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FULL RESEARCH PAPER

Influence of host resistance and phenology on South American leaf blight of the rubber tree with special consideration of temporal dynamics

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Abstract South American leaf blight (SALB), the most dangerous disease of the rubber tree, is responsible for the lack of significant natural rubber production in South America and is a major threat to rubber tree plantations in Asia and Africa. Although the selection of resistant clones is the preferred disease control method, greater knowledge is required of the relationship between host and pathogen, in order to construct more durable resistance. Based on small-scale trials, this study set out to compare the dynamics of SALB on two highly susceptible and one moderately susceptible clone and to analyse the effect of host phenology on disease severity, at leaflet and flush scales. Clonal resistance was found to have a noticeable effect on disease severity, asexual sporulation and stromatal density at both leaflet and flush levels, and on disease dynamics at a leaflet level; time for symptom and sporulation appearance were longer on the moderately susceptible clone than on the susceptible clones. On the moderately susceptible clone, the stromatal density was largely dependent on disease severity. The phenology did not differ among the three clones and could not be considered as a factor in genetic resistance to SALB. However, for the three clones, the position of the leaflet in the flush affected the duration of the immature stages and the disease: the shorter the duration of leaflet development, the lower the disease severity, the sporulation intensity and the stromatal density.

Keywords *Hevea brasiliensis* · *Microcyclus ulei* · Epidemiology · Sporulation

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Introduction

South American leaf blight (SALB) caused by the ascomycete fungus *Microcyclus ulei* is the most dangerous disease of the rubber tree. The disease prevents economically significant natural rubber production in South America (Langford 1945). It would be a major threat to natural rubber if it was ever introduced into Asia and Africa, because of the very high susceptibility of all the clones planted in those areas (Rao 1973; Chee 1985). Current research on SALB mostly focuses on selecting resistant rubber tree clones and identifying resistance mechanisms (Chee 1976b; Darmono and Chee 1985; Junqueira et

al. 1988; de Araujo et al. 2001; Le Guen et al. 2002; Garcia et al. 2004). Several criteria correlate with disease severity and dynamics, such as incubation time, latency, lesion size and number, sporulation, time taken for stromata to appear, and time from inoculation to leaf fall. These criteria have been used to assess partial clonal resistance (Junqueira et al. 1990; Rivano 1992) under controlled conditions but most of them have never been studied in the field, where trees are challenged by a diversified population of fungal strains. Only more easily assessable criteria, such as symptoms, sporulation and stromatal density, are currently used under controlled conditions and in the field (Lespinasse 1999; Le Guen et al. 2002; Mattos et al. 2003). Consequently, the evaluation of clonal resistance does not take into account disease dynamics.

Although SALB has been known since the beginning of the twentieth century and extensively studied by Stahel (1917), some aspects of the life cycle of the fungus remain almost totally unknown, especially sexual reproduction. *Microcyclus ulei* is considered to infect only the genus *Hevea*. Immature leaves are the only susceptible stage. The incubation time is approximately 5 to 7 days. Asexual sporulation occurs on the still immature leaves, principally on their underside, about 7 days after infection, while sexual structures (stromata) appear on the margin of the necrosis once the leaves reach maturity (about 3 weeks after budburst). Ascospores are produced 1 to 5 months after infection (Langford 1945; Holliday 1970; Chee 1976a; Medeiros 1976), but without experimental evidence.

Rubber tree growth is rhythmic in about the first 8 years, because of the succession of dormancy and growth phases, corresponding to periods of meristem inactivity and activity respectively. Consequently, the tree grows by a succession of flushes over the whole year, with production depending on genetical and environmental factors. In general, the development of a flush, from budburst to maturity, lasts about 1 month. During the first 6 months after planting, or after complete pruning, the young rubber tree can quarterly produce one or two flushes, which are synchronized between trees. Once the tree begins to branch laterally, this synchronicity disappears and each tree can produce new flushes uninterruptedly as long as rainfall is sufficient. During the dry season, flush production tends to decrease and stop until the wet season returns. Older trees become mature, with a cyclic phenology characterized by an annual period of defoliation and refoliation (also called wintering). The old leaves fall and are replaced by new leaves at a precise time of the year for all the clones, but the pattern of defoliation and refoliation (precocity, homogeneity, and speed) varies substantially depending on the clone. For certain clones, the defoliation–refoliation process is brief, homogeneous and lasts 1 month. For other clones, it is long for a single tree and the process can start with a delay of several weeks.

Because of the unique phenological behaviour of the rubber tree, its consequences for leaf diseases has to be considered at five scales: the leaf, the flush, the tree, the monoclonal plot and the polyclonal plantation. The clonal differences in the phenology of trees within a plantation may lead to disease escape or severe damage. The presence of neighbouring plots of different clones with their own pattern of defoliation–refoliation may be a factor of conservation and dissemination of the inoculum between neighbouring plots with consequences for disease epidemics (Furtado 1990; Rivano 1992). The influence of phenology on host-pathogen dynamics has never been studied at flush and leaflet scales. The aim of our study was to analyse how the clonal susceptibility of rubber trees affected SALB at both leaflet and flush scales, under field conditions (i.e. with a diversified natural inoculum), considering not only the symptoms but also the dynamics of the disease as a consequence of clonal resistance to *M. ulei*. With a time scale of a week for leaflets, and a month for flushes, it was possible to repeat observations throughout the year, thereby encompassing several different weather and inoculum pressure conditions thought to be representative of the local epidemiological context.

Materials and methods

Plot set-up

The trial was conducted at Pointe Combi (French Guiana, 5°20′N–52°55′W), in the Amazonian region from which the genera *Hevea* and *M. ulei* originate and where they occur in the wild. For the 1980–2004 period the annual mean rainfall was 2,911 mm, with a wet season from December to July and a dry season

from August to November. The mean rainfall over those 25 years was 119, 44, 60 and 135 mm for August, September, October and November respectively (CIRAD, unpublished). Three rubber tree clones, IRCA GY 5 (highly susceptible), PB 260 (highly susceptible), and FX 3864 (moderately susceptible) were planted in January 1999 in small monoclonal plots (7.5×4.5 m), each containing 60 trees (4 rows×15 trees). There were 10 m between two neighbouring plots. To keep the trees small enough for observations and to have young flushes throughout the year, about five trees were cut each month. Phenology and disease were assessed twice a week from November 2000 to December 2002. One to five flushes were observed simultaneously at random, from budburst to maturity, until there was no further advance in disease symptoms (about 9 to 11 days after all the leaflets had reached maturity). Since the observation on a tree depended on the presence of young flushes, not all the trees of the plots were observed and the number of observations was not the same for each tree. Thus, the numbers of trees observed during the experiment were 27 for IRCA GY 5, 34 for PB 260 and 25 for FX 3864. Disease and phenology were assessed on all the leaflets of each flush and the analysis was performed at a leaflet scale because the disease might have evolved differently on the three leaflets of a single leaf. The number of leaflets observed during the trial was 3,126 on 75 flushes for IRCA GY 5, 3,855 on 86 flushes for PB 260 and 4,329 on 89 flushes for FX 3864. The levels of the disease variables and their dynamics as well as the influence of the duration of phenological stages on the disease were compared between the clones at both leaflet and flush scales. At the flush scale, the analysis of the dynamics of the disease aimed especially to answer the two following questions: (1) did the same maximal level of disease on clones with different susceptibilities result from a same disease progress? (2) did the severity of the infections influence the level of the asexual sporulation and the density of the sexual organs? The effect of the position of the leaflets along the flush was also analysed.

Phenology at a leaflet scale

Growth stages were recorded as proposed by Hallé and Martin (1968): B1 (leaflets folded dorsally, pointing upward, reddish), B2a (leaflets partially or totally unfolded, pointing downward, shiny reddish), B2b (leaflets unfolded, pointing downward, dull and light reddish, abaxial surfaces of the limbs lightly pressed against surfaces of other leaflets), C (leaflets unfolded – dull and light green, hanging downward) and D (maturity, leaflets unfolded – horizontal, shiny green with hard limbs). Because of the very short duration of stage B2b, stages B2b and C were merged here into a single stage called B2bC. Reference time T_{0L} was defined as the beginning of stage B2a. For each flush, the leaflets were numbered from bottom to tip.

Phenology at a flush scale

The time (in days) between the observation of the first and last leaflet at stage B1 was noted as $B1_{FL}$; similar notations $B2a_{FL}$ and $B2bC_{FL}$ were used for stages B2a and B2bC respectively. The immaturity period (IMM_{FL}) of a flush was the time from the observation of the first leaflet at stage B1 to the last leaflet at stage C. The reference time T_{0FL} was defined as the first observation when all the leaflets were at stage D. To compare how leaflet position along the flush affected the disease, the leaflets were ranked in ten classes according to their position in the flush: $B1_{POS}$, $B2a_{POS}$, $B2bC_{POS}$ and IMM_{POS} were defined as the durations (in days) of stages B1, B2a, B2bC and period of immaturity respectively, for a given position, and as the means of the respective stage or immaturity duration for all the leaflets in that position class from all the observed flushes together.

Disease at a leaflet scale

The disease index (DI), characterizing the necrotized proportion of a leaflet, was recorded on Chee's scale (1976b), modified as follows: 0=nil; 1=low (<1% leaf area necrotized); 2=medium (1–10% leaf area necrotized); 3=high (11–30% leaf area necrotized); 4=very high (large necrosis, >30% leaf area necrotized); 5=leaflet fall due to SALB (Fig. 1). The sporulation intensity was recorded on both sides of the leaflets as follows: 0=nil, 1=low, 2=abundant; the sporulation index (SpI) of a leaflet was the sum of the sporulation intensities on each side. The stromatal index (StI) was recorded as follows: 0=nil, 1= very low density, 2=low density; 3=medium density; 4= high density of stromata. For each position class in

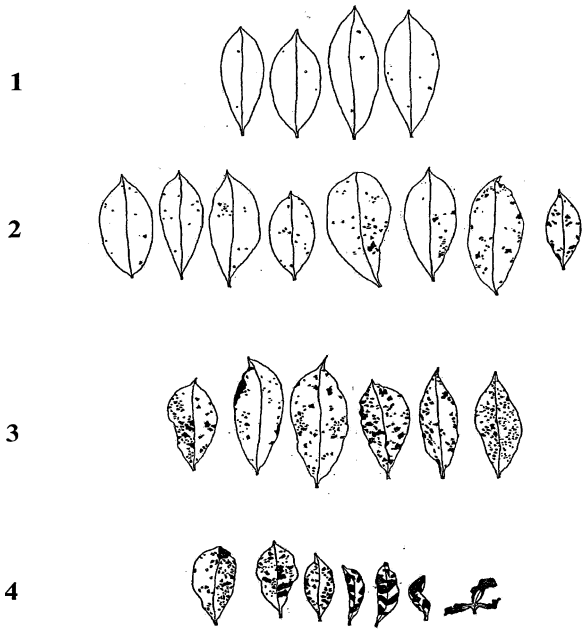

Fig. 1 Scale for disease index assessment on the leaflets (modified from Chee 1976b)

the flush, the disease index DI_{POS}, the sporulation index SpI_{POS} and the stromatal index StI_{POS} were the means of DI, SpI, StI for all the leaflets in that position class from all the observed flushes.

Disease at a flush scale

The indices for the flushes DI_{FL}, SpI_{FL} and StI_{FL} were the means of DI, SpI and StI respectively for all the leaflets in the flush at the same time. Fallen leaflets were not taken into consideration for SpI_{FL} and StI_{FL}. DI_{FLmax} and StI_{FLmax} were the maximum DI_{FL} and StI_{FL} recorded (in general when all the leaflets were mature and symptoms did not progress further). SpI_{FLmax} of a flush was the sum of the maximum upper sporulation intensities for the flush (mean of the sporulation intensities on the upper side of all the leaflets in the flush) and the maximum lower sporulation intensities for the flush (mean of the sporulation intensities on the underside of all the leaflets in the flush). All the phenological and disease variables are listed in Table 1. Considering that the same maximum disease level at a flush scale (DI_{FLmax}) might reflect very different disease dynamics, the analysis was performed with both data for all the flushes and data obtained after classifying the flushes into five groups according to DI_{FLmax}: G5 ($4<DI_{FLmax}\leq5$), G4 ($3<DI_{FLmax}\leq4$), G3 ($2<DI_{FLmax}\leq3$), G2 ($1<DI_{FLmax}\leq2$), G1 ($0<DI_{FLmax}\leq1$). Given the very small amount of data in group G5 for FX 3864, groups G5 and G4 were considered as a single group G4-5 ($3<DI_{FLmax}\leq5$). For IRCA GY 5 there was no flush in group G1 and, for PB 260, that group contained only one flush which was excluded from the analysis.

Several non-linear models (including logistic, monomolecular and Gompertz models) were tested to compare the temporal evolution of the disease index and stromatal index. In most cases, the logistic model gave the best fit, according to:

$$DI_{FL}(t)/StI_{FL}(t) = \frac{P1}{1+\exp(P2-P3t)} \quad (1)$$

where t=number of days before or after T_{0FL}; $DI_{FL}(t)$= disease index on a flush scale at time t; $StI_{FL}(t)$= stromatal index on a flush scale at time t, P1=asymptote parameter, P2=earliness parameter, P3=the rate parameter, P1*P3/4=maximum infection rate and P2/P3=time to inflexion point (Berry and Cilas 1994). The mean infection rate was calculated from first symptoms to the asymptote of the calculated logistic curve. The infection rates were expressed in units of disease index or stromatal index per day and the time to inflexion point was expressed in days. The number of data included in the calculation of the logistic parameters is listed in Table 2. The confidence limits of P1, P2 and P3 were used for the comparisons of the logistic curves, but no statistical comparison was possible for the maximum and mean infection rates and for the time to inflexion point. Statistical analyses were performed using the SAS 9.1. (2002, 2003) and CurveExpert 1.3. (1995, 2001) software.

Results

Influence of clonal susceptibility on disease dynamics

At a leaflet scale, the comparison of the indices for a 5% risk indicated that the disease index did not differ significantly between the susceptible clones IRCA GY 5 and PB 260, but it was higher than for the moderately susceptible clone FX 3864. The sporulation and stromatal indices were significantly higher for PB 260 and lower for FX 3864 (Table 3). The first symptoms appeared about five days earlier for IRCA

Table 1 Summary of the variables used in the text

	Variables	Definition
Phenology		
Leaflet scale	B1	B1 duration=time from beginning to end of stage B1
	B2a	B2a duration=time from beginning to end of stage B2a
	B2bC	B2bC duration=time from beginning of stage B2b to end of stage C
	IMM	Immaturity duration=time from beginning of stage B1 to end of stage C
	$B1_{POS}$	B1 duration according to leaflet position
	$B2a_{POS}$	B2a duration according to leaflet position
	$B2bC_{POS}$	B2bC duration according to leaflet position
	IMM_{POS}	Immaturity duration according to leaflet position
Flush scale	$B1_{FL}$	B1 duration=time from first leaflet at stage B1 to last leaflet at stage B1
	$B2a_{FL}$	B2a duration=time from first leaflet at stage B2a to last leaflet at stage B2a
	$B2bC_{FL}$	B2bC duration=time from first leaflet at stage B2b to last leaflet at stage C
	IMM_{FL}	Immaturity duration=time from first leaflet at stage B1 to last leaflet at stage C
Disease		
Leaflet scale	DI	Disease Index
	SpI	Sporulation index
	StI	Stromatal index
	DI_{POS}	Mean Disease indices of all leaflets present in the same part of a flush
	SpI_{POS}	Mean Sporulation indices of all leaflets present in the same part of a flush
	StI_{POS}	Mean Stromatal indices of all leaflets present in the same part of a flush
Flush scale	DI_{FL}	Disease index=mean disease index of all leaflets at time T
	SpI_{FL}	Sporulation index=mean sporulation index of all leaflets present at time T
	StI_{FL}	Stromatal index=mean stromatal index of all leaflets present at time T
	Di_{FLmax}	Maximun disease index
	SpI_{FLmax}	Maximum sporulation index
	StI_{FLmax}	Maximum stromatal index
Reference time		
Leaflet scale	T_{0L}	Beginning of stage B2a
Flush scale	$T0_{FL}$	First observation of all leaflets at maturity

GY 5 and PB 260 than for FX 3864 (Table 3). For the moderately susceptible clone, the mean time from beginning of stage B2a and first symptoms was 4.6 days shorter on leaflets with sporulating lesions (8.5 days) than on leaflets with only non-sporulating lesions (13.1 days). However, the time from beginning of stage B2a to maximum disease index was approximately the same for the three clones. Differences between time from beginning of stage B2a and maximum disease index and time from beginning of stage B2a to first symptoms indicate that symptoms progressed about 5 days quicker for the moderately

Table 2 Number of data (flushes and observations) used for the calculation of the logistic parameters at the flush scale

Groups	IRCA GY 5		PB 260		FX 3864	
	Flushes	Observations	Flushes	Observations	Flushes	Observations
G1	0	0	1	13	28	342
G2	6	59	6	59	47	511
G3	7	66	15	153	11	103
G4	21	228	20	219		
G5	41	391	44	432		
G4-5					3	28
All data	75	744	86	876	89	984

Table 3 Comparison of the disease level and dynamics at the leaflet scale for the three clones (times in days)

	IRCA GY 5 (S)		PB 260 (S)		FX 3864 (MS)	
	Mean (Min–Max)	SD	Mean (Min–Max)	SD	Mean (Min–Max)	SD
Disease Index	3.86 (0–5)	1.33	3.79 (0–5)	1.38	1.44 (0–5)	1.00
Sporulation Index	1.63 (0–4)	1.25	1.88 (0–4)	1.18	0.33 (0–4)	0.67
Stromatal Index	2.62 (0–4)	1.14	3.08 (0–4)	1.07	1.57 (0–4)	1.08
Time from T_{0L} to first symptoms	6.71 (−1–25)	2.84	7.23 (0–25)	2.84	11.85 (3–32)[a]	4.84
Time from T_{0L} to maximum DI	14.87 (2–47)	6.05	15.81 (0–39)	5.43	15.27 (3–37)	5.59
Time from T_{0L} to sporulation	7.93 (0–18)	2.51	7.58 (0–15)	2.21	8.86 (3–14)	2.49
Time from T_{0L} to maximum SpI	8.24 (0–18)	2.55	7.97 (0–16)	2.35	9.20 (3–14)	2.44
Time from T_{0L} to first stromata	15.49 (7–26)	2.59	15.72 (8–25)	2.43	17.44 (9–35)	3.18
Time from T_{0L} to maximum StI	18.36 (7–40)	3.92	18.92 (10–37)	3.98	20.04 (9–39)	4.20

S susceptible, *MS* moderately susceptible

[a] For leaflets without sporulation: 13.11 (3–32), SD=4.88. For leaflets with sporulation: 8.51 (3–21), SD=2.62

susceptible clone FX 3864 (3.4 days) than for IRCA GY 5 (8.2 days) and PB 260 (8.6 days). For the two susceptible clones, IRCA GY 5 and PB 260, the pattern of symptom appearance (Fig. 2) was similar with a maximum at 7 days (50% of the leaflets) while for the moderately susceptible clone FX 3864, the pattern of symptom appearance was more staggered with only 22.8% of the leaflets at the maximum (7 days). The sporulation dynamics (Table 3) did not differ greatly between the three clones: sporulation began about 1 day later for the moderately susceptible clone FX 3864, and reached its maximum, depending on the clones, 0.3–0.4 days after it began. The stromata appeared about 1.8 days later for FX 3864 and the maximum stromatal index was reached a little more than 1 day later for FX 3864.

At the flush scale, the logistic parameters for the dynamics of the disease index were compared. For all the data, parameter P1 differed significantly, as expected, between the moderately susceptible clone FX 3864 and the two susceptible clones IRCA GY 5 and PB 260. For the same level of disease index, P3 differed only for groups of low disease indices only, (greater for FX 3864 than for IRCA GY 5). When considering all the data, P2 did not differ between clones; for lightly (G2) and very highly (G5, G4-5) infected flushes, it differed between IRCA GY 5 and FX 3864, and for highly infected flushes (G4, G4-5), it differed between IRCA GY 5 and PB 260. Table 4 indicates that, for each clone considered separately, as the maximum disease index decreased, the maximum and mean rates of disease increase tended to decrease. For IRCA GY 5 also, the time from T_{0FL} to the inflexion point tended to be shorter. There was the same tendency when clonal susceptibility decreased, but only for severe infections ($3<DI_{FLmax}<5$). The maximum rate, mean rate and time from T_{0FL} to inflexion point did not seem to vary in relation to clonal susceptibility for lower maximum disease index (<3). Thus, for the dynamics of the disease index at flush scale, PB 260 appeared to rank between IRCA GY 5 and FX 3864 and was more often closer to FX 3864 than to IRCA GY 5. As at a leaflet scale, the maximum sporulation index on a flush scale was easily lowest for FX 3864 and highest for PB 260 (Table 5). Sporulation began earlier for PB 260 and later for FX 3864. The sporulation duration was the same for IRCA GY 5 and PB 260 but it was 6 days shorter for FX 3864. As a distinctive feature, PB 260 showed a decrease in precocity and duration of the sporulation as the disease index became lower (Table 5).

The calculation of the logistic parameters for the stromatal index at the flush scale showed that the increase in P1 was similar to that in P1 for the disease index on the clone FX 3864 only: the more severe the damage, the higher the stromatal density. Indeed, the necrotized area was rarely large on this clone. The consequence of an increase in disease index was a larger number of necrotized points rather than larger areas of necrosis. As stromata emerged on the margin of the lesions, more severe damage was conducive to a high stromatal density. For the other two clones, the rise in disease severity led mainly to a larger area of necrosis; more leaflet fall and thus severe infections were not conducive to an increase in the stromatal

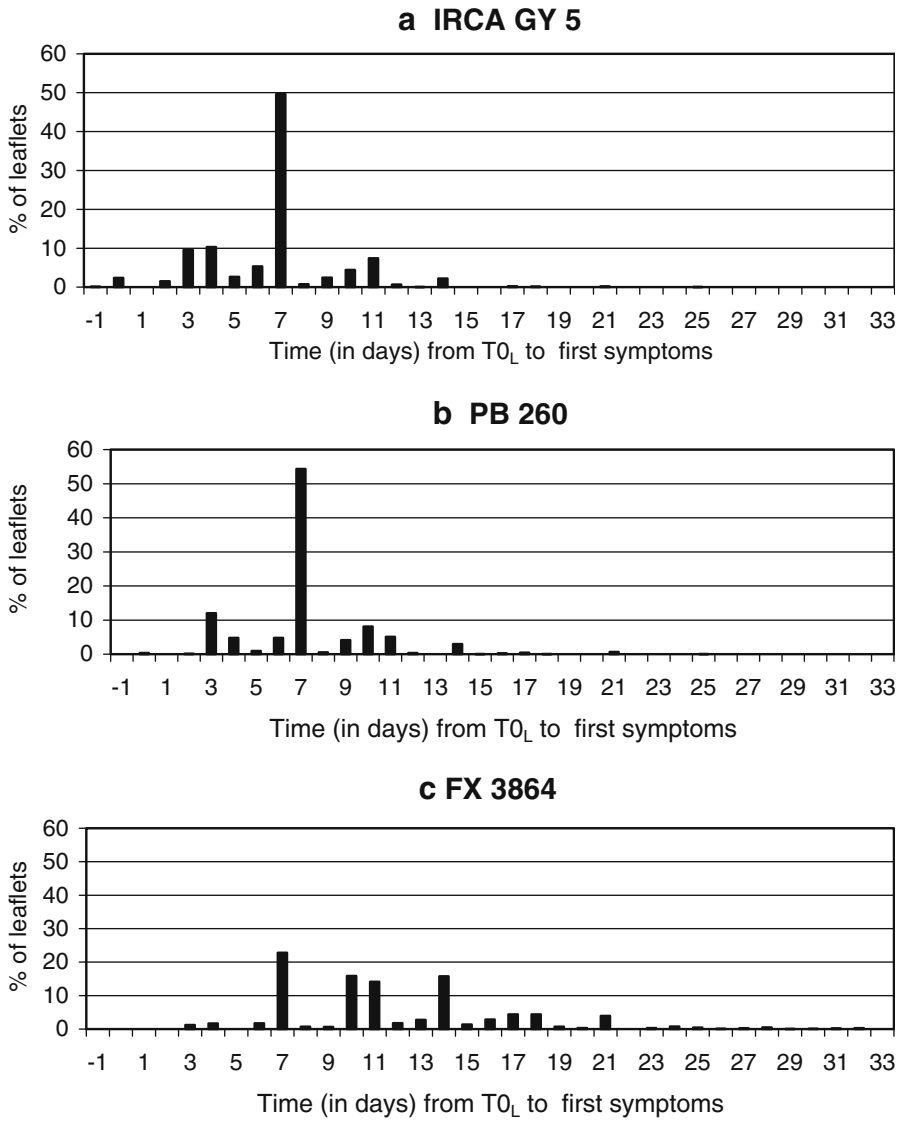

Fig. 2 Percent of leaflets according to time between T_{0L} and appearance of first symptoms for each clone; **a** IRCA GY 5, **b** PB 260, **c** FX 3864

index. P2 and P3 did not appear to be related to disease severity, P3 being identical between all the groups for each clone.

Influence of host phenology on disease dynamics

There were few differences between clones for leaflet immaturity duration (mean=17.6 days for IRCA GY 5, 17.8 days for both PB 260 and FX 3864; Fig. 3). For each phenological stage, the three clones reached their maximum % of leaflets for the same duration (3–5 days for B1, 6–8 days for B2a, 6–8 days for B2bC, 13–18 days for immaturity). For clones IRCA GY 5, PB 260 and FX 3864 respectively, the mean durations, in days, were 4.2, 4.2, and 4.0 for B1, 5.0, 5.4, and 5.6 for B2a, 6.4, 6.6, and 6.5 for B2bC.

An analysis of variance indicated that the position of the leaflet along the flush had a highly significant effect at 99% on the disease index DI_{POS}, the sporulation index SpI_{POS}, the stromatal index StI_{POS} and the duration of all phenological stages, whereas the clone had a significant effect only on DI_{POS}, SpI_{POS}, StI_{POS} and duration of $B2a_{POS}$. DI_{POS}, SpI_{POS}, StI_{POS} and the duration of all phenological stages, except $B2a_{POS}$, which decreased from the bottom of the flush to its tip (Table 6). Table 7 confirms that, as a consequence, the disease variables were, in most cases, strongly positively correlated

Table 4 Statistical comparison, between the three clones for the same level of damage, of the logistic parameters for the disease index (DI_{FL})

Group	Clone	P1	P2	P3	R2	Max rate	Mean rate	Days to IP
All data	IRCA GY 5 (S)	3.5287±0.1380 a	−2.975±0.5747 a	0.3541±0.0581 a	0.85	0.31	0.12	−8.40
All data	PB 260 (S)	3.4928±0.1386 a	−2.094±0.4322 a	0.3107±0.0484 a	0.84	0.27	0.11	−6.74
All data	FX 3864 (MS)	1.3054±0.0667 b	−2.11±0.6201 a	0.3741±0.0855 a	0.75	0.12	0.04	−5.64
G5	IRCA GY (S)	4.4357±0.1529	−3.094±0.4990 a	0.3635±0.0494 a	0.94	0.40	0.15	−8.51
G5	PB 206 (S)	4.4631±0.1702	−2.216±0.4213 a b	0.3119±0.0453 a	0.97	0.34	0.15	−7.10
G4-5	FX 3864 (MS)	3.9641±0.6754	−1.025±1.0184	0.2696±0.1391 a	0.93	0.27	0.13	−3.80
G4	IRCA GY 5 (S)	3.4861±0.1683	−1.978±0.4573 a	0.2681±0.0454 a	0.94	0.23	0.11	−7.38
G4-5	FX 3864 (MS)	3.9641±0.6754	−1.025±1.0184 a b	0.2696±0.1391 a	0.93	0.27	0.13	−3.80
G4	PB 260 (S)	3.5489±0.1582	−1.018±0.2644 b	0.2382±0.0314	0.96	0.21	0.11	−4.27
G3	IRCA GY 5 (S)	2.446±0.2922	−1.187 0.7684 a	0.2751 0.0996 a	0.94	0.17	0.08	−4.31
G3	PB 260 (S)	2.3496±0.1300	−2.326±0.7192 a	0.3759±0.0930 a	0.94	0.22	0.08	−6.19
G3	FX 3864 (MS)	2.4786±0.2087	−1.994±0.8738 a	0.3493±0.1141 a	0.93	0.22	0.08	−5.71
G2	IRCA GY 5 (S)	1.9215±0.2284	−0.603±0.5434 a	0.2309±0.0727 a	0.93	0.11	0.06	−2.61
G1	FX 3864 (MS)	0.7683±0.0611	−0.665±0.4005 a b	0.2369±0.0545 a	0.85	0.05	0.02	−2.81
G2	PB 260 (S)	1.6151±0.1238	−1.5140±0.6706 a b	0.2948±0.0852 a b	0.96	0.12	0.08	−5.14
G2	FX 3864 (MS)	1.4009±0.0476	−1.931±0.3668 b	0.3551±0.0503 b	0.93	0.12	0.05	−5.44

The groups were defined according to maximal disease index of the flushes (DI_{FLmax}). Maximum rate (Max Rate), Mean rate and Number of days between maturity of all the leaves of the flush a(T_{0FL}) to the inflexion point of the logistic curve were calculated from the logistic parameters.

Days to IP, no. days from T0FL (maturity of all the leaves of the flush to inflexion point); *Mean Rate*, daily increase rate calculated from first symptoms to the asymptote on the predicted curve (in units day^{-1}); *S*, Susceptible; *MS*, Moderately susceptible

with the duration of the phenological stages for all the three clones. The only exceptions were for B2a duration of clone IRCA GY 5 with the three disease variables and the B2a duration of clone PB 260 for stromatal density. These correlations were higher for the moderately resistant clone.

Pearson's linear correlations were also calculated between maximum disease parameters and the duration of phenological stages at flush (Table 8). FX 3864 appeared to be the clone for which the disease variables were most correlated with the duration of the phenological stages but in all cases they were negative, i.e. the disease was less when the duration of susceptible stages was longer. There was no straightforward explanation for this observation. For the two susceptible clones, the correlations were poor.

Using the five classes of maximum disease index as previously defined, the mean proportions of the

Table 5 Non-statistical comparison of the sporulation dynamics of SALB at the leaflet stage

Clone	Number of days from To_{FL} to beginning of sporulation	Sporulation duration	Max. sporulation (SpI_{FLmax})
IRCA GY 5 (S)	−15	18	0.82
PB 260 (S)	−18	18	0.92
FX 3864 (MS)	−12	12	0.18
PB 260-G5	−18	18	0.96
PB 260-G4	−15	15	1.02
PB 260-G3	−12	12	0.89
PB 260-G2	−12	9	1.14

The groups for PB 260 were defined according to the maximum disease indices of the flushes (DI_{FLmax})

S, susceptible; *MS*, moderately susceptible

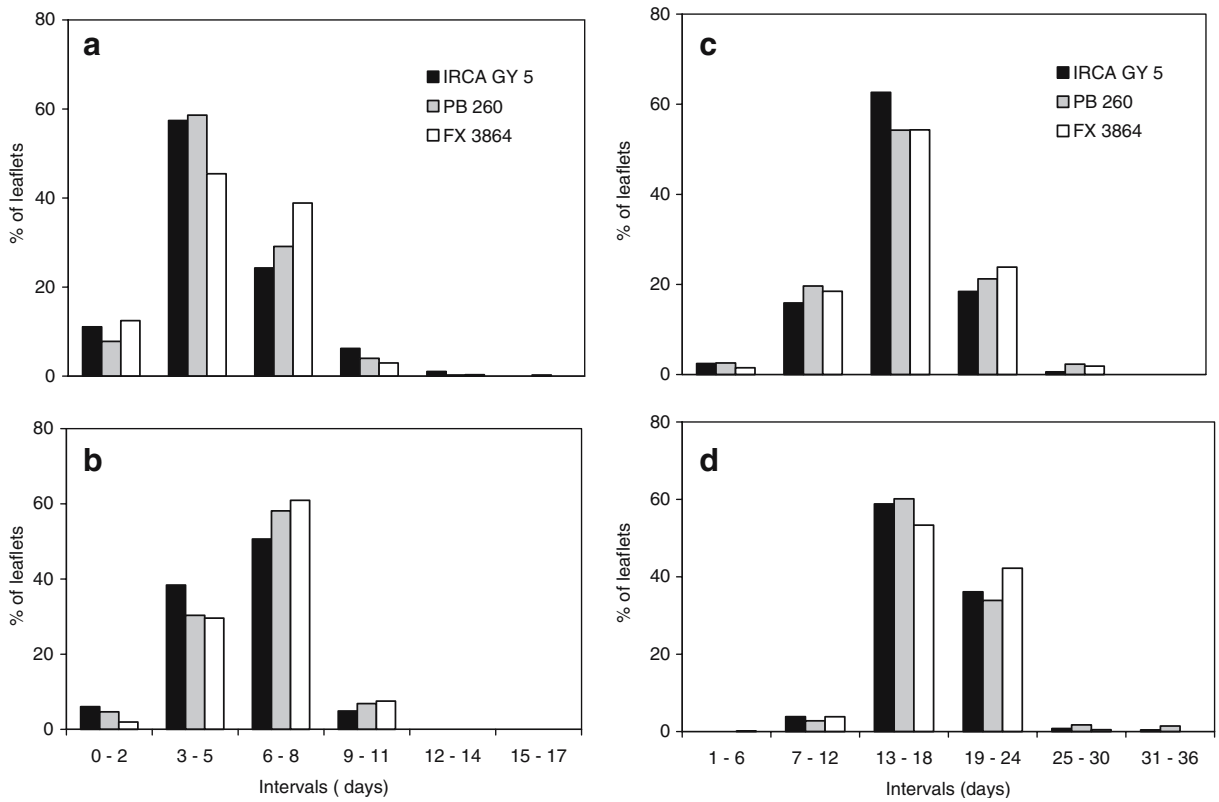

Fig. 3 Phenological stage durations: percent of leaflets in each duration category. **a** Duration of stage B1, **b** duration of stage B2a, **c** duration of stage B2bC, **d** duration of immaturity

phenological stages were plotted against time (Fig. 4). There was evidence that mean growth patterns at a flush scale were similar for the three clones. Phenology did not differ noticeably between the classes for the two susceptible clones IRCA GY 5 and PB 260. For FX 3864, the immaturity duration, IMM_{FL}, was only a little shorter for the most diseased flushes (Group G4-5), with quicker transitions between stages. At a flush scale, disease severity was not related to the duration of the immature stages.

Discussion

Our results show that the partial resistance of clone FX 3864 to *M. ulei,* when compared to the two susceptible clones IRCA GY 5 and PB 260, was reflected in fewer lesions on leaves, a lower sporulation intensity and less dense sexual reproductive structures. Symptoms occurred later and were more staggered in time, suggesting differential resistance to several strains making up the natural population of the pathogen. Rivano (1992) also detected strong interactions between clone and strain factors. The lesions reached a smaller size than for the two susceptible clones, although they developed more rapidly. Sporulation occurred slightly later and was substantially shorter at a flush scale, especially during severe infections. Stromata also appeared slightly later for FX 3864. Our results confirmed those reported by Rivano (1992), who showed, at a leaf scale, with nine clones and two strains, that clonal resistance was expressed as much by the length of the latent period and the time taken for stromata to appear as it was by the level of the disease (attack and sporulation scores, number and diameter of lesions, abscission). The less susceptible clones in terms of disease level were also the less susceptible when considering the rate at which the disease was expressed. Also at a leaflet scale and under controlled conditions, Junqueira et al. (1990) reported differences in disease dynamics depending on the clone.

The dynamics of symptom development at a flush scale followed a logistic model. The rate parameter differed little between clones for the same damage

Table 6 Disease index (DI_{POS}), sporulation index (SpI_{POS}), stromatal index (StI_{POS}) and duration (in days) of phenological stages according to clone and leaflet position along the flushes

Clone	Position class	DI_{POS}	SpI_{POS}	StI_{POS}	$B1_{POS}$	$B2a_{POS}$	$B2bc_{POS}$	IMM_{POS}
IRCA GY 5 (susceptible)	1st tenth	4.33	1.98	2.87	6.19	4.75	7.73	21.22
	2nd tenth	4.08	1.86	2.58	5.96	5.63	7.02	20.59
	3rd tenth	3.95	1.83	2.81	5.40	5.39	6.67	19.50
	4th tenth	4.01	1.90	2.82	4.84	5.30	6.42	18.78
	5th tenth	3.85	1.79	2.69	4.15	4.96	6.50	17.93
	6th tenth	3.71	1.73	2.63	3.89	4.74	6.47	17.53
	7th tenth	3.80	1.53	2.41	3.60	4.61	6.11	17.04
	8th tenth	3.67	1.45	2.49	3.33	4.69	6.15	16.18
	9th tenth	3.71	1.39	2.38	2.98	5.06	5.98	15.80
	10th tenth	3.74	1.13	2.64	3.24	4.69	5.76	14.96
PB 260 (susceptible)	1st tenth	4.05	2.30	3.42	5.30	5.73	7.64	20.93
	2nd tenth	4.15	2.33	3.20	5.67	5.84	7.12	20.94
	3rd tenth	3.87	2.21	3.31	5.34	5.54	7.45	20.49
	4th tenth	3.78	2.15	3.14	5.04	5.48	6.82	19.27
	5th tenth	3.89	2.14	3.17	4.38	5.40	6.92	18.24
	6th tenth	3.59	1.94	3.27	4.11	5.29	6.77	17.62
	7th tenth	3.66	1.84	3.07	3.81	5.10	6.69	17.35
	8th tenth	3.56	1.68	2.98	3.42	5.32	6.13	16.48
	9th tenth	3.70	1.50	2.80	2.93	5.56	5.91	15.70
	10th tenth	3.72	1.04	2.63	2.73	4.89	5.54	14.45
FX 3864 (moderatly susceptible)	1st tenth	1.55	0.43	1.80	5.01	6.20	7.65	20.57
	2nd tenth	1.73	0.51	1.95	5.47	6.03	7.53	20.56
	3rd tenth	1.60	0.38	1.78	5.40	5.96	7.35	20.42
	4th tenth	1.57	0.38	1.83	4.64	5.84	7.22	19.38
	5th tenth	1.45	0.37	1.64	3.91	5.94	6.90	18.36
	6th tenth	1.48	0.30	1.62	3.68	5.54	6.77	17.50
	7th tenth	1.31	0.30	1.35	3.34	5.60	6.26	16.57
	8th tenth	1.33	0.27	1.45	3.38	5.41	6.05	16.35
	9th tenth	1.25	0.25	1.35	3.31	5.25	5.72	15.70
	10th tenth	1.21	0.20	1.09	3.10	4.63	5.42	14.61

DI_{POS}, disease index depending on leaf position; StI_{POS}, stromatal index depending on leaf position; $B1_{POS}$, duration of stage B1 depending on leaf position; $B2a_{POS}$, duration of stage B2a depending on leaf position; $B2bC_{POS}$, duration of stage B2bC depending on leaf position; IMM_{POS}, duration of immaturity depending on leaf position

level but the logistic parameter representative of earliness of infection was more variable between clones. Infections occurred later for the moderately susceptible clone FX 3864, occurring a shorter time before the complete maturity of the leaf flush. The same level of disease severity could result from different precocities of infections according to the clone. Lastly, within the same clone, the more severe the symptoms were, the greater were the maximum and average rates of increase and the infection precocity. The asexual sporulation began later and its intensity was lower for FX 3864. Variation in stromatal density also followed a logistic regression. The level reached for stromatal density for the moderately susceptible clone FX 3864 increased in line with disease severity. On the other hand, for the more susceptible clones, very severe infections disrupted stromatal production due to the wide expansion of lesions and leaflet fall.

The delay observed for the appearance of stromata is only a part of the possible influence of host resistance on the sexual reproduction of the fungus. Clonal resistance was shown to be primarily due to histological reactions that occurred shortly after the fungus penetrated the host cells (Blasquez and Owen 1963; Hashim et al. 1978; Garcia et al. 1995).

Table 7 Table of Pearson's linear correlations at a leaflet scale between disease and stage duration for each clone, calculated from data distributed in position classes

Clone	Durations considered	Disease parameters					
		DI_{POS}		SpI_{POS}		StI_{POS}	
IRCA GY 5 (susceptible)	$B1_{POS}$	+0.93	[a]	+0.85	[a]	+0.71	[b]
	$B2a_{POS}$	+0.39		+0.49		+0.27	
	$B2bC_{POS}$	+0.90	[a]	+0.84	[a]	+0.62	
	IMM_{POS}	+0.90	[a]	+0.92	[a]	+0.64	[b]
PB 260 (susceptible)	$B1_{POS}$	+0.75	[b]	+0.94	[a]	+0.86	[a]
	$B2a_{POS}$	+0.72	[b]	+0.76	[b]	+0.61	
	$B2bC_{POS}$	+0.64	[b]	+0.94	[a]	+0.96	[a]
	IMM_{POS}	+0.72	[b]	+0.95	[a]	+0.88	[a]
FX 3864 (moderately susceptible)	$B1_{POS}$	+0.94	[a]	+0.90	[a]	+0.90	[a]
	$B2a_{POS}$	+0.83	[a]	+0.89	[a]	+0.91	[a]
	$B2bC_{POS}$	+0.95	[a]	+0.93	[a]	+0.96	[a]
	IMM_{POS}	+0.95	[a]	+0.94	[a]	+0.96	[a]

[a] =significant at 99%

[b] =significant at 95%

DI_{POS}, disease index depending on leaf position; SpI_{POS}, sporulation index depending on leaf position; StI_{POS}, stromatal index depending on leaf position

Sambugaro et al. (2004) extended the investigations to different clonal behaviour up to the appearance of sexual structures. Unfortunately, their work did not go beyond the first perithecial primordia. So far, it is not known whether the different susceptibilities between clones also involve the time taken for perithecia to mature, and their viability, along with the quantity of ascospores formed, and their viability. For a pathogen such as *M. ulei*, whose survival in unfavourable seasons and dissemination are generally attributed to sexual structures (Holliday 1970; Chee 1976c), it will be essential to determine the conditions required to achieve that phase of the disease cycle, taking into account clonal and environmental effects.

The average duration of the immature leaf stages did not differ substantially between clones, be it for leaflets or leaf flushes. However, a comparison between clones for same disease levels revealed that the time taken for the twig to reach maturity was little shorter for the moderately susceptible clone than for the other two clones, with more rapid transition between phenological stages. The difference was clearer on the most diseased twigs. This indicated that different phenologies might lead to identical attack levels, at least within the variability range found during our trial. The phenologies of IRCA GY 5 and PB 260 were similar. The variability in the phenology of the flush was not sufficiently marked to explain the differences in

Table 8 Significant correlation coefficients between disease variables and duration of the phenological stages at the flush scale

Clone	Disease variable	Phenological stage	Correlation coefficient	Probability
IRCA GY 5 (susceptible)	Sporulation index	B1 duration	−0.37	0.0080
	Sporulation index	Immaturity	+0.29	0.0435
PB 260 (susceptible)	Stromatal index	B2bC duration	+0.38	0.0009
	Stromatal index	Immaturity	+0.30	0.0424
	Disease Index	Immaturity	−0.31	0.0117
FX 3864 (moderately susceptible)	Sporulation index	Immaturity	−0.27	0.0295
	Sporulation index	B2a duration	−0.41	0.0001
	Sporulation index	B2bC duration	−0.32	0.0023
	Stromatal index	Immaturity	−0.32	0.0081

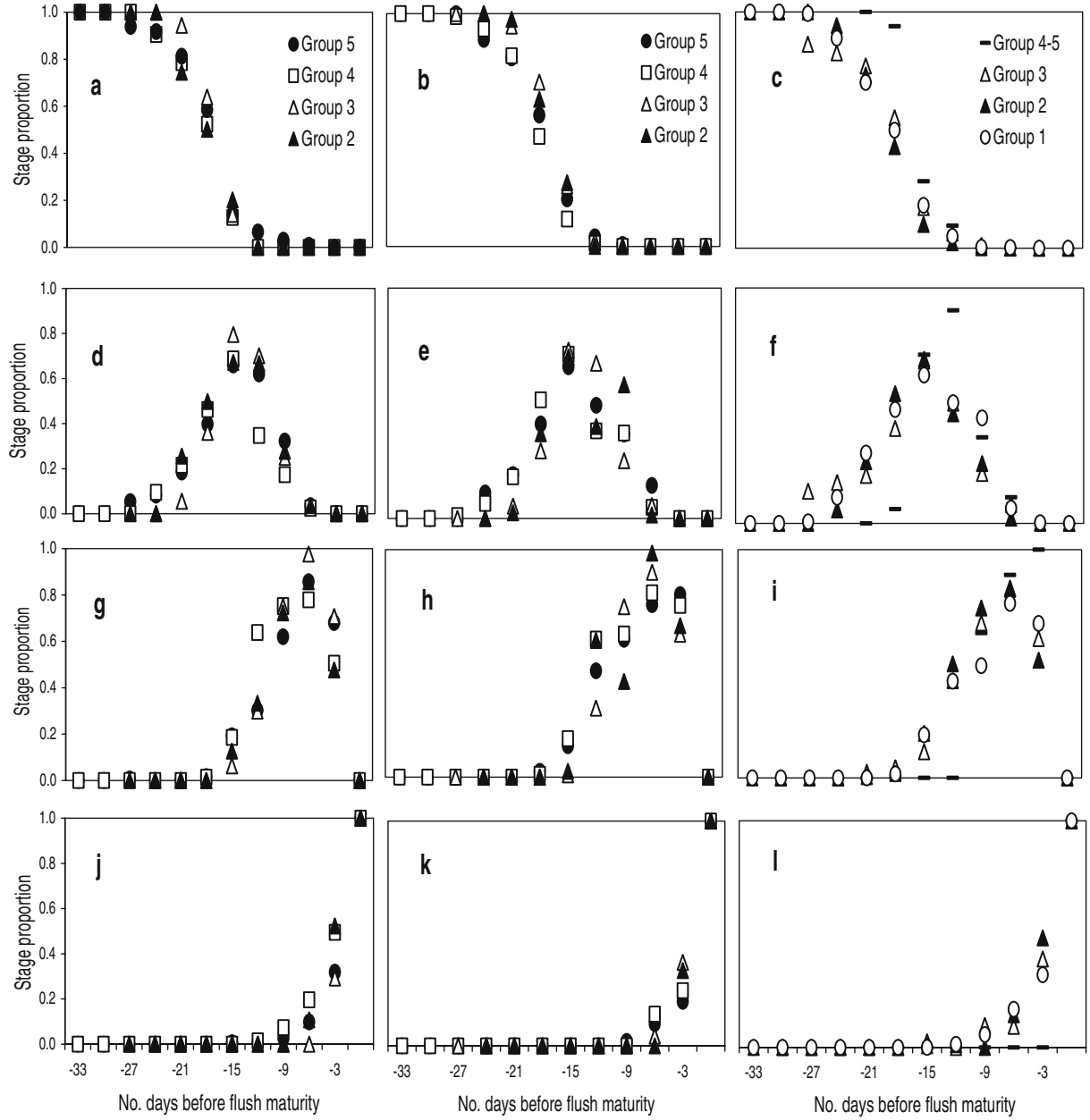

Fig. 4 Proportion of phenological stages for each clone depending on the time (in days) to flush maturity T_{0FL}. The groups are defined according to the maximum disease indices DI_{FLmax} of the flushes. (*x*-axis=days before T_{0FL}, *y*-axis= proportion of each phenological stage). **a** IRCA GY 5 – stage B1, **b** PB 260 – stage B1, **c** FX 3864 – stage B1, **d** IRCA GY 5 – stage B2a, **e** PB 260 – stage B2a, **f** FX 3864 – stage B2a, **g** IRCA GY 5 – stage B2bC, **h** PB 260 – stage B2bC, **i** FX 3864 – stage B2bC, **j** IRCA GY 5 – stage D, **k** PB 260 – stage D, **l** FX 3864 – stage D

disease severity within the same clone. Consequently, at a leaflet and flush scale, the phenological factor was not involved in clonal resistance to the pathogen.

However, at a leaflet scale, and identically for all three clones, there existed a strong relationship between the position of the leaf on the leaf flush and the time taken for it to develop. The different phenological stages and the whole of the immature phase were all shorter the more the leaf was located near the twig apex. At the same time, the proportion of necrotized leaf area, sporulation intensity and stromatal density decreased towards the tip of the

leaf flush. The duration of the leaf stages appeared to affect the disease more for the least susceptible clone FX 3864. Significant linear correlations existed for all three clones between the disease (attack, sporulation, stromata) and most of the leaf stage duration.

The clonal effect on resistance to SALB could have been due to a factor briefly touched upon by Junqueira et al. (1990), who reported a different duration in leaf receptivity depending on the clone, ranging from 10 to 16 days. However, their results did not specify whether these differences occurred at the beginning or end of leaf development, or whether there was any quantitative aspect. This aspect of clonal resistance has not been reported in the literature and, more generally, the susceptibility of the different stages of leaf growth has never been studied, particularly for the young stages between bud burst and stage B2a.

The influence of phenology on the disease must be considered at different stages. Indeed, it is well known that the defoliation–refoliation behaviour plays a major role in some leaf diseases, such as secondary leaf fall due to *Colletotrichum gloeosporioides* (Rao and Azaldin 1973; Sénéchal 1986; Guyot et al. 2001). The observations made by Medeiros (1976), Furtado (1990) and Rivano (1992) indicated that the same applied to SALB. For instance, the heterogeneity of damages in various Brazilian plantations (*pers. obs.*), between neighbouring trees and between very close topographical zones could only be explained by different tree performance.

However, the influence of this behaviour is closely related to environmental conditions, especially rainfall, and therefore depends on the location of the rubber estate and on the climatic conditions prevailing during this process. Consequently, the same behaviour can be favourable to the disease 1 year, or may provide escape conditions another year. However, early and quick defoliation–refoliation generally results in low disease. At the leaf and flush scales, our work clearly showed that shorter duration of the immature stages reduced disease severity, but the duration of the immature stages did not appear to be a genetic characteristic of the three clones studied. The literature does not mention clonal differences in phenological behaviour, either at the leaf scale or at the flush scale. Although no detailed study has been carried out on this point, which is never considered as a criterion for clonal selection, this lack of data probably means that all the clones have similar behaviour at these scales, and, consequently, shorter duration of leaf stages cannot be used for selection against SALB. However, the phenological behaviour at a larger scale (tree, plot, plantation), in relation to climatic factors, has to be taken into account in disease management and in the choice of planting material in order to more effectively define zones that are conducive to SALB, based on strong epidemiological features, not only at a very large scale as proposed by Holliday (1970) and Ortolani et al. (1983), but also at the scale of a geographical zone, or even a plantation. The impact of the dual action of phenology and climate on inoculum production and infection also requires further investigation.

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