

# SURVIVAL OF *PHYLLOSTICTA CITRICARPA*, ANAMORPH OF THE CITRUS BLACK SPOT PATOGEN

by

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## RESUMÉ

Citrus black spot (CBS) is caused by *Guignardia citricarpa*, a member of the *Mycosphaerellaceae* in the ascomycete order, *Dothideales*. *G. citricarpa* infects primarily by ascospores produced on detached senescent leaves, but exists on citrus fruit in the anamorphic or conidial state, *Phyllosticta citricarpa*. Although conidia of *P. citricarpa* are regarded as of little or no significance in the epidemiology of CBS, concern has recently been expressed that infected fruit could serve as source of infection. This report describes the production of inoculum by *P. citricarpa* and provides evidence on the survival of conidia and mycelium of the pathogen.

CBS lesions commonly occurring on citrus fruit were indexed into four distinct categories, viz. lesion type A (hard spot or shot-hole spot), B (false melanose, speckled blotch or inky spot), C (freckle spot), and D (virulent, spreading or galloping spot). This

indexing system allowed the allocation of atypical CBS lesions present on fruit to a particular class. Type C lesions developed first on naturally-infected Valencia fruit, appearing within 5-7 days at 27 °C, high humidity and continuous fluorescent lighting. Pycnidia produced in these lesions after 9-13 days already contained viable conidia. Conidia present in type A lesions remained viable for 14 days on unprocessed fruit stored under optimal conditions, but succumbed to standard packhouse treatments. Mycelium of *P. citricarpa* in the periphery of type A lesions survived packhouse processing.

Conidia of *P. citricarpa* did not infect unwounded packhouse-treated citrus fruit at 0.5 and 25 °C in artificial and natural inoculation studies. A low percentage infection of wounded fruit was evident at 25 °C, but no infection occurred at 0.5 °C. Conidia adhered more readily to the synthetic polyethylene micro-wax layer on fruit than to the natural waxy surface layer. CBS could not be induced in healthy mature Valencia leaves inoculated with conidia of *P. citricarpa*. In the laboratory, conidia germinated optimally at 22 °C, with no germination evident at 0.5 °C and 40 °C. Optimal pH for germination in a citric acid pH-range was 4.0-4.2, the minimum 3.2, and the maximum 4.8. Light had no effect on germination rate.

Seven *P. citricarpa* isolates from different regions of the world were screened *in vitro* for sensitivity towards fungicides and disinfectants registered for use in South African citrus packhouses. Guazatine and *o*-phenylphenol (sodium salt) inhibited conidial germination and appressorium formation completely at recommended rates. Prochloraz, thiabendazole, and two emulsion formulations of imazalil, viz. Chloramizol and Sanazil, reduced germination by more than 98 %. Imazalil sulphate was less effective, with 21 % of the conidia exposed to the compound remaining viable. Chlorine dioxide inhibited conidial germination more effectively, and at lower concentrations, than calcium hypochlorite.

Packhouse procedures which consistently reduced the incidence of *P. citricarpa* in CBS lesions *in vivo* included warm water treatment (43-47 °C for three minutes), chemical tank (imazalil sulphate, guazatine and 2,4-D), and a combination of chlorine, high-

pressure spraying, warm water, the above chemicals, and polyethylene waxing. Conidial viability was reduced to zero by keeping CBS-infected fruit for three weeks at 25 °C, as well as by exposing the fruit to chlorine, warm water, the above chemicals, or all treatments combined. Various fungicides not registered for use on harvested citrus fruit inactivated conidia of *P. citricarpa*, one notable exception being the plant resistance-activating substance, acibenzolar-*s*-methyl. Difenoconazole, although not registered on citrus, substantially reduced mycelial inoculum present in CBS lesions. Cobalt irradiation of up to 400 Gy did not eliminate inoculum of *P. citricarpa* on infected fruit.

# DIE OORLEWING VAN *PHYLLOSTICTA CITRICARPA*, ANAMORF VAN DIE SITRUS SWARTVLEK PATOGEEN

deur

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## OPSOMMING

Sitrus swartvlek (SSV) word veroorsaak deur *Guignardia citricarpa*, 'n lid van die *Mycosphaerellaceae* in die askomiseete-orde, *Dothideales*. *G. citricarpa* infekteer hoofsaaklik deur askospore wat geproduseer word op sitrusblare wat van die boom afgeval het. Die patogeen kom voor op sitrusvrugte in die anamorf- of konidiale fase, *P. citricarpa*. Alhoewel konidium van *P. citricarpa* beskou word as onbelangrik in die epidemiologie van SSV, is kommer onlangs uitgespreek dat geïnfekteerde vrugte kan dien as bron van infeksie. Hierdie verslag beskryf die produksie van inokulum deur *P. citricarpa* en voorsien inligting oor die oorlewing van konidiums en miselium van die patogeen.

SSV letsels wat algemeen voorkom op sitrusvrugte is opgedeel in vier duidelik-onderskeibare kategorieë, nl. letseltipe A (hardevlek of haelgatvlek), tipe B (vals

melanose, spikkelvlek of inkvlek), C (sproetvlek) en D (virulente- of spreïende vlek). Hierdie indekseringsstelsel laat die plasing van atipiese SSV letsels op vrugte in een van die onderskeie kategorieë toe. Tipe C letsels het eerste ontwikkel op natuurlik-geïnfekteerde Valencia vrugte na inkubasie van 5-7 dae by 27 °C met 'n hoë humiditeit en deurlopende fluoresserende beligting. Piknidiums wat na 9-13 dae in hierdie letsels geproduseer is het reeds kiembare konidiums bevat. Konidiums teenwoordig in tipe A letsels het vir 14 dae lewensvatbaar gebly vir onbehandelde sitrusvrugte wat gestoor is onder optimale toestande, maar is totaal uitgewis deur standaard pakhuisbehandelings. Miselium in die periferie van tipe A letsels het die pakhuisbehandeling oorleef.

Konidium van *P. citricarpa* kon nie onbeskadigde pakhuisbehandelde sitrusvrugte by 0.5 en 25 °C infekteer in kunsmatige en natuurlike infeksiestudies nie. 'n Lae persentasie infeksie het voorgekom in verwonde vrugte gestoor by 25 °C, maar geen infeksie is waargeneem by dieselfde vrugte gestoor by 0.5 °C nie. Konidiums het meer gereedelik geheg aan die sintetiese poliëtileen mikro-wakslaag op sitrusvrugte as op die natuurlike wakslaag. SSV kon nie geïnduseer word in gesonde, volwasse Valencia blare geïnkuleer met *P. citricarpa* konidiums nie. In die laboratorium het *P. citricarpa* konidiums optimaal ontkiem by 22 °C en geen waarneembare ontkieming het plaasgevind by 0.5 en 40 °C nie. Die optimale pH grense vir ontkieming in 'n sitroensuur pH-reeks was 4.0-4.2, met 'n minimum en maksimum pH van 3.2 en 4.8, onderskeidelik. Lig het geen effek op die ontkiemingstempo van *P. citricarpa* konidiums gehad nie.

Sewe *P. citricarpa* isolate van verskillende dele van die wêreld is *in vitro* getoets vir sensitiwiteit teenoor swamdoders en ontsmettingsmiddels geregistreer vir gebruik in Suid Afrikaanse sitruspakhuis. Guasatien en *o*-fenielfenol (natriumsout) het ontkieming en appressoriumvorming van konidium geheel en al geïnhibeer by die aanbevole konsentrasies. Prochloras, tiabendasool en twee emulsie-formulasies van imasalil nl. Chloramizol en Sanazil het ontkieming met 98 % geïnhibeer. Imasalil sulfaat was minder doeltreffend, met 'n oorlewingsyfer van 21 %. Chloordioksied het konidiumontkieming meer doeltreffend en teen laer konsentrasies geïnhibeer as kalsiumhipochloriet.

Pakhuisbehandelings wat die voorkoms van *P. citricarpa* in SSV letsels *in vivo* konsekwent verminder het, sluit in: warm water behandeling (43-47 °C vir 3 minute), chemiese dompelbad (imasalil sulfaat, guasatien en 2,4-D) en 'n kombinasie van chloor, hoë-druk spuit, warm water, bogenoemde chemikalieë en poliëtilieen polering van sitrusvrugte. *P. citricarpa* konidiums is totaal uitgewis deur SSV-geïnfekteerde sitrusvrugte te stoor vir 3 weke by 25 °C na behandelings met chloor, warm water, bogenoemde chemikalieë, of 'n kombinasie van al die behandelings. Verskeie swamdoders wat nie geregistreer is vir na-oesgebruik op sitrusvrugte nie, het *P. citricarpa* konidiums gedood. 'n Uitsondering was die plantbestandheidsaktiveerder, asibensolar-*s*-metiel. Difenoconasool wat ook nie geregistreer is vir na-oes sitrusbehandeling nie, het die miseliuminokulum in SSV letsels aansienlik verminder. Kobaltbestraling van tot 400 Gy het nie die *P. citricarpa* inokulum in besmette sitrusvrugte uitgeskakel nie.