Biological Constraints 11

It is noteworthy that unlike other clonal species, *Hevea* is not affected by viral diseases (Simmonds 1989). Apart from South American leaf Blight (SALB), other diseases of economic importance are the Gloeosporium leaf disease (*Colletotrichum gloeosporioides*), powdery mildew (*Oidium heveae*), Corynespora leaf disease (*Corynespora cassiicola*) and the Phytophthora leaf fall (*Phytophthora* spp.). Clonal specificity is evident towards resistance to these diseases (Wycherly 1969). A study with Gloeosporium showed that clones from Malaysia and Indonesia are fairly resistant, while clones from Sri Lanka and China are less resistant. But clones from South America are seen to be highly resistant indicating local adaptation rather than breeding is the cause for the resistance (Simmonds 1989). Ho (1986) gives a good narration of the breeding implications of diseases in *Hevea*. It is imperative that too much susceptible genotypes are rejected at the first instance and the survivors are seen to be moderately resistant.

11.1 South American Leaf Blight

South American Leaf Blight (SALB—caused by *Microcyclus ulei*) that is singularly devastating is a stress factor limiting the yield of *Hevea.* It has played and still plays a major role in the history and in the geographic distribution of rubber industry in the Americas. On the one hand, it prevents Latin America from developing rubber cropping in all the otherwise favourable climatic conditions, and on the other hand, it represents a permanent major threat to the crop in Asia and Africa (Dean 1987; Davies 1997). da Hora et al. (2014) used six genomic regions (LSU rRNA, mtSSU, MCM7, EF-1 α, Act and ITS) for reconstructing the molecular phylogeny of the SALB fungus based on material collected throughout Brazil. The analyses support the classification of the fungus in the family *Mycosphaerellaceae* s. str. and place it firmly within the clade *Pseudocercospora* s. str., now accepted as one of the distinct genera within *Mycosphaerellaceae*. da Hora et al. (2014) proposed new combination of *Pseudocercospora ulei* and the life cycle of the fungus is confirmed, based on both experimental and phylogenetic evidence. The epidemiology and genomics of this fungus needs to be investigated further by the mycologists to reconfirm the taxonomic status of the fungus.

Some amount of breeding work, mainly based on back-cross technique, has been undertaken in the past to incorporate resistance to these diseases in high-yielding clones. However, the efforts were in vain due to unknown polygenic nature of the attributes, high variability of the pathogen and multiple interactions between fungus strains and rubber clones (Rivano 1997a, b). Simmonds (1990, 1991) argue that the pathotype-specific resistance (vertical resistance-VR) has resulted in catastrophic failures. Horizontal resistance (HR) should be more effective and durable (Rivano et al. 1989; Simmonds 1990). Resistance sources appear to be absent in high-yielding Wickham

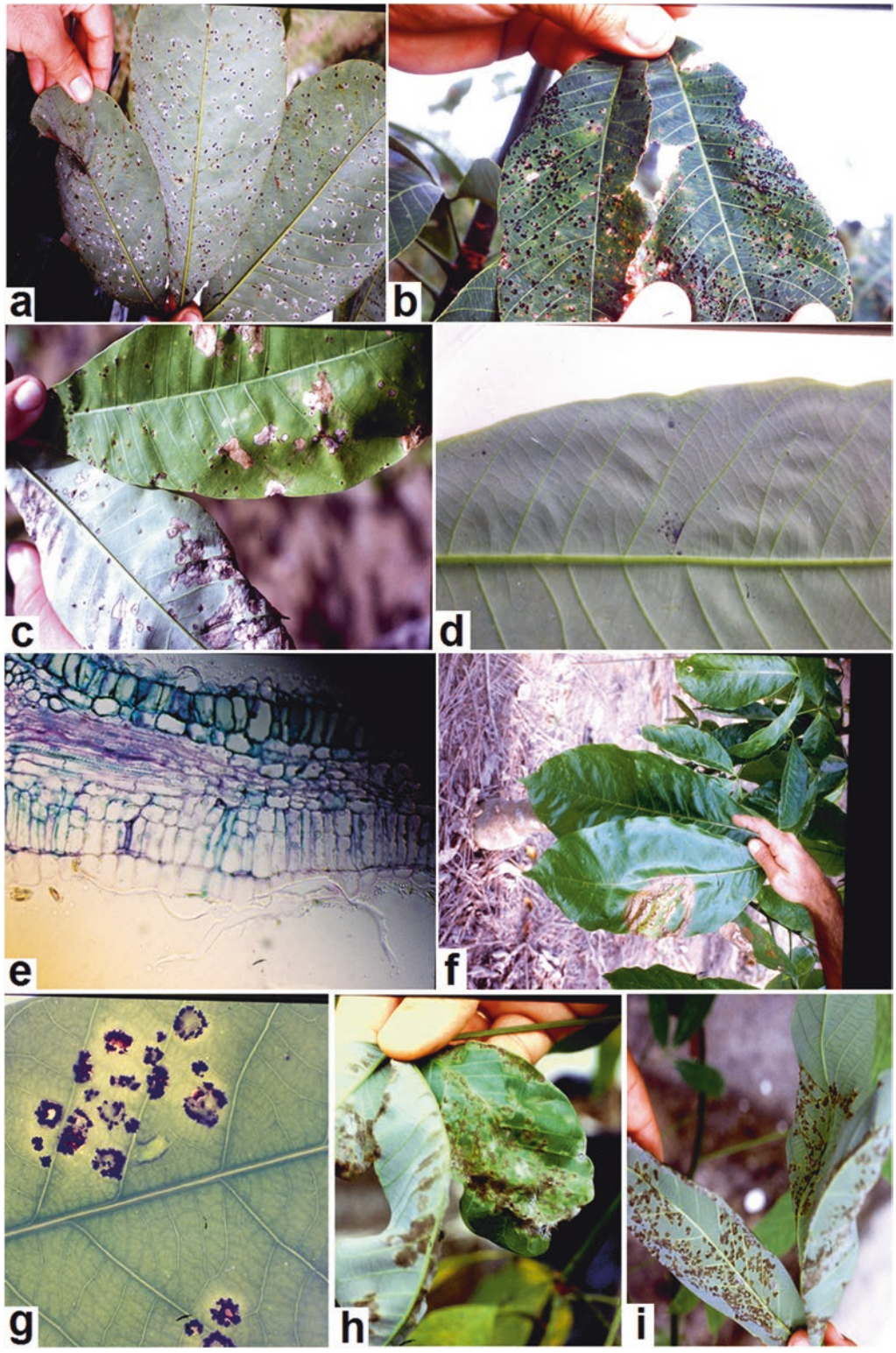
P.M. Priyadarshan, *Biology of* Hevea *Rubber*, DOI 10.1007/978-3-319-54506-6_11

population, but rather frequent within the Amazonian germplasm. However, the wild population is yet to be improved for yield. With these views, efforts have been reoriented towards the analysis of partial resistance components (Junqueira et al. 1990). Recently, the genetic determinism of the resistance source of *H. benthamiana* (F 4542), widely used in many former back-cross programmes, has been characterized by a genetic map (Lespinasse et al. 2000b). A Cirad-Michelin common research and breeding programme is currently carried out in Brazil for reducing the incidence of SALB on rubber cropping. SALB is present in Bolivia, Brazil, Peru, Ecuador, Colombia, Venezuela, Guyana, Surinam, French Guiana, Trinidad, Panama, Costa Rica, Nicaragua, EI Salvador, Honduras, Guatemala, Haiti and Mexico (Holliday 1970; Compagnon, 1986) and has caused the abandonment of ambitious programmes of extensive rubber cultivation in the South American humid tropics.

Under the CIRAD-Michelin-Brazil (CMB) breeding programme of Brazil set up in 1992 to breed SALB-resistant clones, the programme developed CMB genotypes through combining family and individual selections (Rivano et al. 2013). Thirteen genotypes were selected for evaluation of their resistance, girth and rubber production in a trial network covering eight sites in Brazil and Ecuador. Resistance was confirmed after several years and promising yields were obtained for three clones (CDC 312, FDR 5788, PMB 1) against resistant clone MDF 180. FDR 5788 gave an estimated yield of 1.8 ton/ha per year. There were significant differences between clones, sites and clone-site interactions. Nine *Hevea* genotypes resistant to SALB (TP875, FDR5788, MDX608, PMB1, CDC429, CDC312, FDR4461, MDF180, SIAL893) were isolated and evaluated from among 960 accessions in a 12-year trial located in the Michelin plantation (Bahia, Brazil) having quantitative resistance to SALB (Cardoso et al. 2014). They were characterized by partially sporulating lesions (anamorph stage). No sexual form of *M. ulei* (stromata, teleomorph stage) was found in FDR5788, MDF180, CDC312, PMB1. FDR5788, MDX608, CDC312 and PMB1 were with estimated yields of 2.6, 2.0, 1.8 and 1.2 t year^{-1} ha^{-1}, respectively.

Conidia and ascospores cause infection and both are equally important in completing the disease cycle (Langford 1945; Chee 1976a, b, 1977). Rain plays an important role in the spread of leaf blight. It is believed that rain is the most effective disseminator of large masses of spores and wind is the chief means of dispersal. Brookson (1963) observed that conidia survived for 2 weeks under normal laboratory conditions. However, the longevity of conidia decreases as RH increases. Under high humidity, conidia survive for 3 weeks, and at 100% RH, they are killed within 1 week. It seems probable that leaf blight could be spread by conidia carried on plants, plant parts or man himself. Outbreaks of leaf blight occur when the daily temperature is below 22 °C for longer than 13 h, RH over 92% for a period longer than 10 h and rainfall above 1 mm per day for the previous 7 days (Holliday 1969; Chee 1976a). The fungus can affect petioles, green stems, inflorescences and fruits. But the most obvious infection is on young leaves on the abaxial surface of 4–9-day-old, expanding tender leaves. They appear as greyish-black lesions covered with olive-green powdery sporulating masses (Lieberei 2007) (see Fig. 11.1). On the young infected leaves, lamina distortion, growth arrest, crinkling

Fig. 11.1 (**a**) Stromata of *Microcyclus ulei* on *Hevea brasiliensis* leaves, superinfected with *Hansfordia pulvinata*, a potential biocontrol organism. (**b**) Stromata of *M. ulei*, upper leaf surface. (**c**) Mixed infection of rubber tree leaves with *Thanatephorus cucumeris*, causal agent of target leaf spot and small black necrotic spots of partially resistant *Hevea brasiliensis* leaves in a resistance reaction against *M. ulei penetrationta* of *M. ulei.* (**d**) Initial infection step of attack of young rubber tree leaf by *Thanatephorus cucumeris*. Oxidizing latex drops develop at the penetration site. (**e**) cross-section through *Hevea brasiliensis* leaf (young leaf). Violet staining: hyphae of *M. ulei* parallel to vascular bundle and hyphae growing down to lower leaf surface to form conidiophores. (**f**) Leaf of *Hevea pauciflora* with typical lesion or target leaf spot (*Thanatephorus cucumeris*). (**g**) Ring-forming lesions caused by fusion of small globular stromata of *M. ulei*. (**h**) Conidiospore layers on highly susceptible young *Hevea brasiliensis* leaves. (**i**) Conidiospore layers on susceptible young *Hevea brasiliensis* leaves. The small conidiophore layers are not confluent but slightly separated. This is caused by a control factor of the leaves, which might be developed into a resistance component (Photo courtesy: Reinhard Lieberei)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and shrivelling of leaflets, blackening, drying and abscission are the common symptoms. The secondary stage develops on the adaxial surface of the leaves as it hardens. In Trinidad, the conidia have maximum dispersal in June and July and peak ascospore concentration occurred from August to November during the wet season (Chee 1976b). In a mature stand of rubber, a fresh disease cycle probably starts when ascospores are released from leaves which fall due to wintering and also from infected leaves remaining on the trees. As infection builds up on the newly emerging flushes, conidia take over the spread during the wet season to complete the disease cycle.

After penetration of the leaf surface, the hyphae colonize the underlying tissue by intercellular growth (Fig. 11.2). They often enter the tissue layers adjacent to the leaf vascular bundles and spread rapidly along the veins into the leaves. In this biotrophic phase, compatible combinations do not show cell death. However, in resistant clones, the cells in direct contact with the penetration hyphae collapse. Hashim et al. (1978) ascribed this to a hypersensitive reaction and to pre-formed resistance factors (Blasquez and Owen 1957; Figari 1965), and proof was also given for induced defence compounds such as scopoletin (Tan and Low 1975; Giesemann et al. 1986; Garcia et al. 1995). This early detection process of the fungal presence in the attacked tissue of resistant plants leading to a hypersensitive response is a typical defensive reaction (Breton et al. 1997). This reaction is regarded as an indicator for complete or vertical resistance, but this concept is applicable only to mature leaves of *H. brasiliensis* and occurs with most genotypes of the previously uninvestigated host species *Hevea pauciflora* (Junqueira et al. 1988). At the biochemical level of host reactions, a hypersensitive response is often associated with well-described defence reactions such as formation of reactive oxygen-type compounds (Garcia et al. 1999), deposition of autofluorescent compounds in the cell wall (Mevenkamp 1992), synthesis of callose, occurrence of scopoletin as phytoalexin (Giesemann et al. 1986; Garcia et al. 1995), and finally cell death in a restricted area surrounding the penetrating hyphae. Detailed and quantified descriptions have been given by Garcia et al. (1999). Rubber tree leaves are formed in a flush growth pattern. Directly after bud burst, the leaves are thin, have a high respiration rate, no net photosynthesis (Lieberei et al. 1996) and are devoid of any resistance against the virulent isolates of *M. ulei.* In the course of maturation, rubber tree leaves change from susceptible to completely resistant (Chee 1980). This maturation requires 12–20 d after bud burst and the maturation time is genotype dependent.

The causal fungus *Microcyclus ulei* (P. Henn.) von Arx and E. Muller (*Dothidella ulei* P. Henn.) is specific to *Hevea* species only. The pathogen has been recorded on four species, viz. *H. brasiliensis, H. benthamiana, H. guianensis and H. spruceana.* SALB infection results in repeated defoliation, die-back of the shoots and even death of the mature trees (Stahel 1927; Holliday 1970; Rao 1973). An examination of the morphology and an updated taxonomic description of this species has appeared elsewhere (Chee and Holliday 1986). In the South American plantations, it reduced the yield by over 90%. More than 90% of the world's natural rubber requirement is being met by production from the Far East (Holliday 1970). All the planted African and Asian rubber is extremely susceptible and the climatic conditions present in the rubber-growing areas of Asian and African countries are comparable to that of the American tropics. Hence introduction of SALB into these regions could destroy the existing plantations. This has prompted rubber-growing countries to implement quarantine regulations (Altson 1950; Edathil 1986).

Eleven physiologic races (plus an avirulent one) of the pathogen have already been detected. Many clones which were reported to be tolerant/resistant to *M. ulei* have succumbed later with the appearance of more virulent strains. Six species in which natural infection was not reported include H. *camporum, H. microphylla, H. nitida, H. paudflora, H. camargoana* and *H. rigidifolia.* The clones belonging to these species are being used in Brazil for crown bud grafting on high-yielding susceptible clones. Some resistant clones which are being used for crown budding are PA 31, IAN 717, IAN 6486, IAN 7388, IAN 7657,

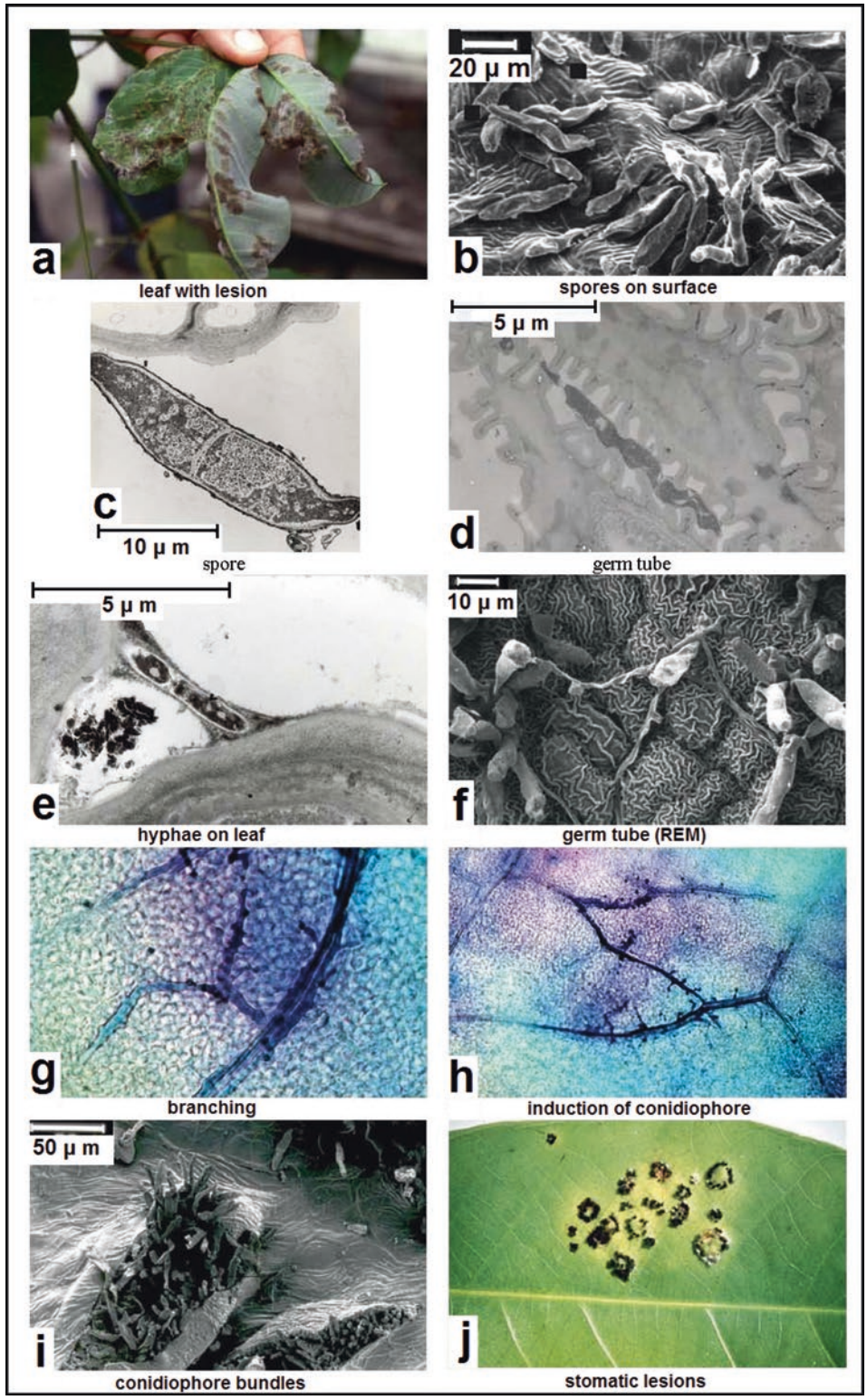

Fig. 11.2 Habit and habitat of South American Leaf Blight (SALB)

FX 25, FX 614 and FX 636 (Holliday 1970; Pinheiro et al. 1982).

Biological control of *M. ulei* using *Hansfordia pulvinata,* a hyperparasite which grows well on conidial lesions, has been attempted (Lieberei et al., 1989). It was reported by Feldman (1990) that mycorrhizal fungi can cause an increase of resistance of the rubber tree against *M. ulei.* The generation period of spores was increased and the sporulation of the pathogenic fungus was decreased. The diameter of lesions was also decreased.

Of late, molecular studies are being pursued on resistance to SALB. According to detailed studies by Seguin et al. (1996) and Lespinasse et al. (2000b), *Hevea brasiliensis* has a diploid genomic organization with rare duplicated loci. These studies led to the identification of 18 basic linkage groups in a rubber genome of 2150 cM total map length. Using the 195 progenies of the population derived from crossing of PB 260 × FX 3899 and their response to six isolated strains of *M. ulei*, eight quantitative trait loci (QTL) with respect to resistance were identified on seven independent linkage groups. Le Guen et al. (2003) and Lespinasse et al. (2000b), on the basis of their molecular data, prepared the mapping of genes conferring field resistance to SALB. For the first time, it was shown that both factors for partial resistance and for complete resistance were quantitatively expressed in the progeny and could be correlated with five loci. The molecular approach to this plant–pathogen combination has greatly enhanced the possibilities of proceeding with marker-assisted breeding and selection. Le Guen et al. (2011) attempted a QTL mapping of MDF 180 for resistance towards SALB. The resistance of progeny from a cross between PB 260 (susceptible) and MDF 180 (resistant) was assessed under controlled conditions with the inoculum of three *M. ulei* isolates and under natural conditions. Microsatellite marker mapping showed no QTL in the susceptible parent. In the resistant parent, Le Guen et al. (2011) identified a qualitative resistance gene against isolates from French Guiana and a major quantitative resistance factor determining the resistance against isolates from the state of Bahia (Brazil). The qualitative resistance gene was denominated *M15md* and was located in the linkage group g15. Two of the four minor resistance QTLs showed an epistatic interaction with *M15md*. RNA-seq high-throughput sequencing technology was used to analyze the differential expression of FX 3864 clone transcriptome at 0 and 48 h post infection (hpi) with the *M. ulei* isolate GCL012 (Páez et al. 2015). They could identify 86 differentially expressed genes associated with the defence response of FX 3864 to GCL012. Seven putative gene members of the AP2/ERF ethylene (ET)-dependent superfamily were found to be downregulated. An increase in salicylic acid (SA) was associated with the up-regulation of three genes involved in cell wall synthesis and remodelling. The defence response of FX 3864 against the GCL012 isolate was associated with the antagonistic SA, ET and jasmonic acid (JA) pathways. According to Fang et al. (2016), in the course of maturation, leaves of *Hevea* become more resistant to leaf diseases, including the South American Leaf Blight (SALB). They sequenced the *Hevea* leaf transcriptome at four developmental stages (I–IV) by Illumina sequencing to understand the underlying mechanisms of this defence and to identify the candidate genes involved (Fig. 11.3). Of the 3905 differentially expressed genes identified for leaf development, 67.8% (2651) were during the transition to leaf maturation that are meant for cyanogenic metabolism, lignin and anthocyanin biosynthesis for developing leaves (stages I–III) and mature leaves (stage IV). Such studies are really beneficial in devising strategies to engineer resistance to leaf diseases.

Several plant protection operations are being carried out for controlling this disease. Aerial spraying (8–10 rounds) is done using benomyl 300 g, thiophanate methyl 200 g or mancozeb 2 kg in 30 L water per ha at intervals of 7–10 days. For fogging 200 g thiophanate methyl or 1 kg mancozeb are being used in 6–8 L of agricultural spray oil per ha at intervals of 4–7 days (Martins and Silva 1979; Chee and Wastie 1980). Systemic fungicides like chlorothalonil (Daconil), triforine (Saprol) and triadimefon (Bayleton) are found promising in small-scale trials (Chee and Wastie 1980; Dos Santos et al.1984). In case of an accidental entry

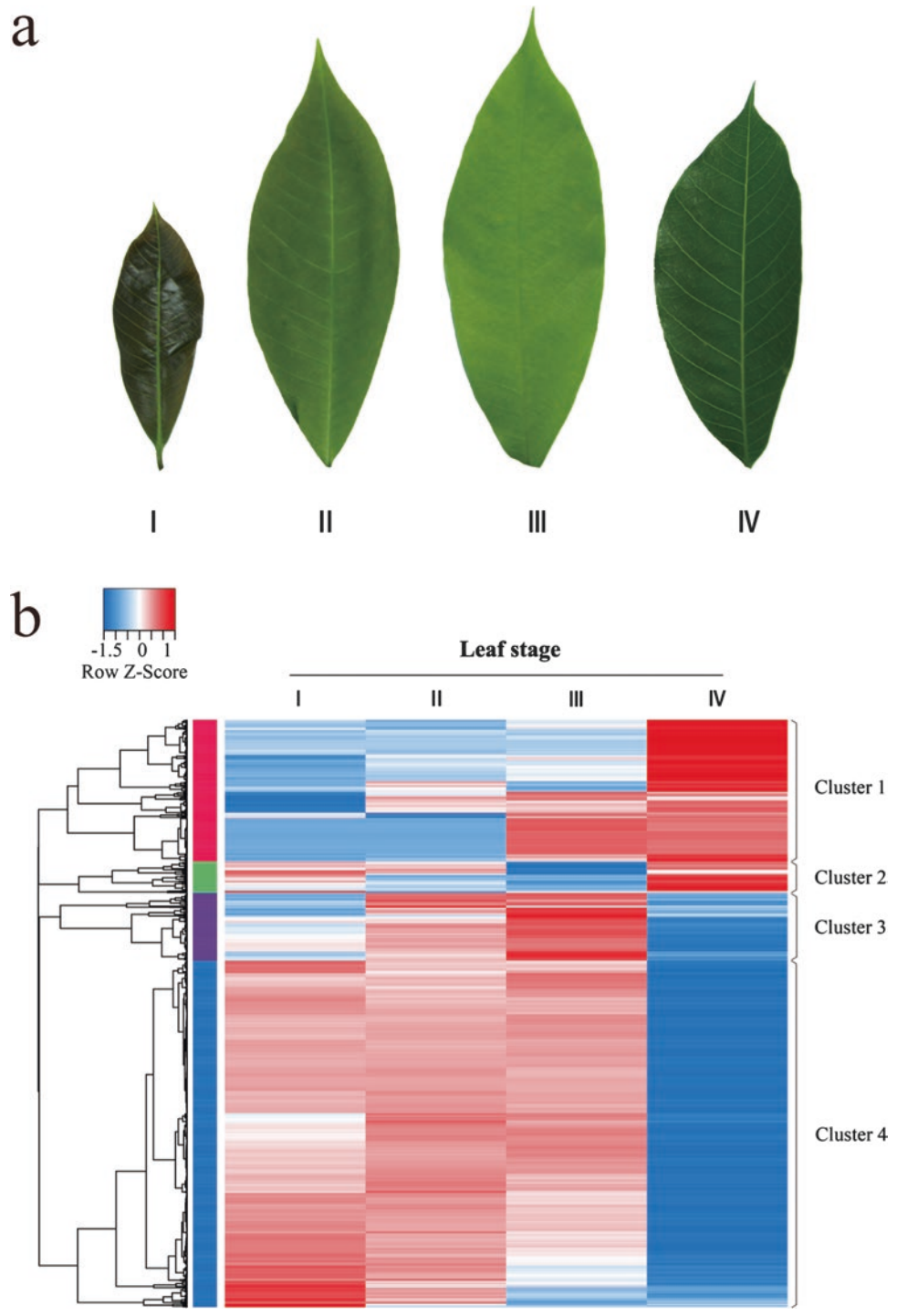

Fig. 11.3 Relationship between exponential leaf growth, transition phase from completely susceptible leaf stage to completely resistant stage. (**a**) Four representative stages of *Hevea* leaf development. I, bronze; II, colour change; III, pale green; IV, mature; (**b**) Expression profile and clustering of 3095 differentially expressed genes across four developmental leaf stages (After Fang et al. 2016) (Photo courtesy: Chaorong Tang, Rubber Research Institute, Chinese Academy of Tropical Agricultural Sciences, Danzhou 571737, Hainan, China)

of this disease, despite the phytosanitary measures, immediate adoption of eradication procedures should receive top priority. First two to three rounds of spraying (aerial application) with protectant chemicals such as mancozeb, benomyl or thiophanate methyl are given, and then the entire area is defoliated using n-butyI2,4,5-T, folex, cacodylic acid or ethephon, so that the trees remain leafless for about 2 months (Abdul Aziz 1976; Lim and Hashim 1977).

11.2 Abnormal Leaf Fall

Abnormal leaf fall is the most destructive disease in India and occurs during the southwest monsoon months of June, July and August. It infects pods, leaves and tender shoots causing heavy defoliation and die-back of tender twigs. The first report on this disease from India was in 1910 from estates near Palapilly, in Trichur district, Kerala state (McRae 1918). In due course, the disease spread to all other rubber-growing districts. Later, the disease was reported from Sri Lanka and Burma (Petch 1921). Subsequently pod rot and leaf fall were also reported from Cambodia, Vietnam, Liberia, Ghana, Nigeria, Cameroon, Congo, Brazil, Peru, Nicaragua, Costa Rica and Venezuela. In Malaysia, a serious outbreak of this disease was noticed during 1966 (Chee et al. 1967; Chee 1969). Pod rot and leaf fall due to *Phytophthora* attack have been reported from Thailand also (Chee and Greenwood 1968). Though this disease occurs in several countries, severe incidence necessitating adoption of control measures every year is observed only in South India.

Rainfall is the most important predisposing factor for the initiation and spread of the disease. In the traditional rubber-cultivated areas in India, a continuous spell of 250–350 mm rain for 7–10 days without intermittent hot sunshine, with minimum and maximum temperatures within the range of 22–25 °C and 26–30 °C, respectively, and relative humidity (RH) above 90% is most congenial for the outbreak of the disease. Under such conditions of low temperature and very high atmospheric humidity, the disease spreads rapidly and assumes epidemic proportions. Under normal monsoon, the disease starts by the middle of June and reaches the peak by the middle of July. However, when monsoon is late, very heavy incidence is noticed from the middle of July to middle of August.

Different species of *Phytophthora* are reported to be causing pod rot, bark rot, patch canker and leaf fall diseases of rubber in various countries. In India, four species of *Phytophthora*, viz. *P. palmivora* (Butler) Butler, *P. meadii* McRae., *P. nicotianae* var. *parasitica* (Dastur) Waterhouse and *P. botryosa* Cheewere are isolated from infected specimens (Thankamma et al. 1968; Edathil and George 1976, 1980). However, the species most common in the traditional areas is *P. meadii.*

Hyphae of the fungus are found to ramify inside the tissues of the infected portions intercellularly or intracellularly. Sporangia are found emerging externally through the stomata. The shape and size of the sporangia vary according to the species. During favourable climatic conditions, the pathogen resorts to the production of profuse asexual sporangia, which aid in quick dispersal and rapid spread of the disease. The sporangia liberate binucleate, biciliate and reniform zoospores, which swim in the available water and on contacting the green tissues, produce germ tubes, thus establishing a fresh infection on the host. Sporangia may also germinate directly producing germ tubes which also causes fresh infection. The pathogen gains entry into the host tissue through stomata (Thankamma et al. 1975). In general, all high-yielding clones and clonal seedlings are susceptible to abnormal leaf fall disease under Indian conditions. Clones like PB 86, PB 235, PB 260, PB 311, PB 28/59, RRIM 600, RRIM 628, RRIM 703, RRII 5, PR 255, PR 261 and Tjir 1 are observed to be susceptible to the disease.

Prophylactic spraying of rubber plants with 0.75% Bordeaux mixture is the very popular method (Ashplant 1928). Later experiments revealed that 1% Bordeaux mixture was more effective for the control of this disease and is being adopted extensively by rubber planters. It was noticed that addition of 0.5% zinc sulphate

to 0.5% Bordeaux mixture could give adequate protection to the clones RRIM 600 and RRII 105 and reduced the cost of spraying by about 35% when compared to spraying with 0.75% or 1% Bordeaux mixture (Idicula et al. 1994). As an alternative to Bordeaux mixture, Copper oxychloride (COC) dispersed in agricultural spray oil sprayed through low-volume applicators proved effective for the control of this disease.

11.3 Powdery Mildew

Powdery mildew disease was first reported from Indonesia. Subsequently, it was reported from Uganda (Small 1924) Sri Lanka (Stoughton-Harris, 1925) and Malaysia (Sharples 1926). In India, the disease was reported in 1938 (Mitra and Mehta 1938). Since then, the disease has been reported from almost all rubber-growing countries. The disease affects the immature leaves of rubber when the trees refoliate after annual wintering and causes leaf fall (Fig.11.4). Tender leaves at the brown or light green stage are highly susceptible. The presence of dull cool weather with intermittent light showers during refoliation predisposes the plants to severe disease attack. Prevalence of mist, dew and cloudy days with 75–80% relative humidity is favourable for disease development. Early wintering clones usually escape from the disease because the climatic conditions during their refoliation period are not favourable for the disease development. Late wintering clones are usually severely affected. Dry weather conditions during wintering period encourage early and rapid wintering and consequent escape from the disease. In India, the disease is severe in Kanyakumari, Idukki and Wayanad districts of South India and in the north-eastern states.

The optimum temperature for germination, infection and sporulation ranges from 25 to 30 °C (Liyanage et al. 1985). The fungus is disseminated by air-borne conidia. The peak sporulation is around noon. *Oidium heveae* Steinm, an obligate parasite, is responsible for the disease. The fungus produces superficial, branched, hyaline and septate hyphae. The hyphae are anchored on the host tissue with haustoria which help in deriving nutrients. The fungus has simple erect conidiophores which bear elliptical or barrel-shaped vacuolated conidia with round ends. The sexual stage has not yet been reported.

Leaf fall due to powdery mildew adversely affects the growth and yield of rubber trees. Wastie and Mainstone (1968) have reported a crop loss of 8.1% in the clone PB 5/51 over a period of 9 months, in Malaysia. Increased bark renewal and girth increment of trees protected against powdery mildew compared to unprotected trees were also observed. Tan et al. (1985) have reported 6.3–10.3% yield increase by controlling

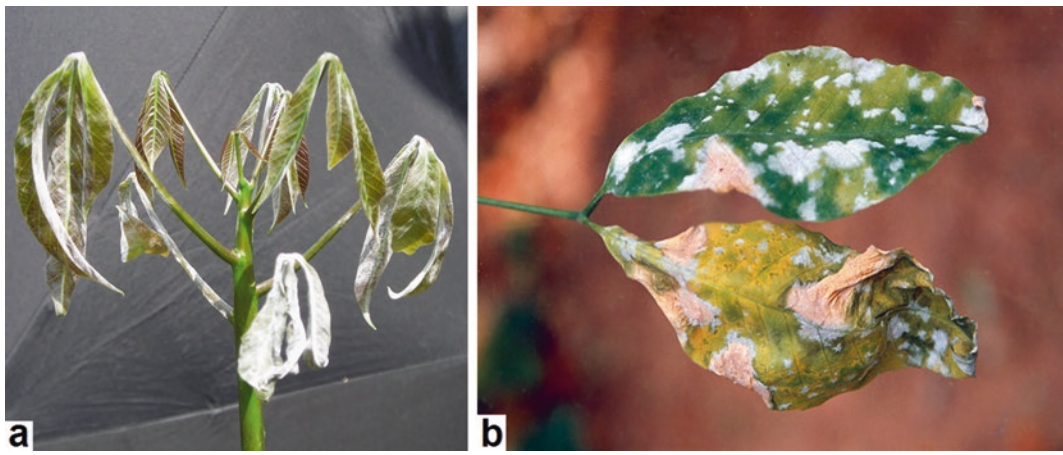

Fig. 11.4 Infestation by *Oidium heveae* in nursery and mature leaves

powdery mildew disease. In India, it was observed that in clone PB 86, 8–12% more disease in unprotected plots when compared to protected resulted in 21–32% crop loss. Similarly, 8–18% more disease in unprotected RRIM 600 caused 14–29% crop loss. The disease caused reduction in yield throughout the year. Disease resistance has been reported only in the low-yielding clone LCB 870 from Sri Lanka. In India, clones PB 86, GT 1, Gl 1, PR 107, RRIM 703, RRII 208 and PB 310 show some tolerance. The clones Tjir 1, PB 5/51, RRIM 605, RRII 105, RRII 118, RRII 300, PR 261, PB 21.7, PB 235, PB 280 and PB 311 are susceptible.

Chinese clone Reyan 7-33-97 is susceptible to powdery mildew. To study the benzothiadiazole (BTH)-induced resistance at gene level, Luo et al. (2013) constructed differentially expressed cDNA library by suppression subtractive hybridization (SSH). There were 23 cDNA sequences matching the function of basic metabolism, signal transduction and secondary metabolism selected randomly from the cDNA library and comparison to nucleic acid sequences in *GenBank*. Seven expressed sequence tags (ESTs) were logged in *GenBank,* and accession numbers were GW873071 and GW874604-GW874610. Their results implicated that BTH could effectively induce resistance to powdery mildew through increasing expression of defence-related genes in leaves. Such approaches should provide new insights for rubber disease management.

Wang et al. (2014) studied the effects of powdery mildew infection on the mitochondrial and chloroplast functions. Powdery mildew damaged the structure and function of mitochondria prior to chloroplasts, causing inner and outer membranes disruption. The intact rate of mitochondria membrane was reduced from 70% in control leaves to 23.1% in the leaves at 5 days after inoculation (dai). Significant decreases in the activities of cytochrome c oxidase, NADH oxidation and malate dehydrogenase (MDH) were observed in the powdery mildew-infected leaves. Tricarboxylic acid cycle (TCA) and electron transfer capacity were seriously impaired after powdery mildew invasion. Chlorophyll contents, maximal photochemical efficiency (Fv/Fm), actual photochemical efficiency of photosystem II (ΦPSII) and electron transport rate (ETR) were dramatically decreased in the infected leaves from 10 days after infection.

Dusting with sulphur gives effective control of powdery mildew disease. Spraying wettable sulphur is preferred only in the nurseries and young rubber plantations as repeated spraying in mature areas is expensive and impracticable. Sulphur dust having a minimum of 70% sulphur is generally used for dusting. The dust should be dry, free flowing and should pass through 325 mesh-sieve (particle size 40 microns). Dusting is done at the rate of 11–13 kg/ha at an interval of 7–10 days. Three to six rounds of dusting are usually required. First round of dusting is done when 10% of the trees start refoliation. Micron duster is employed for this purpose. The duster should be carried along every fourth row of trees at a speed of 3–4 km/h. With one duster, nearly 10–12 ha can be covered in a day. Sulphur dusting should preferably be done early in the morning so that the dew on the leaves helps in sticking of the dust. The still air in the morning hours also helps to raise the dust to reach the canopy. An integrated approach using tridemorph and sulphur in dust form was found to be more effective (Edathil et al. 1992). Carbendazim (Bavistin) 1.5% dust has also proved to be effective and could be used in integration with sulphur.

11.4 Corynespora Leaf Disease

Corynespora cassiicola (Berk. & Curt.) Wei is an important plant pathogenic Ascomycete causing the damaging corynespora leaf fall (CLF) disease. A small secreted glycoprotein named cassiicolin was previously described as an important effector of *C.* cassiicola. *Corynespora* causes leaf spot and leaf fall diseases. First reported in India from seedling nurseries (Ramakrishnan and Pillay 1961b), it was then reported from Malaysia (Newsam 1963), Nigeria (Awodern 1969), Indonesia (Soepena 1983), Sri Lanka (Liyanage et al. 1986) and Thailand (Kajornchaiakul et al. 1987). The disease has now been found in almost

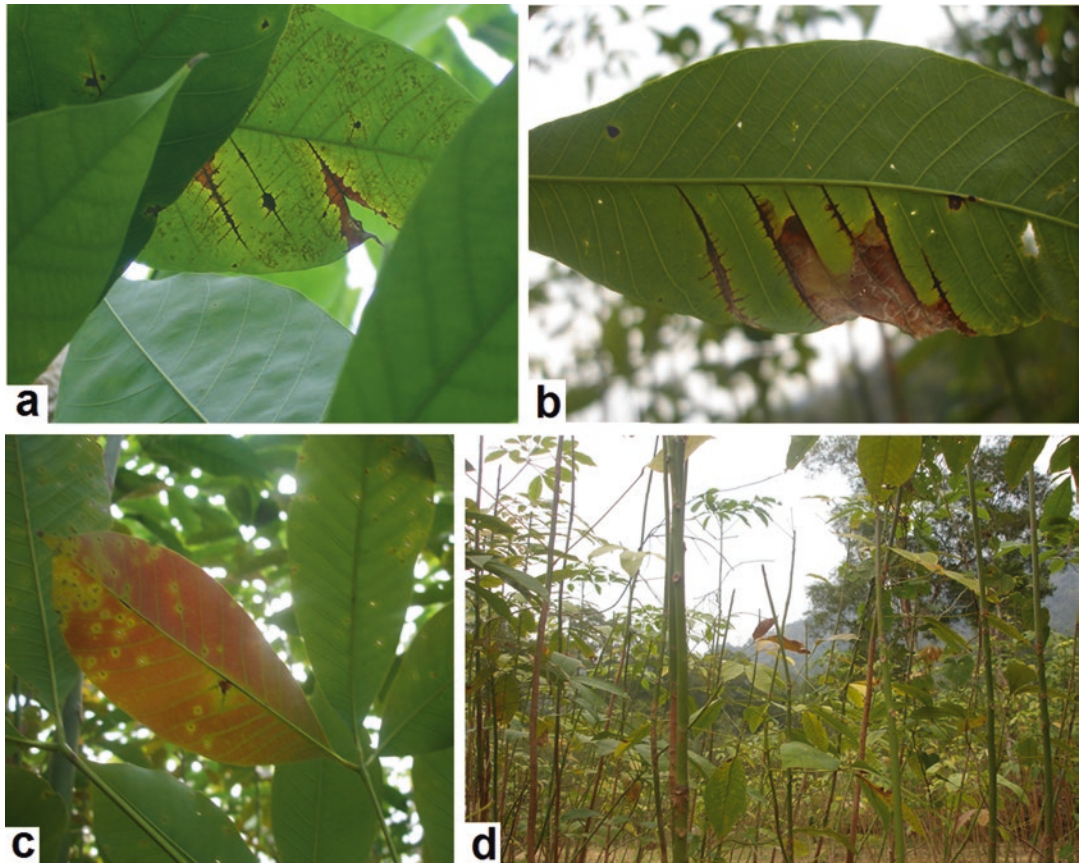

Fig. 11.5 Corynespora leaf disease in Hainan, China (Photo courtesy: Pu Jinji, EPPI, CATAS, China)

all rubber-growing regions (Chee 1988). Severe leaf fall was reported from Malaysia (Tan 1990) and Indonesia (Sinulingga et al. 1996). The disease appears in mature plantation during refoliation period infecting young leaves. The environmental factors favouring disease development are high humidity, a temperature of 28–30 °C, humid air and cloudy weather (Situmorang et al. 1996). The conidia of the fungus, produced abundantly on infected leaves, are carried by wind and cause rapid spread of the disease. The spore release increases steadily from morning and reaches the peak by noon and thereafter falls to very low levels (Chee 1988). The spore load in air has been negatively correlated to rainfall (Radziah et al. 1996). Conidia remain viable for about a month. Although the host range of *Corynespora* is wide (Liyanage et al. 1986), cross infectivity is doubtful (Chee 1988). In the severe form of the disease, a characteristic browning and blackening of veins gives a 'fishbone'- or 'railway track'-like appearance (Fig. 11.5). Even a single leaf spot can cause defoliation. Severe infection on the midrib causes leaf blight. When leaf petioles are infected, greyish-black lesions are formed causing defoliation without any symptoms on the lamina. Repeated defoliation and refoliation lead to shoot die-back. However, there is a relationship between exponential leaf growth, transition phase from completely susceptible leaf stage to completely resistant stage (Fig.11.6).

M. Déon, et al., Diversity of the cassiicolin gene in Corynespora cassiicola and relation with the pathogenicity in Hevea brasiliensis, Fungal Biology (2013) studied the diversity of the cassiicolin-encoding gene in *C. cassiicola* isolates sampled from various hosts and geographical origins. A cassiicolin gene was detected in 47%

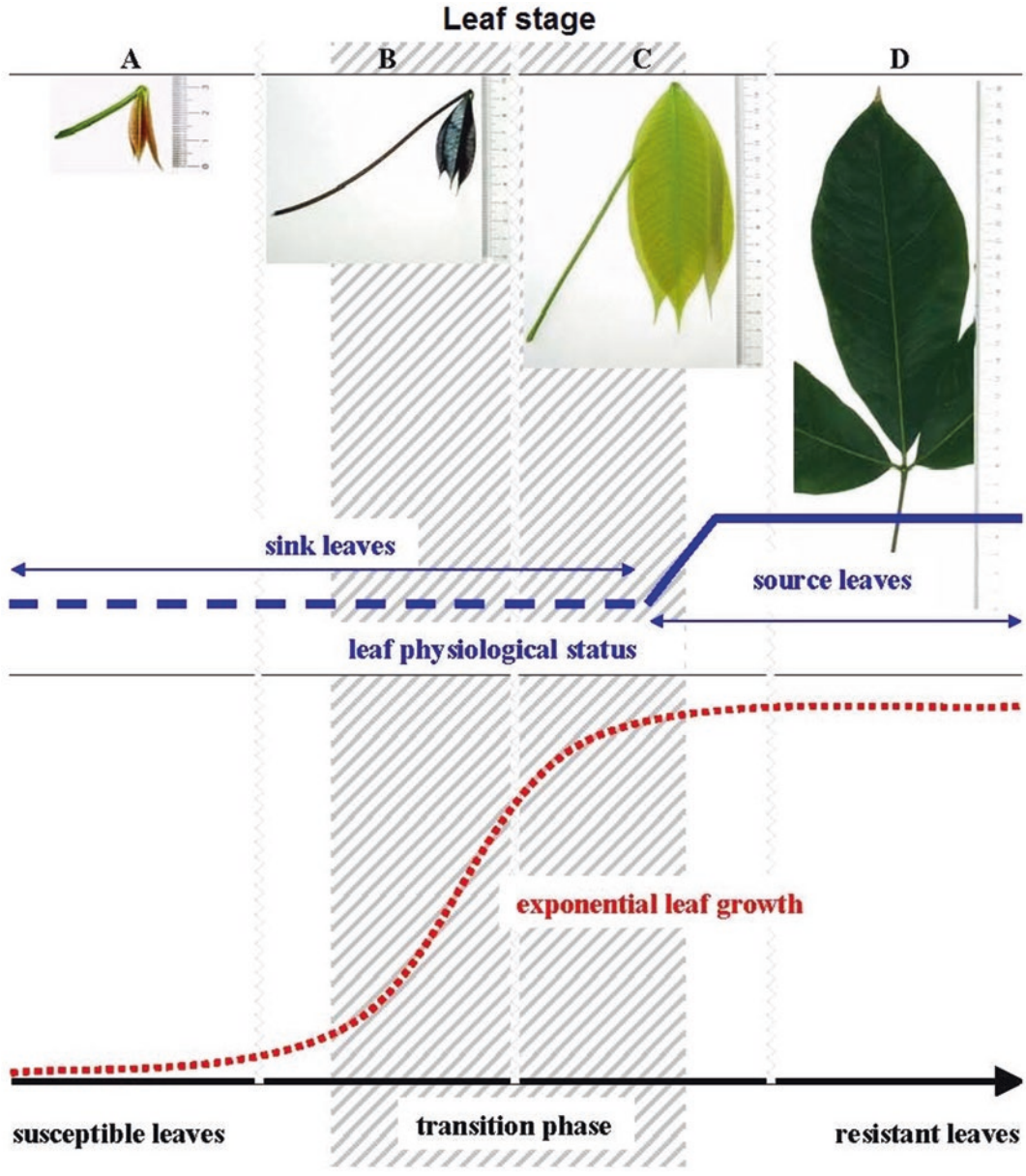

Fig. 11.6 Relationship between exponential leaf growth, transition phase from completely susceptible leaf stage to completely resistant leaf stage and the short physiological step from sink to source leaves. This attempts to he different developmental processes that turn a susceptible leaf into completely age resistant. The hatched area shows the resistance factors that interact in various ways and do not allow the correlation of the QTLs developed with the leaf properties in transit

of the isolates, encoding up to six distinct protein isoforms. In three isolates, two gene variants (Cas2 and Cas6) encoding cassiicolin isoforms were found in the same isolate. A phylogenetic tree based on four combined loci and elucidating the diversity of the whole collection was strongly structured by the toxin class, as defined by the cassiicolin isoform. The isolates carrying the Cas1 gene (toxin class Cas1) were all grouped in the same highly supported clade and were found

the most aggressive on two rubber tree cultivars. The study of Déon et al. (2013) provides a platform for future studies of *C. cassiicola* population biology and epidemiological surveys. RRIC 103, RRIC 104, RRIM 600, RRIM 725, Tjir I, RRIC 110, RRIC 133, RRIM 600 GT 1, PB 5/51, PB 217, PB 235, PB 260, PR 107, RRIM 901, RRIM 905 and Tjir 1 are seen susceptible (Tan 1990; Jayasinghe and Silva 1996). AVROS 2037, BPM 24 and RRIC 100 are reported as tolerant from Indonesia (Azwer et al. 1993). Studies conducted in France indicated PB 260 to be highly susceptible and GT 1 to be tolerant (Breton et al. 1997).

Several fungicides have been recommended for the control of *Corynespora* leaf disease. Spraying of benomyl, mancozeb, captan or propineb is recommended for affected nursery plants (Jayasinghe and Silva 1996; Hashim 1994). Four to five rounds of spraying with tridemorph (Calixin 0.6/ha) or mancozeb (Dithane M45 1.5–3 kg/ha) are recommended for *Corynespora* control in Indonesia (Soepena et al. 1996).

11.5 Shoot Rot

Initial symptom of this disease is noticed on the terminal portions, especially on the purple-coloured leaflets. Within 24–48 h, the leaflets become dark-coloured and the rotting extends up to the petiole. In a short time, infection spreads to other leaflets also. Subsequently, infection spreads to the stem and progresses from apex to downwards. The affected portions of the stem are initially dark brown but later turn black and shrunken. The rotting of the shoot may extend from 15 to 75 cm in length. The diseased portion dries up and later new branches arise from below the infected portion. Clones that are susceptible to abnormal leaf fall are also susceptible to shoot rot.

The disease could be controlled by prophylactic spraying with copper fungicide for mature and immature plants in the field. Repeated spraying with 1% Bordeaux mixture or 0.5% Bordeaux mixture +0.5% zinc sulphate at an interval of 7–10 days is required to protect the young plants in the nursery and field during the monsoon period. Phosphorous acid at 0.16% and metalaxyl MZ at 0.2% are also effective in checking the disease (Idicula et al. 1998).

11.6 Gloeosporium Leaf Disease

Even though the disease is not a serious problem in mature rubber, it has been observed throughout the rubber-growing regions. Though confined to seedling and budwood nurseries, immature plants are also being seriously affected. The disease is generally noticed during April to May, before the onset of southwest monsoon and in August, September and October or whenever wet weather is prevailing. High humidity is a pre-requisite for the formation of sporocarp. Free water is necessary for optimum germination of the fungus. Germination of spores occurs in a few hours at 100% humidity and longer time is taken at lower levels of humidity (Wastie 1972).

Tender leaves produced soon after bud burst are more susceptible to infection. Under extensive damage, leaves become distorted, turn black, shrivel and fall off, leaving the petioles on the stem. The infection usually starts at the tip of the leaf and spreads towards the base. If the leaf gets infected at a later stage, it becomes either highly spotted or may be partially damaged along the tip and margin. As the leaf ages, the margins of the leaf spots become thick and raised above the surface as conical projections, this being the most important diagnostic feature of the disease. The pathogen is *Gloeosporium alborubrum* retch. The hyphae of the fungus penetrate the tissues of the affected part. Intraepidermal or subepidermal stromata are formed on the infected region.

A severe incidence in the immature plants in the field may lead to heavy defoliation and shoot die-back resulting in the girth retardation and extension of immaturity period. In Indonesia, the persistence of this disease over a long period resulted in loss of yield up to 50% and delay in maturity up to 3 years (Basuki 1992). After 3 years of continuous artificial defoliation to control SLF, a yield increase exceeding 30% was achieved in Malaysia (Radziah and Hashim 1990).

PB 217, PB 260 and RRIM 600 are the clones having some tolerance.

Copper oxychloride spraying carried out as a prophylactic measure in April/May keeps this disease under check. Mancozeb (0.2%), carbendazim (0.05%), bitertanol (0.025%) and Bordeaux mixture (1%) were found effective in controlling the disease in young rubber plantations (Joseph et al. 1994). Mechanical fogging of captafol in oil (0.6 kg/ha) thrice at weekly intervals during refoliation gave good control of the disease in Malaysia (Tan et al. 1985). In Malaysia, artificial defoliation by aerial spraying of several chemical defoliants mixed in water is practiced (Radziah and Hashim 1990). In Cameroon, ethephon (3 l/ha)-induced defoliation and early refoliation helps in avoiding secondary leaf fall (Senechal and Gohel 1988).