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Natural occurrence and distribution of stem cankers caused by *Phytophthora megakarya* and *Phytophthora palmivora* on cocoa

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Abstract

Epidemiological studies were conducted in five cocoa growing districts in the Eastern Region of Ghana solely infected by *Phytophthora palmivora* and five districts in the Ashanti and Brong Ahafo Regions prevalently infected by *Phytophthora megakarya* to determine the natural incidence, the vertical distribution on trees and the probable sources of stem canker infections, and to isolate and identify the causal pathogens. The incidence of canker in the solely *P. palmivora* infected area was higher (between 0% and 16.0%) than in the area mainly infected with *P. megakarya* (0.5–8.0%). Differences were found in the natural height distribution of cankers in the two areas, whilst the areas solely infected with *P. palmivora* showed a near normal curve, those prevalently infected with *P. megakarya* were positively skewed. Most of the cankers caused by *P. megakarya* were found at the base or near the base of the tree trunks (1–40 cm above ground level), while those of *P. palmivora* were concentrated between 41 and 100 cm from the ground level. The majority (71.8%) of cankers in the solely *P. palmivora* infected area were cushion-borne, followed by 24.3% from unknown sources and only 3.9% from the soil. In contrast, a significantly large proportion (32.6%) of the cankers in the prevalently *P. megakarya* infected area were soil-borne, although cushion-borne cankers formed the majority (48.4%) due to the presence of *P. palmivora* infection whilst those of unknown sources constituted 19.0%. *Phytophthora megakarya* was frequently isolated from all the three sources of canker infections, indicating *P. megakarya* readily causes stem canker on cocoa. These results emphasise the importance of different reservoirs as sources of primary inoculum for diseases caused by the two *Phytophthora* species particularly pod rot infection on cocoa.

Introduction

Cocoa (*Theobroma cacao*) is the most important cash crop in many West and Central African countries and is largely produced (more than 80%) by small-scale farmers (Assoumou, 1997). The most serious constraints to cocoa production in this region include pest and diseases (Duguma et al., 2001), of which *Phytophthora* pod rot, commonly called 'black pod' is the most economically important. Until the mid 1980s, only *Phytophthora palmivora* was known as the causal agent of *Phytophthora* diseases on cocoa in Ghana and it is found in all the cocoa growing regions. Crop losses attributed to this species were estimated between 4.9% and 19% (Blencowe and Wharton, 1961;

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Dakwa, 1984). However, in 1986 *P. megakarya* was found in the Akomadan cocoa district in the Ashanti Region of Ghana (Dakwa, 1988). This species causes severe crop losses ranging between 60% and 100%. Recent surveys (Anonymous, 1995; Opoku et al., 1997a) have indicated a rapid spread of *P. megakarya* to other cocoa districts of the country (Appiah, 2001), threatening the livelihood of many cocoa farmers (Opoku et al., 2000).

All parts of the cocoa tree are susceptible to *Phytophthora* species although to different extents (Appiah, 2001). Unlike pod infections, the effect on root, stem and leaves are rather indirect and difficult to quantify, therefore both farmers and researchers alike have tended to ignore their impact and epidemiological importance. Canker is the symptom developed on stems covered with matured bark following *Phytophthora* infection (Firman, 1974). Stem canker is the next important *Phytophthora* disease of cocoa after black pod but has been described as the forgotten disease of cocoa (Vernon, 1971).

In West Africa, stem canker has not caused much concern, although it is very important in Papua New Guinea (Prior and Sitapai, 1980; Prior, 1981). However, during a nationwide survey of *Phytophthora* species on cocoa in Ghana (Opoku et al., 1997a) and specific visits to farms in the Brong Ahafo and Ashanti Regions following reports of canker outbreaks from both extension staff and farmers, an upsurge in canker infection in Ghana was realised. It was particularly interesting to observe that many of the large and multiple cankers, in the areas infected with *P. megakarya* were found at the base or near the base of the trees. The present epidemiological study was undertaken to estimate the extent of canker infections in the field, its height distribution on the trees, and to determine the possible sources of the canker infection and the causal species involved.

Materials and methods

Epidemiological survey

The epidemiological studies were conducted in the Eastern Region (a cocoa growing region solely infected by *P. palmivora*) and in the *P. megakarya* prevalent areas of Ashanti and Brong Ahafo Regions of Ghana (Opoku et al., 1997a). The two areas (Eastern and Ashanti/Brong Ahafo Regions) used lie within the same agro-ecological zone of Ghana and experience similar meteorological conditions and farming practices. *Phytophthora palmivora* infection on cocoa is established in all the cocoa growing regions of the country while *P. megakarya* infection is fairly recent, still in the invasive phase and spreading through the cocoa growing regions (Opoku et al., 1997a, 2000). Twenty-one farms in each of the two study areas were randomly selected and inspected between May and December, 1995. On each farm, 400 trees of mixed Amazon and Amazon × Amelonado hybrid parentage were randomly inspected. Each tree was inspected from the ground level up to 220 cm height. Inspections into the tree canopy were excluded because cankers are known to be restricted to the lower parts of the tree and occur mainly on main trunk (Schieber and Zentmyer, 1978; Maddison and Griffin, 1981). The following records were taken: (1) the number of cankers on each tree, (2) the position of the canker in relation to height from the ground and (3) the source of infection. On the basis of visual inspection and bark scrapping, the cankers were classified as follows: cushion, if the canker could be traced to a flower cushion on the stem; soil-borne, if the lesion was in direct contact with the soil or extended into the roots; and unknown source, if the lesion could neither be traced to the soil nor cushion.

Canker sample collections

Since *P. palmivora* infection on cocoa occurs in all the cocoa growing regions of Ghana, samples of stem canker infections were taken for isolations and identification of causal agents from the area known to have prevalent *P. megakarya* infection (the Ashanti and Brong Ahafo Regions). The samples were taken during the survey at the time of assessment of the sources of infection. Diseased barks were removed to include healthy tissues from areas extending about 1 cm beyond the lesions. Two pieces of approximately 2.5×2.5 cm from each canker lesion were collected in polythene bags and sent to the laboratory for isolations. A total of 120 samples: 35 from soil-borne cankers, 61 from cushion-borne cankers and 24 from unidentified sources were collected.

Isolations and identification

The method of isolation was similar to that described by Manço (1966) and Dakwa (1972). Each piece of bark about 1.5 × 1.5 cm was inserted into the husk of a whole matured green cocoa pod and incubated in a humid transparent polythene bag for 3 – 5 days. Subsequent lesions developed on the pods were surface sterilised in 10% sodium hypochlorite solution and a disc of 10 mm diameter from each pod transferred onto $P_{10}VP$ (Tsao and Ocana, 1969) from which subcultures were made onto fresh 20% V_8A plates. Three replicated plates per isolate were inoculated with 5-day old cultures for identification.

The *Phytophthora* isolates were identified by their colony morphologies and sporangial characteristics including the length and nature of their pedicels (Brasier and Griffin, 1979). The same cultures used for the colony morphological characterisation were flooded after 9–15 days with 20 ml sterile distilled water and then agitated gently to dislodge the sporangia from the sporangiophores. The suspensions were decanted carefully into 50 ml Erlenmeyer flasks and the pedicels of 30 sporangia per isolate measured using a microscope fitted with an eye-piece graticule, which had been calibrated with a stage micrometer.

Statistical analysis

Analysis of variance (ANOVA) was performed on the mean values obtained from the probable sources of canker infection data from the two study areas using GenStat Release 4.1 (VSN International, Hemel Hempstead, United Kingdom) followed by Duncan's multiple range test at 1% confidence interval to determine which of the means were significantly different.

Results

Incidence of stem canker

The symptoms of cankers observed were largely reddish water-soaked lesions with dark brown to black margins. In some cases, reddish-brown liquid oozed from these lesions, usually through cracks in the bark. The incidence of canker was higher in the solely *P. palmivora* infected area than in the *P. megakarya* prevalent area. In the solely *P. palmivora* infected area, out of the 8400 plants inspected 586, (7.0%) were infected with the disease (Table 1). Two hundred plants (2.4%) had multiple canker infections and 34 of those trees were completely girdled by the canker lesions. The situation in the prevalently *P. megakarya* infected farms was similar, although fewer trees (370, 4.4%) were infected. Eighty-four trees representing 1.0% of the total count had multiple canker lesions (Table 1) and 62 trees (nearly twice the number recorded in the solely *P. palmivora* infected area) were completely girdled, most of which were soil-borne infections. The number of cocoa trees (200) with multiple canker infections in the solely *P. palmivora* infected area was twice more than that recorded in the prevalently *P. megakarya* infected farms (84). Similarly, the total count of canker lesions in the solely *P. palmivora* infected area (922) was nearly twice of the number (514) recorded in the prevalently *P. megakarya* infected area (Table 1).

Vertical distribution of cankers on cocoa trees

The distribution of canker on the cocoa trees in the two areas studied was completely different from each other (Figure 1). The frequency distributions represent the numbers of discrete cankers counted within different height ranges on the tree stems along the vertical axis in the two areas. The canker distribution in the Eastern Region showed a near normal curve with an adjusted third-degree polynomial trend ($R^2 = 0.9234$) whilst that of the Ashanti and Brong Ahafo Regions showed a typical positively skewed curve with a sixth-degree polynomial trend ($R^2 = 0.9761$). The majority of the cankers were concentrated between 41 and 100 cm above ground level in the solely *P. palmivora* infected area and close to the soil between 1 and 40 cm above ground level in the prevalently *P. megakarya* infected areas. The only apparent similarity in the canker distribution in the two situations was that fewer cankers were observed beyond 160 cm height above ground level.

Sources of canker infection

The probable sources of the canker infections indicated that the cushion cankers were more common than either soil or unknown in both study

Table 1. Canker infected cocoa trees in the solely *P. palmivora* and the prevalently *P. megakarya* infected areas

	Number of trees in the solely *P. palmivora* area:				Number of trees in the *P. megakarya* prevalent area:			
Farm[a]	Infected with canker	With multiple infection	No. of cankers observed	% of trees with canker	Infected with canker	With multiple infection	No. of cankers observed	% of trees with canker
A	22	8	36	5.5	24	4	30	6.0
B	8	4	16	2.0	32	8	40	8.0
C	6	0	6	1.5	10	0	10	2.5
D	20	2	26	5.0	30	4	34	7.5
E	48	10	64	12.0	22	6	28	5.5
F	52	8	64	13.0	22	2	24	5.5
G	20	4	28	5.0	20	4	26	5.0
H	40	14	58	10.0	12	4	16	3.0
I	64	24	118	16.0	22	2	30	5.5
J	44	22	76	11.0	14	0	14	3.5
K	20	10	44	5.0	30	2	32	7.5
L	22	12	44	5.5	2	0	2	0.5
M	22	8	30	5.5	16	10	36	4.0
N	26	8	38	6.5	24	4	34	6.0
O	44	10	60	11.0	12	8	22	3.0
P	32	12	48	8.0	14	0	14	3.5
Q	0	0	0	0.0	8	2	10	2.0
R	12	0	12	3.0	12	8	30	3.0
S	32	14	66	8.0	16	4	22	4.0
T	22	12	38	5.5	12	4	16	3.0
U	30	18	50	7.5	16	8	44	4.0
Mean	27.9	9.5	43.9	7.0	17.6	4.0	24.5	4.4

[a] Each farm consisted of 400 trees; % infection was calculated as number of trees infected over the total number of trees (400) × 100.

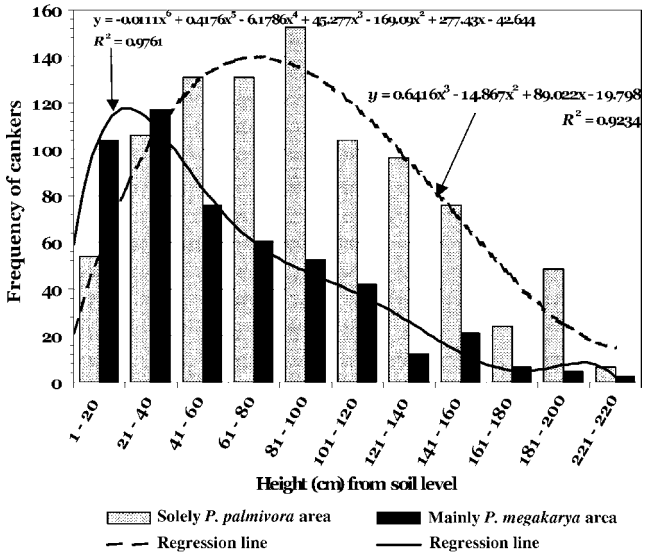

Figure 1. Distribution of naturally occurring canker on cocoa trees in the solely *P. palmivora* and prevalently *P. megakarya* infected areas of Ghana.

Table 2. Sources of canker infection in the solely *P. palmivora* and prevalently *P. megakarya* areas

	P. palmivora solely infected area			*P. megakarya* prevalent area		
Farm[a]	Soil	Cushion	Unknown	Soil	Cushion	Unknown
A	2	26	8	8	14	8
B	0	10	6	10	20	10
C	0	4	2	2	8	0
D	2	24	0	8	22	4
E	0	46	18	10	16	2
F	0	54	10	10	8	6
G	0	18	10	10	12	4
H	0	38	20	6	4	6
I	6	70	42	12	8	10
J	0	42	34	8	6	4
K	0	24	20	20	10	2
L	4	34	6	2	0	0
M	0	30	0	12	18	6
N	4	34	0	10	20	4
O	4	46	10	6	14	2
P	2	40	6	0	12	2
Q	0	0	0	4	6	0
R	0	10	2	12	10	8
S	4	46	16	2	14	6
T	0	28	10	6	4	6
U	8	38	4	10	24	10
Mean[b]	1.7 c	31.5 a	10.7 b	8.0 b′	11.9 a′	4.8 c′

[a] See Table 1.
[b] ANOVA was performed on mean values for probable sources of canker infection. Figures followed by a,b,c or a′,b′,c′ are significantly different from each other after Duncan's multiple range test at 1%.

areas (Table 2). The number of cushion borne cankers recorded in the solely *P. palmivora* infected area was twice higher than in the prevalently *P. megakarya* infected area. Significantly, more soil-borne cankers (about 5 times) were recorded in the Ashanti and Brong Ahafo Regions than in the Eastern Region. The Duncan multiple range test analysis showed that in the solely *P. palmivora* infected area, cushion cankers were significantly ($P < 0.01$) more than those from unknown sources (Table 2). Likewise, in the prevalently *P. megakarya* infected areas, cushion cankers were significantly ($P < 0.01$) more than the soil-borne cankers, which were also significantly ($P < 0.01$) more than those from unknown sources (Table 2).

Isolations and identifications

From the 120 canker samples collected, 101 isolations of *Phytophthora* were made (Table 3). The majority of the isolations (74) were identified as *P. megakarya*, 27 as *P. palmivora*, and 19 as other

Table 3. Isolations of *P. palmivora* and *P. megakarya* from different sources of canker infection on cocoa in the area mainly infected by *P. megakarya*

Source of infection	No. of samples	No. of isolates identified as		
		P. palmivora	*P. megakarya*	Other fungi
Soil	35	6	20	9
Cushion	61	16	38	7
Unknown	24	5	16	3
Total	120	27	74	19

fungi, mostly *Fusarium* sp. and *Trachysphaera fructigena,* with a few that could not be identified. The numbers of *P. megakarya* isolations made from all the three sources (soil, cushion and unknown sources) were significantly ($P < 0.01$) greater than those of *P. palmivora*.

Discussion

The earliest report on cocoa stem canker studies in Ghana dates back to the 1920s (Dade, 1927), but compared to pod rot disease, little research attention has so far been devoted to it. The lack of research attention on cocoa stem canker was probably due to that fact that it was not considered as a major disease. However, the recent reports by farmers of severe canker infections that resulted in the death of many trees (Opoku and Akrofi, 2000), particularly in the prevalently *P. megakarya* infected cocoa growing areas of Western, Ashanti and Brong Ahafo Regions of the country, suggest an upsurge of the disease. This study shows that though *Phytophthora* stem canker is still not as major a threat to the cocoa industry as black pod disease (Opoku et al., 2000), in certain areas the level of canker infections are serious. While black pod incidence in Ghana attributed to *P. palmivora* ranges between 18% and 27% and that for *P. megakarya* between 60% and 100% (Dakwa, 1984, 1988), the incidence of stem cankers caused by the two species were 7.0% and 4.4%, respectively.

However, since the economic losses due to cankers are indirect and difficult to assess, these figures may be deceptive. Stem cankers are known to play major role in primary infection of cocoa pods (Dade, 1928, 1929; Wharton, 1954; Thorold, 1955). Mohanan (1978) reported that when canker girdled the main stem or branch, the pods on the tree wilted, the leaves discoloured and defoliated, the branches died-back and eventually the tree died. Stem cankers therefore reduce tree vigour and consequently the economic yield. Control at the early stages of stem cankers is simply achieved by scrapping the bark to expose the canker lesions to dryness or in addition painting the scrapped lesions with fungicides. This practice generally halts the advancement of the canker under dry conditions. Nevertheless, under conditions of high rainfall and humidity the canker may quickly girdle the stem and kill the tree.

The present study is the first epidemiological evidence of the natural and vertical distribution of cankers caused by both *P. megakarya* and *P. palmivora* on cocoa trees. The study confirms earlier reports by Schieber and Zentmyer (1978) and Gregory and Maddison (1981) that canker is evident mainly on the lower part of the main trunk and rarely found on older branches and in the canopy. Although, the studies were restricted to 220 cm height above ground level, only a few cankers (9.3% and 8.5%) were observed beyond 120 cm in the area where *P. megakarya* prevailed or 160 cm in the area solely infected by *P. palmivora*, respectively. It should also be noted that in the *P. megakarya* prevalent area, the highest concentration of cankers (the top three frequencies) occurred from the soil level up to 60 cm, while in the solely *P. palmivora* infected area it was between 41 and 100 cm above soil level. In addition, more soil-borne cankers were recorded in the mainly *P. megakarya* infected area than in the solely *P. palmivora* area. This confirms the importance of the soil phase in the life cycle and epidemiology of *P. megakarya*, which is known to have a self-sustaining reservoir in plantation soils (Gregory and Maddison, 1981). The low record of soil-borne cankers in the solely *P. palmivora* infected area (Table 2) and the fewer isolations of *P. palmivora* from soil borne cankers (Table 3) suggest the soil as not a major source of primary infection for this species. Dakwa (1974) reported that the soil inoculum was relatively unimportant for black pod disease caused by *P. palmivora* and as indirect evidence, Dakwa (1974) observed that it was common to find pods in the field with their distal ends buried in the soil in *P. palmivora* infected areas without infection even during the main black pod period. In contrast, virtually all pods associated with soil in one form or another tend to become infected with black pod disease in areas having *P. megakarya* during the main black pod season from June to October (personal observations). Cushion borne infections seem to be more important in *P. palmivora* diseases on cocoa than in *P. megakarya*. Although significantly higher cushion borne cankers were recorded in the *P. megakarya* prevalent area compared to soil-borne cankers, this included cankers caused by both *P. palmivora* and *P. megakarya* as shown in

the high numbers of isolations of both species from the cushion canker samples (Table 3). Also, most farmers fail to remove infected pods from the trees allowing the pathogen to grow into flower cushion points on the main trunk. These data confirm the importance of different sources as primary inoculum in the epidemiology of the diseases caused by the two *Phytophthora* species on cocoa.

Development of canker is generally attributed to the spread of mycelia of *Phytophthora* from an infected pod along the peduncle and into the flower cushion (Dade, 1928; Firman, 1974; Wood and Lass, 1985). On peasant farms in Ghana where maintenance is generally poor, farmers tend to leave diseased pods on the trees, particularly in the canopy unharvested. These pods rot, dry up and become mummified and could remain on the trees for months. By definition, these should normally result in several cankers. It was therefore surprising that cankers tend to be concentrated on the stem along the main trunk and rarely found in the canopy. The role of mummified pods in canker formation needs to be critically examined.

Maddison and Griffin (1981) and Gregory and Maddison (1981) reported that the ability of *Phytophthora* species to form canker and flower cushion infection differs and that *P. megakarya* is less able to infect woody tissue than *P. palmivora*. The data presented in Table 3 clearly show that *P. megakarya* readily causes stem cankers. *P. megakarya* was readily isolated from samples taken from the three different sources (cushion, soil and unknown) of canker. Also, in an earlier study where stem inoculations of cocoa trees were carried out using mycelia plugs of *P. palmivora* and *P. megakarya*, the abilities of the two species to cause cankers were not significantly different (Opoku et al., 1997b). The symptoms of the cankers observed in the field were generally similar in the two areas studied, which confirm earlier observations of Maddison and Griffin (1981). The cankers caused by both species tend to be larger along the shoot-root axis than along the tangential plane, and this perhaps explains why only a small proportion of infected trees eventually become girdled. However, most of the large and multiple cankers that occurred at the base or near the base of the cocoa trees were predominantly found in the mainly *P. megakarya* infected areas.

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