Investigation into the Cause of Leaf Tumours of Tea Seedlings.

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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

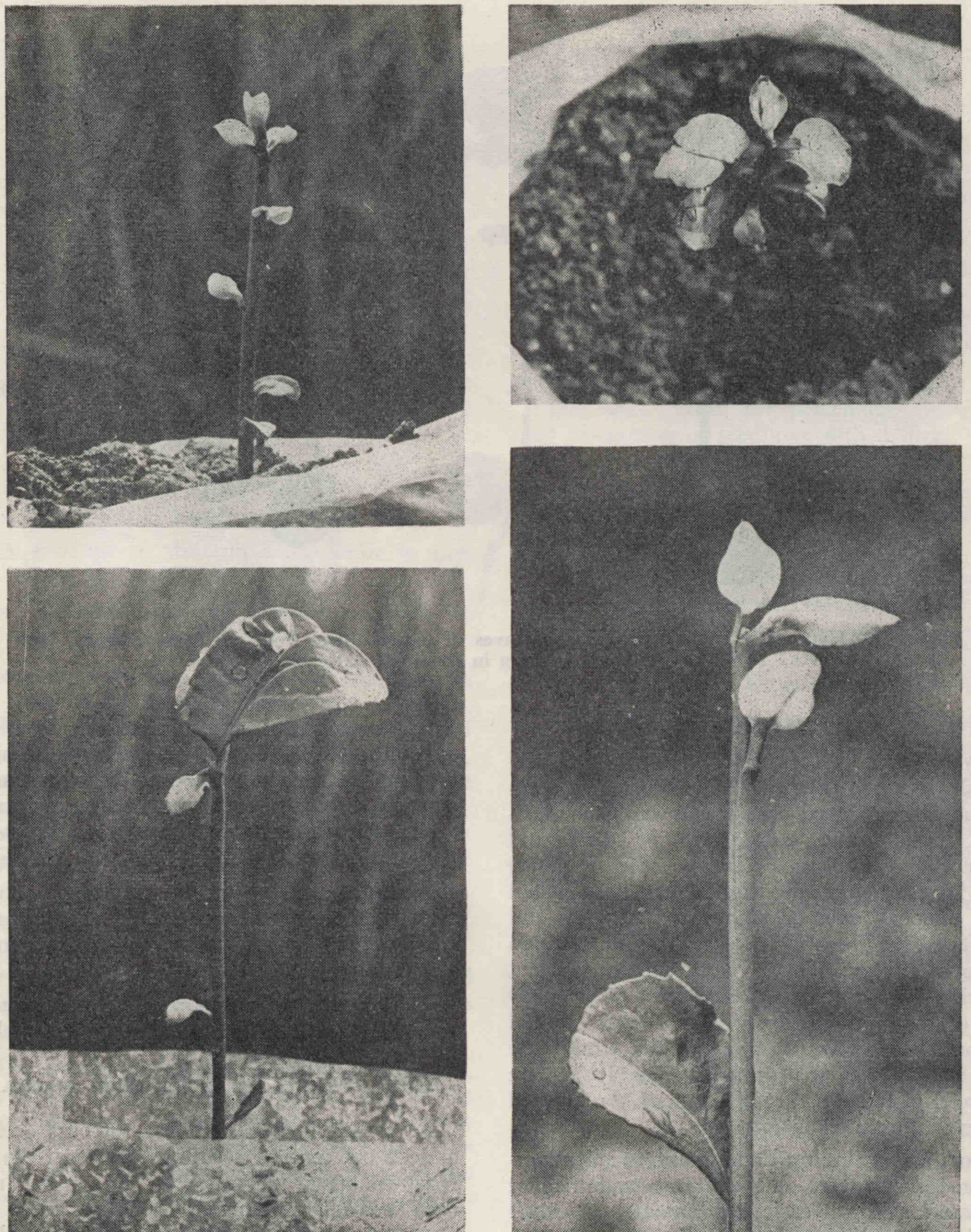


Plate II.—Hyperplastic malformations of early true leaves of tea seedlings at Laloki, Papua, from Indian seed.

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.

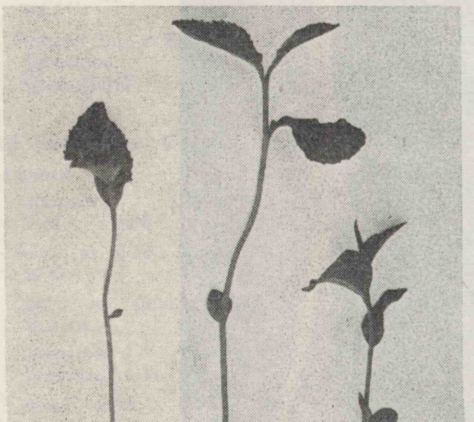


Plate III.—**Tea seedlings with shoots with malformed leaves below soil level and later produced normal shoots extending above soil level.**

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

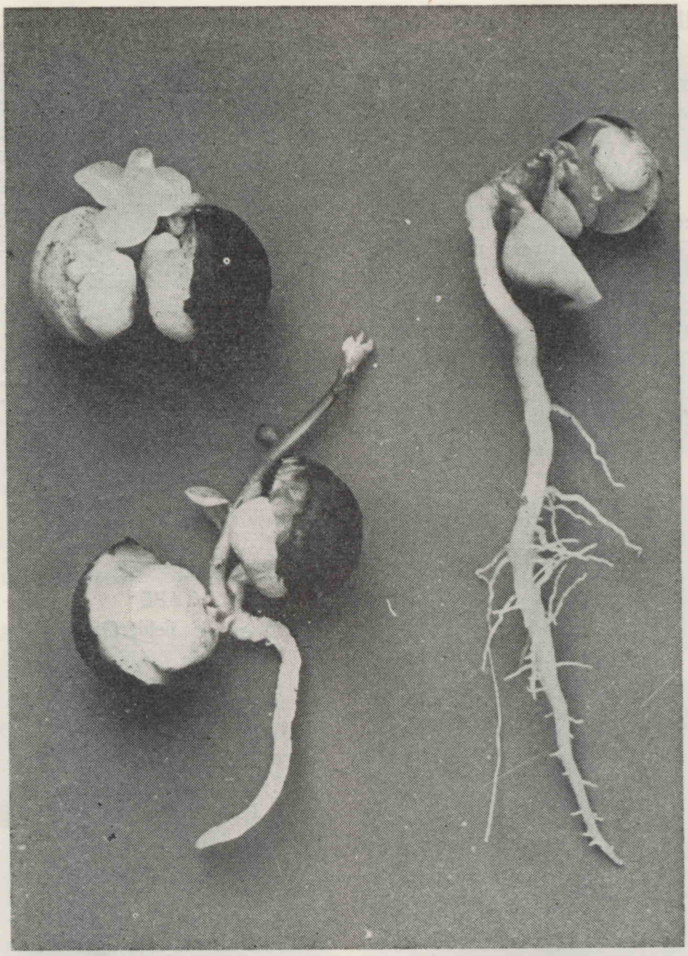


Plate IV.—Three tea seedlings with large tumours on the coty-ledonary faces and tumourous true leaves.

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
	3½ oz./2 gall. ½ 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.

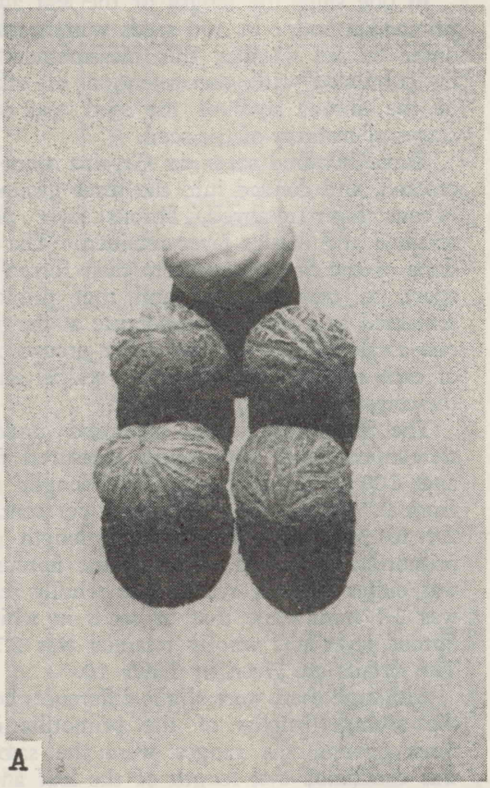
The seeds were sown in crushed quartz in plastic pots, watered with tap water from the base, and covered with plastic bags to prevent too rapid drying out. The results are given in *Table* 8.

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



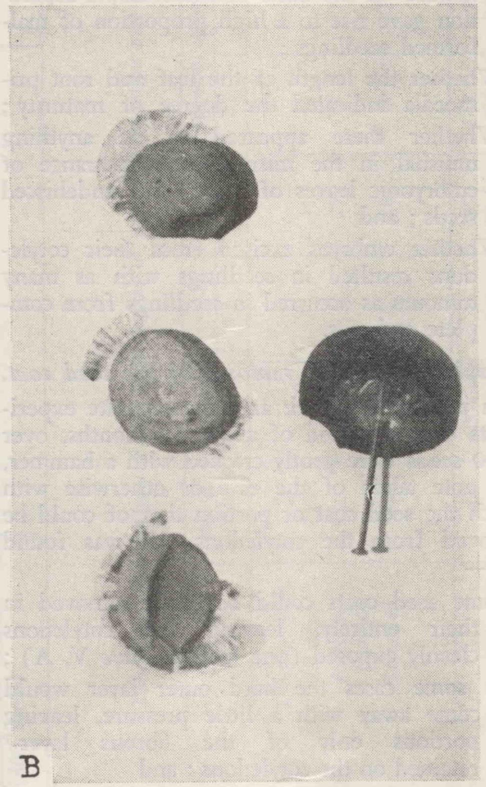


Plate V.—A. One seed with the seed coat completely removed exposing the bare cotyledons ; four seeds with the outer layer of the seed coat removed, revealing the vascular strands on the outside of the inner layer.

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).

[1] Refer Appendix.

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

[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

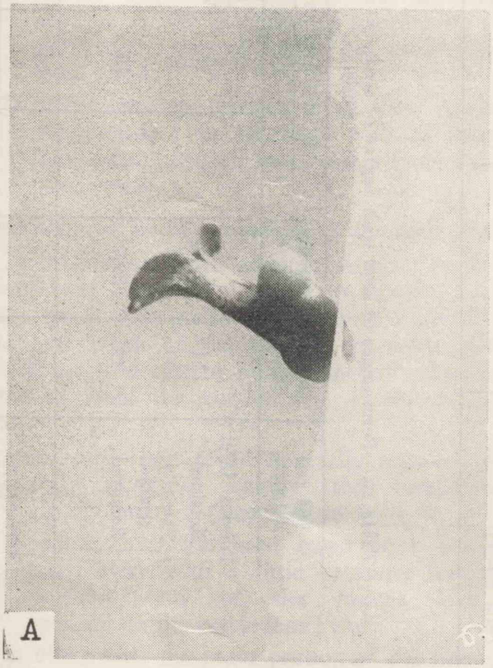


Plate VI.—A. 'Seedling' grown on nutrient agar slant after excision of the embryo from its cotyledons; photographed *in situ*.
B. Another 'seedling' grown on nutrient agar slant after excision of the embryo from its cotyledons; photographed after removal to wet cotton wool.
Leaves of both 'seedlings' 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 Range °F.	Minima Average °F.	Maxima Range °F.	Maxima 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 Range °F.	Minima Average °F.	Maxima Range °F.	Maxima 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			$3\frac{3}{4}$	50.0+/—	9.5	
India	Nov.	o	42.7	30.5	$6\frac{3}{4}$	58.6	28.7	h
Malawi	July	o			$9\frac{1}{4}$	38.7	4.5	
Aust. (14)	May	o	a) 70.0	37.2	$3\frac{3}{4}$	88.0	46.6	
	May	o	b) 91.0	8.8	$3\frac{3}{4}$	97.0	11.3	
Exp. 1	Aug.	o			4	22.9	5.7	
Exp. 2	Jan.	o	10.6	30.4	$6\frac{1}{4}$	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	$7\frac{3}{4}$	29.9	18.8	h
Exp. 6	April	l	56.3	56.9	$3\frac{1}{2}$	63.5	54.3	h
Exp. 7	May	l	65.5	13.7	$6\frac{1}{4}$	79.5	17.6	
Exp. 8	June	l	43.3	5.8	$5\frac{1}{4}$	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	$5\frac{1}{4}$	75.0	43.1	h
Exp. 13	Jan.	o	38.5	46.8	$4\frac{1}{2}$	46.5	40.8	h
Exp. 15	June	B	a) 38.5	28.6	$6\frac{1}{4}$	55.3	25.9	h
		o	b) 47.8	0.0	$6\frac{1}{4}$	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

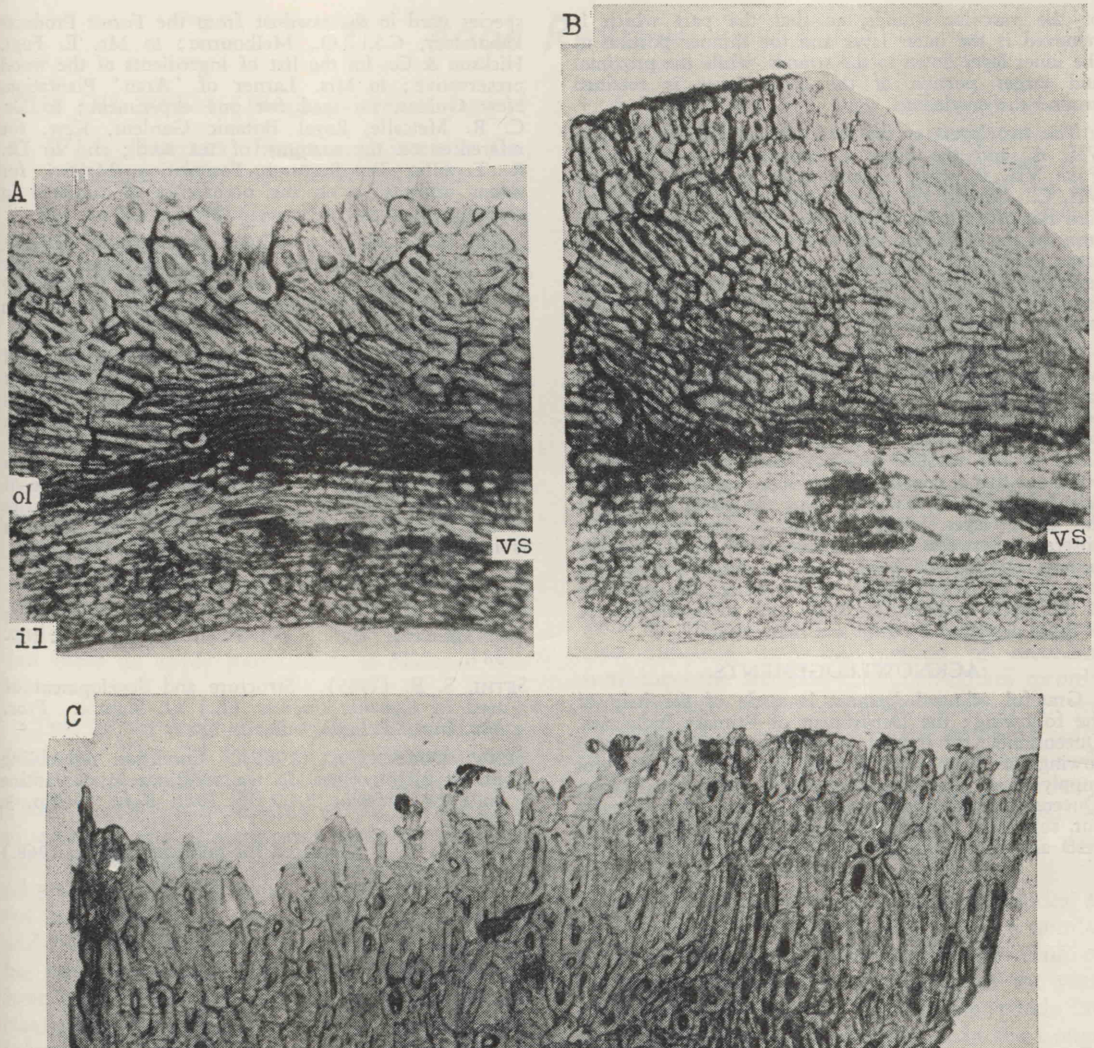


Plate VII.—A. Section through seed coat showing inner layer ('il') bisected by the vascular strands ('vs'), and the outer layer ('ol').
B. Section through seed coat showing splitting of the inner layer in the plane of the vascular strands.
C Section through seed coat showing more advanced splitting of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