African Scientist Vol. 7, No. 3, September 30, 2006
Printed in Nigeria

1595-6881/2006 $12.00 + 0.00

http://www.klobex.org

AFS 2004013/7301

Thread blight disease of tea [*Camellia sinensis* (L.) O. Kuntze] caused by *Marasmius pulcher* (Berk & Br.) Petch in the South Western Nigeria.

A. R. Adedeji

Plant Pathology Division, Cocoa Research Institute of Nigeria, P.M.B. 5244, Ibadan, Nigeria.

E-mail: aradeji@yahoo.co.uk

(Received March 22, 2004)

ABSTRACT: The causal organism(s), and percentage infection of thread blight disease observed on some tea stands established in 1985 at the Cocoa Research Institute of Nigeria (CRIN), Ibadan, experimental plot was investigated for a period of one year (12 months).

For the first months, the percentage infection ranges between 41.4% and 47.9%. Out of three fungi isolated (*Botryodiplodia theobromae, Fusarium sp.* and *Marasmius pulcher*) only *M. pulcher* produced the characteristic symptom observed in the field. The fungal hyphae infected through the stomata pores that were found only on the abaxial surfaces of the leaves. Clone 35 was found to be most susceptible followed by clones 143 and 318 while the least infection was recorded in clone 236. Pruning as a cultural control method proved effective by reducing the percentage infection by 100% in the first 3 months. However, re-infection of 5.4 and 5.9% were recorded in the 4th and 5th months respectively.

Key Words: Tea (*Camellia sinensis*); Thread blight disease; *Fusarium; Marasmius; Botrydiplodia.*

Introduction

Tea (*Camellia sinensis* (L) O. Kuntze) was introduced into the country by de Bouley from West Cameroon in 1952 (Kassbol-Smith, 1965). However, the first commercial clones were imported into the mambilla Plateau in 1975 (Hainsworth, 1981). Since then, more clones have been imported to establish large commercial plantations.

Since 1983 when regular survey for tea diseases started by the Cocoa research Institute of Nigeria (CRIN), five major diseases have been reported (Filani *et al.,* 1989). These diseases are leaf spot caused by *Helminthosporium sp.* and *Pestalotia theae* (Olunloyo, 1985); shoot rot disease caused by *Fusarium, Helminthosporium, Cercospora, Botryodiplodia* and *Rhizoctonia spp.* and *Pestalotia theae* (Olunloyo, 1985 and Filani *et al*., 1989), rot diseases of tea cutting caused by *Fusarium solani; F. oxysporum* and *Pythium*

ultimum (Olunloyo *et al*., 1987), root rot caused by *Fomes lignosus* and leaf blight caused by *Pestalotia theae, Collectorichum camellia, Helminthosporium* and *Cercospora spp.* (Olunloyo, 1985).

Apart from five major diseases reported so far on Mambilla Plateau, a host of other destructive diseases have been reported on tea all other the world. These include root rot caused by *Armilaria mellea* (VAHL) Pat., grey blight caused by *Pestalotia theae*, sectorial die-back caused by *Phomosis sp.* (Onsando *et al*., 1997; Annon, 1991); *Hypoxylon* wood rot caused by *Hypoxylon serpens* (Pers. Exfr.) Kickx (Otieno, 1996), wood rot caused by *Poria hypolateritia* (BERK), branch cancer caused by *Macrophoma thiecola* (Petch) Annon (1991).

Due to limited land area for tea on Mambilla Plateau, tea adaptation trial plots were established in seven lowland locations in Nigeria (Omolaja, *et al*., 2000). This has called for constant monitoring of disease occurrence and incidence in these zones. In August 2000, occurrence of leaf blight, which resulted in heavy defoliation, was observed on some tea stands at the CRIN headquarters, Ibadan (7° 25'N and 3° 52'E). On closer observation, the myceliastrands of the causal organism were found ramifying the leaf blade of the affected leaves forming network of threads. This symptom was quite different from the previously reported blights in that it has network of threads and infects through the lower surface of the leaf blade, hence the need to investigate its causal organism(s), percentage infection and to develop safe control method.

Materials and Methods

Field Observations

Infected tea stands (15 years old) were thoroughly monitored in the field to determine the mode of infection. Percentage infection was calculated by counting the number of infected and non-infected leaves. The rate of spread of hyphae on both young and old leaves was measured weekly.

Penetration

Mode of penetration of the fungus was studied in the laboratory through sectioning of infected leaves. The leaves were decolourised in ethyl alcohol (7%%) for 24 hours in covered Petri dishes, then stained in glycerin in cotton blue. The sections were then examined under microscope.

Presence and frequency of occurrence of stomata were determined using Mustapha 91984) method in the relation;

$$\frac{x}{y} \times 1 \text{ (stomata mm)}^{-2}$$

Where x = average number of stomata per field of view
y = calculated area of field of view (πr^2)
r = 0.65

Isolation and identification of Fungi

Mycelial strands of the fungus and infected leaves were collected into sterile polythene bags and brought to the laboratory. Fungi isolations were carried out by plating each sample on Potato Dextrose Agar (PDA) and Oat meal agar. Plates were incubated at 25°C for 10 days and pure cultures were obtained through sub-culturing. The isolated fungi were identified in accordance with the description of Ainsworth and Sussman (1965, 1966 and 1968), Turgen *et al*., (1977).

Pathogenicity test

Four tea clones (35, 143, 236 and 318) were tested in the screen house for their susceptibility to the fungal attack using both hardened and fresh young leaves. The two surfaces (adaxial and abaxial) were inoculated with 3mm disc of the pathogens' culture. The tips of the leaves were then attached to the petiole using an adhesive tape to enclose the inoculum in the fold. Five of such leaves were treated per clone. Samples were collected after 10 days from the infected among the treated leaves for re-isolation and re-

examination in the laboratory. The re-isolated pathogen was then compared with the previously isolated ones.

Control

Since the use of fungicide to control tea folial diseases have been discouraged (Hainsworth, 1981), 60% pruning of infected twigs (Shurtleff, 1962) was carried out. The disease incidence was then evaluated after 5 months.

Results and Discussion

Field Observation

The white mycelial strands grew up from the main stems, extend to branches and send whitish fungal 'thread' (hyphae) to the leaf stalk where they extend to the leaf blades. The fungus was found only on the abaxial surfaces of the leaves (Plate 1).

In most cases, necrosis did not form until the whole leaf blade is covered with the strands. Whenever the necrosis forms, it spreads within 21 days to every parts of the leaf blade.

Unlike the finding of Asare-Nyako (1977), the pathogen was found to infect both young and old leaves, however necrosis formed early on the older leaves. This is because the stomata of old leaves are more wider and easily penetrated by the fungal – hyphae. The leaves stop to develop as soon as necrosis form and may become dried and detach within 2 weeks due to the release of toxic exudase into the leaf tissue. Interestingly, the fungal hyphae can be easily removed without leaving any sign on the leaf if the necrosis did not form. This indicated that necrosis only formed after the pathogen hypha might have penetrated the leaf blade.

The infection of healthy leaves was either through direct contact with infected ones or 'Sticky hyphal clump', which extends from dead detached and lossely hanging leaves, thus making pruning an effective control measure of this diseases.

During the first seven months (August – February) of monitoring, the maximum percentage infection (47.9) was recorded in January while the minimum (41.3%) was recorded in February (table 3)..

Table 1: Presence and frequency of stomata on the leaf surfaces of four tea (*Camellia sinensis*) clones.

S/No.	Cones	Distribution of Stomata		Stomata Frequencies
		Upper surface	Lower surface	
1	35	-	+	21*
2	143	-	+	28
3	236	-	+	20
4	318	-	+	27

\- Absent
\+ Present
*Per $1mm^2$ view.

Table 2: Results of inoculation on the lower and upper leaf surfaces of four tea (*Camellia sinensis*) clones.

Tea	Lower leaf surface replications						Upper leaf surface Replications					
	1	2	3	4	5	Disease Incidence [%]	1	2	3	4	5	Disease incidence [%]
35	+	+	+	+	+	100	-	-	-	-	-	0
143	+	-	+	+	+	80	-	-	-	-	-	0
236	-	+	+	+	-	60	-	-	-	-	-	0
318	+	+	+	_	+	80	-	-	-	-	-	0

\+ Infected
\- Non-infected

Table 3: Percentage infection for both pre-control and post-control periods

Month	Percentage Infection	
	Pre-control period	Post-control period
August 2000	46.2	-
September 2000	46.4	-
October 2000	46.7	-
November 2000	46.4	-
December 2000	46.0	-
January 2001	47.9	-
February ”	41.3	-
March “	-	0
April “	-	0
May “	-	0
June “	-	5.4
July “	-	5.9

\- Not Applicable.

Table 4: Effect of pruning on disease incidence.

Month	Percentage Infection		Percentage Reduction	
	Untreated	*Treated*	*Untreated*	*Treated*
March	46.3	0	0	100.0
April	46.8	0	0	100.0
May	46.7	0	0.1	100.0
June	46.9	5.4	0	94.6
July	46.9	5.9	0	94.1

Penetration

Plate 2 shows the fungal hyphae penetrating thestomata pore of the leaf. Necrosis was induced after penetration, while the epidermal cells of the infected leaves were observed to be disorganized. This is an indication that a toxic substance was exuded from the invading strands. The stomata were found only on the abaxial surface of the leaf (Plate 1). The presence and frequency of stomata on the four tea clones examined were presented in table 1. It is therefore understandable that the infection of all the tea clones tested were on the abaxial surface of the leaf. Clone 143 had more stomata (28) per $1mm^2$ of leaf surface followed by clone 318 (27) while clones 35 and 236 had 21 and 20 stomata per $1mm^2$ respectively.

Disease incidence

Marasmius pulcher, family *Agaricaceae* (Ainsworth and Sussman, 1965, 1966, 1968 and Clement and Shear, 1964) was isolated when Oat meal agar was used. The organisms covered the culture plate 13 days after inoculation. The pathogen gave the characteristic synmptom observed in the field when the abaxial surface of the leaf was inoculated with 3mm disc of the pathogen's culture. This agreed with the report of Turgen *et al*., (1977).

Other two fungi (*Botryodiplodia theobromae* and *Fusarium sp.*) were frequently isolated from the necrotic and dead parts of infected leaves when potato dextrose agar (PDA) was used. However, none of them gave the well-marked thread network symptom observed in the field. It is possible that these organisms were saprophytes.

All the four clones (35, 143, 318 and 236) tested were found to be susceptible to the pathogen attack (Table 2), clone 35 was mostly susceptible (100%), clones 143 and 318 had 80% susceptibility while clone 236 was least susceptible (60%). However, considering the level of susceptibility, none of these clones can be said to be resistant or tolerant to this disease. This situation requires adequate attention. The susceptibility of clone 35 to other diseases has been reported earlier (Fawole, 2000).

The fungus did not infect through the adaxial surfaces of the leaves of all the clones tested. This could be due to the absence of stomata on this surface as it was found that the fungal hyphae penetrate the leaf surface through the stomata pore (Plate 2).

Control

Hundred percent control was observed for the first three months after 60% pruning of the affected twigs (Table 4). This was possible since the disease spread from infected to healthy twigs through direct contact. However, 5.4 and 5.9% infection were recorded in the 4^{th} and 5^{th} months respectively (Table 3). This was suspected to be due to the presence of remains of mycelia strands on the stalk of pruned twigs.

Conclusion

This is the first time that the thread blight disease would be reported on tea in Nigeria. The disease attacks both young and old leaves that might make it a delimiting factor to tea production in the low land areas of the country since the leaf is the harvested part of tea crop that is processed into "made tea". Interestingly, the result obtained in this work showed that 100% control could be achieved with 60% pruning of the affected twigs thereby allaying this fear (Table 4).

It is therefore recommended that the pruning should be done immediately the disease is noticed and the pruned parts should be burnt outside the plot to avoid re-infection of healthy strands. However, regular monitoring should be carried out to detect any further re-infestation of the healthy twigs.

ACKNOWLEDGEMENT:The author is grateful to The Director/Chief Executive of CRIN for the permission to publish this work.

References

Ainsworth, G.C. and Sussman, A.S. (1965). The fungi – vol. I, The Fungi cell – an advance treatise. Acad. Press Inc., p. 500

Ainsworth, G.C. and Sussman, A.S. (1966). The Fungi – vol. II – The fungi organism. Acad. Press Inc., p. 446.

Ainsworth, G.C. and Sussman, A.S. (1968). The Fungi – vol. III – The fungi population. Acad. Press Inc., pp. 537.

Anonymolus (1991). Tea – The tropical agriculturist series. Macmillan Edn., pp. 69 – 73.

Asare-Nyako, A. (1997). White thread blight of cocoa. Proceedings of 1st Intl. Cocoa Pests and Disease Seminar, Accra – Ghana, pp. 132 – 138.

Clement, F.E. and Shear, C.L. (1964). The general of fungi. Hafner Pub. Inc.

Fawole, E.A. (2000). Major diseases identified on the tea bushes on the Mambilla Plateau (Unpublished data).

Filani, G.A.; Olunloyo, O.A. and Idowu, O.L. (1989). Diseases and insect pests of tea in Nigeria. Progress in tree crop research, 2nd edition, pp. 201 – 208.

Hainsworth, E. (1981). Tea production on the Plateau, Gongola State of Nigeria. A report on the project by the consultants for the Nigeria Beverages production Company Ltd. Pests and Disease, pp. 120.

Kaasboll-Smith, S.A. (1965). Notes on cultivation of Arabica coffee on mambilla District. Ministry of Agriculture, Northern States, Nigeria, 21pp.

Mustapha, O.T. (1984). Biosystematic studies of *Pancratium hirtum* A. Chev. Complex (*Amaryllidaceae*) in Nigeria. M.Sc. Botany Thesis, University of Ilorin (Unpublished).

Olunloyo, O.A. (1985). Field survey of tea diseases on Mambila Plateau. Ann. Rept. CRIN, Ibadan, Nigeria, pp. 91 – 93.

Olunloyo, O.A.; Obatolu, C.R. and Esan, E.B. (1987). Interim report on the diseases out break in Lever Brothers' Tea project Nursery at Maisamari, Mambilla, Plateau, pp. 1-7.

Omolaja, S.S.; Esan, E.B. and Obatolu, C.R. (2000). Adaptability trial of three tea clones in lowland areas of Nigeria (Unpublished data).

Onsando, J.M.; Wargo, P.M. and Wando, S.W. (1997). Distribution, severity and spread of *Ammilaria* root disease in kenya tea plantations. Plant Dis. 81: 133 – 137.

Otieno, W. (1996). Hypoxylonwood rot of tea pathogenesis and control. Tea 17(1): 5 – 6.

Shurtleff, M.C. (1962). How to control plant disease. Iowa State University Press.

Turgen, K.; Heinz, S. and Werner, K. (1977). Diseases, pests and weeds in tropical crops. John Wiley and Sons Ltd., pp. 101.