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Selection of species resistant to the wood rot fungus *Phellinus noxius*

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Abstract Brown root rot, caused by the pathogen *Phellinus noxius*, has been reported throughout South-east Asia, Oceania and Africa as a cause of tree decline and mortality. This paper describes how a field survey can be used to quickly select species that are locally resistant to *Phellinus noxius* by performing qualitative and quantitative analyses.

Keywords Brown root rot · Resistant plant · Pathogen

Phellinus noxius (Corner) G. Cunn. is widespread in tropical countries in Southeast Asia, Oceania, Central America and Africa (Corner 1932; Cunningham 1965; Larsen and Cobb-Poule 1990; Chang and Yang 1998; Ann et al. 2002), where it causes a brown root rot disease that is responsible for the decline of various orchard and forest tree species (Hodges and Tenorio 1984; Neil 1986; Nandris et al. 1987; Chang 1995; Ann et al. 1999a; Sahashi et al. 2007). Usually the leaves of infected plant wilt and die within a few months because the fungus destroys roots and cuts off the plant's supply of water and nutrients (Ann et al. 1999a). Therefore, it is important to effectively manage the disease to reduce economic losses and environmental disturbance caused by the pathogen. However, the root rot disease caused by *P. noxius* has not been easy to manage (Durand-Gasselin et al. 2005; Hood et al. 2006) because the disease spreads by root-to-root contact and can persist in the roots and stumps of infected plants for more than 10 years after the death of the host (Chang 1996). The most efficient physical method of destroying residual inoculum in the soil in infested fields is by flooding the field (Chang 1996), although this is difficult to implement. The most practical chemical way of destroying inoculum is to fumigate infested soil with ammonia, which can be generated by amending soil with urea under alkaline conditions (Chang and Chang 1999; Ann et al. 2002). However, ammonia is ineffective at killing *P. noxius* growing in pieces of wood more than 3 cm in diameter and can also pollute the environment (Chang 2002). Another approach would be to replant the infected areas with resistant plant species (Ann et al. 1999b), although an effective method of selecting

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species that are resistant to *P. noxius* infection has not been reported. The purpose of this study was therefore to develop a method of quickly selecting locally-occurring species that are resistant to *P. noxius* by carrying out a field survey.

All the survey sites were located on the Colina da Guia Hill (113°32′ E longitude, 22°11′ N latitude), Macao (China) where the brown root rot disease associated with *P. noxius* is a major constraint to plantation forestry. The average annual temperature and rainfall for this 143,000 m^2 area are respectively 22.3°C and 1970.4 mm. Six survey sites were selected because these sites were infested with *P. noxius* and had a sufficient area for transecting. The total area sampled was 1780 m^2: transect 1 measured 30 m×10 m; transect 2 measured 25 m×25 m; transect 3 measured 30 m×15 m; transect 4 measured 20 m×15 m; transect 5 measured 15 m×5 m; and transect 6 measured 20 m×15 m. We recorded for each tree species within the transect areas that had a diameter at breast height (DBH) of >2 cm, the crown radius, DBH, position (*x, y* coordinates) within the transect, and disease severity due to *P. noxius* rated on a scale of 0–3. Uninfected individuals were classified as disease severity 0. The next rating in the scale reflected the initial stages of *P. noxius* infestation where it spreads when roots of healthy trees contact roots, stems, or stumps of infected trees. This corresponded to the presence of a dark brown mycelial mat on the surface of the roots and the base of stems (disease severity 1), which was also used for the field identification of *P. noxius* (Chang and Yang 1998). Once plants were infected, the fungus grew in fine mats beneath bark and destroyed the vascular cambium. In advanced stages of decay, leaf yellowing (chlorosis) (disease severity 2) were followed by wilt and branch dieback. Occasionally the whole canopy appeared chlorotic, and the affected plants died (disease severity 3) within a few months (Ann et al. 1999a; Ann et al. 2002).

The recorded variables were subjected to qualitative and quantitative analysis described in the electronic supplementary material to assess the resistance of a tree species to *P. noxius* infection. Qualitative analysis was carried out by calculating the prevalence of tree species with a root range that intersected with the root range of diseased trees. This analysis is based on the spreading characteristics of *P. noxius*. Because the fruiting bodies of this fungus is rarely found in the field (Ann et al. 1999a; Ann et al. 2002), the fungus is thought to spread primarily via root-to-root contact (Hodges and Tenorio 1984; Nandris et al. 1987; Ann et al. 2002; Sahashi et al. 2007). A tree may thus “have the chance to be infected” if its root range intersects with that of diseased trees.

Quantitative analysis was performed by assessing the resistance of a tree species to *P. noxius* infection according to the susceptibility index of the species. The formula for the susceptibility index was derived from Hegyi’s competition index model for individual trees (Hegyi 1974). Susceptibility index was calculated using the formula:

$$Z_k = \frac{R_k}{\overline{S_k}}$$

where Z_k *is* the susceptibility index of target species *k*, $\overline{S_k}$ is the average infection pressure of target species *k*, and R_k is the prevalence of brown root rot in target species *k*. The higher the value of Z_k, the more susceptible species *k* is to brown root rot disease; the lower the Z_k, the more resistant species *k* is to the disease. Infection pressure of a tree was calculated using the formula:

$$S_i = \sum_{j-1}^{n} \frac{D_i \times D_j \times G_j}{L_{ij}}$$

where S_i=the infection pressure of the target tree *i*, D_i=DBH of the target tree *i*, D_j=DBH of tree *j* (a tree in the same transect as tree *i*), G_j=disease severity of tree *j*, and L_{ij}=distance between tree *i* and tree *j*. The $\overline{S_k}$ of a tree species is an average of the S_i of target trees of the same species.

In the field survey, the tree species was not considered representative where replication was <3 or present in <3 transects. Therefore, we have only drawn conclusions from those species where replication was ≥3 and present in ≥3 transects in the results for both the qualitative and quantitative analysis.

The results of our qualitative analysis showed that the prevalence of brown root rot in trees of *Mallotus apelta* (Lour.) Müll.Arg., *Mallotus paniculatus* (Lam.) Müll.Arg., and *Syzygium jambos* (L.) Alston, with a root range that intersected with the root range of diseased trees, was 0%. The prevalences of brown root rot in *Sterculia lanceolata* Cav. and *Aleurites* *moluccana* (L.) Willd. were 37.5 and 50%, respectively; and the prevalence of infection in *Cinnamomum burmannii* (Nees & Th. Nees) Nees ex Blume and *Litsea monopetala* (Roxb.) Pers. was greater than 75% (Table 1). Similarly, the results of the quantitative analysis demonstrated that the susceptibility index of *M. apelta*, and *S. jambos* to brown root rot was 0. The susceptibility index of *M. paniculatus, Microcos paniculata* L.*, A.moluccana* and *L. monopetala* was 2.21–3.83, while the susceptibility indices of *C. burmannii* and *S. lanceolata* were 5.05 and 7.27, respectively (Table 2).

P. noxius is thought to have a very wide host range of more than 200 woody plant species representing 59 families (Ann et al. 2002; Sahashi et al. 2007). It was reported that *Acacia confusa* Merr. and *Salix babylonica* L. showed lower mortality than other hosts, indicating these species may have some level of resistance to the disease (Chang 1995; Chang and Yang 1998). According to field observations, tree species such as *Melaleuca leucadendra* (L.) L. and *Alstonia scholaris* R. Br. were free of disease when they were growing in close proximity to diseased trees of other species (Chang 2002). Our study also showed that *M. apelta* and *S. jambos* in the Colina da Guia Hill survey sites were highly resistant to *P. noxius*. Therefore, because of the different degrees of resistance to the fungus, selection of resistant species is of great importance for managing areas infected with *P. noxius* (Ann et al. 2002). Planting infested areas with resistant species is a measure of disease control, as the presence of resistant species may create an isolation region around diseased trees and prevent the disease spreading.

Table 1 Prevalence of brown root rot in tree species with a root range that intersected with the root range of diseased trees

Species	Prevalence of brown root rot (%)	Total number of trees	Serial number of transects in which species present
Aralia chinensis	0	1	2
Helicteres angustifolia	0	1	3
Syzygium levinei	0	1	2
Schefflera octophylla	0	3	1
Ligustrum sinense	0	4	1
Mallotus apelta[a]	0	6	2, 5, 6
Mallotus paniculatus[a]	0	8	1, 2, 6
Syzygium jambos[a]	0	11	1, 5, 6
Ficus simplicissima	16.7	6	2, 4
Antirhea chinensis	33.3	3	2, 5
Sterculia lanceolata[a]	35.7	14	1, 2, 3, 4, 5
Aleurites moluccana[a]	50	10	1, 4, 5, 6
Ficus variegata	75	4	2
Litsea monopetala[a]	76.9	13	2, 3, 6
Cinnamomum burmannii[a]	81.8	121	1, 2, 3, 5, 6
Averrhoa carambola	100	1	4
Bridelia monoica	100	2	3, 6
Microcos paniculata	100	3	2
Casuarina equisetifolia	100	4	5
Livistona chinensis	100	4	5

[a] Total number of trees ≥3 and present in ≥3 transects

Our results indicated that of the tree species examined, *C.burmannii* was most susceptible to *P. noxius* in Colina da Guia. Brown root rot infection was highly prevalent in this species as more than 50% of the trees sampled in the transects were infected with the fungus. *C.burmannii* also had a susceptibility index of more than 5. The tree species could therefore be invaluable for disease management because of its potential application as an indicator for the presence of *P. noxius*.

Previously reported methods of selecting resistance in trees have been based on the prevalence of brown root rot in all samples or by observing which species were free of disease when growing in close proximity to diseased trees of other species (Chang 2002). However, in this study we considered both the prevalence of brown root rot and whether trees were growing in close proximity to diseased trees. Our qualitative analysis allowed us to evaluate and identify species tree that had the opportunity to be infected by *P. noxius* as well as species that were resistant to *P. noxius* infection, while our quantitative analysis allowed us to determine the susceptibility indexes that included parameters for prevalence and distance between target trees. Therefore, the results in our survey may be more accurate. We first judged the resistance of tree species to *P. noxius* according to their susceptibility index calculated by quantitative analysis (appendix 2, Hegyi 1974). Accordingly, tree species that were resistant to *P. noxius* infection had a susceptibility index of 0 because the infection pressure (*i.e.*, the denominator in the susceptibility index formula) cannot be equal to zero. In other

Table 2 Susceptibility index and average infection pressure of species in quantitative analysis

Species	Susceptibility index (Z)	Average infection pressure (S)	Prevalence of brown root rot in all transects (R)	Number of trees sampled	Serial number of transects in which sampled trees present
Clausena lansium	–	0.00	0.00	1	3
Dimocarpus longan	–	0.00	0.00	1	3
Psychotria rubra	–	0.00	0.00	1	1
Aralia chinensis	0.00	0.02	0.00	3	2,3
Mallotus apelta[a]	0.00	0.12	0.00	6	2,5,6
Syzygium jambos [a]	0.00	0.19	0.00	8	1,2,5,6
Craibiodendron kwangtungense	0.00	0.00	0.00	2	3,6
Ficus hispida	0.00	0.01	0.00	1	3
Helicteres angustifolia	0.00	0.03	0.00	1	3
Ligustrum sinense	0.00	0.01	0.00	2	1
Schefflera octophylla	0.00	0.01	0.00	2	1,6
Syzygium levinei	0.00	0.05	0.00	1	2
Casuarina equisetifolia	0.71	1.41	1.00	2	5
Averrhoa carambola	1.92	0.26	0.50	1	4
Mallotus paniculatus [a]	2.21	0.02	0.04	20	1,2,3,4,6
Ficus variegata	2.63	0.19	0.50	5	2, 4
Microcos paniculata[a]	3.02	0.19	0.57	6	2,3,6
Aleurites moluccana[a]	3.29	0.19	0.62	4	2,4,5,6
Antirhea chinensis	3.38	0.10	0.33	1	2
Litsea monopetala[a]	3.83	0.18	0.69	7	2,3,6
Ficus simplicissima	4.56	0.04	0.17	3	2
Cinnamomum burmannii [a]	5.05	0.12	0.60	63	1,2,3,4,6
Sterculia lanceolata[a]	7.27	0.03	0.19	29	1,2,3,4,5
Bridelia monoica	7.67	0.04	0.30	4	3,6
Livistona chinensis	27.15	0.04	1.00	2	5

[a] Z=R/S. Number of trees sampled ≥3 and present in ≥3 transects

words, a tree species remained uninfected despite infection pressure when the susceptibility index was 0. This method may also be useful for the forest management and replanting of areas infected by other diseases spread by root-to-root contact.

Inoculation tests have been used in other studies to assess the resistance of tree species to *P. noxius* infection (Chang 1995; Ann et al. 1999b; Chang 2002; Mohd Farid et al. 2009; Sahashi et al 2010). These tests showed that different tree species show different degrees of resistance to *P. noxius* infection, with some species showing lower levels of mortality than others (Chang 1995; Chang and Yang 1998; Ann et al. 1999b; Mohd Farid et al. 2009; Sahashi et al 2010). However, these tests did not select any species with a zero mortality level. This might be because only a small number of species were inoculated and it was not known before testing whether any of the species selected were resistant. However, it would not be feasible to test all species for resistance using inoculation tests. It would be more practical to select target species that might be resistant using the field survey method described in this study, and then confirm whether or not these species are resistant to *P. noxius* by performing inoculation tests. This should enable resistant species that occur locally to be easily and accurately selected, which would be better for managing local infested areas and would avoid the risk of invasion by exotic resistant species.

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