Asian Wart bark, blister canker, ring rot, Physalospora canker of pear and apple - *Botryosphaeria berengeriana* f. sp. *pyricola*

This fungus is an ascomycete causing leaf spots, warty growths on stems, and "ring rot" of fruits of pears and apples in eastern Asia. Morphologically, it is similar to the "white rot" pathogen of pome fruit, *B. dothidea*, and the species *B. berengeriana* itself has been synonymized with that more widespread species. However, the production of different symptoms by the Asian fungus suggests physiological differences that are not understood or defined and that could still constitute a threat in pome fruit-growing areas where white rot already occurs. Other than by inoculation of trees, no assay exists to distinguish this fungus easily and quickly from closely related *Botryosphaeria* pathogens that might also be carried in fruit or in fruit tree propagating material.

*Botryosphaeria berengeriana* f. sp. *pyricola* (Nose) W. Yamam. 1961 (B. berengeriana f. sp. pyricola invalidly published)

No regional plant protection organization has considered *B. berengeriana* f.sp. *pyricola* to be a quarantine pest, but it is listed in the regulations of the European Union, and also those of the USA (EPPO/CABI,1997b). Though mainly occurring on *Pyrus pyrifolia* in Japan, the fungus has been recorded damaging *P. communis* (Kobayashi, 2007). In other countries, apple is the crop of primary concern (Lee and Yang, 1984; Zhao et al., 1998). In Japan, *B. berengeriana* f.sp. *pyricola* has been thought to be more important than *B. dothidea* and to cause different symptoms. In principle, apple and pear-growing countries around the world are at risk. However, different names for the pathogens are used in different countries, and it is not clear whether the published information all refers to the same fungus, or whether *B. berengeriana* f.sp. *pyricola* can really be distinguished from *B. dothidea*, or how feasible it might be to take regulatory measures against a physiologically specialized form alone. In addition, it may be noted that the Japanese fungus, like *B. dothidea* in south-eastern USA (Brown and Britton, 1986), is favoured by rather warmer and more humid conditions than those which prevail in most apple and pear-growing areas (EPPO/CABI, 1997b).

According to Koganezawa and Sakuma (1984), the morphology of the fungus is identical to that of *B. dothidea*. The sizes of the stroma, asci and ascospores are variable. Asci are 80-130 x 14-23 µm, and ascospores 19-26 µm long. The conidia, of the *Fusicoccum* type, are 23-29 x 6-8 µm. In China, conidia of ten isolates measured 20-31.5 x 4.3-7.2 µm (Lee and Yang, 1984).

In the dark, colonies of all isolates on PDA were initially white, turning grey and then black. In diffuse light (Koganezawa and Sakuma, 1984), isolates of *f.sp. piricola* remained grey, while isolates of *B. dothidea* from various hosts (including *Malus* and *Pyrus*) were reported to turn yellowish-brown.

**Host:** Principal host: *Pyrus pyrifolia*. Additional hosts: *Pyrus spp.*, *Malus* spp., *Chaenomeles japonica* (Rosaceae).

**Distribution:** Asia.

**Notes:** Slippers et al. (2004) were unable to find morphological differences between the type specimens of *B. berengeriana* and *B. dothidea*, but did observe differences between *B. dothidea* and *B. ribis*. Comparison of the sequences of the ITS, 5.8S, beta-tubulin and EF1-alpha regions of nuclear DNA supported the latter distinction, but no isolates of *B. berengeriana* were included in the molecular analysis. On the basis of morphology, then, *B. berengeriana* was placed in synonymy with *B. dothidea*. Grouping of fruit tree isolates of *Botryosphaeria* in Japan by symptoms caused, conidium size, and ITS sequences placed both *Physalospora piricola* and *B. berengeriana* f. sp. *pyricola* together with white-rot-causing *B. dothidea* isolates from the USA (Ogata et al., 2000). Isolates from fruit trees in Japan were included in the further study of ITS sequences and RFLP patterns by Slippers et al. (2007), which found them to group with isolates of either *B. dothidea* or the anamorph of *B. parva*, *Neofusicoccum parvum*. Thus, while the pathogen population on fruit trees in Japan continues to be complex (Ogata et al., 2000), the identity of the wart bark-causing fungus and the white rot-causing *B. dothidea* appears confirmed. *Macrophoma Kawatsukai* Hara and *Macrosporum pirorum* Cooke have also been listed as anamorphs of *B. berengeriana* in East Asia (Tai, 1979; Kobayashi, 2007).

As a physiologically specialized variant, *B. berengeriana* f.sp. *pyricola* has only been distinguished by the different symptoms, warts rather than cankers, that it causes on twigs and stems of apple. Examining a number of *Botryosphaeria* isolates from fruit trees in Japan, Ogata et al. (2000) found a group that caused the wart symptom, produced conidia within a certain size range, and could be distinguished by the sequence of the ITS rDNA region. The group included isolates identified as *Physalospora piricola*, *B. berengeriana* f. sp. *pyricola*, or *B. dothidea*. Other isolates had smaller or larger conidia and different ITS
sequences.

Punithalingam and Holliday (1973) have described the morphology of *B. ribis*, and Jones (1990) used the figure from that description in his description of *B. dothidea*. The anamorph of *B. dothidea*, *Fusicoccum aesculi*, is also described by Sutton (1980).

A data sheet on *Botryosphaeria berengeriana* f.sp. *pyricola* published by EPPO/CABI (1997b) highlights the doubts about the separate identity of this fungus. An explanation of the taxonomic situation is provided here, but the data sheets on *B. ribis* and *B. dothidea* effectively provide a full account of the white-rot disease of pome fruits.

In Japan, the pathogen has been known as *Physalospora pyricola* since it was described in 1933 although the name *Guignardia pyricola* (used in the EU Plant Health Directive) was proposed by Yamamoto (1961). *Guignardia* species, however, have ascospores with a mucus cap and anamorphs of the *Phyllosticta* type, while *Botryosphaeria* species have larger ascospores without caps and conidial forms in *Fusicoccum* (Denman et al., 2000). Koganezawa and Sakuma (1980, 1984) compared *P. pyricola* with another fungus causing fruit rot in Japan, which they called *Botryosphaeria berengeriana*, and concluded that the two fungi were identical morphologically. The anamorph of *B. berengeriana* is a *Fusicoccum* (Sutton, 1980). Recently, *B. berengeriana* has been considered synonymous with *B. dothidea*, a fungus which is widespread as a pathogen of many plants in warm temperate regions (von Arx and Muller, 1954; Slippers et al., 2004). However, the Japanese authors regarded it as a synonym of *B. ribis*, which they and some others consider to be distinct from *B. dothidea* and which is also widespread in temperate regions. Other authors consider *B. dothidea* and *B. ribis* to be synonymous (Brown and Britton, 1986). The disease of pome fruits caused by *B. dothidea* is called “white rot” (Jones, 1990).

Because the Japanese isolates of *B. berengeriana*, previously known as *P. pyricola*, cause some symptoms (“wart bark”) distinctly different from the cankers due to typical *B. berengeriana*, Koganezawa and Sakuma (1984) proposed the name *B. berengeriana* f.sp. *pyricola* for the fungus causing apple wart bark and *B. berengeriana* f. sp. *persicae* for a similar fungus causing blister canker of peaches. These names have not been used outside Japan. Elsewhere in Asia, the agent of apple ring rot has been called *B. dothidea* (Kim and Kim, 1989), *B. berengeriana* (Lee and Yang, 1984), or still *P. pyricola* (Zhang and Huang, 1990). A fungus on apples in Brazil has also been recorded as *B. berengeriana* (Melzer and Berton, 1986), but the name is probably being used in this case as a simple synonym of *B. dothidea*. Jones (1990) regards *B. berengeriana* f. sp. *pyricola* as a synonym of *B. dothidea*. Furthermore, it is not usual to designate as a forma specialis a fungus which attacks species in more than one host genus, and such a name has no status under the rules of nomenclature (Farr and Rossman, 2009).

**DISTRIBUTION**

Strictly speaking, *B. berengeriana* f.sp. *pyricola* has only been recorded in Japan, except that the name has also been used in recent publications from China. Apple ring rot (under various names for the pathogen) has been recorded from several East Asian countries (China, Japan, Korea, and Taiwan) (CABI/EPPO, 1998). The white rot pathogen *B. dothidea*, on the other hand, occurs very widely (Sutton, 1990). In the absence of a universally agreed-upon taxonomy, geographical distribution cannot be reported meaningfully. The name *B. berengeriana* has also been used for an apple pathogen in Brazil (Melzer and Berton, 1986), but presumably this refers to *B. dothidea*, given that cankers, rather than warts, are the symptom on stems (see Valdebenito-Sanhueza et al., 2005).

**DETECTION AND INSPECTION METHODS**

The symptoms caused on pear and apple twigs and stems include warts and dieback. The fungus may or may not fruit in lesions, producing straight hyaline aseptate fusiform conidia in locules contained in a dark stroma (Sutton, 1980).

**DIAGNOSTIC METHOD**

No DNA sequence linked to a published report has been deposited in GenBank for *B. berengeriana* f.sp. *pyricola* (NCBI, 2009). Sequences for strains identified as causing the wart symptom by Ogata et al. (2000) are deposited as *B. dothidea*. Slippers et al. (2007) report a protocol for distinguishing among *Botryosphaeria* species from fruit trees using a RFLP map based on results with two restriction enzymes. Vilas-Boas et al. (2007) found that 200 bp sequences from the beginning of ITS 1 or the end of ITS 2 were the minimum necessary to distinguish among 11 of 13 species of *Botryosphaeria*.

**NOTES ON CROPS/OTHER PLANTS AFFECTED**

The main host is Japanese pear (*Pyrus pyrifolia* (Burm. f.) Nakai), but European pear (*Pyrus communis* L.) and apple are also attacked. Other hosts mentioned by Kato (1973) are *Chaenomeles japonica* and *Malus micromalus*. Ogata et al. (2000) identified a group of similar pathogenic isolates that included *B.
Berengeriana f. sp. pyricola; hosts for these Japan isolates included grapevine and peach as well as apple and pear.

Both B. dothidea and B. ribis occur on a wide range of woody hosts (Punithalingam and Holliday, 1973; Farr and Rossman, 2009).

**Symptoms**

On Japanese pears (Kato, 1973) and apples in China (Lee and Yang, 1984), the fungus forms wart-like protuberances (wart bark) on the surface of trunks and branches, rather than typical Botryosphaeria cankers. These are subsequently surrounded by dark-brown spots. Infected twigs eventually wither and die back. Large contoured dark-brown spots are formed on the leaves and the fruits. The warts on trunks and branches damage the tree, reducing its growth and productivity. The leaf spots are of minor importance and do not affect yield.

Fruit spots are circular, slightly sunken, and may be surrounded by a red halo; alternating light and dark brown rings develop as the lesions enlarge (Jones, 1990). The fruit spots develop further after harvest (Al-Haq et al., 2002, 2003), and thus cause a loss of fruit quality.

On apples, the fungus causes similar symptoms of rough bark (Koganezawa and Sakuma, 1980) and apple ring rot (Koganezawa and Sakuma, 1984).

**Biology and Ecology**

Botryosphaeria berengeriana f. sp. pyricola infects the branches, shoots, leaves and fruits of its hosts. Stroma containing conidiomata form on diseased branches and shoots after these have withered, during the period from April to September, but mainly in August and September (Dong and Zhou, 1985; Koga and Ohkubo, 1994). Sporulation is most abundant on 2-3-years-old infected shoots and less on older wood. The conidia are rain-dispersed, usually up to about 10 m, but exceptionally up to 20 m by strong wind-driven rain. Most germinate within the first 24 h, and infection is favoured by warm humid conditions (optimum temperature 28°C). Infection of young fruits requires 5 h of surface wetness, while a longer period is needed on older fruits (Koga and Ohkubo, 1994).

Under experimental conditions, artificial wounding is needed for infection of branches, although shoot tips and young leaves can be infected in the absence of wounds. Natural infection of shoots probably occurs through the shoot tip. Similarly, young fruits can be infected early in the season (up to mid-July) through stomata or lenticels (Kishi and Abiko, 1971; Dong and Zhou, 1985). Thereafter, wounds, such as the punctures made by Grapholita molesta, the oriental fruit moth, are needed for infection of fruits (EPPO/CABI, 1997a).

The incubation period for infection of shoots is 90-120 days, so that shoots infected during April-August show symptoms in September-November, and will provide inoculum in the following year. Leaves are infected in July-August, with an incubation period of about 30 days. The occurrence of the disease on fruits can be predicted from the number of rainy days in May using a quadratic regression equation (Kato, 1973).

Ascomata are found on withered branches, but ascospores are not reported to play a significant role in disease spread.

This description of the biology of B. berengeriana f. sp. pyricola is taken from the Asian literature. It is, however, very broadly similar to that of B. dothidea, for example in the south-eastern USA (McGlohon, 1982; Brown and Britton, 1986; Jones, 1990), or in Korea (Kim and Kim, 1989).

**Movement and Dispersal**

Natural dispersal: Conidia are dispersed by rain and wind (Kishi and Abiko, 1971; Dong and Zhou, 1985). Ascospores of Botryosphaeria species are carried in the air (Sutton, 1990).

Vector transmission: None reported.

Accidental introduction: None reported, but the fungus could be carried by infected fruit or in the bark of infected fruit tree propagating material.

**Impacts**

Economic impact

As Physalospora pyricola, the fungus was listed as one of the economically important pests of apples and pears in Japan (Anon., 1984), being responsible for branch dieback (of the "mal secco" type) and fruit rot. According to Koganezawa and Sakuma (1984), it became even more important, causing apple fruit rot in the 1980s, after Bordeaux mixture was less frequently used in orchards and the practice of bagging fruits had declined. (In Japan, high quality pome fruits are often individually bagged on the tree to protect them
from all kinds of damage). Presumably, the fungus was previously controlled well by copper fungicides. Losses of up to 50% in susceptible cultivars of apples were reported in China (Kexiang et al., 2002).

*Botryosphaeria dothidea*, in the wide sense, also causes a branch canker of pome fruit trees, and white rot of fruits. It is not regarded as very important over most of its range, but has been more severe in the south-eastern USA, where losses could be as high as 100% (McGlohon, 1982; Brown and Britton, 1986; Sutton, 1990).

**MANAGEMENT**

**Productive Uses**

Sassa et al. (1998) identified antifungal compounds produced by a fungus identified as *B. berengeriana*. The macrophorins E, F and G inhibited growth of both the producing species and *Gibberella fujikuroi*, a pathogen of rice.

**Prevention**

**SPS measures**

Unless a specific test is found for the Japanese or Asian isolates of the pathogen, particular exclusion of the specialized form of the fungus will not be feasible when materials not exhibiting the "wart bark" symptom are involved. Nevertheless, even though *B. dothidea* may be present already as a pathogen of fruit trees in a particular region, exclusion of foreign strains, which may differ in host range, aggressiveness or resistance to pesticides, should still be enforced.

**Control**

**Cultural control and sanitary measures**

In general, measures to reduce the conidial inoculum are recommended. Branches showing symptoms of infection should be pruned. The warts on shoots can be shaved away. Affected fruits should be removed and destroyed.

**Physical/mechanical control**

Al-Haq et al. (2002) found that EO (electrolyzed oxidizing) water reduced but did not eliminate post-harvest rot in pears. Hot water treatment, on the other hand, consisting of immersion of fruit for three minutes at 54° C, did reduce disease incidence by 85% in experimental trials in Japan (Al-Haq et al., 2003). It was noted that is unlikely to be effective against deeper wounds, especially those which became inoculated during harvest.

**Biological control**

Because frequent application of fungicides has resulted in development of resistance in the pathogen, Kexiang et al. (2002) investigated the use of *Trichoderma* species for control of ring rot of apple. Treatment with suspensions of conidia of *T. harzianum* T88 or *T. atroviride* T95 reduced both the incidence and severity of disease, as well as reducing survival of the fungus, in detached branch sections. Disease on stems and fruit of orchard trees was reduced to a level similar to that obtained with regular applications of Bordeaux mixture and carbendazim. Other efforts at biological control in China have involved application of the bacterium *Bacillus subtilis* (Zhao et al., 2008) and an antibiotic (Zhu et al., 2004).

**Chemical control**

Copper fungicides have proved effective in Japan, and the reduction in their use has led to a resurgence of apple fruit rot. Captan, benomyl, captan, difolatan, polyoxin and 8-hydroxyquinoline are other fungicides which have been shown to be effective in Japan (Kishi and Abiko, 1971; Kato, 1973). Both the copper-containing Bordeaux mixture and the organic carbendazim are use in China (Kexiang et al., 2002). Organic fungicides are recommended against *B. dothidea* in the USA (McGlohon, 1982) and Korea (Kim and Kim, 1989). Organic arsenic emulsion has been recommended in Japan for treatment of the warts on the shoots, though it is doubtful whether such products would now be authorized for this use. Sato et al. (1987) have recently investigated eradicant fungicides for trunk lesions.

**Host resistance**

Some cultivars are reported to have resistance (Cho et al., 1986; Kim and Kim, 1989; Li et al., 1997). Some varieties resistant to infection have fruit with smaller lenticels that are more prone to cracking (Kim and Kim, 1989).

**GAPS IN KNOWLEDGE/RESEARCH NEEDS**
An assay is needed to distinguish the physiologically different fungus easily and quickly from closely related *Botryosphaeria* pathogens that might also be carried in fruit or in fruit tree propagating material. This assay will likely also elucidate the nature of the difference in the Asian pathogen, as well as allowing for a clear determination of its geographic distribution.

References


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