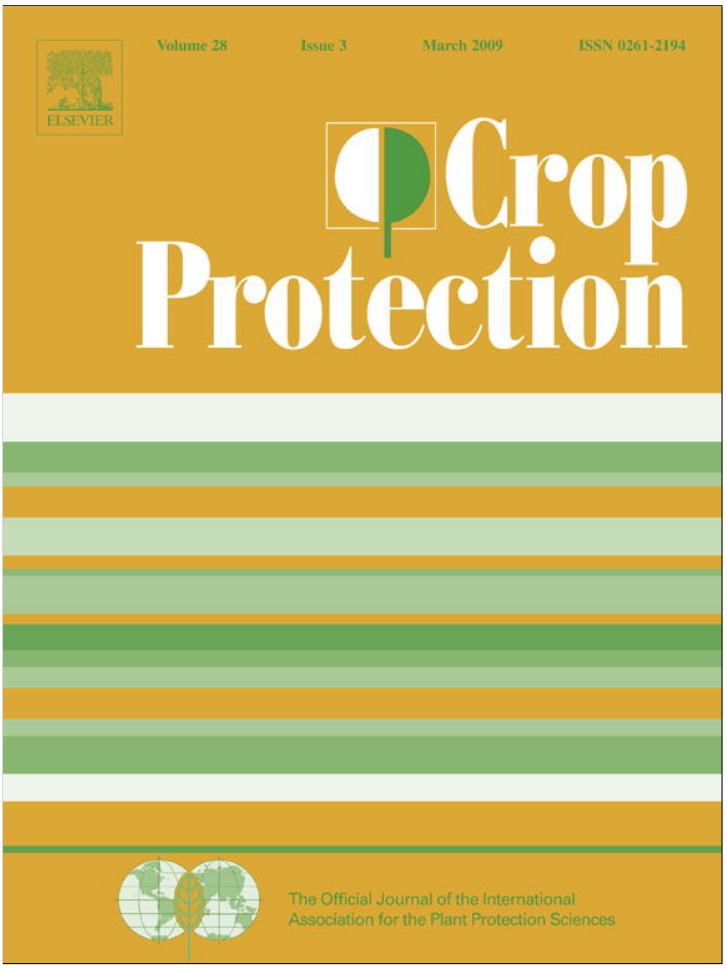

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Some spatial, temporal and spatio-temporal considerations of wood decay of tea (*Camellia sinensis*), caused by *Nemania diffusa* (Syn. *Hypoxylon vestitum*)

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ABSTRACT

Tea stem wood decay caused by *N. diffusa* is confirmed here to be a soft rot. The activity of periodic pruning of tea bushes contributed to dissemination of disease through the contaminated pruning knife. Once contaminated, the infection can spread linearly in either direction at approximately 1.2 cm per annum. This compared well with the rates experienced under *ex situ* inoculation tests, which worked out to be 1.7 cm per annum.

Field resistant tea cultivars recorded a relatively lower number of fibre cells than the field susceptible cultivars. The natural susceptibility (decay potential) of tea stem wood due to *N. diffusa* infection was around 69 g cm^{-3} yr^{-1}, with the capacity to cause *in vitro* wood loss/decay in the range of 62–75%.

Under natural field conditions, long-term infections accounted for an increase in disease score of about 0.3 for every 1 m rise in altitude starting from a base level of 1500 m, commensurate with a rate of increase of disease of approximately 0.01% m^{-1} rise per year.

1. Introduction

Many species of *Hypoxylon* have been reported as either associated organisms or ones capable of inflicting heavy casualties in tea. The first record of *Hypoxylon vestitum* in Sri Lanka was by Petch in 1924, and much later in 1991 (Arulpragasam and Balasuriya, 1991) as a spreading disease. India reported this as a disease in certain tea districts in 1935 (Anon, 1935). Much later, Kenya (Laycock, 1978; Onsando, 1985) and Malawi (Rattan, 1988) reported similar disease situations separately. Ju and Rogers (1996) after an exhaustive coverage of all the known *Hypoxylon* species described them as being weak to damaging pathogens. *H. vestitum* was renamed as *Nemania diffusa* (Sowerby) by Ju and Rogers (1996).

Infection of various host plants by *Hypoxylon* spp., probably takes place by germinating ascospores or possibly by conidia through wounds (Venkata Ram, 1970a,b; Alexopoulos and Mims, 1979). The fungus enters the aerial parts of the tea bush through pruning cuts or sun-scorched lesions, rendering the main stem and branches either partially or wholly defunct (Venkata Ram, 1974). The fungus can occur a distance away from the necrotic lesions in the wood of branches that show symptoms, and therefore, it can be disseminated from one bush to another by direct transfer of mycelium through pruning knives (Otieno, 1993).

Decay by fungi can happen in wood in service, in standing trees and logs and branches on the forest floor. The principal decay types under all these situations are similar although the causal fungal species may differ (Cartwright and Findlay, 1958). Brown rotted wood is reddish brown to dark brown in appearance and soft to some depth when wet. Brown rot fungi are unable to degrade lignin (Davidson et al., 1938). The white rot fungi too grow principally in the wood cell lumina. The third, 'soft rot' decay occurs under conditions where the growth and activities of the generally more active and competitive basidiomycetes fungi are retarded (Eaton and Hale, 1993). Soft rot fungal attack differs from that of basidiomycetes as the fungi grow in the S_2 layer of the secondary cell wall, forming chains of cavities with pointed ends, which are arranged in a helix parallel to the orientation of cellulose microfibrils (Savory, 1954).

The most fundamental aspect of wood decay assessment is the direct examination of the arrangement of columns of decay and discolouration in the internal wood matrix (Rayner and Boddy, 1988). These patterns can be recorded by drawing, photography or by using computerised techniques (Mercer, 1979; Blanchette, 1982). The distribution of fungal hyphae in decomposing wood can be readily ascertained using routine sectioning and staining methods (Wilcox, 1964).

Weight loss remains the most common index of the state and the rate of rotting. Here, either the percentage loss of weight as compared with an undecayed sample or the percentage of original weight remaining is used as the measure of decay (Rayner and

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Boddy, 1988). For situations where original dry weights are not available, Christensen (1984) used the density of wood to quantify the amount of decay.

This paper studies the mode of disease dissemination, the rate of spread within individual branches, the rate of wood decay, and the rate of dissemination of wood decay under natural field conditions of large tea gardens.

2. Materials and methods

2.1. Pruning knife as a means of pathogen dissemination (spatial)

Cotton wool swabs of about 1 cm diameter and the instruments like forceps, lances, needles, *etc*., were wrapped in aluminium foil and oven sterilized at 180 °C for 20 min. A formaldehyde solution (1%) was used to surface sterilize the instruments in the field. Fresh cuts were made across infected stems using a pruning knife to contaminate it with the inoculum. The entire blade of the pruning knife was cleaned, dipping in 1% formaldehyde solution and wiping it dry with cotton wool before and after the pruning of each individual bush. Immediately following cutting, the cutting edge of knife was swabbed with a fresh, sterilized cotton wool plug. The swabs were transferred immediately on to the agar surface in petri plates, applying a slight pressure to ensure a close touch between the two surfaces. Three swabs were placed in one plate at equidistant points. This activity was repeated 18 times (in six petri plates) across infections and 3 times using a sterilized knife blade as controls. The plates were incubated for 5 days at 25 °C to ensure the full complement of fungal colony development. Most of the prominent colonies were sub-cultured for confirmation of the identity of fungi. This study was repeated in the two estates, Nuwara Eliya (6°57′59″N, 80°45′30″E) and Diyagama West (6°50′26″N, 80°42′20″E).

2.2. Internal spread of natural decay in intact branches (spatio-temporal)

Tea branches that were at the end of second pruning cycle and were therefore, approximately 10 years (the estate was on 5 year pruning cycles) with active infections were removed with the entire infection. Care was taken to include only those with a centrally located clear entry point, which constituted a previous pruning cut. Detached branches were brought to the laboratory and sawed into two equal longitudinal halves. The internal spread of infections was determined by measuring the length and the breadth of decay, following the natural discolouration patterns. When the cuts were fresh, the progressive fronts of the patches were easily measurable in each direction (towards base and top) separately, starting from the point of entry.

2.3. Rate of spread of infection following ex situ inoculation (temporal)

The isolate of *N. diffusa* obtained from Nuwara Eliya Estate was grown on Czapek Dox Agar (CDA). Healthy tea bushes of the cultivar K 145, which were approximately 20 years old, located in St Coombs Estate were selected as the host. Inoculum by way of mycelial discs, cork borer No. 6 (10 mm diameter) obtained from the edge of a 2-week-old culture was placed on the freshly cut surfaces of a branch wound, about 2 cm in diameter. This was repeated 6 times. The inoculations were protected from rain with white polythene strips for a period of 1 month. Regular observations were made at monthly intervals until the branches/snags were removed for examination at the end of 1 year.

2.4. Rate of wood decay (temporal)/inoculation of miniature wood blocks

Miniature wood blocks ($30 \times 20 \times 5$ mm^3) of two field resistant (TRI 2025 and HS 10) and two field susceptible (PK 2 & K 145) cultivars (Arulpragasam and Balasuriya, 1991) and a standard in Birch (*Betula pendula*) wood of approximately 1 g each were oven dried overnight at 105 °C for their dry weight measurements. Three wood blocks of each cultivar were placed on sterile plastic meshes, and rested on 1-week-old cultures of *N. diffusa*, grown on mineral nutrient agar (MNA) (Gersonde and Kerner-Gang, 1975) as modified by Gray (1983). pH of the MNA medium was adjusted to 5.0 using either diluted H_2SO_4 or NaOH. Miniature wood blocks were incubated at 22 °C for 8 weeks. Wood blocks were oven dried overnight, at 105 °C for the dry weight measurements. Data were analysed using ANOVA and DMRT.

2.5. Decay potential (temporal)/Inoculation of stem sections

Stem sections of two cultivars, one highly susceptible (PK 2) and one highly resistant (HS 10) were used. Freshly harvested immature (where the colour of wood had just turned brown, either in secondary or tertiary branches, normally <1 cm in diameter) and mature (where the colour of wood was firm brown, primary or tertiary branches, normally ≥ 2 cm in diameter) stem sections of about 10 cm long were placed inside conical flasks and autoclaved at 1.05 kPa (15 psi) for 20 min. The fresh weights of stem sections were obtained prior to autoclaving and were adjusted to dry weight basis by oven drying reference samples from each category overnight at 105 °C. The density of wood was obtained by measuring respective volumes, immersing reference stem sections in water in a measuring cylinder.

Autoclaved stem sections were inoculated using mycelial discs of cork borer No. 2 (5 mm) cut from the margin of 7-day old *N. diffusa* colonies grown on CDA. The test included six stem sections each in the immature category and three sections each in the mature category. These were incubated over a period of 10 months at room temperature (approximately 25 °C). At the end of incubation, the inoculated samples were re-moistened inside the flasks and incubated for a further week to confirm their viability. These were re-autoclaved before oven drying overnight at 105 °C for final dry weight measurements.

2.6. Anatomy of decay (spatial)

Thin sections were obtained of the four tea cultivars using the miniature wood blocks of similar weight and dimension, as above. The wood blocks were imbibed with water under 700 mm Hg pressure for 30 min. Transverse and tangential thin sections of 25 μm were made using a sledge microtome (Spencer Lens Co.). Thin sections were transferred on to moistened filter papers, running into several alternating layers and were placed inside 9 cm glass petri plates. A wet filter paper was placed on top covering the stack. These petri plates with lids were wrapped in aluminium foil before autoclaving at 1.05 kPa for 20 min (Lundstrom, 1970).

Fresh cultures of *N. diffusa* and *Chaetomium globosum*, a standard soft rot fungus (Eaton and Hale, 1993) were grown on 2% malt extract agar (MEA). Since the *N. diffusa* did not produce spores on culture medium, the fungal mycelium was physically shredded along with the agar medium in the presence of a small amount of sterile distilled water (SDW) to make a uniform inoculum on test agar plates. With *C. globosum* a spore suspension (2.5×10^5 ml^{-1}) in SDW was used. Freshly prepared MNA plates were inoculated by streaking their surfaces with loops containing either inoculum. The plates were incubated at 22 °C for 5 days prior to introduction of thin wood sections. Four thin stem sections, one from each from the

four cultivars, were transferred onto four sectors of the surface of each plate containing MNA medium.

Harvesting of thin stem sections commenced after a week and continued at weekly intervals up to 5 weeks. They were preserved immediately in FAA (6%–40% formaldehyde, 4% – glacial acetic acid, 90%–70% ethyl alcohol) (6:4:90). When all the sections were harvested, they were first washed with 50% ethyl alcohol and double stained successively with 1% aqueous safranine for 2–3 min and cotton blue in lactophenol for 1–2 min. The sections were destained using a series of ethyl alcohol at 50%, 70%, 90% and 100% strength. They were then fixed using about two drops of 'euparal' essence on a microscopic slide, mounted with 'euparal' mounting medium and covered with a cover slip. Mounted sections were kept in position with the aid of clothes pegs and dried on a hot plate at 40 °C for about 3 days before observing under microscope ×400 magnification. Five field views were examined in each treatment for the count of soft rot attack in fibre cell walls using the following key as modified from Wyles (1987):

1. 1–2 Pin-pricks	6. Many pin-pricks and a few small cavities
2. 3–4 Pin-pricks	7. Many pin-pricks and many small cavities
3. 5–6 Pin-pricks	8. Many pin-pricks and a few large cavities
4. >6 Pin-pricks	9. Many pin-pricks and many large cavities
5. Few pin-pricks and a few small cavities	

2.7. Natural incidence and spread (rate) of disease due to N. diffusa in Nuwara Eliya Estate (spatial and temporal)

Two fields with natural infections were selected in the Nuwara Eliya Estate (6°57′59″N, 80°45′30″E), Field 3 of the upper division, (altitude 1880 m) and Field 8A of the lower division (altitude 1740 m). General slopes of fields were 24° and 27°, respectively (comparison between fields of same estate and environment). At each site a contiguous block of 50 bushes was identified. Individual bushes were closely examined to record disease parameters such as Bushes: free of decay, moderately affected, severely affected and dead due to disease. Number of primaries: presently affected, previously affected, diameter of the affected branches and the bushes affected at collar, number of healthy branches and their diameter, number of diseased patches and their lengths (whenever they were standing out with discrete borders), clearance of collar from soil line and such numbers, *etc.*, in assessing the status of disease on an individual bush. All the disease parameters were reassessed 2 years later using the same bushes in the two blocks.

2.8. Natural incidence and spread (rate) of disease due to N. diffusa in Diyagama East Estate (spatio-temporal)

In Diyagama East Estate (6°50′N, 80°42′47″E) one contiguous block in Field No. 3 of the Second Division was selected with a range of disease intensities from top of the field at 1480 m to the bottom at 1460 m altitude (comparison within the slope of same field). This consisted of 50 rows of tea, spaced at 1.2 m with a slope of 18°. Thirty bushes from each fifth row of this block were assessed accounting for 10 rows of 30 bushes each, thus making a total of 300 bushes. The same disease parameters as above were assessed 3 years after the first. The disease status of individual bushes was recorded using the following rating:

Healthy – 0 (apparently healthy, no visual symptoms).
Slightly infected – 1 (very light to light infections on, up to 3 main branches).
Moderately infected – 2 (moderate to heavy infections on, up to 3 main branches).
Heavily infected – 3 (as above, plus several broken snags due to disease).
Dead due to decay – 4 (only a stub of a bush without any live branches).

Where appropriate, for the statistical analysis of data, the version 6 of the SAS statistical package was used.

3. Results

3.1. Pruning knife as a mode of disease dissemination (spatial)

In the two estates, the pathogen *N. diffusa* was recovered, 69% of times from the blade of the pruning knife while cutting across infections, an action that simulated pruning. In the Nuwara Eliya Estate this was lower at 39% while in Diyagama West Estate there was a positive recovery of inoculum 100% of times. Similarly, other contaminant fungi were recorded at the proportions of 2 and 1.5 in the two estates, respectively (Table 1).

3.2. Natural distribution of decay in intact branches (spatio-temporal)

With the limited number of natural two-way infections and assuming (see Fig. 1) that the infection takes place through the pruning cut, at the end of two pruning cycles the two directional linear decays measured up to 12.0 upward and 11.7 cm, downward. These did not differ significantly (standard error, 2.04). The same was true for the average depth (width) of infections at 2.1 and 2.7 cm, thus indicating, given the environment, the decay can take place in either direction with equal ease. This worked out to an approximate annual spreading rate of the patch of 1.2 cm tangentially and 0.2–0.3 cm axially.

3.3. Rate of spread of infection (temporal)/direct inoculation

An average linear spread of 1.68 cm per annum, with a standard error of 0.17 was recorded through the six inoculation tests. It is worth comparing the rate of spread, which was under a regulated environment with that of the rate of decay at 1.2 cm per year under general field conditions (section above).

3.4. Rate of wood decay (temporal)/ Inoculation of miniature wood blocks

The rate of decay of the two field resistant cultivars was comparable with the above at 65% and 52% per annum (Table 2). However, in the two field susceptible cultivars the rates were less at

Table 1
Potential of the blade of pruning knife in disseminating *N. diffusa*.

Location	Treatment	No. of attempts	No. of colonies and their proportions	
			Contaminations	*N. diffusa*
Nuwara Eliya Estate	Control	3	5 (1.67)	3 (1.00)
	Through infections	18	36 (2.00)	7 (0.39)
Diyagama West Estate	Control	3	4 (1.33)	1 (0.33)
	Through infections	18	27 (1.50)	18 (1.00)
Overall	Control	6	9 (1.50)	4 (0.67)
	Through infections	36	63 (1.75)	25 (0.69)

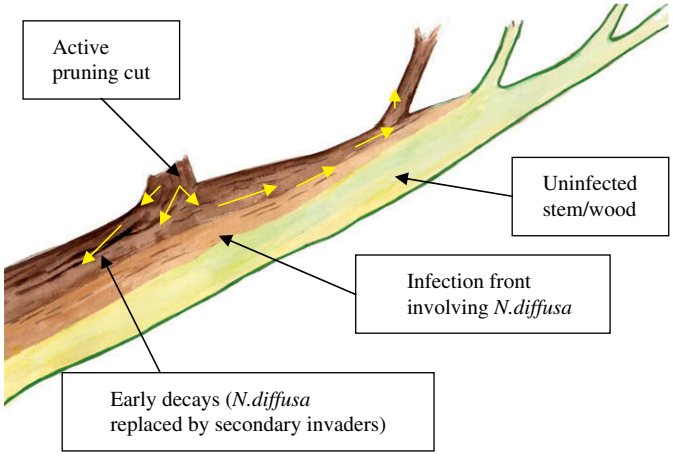

Fig. 1. Sketch of tea stem wood, showing the pattern of upward and downward directional spread of decay from the pruning wound.

29% and 33% per annum. Under similar conditions, the birch wood (a highly susceptible test wood for soft rot) showed a decay rate of 11% per annum due to *N. diffusa*.

3.5. Decay potential (temporal)/Inoculation of stem sections

Whole stem sections of all categories, whether the cultivar was susceptible or resistant in the field or whether the wood was immature or mature were subjected to decay under *in vitro* conditions. However, decay potentials of mature stem wood were slightly less at 64.2–66.8 g cm^{-3} yr^{-1} compared to the immature stem wood at 72.8–73.7 g cm^{-3} yr^{-1} (Table 3).

3.6. Anatomy of decay (spatio-temporal)/thin stem sections

The initial fibre cell counts indicated a potentially higher number of fibre cells in the field susceptible cultivars such as PK 2 and K 145 than in those resistant to the disease in the field (TRI 2025 & HS 10). Soft rot cavities increased progressively on the thin sections of all cultivars with each passing week. The rate and the formation of cavities by *N. diffusa* was comparable with those caused by the soft rot causing fungus *C. globosum* (Table 4).

By the 4th week, the resistant cultivars showed a slightly higher number of cavities by *N. diffusa* under *in vitro* conditions, probably due to loss of biochemical resistance as discussed above. *C. globosum* proved capable of making more cavities in tea stem wood than *N. diffusa* (Table 5).

At the end of 4 weeks, tea wood of field resistant and susceptible cultivars achieved an intensity of decay (number and the size) of 6 and 8 on a scale of 1–9, by *N. diffusa* and *C. globosum*, respectively (Table 6).

Table 2
Weight loss of stem wood of different tea cultivars, in comparison to Birch wood, due to the action of *N. diffusa*, after 8 weeks of exposure.

Tea cultivar/wood sp.	Oven dry weight		% Wood loss[a]	Rate of wood loss/annum (%)
	Initial (g)	Final (g)		
TRI 2025	1.060	0.954	10.03a	65
HS 10	1.056	0.973	8.00b	52
PK 2	0.741	0.708	4.52d	29
K 145	0.667	0.634	5.04cd	33
Birch	0.876	0.861	1.75e	11
Probability			<0.001%	
CV%			18.4	

[a] Figures followed by the same letter do not differ significantly.

Table 3
Decay potential (natural susceptibility) of tea stem wood in immature and mature stems of a susceptible and a resistant tea cultivar, due to infection by *N. diffusa*.

Tea cultivar	% Loss (in 10 months)	%Weight/ wood loss yr^{-1}	Density of wood g cm^{-3}	[a]Decay potential g cm^{-3} yr^{-1}
Immature wood				
PK 2	61.94	74.3	0.98	72.8
HS 10	62.48	75.2	0.98	73.7
Average	73.2	74.8	0.98	73.3
S.E. mean	*0.97*			
Mature wood				
PK 2	52.28	62.9	1.02	64.2
HS 10	56.03	67.5	0.99	66.8
Average	65.3	65.2	1.01	65.9
S.E. mean	*1.37*			
Overall average decay potential				69.6

[a] Decay potential (DP) = density of wood × % wood loss (Butcher, 1980).

3.7. Natural incidence and spread (rate) of disease due to N. diffusa, Nuwara Eliya Estate (spatial and temporal)

This exercise compared the two aspects 'elevation and time', using the two distantly located fields (approx. 1 km on a straight line) within one estate, but situated at two elevations which differed by 140 m.

3.8. Influence of elevation on the incidence of disease (spatial)

On a total disease score basis, the field at higher elevation (1880 m) showed more disease (scores of 135 and 137) than that at lower elevation (1740 m) (scores of 95 and 96) thus highlighting the influence of elevation and the accompanying weather conditions which are considered to be more favourable and thus contributory to the disease incidence (Table 7). This worked out to an approximate increase of the disease score by 0.3 for every 1 m increase in elevation.

Reinforcing this, there were more bushes affected at the collar at the higher elevation (11 and 26) than at the lower (4 and 5). The high potential of disease was reflected with 192 and 209 snags of previously affected branches at the higher elevation and relatively a lower potential with 102 and 129 snags, at the lower elevation field. These can account for an increase in the range of 0.05–0.15 bushes infected at the collar for every increase in elevation by 1 m.

Average diameter of affected branches was recorded to be smaller (3.35 and 3.08 cm) at the higher elevation than at the lower elevation (4.71 and 4.48 cm). This would amount to a decrease in diameter of affected branches by 0.01 cm, for every 1 m rise in elevation. The average diameter of healthy branches followed a similar trend, smaller diameters (2.21 and 1.66 cm) at the higher elevation and larger diameters (4.05 and 3.14 cm) at the lower

Table 4
Number of fibre cells showing soft-rot cavities in thin sections of different tea cultivars on MNA medium, due to the activities of *N. diffusa* and *C. globosum*.

Tea cultivar	Fibre cell count	After 1 week	After 2 weeks	After 3 weeks	After 4 weeks
Activity of *N. diffusa*					
TRI 2025	159	89	97	121	157
HS 10	127	53	69	89	111
PK 2	297	8	18	50	80
KO 145	241	18	45	64	119
S.E. mean		*4.8*	*4.7*	*7.3*	*12.7*
Activity of *C. globosum*					
TRI 2025	159	63	94	126	156
HS 10	127	25	47	77	125
PK 2	297	26	42	90	157
KO 145	241	29	113	159	189
S.E. mean		*4.9*	*9.3*	*11.1*	*14.7*

Table 5
Comparison of soft-rot cavity formation in thin sections of different tea cultivars on MNA medium, due to the actions of *N. diffusa* and *C. globosum*.

Tea cultivar	After 1 week	After 2 weeks	After 3 weeks	After 4 weeks
N. diffusa				
Resistant	71	83	105	134
Susceptible	19	57	102	138
S.E. mean	*10.3*	*18.1*	*21.9*	*26.0*
C. globosum				
Resistant	44	71	102	141
Susceptible	28	109	125	173
S.E. mean	*10.0*	*16.6*	*15.8*	*17.8*

elevation. There was a similar rate of reduction in the diameter of healthy branches by 0.01 cm for every 1 m rise in elevation.

Similarly, the lengths of diseased patches on live branches were recorded to be lower at the higher elevation (14.1 and 4.25 cm) than at the lower elevation (15.0 and 13.53 cm). These amounted to a decrease in the length of diseased patches in the range of 0.006–0.06 cm m^{-1} rise in elevation.

Average clearance in the collar of tea bushes (proximity of disease patch to soil line) also showed a similar trend with smaller clearances (9.0 and 4.2 cm) at the higher elevation and larger clearances (12.2 and 5.89 cm) at the lower elevation (Table 7). These averaged a decrease in the clearance from ground to the edge of a patch of 0.01–0.02 cm m^{-1} rise in elevation.

3.9. Influence of time on the incidence of disease (temporal)

During a span of 2 years there was an overall increase in the disease incidence in the higher elevation field of 2% while in the lower elevation this increase was 1% (Table 7). These were responsible for 1% and 0.5% rate of increase of disease per annum at higher and lower elevations, respectively.

The increase in the percentage of bushes affected at the collar at the higher elevation was as high as 136% while at the lower elevation this increase was restricted to 25%.

Average diameter of affected branches reduced with time because it was found that at the higher elevation there was a higher rate of turnover of new branches (2.2) than at the lower elevation (1.5) replacing those detached due to disease. These accounted for annual reductions in diameter of 4% and 2.5% at higher and lower elevations, respectively. Similar but more marked and significant reductions were seen in the diameters of healthy branches at 12.5% and 11.5% per annum at the higher and lower elevations, respectively.

Average lengths of diseased patches over time also reduced contrary to expectations because branches tended to collapse and be detached before the disease could spread further. These were recorded at 35% and 5% per annum at higher and lower elevations, respectively.

The proximity of the infection front to the soil line reduced by 26.5% and 26% per annum at higher and lower elevations, respectively.

Table 6
Intensity of soft rot cavities in thin sections of different tea cultivars on MNA medium, due to the actions of *N. diffusa* and *C. globosum*.

Tea cultivar	*N. diffusa* (NE)	*N. diffusa* (DG)	*N. diffusa* (Mean)	*C. globosum*
TRI 2025	6	9	7.5	8
HS 10	6	8	7.0	7
PK 2	5	8	6.5	7
KO 145	6	7	6.5	8
Mean	5.8	8.0	6.9	7.5

3.10. Natural incidence and spread (rate) of disease due to N. diffusa, Diyagama East Estate (spatio-temporal)

Different from Nuwara Eliya Estate, this study compared the two aspects 'elevation and time' in the same field sharing a common slope, thus yielding to an interaction effect of time (3 years) × elevation (20 m).

3.11. Influence of elevation on the incidence of disease (spatial)

The total disease score at the higher elevation (1480 m) was less (scores of 40 and 52) than at the lower elevation at 1460 m (scores of 83 and 89), highlighting the opposite influence of elevation within field due to the possible roll over effect of inoculum and their accumulation at lower positions of the field. Weather across the field, and therefore its influence on the disease was considered uniform. This worked out to an approximate increase of the disease score by approximately 2 m^{-1} decrease in elevation. Reinforcing this point there were more number of bushes free of any disease at the higher elevation (13 and 5) than at the lower elevation (1 and 0). There were 8 and 9 heavily infected bushes at the higher elevation and 21 and 24 at the lower elevation. These amounted to an increase in the range of 0.7–0.8 bushes heavily infected for every 1 m decrease in elevation (Table 8).

3.12. Influence of time on the incidence of disease (temporal)

Over a period of 3 years the location at higher elevation (first row) at 1480 m showed an increase in the disease severity of 10% per annum possibly without any interaction effect. However, this remained fairly low at 2.3% per annum at the lower elevation (last row), probably due lack of potential to increase further, because this row already had 21 heavily infected bushes at the beginning of the study (Table 8).

The overall severity of disease in the field increased by 32.8% over a period of 3 years, thus averaging a rate of increase of 11% per annum (Table 9).

4. Discussion

The natural infestations in Nuwara Eliya and Diyagama West Estates were assessed to be at 100% and 85%, respectively. In the former, the majority of bushes had infections reaching beyond the collar region with debris of infected material strewn thickly over the area. The majority of intact infections also proved to be old. Therefore, it is possible that in the Nuwara Eliya Estate at 100% field infestation, the knife cuts had been through older infections in which the pathogen *N. diffusa* would have been replaced with secondary invaders (Dix and Webster, 1995). This could be why the recovery of *N. diffusa* through the pruning knife was relatively low in the Nuwara Eliya Estate. In Diyagama West Estate, with an infestation level yet to reach 100%, the majority of infections could have been active, providing more opportunities for the inoculum to come in contact with the pruning knife while making a pruning cut of infected bushes there. This suggests that there is a higher potential of pruning knives contributing to the dissemination of wood decay caused by *N. diffusa*, through the activity of pruning in those fields that are in early stages of infestation.

Any infection starting through a pruning cut can work its way through, in either direction, with equal ease to cause decay of wood in standing bush/tree with an annual rate of spread of about 1.2 cm. A comparable or a better rate of spread of decay at 1.7 cm per annum was observed through the more specific *ex situ* inoculation tests.

The field susceptible and field resistant cultivars (two in each) behaved similarly in the *in vitro* tests, whether they were in the form of stem sections, miniature wood blocks or thin sections. The

Table 7
Percentage changes of wood decay parameters due to *N. diffusa* in the Nuwara Eliya Estate, at two different elevations under natural field conditions, over a period of 2 years.

Disease parameter	Field 1 (at 1880 m elevation)			Field 2 (at 1740 m elevation)		
	1st Assessment	2nd Assessment	% Increase (decrease)	1st Assessment	2nd Assessment	% Increase (decrease)
Bushes free of decay	0	0	–	6	6	–
Bushes affected	50	50	–	44	44	–
Moderately	23	21	(9)	38	37	(3)
Severely	19	21	11	5	6	20
Dead due to decay	8	8	–	1	1	–
Disease score	135	137	2	95	96	1
No. of primaries						
Presently affected	55	25	(55)	80	54	(33)
Previously affected	192	209	56	102	129	27
Affected at collar	11	26	136	4	5	25
Affected branches						
Number	55	25	(55)	80	54	(33)
Diameter (cm)	3.35	3.08	(8)	4.71	4.48	(5)
S.E. mean	*0.17*	*0.23*		*0.17*	*0.18*	
Healthy branches						
Number	83	132	59	144	125	(13)
Diameter (cm)	2.21	1.66	(25)	4.05	3.14	(23)
S.E. mean	*0.09*	*0.06*		*0.11*	*0.13*	
Diseased patches						
Number	21	26	19	42	51	21
Length (cm)	14.10	4.25	(70)	15.0	13.53	(10)
S.E. mean	*1.79*	*0.44*		*1.47*	*1.51*	
Clearance from ground						
Number	46	46	–	39	39	–
Height (cm)	9.00	4.20	(53)	12.2	5.89	(52)
S.E. mean	*0.15*	*0.55*	–	*1.10*	*0.91*	–

ability of *N. diffusa* to produce a range of enzymes such as phenoloxidases, peroxidase, acid phosphatase, laccase, *etc.* (Balasuriya, 1998), which are capable of neutralising fungitoxic compounds like phenols, ferulic acid, tannic acid, *etc.* (Rayner and Boddy, 1988), suggested the presence of a probable biological/biochemical barrier to infection by *N. diffusa* of intact tea stems of certain cultivars, which they may have lost when the tissues were killed in detached branches after autoclaving. This could be the reason why field resistant cultivars showed a relatively higher rate of decay, *in vitro*.

Boddy and Swift (1984) recorded a weight loss in the range of 10.4% in a *Betula* spp., stand. A rate of approximately 11%, achieved using the combination of *N. diffusa* and *B. pendula* did well to assess the ability of *N. diffusa* to cause similar decay on standing tea bush stems. Under *in vitro* conditions such wood losses constituted 62–75%, which amounted to a rate 5–7 times faster than the *N. diffusa* and *B. pendula* combination.

The decay potential is a measure of natural susceptibility of wood (Butcher, 1980). At about 69 g cm^{-3} yr^{-1} the tea stem wood and *N. diffusa* interaction fell within the lowest third of the natural susceptibility (decay potential) category, which is under 100 g cm^{-3} yr^{-1}, the 'first' and the 'second' being in the ranges of 150–200 and 100–150, respectively (Butcher and Nilsson, 1982).

The characteristic pinheads (decay initials) followed by much larger decay cavities on fibre cell walls is proof that *N. diffusa* is causing soft rot type of decay in tea stem wood of standing bushes. These characteristics and the rates of decay of individual cells compared very well with the known soft rot fungus *C. globosum* (Eaton and Hale, 1993; Dix and Webster, 1995).

Under Sri Lankan conditions, *N. diffusa* is capable of causing disease only at altitudes around or exceeding 1500 m and even then only in some selected cultivars (Balasuriya and Adikaram, 1998; 2002). Comparing different fields situated at different altitudes, they could possibly account for a rate of increase of disease of 0.01% m^{-1} rise per year, starting from the base level of 1500 m, when the weather and the cultural conditions remained favourable.

In addition at higher altitudes, due to the faster rate of decay of branches, they tend to collapse early thus limiting the disease patch from reaching its maximum potential length. The new replacement branches in turn will result in reduced diameters of healthy branches. Working on forest wood litter, as explained by Yoneda (1982) reduced diameters could lead to faster rates of decay. This can go on, leading to a tea bush frame consisting of only the current cycle's branches as evidenced in places with highly advanced state of infestations due to *N. diffusa*.

Table 8
Percentage changes of wood decay parameters of bushes in two rows approximately 59 m apart, due to *N. diffusa* in the Diyagama East Estate, under natural field conditions, over a period of 3 years.

Disease parameter	At 1st row (1480 m elevation)			At 50th row (1460 m elevation)		
	1st Assessment	2nd Assessment	% Increase (decrease)	1st Assessment	2nd Assessment	% Increase (decrease)
Bushes free of decay	13	5	(62)	1	0	(100)
Slightly infected	2	7	250	2	1	(50)
Moderately infected	7	9	29	3	2	(33)
Heavily infected	8	9	13	21	24	14
Dead due to decay	0	0	–	3	3	–
Total bushes	30	30	–	30	30	–
Disease score	40	52	30	83	89	7

Table 9
Percentage changes of wood decay parameters of bushes under natural field conditions, due to *N. diffusa* in the Diyagama East Estate, over a period of 3 years.

Disease parameter	1st Assessment	2nd Assessment (3 years later)	% Increase (decrease)
Bushes free of decay	91	30	(67.0)
Slightly infected	43	47	9.3
Moderately infected	32	44	37.5
Heavily infected	128	167	30.5
Dead due to decay	6	12	100
Total bushes	300	300	–
Disease score	515	684	32.8

In an individual field, the rate of spread of the disease could be confounded by the roll over effect of the inoculum. The study in Diyagama East Estate helped establishing the spread of disease at an increasing rate of approximately 1.0% m^{-1} decline in altitude per year. Thus the combined effect on the field as a whole was a rate of increase of the disease at a staggering 11% per annum.

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