Pathogenic *Penicillium* spp. on apples and pears

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Numerous *Penicillium* spp. have been associated with postharvest fruit spoilage. This study investigates pathogenicity and aggressiveness of selected *Penicillium* spp. previously isolated from South African and European Union fruit export chains. *Penicillium expansum* was the most- and *P. crustosum* the second most aggressive on all apple cultivars (Royal Gala, Granny Smith, Golden Delicious, Topred and Cripps Pink) and two pear cultivars (Packham's Triumph and Forelle) tested. *Penicillium digitatum* was the most aggressive on Beurre Bosc, Beurre Hardy and Sempre (Rosemarie) pear cultivars and the third most aggressive on Granny Smith and Cripps Pink apple cultivars. To our knowledge this is the first report where *P. digitatum* has been described as aggressive on certain pome fruit cultivars. These cultivars are also the most commonly associated with decay on the export markets resulting in considerable end-market losses. *Penicillium brevicompactum* was detected pathogenic on pears, but was not further evaluated in the study. *Penicillium solitum* was observed more pathogenic (broader cultivar range and higher disease incidence) and aggressive (larger lesions) on pear cultivars than on apple cultivars. This study provides new information on host specificity and the importance of pathogenic *Penicillium* spp. isolated from various environments in the shipping and marketing channels.

Additional keywords: blue mold, green mold, virulence, PCR-RFLP

INTRODUCTION

The South African pome fruit industry is economically important in terms of global trade. The industry produces over 1.1 million tons of fruit of which 46.6% are exported as fresh produce, earning close to $431.6 million in net export realization in 2012 (9). Postharvest losses have been reported as high as 50% in developing countries (5, 21, 26). Significant losses have been attributed to decay caused by *Penicillium* spp. (12, 14, 16, 20, 22, 32).

A report by Sanderson and Spotts (22) indicates that numerous *Penicillium* spp. naturally inhabit pome fruit environments (packinghouses and storage facilities) and are prominent on fruit bins. Of these species, *P. expansum* Link ex Gray, *P. crustosum* Thom, and *P. solitum* Westling have been described as the most important on apples (*Malus domestica* L. Borkh.) and pears (*Pyrus communis* L.) in causing decay (14, 22, 32). Other pathogenic species reported include *P. aurantiogriseum* Dierckx., *P. brevicompactum* Dierckx., *P.commune* Thom, *P. griseofulvum* Dierckx., *P. verrucosum*, Dierckx. and *P. carneum* Frisvad (2, 12, 16, 18, 23). However, these species have less often been associated with decay of pome fruit.

Several other *Penicillium* spp. isolated from pome fruit environments (2, 22) are best known as pathogens on other fruit crops. These include *P. digitatum* (Pers.:Fr.) Sacc. on citrus (8) and *P. glabrum* (Wehmer) Westling on pomegranates (3). Complex fruit trade networks result in a large variety of fruit from different countries being retained together in storage or holding facilities (34). In addition, citrus and pome fruit are sometimes re-packed in the same facility to remove decaying fruit. This practice can potentially introduce a wider range of pathogens to the hosts that would not otherwise encounter these pathogens. Recently, Vilanova *et al*. (35) reported *P. digitatum* (Pers.:Fr.) Sacc. able to infect ‘Golden Smoothee’ apples but with lesions not developing beyond the initial infection site. Infectivity was described as limited to specific fruit

maturity conditions (commercial and over-mature) and inoculum load, resulting in peel infection reactions of up to 6 mm diameter (including wound site).

The lack of transparency in the fruit industry and retail practices towards the end of the supply chain makes it difficult to establish the impact of the causal agents. Costs associated with market-end losses are often passed back to the farmer without verification of the causal agent or possible point of contamination. End-of-the-season fruit, and long-term storage and extended transit periods can result in physiologically older end market produce that may be more susceptible towards decay (4, 10, 15, 36). In this context, opportunistic pathogens encountered further down the supply chain may attack fruit previously considered non-hosts for those pathogens.

The aim of this research project was to evaluate representative isolates from dominant *Penicillium* spp. isolated from various environments in the shipping and marketing channels of citrus- and pome fruit supply chains and to determine pathogenicity, aggressiveness and host specificity of these isolates. This knowledge will provide a better understanding of the causal agents, inoculum buildup potential and fruit decay at the market-end of the supply chain.

MATERIALS AND METHODS

Fungal cultures. The *Penicillium* spp. isolates were obtained from the fungal culture collection at the University of Pretoria. Isolates came from pear (2010/2011) and citrus (2009/2010) export chain studies (unpublished data) (Table 1). The criterion for initial selection of species from the culture collection for the present study was based on the identified species being well-known pathogens or the species representing dominance in the supply chain environment. Cultures of the isolates for experimental trials were prepared by single spore isolation. Cultures were grown for up to three weeks on malt extract agar (MEA) (Merck, Biolab

Diagnostics (Pty) Ltd, Johannesburg, South Africa) plates in darkness at 25°C. Original isolates were previously purified, morphologically grouped, molecularly identified and preserved in water as working cultures and in 10% glycerol for long-term storage. Water preservations were made by placing five to six pieces of the fungal mycelial growth culture on MEA into double-sterilized distilled water in McCartney bottles, parafilmed and stored at room temperature. Cryo-preservation was made by placing five to six pieces of MEA grown cultures into 10% glycerol (autoclaved five times) in cryotubes and stored at -72°C in a freezer.

The isolates were identified by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method and identity was confirmed by sequencing. DNA was extracted using Neuclospin® Plant II kit (Macherey-Nagel GmbH and Co. KG, Düren, Germany), PCR amplified, restriction digested (11, 17, 20) and submitted for sequencing to conduct final identification. The PCR reactions were performed in a 2700 Prekin-Elmer PCR thermocycler using beta-tubulin (β-tubulin) primers (Bt2a and Bt2b) (7). The cycle conditions included a preliminary three minute denaturation step at 95ºC, 35 cycles of denaturation (94ºC for 30 sec), annealing (57ºC for 45 sec) and elongation (72ºC for two min), followed with a final elongation step (72ºC for seven min) and samples held at 4 ºC upon completion. The yield, purity and quality of PCR amplicons were verified on an agarose gel with a 1kb ladder/marker.

A restriction enzymes BfaI (isochitzomer - FspBI) (Inqaba, Pretoria, South Africa) was used to restriction digest the PCR amplicons. The fragments were separated by electrophoresis on 3% agarose gel run at 75 V between three to five hours (minimum and maximum run time) with a 100bp ladder/marker to separate the fragment and determine polymorphisms. For sequencing, the PCR amplicons were purified according to MSB® Varo CleanUp (Invitek GmbH, D13125 Berlin, Germany). The sequencing of PCR samples was conducted with the BigDye® Terminator

V3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) including the forward primer (Bt2a). Sequence cycle profiles followed in a 2700 Prekin-Elmer PCR thermocycler. The cycle conditions were 96ºC for one min, trailed by 25 cycles (96ºC for 10 sec, 50ºC for five sec and 60ºC for four min) and samples held at 4 ºC. The sequenced PCR products were analyzed with an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, USA).

Confirming pathogenicity and comparing citrus isolates to pear isolates. The *Penicillium* spp. selected for pathogenicity trials included isolates from the citrus environment (*P. expansum*, *P. crustosum*, *P. solitum*, *P. digitatum* and *P. brevicompactum*). Spore suspensions were prepared in sterilized Ringers (Merck) solution containing 0.05% Tween 80 (Associated Chemical Enterprises, Johannesburg). Concentration of the spore suspensions were determined with a haemocytometer and the suspensions were diluted to the final concentrations of 6.3×10^4 conidia/ml. Freshly harvested untreated commercial grade mature apples ('Golden Delicious') and pears ('Beurre Hardy') from a packinghouse in the Western Cape Province with different postharvest practices were used in the pathogenicity trials (Table 2). Fruit were surface sterilized by dipping into 0.002% sodium hypochlorite solution for approximately ten min and allowed to air dry. Ten apples and 10 pears were inoculated with each isolate of *Penicillium* spp. Each fruit was wounded on opposite sides (1.5 mm x 1.5 mm x 3 mm) by gently piercing the fruit surface with a sterile yellow micropipette tip. Inoculation was conducted by depositing 20 µl of spore suspension into each wound using a pipette. Controls consisted of un-inoculated wounded fruit. All wounds (inoculated and un-inoculated) were taped with Parafilm to prevent cross-contamination during the experiment. Fruit were randomized on a disinfected table and incubated under ambient conditions (24.1±0.9°C; 48.8±5.8% RH) for seven days. The decay was assessed three, five and seven days post-inoculation by measuring the horizontal and vertical

(stem-calyx axis vertical) diameters of lesions. Control wound sizes were also measured, and mean diameter of control wounds were subtracted from the measured lesion diameters. The experiment was repeated and arranged according to the complete randomized designs.

The citrus isolates that were confirmed pathogenic to apples and pears were further evaluated to observe if they can be considered pathogenic representatives of their species by comparing them with environmental isolates from a pear chain study. The isolates included *P. expansum*, *P. crustosum*, *P. solitum*, and *P. digitatum*. This comparative trial was conducted by inoculating five surface sterilized pears ('Beurre Hardy') per isolate using the spore suspensions method. Inoculation and incubation of the fruit and the measuring of lesion diameters were as previously described. The isolate comparison trial was repeated and using a complete randomized design.

Decay caused by *Penicillium* spp. under cold storage conditions. The cold storage trial was performed to determine effect of temperature on disease expression on 'Golden Delicious' apples (Table 2) inoculated with *P. expansum*, *P. crustosum*, *P. digitatum* and *P. solitum* isolated from citrus environment. Each pathogen was inoculated to ten surface sterilized apples using the spore suspension method. One set of inoculated apples was incubated under ambient conditions whilst the second group was incubated under refrigerated conditions (6.2±1.7°C; 63.3±3.0% RH).During the 43-day incubation period, the lesion diameters were recorded (as previously described) every second day starting from the third day after inoculation. Mean wound sizes for controls were also subtracted from the measured lesion diameter means to present decay lesion diameter alone. The trial included a repeat with factorial arrangements on a complete randomized block design.

Aggressiveness of *Penicillium* spp. on apple and pear cultivars. Five different apple- ('Golden Delicious', 'Granny Smith', 'Royal Gala', 'Topred' and 'Cripps Pink') and five

different pear (‘Beurre Hardy’, ‘Beurre Bosc’, ‘Forelle’, ‘Packham’s Triumph’ and ‘Sempre’ (‘Rosemarie’)) cultivars were inoculated with citrus environment isolates of *P. expansum, P. crustosum, P. digitatum* and *P. solitum* using the spore suspension inoculation method. Fruit for the test originated from two packinghouses in the Western Cape Province, but were collected from various sectors in the supply chain ranging from freshly harvested (fully mature) to packed or commercially stored (Table 2). Ten surface sterilized fruit per *Penicillium* spp. per cultivar were inoculated with the pathogens, incubated under ambient conditions and the lesion diameters were measured as previously described. Mean wound sizes for the controls were subtracted from the measured lesion diameter means in the results. Symptom expression (necrotic pattern, visual fungal growth and sporulation) and disease progression were recorded. The experiment was repeated and organized according to a factorial arrangement on a complete randomized design.

Isolations from lesions, culture preservation and *Penicillium* spp. identification. Two to three fruit from the aggressiveness tests were selected for each cultivar for re-isolation of the pathogen. Isolations were made onto MEA medium and incubated as previously described. Once sufficient growth occurred the cultures were observed for morphological similarity, purity and one culture for each *Penicillium* spp. from each cultivar was preserved and identified using the genetic approach as previously described.

Statistical analysis. Statistical analysis was performed with SAS (version 9.2; SAS Institute Inc., Carry, NC, USA). Each fruit in the trials was treated as a replicate, with its mean derived from four measurements (two wounds with horizontal and vertical diameter measurements). Where means follow with ±*x*; *x* refers to standard deviation. Least-square mean t-test was used to analyze similarity between repeated tests. The independent experiments for all the trials proved

not significantly different and were thus pooled. Fisher's Least Significant Difference (LSD) was used to separate means.

Lesion diameter (mm) and disease incidence (%) of the inoculated wounds from the pathogenicity trials were recorded and used to calculate disease intensity, a measure that represents the combined effect of disease severity and disease incidence. Lesions significantly different from the control were regarded as pathogenic reactions and used in calculating disease incidence. Disease intensity can be used to compare the potential importance of each *Penicillium* sp. on the respective crop, indicating possible disease-associated concerns within the fresh produce chain. The disease intensity relation is as follow: Disease intensity = $[(d \times F)/(T_n \times D)] \times 100$; d represented degree of disease severity assessed or specific lesion size classified on the empirical scale, F frequency, T_n total number of fruit examined and D highest numerical number value of the empirical scale used (33). The relation was applied with mean lesion diameter (d), number of pathogenic reactions observed (F), total number of inoculated wounds examined (T_n) and maximum lesion diameter measurable (D).

RESULTS

Confirming pathogenicity and comparing citrus isolates to pear isolates. The independent pathogenicity experiments (trial and repeat) for both apples and pears did not differ significantly ($P = 0.57$ and $P = 0.89$). Different host-pathogen interactions did however differ significantly (Table 3). All of the *Penicillium* spp. originating from citrus environment were pathogenic on pears. Fewer *Penicillium* spp. were pathogenic on apples compared to pears. *P. brevicompactum* was not pathogenic, whereas *P. solitum* and *P. digitatum* expressed low incidence and small lesions on apples within the seven day period assessed. Mean lesion diameters caused by *P.*

solitum and *P. digitatum* on apples did not differ significantly when compared to the control, but some significantly different lesions were noted to calculate disease intensity (Table 3).

Lesion sizes were not significantly different in two independent experiments for isolate comparison on pears ($P = 0.85$). The pear and citrus isolates also did not differ significantly in terms of lesion sizes (Fig. 1).

Decay caused by *Penicillium* spp. under cold storage conditions. The temperature had a significant impact on rate of lesion development ($P < 0.0001$). The cold storage trial and repeat did not differ significantly ($P = 0.7954$). *P. expansum* was the only species able to cause symptoms on 'Golden Delicious' under cold storage conditions over the 43 day storage period (lesion diameter (*ld*) = 49.5±17.3 mm). *P. crustosum* did however produce small lesions of 4.0±0.5 mm (mean wound diameter of the control was already subtracted) at low incidence (10%) under the cold storage conditions. Under ambient conditions (17 days) large lesions were caused by *P. expansum* (73.9±9.2 mm) and *P. crustosum* (42.2±12.4 mm). *P. digitatum* and *P. solitum* again caused low incidence and small lesions on the 'Golden Delicious' apples. The experiment was terminated on the 17th day due to total decay of fruit by *P. expansum* at ambient conditions. Under cold storage conditions lesions began to develop seven days post-inoculation while fruit kept under ambient conditions developed decay two days post-inoculation. No surface mycelium or sporulation was observed on inoculated fruit kept in cold storage. Fruit inoculated with *P. expansum* and stored under ambient conditions displayed mycelial growth on the fruit surface after six days (no sporulation noted).

Aggressiveness of *Penicillium* spp. on apple and pear cultivars. The *Penicillium*-cultivars interactions were significantly different ($P < 0.0001$). The repeated experiments were not significantly different, except *P. digitatum* on 'Beurre Bosc' pears where in the initial

experiment small lesions (seven day *ld* = 7.6±3.7 mm) were observed compared to large lesions (fifth day *ld* = 84.0±5.2 mm) in the second experiment. A similar shift in aggressiveness occurred on 'Beurre Bosc' inoculated with *P. crustosum* between the two experiments, but differences were not as large (25.0±7.7 mm vs. 40.3±3.0 mm) (Fig. 2). The same cultures were used for inoculation, but in the second experiment the fruit were presumed riper as they were kept in cold storage four days longer (Table 2). Due to differences in production practices, cultivar availability, and/or seasonality, the trial experiments could not be completed simultaneously (15, 36).

Decay development by *P. expansum* and *P. crustosum* over the cultivar ranges were more consistent compared to *P. digitatum* and *P. solitum*. *P. digitatum* and *P. solitum* did not cause decay on 'Royal Gala' apples (Fig. 3). The mean of lesions caused by *P. digitatum* on 'Packham's Triumph' (Region 1) were not significantly different from the control, but few independent lesions were noted significantly different; 10.5% of the inoculated wounds had lesions 4.7±1.3 mm in diameter. *P. digitatum* lesion decay on the remaining cultivars were significantly different from the control, however incidences (only lesions significantly different from the control were regarded as pathogenic reactions) varied with some (Table 4). *P. digitatum* was the most aggressive species on 'Beurre Bosc' (100.0% fifth day incidence with 83.9±5.2 mm *ld* for the second experiment), 'Beurre Hardy' (100.0% seventh day lesion incidence with 84.7±8.4 mm *ld*) and 'Rosemarie' (100.0% seventh day incidence with 73.6±11.5 mm *ld*). *P. solitum* lesions on all the cultivars, except 'Royal Gala', were significantly different compared to the control, but incidence also varied (Table 4). In general, greater incidence and larger lesions were observed from the *Penicillium* spp. on pears than apples (Fig. 3).

Symptom expression on apple and pear cultivars. Expressions of decay symptoms on various cultivars were compared to previously described symptoms caused by *P. expansum*, *P.crustosum*, *P. solitum* and *P. digitatum*. This study noted additional symptoms not previously described from various *Penicillium* pathogens screened on different cultivars. Symptom expression was not uniform on different cultivars. The lesions produced on all apple and pear cultivars tested were brown, circular, and slightly sunken. The shade of the brown lesions differed depending on the *Penicillium* spp. and the cultivar host they infect. Thus, lesions coloration cannot be associated with either a specific species or cultivar, but rather to an interaction between *Penicillium* sp. and fruit cultivar. (Fig. 4 and Fig. 5). *P. digitatum* also produced not distinctly defined lesions with brown blotching and softening and swollenness of the tissue, later resulting in a bulged appearance of browned skin on sensitive hosts (Fig. 6). These lesions can be seen during development from a central infection point or as in the case of 'Granny Smith' appearing five to six days after inoculation, from below the skin surface. In the latter case the skin of the apple will appear healthy during the first few days, but the application of pressure reveals a hollowing or softening underneath the skin surface. *P. expansum* and *P. crustosum* frequently produced bull's-eye rot type symptoms as the growth progressed on all cultivars (Fig. 4 and Fig. 5). *P. solitum* produced very small lesions on apples, when inoculated into susceptible hosts. Some apple cultivars displayed typical resistant response reactions (avirulence as described by Agrios (1)) as skin and/or tissue darkening was restricted to the inoculated wound areas (Fig. 4: *P. solitum* reactions on 'Granny Smith', 'Golden Delicious' and 'Topred'; *P. digitatum* reactions on 'Golden Delicious' and 'Topred'). Avirulent reactions were also observed on a few independent 'Packham's Triumph' pears inoculated with *P. digitatum*.

P. expansum and *P. crustosum* produced white mycelia on all apple cultivars, although the amount produced by *P. expansum* made it more apparent. *P. solitum* and *P. digitatum* produced only small amounts of mycelia when infecting 'Granny Smith' and 'Cripps Pink'. No *Penicillium* spores were observed on any apple cultivars during the duration of the experiment. Mycelial growth with limited sporulation was observed on all pear cultivars inoculated with *P. expansum* and *P. crustosum*. *P. solitum* never sporulated, but mycelial growth was observed on all pear cultivars. *P. digitatum* produced very limited mycelia and spores on the large lesions produced on 'Beurre Bosc'. More sporulation was noted on 'Beurre Hardy' with 'Rosemarie' supporting profuse sporulation after seven days incubation. No mycelial growth or spores were on the remaining pear cultivars inoculated with *P. digitatum*. *P. expansum* and *P. crustosum* typically produced blue spores (blue mold) whereas *P. digitatum* produced lime green spores (green mold) (Fig. 6).

Confirmation of identity. Koch's postulates were confirmed on all species tested in the trails. The identity of the *Penicillium* spp. re-isolated from infected fruit were confirmed using PCR-RFLP and sequencing as the same species that were used for the inoculation of the fruit (Table 5).

DISCUSSION

The most important pathogenic *Penicillium* spp. in terms of decay on pome fruit identified in this study were *P. expansum*, *P. crustosum*, *P. digitatum* and *P. solitum*. These species have formerly been described in pome fruit environments (2, 22) and pathogenic (excluding *P. digitatum*) on pome fruit (14, 22, 32). Sanderson and Spotts (22) previously reported isolating *P. digitatum* with low incidence (1.8% of 57 pears) from decay lesions on winter pears ('d'Anjou', 'Bosc', 'Comice' and 'Red Anjou'). In their study, decaying apples and pears were sampled

from both packinghouses and local markets. The sampling from the packinghouses were regarded as biased because samples were selected based on a specific type of decay. Nonetheless, *P. digitatum* was only isolated from the market winter pears and were not pathogenic when inoculated into fresh ‘d’Anjou’ pears. The authors never demonstrated, nor explicitly stated *P. digitatum* pathogenic on pears, but theorized that the species may be able to colonize “over-mature” fruit (market fruit).The authors did not screen a spectrum of different cultivars or test physiologically older fruit obtained from the market (22). *P. brevicompactum* was found to be non-pathogenic on apples and were not tested further in this study.

P. digitatum produced the largest lesions on three cultivars (Rosemarie, Beurre Bosc and Beurre Hardy) indicating its importance as a postharvest pathogen on pears. These cultivars were also identified by the industry as the cultivars experiencing concerning losses on the export markets. To our knowledge, this is the first report where *P. digitatum* was described as a highly aggressive pathogen on selected pear cultivars. *P. digitatum* infections are more prominently associated with over-mature fruit (22, 35), possibly causing problems much later in the market chain, similar to our results in the second experiment on ‘Beurre Bosc’. Although fruit for both trials were selected at commercial harvest maturity stage, fruit for the second experiment was refrigerated (6.2±1.7°C) four days longer (total of 12 days) before inoculation trials. Storage at 5-10°C for three, five or seven days induces pear ripening (36), thus influencing susceptibility toward decay (10, 35). The prolonged storage at the specified temperature is a potential explanation for the fruit being more mature and thus more susceptible toward decay. *P. expansum* lesion development was less affected by the prolonged stored fruit, although *P. crustosum* was influenced. This raises the question if *P. crustosum* should be regarded more problematic on pears after suboptimum storage or transport of the fruit? It also illustrates that

stored or transported fruit may become more susceptible towards certain pathogenic *Penicillium* spp. Effective cold chain management and reduction of inoculum loads still remain important for disease control.

Although fruit maturity indexes for each cultivar were not tested in the lab prior to inoculation in this study, the fruit were all commercially handled and as a batch officially inspected for compliance with national quality standards and maturity according to the Agricultural Products Standards Act No 119 of 1990 (28, 29). Through this study we have come to understand the importance of taking fruit seasonality and postharvest changes into account in pathogenicity/aggressiveness studies. Some reports dealing with pathogenicity or aggressiveness do not test or report on the physiological status of the host (2, 16, 31). However, this aspect should be included to more effectively assess host-pathogen interactions in the future.

It is important to consider further steps in the supply chain when fruit is stored for months prior to packaging and marketing. Fruit designated for export markets with long distribution systems will be more prone to decay, because it will be more mature after a long sea shipment (16 days or longer) and may be exposed to pathogens from other fruit species (e.g. citrus and apples when seasons overlap) that may be handled in the same central facility. In addition, during re-packing the cold chain is broken and the fruit can be wounded and exposed to *Penicillium* inoculum that can cause decay (34) at the consumer-end of the chain. *P. digitatum* in this study has been identified as a pathogen that has the potential to cause rapid decay at the market-end (total fruit decay within five days), especially when fruit are riper. Pathogenicity trials on market aged fruit are therefore required to identify the pathogens that can cause rapid decay and extensive losses at the end of market chain.

Sanderson and Spotts (22) noted that *P. digitatum* probably colonize over-mature fruit because they isolated the species from market pears, but could not reproduce infections on newly harvested pears. Unfortunately no symptom descriptions were given of the isolated lesions from the market fruit. Vilanova *et al*. (35) described reactions of *P. digitatum* infections on 'Golden Smoothee' apples, but restricted the descriptions to "no decay development" with limited disease symptoms and or a hypersensitive reaction. The host resistance responses and infection reactions of *P. digitatum* described on 'Golden Smoothee' were very similar to symptoms produced by *P. digitatum* on 'Golden Delicious' apples in our study. *P. digitatum* was not isolated from the described infection sites on 'Golden Smoothee' to confirm the identity of the organism causing the reaction. Books or publications with pome fruit *Penicillium* pathogen symptom descriptions are usually limited to descriptions or illustrations of one or two cultivars (13, 25, 35). A compendium describing symptoms associated with different *Penicilliums* spp. infections on various commercial cultivars is not readily available for industry and fruit inspectors. Similar to green- and blue mold of citrus, there is a need to distinguish between decay caused by *P. digitatum* (distinct green mold) and the other blue mold causing *Penicillium* spp. as described in this study. This study further provides a more detail description of blue mold symptoms over a range of apple and pear cultivars. *P. expansum* and *P. crustosum* symptoms were frequently related to Bull's-eye rot symptoms which are commonly associated with *Neofabraea* (Jacks.) spp. (6, 31). This may lead to the misidentification of the causal pathogen if no isolation and identification of the fungus is made. *P. digitatum* symptom expression on pears and advanced symptom expression on apples were reported for the first time in this paper.

Pears were in general more susceptible and sensitive to *Penicillium* decay than apples. Pears are naturally more perishable (deteriorate faster) than apples (13), adding to susceptibility and

sensitivity. *P. expansum* and *P. crustosum* expressed pathogenicity over the cultivar ranges tested. *P. expansum* was considered the most aggressive, except on 'Beurre Bosc', 'Beurre Hardy' and 'Rosemarie'. *P. crustosum* closely mimicked the pathogenic and aggression profile of *P. expansum*, generally expressing the second highest aggressiveness on the majority of cultivars tested (excluding 'Beurre Bosc', 'Beurre Hardy' and 'Rosemarie'). *P. solitum* was more pathogenic (covering a broader cultivar range with a higher incidence) and aggressive (larger lesions) on pear cultivars than on apple cultivars.

Penicillium spp. isolates from different environments (isolated from pear and citrus chains) did not produce significantly different lesion sizes on pears. This finding confirms that environmental isolates from different origins represent a source of natural inoculum (30) for varying fruit types. The compilation of fruit from different origins, received in a central port or holding facility and sometimes even being re-packed prior to further distribution in the same supply chain may increase the likelihood of cross-contamination. It also shows that *P. digitatum* and *P. expansum* originating from the citrus chain environment, produced similar lesion sizes and symptoms as the *P. digitatum* and *P. expansum* isolates from the pear environment.

The cold storage test results showed that a change in environmental conditions can have a significant influence on pathogenicity, aggressiveness and symptom expression. *P. expansum* was the only species that produced large lesions under cold storage conditions that simulate commercial practices. The results agree with findings from Vilanova *et al*. (35), indicating that the pathogen can infect, invade and produce symptoms whilst in the cold chain. *P. crustosum* was unable to produce large lesions at the cold storage conditions tested compared to the big lesions under room storage conditions. *Penicillium* spores are able to tolerate extremely low temperature conditions and germinate when conditions become favourable for growth (24, 27).

This can occur when the cold chain is broken during the extended supply chain. *P. crustosum* may thus be able to produce lesions on 'Golden Delicious' apples once the fruit exit cold storage. *P. digitatum* is able to germinate at 4°C (19) and *P. solitum* is able to produce symptoms at -1°C (22), but the low sensitivity of 'Golden Delicious' towards the two *Penicillium* spp. made it difficult to evaluate temperature based disease sensitivity of the species on the apple cultivar. The growth patterns produced by *P. expansum* and *P. digitatum* in cold storage were similar to that described by Vilanova *et al*. (35). The results are thus in agreement although the higher temperature in this study (6.2±1.7°C vs. 0°C) resulted in a shorter lag phase and faster growth rate for *P. expansum*. Future research should include cold storage trials with a range of cultivars with varying susceptibility levels. This will allow the identification of temperature based disease sensitivity of specific *Penicillium* spp. depending on host susceptibility.

Fruit designated for export remains in the supply chain for an extended period of time and often is re-packed in facilities that do not always comply with the same quality standards as is required of packinghouses at the beginning of the supply chain. It is therefore hypothesized that losses at the consumer-end of the chain can be partly due to improper handling, breaking of the cold chain and sometimes re-packing of fruit in facilities that do not comply with the same quality standards as the primary producer and packer.

CONCLUSION

All tested species; *P. expansum, P. crustosum, P. digitatum, P. solitum* and *P. brevicompactum* were pathogenic on pears, but only the former four proved pathogenic on apples. *P. digitatum*, a known citrus pathogen, caused lesions on several pear and apple cultivars tested. This is the first report of *P. digitatum* obtained from a citrus chain environment showing high aggressiveness (exceeding that of *P. expansum*) on pears ('Rosemarie', 'Beurre Bosc' and

‘Beurre Hardy’) and causing rot on apples (‘Granny Smith’ and ‘Cripps Pink’). These cultivars have been described by the fruit industry as the most prone to decay at the retail-end of the supply chain resulting in significant financial losses. Differences in aggressiveness and host specificity were found among the isolates. *P. expansum* and *P. crustosum* were pathogenic on all cultivars tested whereas *P. digitatum* and *P. solitum* had narrower host cultivar ranges. *P. expansum* and to a lesser extent *P. crustosum* are known as the more typical postharvest pathogens on pome fruit. *P. digitatum* was identified as an opportunistic pathogen on pome fruit that can result in rapid pome fruit losses at the market-end of the supply chain depending on sanitation in the storage/shipping/marketing environments, temperature management during shipping and marketing, and host susceptibility as related to cultivar and fruit maturity.

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Table 1. *Penicillium* isolates used in the pome fruit studies

Isolate code	*Penicillium* spp.	Fruit chain	Year	Country of origin	Source (location of isolation)
P.eC	*P. expansum*	Citrus	2009/2010	Germany	Distributer/repack facility-wall. Koch postulates confirmed on apples (2011).
P.eP	*P. expansum*	Pear	2011	United Kingdom	Cold storage facility-air.
P.cC	*P. crustosum*	Citrus	2009/2010	Germany	Cold storage-air.
P.cP	*P. crustosum*	Pear	2011	South Africa	Packinghouse holding area-wall.
P.sC	*P. solitum*	Citrus	2009/2010	Germany	Distributer/repack facility-wall.
P.sP	*P. solitum*	Pear	2011	United Kingdom	Retail storage facility-wall.
P.dC	*P. digitatum*	Citrus	2009/2010	Netherlands	Distributer/repack facility-floor. Koch postulates confirmed on plums (2011).
P.dP	*P. digitatum*	Pear	2011	United Kingdom	Repack facility area small waste bins.
P.bP	*P. brevicompactum*	Pear	2010	United Kingdom	Distribution center-air.

Table 2. Pome fruit origin and handling practices

Cultivar	Time of harvest (season)[z]	Postharvest practices	Cold storage before inoculation (days)
Pathogenicity trials/Inoculation comparison method trial/Cold storage trial			
Golden Delicious	Mid	A	18/20
Beurre Hardy	Late	A	12/13
Isolate comparison trial			
Beurre Hardy	Late	A	32
Aggressiveness trials on different cultivars			
Golden Delicious	Mid	A	12/13
Granny Smith (2012)	Late	A	31
Granny Smith (2011)	Previous year (long term storage)	B	12/13
Cripps Pink	Late	B	31
Topred	Late	C	1/2
Royal Gala	Mid/Late	D	8/12
Packham's Triumph (Region 1)	Early	D	8/12
Packham's Triumph (Region 2)	Mid	E	1/2
Forelle	Early/Mid	E	1/2
Beurre Hardy	Late	A	12/13
Beurre Bosc	Mid	D	8/12
Sempre (Rosemarie)	Mid	D	8/12

A, Standard packaging (8 1.5kg bags per box) and conventional transport (commercial transport via truck) to Tshwane fresh produce market; **B,** Standard packaging (layering in box) and commercial transport to Tshwane fresh produce market; **C,** Sent directly from the farm (loose packed in box) and commercial transport to Tshwane fresh produce market; **D,** Standard packaging (layering in box), flown (cargo) to O.R. Tambo International Airport; **E,** Standard packaging (layering in box) and commercial transport to Tshwane fresh produce market; **z,** The period when the fruit was harvested within the season.

Table 3. Apple and pear pathogenicity and disease intensity results

Cultivar	*Penicillium* spp.	Mean of all inoculated wounds[y] (mm)	Mean of significant lesions[y,z] (mm)	Incidence (% significant lesions)	Disease intensity (%)
Beurre Hardy	*P. expansum*	34.1 ± 3.2 b	34.1 ± 3.2	100	36.3
	P. crustosum	29.5 ± 4.5 b	29.5 ± 4.5	100	31.1
	P. solitum	8.1 ± 3.3 dc	9.8±2.2	70	7.3
	P. digitatum	81.7 ± 11.1 a	81.7 ± 11.1	100	87.0
	P. brevicompactum	3.7 ± 2.7 de	8.4 ± 0.9	20	1.8
	Control	0.1 ± 0.2 e	0	0	0
Golden Delicious	*P. expansum*	27.3 ± 3.9 a	27.3 ± 3.9	100	27.1
	P. crustosum	12.5 ± 3.5 b	12.5 ± 3.5	100	12.4
	P. solitum	1.5 ± 1.1 c	2.6 ± 0.5	40	1.1
	P. digitatum	1.5 ± 1.4 c	4.0 ± 0.6	20	0.8
	P. brevicompactum	0.2 ± 0.1 c	0	0	0
	Control	0.0 ± 0.1 c	0	0	0

Disease intensity = $[(d \times F)/T_n \times D] \times 100$: D pears = 93.95mm and apples = 100.48mm

y, Mean wound diameter for controls were already subtracted from measured lesion diameters; **z,** Only lesions significantly different from the control was used to calculate these means (non-significant lesions were excluded).

*****Means followed by ±*x*; *x* refers to standard deviation.

Table 4: *Penicillium digitatum* and *P. solitum* disease interactions displaying incomplete incidence (>100%) after seven days incubation on tested apple and pear cultivars

***Penicillium* spp.**	**Cultivar**	**Mean of significant lesions[z]**	**Incidence (%)**
P. digitatum	Topred	4.0 ± 2.3	27.8
	Golden Delicious	3.1 ± 1.1	27.5
	Cripps Pink	6.6 ± 3.9	81.8
	Granny Smith 2011	20.3 ± 12.7	97.3
	Granny Smith 2012	19.1 ± 8.8	96.8
	Packham's Triumph (Region 2)	7.0 ± 2.7	51.3
	Forelle	7.1 ± 3.1	64.5
P. solitum	Topred	3.4 ± 0.8	30.0
	Golden Delicious	3.0 ± 1.1	35.0
	Cripps Pink	4.5 ± 1.8	40.0
	Granny Smith 2011	5.0 ± 2.4	89.5
	Granny Smith 2012	4.3 ± 1.9	40.0
	Packham's Triumph (Region 1)	5.8 ± 2.6	70.0
	Packham's Triumph (Region 2)	6.9 ± 3.4	85.0
	Beurre Hardy	8.4 ± 3.4	90.0
	Rosemarie	8.4 ± 3.2	88.2

z, Only lesions significantly different from the control was used to calculate these means (non-significant lesions were excluded).

*****Means followed by ±*x*; *x* refers to standard deviation.

Table 5. Identified isolate accession numbers as allocated by GenBank

Isolate no.	Sequence identification	GenBank accession no.
8	*P. solitum*	KF303072
28	*P. expansum*	KF303073
32	*P. expansum*	KF303074
54	*P. digitatum*	KF303075
58	*P. digitatum*	KF303076
78	*P. crustosum*	KF303077
109	*P. brevicompactum*	KF303079
110	*P. brevicompactum*	KF303080
111	*P. brevicompactum*	KF303081

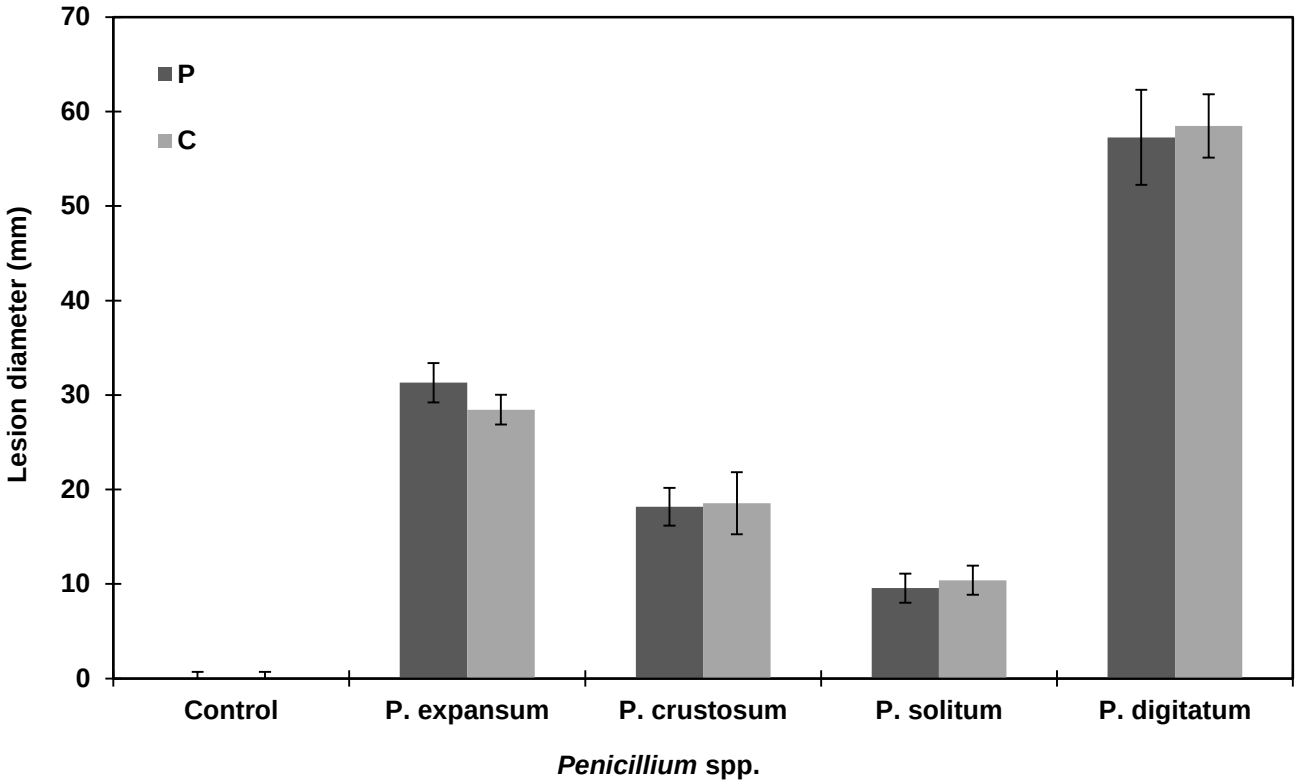

Fig. 1. Lesion diameters (seven days incubation at ambient conditions) caused by different *Penicillium* spp. isolates infecting 'Beurre Hardy' pears; **P,** Pear supply chain isolate; **C,** Citrus supply chain isolate.

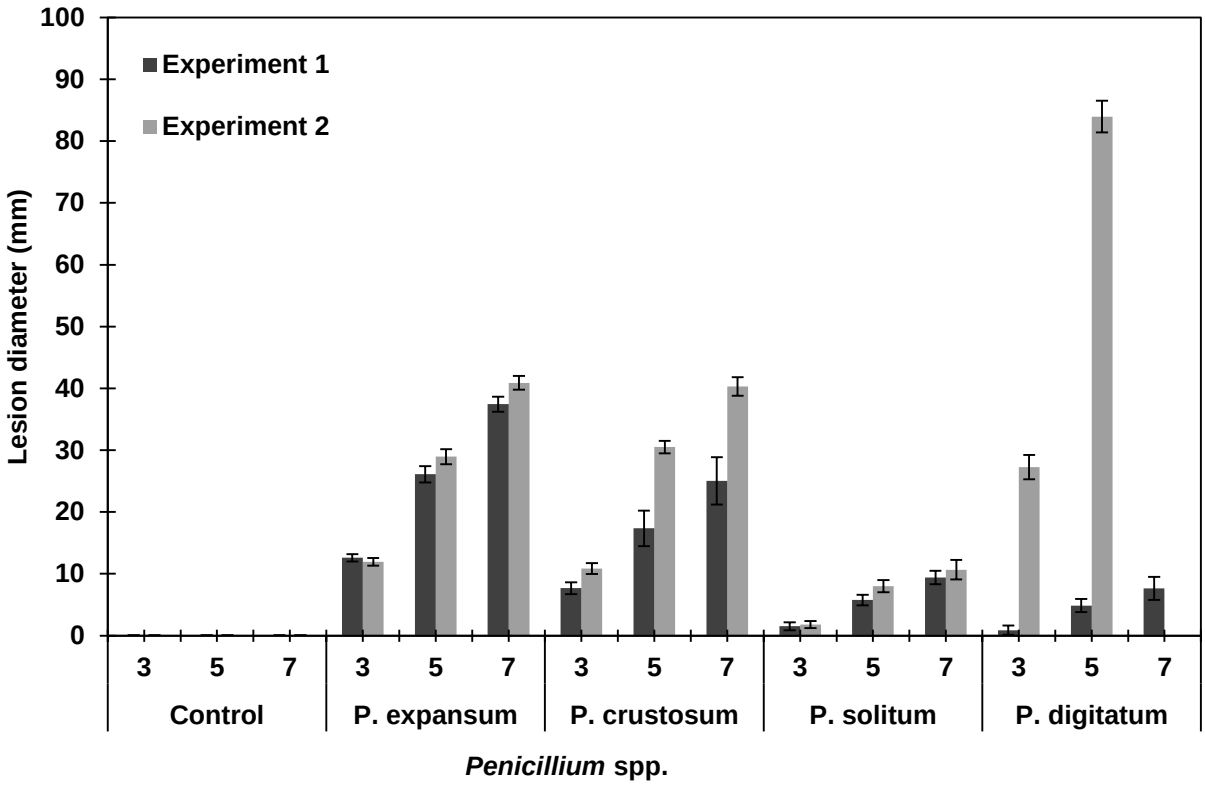

Fig. 2. *Penicillium* spp. lesion diameter growth on Beurre Bosc pears over seven days incubation at ambient conditions in two individual experiments.

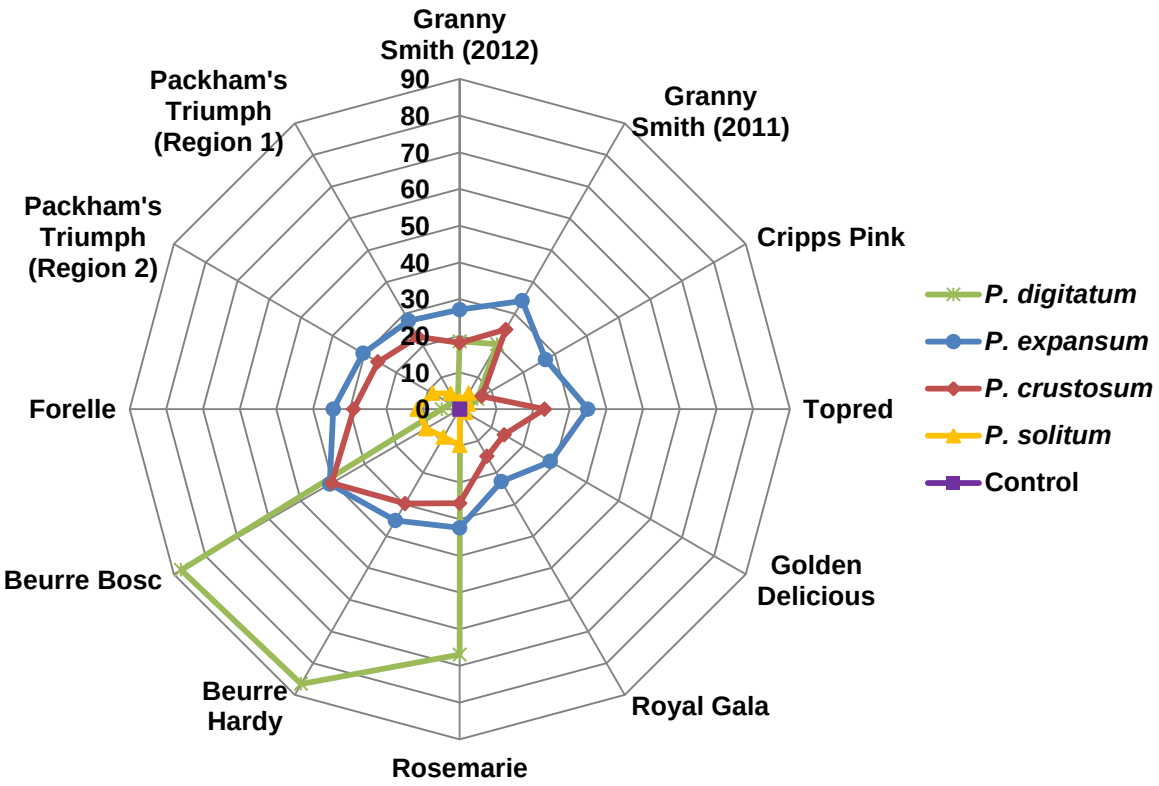

Fig. 3. Lesion diameter (mm) of *Penicillium* spp. inoculated apple and pear cultivars after seven days incubation at room conditions.

Fig. 4. *Penicillium* spp. (columns left to right: *P. digitatum, P. crustosum, P. expensum, P. solitum*) symptom expression on apple cultivars (rows top to bottom: Granny Smith, Golden Delicious, Cripps Pink, Royal Gala), cultures (Malt Extract Agar) and PCR-RFLP (fragments of restriction digested DNA separated on 3% agarose gel).

Fig. 5. *Penicillium* spp. (columns left to right: *P. digitatum, P. crustosum, P. expensum, P. solitum*) symptom expression on pear cultivars (rows top to bottom: Rosemarie, Beurre Bosc, Forrel, Packham's Triumph), cultures (Malt Extract Agar) and PCR-RFLP (fragments of restriction digested DNA separated on 3% agarose gel).

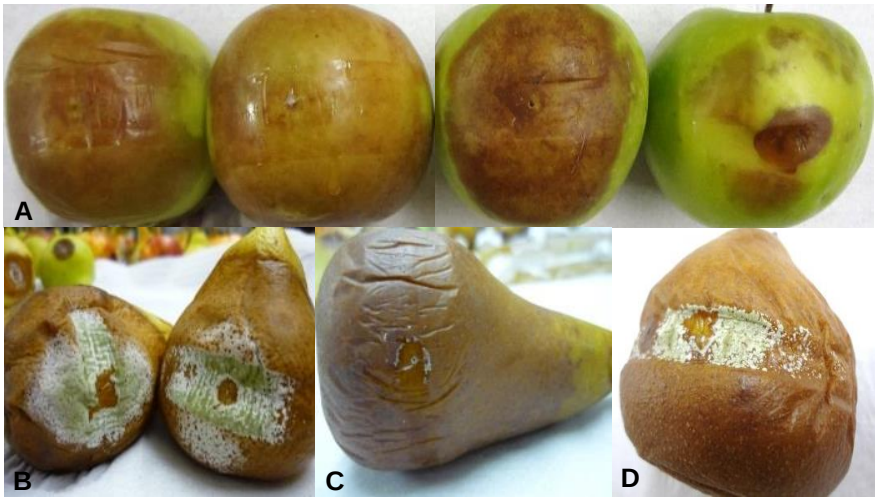

Fig. 6. *Penicillium digitatum* disease symptoms on pome fruit. **A,** 'Granny Smith' (11 days incubation); **B,** 'Rosemarie'; **C,** 'Beurre Bosc'; **D.** 'Beurre Hardy' (B-D; seven days incubation).