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Mathematical modeling to improve control strategies in the cocoa and black pod disease pathosystem

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ABSTRACT

Black pod rot of cocoa, due to *Phytophthora* spp. is a major concern in cocoa production and much research attention has been dedicated to it. However, there are still many questions regarding the factors that govern the disease's dynamics. Here, we explore the use of mathematical modelling to understand the spatial dynamics of black pod disease caused by *P. megakarya* and notably the impact of shade on disease dynamics. The results were exploited as a base for an *in-vitro* investigation on the impact of light on growth and sporulation of *P. megakarya.* A mechanistic–statistical approach was used to estimate spatio-temporal model parameters from real observations of a specific cocoa plot. Shading data collected in the cocoa plot led to refined numerical simulations of disease dispersion and to identify a greater number of infected pods located in areas of the plot with higher shading values. Following the investigation on the effect of shading on system dynamics, the effects of different light wavelengths on *P. megakarya* biology (growth and sporulation) was assessed. Experiments revealed that all the studied strains grew relatively better in the dark compared with exposure to light. However, it was noted that in general, light was a stimulating factor for *P. megakarya* sporulation. The increase in the growth rate of *P. megakarya* in the dark and increased sporulation under light conditions may help explain why shaded or heterogeneously shaded systems are more favorable to *P. megakarya* development. Based on these findings recommendations in terms of cocoa farming systems with specific (homogenous) shading regimes are discussed

Keywords: Cocoa, black pod disease, epidemiological model, shade, light

1. Introduction

Modelling plant disease spread

Mathematical models represent essential tools allowing a quantitative analysis of an epidemic system with the consequent identification of possible strategies to control a disease outbreak or even to prevent it (Brunetti *et al*, 2020). Mathematical modelling is useful for the study of complex phenomena, because models show how separate measurements can be seen as manifestation of the same underlying processes (de Jong, 1995). Recently, the spatial aspects of plant disease have been incorporated into mathematical models of epidemics. Simulating the spatial pattern of plant pathogens, or that of disease, and, more particularly, the way that such patterns change, have become an increasingly important component of the study of plant-disease epidemics (Hughes *et al*, 1997).

Shade and the Black Pod Disease pathosystem

Shading in plant production system is a complex concept widely used but vaguely defined (Bellow and Nair, 2003). Cocoa is generally produced in agroforestry systems where associated trees provide shade (Mossu, 1990). In Cameroon, cocoa is generally produced in dense and highly diversified multi-layered agroforestry systems (Laird *et al*, 2007; Sonwa *et al*, 2007). In such systems, described as agroforests (Torquebiau, 2007), the canopy of cocoa trees is generally intertwined and forms a thick layer of foliage, which is covered by canopy shading of associated trees (Babin *et al*, 2010). Agroforests are thus characterized by a great biodiversity with many species associated with the cocoa tree. However, it should be noted that associated shade trees are not always beneficial for cocoa growing. Some plant species can e.g. constitute alternative hosts or reservoirs of infectious agents and

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cocoa pests (Schroth *et al*, 2000; Opoku *et al*, 2001). Despite some studies conducted in Cameroon to assess shading effect on disease incidence (Kankeu, 2010), as well as methods for estimating shading (Cuissu, 2013), the real link or a direct correlation with the disease has not yet been clearly established. Yet, it has been shown that excessive shading increases the black pod rot disease while a lack of shade or heterogeneous shade favors the damage caused by mirids (Babin *et al*, 2010; Gidoin *et al*, 2014).

Light effect on *P. megakarya*

Shade trees influence light availability and thus cocoa photosynthesis in the field (Beer *et al*, 1998). However, to date the direct role played by light on the development of *P. megakarya* remains poorly studied and seems ambiguous. Since we know relatively little about the optimal light conditions for the growth and sporulation of *Phytophthora* species it is also unclear how shade from associated and and cocoa trees influence the pathogen. Brasier (1969) demonstrated the favorable influence of light on spore production as well as an inhibition of oospore formation in two species of *Phytophthora* (*P. palmivora* and *P. hevea*). Along the same line, the work of Englander *et al*, (2006) revealed poor growth of *P. ramorum* strains exposed to increasing doses of light and ultraviolet. The only study conducted in this way on *P. megakarya* by Blaha (1983) allowed to demonstrate a strong inhibition of growth in continuous white light and in alternating green light 12H /12H as well as photoperiodism under alternating white light/dark 12H/12H conditions, resulting in a characteristic zonation of the thallus. Similar work has revealed that the quality (types of illumination or wavelengths) and the amount (duration of illumination) of light can have dramatic effects on many fungal organisms and oomycetes (Cohen *et al*, 1975; Cohen, 1976; Cohen and Eyal, 1977).

2. Study objectives

In this paper, the main focus is to demonstrate how mathematical modeling can be useful in understanding the mechanisms behind black pod rot epidemics and in identifying action levers on which we could act to control the disease. Basically, our goal is to show how a mathematical model previously built can be used to investigate the role played by a specific environmental factor (plot shading) on *P. megakarya* and provide ideas on potential mechanisms that should be studied in more detail to better understand disease dynamics, in this case light availability, and ultimately how this information can help in developing new ideas for disease control.

3. Methodology

3.1 The spatio-temporal model

Following Nembot *et al*, (2017; 2018), the spatio-temporal model developed here subdivides hosts (cocoa pods in the plot) in two epidemiological states: susceptible (S) and infectious (I). The infectious pods compartment (I) has two sub-compartments according to disease transmission pathway, i.e., spores produced by infected pods and released in the environment, responsible for environmental infections (P2), and spores produced by infected pods and directly responsible for secondary infections. The spatial spread of black pod epidemic in a cocoa plot is described by the compartmental diagram presented in Fig. 1.

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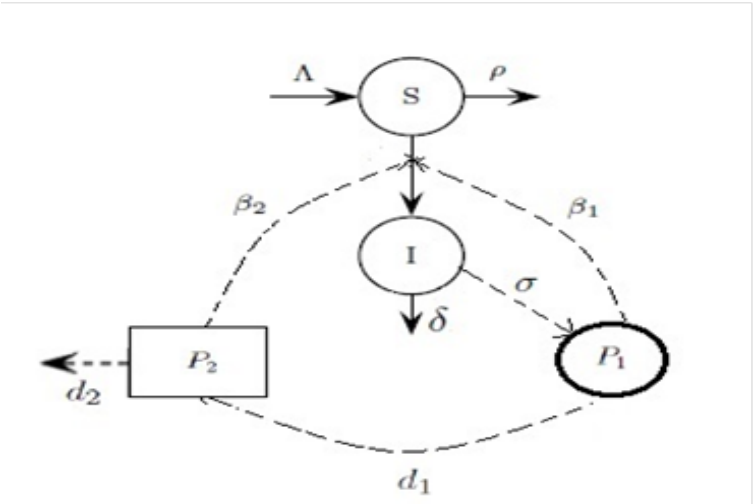

Figure 1 Diagrammatic representation of the spatio-temporal model for black pod rot epidemics.

The infection forces β_2 and β_1 (respectively primary and secondary infection) are modelled in a simple linear form. Disease spread in the plot is taken into account by introducing two dispersal coefficients in the two spore compartments (P_1) and (P_2). Altogether, based on the compartmental diagram in Fig. 1, we built a Partial Differential Equation in the form of a parabolic quasimonotone system model (Nembot *et al*, 2021).

3.2 Field Observation

Data on the shading levels (*X)* in the plot were obtained using a densiometer measuring the canopy closure in the plot. Values obtained through 205 collection point displayed in the plot were interpolated and scaled (computing the rational division over the maximal shade value); thus, almost all the zones of the plot were characterized by densiometer values between 0 and 1. Data regarding black pod disease evolution in the plot was also exploited in our modelling study. For details on the spatiotemporal data collection, see Nembot *et al*, (2021).

3.3 Assessing the impact of shade on the system

To assess the impact of shade on the system, the initial amount of environmental spores in the plot (P_2^0) was modelled as a function of a spatial co-variable, namely the level of shading (X) in the plot. We applied an estimation strategy based on a mechano-statistical approach, which consists in the linkage of the spatio-temporal model to a statistical method for estimating parameters by coupling the mechanistic vision of the phenomenon studied, with the stochastic vision of the observation process (Soubeyrand and Roques, 2014). Two additional coefficients describing the link between the initial amount of environmental spores in the plot and the shading data were therefore estimated. Parameter estimation of model parameters was performed by approximating system solutions by numerical resolution (see Nembot *et al*, 2021). Final positive values were obtained for the two coefficients, which tends to show that higher shade intensities in the cocoa plot led to higher initial inoculum density levels (P_2^0). This was also confirmed by numerical simulations of disease spatiotemporal evolution where sequential images of the cocoa plot show that first infections and black pod disease outbreaks occurred in places of the plot characterised with higher shading intensities (Nembot *et al*, 2021). From this confirmation of shading effect on the pathosystem, we can hypothesize about the exact relationship(s) that exists between shading and disease epidemics. For instance, shading can influence disease dynamics in the plot by influencing temperature, relative humidity, rainfall interception/kinetic energy of raindrops thereby influencing dispersal but also by influencing the light availability (quantity and quality of light). It is likely that in the field these parameters interact, however, we can study some of these parameters individually to provide us with an idea of the specific response of *P. megakarya*. To date, the direct role played by light on the development of

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Phytophthora species remains poorly studied and is even less studied in cocoa. Therefore, we proposed in this study to put in place an experimental design to study the effects of different light wavelengths on *P. megakarya* evolution (growth and sporulation) in order to broaden existing knowledge on shading associated (light availability) effects on this pathosystem.

3.4. Sampling: Description of study strains

A total of 27 strains of *Phytophthora megakarya*. (14 from Cameroon and 13 from Côte d'Ivoire) were selected for the experiments. The selected strains of *P. megakarya* originated from two different agro-ecological environments, namely forest (15 from shaded cocoa systems) and savannah (12 from full sun cocoa plots). Two particular strains of *Phytophthora* (*P. megakarya*-KP58 and *P. palmivora*-TRI1) were present in all experiments. These 2 reference strains were chosen as control strains because of prior homogeneous growth during similar experiments. For each experiment, 3 replicates of each of the strains were used. The *Phytophthora* strains used for the experiments were cultured and subcultured on a V8 agar medium and were stored until further use under controlled conditions: 25 °C and 62% RH

3.4.1. Studied factors

All isolates were grown under different light conditions. For each experiment, the control consisted of 24h dark conditions. The agroecological zone of origin of the studied strains was considered as a factor for the analysis. All the studied factors are depicted in the following table (Table 1.)

Table 1 Details on factors studied

Light Conditions	*Experimental control*	*Modalities*
White light photoperiod	*Obscurity*	*12h/ 12h – 24h/24h*
Light intensity	*Obscurity*	*2 tubes – 4 tubes – 6 tubes*
Ultraviolet radiation (UV)	*Obscurity*	*UV-a*
Light colors	*Obscurity*	*Blue-Yellow-Red*
Strain origin	*Obscurity*	*Forest - Savannah*

Remarks

- **12h/12h** represents light-dark alternation at 12 hours intervals
- **24h/24h** represents continuous lighting.
- **UV-a** stands for type A ultraviolet rays (320-400 nm)
- **Obscurity** Experimental control for light in all manipulations

3.4.2. Strain development monitoring and data analysis

Radial growth along two perpendicular axes after 2, 3 and 6 incubation days and the daily growth rate (in mm day^{-1}) was determined. After 10 days, zoospore production was quantified using the cold shock method (Denlinger *et al*, 1991) using 20 ml of distilled water. Mean zoospore production was determined using a haematocymeter. Data collected were subjected to descriptive statistics and analysis of variance regarding mean daily (radial) growth rates, as well as zoospore production as a function of studied factors such as light color, light intensity, illumination photoperiod, and agro-ecological origins for the study strains.

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4. Results

4. 1 Strains growth rate in white light

Experiments carried out revealed that globally all the studied strains grew better in the dark (p-value < 0.001) compared to light exposure. In the presence of white light, all strains had significantly reduced growth rates (p-value < 0.001) when exposed to increasing photoperiods and illumination intensity (Fig.5). We also note that during this experiment, the control strain KP58 seem to have not been influenced by light duration and intensity. Furthermore, the second control strain TRI1 (*P. palmivora*) seem to act slightly different from the others.

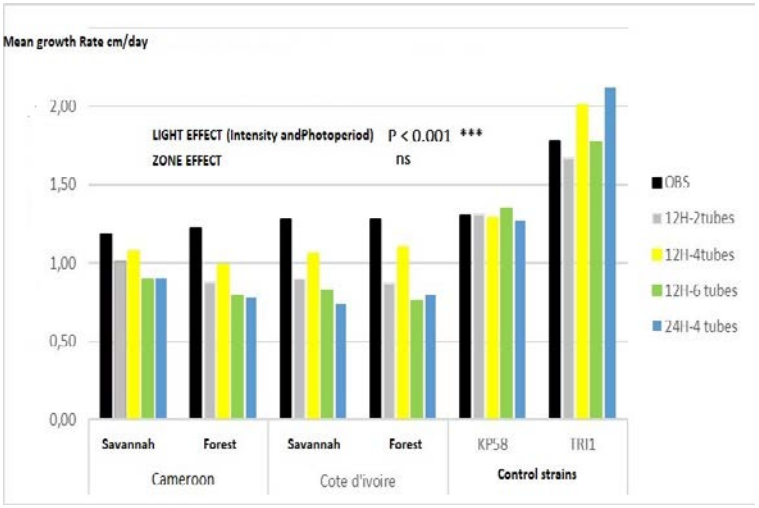

Figure 5. White light effect on strains growth (Photoperiod and light intensity)

4. 2 Strains growth rate in other colors (wavelengths)

In the first experiment, it was observed that ultraviolet light (UV-A) significantly (p-value < 0.001) led to a reduction in the growth rate compared to obscurity (Fig.6).

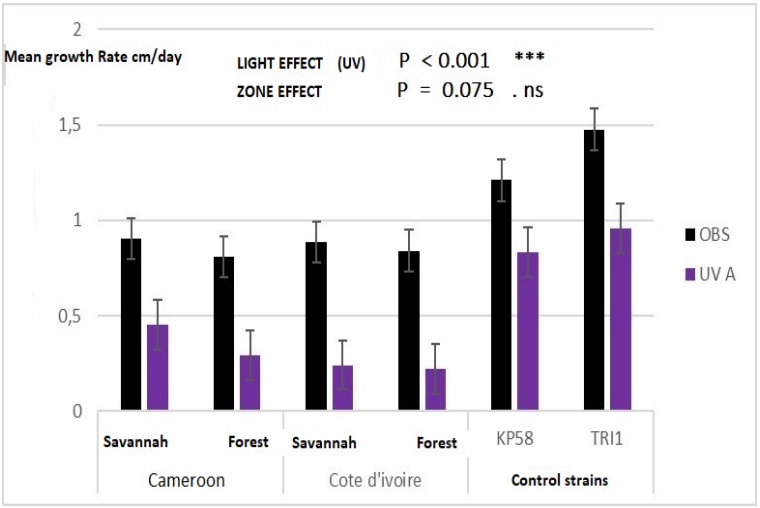

Figure 6. UV light effect on strains growth

In the second experiment, we noted clearly that blue light significantly induced (p-value < 0.001) a reduction of strains growth in comparison to red color and obscurity (Fig.**7**).

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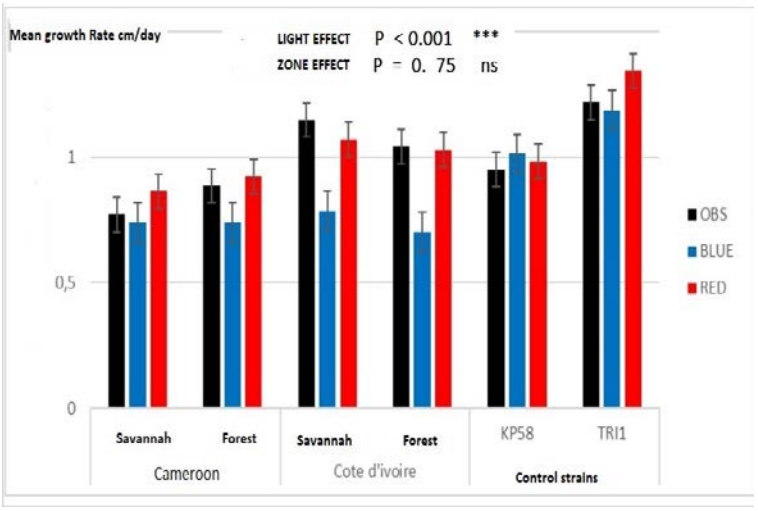

Figure 7. Blue and red light effect on strains

For the last experiment, we observed also a significant reduction of radial growth rate in presence of the blue colors (p-value < 0.001) in comparison to obscurity and yellow color which seemed to have a rather stimulating effect on growth (Fig.8).

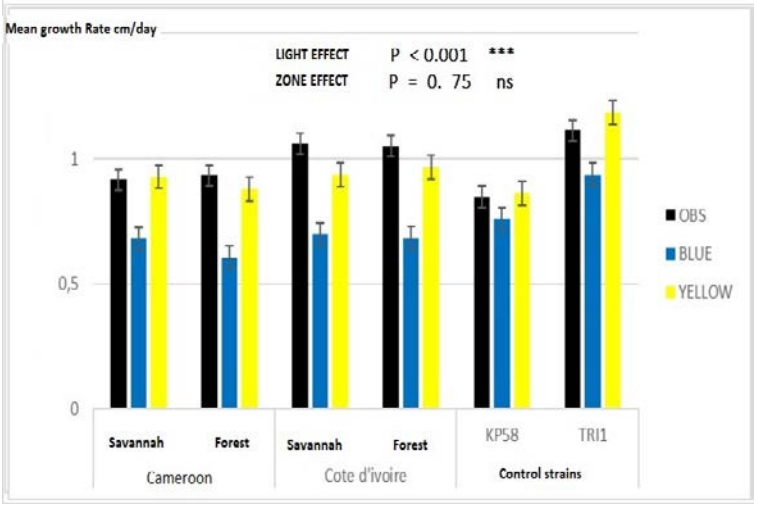

Figure 8. Blue and yellow light effect on strains growth

4. 3 Zoospore production

In all experiments light induced significantly, higher spore-production (p-value < 0.001) compared with complete darkness. A kind of a production peak was observed for zoospore production at low intensities of white light (2 tubes). It was also noted that zoospore production was significantly lower (p-value < 0.001) in presence of blue color and ultraviolet (UV-A) when compared with other light conditions.

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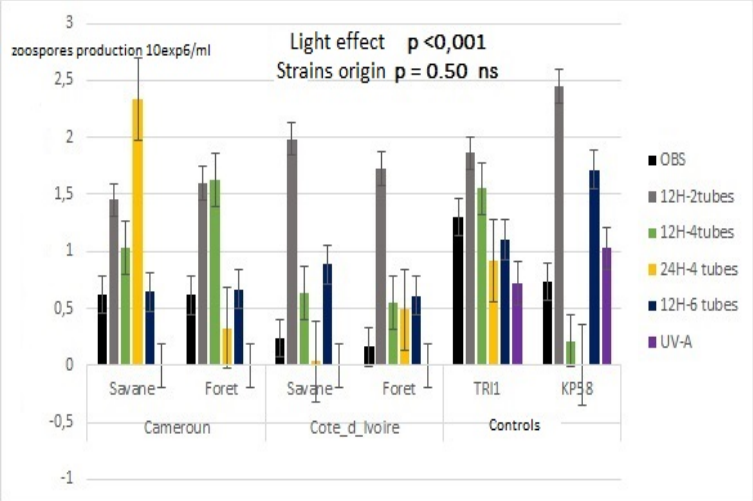

Figure 10. Zoospore production under different light conditions

4. 4 Effect of the agro-ecological origin of strains

Overall, the origin and the ecological environment did not seem to significantly influence (p-value > 0.05 ns) the response of the strains in terms of growth and sporulation in different light conditions. The savannah (full sun cocoa plots) and forest (shaded cocoa systems) strains of the two countries globally reacted in a similar way to light regardless of their ecological zone of origin.

5. Discussion and Conclusions

The model previously developed and the estimation strategy proposed contributed to expand existing knowledge on the role played by the primary inoculum and shading in the disease dynamics (Nembot *et al*, 2021). Assessing the impact of shading (canopy closure) on system dynamics revealed a positive effect on the initial density of primary inoculum, meaning that high shading intensities in the plot are linked to higher quantities of environmental spores. The positive link shown between shading in the plot and the amount of environmental spores can be explained by more favorable environmental conditions (lower temperatures, high humidity or light availability for example) more conducive to the development of spores. Whereas temperature and humidity levels have been identified as important parameters for *Phytophthora* (ten Hoopen and Krauss, 2016; Puig *et al*, 2018) development/epidemics, the influence of light availability remains largely unexplored. This study is an attempt to broaden existing knowledges on light availability effects (linked to plot shading) on the pathoystem by investigating the *in vitro* effect of light on *P. megakarya* strains evolution.

The experimental design put in place allowed to characterize *P. megakarya* strains (growth and production of spores), in response to different lighting conditions. Conditions stimulating or inhibiting growth and sporulation of *P. megakarya* strains were identified. Data showed that all studied *P. megakarya* strains grew relatively better in the dark (compared to exposure to light). This result confirms partially why black pod disease would proliferate in shaded systems (less light than full sun systems). It was also noted that in the presence of white light, all strains studied had reduced growth rates when exposed to increasing photoperiods and light intensity. Similar results were obtained by Blaha (1983). Regarding other wavelengths, blue light and ultraviolet (UV-A) significantly reduced *P megakarya* growth compared to other wavelengths tested (green, red, yellow). Transposing this result to the field could be explained by Ross (1975), who stated that light under the plant canopy may be rich in wavelengths in the near infrared. This enhancement of infrared light is thought to be the result of the spectral properties of green leaves which transmit and reflect strongly in the near infrared region. This could explain why *P megakarya* strains grew relatively well under wavelengths relatively close to infrared such as yellow and red for instance. Experiments carried out also revealed that light in general was a stimulating factor for sporulation (production of zoospores) and we also noted

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savannah and forest strains globally reacted in a similar way to light regardless of their ecological zone of origin, the ecological origin (forest or savannah) did not seem to influence the response of the strains in terms of growth and sporulation. This is in contrast with the hypothesis we had of adaptation of the strains in relation to the availability of light according to their environment.

Nonetheless, results obtained here corroborate the hypotheses highlighted in the spatio-temporal model, namely a positive effect of shading on disease dynamics. This shows once again that shaded systems are quite conducive to the development of *P. megakarya.* Strategic management of shade in the field is therefore important for black pod disease control. This study can be seen as a contribution to lay foundations for risk prediction models. Nevertheless it should still be noted that the *in vitro* growth and sporulation of *P. megakarya* cannot accurately reflect the behavior of strains in their natural environment and also in the presence of their host. Further investigations need to be carried out to refine these results and to clarify in an explicit and thorough manner the effect of light and shading on disease dynamics.

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