings developed tumours at high temperatures in the glasshouse than in a coolroom. There were ten times more tumours on seedlings grown from immature 'ripe' seed from capsules picked green from the seed bearers than from mature seed which had dehisced naturally from the capsule.

It is suggested that the cause of the tumours may be a growth substance found in immature 'ripe' seed, with little or no residual substance present in mature seed. The concentration or activity of the substance appears to be affected by the removal of the seed coat and by temperature.

A Lightning Strike of Coconuts in New Guinea *

DOROTHY E. SHAW.†

ABSTRACT.

The effects are recorded of a lightning strike on coconuts in a plantation on an island off the New Guinea coast. There was a delayed expression of symptoms (mainly frond break and frond droop), the most severe damage being apparent on 19 *palms (including the struck palm) four months after the strike. Six palms died within* 100 *ft. of the struck palm but others within this radius were either apparently unaffected or showed only mild to severe symptoms. The recovery period of the* 12 *affected palms which did not die coincided in the main with a four-month drought which may have prevented the development of secondary rots. The site was under observation for* 31 *months.*

INTRODUCTION.

In response to an appeal to planters to advise the Department of the occurrence of lightning strikes in coconut plantations or in cacao, one planter reported that a strike had occurred on his plantation and kindly agreed to keep the area under observation and to report regularly on the onset of symptoms. The following notes and diagrams have been prepared from the data made available by him.

The strike was reported by indigenous eye witnesses to have occurred during a very severe electrical storm on 20th April, 1965, on a coconut plantation on an island off the north coast of New Guinea. The struck palm was one of a pair growing close together side by side, both of approximately the same height as far as could be remembered and part of a planting of palms at 30 ft. by 32 ft. spacing, although a little out of alignment near the seashore.

Notes on the symptoms during the subsequent four months and at seven, eleven, 23 and 31 months, are as follows :—

24*th April*, 1965.

Very little aerial damage was apparent, but the struck palm (marked S in *Figure* 1, A) had two large pieces torn out of its rather exposed root system. Three palms in its vicinity showed slight frond droop.

A few days later there was little change and local New Guineans living close to the strike reported to the planter that visible damage [1] was not observed until well into the second week.

26*th May*, 1965.

At this inspection the struck palm was scored as dead, with another dead palm next but one away, and a third palm (the right hand lower corner palm in *Figure* 1, B) showing very heavy damage. Eight palms including the 'twin' of the struck palm were severely affected and three were mildly affected, as shown in *Figure* 1, B.

19*th June*, 1965.

Only a little further change had occurred at the time of this inspection. It appeared to the planter, therefore, as if the greatest visible change occurred during the first two to four weeks after the strike. A few trees showed serious deterioration and a few more showed mild effects (*Figure* 1, C). At this stage the planter was convinced that quite a number of palms would eventually die.

19*th July*, 1965.

At this inspection three more palms were dead, one of them (right hand top corner in *Figure* 1, D) being some distance from the struck palm and another being removed by one palm. Three

* Compiled from notes submitted by a New Guinea planter.

† Chief Plant Pathologist, Department of Agriculture, Stock and Fisheries, Port Moresby.

1 Affected palms showed either frond break and/or frond droop, although precise numbers of affected fronds were not recorded by the planter. Unfortunately, no detailed examination by a pathologist of the affected parts of the struck and surrounding palms was possible at any time during the recordings because of the relative inaccessibility of the plantation and the distance from Port Moresby.

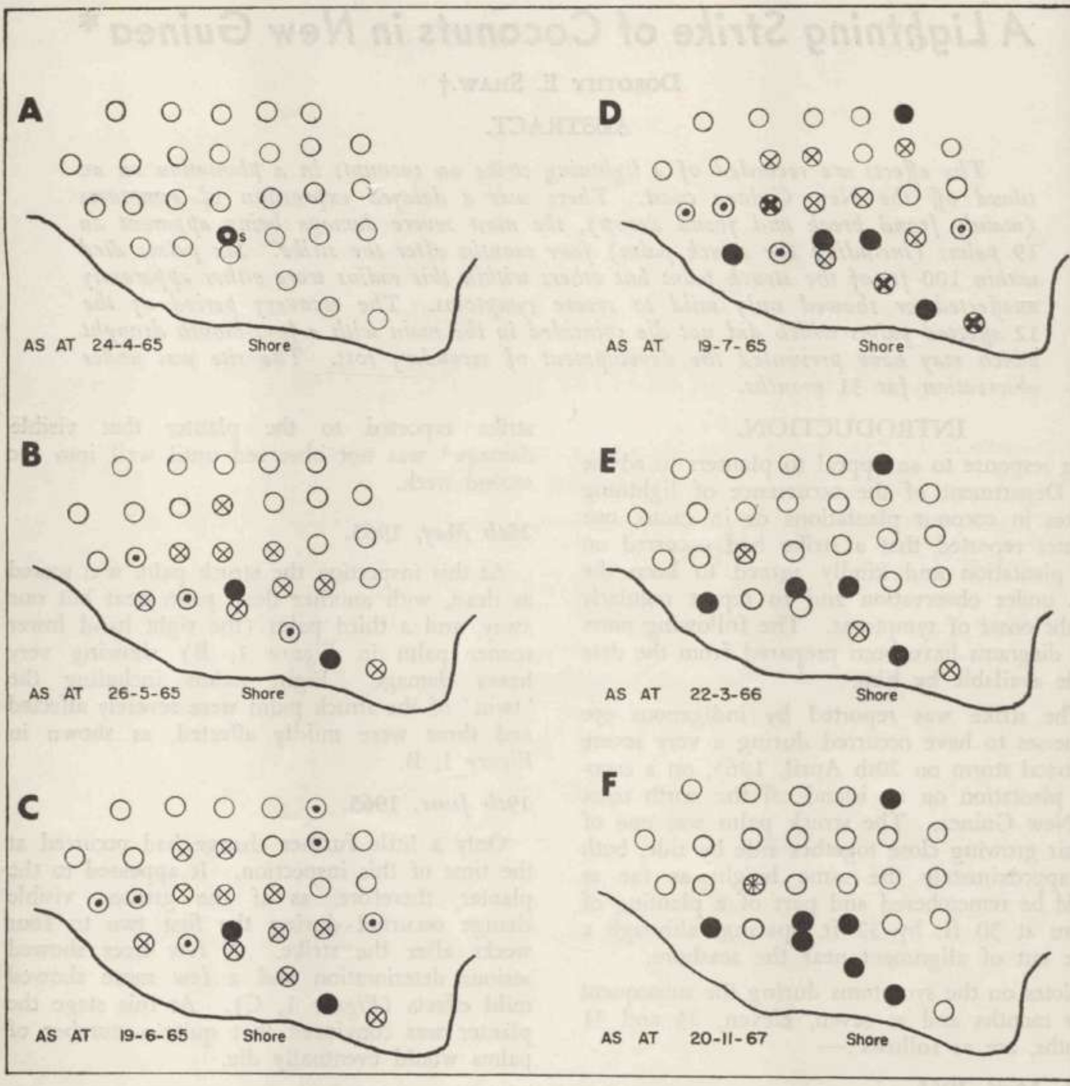

Figure 1.—The effect of ligtning strike on coconut palms over a period of 31 months.

other palms considered dead at this stage later recovered, and these are distinguished in *Figure* 1, D. One other palm which had been only mildly affected previously showed more severe damage. The 'twin' to the struck palm, however, appeared no worse than at the previous two readings, although damage was still severe. At this stage 19 palms were affected.

19th August, 1965.

No material change in the position was observed except that some of the affected palms did not appear to be quite as severely damaged as at previous inspections and the planter thought that some might even recover.

22nd November, 1965.

Observations were resumed in November, when the planter returned from leave. From previous experience of strikes, which are frequent in this area, the planter expected considerable deterioration to have occurred. He reported amazement, however, at the lack of deterioration in affected palms. The 'twin' of the struck tree appeared certain to survive, and the palm (right hand side lower corner *Figure* 1) which in July had appeared certain to die was developing a new shoot.

It should be mentioned that the four months prior to this reading constituted the worst recorded drought in the area, with the entire period practically rainless ; usually at least four inches per month is recorded during that period. The drought reduced the total rainfall for 1965 to 112 in. whereas the average rainfall is said to be approximately 145 in. per annum. During the drought period, however, the sandy soil in which the plotted palms were growing had ample ground water draining to the sea from a nearby swamp.

The relatively dry period following the strike perhaps prevented or reduced secondary fungal and bacterial attacks which might, under conditions of higher rainfall, have administered the *coup de grace* to the affected palms.

22nd March, 1966.

It was evident at this inspection that deaths were confined to the struck palm plus four others, only one of which was within 30 ft. of the struck palm and the furthest was about 100 ft. away on the outskirts of the affected area. All the slightly damaged palms had recovered, while some which at earlier inspection appeared certain to die were producing new fronds. The three palms which had appeared dead in July, 1965, had recovered but were far from healthy-looking and were still devoid of nuts. One palm (unspecified) had what may have been 'stem bleeding' as described by Sharples (1933) —in the present case the 'stain' was stated to "transfer easily enough to a damp handkerchief and if not applied too heavily was light brown in colour ".

The planter was again amazed at the recovery of the palms, many of which he was sure would die when examined nine months previously.

31st March, 1967.

The palm in the right hand lower corner of *Figure* 1 survived, and although its canopy was still in very poor shape it had started to bear nuts again. Two of the palms which had appeared dead in July, 1965, were now carrying a reasonable crop of young nuts, although the cabbages were less dense than those of the surrounding palms. The third palm which had appeared dead in July, 1965, had been blown over by the wind. The 'twin' to the struck palm appeared to be in reasonable condition (*Figure* 1, E).

20th November, 1967.

After an interval of eight months, the planter revisited the site and found that a further two palms had died and their tops had fallen off. They were the twin of the struck palm and the one to the right of it on the beach. The New Guineans living nearby stated that no further strikes had occurred. A third tree, the palm on the left of the struck tree, appeared healthy to the planter although the locals stated that it would die too. When pieces of the cabbage were collected and examined by the planter he found them badly attacked by Hispid.

DISCUSSION.

It should be noted from the above records and the *Figure* that—

- there was an effect on surrounding palms as well as on the palm said to have been struck by lightning ;
- three months after the strike, symptoms were beginning to show upon hitherto apparently unaffected surrounding palms. This is similar to delayed effects of strikes as previously reported by Sharples (1933) for Estate A, by Dwyer (1936), and by Charles (1960) ;
- six palms which ultimately died were growing from three to one hundred ft. away from the struck palm, which died first. Twelve other palms within that radius, however,

showed only mild to severe symptoms ; the 'twin' to the struck palm was one of them, although it eventually died within two-and-a-half years ; ten palms were apparently unaffected ;

the recovery of some of the 12 affected palms which did not die was evident by the fourth month, and the recovery increased with time. Recovery of affected palms was not recorded by Sharples (1933) or by Charles (1960) ; and

the recovery period coincided with a drought which may have prevented secondary organisms from causing rots in some of the affected palms.

ACKNOWLEDGEMENTS.

The planter who provided the notes on the symptoms is gratefully thanked for his co-operation. The Soil Survey Field Assistants helped with the preparation of the figures.

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(Accepted for publication July, 1968.)

A Note on the Non-transmission of Centrosema *Mosaic Virus through Seed.*

DOROTHY E. SHAW.*

ABSTRACT.

No symptoms of Centrosema *mosaic virus were recorded on* 1,252 *plants of* Centrosema pubescens *raised from seed derived from* C. pubescens *plants with mosaic symptoms in three tests carried out in Port Moresby. New leaves produced on* C. pubescens *transplants infected with* Centrosema *mosaic virus grown in the same vicinity as the test seedlings had characteristic symptoms under the same conditions.*

INTRODUCTION.

Centrosema pubescens Benth. has been recorded with mosaic symptoms in the field in Papua and New Guinea since 1954 (Magee 1954), and has been studied by van Velsen and Crowley (1962).

The latter authors found that sap from mosaic-infected leaves of *Crotalaria anagyroides* produced mosaic symptoms on *Centrosema pubescens,* some other species of *Crotalaria, Calopogonium mucunoides, Desmodium distortum, Medicago orbicularis, Stizolobium deeringianum* and *Trifolium subterraneum.* Thirty-nine other species of 23 genera, including species of *Phaseolus* and *Pisum,* and *Vigna sinensis,* were found to be non-susceptible in these tests.

Van Velsen and Crowley reported that no symptoms occurred in 100 seedlings of each species raised from seed from naturally infected plants of *Centrosema pubescens, Crotalaria anagyroides, Crotalaria retusa, Crotalaria goreensis* and *Calopogonium mucunoides* kept under observation for 56 days.

EXPERIMENTAL.

As it was considered important to know whether the causal virus was seed-borne even at low percentage transmission, the following tests were carried out.

Seed from *C. pubescens* plants showing characteristic symptoms of mosaic were collected by two agronomists in the Markham Valley. The seed was lightly abraded with sandpaper in Port Moresby, and sown in pots kept in insect-proof cages. Three such tests were carried out in 1967 and 1968.

In the first two tests with seed from infected plants, 968 seedlings were kept under observation for twelve weeks while in the third test, 284 seedlings were observed for five weeks. No symptoms of mosaic were observed in any of the 1,252 seedlings.

During one of the tests *C. pubescens* plants with mosaic symptoms from around Port Moresby and the Markham Valley were transplanted to pots and kept within a few yards of the insect cage under similar conditions of temperature and shade as the test seedlings in order to determine whether symptoms were still produced on the new leaves in this environment. Young leaves produced after transplanting had characteristic mosaic symptoms.

From the work of van Velsen and Crowley (1962) and from the above tests, it appears that *Centrosema* mosaic virus is not seedborne or that it is of low percentage transmission.

ACKNOWLEDGEMENTS.

Grateful thanks are extended to Mr. C. Edwards and Mr. G. Hill, Senior Pasture Agronomist and Agronomist with the Department of Agriculture, Stock and Fisheries, for obtaining seed from *C. pubescens* plants with mosaic, and to the latter and to Mr. D. Murray of the same Department for obtaining infected plants. Thanks are also extended to Mr. R. M. Burnett for watering the plants during the author's absence from Port Moresby.

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(Accepted for publication July, 1968.)

* Chief Plant Pathologist, Department of Agriculture, Stock and Fisheries, Port Moresby.

Wellman's Leaf and Fruit Spot of Arabica Coffee in New Guinea.

DOROTHY E. SHAW.*

ABSTRACT.

A leaf and fruit spot of Arabica coffee, variety 'Bourbon', in New Guinea is similar to Wellman's blister spot of coffee in Costa Rica, attributed to a virus, and to Bitancourt's oily spot in Brazil. The leaf symptoms appear distinct from those of ring spot of coffee in the Philippines.

In experiments, spots occurred on leaves produced on tips of affected bushes kept under protective insecticide cover, indicating the probable systemic nature of the condition. Spots occurred on the cotyledons of volunteer seedlings in the field at 5,000 ft. altitude but not on the subsequent leaves of such seedlings transplanted to the glasshouse at sea level. In other experiments at sea level, spots were only rarely observed on new leaves grown after transplanting of older affected seedlings and a mature bush or on the new leaves of cuttings taken from affected plants. Nor were spots seen on leaves grown on healthy scions grafted onto affected bushes in the Highlands, except for four spots on three leaves of two grafts.

The condition was not transmitted through 477 seeds from affected bushes, the seedlings having been kept under observation for from 18 to 24 months.

In five areas totalling 629 trees with five per cent. of trees initially affected, only two additional trees (0.3 per cent. of the total) were observed to become newly infected during observation for one year.

No particles which could be ascribed to a virus were observed in preparations of sap extracts examined in the electron microscope.

As at least three common names are being used for conditions with similar symptoms in America, in New Guinea the condition is being designated as 'Wellman's leaf and fruit spot'.

INTRODUCTION.

In 1962, specimens were first received of a leaf spot of Bourbon coffee from the Eastern Highlands of New Guinea and later investigations showed that fruit from these trees was also affected.

The condition resembles blister spot of Arabica coffee described by Wellman (1957) in Costa Rica [1] and attributed to a virus which was not seed-borne but which was mechanically transmitted by the aphid *Toxoptera aurantiae* Koch, symptoms appearing in from two months and nine days up to fourteen months, and by grafting, the first symptoms appearing in two and a half months. The variety of coffee affected was not designated but Wellman (personal communication) stated that it was 'var. *typica*'. Rate of spread was up to 15 per cent. in one year.

Bitancourt (1958) recorded that he first observed a similar disease on coffee leaves and fruit in Brazil in 1938, and called it oily or greasy spot. Experiments on transmission carried out in Sao Paulo were negative. After two years the disease had not spread from the trees where it had been found towards neighbouring trees, which indicated very slow dissemination. The damage did not seem of sufficient importance to warrant further investigation into the disease. No further experimental work was reported in a recent review of coffee

* Chief Plant Pathologist, Department of Agriculture, Stock and Fisheries, Port Moresby.

[1] In October, 1965, the author examined two bronze-tipped plants with blister spot at Turrialba in Costa Rica. The symptoms were identical with those under study on green tipped Bourbon in New Guinea. A few days later the author inspected many bronze-tipped spotted coffee bushes near San Jose with Dr. Luis C. Gonzales, the symptoms again being identical with those of the New Guinea condition.

diseases in Brazil (Silberschmidt and Bitancourt 1965).

The leaf symptoms of the blister or oily spot disease as described by Wellman and Bitancourt, and as seen in New Guinea (see description below), appear to the author to be distinct from those of the ring spot of coffee described by Bitancourt (1939) in Brazil (the transmissibility of which was proved by grafting by Silberschmidt (1941)), and by Reyes (1959, 1961) in the Philippines, where it was recorded affecting Arabica, Excelsa, Robusta and Liberica leaves and fruit, and stems of Excelsa. Reyes, however, considered ring spot to be identical with Wellman's blister spot disease, an opinion not supported by Valdez (1966).

Studies on the New Guinea conditions are reported below.

HOST.

The condition was found on Bourbon coffee in the Asaro Valley in the Eastern Highlands of New Guinea at an altitude of approximately 5,000 ft. and 260 miles from Port Moresby.

Coffee plantations in the Highlands grow only *Coffea arabica* L., consisting nearly exclusively of mixed stands of *C. arabica* var. *arabica* (syn. var. *typica* Cramer), locally called Typica coffee, and of *C. arabica* var. *bourbon* (B. Rodr.) Choussy., locally called Bourbon coffee ; in some cases the former predominates, and in others the latter. A little 'Maragogipe' coffee (sometimes given as *C. arabica* var. *maragogipe* Hort.) is also grown. The condition is confined to Bourbon coffee, even in those plantations where Bourbon and Typica are both growing, sometimes with intermingled branches [1]. Affected bushes are irregularly scattered throughout the plantations although adjacent bushes are occasionally affected.

SYMPTOMS AND MICROSCOPIC EXAMINATION OF LESIONS.

Leaves.

The leaf spots occur mainly on the upper surfaces of the leaves, only occasionally being evident on the under surfaces. They are circular, up to four mm. in diameter, either the same colour or slightly paler green than the unaffected tissue, and if few in number are inconspicuous. To the naked eye they usually have a dull rather than a shiny appearance ; the surface itself appears almost imperceptibly depressed, often with a faint stippling discernible only by moving the lesion under a strong light. Some lesions have a light brown spot the size of a pin prick in the centre. If the leaf is held against the light, the lesions are evident as translucent spots (compare *Plate* I, A and B, showing the same leaf by reflected and by transmitted light).

Apart from the tiny area of dead cells in the centre of some spots only, the tissue of the spots does not become necrotic. In fact, on aged yellow leaves the lesions are usually striking as green spots on the yellow tissue (*Plate* II). On faded leaves found beneath affected bushes the lesions stand out as distinct reddish-brown spots on the dead tissue. The surface of the spot is not convex as in the young leaves described by Wellman, but occasionally heavily affected young leaves have a slightly puckered appearance.

In some cases only a few spots occur per leaf, while in others they cover most of the lamina (*Plates* I and II). In the cases examined the young leaves just expanding were usually free from spots while the first expanded leaves were often spotted. Sometimes leaves without spots were found on the same branch as leaves with spots while occasionally whole branches without affected leaves were found on affected bushes.

The spots were also found on the cotyledons of some volunteer seedlings even before the appearance of true leaves. At that stage the plants would not have been more than six weeks old.[2]

In *Plate* III, a young seedling is shown with about 64 spots on the cotyledons, many being confluent. On other seedlings collected at the same time with spots on the cotyledons, the first true leaves had formed but were unspotted. The behaviour of such seedlings when removed from the field to the greenhouse is discussed later.

[1] In July, 1966, five spots were found on three leaves (which were removed for examination) on a well-grown bronze-tipped bush, presumably Typica, on one of the plantations, although no Bourbon with symptoms occurred in the immediate vicinity. Re-inspection of the plant and about 200 immediately adjacent coffee bushes eight months later did not reveal any spotted leaves.

[2] Estimated after comparison of growth records obtained by Mr. R. S. Carne, Agronomist-in-Charge, Highlands Agricultural Experiment Station, at the request of the author for the age of seedlings before the appearance of the first true leaves.

A

B

Plate I.—A. Leaf of Bourbon coffee with leaf spots, viewed by reflected light. B. Same leaf viewed by transmitted light.

Microscopically the affected tissue is very difficult to distinguish from the unaffected. The cells appear indistinguishable in shape, malformation of pallisade and spongy parenchyma tissue not being detected in sections. However, from the appearance of the spots, which remain green as the leaves yellow with age, it seems as if the main effect is in the chloroplasts, as stated by Wellman, as the spots usually retain their chlorophyll longer than normal leaf tissue. That a change of some magnitude has occurred is evident from the persistence of the spots, even on faded brown leaves.

No hyphae or bacteria were detected in sections or teasings of the leaf spots. Miscellaneous fungal spores were sometimes found on the leaf surfaces, but on unaffected as well as affected tissues.

Stems, Flowering and Fruit Setting.

No lesions have been noted on stems. The length of the internodes and the number of flowers and fruit set appeared to be about normal, whereas Wellman reported that in Costa Rica there was some dwarfing of growth and shortening of internodes, with a reduced number of flowers which set very few fruit. Blackening and early abscission of many of the fruit means that heavily affected bushes in New Guinea are often left with branches with few or no fruit at a time when the unaffected bushes are still carrying their full maturing crop.

Fruit.

Lesions on the fruit are similar to those described and illustrated by Wellman. The New Guinea spots are round, up to two mm. in diameter, light brown in colour and definitely concave. *Plates* IV and V show fruit with various degrees of spotting, and seeds from slightly and severely affected fruit.

All the fruit on an affected branch does not always become spotted, especially on bushes with only a few spots on the leaves (such bushes presumably being fairly recently infected), but most cherries carry from one to about fourteen spots, the spots not being confined to one side although occasionally a preponderence does occur on the upper surface. A dark brown rot sets in on most fruit, often around a depressed spot, sometimes two or more rotted areas occurring on the same fruit and not necessarily towards the terminal half of the branches, as mentioned by Wellman. As

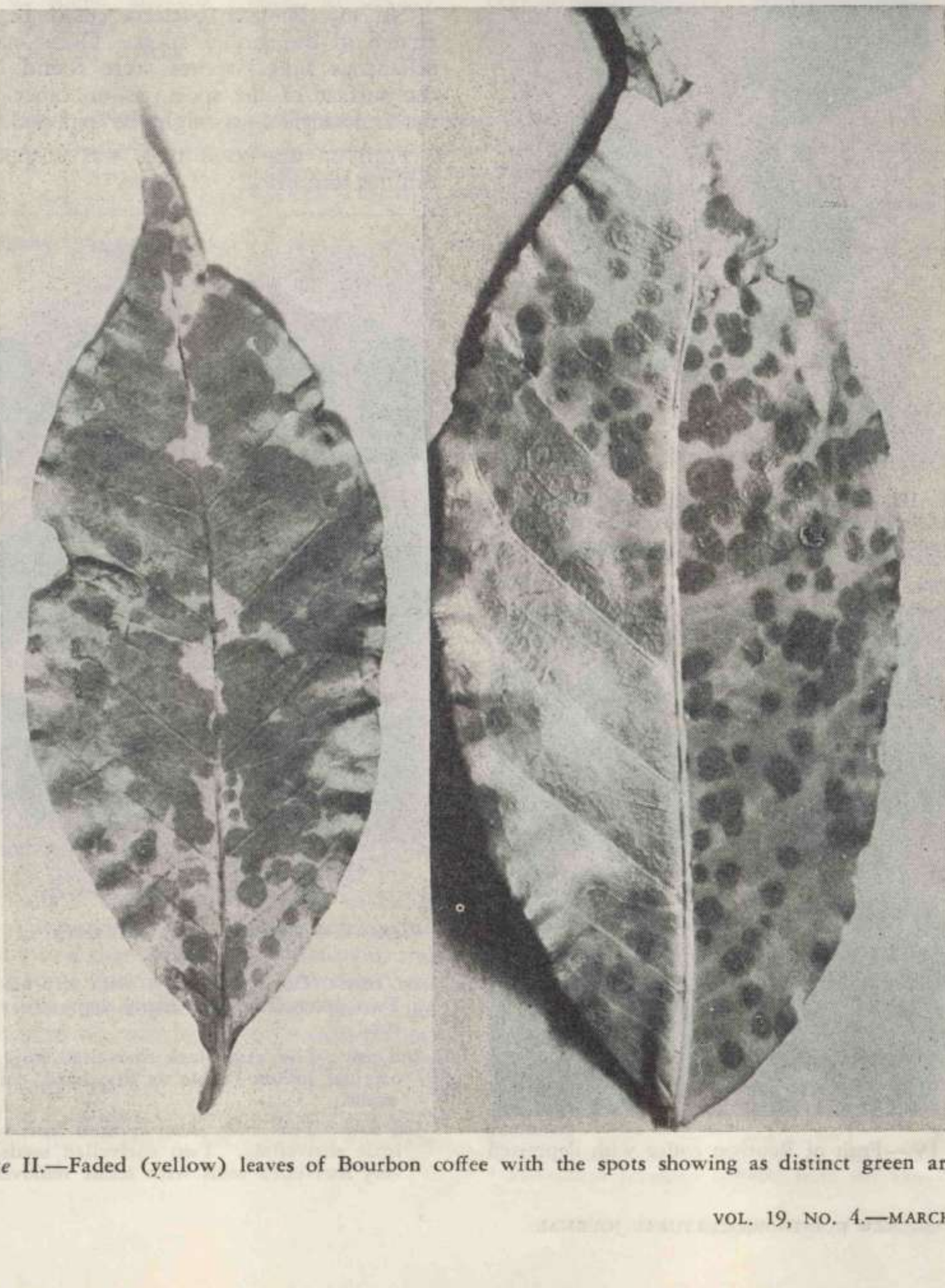

Plate II.—Faded (yellow) leaves of Bourbon coffee with the spots showing as distinct green areas.

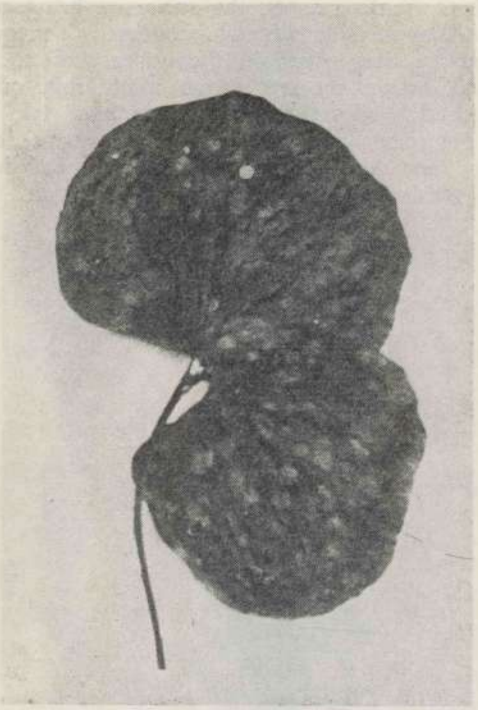

Plate III.—Cotyledons of self-sown Bourbon coffee from around affected bush, with separate and confluent leaf spots, after pressing for 24 hours.

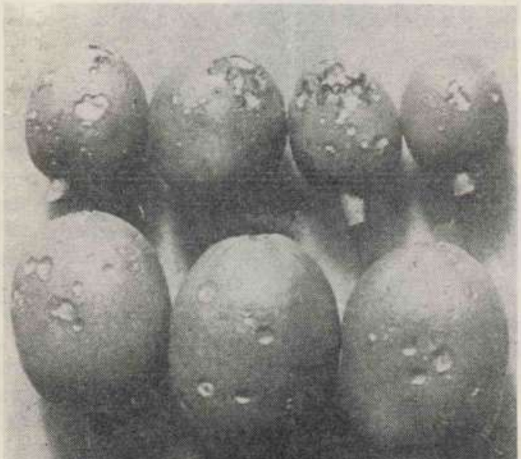

Plate IV.—Fruit of Bourbon coffee with depressed spots.

the fruit blackens and shrinks, the small depressed spot sometimes takes on the appearance of a small light-brown-coloured scab.

Sections through the depressed spots show a collapse of the tissue to a depth of about six to eight rows of cells, the area being most prominent when the fruit is red as the collapsed cells are without pigment.

No mycelium or bacteria could be demonstrated in the smaller spots. Occasionally miscellaneous fungal spores were found lying on the surface of the spots, as on other parts of the fruit surface, as might be expected.

Fruit on unaffected trees was unspotted and did not turn black.

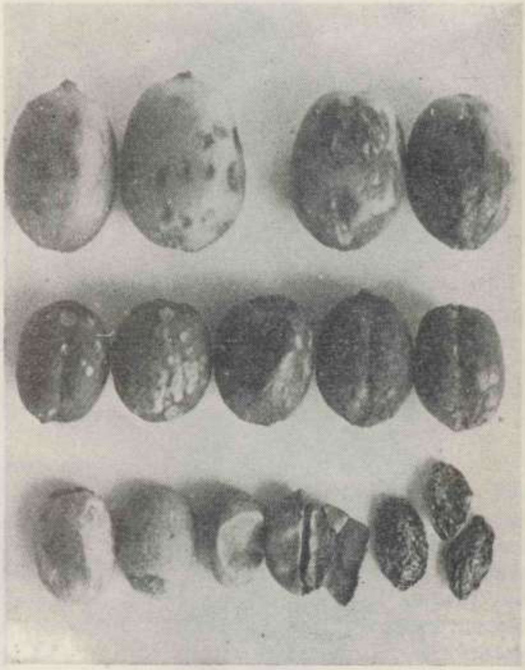

Plate V.—Affected fruit and seeds of Bourbon coffee.

1st row : Two green fruit with depressed spots. Two green fruit with many depressed spots and rot.

2nd row : Five hard black shrivelled fruit with the original lesions visible as dry, hard, pale brown scabs.

3rd row : Two seeds from spotted fruit with very little shrivelling. Two shrivelled seeds. Three very shrivelled seeds with testae removed.

INCIDENCE.

The disease to date has only been found in the Asaro Valley of the Eastern Highlands.

The incidence of the disease on affected plantations as noted in checks carried out at intervals since the condition was first reported has not been high. Growers have been continually advised to rogue affected bushes, to destroy volunteer seedlings with spotted leaves, and to carry out regular checks in order to eliminate possible sources of inoculum. This was done sporadically on some plantations and apparently not at all on others. It was estimated by officers that probably not more than 700 bushes were rogued prior to September, 1967.

In a survey carried out by agricultural officers (Mr. J. J. Nitsche and Mr. W. G. Cowie and assistants) in late 1967, each tree on seven out of the eight known affected plantations was checked and affected trees rogued ; a partial check was carried out on the eighth.

The results of the survey are shown in *Table* 1. Out of approximately 292,000 trees inspected it was estimated that 200,000 were Bourbon, and of these 680 trees were showing symptoms ; no Arabica was found affected. The percentage Bourbon infection, which varied on each plantation, ranged from 0.03 per cent, to 1.2 per cent., the total average being 0.3 per cent. If the estimated number of previously rogued trees is included, the estimated total average infection is 0.7 per cent.

The results of a survey of some smallholdings in the same valley are also shown in *Tables* 1 and 2. Infected coffee was found at 159 out of 931 sites inspected, involving an estimated 399,700 total trees of which the estimated number of Bourbon was 220,600. Of these 1,984 were found affected, giving an average infection of 0.5 per cent. of total trees and 0.9 per cent. of Bourbon (*Table* 1) with the greatest number of infections ranging between 1.1 and 5.0 per cent. (*Table* 2). Special checks carried out by the officers at the few sites with high infections failed to reveal any reason to account for the occurrence. No Arabica was found with symptoms during the survey.

Table 1.—Number of trees inspected and percentage infection in three coffee growing areas.

Area.	Property.	Number inspected.	Number infected.	Total trees inspected.*	Estimated number of Bourbon.	No. of infected Bourbon.	Infected Bourbon. Percentage.	Infected Arabica. Percentage.
Asaro Valley	Plantation	8	8	292,000	200,000	680 (est. 1,380) †	0.3 (0.7) †	0
	Smallholdings	931	159	399,700	220,600	1,984	0.9	0
Kainantu Subdistrict	Plantation and Station	8	0	149,750	24,000	0	0	0
	Smallholdings	4	0	500	200	0	0	0
Wahgi Valley	Plantations	10	0	49,680	16,800	0	0	0
	Smallholdings	172	0	76,200	33,650	0	0	0

* Round figures.

† Including number of trees said to have been rogued previously.

Table 2.—Classes of estimated percentage infection in smallholders' Bourbon coffee in the Asaro Valley.

Percentage of infected Bourbon * in the following classes.	No. of small holdings.
0.1-0.5	32
0.6-1.0	26
1.1-5.0	73
5.1-10.0	13
10.1-15.0	4
15.1-20.0	4
20.1-25.0	1
25.1-30.0	1
35.1-40.0	1
50.1-55.0	1
55.1-60.0	1
75.1-80.0	1
90.1-95.0	1

* Percentage infection of total trees not shown.

Spot checks by the same officers were made at ten plantations and stations and at 172 smallholdings in the Wahgi Valley, as shown in *Table* 1. Of the estimated 126,000 trees inspected, of which 50,500 were estimated to be Bourbon, none was found with symptoms.

In spot checks by Mr. R. S. Carne and assistants of eight plantations and stations and four smallholdings in the Kainantu Subdistrict of the Eastern Highlands, involving approximately 150,250 trees of which an estimated 24,200 were Bourbon, none was found infected (*Table* 1).

No special surveys have been carried out on coffee in the other parts of the Territory, but no specimens or reports of the disease have been received from growers or agricultural officers in these areas.

The incidence of the disease in New Guinea, therefore, has to date been much lower than that reported by Wellman (1957) in Costa Rica, or noted by the author during a visit to Costa Rica in 1965.

As a result of the surveys carried out and the publicity given to the disease in New Guinea especially in the last few years, it is hoped that the recommended control measures—roguing of infected bushes followed by regular inspections—will now be vigorously carried out by growers in the Asaro Valley. Growers elsewhere are urged to keep their crop under close observation and report any symptoms if these are detected.

SPREAD OF THE CONDITION.

While many coffee plantings in the Asaro Valley were started from seed, many others were started from seedlings raised in thousands at several plantations and at other centres. As seedlings up to one foot high have been found with leaf symptoms in nurseries, it is considered that distribution of such seedlings may have acccounted in some measure for the occurrence of the disease throughout the Asaro Valley.

In order to check rate of spread at an infected site four areas chosen at random consisting of 79, 103, 125 and 262 trees (totalling 569 trees, in a block of several thousand Bourbon [1]) were scored and kept under observation for one year in 1966-1967 during which three inspections were carried out. Twenty-nine of the 569 trees (5 per cent.) were affected at the begining of the period and thirty at the last inspection, the newly affected tree (an increase of 0.2 per cent.) having very few spots on some leaves of a few branches only. A further 60 trees were kept under observation for nine months. These originally included one affected tree (1.6 per cent.) but another was found with very few spots on a few leaves of a few branches at the end of the period, giving an increase of 1.6 per cent. If these two bushes had been even more lightly affected at previous inspections, the spots may have been overlooked, since the trees were up to ten feet high and about twenty feet in circumference, so that inspection of every leaf was impossible. A few affected trees were adjacent to one another, but on the whole distribution was non-aggregated (*Figure* 1).

Wellman (1957) found that in a large field in Costa Rica with 49 (10 per cent.) of the 497 trees affected with blister spot, 76 (15 per cent.), 42 (8 per cent.) and 52 (10 per cent.) of the trees were *newly* affected in the three following years, at the end of which period 42 per cent. of the total trees were affected, although ten had apparently recovered.

Bitancourt (1958), however, reported that in Brazil the 'oily' spot disease had not spread after two years from the trees where it had been found towards neighbouring trees.

No recovery of marked trees was apparent for the observation period in New Guinea. The apparent rate of spread was markedly less than that reported by Wellman in Costa Rica, being approximately only 0.3 per cent. for the year in the area under study.

ISOLATIONS.

Isolations were attempted from both diseased leaves and young spots on the fruit, as well as from blackened berries after surface sterilization. Only occasional fungi (particularly *Gloeosporium* sp.) were obtained from the leaf and cherry spots, although various saprophytes were isolated from the blackened cherries. Bacteria obtained from unsterilized leaf tissue did not produce

[1] This block, which, except for a few Typica, consisted entirely of Bourbon, had the highest percentage infection known to the author at the time; infected trees were very irregularly distributed throughout the block.

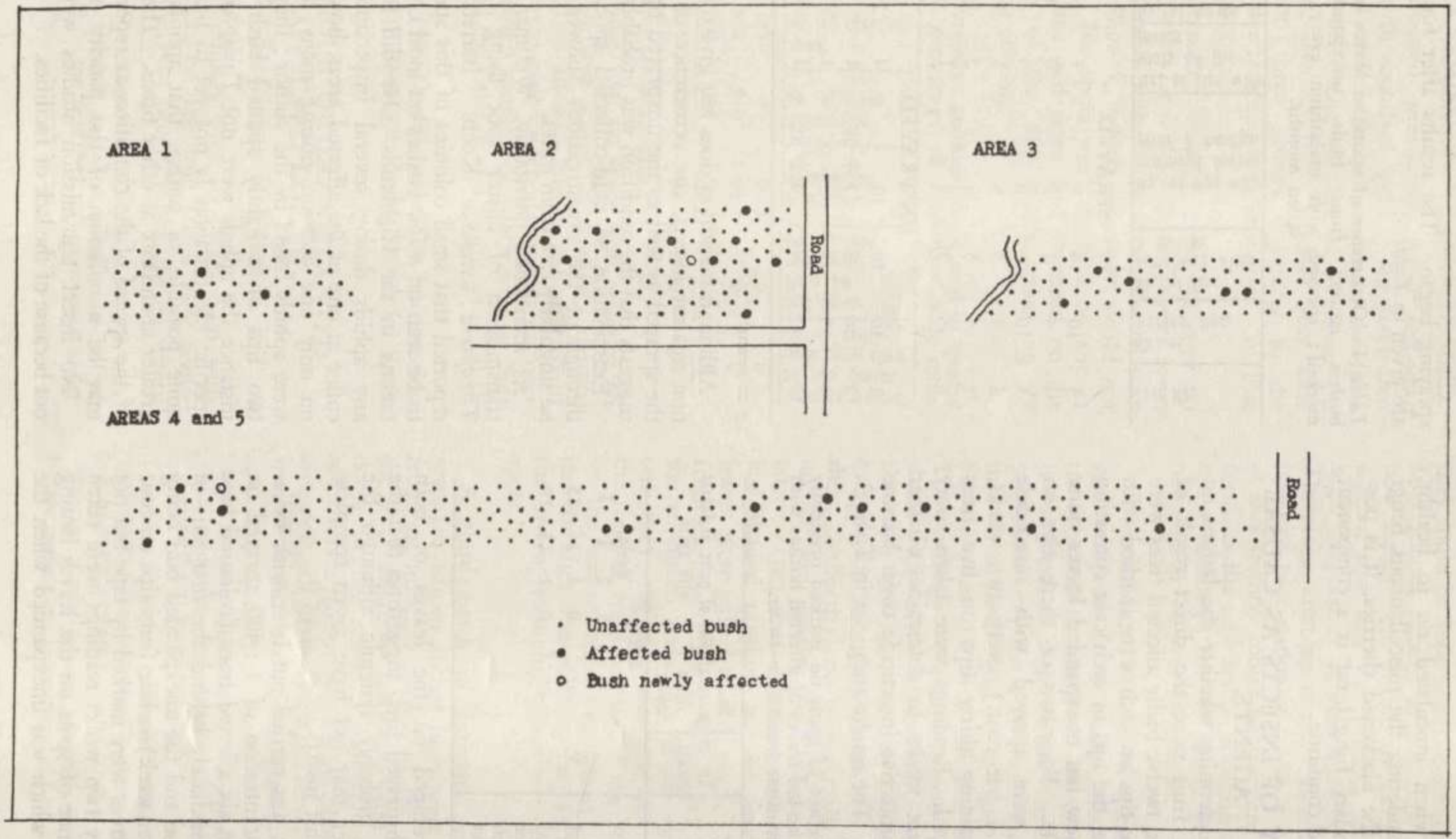

Figure 1.—Distribution of affected and newly affected bushes in five area s of the one block in approximately one year; all plants Bourbon except four Typica in Area 2.

the condition when inoculated on to healthy seedlings. Considering the miscellaneous fungi obtained from the blackened cherries, it is possible that secondary fungal rot is a component of the blackened condition.

POSSIBILITY OF INSECTS AS CAUSAL AGENTS.

In order to determine whether the lesions on the leaves and fruit were the direct result of insect punctures, twelve badly affected trees were selected and six tips on each were marked with identifying tape, the tips in each case consisting of a pair of new but unexpanded leaves quite free from spots. Forty-two of these tips on seven bushes were sprayed with insecticide (dieldrin at 0.4 per cent.) every two weeks, while the remaining thirty tips on five trees were not sprayed. Readings were taken every fortnight for six weeks to determine whether the tips with protective insecticide cover became spotted or not. The results are given in *Table* 3.

Table 3.—Occurrence of spots on marked originally unspotted, unexpanded leaves of affected bushes with and without insecticide cover.

—	No. of bushes.	No. of pairs of young leaves.	No. of pairs of leaves with spots			
			Weeks.			
			0	2	4	6
Tips not sprayed	5	30	0	4	16	25
Tips sprayed with 0.4 per cent. dieldrin every 2 weeks	7	42	0	1	13	18

Lesions developed on the leaves of both sprayed and unsprayed tips, suggesting that the condition was probably systemic although the insecticide cover may not have given complete protection against insects.

Another test was carried out later with malathion at a concentration of 1 : 400 sprayed on to forty tips on six affected trees (coloured tape being tied immediately behind the first pair of leaves and these and the unexpanded bud being sprayed once per week), while forty tips on four other affected trees were marked by tape but not sprayed. Every two weeks readings were taken on the occurrence of spots on the leaves issuing from the bud which was unexpanded when the spraying began. The results after four months are given in *Table* 4.

Table 4.—Occurrence of spots on leaves on affected bushes issuing from buds unexpanded when marked; sprayed with malathion every week for seven months.

Tree No.	No. of marked tips.	No. of tips remaining after 7 months.	No. of tips with expanded leaves after 7 months.	No. of tips with spots on the leaves.	No. of leaves on affected tips.	No. of leaves with spots.
			SPRAYED.			
3	10	10	5	3	16	6
4	5	3	2	0	0	0
5	10	9	6	4	18	9
6	5	5	5	3	12	6
7	5	5	4	2	12	5
8	5	5	4	1	6	2
			UNSPRAYED.			
1	10	10	10	4	24	12
2	10	9	5 + 3y	1	4	2
9	5	4	2	1	5	1
10	15	12	5 + 4y	1	4	1

y = young

Although malathion does not give total protection against insects, the occurrence of spots on the sprayed as well as the unsprayed tips further suggests that the condition was probably systemic.

Examination of field-collected spotted leaves throughout the investigations showed them to be singularly free from mites.

As mentioned previously, Wellman obtained transmission of blister spot using the aphid *Toxoptera aurantiae* Koch. Barrett (1966) reported that small colonies of this species were to be seen on a few bushes on most coffee plantations in the Highlands. He did not record any aphids during several inspections of the coffee at one of the affected areas, however, and on only one occasion during many inspections were aphids found by the author; they were on two tips of a lightly spotted bush. In this instance the aphids were not *Toxoptera* but the specific identification is not yet to hand. It is quite possible, of course, that aphids occur in greater abundance at other times. The slowness of the spread of the condition as reported above may be a reflection of the paucity of vectors.

No insect transmission studies were carried out because of the lack of facilities.

NON-TRANSMISSION THROUGH SEED.

As mentioned previously, many self-sown seedlings with abundant spots on the cotyledons were found under affected bushes in the field. In order to determine whether the condition was seed borne, cherries from affected trees were divided into three groups according to the amount of surface spotting and sown [1] in flats at Port Moresby.

The germination in each class was over 90 per cent. (refer *Table* 5) but no cherries sown were comparable with the severely affected ones shown in *Plate* V, which obviously would not germinate. No spots were recorded on any of the cotyledons and none had appeared on the true leaves of the 410 plants remaining 29 months after sowing.

Table 5.—Percentage germination of seed in spotted cherries and percentage of cotyledons with spots.

Amount of spotting on cherries.	No. of cherries sown.	No. of seeds germinated.	Germination percentage.	No. of seedlings with spots on cotyledons.	No. of seedlings with spots on true leaves after 18 months.
Slight	35	33	94.3	0	0
Medium	340	310	91.2	0	0
Severe *	70	67	95.7	0	0
	445	410	92.1	0	0

* But shrivelled seeds excluded.

In a previous test, no spots were recorded on cotyledons or true leaves of seedlings derived from seed from affected bushes, although 67 of the plants were kept under observation for over two years, seven months of which were at Port Moresby and the balance at a Station at 1,700 ft. altitude.

Wellman (1957) did not obtain transmission of blister spot through Arabica seed in Costa Rica.

BEHAVIOUR OF TRANSPLANTED AFFECTED COFFEE.

Transplanted Seedlings with Spots.

Affected seedlings over six inches high with abundant spots on the true leaves were transplanted to the Port Moresby laboratories, where they could be kept under continuous observation. Leaf lesions present at that time remained, but new leaves were devoid of spots. It was considered that this was possibly an effect of the high temperature at Port Moresby [2] at sea level. Therefore, after six months these seedlings were removed to a station at 1,700 ft. altitude. Even at this slightly cooler temperature spots did not form on the new leaves, totalling 403 at the last count, although the 59 plants were kept under observation for over 34 months.

A second batch of affected volunteer seedlings was removed from under affected trees and transplanted to the Port Moresby greenhouse. It consisted of eighteen seedlings, mainly with cotyledons only but a few with the first pairs of true leaves just beginning to expand. The seedling in *Plate* III was included in this group. The seedlings were kept in pots, mainly in the shade with only a little morning sun. Although the spots were abundant on the cotyledons (up to approximately 64 spots on one pair, many of them confluent), no spots at all had appeared on the true leaves (totalling 41) two months later. They were then moved into a slightly less shaded position but six weeks later true leaves were still devoid of spots. Seven months from the time of transplanting 219 true leaves on the seventeen remaining plants were still without spots ; the same was true nine months from the time of transplanting of the 260 true leaves on the sixteen remaining plants ; and fourteen months after transplanting the 353 true leaves on the fifteen remaining plants were still devoid of spots.

A third batch of infected seedlings from seven to fifteen inches high were transplanted from an infected garden in the Highlands to the Port Moresby laboratory and kept out of doors in the shade. Although some of the older leaves still present on the plants were without spots, all of the younger leaves were heavily spotted at the time of transplanting.

[1] The cherries (with seed enclosed) were sown *in toto*, in order to simulate as far as possible the fall of affected fruit in the field and the germination under affected trees.

[2] Port Moresby (Jackson's Airstrip), mean maximum and minimum temperatures : 88 degrees and 73 degrees F (20 years' average).

Goroka, 5,200 ft. (Airstrip), mean maximum and minimum temperatures : 78 degees and 57 degrees F (7 years' average)

Station, 1,700 ft.—no temperature records, but a little cooler than Moresby.

Four months later 66 fully expanded or new leaves had been produced on 13 plants, one plant having died. Small and not very definite spots were present on six leaves [1] of four of the plants ; all of the other new leaves were unspotted (*Plate* VI). At that stage three of the plants, including two of those with faint spots on the leaves, were placed in an air conditioned laboratory whose temperature range was 65 to 73 degrees F compared with the outside temperature range of 72 to 94 degrees F. The plants, however, were placed outside the laboratory in the same environment as the remaining plants, for eight hours each day on five days of each week. This arrangement subjected the plants to a temperature range which was the nearest which could be obtained to the range operating in the field in the Highlands.

The plants were again examined three months later. The small faint spots were still present on the same few leaves as noted previously, but no other spots occurred on the other new leaves which totalled 126.

In another instance one lightly infected coffee tree in the Highlands with a trunk diameter of two and one half inches at ground level was cut back to fifteen inches above ground level and transplanted into soil outside the Port Moresby laboratory. Seven months later 336 new leaves had been produced but none was spotted.

All the transplanted seedlings and the tree reported above were transferred in a quantity of the soil in which they had been growing.

Cuttings from Affected Bushes.

Cuttings from affected bushes were established in the Highlands and five months later were transferred to Port Moresby. Four months later faint spots were found on a few leaves of three of the twenty-five surviving plants. As it was thought that the high temperatures at Port Moresby may have been responsible for suppression of symptoms, the plants were removed to the Station at 1,700 ft. Fourteen months later, however, no symptoms were found on any of the 163 leaves then present on these plants, nor had any spots appeared 30 months after removal to this altitude.

[1] Affected new leaves were the second pairs expanded after transplanting.

Transplanted Healthy Seedlings.

Healthy seedlings transplanted from the Highlands to Port Moresby kept within feet of the diseased seedlings and cuttings mentioned above remained free from spots until discarded 34 months later.

GRAFTING EXPERIMENTS.

Investigations were hampered by the lack of suitable facilities such as an insect-proof house in the Highlands at about 5,000 ft., the difficulty of transporting material for grafting to the laboratories at Port Moresby 260 miles away, and, when transplants and seedlings were established at Port Moresby, the high temperature conditions on the coast.

The graftings and buddings first attempted at Port Moresby with material flown from the Highlands failed to survive. Another set of grafts was made in the plantation by agricultural officers, but the majority of the grafts failed or were broken off by labourers during harvesting and maintenance operations. On the three grafts which remained seven months later (two scions from diseased trees onto healthy stocks and one healthy onto diseased stock), no transmission of symptoms occurred.

As the plantations were roguing affected trees, it was decided to try to establish grafts and buddings at Port Moresby so that the material could be kept under closer observation, although it was realized that the change in temperature would adversely affect the host and possibly mask the symptoms.

Affected seedlings and cuttings which had been established at Goroka from affected bushes, as well as healthy seedlings were transferred to Port Moresby and then to the nearby Station at 1,700 ft., where a third set of grafts was attempted by Mr. A. van Haaren. They consisted of healthy scions grafted onto the following stocks :—

- Ten of the transplanted seedlings which had previously had abundant spots on the true leaves, although no spots had appeared on the new leaves since the transfer to Port Moresby ; and
- Three of the cuttings established from branches carrying the condition, being the only ones which had produced a few affected leaves.

These grafts were kept under close observation but no spots appeared on the scion leaves,

Plate VI.—A. Seedling with two old spotted leaves (os) visible and six unspotted leaves, the latter having been produced after transplanting from an altitude of 5,000 ft. to sea level.
B. Seedling with four old spotted leaves (os) visible and four unspotted leaves, the latter having been produced after transplanting from an altitude of 5,000 feet to sea level.

of which there were 86 five months after grafting. Further checks at eight and thirteen months were still negative.

By that time very few of the plantations were roguing regularly, so a fourth series of grafts was carried out by Mr. A. van Haaren in a section of an affected Highland plantation that could be kept under close surveillance. The grafts were examined every two weeks by the local agricultural officer, Mr. R. N. Amos, who reported on the successful ' takes ', the emergence of new leaves and fruit, and the occurrence of symptoms. From time to time the author checked the results in the field.

The details of this series are as follows :—

Forty-five healthy Bourbon scions from an unaffected plantation were grafted onto eight affected trees using a single node wedge or cleft graft, 26 being plagiotropic and 19 orthotropic.

Twenty-two healthy Bourbon scions from an unaffected plantation were grafted on to six presumably healthly Bourbon bushes on the affected plantation, 17 grafts being plagiotropic and five orthotropic.

Very wet weather occurred immediately after the grafts were made, but after two months 16 grafts were still alive ; after three months one of the 13 surviving scions had one new pair of leaves. At this stage fortnightly spraying began with 0.4 per cent dieldrin and was continued for the next twelve months in order to provide some protection against insects, including possible vectors, so that if lesions did appear on the scions there would be a good chance that it was the result of transmission through the graft and not the result of any subsequent factor. Readings were as follows :—

After six months eight grafts remained, all except one having leaves, and two were bearing fruit ;

After seven months two spots were found on one leaf on Tree M, and the leaf was removed ;

After nine months one spot was found on each of two leaves of Tree J and the leaves were removed ; and

After 13 months—

(i) three healthy scions on affected stocks remained, with 110 scions leaves altogether, all being quite free from spots ; and

(ii) two healthy scions on healthy stocks remained, with eight scion leaves and one cherry, all being quite free from spots.

After 15 months, all the remaining 127 scion leaves were still free from spots.

A summary of the results of the transplantings and graftings is given in *Table* 6.

ELECTRON MICROSCOPE CHECK FOR VIRUS PARTICLES.

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

The sap extract sources were as follows :—

Unaffected coffee.

Strips of tissue from the underside of Bourbon leaves and from cotyledons and fruit.

Affected coffee

Strips of tissue from the underside of a spot on a Bourbon leaf (samples from two plantations).

Strips of tissue from the underside of a spot on a Bourbon cotyledon (samples from two plantations).

Strips of tissue from a spotted Bourbon fruit (samples from two plantations).

Gibbs (personal communication) found no virus-like particles in the preparations, although extracts of *Crotalaria anagyroides* leaves showing mosaic symptoms, mounted in the same way and at the same time, contained filamentous virus-like particles. In Dr. Gibbs' opinion these results suggest that there was little likelihood of a virus with elongated particles being present in the affected coffee trees. A virus with isometric particles whose concentration was lower than 10^{11} or so per millilitre might have been present, however, because small isometric virus particles are not easily distinguished from round plant sap constituents in the electron microscope, unless they are abundant.

DISCUSSION.

The leaf and fruit spot of Bourbon coffee in New Guinea is dissimilar to ring spot described

Table 6.—Summary of results after transplanting and grafting affected Bourbon coffee.

Treatment.	Location.	No. surviving.	Period of observation (months).	Results.
Grafts—				
Grafts and buddings on potted seedlings	Port Moresby	None	—	—
Grafts on to plantation bushes	Goroka	3	7	No spots on leaves.
Grafts on to transplanted seedlings	Station at 1,700 ft.	10	5	No spots on 86 leaves.
Grafts on to transplanted cuttings	Station at 1,700 ft.	3	5	
Grafts on to plantation bushes	Goroka	8	7	Two spots on 1 leaf.*†
		8	9	Two spots on 2 leaves.*†
		5	13	No spots on 110 leaves.
		5	15	No spots on 127 leaves.
Transplants—				
Affected seedlings over 6 in. high	Goroka to Port Moresby to Station at 1,700 ft.	59	34	No spots on 403 leaves.
Affected seedlings mainly with spotted cotyledons only	Goroka to Port Moresby	18	2	No spots on 41 leaves.
		17	7	No spots on 219 leaves.
		16	9	No spots on 260 leaves.
		15	14	No spots on 353 leaves.
Affected seedlings 7 to 15 in. high	Goroka to Port Moresby	13	4	13 small faint spots on 6 new leaves ‡ of 4 plants, no spots on 60 other new leaves.
		13	7	19 faint spots on 6 new leaves ‡ of 4 plants, no spots on 126 other new leaves.
Mature tree stubbed to 6 in. above soil level	Goroka to Port Moresby	1	7	No spots on 336 new leaves.
Affected cuttings	Goroka to Port Moresby to Station at 1,700 ft.	25	4	Faint spots on a few leaves of 3 plants.
			18	No spots on 163 leaves.
			34	No spots on many leaves.

* Spotted leaves removed after inspection.

† Total leaves not counted.

‡ Affected new leaves were the second pairs expanded after transplanting.

by Bitancourt (1939) in Brazil and by Reyes (1959, 1961) and Valdez (1966) in the Philippines.

The symptoms are similar to those of blister spot of coffee in Costa Rica, attributed to a virus (Wellman 1957), and to oily spot in Brazil (Bitancourt 1958).

Although Bitancourt's grafting experiments were negative, Wellman reported transmission of symptoms by aphids and by grafting. Difficulty was experienced in the present study, however, in obtaining symptoms on new leaves of transplanted affected cuttings, seedlings and one tree and after grafting, even though some of the plants were under observation for nearly three years. Most of the mainly negative results obtained could possibly be attributed to the high temperature at the Port Moresby laboratories (260 miles away and 5,000 ft. lower in altitude than the infected area) where most of the work was carried out. Lack of facilities prevented feeding experiments with aphids.

Wellman (1957) reported apparent recovery of over four per cent. of affected trees in an observation block in a three year period in Costa Rica, but did not report any apparent recovery of affected plants under study in the glasshouse.

No symptoms were obtained at Port Moresby on the cotyledons or true leaves of 410 plants raised from spotted fruit and kept under observation for over 29 months, or on 67 plants derived from seed from affected bushes, kept under observation for over two years. Wellman likewise reported non-transmission through seed.

It must be pointed out, however, that under affected bushes in the field, the cotyledons of seedlings with no true leaves were covered with spots, such seedlings being probably not more than six weeks old. No spots occurred on the true leaves of these seedlings when they were transplanted to Port Moresby.

True leaves issuing from buds which were kept under protective insecticide cover from before expansion of the leaves throughout months of observation became spotted, indicating the probable systemic nature of the condition.

No virus-like particles were found in preparations of sap extracts from affected fruit, leaves or cotyledons when examined in the electron microscope by Dr. A. Gibbs. This does not rule out the possibility that a virus with isometric particles at a low concentration might have been present.

Wellman called the disease 'blister' spot, Bitancourt referred to the Brazil disease as 'oily' or 'greasy' spot, and apparently in parts of Costa Rica it is being referred to as 'buttery' spot. As none of these designations seems fully appropriate to the author, the disease in New Guinea is being referred to as 'Wellman's leaf and fruit spot', as Wellman was the first to publish a description of the condition and the first to conclude that it was apparently of virus causation.

ACKNOWLEDGEMENTS.

Grateful acknowledgement is made for the assistance given by officers of the Department, particularly to Mr. J. Kingston, Mr. R. N. Amos and Mr. J. J. Nitsche at various times at Goroka during the investigations ; to Mr. Nitsche, Mr. G. W. Cowie and Mr. R. S. Carne for the coffee surveys ; to Mr. A. J. van Haaren for most of the grafting ; to Mr. Carne for checking the time of emergence of the true leaves of coffee seedlings in the Highlands ; to other officers for assistance in the compilation of the survey results ; and to Mr. A. W. Charles for critically reading the manuscipt.

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Investigation into the Cause of Leaf Tumours of Tea Seedlings.

DOROTHY E. SHAW * AND W. M. BURNETT †.

ABSTRACT.

Tumours occurred on the early true leaves of tea seedlings derived from seed from two localities in New Guinea, and from Ceylon, India, Malawi and Australia, grown in a glasshouse and a laboratory at Port Moresby and in the field in New Guinea, New Britain and Australia. Leaves produced later on these plants were unaffected. Recently a report of leaf tumours of tea seedlings in nurseries in Malawi has also been received.

Experiments reported herein seem to eliminate the following as possible causes of the condition : micro-organisms, mites, insecticide, copper fungicide, hormone herbicide, charcoal packing, sawdust used in pregermination beds, timber used in pregermination boxes, the water supply and the local soil.

Slightly fewer seedlings with tumours occurred with whole or partial removal of the seed coat before germination than when seed remained intact, and slightly fewer with soil than with inert crushed imported quartz. No malformed leaves occurred on 66 'seedlings' developed from embryos excised from their cotyledons and grown to the two to six leaf stage on nutrient agar. Ten times more seedlings developed tumours at high temperatures in the glasshouse than in a coolroom. There were ten times more tumours on seedlings grown from immature 'ripe' seed from capsules picked green from the seed bearers than from mature seed which had dehisced naturally from the capsule.

It is suggested that the cause of the tumours may be a growth substance found in immature 'ripe' seed, with little or no residual substance present in mature seed. The concentration or activity of the substance appears to be affected by the removal of the seed coat and by temperature.

INTRODUCTION.

Hyperplastic malformations of young true leaves of tea seedlings derived from seed from Ceylon raised in quarantine in Port Moresby were described by Shaw (1965). Although the malformations somewhat resembled symptoms of blister blight of tea, no mycelium, basidia or basidiospores were found in or on the tumours. Later, malformations were also found on seedlings derived from seed produced in Malawi, a non-blister blight area. The following investigations were undertaken to determine the cause of the condition.

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EXPERIMENTAL METHODS.

The various factors considered as possible causes of the condition were as follows :—

1. Presence of *Exobasidium vexans ;*
2. A factor connected with the source of the seed ;
3. A carcinogenic substance present in the timber species of the sawdust and shavings used for pregermination ;
4. A compound used in the preservation treatment given to some of the timber from which the sawdust was derived ;
5. The insect fumigation treatment used on one consignment of seed with a high percentage of tumours in the seedlings ;

6. The effect of the copper oxychloride used by the quarantine staff as a fungicidal treatment on the seed ;

7. The effect of charcoal or crude wood ash used as packing for seed during transportation ;

8. Hormone herbicide contamination at the Laloki Plant Introduction Station ;

9. The effect of the Laloki River water used for watering at the Quarantine Station, and of the laboratory water supply derived from the town water supply, which is treated for bacterial contamination ;

10. A substance present in the soil (ex Goldie River alluvial flats) used at the Laloki Quarantine Station ;

11. The effect of insects such as mites ;

12. The effect of a micro-organism ;

13. Damage to the cotyledons prior to germination ; and

14. A factor associated in some way with the seed itself.

Seed from overseas was obtained from Ceylon (as reported previously by Shaw, 1965), Malawi, India and Australia. Local seed was obtained from the Government Tea Plantation at Garaina, approximately 130 miles direct from Port Moresby, and from a private plantation, 'Arau', approximately 240 miles direct from Port Moresby.

The 'laboratory' experiments reported in the following section were conducted at Port Moresby either in the glass house, air-conditioned laboratory or in the open outside the laboratory, and at the Quarantine Station at Laloki, 12 miles from Port Moresby.

The sowing reported from Brisbane, Australia, was kindly made on request by the staff of the Plant Pathology Section, Department of Primary Industries, at Indooroopilly, near Brisbane. The results were recorded by that staff although the senior author examined the plants when germination was complete.

The percentage germinations are known for all the above experiments except those for the seed from Ceylon and the seed in Experiment 4. The precise number of seeds sown in those two cases was not recorded by the quarantine and chemistry staff respectively (who carried out the sowings), so both figures are shown as '50 per cent +/—'.

Readings on each experiment were made weekly, affected seedlings (with one or more leaves affected) being ringed by coloured wire which remained in place until the conclusion of the experiment.

All the experiments were maintained until percentage germination remained stationary for approximately three weeks, except for Experiment 10, the seedlings of which were required by the chemists three months after sowing.

The final figures for percentage germination and percentage of seedlings with tumours on the leaves are given for each experiment; weekly recordings are given only for those experiments where differences in speed of germination were important.

While specific experiments with controls were planned for each of the factors given above, some treatments were repeated in later experiments; the combined results are marked 'TAPE' (Total All Pertinent Experiments) in the Tables.

The seedlings with tumours in the field (in nurseries) at Garaina, New Guinea (*Plate I*), and at South Johnstone in North Queensland, were recorded by the senior author during visits to those sites. As precise details were not known as to numbers of seeds sown, seeds germinated and seedlings with tumours no percentages can be given, but a positive recording is shown as '+' in the column under 'Tumours. Percentage' in the Tables. The staff at both places had not noted the tumours until they were pointed out.

The seedlings with tumours in the field at Vudal, New Britain, (500 miles from Port Moresby) were noted in November, 1967, by Mr. Kana Aburu, who had been on the staff at the Quarantine Station, Laloki, when the first tumours were noted in 1964. Mr. Aburu reported that 1,000 seeds were planted, and 50 to 60, or more than five per cent., of the resulting seedlings had tumours on one or more of the early produced leaves but as the precise figures are not known the symbol '+' is shown in the 'Tumours. Percentage' column in the Tables.

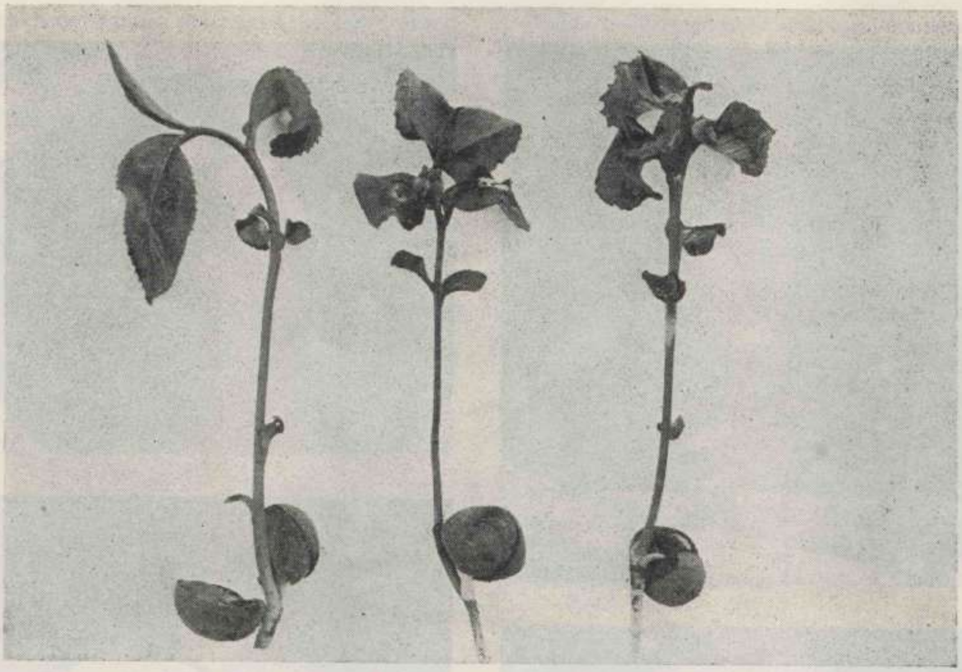

Plate I.—Hyperplastic malformations on leaves of tea seedlings from the field, Garaina, New Guinea, resulting in abnormal leaf curl.

RESULTS.

Hyperplastic malformations or tumours as previously described (Shaw 1965), occurred on some of the first-produced true leaves of some seedlings, usually one, often two or three and occasionally more leaves being affected (*Plate* I). No leaf past the eighth has been noted with tumours, even though many affected and unaffected seedlings were kept under observation for over a year. Leaves were usually but not invariably consecutively affected and occasionally one or more of the early leaves were normal whereas later leaves (although still within the first eight) were malformed (*Plate* II). The chlorotic globular nature of some of the affected leaves is illustrated in *Plate* II.

From and including Experiment 15 all ungerminated seeds were dug up and examined at the conclusion of each experiment. A small number of seeds were found to have germinated, but the malformations of the leaves were such that they had not penetrated above soil level at the time when germination of the other seeds had apparently ceased.

Occasionally a seedling whose first shoot with tumourous leaves did not emerge above soil level produced immediately above the cotyledonary junction a second shoot which bore only normal leaves. Three such plants are shown in *Plate* III.

On only three occasions were tumours found on the flat surfaces of the cotyledons. In all cases they were on apparently 'ungerminated' seeds dug up at the conclusion of experiments. They are shown in *Plate* IV.

The results of the experiments, arranged according to the factor tested, are given below :

Possible Presence of Exobasidium vexans.

As shown in *Table* 1, leaf tumours occurred in 18.2 per cent. of seedlings derived from seed from countries where blister blight has never been recorded (Malawi, Australia and New Guinea) as well as in 19.2 per cent. of seedlings from seed from countries with blister blight (Ceylon and India). It is concluded, therefore, that the blister blight fungus was not responsible for the condition, even though some of the

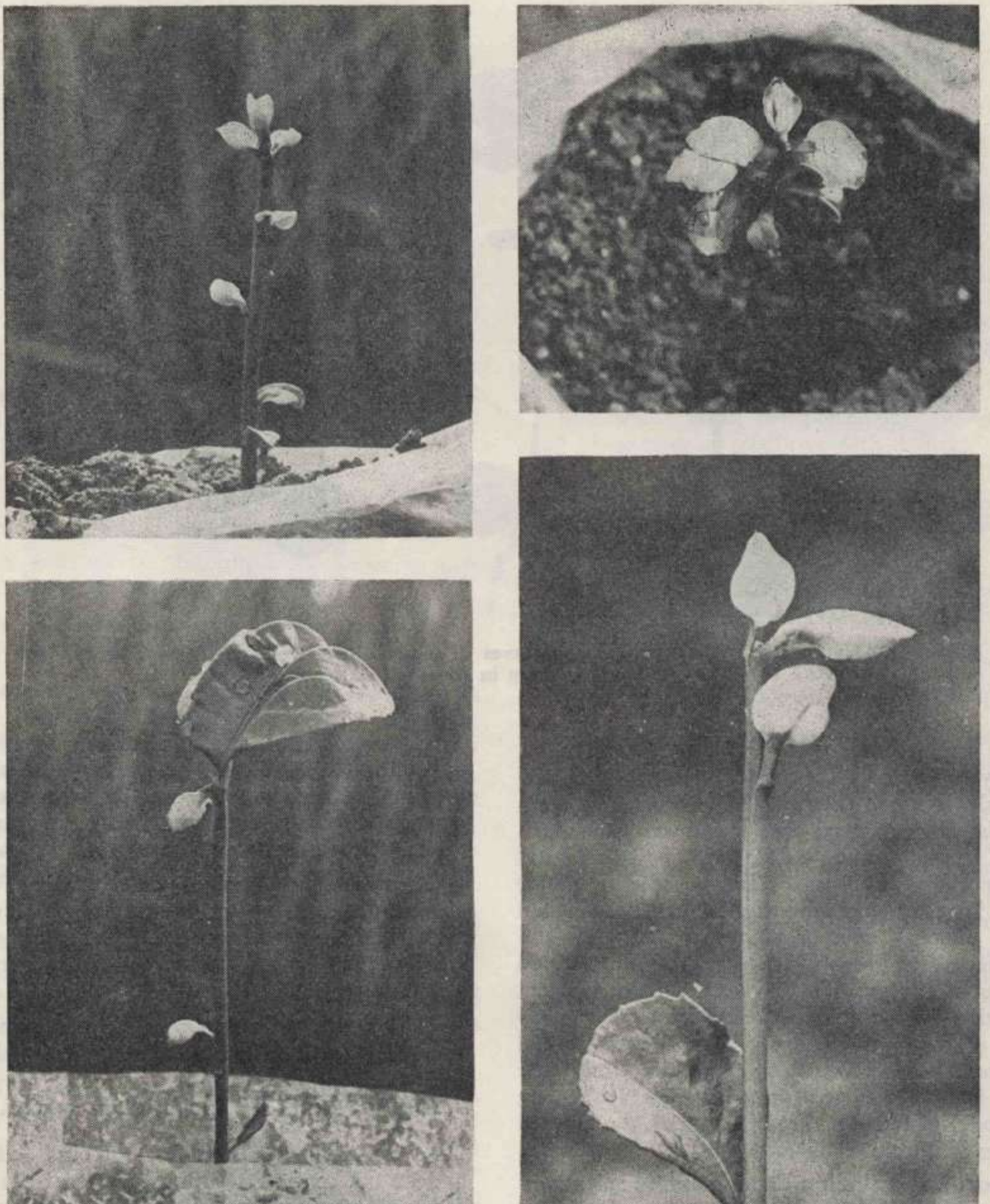

Plate II.—Hyperplastic malformations of early true leaves of tea seedlings at Laloki, Papua, from Indian seed.

tumours superficially resembled blisters caused by *E. vexans*.

Microscopic examination of tumours failed to reveal mycelium, basidia, or spores on or in the malformations.

Source of Seed.

Tumours occurred in 13.6 per cent. of seedlings derived from seed from overseas (Ceylon, India, Malawi and Australia) as well as in 26.5 per cent. of seedlings germinated from New Guinea seed, as shown in *Table* 2. It is concluded, therefore, that the factor causing the tumours was not confined to seed from local sources.

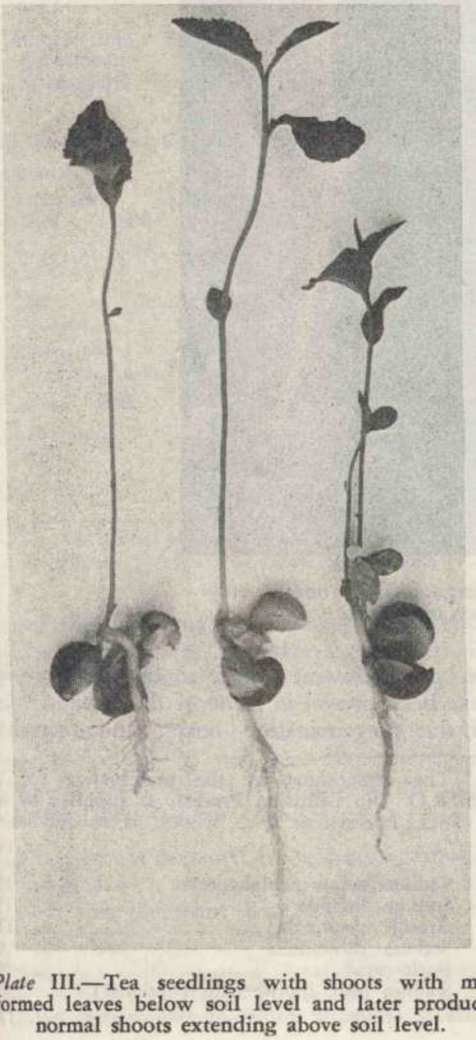

Plate III.—Tea seedlings with shoots with malformed leaves below soil level and later produced normal shoots extending above soil level.

Table 1.—Percentage of seedlings with tumours derived from seed from countries with and without *Exobasidium Vexans.*

Factor being tested.	Total No. of seeds germinated.	Germination percentage.	Seedlings with tumours. Percentage.
Sources of seed with E. vexans—			
Ceylon	1235	50+/—	9.5
India	1376	58.6	28.7
	2611	54.0	19.2
Sources of seed without E. vexans—			
Malawi	1807	38.7	4.5
New Guinea—			
Garaina			
Laboratory sowings, P.M.[1] TAPE[2]	2626	40.4	28.9
Sown in field, Garaina			+
Sown in field, New Britain			+
Laboratory sowing, Brisbane, Qld.	343	74.2	0.0
" Arau "			
Laboratory sowings, P.M.	186	46.5	40.8
Australia—			
Laboratory sowings, P.M.	416	83.9	14.7
Sown in field, South Johnstone, North Queensland			+
	5378	42.7	18.2

1. P.M.=Port Moresby.
2. TAPE=Total all pertinent experiments.

Carcinogenic Substance in the Timber Species of the Sawdust.

In case a carcinogenic substance was present in the timber from which was derived the sawdust in which the seed from Ceylon and Malawi was pregerminated, an early experiment was designed to determine the percentage malformations of seedlings pregerminated in sawdust in comparison

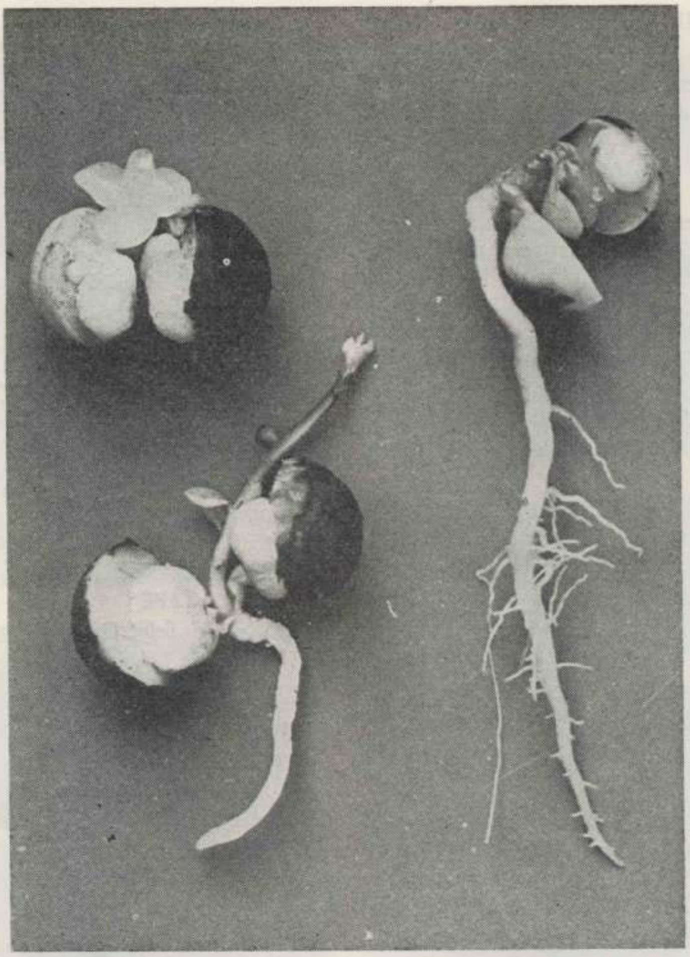

Plate IV.—Three tea seedlings with large tumours on the cotyledonary faces and tumourous true leaves.

with other media. It will be seen from *Table* 3 (*a*), that malformations occurred in seedlings germinated in media other than sawdust (total 26.1 per cent.) as well as in those pregerminated in sawdust (total 13.1 per cent.) and it is concluded that the cause of the malformations was not associated with the timber species from which the sawdust was derived.

A Preservative used on the Timber Providing the Sawdust.

When the malformations were also found on the Malawi seed, it was thought that a preservative used on some timber in the Territory may have been present in some of the sawdust [1] used in the pregermination boxes, and may have

1 The composition of the preservative for the C.S.I.R.O. Dip Diffusion Process, as supplied by Mr. E. Fogl, Hickson & Co., Sydney, is as follows :—

	Per cent.
Sodium borate pentahydrate	25
Sodium fluoride	15
Arsenic pentoxide	11
Sodium dichromate	9
Boric acid	40
Sodium pentachlorphenate (optional)	0.5

Footnote continued at foot of first column, page 173.

Table 2.—Percentage of seedlings with tumours derived from seed from overseas and New Guinea.

Factor being tested.	Total No. of seeds germinated.	Germination percentage.	Seedlings with tumours. Percentage.
Seed from overseas sources—			
Ceylon	1237	50+/—	9.5
India	1376	58.6	28.7
Malawi	1807	38.7	4.5
Australia			
Laboratory sowings, P.M.[1]	416	83.9	14.7
Sown in field at South Johnstone, North Queensland			+
	4836	48.4	13.6
Seed from New Guinea—			
Garaina seed			
Laboratory sowings, P.M.[1] TAPE[2]	2626	40.4	28.9
Sown in field, Garaina N.G.			+
Sown in field, New Britain			+
Laboratory sowing, Brisbane, Qld.	343	74.2	0.0
"Arau" seed			
Laboratory sowings, P.M.	186	46.5	40.8
	3155	31.6	26.5

1. P.M.=Port Moresby.
2. TAPE=Total all pertinent experiments.

caused the malformations. From the percentage malformations given in *Table* 3 (*b*), it will be seen that tumours occurred in seedlings germinated in media other than sawdust (soil or crushed quartz) (total 26.1 per cent.) and in sawdust known to be free from preservative (total 27.9 per cent.) as well as in the suspect sawdust (total 6.4 per cent.).

No tumours occurred in seedlings germinated in sawdust with preservative, but total germination in this treatment was only three per cent. This drastic reduction in germination indicates that it was unlikely that, even if preservative were in the suspect sawdust, it was there in any considerable portion of it. It is concluded that the preservative was not responsible for the condition.

Fumigation of Seed.

One of the early seed lots (from India) had a high percentage of seedlings with malformations (28.7 per cent.) and, as this seed had been fumigated[1] against insects, passing consideration was given to the insecticide as a possible cause of the tumours. It will be seen from *Table* 3 (*c*), however, that malformations were recorded also when seed was not treated with insecticide (16.6 per cent.).

Charcoal or Crude Wood Ash as Transport Packing.

From the figures given in *Table* 3 (*d*), it will be seen that seedlings with tumours occurred not only with seed which had been consigned in charcoal or wood ash (total 19.8 per cent.), but also with seed not packed in these materials (total 17.9 per cent.). It is concluded that this packing material was not implicated as a tumour-producing agent.

Copper Fungicide Treatment of Seed Prior to Germination.

As the seed ex Ceylon, Malawi and India had been soaked in a copper fungicide by the quarantine staff prior to sowing, experiments were conducted with copper oxychloride at different concentrations with soaking for different periods of time, and with copper sulphate. As will be seen from *Table* 4, however, malformations occurred with seed soaked in water for three hours and unsoaked (total 27.3 per cent.) as well as with seed soaked in copper fungicide at various concentrations and times, the total percentage malformations of the combined copper treatments

In order to determine whether this batch of sawdust had been treated by preservative, a chemist of the Department of Forests carried out a test for boron, which proved negative, and a chemist, Department of Agriculture, Stock and Fisheries, carried out a test for arsenic, which proved positive at a very low level of concentration. As the compounds used in the preservative are water soluble and the sawdust had been subjected to watering for various periods, it was considered that some at least of the timber contributing to the sawdust and shavings had probably been treated.

[1] Fumigated with ethylene dichloride 75 per cent. and carbon tetrachloride 25 per cent. mixture at 20 cc. per kg. of seed, exposed for 48 hours.

Table 3.—Percentage of seedlings with tumours grown in media with and without sawdust, preservative, and with and without fumigation and wood ash packing of seed.

Factor being tested.	Treatment.	Total No. of seeds germinated.	Germination percentage.	Seedlings with tumours. Percentage.
(*a*) Carcinogenic substance in the timber species providing the sawdust	Seed germinated in sawdust or shavings TAPE *	4614	45.1	13.1
	Seed not germinated in sawdust TAPE	3377	47.4	26.1
(*b*) A preservative used on the timber providing the sawdust	Seed germinated in sawdust with preservative	6	3.0	0.0
	Seed germinated in sawdust with slight evidence of preservative.	3132	42.6	6.4
	Seed germinated in sawdust known to be without preservative	1420	55.8	27.9
	Seed germinated in soil or crushed quartz TAPE	3433	47.3	26.1
(*c*) Fumigation of seed	Seed fumigated with insecticide	1376	58.6	28.7
	Seed not fumigated TAPE	6615	44.1	16.6
(*d*) Charcoal or wood ash as transport packing	Seed packed in charcoal or wood ash TAPE	3226	47.3	19.8
	Seed not packed in charcoal or wood ash TAPE	4765	45.2	17.9

* TAPE=Total all pertinent experiments.

Table 4.—Percentage of seedlings with tumours with and without various copper treatments.

Factor being tested.	Treatment.	Total No. of seeds germinated.	Germination percentage.	Seedlings with tumours. Percentage.
Copper fungicide treatment of seed prior to germination	Soaked in "Cuprox" 1 lb./20 gall. 3 hours	1237	50+/—	9.5
	1 oz./gall 1 hour	1807	38.7	4.5
	$3\frac{1}{2}$ oz./2 gall. $\frac{1}{2}$ hour	1376	58.6	28.7
	1 lb./20 gall. 3 hours	229	22.9	5.7
	1 lb./10 gall. 3 hours TAPE *	31	31.0	19.3
	2 lb./10 gall. 3 hours TAPE	253	31.6	17.0
	2 lb./10 gall. 6 hours	12	32.4	41.7
	Copper sulphate 2 lb./10 gall. 3 hours	32	32.0	15.6
Not treated with copper fungicide	Soaked in water for 3 hours TAPE	221	31.6	25.8
	Not soaked TAPE	2793	54.5	27.5
Total all above experiments	Copper fungicide treatment of seed	4977	43.2	13.4
	Not treated with copper fungicide	3014	51.8	27.3

* TAPE=Total all pertinent experiments.

being 13.4 per cent. It is concluded that copper was not the tumour-inducing agent.

Hormone Herbicide Contamination.

Although the staff at the Quarantine Station stated that neither soil nor containers nor implements could have been contaminated with hormone herbicide, it was considered that this possibility could not be overlooked. It will be seen from *Table* 5 (*a*), however, that the malformations occurred in seedlings grown under the strictest experimental conditions outside and inside the glasshouse at Port Moresby, as well as inside the air-conditioned laboratories, where many of the plants were further protected by plastic bags placed over the pots when the seed was sown and not lifted until after the first malformations occurred, watering being from the base and the growing medium imported crushed quartz. No hormone herbicide was ever used in the vicinity of the laboratories, where the total percentage of seedlings with tumours was 20.5 per cent., the total at Laloki being 14.5 per cent.

Laloki River Water or the Laboratory Water Supply.

As plants at the Quarantine Station are watered with a supply drawn directly from the Laloki River, and as plants at the Port Moresby laboratories are normally watered with the town water supply, which is also drawn from the Laloki River and treated against bacterial contamination, experiments were designed to determine whether a decrease in malformations occurred if the plants were watered with de-ionized water. As will be seen from *Table* 5

Table 5.—Percentage of seedlings with tumours with and without possible hormone contamination and with different water sources and growing media.

Factor being tested.	Treatment.	Total No. of seeds germinated.	Germination percentage.	Seedlings with tumours. Percentage.
(*a*) Possible hormone herbicide contamination	Seed germinated at Laloki, perhaps with herbicide contamination TAPE *	5284	42.5	14.5
	Seed germinated in laboratories, or inside or outside glasshouses, with no possiblity of herbicide contamination TAPE	2707	55.0	20.5
(*b*) Water Source	Laloki River water or laboratory water supply TAPE	6665	43.3	17.1
	De-ionized water TAPE	983	46.6	35.1
	Laboratory water, Brisbane	343	74.2	0.0
	Rain in field at Garaina, New Guinea			+
	Rain in field at Vudal, New Britain			+
	Rain and perhaps local water supply, South Johnstone, North Queensland			+
(*c*) Growing medium and/or an agent in the dust	Goldie River soil or in soil from around Port Moresby TAPE	5476	42.7	14.4
	Crushed imported quartz, but exposed to Port Moresby dust TAPE	851	37.5	46.8
	Crushed imported quartz in air-conditioned laboratory, Port Moresby TAPE	1321	73.6	22.5
	Sand/peat mixture at laboratory, Brisbane, 1300 miles from Port Moresby	343	74.2	0.0
	In field at Garaina, New Guinea, 130 miles from Port Moresby			+
	In field at New Britain, 500 miles from Port Moresby			+
	In field at South Johnstone, North Queensland, 570 miles from Port Moresby			+

* TAPE=Total all pertinent experiments.

(*b*), however, malformations occurred in seedlings watered with de-ionized water (total 35.1 per cent.) as well as in those receiving Laloki River water direct or from the town water supply (total 17.1 per cent.). Tumours also occurred in seedlings germinated in the field at two localities in New Guinea (Garaina and Vudal, New Britain) and at South Johnstone, North Queensland. It is concluded that no tumour-inducing agent occurred in the Laloki River water or in the laboratory water supply.

Goldie River Soil, or an Agent in the Dust Derived from the environs of Laloki or Port Moresby.

In case a tumour-inducing agent was occurring in the soil used at the Quarantine Station or in the sowings in the Port Moresby glasshouse, other sowings were carried out in crushed imported (Norwegian) quartz [1] in plastic pots washed with alcohol and then de-ionized water, the pots being covered with plastic bags and watered from the base so that there was little dust contamination ; they were then kept in the air-conditioned laboratories. From *Table* 5 (*c*) it will be seen that tumours occurred in seedlings grown in crushed quartz under the above conditions (22.5 per cent.) as well as when grown in quartz but exposed to Port Moresby dust (46.8 per cent.) or when grown in local soil (14.4 per cent.) ; more malformations occurred in quartz than in soil. They also occurred in seedlings raised in the field at two sites in New Guinea (one 130 miles away and the other 500 miles away in New Britain) as well as in the field at South Johnstone, North Queensland, (570 miles away). It is concluded therefore, that the tumour-inducing agent was not in the local soil.

Effect of Insects such as Mites.

In case the malformations were the result of mite attack, two experiments were designed to see if a reduction in tumours occurred when a miticide was used on the seed and on the growing plants.

In the first experiment, 200 seeds of a 400 seed lot from Garaina were sprayed with miticide [dicofol ('Kelthane') 1 in 500], 100 being sown in autoclaved quartz in plastic pots and 100 in unsterilized soil in plastic pots, the surface of the media of both sets of pots also being sprayed with miticide every 14 days from the time of sowing. The remaining 200 seeds received no miticide treatment ; 100 were sown in unsterilized quartz and 100 in unsterilized soil. The full details of the results are shown in *Table* 6 (*a*), (*b*) and (*c*).

Seed germinated quicker in quartz than in soil (24.0 per cent. against 3.5 per cent. germination six weeks after sowing [*Table* 6 (*a*) and (*b*)]) and total germination was higher in quartz [*Table* 6 (*a*) and (*b*)]. Percentage of seedlings with tumours was also higher in quartz than in soil, being 12.3 per cent. against 3.6 per cent. [*Table* 6 (*a*) and (*b*)].

Apart from the nutritive level, the main difference in the two media was the quick draining properties of the quartz—the bulk of the water applied to its surface drained through within seconds. It was for that reason that plastic bags were inverted over the surface to prevent excessive drying out.

Tumours occurred in treatments receiving miticide (5.0 per cent.) as well as in those without miticide (12.4 per cent.) [*Table* 6 (*b*)]. The higher figure in the non-miticide treatments was related more to the speed of germination in unsterilized quartz than to the absence of miticide treatment, the number of seedlings with tumours being approximately equal at the same stage of germination, as shown in *Table* 6 (*a*).

Miticide treatment was also incorporated into a later small experiment (Experiment 12) involving whole or partial peeling of the seed coat prior to sowing in quartz. The results set out in *Table* 6 (*c*) show that seedlings with tumours occurred in the miticide treatment (35.7 per cent.) as well as in the non-miticide treatments (28.0 per cent.) with partially or wholly peeled seed ; the figure with unpeeled seed was 51.1 per cent.

From the above two experiments it was concluded that mites were not the cause of the condition.

Close observation of seedlings grown in the laboratory experiments failed to reveal evidence of mite attack.

The effect of a Fungus, Bacterium or Virus.

As shown previously (Shaw 1965) no mycelium was found in sections of malformed

[1] The quartz consisted of angular chips about 0.5 to 3.0 mm. long by 0.5 to 1.5 mm. wide and thick.

Table 6 (*a*).—Percentage germination and seedlings with tumours with and without miticide.

—	Seeds treated with miticide, and miticide applied * fortnightly from date of sowing.				No miticide.			
Date.	Autoclaved quartz.		Unsterilized soil.		Unsterilized quartz.		Unsterilized soil.	
	Germination percentage.	Seedlings with tumours. Percentage.	Germination percentage.	Seedlings with tumours. Percentage.	Germination percentage.	Seedlings with tumours. Percentage.	Germination percentage.	Seedlings with tumours. Percentage.
3.8.1966	23.0	0.0	3.0	0.0	25.0	8.0	4.0	0.0
11.8.1966	35.0	2.9	15.0	0.0	37.0	5.4	11.0	0.0
25.8.1966	48.0	4.2	15.0	0.0	47.0	6.4	19.0	0.0
8.9.1966	51.0	3.9	23.0	0.0	58.0	8.6	23.0	0.0
15.9.1966	52.0	3.8	25.0	0.0	60.0	11.7	28.0	0.0
22.9.1966	54.0	3.7	26.0	0.0	63.0	12.7	30.0	0.0
29.9.1966	56.0	5.4	29.0	3.4	68.0	13.2	31.0	0.0
6.10.1966	56.0	5.4	35.0	2.9	68.0	14.7	34.0	2.9
13.10.1966	57.0	7.0	36.0	2.8	71.0	16.9	37.0	5.4
25.10.1966	58.0	6.9	40.0	2.5	79.0	16.5	39.0	5.1
3.11.1966	58.0	6.9	40.0	2.5	79.0	16.5	40.0	5.0
17.11.1966	59.0	6.8	41.0	2.4	79.0	16.5	41.0	4.8
24.11.1966	59.0	6.8	41.0	2.4	79.0	16.5	41.0	4.8
1.12.1966	59.0	6.8	41.0	2.4	79.0	16.5	42.0	4.8

* Miticide applied fortnightly to surface of growing medium, and to seedlings.

Table 6 (*b*).—Analysis of Table 6 (*a*).

—		Germination percentage.	Seedlings with tumours. Percentage.
Growing medium			
Quartz	6 weeks after sowing	24.0	0.0
	20 weeks after sowing	69.0	12.3
Soil	6 weeks after sowing	3.5	0.0
	20 weeks after sowing	41.5	3.6
Pest control			
Miticide		50.0	5.0
No miticide		60.5	12.4

leaves and it was concluded that no internal fungus was responsible for the condition. Examination of further sections also failed to reveal any bacterial masses or fungal hyphae which could hardly have been missed unless in an extremely dilute condition and confined in action to the first few leaves.

High tumour percentages occurred in seedlings grown in crushed quartz which had been vigorously washed with tap water, as well as in soil. Tumours also occurred in seedlings grown in autoclaved crushed quartz which was watered from the base with de-ionized water, the pots being covered by plastic bags which were not

Table 6 (c).—Percentage germination and seedlings with tumours with and without miticide in second experiment.

Treatment.	Germination percentage.	Seedlings with tumours. Percentage.
Miticide		
Seed coats partially or wholly removed (100 seeds)	93.3	35.7
No miticide		
Seed coats partially or wholly removed (100 seeds)	83.0	28.0
Seed coat intact (200 seeds)	69.5	51.1

removed, the pots and saucers having been previously washed as described in an earlier experiment.

While the above experiments do not completely rule out the possibility of external fungi or bacteria causing the malformations, it is considered that it is unlikely that they are involved. Moreover, seeds from which the seed coats were removed produced seedlings with slightly fewer malformations than those from seeds with the seed coat intact, a reverse situation from what might have been expected if external organisms were responsible for tumour formation.

In another experiment four newly-emerged malformed leaves were macerated in de-ionized water and applied to the growing tips of six healthy young tea seedlings, the tips covered with small pads of wet cotton wool and the plants held in a humid chamber for 30 hours. Young newly-emerged leaves of healthy seedlings were also macerated in de-ionized water and applied to the growing points of six healthy seedlings and treated as above. Small pads of wet cotton wool were also applied to the tips of six other healthy plants which were held in the humid chamber. No malformations occurred on the subsequent leaves of any of the seedlings. It was concluded that if a virus were causing the condition it was not mechanically transmitted by the method used.

It is also considered unlikely that a virus would cause symptom expression on the first few true leaves of seedlings but not on the later leaves.

As shown in *Plate* III, some seedlings occurred where the first shoot had severely malformed leaves but a second shoot produced immediately adjacent to it was free from the condition. From this observation it seems unlikely that an organism was involved as the causal agent.

As recorded later, no malformations occurred in embryos excised from the cotyledons and grown on nutrient agar ; if a virus were the cause of malformations it would probably have been present in the leaf primordia and would not have been excluded by the excisions.

In the early experiments many seedlings were grown in the same flat and in later experiments seedlings were usually grown ten to a pot. In each case affected seedlings were randomly distributed, which suggests that a soil-borne organism was not responsible for the condition.

It has not been demonstrated that a microorganism was involved as the causal agent of the malformations and from the evidence given above it is considered unlikely that one is responsible for the condition.

Damage to Cotyledons prior to germination.

If a random sample of sinkers of seed picked in the usual way at Garaina is examined before germination, it will be found that often a very small percentage of such seed has cotyledons showing damage. The damage may consist of a pinpoint of discoloured tissue, or a larger area of discolouration with obvious difference between the consistency of the normal cotyledon tissue and the discoloured tissue. In case such damage led to the production of tumours on the leaves of the seedlings, the cotyledons of a number of affected seedlings were examined, but no sign of such damage was noted.

It is concluded that tumours can be produced on seedlings whose cotyledons are free from macroscopic damage.

A Factor associated with the seed itself.

It was at first thought unlikely that any factor intimately connected with the seed itself could have been causing the tumours, as they occurred in seedlings derived from seed ex Ceylon, Malawi and India, where huge quantities of seed have been germinated since the inception of the tea industries in those countries, without, as far as the authors are aware, such tumours having been reported.

After the experiments described in the previous sections had apparently ruled out any association with external factors, the possibility was considered that the tumour-inducing agent was in some way associated with or inherent in the seed itself. This view was also supported by the following observations :—

Tumoured leaves were often visible as soon as the first sign of germination occurred ;

The tumours mainly involved the first few leaves, the first, second and often the third usually being affected although occasionally up to the seventh and on one occasion up to the eighth leaf was affected. Very occasionally a lower leaf or even leaves appeared normal, with a later leaf (but still within the first eight) being affected ; and

Although some plants, e.g., malformed ones ex Malawi seed, were kept in the same environment for over a year, no further tumours developed, and even malformed seedlings ex Indian seed (which had a high percentage of malformations) kept for ten months showed no further sign of tumours.

Experiments were therefore set up to test the hypothesis that the tumour-inducing agent was present in the seed. At the same time an enquiry was made into the maturation of the seed at Garaina.

It was found that seed is produced all the year round at the Garaina Station, but the staff state that if it is allowed to remain to full maturity on the trees it is attacked by insects and moulds and loses its viability. This statement is given without comment, as the matter has not been studied by the authors. The local practice, however, is to pick the capsules from the trees when fully formed but before natural dehiscence takes place, although the seed is capable of germination. The seed ex Garaina reported in the experiments described so far was prepared by picking capsules from the bearers while still green, floating the seed in water and forwarding the sinkers to Port Moresby by air.

A. Mature and immature seed.

In order to test whether a tumour-inducing agent was associated with the seed itself, seed selected to give a difference in maturity was obtained from the Garaina Tea Station as follows :—

Dehisced ripe seed.

Twelve seed bearing trees were shaken and the material which fell was caught on covers laid underneath on the ground. From this material the dried ripe seed was selected, immersed in water and the floaters discarded. Such seed was difficult to obtain in quantity as mentioned above, and only 100 seeds were available.

Undehisced 'ripe' seed.

Undehisced capsules were picked from the trees, the seeds, however, being sufficiently 'ripe' in the Manager's opinion to germinate. (Apparently seed from a few capsules at the same visual stage of maturity which had fallen from the tree, were also included.) (100 seeds.)

Both the above seed lots which had been forwarded in cardboard boxes to Port Moresby without charcoal packing were washed once in tap water and twice in de-ionized water, and the plastic germination pots and saucers washed three times in tap water and once in de-ionized water. The seed was sown in crushed imported quartz (usually used as an inert medium for nutritional studies) and kept in an air conditioned laboratory, each pot being covered by a plastic bag as soon as the seed was sown and watered with de-ionized water from the bottom, so that the plastic bag did not have to be removed.

The results are shown in *Table* 7, from which it will be noted that—

germination was quicker from ripe seed which had dehisced naturally than from 'ripe' seed not naturally dehisced, (44 per cent. as against 11 per cent. eight weeks after sowing) although the final percentages were of the same order ; and

the number of malformations occurring with seed derived from green 'ripe' capsules picked from the trees was over ten times as great as that which occurred with seed which had dehisced naturally (30.6 per cent. against 2.7 per cent.).

In order to test the maturity factor further, the Queensland Department of Primary Industries (Australia) kindly supplied from South

Table 7.—Percentage germination and seedlings with tumours derived from seed of different maturities.

Date of reading.	Seed from dry ripe capsules which fell from tree when shaken.		Seed from green 'ripe' capsules picked from the trees (plus a few green capsules which fell from the trees).	
—	Germination percentage.	Seedlings with tumours. Percentage.	Germination percentage.	Seedlings with tumours. Percentage.
20.7.1966	44.0	0.0	11.0	36.4
3.8.1966	66.0	1.5	27.0	37.1
11.8.1966	71.0	2.8	38.0	29.9
25.8.1966	74.0	2.7	57.0	28.1
6.9.1966	74.0	2.7	65.0	29.2
15.9.1966	74.0	2.7	69.0	29.0
22.9.1966	74.0	2.7	72.0	30.6
29.9.1966	74.0	2.7	73.0	30.2
6.10.1966	74.0	2.7	74.0	29.8
13.10.1966	74.0	2.7	76.0	28.9
25.10.1966	74.0	2.7	82.0	30.5
3.11.1966	74.0	2.7	84.0	29.8
17.11.1966	74.0	2.7	85.0	30.6
24.11.1966	74.0	2.7	85.0	30.6
1.12.1966	74.0	2.7	85.0	30.6

Tablbe 8.—Percentage germination and seedlings with tumours derived from seed of different maturities.

Date of reading.	Dry ripe seed which was picked up from ground after natural dehiscence (100 seeds)		Seed from green 'ripe' capsules picked from the tree and placed in sun to induce splitting of capsules (100 seeds)	
—	Germination percentage.	Seedlings with tumours. Percentage.	Germination percentage.	Seedlings with tumours. Percentage.
26.6.1967	0.0	0.0	0.0	0.0
7.7.1967	8.0	0.0	9.0	11.1
14.7.1967	26.0	3.8	21.0	23.8
21.7.1967	53.0	9.4	34.0	29.4
28.7.1967	67.0	8.9	40.0	32.5
4.8.1967	85.0	9.4	51.0	23.4
11.8.1967	88.0	9.1	53.0	32.1
18.8.1967	89.0	9.0	60.0	35.0
25.8.1967	89.0	9.0	67.0	34.3
1.9.1967	91.0	8.8	70.0	37.2
8.9.1967	92.0	8.7	72.0	36.1
15.9.1967	93.0	8.6	75.0	36.0
22.9.1967	93.0	8.6	77.0	35.1
22.9.1967 *	97.0	11.3	88.0	46.6

* "Ungerminated" seed dug up, and germinated seeds with and without tumours recorded.

Johnstone, North Queensland, tea seed which had dehisced naturally and fallen to the ground over a period of several days, and 'ripe' tea seed from green capsules which had been picked from the trees. Unfortunately it was found later that after the green 'ripe' capsules were picked from the tree, they were placed in the sun (for less than a day) to cause splitting of the capsules, and this forced maturation may also have induced chemical changes in the seed. These seeds, therefore, could probably not be considered as 'immature' as the undehisced 'ripe' seeds described in the previous experiment.

The seeds were sown in crushed quartz in plastic pots, watered with tap water from the base, and covered with plastic bags to prevent too rapid drying out. The results are given in *Table* 8.

It will be noted that—

germination of the seed which had dehisced naturally was again a little quicker than that of the seed from the green 'ripe' capsules ; and

the number of malformations occurring with seed derived from green 'ripe' capsules was four times as great as that which occurred with seed which had dehisced naturally (46.6 per cent. against 11.3 per cent.).

From the above experiments it seems that the factor causing hyperplastic malformations on up to the first six or so true leaves of some tea

Plate V.—A. One seed with the seed coat completely removed exposing the bare cotyledons ; four seeds with the outer layer of the seed coat removed, revealing the vascular strands on the outside of the inner layer.

B. Three seeds with testae removed showing the papery skin, probably the remains of the inner integument, peeled back ; half a seed coat with portion of the thin skin still in place with point of pin visible through the transparent skin.

seedlings may be a growth substance present in fully-formed but not fully ripened tea seed which decreases with further maturity, so that little if any is left in physiologically fully mature seed. The authors are unaware of any work on growth regulators or inhibitors governing maturation and dehiscence of tea seed.

B. The seed coat and embryo in relation to maturity and to the tumour-inducing factor.

Examinations were carried out on a random seed lot ex Garaina to determine the following :—

- Whether the more immature seeds could be distinguished macroscopically by examination of the seed coat and embryo ;
- If so, whether seed which was the more immature on the basis of macroscopic separation gave rise to a high proportion of malformed seedlings ;
- Whether the length of the leaf and root primordia indicated the degree of maturity ;
- Whether there appeared to be anything unusual in the microscopic appearance of embryonic leaves of 'ripe' but undehisced seeds ; and
- Whether embryos excised from their cotyledons resulted in seedlings with as many tumours as occurred in seedlings from complete embryos.

Complete or partial removal of the seed coat.

In preliminary work and five separate experiments over a period of about 18 months, over 1,000 seeds were gently cracked with a hammer, and note taken of the ease or otherwise with which the seed coat or portion thereof could be removed from the cotyledons. It was found that—

- some seed coats could be easily removed in their entirety, leaving the cotyledons cleanly exposed (one seed in *Plate* V, A) ;
- in some cases the hard outer layer would come away with a little pressure, leaving portions only of the fibrous layer [1] retained on the cotyledons ; and
- in other cases the inner portion of the fibrous layer was completely retained on the seed, covering the whole surface of the cotyledons (*Plate* V, A).

The percentage of seeds which fell into the above three groups in five experiments and in the embyro study are shown in *Table* 9 under 'Percentage of sample.'.

As will be seen from the table the samples varied considerably. However, the general trend was that the more mature the sample and the higher the germination, the more seeds there were with complete split in the fibrous layer, resulting in more seeds with the inner portion of the fibrous layer still adhering and less seeds with completely exposed cotyledons.

As was shown previously, and is somewhat evident from *Table* 9, the more mature the seed and the higher the germination the lower the percentage of seedlings with tumours.

Length of leaf and root primordia.

In one study the length of the leaf and root primordial body [2] in 300 seeds was measured in order to see whether this measurement could be correlated with the retention or otherwise of the fibrous layer of the seed coat or with stages of maturity of the seed.

Three hundred seeds ex Garaina were gently cracked, and divided into the three groups, viz., fibrous layer removed, fibrous layer partially retained and fibrous layer retained. The cotyledons of the first group were then forced gently apart, so that the leaf and root primordium remained as a small protuberance at the base of one cotyledon ; in the two other groups the top of each seed was cut off with a scalpel and then the cotyledons were forced apart.

The primordia were measured under the stereomicroscope, 200 being measured *in situ* and 100 after excision. The length ranged from 0.7 to 4.0 mm. with 92.9 per cent. from 2.0 to 3.0 mm. The average length of the primordia from seed in which the fibrous layer was either partially retained or wholly removed was 2.5 mm. while that of seeds in which the fibrous layer was wholly retained was 2.7 mm. The details are given in *Table* 10.

Although there were slight differences between the average lengths of the primordia in the three groups, the ranges were the same. It was concluded that length of the leaf and root primordial body of this seed lot ex Garaina did not correlate with the type of seed coat retention and could not be used as a measure of maturity. It is possible that the leaf and root

[1] Refer Appendix.

[2] The embryo less the cotelydons.

Table 9.—Percentages of seed coat retention groups, germination and seedlings with tumours, with different seed lots.

—		Seed coat intact.			Seed coat partially or wholly removed. Fibrous layer retained.				Fibrous layer partly retained.				Fibrous layer removed.			
Exp.	Source.	No. of seeds.	Germination percentage.	Seedlings with tumours. Percentage.	No. of seeds.	Percentage of sample.	Germination percentage.	Seedlings with tumours. Percentage.	No. of seeds.	Percentage of sample.	Germination percentage.	Seedlings with tumours. Percentage.	No. of seeds.	Percentage of sample.	Germination percentage.	Seedlings with tumours. Percentage.
12a	Garaina, New Guinea	200	69.5	51.1	40	40.0	95.0	50.0	20	20.0	85.0	23.5	40	40.0	77.5	9.7
13	Arau, New Guinea	200	44.5	50.6	120	60.0	55.8	41.8	55	27.5	47.3	7.7	25	12.5	16.0	25.0
14a	South Johnstone, Queensland (Undehisced)	100	88.0	46.6	97	64.7	84.5	7.3	18	12.0	77.8	0.0	17	11.3	76.5	7.7
14b	South Johnstone, Queensland (Dehisced)	100	97.0	11.3	110	75.3	81.8	2.2	8	5.5	75.0	0.0	12	8.2	0.0	0.0
15	Garaina, New Guinea	200	53.5	27.1	28	28.0	71.4	15.0	25	25.0	48.0	16.7	47	47.0	36.2	53.0
Embryo Study	Garaina, New Guinea ...... (400 seeds)				162	40.5			124	31.0			114	28.5		

Table 10.—Percentages of seed with degree of seed coat retention and length of seed primordial body.

Seed coat retention.	No. of seeds.	Percentage of sample.	No. of leaf and root primordial bodies in length class (mm.). 1.5	2.0	2.5	3.0	3.5	4.0
Fibrous layer :								
Retained	111	37.0		16	54	30	11	
Partially retained	100	33.3	1	25	51	21	2	
Removed	89	29.7	1	18	43	21	3	3
	300	100.0	2	59	148	72	16	3
Percentage			0.7	19.6	49.3	24.0	5.3	1.0

primordia of fully physiologically mature seeds which dehisce naturally from the tree may be longer than those of the sample studied but insufficient mature seeds were available for such measurements.

Microscopic examination of embryonic leaves.

In another experiment 30 leaf and radicle embryonic bodies were excised from seed, mounted on slides in lacto-phenol and examined microscopically. Although 200 other whole seeds from this sample gave rise to seedlings 51 per cent. of which had leaves with tumours, and 40 other seeds whose testae were removed gave rise to 9.7 per cent. seedlings with tumours, no abnormalities could be recognized in the embryonic leaves visible when examined.

Excision of leaf and root primordia.

An experiment was conducted to determine whether leaf and root primordia excised from seed gave rise to seedlings with tumours on the leaves.

The hard seed coats of 100 seeds were gently cracked with a hammer and the seed was divided into three groups depending on whether the coat could be wholly or partially removed, the groups being 'fibrous layer removed, fibrous layer partly retained and fibrous layer wholly retained'.

The cotyledons of the first group were forced gently apart, the leaf and root primordia remaining attached to the face of one cotyledon as a small protuberance; in the other two groups the top of each seed was cut off with a scalpel and then the cotyledons were forced apart.

The excisions were carried out under the stereomicroscope, a dissecting needle being inserted beneath the leaf and root primordial body which was then forced gently upwards; in most cases it lifted cleanly from the cotyledons with only slight rupturing of the attached tissues.

Each primordial body was surface sterilized in mercuric chloride (1 in 1,000) for 5 to 10

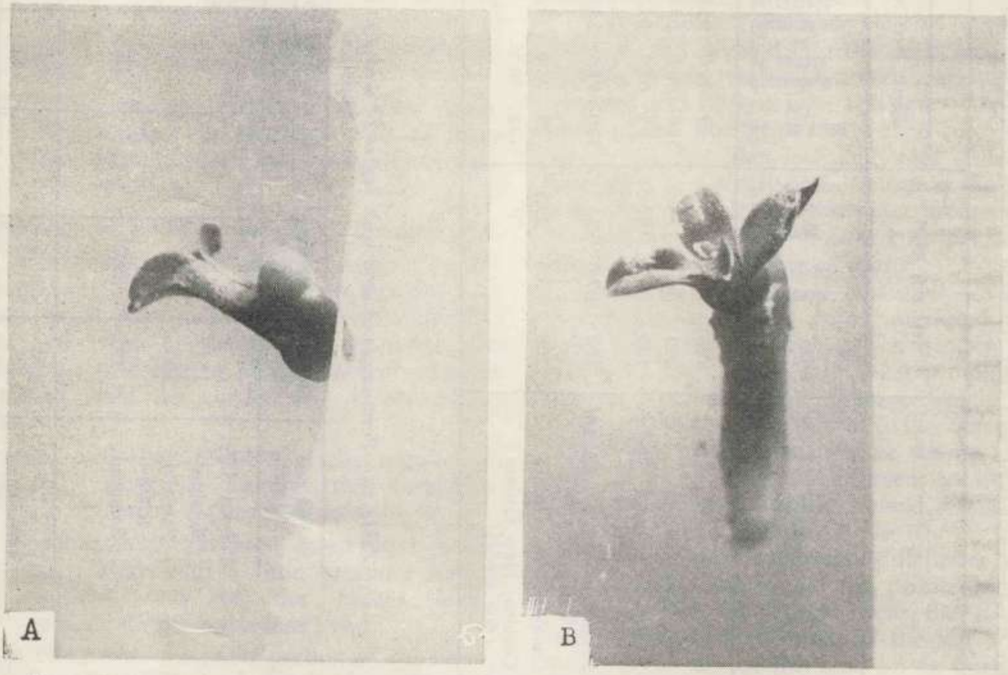

Plate VI.—A. 'Seedling' grown on nutrient agar slant after excision of the embryo from its cotyledons; photographed *in situ*.
B. Another 'seedling' grown on nutrient agar slant after excision of the embryo from its cotyledons; photographed after removal to wet cotton wool.
Leaves of both 'seedlings' without malformations. Scale of A and B. 1 div. = 1 mm.

seconds and placed on the surface of a slant of potato dextrose agar. Some slants became contaminated with *Penicillium* sp. and some primordia dried out because of inadequate contact with the medium. The majority, however, began to green and produced from two to six leaves within two months. The primordia were nearly perpendicular on the surface of the slant, and development of the plumule mainly occurred at right angles to this, as shown in *Plate* VI, A ; there appeared to be no or very little geotropic orientation. In a few cases where the primordia were accidently inserted upside down, growth of the plumule proceeded downwards. There appeared to be no delayed germination as occurs with whole seed but growth of the plumules was slow and appeared to come to a halt after about two months, probably because of the sub-optimal medium. No root development occurred from any primordium, also probably due to inadequate nutrition.

Disorganized tissue development occurred in only two cases out of sixty-six, the white amorphous mass of cells apparently occurring in the region of the ruptured tissue although the origin was difficult to determine.

The seedlings were examined mainly at the four-to-six-leaf stage, when they were up to half an inch long. They were examined under the stereomicroscope, but no tumours or malformations were detected on any leaf (*Plate* VI, B). It is possible but unlikely that malformations may have occurred on later leaves.

A portion of the same seed lot was sown in crushed quartz on the day the excisions were carried out. The percentage germination and malformations with this sowing are shown in *Table* 11 together with the details of the embryonic primordial excisions.

It will be noted that whereas tumours ocurred in 24 out of 60 seedlings raised from whole seed, no malformations were detected at all on leaves of 66 'seedlings' derived from the leaf and root primordial body excised from the cotyledons of seed from the same sample and grown on nutrient agar. Some of the leaves of the seedlings from whole seed were as severely tumourous as those shown in *Plate* II.

It would seem therefore, that if a growth substance is involved in tumour formation it is concentrated in the cotyledons and not in the leaf and root primordia.

Miscellaneous Experiments—Sowing at Brisbane, Australia, and parallel sowing at Port Moresby.

Special mention must be made of the results of a sowing kindly carried out by the Plant Pathology staff of the Department of Primary Industries, at Indooroopilly, near Brisbane.

The sowing consisted of 462 seeds ex Garaina, New Guinea. It was made in 50 per cent. fine sand, 50 per cent. peat moss mixture with fertilizer (blood meal, potassium nitrate, potassium sulphate, single superphosphate, dolomite lime and calcium carbonate lime). Records were made weekly of the number of seeds germinated and of seedlings with tumours, as was the practice in all experiments in New Guinea.

Final germination was 74.2 per cent. and no tumours were recorded (*Table* 12).

A parallel sowing of 300 seeds of the same seed lot was made at Port Moresby, 200 being sown with seed coat intact and 100 with the seed coat wholly or partially removed. The sowing took place two weeks before the Brisbane sowing and was made in crushed quartz, the pots being kept outside the glasshouse.

Tumour percentages were 27.1 for seedlings derived from whole seed and 23.7 for seedlings

Table 11.—Percentage of seedlings with tumours from seed and from embryos excised from their cotyledons.

Seedlings from seed.			'Seedlings' from embryos excised from their cotyledons.		
No. of seed sown.	No. of seeds germinated.	No. of seedlings with tumours.	No. of primordia excised.	No. grown to 2 to 6 leaf stage.	No. of 'Seedlings' with tumours.
100	60	24	100	66	0

Table 12.—Percentage germination and seedlings with tumours in sowings at two sites with the same seed.

BRISBANE (462 seeds sown) Whole seed		PORT MORESBY (300 seeds sown)			
		Whole seed (200 seeds)		Seeds with seed coats partially or wholly removed (100 seeds)	
Germination percentage.	Seedlings with tumours. Percentage.	Germination percentage.	Seedlings with tumours. Percentage.	Germination percentage.	Seedlings with tumours. Percentage.
74.2	0.0	53.3	27.1	59.0	23.7

derived from seeds whose testae had been wholly or partially removed.

A comparison of the results of the Brisbane and the Port Moresby sowings is given in *Table* 12. Tumours were present on seedlings grown in New Guinea, but not on those grown in Brisbane. If tumours had not been found in the field in Queensland, it would have been necessary to consider whether something in the Australian environment was inimical to the production of tumours, or conversely, whether something in the New Guinea environment was responsible for them. However, tumours were found on seedlings in the field at South Johnstone, Australia, so that the environment of the Brisbane sowing was examined to see if any explanation could be found to account for the lack of tumours.

The Brisbane peat/sand mixture had been steamed at 180 degrees F. for one half hour from two to seven days before sowing—no record was kept of the precise time. No precautions were taken after sowing to prevent aerial or other contamination. When the Brisbane results became available it was wondered whether the heat treatment of the medium may have destroyed a micro-organism or substance responsible for the malformations in seedlings in other instances. However, as tumours were still produced on seedlings grown in autoclaved quartz in the Port Moresby laboratory where special precautions were taken to prevent or reduce subsequent contamination, it was concluded that lack of tumours on the leaves of the Brisbane seedlings was not due to the steam heating of the peat/sand mixture.

Sowings of tea seed in the Port Moresby laboratories were generally made within a few days of the seed being harvested, the period before sowing being longer with foreign seed. The seed ex Ceylon, for example, was nine days in transit, and was probably harvested at least several days before despatch. A period of nineteen days occurred between harvest and sowing of the seed in Brisbane, which may have been one of the longest periods for any seed lot.

Investigations are still under way to determine whether the length of time between harvest and sowing affects the number of seedlings with tumours ; at this stage it is not known whether the long period before sowing of the seed in Brisbane contributed to the lack of tumour formation.

As shown in *Tables* 5 (*c*) and 6 (*a*) and (*b*), the percentage of seedlings with tumours was generally lower for seed sown in soil than in quartz. The Brisbane sowing was made in peat/sand, with good water-holding capacity, whereas the Port Moresby sowing was made in quartz, which was very quick draining ; this difference may have contributed in some way to the lack of tumours.

The temperatures at Port Moresby during the experiment are given in *Table* 13, together with information available from Brisbane. It will be noted that the minimum range was lower for Brisbane than for Port Moresby, especially during the first part of the experiment, and that the maximum range was lower except for the upper limit. It is shown in the next section that seedlings grown at lower temperatures had fewer tumours than seedlings grown at higher temperatures. The lower temperatures at Brisbane may have been another factor contributing to the lack of tumours.

Table 13.—Temperatures at Brisbane and Port Moresby glasshouses.

BRISBANE			PORT MORESBY				
	Minimum Range °F.	Maximum Range °F.		Minima		Maxima	
				Range °F.	Average °F.	Range °F.	Average °F.
Aug. 17 to Oct. 2	48-67	74-91	July	64-74	70.5	80-89	84.9
			August	61-75	71.0	82-89	85.8
			September	58-74	69.5	81-92	86.0
Nov. 11 to Dec. 24	63-74	76-105	October	67-76	72.7	81-90	87.8
			November	62-75	71.2	80-85	90.6
			December	68-74	71.7	88-97	92.7

Influence of temperature on germination and production of tumours.

This experiment involved 800 seeds, 400 being Boh jat from Garaina and 400 being originally South Johnstone jat which had been grown at Garaina for many years. Each seed lot was further divided into two groups, one half being sown in crushed quartz in the coolest room available (a little-used air-conditioned laboratory) and the other half in the same medium in the glasshouse. Each group was again divided into the one hundred smaller seeds and the one hundred larger seeds.

Maximum and minimum thermometers installed in both the glasshouse and the cool room at the beginning of the experiment were read each workday for five and one-half months. From the ranges and averages of these readings, as shown in *Table* 14, it will be seen that the averages for the minimum and maximum temperatures were 13.5 degrees F. and 30.3 degrees F. respectively lower in the cool room than in the glasshouse.

The light intensity in the glasshouse was higher than in the cool room, but the authors consider that this was of minor importance compared with the temperature differences.

Table 14.—Minima and maxima temperatures at the two sites of germination of Experiment 16 during 5½ months.

	Minima		Maxima	
	Range °F.	Average °F.	Range °F.	Average °F.
Glasshouse	69-78	74.9	89-105	98.5
Cool room	61-64	61.4	66-74	68.2
Difference	8-14	13.5	23-31	30.3

The final recordings of percentage germination and seedlings with tumours are given in *Table* 15. It will be noted that—

- there was virtually no difference between the germination and tumour percentages with the small and large seeds [*Table* 15 (*a*)] whose results could then be bulked ;
- germination was slightly quicker during the first six weeks in the glasshouse (records not reproduced), but final germination was higher in the cool room (*Table* 15) ;
- Boh jat had slightly higher germination and slightly lower tumour percentages than South Johnstone jat (*Table* 15) in both the glasshouse and cool room ; and
- the percentage of seedlings with tumours was far higher in the glasshouse (55.5 per cent.) than in the cool room (5.0 per cent.) [*Table* 15 (*c*)]

The decrease in tumours with low temperatures may explain why seedlings with tumours have not been recorded (to the authors' knowledge) in the main tea growing countries,[1] where temperatures during germination at higher altitudes are lower than at Port Moresby. As mentioned previously, however, tumours were also found in the field at Garaina at 2,350 feet.

[1] Dr. R. T. Ellis, Director of the Tea Research Foundation of Central Africa, Malawi, informed the authors in a letter dated 24.5.1968 that the condition has now been found on seedlings in nurseries in several places in Malawi.

Table 15.—Percentage germination and seedlings with tumours with large and small seed of two types in two environments.

(a) *Details of results*

Treatment.	Germination percentage.	Seedlings with tumours. Percentage.
Boh seed :		
Cool room		
small seed	66.0	1.7
large seed	67.0	1.5
Glasshouse		
small seed	44.0	52.3
large seed	42.0	47.7
South Johnstone seed :		
Cool room		
small seed	47.0	10.6
large seed	58.0	8.6
Glasshouse		
small seed	38.0	68.4
large seed	38.0	55.2

(b) *Large and small seed bulked*

Boh seed :		
Cool room	66.5	1.5
Glasshouse	43.0	50.0
South Johnstone seed :		
Cool room	52.5	9.5
Glasshouse	38.0	61.8

(c) *Coolroom and glasshouse results combined*

	Germination percentage.	Seedlings with tumours Percentage.	Seedlings with tumours (Average percentage.)
Cool room :			
Boh seed	66.5	1.5	5.0
South Johnstone seed	52.5	9.5	
Glasshouse :			
Boh seed	43.0	50.0	55.5
South Johnstone seed	38.0	61.8	

Germination and percentage malformations.

An examination of the results of the experiments revealed that the speed of germination and germination percentages of the various seed lots varied considerably. In order to determine whether high or low germination percentage was correlated with high or low percentage of tumours, the results of the experiments were re-examined on the basis of the following recordings :—

Final tumour percentage, i.e., at the conclusion of the experiments when germination percentage was stationary ;

Tumour percentage at three months (if available) ;

Percentage tumours at the point where germination was nearest to 30 per cent., no matter what the time after sowing ; and

Percentage tumours according to the month of sowing.

The results are shown in *Table* 16. It was found that—

final germination percentage usually reflected percentage at three months after sowing so that the quicker the germination usually the higher was the final germination percentage ;

the percentage of seedlings with tumours did not seem to be invariably correlated with high or low germination, although the seed lot with the highest germination gave one of the lower percentages of tumour incidence ;

while the seed lots giving the lowest incidence of tumours were generally those sown during the coolest times of the two-year period, the order being 0.0 per cent. (June) ; 4.5 per cent. (July) ; 5.0 per cent. (July) ; 5.7 per cent. (August) ; 9.1 per cent. (June) ; 9.5 per cent. (July) ; 11.3 per cent. (May) ; 17.6 per cent. (May) ; 18.8 per cent. (March), some high percentages did occur in seed sown in the cooler months, e.g., 55.5 per cent. (July) ; 25.9 per cent (June) ; 61.5 per cent. (August) and 46.6 per cent. (May) ; and

from *Table* 16 it will also be noted that in fifteen experiments where comparison was possible, ten results showed a higher percentage of seedlings with tumours at three months after sowing than at the conclusion of the experiment. From this it would seem that there is a tendency for tumours to occur more frequently in seed which germinates quickly than in seed which germinates slowly, at least in sowings of 'ripe' undehisced seed.

Table 16.—Percentage germination and seedlings with tumours three months after sowing and at conclusion of germination.

Experiment.	Month sown.	Location.*	Three months after sowing.		At conclusion of germination.			Comparison of tumour per cent. †
			Germination percentage.	Seedlings with tumours. Percentage.	Time after sowing (months).	Germination percentage.	Seedlings with tumours. Percentage.	
Ceylon	July	o			3¾	50.0+/—	9.5	
India	Nov.	o	42.7	30.5	6¾	58.6	28.7	h
Malawi	July	o			9¼	38.7	4.5	
Aust. (14)	May	o	a) 70.0	37.2	3¾	88.0	46.6	
	May	o	b) 91.0	8.8	3¾	97.0	11.3	
Exp. 1	Aug.	o			4	22.9	5.7	
Exp. 2	Jan.	o	10.6	30.4	6¼	36.5	22.1	h
Exp. 4	Feb.	o		80.6	6	50.0+/—	58.9	h
Exp. 5	March	o	12.4	25.6	7¾	29.9	18.8	h
Exp. 6	April	l	56.3	56.9	3½	63.5	54.3	h
Exp. 7	May	l	65.5	13.7	6¼	79.5	17.6	
Exp. 8	June	l	43.3	5.8	5¼	55.3	9.1	
Exp. 10	July	o	12.5	38.8				
Exp. 11	Aug.	o	34.7	63.5	5	43.3	61.5	h
Exp. 12	Dec.	l	44.0	51.2	5¼	75.0	43.1	h
Exp. 13	Jan.	o	38.5	46.8	4½	46.5	40.8	h
Exp. 15	June	B	a) 38.5	28.6	6¼	55.3	25.9	h
		o	b) 47.8	0.0	6¼	74.2	0.0	
Exp. 16	July	c	24.3	4.1	5	59.5	5.0	
		o	18.3	60.3	5	40.5	55.5	h

* Location : O = outside glasshouse ; 1 = in air-conditioned laboratory ; B = in Brisbane glasshouse ; c = coolest air-conditioned lab.

† Comparison of tumour percentages : h = percentage higher at 3 months than at conclusion of experiment.

DISCUSSION AND CONCLUSIONS.

The experiments described in this paper show that the tumours on the early leaves of some tea seedlings were probably not caused by a fungus or bacterium, and were not caused by mites, by damage to the cotyledons prior to germination, by a tumour-inducing agent in the sawdust used in some of the pregermination boxes or a wood preservative in some of the sawdust, by the charcoal or wood ash packing, by an insecticide mixture used in one of the seed lots, by hormone herbicide contamination, or by copper fungicide treatments used on some of the seed. Moreover they were not caused by local soil conditions or local water supply, as they occurred with soil in New Britain and in Australia as well as at two field sites in New Guinea, and with imported crushed quartz in laboratory experiments.

As the tumours occurred on leaves of seedlings derived from seed produced in Ceylon, India, Malawi, Australia and New Guinea, and as external causes of the condition seem to have been ruled out, it is suggested that the cause of the tumours is inherent in the seed. As from four to ten times as many tumours occurred with 'ripe' immature seed (i.e., undehisced seed picked from green capsules but capable of germination) as with mature seed shed from the tree after dehiscence, it is suggested that the tumours may be caused by a growth substance present in fully formed, immature (undehisced) seed, but not present, or present in only a small quantity, in physiologically mature seed.

An examination of the ease of removal of the seed coat partially or in its entirety revealed considerable variation in six seed lots. The general trend appeared to be that the more mature the seed lot, the more frequently did the inner layer of the testa adhere to the seed when peeled manually. The percentage of seedlings with tumours was generally less with whole or partial removal of the seed coat.

The lengths of the leaf and root primordial body of 'ripe' undehisced seed were mainly in the range 2 to 5 mm. and could not be used as a measure of the maturity of the seed, nor did it correlate with the type of seed coat retention.

No abnormalities could be recognized in the leaf primordia of thirty excised embryos examined microscopically.

In ten out of fifteen experiments where a comparison was possible, a higher percentage of seedlings with tumours occurred three months after sowing than at final germination. It would seem, therefore, that there is a tendency for more tumours to occur on the first seedlings germinating than on those germinating later, at least as far as 'ripe' undehisced seed is concerned.

There were no differences in percentages of germination or of seedlings with tumours in larger and smaller seed of one seed lot. The percentage of seedlings with tumours was greater with seed germinated in quartz than in soil and germination was also initially quicker in quartz. The percentage with tumours was ten times greater when the seedlings were grown at high temperatures than at low temperatures within the range of temperatures tested.

Tumours of leaves as described by Shaw (1965) and in the present paper have not, to the authors' knowledge, been described on tea seedlings overseas.[1] It is interesting to note, however, that seedlings with tumours on the leaves were occurring in the field in New Guinea and Australia when an examination was made by the senior author, although previously unreported by the resident staff.

It is possible that if some have occurred overseas, they were mistaken for blister blight tumours. It is also claimed, we understand, that the main tea-growing countries usually use physiologically mature seed dehisced naturally from the capsules, and such seed gave a lower percentage of tumours than more immature seed in these experiments. However, seed obtained from India and Ceylon gave 28.7 per cent. and 9.5 per cent. seedlings with malformations respectively when it was grown in New Guinea and it is unlikely that the seed exported would have been different in maturity to that used locally in these countries.

[1] Tumours have also been reported to occur in seedlings in several nurseries in Malawi (Dr. R. T. Ellis, personal communication, 24.5.1968).

APPENDIX.

A NOTE ON THE COMPOSITION OF THE SEED COAT OF TEA.

During investigations into the factors causing tumours on the early leaves of some tea seedlings, the authors examined the testae of nearly and fully mature seed; no attempt was made to study earlier development. The following note records their findings in comparison with previously published work.

Keng (1962) described the mature seed coat as follows :—

"The seed coat [his *Figure* 29b] has a very thick outer cuticle and is clearly differentiated into two parts. The outer part, 8 to 10 cells thick, is composed of cells more or less isodiametric in transection, intermixed with sclereids; the inner part, 20 to 25 cells thick, consists of elongated and enlarged, obliquely oriented cells with thickened walls, in which the innermost three or four layers are strongly lignified and have prominent plasmadesmata."

Sethi (1965) described the testa as follows :—

"The outer epidermis (of the outer integument) becomes stretched and flattened. The parenchymatous zone below the epidermis is separated into two portions by the ramifying vascular strands which consist of spiral tracheids and elongated parenchymatous cells [his *Figure* 3B]. The cells of the outer region show granular contents and begin to develop sclerenchymatous thickenings; some of them also contain crystals of calcium oxalate. The thickenings increase further so that the lumen of the cells is considerably reduced [his *Figure* 3D]. The cells of the inner zone also become slightly thickened and contain tannin. Contrary to this, the cells of the inner integument elongate, become vacuolate and begin to degenerate [his *Figure* 3C at ii]."

The present authors found that if the seed coat is gently cracked with a hammer, the seed coat of some seeds can be lifted off in its entirety, leaving the cotyledons cleanly exposed (*Plate* V, A). In that case the inside of the seed coat is smooth and shiny. In some seeds a thin papery layer which would probably be interpreted as the remains of the inner integument according to Sethi (1965) can still be seen nearest to the cotyledons (*Plate* V, B).

In other seeds an outer portion peels off, leaving an inner portion of the seed coat still retained around the cotyledons (*Plate* V, A) the pattern of the ramifying vascular strands being clearly evident on the surface. These strands, consisting of spiral tracheids, issue like a flattened cord about 4 mm. wide from the base of the seed, and travel for 15 to 20 mm. along the back of the seed before dividing into numerous strands which splay out over the surface until they reunite at the base.

Sections through the seed coat show that the testa consists of—

an outer layer about 0.5 to 0.6 mm. wide composed of cells which are elongated and enlarged, obliquely oriented with thickened walls, shown as 'ol' in *Plate* VII, A; and

an inner layer ('il' in *Plate* VII, A) from 0.13 to 0.19 mm. wide composed of cells which are more or less parallel to the inner surface, unthickened, and divided by the vascular strands ('vs') into a narrower outer portion and a wider inner portion.

When the seed coat is peeled off and a part is left around the cotyledons, the break occurs in the plane

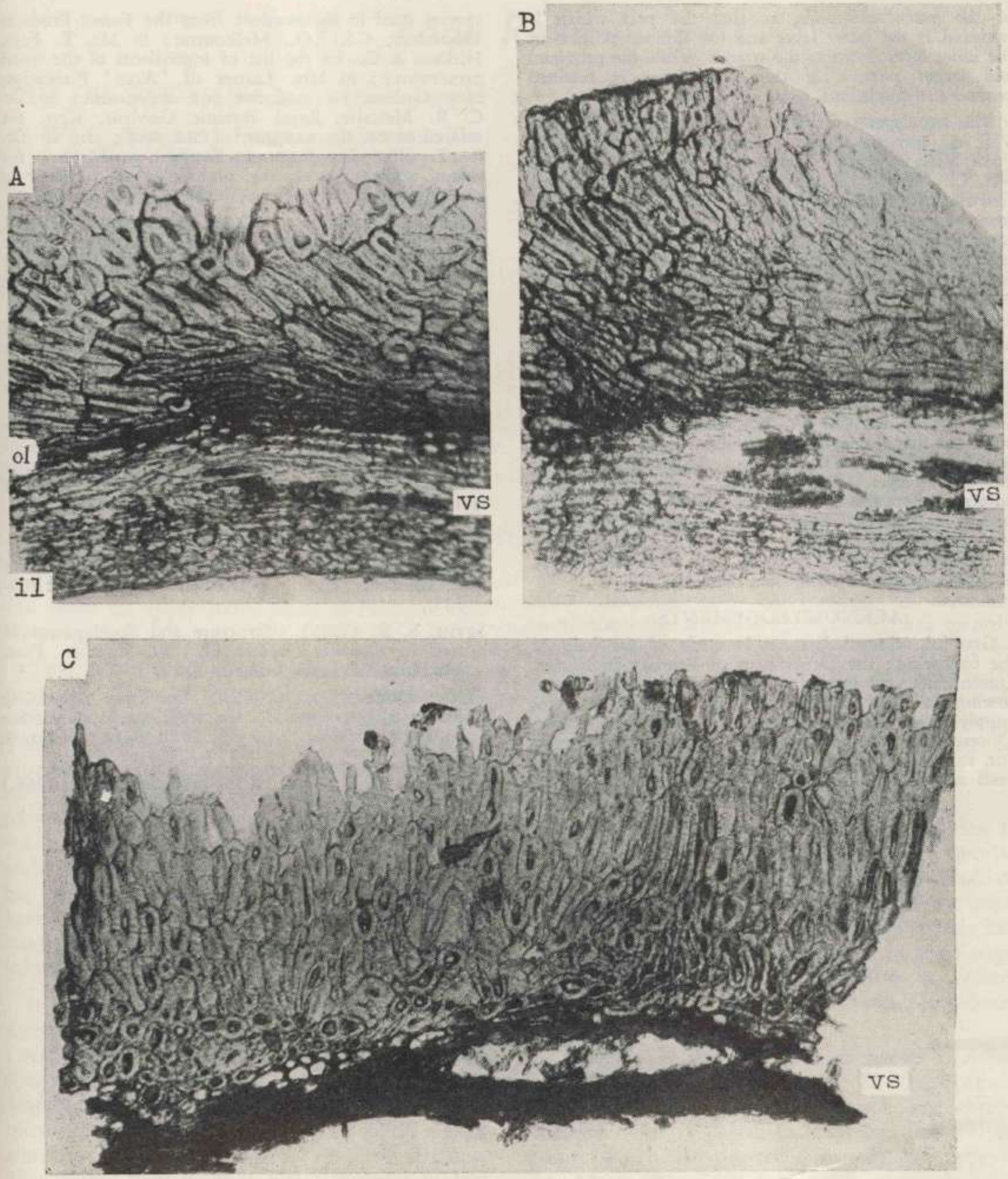

Plate VII.—A. Section through seed coat showing inner layer ('il') bisected by the vascular strands ('vs'), and the outer layer ('ol').
B. Section through seed coat showing splitting of the inner layer in the plane of the vascular strands.
C Section through seed coat showing more advanced splitting of the inner layer of the seed coat in the planes of the vascular strands. All sections x 100.

of the vascular strands, so that the part which is removed is the outer layer and the thinner portion of the inner layer down to the strands, while the proximal and larger portion of the inner layer is retained around the cotyledons.

The two layers of the seed coat are shown in *Plate* VII, A, and the break is shown just occurring in *Plate* VII, B, while a later stage is shown in *Plate* VII, C. Usually the strands remain on the inner portion but often some at least are pulled off and can be seen on the lining of the portion peeled off. In some cases portions of the inner layer are also pulled off, revealing parts of the cotyledons, this group being termed 'partially peeled' in an earlier section of this paper.

The sections examined by the authors agree well with *Figure* 3D of Sethi (1965). A slight difference in interpretation or wording is that Sethi terms the portion distal to the vascular strands the 'outer layer' whereas the authors, while agreeing that it is the part distal to the vascular strands which peels off easily, point out that it consists of the outer layer of obliquely oriented and thickened cells plus the outer portion of the inner layer. There seems to be considerable difference between the illustrations of Sethi and the authors on the one hand, and the description of the seed coat by Keng (1962) on the other; the positions of the layers described by Keng seem to be the inverse of those described by Sethi and the authors.

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Book Review.

THE OIL PALM (*Elaeis guineensis* Jacq.).

C. W. S. HARTLEY, Longmans, Green & Co., London, 1967. 692 pp. $Aust.12.00.

This is by far the most valuable general book on the oil palm to appear so far, and it should become a standard text for all concerned with the crop. The author spent most of his time in Africa, particularly as Director of the West African Institute for Oil Palm Research, which was one of the most important research centres for the crop. However, he has travelled widely in all the major oil palm growing areas in West and Central Africa, Malaysia, Indonesia, and South and Central America, frequently advising on research programmes and he is regarded as on of the foremost authorities.

It is this world-wide approach which is of particular interest. There have been several quite good books published in Malaya recently, but these do apply particularly to Malayan conditions.

Two of the most interesting and useful chapters are "The Oil Palm and its Environment" and "Factors Affecting Growth, Flowering and Yield". The characteristics of the environment in each of the present and potential growing areas is discussed in relation to its effects on oil palm growth and yield, and this is most useful to workers in a new country such as Papua and New Guinea. The emphasis in this work has been on Africa, where more detailed studies have been carried out, and where there are more clear-cut environmental variations, but available information from other countries is also reviewed.

It seems that the oil palm is well adapted to surviving in marked seasonal climates, as it has a very efficient mechanism regulating the stomatal opening, and hence water loss. If soil moisture drops below the level required for normal transpiration, the stomata close and the palm almost ceases growing, and hence suffers little damage. This means though, that yields fluctuate widely under such conditions and are less than under more even climates. In West Africa, yields are related to rainfall, and to 'effective sunshine' or only the sunshine received when soil moisture is adequate for growth.

A useful test has been developed to show when soil moisture is limiting transpiration and growth and uses a range of alcohol/water mixtures to test the degree of opening of the leaf stomata. A higher proportion of alcohol reduces the surface tension of the mixture, and hence increases its ability to penetrate smaller stomatal openings. This test has been used to show the effects of different soil types and rainfall regimes and also the effect of different ground covers on palm growth. With a severe dry season, a pueraria cover competed strongly for moisture and markedly reduced palm growth compared to either bare soil or a maize crop.

Under very wet conditions, as in Colombia where they have areas with 250 inches of rain and only 1,250 hours of sunshine per year, yields are much better than in West Africa, and it seems that low sunshine does not have as great an effect as low rainfall on yields. There is also difficulty with the method of recording sunshine—the standard Campbell-Stokes recorder only records bright sunshine, whereas in wet climates a large proportion of the incoming radiation usable by palms may be under conditions of light cloud. On the other hand, under hazy, dusty conditions in the West African dry season, the recorder may burn 6 to 7 hours per day, but radiation is not high.

Low winter temperatures in higher latitudes, as in Honduras (15 degrees N), have a similar effect to a dry season, and there 90 per cent. of the crop is harvested in six months of the year. At higher altitudes near the equator, yields can be reduced over the whole year, as in the Congo Basin, or in Sumatra above 1,500 ft., where palms may take a year longer to come into bearing also.

There is a marked contrast between Asian and African oil palm areas. In the former, there is a largely uniform climate, but abrupt changes occur in parent material and the derived soils (which are, however, mainly clay types). In Africa there are vast areas of sandy soils from similar parent materials, but with marked climatic changes. Conditions in parts of America seem to be rather more similar to New Britain, and in Ecuador, palms are being planted on young volcanic ash soils with buried organic horizons, and under a high rainfall. Our conditions correspond closely to this.

A full discussion is given of work on the effects of climate on sex ratio, and hence yield cycles. In seasonal climates, the optimum sex ratio may only coincide with optimum conditions every three years. The sex ratio seems to vary when the balance of photosynthetic assimilation and uptake of water and nutrients is upset, as in a period of low light intensity or soil moisture stress, or with heavy pruning.

The selection and breeding chapter starts with an interesting account of early selection work. The best work was done in the Congo and resulted from careful prospection amongst the palm groves for good fruit types, and then a sound breeding programme based on this material. The work was of a high standard, and it was eventually realized that the *tenera* palm was a hybrid, and all the sterile palms appearing in plantations established with *tenera* progeny were part of a segregating generation. This led to the discovery of the mechanism of inheritance of shell thickness and to commercial *tenera* production.

One particular *tenera* type developed had excellent fruit characters, and gave rise to the Sumatran *tenera* SP540, which in turn is the basis of much of the Sumatran and Malayan commercial seed today, including that being planted in Papua and New Guinea. This line has also excelled in America, so has shown its worth under widely differing conditions.

Modern breeding methods and the programmes undertaken in various countries are discussed. For some time, the emphasis was on programmes similar to that used for maize hybridization, where large numbers of lines are inbred and tested for combining abilities, and the best crosses are used as hybrids. It was realized though, that this was not really suitable for a perennial such as the oil palm, where each individual occupies such a large space. Ideas turned more towards animal breeding methods, where a parent can be both performance and progeny tested, and then used for breeding purposes for many years Often both the parent and its progeny are being used concurrently.

The sections on practical oil palm growing have similar content to books published in Malaya, with a somewhat wider perspective.

In the nutrition section, some work is discussed showing that the minimum number of palms to be sampled for chemical analysis to detect a given difference in levels (say 5 per cent.), varies widely between different nutrients. Also, the best leaf for sampling varies—the 17th is best for N, P, Ca, but the first for Mg and K. An intelligent approach to leaf sampling should be used, and the arbitrary methods now in use closely examined.

A large number of fertilizer experiments have shown that responses are generally small in young palms, unless they are replants or on very poor soil. Large quantities of fertilizers however, can be needed later on. Very small increases in yield, even if not statistically significant in some trials, can be very profitable in high yielding areas.

One chapter is devoted to intercropping, which may be desirable, particularly on smallholdings, while waiting for palms to come into bearing. In the early years, production of annual crops such as maize can be quite successful, and not detrimental to the main crop if precautions such as fertilizing are taken. Cattle grazing is another form of intercropping, and has been mainly tried in America. With a slightly wider palm spacing to give better pasture growth, and with careful management, the combination could be very productive. Cattle would need to be kept out of young areas unless the palms could be protected.

Most diseases and pests are discussed although it is inevitable that we shall have some different ones to contend with in Papua and New Guinea. New pests are appearing in America, and of note is 'Red Ring', a nematode condition similar to the coconut disease of the same name found there.

N. J. MENDHAM.

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