CHAPTER 1

GENERAL INTRODUCTION

Occurrence and significance of citrus black spot. Citrus black spot (CBS) causes substantial economic losses in all major citrus-producing countries subject to summer-rainfall (Kotzé, 1981). Countries in which CBS occurs include Argentina, Brazil, Peru, Venezuela, Uruguay, summer-rainfall regions of South Africa, Nigeria, Uganda, Kenya, Zimbabwe, Swaziland, Mocambique, India, China, Hong Kong, Taiwan, Japan, Philippines and coastal regions of Australia (Kiely, 1948; Sutton & Waterston, 1966; Kotzé, 1981; Whiteside *et al.*, 1988; Schutte *et al.*, 1996). The disease is absent in Mediterranean regions subject to winter-rainfall, e.g. Israel, Italy, Spain, Greece, Cyprus, Chile, and California in the USA (Kotzè, 1981; Schutte *et al.*, 1996).

Citrus orchards in the northern, eastern and central Transvaal, Natal, and lemon orchards in the eastern Cape represent approximately 50% of the total citrus plantings in South-Africa (Schutte, 1995). These areas must continuously be protected against CBS with registered fungicides. The cost of control measures of CBS in South Africa during the 1995 season amounted to between R11 million and R16.5 million (Schutte, 1995). In the 1997 season, South Africa spent in the region of R30 - 50 million on fungicides alone (mainly mancozeb and benomyl) for preharvest control of CBS (G.C. Schutte, personal communication). This estimate excluded the indirect losses due to CBS, viz spray cost (labour, tractor and spraying equipment maintenance, etc.) and rejection of exportable fruit due to the development of CBS in transit (packhouse processing and packing material costs, etc.).

Causal organism. McAlpine (1899) assigned the imperfect state of the CBS pathogen to the genus *Phoma* and described it as a new species, *Phoma citricarpa* McAlp. Sutton & Watterson (1966) reclassified the imperfect state in 1966 as *Phyllostictina citricarpa*

(McAlp.) Petrak, and Van der Aa (1973) changed it to *Phyllosticta citricarpa* (McAlp.) Van der Aa. The latter classification was confirmed on basis of the conidial appendages by Punithaingam & Woodhams (1982) and is currently still accepted.

Pycnidia of *P.citricarpa* may occasionally be found in lesions on green leaves and dead twigs attached to the tree (McOnie, 1964). Appreciable numbers of conidia can develop on old fruit lesions (hard spots), whereas numerous conidia are present on fallen decaying citrus leaves (Kiely, 1948; Kotzé 1963). Conidia are exuded in a sticky gelatinous mass in the presence of moisture and depend on running water for dispersal (Darnell-Smith, 1918; Kiely 1948; McOnie, 1965). The condia are borne in tandem in large numbers on short conidiophores in the pycnidium and are released upon contact of the conidioma with water (Darnell-Smith, 1918; Kiely, 1948). The dimensions of the conidia are in the order of 8.0–10.5 μm x 5.5–7.0 μm (Sutton & Watterson, 1966). Spermatia, measuring 7.5 x 1.6 μm, are sometimes present (Darnell-Smith, 1918). Lesions on mature fruit (hard spots) can contain one to more than 50 pycnidia.

The parasitic relationship between *P.citricarpa* and citrus was studied by Darnell-Smith (1918), Kiely (1948), Wager (1949) and Kotzé (1963). Since then little new information has been gathered in this regard. There is particularly a lack of basic knowledge concerning conidium attachment, germination and appressorium formation by the fungus.

Conidia of *P. citricarpa* are not airborne and do not play an important role in the epidemiology of CBS in orchards (Kiely, 1948; McOnie, 1964; Kotzé 1981). They only contribute to the epidemiology of CBS if infected out-of-season fruit remaining on the trees (Kiely, 1948; Wager, 1949; McOnie, 1964; Kotzé, 1981). Conidia may cause infection on young susceptible fruit and leaves when splashed from out-of-season fruit by rain (Wager, 1953). Fruit are only susceptible during the first few months after fruitset, whereafter they become resistant to infection (Wager, 1953; Kotzé, 1981).

Ascospores produced in ascomata (perithecia) by the teleomorphic state of the pathogen are the primary source of inoculum (McOnie, 1964; Kotzé, 1981). Perithecia are found on dead leaves on the orchard floor (Kiely, 1948; Wager, 1949; Kotzé 1963; McOnie, 1964; Kotzé,

1981). Spermatia referred to by Darnell-Smith (1918) as X-spores are present on fallen decaying citrus leaves and can also be produced in culture. There is strong evidence that spermagonia produce functional male gametes, entitled to be called spermatia. Their appearance always precedes perithecium formation on decaying citrus leaves and they therefore have a distinct sexual function (Kiely, 1948). This supports the view that, although pycnidia mature on dead leaves several weeks before perithecia are produced, detectable infection in orchards does not occur before ascospores are available (McOnie, 1964). To date, ascospores could not be retrieved from fruit lesions (Kiely, 1948; Kotzé, 1963; McOnie, 1965; Kotzé, 1981).

Ascospores on dead leaves are forcibly discharged into air currents after rainy spells and are disseminated by wind (Kotzé, 1981). Infection of citrus fruit by ascospores takes place early in the season in the presence of moisture when the spores germinate and produce appressoria. A thin infection peg penetrates the cuticle and expands, forming a small mat of mycelium between the cuticle and the epidermis (McOnie, 1967). This is referred to as latent infection and ends when the fruit becomes mature. Infection depends on prevailing environmental conditions and the stage of fruit development (Brodrick, 1969). Light and temperature play an important role in lesion development on the fruit, and hence on the development of pycnidia and conidia. Black spot symptoms and sporulation occur optimally on citrus fruit and flavedo pieces exposed to light at 27 °C (Brodrick & Rabie, 1970).

The host. Except for sour orange (*Citrus aurantium* L.) and its hybrids, all commercially grown citrus varieties are susceptible to CBS (Kotzé, 1981). Lemons are particularly prone to the disease, although heavy losses may also occur on Valencia and Navel oranges (*C. sinensis* (L.) Osbeck) as well as in grapefruit (*C. paradisi* Mart.) (Kiely, 1948; Kotzé, 1981). The flavourless glucoside, hesperidin, ($C_{28}H_{34}O_{15}$) occurs abundantly in oranges and other citrus varieties, especially in young fruit (Hendrickson & Kesterson,1961) and can readily be isolated from chopped citrus peel by methanol extraction. Heperidin was found to be the best carbon source for supporting growth of *P. citricarpa* (Frean,1964). All citrus fruit furthermore produce oil which is contained in numerous oil sacs in the rind. Oil yield of citrus fruit at different developmental stages was directly correlated with the increase in fruit surface area up to maturity. Oil can be separated into two basic chemical groups, the terpenes and the

terpenoids (Bernard, 1961). The main terpene in citrus oil is d-limonene ($C_{10}H_{16}$), which comprises 90% of the total constituents in the oil (Braverman, 1949). Brodrick (1971) showed that d-limonene as such is toxic to fungi and that it inhibits growth of *P. citricarpa*.

Export conditions. Export procedures have changed appreciably since the first detailed description of CBS in 1899. It now takes up to a week from harvesting to pack fruit for the export market. Degreening of fruit is an additional time factor, extending handling of fruit with 2-4 days (A Du Pisanie, personal communication). The packhouse procedures ($Ca(OCl)_2$ or chlorine dioxide dump trough, high pressure spray, warm water bath, fungicide dip tank, and wax application) minimise the risk of decay. The time it takes for citrus fruit to reach the harbour depends on several factors, including the type of transport available. For instance, fruit packed at Letaba Estates are transported by rail and reach Durban harbour within 2-3 days (A Du Pisanie, personal communication). When the consignment reaches the harbour, the fruit are kept under cool conditions. Loading it onto the ship can take another day during which the cold chain is broken. It takes another 20-30 days on the ship under refrigeration (specific temperatures for specific citrus cultivars) to reach the destination and another week before the fruit is sold. Although Wager (1949) reported that conidia of *P. citricarpa* on CBS-infected fruit have a short lifespan, the fate of these conidia during processing and transit has not been investigated.

Objectives of the study.

- To determine the viability of conidia and mycelium of the CBS pathogen on citrus fruit.
- To categorise the different types of CBS lesions according to appearance and risk as source of infection.
- To determine the time needed for pycnidia to develop in newly-formed lesions and for the production of conidia in the pycnidia.
- *In vitro* studies concentrating on the physical and chemical conditions influencing conidial germination, and on the efficacy of existing packhouse procedures and alternative fungicides to suppress germination.
- *In vivo* experiments determining the possibility for CBS fruit to cross-contaminate clean (symptomless) packhouse-treated fruit.
- Evaluating existing packhouse treatments *in vivo* for their effect on *P. citricarpa* mycelium

and conidia present in the lesions. The entire process from harvesting in the orchard to reaching the final destination on foreign markets (incubation time) will be simulated in the lab. This will be done to determine if conidia from CBS fruit are still viable.

- Determination of the effect of cobalt irradiation on the viability of CBS propagules.

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