



Symptomatology and pathogenicity of *Phytophthora* pod rot disease associated with Cacao plants in selected municipalities in the Province of Cagayan, Philippines

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Abstract

The study aimed to describe the *Phytophthora* pod rot (PPR) symptoms disease associated with cacao plants and its pathogenicity to cacao. Specifically, it aimed to: (a) describe the PPR disease symptoms associated with cacao plants; (b) determine the pathogenicity of PPR disease to cacao seedlings, cacao flower and unripe and ripe pods; (c) document the reaction of cacao varieties to PPR disease. Samples isolated from infected cacao pods of five cacao farms revealed 14 isolates of *Phytophthora* spp. Pathogenicity test showed development of typical symptoms of *Phytophthora* of isolates from infected tissue of cacao pod. Incubation period was recorded between 4 days to 14 days after inoculation. The pathogen associated with *Phytophthora* pod rot belongs to *Phytophthora palmivora*. Cacao seedlings from 21-days old to 6 months manifested water-soaking leaves to leaf blighting symptoms, while infected flowers were found to have blighting and necrotic symptoms. Ripe variety could easily infected compared to unripe pod as shown by oily brown appearance and it changes from brown to black. White leathery structure or whitish appearance (sporangia) on the surface of the pods and mummified pods in advance stages.

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Introduction

Cagayan valley region contributes 4% of the national cacao production from the 10% shared of Luzon and Visayas region outside of Mindanao which is 80% of the total production of the country. In Cagayan province, there are more than 73 cacao farmers growing cacao with cacao production areas ranging from 1-15 has. Cacao nurseries are also operational in the region. Cacao Nurseries and farms are located in Santa Praxedes, Sanchez Mira, Gonzaga, Lal-lo, Lasam, Gatarran and Penablanca (Fig. 1.). Visits of these cacao-producing towns and nurseries, it has been observed that a number of the cacao plants of various

stages of growth show various deviations from normal and symptoms: *i.e.*, seedling blight that starts with vein clearing on the younger and defoliation in older leaves of up to to 4 months old in polybags; flowers turned brown; in fruits, chocolate brown necrotic lesions is commonly observed on infected pods which usually are accompanied by seed rot are common in cacao pods.

Then pathogen appears on the surface of the pod as a whitish down on which masses of sporangia are produced. Diseased cacao fruits are commonly observed in the municipality of Lasam and Gattaran, Cagayan, Philippines.

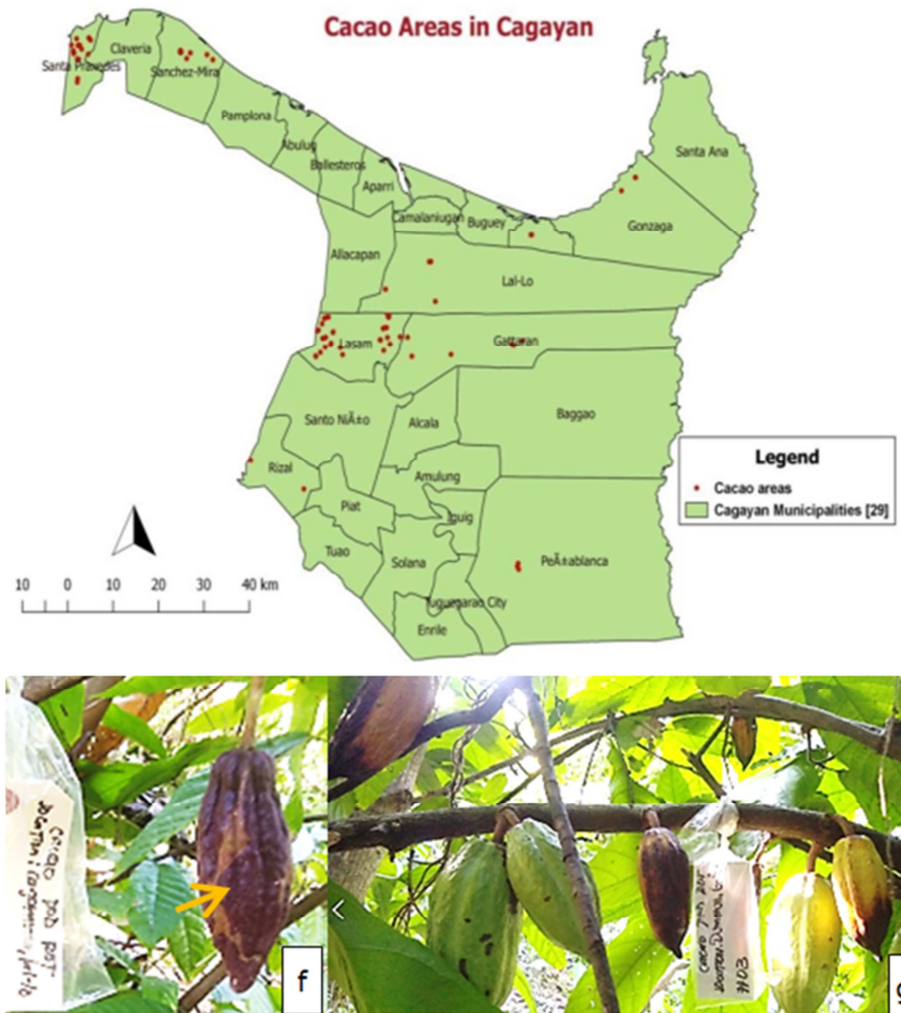


Fig. 1. (a). Maps of cacao nurseries and farms located in Santa Praxedes, Sanchez Mira, Gonzaga, Lal-lo, Lasam, Gatarran and Penablanca; (b-c) plant tagging and *Phytophthora* pod rot disease collection.

To date, there are four most common cacao pests and diseases in the Philippines, *i.e.*, black pod rot, vascular streak dieback, cacao borer, *Helopeltis* and

cacao stem borer (Department of Agriculture Bureau of Agricultural Research as cited by Tan *et al.*, 2016). *Phytophthora* pod rot is a major disease of cacao and

causes 20 to 30% pod losses, and kills up to 10% of trees annually through stem cankers (Bowers, J. H, *et al* 2001; Guest, 2007, and Adomako, 2007). The disease was first reported as cacao canker in Java in 1924 (Hartley and Rands, 1924).

Phytophthora pod rot is caused by four different species in the *Stramenopile* genus *Phytophthora*: *P. capsici*, *P. citrophthora*, *P. megakarya*, and *P. palmivora* which occur in almost all cacao growing region in the world (Guest, 2007). *P. megakarya*, which is restricted to Africa is the most aggressive species, causing 60-100% yield loss compared to the 15-30% losses attributed to *P. palmivora* (Nyassé *et al.*, 2002; Tahi *et al.*, 2006). Eight species of *Phytophthora* have been isolated from cacao: *P. palmivora* (Butler) Butler, *P. megakarya* (Brasier and Griffin), *P. capsici* (Leonian emend.) (*P. tropicalis*), *P. katsurae* (Ko and Chang), *P. citrophthora* (R.E. Smith and E.H. Smith), *P. arecae* (Coleman) Pethybridge, *P. nicotianae* (van Breda de Haan) and *P. megasperma* (Dreschler) (Erwin and Ribeiro 1996; Iwaro *et al.* 1997; Appiah *et al.* 2003).

In the Philippines, cacao had been attacked by disease caused by *Phytophthora faberi* (Reinking, 1918; Tangonan (1999) host index listed *Phytophthora* pod rot disease in cacao. In addition, a review of Solpot (2001) enumerated the following diseases of cacao. Similar symptoms or disorders were reported by Panguntalan *et al.*, (2022) on cacao plants of an orchard in Calauan, Laguna.

However, no report is available if the disease is occurring in the cacao nurseries and cacao farms in the province of Cagayan. Thus, this study was conducted to validate if *Phytophthora* spp. is involved and caused cacao pod rot disease in the cacao farms in Cagayan. This study is undertaken to: (a) collect asymptomatic and symptomatic cacao parts; and (b) isolate, characterize and identify pathogen associated with seedlings, flower, stems, and young and mature pods; (c) and observe and document if the tentative PRR isolates incite PRR disease in seedlings, flowers, and young and matured cacao pods.

Materials and methods

Visits to cacao nurseries and collection of samples

Cacao farms and nurseries located at Santa Praxedes, Sanchez Mira, Gonzaga, Lal-lo, Lasam, Gatarran and Penablanca were visited. Sample plants and associated diseases and related pathogen collected from the area were observed. These were vegetative or seedlings stage; flowers, and pods (immature and mature pods) were collected, described and brought to the Cagayan State University Lal-lo Campus - College of Agriculture Plant Pathology Laboratory for further study (Fig. 1f-g).

Isolation

To properly identify the *Phytophthora* associated in cacao pods, isolation trials were carried out on different non-diseased and diseased flowers, young and fully developed pods collected from five municipalities of Santa Praxedes, Sanchez Mira, Gonzaga, Lal-lo, Lasam, Gatarran and Penablanca, Cagayan. Cacao seedlings and flowers with and without disease symptoms while, young and matured cacao pods with or without rotting symptoms were used to isolate associated pathogens. The *Phytophthora* species were isolated following the baiting methods of Chee and Foong (1968); Lee and Varghese, (1974); and Newhook and Jackson (1977). Identification was carried out based on diagrams and micrographs reproduced from Erwin and Ribeiro (1996).

Pathogenicity tests

Fungal isolates recovered from the diseased cacao plants parts were inoculated to 21- day old, 3-month old and 6-month old cacao seedlings (Fig. 3.a-c); cacao flowers (Fig.3.d-e.); and young and ripe cacao pods (Fig.3.g-f). These inoculated cacao plant parts were then incubated after the method of Drenth & Sendall (2001). The fate of the infection was carefully monitored and observed in seedlings (at different stages), flowers and unripe and ripe cacao pods and beans. After inoculation of PPR pathogen to healthy cacao plants, seedlings, leaves, flowers, young and matured cacao pods, resulting infected seedlings, cacao leaves, flowers, young and fully developed pods were recorded, collected, examined and confirmed for

pathogen-causing the same disease symptoms (Fig. 2). Disease expression development was observed, recorded and described.

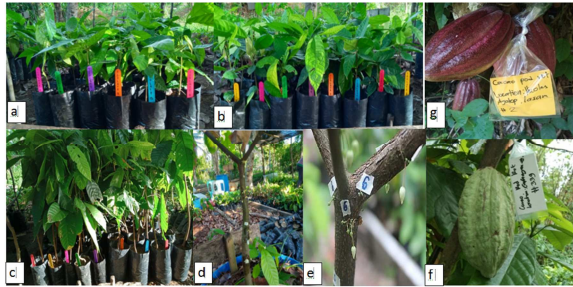


Fig. 2. (a.) 21-day old cacao seedlings; (b) 3-month old cacao seedlings; (c) 6-month old cacao seedlings; (d-e) cacao flower; (f) unripe cacao pods; and (g) ripe cacao pods.

Results and discussion

Visit to Cacao Nurseries and Cacao Farms

Cacao farms visits were conducted on five major cacao farm locations in the province of Cagayan, namely: Lal-lo, Gattaran, Lasam, Penablanca and Sta. Praxedes. Observation and description of collected disease symptoms associated from the different parts of cacao plants showed that: (a) Cacao seedlings infected with *Phytophthora* pathogen generally produce symptoms such as wilting, leaf prematurely fall off/drop, followed by browning of the vascular system and necrosis. *Phytophthora* infected seedlings wilt starts out looking like vein clearing on the younger and even in older leaves and drooping of the older lower leaves; (b) diseased cacao flowers are brown in color and advanced disease observed to have dead or necrotic tissues (Fig. 2 c); Diseased cacao pods observed in the field show: (a) translucent to oily spots on pod surface, which usually developed into small dark hard spots; (b) in advanced disease occurrence the entire pod becomes dark brown to black and turned the whole pod necrotic (Fig. 2.d-g). However, if the infected pod is still young, it is not harvestable and usually left attached in the tree.

Isolates

Colonies of recovered isolates are woolly, usually with pale whitish mycelial growth with no pattern while, asexual spore has papillate; caduceus with short to

medium pedicel; some spores are globose, some are globose to ovoid and irregular in shape; some asexual spores with elongated necks and tapered bases. Hyphal swellings are also observed (Brasier, C. M *et al.*, 1979; Aragaki *et al.*, 2001).

Pathogenicity and Symptomatology

The fate of the infection was carefully monitored and observed in seedlings (at different stages), flowers and unripe and ripe cacao pods and beans. After inoculation of PPR pathogen to healthy cacao plants, seedlings, leaves, flowers, young and matured cacao pods, resulting infected seedlings, cacao leaves, flowers, young and fully developed pods were recorded, collected for examination of the disease (Table 1; Fig. 2. a-h).

From the day of inoculation, development of typical symptoms of pod rot disease of cacao was observed in cacao seedlings, flowers, young and matured cacao pods. Wilting and necrotic symptoms are manifested by seedlings 3-6 days after inoculation. Necrotic tissues and plant death are observed as the disease infection advanced in cacao seedlings (Fig. 2.i-j). Cacao flowers are also susceptible to the attack of the fungus. Four to seven (4- 7) days of inoculation, cacao flowers turned brown, followed by death of the tissues of the cacao flowers (Fig. 2.k). In fruits, brownish margins from the infected disc to healthy tissue were observed 4-5 days after inoculation (Fig 2.l-m.). The very obvious changes on the surface of the pods are the circular spread from the point of inoculation, and the spread is rapid across the surface. It is observed that in 14 days, the entire surface of the cacao pod is covered with infection. The infected area turns oily brown and as the disease advances, it changes from brown to black. For 10-14 days, as the symptoms advances, the disease progresses and gets inside the beans, from mesocarp, and endocarp and into the beans. A visible rotted and blackened infected bean is obviously observed, while the internal tissues become dried and shriveled resulting to mummified pods. Results from the observation of symptoms of infected cacao pod shows that PPR disease could happen in various age, from young pod (Cherelle wilt) to ripe pod.

Resolution and Disease Progress

The resulting disease symptoms were used, processed and artificially grown through baiting technique. The result shows that the isolates are the similar with the virulent isolates used to inoculate the plants for the pathogenicity test. For the PPR disease progress, the

disease can incite disease to healthy leaves, detached leaves incubated with the solution mixed with infected tissue registered faster growth & manifested higher degree of disease after inoculation from 7-15 days compared to leaves inoculated with the mycelial solution (Fig. 3).

Table 1. *Phytophthora* pod rot disease symptoms on cacao seedlings, cacao flower and cacao beans.

Cacao Parts	Disease Expression After Inoculation	Symptoms
Cacao seedlings		
21-day old	4 days	wilting, leaf prematurely fall off/drop , followed by browning of the vascular system and necrosis
30-day old	7-14 days	
90-day old	7-14 days	
Cacao flower	2 days	cacao flowers turned brown, followed by death of the tissues of the cacao flowers
Cacao pod		
Unripe	14 days	infected area turns oily brown and as the disease advances, it changes from brown to black. It is observed that white leathery structure or whitish appearance (sporangia) on the surface of the pods; as the symptoms advances, the disease progresses and get inside the beans, from mesocarp, and endocarp and into the beans
Ripe	2-4 days	

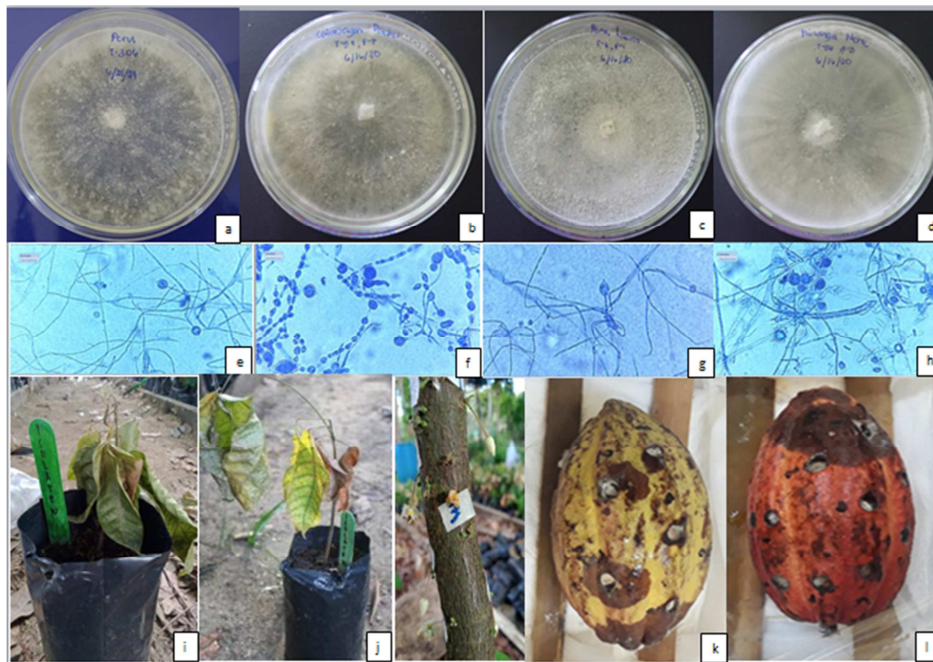


Fig. 3. (a-d). Some colony morphologies of *Phytophthora* spp: (a-d) with pale whitish mycelial growth and with no growth pattern; e-h. Terminal chlamydospore (e & f); (i-j) Cacao flowers and young and matured cacao pods.

The findings that pod rot disease caused by *Phytophthora* spp associated with the cacao farms in the selected municipalities of Cagayan province corroborate with many authors. Once established in cacao farm, the *Phytophthora* pod rot (PPR) disease spread or dispersed from infected pods to adjacent

healthy pods, young and mature through the aid of available moisture, and then continuous to progress throughout the year. The fate of infection as a result of series of studies corroborates with the study of Thurson (1984) that *Phytophthora* spp. infect the roots, stems, leaves, flowers, cherelles, and pods of

cacao plants. Apart from the practice by the farmers in Lasam and Gattaran, non-marketable cacao beans are just thrown in the surrounding or on different locations on farmer's fields. This serves as the sources of inoculum of the pathogen of PPR disease. The spores of *Phytophthora* species on infected cacao pods are usually left on the field after extraction of the beans and are reactivated under suitable conditions to infect fresh cacao pods. Also, according to them, after planting, they do not implement any sanitation measures like pruning, and disease infected parts removal and weeding.

Accordingly, in cacao farms, *Phytophthora* is dispersed by vectors such as flying insects and ants (Evans 1971, 1973a,b; Gregory *et al.*, 1984; Konam and Guest 2004) and by rainsplash (from the soil, usually as sporangia, and from infections on the plant); and by the most important infective propagules of *Phytophthora*, which are the motile zoospores. Rain splash probably disperses sporangia and followed by release of zoospores. Encysted (dormant) zoospores, chlamydospores and hyphae might be other forms of inoculum (Turner 1961; Gregory *et al.*, 1981). Both *P. palmivora* and *P. megakarya* can survive for up to 4 months in cacao soil and roots, as discussed by Opoku, 1998; and Konam and Guest 2002).

Accordingly, pod rot infection success is determined by several factors, including pod wetness. It has been reported that the presence of a film of water over the pod is required for spore germination (Porrás-Umaña and Galindo 1985). High relative humidity (80 to 100%) and warm temperatures (20 to 27°C) are suitable conditions for spore germination and for the fungal penetration (Campuzano Londoño, 1980).

Favorable condition for sporulation is also dependent on fruit moisture and warm temperatures (20°C to 28°C) but according to Porrás VH, González L, 1982 and Herrera 1988. This last factor must fluctuate in order to stimulate the sporulation process. Light is also another important factor in the sporulation process. Alternate periods of light and darkness

promote the spore formation in in vitro conditions. In similar situation, in the major cacao producing countries, the *Phytophthora* species affect different parts of cacao, but infection of the pod is the major economic loss as pods or cherelles may be infected at any parts on the surface. Observation of the disease indicates a firm, spreading, chocolate-brown lesion which eventually covers the whole pod. The beans inside the pod remain undamaged for several days after initial infection of the husk, but in advanced infections, *Phytophthora* invades the internal pod tissues and causes discoloration and shrivelling of the cocoa beans, thus tampering with the mucilage colouration and affecting quality of the cacao bean.

Conclusion and recommendation

The study aimed to describe the *Phytophthora* pod rot (PPR) symptoms disease, and its pathogenicity to cacao. Specifically, it aimed to: (a) describe the PPR disease symptoms associated with cacao plants; (b) determine the pathogenicity of PPR disease to cacao seedlings, cacao flower and unripe and ripe pods; (c) document the reaction of cacao varieties to PPR disease. Cacao infected seedlings, flowers were grown in a staggered basis; cacao pods were collected, in a synchronize manner to make them available at time of inoculation. The PPR disease symptoms were described after inoculation was observed and recorded. The pathogen was re-isolated, confirmed and described by using the taxonomic key. Pathogenicity was carried out in getting daily disease progress in ripe, unripe cacao varieties and cacao seedlings and cacao flower.

Symptoms manifested by cacao infected with PPR suggested all parts of cacao can be infected by the pathogen from cacao seedlings from 21-days old to 6 months shown by water-soaking cacao leaves can incite a leaf blighting symptoms, while infected flowers were found to have blighting and necrotic symptoms. It is generally observed that ripe variety could easily infected compared to unripe pod as shown by oily brown appearance and it changes from brown to black. White leathery structure or whitish appearance (sporangia) on the surface of the pods

and mummified pods in advance stages. Pathogenicity test shows that the PPR is aggressive to incite disease as early as 4 days to 14 days after inoculation. The results, therefore confirm that *Phytophthora* is the causal organism of cacao pod rot disease observed associated with cacao plants, including the flowers and pods in Cagayan.

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