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Seasonal and altitudinal differences in coffee leaf rust epidemics on coffee berry disease-resistant varieties in southwest Ethiopia

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Original Article

Seasonal and altitudinal differences in coffee leaf rust epidemics on coffee berry disease-resistant varieties in southwest Ethiopia

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Abstract

Coffea arabica is native to the Afromontane forests of southwestern Ethiopia, the leading African country in Arabica coffee production. The intensity of coffee leaf rust (CLR), a fungal disease of growing concern to coffee farmers, was assessed in eight coffee berry disease-resistant *C. arabica* varieties planted at three different altitudes. Disease variables assessed were CLR prevalence (percent of infected plants), incidence (percent rusted leaves) and severity (percent leaf area affected) at four times (Nov, Jan, Apr and Jun). The intensity of CLR epidemics was highest at the lowland and lowest at the highland location. We hypothesize that inoculum may be limited by cooling conditions that negatively affects fungal sporulation. CLR intensity was highest at harvest time (Nov) and decreased during the following dry season, reaching zero values in Apr and Jun. All coffee varieties were similarly susceptible to CLR. Our data suggest that more efforts should be employed by breeders to develop CLR-resistant coffee varieties, and that shifting cultivation from lower to higher altitudes will lead to higher yields due to lower impact of CLR epidemics.

Keywords *Coffea arabica* L., *Hemileia vastatrix*, Epidemics, Host plant resistance

Introduction

Among all natural commodities, the monetary value of the coffee trade is only exceeded by that of oil (DaMatta and Ramalho 2006; Gichimu 2012). The crop is also an essential export revenue and provides labor opportunities in many tropical countries (Kushalappa and Eskes 1989;

Ribeyre and Avelino 2012; Talhinhas et al. 2017). *C. arabica* is native to the Afromontane forests of SW Ethiopia and the country ranks first in Africa and fifth in the world in terms of Arabica coffee production. The average annual Ethiopian coffee production is estimated to be more than 350,000 tons, of which 90% comes from home gardens and semi-natural agroforestry systems, and the remaining 10% originates from large-scale intensively managed shade plantations (Alemu et al. 2016).

Despite its huge economic importance, Ethiopian coffee production faces many challenges, including weather, pests and diseases constrains together with poor agronomic practices (Aerts et al. 2017; Alemu et al. 2016; Davis et al. 2012; Moat et al. 2017). Plant diseases are a major cause of yield losses in Ethiopia, including coffee berry disease (caused by *Colletotrichum kahawae)*, coffee wilt disease (caused by *Gibberella xylarioides)*, and coffee leaf rust. These diseases may account for yield losses of 24-30%, 29% and 20-25%, respectively (Alemu et al. 2006; Rutherford 2006; Zeru et al. 2012).

Coffee leaf rust (CLR) is a fungal disease caused by *Hemileia vastatrix* (Basidiomycota, Pucciniaceae) (Arneson 2000; Matovu et al. 2013). *H. vastatrix* is a hemicyclic fungus and the urediniosporic life cycle is the most important source of inoculum (Talhinhas et al. 2017). Chlorotic spots are the first macroscopic symptoms, preceding the differentiation of suprastomatal uredinia. Ultimately, the disease leads to defoliation, and when acute it can lead to the death of branches and heavy crop losses (Avelino et al. 2015). The peak of the CLR symptom development in Ethiopia occurs during fruit harvesting in the dry season but primary crop losses during initial infection are generally low. Secondary crop losses as a result of decreased plant fitness following previous infections tend to be more substantial (Talhinhas et al. 2017).

CLR was first reported in Ethiopia in 1934 (Sylvain 1958). The current prevalence of CLR in Ethiopia was estimated in 27% at Mechara (Hararghe) in the eastern part of the country, and from 33% to 96% in the montane rainforests of SW Ethiopia (Zeru et al. 2012). Furthermore, the prevalence of CLR is currently increasing in Ethiopia; percentage of infected trees increased from 12.9% in 2000 year to 36.0% in 2010 (Zeru et al. 2012). The growing CLR incidence has been associated with a changing climate, especially increased temperatures and annual rainfall, earlier rain in the season and sunshine duration reduction, which favor the life cycle of *H. vastatrix (*Avelino et al. 2015). An early onset of the rainy season favors early development of the disease, as reported for CLR epidemics in Nicaragua *(*Avelino et al. 2015*).* A reduction in in the daily thermal amplitude during the rainy season onset further shortens the CLR latent period (Avelino et al. 2015; Boudrot et al. 2016). In the Americas, CLR prevalence is strongly linked to rainfall, with severe outbreaks of CLR occurring during the two annual rainy seasons (Boudrot et al. 2016; Vandermeer et al. 2017). After each rainy season, CLR strongly declines and the coffee shrubs shed the infected leaves (Vandermeer et al. 2017).

Detailed information on the seasonality of CLR in Afromontane forests at different altitudes has not yet been documented for SW-Ethiopia. In Rwanda, CLR was reported to decrease with altitude; CLR severity (percent leaf area damaged) decreased by 1.5 % per 100 meter increase in altitude, when limited between 1400 and 1800 m a.s.l. (Bigirimana et al. 2012). The increasing altitude would be a surrogate for lowering temperature, negatively affecting sporulation, thus reducing inoculum levels (Bigirimana et al. 2012). Whether the same pattern occurs in SW Ethiopia is not not entirely known, but data from Bonga, Berhane-Kontir,Yayu and Harenna areas in Ethiopia corroborates this hypothesis (Jefuka et al. 2010; Zeru et al. 2012).

The most commonly used *C. arabica* varieties in Ethiopia exhibit resistance to CBD, as other means to control the disease are scarce (Belachew et al. 2015; Van der Graaff 1981). Out of the 40 *C. arabica* varieties used in Ethiopia, 13 are known to be CBD-resistant (G. Bekele Personal communication; Belachew et al. 2015). These CBD-resistant varieties were identified through long-term observational and experimental research during the 1970s and 1980s (Van der Graaff 1981). Although several CBD resistant *C. arabica* variaties from East Africa (such as Ruiru 11 or Batian in Kenya) are known to also express some level of resistance to CLR (Gichimu, 2015), the susceptibility of CBD resistant *C. arabica* varieties to CLR is not known for the Ethiopian varieties. Given the high prevalence of CBD in SW-Ethiopia, almost all newly planted coffee is CBD resistant (Getu et al. 2015). Hence, it is critical to assesses and compare their susceptibility related to CLR before starting new plantations. Also identifying the optimal time of assessment for CLR infection is important for its management strategy. The objective of this study was to monitor and compare CLR epidemics in eight CBD-resistant *C. arabica* varieties planted at three locations (altitudes) throughout one annual seasonal cycle in SW Ethiopia. Both single and interaction effects were of interest.

Material and Methods

Study area and experimental setting

Our study was conducted at three sites in the Mana district, Jimma Zone, Oromia Regional State, Southwestern Ethiopia (Table 1 and Fig.1). The average annual rainfall, mean daily minimum temperature, and maximum temperature are 1523mm, 13°C and 24.8°C, respectively (Bote and Jan 2017). Mana is one of the major coffee producing districts in the Jimma zone with a total cultivated area of 48,000 ha of which 23% is in lowland areas, 65% in midland, and the remaining 12% in highlands (Bote and Jan 2017). There are two seasons in Ethiopia (the dry and the rainy season). The onset of the dry season is in September and the onset of the rainy season is in June. In June 2012, eight different CBD resistant *C. arabica* varieties which are the most commonly used in the region (74-1, 75-227, 74-54, 74-112, 74-140, 74-158, 74-165 and 74-148) (Table 2) were planted at three different sites with different altitudes: Degelu (Low: 1450 m), Gembe (middle:1610 m) and Buture (High: 2063 m) (Table 1, Fig.1). The study was conducted in semi-plantation coffee systems where the coffee management practices were uniform. Agronomic practices such as weeding, slashing, pruning and planting distance were identical. Addition of fertilizer in the coffee farms is not practiced and fertilizers were not applied to the experimental sites in all the three locations. The dominant shade tree species in all the three locations were *Cordia Africana, Acacia abyssinica, Albizza grandibtateata and Vernonia amygdalina.* The shade level in all the three locations was uniform. At each altitude, a randomized complete block design was established, with three blocks per location. Each block consisted of 8 plots (10 x 10 m each), totalling 24 plots per location (Fig. 2). Each variety was randomly assigned to the plots, with each plot composed of 25 plants of the same variety spaced at 2-m from each other (Fig.2).

Disease assessments

Three disease variables**,** CLR prevalence, incidence and severity (Jefuka et al. 2010) were assessed four times during the seasons: Nov 2015, Jan 2016, Apr l 2016 and Jul 2016 on five coffee plants selected at random in each plot. CLR prevalence was operationally defined as the percentage of plants affected by CLR per plot. For assessing incidence, six branches, one pair in the upper, middle and lower canopy layer, were then selected and marked and the percentage of diseased leaves per branch was recorded. CLR severity refers to the percent leaf area affected, which as estimated with the aid of a diagrammatic scale with five severity levels: 1, 3, 5, 7 or 10% (Kushalappa and Chaves 1980). Both incidence and severity were averaged across branches to obtain one value per shrub. All measures were then expressed (averaged) at the plot level.

Data analyses

The effects of *C. arabica* varieties, altitude and assessment time on the disease were assessed by fitting generalized linear mixed effects models (GLMM) with maximum likelihood estimation to data from each experimental site. For ratio variables such as proportions/percentages that are constrained between 0 and 1, a beta rather than a normal distribution was used, by setting a logit link function. Because proportional boundaries 0 and 1 are not allowed in a beta distribution, the original responses (Y) were transformed using Y' = (Y x (*N*-1) + 0.5) / *N*, where *N* is the sample size (Cribari-Neto and Zeileis 2009). Measures assessed in the same individuals at different times lack independence. A plot ID variable, since the same plot was assessed through time, was included as a random intercept in each GLMM. Block ID was furthermore included as a second random intercept. Altitude (lowland, midland or highland), *C. arabica* variety and time of evaluation and their first order interactions were included as fixed factors. Final models were obtained after stepwise model reduction, based on Akaike's information criteria (AIC). All models were fitted using the *glmmTMB* packag (Magnusson et al. 2017) under R 3.3.3 statistical environment (R Development Core Team, 2017). Pairwise comparisons between factor levels were based on Tukey test at 5% significance. Note that due to the nested block design of the experiment, collinearity problems across the fixed factors did not occur.

Results

The mixed models results showed that coffee varieties did not affect CRL epidemics, while time, location and their interaction effects did affect (*P*<0.0001) all CLR variables (Table 3). On average, CLR tended to decrease during the dry season (January) following harvest (Table 3, Fig. 3), reaching zero values in April and July (data not shown). Disease intensity was highest at the lowland locations and lowest for highland locations during November (Fig. 3). However, the significant location x time interaction for all disease measures showed significant reduction in disease only for lowland and midland locations, especially CLR prevalence (lowland z=12.38, p<0.001; midland z=7.80, p<0.001) and incidence (lowland z=11.76, p<0.001; midland z=4.56, p<0.001), and only for lowland locations for CLR severity (z=9.21, p<0.001) (Table 4, Fig.3a, b,c).

The differences in disease intensity among the three locations in November were reduced in January, with only significant differences between highland and midland (z=-3.70, p<0.001); between highland and lowland (z=-2.37, p=0.046) for CLR prevalence (Table 4 and Fig. 3c); between highland and midland (z=-2.58, p=0.027) for CLR incidence (Table 4, Fig. 3b); and between highland and midland (z=-2.63, p=0.024) for CLR severity (Table 4, Fig. 3b). Neither the main effect of coffee variety, nor the interaction between coffee variety and location significantly affected any of the disease measures (Fig. 4a, b, c). Note that because of this non-significance (*P* <0.05), these factors were removed from the final GLMM models presented in Table 3, following the stepwise model reduction.

Discussion

Our findings add new knowledge to CLR epidemics in SW Africa. We corroborate previous reports of the effect of altitude shaping CLR development on Arabica coffee and further demonstrated that

CBD-resistant *C. arabica* behaved similarly in relation to CLR epidemics. The decrease in CLR intensity with increasing altitude has been linked to decreasing temperatures that affect rust spore germination (Kushalappa and Eskes 1989). The optimal temperature for *H. vastatrix* spore germination is 22°C, or in the range of 20-25°C (Bigirimana et al. 2012; Ribeyre and Avelino 2012; Talhinhas et al. 2017; Toniutti et al. 2017). This matches the average temperatures at our lowland site (21.5°C). Bote and Jan (2017) reported average temperatures at our highland and midland locations of 17.1°C and 18.8.°C, respectively. These lower temperatures at both locations likely resulted in a longer latent period and slower disease development. Also larger seasonal and daily temperature fluctuations and lower night temperatures, commonly observed at higher latitudes, have been associated with reduced CLR incidence (Kushalappa and Eskes 1989). The observed gradual decrease in CLR with increasing altitude has also been observed in the Americas (Avelino et al. 2015). Although out study did not allow to disentangle the effects of altitude and inoculum pressure, and although we have no measures of ambient spore amounts, we hypothesize that the higher altitude is a surrogate variable for lower inoculum pressure.

As to the seasonal variation of CLR, we found higher intensity during coffee harvest time (November), decreasing during the following dry season (January). The disease was not observed during the subsequent rainy seasons (April and July evaluation times). The peak of CLR epidemics in November may be related to spore release due to the physical disturbance introduced by the operations of coffee harvesting, which occurs from late September to October. Similar findings were reported in coffee farms in Costa Rica and Mexico, where secondary infection cycles occur right after harvesting time and dramatically expands from November to January (Boudrot et al. 2016; Vandermeer et al. 2017). During our study, weather was generally dry in January and probably there was no free water to support rust disease development. This is in agreement with previous research conducted in Ethiopia (Jefuka et al. 2010).

We found no significant differences related to CLR intensity among the *C. arabica* varieties across the altitudinal and seasonal gradients. However, since only a limited number of potentially rather genetically similar, *C. arabica* varieties were tested, the possibility remains that other coffee varieties exhibit some level of resistance to CLR. The Ethiopian montane rainforests indeed contain the wild gene pool of Arabica coffee, although this gene pool is currently highly underexploited (Aerts et al. 2017). Future research should therefore focus on systematically screening larger numbers of cultivated and wild coffee varieties to identify potential CLR disease resistance. Apart from genetic make-up, the susceptibility of coffee varieties to CLR is influenced by crop management, physiological status of the plant, and the production situation (Ribeyre and Avelino 2012; Kushalappa and Eskes 1989; Toniutti et al. 2017). High berry yield and high fruit load, for example, have been reported to enhance *H. vastatrix* infection, as well as a high leaf-to-fruit ratio (Ribeyre and Avelino 2012; Kushalappa and Eskes 1989; Toniutti et al. 2017). Our results suggest that moving coffee plantations to higher altitudes may help to protect against CLR. Producing coffee at higher altitude will furthermore also provide producers with better coffee bean qualities and better prices (Tolessa et al. 2017; Worku et al. 2018). Yet, CLR disease was recorded at the highland site but at reduced levels. At the same time, the eight *C. arabica* varieties evaluated here showed equal susceptibility to CLR, suggesting that further efforts need to be directed towards developing CBD resistant varieties that are also resistant to CLR.

Coffee in Ethiopia is grown across a huge area, under a wide variety of production systems and cultivation practices. Since this research was limited to one production system and particular sites in SW Ethiopia, further assessment of CLR in relation to altitude and inoculum pressure is recommended. Although our study was limited to one production cycle, we do not expect that interannual variability in weather would drastically influence the results, but it will be instructive to keep collecting and analysing data in the following seasons in order to confirm our findings. Finally, if CLR epidemics are confirmed to not occur during April and June, evaluations in November and January may suffice for collecting epidemiological data.

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Author contribution

GD, AD and GB designed the study. GD performed the field work. OH and GD drafted the manuscript. All authors contributed to data interpretation. KH and GD performed the statistical analyses. All authors gave final approval for publication.

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Table 1 Description of the experimental sites

Location	Altitude	Altitude (m.a.s.l.)	Longitude	Latitude	T max (°C)	T min (°C)	T mean (°C)	Humidity (%)
Degelu	Lowland	1450	37°02’43” E	08°67'96” N	26.51	17.61	21.5	69.57
Gembe	Midland	1610	37°07’44” E	08°67'10” N	22.17	13.01	18.8	72.8
Buture	Highland	2063	37°02’50’’ E	08°56'96” N	20.86	10.37	17.1	75.64

Table 2 Coffee berry disease resistant *Coffea arabica* varieties used in this study, including typical grain yield and growth habit

Variety	Grain yield (kg ha^{-1})		Habit
	On research station	On farm	
74-1	1200	600-700	Open
75-227	1700	800-900	Open
74-54	1800	800-900	Intermediate
74-112	1800	900-1000	Compact
74-140	1900	900-1000	Compact
74-148	1800	600-700	Compact
74-158	1900	900-1000	Compact
74-165	1700	800-900	Compact

Table 3 Parameter estimates of the generalized linear mixed effect models (GLMM) on the coffee leaf rust (CLR) infection measures. Beta coefficients, marginal means (MM) and test statistics of contrast predictor=0 (z) given for each retained predictor after model reduction (N= 144)

Coefficient		**CLR Incidence**			**CLR Severity**			**CLR Prevalence**		
		beta	MM	z	beta	MM	z	beta	MM	z
Location	Highland	-3.59	0.028	7.29***	-3.84	0.022	5.73***	-2.10	0.11	16.51***
	Midland	-1.89	0.086	23.28***	-2.79	0.052	14.39***	-0.87	0.47	36.48***
	Lowland	-0.57	0.13	27.96***	-0.98	0.092	16.95***	2.85	0.66	36.12***
Time	November	-3.59	0.12	28.9***	-3.84	0.074	20.30***	-2.10	0.63	45.34***
	January	-3.51	0.041	12.96***	-3.77	0.030	8.79***	-2.15	0.17	25.94***
Location*Time	Highland:Nov	-3.59	0.027	5.88***	-3.84	0.021	4.66***	-2.10	0.11	13.09***
	Highland:Jan	-3.51	0.029	6.33***	-3.77	0.023	5.03***	-2.15	0.10	12.48***
	Midland:Nov	-1.89	0.13	23.66***	-2.79	0.058	12.23***	-0.87	0.70	32.23***
	Midland:Jan	-4.52	0.056	12.70***	-4.09	0.046	10.50***	-4.10	0.25	20.64***
	Lowland:Nov	-0.57	0.36	46.88***	-0.98	0.27	36.01***	2.85	0.95	29.37***
	Lowland:Jan	-6.17	0.041	9.17***	-6.47	0.027	4.97***	-6.43	0.18	17.72***

Significance: *:0.05 ≥ *P*> 0.01; **: 0.01 ≥ *P*> 0.001; ***: 0.001 ≥ *P*.

Table 4 Results of the pairwise contrasts (Tukey tests) for the generalized linear mixed effect models (GLMM) on the coffee leaf rust (CLR) infection measures. Test statistics (z) and p-values given for each pairwise contrast (N= 144)

		CLR Prevalence		**CLR Incidence**		**CLR Severity**	
		z	p	z	p	z	p
Location	highland-midland	-9.67	<0.0001	-6.01	<0.0001	-4.23	<0.0001
	highland-lowland	-11.19	<0.0001	-8.69	<0.0001	-7.73	0.0001
	lowland-midland	3.93	0.0003	3.41	0.0019	3.41	0.0019
Time	November-January	11.77	<0.0001	8.57	<0.0001	6.36	<0.0001
Location*Time	highland:Nov-Jan	0.18	0.86	-0.32	0.75	-0.28	0.78
	midland:Nov-Jan	7.80	<0.0001	4.56	<0.0001	1.12	0.26
	lowland:Nov-Jan	12.38	<0.0001	11.76	<0.0001	9.21	<0.0001
Time*Location	Nov: highland-midland	-10.47	<0.0001	-6.73	<0.0001	-3.94	<0.0001
	Nov: highland-lowland	-13.03	<0.0001	-11.69	<0.0001	-10.65	0.0002
	Nov: Lowland-Midland	6.21	<0.0001	8.16	<0.0001	8.56	<0.0001
	Jan: highland-midland	-3.70	0.0006	-2.58	0.027	-2.63	0.024
	Jan: highland-lowland	-2.37	0.046	-1.34	0.37	-0.58	0.83
	Jan: lowland-midland	-1.38	0.35	-1.38	0.35	-1.87	0.15

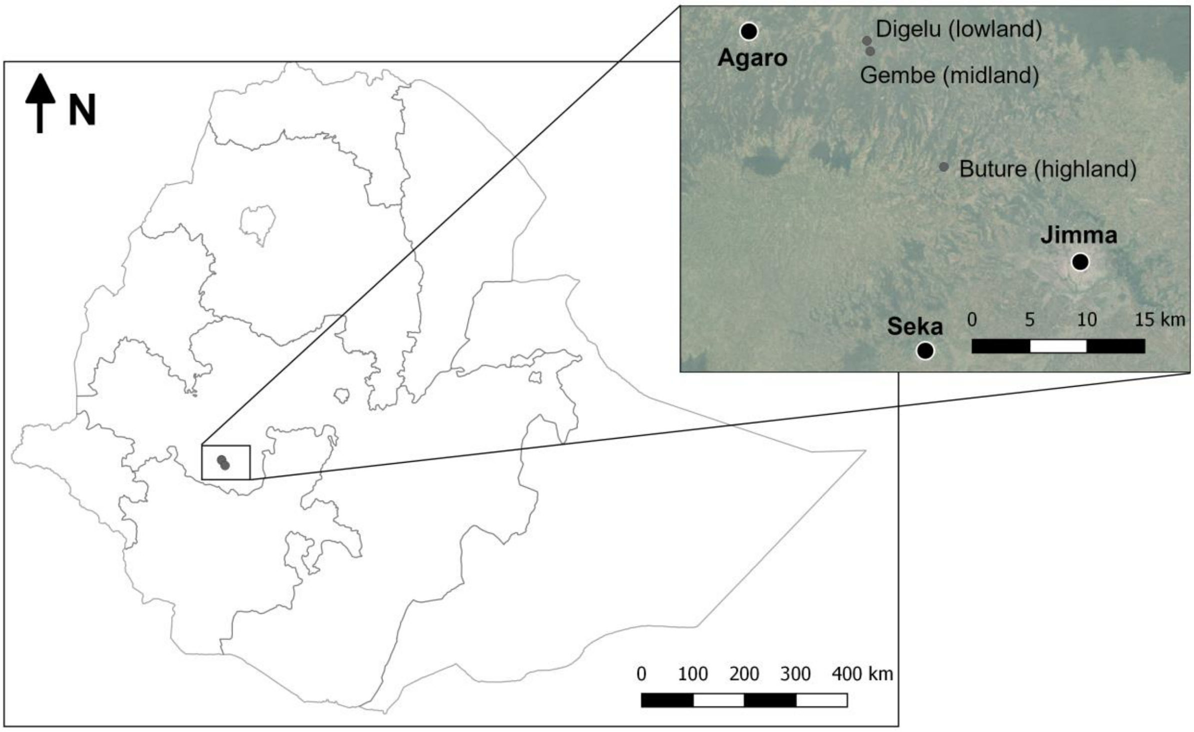

Fig.1 Map of the study sites in the Mana district, Southwestern Ethiopia

Block-I	**74-112**	**74-165**	**75-227**	**74-1**	**74-140**	**74-158**	**74-148**	**74-54**
Block-II	**74-148**	**74-1**	**74-158**	**74-112**	**74-54**	**75-227**	**74-140**	**74-165**
Block-III	**74-158**	**74-140**	**74-54**	**74-148**	**74-165**	**74-112**	**75-227**	**74-1**

Fig. 2 Experimental set-up. Eight C. arabica varieties are replicated three times at each location (lowland, midland and highland). Twenty-four plots at each location, each plot having a 10x10m size. Numbers indicate the *C.arabica* variety ID

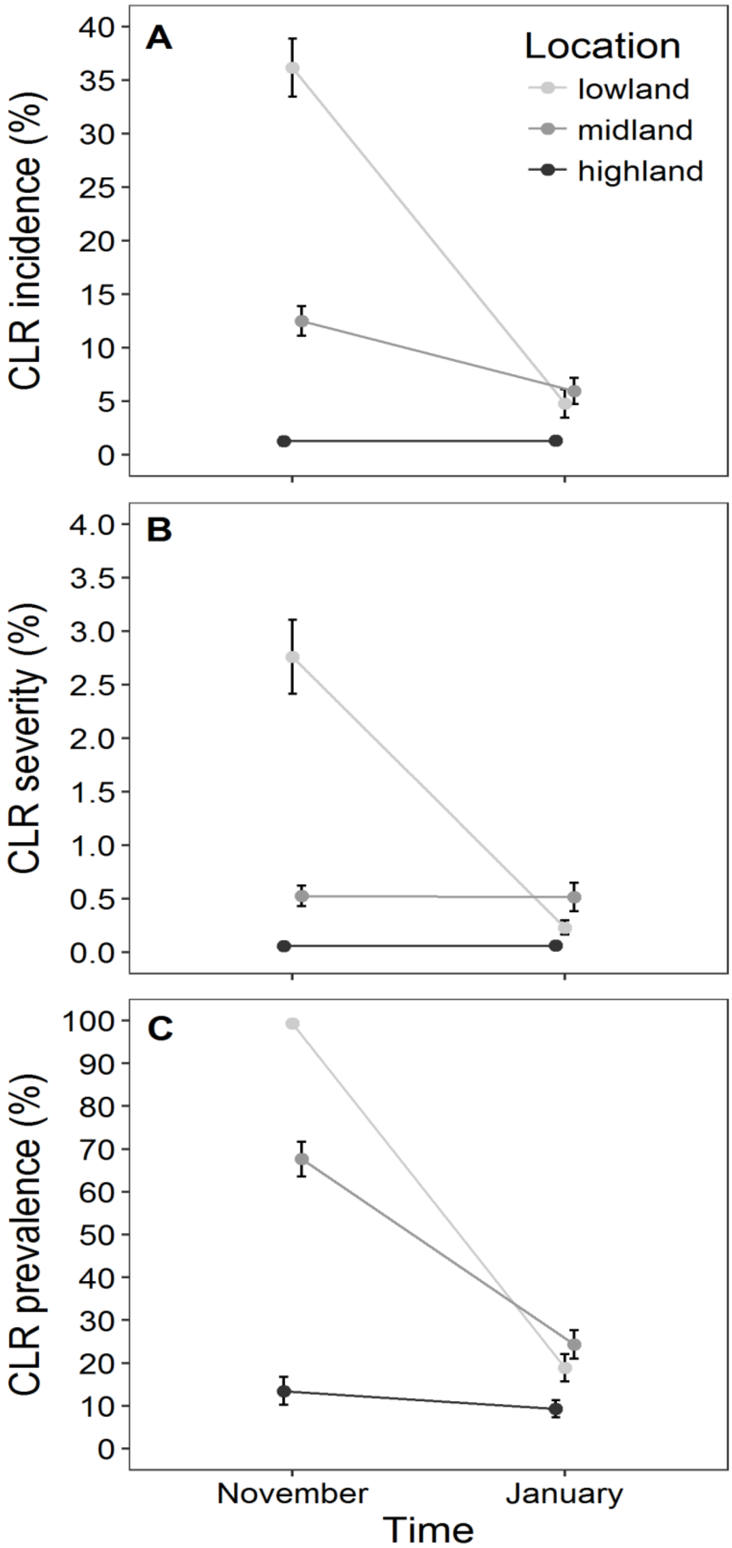

Fig.3 Incidence (a), Severity (b) and Prevalence (c) of CLR as influenced by the interaction effect of altitude and time (all interactions were significant, $p<0.0001$). Error bars represent standard error of the mean (N=144)

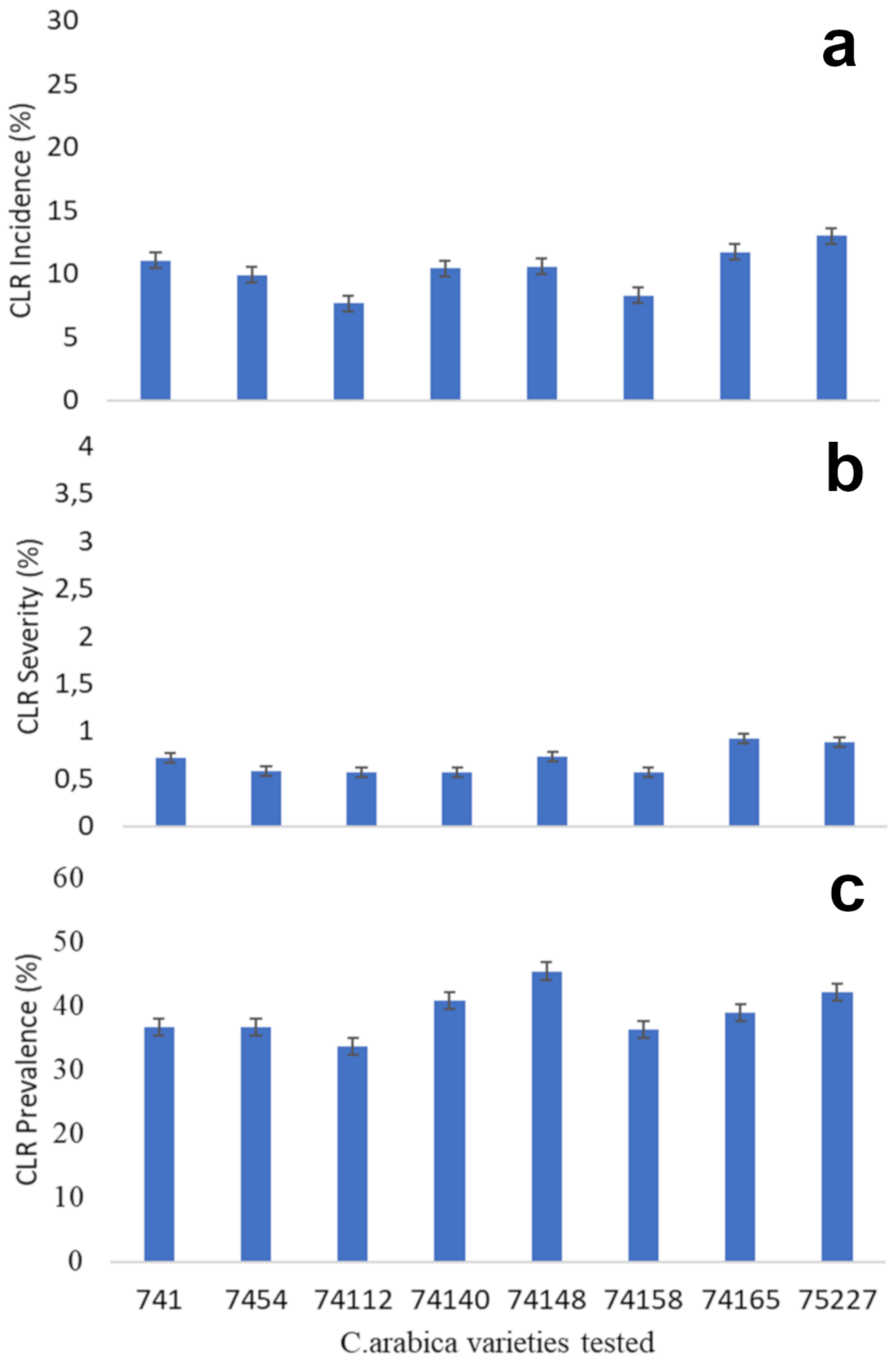

Fig.4 Incidence (a), Severity (b) and Prevalence (c) of CLR among a number of C. arabica varieties with resistance against coffee berry disease. There were no significant differences among varieties. Error bars represent standard error of the mean (N=144)