Wood Rot Associated Fungal Species in Low-Country Live-Wood Termite (*Glyptotermes dilatatus*) Infestation in Susceptible (TRI 4042) and Tolerant (TRI 3055) Tea Cultivars

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Abstract

The Low-Country Live-Wood Termite (*Glyptotermes dilatatus*) is a major pest of tea cultivation in the Low Country Sri Lanka. They attack mostly on unhealthy tea bushes with die-back followed by wood rot. Understanding of the relationship between wood rot associated fungal species and LCLWT infestation is important to formulate alternative methods for managing LCLWT. This study was undertaken to identify wood rot associated fungal species in tea and to determine the relationship between wood rot associated fungal species and LCLWT infestation. The fungi from three stem portions of tea, both on LCLWT infested and non-infested susceptible and tolerant cultivars were isolated on sterilized PDA medium and identified following an identification key. Thirty nine fungal species were isolated from susceptible cultivar (TRI 4042). Among them, 81% was isolated on infested stems and 19% was isolated from non-infested stems. Many fungal species (39) were isolated from tolerant cultivar (TRI 3055). Among them, 38% was isolated from infested stems and 35% was isolated from non-infested stems. *Acremonium* spp. was the most prominent fungal species in both infested non-infested susceptible and tolerant cultivars followed by *Verticillium* spp and *Nectria* spp. There was high variation among fungal species isolated from tolerant and susceptible cultivars as well as infested and non infested stems. Remarkable difference was observed in the numbers of fungal species, isolated from infested and non-infested stems in susceptible cultivar. Number of fungal species isolated from the wood closer to the pruned surface was higher in both infested and non-infested branches of susceptible cultivar. Contrasting difference between fungal species in infested and non-infested bushes of susceptible cultivar provide a clue to develop new control strategies for LCLWT.

Keywords: *Glyptotermes dilatatus,* Low-Country Live-Wood Termite, TRI 3055, TRI 4042, Wood rot

Introduction

Tea [*Camellia sinensis* (L) O. Kuntze] is one of the major plantation crop grown in Sri Lanka. Among the key pests of tea, Low-country Live-wood Termite *(Glyptotermes dilatatus,* Bugnion and Pop off, 1910) is the predominant one (Vitharana *et al.,* 2003). LCLWT is mainly distributed at low tea grown areas approximately below 1000 m elevation. As the colony population increase and gallery system of advanced spreads, gallery would extend into conducting tissues. This results in wilting of single branches with the mature leaves acquiring a coppery texture. Old fields which are heavily infested should immediately enter a programme of replanting. If the LCLWTs' galleries are detected in branches in young VP clearings it should be cut progressively until healthy wood is exposed, thereby removing the colony completely (Vitharana and Mohotti, 2008).

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The natural process of healing wound in tea is very slow. Every tea bush undergoes pruning at 3 years intervals. So tea bush has parts of its body frame badly rotted. This condition is commonly called Wood Rot. Wood Rot weakens the affected branch and breaks it easily. (*Camellia sinensis*, 2011). Rotted wood resulting from pruning, sun-scorch damage, fungal damage such as canker and shot-hole borer damage are normally favours to LCLWT attack.

In a preliminary study, a group of fungal species were isolated from LCLWT attacked tea of a susceptible cultivar TRI 4042 (Menaka, 2011). However, this study has not covered to understand the role of Wood Rot associated fungal species in LCLWT and their infestation levels in both susceptible and tolerant cultivars. As the role of Wood Rot associated fungal species in LCLWT is important to formulate new and alternative control methods for LCLWT, the present study was planned to Identify fungal species associated with wood rot of tea and to find out the Wood Rot associated fungal species in LCLWT infestation.

Materials and methods

Study site and sample collection

The experiments were carried out at the Entomology laboratory of the Tea Research Institute, Low country station, Rathnapura, from July to December 2012. St. Joachim Tea Estate, Ratnapura was selected for the sample collection. TRI 4042 selected as the susceptible cultivar and TRI 3055 selected as the tolerant cultivar for the LCLWT. Twelve year old tea bushes were chosen to obtain stem parts. Among 50 tea bushes from each tolerant and susceptible cultivars, 10 termite infested and non-infested (only with wood rot) stem parts were collected randomly.

Isolation of fungi from termite infested and non-infested tea stems

Only one stem was select from each sample (TRI 4042/ Infested, TRI 4042/Non-infested, TRI 3055/Infested, TRI 3055/Non-infested). Stems were divided into three portions as top (near to prune cut), middle and bottom (near to healthy wood). Five pieces were taken from each stem parts. The pieces were cleaned in tap water and then submerged in sterilized distilled water for 30 seconds followed by, 30 seconds in 95% ethanol, 30 seconds in 0.05% NaOCl and finally 30 seconds in 70% ethanol. The samples were kept on a sterilized filter paper to drain excess moisture and cultured on sterilized PDA medium and incubated at 27 °C for 7-10 days.

Slides containing fungal particles of the fungi isolates were prepared using sticky tape method and observed under the microscope and were identified using identification key up to genus level and Koch's postulate was performed to prove the pathogenicity.

Percentages of study of isolated fungi

The percentage of occurence of fungi from each sten was calculated as follows.

$$\% \text{ of occurrence} = \frac{\text{No. of stem pieces produced colonies}}{\text{Total no. of stem pieces}}$$

Result and discussion

Totally, thirty nine fungal species were isolated from both infested and non infested susceptible and tolerant cultivars. From the isolated fungi species, seven species were identified using identification key up to the genus level. Three species were not identified, since they had unknown spore structures. Twenty nine non-spore forming species were not able to identify. Among non-spore forming unidentified species, twenty species produced whitish and nine species produced blackish mycelia on PDA.

Table 1. Isolated fungal species and their percentage occurrence

No of species	Tolerant		Susceptible	
	Infested (%)	Non-infested (%)	Infested (%)	Non-infested (%)
	26	6	15	14
Acremonium spp. large	26.6	20	40	33.3
Acremonium spp. small	13.3	6.6	33.3	6.6
Nectria spp.	20	0	6.6	20
Morteilla spp.	6.6	6.6	0	6.6
Fusarium spp.	13.3	0	0	13.3
Verticillium spp.	6.6	6.6	26.6	13.3
Aspergillus spp.	0	0	13.3	33.3
Unidentified spp.1	0	0	6.6	0
Unidentified spp.2	0	0	6.6	0
Unidentified spp.3	0	6.6	0	0
Un.N.W.spp.1	6.6	0	6.6	13.3
Un.N.W.spp.2	0	6.6	0	0
Un.N.W.spp.3	0	0	6.6	26.6
Un.N.W.spp.4	6.6	0	6.6	13.3
Un.N.W.spp.5	6.6	0	0	6.6
Un.N.W.spp.6	0	0	13.3	0
Un.N.W.spp.7	6.6	0	0	0
Un.N.W.spp.8	6.6	0	0	0
Un.N.W.spp.9	0	0	0	6.6
Un.N.W.spp.10	0	0	0	6.6
Un.N.W.spp.11	0	0	0	6.6
Un.N.W.spp.12	0	6.6	0	0
Un.N.W.spp.13	0	0	0	0
Un.N.W.spp.14	13.3	0	0	0
Un.N.W.spp.15	6.6	0	6.6	0
Un.N.W.spp.16	0	0	0	6.6
Un.N.W.spp.17	0	13.3	13.3	0
Un.N.W.spp.18	6.6	0	6.6	0
Un.N.W.spp.19	6.6	0	0	0
Un.N.W.spp.20	0	6.6	0	0
Un.N.B.spp.1	13.3	6.6	0	0
Un.N.B.spp.2	20	0	13.3	0
Un.N.Bspp.3	13.3	0	0	0
Un.N.B.spp.4	6.6	0	6.6	0
Un.N.B.spp.5	13.3	0	0	0
Un.N.B.spp.6	13.3	0	0	6.6
Un.N.B.spp.7	13.3	0	6.6	0
Un.N.B.spp.8	13.3	0	0	0
Un.N.B.spp.9	6.6	0	0	0

The isolated identified fungal species were *Acremonium* spp. Large, *Acremoniuam* spp. Small, *Nectria* spp., *Verticillium* spp, *Fusarium* spp., *Motriella* spp. and *Asppergillus* spp.

The unidentified species with unknown spore structures were named as unidentified spp.1, spp.2 and spp.3. The unidentified non-spore forming whitish species were named Unidentified non-spore forming whitish spp.1 to 20. The unidentified non-spore forming blackish species were named as unidentified non-spore forming blackish spp.1 to 9. Those unidentified non-spore forming species were categorized according to their culture characteristics.

The highest number of fungal species (26) were identified on infested susceptible cultivar. It is 81.2% from the total number of fungal species identified on susceptible cultivar. The lowest number of fungal species (6) were identified by non-infested susceptible cultivar (18.7%) from total number of fungal species which were identified on susceptible cultivar. There is a difference between numbers of fungal species isolated from infested and non-infested stem pieces in susceptible cultivar. Many non-spore forming blackish fungal species (9) were isolated from susceptible cultivar infested stem pieces. Hence, there is a doubt whether there is a relationship between fungal species which cause to wood rot (non-infested) and LCLWT infestation in susceptible cultivar.'

When considering tolerant cultivar, there is no difference between numbers of fungal species isolated from infested (15 species) and non-infested (14 species) stem pieces. But there is a difference between fungal species isolated from infested and non-infested stem pieces in tolerant cultivar.

Acremonium spp. was the most prominent fungal species as it was identified on both susceptible and tolerant cultivars of infested and non-infested stem parts. The genus *Acremonium* currently contains 100 species, of which, most are saprophytic, being isolated from dead plant material and soil. A number of species are recognized as opportunistic pathogens of man and animals, causing mycetoma, onychomycosis, and hyalohyphomycosis, those include *A. falciforme, A. kiliense,A. recifei, A. alabamensis, A. potroni, A. roseo-griseum* and *A. strictum*. However, many previous studies identified *Acremonium* species only to the genus level. Further studies are required before concluding *Acremonium* spp is specific for wood rot in tea. According to Menaka, (2011) *Acremonium* spp was isolated from termite body, gut and termite galleries too. Because some termites prefer certain fungi while others do not prefer those. If *Acremonium* spp was a preferred fungi by termites, there is a possibility to formulate new control method for LCLWT. When using isolated fungi for the control of LCLWT, we need to check whether those fungi are symbiotic, synergistic, antagonistic, pathogenic or parasitic with them.

Every fungus cannot penetrate via lignin layer of the stem. Those who can penetrate lignin layer, infect the stem first and go through it. After that, other fungi are able to penetrate via cellulose and infect the stem. The fungi isolated near to the healthy wood may be lignin feeding fungi. Their feeding behavior is needed to understand in future studies.

Conclusion

Among fungal species isolated, the most prominent fungal species is *Acremonium* spp. The number of fungal species isolated from susceptible and tolerant cultivars varied. Number of fungal species isolated from infested tea bushes was remarkably high

compared to non-infested bushes of susceptible cultivar. There is a similarity between number of fungal species isolated from infested and non-infested bushes in susceptible cultivar. Number of fungal species isolated from the wood closer to pruned surface was higher in both infested and non-infested bushes of susceptible cultivar. Infection by different fungal species was more or less equally distributed among three layers near to prune cut, middle of stem and near to healthy wood on both infested and non-infested bushes of tolerant cultivar.

References

Menaka PH 2011 Estimation of fungal associations with the Low-country live-wood termitefor potential application in IPM program B Sc Thesis University of Ruhuna

Vitharana SI 2003 75 Years of Research and Development in Tea Entomology, Acarology and Nematology", in Modder (eds) Twentieth Century Tea Research in Sri Lanka Tea Research Institute Sri Lanka :111-150.

Vitharana SI and Mohotti KM 2008 Management of Tea Termites. Handbook on Teain Zoyza AKN (eds) Tea Research Institute Sri Lanka 231-238.