DISPERSAL AND DISTRIBUTION OF PHYTOPHTHORA MEGAKARYA IN YOUNG CACAO PLANTATIONS

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SUMMARY

The arrival and subsequent dispersal of *Phytophthora megakarya* within young cacao plantations, previously free of black pod and containing cacao germplasm that is evaluated for its resistance to *P. megakarya*, is the subject of an ongoing study in Cameroon. A total of 12 plantations, established in 2006 on lands free of cacao cultivation for at least 20 years have been closely monitored on a weekly basis since June 2009. Near the end of 2009, the first infections were observed in three plantations, with a slow but gradual increase over 2010 and 2011 in the number of plantations, trees and pods that were affected. The spatial as well as temporal distributions of infections seem to indicate that multiple *P. megakarya* introductions occurred and that dispersal by humans plays an important role. Moreover, spatial distribution is not only a function of dispersal mechanism(s) but also depends on other factors such as microclimate and cacao genotype. Understanding the factors that govern the establishment and subsequent dispersal of *P. megakarya* in newly established cacao plantations will help farmers to delay the onset of cacao black pod epidemics.

INTRODUCTION

It is commonly assumed that *Phytophthora megakarya,* the causal agent of Phytophthora Pod Rot (PPR) of cacao (*Theobroma cacao*) in Cameroon, is dispersed primarily through rain splash (Ristaino and Gumpertz, 2000; Gregory et al., 1984). In Nigeria, where the predominant *Phytophthora* pathogen is also *P. megakarya*, a long-term research study on PPR demonstrated that rainsplash from or contact with infected pods accounted for more than 71% of pod losses (Gregory et al. 1984). Other sources of infection included soil (5%), ant tents (5.8%) and pod damage due to insects and rodents (4.9%) with 10.9% attributed to 'no obvious' sources. Rather than disease spreading from a few initiator pods, it appeared it spread from numerous 'initiator' pods with sources for these initial infections being partly derived from the soil and ant tents, but also largely (40%) from 'no obvious sources' (Griffin et al. 1981; Gregory et al. 1984). A study in Cameroon (Ten Hoopen et al., 2010), confirmed some of these findings and the authors, based on their results, hypothesized that the distribution of primary inoculum in the soil is the main determinant for the spatial and temporal development of an epidemic at the plantation level and that secondary inoculum is mainly responsible for the within-tree temporal development of an epidemic.

Yet, all these results originated from observations and experiments in cacao plantations where PPR was already well established. To date, there is actually little knowledge about the relative importance of dispersal mechanisms at different scales and most interestingly, not much is known about the w ay *P. megakarya* spreads to plantations/areas devoid of the pathogen. The objective of this ongoing study is to determine the pathways behind first *P. megakarya* infections in newly established cacao plantations. The knowledge thus generated will help improve our understanding of the mechanisms of dispersal of *Phytophthora megakarya* propagules and provide information on the physical and biological factors that are important for the spread of the pathogen. Such knowledge is necessary in order to improve disease management.

MATERIALS AND METHODS

In 2006 numerous innovative cacao plantations have been installed in the centre region of Cameroon on fallow land, devoid of cacao for at least 20 years. These cacao plantations, approximately 0.3 to 0.45 ha, contain cacao germplasm being evaluated for its resistance against PPR. Cacao was planted at 3 x 3 meter intervals either interspersed with coconut or oil palm, as shown in Figure 1A, or with fruit trees (*Persea americana*, *Citrus* spp. & *Dacryodes edulis*), as shown in Figure 1B. Since the cacao trees, fruit trees and coconut or oil palm were all planted at the same time, these plantations, for the time being, are considered to be unshaded except for those areas located near forest edges or old cacao plantations.

A total of 12 cacao plantations were selected in the framework of this study: four plantations in Ngat and eight near Bokito (4 in Bakoa and 4 in Kédia). The climate is characterized by a bi-modal rainfall pattern. However, Ngat is located in a forest zone whereas the other sites are located in a savanna

zone with gallery forests. From June 2009 till December 2011, these 12 plantations have been closely monitored in order to detect *P. megakarya* infections. Local observers passed once a week and noted for each tree i) the presence or absence of cacao pods and ii) the presence or absence of PPR. When PPR was present, rotten pods were removed from the tree and send to the IRAD Phytopathology Laboratory of IRAD in Nkolbisson where the presence of *Phytophthora megakarya* was confirmed. Additionally, in June 2009, 10 compound soil samples of each plantation (n=120) were checked for the presence of *P. megakarya*.

The presence of PPR was visualized using the plantation lay-out maps as shown in Fig 1 for 2009, 2010 and 2011. When a plantation showed an aggregation of at least four contiguous trees with PPR, possible factors that could explain this aggregation were identified. Special attention was given to cacao genotype, temperature and relative humidity, presence of tent building ants and human activities.

Temperature and Relative humidity

In 2012, four Tinytag data loggers, capable of measuring temperature (Temp.) and relative humidity (RH) were installed in a plantation in Ngat. This plantation has the peculiarity that it is enclosed on three sides by secondary forest/abandoned cacao plantations. Three data loggers were installed in the canopy of cacao trees, approximately 2 meters above ground level, one near the centre of the plantation, one near a border with an abandoned cacao plantation and a third in a corner tree of the plantation (Fig 1b). An additional data logger was installed approximately 75 cm above ground level in this particular corner tree (Fig. 1B). Temperature and RH data were registered every 30 minutes starting the 7th of February till the first of June. This period coincides with the first rainy season and is normally the period where the first PPR infections occur. Differences between average Temp. and RH between the four dataloggers were analyzed with the appropriate generalized linear model using SAS (Statistical Analysis System, version 9.2).

RESULTS

All soil samples collected in June 2009 tested negative for the presence of *P. megakarya*. Near the end of 2009, the first PPR infections were observed in three plantations (two in Ngat, one in Kédia). In 2010, a total of five plantations had PPR infections (two in Ngat, one in Kédia and two in Bakoa) and in 2011, a total of eight plantations (three in Ngat, three in Kédia and two in Bakoa) were affected by PPR. When comparing the plantations that had PPR infections in 2010 as well as in 2011, only four out of 15 cacao trees with PPR in 2010 had PPR in 2011, whereas 56 additional trees showed PPR in 2011.

In 2011, there were two plantations that showed infection sites containing at least four contiguous cacao trees, one in Bakoa and one in Ngat. In both plantations, tent building ants were basically absent, although in Ngat, one isolated case of PPR was likely due to the presence an ant tent.

In Bakoa, the main infection site was located in an area containing the cacao clone SNK10 (Fig 1A), a clone known for its susceptibility to *P. megakarya*. Moreover, this clone was located closely to an old traditional cacao plantation where *P. megakarya* is well established.

In Ngat, the main infection site was located in a corner of the plantation surrounded on one side by heavily overgrown fallow land and on the other by an old abandoned cacao plantation containing a few remnant cacao trees. Through this area runs a small footpath (Fig 1B) which is frequently used by local farmers and was always used by the local observer when entering the plantation for his weekly observations. Interestingly, our observer always started his observations at the main infection site.

Microclimatic conditions at the main infection site in Ngat were better suited to *P. megakarya* development compared with the other two locations. Mean RH was significantly ($P<0.0001$) higher (89.0%) and mean temp was significantly ($P<0.0001$) lower (23.8 °C) compared with the centre of the plantation (85.6% and 24.4 °C, respectively). More importantly however, were the differences in mean daily fluctuations of RH and temperature at the infection site when compared with the centre of the plantation. Mean daily RH, fluctuated more than 50% near the centre of the plantation and slightly less than 40% near the main infection site. Mean daily temperature fluctuations were 15.3 °C for the centre of the plantation compared with only 10.8 °C for the infection site. These differences were both significant at $P<0.0001$.

DISCUSSION

Given that all soil samples collected in 2009 tested negative for the presence of *P. megakarya*, together with the fact that the cacao plantations were established on terrain devoid of cacao for at least 20 years, the soil was discarded as a possible origin for the first infections occurring in a specific plantation. These infections are considered to be of exogenous origin to the plantations under study. Moreover, since it is known that the dispersal capacity of *P. megakarya* through rain-splash is fairly limited in range (approximately 6-10 m, Mfegue, 2012; Ten Hoopen et al. 2010), the occurrence of PPR in trees located at distances from other trees with PPR surpassing this range, are also considered to be of exogenous origin.

Thus, when looking at the data, it becomes clear that multiple introductions of *P. megakarya* must have occurred within as well as between years.

Although the first PPR infections can occur seemingly everywhere, only a very few of these infection sites seem to be persistent over time. This is probably due to the fact that certain locations in the plantations are less conducive to *P. megakarya* survival and establishment compared with others. An example is provided by the plantation in Ngat where the infections of 2009 did not give rise to PPR infections in the following years. The PPR infections of 2010 however, are probably at the origin of the infections in or near the same trees in 2011. The same seems to be true for the plantation in Bakoa. Certain isolated infections in 2010 are not repeated in 2011, whereas the SNK10 trees affected in 2010 are probably at the origin of the infections in the same and neighbouring trees in 2011.

The microclimate data from Ngat also help support this idea since the microclimate in the main infection site was more conducive for *P. megakarya* than the microclimate surrounding the trees farther away from the plantation borders. The additional observation that a footpath passes through this infection site as well as the fact that our local observer always started his observations in this part of the plantation also lead us to believe that human vectored dispersal is taking place.

The preliminary findings of this study seem to indicate that human vectored dispersal, cacao genotype as well as microclimate play an important role in the establishment of *P. megakarya* in cacao plantations. However, additional data is needed to verify our findings more conclusively. Currently, using recently developed microsatellite markers (Mfegue et al., 2012) a more detailed molecular based study is underway in order to do so. Understanding the pathways of *P. megakarya* introductions in cacao orchards and identifying the factors that determine the successful establishment of *P. megakarya* in new areas, previously free of the pathogen are needed to reduce the spread of the pathogen and to help farmers delay the onset of PPR epidemics in their newly established cacao plantations.

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1a) Bakoa: cacao plantation intercropped with coconut

1b) Ngat: cacao plantation intercropped with fruit trees

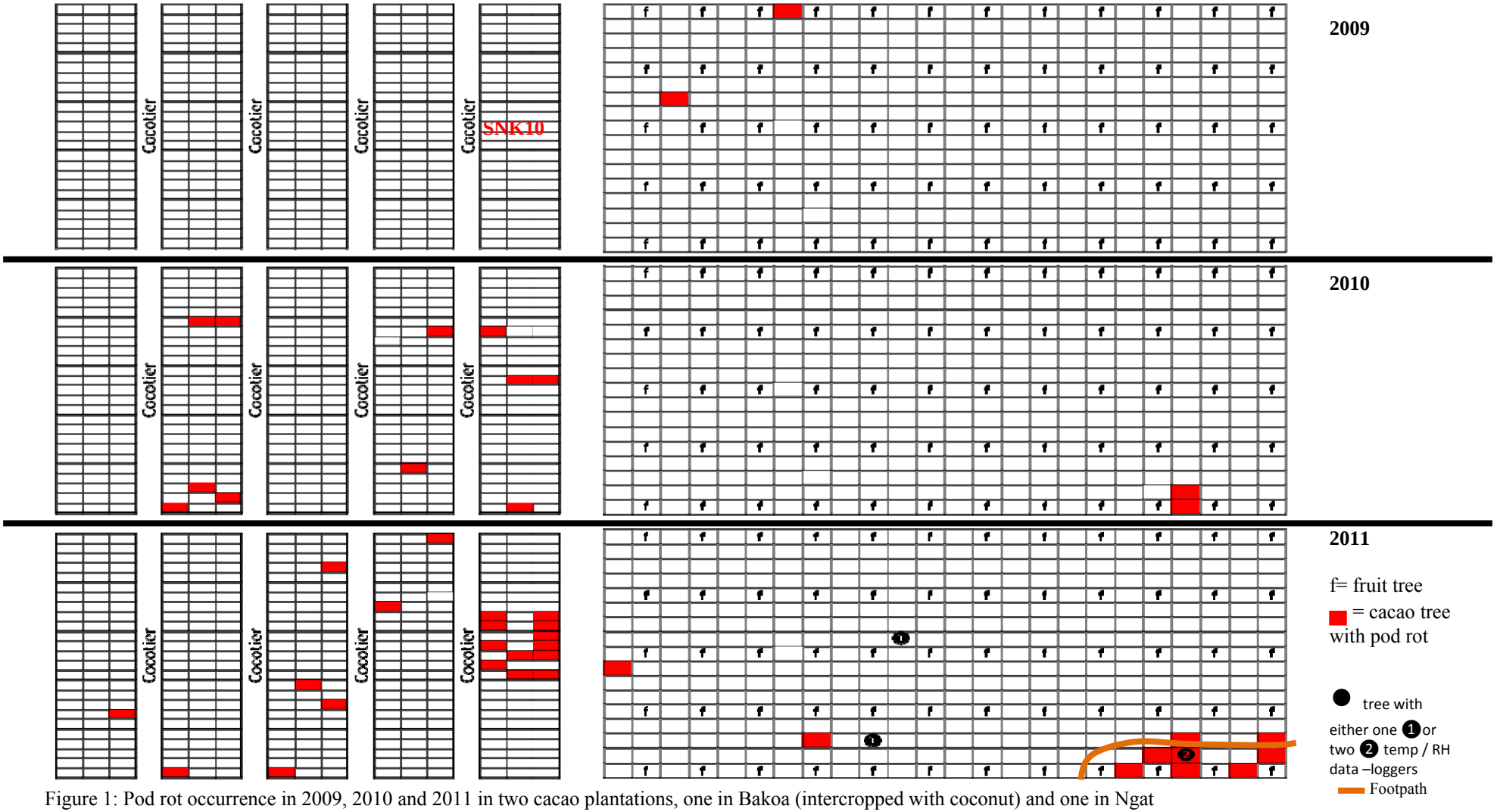

Figure 1: Pod rot occurrence in 2009, 2010 and 2011 in two cacao plantations, one in Bakoa (intercropped with coconut) and one in Ngat (intercropped with fruit trees: *Persea americana*, *Citrus* spp., & *Dacryodes edulis*), both located in the centre region of Cameroon and established in 2006.