

Full Length Research Paper

***Phytophthora* seedling blight disease of cacao and its control measures**

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Symptoms of *Phytophthora* seedling blight disease of cacao were found on 3 weeks old cacao seedlings at the nursery of Seed Production Division of Ghana Cocoa Board in New Tafo-Akim during routine surveys of cacao diseases in the Eastern Region of Ghana. Survey of seedlings in the nursery was conducted to determine disease incidence (DI). Heat sterilization of soil and drenching with Ridomil Gold plus 66 WP (6% metalaxyl-M and 60% copper (I) oxide) and Champion (77% cupric hydroxide) fungicides were evaluated for disease management. Symptoms of the disease started as vertical brown lesions above the cotyledon toward the top of cacao seedlings, causing the stems and leaves to wilt. Out of a total of 135,000 inspected cacao seedlings at the nursery, 2,525 (DI = 1.8%) seedlings were infected within one week of disease identification. Isolations from samples of infected tissues yielded *Phytophthora palmivora*. The fungicides were effective in disease control but heat sterilization of nursery soil before sowing cocoa beans was found to be the ideal control method.

Key words: Seedling blight, cacao, *Phytophthora palmivora*, fungicides.

INTRODUCTION

Globally, the genus *Phytophthora* causes more losses than any other disease of cacao (Bowers et al., 2001; Ploetz, 2016). Losses due to *Phytophthora* pod rot was estimated at 700,000 metric tons in 2012 (Ploetz, 2016). *Phytophthora* causes seedling blight (Guest, 2007) and the disease can cause 100% loss if not controlled (Peter and Chandramohan, 2014). Seedling blight caused by *P. palmivora* results in wilting of stems and leaves, defoliation and eventual death within seven days after infection (Nur'Aini et al., 2016).

The survival of *P. palmivora* as mycelia and

chlamydospores (thick-walled resistant spores) in the soil (Gregory and Maddison, 1981) makes it difficult to control and it is usually transported through partially infected seedlings or contaminated soil to newly established fields or increase inoculum population in already infected fields during transplanting of cacao seedlings. Combination of row cover and 0.2% copper oxide protects seedlings from *P. palmivora* attack (Nur'Aini et al., 2016). *Phytophthora* seedling blight is managed through integrated approach involving the use of phytosanitary practices, biocontrol agents and fungicides (Peter and Chandramohan,

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2014).

In Ghana, cacao is usually cultivated using seedlings. To encourage the planting of improved planting materials for the sustainability of Ghana's cocoa production, the Seed Production Division (SPD) and Cocoa Health and Extension Division (CHED) of Ghana Cocoa Board (COCOBOD) collaborate to raise and distribute hybrid seedlings at no cost to farmers across the cocoa growing regions of the country for planting every year. The seedlings are raised in polythene bags filled with top soil under shade and watered periodically for 3 to 6 months before transplanting to the field (Opoku-Ameyaw et al., 2010). Survival of seedlings at the nurseries is threatened, among other factors, by a host of diseases including *Phytophthora* blight.

Phytophthora seedling blight is a sporadic disease but causes great loss in Ghana due to little knowledge on disease identification, cause and management among workers. The goal of this study was to make it easy for cocoa extension agents, workers and farmers to identify and manage *Phytophthora* seedling blight of cacao. The specific objectives were to describe the symptoms and confirm the pathogen of *Phytophthora* seedling blight identified at the nursery of Seed Production Division of Ghana Cocoa Board in New Tafo-Akim during routine surveys of cacao diseases in the Eastern Region of Ghana. It was also to evaluate heat-treated soil and fungicides for disease management.

MATERIALS AND METHODS

Symptomatic characterization, disease incidence and sampling

Symptoms resembling *Phytophthora* seedling blight disease of cacao were found on 3 weeks old cacao seedlings raised in black polythene bags under shade with a watering regime of 3 times per week at the nursery of Seed Production Division of Ghana Cocoa Board in New Tafo-Akim (06°22' N and 00° 36' W) in the East Akim district of the Eastern Region of Ghana. Each seedling was visually inspected for the symptoms. Disease incidence was expressed as a percentage of seedling infection. Infected stems and leaves of randomly selected cacao seedlings were collected in sterile polythene bags and taken to the laboratory for isolation of *P. palmivora*.

Isolation and characterization of *P. palmivora*

P. palmivora was isolated from infected stems and leaves following the procedure of Agrios (2005). Pieces of 4 mm from advancing margins of infected stems and leaves were surface sterilized in 70% ethanol for 60s and blot-dried between sterile filter papers. With a pair of sterile forceps, the pieces of infected tissues were placed on water agar and incubated in the dark at 28°C. After 48 h of incubation, tips of emerging fungal colonies were aseptically transferred onto V8 juice agar medium (200 ml V8 juice, 800 ml sterile distilled water, 20 g agar and 2.5 g⁻¹ CaCO₃) amended with 10 mg⁻¹ pimaricin, 100 mg⁻¹ vancomycin and 10 mg⁻¹

pentachloronitrobenzene to inhibit bacterial growth. Colony morphology of a 5-day old pure culture was recorded as growth pattern. For sporangial form and dimensions, mycelia mat from a 7-day old culture under continuous light at 28°C was placed on glass slide and stained with lactophenol blue. Description of sporangial shape, measurement of pedicel length, length (*l*) and breadth (*b*) of 50 caducous sporangia from each of 3 replicated plates were carried out under Leica CME compound microscope at ×400 magnification and *l/b* ratio calculated.

Pathogenicity test

Apparently healthy 3 to 4 months old green hybrid cacao pods were centrally inoculated with 10 mm disc plugs taken from the periphery of 3-day old pure culture of *P. palmivora*. The pods were placed on foam soaked with 500 ml sterile distilled water in plastic tray (70 cm × 60 cm × 15 cm) and incubated at 28°C for 7 days. Ten (10) cacao cores of 10 mm in diameter taken at 7 mm depth with sterile cork borer from *P. palmivora* infected cacao pods were completely buried in each of 10 bags of heat-treated soil (autoclaved at 121°C for 15 min) and watered (100 ml/bag) at 2 days interval for 2 weeks prior to the sowing of hybrid cocoa beans. Ten (10) bags of heat-treated soil without *P. palmivora* infected cacao cores were sowed with hybrid cocoa beans and included as control. The beans were watered (100 ml/bag) at 2 days interval. There were ten (10) replicated bags per treatment. The treatments were labeled and arranged in a completely randomized block design on raised platform in the greenhouse with alternating day and night at maximum and minimum temperature of 33°C and 21°C (CRIG Meteorological data) respectively. The number of infected cacao seedlings in each treatment were recorded and expressed as percentage of infection. Infected stems were sampled and taken to the laboratory for re-isolation of *P. palmivora*.

Baiting of *P. palmivora* from soil

Apparently sterile soil without cacao seedlings and *P. palmivora*-contaminated soil with infected cacao seedlings were collected from the nursery, where the disease outbreak occurred, for the test. Cacao cores of 10 mm in diameter taken at 7 mm depth with sterile cork borer from apparently healthy 3 to 4 months old green hybrid cacao pods were surface sterilized in 70% ethanol for 60s, rinsed in three changes of sterile distilled water and air dried on sterile filter papers under laminar air flow. Fifteen cacao cores were completely buried in each soil type and moistened with 10 ml of sterile distilled water. There were four replicated Petri dishes for each soil type containing 100 g of soil sampled from 5 nursery bags (20 g of soil from each bag). The plates were placed in plastic trays lined with wet foams and incubated at room temperature for 7 days. Cacao cores from the four plates were pooled together for each soil type and washed in four changes of sterile distilled water. They were air dried on sterile filter papers under laminar air flow. The number of uninfected, infected and disintegrated cacao cores were counted and expressed as percentage of infection.

Recovery of *P. palmivora* from infected cacao cores

P. palmivora infection of cacao cores was confirmed through its recovery on V8 juice agar medium amended with 10 mg⁻¹ pimaricin, 100 mg⁻¹ vancomycin and 10 mg⁻¹ pentachloronitrobenzene to inhibit bacterial growth. Tissue bits of

approximately 0.4 mm² were excised from uninfected, infected and disintegrated cacao cores, surface sterilized in 70% ethanol for 60 s and rinsed in three changes of sterile distilled water. There were three replicated plates containing 5 tissue bits for each category of cacao core and incubated under light at 28°C for 3 days. Growth pattern of *P. palmivora* was recorded. Sporangial shape and dimensions were used to characterize the pathogen.

Disease control in a greenhouse study

In an attempt to prevent further spread of the disease, cacao seedlings showing symptoms of the disease including the polythene bags containing apparently *P. palmivora*-contaminated soils were destroyed by burning the seedlings and exposing the soil to sun. In a greenhouse experiment, each of ten (10) bags of apparently *P. palmivora*-contaminated soil collected from the nursery, where the disease outbreak occurred, were sowed with hybrid cocoa beans (1 per bag) and drenched with 100 ml/bag of either 50 g/15 L water of Ridomil Gold plus 66 WP (6% metalaxyl-M and 60% copper(I) oxide) or 100 g/15 L water of Champion (77% cupric hydroxide). In another treatment, ten (10) bags of apparently *P. palmivora*-contaminated soil were heat treated by autoclaving at 121°C for 15 minutes, allowed to cool and sowed with hybrid cocoa beans. Ten (10) bags of untreated apparently *P. palmivora*-contaminated soil sowed with hybrid cocoa beans were included as control. There were ten (10) bags/replicates per treatment and arranged in a completely randomized block design on raised platform in the greenhouse with alternating day and night at 33 and 21°C (CRIG Meteorological station) respectively. The beans were watered at 2 days interval. Number of seeds that germinated and the number of seedlings that showed symptoms of blight in each treatment were recorded and expressed as percentage of germination and infection respectively. Number of days to germination of cocoa beans in each treatment was recorded. Heights (cm) of cacao seedlings above cotyledon in the various treatments were measured with a ruler. The number of leaves was counted and girths (cm) at 2 cm above cotyledon were measured with a Vernier caliper at 8 weeks after sowing.

Data analysis

To determine the influence of treatments on height, girth and number of leaves on cacao seedlings, recorded data were statistically analyzed for significance using Analysis of Variance (ANOVA) in GenStat 11th Edition statistical software (VSN International Limited). The level of significance was taken at 5% probability. Means were separated whenever the *F* test values were significant ($p < 0.05$) using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Disease incidence and characterization of disease symptoms

Symptoms of seedling blight disease were found on 3 weeks old cacao seedlings. Out of a total of 135,000 inspected cacao seedlings at the nursery, 2,525 seedlings were infected, representing 1.8% of disease

incidence within one week of disease identification. This poses a threat to cacao cultivation in Ghana since almost half of the seedlings may be lost to the disease before the recommended transplanting age of 6 months. Symptoms of the disease started as a vertical brown lesion above the cotyledon (Figure 1a) or at the cotyledon (Figure 1b) toward the top of the cacao seedling. The lesion darkened as it spread toward the top causing the stem and leaves to wilt (Figure 1c). The stems weakened and bent down (Figure 1d). The symptom appeared as blight on leaves (Figure 1e). Defoliation (Figure 1f) and eventual death (Figure 1g) of cacao seedlings occurred at the later stage of infection. However, roots were not infected (Figure 1h). *Phytophthora* seedling blight disease of cacao is a major constraint to cacao seedling production and disease incidence decreases with increase in age of seedlings (Peter and Chandramohan, 2014). Seedlings up to 4 months old are susceptible to blight (Lim, 1980) but escape the disease when they are 5 to 6 months old (Peter and Chandramohan, 2014). Symptoms of the disease were similar to that reported by Nur'Aini et al. (2016).

Characterization of *P. palmivora*

Pure culture of the isolate produced a stellate-striate colony pattern with no aerial mycelium. Elliptical to ovoid, papillated and caducous sporangia with short pedicel ranging from 1.3 µm to 4.8 µm were observed. Sporangial length (*l*) and breadth (*b*) ranged from 35.0 µm to 57.5 µm (average 45.3 µm) and 30.0 µm to 40 µm (average 34.5 µm) respectively with an average *l/b* ratio of 1.3 µm. Numerous chlamydospores were observed with an average diameter of 36.1 µm. Identification of *P. palmivora* as the cause of seedling blight confirms earlier reports (Peter and Chandramohan, 2014; Nur'Aini et al., 2016). Colony morphology of the *P. palmivora* isolate was in agreement with previous observations (Opoku 2004). Sporangial shape and dimensions were within the ranges reported for *P. palmivora* (Erwin and Ribeiro, 1996; Drenth and Sendall, 2001). All major organs of cacao, including pods, stems, leaves and roots can be attacked by *P. palmivora* (Firman, 1974). *P. palmivora* is widely distributed (Bong et al., 2000; Thanh et al., 2004) and less virulent than *Phytophthora megakarya*, which is restricted to West Africa (Opoku et al., 2000). However, due to its presence in all cocoa producing areas, *P. palmivora* causes the greatest yield loss of cocoa (Ploetz, 2016). The pathogen requires wet conditions and high humidity for infection (Gregory and Maddison, 1981). Seedling infections were reported in June during the rainy season in India (Peter and Chandra, 2011, 2014). On the contrary, seedling blight was identified in the dry season of December at New Tafo-Akim in the Eastern Region of Ghana. Generally, the prevalence of *Phytophthora*



Figure 1. Symptoms of *Phytophthora* seedling blight disease showing brown lesion on the stem above the cotyledon toward the top (a), brown lesion from the cotyledon toward the top (b), wilting of stem and leaves (c), bending of seedling stem at the brown lesion toward the top (d), leaf blight (e), defoliation (f) die-back (g) and uninfected roots (h).

diseases of cacao is less in Ghana during the dry season (November to May annually). However, *Phytophthora* infections occur in West Java in the absence of rain as the humidity of nearly 100% that occur for a few hours during the night provide enough free water to initiate infection (Purwantara and Pawirosoemardjo, 1990; Purwantara, 2003). Perhaps, the cacao seedlings were infected in the dry season of December following continuous watering of seedlings under shade which enhanced germination and spread of propagules of *P. palmivora*.

Pathogenicity test

P. palmivora infected cacao seedlings were characterized by vertical brown lesions on stems (Figure 2) above soil

level toward the cotyledon 11 days after germination. About 70% of infected cacao seedlings wilted and died. However, seedlings in sterilized soil remained healthy 44 days after germination. *P. palmivora* was successfully re-isolated from infected stems. Infections of stems above soil level toward the cotyledon contradicted field observations where the lesions appeared above the cotyledon or at the cotyledon toward the top of the cacao seedling. However, observed symptoms in both instances were caused by *P. palmivora*. It is unclear how the pathogen infected stems above cotyledon without causing root rots of seedlings at the nursery. It is suggested that water splash of contaminated soil might have introduced the pathogen to infect the aerial part of the stem above the cotyledon. The epicotyls or cotyledons might have also been infected by the pathogen before emerging from the soil.



Figure 2. Vertical brown lesion on the stem above soil level toward the cotyledon of *P. palmivora* infected cacao seedling.

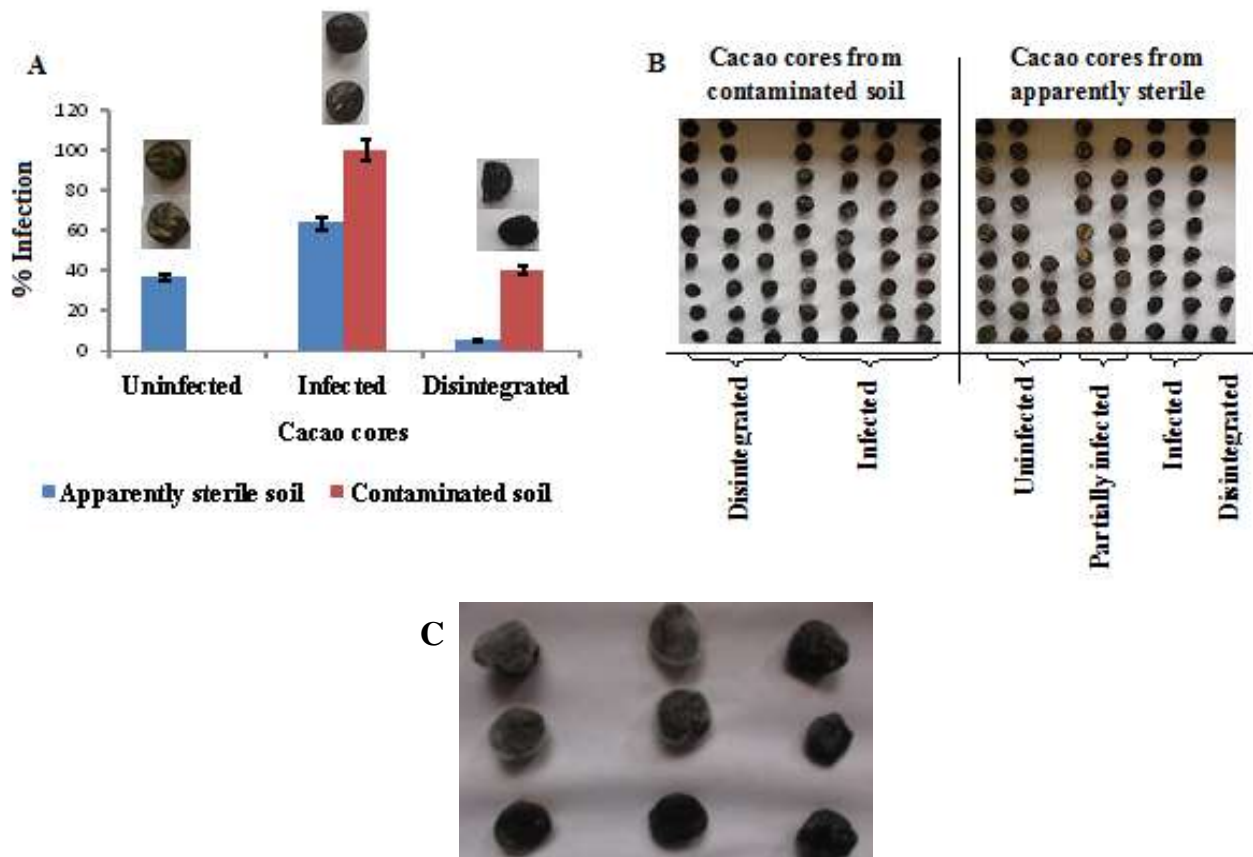


Figure 3. Graphical presentation of percentage of uninfected, infected and disintegrated cacao cores in apparently sterile nursery soil and *P. palmivora*-contaminated soils (A), Photographic presentation of uninfected, infected and disintegrated cacao cores in apparently sterile nursery soil and *P. palmivora*-contaminated soils (B), Infected cacao cores covered with bloom mass of *P. palmivora* after 24 h of incubation (C).

Table 1. Percent germination and infection of cacao seedlings in contaminated, sterilized, Champion and Ridomil Gold plus 66 WP treated soils.

| Treatment | Cacao seedlings | |
|-------------------------|-----------------|-------------|
| | % Germination | % Infection |
| Contaminated soil | 70 | 40 |
| Sterilized soil | 90 | 0 |
| Champion | 90 | 10 |
| Ridomil Gold plus 66 WP | 60 | 0 |

Baiting of *P. palmivora* from soil

Infected cacao cores were characterized by brown lesions typical of *P. palmivora* infection (Opoku, 2004). All cacao cores in apparently *P. palmivora*-contaminated soil were infected, resulting in tissue disintegration of 40%. However, 36.7% of cacao cores which were not infected in apparently sterile soil remained green (Figure 3A). Out of 38 infected cacao cores in apparently sterile soil, only 3 were disintegrated (Figure 3B). Infected cacao cores were covered with bloom mass of hyphae (Figure 3C) after 24 h of incubation. Successful baiting of *P. palmivora* from the soil in 7 days demonstrates survival of the pathogen in soil. Baiting technique has been used to isolate *P. palmivora* from soil in 4 days (Okaisabor, 1971). It provides a quick and simple way of detecting the presence of the pathogen in the soil. Reports suggest that *P. palmivora* can survive in the soil up to 10 months (Enriquez and Zentmyer, 1980). This is an indication that propagules of the pathogen can be found in the soil all year round. In the dry season, the pathogen survives as chlamyospores (thick-walled resistant spores) or mycelia (Gregory and Maddison, 1981), making the soil a reservoir of the pathogen's inoculum. Under favourable conditions, the propagules germinate to infect roots of cacao seedlings and subsequently infect aerial parts of the seedling through water splashes of contaminated soil during watering. Inoculum size of the pathogen increase in the soil after root infections and this was evidenced in the infection of all cacao cores in the apparently *P. palmivora*-contaminated soil. Due to increase in population size of the inoculum in the contaminated soil, cacao cores were probably infected in a few days leading to the disintegration of 40% after 7 days of incubation.

Recovery of *P. palmivora*

P. palmivora was successfully recovered from infected and disintegrated cacao cores. Pure cultures of isolated *P. palmivora* produced stellate-striate colony patterns on V8 juice agar medium. However, no growth was observed on V8 juice agar plates inoculated with tissue

bits excised from uninfected cacao cores 3 days after incubation.

Disease control in a greenhouse

In Ridomil Gold plus 66 WP treated soils, 60% of cocoa beans germinated and none of the seedlings was infected. Similarly, all seedlings in sterilized soils remained healthy 43 days after germination. On the contrary, 40% of seedlings were infected in contaminated soil (Table 1). There were no significant ($p>0.05$) differences in the number of days for germination of cocoa beans, heights and number of leaves on cacao seedlings in the various treatments. However, girths of cacao seedlings in sterilized soil were significantly ($p=0.027$) bigger than those in the other treatments (Table 2). As a soil-borne pathogen, control of *Phytophthora* is a challenge (Erwin et al., 1983). Burning of infected cacao seedlings, including the polythene bags containing apparently *P. palmivora*-contaminated soils, and exposing the soil to sun prevented further spread of the disease at the nursery. Opoku et al. (2007) reported that fungicide application combined with crop sanitation practices were effective in the management of *Phytophthora* on cacao. In this study, drenching of soil with Ridomil Gold plus 66 WP (6% metalaxyl-M and 60% copper (I) oxide) and Champion (77% cupric hydroxide) were effective in the control of the disease even though 10% of the seedlings were infected in Champion-treated soils. Despite the effectiveness of Ridomil Gold plus 66 WP in the control of the disease, only 60% of the cocoa beans germinated after 13 days of sowing. Low germination of cocoa beans in Ridomil Gold plus 66 WP treated soil could be due to the fungicide since negative influence of long-term use of pesticides on microbial growth and activity, leading to reduced soil fertility and productivity has been reported (Wang et al., 2006). Braun and Supkoff (1994) reported that soil treatment by steaming at 80 – 100°C for half an hour effectively controls most soil-borne pathogens. In this study, *P. palmivora* was effectively controlled as all the germinated cocoa beans (90% germination) in the sterilized or heat-

Table 2. Number of days for germination of cocoa beans, girth, height and number of leaves on cacao seedlings in contaminated, sterilized, Champion and Ridomil Gold treated soils.

| Treatment | Number of days for germination of cocoa beans | Girth (cm) | Height (cm) | Number of leaves |
|-------------------------|---|-------------------|-------------|------------------|
| Ridomil Gold Plus 66 WP | 12 | 30.6 ^b | 24.3 | 10 |
| Champion | 13 | 32.0 ^b | 21.6 | 11 |
| Sterilized soil | 13 | 36.5 ^a | 23.8 | 11 |
| Contaminated soil | 12 | 30.5 ^b | 21.9 | 10 |
| Isd | Ns | 4.2 | ns | ns [#] |

Mean followed by a different letter is significantly different at $p=0.027$. ns[#] = Values are not significantly different from each other at $p=0.05$.

treated soil survived for 43 days without infection, making it the ideal control method.

Conclusions

The importance of seedling blight disease of cacao is highlighted in this study. Symptoms of the disease have been described in this paper to make it easy for cocoa extension agents, workers and farmers to identify it. Heat sterilization of soil and the use of fungicides for disease management have been demonstrated. However, sterilization of nursery soils is recommended to kill inoculum of *P. palmivora* and any other pathogen that may infect cacao seedlings.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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