Prepared by CABI and EPPO for the EU under Contract 90/399003

Data Sheets on Quarantine Pests

Guignardia citricarpa

IDENTITY

Name: *Guignardia citricarpa* Kiely
Anamorph: *Phyllosticta citricarpa* (McAlpine) Van der Aa (macroconidial state)
Synonyms: *Phoma citricarpa* McAlpine
Phyllostictina citricarpa (McAlpine) Petrak
Synanamorph: *Leptodothiorella* sp. (microconidial state)
Taxonomic position: Fungi: Ascomycetes: Dothideales
Common names: Black spot, hard spot, shot-hole, freckle spot, virulent spot, speckled blotch of citrus (English)
Maladie des taches noires (French)
Schwarzfleckenkrankheit (German)
In recent publications citrus black spot has been referred to by the acronym CBS (Kotzé, 1981; Herbert & Grech, 1985).
Notes on taxonomy and nomenclature: On the basis of gross morphology, the name *G. citricarpa* has been applied to several *Guignardia* samples and isolates derived from citrus and non-citrus hosts, irrespective of whether or not they can cause black spot disease of citrus (Kiely, 1949a; McOnie, 1964a; Van der Aa, 1973; McMillan, 1986;). McOnie (1964a; 1964c) reported that two *Guignardia* species, the black-spot-causing *G. citricarpa* and a non-pathogenic *Guignardia* sp., occur on citrus. Furthermore, on some of the non-citrus hosts, several different *Guignardia* species have been described (Petch, 1923; Roy, 1968; Pande, 1969; Van der Aa, 1973; Punithalingam, 1974; Ullasa & Rawal, 1984). This has resulted in confusion over taxonomy, identity and host range of *G. citricarpa*. It is therefore imperative that, until a thorough reassessment and characterization of the *Guignardia* isolates causing black spot of citrus and those assumed to be found on alternate hosts are carried out, the name *G. citricarpa* is exclusively applied to the fungus causing black spot of citrus.
Bayer computer code: GUIGCI
EPPO A1 list: No. 194
EU Annex designation: II/A1 (strains pathogenic to citrus)

HOSTS

The principal hosts are *Citrus* species: *C. limonia*, *C. nobilis*, *C. poonensis*, *C. tankan*, grapefruits (*C. paradisi*), lemons (*C. limon*), limes (*C. aurantifolia*), mandarins (*C. reticulata*), oranges (*C. sinensis*). Sour oranges (*C. aurantium*) are not susceptible.

Non-citrus hosts reported to harbour *G. citricarpa* include almonds (*Prunus dulcis)*, avocados (*Persea americana*), *Eucalyptus* spp., guavas (*Psidium guajava*, *P. montanum*), mangoes (*Mangifera indica*), passionfruits (*Passiflora edulis*), *Rubus* spp. and a variety of ornamentals such as *Caesalpinia pulcherrima*, *Callistemon citrinus*, *Camellia japonica*, *Dendrobium speciosum*, holly (*Ilex aquifolium*), *Magnolia* sp., *Smilax* sp. (Kiely, 1948; Kiely, 1949b; McOnie, 1964a). Other recorded hosts are cardamoms (*Elettaria*

cardamomum) (Allen, 1971), *Cola nitida*, *Dioscorea pentaphylla* (Roy, 1965), *Eucalyptus deglupta* (FAO, 1960) and sugarcane (*Saccharum officinarum*) (Hudson, 1962).

The non-citrus host list is controversial and doubtful for two main reasons: 1) adequate cross inoculation details are lacking and 2) on *Camellia*, *Dioscorea*, *Ilex*, *Persea*, *Psidium*, *Mangifera* and *Smilax*, species have been described under the names *Guignardia camelliae* (Cooke) Butler, *G. dioscoreae* A.K. Pande, *G. philoprina* (Berk. & M.A. Curtis) Van der Aa, *G. perseae* Punithalingam, *G. psidii* B.A. Ullasa & R.D. Rawal, *G. mangiferae* A.J. Roy and *G. smilacis* A.J. Roy, respectively.

GEOGRAPHICAL DISTRIBUTION

The geographical distribution is given for true *G. citricarpa* and then for doubtful or non-pathogenic records, which are probably other species (see Notes on taxonomy and nomenclature). Citrus black spot, originating in south-east Asia, spread to Australia, South Africa and China many years ago.

- ***Guignardia citricarpa***
  EPPO region: Absent.
  Asia: Bhutan, China (Fujian, Guangdong, Sichuan, Yunnan, Zhejiang; Fawcett, 1936), Hong Kong, Indonesia (Java), Philippines, Taiwan.
  Africa: Kenya, Mozambique, South Africa (Wager, 1952; Herbert & Grech, 1985), Zambia, Zimbabwe.
  Oceania: Australia (New South Wales, Queensland, Victoria; McAlpine, 1899), New Zealand, Vanuatu.
  EU: Absent.
  Distribution map: See CMI (1990, No. 53).
- **Doubtful *G. citricarpa* or *Guignardia* spp. non-pathogenic to citrus**
  EPPO region: Egypt, Israel, Italy (Sicily), Lebanon, Spain (McOnie, 1964c).
  Asia: Georgia, India (Assam on *Dioscorea pentaphylla*), Iran, Israel, Japan (including Ryukyu Archipelago), Korea Democratic People's Republic, Korea Republic (intercepted in USA), Lebanon, Malaysia (peninsular, Sabah, Sarawak; on *Eucalyptus deglupta*), Myanmar, Pakistan (intercepted in USA), Singapore (intercepted in USA), Sri Lanka (on tea), Thailand (intercepted in USA), Viet Nam (intercepted in USA).
  Africa: Egypt (intercepted in USA), Nigeria (on *Cola nitida*), South Africa (Cape Province), Swaziland, Tanzania (on cardamoms; Allen, 1971), Uganda.
  North America: USA (Florida on mangoes, McMillan, 1986; Farr *et al*., 1989; Hawaii, intercepted in mainland USA). The report from Florida failed to show that black spot of citrus could be produced with the mango isolate and made no comparison with *G. mangiferae* naturally found on mangoes.
  Central America and Caribbean: Belize, Cuba (various hosts but not citrus), Honduras, Jamaica, Trinidad.
  South America: Argentina, Brazil (São Paulo), Peru, Venezuela (on *Tabebuia pentaphylla*).
  Oceania: Cook Islands, Fiji (Dingley *et al*., 1981), Niue, Papua New Guinea (on tea), Samoa, Tonga.
  EU: Present.
  Distribution map: See CMI (1990, No. 53).

BIOLOGY

The asexual states of the citrus black spot fungus are *Phyllosticta* (macroconidial) and *Leptodothiorella* (microconidial). The latter is sometimes referred to in the literature as the

'spermagonial' state and the dumb-bell shaped microconidia as spermatial cells (Kiely, 1949a; Van der Aa, 1973) but to date no evidence has been found to substantiate the claim that it has this function. The *Phyllosticta* state occurs on fruit lesions, leaf lesions, dead twigs, fruit stalks and in abundance on leaves on the orchard floor (Kiely, 1949a; Kotzé 1981). The *Leptodothiorella* state usually appears on fallen leaves before ascocarps develop; the role of the microconidia is unclear. Ascocarps occur throughout the year on leaf litter lying on the orchard floor.

Cultures of *G. citricarpa* grow well on agar media; the optimal temperature for growth has been reported to be 24-27°C (Wager, 1952) and optimum growth in liquid basal synthetic medium has been reported to be at 27°C (Kotzé, 1981). Germination of macroconidia has been reported to be stimulated by citric acid solutions at concentrations of 0.1-0.5%. Maximum germination, nearly 80%, has been obtained using 0.3% citric acid solution and incubating conidia for 4 days at 25°C in a damp chamber (Kiely, 1949a). Germination of macroconidia in tap water has been reported in South Africa (Wager, 1952). Longevity of macroconidia differs from country to country. In Australia, freshly exuded mature macroconidia have been reported to lose their ability to germinate 1 month after they were produced (Kiely, 1949a) but in South Africa macroconidia have been reported to retain their germinative capacity up to 5 months (Wager, 1952). Macroconidia on germination enter both unwounded and wounded fruits, and through abrasions caused by hail or insect damage (Kiely, 1949a; Lee, 1969). In field trials carried out in Australia, young fruits inoculated with conidial suspensions after petal fall in October produced black spot disease after nearly 1 year (Kiely, 1949a). In South Africa, young citrus fruits inoculated with high concentrations of macroconidia near mid-November showed speckled blotch lesions by the end of January the following year (McOnie, 1964e). The role of macroconidia in spreading the black spot disease is considered to be of minor importance when compared with airborne ascospores which are regarded as the primary source of inoculum (Kiely, 1949a; McOnie, 1964b; Kotzé, 1981; Zheng, 1983).

The disease spreads in orchards by infection coming from macroconidia and ascospores. It takes several years from the time the first symptoms are noticed until the disease reaches epidemic proportions in South Africa (Kotzé, 1981). Macroconidia are water-borne and require droplets of water for their emergence and dispersal (Wager, 1952) and pycnidia have no special release mechanism for expelling conidia into the atmosphere (Kotzé, 1981). Macroconidia are washed down or rain-splashed from dead twigs and old fruit stalks to infect susceptible fruits in Zimbabwe (Whiteside, 1967). Ascospores are forcibly ejected vertically up to 1 cm and carried by wind and water. Dew, rain and high temperature promote the release of ascospores from ascocarps developed on leaf litter from the floor of orchards during May to October in Taiwan (Huang & Chang, 1972), from November to June in South Africa (McOnie, 1964d), and throughout the year in Australia (Kiely, 1949a). Release of ascospores commences within the first hour after the leaf litter has become wet (Kiely, 1949a; Kotzé, 1981). Rainfall as little as 3 mm can bring about the discharge of ascospores from mature ascocarps (McOnie, 1964d). Significant fruit infection has been correlated with an abundance of ascospore inoculum in the atmosphere (McOnie, 1964b).

In Australia, ascospores take more than 24 h to germinate at 25°C and 4 days to reach 98% germination (Kiely, 1949a), whereas in South Africa peak germination approaching 100% has been observed in 24 h (McOnie, 1967). Ascospores on germination produce appressoria with infection pegs that penetrate the cuticle. The infection pegs produce at their tips, between the cuticle and the upper epidermal cells, knots of fungal tissue which are considered to establish latent infection (McOnie, 1967; Kotzé, 1981). Flowers and fruits are susceptible to infection from anthesis until approximately 16 weeks later (Kellerman & Kotzé, 1979). Infection is usually followed by a long period of latency which

may last 12-36 months in Australia and about 3-12 months after anthesis for fruit infection in South Africa (McOnie, 1967; Kellerman & Kotzé, 1979). Disease symptoms first develop on fruits which are at or near maturity depending on several factors: *Citrus* species or variety, prevailing weather conditions and duration of latent period (McOnie, 1967). In Australia primary infections of young leaves are initiated by wind-borne ascospores during September and October and young fruits from October to February. Latent infections on green leaves provide ascospore inoculum, whenever such leaves fall, over a period of 1 to approximately 3 years. The presence of *G. citricarpa* mycelium in healthy green citrus leaves in commercial nurseries has been demonstrated and mycelium in latent infections has been reported to survive 18 days in wilted air-dried citrus leaves and then to produce fructifications when such leaves were moistened and incubated at 30°C (Kiely, 1949a).

The existence in Australia of a primary and secondary infection cycle and their relationship have been demonstrated by Kiely (1949a). Primary latent infection of young fruits gives rise to pycnidia with macroconidia after 12-15 months, and at maturity they develop black spot lesions, which in turn initiate a secondary infection cycle. In South Africa, the seasonal cycle of the pathogen, the climatic conditions and the cycle of citrus fruit and leaves are regarded as the three primary components affecting a black spot epidemic (Kotzé, 1981). The leaf litter on the floor of the orchard acts as the reservoir for ascocarps which develop within 50-180 days but maturation depends on intermittent wetting and drying of leaves and prevailing temperature (Lee & Huang, 1973; Kotzé, 1981). Heavy dew alone has been reported to be sufficient for maturation and release of ascospores in New South Wales (Kiely, 1950), but in South Africa irrigation and dew have been reported to have little or no noticeable effect on ascocarp development or ascospore release (McOnie, 1964d). Cool, dry weather has been reported to prolong ascocarp maturation up to 6 months under South African conditions (Kotzé, 1981). Rainfall pattern has been reported to influence the release of the primary inoculum (ascospores) into the atmosphere; 3 mm of rain is considered sufficient for the release of large numbers of ascospores but continuous heavy showers are reported to affect ascospore discharge adversely and reduce ascospore load in the air (McOnie, 1964d; Kotzé, 1981).

In Australia, Kiely (1950) has reported the existence of a positive correlation between disease development and rainfall during the susceptible period and a negative one during the period after petal fall when infection occurred. Mean maximum temperature for disease development is between 24 and 25°C. Disease development in fruits of orange cv. Valencia in coastal New South Wales is favoured by high temperature and low soil moisture (Kiely, 1969). In South Africa symptom expression is favoured by high temperature (McOnie, 1964e) and late-hanging fruit in orange cv. Valencia (Kellerman & Kotzé, 1979). Once re-greening of the rind starts black spot development ceases. Other fungi which are sometimes associated with speckled blotch of citrus are *Alternaria* sp. and *Glomerella cingulata* (Kiely, 1960).

DETECTION AND IDENTIFICATION

Symptoms

Black spot of citrus was first officially noticed in Australia in 1895 on fruits in the citrus-growing areas around Sydney (Kiely, 1949a). Spot development on oranges cv. Valencia goes through several stages and Australian growers refer to these phases of fruit lesions as hard spot and shot hole spot; freckle spot; and spreading or virulent spot (Kiely, 1949a). In Australia, hard spot and shot-hole spot usually develop on oranges cv. Valencia in mid-August (late winter), with 50 or more lesions per fruit. Lesions are at first circular and brown with slight depressions, which gradually sink in the centre developing into crater-like depressions, grey-white in the centre with black margins encircled by green rind tissue.

The smaller, orange to brick-red freckle spots usually appear on the half of the fruit exposed to the sun, with several hundred spots per fruit. When conditions are favourable for spotting, hard spot development is replaced by freckle spot which drastically reduces the keeping quality of fruits. Virulent spots are common, developing 2-3 weeks after freckle spot development with the onset of warmer conditions. They spread rapidly becoming confluent, involving approximately two thirds of the fruit surface in 4-5 days and assuming irregular shapes. Freckle spots may develop into virulent lesions and virulent spots may engulf freckle and hard spot lesions. Virulent spots extend deeper into the rind. An epidemic of virulent spots may occur when orange cv. Valencia is at the peak of maturity (Kiely, 1949a). Another development phase of black spot, speckled blotch, has been reported on citrus fruits in Australia (Kiely, 1960) and South Africa (McOnie, 1964e).

In inoculation tests carried out in Taiwan, only freckle spots and virulent spots were produced by isolates from fruit with hard spot or black speckle lesions; freckle spots developed into virulent spots on fruits in continuous storage (Lee, 1969).

Lesions on leaves of orange cv. Valencia are rare in the Gosford area of New South Wales but common in Queensland (Kiely, 1949a). Lesions usually occur on lemons as sunken spots, 1.5-3 mm in diameter. Black spot leaf lesions are very common in Transvaal and Natal (Wager, 1945; Kiely, 1949a).

Morphology

Phyllosticta state: macroconidial state pycnidial, immersed, dark-brown to black, globose 115-190 µm. Macroconidia hyaline, variable in shape, obovoid to broadly ellipsoid or pyriform, aseptate, (6-)8-10.5(-13) x (5-)5.5-7(-9) µm, surrounded by a colourless gelatinous coat and with a subulate, apical appendage 5-15 µm long, caducous. *Leptodothiorella* state: microconidial state pycnidial, resembling the pycnidia of the *Phyllosticta* state in general morphology, usually developing prior to ascocarp formation. Microconidia hyaline, dumbbell-shaped, 5-8 x 0.5-1 µm. Similar pycnidia develop in agar culture. Ascocarps solitary or in groups; solitary ascocarps globose, 125-135 µm, papillate, ostiolate; aggregated ascocarps 220-360 µm. Asci clavate-cylindrical, bitunicate, 45-85 x 12-15 µm, 8-spored, uniseriate. Ascospores hyaline, aseptate, broader in the middle, cylindrical, 8-17.5 x 3.3-8 µm, ends obtuse with colourless terminal mucoid appendage. Paraphyses and periphyses absent.

Detailed descriptions are given by McAlpine (1899), Kiely (1949a), Sutton & Waterston (1966) and Van der Aa (1973).

Detection and inspection methods

Citrus trees introduced to new sites can be tested for latent infection by sampling green leaves as suggested by Kiely (1949a).

MEANS OF MOVEMENT AND DISPERSAL

G. citricarpa is dispersed naturally only over short distances. It has been introduced to new sites in South Africa by distributing nursery trees with latent infection from Pietermaritzburg where citrus black spot was first reported in 1929 (Wager, 1952). It has been claimed that the disease was spread in Zimbabwe on bud wood or nursery trees before restriction was placed on material from South Africa (Whiteside, 1965). Grafting infected twigs on healthy trees is considered to be a means of spreading *G. citricarpa* to new trees (Schüepp, 1961). The fungus has been intercepted in the USA on fruits from several countries (see Geographical Distribution). However, only pycnidiospores form on fruit and these are not airborne, so the risk of spread on fruit is relatively low (Whiteside *et al*., 1988).

PEST SIGNIFICANCE

Economic impact

Black spot of citrus is a serious disease of citrus cultivars in Australia (Kiely, 1949b; 1969), Guangdong province in China (Fawcett, 1936) and South Africa (McOnie, 1964b). In the Windsor and Hawkesbury River areas of Australia in 1931, all orchards of orange cvs Washington Navel, Joppa and White Siletta were severely affected and losses of 80% were common in individual orchards (Kiely, 1960). Before the adoption of control measures, heavy losses in orange cv. Valencia had been reported in the coastal orchards in New South Wales (Kiely, 1949a; 1949b). In South Africa 90% of fruits from unprotected trees were claimed to be unfit for export (McOnie, 1964b) and losses of more than 80% of unprotected fruits were reported to be common (McOnie, 1964d). In Zimbabwe black spot was known from 1965 but reached epidemic proportions in 1978 (Kotzé, 1981). In South Africa, summer rains on lemon orchards is the most important factor in establishement of an epidemic and so far black spot has not been known to disappear or decline once it has reached the epidemic stage (Kotzé, 1981). It is mainly a fruit disease and the unsightly lesions do not cause post-harvest decay but render the fruits unmarketable. During the period between 1929 and 1939, when epidemics were at their worst in Australia, the wholesale market for oranges in Sydney was depressed due to growers creating a glut for fear of their fruits developing disease.

G. citricarpa is considered to be the most important pathogen of citrus in China, Australia and South Africa, where the citrus industry is of major importance (McOnie, 1967). Recent statistics on crop losses are unavailable.

Control

Since all commercially grown *Citrus* species except *C. aurantium* (sour oranges) are susceptible (McOnie, 1964d; Kotzé, 1981), a range of fungicides has been tested and recommended for protection and control. The use of preventive sprays such as Bordeaux + white oil, Bordeaux and zineb or mezineb (Kiely, 1949a; 1950; 1963; 1969; Wager, 1952), mancozeb (Kellerman & Kotzé, 1979), benomyl + mineral oil (McOnie *et al*., 1969; Kellerman & Kotzé, 1973; 1979; Kiely, 1976; Tsia *et al*., 1977; Bertus, 1981; Kotzé, 1981) have been reported to give adequate control. Since 1971, black spot of citrus has been controlled in South Africa by a single application of benomyl but recently in orchards of orange cv. Valencia in Eastern Transvaal, benomyl became ineffective due to tolerance by some *G. citricarpa* strains (Herbert & Grech, 1985). The benomyl-tolerant strains were also found to be tolerant of other benzimidazoles but sensitive to mancozeb. The benomyl-tolerant isolates have been reduced from over 90% to 30% by the use of mancozeb. Another control measure is orchard sanitation, including the removal of mature fruits before the new crop set, to prevent pycnidial inoculum getting washed down. Stripping nursery trees of leaves before being sold has also been recommended (Wager, 1952).

Phytosanitary risk

G. citricarpa has recently been added to the A1 quarantine list of EPPO; it is an A1 pest for CPPC and an A2 pest for APPPC and IAPSC. True *G. citricarpa* is absent from the EPPO region. Though of tropical origin, the fungus has established itself and causes serious damage in subtropical climates, e.g. China, New South Wales (Australia) and South Africa. It could be expected to establish and cause significant losses if introduced into the Mediterranean citrus-growing areas.

PHYTOSANITARY MEASURES

Importation of planting material of citrus from countries where true *G. citricarpa* occurs should be prohibited (as it is on account of several other non-European citrus pests). The fungus can readily be carried on imported citrus fruits, but the risk of spread from these is relatively low. Fruits from infested countries should come from orchards found free from, or treated against, the pest.

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