STUDIES ON COLLAR AND BRANCH CANKER OF YOUNG TEA (*PHOMOPSIS THEAE* PETCH)

II—INFLUENCE OF SOIL MOISTURE ON THE DISEASE

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Young tea was inoculated with *Phomopsis theae* at different times of the year, on four different locations, and in more than 75% of the plants, infection occurred. There was evidence of the existence of a seasonal variation in the susceptibility of young tea to infection by *P.theae*. Although infection could take place any time during the year, maximum canker development occurred only when plants were infected during the drier months ; the period of maximum canker activity, therefore, coincided with the drought.

The final mean length of the cankers was inversely correlated with rainfall, soil moisture and bark moisture. Bark moisture, measured according to the method described by Bier (1961) was positively correlated to soil moisture and appeared to be dependent on the latter.

In pot experiments, the size of the cankers proved to be closely correlated to soil moisture content. Low soil moisture favoured maximum canker development and, in this regard, a post-infection dry period appears to be highly critical.

Introduction

Collar and Branch Canker disease of tea caused by *Phomopsis theae* is serious only in young plantations up to about eight years in age. Cankers are generally found on the branches, the collar and at the base of twigs, leaf-scars and prune cuts, and are invariably associated with wounds or injuries caused by mechanical damage, wind or sun-scorch. Young plants are killed if cankers girdle the collar completely. The death of branches caused by branch cankers is also a common feature (Shanmuganathan 1965). Recently, *P.theae* was also found to infect young, apparently uninjured, succulent, green shoots, although such infection rarely caused serious damage to the bush (Shanmuganathan & Rodrigo (1966).

The disease occurs only at high elevations, usually over 4000 ft above sea level, and is of economic importance only in the north east monsoon zone, which experiences a pronounced drought from June to August, and in some of the drier parts of the south west monsoon zone. Further, canker attacks are generally more severe on poor soil, showing that dry weather and poor soil are predisposing factors.

Several investigators have shown in the past that climatic and site factors, age of the host and seasonal changes may exert a strong influence on the intensity of canker disease of forest trees, caused by facultative parasites like *Phomopsis* spp., *Hypoxylon pruinatum, etc* (Boyce (1961); Anderson (1964); Wright (1957). It has also been observed that bacterial canker of stone fruits caused by *Pseudomonas mors-prunorum* is more severe during dormancy than during the active growing period, and this is attributed to the inability of the host to produce wound periderm during dormancy (Shanmuganathan 1962). Further, Bier (1959 a ; b) has demonstrated a close correlation between the development of bark cankers and the relative turgidity levels of young, living bark. Bier & Rowat (1962) also maintain that the saprophytic flora of the bark may play a significant role in the onset and severity of canker diseases of poplar and willow. In the case of Collar and Branch Canker of tea, however, there is much information from field observations which suggests that soil moisture is an important factor influencing the disease. To determine how

FIGURE 1 — *Death of branches following branch inoculation with* P. theae

significant this is and to confirm the field observations, a number of field and greenhouse experiments were conducted, and this paper outlines the results of these investigations. The field experiments also served to find out whether there were seasonal variations in the susceptibility of tea to cankers caused by *P. theae.*

Materials and methods

Four inoculation experiments were conducted in the field on estates situated in the north east monsoon zone. The estates and other relevant data are given below.

Experiments No.	*Estate*	*Elevation* (ft)	*Clone inoculated*	*Age* (Years)
UP3	Nayabedde	6500	N 3	2
UP4	Demodera	3500	TRI 2024	5
UP5	Aislaby	4400	TRI 2024	5
UP6	TRI Gonakelle Substation	3600	TRI 2023	2

In these experiments young tea plants were inoculated with *P. theae* at monthly intervals over a period of one year. Inoculations were made about the middle of each month and the resulting cankers were measured three months later when cankers had ceased growth. Twenty plants were inoculated each month, and on each plant one inoculation was made on the collar and one on each of three different branches, making a total of four inoculations per plant.

Methods of inoculation

The collar inoculations were made by removing a disc of bark with a No 3 cork borer and placing a disc of agar culture of the fungus on the exposed wood. The branches were inoculated by making a clean ∧-shaped cut with a sharp scalpel deep enough to penetrate into the wood and then placing a disc of the fungal culture inside the wood. Soon after inoculation all wounds were bound with polythene tape to prevent the inoculum drying up or being washed away. On each occasion five plants were wounded but left uninoculated, and these served as controls. The fungus was grown on potato dextrose agar for about two weeks at 25°C, and discs were taken from the outer edge of the culture for inoculation.

Soil and bark moisture determinations

In experiments **UP3** and **UP4**, soil moisture was determined on an oven-dry basis once every month on the day of inoculation by sampling around the inoculated plants. Bark moisture was measured according to the method described by Bier (1964) using 20 bark discs (approximate diameter 0.7 cm) for each determination.

In experiments **UP5** and **UP6**, soil and bark moisture were determined weekly instead of monthly, during the last six months of the inoculation period. Rainfall and temperature were also recorded on the experimental sites.

Results

Typical cankers developed from the inoculations at all four locations, the longest cankers being formed at Nayabedde. The percentage of successful inoculations for all four locations was 78.0. At Gonakelle the percentage of successful inoculations (62.9) as well as the length of cankers was low compared with the other three locations. Cankers rarely girdled the collar completely except in one case at Nayabedde where the bush died. Branch inoculations resulted in the death of several branches, and in many instances cankers extended to the tip of the branches (Figure 1). Collar inoculations were on the whole less successful than the branch inoculations, presumably because of the difference in the method of inoculation. About eight per cent of the control inoculations also developed cankers, but many of these were not typical of *P. theae* and were possibly caused by allied pathogens like *Macrophoma theicola* and *Botryodiplodia theobromae*. These cankers were all small and appeared at random throughout the inoculation period without showing any definite seasonal pattern.

Inoculation experiments at Nayabedde Estate

Table 1 shows the mean lengths of the cankers obtained at Nayabedde, together with the figures for rainfall, and bark and soil moisture. These results show clearly that there is a marked seasonal variation in the susceptibility of tea to canker development. Although stems can be infected at any time during the year, the largest cankers are produced only when infection takes place during the drier months. The period of maximum invasive activity of the pathogen, therefore, coincides with the drought. It is interesting to note that during the period of maximum canker activity both soil and bark moisture are at their lowest, the only exception being January, when canker development was restricted, although rainfall and bark and soil moisture were low. It is possible that the low mean temperatures (monthly mean 55°F) prevailing at this time of the year was limiting canker activity.

TABLE 1—*Relationship between size of canker, time of inoculation, rainfall and soil and bark moisture — Experiment* **UP3** *at Nayabedde Estate, Bandarawela*

Month	Rainfall (in)	Soil moisture (%)	Bark moisture (Relative turgidity) (%)	Mean canker length (cm)
November ..	5.45	34.7	94.0	1.7
December ..	4.74	30.3	92.4	1.8
January ..	2.83	20.5	89.5	1.8
February ..	6.08	30.8	99.2	2.2
March ..	4.68	20.2	98.7	2.5
April ..	15.02	29.0	93.5	0.5
May ..	10.14	25.0	94.2	2.3
June ..	0.06	21.0	90.0	15.7
July ..	3.34	21.0	—	10.3
August ..	8.79	32.1	96.3	2.1
September ..	2.88	33.7	97.3	3.7
October ..	14.59	36.7	100.0	1.9

Inoculation experiments at Demodera Group

Table 2 shows the mean lengths of the cankers obtained at Demodera, together with the figures for rainfall and bark and soil moisture. It is seen that the general features observed at Nayabedde are also evident at Demodera. Maximum canker development is again found to be associated with low rainfall and low soil and bark moisture. The largest cankers at Demodera were obtained from the January inoculations unlike at Nayabedde where June and July appeared to be the most favourable months for canker development. This may be because the lower elevation, and, therefore, the higher mean temperatures (72-73°F) at Demodera during June and July were not very favourable for maximum canker activity. Nevertheless, reasonably large cankers were still obtained during June and July. The mean monthly temperature for January was 66°F (*cf* the mean temperature for June and July at Nayabedde were 62 and 61°F respectively).

TABLE 2—*Relationship between size of canker, time of inoculation, rainfall and soil and bark moisture — Experiment* **UP4** *at Demodera Group, Demodera*

Month	Rainfall (in)	Soil moisture (%)	Bark moisture (Relative turgidity) (%)	Mean canker length (cm)
November ..	4.50	16.6	95.5	—
December ..	5.42	19.0	94.1	1.7
January ..	1.61	10.9	93.1	5.5
February ..	5.40	19.0	97.2	1.8
March ..	3.47	11.4	89.7	2.5
April ..	10.20	21.5	98.8	1.0
May ..	8.76	25.2	95.9	1.7
June ..	0.05	13.4	77.0	3.0
July ..	3.37	12.4	71.6	3.7
August ..	8.23	24.6	95.4	1.6
September ..	8.39	24.6	94.2	1.8
October ..	9.33	25.4	93.9	1.6

Inoculation experiments at Aislaby Estate

Table 3 shows the mean lengths of the cankers obtained at Aislaby Estate, together with the figures for rainfall, and bark and soil moisture. These results show similar trends to those observed at Nayabedde although cankers were generally smaller, probably because the plants were older at the time of inoculation. The largest cankers at Aislaby were obtained from the July inoculations coincident with low rainfall and low bark and soil moisture. June inoculations, however, resulted in smaller cankers in spite of the rainfall and soil and bark moisture being low. This is difficult to explain.

TABLE 3—*Relationship between size of canker, time of inoculation, rainfall and soil and bark moisture — Experiment* **UP5** *at Aislaby Estate, Bandarawela*

Month	Rainfall (in)	Soil moisture (%)	Bark moisture (Relative turgidity) (%)	Mean canker length (cm)
December ..	9.71	22.8	98.8	0.44
January ..	5.34	22.2	98.7	0.27
February ..	0.82	16.4	94.5	1.84
March ..	7.90	27.3	91.8	0.24
April ..	3.19	17.4	85.2	0.77
May ..	1.67	16.7	90.5	2.01
June ..	2.03	14.5	85.9	1.00
July ..	0.94	13.6	79.8	4.05
August ..	6.79	19.2	80.7	1.58
September ..	5.35	22.8	91.9	1.46
October ..	9.85	24.2	94.6	1.14
November ..	4.79	20.1	92.0	1.88

Inoculation experiments at Gonakelle Group

Table 4 shows the mean lengths of the cankers obtained at Gonakelle together with the figures for rainfall and bark and soil moisture. Although these results are somewhat erratic, the association between low rainfall and maximum canker activity is still discernible. Cankers obtained here were the smallest, and one important reason for this may be that the clone used (TRI 2023) is highly resistant to *P. theae* (Shanmuganathan 1967).

TABLE 4—*Relationship between size of canker, time of inoculation, rainfall and soil and bark moisture — Experiment* **UP6** *at Gonakelle Group, Passara*

Month	Rainfall (in)	Soil moisture (%)	Bark moisture (Relative turgidity) (%)	Mean canker length (cm)
December ..	14.24	19.7	96.5	0.47
January ..	7.02	27.0	98.1	0.69
February ..	1.39	21.2	94.5	1.26
March ..	11.38	18.7	97.1	0.06
April ..	7.57	28.6	92.3	0.77
May ..	2.83	20.7	90.0	1.34
June ..	1.59	14.5	82.4	1.24
July ..	1.73	17.1	88.3	2.38
August ..	16.27	22.3	94.4	1.33
September ..	5.71	21.7	93.1	0.47
October ..	11.36	24.4	98.1	1.19
November ..	22.84	24.6	99.7	1.18

Relationship between canker length and rainfall and soil and bark moisture

The coefficient of correlation (r) between canker length and (1) rainfall, (2) soil moisture and (3) bark moisture is given in Table 5 for the four inoculation experiments. It will be seen that r is negative for all the three correlations determined, indicating a negative correlation between disease and rainfall, soil moisture and bark moisture. The negative correlations between (1) canker length and soil moisture and (2) canker length and rainfall are significant at Demodera and Aislaby and are nearly so at Nayabedde. The failure to obtain a significant correlation between canker length and rainfall, and canker length and soil moisture at Gonakelle is incomprehensible. Bark moisture also shows a significant negative correlation with canker length at Aislaby and Gonakelle. It should be mentioned that in the case of the Nayabedde and the Demodera inoculations, many of the bark moisture determinations were made on the day following sampling because of certain unavoidable difficulties and this may account for the poor correlations obtained there.

A close correlation seems to exist between soil moisture and bark moisture as is apparent from the weekly determinations of these factors carried out at Gonakelle and Aislaby over a six-month period. The respective r values for the two series are, Gonakelle 0.8318 ($P < 0.001$) and Aislaby 0.6737 ($P < 0.001$). Bark moisture appears, therefore, to be significantly dependent on soil moisture.

TABLE 5—*Relationship between size of canker, rainfall and soil and bark moisture*

	Correlation coefficient (r)			
	Nayabedde	Demodera	Aislaby	Gonakelle
Rainfall	—0.572	—0.713*	—0.582*	—0.314
Soil moisture	—0.506	—0.793**	—0.652*	—0.341
Bark moisture	—0.324	—0.463**	—0.584*	—0.718**
LSD - P = 0.05*	—0.576	—0.602	—0.576	—0.576
P = 0.01**	—0.708	—0.735	—0.708	—0.708

Greenhouse experiments

Two experiments were conducted in the greenhouse to study the precise effect of soil moisture on the disease. In these experiments two-year-old potted plants of clone TRI 2024 were inoculated with *P. theae* and the size of the resulting cankers was measured three months later. There were ten plants per treatment and each plant was inoculated once on the main stem. The treatments in the first experiment (**P50**) were :

1 — Water witheld for two weeks before and after inoculation.

2 — Water witheld for two weeks after inoculation.

3 — Water witheld for two weeks before inoculation.

4 — Plants watered daily.

Results are shown in Table 6 from which it is abundantly clear that soil moisture has a very marked influence on the size of the cankers resulting from infection by *P.theae*. For maximum canker activity, a pre- and a post-inoculation period of low soil moisture seems necessary and, in this regard, a post-inoculation dry period appears to be more critical than a similar pre-inoculation dry period. A pre-inoculation dry period did not appear to have a significant effect on canker activity unless it was followed by a similar post-inoculation dry period.

TABLE 6—*Relationship between size of canker and soil moisture in potted plants subjected to dry periods of varying duration*

Treatments	Mean canker length (cm)
wet/wet	1.78
dry/wet	2.06
wet/dry	6.89
dry/dry	11.16
Significant difference	P = 0.001 — 5.14
	P = 0.01 — 3.90
	P = 0.05 — 2.91

In the second experiment (**P51**), the effect of three different levels of soil moisture was studied in three soils. The soils used were collected from St Coombs, Gonakelle and Maturata estates.

The St Coombs soil was a clayey loam with a high content of organic matter and with good texture. The Gonakelle and Maturata soils were sandy loams with much gravel and low organic matter, the Maturata soil being distinctly poorer than the Gonakelle soil.

The treatments were :

1 — Plants watered daily
2 — Plants watered once a week
3 — Plants watered once every two weeks.

The treatments commenced a month before inoculation and were continued for three months after inoculation until the cankers were measured. The results are presented in Table 7.

TABLE 7—*Relationship between size of canker and soil moisture in potted plants watered differently*

Treatments		Mean length of canker (Transformed data)
Watered daily		0.173
Watered once a week		0.425
Watered once in two weeks		0.618
Significant difference	P= 0.001	— 0.263
	P= 0.01	— 0.183

It will be seen that when plants were watered once a week and once in two weeks larger cankers were formed than when plants were watered daily. Further, when plants were watered once in two weeks cankers were significantly larger than when plants were watered once a week indicating that the lower the soil moisture, the greater the canker length. There was no significant difference in the size of the cankers produced in the three different soils, which was somewhat contrary to what was expected. It is possible that the moisture levels tested were not low enough to bring out any possible differences due to soil type.

Discussion

The results of the field experiments show clearly the existence of a seasonal variation in the susceptibility of young tea plants to infection by *P.theae*. Once-monthly inoculations carried out over a period of one year at four different locations indicated that maximum canker development was associated with low rainfall. This seasonal pattern is particularly evident at Nayabedde where outbreaks of *P. theae* have been frequent in the past. Although stems can be infected any time during the year, some factors restrain canker development during the wet months.

Temperature also appears to have some influence on canker development, the ideal range being 60-70°F. This explains the restriction of the disease to high elevations in the range of 4000 to 6000 ft. The failure to obtain large cankers at Gonakelle and Demodera, comparable with those occurring naturally, may be because these two estates are situated outside the elevation range in which outbreaks of *P. theae* normally occur. At Aislaby the probable reason for the similar trend was because the plants were old. Although attacks of *P. theae* can occur up to eight years, they are generally more severe in the second - third-and fourth-year clearings. It should also be mentioned that different isolates of the fungus were used for inoculation at the four different sites, and inherent differences in virulence between these isolates may also have contributed to the differences in the size of the cankers obtained at the four locations (Shanmuganathan 1967).

Seasonal variation in host susceptibility is a feature common to many diseases involving stems and branches of trees originating from wound infections, *eg* Bacterial Canker of stone fruits (Shanmuganathan 1962) and *Gloeosporium* Canker of apples (Corke 1956), and is obviously related to host activity. In temperate plants, which experience a well-defined dormant season, it is possible that during dormancy, the ability to form defensive barriers like periderm may be lost, and this may explain the greatest invasive activity of the pathogen at this time. In the case of Collar and Branch Canker, however, it is not yet known whether any significant reduction in host activity occurs during the drought, which could retard the development of such barriers. It is, of course, well known that the flushing capacity of even the most vigorous clones drops considerably during the drought so that plants can be assumed to be almost dormant at this time.

The relationship between soil moisture and canker development is amply demonstrated in the greenhouse experiments. It now seems clear that low soil moisture is an important factor favouring canker activity and that when plants are subjected to a water stress they are more vulnerable to canker infection. This is in accordance with field observations which indicate a close parallel between deaths caused by drought and deaths caused by *P.theae* cankers. On several estates we have observed that clearings and clones which show a high incidence of deaths caused by *P.theae* also show heavy casualties caused by drought periods.

One of the manifestations of low soil moisture appears to be a reduction in bark moisture, a factor which Bier (1964) considers vital for the development of canker diseases. In this work we have demonstrated a strong positive correlation between soil moisture and bark moisture and the latter appears to be dependent on the former. It thus seems reasonable to assume that dry weather leads to a reduction in bark moisture, through its effects on soil and plant, thereby causing certain physiological changes in the bark tissues which are favourable for canker development. It has been shown that one of the effects of water stress in plants is the hydrolysis of the protein in the leaves resulting in an enrichment of the phloem sap with soluble nitrogen (Kennedy & Booth 1959). If such an enrichment also occurred in the bark tissues, there is little doubt that it will greatly facilitate the growth of the pathogen. Obviously further investigations are required to elucidate fully the close association between soil moisture and the disease.

The poor correlation between bark moisture and canker length at Nayabedde and Demodera may be due to errors in bark moisture determinations, as explained earlier. Further, a single determination per month may also have been inadequate. There are also other errors inherent in the method itself such as leaching of the bark samples when placed in distilled water to determine saturated weight. The same criticisms probably hold true for the soil moisture determinations. It is now realized that soil and bark moisture should have been recorded at least weekly in all four experiments throughout the inoculation period, although this may have been laborious.

Acknowledgements

We wish to thank the Managers of Nayabedde, Demodera and Aislaby Estates for providing sites for experiments and for their co-operation. Thanks are also due to Messrs L. M. de W. Tillekeratne and D. N. R. Wijewardena of the TRI Uva Station for much help with the field experiments, and to Mr P. Kanapathipillai for the statistical analyses.

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(Accepted for publication—25th August 1967)