European and Mediterranean Plant Protection Organization
Organisation Européenne et Méditerranéenne pour la Protection des Plantes

Data sheets on quarantine pests
Fiches informatives sur les organismes de quarantaine

Sirococcus clavigignenti-juglandacearum

Identity

Name: *Sirococcus clavigignenti-juglandacearum* Nair, Kostichka & Kuntz
Taxonomic position: *Fungi*: anamorphic *Ascomycota*
Notes on taxonomy and nomenclature: the form-genus *Sirococcus* is probably heterogeneous (Castlebury *et al*., 2003), and the affinities of this fungus are not clear
Common names: canker of butternut (English), chancre du noyer cendré (French), Sirococcus-Krebs der Walnuss (German)
EPPO code: SIROCJ
Phytosanitary categorization: EPPO A1 action list no. 329

Hosts

The main host is *Juglans cinerea* (butternut, white walnut, oilnut), which is native to North America. It is the only species that is killed by the pathogen, though other *Juglans* species and hybrids are diseased to varying degrees. Two other hosts are naturally infected in North America: *Juglans nigra* (black walnut) and *Juglans ailantifolia* var. *cordiformis* (heartnut), but infrequently and only when standing among heavily infected butternut trees (Kuntz *et al*., 1979; Ostry, 1997a; Ostry *et al*., 1997). Some other *Juglandaceae* were found to be susceptible on artificial inoculation in laboratory experiments: *Juglans regia*, *J. ailantifolia*, and various hybrids (Orchard *et al*., 1982). Laboratory experiments by Ostry (1997b) indicated that *S. clavigignenti-juglandacearum* might be able to survive on other Juglandaceous hosts (*Carya* spp.) and possibly other trees (*Quercus*, *Prunus*).

The most important potential host in the EPPO region is *Juglans regia* (walnut), an Asian species which is widely planted throughout most of Europe except the north. It needs mild winters without late frosts, and a climate which is not too dry. It can grow up to an elevation of 800 m, in the Alps up to 1200 m. Hungary, Turkey, Italy and France have commercial plantations of *J. regia* (Bernyi *et al*., 1991; Schütt *et al*., 1992), to produce nuts, oil or the valuable wood. In these countries and in many others, individual trees of *J. regia* are common in farms and gardens, providing nuts locally. *Juglans nigra* from North America, and *J. x intermedia* (*J. regia* × *J. nigra*), are also planted on a smaller scale, and used as rootstocks for *J. regia*.

Juglans spp. are also grown as amenity trees in parks and sold in nurseries (e.g. *J. nigra*, *J. ailantifolia*). The main host of *S. clavigignenti-juglandacearum* (*J. cinerea*) is, however, only grown to a very limited extent in Europe.

Geographical distribution

S. clavigignenti-juglandacearum was first reported in Wisconsin (US) in 1967 (Renlund, 1971). A pest alert announcing butternut decline was issued in 1976 (USDA, 1976). The origin and the original host plant of the fungus are not known, but there is evidence that it was introduced into the USA as a single isolate in the 1960s or earlier. The American population has a low level of genetic diversity, as confirmed by RAPD-PCR (Ostry & Skilling, 1995; Ostry, 1997b; Furnier *et al*., 1999). The pathogen spread rapidly across the USA and is now present in the entire native distribution area of *J. cinerea* (Tisserat & Kuntz, 1983a; USDA, 1995). In Canada (Ontario, Québec), it was detected for the first time in 1991 (Davis *et al*., 1992; Innes & Rainville, 1996). In 1995, infestation of a forest nursery was reported in Québec. In New Brunswick, the fungus was confirmed present for the first time in 1997 (Harrison *et al*., 1998).

Since the genus *Juglans* and the *Juglandaceae* are, besides North America and Europe, distributed in Asia and in South America, it must be presumed that *S. clavigignenti-juglandacearum* originates in one or other of the latter.
EPPO region: absent
North America: Canada (at least in Ontario, Québec and New Brunswick), USA (spread across the entire range of butternut, see above)

Biology

S. clavigignenti-juglandacearum was first described by Nair *et al*. (1979), and its pathogenicity has been demonstrated according to Koch's postulates. Up to now, the teleomorph of the fungus have not been discovered (Furnier *et al*., 1999). Conidia develop beneath infected bark in sticky masses. Stromatal pegs lift and rupture the bark and, under moist conditions, millions of conidia are extruded in colourless cirri from globose to flattened, single or confluent pycnidia borne in the stroma among the hyphal pegs. These conidia are dispersed by rain splash and wind in little droplets or as aerosols during rainfall (Kuntz *et al*., 1979; Tisserat & Kuntz, 1983b). Relative humidity of 95–100% was found to aid successful infection greatly (Federspiel & Nair, 1982). Conidia are transported by run-off water from infected branches to tree trunks, infecting wounds and other openings. This results in multiple stem cankers.

The tree is first infected through buds and openings like lenticels, cracked bark, or leaf scars (Kuntz *et al*., 1979; Ostry, 1997b). Cankers on twigs start to develop, usually in the lower crown, due to the favourable microclimatic conditions in this part of the tree (the branches in the lower crown tend to retain the moisture necessary for spore germination for longer periods than in the upper crown; Tisserat & Kuntz, 1984). Spores of *S. clavigignenti-juglandacearum* require at least 16 h of dew at 20°C to germinate on the bark of *J. cinerea*.

In infected suckers, twigs and branches, cell walls of the bark are degraded, macerated and rapidly broken up entirely, resulting in a dark brown sticky residue and a brown exudate. The fungal hyphae penetrate the phloem parenchyma intracellularly and between the phloem fibre elements intercellularly. They advance intracellularly through the uni- and multiseriate xylem ray cells and parenchyma cells. In infected *J. cinerea* and *J. nigra*, hyphae in the xylem ray cells appeared larger and darker than those in xylem parenchyma cells. From the xylem, hyphae progress finally into other bark and wood tissues (Kuntz *et al*., 1979; Nair, 1999). Hyphae grow also beyond cankered tissue, creating new cankers when reaching the cambium (Sinclair *et al*., 1987).

The fungus is able to sporulate on standing or felled dead trees for at least 20 months (Tisserat & Kuntz, 1982, 1984). Outside the host, conidia can survive for at least 8 h in cool and cloudy weather (Tisserat & Kuntz, 1983c). The pathogen remains viable in diseased tissue and in culture down to 0°C and below. The fungus is seed-borne, at least in seeds of *J. cinerea* and *J. nigra* (Orchard, 1984; Innes, 1997). This partially explains why *J. cinerea* does not regenerate after early infection and killing of trees (Ostry, 1997b). In a glasshouse experiment, seedlings of *J. cinerea* were killed soon after emergence from seeds derived from infected trees that were stratified with intact husks (Orchard, 1984). Additionally, the seedlings are highly susceptible to the fungus and die quickly after infection. It is accordingly not likely that young plants would carry the pathogen if traded as plants for planting. There is no evidence that the fungus can spread on seed or seedlings of *J. ailantifolia*.

Detection and identification

Symptoms

Cankers can occur on all woody parts of *J. cinerea*, including twigs, branches, stems and buttress roots. Stem cankers, usually many per tree, separate or joined together, can be present on all sides of the trunk, especially the lower portion. Trees of all ages and sizes, regardless of site conditions, can be infected. Tisserat & Kuntz (1984) found that the disease develops in a characteristic pattern. Young cankers are elongated, sunken areas, which usually originate at leaf scars, lenticels, lateral buds, stomata, bark wounds, natural bark cracks, and even sporadically at points apparently free of any injury. These initial cankers are generally found in the lower crowns. In spring, an inky black, watery exudate issues from canker fissures. In summer, dry sooty black patches remain, often with a whitish margin. If the bark is removed, a brown to black elliptical area of killed cambium can be seen. Small branches and young saplings die rapidly, but most stem cankers of older trees become perennial. These occur mostly on the lower stem and on exposed parts of the roots. Older cankers are surrounded by layers of callus and are vertically oriented, large, open or partly covered by shredded bark. Trees are killed progressively as cankers girdle the stem or kill the crown of the tree. Trunk sprouts and branch suckers often emerge below the girdled area but are infected subsequently and rapidly killed (Kuntz *et al*., 1979; Ostry, 1997b). Fruits from infected trees are sound and edible according to Kuntz *et al*. (1979). For a detailed description of symptoms on *J. regia* and *J. nigra*, see Orchard (1984).

Another fungus, *Melanconis juglandis* (Ellis & Everhart) Graves (anamorph: *Melanconium oblongum* Berk.), often causes secondary infection (Nicholls *et al*., 1978), and has been confused with *S. clavigignenti-juglandacearum*. *M. juglandis* does not cause cankers. Its anamorphic fruiting bodies appear as very small, dark, smooth bumps on the bark, releasing inky black masses of spores. Spores are usually asymmetrically ovoid, dark, and non-septate (Nicholls *et al*., 1978).

Morphology

The pycnidia form in a thin, dark brown to black stroma of abundant, septate, branched mycelium beneath the outmost bark layer. Prominent hyphal pegs, measuring $1550–1900 \times 450–510$ µm, arise from this stroma. The pycnidia arise from the eustroma and are glabrous, non-rostrate, innate to erumpent and without a clypeus, globose to flattened. Pycnidia measure $90–320 \times 100–375$ µm, reaching $90–320 \times 200–490$ µm if confluent or multilocular. Walls multilayered, the outer becoming dark and coriaceous with age; cavity simple, convoluted or multilocular, uni- or multiostiolate; ostioles 8–30 µm in diameter; conidiophores 5–24 µm, simple or branched, septate with monophialidic, conidiogenous cells; conidia hyaline, two-celled, median-septate and fusiform, $9–17 \times 1.0–1.5$ µm, extruded singly or in chains through the ostiole in a glutinous beige to tan matrix, in cirri under moist conditions; cirri composed of coherent, vertically arranged spores, colourless and either thin or thick; during germination, germ tubes arise from the swollen cells either terminally or laterally (Nair *et al*., 1979).

Detection and inspection methods

S. clavigignenti-juglandacearum is easily isolated on common nutrient agar media (e.g. potato dextrose agar, malt agar, nutrient dextrose agar) from bark and wood. It grows best at

24–28°C, in light or dark, producing beige to tan colonies, commonly advancing as irregular fans along the margins, darkening the underlying agar subsequently from amber to dark brown. The fungus forms hyphal pegs and pycnidia in culture. It sporulates profusely in the light or dark especially on thin, drying agar cultures (Nair *et al*., 1979; Kuntz *et al*., 1979; Nair, 1999). In water, the spores swell slightly in width and shrink in length and look like two spear heads butted together.

Artificial inoculation of *J. cinerea*, as described by Federspiel & Nair (1982) and Gabka (1996), leads to blackening of the inner bark tissue in 6 days (8 for *J. nigra*). Removal of the bark reveals a brown to black elliptical area of killed cambium (Ostry, 1997b).

Pathways for movement

The fungus apparently spread rapidly in the USA and Canada. However, infected trees and/or seed were probably moving freely in trade long before the disease was recognized. The pathogen may have gone undetected, as *J. cinerea* is not very commonly grown. After identification of the fungus, survey intensity increased and this may have given an impression of rapid spread (Cree, 1995).

Spores are dispersed by rain or mist over short distances. By wind, spores can be transported up to 45 m (Tisserat *et al*., 1981; Tisserat, 1982). Small droplets or aerosols with conidia may be swept above the tree canopy by turbulent air and may possibly spread by wind over distances of more than 1 km (Tisserat & Kuntz, 1983c). Insects, including sap and tissue feeders, are thought to be responsible for long-distance spread of the fungus. Birds may also be vectors. Additionally, insects or birds may, by causing wounds, increase points for infection (Ostry *et al*., 1994; Nair, 1999). Ostry (1997b) found the fungus on the beetles *Laemophlaeus fasciatus*, *Laemophloeus testareus* (Laemophloeidae) and *Bactridium* spp. (Monotomidae). Halik & Bergdahl (1996; 2002), Bergdahl & Halik (1997) and Katovich & Ostry (1998) found it on *Acoptus suturalis* (Curculionidae), *Astylopsis macula* (Cerambycidae), *Cossonus platalea* (Curculionidae), *Eubulus parochus* (Curculionidae), *Hyperplatys maculata* (Cerambycidae), *Laemophlaeus biguttatus* (Laemophloeidae), and *Glischrochilus quadrisignatus* (Nitidulidae). Up to 44% of the collected specimens of *A. macula* and 38% of the specimens of *E. parochus* carried conidia of *S. clavigignenti-juglandacearum*. However, disease transmission by these insects does not as such seem to have been demonstrated.

According to Orchard (1984) and Innes (1997), the fungus can be internally seed-borne on *J. cinerea* and *J. nigra*, surviving in the cotyledons of infected seed stratified at 4°C up to 18 months (Prey *et al*., 1997). Seedlings from infected seeds develop basal lesions, stem cankers, stroma, pycnidia, and hyphal pegs and die rapidly (Orchard, 1984). Thus, internally infected seeds from infected trees could be present an important pathway for moving *S. clavigignenti-juglandacearum* to new areas. Movement of scion wood and other propagative material, as well as untreated logs and firewood with bark from diseased trees, may add to the dispersal of the fungus.

Pest significance

Economic impact

S. clavigignenti-juglandacearum is a very aggressive plant pest. From 1981 to 1996, in the USA, it killed 58% of the trees of *J. cinerea* in Wisconsin and 84% in Michigan (Ostry, 1997b). Cummings-Carlson (1993) reported that 91% of *J. cinerea* trees in Wisconsin were already infested at the time of her survey. In North and South Carolina, the fungus had eliminated *J. cinerea* to a large extent by 1977 (Anderson & Lamadeleine, 1977) and 77% of *J. cinerea* trees had been killed in North Carolina & Virginia by 1994 (Usda, 1995; Tainter & Baker, 1996). In USA, good-quality wood of *J. cinerea* is second in economic value only to *J. nigra* (Peterson, 1977), for use in interior woodwork, household woodenware, furniture, cabinetry and dye production. Nuts present an important source of wildlife food.

S. *clavigignenti-juglandacearum* presents a threat to biodiversity in North America. Due to the extensive mortality caused by the fungus and the large amount of already infected trees, *J. cinerea* was listed as a threatened and endangered species in the USA (Category 2 – more information required prior to formal listing; Ostry *et al*., 1994), but this category has been eliminated and currently *J. cinerea* has no official listing status. In Canada, butternut was listed as an endangered species by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in November 2003.

Control

Up to now, there is no effectively applied control against *S. clavigignenti-juglandacearum*. The fungicides benomyl and chlorothalonil have been found to be effective preventive products in laboratory experiments (Nicholls, 1979), but were not approved in the field. Even if an effective mechanical method, fungicide or biocontrol agent existed, it would be difficult to apply it due to the scattered distribution of susceptible trees. Removal of recently killed and visibly infected trees may reduce the amount of inoculum only locally (Nair, 1999).

The only possibility for successful control of the fungus seems to be the selection of resistant forms. Resistance varies greatly between individual trees and is possibly heritable (Ostry, 1997b). Studies are in progress to find, graft, propagate, and plant resistant trees of *J. cinerea* in the USA (Ostry, 1997b).

Phytosanitary risk

The status of the host plants of *S. clavigignenti-juglandacearum* in Europe, and their susceptibility to the fungus, has been reviewed under Hosts (above). Economically, *J. regia* and *J. nigra* have the most valuable wood in Central Europe (Kucera, 1991; Beyse, 2001) and high-quality veneers make profits of more than 5000 EUR per m^3. Wood of *Juglans regia* is used for high-quality cabinet making, musical instruments, crafts, stocks, etc. In France (Becquey, 1991), a campaign, funded by the EU, aims to produce high-quality *Juglans* wood for the furniture industry, by increasing the number and quality of walnut trees in Europe and standardizing walnut wood according to its use. It is planned to afforest agricultural lands with *Juglans* spp., according to new intensive sylvicultural systems. In Germany, walnut is also being promoted beyond its typical cultivation areas by the 'Interessengemeinschaft Nuss' (Kronauer, 1991; Anonymous, 1999). Nut and oil production are important industries in southern European countries (France, Hungary, Italy, Turkey).

It seems very likely that the fungus would, in southern Europe, encounter similar conditions to those in which it has been so damaging in North America. European *Juglans* spp. carry insects of the same families as those thought to act as vectors in North America (Schwenke, 1974) (see above). As in North America, reliable control measures are not available. Current American research on resistance in *J. cinerea* would not protect the *Juglandaceae* which grow in Europe.

According to Patterson (1993), *S. clavigignenti-juglandacearum* is much more aggressive than the fungi which caused chestnut blight (*Cryphonectria parasitica*; EPPO/CABI, 1997) and Dutch elm disease (*Ophiostoma ulmi*/*Ophiostoma novo-ulmi*). Furthermore, though the main pathways which were involved in the spread of these fungi (plants for planting, wood) are probably not of great importance in practice for *S. clavigignenti-juglandacearum*, the latter can be carried in infected nuts which carry a significant risk of casual introduction. There is general agreement among experts on the disease (e.g. Orchard, 1984; Fleguel, 1996; Nair, 1999) that the fungus presents a high risk to other continents and justifies phytosanitary measures. The seed transferability of the pathogen is one of the main risk factors to be considered when measures against the introduction of the fungus are taken. Thus, *S. clavigignenti-juglandacearum* presents a high risk to the EPPO region (Schröder *et al*., 2002) in that it is likely to kill large numbers of valuable trees used for economic production of wood, nuts and oil. It also threatens walnut as an amenity species widely planted throughout the EPPO region.

Phytosanitary measures

S. clavigignenti-juglandacearum was added in 2005 to the EPPO A2 action list, and EPPO member countries are thus recommended to regulate it as a quarantine pest. Suggested measures could include the prohibition of import of host plants for planting (including seeds) from countries where the pest occurs, or as appropriate origin from a pest-free area or pest-free place of production. It may be noted that *J. regia* is produced in USA nearly entirely in California, where natural infestation of *J. regia* has not yet been observed and the fungus does not occur (movement of *Juglans* spp. from eastern USA to California is restricted).

As a preventive measure in the EPPO region, countries could restrict the planting of *J. cinerea* and avoid planting other *Juglans* spp. in the vicinity of *J. cinerea*. If the fungus is locally introduced into Europe, measures may also be needed for movement of walnut wood.

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