EPPO Datasheet: *Botryosphaeria kuwatsukai*

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IDENTITY

Preferred name: *Botryosphaeria kuwatsukai*
Authority: (Hara) G.Y. Sun & E. Tanaka
Taxonomic position: Fungi: Ascomycota: Pezizomycotina: Dothideomycetes: Botryosphaeriales: Botryosphaeriaceae
Other scientific names: *Botryosphaeria berengeriana f. sp. pyricola* (Nose) Koganezawa & Sakuma, *Guignardia pyricola* (Nose) Yamamoto, *Macrophoma kuwatsukai* Hara, *Macrophoma pyrorum* Cooke, *Physalospora pyricola* Nose
Common names: blister canker of pome fruits, physalospora canker of pome fruits, ring rot of apple, wart bark of apple
view more common names online...
EU Categorization: A1 Quarantine pest (Annex II A)
EPPO Code: PHYOPI

Notes on taxonomy and nomenclature

Ring rot is one of the most destructive apple diseases worldwide and it is caused primarily by *B. kuwatsukai* and *B. dothidea* which were considered to be synonyms for a long time (Sinclair & Lyon, 2005). The pathogen *B. kuwatsukai* was initially reported as *Physalospora piricola* in Japan, while the name *Guignardia piricola* was proposed by Yamamoto (1961) for the same pathogen, but not accepted. Later, *P. piricola* was synonymized with *Botryosphaeria berengeriana*, another fungus causing fruit rot in Japan (Koganezawa & Sakuma, 1980). However, despite *P. piricola* and *B. berengeriana* are indistinguishable in terms of morphology, isolates of these species caused different canker symptoms, therefore, a new name, *B. berengeriana* f. sp. *pyricola*, was proposed (Koganezawa & Sakuma, 1980; Xu *et al.*, 2015). *B. berengeriana* f. sp. *pyricola* was generally considered to be a synonym of *B. dothidea* (Jayasiri *et al.*, 2015). However, *B. berengeriana* f. sp. *pyricola* demonstrates substantial genetic, morphological and biological distinctions from *B. dothidea*, such as different number and length of group I introns in the primary structures of the 18S rDNA, and different structure of aerial mycelia and pathogenicity tests on pear stems (Jayasiri *et al.*, 2015; Xu *et al.*, 2015). Based on morphological, phylogenetic, pathological, and molecular analyses of reference isolates of *B. berengeriana* f. sp. *pyricola* and *B. dothidea* from Japan, New Zealand, and Switzerland Xu *et al.* (2015) showed the existence of two species within the *Botryosphaeria* isolates: one species included an ex-epitype isolate of *B. dothidea* and the other an isolate previously designated as *B. berengeriana* f. sp. *pyricola*. Thus, *B. berengeriana* f. sp. *pyricola* was described as a new species, namely *B. kuwatsukai*, causing fruit ring rot and extensive cankers and/or warts on pear stems, whereas *B. dothidea* was recognized to occur non-pathogenically on pear stems (Xu *et al.*, 2015). However, both species can infect apple stems and fruits and cause similar symptoms in apples (Xu *et al.*, 2015).

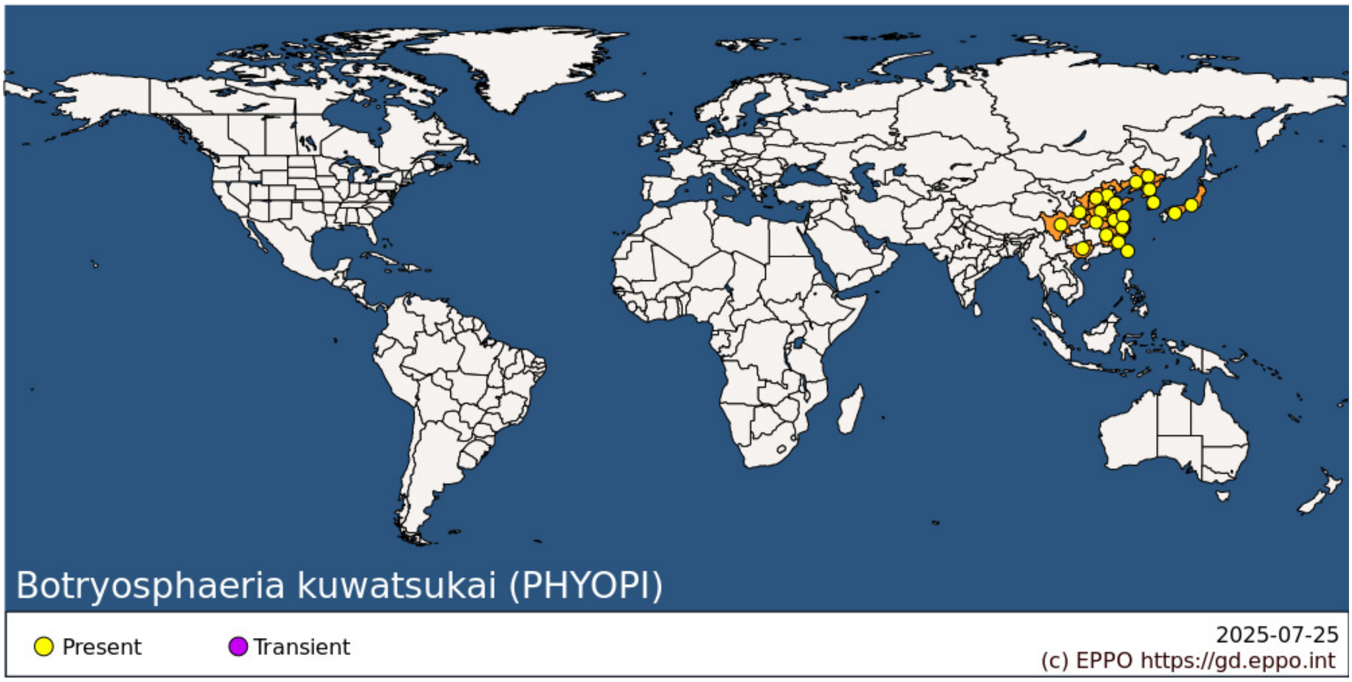
HOSTS

The main host is Japanese pears (*Pyrus pyrifolia*), but European pears (*P. communis*) and apples (*Malus* spp.) are also be affected (Xu *et al.*, 2015). Other hosts are *Chaenomeles japonica* and *Malus micromalus* which were mentioned by Kato (1973) and *Cydonia oblonga* (CABI, 2023), but no additional references were found to confirm susceptibility of these plants to the pathogen, so there is still uncertainty about these hosts (Jayasiri *et al.*, 2015; EFSA, 2017).

Host list: *Chaenomeles japonica*, *Cydonia oblonga*, *Malus domestica*, *Malus x micromalus*, *Pyrus communis*, *Pyrus pyrifolia var. culta*

GEOGRAPHICAL DISTRIBUTION

B. kuwatsukai has been recorded only from Eastern Asia and has not apparently widely spread from there. The name *B. berengeriana* has also been used for an apple pathogen in Brazil, but presumably refers to *B. dothidea*.



Asia: China (Anhui, Fujian, Guangxi, Hebei, Henan, Hubei, Jiangsu, Jiangxi, Jilin, Liaoning, Shaanxi, Shandong, Shanxi, Sichuan, Zhejiang), Japan (Honshu, Shikoku), Korea, Democratic People's Republic of, Korea, Republic of, Taiwan

BIOLOGY

B. kuwatsukai infects branches, shoots, leaves and fruits of its hosts. The fungus causes large cankers and bark blisters on pear (*Pyrus communis*), whereas on apple shoots, it induces large wart-like bark swellings (Xu *et al.*, 2015). The disease is characterized by black pycnidial stromata that differentiate beneath the surface of killed bark and break through at maturity (Sinclair & Lyon, 2005). Stromata vary in shape, pycnidia form on diseased branches and shoots after these have withered, during the period from April to September, but mainly in August and September (Ogata *et al.*, 2000; EPPO, 2023). Sporulation is most abundant on infected shoots which are 2–3 years old and less on older wood (Sinclair & Lyon, 2005). Under wet weather conditions when infected bark is moist, one-celled, colourless conidia 17–25 x 5–7 µm, somewhat narrowed at each end, extrude in a white mass from the pore at the top of each pycnidium (Sinclair & Lyon, 2005). The conidia are rain-dispersed, usually up to about 10 m, but exceptionally up to 20 m by strong wind-driven rain (Sinclair & Lyon, 2005). They mostly germinate within 24 h, and the peak of conidia release occurs after 4 h of moisture retention and maintains a high level for 12 h (Shutong *et al.*, 2012). The infection is favoured by warm humid conditions (optimum temperature 28°C) and infection of young fruits requires 5 h of surface wetness, while older fruits need longer (Shutong *et al.*, 2012).

The pycnidial stromata develop throughout the year as temperature permits, usually beginning within days to weeks after diseased tissues die and stromata development depends upon sufficient moisture in the killed tissues (Sinclair & Lyon, 2005).

Under experimental conditions, artificial wounding is needed for branches to be infected, although shoot tips and young leaves can be infected without wounding. 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 & Abiko, 1971). Thereafter, wounds are needed for infection of fruits (e.g. punctures of *Grapholita molesta*; EPPO/CABI, 1996).

The incubation period for infection of shoots is 90–120 days, so that shoots infected during April–August show symptoms in September–November and 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 by a quadratic regression equation (Kato, 1973).

Mature pseudothecia of the sexual stage may develop in the same stromata that previously produced conidia or in new stromata, usually on tissue which has been dead for several months to a year or more (Sinclair & Lyon, 2005). When pseudothecia are developed, ascospores are dispersed by air and water during much of the growing season and, like conidia, are most abundant during late spring to early summer, but ascospores are not reported to play a significant role in disease spread (Sinclair & Lyon, 2005).

Morphologically, however, it is hard to discriminate *B. kuwatsukai* from *B. dothidea* as they produce similar conidia and affected the same hosts inducing similar symptoms (Xu *et al.*, 2015).

B. kuwatsukai can exist as endophyte in healthy plant tissues for extended period of time (Slippers *et al.*, 2017).

This description of the biology of *B. kuwatsukai* is taken mostly from the old Japanese literature; it is, however, very broadly similar to that of *B. dothidea*, for example in South-Eastern USA (McGlohon, 1982; Koganezawa & Sakuma, 1984; Brown & Britton, 1986; Jones & Aldwinkle, 1990).

DETECTION AND IDENTIFICATION

Symptoms

Symptoms caused by *B. kuwatsukai* may vary in size and extent on apple and pear trees (Sinclair & Lyon, 2005, Dong *et al.*, 2021). The disease characterized by ring rot on fruit and restricted warts on branches is known as ring rot because of the alternating light and dark concentric rings in the fruit rot lesions (Dong *et al.*, 2021). On Japanese pears and apple (*Malus* spp.), the fungus forms wart-like protuberances (wart bark) on the surface of trunks and branches, rather than typical Botryosphaeria cankers (Jones, 2014). The warts on trunks and branches damage the tree, reducing its growth and productivity. Lesions on branches, twigs and trunk vary from tiny and superficial spots on bark to sunken cankers delimited by vigorous callus ridges or spreading lesions without marginal callus (Sinclair & Lyon, 2005; Jones, 2014, Dong *et al.*, 2021). Usually, infected twigs die, but trunks and branches may have no symptoms or contain the pathogen within discrete cankers (Sinclair & Lyon, 2005; Jones, 2014). The leaf spots are of minor importance and do not affect yield. The fruit spots progress after harvest, alternating light and dark brown rings develops in the decayed tissue, and thus cause a loss of fruit quality (Koganezawa & Sakuma, 1980, 1984).

To distinguish *B. kuwatsukai* and *B. dothidea*, pathogenicity tests could be used showing that on pear stems *B. kuwatsukai* caused large-scale cankers along with blisters whereas *B. dothidea* was non-pathogenic (Xu *et al.*, 2015). There are two distinct symptoms on apple (*Malus domestica*) that can be also used to distinguish *B. kuwatsukai* and *B. dothidea*. The first one is causing ring rot on fruit and restricted wart-like prominences or canker-like protrusions the year after infection, while the other causes expanding cankers on branches (Dong *et al.*, 2021).

Morphology

According to Xu *et al.* (2015), most of morphological features of the fungus are identical to those of *B. dothidea.* Xu *et al.* (2015) also provide the following description of the cultural and morphological characteristics of *B. kuwatsukai* : colonies on potato dextrose agar (PDA) media attaining 52 mm diameter after 4 days at 25°C in the dark, initially white with moderately dense, appressed mycelial mat and aerial mycelium without columns, gradually becoming grey to dark grey (Jayasiri *et al.*, 2015). The reverse side of the colonies at first is white, but after 2–3 days it becomes dark green to olive-green from the centre. This colouration gradually spreads to the edge and becomes darker from the centre until the entire underside of the colony is black (Jayasiri *et al.*, 2015). Conidiomata in culture are superficial, dark brown to black, globose, mostly solitary and covered by mycelium (Jayasiri *et al.*, 2015). Conidiogenous cells holoblastic, hyaline, sub-cylindrical, 7–18 × 2–4 ?m, conidia produced in culture similar to those formed in nature, narrowly fusiform, or irregularly fusiform, base subtruncate to bluntly rounded, smooth with granular contents, widest in the middle to upper third, (18.5–)20…24.5(–26) × 5…7(–8) ?m (mean ± SD = 22.3 ± 2.1 × 6.2 ± 0.9 ?m, n = 60, L/W ratio = 3.6), forming 1–3 septa before germination (Jayasiri *et al.*, 2015). The pycnidial stromata in nature vary in size, they are 1–4 mm in the longest dimension and contain one to several pycnidial cavities 150–250 µm in diameter with colourless contents that appear white when sliced (Sinclair & Lyon, 2005). Microconidiomata globose, dark brown to black. Microconidiophores hyaline, cylindrical to sub-cylindrical, 3–10 × 1–2 ?m, microconidia unicellular, hyaline, allantoid to rod-shaped, 3–8 × 1–2 ?m. Sexual state not observed in culture (Jayasiri *et al.*, 2015).

Detection and inspection methods

B. kuwatsukai, which has for many years been confused with *B. dothidea*, can be identified based on multiple methods. Biological characteristics including the aerial mycelia growth, mycelial growth rate and pathogenicity also supported the segregation of these two species. Morphologically, however, it is difficult to discriminate *B. kuwatsukai* from *B. dothidea* as they produce similar conidia. As a physiologically specialized taxon, *B. kuwatsukai* has only been distinguished by the different symptoms, warts rather than cankers, that it causes on twigs and stems of apple (*Malus* spp.). Examining a number of *Botryosphaeria* isolates from fruit trees in Japan, Ogata *et al.* (2000) found a group that caused the wart symptom on twigs, size of the conidia within a certain size range, and could be distinguished by the nucleotide sequences of rDNA ITS 1, ITS 2 and 5.8S rDNA. Molecular data are available in GenBank for the epitype of *B. kuwatsukai*, HMAS 245112 (PG 2) and GenBank contains sequences of different region: ITS: KJ433388 (ITS1/ITS4); EF1-?: KJ433410 (EF446f/EF1035r); HSP: KJ433456 (HspF3/HspR); HIS: KJ433432 (HisF3/HisR) (Jayasiri *et al.*, 2015).

Imported host plants for planting and dormant plants of apple (*Malus* spp.) and pear (*Pyrus* spp.) trees from the countries where the disease occurs should not have any symptoms of cankers and bark blisters or wart-like bark swellings cankers on apple and Japanese pear during inspection.

Any material with canker lesions should be carefully inspected. Particular attention should be paid to the fruit (apples and pears) because they can have black conidiomata scattered on the lesions (Jayasiri *et al.*, 2015). Infection occurs on young fruit, and would be detectable on harvested fruit, rather than only appearing later in storage (post-harvest rot). Accordingly, infected fruit are relatively unlikely to be traded. However, it is possible that pathogen can survive in symptomless branches and trunks which may contain mycelium or stromata, thus inspection may be ineffective.

PATHWAYS FOR MOVEMENT

The pathogen can spread by both natural and human-assisted means. Under natural conditions, *B. kuwatsukai* is locally dispersed by rain over relatively short distances. Nevertheless, uncertainty exists on the distance over which the ascospores of the pathogen could be wind disseminated because of lack of information (EFSA, 2017). The fungus can potentially spread over long distances through the movement of infected (symptomatic and asymptomatic) timber, bark, plants for planting (rootstocks, grafted plants, scions, etc.), and fresh fruit (EFSA, 2017), although it seems improbable that infected fruit could be traded. As mentioned above, *B. kuwatsukai* can exist as endophyte in healthy plant tissues for extended periods of time, thus it can potentially spread freely with healthy plant material, including fruit (Slippers *et al.*, 2017).

PEST SIGNIFICANCE

Economic impact

B. kuwatsukai is the pathogen responsible for apple ring rot and pear canker and it can cause rot on fruit, especially during storage, and extensive cankers and/or warts on branches and trunks, resulting in serious economic losses to fruit farmers in China, Japan, and Koreas on apple and Japanese and European pears (Ogata *et al.*, 2000; Jayasiri *et al.*, 2015; Zhao *et al.*, 2015, Dong *et al.*, 2021). According to Koganezawa & Sakuma (1984), it has become more common, causing apple fruit rot in the 1980s, when Bordeaux mixture began to be used less frequently in orchards and the practice of bagging fruits declined (in Japan, high quality pome fruits are often individually bagged on the tree to protect them from all kinds of damage). A survey in 2008 in the seven main apple production provinces in China has showed that the average incidence of apple ring rot was 77.6% (Dong *et al.*, 2021).

Control

Copper fungicides have proved effective in Japan, and the reduction in their use has led to a resurgence of apple fruit rot (Kexiang *et al.*, 2002; Bhusal *et al.*, 2016). Organic arsenic emulsion was previously 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 (Liu *et al.*, 2011).

Biological control of this pest has also been tested. Laboratory and field trials were carried out in Hebei province (China) to evaluate the potential of *Trichoderma harzianum* and *T. atroviride* (Ascomycota: Hypocreaceae) to control *B. kuwatsukai*. In the laboratory, both *Trichoderma* species inhibited the *B. kuwatsukai*, apparently by direct antagonism with minor inhibition by antibiosis. In the field, where apple trees were severely affected by the disease, the application of spore suspensions of both fungi gave satisfactory results and the efficacy was similar to that of routine chemical control (Kexiang *et al.*, 2002).

In general, it is recommended to take the following agricultural practices and sanitary and chemical measures to reduce the inoculum source: pruning of symptomatic and dead plant parts and shaving of warts on shoots; sprays with copper-based fungicides; sanitation measures to reduce inoculum sources in the orchards (Cho *et al.*, 1986; Kim & Kim, 1989; EFSA, 2017).

Phytosanitary risk

B. kuwatsukai has never been reported in the EPPO region. In Japan, China, and Koreas the fungus is reported to be important. Though mainly occurring on Japanese pears, the fungus has been recorded in Japan damaging European pears and apples. The risk of entry (for the EU) was assessed by EFSA as the pest could potentially enter, establish and spread in the EU. It is not clear, however, whether the fungus can be distinguished from *B. dothidea* during inspection, and how feasible it is to take measures if the pest identification is difficult (EFSA, 2017).

PHYTOSANITARY MEASURES

Phytosanitary measures were suggested to prevent the entry of the pathogen into the EPPO region (e.g. sourcing host plants for planting and fruit should originate from pest-free areas or pest-free places of production). In the case of *B. kuwatsukai*, inspections at the place of origin and the entry point might be not fully effective to prevent the entry of the pathogen because of it is difficult to ensure that imported plants for planting of *Malus* and *Pyrus* from countries where the pathogen is known are free from a latent infection As a general approach, it has also been recommended that when importing plants for planting (except seeds) from the countries where *B. kuwatsukai* occurs, precautions should have been taken to avoid any infestations while the plants or fruits are transported through possibly infested areas (Kim & Kim, 1989; EFSA, 2017).

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Datasheet history

This datasheet was first published as *Botryosphaeria berengeriana* f. sp. *piricola* in the first edition of 'Quarantine Pests for Europe' in 1992 and revised in the second edition of the book in 1997, as well as in 2023. It is now maintained in an electronic format in the EPPO Global Database. The sections on 'Identity', 'Hosts', and 'Geographical distribution' are automatically updated from the database. For other sections, the date of last revision is indicated on the right.

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