APPENDIX 20 - Guidelines on Brown Root Rot Disease
GUIDELINES ON BROWN ROOT ROT DISEASE

Issue Date: December 2012

TREE MANAGEMENT OFFICE
GREENING, LANDSCAPE AND TREE MANAGEMENT SECTION
DEVELOPMENT BUREAU
THE GOVERNMENT OF THE HONG KONG SPECIAL ADMINISTRATIVE REGION
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**Annexes**

1. Pictorial Guide of Brown Root Rot (BRR) Disease
2. Frequently asked questions on BRR Disease

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Guidelines on Brown Root Rot Disease

1. Purpose

1.1. This guideline on Brown Root Rot (BRR) Disease focuses on disease management strategy, identification of suspected cases and removal procedures of infected trees.

2. What is Brown Root Rot Disease?

2.1. *Phellinus noxius* is an aggressive fungal pathogen that causes BRR disease. The disease mainly spreads through root-to-root contact or through infested wood debris in soil. There may also be a possibility of spreading the disease through the dissemination of basidiospores from fruiting bodies. It is prevalent in tropical and subtropical regions and has a wide host range covering over 200 plant species in 59 families. In Hong Kong, a number of trees species such as *Aleurites moluccana*, *Bombax ceiba*, *Celtis sinensis*, *Delonix regia*, *Ficus microcarpa*, *Ficus benjamina*, *Gleditsia fera*, *Lophostemon confertus*, and *Mangifera indica* have recently been confirmed to have contracted the disease. There is currently no effective cure to the disease.

2.2. Due to the highly pathogenic and infectious nature of *Phellinus noxius*, it is essential to step up measures to avoid the local establishment of BRR disease. Present literature and overseas experience indicate that the most effective way of prevention is through the promotion of tree health and the reduction of the inoculum of *Phellinus noxius*. These are achievable by the implementation of a vigilant surveillance programme. This programme should consist of three components, namely, a management strategy to prevent local spread, a referral mechanism for reporting of suspected cases and a removal procedure of infected trees.

3. Identification of Suspected BRR Cases

3.1. The following steps should be followed in identifying BRR disease.

- Step 1. Look for trees with the following abnormality at the crown:
  - sparse foliage density;
  - abnormal foliage colour;
- abnormal leaf size; and
- dieback twigs.

Section A of Annex I contains a pictorial guide on crown abnormality.

- Step 2. Then, further examination of the entire lower trunk, root collar and individual roots of the trees is required to ascertain whether the trees initially identified have one or more of the typical BRR signs, i.e. fruiting bodies of *Phellinus noxius*, mycelial encrustation, soil aggregates, mycelial nets. Section B to E of Annex I contains photographic records of fruiting bodies of *Phellinus noxius*, mycelial encrustation, soil aggregates and mycelial nets. Root excavation with appropriate tools (e.g. handheld adze, digger, air spade, etc.) may be required to expose the root collar and roots for further examination of typical signs of BRR disease. Soil aggregate and mycelial nets, observable after scraping off bark tissue using appropriate tools (e.g. knife) are indicative of BRR. Please note that bark tissue should only be scraped off from decayed, damaged or dead wood/roots. The use of mallets may assist to differentiate healthy wood/roots from decayed, damaged or dead wood/roots. Damage to healthy wood/roots should be avoided as this may cause unnecessary damage to the tree, which may also create open wounds for fungal invasion.

- Step 3. If a tree with crown abnormality identified in step 1 contains one or more of the typical signs of BRR disease in step 2 examination, the tree is considered a suspected BRR infection case.

3.2. The confirmation of BRR disease can be made through further field diagnosis based on observable signs, or field diagnosis followed by laboratory diagnosis based on culture and/or molecular techniques. Upon confirmation, recommendations should be made based on the information provided in para. 4.1.2 on handling the confirmed case.

4. Management Strategy

4.1. We adopt a dual-pronged management strategy comprising of precautionary and preventive measures. The objectives of this approach
are to keep our trees healthy, and at the same time to minimize the source of BRR inoculum as far as possible.

4.1.1 Precautionary Measures

- Proper tree planting and maintenance practices are the best precaution one can take against BRR disease. These practices include planting the right tree at the right place, providing sufficient growing space, planting at the right depth, proper irrigation and fertilization regimes, and mulching of root zone, not to mention regular and proper pruning. In particular, number and size of pruning wounds as well as damage to the roots should be kept to a minimum. This reduces the surface area where infection may start off. For details on tree maintenance, please refer to the relevant guidelines issued by the Tree Management Office.

4.1.2 Preventive Measures

- Preventive measures are necessary to minimize the source of BRR inoculum and control the spread of the BRR through removal of diseased parts. Trees in the Category I of Tree Risk Management Zone (i.e. areas of high traffic flow and high pedestrian flow such as public parks, playgrounds, roadside etc) infected with BRR disease should be removed entirely, including fruiting bodies, stumps, wood debris and associated fine roots in soil medium. For details on the Tree Risk Management Zones, please refer to the Guidelines for Tree Risk Assessment and Management Arrangement on an “Area Basis” and a “Tree Basis” which may be downloaded at http://www.trees.gov.hk.

- There are however occasions where preservation of a tree is warranted e.g. Old and Valuable Trees or trees that draw strong public sentiment for preservation. The tree concerned should be quarantined to avoid local spreading. The structural stability of the retained infected tree should first be ascertained by conducting a thorough tree risk assessment. This should include, among other normal produces, soil excavation with proper tools (e.g. adze, digger or air spade, if applicable) and advanced examination techniques including tomography and resistography, to examine the extent of the infection and decay at critical locations, such the root collar and
subsoil surface levels.

- For structurally stable trees at the early stage of BRR infection (i.e. trees confirmed with laboratory diagnosis based on culture and/or molecular techniques showing no foliage abnormality and typical signs/symptoms of BRR), treatment efforts can still be made through the creation of physical barrier by digging a trench and the use of chemicals as a means to retard the spread of BRR. It must be borne in mind that treatment through chemical means is only an interim measure for suppressing early stage of BRR infection, and will not revitalize the long term health and structural stability of the trees. Regular monitoring and assessment are still necessary. Trees that are structurally safe should be closely monitored on at least a quarterly basis. The tree should also be lodged under the Tree Register.

5. Removal Procedures for BRR Disease Infected Trees

5.1. For confirmed cases that require removal, the proper disposal of diseased parts (i.e. woody stumps, roots systems, fruiting bodies, fine roots in soils, debris) and subsequent removal or sanitation of the soil medium is very important. The removal procedures of trees infected with BRR disease are summarised below:

- Step 1. The above ground parts of BRR infected trees could be removed initially before proceeding to the removal of the tree stump and roots. The entire tree stump, infected roots in the soil, and the fruiting bodies should be incinerated or properly disposed of. Measures needed to be taken to prevent accidental dissemination of contaminated soil/infected tissues to the surrounding environment during transportation to landfill area. Removal of tree stump and large root pieces may require machines, while fine root or root pieces larger than 1cm in diameter in soil need to be manually removed and packed in strong plastic bags before disposal to landfill area. To ensure complete removal of the source of inoculum, other vegetation (i.e. shrubs, perennials, herbaceous) within the dripline area and/or growing in the root zone area of the infected tree should also be removed. Trees in the vicinity should be checked for BRR infection as well.
• Step 2. According to the literature, mycelia of *Phellinus noxius* could survive in decayed root tissues in the soil for 10 years or more. As there may be infected roots in the soil, treatment should cover also the soil. Depending on site conditions, the soil medium containing the infected debris could either be disposed of or disinfected with a soil fumigant. Application dosage and safety measures from the manufacturers should be carefully read and followed before using a specific soil fumigant. If disinfection or replacement of soil could not be done due to site constraint (i.e. on slope or inaccessible area), tree replanting should be avoided.

• Step 3. Tools like adze, knives, scissors, shovels etc. and transportation equipment (such as car hopper etc.) used in the infested area should be thoroughly disinfected (e.g. with 70% ethanol or 1:49 bleach) after operation.

6. Frequently asked questions on BRR Disease

6.1. A list of frequently asked questions at Annex II is provided to facilitate the understanding of BRR’s biology background, diagnostic methods, identification of suspected cases, management strategy, and removal procedures of trees infected with BRR disease.

7. List of Reference Documents


(d) Chang, T.T. and Yang, W.W. 1998. *Phellinus noxius* in Taiwan:
distribution, host plants and the pH and texture of the rhizosphere soils of infected hosts. Mycological Research. 102: 1085-1088.


(v) 張東柱、傅春旭、呉孟玲 2009. 褐根病診斷鑑定與防治標準作業程序。行政院農業委員會林務局、林業試驗所。

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蔡志濃、謝文瑞、安寶貞、楊淨棉 2007. 褐根病菌 *Phellinus noxius* 檢測用專一性引子對之開發 植病會刊 16:193-202。
A. Crown Condition

Photo 1 *Ficus microcarpa* (OVT E/2): branch dieback, defoliation and leaf size reduction.

Photo 2 *Ficus microcarpa* (OVT WCH/10): branch dieback, defoliation and leaf size reduction.

Photo 3 and 4 *Ficus microcarpa*: yellowing, leaf size reduction on the upper portion of the tree, defoliation and dieback.

Photo 5 and 6 *Celtis sinensis*: branch dieback, defoliation, leaf size reduction and yellowing.

Photo 7 *Ficus microcarpa* showing normal healthy crown condition.
B. Fruiting Bodies of *Phellinus noxius*

*Photo 8* Fruiting bodies on *Ficus microcarpa*. *Photo 9* Primordial stage of fruiting bodies (with white advancing margin and drops of exudates). *Photo 10* Senescent fruiting body.

*Photo 11 and 12* Primordial stage of *Phellinus noxius* fruiting bodies on *Celtis sinensis* (with white advancing margin and drops of exudates).
B. Fruiting Bodies of *Phellinus noxius* (Con’t)

**Photo 13 and 14** Primordial and premature senescent stages of fruiting bodies on lower trunk of *Ficus microcarpa*. Photos were taken at two weeks interval.

**Photo 15 and 16** Mature fruiting bodies (resupinate/bracket forms) on dead stump and root of *Ficus microcarpa*.

**Examples of Non-*Phellinus noxius* fruiting bodies Photo 17 and 18** Fungus with pinkish colour on root collar of *Ficus microcarpa*. Photos were taken at two months interval. **Photo 19, 20 and 21** Fruiting bodies of *Ganoderma* spp. (Hong Kong).
C. Mycelial encrustation of *Phellinus noxius* (flecky appearance on the outer surface of structural roots, root collar and lower trunk)

*Photo 20* Mycelial encrustation on lower trunk of *Ficus microcarpa*. *Photo 21 and 22* Mycelial encrustation on root collar of *Ficus microcarpa*.

*Photo 23 and 24* Mycelial encrustation on structural root of *Ficus microcarpa*.

*Photo 25* Mycelial encrustation on root collar of *Delonix regia*.

*Example of Non-Phellinus noxius* *Photo 26* Algal growth on root collar of *Ficus microcarpa* (dark green to dark brown in appearance).
D. Soil aggregates of *Phellinus noxius* infected trees: A layer of adhering soil particles and fungal mycelia. The outer bark surface appears rough.

**Photo 27 and 28** Soil aggregates on root collar of *Ficus microcarpa*.

**Photo 29, 30 and 31** Soil aggregates on roots of *Ficus microcarpa*.

**Photo 32** Soil aggregates on roots of *Delonix regia*.

**Photo 33** Soil aggregates on roots of *Celtis sinensis*.

**Example of Non-*Phellinus noxius***

**Photo 34** Sandy soil adheres on the root collar of *Ficus variegata var. chlorocarpa*.
E. Mycelial nets of *Phellinus noxius* infected trees: Ectotrophic mycelium (brownish or black lines) located between the bark and sapwood

**Photo 35 and 36** Inner bark of *Ficus microcarpa* covered with brownish mycelial nets.

**Photo 37 and 38** The presence of mycelial nets underneath the bark of root collar of *Aleurites moluccana*.

**Photo 39** Mycelial nets on root of *Celtis sinensis*.

**Photo 40** Peeling of the bark of a soil aggregated root reveals the presence of mycelial nets.
Annex II

Frequently asked questions on Brown Root Rot Disease

Biological Background:

Q1. What is *Phellinus noxius*?

A1. *Phellinus noxius* is a fungus that causes BRR disease on trees. Belonging to the genus *Phellinus*, *P. noxius* is placed under the family of Hymenochaetaceae within the Phylum Basidiomycota. 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 *P. noxius* is one of the strongest among them. It prefers acidic, hot and humid conditions. It is characterised by its brownish black fruiting bodies (which will turn black with a drop of 3-5% KOH) with no clamp connections in its vegetative hyphae. The species within *Phellinus* are the typical white roters, which can release enzymes through the action of microhyphae and decompose lignin and polysaccharides such as cellulose, hemicellulose and pectic substance, resulting in wood decay. *Phellinus noxius* causes white simultaneous rot in which the major components of wood (i.e. cellulose, hemicellulose and lignin) degrade at approximately the same rate.

Q2. What is the host range of *Phellinus noxius*?

A2. It has been reported that over 200 plant species in 59 families are hosts to *Phellinus noxius*. In Hong Kong, we know that trees species such as *Aleurities moluccana*, *Bombax ceiba*, *Celtis sinensis*, *Delonix regia*, *Ficus microcarpa*, *Ficus benjamina*, *Gleditsia fera*, *Lophostemon confertus*, and *Mangifera indica* are host to BRR disease.

Q3. What are the potential infection routes of BRR disease in tree?

A3. The disease mainly spreads through root-to-root contact or through infested wood debris in soil, though there may be the possibility of spreading of the disease through the dissemination of basidiospores from fruiting bodies. According to literature, mature fruiting bodies of *Phellinus noxius* seldom form in nature, though their basidiospores may assist in the long range dissemination of the fungus. In Hong Kong, there were observations that fruiting bodies of *Phellinus noxius* produced massive basidiospores on infected trees. The prevalence of the fruiting bodies in this region remains unknown. The potential infection route of BRR disease in trees is shown
in diagram 1.

A diseased tree can infect healthy trees through root-to-root contact

Infected tree died of brown root rot disease

Diseased roots with brownish-black mycelial encrustation can infect healthy roots

Phellinus noxius can survive in dead tree, stump and decaying roots

Fruiting bodies formed at the base of the main trunk

Stump infected by basidiospores

Basidia with basidiospores

The leaves of the diseased tree wilt and thin out

Brownish-black mycelial encrustation can spread upward from the roots to the base of the main trunk


Q4. What are the geographical ranges of BRR disease?

A4. The disease is prevalent in tropical and subtropical regions in different part of the world and has been found in Asian countries & regions such as Japan, Mainland China, Hong Kong, Taiwan, Malaysia, Singapore as well as Central America, Africa and Oceania.
Q5. Is there an effective cure to BRR disease?

A5. According to literatures and expert opinion, there is yet to be an effective cure for BRR disease.

Diagnostic Methods:

Q6. What are the methods available for diagnosis of BRR disease on trees?

A6. There are currently two main levels of diagnostic methods available for determination of BRR disease in trees, namely **field diagnosis through visual tree assessment** and **laboratory diagnosis through fungal isolation method and/or molecular diagnosis**.

Q7. How is BRR disease detected through field diagnosis?

A7. Field diagnosis through visual tree assessment is based on observable symptoms and signs of BRR. There are two steps. Step 1: Identify abnormal crown symptoms (e.g. sparse foliage density, abnormal foliage colour (chlorosis), abnormal leaf size, dieback twigs) as these are exhibited in infected trees. Step 2: examine the entire lower trunk, root collar and individual roots of the trees to look for typical signs of BRR disease, i.e. a) fruiting bodies of *Phellinus noxius*, b) mycelial encrustation, c) soil aggregates and d) mycelial nets. If a tree with a crown abnormality contains one or more of the typical signs of BRR disease under step 2 examination, the tree is considered infected with BRR disease.

Q8. What crown symptoms are observed on trees with BRR disease?

A8. Symptoms of the disease are of two types, namely slow decline and rapid decline. For trees experiencing slow decline, the most noticeable symptoms may include crown thinning out gradually and turning yellowish and their leaves reduced in size or even dropped as a result of early senescence. The trees could be dead in months or one to two years and structurally become unstable. Trees suffering from quick decline will wilt rapidly. Their leaves become brownish in color and the trees will die within weeks. The leaves of the dead trees will not fall immediately but remain attached on the branches for months. **Please refer to section A of Annex I in pictorial guide for general symptom of trees infected with BRR disease.**
Q9. Are the general symptoms observed on tree crown unique to BRR disease?

A9. No, the abnormal crown symptoms are commonly associated with root diseases and malfunctions, and are not unique to BRR disease. Thus, it is important to further examine the lower trunk, root collar and roots for typical signs of BRR disease.

Q10. Elaborate on the typical signs of BRR disease to look for at the lower trunk, root collar and individual roots of the trees.

A10. The typical signs of BRR disease are a) fruiting bodies of *Phellinus noxius*, b) mycelial encrustation, c) soil aggregates and d) mycelial nets.

(a) The appearance, on lower trunk or roots, of brownish-black/dark greyish-brown colored imbricate or resupinate fruiting bodies of *Phellinus noxius* with their characteristic porous hymenium surface up-facing is an obvious sign of BRR infection. The sizes of fruiting bodies vary greatly ranging from 3-10 cm in length to 8-20 cm in width. The fruiting bodies of *Phellinus noxius* are the sexual stage of the fungal lifecycle and their development, under the right conditions, begin with the formation of the primordial stage. The developing fruiting bodies would continue to grow in size, reach a stage of maturity in bracket and/or resupinate forms from which basidospores are formed for dissemination, and end at senescence. There are occasions where the developing fruiting bodies become abortive and reach premature senescence without forming basidospores. **Please refer to section B of Annex I in the Pictorial Guide for fruiting bodies of *Phellinus noxius* on infected trees.**

(b) If the mycelia of *Phellinus noxius* are spreading under the bark, or under the outer layer of roots, these parts can peel off easily. The diseased parts look rough with flaky appearance on their surfaces covered by a brownish-black mycelial encrustation. Normally, the mycelial encrustation can extend from the root collar to 1 m high on the tree trunk. There are also reported cases from the literature that mycelial encrustation can reach 2-3 m in height. **Please refer to section C of Annex I in the Pictorial Guide for mycelial encrustation.**

(c) and (d) If fruiting bodies of *Phellinus noxius* and mycelial encrustation cannot be found, the bark of the entire lower trunk/root collar and the outer layer of all the roots of the suspected diseased tree should be examined. If necessary, cut open with appropriate tools (e.g. handheld adze, knife, etc.) upon soil
excavation to check for soil aggregates and yellow, dark brown or brownish-black mycelial netting on the inner surface between the bark and the wood tissues. Please refer to sections D and E of Annex I in Pictorial Guide for soil aggregates and mycelial nets.

Q11. Are tools required in checking the lower trunk, root collar and individual roots of trees?

A11. Yes, root excavation with appropriate tools (e.g. handheld adze, digger, air spade, etc.) may be required to expose the root collar and roots. The exposed root collar and roots could subsequently be examined for the typical signs of BRR disease. Scraping off of bark tissue should only be conducted on decayed, damaged or dead wood/roots. Mallets may be used to differentiate healthy wood/roots from decayed, damaged or dead wood/roots. Damage to healthy wood/roots should be avoided as this may cause unnecessary damage to the tree, which may also create open wound for fungal invasion.

Q12. Is it difficult to diagnose BRR disease on trees in early stage of infection?

A12. Yes, early diagnostic symptoms of BRR disease are often difficult to detect, despite the fact that the disease can cause a rapid decline in tree growth conditions within a short time. More often than not, obvious symptoms will only be visible at a late stage of infection. Once symptoms such as abnormal crown symptoms (e.g. sparse foliage density, abnormal foliage colour (chlorosis), abnormal leaf size, dieback twigs are discernible in the above ground portion of the tree, the majority of its roots are likely to have been infected and the tree basically cannot be cured.

Q13. Under what circumstances are laboratory diagnosis used in BRR detection?

A13. Laboratory diagnosis would be required to confirm disease status of trees exhibiting no detectable symptoms and signs of BRR disease (i.e. trees in the root zone area of another tree infected with BRR). Laboratory diagnosis would also serve as a tool to verify the findings of field diagnosis on trees of special significance (i.e. Old and Valuable Trees).
Management Strategy of BRR Disease

Q14. What are the management strategies of BRR disease in Hong Kong?

A14. We adopt a dual-pronged management strategy comprising precautionary and preventive measures. The objectives of this approach are to keep our trees healthy, and at the same time to minimize the source of BRR inoculum as far as possible.

Q15. What are the precautionary measures for BRR disease management?

A15. Proper tree planting and maintenance practices are the best precaution one can take against BRR disease. These practices include planting the right tree at the right place, providing sufficient growing space, planting at the right depth, proper irrigation and fertilization regimes, and mulching of root zone, not to mention regular and proper pruning. In particular, number and size of pruning wounds as well as damage to the roots should be kept to a minimum. This reduces the surface area where infection may start off.

Q16. What are the preventive measures for BRR disease management?

A16. Preventive measures are necessary to minimize the source of BRR inoculum and control the spread of the BRR through removal of diseased parts. Trees in the Category I of Tree Risk Management Zone (i.e. areas of high traffic flow and high pedestrian flow such as public parks, playgrounds, roadside etc) infected with BRR disease should be removed entirely, including fruiting bodies, stumps, wood debris and associated fine roots in soil medium.

Q17. How are trees of special value and significance infected by BRR disease treated?

A17. There are occasions where preservation of a tree warrants retention (e.g. Old and Valuable Trees or trees that draw strong public sentiment for preservation). In these circumstances, the structural stability of the infected tree should be ascertained by conducting a thorough tree risk assessment, followed by soil excavation with proper tools (e.g. adze, digger or air spade, if applicable) to examine the extent of the infection and decay at critical locations, such as at the root collar and subsoil surface levels. The use of advanced examination techniques such as tomography and resistography will offer additional information about the extent of internal decay at
the root collar and sub-soil levels. Trees that are structurally unstable should be cordoned off and removed as soon as possible. Trees that are justified for retention from a structural standpoint should be closely monitored on a quarterly basis to re-assess their structural integrity/stability.

Q18. How the stability of trees infected with BRR disease may be ascertained?

A18. Since BRR disease causes root rot at the lower trunk above grade level (e.g. it could be observed up to 2 m in some tree species) and/or root collar and/or roots at the sub-soil level, it is essential to evaluate the thickness of sound wood of the tree at horizontal plane at critical levels (i.e. area showing decay and/or specific signs/symptoms of BRR disease) of the lower trunk, as well as at the trunk base, through the use of tomography and/or resistography. For sub-soil level evaluation, the trunk base or even at lower levels (e.g. after soil excavation using spade and/or air spade without affecting tree stability) could be assessed using resistography drilling at an angle (45 degree) downwards which could provide some indication on the relative soundness of tree roots.

Q19. What is the effectiveness of chemical control on BRR infected trees?

A19. Treatment through chemical means is only an interim measure. It will not revitalize a tree or improve its structural stability. Interim measures may include applying fungicides to trees at the early stage of BRR infection showing no foliage abnormality or major symptoms of BRR.

Removal Procedures of BRR Infected Trees

Q20. Is the installation of trench recommended to prevent spread of BRR disease to neighboring trees?

A20. Yes, this is because root to root contact is still the main route of transmission of BRR disease. It is important to prevent the spread of the disease through digging of a trench ideally of 1 m depth by 1 m width at the dripline of an infected tree, followed by application of a root barrier to separate it from healthy ones. Such application, however, may not be feasible for trees with restricted root zone such as trees on planter or trees grown on slopes.
Q21. Will annuals and shrubs be carriers of BRR disease?

A21. Yes, annuals and shrubs may be carriers of BRR disease. As such, it is advisable to avoid planting such vegetation near the root zone, particularly under Old and Valuables Trees or mature trees in confined, restricted or graded areas. This is to minimize the inoculum of BRR disease. Please also refer to “Proper planting Practice-Keep Sufficient Space Clear of Vegetation at the base of Tree” available at www.greening.gov.hk

Q22. Is the removal of fruiting bodies of Phellinus noxius an effective way to prevent the spread of the disease?

A22. While fruiting bodies of Phellinus noxius should be removed and properly disposed of as soon as possible, they are the sexual stage of fungi and their removal can only prevent the dissemination of basidiospores, but could not remove the mycelia that are present in the infected trees.

Q23. How should the soil medium containing the infected debris be handled?

A23. Depending on the amount of soil that needs to be handled, the soil medium could either be disposed of at landfills or disinfected with a soil fumigant on-site. For instance, if a manageable amount of soil could be replaced (i.e. in a planting strip or confined planter), the contaminated soil should be disposed of at landfills.

Q24. Do we need to sterilise the tools after examining trees with BRR disease?

A24. Yes, tools like adze, knives, scissors, shovels etc. and transportation equipment (such as hopper car etc.) used in the infected area should be disinfected (e.g. with 70% ethanol or 1:49 bleach) after operation.