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Variation in susceptibility to *Phyllosticta capitalensis*-associated leaf disease among inter-specific hybrids, half-sibs and high-yielding clones of Para rubber tree (*Hevea*)

Narayanan C* and Reju MJ

Botany Division, Rubber Research Institute of India, Kottayam 686 009, Kerala, India

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Abstract

High susceptibility of many commercial clones of Para rubber tree (*Hevea brasiliensis*) makes disease management very difficult warranting development and use of disease tolerant clones. Hence, hybridization was carried out using high-yielding and susceptible clones of *H*. *brasiliensis viz*. RRII 105, RRII 414 and RRII 430 and two disease tolerant species *viz*. *H*. *spruceana* and *H*. *camargoana* and two wild Rondônia germplasm accessions *viz*. RO 380 and RO 2871. The progenies from the breeding are under various stages of evaluation for their growth and rubber yield. During the course of evaluation, it was observed that many progenies were affected by a serious leaf disease in the form of spots which affected only mature leaves. In all leaf samples examined, conidiomata and ascomata were observed from the initial stages of leaf spot symptoms. Based on characteristic morphology of conidiomata and ascomata, the fungus which was consistently detected in the spots was identified as *Phyllosticta capitalensis*. Occurrence of *P*. *capitalensis* in both of its conidiomata and ascomata states with appendaged conidia and appendaged ascospores respectively, in association with the characteristic leaf spots in either *H*. *brasiliensis* or its inter-specific hybrids, is a novel report from India or elsewhere. In very few progenies, leaf blight associated with *Pestalotiopsis* sp., was also observed. In order to find variability in response to the leaf spot disease associated with *P*. *capitalensis*, all progenies were assessed for disease incidence and severity. Progenies of crosses *viz*. RRII 430 x RO 2871, RRII 414 x *H*. *spruceana*, RRII 414 x RO 380 and RRII 105 x *H*. *spruceana* had more than 80 percent disease incidences in their progenies. Progenies of cross RRII 430 x RO 380 showed minimum disease incidence (I = 61%). Half-sib progenies of a disease tolerant clone Fx 516 showed lesser disease incidences (I = 25-27%) and very low disease index (*DI*, 0.8-0.9) indicating better tolerance. In addition to the above, few other high-yielding commercial rubber clones were assessed for their susceptibility to *P*. *capitalensis* associated leaf spot. In an on-farm trial, two clones *viz*. RRII 430 and RRII 417 recorded maximum (I = 60%) disease incidence. Clone RRII 422 (I = 10%) followed by RRII 414 (I = 20%) showed minimum disease incidence. Two other clones *viz*. RRII 105 and RRII 429 showed moderate incidence (I = 50%). *P*. *capitalensis* was consistently detected and associated in all of the infected leaf samples from all the host species observed from early stages of disease development. However, more detailed studies are required to ascertain its specific role in disease development in order to develop suitable management strategies.

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Corresponding Author: Narayanan Chaendaekattu – e-mail – narayanan.chaendaekattu@gmail.com

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Introduction

Hevea brasiliensis (Willd. ex. A. de. Juss.) Müell. Arg., belonging to family Euphorbiaceae (chromosome number, 2n=36), and most commonly known as Para rubber tree, is naturally distributed in the Amazon forest ranges of South America. The tree yields natural rubber latex which is a major strategic raw material for more than 40,000 daily-use products including tyres and hundreds of medical devices and life-saving accessories like latex gloves (Mooibroek & Cornish 2000). The Para rubber tree is susceptible to many leaf and stem diseases caused by phyto-pathogenic fungi including *Phytophthora*, *Colletotrichum*, *Corynespora*, *Oidium* and *Corticium*, which mostly lead to severe economic loss in rubber yield (Liyanage & Jacob 1992, Jacob 1997, Jayasinghe 1999, Narayanan & Mydin 2012). *Pseudocercospora ulei* (Henn.) Hora Junior & Mizubuti (= *Microcyclus ulei* (Henn.) Arx.) is a devastating fungal disease causing South American Leaf Blight (SALB) in Brazil and it is a looming threat to global rubber cultivation (Chee 1990, Hora Júnior et al. 2014). Recently, serious disease epidemics due to fungal pathogens including *Neofusicoccum*, *Fusicoccum* and *Pestalotiopsis* reportedly caused huge losses in natural rubber production in many countries including China, Cameroon, Indonesia, Malaysia and Sri Lanka (Ngobisa et al. 2013, Liu et al. 2017, Ngobisa 2018). Based on a recent study on *H. brasiliensis* in Sri Lanka, few foliar fungal pathogens including *Phyllosticta capitalensis* Henn. were found to have host-pathogen association (Herath et al. 2019a, b). However, the above report lacked details on symptomatology of the foliar diseases and there were no morphological descriptions of the fungi.

Development and use of disease tolerant clones is the only long term strategy for effective and sustainable management of the diseases (Fernando 1969). In a disease resistant breeding, three high-yielding clones of *H. brasiliensis* were hybridized with other disease tolerant *Hevea* species *viz*. *H. spruceana* (Benth.) Müell. Arg. and *H. camargoana* Pires and wild Rondônia germplasm accessions collected from Amazon forests of Brazil. While *H. spruceana* and *H. camargoana* have been used in hybridization for recovering progenies with high level of resistance to SALB and *Corynespora cassiicola* (Berk. & M.A. Curtis) C.T. Wei, the two wild accessions *viz*. RO 380 and RO 2871 are putatively tolerant to a few fungal diseases including those caused by *Oidium* (Goncalves et al. 1980, Goncalves et al. 1982, Tran et al. 2016, Adifaiz et al. 2017). Open pollinated progenies were collected from another disease tolerant Brazilian clone Fx 516 which was earlier used in breeding for resistance to SALB and other major fungal diseases in Sri Lanka and India (Senanayake & Wijewantha 1968, Fernando & Liyanage 1975, 1980, Narayanan & Mydin 2012). During course of study, a serious leaf disease was found to affect mature leaves of many progenies. Detailed studies were carried out on the infected leaf samples to identify involvement of any fungal pathogen. Based on characteristic morphology of conidiomata and ascomata, the fungus which was consistently detected in the spots was identified as *Phyllosticta capitalensis* Henn. *Phyllosticta* sp., in either ascomatal sexual state (*Guignardia heveae* Syd. and Syd.) or conidiomatal asexual state, has been forecasted as an economically important pathogen of *H. brasiliensis* for Asian and African regions (Jayasinghe 1999). In subsequent taxonomic treatments, *G. heveae* has been grouped under *G. mangiferae* 'species complex' and the name is now considered as synonym of *P. capitalensis* (Wulandari et al. 2009). Detailed morphological descriptions of both conidiomatal as well as ascomatal states of *P. capitalensis* in association with the characteristic infectious leaf spots as observed in the present study, has not so far been reported in *Hevea*. This paper reports the findings on symptomatology of leaf spot disease, morphology of *P. capitalensis* and variation in susceptibility among inter-specific hybrids, half-sib progenies as well as high-yielding clones of *H. brasiliensis*. In addition to the above, occurrence of *Pestalotiopsis* sp. in association with a minor incidence of leaf blight, is also reported as this fungus is of quarantine importance in many rubber growing countries.

Materials & Methods

Plant material and location

The parental clones were selected from breeding trials located in the Central Experimental Station of Rubber Research Institute of India (Ranni, Kerala State, India). Through hand pollination in various cross combinations, high-yielding and susceptible clones of *H. brasiliensis viz*., RRII 105, RRII 414 and RRII 430 were hybridized with two disease tolerant *Hevea* species *viz*. *H. spruceana* and *H. camargoana* and two wild Rondônia germplasm accessions *viz*. RO 2871 and RO 380, during 2014 to 2016. Two sets of open-pollinated polycross progenies (or half-sibs) were collected from the disease tolerant inter-specific hybrid clone Fx 516 during 2015 and 2017. Details of the clones, parentage and country of origin are given in Table 1. All progenies were grown and maintained in nursery trials (1 x 1 m spacing; C.R.D.) at the experimental station of Rubber Research Institute of India (RRII; Kottayam, Kerala State, India). RRII is located in Kerala (09^{o}32'N, 76^{o} 36'E; 73 m above MSL) with a mean annual rainfall of more than 3000 mm. In addition to above, three more clones *viz*. RRII 417, RRII 422 and RRII 429, were assessed for the leaf disease incidence in an on-farm block trial located at Chithalvetty (Punalur, Kerala State, India). Since the above three clones share same parentage as that of RRII 430 and RRII 414, their susceptibility to leaf disease gains importance (Table 1). All the above observations were made during 2019.

Table 1 Details of parental clones and other *Hevea* species.

Clone (pedigree)/Species	Origin, distribution and habit	Remarks
RRII 105 (Tjir 1* x Gl 1#); RRII 414, RRII 430 (RRII 105 x RRIC 100); Hybrids of *H. brasiliensis.*	India. *H. brasiliensis* naturally occurs in South of Amazon river (Brazil, Bolivia, Ecuador and Peru). Large trees.	High-yielding clones classified under Category-I of planting advisory.
Fx 516 (F 4542 x AVROS 363$) (Fx, Ford Cross; F 4542 is a selection of *H. benthamiana* Müell. Arg.)	Par´a (Brazil). *H. benthamiana* naturally grows in areas from Colombia to N. Brazil. Medium to large sized trees.	Tolerant to leaf diseases caused by *Phytophthora* and *Corynespora*.
H. camargoana Pires	Brazil. Distributed in Marajo island of Amazon river delta. Trees of 2-25 m ht.	Variable tolerance to SALB disease.
H. spruceana (Benth.) Müell. Arg.	Brazil. Naturally occurs in banks of Amazon, Rio Negro and lower Madeira. Medium sized tree.	Variable tolerance to SALB disease.
RO 2871 (Unknown parentage) (RO, Rondônia)	Brazil. Collected from Rondônia in 1981. Large trees.	Tolerant to *Oidium* leaf disease.
RO 380 (Putative hybrid of *H. pauciflora* (Spruce ex Benth.) Müell. Arg.)	Brazil. Collected from Rondônia in 1981. Medium to large trees.	Putatively tolerant to various leaf diseases.

**H. brasiliensis* clone, Indonesia; *#H. brasiliensis* clone, Malaysia; $AVROS, Al-gemene Verneiging Rubber planters Oostkust Sumatra, Indonesia.

Symptomatology, fungal morphology and percent disease incidence

Progenies and parental clones were affected by a leaf disease in the form of spots of variable sizes. The disease affected only mature leaves and there was considerable variation in incidence and severity of the leaf disease among progenies and parental clones. In very few progenies, leaf blight was observed. Detailed studies were carried out on the symptomatology of the leaf spot and leaf blight and also on morphology of fungus present in the leaf spots and blights through microscopic observation using thin sections (15-20 µm). Morphological identification of the leaf spot fungus was confirmed by the Ajrekar Mycological Herbarium of the National Fungal Culture Collection of India (Pune, Maharashtra, India), where the infected leaf specimens were deposited with unique accession number.

Percent disease incidence ($I = (\sum x/N)*100$) was estimated as a proportion of diseased plants where x was number of affected plants and N was total number of plants. Disease severity was recorded following a modified disease rating scale (Horsfall & Barratt 1945) as follows: 0, spots absent; 1, spots in less than 25 percent of leaves; 2, spots in 25 to 50 percent of leaves; 3, spots in 51 to 75 percent of leaves; 4, spots in more than 75 percent leaves along with premature leaf fall. Disease index ($DI = \sum(x_i n_i)/N$) was estimated as the product of incidence and severity of disease where, where x represented disease rating (0-4), n_i represented the number of diseased plants on the ith grade of the disease scale and n was the total number of diseased plants assessed (Groth et al. 1999). Based on the disease index, the progenies could be rated as tolerant (DI = 0.1-1.0), moderately tolerant (DI = 1.1-2.0), moderately susceptible (DI = 2.1-3.0) and highly susceptible (DI = 3.1-4.0). Similarly, observation was also made on disease incidences in the parental clones located at CES.

Results

Symptomatology of leaf spot disease

Mostly, spots had distinct ash gray, pale or light brown central portion bordered by a narrow necrotic margin (Fig. 1). The size of the spots was variable according to the development stages but mature spots were mostly in the range of 8 to 14 mm in diameter. Often, two or more spots coalesced and gave an appearance similar to that of small blotches thus affecting larger leaf area.

Fig. 1 – Symptoms of leaf spot. A, B Leaf spots in 2-year-old seedling. C Leaf spots in 4-year-old tree. D Paling of affected leaves. E Infected leaves with typical spots. F Severely infected plant affected by premature leaf fall. G Leaf in yellowing stage with fungal fruit bodies (arrow). H Concentric lesions. I Nearby spots colaescing to form small blotches. J, K Spots showing initial stages of development of fungal fructifications in margin (arrows). L Prolifereation of more fructifications in the margin of spot. Scale bars: I = 5 mm, J-L = 3 mm. (Copyright: Narayanan Chaendaekattu)

In severe cases, the affected leaves turned greyish brown or pale yellow and withered prematurely. It was observed that the disease affected only mature leaves. Leaf spots in different stages of disease development revealed several minute blackish fungal fructifications in the form of pycnidia and perithecia in various developmental stages (Figs 2, 3). Initially, few isolated fructifications could be observed in area lining the advancing lesion of the spots (Figs 2, 3). Subsequently, as the disease progressed into more advance stages, more fructification was observed.

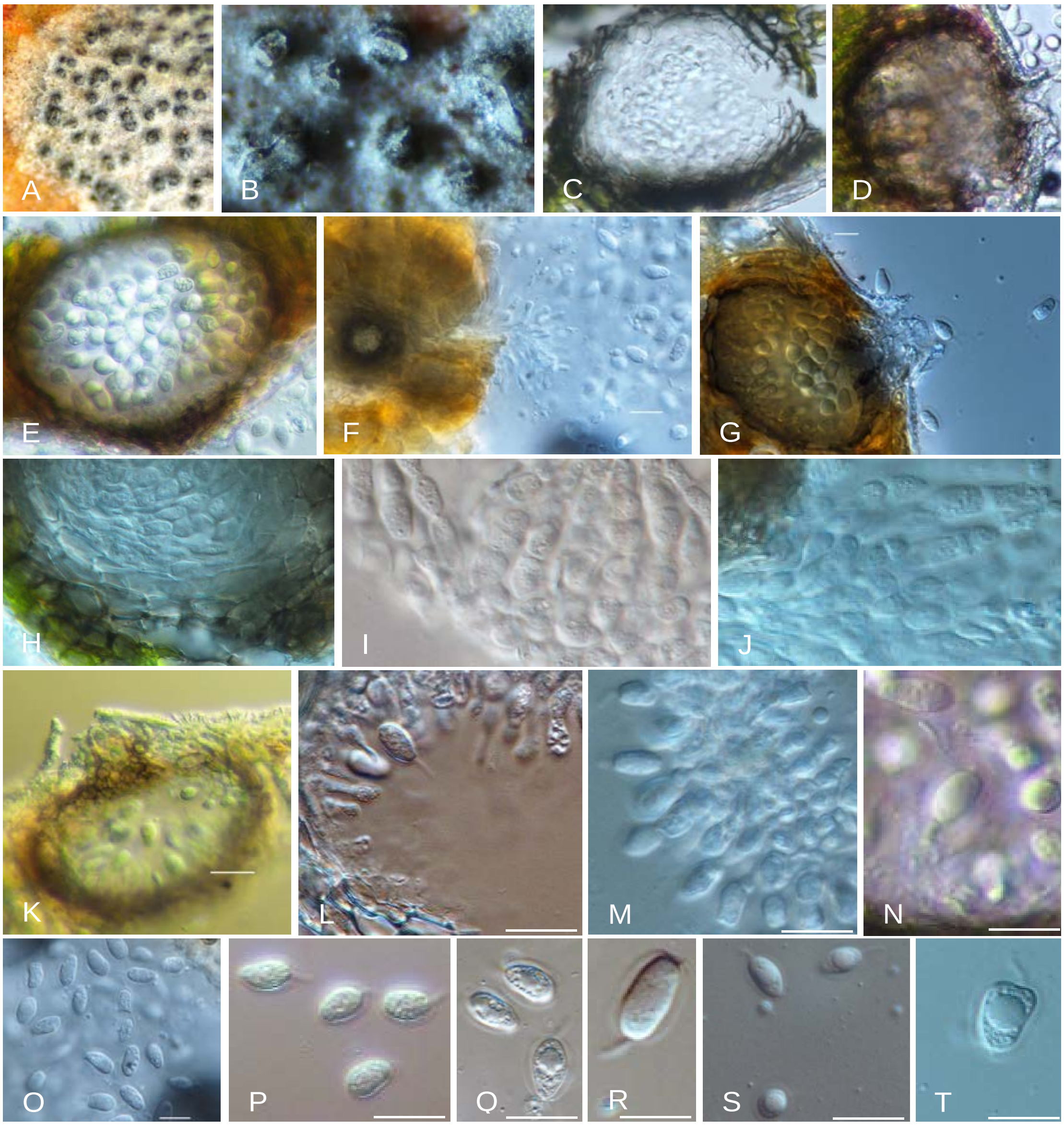

Fig. 2 – Conidiomata of *P. capitalensis*. A, B Pycnidia in leaf spot. C-E Conidiomata showing conidial formation (vertical section). F, G Top view and vertical section view, respectively of ostiole. H-J Conidiogenous cells. K Finer details of ostiole and ruptured leaf tissue. L-N Conidiogenesis. O-T Conidia. (Note characteristic single apical appendage and mucilaginous sheath). Scale bars: K = 18 µm, L, M = 20 µm, N = 10 µm, O = 17 µm, P = 20 µm, Q = 10 µm, R = 5 µm, S = 20 µm, T = 10 µm. (Copyright: Narayanan Chaendaekattu)

Morphological description of the fungus associated with leaf spot

Based on the characteristic morphological features of the anamorphic and teleomorphic stages detected in the spots, the fungus was identified as *Phyllosticta capitalensis* Henn. (Barnett & Hunter 1998, Glienke et al. 2011, Wikee et al. 2011, Wikee et al. 2013a, 2013b). The fungus produced conidiomata and ascomata with characteristic conidiospores and ascospores, respectively (Figs 2-3).

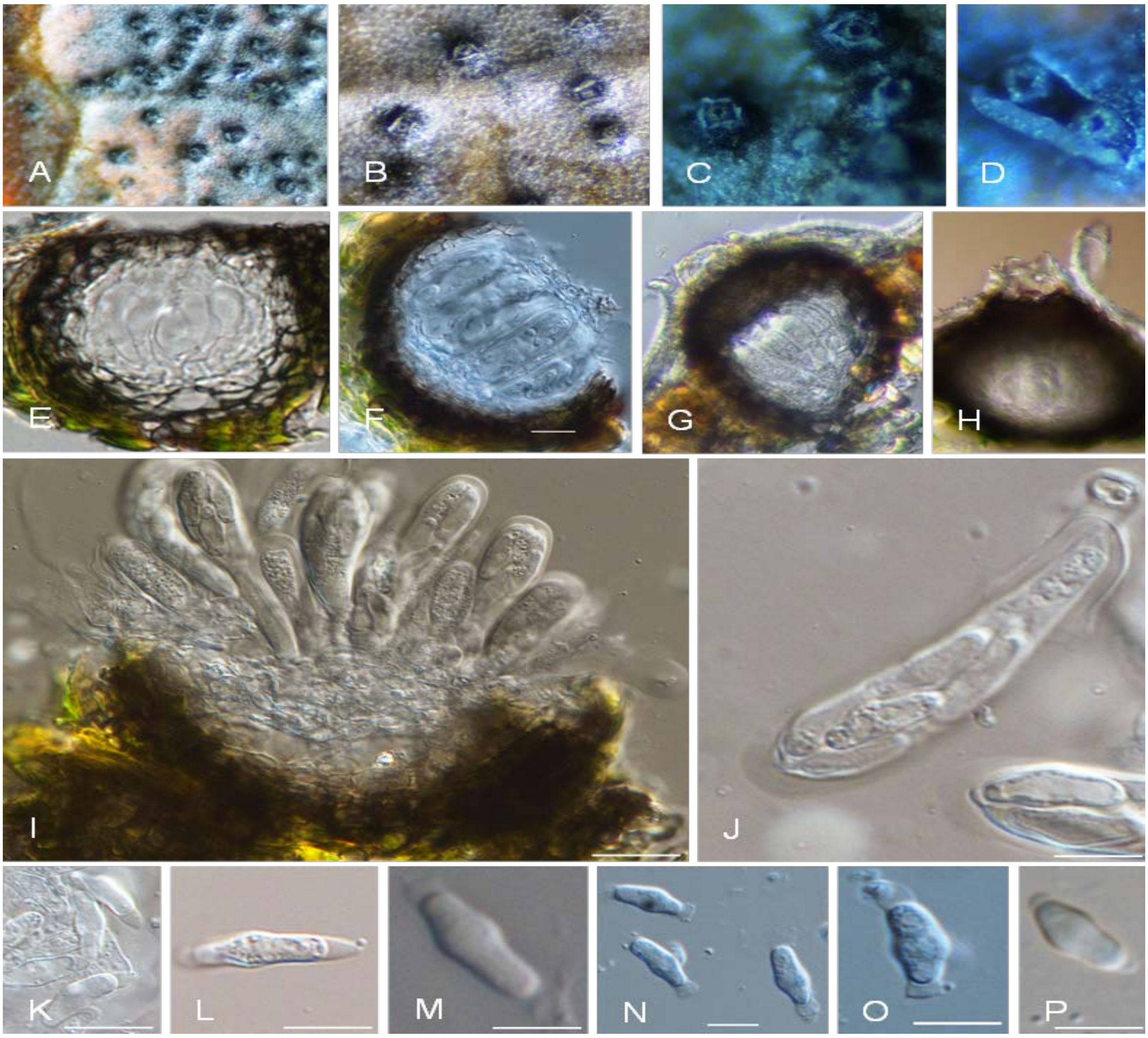

Fig. 3 – Ascomata of *P. capitalensis*. A-D Ascomata in host leaf spots. E-H Vertical sections showing details of ascomata development. G Mature ascomata with ostiole. H Ascomata showing ostiole and withest. I, J Asci and ascospores. K-P Ascospores (note the characteristic mucilaginous appendages of ascospores). Scale bars: F = 15 µm, I = 15 µm, J = 7.5 µm, K = 15 µm, L-M = 10 µm, N = 9 µm, O = 8 µm, P = 10 µm. (Copyright: Narayanan Chaendaekattu)

Phyllosticta capitalensis Henn. Hedwigia 48: 13 (1908) Figs 2-3

Ajrekar Mycological Herbarium (AMH10221)

Conidiomata in leaves pycnidial, epiphyllous, brown or black with a central ostiole. Peridium thick, lined by brown cells of *textura angularis*. Conidiogenous cells lining the walls of pycnidium phialidic, almost cylindrical and hyaline. Conidia ellipsoidal, hyaline, variously guttulate, single celled, smooth, 8-11×5-6 µm, surrounded by mucilaginous sheath with a single apical appendage, appendage 4-6 µm long. Ascomata in leaves black, globose to subglobose, unilocular, solitary to clustered, ostiolate. Pseudoparaphyses absent. Asci bitunicate, fissitunicate, broadly cylindrical to cylindro-clavate, rounded at the apex, tapering gradually to a long pedicel attached to the basal peridium, 45-66 × 7-14 µm, 8-spored. Ascospores ellipsoidal, swollen in the centre, single celled, coarse-guttulate, smooth-walled, 9-16 × 5-8 µm in size, with mucilaginous appendage at each end.

Material examined - in spots of mature leaves of *H. brasiliensis*, *H. brasiliensis* x *H. spruceana*, *H. brasiliensis* x *H. camargoana*, India, Kerala State, Kottayam, farm of Rubber Research Institute of India, 4 Jan 2020, C. Narayanan, 04012020/Ktym/India/1-5, Ajrekar Mycological Herbarium, AMH10221.

Variation in incidence of *Phyllosticta capitalensis*-associated leaf spot disease

Details regarding age, number of progenies from each crosses, number of half-sibs, disease incidence (*I*) and disease index (*DI*), are given in Table 2. Based on *DI*, the families were rated as tolerant or susceptible. While progenies of RRII 430 x RO 2871, RRII 414 x *H. spruceana*, RRII 414 x RO 380 and RRII 105 x *H. spruceana* had more than 80 percent disease incidence, RRII 430 x RO 380 showed minimum disease incidence (61%). Comparatively, progenies of Fx 516 had more tolerance with low disease incidences (25-27 %). Regarding disease index also, progenies of Fx 516 showed very low rating (*DI*, 0.8-0.9) when compared to hybrids (*DI*, 2.0-2.7).

Table 2 Percent disease incidence (*I*), and disease index (*DI*) of progenies

Progeny type	Age (yr.)	$n^{\#}$	*I* (%)	*DI*	Clone	$n^{\#}$	*I* (%)
RRII 105 x RO 380	4	6	69	2.2	RRII 105	143	50
RRII 105 x *H. spruceana*	4	19	83	2.5	RRII 414	243	20
RRII 414 x RO 380	4	6	84	2.7	RRII 417	397	60
RRII 430 x RO 380	5	95	61	2.0	RRII 422	481	10
RRII 430 x *H. camargoana*	5	6	74	2.3	RRII 429	240	50
RRII 430 x *H. spruceana*	5	49	84	2.6	RRII 430	333	60
RRII 430 x RO 2871	5	11	85	2.7			
Half-sib of Fx 516	4	91	25	0.8			
Half-sib of Fx 516	2	273	27	0.9			

$^{\#}$number of plants

All female parental clones of *H. brasiliensis viz*. RRII 105, RRII 414 and RRII 430, showed symptoms of leaf spot disease. Most of their hybrid progenies also showed high disease incidence and disease index (Table 2). Among the male parental clones, *H. camargoana* and *H. spruceana* were asymptomatic without any visible leaf spots. Similarly, wild germplasm male parental clones (RO 380 and RO 2871) were also asymptomatic, apparently not affected by the disease. Regarding disease incidence of clones in the on-farm trial, clones RRII 430 and RRII 417 (*I* = 60%) followed by RRII 105 and RRII 429 (*I* = 50%) had high disease incidences indicating less tolerance to the disease (Table 2). In contrast, clones RRII 422 and RRII 414 showed minimum disease incidences of 10% and 20% respectively, indicating better tolerance to the disease.

Minor incidence of *Pestalotiopsis* leaf spot and blight

In very few progenies, ash coloured, irregular, necrotic leaf spot and leaf blights, which were contrastingly different from those associated with *Phyllosticta* sp., were observed (Fig. 4).

Microscopic observation of the infected leaves revealed association of *Pestalotiopsis* sp. with blights. Conidia were fusoid, ellipsoid, straight or slightly curved (18.1 - 24.9 µm x 5.3 - 6.6 µm) with four septa and a hyaline, thin-walled and sub-cylindrical apical cell with two to four hyaline filamentous apical appendages and a basal cell with one hyaline appendage.

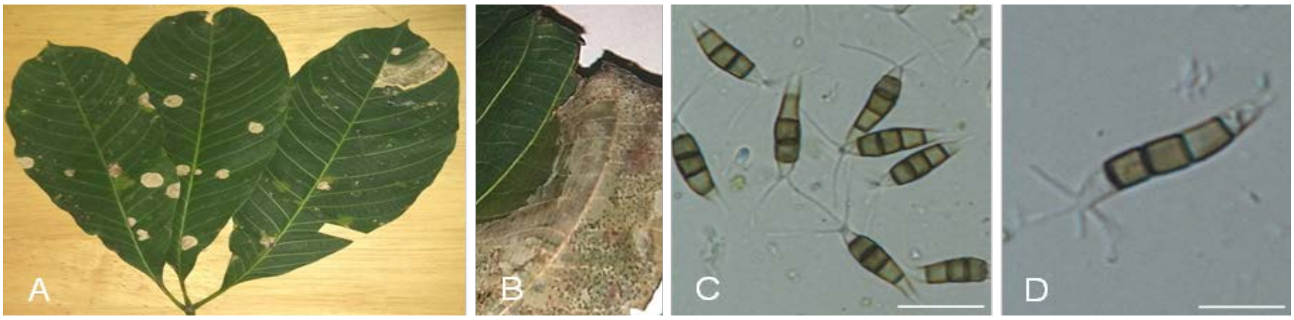

Fig. 4 – *Pestalotiopsis* leaf blight. A Leaf with spots and blight. B Closer view of blight. C, D Conidia. Scale bars: C = 15 µm, D = 10 µm. (Copyright: Narayanan Chaendaekattu)

Discussion

P. heveae Henn. was described in its conidiomatal state from leaves of *H. brasiliensis* in 1904 (Hennings 1904). *G. heveae* Syd. and Syd., as the teleomorph state, was described in leaves of *H. brasiliensis* from Philippines in 1916 (Sydow & Sydow 1916). *G. heveae* was later reported from leaves and seedlings of *H. brasiliensis* from Selangor, Kuala Lumpur and Peninsular Malaysia during period 1982 to 1987 (Lee et al. 2012). Later, based on a combined phylogenetic tree generated through DNA sequence analysis of the internal transcribed spacer region (ITS1, 5.8S, ITS2), translation elongation factor 1-alpha (TEF1) and actin genes, *G. heveae* was grouped under *G. mangiferae* 'species complex', along with several other *Guignardia* species (Wulandari et al. 2009). In subsequent taxonomic treatments, *G. heveae* was synonymized with *P. capitalensis* (Slippers et al. 2013, Wulandari et al. 2013a, b). Although *P. capitalensis* was recently reported to have host-pathogen relationship in *H. brasiliensis* in Sri Lanka, the report was devoid of symptomatology of the foliar disease or morphological descriptions of *P. capitalensis* (Herath et al. 2019a, b). The morphological features of the conidiomata and ascomata states with characteristically appendaged conidia and ascospores respectively, as described and illustrated in the present study, were clearly in conformity to those described for *P. capitalensis* Henn. (= *G. heveae*) (Hennings 1908, Wikee et al. 2011, Wikee et al. 2013a, 2013b, Wulandari & To-anun 2014). Based on the existing literature, and based on descriptions from earlier records of *P. heveae* or *G. heveae* reported in *H. brasiliensis*, occurrence of both conidiomatal and ascomatal states of *P. capitalensis* with appendaged conidia and ascospores, in association with the characteristic leaf spots of *H. brasiliensis* and its interspecific hybrids with *H. spruceana* and *H. camargoana*, has not so far been reported from India. Also, detailed symptomatology of leaf spots and morphological descriptions of anamorphic and teleomorphic states *P. capitalensis* has also not been reported so far in *Hevea.*

Our study using several leaf spot specimens collected from different species and clones of *Hevea* growing at various locations showed consistent occurrence of *P. capitalensis* in all samples. Thus, the study clearly established the association of *P. capitalensis* in leaf spot symptoms. However, more specific pathological tests are to be carried out to conclusively establish pathogenicity and virulence of *P. capitalensis* in causing infection in different rubber plants under laboratory conditions. Also, more commercial clones and other species of *Hevea* should be examined for occurrence of the above disease in various other rubber growing locations within India and outside the country, in order to identify potential sources of disease resistance for use in breeding. Outcome of such study will also help in developing appropriate disease management strategies including quarantine regulations, particularly in the event of future large-scale infections in commercial plantations within India and other rubber growing countries. *Phyllosticta* belong to fungal family Phyllostictaceae most of which typically infect leaves and fruit, rather than woody tissue, sometimes causing serious damage (Glienke et al. 2011, Wong et al. 2012). As a plant pathogenic fungus, *Phyllosticta* is capable of causing serious damage to the host plants by reducing photosynthetic ability sometimes even leading to premature leaf or fruit fall (Glienke-Blanco et al. 2002, Baldassari et al. 2008). Several species of *Phyllosticta* (with or without *Guignardia* teleomorph state) have been reported to cause spots on leaves, as well as dark spots and necrotic lesions on fruits of several plant species (van der Aa & Vanev 2002). As far as potential threat to global natural rubber plantations, *Phyllosticta* sp. in either sexual or asexual state has already been forecasted as an economically important pathogen of *H. brasiliensis* in Asian and African regions (Jayasinghe 1999).

Conidial morphology of *Pestalotiopsis* sp. detected from leaf blight specimens (in the present study) strongly resembled *Pestalotiopsis microspora* (Speg.) G.C. Zhao & Nan Li which caused similar leaf blight of *H. brasiliensis* in Cameroon (Ngobisa 2018). However, more detailed studies, including molecular analysis etc., are required to confirm the above. Nevertheless, *Pestalotiopsis* sp. is a common plant pathogen prevailing in both tropical and temperate climatic conditions and is regarded as a non-host specific opportunistic pathogen invading mature leaves of a wide variety of host plants. Although *Pestalotiopsis* sp. has been documented as a minor pathogen in *Hevea*, leaf blight disease caused by *P. microspora* affected 80% of rubber trees in smallholder's fields in South West Cameroon causing huge losses (Ngobisa 2018). The pathogen also affected 382,000 hectares of rubber plantations in Indonesia especially South and North Sumatra, Bangka Belitung as well as South, Central and West Kalimantan causing 15% reduction in rubber yield (Ngobisa 2018). Possibilities of more epidemic outbreaks in Sri Lanka due to *Pestalotiopsis* and *Fusicoccum* and precautions to be taken have also been highlighted (http://www.rrisl.gov.lk/annunce_t.php?id=101). In our study, *Pestalotiopsis* sp., which was tentatively identified as *P. microspora,* was observed in leaf spots and blights of very few progenies and its pathogenic role in development of spots and associated disease symptoms was also not confirmed. Systematic disease surveys supported by detailed pathological investigations are required to detect large-scale disease incidences due to *Pestalotiopsis* sp., if any, as many high-yielding clones possess unknown levels of tolerance to the pathogen.

The four high-yielding female parental clones of *H. brasiliensis viz*. RRII 105, RRII 414 and RRII 430 and their hybrids were susceptible to leaf spot associated with *P. capitalensis*. Hence, more resistant parents are to be identified and used in breeding for developing tolerant clones. Although other *Hevea* sp. (*H. camargoana* and *H. spruceana*) and wild germplasm (RO 380 and RO 2871) were asymptomatic and could be inferred as relatively tolerant to the leaf spot disease, more studies are required to ascertain their tolerance. Clone Fx 516 was found to be highly tolerant to the leaf spot disease as there was no single incidence of visually detectable leaf spot in any of the trees. Clone Fx 516 possesses tolerance to many other diseases including *Phytophthora* abnormal leaf fall and *Corticium* pink disease and has been used in breeding for resistance to SALB in Brazil and Sri Lanka (Senanayaka & Wuewantha 1968, Fernando & Liyanage 1975, 1980). Results from the present study also indicated that Fx 516 could be a potential parental clone for developing horizontal resistance to important fungal diseases in *Hevea*.

Conclusion

Until recently, three genera of fungal pathogens *viz. Pestalotiopsis, Neofusicoccum* and *Fusicoccum* have been mainly reported to cause leaf spot epidemics in *Hevea*. In the present study, *P. capitalensis* was consistently identified from leaf spots which seriously affected intra- and inter-specific hybrids as well as high-yielding clones of *Hevea*. Since *Phyllosticta* sp. has been forecasted as an economically important pathogen for Asian and African regions, findings from the present study assume more significance. Detailed follow-up studies are also required to confirm the primary pathogenic role of *P. capitalensis* in establishment and spread of leaf spots in order to develop appropriate disease management strategies. Although *Pestalotiopsis* sp. was associated with a minor incidence of leaf blight, its current sporadic occurrence in India merits continuous surveillance as this fungus has been declared as a quarantine pathogen in many rubber growing countries.

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