The infection of the fruit of 'Cavendish' banana by *Pseudocercospora fijiensis*, cause of black leaf streak (black Sigatoka)

R. A. Fullerton · S. G. Casonato

Abstract Black leaf streak (black Sigatoka) caused by *Pseudocercospora fijiensis* (teleomorph *Mycosphaerella fijiensis*) is the most destructive leaf disease of banana world-wide. It is capable of destroying plantations unless controlled and successful commercial production is dependent on frequent applications of fungicides. A study was made to determine whether the pathogen is capable of infecting the fruit of 'Cavendish' banana and completing its life cycle on banana skin as the sole substrate. Emerging and recently emerged bunches of 'Cavendish' banana were inoculated in the field with carbendazim-resistant strains of *P. fijiensis*, incubated in situ in a humid environment for 6 days and then allowed to develop and mature naturally. The fruit were harvested approximately 16 weeks after inoculation, ripened to green tip stage, and skins incubated either as whole skins in humid containers or as thin strips of skin removed from the fruit and incubated on water agar modified with penicillin, streptomycin and carbendazim in Petri dishes. Supradermal fascicles of *P. fijiensis* were found on the incubated skin strips and whole skins within 5 days. From a total of approximately 300 field inoculated fruit, 18 colonies of *P. fijiensis* were detected. Small pieces of skin, each supporting a single fascicle, were excised, incubated individually on modified water agar and monitored for the production of conidia. It was shown that the fungus colonised, and was able to sporulate profusely on the excised skin pieces. This finding has implications for the transfer of strains of *P. fijiensis* between countries via commercial banana shipments and represents a potential pathway of entry to countries currently free of the disease.

Keywords Life cycle · Inoculation · Saprophytic colonisation · Sporulation

Introduction

Black leaf streak (black Sigatoka) of banana, caused by the fungus *Pseudocercospora fijiensis* (Morelet) Deighton (teleomorph *Mycosphaerella fijiensis* Morelet), is the most destructive leaf disease of banana worldwide with relatively few countries remaining free of the disease. The pathogen is spread locally by rain-splashed and wind-borne conidia, and by airborne ascospores (Meredith and Lawrence 1969; Meredith et al. 1973; Gauhl 1993). Infection occurs primarily via the stomata (Hernandez et al. 2006) on young, unfurling leaves. Conidia have been shown to remain viable for up to 60 days on leaf surfaces but for only 18 days on the skin of fruit (Hanada et al. 2002). Although the disease is highly destructive to leaves, evidence of fruit infection is rare. A recent comprehensive review the *M. fijiensis* by Churchill (2011) has no record of infection or disease

R. A. Fullerton (✉)
The New Zealand Institute for Plant and Food Research Limited, Private Bag, Auckland 92169, New Zealand
e-mail: bob.fullerton@plantandfood.co.nz

S. G. Casonato
Lincoln University, PO Box 85084, Lincoln, Christchurch 7647, New Zealand

development on fruit. The pathogen has been shown to infect the fruit of 'Harton' plantain, infecting via the stomata and causing minute, dark brown to black specks, each with a water-soaked halo (Cedeno et al. 2000). No symptoms or evidence of infection has been reported on the skin of 'Cavendish' despite heavy inoculum pressure in severely diseased plantations. This suggests that the skin of the fruit of 'Cavendish' is relatively resistant to infection and, as a result, fruit have never been considered to represent a risk for long distance transmission of the disease.

P. fijiensis has a relatively long incubation period compared with many other plant pathogens. The first symptoms, small chlorotic flecks, appear on the abaxial side of the leaf 10 to 14 days after infection but often do not progress to rusty streaks for over 20 days after infection even on highly susceptible 'Cavendish' types (Meredith and Lawrence 1969; Meredith et al. 1973; Jones 2000). The incubation period can be as long as 35 days in more resistant cultivars such as 'Ducasse', 'Pahang' and 'Pisang Mas', with symptoms sometimes not appearing until the leaves senesce (unpublished data). This pattern of symptom expression on more resistant genotypes raised the question of whether the pathogen could infect and colonise the 'perceived resistant' skin of 'Cavendish' banana but remain latent within the skin until the fruit senesced.

In a study of fruit from heavily diseased plantations in Samoa in 2006, *P. fijiensis* was isolated into pure culture from the skin of 'Cavendish' (Fullerton and Casonato 2009). However, because of rapid overgrowth of the skin tissue and culture plates by other fungi, particularly *Colletotrichum* spp., it was not possible to determine whether the pathogen could complete its life cycle (defined by producing conidia) on the fruit skin.

The rate with which it is overgrown by other faster growing skin-inhabiting fungi presents a serious obstacle to the study of the biology of *P. fijiensis* on fruit skin. To overcome this constraint, a method was required that would limit the growth of skin inhabiting fungi such as *Colletotrichum* spp. without affecting the growth of *P. fijiensis*. Resistance to the benzimidazole fungicides (benomyl, carbendazim) by *P. fijiensis* was first reported in the Pacific Islands in Samoa in 1983 (Fullerton and Tracey 1984) and detected soon after in the Kingdom of Tonga and the Cook Islands (unpublished data). An analysis of 49 randomly collected strains of *P. fijiensis* on Tongatapu, Kingdom of Tonga, in 2008 yielded a proportion (4.6%) of isolates resistant to carbendazim

(unpublished data). This offered the opportunity to utilise a strategy in which fruit could be inoculated in the field with carbendazim-resistant strains of *P. fijiensis* to increase infection frequency, and observations made on skin incubated on carbendazim-amended medium to limit the growth of *Colletotrichum* spp.

The objectives of this study were to confirm that *P. fijiensis* could infect the fruit of 'Cavendish' and to determine whether it can complete its life cycle on the skin of the fruit. Knowledge of the life cycle of this pathogen on fruit is important as it has implications for the transfer of specific strains e.g. fungicide resistance, between countries and its spread to countries currently free of disease.

Materials and methods

The study was conducted over the period February – September 2009 during a series of short-term visits, ranging from 7 to 18 days, to the Kingdom of Tonga, and in the laboratories of the New Zealand Institute for Plant and Food Research Limited, Auckland.

Collection of carbendazim resistant strains

In February 2009, samples of leaves with early symptoms of black leaf streak were collected from 49 subsistence stands of banana plants in 24 locations within a 2 km radius of Vaini Research Station, Tonga Ministry of Agriculture Forestry Fisheries and Food (Tonga MAFFF). Using the method of Fullerton and Tracey (1984) small strips of epidermis (~2 × 5 mm), each containing a 'rusty streak' (the first visible sign of infection), were aseptically excised from the abaxial surface of the leaf, placed cut-surface down on Oxoid™ potato dextrose agar (PDA) modified with 100 ppm each of penicillin and streptomycin and 5 ppm methyl benzimidazole carbamate (MBC) (carbendazim as NuChem™ Carbendazim) (PDAPSC) and incubated at room temperature (c. 25 °C). Epidermal strips that exhibited clusters of light grey, 'looped' mycelium (characteristic of *P. fijiensis*) associated with stomata after 2 days were transferred to fresh plates of PDAPSC. From a total of 894 epidermal strips, 84 MBC resistant strains were obtained. A representative selection of these strains is held in the culture collection of Plant and Food Research, Auckland, New Zealand.

Production of conidia for field inoculation

The resistant strains were imported into New Zealand under New Zealand Ministry of Agriculture and Forestry Import Permit No. 2009037420. The strains were assessed for their sporulation ability by spreading on half-strength PDA and incubating under an alternating 12/12 h light/dark cycle (2x warm white fluorescent, 1x black light blue (near UV fluorescent)). Approximately half the strains produced conidia in culture and, of those, 12 were selected for the mass production of conidia for inoculum.

Four growth media (PDA, half-strength PDA (1/2 PDA), 1/5 PDA and V8 juice agar (Hine and Aragaki 1963)) were used to produce inoculum. All media were modified with 100 ppm each of penicillin and strepto-mycin to maintain freedom from bacterial contaminants. Plates were inoculated by spreading a suspension of conidia and hyphal fragments of each strain over the surface of the target plates with a glass spreader and incubating under light as above. In general, the most profuse sporulation occurred on ½PDA, 1/5 PDA and V8 juice agar. From a total of 757 plates prepared, 312 plates (collectively including all 12 strains) exhibiting heavy sporulation were selected and returned to Tonga to provide inoculum for field inoculation studies.

Inoculation of fruit

Inoculations were made on bunches in a small plantation of 'Cavendish' banana on the Vaini Research Station (Tonga MAFFF) during the period 27 April - 7 May 2009. Ten bunches were inoculated. Four plants were selected at the stage that the inflorescences were just emerging and the first bracts lifting to expose the juvenile fingers and flowers (Fig. 1). As those bunches developed, new hands were exposed daily, allowing for all fingers to be inoculated several times during the course of a week. The other six plants had bunches on which all fingers were fully exposed with bracts fallen at the time of inoculation.

Conidial suspensions were prepared by flooding the inoculum plates with commercially bottled water, scraping the surface lightly with a scalpel blade then straining the suspension through terylene gauze. Carbendazim was routinely added to the conidial suspension (to a final concentration of 5 ppm) to inhibit other fungi (e.g. Colletotrichum spp.) that would normally colonise the skin of the fruit, thereby increasing the chances of locating P. fijiensis in the fruit at maturity. The conidial

Fig. 1 Stage of emerging 'Cavendish' banana inflorescence at the time of inoculation with *Pseudocercospora fijiensis*

concentration, measured by haemocytometer on each occasion, ranged from 2×10^5 to 5×10^5 conidia/mL. Conidial viability was confirmed each day by spreading a drop of inoculum over PDAPSC and examining microscopically for germination after 24 h.

The inoculum suspensions were applied to the fingers to the point of runoff using a hand-held 'trigger' spray bottle. The fruit were allowed to dry for approximately 20 min to ensure conidia were adhering to the surface then misted lightly with the remaining inoculum suspension to provide the moisture and humidity needed for germination. The bunches were covered with a black polythene bag which was tied to the stalk above and at the bottom of the bunch and surrounded by two layers of shade cloth in an attempt to reduce the occurrence of high temperatures within the bag. Inoculations were routinely made after 15:00 when the temperature and sunlight intensity had started to decline. The fruit on each bunch were inoculated on six successive days. This procedure meant that fingers on bunches that were fully exposed received six applications of inoculum. Fingers on bunches that were progressively opening received between four and six applications of inoculum.

Observations on conidial deposition and germination were made on fingers of similar age that were inoculated and incubated in a humid chamber in the laboratory, and on two occasions on inoculated fingers collected from the field. Thin (transparent) epidermal strips were excised from the fruit, mounted in lactic acid/aniline blue and examined microscopically.

Care of inoculated bunches

The bunch covers were opened each morning and the bunch misted with water to maintain surface wetness and humidity. For the first 2 days the weather was clear and sunny and the covers were opened during the hottest part of the day (approximately 11:00 to 15:00 h) to prevent overheating of bunches. Thereafter the weather was cooler and cloudy, allowing bunches to remain enclosed under constant high humidity conditions. The bunch sleeves were removed 7 days after the first inoculation, although the weather remained cloudy with frequent showers for a further 2 days, providing the bunches with a further 2 days of wetness and high humidity. Bunches were then left to develop and mature under natural conditions. No fungicides were applied to any of the plants in the block.

Processing of fruit and examination of skins
for the presence of *P. fijiensis*

The first inoculated fruit were harvested on 20 August 2009, approximately 16 weeks after the first inoculations were made. As a result of severe black leaf streak disease of the foliage, there were no green leaves remaining on any of the experimental plants at the time of harvest. At harvest, all fruit were in a hard green condition and free from visible disease symptoms. A number of the fruit had been damaged by banana scab moth (*Naceoleia octasema* Meyr.) and were discarded from the study.

The first fruit harvested were held in plastic bags with ripe apples for 4 days at ambient temperature (c. 25 °C) to trigger ripening. Later harvested fruit were allowed to ripen naturally in the laboratory.

As far as possible, fruit were processed at the 'green tip' stage of ripeness (when the body of the fruit is a clear yellow colour and the flower and stalk ends are still green) although sometimes ripening had advanced to the brown spot stage before they could be processed. The surface of each finger was surface sterilised by wiping with a paper towel saturated with 95% ethanol to

remove surface dust and debris then flooded with ethanol and allowed to evaporate dry in a sterile work station. This method had been shown previously to be effective at eliminating surface contaminants and not detrimental to the pathogen within the tissues (Fullerton and Casonato 2009). This treatment would have eliminated any spores of *P. fijiensis* that may have been recently deposited on the fruit from the surrounding infected leaves in the plantation.

Processing of the inoculated fruit was carried out in two series. The first series involved 164 fruit from three bunches that were inoculated at the time of inflorescence emergence. The second series involved 124 fruit from four bunches that had been fully exposed at the time of inoculation. Time constraints prevented all inoculated bunches from being processed.

Two methods were used for the incubation of the skins:

1. Shallow strips of skin approximately 10 mm wide comprising the epidermis and several layers of cells beneath were excised and placed cut surface down on water agar amended with penicillin (100 ppm), streptomycin (100 ppm) and carbendazim (5 ppm) (WAPSC). In most cases two 9-cm diameter Petri dishes accommodated the entire skin surface from a single fruit. The skins from 260 fruit were processed in this way. The Petri dishes were incubated at 23–25 °C under continuous light under banks of fluorescent/near UV lights in a tissue culture growth room.
2. In an attempt to simulate more closely natural conditions of skin senescence, the complete skins of a number of fruit were incubated in humid chambers. Fruit were surface sterilised as above, and the skin peeled off. The skin from each fruit was then dipped in a solution of 5 ppm carbendazim and 100 ppm each of penicillin and streptomycin and placed in individual pre-sterilised tissue culture pottles with sterile filter paper in the bottom that had been saturated with a solution of carbendazim and antibiotics as above. The pottles were incubated in the laboratory under ambient conditions. Skins from 56 fruit, all from bunches that had been inoculated at emergence, were treated in this way.

Detection and incubation of colonies

The surfaces of all skins (in Petri dishes and pottles) were scanned using a stereomicroscope, starting

approximately 4 days after processing and thereafter approximately daily for a further 4 days. Although the WAPSC medium inhibited most fungi on the skin slices and whole skins, some species e.g. *Rhizopus stolonifer* (particularly on the whole skin in pottles), and *Colletotrichum* spp. presented a risk of overgrowing some samples. For that reason all putative colonies of *P. fijiensis* observed on the skins were retrieved by excising a small block of skin tissue, approximately 5×5 mm with the colony at the centre, and transferring them to fresh WAPSC plates. The Petri dishes were sealed with Parafilm™ to prevent dehydration and incubated under the light banks for 4 days to induce sporulation.

To confirm the identity of the colonies, small portions of mycelium were removed with the tip of a hypodermic needle, mounted in lactic acid + aniline blue and examined microscopically. Identifications were based on the morphological characteristics of conidia and conidiophores.

A total of 13 colonies identified using morphological techniques were returned to Auckland, New Zealand, for molecular confirmation of identity. These included eight skin pieces with associated colonies, which were incubated for a further 5 weeks to observe whether spermagonia or pseudothecia developed in the skin tissue.

Confirmation of identity of colonies

Thirteen of the Tongan cultures identified on the basis of conidiophore and conidial morphology, and control cultures of *P. fijiensis* (CC298 *ex* banana, Papua New Guinea) and *P. musae* Zimm. (teleomorph *Mycosphaerella musicola* Leach) (Yellow Sigatoka) (CC589 *ex* banana leaf, South Johnston, Queensland) were grown on PDA for polymerase chain reaction (PCR) identification.

DNA was extracted from samples using a QIAGEN DNeasy Plant Mini Kit, following the manufacturer's instructions, or a Chelex-100 (BioRad) 10% solution. Samples were tested using primers R635-mod (5' GGTCCGTGTTTCAAGAC 3') and MfFor (5' CGGAGGCCGTCTAAACACT 3') (Henderson 2006) and MF137 (5' GGCGCCCCCGGAGGCCGTCTA 3') and R635 (5' GGTCCGTGTTTCAAGACGG 3') (Johansen and Jeger 1993). The quantity of DNA was determined using a NanoDrop™ spectrophotometer. The cycling parameters were: initial denaturation of 94 °C for 10 m, 40 cycles of 94 °C for 20 s, annealing of 64 °C for 30 s followed by extension at 72 °C for 1 m. This was followed by a final extension at 72 °C for 3 m. Each of the 25-μL reactions contained: 2.5 μL of DNA (final concentration approximately 4 ng), 2.5 μL 10x buffer (Platinum® Taq DNA Polymerase, Invitrogen), 100 μM dNTPs, 3 mM MgCl$_2$, 0.8 μM of each primer, 1 U of Platinum® Taq DNA Polymerase (Invitrogen) with the final volume made up with Milli-Q water. PCR products were separated by electrophoresis on 1.5% or 2% agarose-Tris-Acetate-EDTA (TAE) (100 V for 45 m) gels and visualised by ethidium bromide staining. When assaying the extractions, a PCR product of approximately 1050 bp indicated a positive amplification of *P. fijiensis* using primers R635-mod and MfFor (Henderson 2006). A product of approximately 10000 bp was also amplified using primers MF137 and R635. For confirmation of the amplified product, the resultant PCR product was sequenced.

Results

Conidial viability and germination on fruit surfaces

Germination of conidia and growth from hyphal fragments were observed on the inoculated fruit in the laboratory and on field fruit 3 days after the first inoculation (Fig. 2). Although quantitative data were not recorded, in terms of numbers of germinated conidia and lengths of germ tubes observed, germination was more rapid on the laboratory-incubated fruit than on the field fruit. This was attributed to the constant high humidity and relatively lower temperatures in the laboratory (c. 24 °C) compared with those in the field, where fruit were exposed to fluctuating temperatures and where bunches were uncovered and remained dry for several hours of the first 2 days. Observations showed that the cooler showery conditions and continuous wetness of the bunches for the final 3 days before the bunch covers were removed resulted in a high proportion of spores germinating on field fruit.

Detection of *P. fijiensis* on fruit skin

The fruit ranged from green tip to fully ripe at the time of processing. Incubated whole skins continued to ripen over succeeding days, with the skin of some fruit reaching the brown spotting stage. Skin strips in the Petri dishes either remained yellow or gradually

Fig. 2 Germinated spores of *Pseudocercospora fijiensis* on 'Cavendish' banana fruit surface

Fig. 3 Fascicle of hyphae of *Pseudocercospora fijiensis* on senescent skin of 'Cavendish' banana

senesced, becoming translucent and turning brown. Some of the skins in Petri dishes could not be assessed because of contamination with *R. stolonifer*, others because of overgrowth by other fungi that had been endophytic in the skin. Overall those losses represented only a very small proportion (< 5%) of the plates. A higher proportion (~20%) of whole skins in humid chambers were lost as a result of overgrowth by *R. stolonifer* and *Colletotrichum* spp.

The first colonies of putative *P. fijiensis,* which appeared as small clusters (fascicles) of light grey hyphae often associated with a small dark stroma, were found on the whole skins after 4 days of incubation and on skin strips in Petri dishes after 5 days (Fig. 3).

The fascicles of *P. fijiensis* were characterised by a distinctive inwards looping of the first hyphae formed. New colonies continued to appear over the next 4 days. On some occasions the fascicle was located in a 'brown spot' of senescent skin tissue. This was considered to be a coincidental association as the skin tissue progressed to the 'brown spot' stage of senescence.

A total of 28 colonies (24 from skin strips; four from whole skins) suspected of being *P. fijiensis* were excised from the parent skin pieces for further incubation. Eighteen were subsequently determined, on the basis of colony morphology, to be *P. fijiensis*. Of the 18

confirmed recoveries, 16 were made from three bunches that were just emerging at the time of inoculation. Only two colonies were found on skins of fruit that had been fully exposed at inoculation.

The sequencing of PCR products confirmed that of the 13 cultures tested, nine amplified as positive for *P. fijiensis,* with either MF137 and R635 or MfFor and R635-mod combination primer pairs. The primer pair, MFFor and R635-mod, was routinely used in preference to the MF137 and R635 primer pair because of better primer efficiency and amplification. Four samples failed to amplify, despite the extraction being repeated, because of suspected poor quality DNA. Nevertheless, the result was taken as confirmation that the morphological identifications had been correct.

Colony characteristics and sporulation on banana skin

During incubation, the excised colony-bearing skin pieces turned brown as the tissue senesced. Fascicles of typically grey, inward looping aerial hyphae of *P. fijiensis* were associated with dark supradermal stromata. The first conidia were found on colonies on the excised skin blocks 5 days after the colonies were first detected (approximately 10 days after incubating the skins). Conidia typical of *P. fijiensis* were found on 14

of the 18 colonies of *P. fijiensis* detected. One colony of *P. fijiensis* was found within a spore mass of *Colletotrichum* sp. Conidia were also found on that colony, indicating that the pathogen was able to survive and reproduce on skin tissue in the presence of this faster-growing competitor.

Dissection of the fascicles on skin tissue showed that each comprised a dark, supradermal stroma and light brown aerial hyphae (these hyphae appear light grey under stereomicroscopy). The stromata were anchored in the skin by dark hyphae ramifying through outer skin cells. In some cases fine hyphae also radiated from the fascicle and across the epidermis, some later developing into a loose mass of fine white aerial hyphae growing above and around the original fascicle. Numerous hyphal anastomoses were observed among these fine hyphae. Looped hyphae, typical of *P. fijiensis,* were subsequently observed growing from the cut edges of the skin fragments, indicating that the fungus had colonised the whole of the piece of skin that had been excised. Conidia were formed in abundance on typical, dark conidiophores that were produced both on the surface of the stroma (Figs. 4 and 5) and also on the primary hyphae of
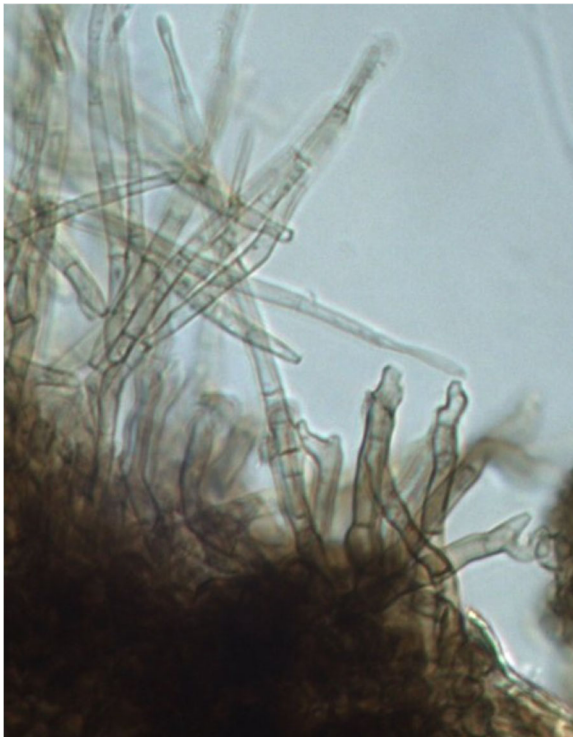

Fig. 5 Conidia of *Pseudocercospora fijiensis* produced on senescent skin of 'Cavendish' banana

the fascicle. Later, as the mass of fine hyaline aerial hyphae developed, conidiophores and conidia were formed on those hyphae also (Fig. 6). The formation of

Fig. 4 Conidiophores and conidia of *Pseudocercospora fijiensis* on stroma on the skin of senescent skin of 'Cavendish' banana

Fig. 6 Conidiophores on aerial hyphae of *Pseudocercospora fijiensis* growing on senescent skin of 'Cavendish' banana

conidiophores and conidia on aerial hyphae has not been recorded previously for this pathogen either on leaves or in culture. In some colonies the stroma remained as a compact dark hyphal mass. In others a number of dark globose structures formed in association with the stroma (Fig. 7). Microscopically some of these structures appeared to be hollow, suggesting that they may have been spermagonial or pseudothecial initials. During a further 5 weeks of incubation of eight colonies, all produced an abundance of conidia but none of the suspected spermagonial or pseudothecial initials developed further.

Discussion

This study has confirmed that *P. fijiensis* can infect the skin of juvenile fruit of 'Cavendish' without causing symptoms, survive in the skin until fruit maturity, and form stromata, conidiophores, and conidia on dead skin tissue as the sole substrate. In contrast to its habit in leaves, where conidiophores typically emerge from the
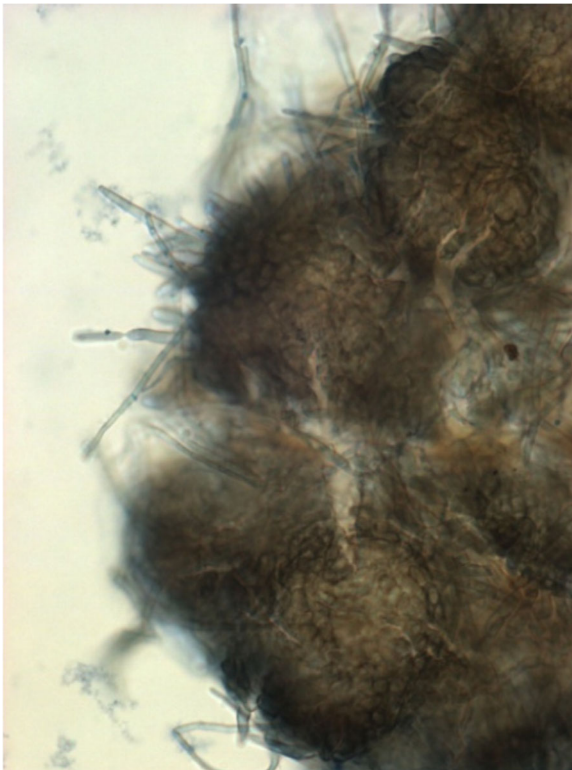

Fig. 7 Globose structures associated with stroma of *Pseudocercospora fijiensis* on senescent skin of 'Cavendish' banana

stomata, on fruit the pathogen forms supradermal stromata bearing hyphae and conidiophores, and subsequently fine aerial hyphae which can also bear conidiophores and conidia. Although conidia were produced in abundance by the colonies on the skin pieces the teleomorph was not found. Formation of pseudothecia and ascospores in *P. fijiensis* is dependent on the pairing of compatible mating types (Etebu et al. 2003), suggesting that each colony isolated in this study was of a single mating type.

The competitive saprophytic ability of *P. fijiensis* in relation to other skin-colonising fungi would be an important factor in its ability to complete its life cycle in skin under natural conditions. *P. fijiensis* has been shown previously to saprophytically colonise dead banana leaf tissue (Balint-Kurti et al. 2001). The saprophytic colonisation of fruit skin tissue was observed in this study. Although its competitive saprophytic ability was not specifically addressed, there was some evidence in this study that the pathogen is capable of colonising and surviving and sporulating on the skin even if overgrown by faster-growing species such as *Colletotrichum* sp.

In this study, bunches were inoculated at different stages of development ranging from bunch emergence (when bracts were just lifting) to bunches with all hands fully exposed. The majority of colonies were recovered from bunches that had been inoculated just as the bracts were lifting. These results indicate that the fingers were most susceptible to infection just as the bracts are lifting and exposing the flowers and young fingers. That timing is significant in terms of fruit infection in commercial plantations. Juvenile fruit would be initially protected from aerially applied fungicides by the bracts as they progressively open and fall over the course of approximately 1 week. Soon after the last bracts fall, the fruit are de-flowered, the bell removed and the bunch covered with a plastic sleeve that would protect the fruit from further fungicide contact. Thus the pathogen, by infecting as the bracts are lifting, would have minimal exposure to fungicides and potentially survive in the fruit until harvest.

The ability of *P. fijiensis* to infect 'Cavendish' fruit and to reproduce on the senescing fruit skin has significant implications for the long distance transfer of specific strains of *P. fijiensis*, e.g. fungicide resistant, via commercial banana shipments. It also represents a potential entry pathway to fruit importing countries currently free of the disease.

The inoculation of fruit in the field with *P. fijiensis*, locating the pathogen in the skin of the mature fruit, and inducing sporulation has not, to our knowledge, been previously attempted. The strategy of using carbendazim-resistant strains of the pathogen was successful in allowing this slow growing pathogen to be detected on the skin before being overgrown by other faster growing fungi such as *Colletotrichum* spp. The methodology developed here can be applied to any further fruit infection studies to determine the competitive saprophytic ability of the pathogen, its ability to reproduce on discarded skins under natural conditions, and for assessing the potential for the production of pseudothecia and ascospores on skin.

Compliance with ethical standards   This paper reports original research by the authors which has not been published elsewhere nor submitted for publication elsewhere.

Conflict of interest   The authors declare they have no conflicts of interest in the presentation of this work.

Research involving human participants and/or animals   No human participants nor animals were involved with this research.

Informed consent   Not relevant to this work.

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