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Development of Brown root disease in Sri Lankan rubber plantations: possible involvement of other tree species

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Abstract

Brown root disease caused by Phellinus noxius is becoming an important root disease of Sri Lankan rubber plantations. Hence, this study was conducted to identify the alternative host species of the pathogen and to determine the role of those species in the incidence and the severity of brown root disease in rubber plantations. Seventy brown root disease incidences were used to assess the host range and the disease transmitting pattern. A structured questionnaire was used to collect the background information from the growers and the extension officers. In association with the roots of the diseased rubber trees, roots/root pieces of twenty six alternative species were identified and pathogen was isolated from seven alternative species only. The signs and the symptoms of the collar and the root systems of rubber and the associated tree species were recorded. The pathogenicity of those cultures were proved by Koch's postulates and the seven different isolates showed cross infection ability among the species from which they were isolated and with rubber. The seven isolates showed a variation in their morphological and growth characteristics. It was observed that in all the brown root disease incidences on rubber, the primary inoculum was from a non-rubber species and the land had been previously either under the forest or abandoned for years with trees and shrubs of forest origin. With the findings of the study, it was concluded that the infection of brown root disease to rubber in these areas has an association with the other tree species such as Cereya arborea, Gmelina arborea and Bridelia retusa in the root contact.

Key words: brown root disease, forest-origin tree species, *Hevea brasiliensis*

Introduction

Brown root disease caused by *Phellinus noxius* is one of the important root disease of rubber plantations in Sri Lanka. *Phellinus noxius* (Corner) G. Cunn. is reported to be a widespread pathogen in tropical countries of Southeast Asia, Oceania, Central America, Caribbean, and Africa, where it causes brown root rot in a variety of trees of all ages and health conditions (Singh *et al.*, 1980; Bolland 1984; Hodges and

Tenorio 1984; Neil 1986, 1988; Nandris *et al.*, 1987; Arentz and Simpson 1989; Bolland *et al.*, 1989; Dennis 1992; CABI/EPPO 1997; Chang and Yang 1998; Larsen and Cobb-Poulle 1990; Ann *et al.*, 2002; Brooks 2002; Albrecht and Venette 2008). The name brown root rot refers to a brown to black mycelial crust formed by the fungus on the surface of infected roots and stem bases (Chang & Yang 1998). *Phellinus noxius* is placed under the family of Hymenochaetaceae within the Phylum Basidiomycota. It is characterized by its brown fruiting bodies with no clamp connections in its vegetative hyphae. However, *P. noxius* is generally considered a white rot fungus because of its ability to degrade lignin, a basic component of wood (Adaskaveg & Ogawa 1990, Chang & Yang 1998). Most of the species within the genus *Phellinus* act as saprotrophs in nature or as weak pathogens on trees. Only very few species are pathogenic with strong virulence, and *Phellinus noxius* is one of the strongest among them.

Brown root disease has a wide host range including, most of the economically-important plantation and other crop species such as, *Camellia sinensis* (tea), *Coffea* spp. (coffee), *Artocarpus altilis* (breadfruit), *Cinnamomum* spp. (cinnamon), *Theobroma cacao* (cocoa), *Cocos nucifera* (coconut), *Garcinia mangostana* (mangosteen), *Citrus* spp. (citrus), *Mangifera indica* (mango), *Artocarpus heterophyllus* (jack), *Tectona grandis* (teak) and *Swietenia mahogani* (mahogany). *Phellinus noxius* spreads by root contact and persists in roots and stumps of infected plants for more than 10 years even after the death of the host (Chang 1996). The economic impact of *P. noxius* is highly variable and a loss of up to 60% has been observed in some other rubber growing countries (Nandris *et al.*, 1987).

The rubber industry is now being extended to non-traditional rubber growing areas in *Uva*, Northern and Eastern provinces of Sri Lanka. It has been observed that the disease incidence is much higher in these non-traditional rubber growing areas where the environmental factors are different and consequently the natural vegetation is also dissimilar to the traditional rubber growing areas.

In this background, the objectives of the current study were to identify the local host species of the disease and to evaluate the potential role of those species in the development of brown root disease in rubber plantations when planted in non-traditional areas.

Methodology

Collection and maintenance of isolates

Seventy brown root disease incidences, which have been reported to the Department of Plant Pathology and Microbiology, Rubber Research Institute of Sri Lanka, for a period of three years (from 2014 to 2016) were used for the study. These incidences were from different agro-ecological regions of the country.

In order to identify the possible alternative host species of the brown root disease of rubber, the presence of infected non-rubber species in the vicinity of the infected rubber trees was investigated. In each of the incidence, the firstly-diseased rubber tree was traced and the root system of the tree was exposed in order to find out the association of the roots of the neighboring trees with the respective rubber tree. Diseased root samples were collected from the other host species which were having root contacts with the diseased rubber tree. The signs and the symptoms of the collar and the root systems of both the rubber tree as well as the associated tree were recorded.

Koch's postulates and cross infection studies

In order to confirm the pathogenicity of the isolated fungi, Koch's postulates was proven with each isolated fungus. For the artificial inoculation, an artificial medium was used. In the medium, 100g of rice bran and saw dust (1:2 w/w) was used as the substrate of the fungus. The prepared medium was autoclaved for 45 minutes at 121ºC in polypropylene bags and the fungal isolate grown on MEA was used on the inoculation. Two agar blocks of 30 cm^2 from the advancing margin of the above cultures were transferred aseptically into each bag of autoclaved medium and incubated for 12 weeks at RT (28±2ºC) under dark conditions. Four six-month old healthy seedlings of each species were grown in pots in a greenhouse and artificially inoculated using the inocula prepared as described above. After four months of inoculation, signs and symptoms of the seedlings of each species were recorded and the pathogens were re-isolated onto MEA from the roots of the artificially inoculated seedlings.

The seven different isolates were tested for the cross infection ability among the species from which they were isolated as well as with rubber. The same artificial inoculation technique which was used to prove the Koch's postulates was used for this study. All the seven isolates from alternative species were tested for the ability to infect four six-month old rubber seedlings and a *Phellinus noxius* isolate obtained from rubber (which was in the culture collection) was tested for the ability to infect four six-month old seedlings of the each respective species. After four months of inoculation, signs and symptoms of all seedlings were recorded and the fungus was re-isolated onto MEA from the roots of the artificially inoculated seedlings.

Cultural characteristics and the rate of growth

For the cultural characteristics and the rate of growth studies, a 9.0 mm mycelial plug taken from the advancing margin of a three-day old culture of the test isolate was placed at the center of Petri plate. The plates were incubated at room temperature under 12 hours light and dark regimes.

Growth of the isolates was evaluated on four media; Malt extract agar (MEA), Potato dextrose agar (PDA), Czapec-dox agar (CDA) and Lima bean agar (LBA) (Oxoid). The growth was determined after 04 days by measuring colony diameter along two perpendicular lines of each Petri plate. Five replicates were used in the experiment.

Cultural characteristics were evaluated on MEA (Oxoid). Coloration, fluffiness and the density of the cultures were

recorded after four days of inoculation. Ranks from 0-5 were given for the brownish colouration developed in the culture, as the rank zero and five being the lowest and highest colourations of the cultures respectively. The fluffiness was ranked from 1-4 as the rank one and four being the lowest and highest fluffiness respectively. The density was ranked from 1-3 as the rank one and three being the lowest and highest densities of the cultures respectively. Five replicates were used in all experiments.
The fungal cultures were subjected to identification based on the cultural characteristics after four days of inoculation. Commonwealth Mycological Institute (CMI) description sheets were used as a guide to identify the isolated fungus.

Studies on the land history and associated species

In order to identify the mode of disease spread and the origin of the pathogen innoculum, the species history of the land was investigated in all seventy disease incidences. Moreover, information was gathered on the cut-trees having root contacts with the firstly-diseased rubber tree. A structured questionnaire was used to gather the information.

Results and Discussions

The host species reported

Decayed roots of twenty six species which were having root contacts with the diseased rubber trees were identified. The pathogen isolates could be obtained from seven alternative species (Table 1).

Table 1. P. noxius *isolates collected from species other than rubber*

Name of the isolate	Host species	Location	Agro-ecological zone
AH 1	*Cereya arborea* (‘kahata’)	Warakapola, Sri Lanka (7°08'22.8"N 80°14'04.2"E)	WL2b
AH 2	*Gmelina arborea* (‘eth demata’)	Badalkumbura, Sri Lanka (6°55’1.165"N 81°13'31.246"E)	IM2b
AH 3	*Bridelia retusa* (‘keta kela’)	Bulathkohupitiya, Sri Lanka (7°05' 27.838"N 80°20' 9.449"E)	WL1a
AH 4	*Mangifera indica* (mango)	Moneragala, Sri Lanka (6°53' 30.663 "N 81°18' 31.948"E)	IL1c
AH 5	*Artocarpus heterophyllus* (jack)	Gampaha, Sri Lanka (7°06 2.652 "N 79°59'42.359"E)	WL3
AH 6	*Tectona grandis* (Teak)	Hopton, Sri Lanka (6°59' 31.56"N 81°11'55.68"E)	IL1c
AH 7	*Cinnamomum zeylanicum* (Cinnamon)	Polgahawela, Sri Lanka (7°20’ 26.967”N 80°16’ 41.978”E)	IL1a

In addition to the above hosts, nineteen species were reported to be present in association with the firstly-diseased rubber tree (Table 2). The decayed root pieces were present *in situ* having root contacts with the diseased rubber tree(s). However, the pathogen isolates could not be obtained from them, as the root segments were too decayed.

Symptomatology of the disease on rubber and alternative hosts

Slow plant growth, yellowing and wilting of leaves, defoliation and branch dieback were the major above-ground symptoms (Figs. 1a & 1b). Roots of rubber infected with *P. noxius* initially exhibited a brown discoloration of the wood just beneath the bark (Figs. 1c). On the roots, tawny brown gummy mycelia were observed firmly fixed to the outer bark surface with an encrustation of sand and stones on the root surface. They were identified as the characteristic diagnostic symptom of *P. noxius* on rubber (Fig. 1d). The dead wood becomes white, dry, and honey-combed (Fig. 1e). Bracket-like hard fructifications are rarely observed on the basal trunk of diseased trees. The fructification are dark brown on the upper surface and dark grey on the lower surface (Fig. 1f & 1g).

Table 2. *Forest tree species observed in association with the firstly-diseased rubber tree*

Serial no	Host species	Vernacular name
1	*Elaeocarpus* spp.	Weralu
2	*Artocarpus nobilis*	Wal del
3	*Michelia champaka*	Ginisapu
4	*Swietenia mahagoni*	Mahogani
5	*Stereospermum personatum*	Lunumidella
6	*Stereospermum chelonoides*	Palol
7	*Adina cordifolia*	Kolon
8	*Vitex pinnata*	Milla
9	*Chloroxylon swietenia*	Burutha
10	*Schleichera oleosa*	Kon
11	*Gliricidia sepium*	Gliricidia
12	*Ficus religiosa*	Bo
13	*Albizia odoratissima*	Sooriya Maara
14	*Syzygium assimile*	Domba
15	*Semecarpus coriaceae*	Badulla
16	*Hybanthus enneaspermus*	Makulla
17	*Schleichera oleosa*	Khone
18	*Macaranga peltata*	Kenda

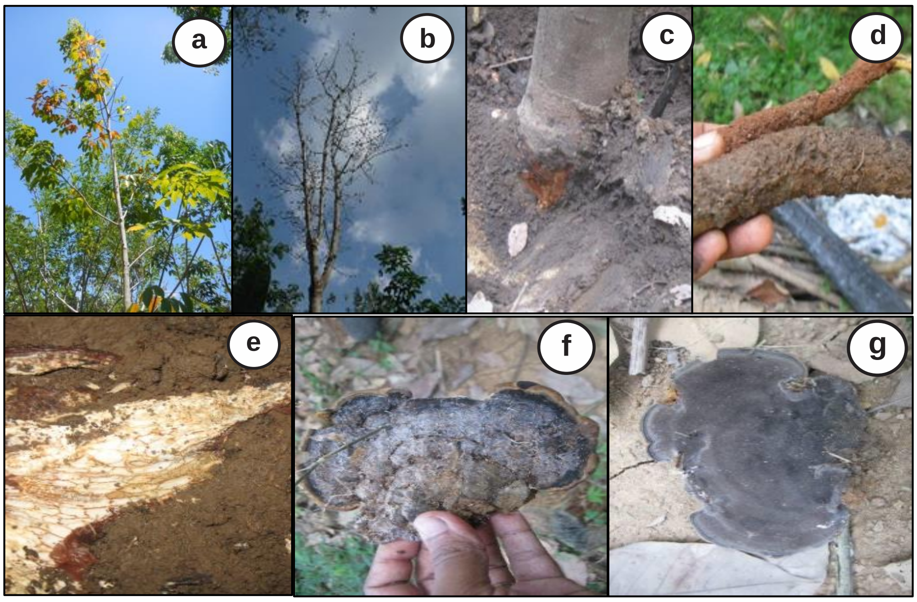

Fig. 1. Signs and symptoms of the brown root disease on rubber

As the disease incidences were reported at the latter stages of the infection of the rubber trees, all the associated species were diagnosed to have infected after the death. Therefore, no foliar symptom could be identified from those species. However, encrustation of sand and stones on the root surface could be observed due to the gummy rhizomorphs firmly fixed to the outer bark surface (Fig. 2a). The dead wood had become dry and honey-combed (Fig. 2b). Infected roots had exhibited a brown discoloration of the wood just beneath the bark (Fig. 2c). The inner bark was covered with the white to brownish mycelial mat (Fig. 2d). Fructifications were observed on the basal trunk of only two species *Cereya arborea* and *Stereospermum personatum* (Fig. 2e).

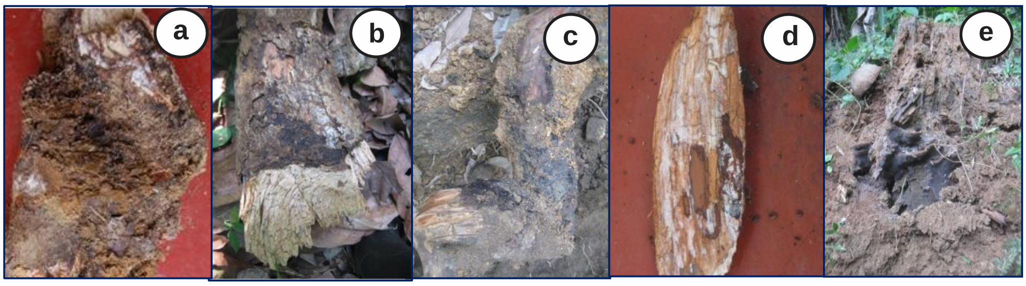

Fig. 2. Signs and symptoms of the brown root disease on non-rubber species

Koch's postulates and cross infection studies

Signs and symptoms of the plants of the seven species resulted after the Koch's postulates were similar to those observed under the natural conditions. As the above-ground symptoms, the turning of the leaves to yellowish brown color and in advanced stages, shedding of the leaves and die-back of the affected branches. The roots were covered with tawny brown gummy rhizomorphs and the encrustation of sand and stones on the root surface was also observed. The cultural characteristics of the re-isolated fungus was similar to those of the original isolate. Moreover, the seven different isolates showed cross infection ability among the species from which they were isolated as well as with rubber.

Growth rate of the isolates

The growth of seven different isolates showed variability on different culture media. Based on the growth rate, MEA was selected for the colony morphology studies. Isolates AH1 and AH2 showed a fastest growth on all media tested (2.14 cm/day and 2.13 cm/day respectively on MEA) while the isolate AH4 showed the slowest growth rate (1.8 cm/day) on MEA (Fig. 3).

Morphological variation of the isolates

Colonies grown in the laboratory showed distinctive raised brown and white plaques (patches) characteristic to *Phellinus noxius* and produced arthrospores, which are asexual spores formed by the division of special hyphal segments. The fungus was identified as *Phellinus noxius*: the causative fungus of the brown root disease of *Hevea brasiliensis* with the guidelines of the Commonwealth Mycological Institute (Pegler and Waterston, 1968).

The different isolates of *Phellinus noxius* showed variations in colony morphology (Fig. 4).

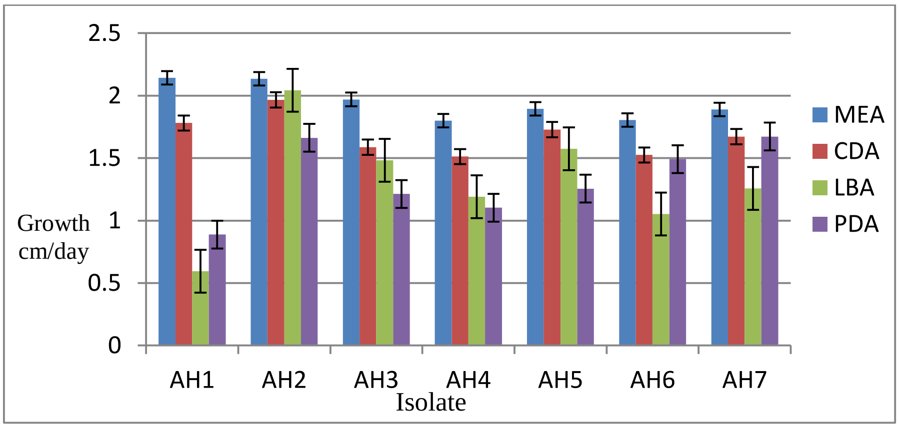

Fig. 3. Growth rates of the seven *Phellinus noxius* isolates on different culture media MEA, CDA, LBA and PDA (Error bar represents the variation around the mean values)

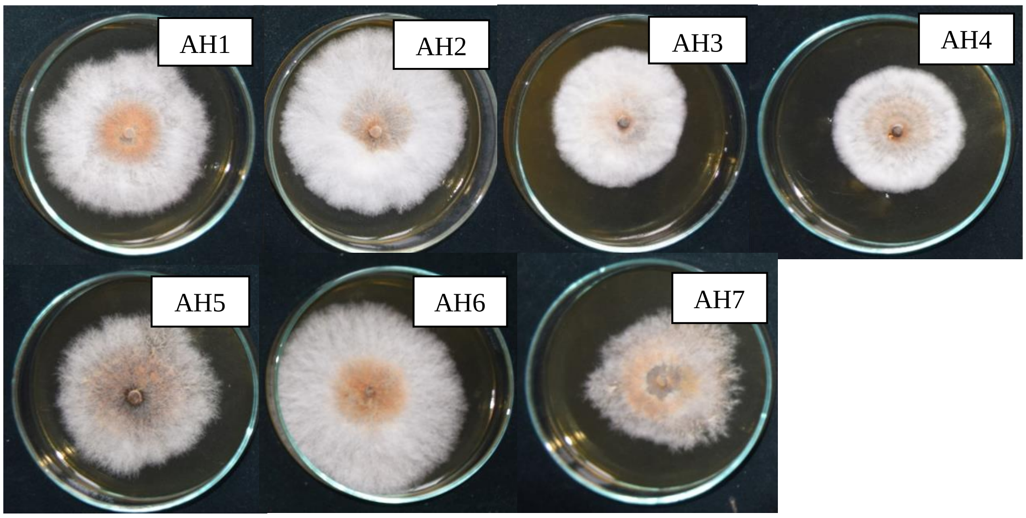

Fig. 4. Variability in cultural morphology *Phellinus noxius* - 03-day old cultures

Land and root zone studies

Seventy brown root disease records on rubber were received from various parts of Sri Lanka during the study period. According to the information, it was observed that the initially diseased rubber tree had a root contact with a diseased root of some other species in all seventy disease incidences. When the host species profile is considered, except for a few cultivated species such as cinnamon, mango and teak, almost all the other species were of forest-origin. Moreover, it could be observed that even these three species were not intentionally-planted in these lands and had a wild origin. When the species history of these lands was considered, it was noticed that 11 (84.28% of the total) of the reported incidences being rubber established after clearing native forests. The rest of the lands has been abandoned for years with trees and shrubs of forest origin or having an neighboring forest land by which the rubber trees can get root contacts.

When E. J. Corner described *Fomes noxius* (currently *Phellinus noxius*) as a new species in 1932, he mentioned that it was usually found in cleared or disturbed areas. Since the beginning of the 20th century, many plantations of rubber, tea, cocoa, coffee, oil palm, and mahogany established on cleared forest sites had been damaged or destroyed by *P. noxius* (Pegler *et al.*, 1968). According to these facts, it can be expected that the innoculum is present in native tropical forests of Sri Lanka. New infection centers may have been initiated when roots of the newly planted trees make contact with infected stumps or other woody debris of cleared native forest.

When the features of land preparation is concerned, it could be observed that all these rubber cultivations had been established after a minimum land preparation and consequently, roots of forest species have not been uprooted (Fig. 5).

Fig. 5. Infected roots and stumps of forest species in rubber clearings: note the disease signs and symptoms on roots of non-rubber species

Furthermore, in 67 incidences (95.71% of the total), the disease was from the smallholder rubber fields, where rubber is planted after a minimum land preparation or rubber is planted in mixed cropping systems with some other crop or garden species.

According to the past records in Sri Lanka, brown root disease causing fungus was of very common occurrence in the earlier days of rubber planting in Ceylon, when rubber was inter planted in cocoa and tea fields. The dead stumps remained after the removal of cocoa and tea by cutting off the bushes and leaving the stumps, the rubber trees later get infection (Advisory Circular of RRISL, 1954). The initial infection occurs when roots of the newly planted trees make contact with stumps or other woody debris that contain the fungus (source of inoculum). Further spread from the initial infection centers is through root contact. *P. noxius* can persist in the roots and stumps of infected plants for more than ten years after the death of the host (Chang, 1996).

Conclusion

Based on these facts, it could be concluded that the infection of brown root disease to rubber in the studied locations in Sri Lanka was found to be due to root contact with diseased other tree species. Moreover, it was found that the innoculum was present in native forests on infected roots or woody debris. The results of this investigation indicated a variation in morphological and physiological characters and symptoms amongst the population of *Phellinus noxius* isolates from non-rubber species in Sri Lanka.

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