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FULL RESEARCH PAPER

Effect of cultural practices on the development of arabica coffee berry disease, caused by *Colletotrichum kahawae*

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Abstract In the high altitude regions of Africa, coffee berry disease (CBD), caused by *Colletotrichum kahawae*, is the main constraint for arabica coffee (*Coffea arabica*) production. However, certain agricultural practices can reduce losses caused by the disease and thereby promote optimum production. On small family farms in Cameroon, mixed cropping with fruit trees, intercropping with food crops and maintenance pruning of coffee trees are very widespread agricultural practices that can affect CBD epidemics. Consequently, an epidemiological study was conducted to assess how cultural practices affected the disease in an arabica coffee smallholding in Cameroon. The disease was monitored on a weekly basis over four successive years (2002–2005) on coffee trees in diverse cultural situations. Cultural practices likely to reduce losses due to CBD were identified. The infection rate was significantly lower on coffee trees grown intensively than on coffee trees grown in the traditional manner. Coffee trees located under the shade of fruit trees were significantly less attacked than those located in full sunlight. In addition, berries on the leafless parts of branches, near the main trunk of the coffee tree, were less infected than those on leafy sections. These results show that maintenance pruning, removal of mummified berries, and mixed cropping with shade plants are cultural practices which create environmental conditions that limit CBD development.

Keywords *Colletotrichum* · Micro-environmental conditions · Disease dispersal

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Introduction

Coffee berry disease (CBD) is a typically African disease of the main Coffea arabica growing zones. The disease is caused by *Colletotrichum kahawae*, a fungus with vegetative multiplication that is specific to the fruits (Van der Graaff 1992; Waller et al. 1993). Dark necrotic spots with clear contours on the pericarp of immature berries are formed. The spots may coalesce causing the entire fruit to rot. However, fruits fall rapidly once they are infected. Necrotic tissues occasionally display orange gelatinous masses arranged in concentric circles corresponding to the acervuli of the pathogen. In the absence of berries during the dry season, the fungus would seem to survive on organs not affected by the disease, such as branches and leaves. Little is known about its survival in the absence of berries, under adverse environmental conditions during the dry season. Flower cushions, branch bark and mummified berries are considered to be the main sources of primary inoculum (Nutman and Roberts 1960; Gibbs 1969; Muller 1980; Van Der Graaff 1992). *Colletotrichum kahawae* begins its parasitic activity in rainy seasons as soon as fruits appear. The first disease symptoms are seen between the 8th and 10th weeks after flowering. New infections then occur gradually up to the fruit hardening stage, towards the 22nd week after flowering (Muller 1980; Van Der Graaff 1992). The pathogen is primarily dispersed by rainfall through splashing, with conidia being transported by droplets caused by the impact of raindrops on the acervuli (Waller 1972). However, some authors have reported the existence of conidial dispersal by coffee insect pests (Waller 1972; Nemeye et al. 1990). Near-saturation humidity and temperatures between 20°C and 22°C are conducive to conidial germination and appressorial formation (Gibbs 1969; Masaba and Waller 1992).

The agricultural techniques used in all cropping systems are intended to optimize development conditions for cultivated plants, encourage expression of their production potential, and limit pest and disease pressure. A wide range of cultural methods (crop rotations, harvest residue management, choice of sowing dates, etc.) are available for plant disease control, but the choice is more limited for tree crops diseases (Palti 1981). However, on tree crops, pruning dead or unproductive branches and removing mummified fruits are very widespread practices. They have helped to limit the levels of various diseases such as fire blight caused by *Erwinia amylovora* on pear (Shtienberg et al. 2003), grapevine black rot caused by *Guignardia bidwellii* (Hoffman et al. 2004), apple scab due to *Venturia inaequalis* (Holb 2005), and pistachio panicle and shoot blight caused by *Fusicoccum* spp. (Mila et al. 2005). On coffee trees, such agricultural operations are very frequent on small family farms in the highland region of West Cameroon, which is severely attacked by coffee berry disease. They are generally carried out in the off-season, corresponding to the dry season. They are also sometimes implemented alongside other cultural practices, such as mixed cropping with fruit trees (mango, guava, kola, banana, etc.) and inter-cropping with low-growing food crops (bean, potato, maize, etc.). These different agricultural practices modify microenvironmental conditions and may affect the growth and dispersal of *C. kahawae*. The purpose of the present study was to determine how those cultural practices affect CBD development and to ascertain how factors, such as shading, affect losses caused by the disease. It was conducted over four successive years from 2002 to 2005 on a farm located in a region of Cameroon with high CBD incidence.

Materials and methods

Planting material

This work was undertaken over four successive years on a farm in North-West Cameroon at Santa (05°47.190 N, 10°09.672 E, 1750 m above see level), located in a region with high CBD incidence. The plantation consisted of *Coffea arabica* cv. Jamaica trees grown in a topped single stem system, which were highly susceptible to the disease. It was divided into two plots, one of which was managed intensively and the other managed in the traditional manner. Each plot contained 100 vigorous, high-yielding coffee trees, of which 50 were preferentially located under the shade of different fruit trees (mango, avocado, kola) and the other 50 in full sunlight. The intensively managed coffee trees required the following main agricultural operations: (1) maintenance pruning, which consisted of removing dead branches and mummified berries at the end of the season, (2) two applications of mineral fertilizer (N-P-K: 20-10-10) at a rate of 200 g tree^{-1}, the first being applied after fruit set, the second in mid-season, (3) two applications of herbicides (GLYPHOSATE—Round-Up) at a rate of 2.4 l ha^{-1}. The coffee trees in this plot were not intercropped with food crops for the duration of the experiment. The traditional management method varied depending on the food crops intercropped with coffee (beans or potatoes). There were generally two manual weeding rounds when the intercrop was hoed and ridged, and irregular application of organic fertilization at variable rates. The fertilizers came from domestic waste and livestock manure (pigs and poultry). However, light mineral fertilization with N-P-K: 20-10-10 was sometimes given when the intercrop was potatoes.

Experimental design

Two main factors were studied: the coffee plantation management method (intensive and traditional) and the type of light (shade or sunlight). Those factors were studied in a completely randomized design of 50 replicates in which the experimental plot comprised a single coffee tree. Each coffee tree was given a specific identity number for the four successive years of observations. Three plagiotropic branches were marked in the upper, middle and lower sections of the coffee trees as soon as fruits reached the pinhead stage, precisely 6 weeks after flowering. In order to measure disease development kinetics on the branch, a distinction was made between the leafy section and the leafless section in the last two years of the experiment (2004 and 2005). Weekly observations were made on three branches at different positions in each coffee tree (top, middle, and bottom). Starting from the third year of the experiment, separate observations were carried out on the leafy and leafless zones of each branch. All the observations were carried out from the 6^{th} week after flowering up to the 25th week. They consisted of counting: (a) the total number of berries (Btot), (b) the number of new infected berries, marking them with small labels to avoid counting them again in subsequent assessments (Bdis), (c) the number of old infected berries (Bmk).

Assessment of losses and statistical analyses

For each coffee tree studied, different harvest losses were estimated by:

(a) The % total losses (Ptot) observed throughout the year of observations, which expressed all losses due to CBD or not, recorded over one year. It was calculated by the formula:

$$\mathrm{Ptot} = \frac{\mathrm{Btot}_1 - (\mathrm{Btot}_n - \mathrm{Bmk}_n - \mathrm{Bdis}_n)}{\mathrm{Btot}_1} * 100 \quad (n = 25) \tag{1}$$

Where Btot_1 is the total number of berries on the first observation, and the expression ($\mathrm{Btot}_n - \mathrm{Bmk}_n - \mathrm{Bdis}_n$) is the number of healthy berries in the 25th week of observations. The terms Btot_n, Bmk_n and Bdis_n are the total number of berries, the total number of old disease berries, and the total number of new infected berries on the final observation.

(b) The % diseased berries (Pdis) which was the ratio between the sum of new diseased berries counted weekly, from the first to the 25th week of observations [$\mathrm{Bdis}_{(1-n)}$] and the initial number of berries (Btot_1). This represents the total diseased berries observed throughout the year of observations.

$$\mathrm{Pdis} = \frac{\sum_{n=25}^{1} \mathrm{Bdis}_n}{\mathrm{Btot}_1} * 100 \tag{2}$$

(c) The % losses not due to CBD (Pfall) which was expressed by the difference between the total losses and losses due to CBD (Ptot–Pdis). Those losses mostly attributed to the fall of apparently healthy berries were considered to be physiological fall. Such losses are commonly observed from the 6th week after flowering up to the 24th week. These two variables (Pdis and Ptot) were calculated for observations obtained at each week.

The % diseased berries and the % physiological fall underwent $\arcsin\sqrt{x}$ transformation in order to meet the conditions for applying the analysis of variance. The analysis of variance was carried out using the GLM (General Linear Model) procedure of the SAS (Statistical Analysis System) software, version 9.1, and the means between treatments were compared using the Student-Newman-Keuls test at the 5% level.

Results

Assessment of annual harvest losses

Losses caused by CBD and those caused by physiological fruit fall varied significantly ($P < 0.0001$) from one observation year to another (Table 1). The disease was severe in 2005 with more than 50% of berries infected on average, compared to around 40%

Table 1 Analyses of variance of the losses caused by CBD and by physiological fruit fall depending on cultural practices (2002–2005)

Source	DF	Losses due to CBD (Pdis)		Physiological fruit fall (Pfall)	
		F value	*Pr* > *F*	*F* value	*Pr* > *F*
Years	3	24.73	<0.0001	9.05	<0.0001
Coffee tree branches	2	1.14	0.3211	1.64	0.1935
Crop management	1	60.82	<0.0001	1.19	0.2764
Shade	1	134.2	<0.0001	79.64	<0.0001
Shade × Management	1	0.76	0.3831	0.45	0.5005
Year × Management	3	2.84	0.0364	23.53	<0.0001
Year × Shade	3	15.76	<0.0001	16.45	<0.0001
Error	2175	–	–	–	–

in 2002 and 45% in 2003. The lowest infection level was in 2004 with around 36% of berries diseased (Table 2). However, the proportion of berries attacked by CBD was always higher than that for physiological fruit fall, irrespective of the observation year (Table 3). For all years, losses caused by physiological fruit fall were always fewer than those due to CBD. They gradually decreased over the four years of the experiment. The highest physiological fruit fall rate, estimated at about 35% of berries, was found in the first year of the experiment (2002). The lowest physiological fruit fall rate, about 25%, was recorded in 2005.

The overall analysis of the data gathered over the four years showed that the level of CBD infection differed significantly ($P < 0.0001$) depending on the crop management methods (Table 1). The trees managed traditionally were more severely attacked than those managed by the intensive technique (Table 3). However, significant ($P = 0.037$) interactions were found between the year and the management techniques. It was reflected in an equivalent disease level in both types of plots in 2005, but greater disease severity in the traditional plot than in the intensified plot from 2002 to 2004 (Table 3). The physiological fruit fall seen on the coffee trees managed intensively from 2003 to 2004 was greater than on coffee trees managed traditionally, which was the opposite to 2002 (Table 3).

Table 2 Annual losses due to CBD and to physiological fruit fall

Year	Number of branches observed	Losses due to CBD* (Pdis)	Physiological fruit fall* (Pfall)
2002	600	40.12 c	34.67 a
2003	435	44.08 b	31.81 b
2004	575	36.46 d	29.04 c
2005	443	53.55 a	25.46 d

* Means with the same letters are not significantly different

CBD intensity strongly depended on coffee tree shading conditions ($P < 0.0001$) (Table 1). The coffee trees exposed to sunlight were more severely attacked by CBD than those trees under shade, irrespective of the management method applied in the plots. However, the infection levels on the coffee trees varied from year to year. It was non-significant in 2003 and quite high in 2002, 2004 and 2005. In 2004–2005, the average disease level on coffee trees exposed to sunlight was about 50% greater than that on the coffee trees under shade (Table 4). Disease intensity differed between the shade and sunlight plots in the same year. However, the disease progress curve showed largely the same trend, irrespective of the type of light received by the coffee trees (Fig. 1). For instance, in the year of high disease intensity (2005), the first diseased berries were recorded in the 8th week after flowering and the maximum infection level was reached around the 15th week. However, in a year less conducive to CBD development (2004), the first symptoms were observed in the 10th week after flowering and the asymptotic infection level was reached around the 20th week.

Physiological fruit fall also varied with tree shading but the treatment differences varied with years. There were no differences in 2004; in contrast it was less in full sunlight in the other years. In 2004,

Table 3 Means of the different annual losses in relation to crop management methods

Year	Crop management	Number of branches observed	Losses due to CBD* (Pdis)	Physiological fruit fall* (Pfall)
2002	Traditional	300	43.92 a	37.12 a
	Intensive	300	36.31 b	32.22 b
2003	Traditional	215	50.62 a	28.22 b
	Intensive	220	37.69 b	35.32 a
2004	Traditional	300	39.58 a	24.75 b
	Intensive	275	33.06 b	33.71 a
2005	Traditional	277	51.57 a	25.22 a
	Intensive	166	56.86 a	25.60 a
Overall means	Traditional	1092	45.99 a	29.02 a
	Intensive	961	39.24 b	31.92 a

Table 4 Means of annual losses under different light conditions

Year	Light received by coffee trees	Number of branches observed	Losses due to CBD (Pdis)	Physiological fruit fall (Pfall)
2002	Shade	300	33.94 b	38.36 a
	Sunlight	300	46.29 a	30.98 b
2003	Shade	226	42.45 a	33.98 a
	Sunlight	209	45.84 a	29.46 b
2004	Shade	276	29.28 b	29.28 a
	Sunlight	299	43.09 a	28.82 a
2005	Shade	181	39.20 b	26.83 a
	Sunlight	262	63.47 a	24.51 a
Overall means	Shade	983	35.56 b	32.68 a
	Sunlight	1070	49.52 a	28.50 b

* Means with the same letters are not significantly different

physiological fruit fall began two weeks before the first infected berries appeared. It occurred one week after the start of the disease in 2005. In addition, the period corresponding to maximum fruit fall always coincided with the period of maximum disease intensity (Fig. 1).

Disease development in relation to fruit position on the branch (2004 and 2005)

No significant difference was found between the different branches of the coffee trees either in the disease incidence (P = 0.321) or the incidence of physiological fruit fall (P = 0.193) (Table 1). The position of the berries in the leafy zone or leafless zone of the branch had a significant effect ($P = 0.017$) on the losses due to CBD. However, it did not have a significant effect (P = 0.080) on the losses due to physiological fruit fall (Table 5). For instance, on the same branch, berries in the leafy zone were always more severely attacked by CBD at the end of the season than berries in the leafless zone, irrespective of the management method in the plots and tree shading conditions (Table 6).

In 2004, CBD occurred in a relatively synchronous manner on both parts of the branches, around the 9th week after flowering. The disease progress curves indicated that the cumulated % infected berries was higher in the leafless zone than in the leafy zone from the 9th week to the 16th week, but lower from the 18th week to the 26th (Fig. 2a). In 2005, infection occurred very early on the leafy sections of the branches: the first symptoms were found on the 7th week after flowering. In contrast, symptoms occurred three weeks later on the leafless section, i.e. 10th week after flowering. The cumulated % diseased berries on the leafy section of the branches were always higher than on the leafless section (Fig. 2b).

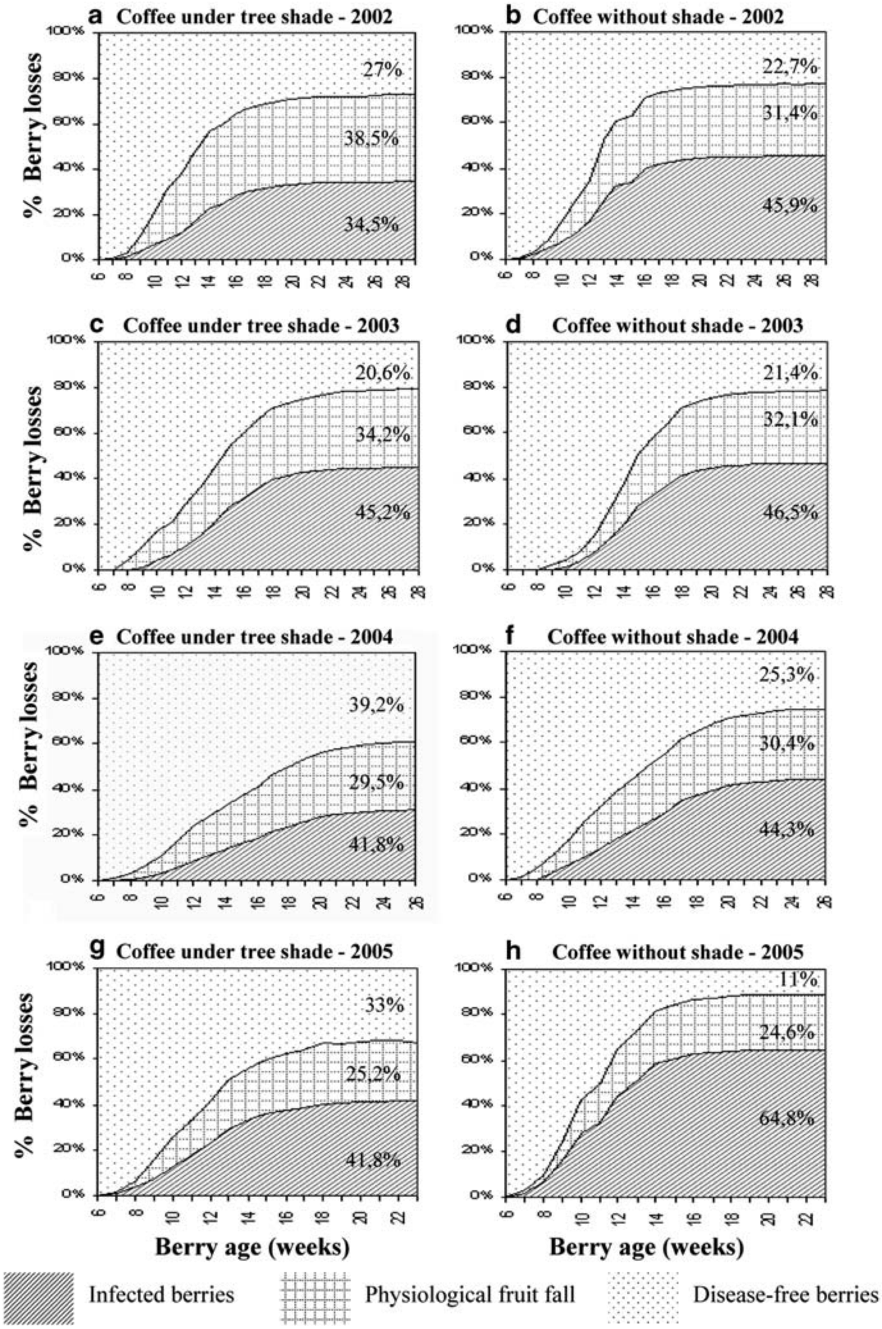

Fig. 1 Cumulated losses at harvest on coffee trees under shade and in full sunlight from 2002 to 2005. (The numbers at the right of each diagram are corresponding final losses)

Discussion

Our results indicated that some agricultural operations affect disease development. Losses caused by CBD were significantly higher on coffee trees managed traditionally when compared to trees managed intensively. Each year, mummified berries and dead branches were systematically removed at the end of the season, from all the coffee trees in the intensively managed plots. Removing those organs helped to

Table 5 Analysis of variance of the losses due to CBD and to physiological fruit fall in relation to berry position on the branches (2004–2005)

Source	DF	Losses due to CBD (Pdis)		Physiological fruit fall (Pfall)	
		F value	*Pr* > *F*	*F* value	*Pr* > *F*
Years	1	25.37	<0.0001	16.28	<0.0001
Branch zones	1	5.75	0.0168	0.08	0.7741
Management methods	1	11.24	0.0008	0.29	0.5902
Shade	1	110.95	<0.0001	44.65	<0.0001
Zone × year	1	0.02	0.8779	6.91	0.0088
Zone × management method	1	0.5	0.48	0.12	0.7255
Zone × shade	1	0	0.9839	0.12	0.7263
Error	646		–	–	–

Table 6 Means of the different annual losses at different branch positions on the tree and berry positions on branches

Branch parameters	Parameter modalities	Number of branches observed	Losses due to CBD* (Pdis)	Physiological fruit fall* (Pfall)
Coffee tree branches	Upper branches	694	43.84 a	29.85 a
	Middle branches	684	43.51 a	30.88 a
	Lower branches	694	41.11 a	30.77 a
Branch zone[a]	Leafless zone	311	39.69 b	32.46 a
	Leafy zone	343	46.08 a	32.33 a

[a] Means calculated using data from 2004 and 2005

* Means with the same letters are not significantly different

reduce the potential sources of primary inoculum when the disease began, since the mummified berries and branches harboured the pathogen during the off-season (Nutman and Roberts 1960; Gibbs 1969; Muller 1980). Pruning is an agricultural operation commonly practised in tree crops. It is used to model tree architecture, renew the assimilating system and stimulate new reproductive organs. It is also recommended for controlling numerous diseases, such as apple scab due to *V. inaequalis* (Holb 2005) or pistachio panicle and shoot blight due to *Fusicoccum* spp. (anamorph of *Botryosphaeria dothidea*) (Mila et al. 2005). Likewise, pruning during the vegetative growth period has led to the effective control of black rot of grape caused by *Guignardia bidwellii* (Hoffman et al. 2004). In the final year of the present experiment (2005), the CBD level was the same for both types of management method, with 53% of infected berries on average. However, between 2002 and 2004, the intensively managed coffee trees were less attacked than those managed traditionally. Nevertheless, the disease incidence remained relatively high, despite a reduction in initial inoculum sources through maintenance pruning and mummified berry removal. This would seem to indicate the limitation of carrying out these agricultural operations alone for effective CBD control. The techniques might be more efficient when combined with other agricultural practices that are detrimental to disease development. For example, pruning has been used to substantially improve the efficiency of chemical treatments on pear trees against fire blight caused by *E. amylovora* (Shtienberg et al. 2003). The coffee trees in the intensively managed plots were not intercropped with food crops, unlike those in the traditionally managed plots. This agricultural practice subjected the coffee trees to competition from the other cultivated plant species for various nutrients in the soil. It also exposed them to physiological stress, due to upkeep operations (hoeing and ridging) in the intercrops, which remained particularly damaging for the root system. Very little is yet known about the involvement of edaphic factors and stress in CBD development. It has been reported that an excess of nitrogen fertilizers can be conducive to *Alternaria alternata* infections in some citrus plantations in Florida (Timmer et al. 2000).

Our results clearly revealed that shading had a highly significant effect on disease severity. The %

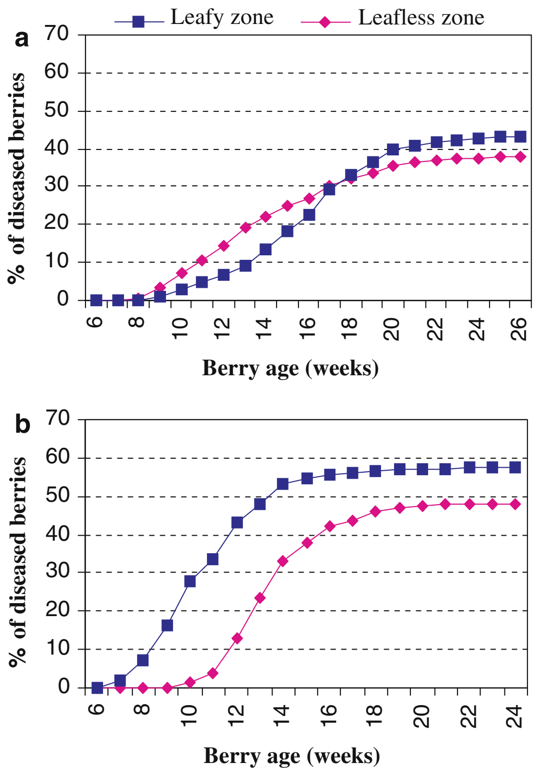

Fig. 2 Cumulated % infected berries at different positions on the branches in 2004 (**a**) and 2005 (**b**)

diseased berries was significantly higher on coffee trees exposed to sunlight than on those located under the shade. However, the effect of shading on disease development varied substantially among years. It was very marked in 2004 and 2005, which corresponded to the years of least and most infection respectively. Shading created microclimatic conditions that helped to delay fruit ripening (Vaast et al. 2006), which might have led to a shift in the period of berry susceptibility in relation to the period of high disease pressure. Moreover, contradictory results were reported by Phiri et al. (2001), regarding the effect of shade plants on CBD incidence, based on a survey conducted on smallholdings in Malawi. Those authors estimated that shading did not have any significant effect on CBD incidence. Yet, in the same study, they found that coffee trees interplanted with banana plants were less infected than coffee trees in a monoculture. They explained this apparent contradictory result by suggesting that the banana plants might have acted as a physical barrier against disease spread. This hypothesis could also be extended to all coffee tree shade plants (banana, fruit trees, legumes, etc.). Indeed, on tree crops, infection severity is very often positively correlated with rainfall, such as in citrus for *Diaporthe citri* infections (Agostini et al. 2003) and *Alternaria alternata* infections (Timmer et al. 2000), and in peach trees for *Taphrina deformans* infections (Rossi et al. 2006). Likewise, *C. kahawae* infections on arabica coffee are highly dependent upon rainfall (Van der Graaff 1992; Muller 1980). The effect of shade plants would appear to consist of modifying certain rainfall characteristics, which might influence conidial dispersal. The canopy of these plants might intercept certain raindrops, divert others from their trajectory or reduce the speed of those droplets that were to reach the coffee trees. Sometimes, the raindrops that fall onto coffee trees via the foliage of shade plants might not be energetic enough to dislodge and disperse conidia. Lovell et al. (2002) showed that the kinetic energy of raindrops was a decisive factor in spore dispersal.

CBD intensity did not vary significantly with the position of coffee tree branches. However, the close proximity of the branches due to the umbrella architecture of the coffee trees studied may have masked potential effects. Most of the fruit-bearing branches were located towards the coffee tree canopy, which guided the choice of branches to be studied towards that part of the trees. This resulted in an ascending gradient in disease severity though it was not statistically significant. This type of gradient was clearly found in epidemiological studies conducted on coffee trees with branches that were distant from each other (Mouen Bedimo 2006). On a branch scale, infections due to CBD proved to be greater on the leafy section. The leafless section of branches, near the trunk, seemed to be less exposed to disease dispersal factors (rain, wind) than the leafy section. Logically, the first berries infected ought to have appeared on the leafless section of branches, nearest to the old wood that had previously been in contact with the pathogen. Present results showed that the disease began on either part of the branch. In 2004, it began simultaneously on both sections of the branches. However, in 2005 the first symptoms occurred on the leafy section, three weeks before those on the leafless section. This result may suggest that the primary inoculum is randomly distributed within the coffee trees.

Substantial variations in disease intensity were found between years. The disease incidence was similar in the first two years, decreased in the third year, and increased substantially in the final year. This oscillating trend seemed to be mainly due to annual variations in climatic factors affecting *C. kahawae* development and coffee tree physiology. Temperature and relative humidity are highly important factors for *Colletotrichum* spp. growth. Near saturation humidity is conducive to conidial germination, germinative tube elongation and appressorial formation. (Goos and Tschirsch 1962; Gupta and Pathak 1990; Leandro et al. 2003). Conidia can also lose viability when exposed to long dry periods (Denham and Waller 1981; Estrada et al. 1993). In addition, rainfall, which is the main factor affecting the dispersal of *C. kahawae* propagules (Griffiths and Waller 1971; Waller 1972; Muller 1980; Masaba and Waller 1992), varies substantially among years. Arabica coffee berries are highly susceptible to CBD during their expansion period from the 10th week to the 20th week after flowering (Muller 1980; Van der Graff 1992). However, it is important for climatic conditions conducive to *C. kahawae* development to occur during this critical phase of the coffee tree phenological cycle for the maximum number of berries to be attacked. Climatic fluctuations appear to strongly influence the development of diseases when host organs become highly receptive to the pathogen. Such is the case for temperature and relative humidity in *Fusarium* spp. infections on wheat during anthesis (Xu 2003) or for *Phomopsis amygdali* on peach trees during the critical period of fruit susceptibility (Lalancette et al. 2003).

This study showed that maintenance pruning, mummified berry removal and interplanting with shade plants helped to significantly reduce disease intensity on coffee trees. Pruning coffee trees and removing mummified berries destroyed numerous sources of primary inoculum and thereby helped to reduce disease pressure at the beginning of infection. Shade plants hindered effective conidial dispersal. These results show the important role played by human beings in the complex interactions between *Coffea arabica*, *C. kahawae* and the environment.

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