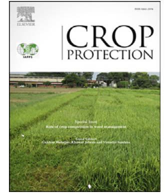
Crop Protection 96 (2017) 265–272

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro



Host ranges of *Penicillium* species causing blue mold of bulb crops in Washington State and Idaho

F.M. Dugan [a, *], S.L. Lupien [a], C.M. Vahling-Armstrong [b, 1], G.A. Chastagner [c], B.K. Schroeder [d]

[a] USDA-ARS Western Regional Plant Introduction Station, 59 Johnson Hall, Washington State University, Pullman, WA 99164-6402, USA
[b] Department of Plant Pathology, Washington State University, Pullman, WA, USA
[c] Department of Plant Pathology, Washington State University, Puyallup, WA, USA
[d] Plant, Soil and Entomological Sciences, University of Idaho, Moscow, ID, USA

ARTICLE INFO

Article history:
Received 12 September 2016
Received in revised form
29 December 2016
Accepted 4 March 2017

Keywords:
Blue mold
Garlic
Iris
Onion
Ornamental onion
Penicillium albocoremium
P. crustosum
P. expansum
P. glabrum
P. paraherquei
P. polonicum
P. radicicola
Taxonomy
Tulip

ABSTRACT

First reported from the Pacific Northwest of U.S.A. as causal agents of blue mold on edible and/or ornamental bulbs are *Penicillium albocoremium* (from *Tulipa* sp.; pathogenic on *Allium sativum*, *A. cepa*, *A. stipitatum*, *Iris hollandica* and *Tulipa* sp.), *P. crustosum* (from *Narcissus*; pathogenic on *A. cepa* and *I. hollandica*), *P. paraherquei* (from *A. cepa*; pathogenic on *A. sativum* and *A. cepa*), and *P. radicicola* (from *Iris* Casablanca; pathogenic on *A. sativum*, *A. cepa* and *I. hollandica*). *Penicillium expansum* and *P. glabrum*, from *A. cepa*, were verified as pathogens of *A. sativum*, *A. cepa* and *Iris hollandica*, and *P. expansum* was also pathogenic on *Tulipa* sp. Pronounced differences between *Penicillium* agents of blue mold in host range and in virulence have implications for crop rotation, postharvest storage and marketing.

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1. Introduction

In recent years there have been advances in documenting taxonomy and behavior of species of *Penicillium* causing blue mold of edible and ornamental bulb crops. Extensive collections of *Penicillium* species of the section Viridicata, many formerly placed in series Corymbifera (essentially segregates of *P. corymbiferum* Westling, syn. = *P. hirsutum* Dierckx) and primarily from Europe, have been extensively researched by Overy et al. (2005a, 2005b, 2005c). A collection of lesser depth but exclusively from the United States and concentrated in the Pacific Northwest (PNW) has been similarly addressed (Dugan et al., 2011, 2014) with a detailed synopsis of taxonomy and nomenclature (Dugan et al., 2014).

As early as Smalley and Hansen (1962) there were indications that *Penicillium* isolates causing blue mold of a given bulb crop (garlic in that instance) and overtly similar in morphology and cultural characters were not all of the same taxon. Such isolates have subsequently been shown to differ in host range, environmental preference and species assignment (Dugan et al., 2011, 2014; Overy et al., 2005a, 2005b, 2005c). We have received anecdotal information from horticulturists that additional *Penicillium* taxa are involved in bulb rots, and also have recovered from regional bulb crops *Penicillium* taxa not listed as attacking these hosts in extant literature, or at least novel with regard to the PNW.

New isolates from the PNW were provisionally assigned to two

* Corresponding author.
E-mail address: fdugan@wsu.edu (F.M. Dugan).
[1] Present address: USDA-ARS Subtropical Plant Pathology Research, Fort Pierce, FL 34945 USA.

http://dx.doi.org/10.1016/j.cropro.2017.03.002
0261-2194/Published by Elsevier Ltd.

species inside the traditional series Corymbifera (*P. albocoremium* and *P. radicicola*), to *P. crustosum* (series Camemberti), *P. expansum* (series Expansa) and *P. polonicum* (series Viridicata), all in subgenus Penicillium section Viridicata (= section Fasciculata) (Frisvad and Samson, 2004; Houbraken et al., 2011). We also recovered isolates provisionally assigned to *P. glabrum*, traditionally placed in subgenus Aspergilloides section Aspergilloides, or to *P. paraherquei* in subgenus Furcatum section Furcatum (Pitt, 2000). Assignment of *P. parahequei* to section has been recently revised to section Lanata-Divaricata (Visagie et al., 2014).

Given the management implications of these findings, we resolved to confirm identifications, and to use these additional isolates recovered from edible *Allium* and ornamental bulb crops in our region for further investigations of host ranges. Intra-specific variability in pathogenicity was largely not assessed due to lack of multiple, well-identified isolates of a single taxon from regional (PNW) bulbs, but see remarks on *P. polonicum* below (*4.2*).

2. Materials and methods

2.1. Fungal isolates

Isolates (Table 1) were recovered by us either from field surveys of onion (Vahling-Armstrong, Schroeder) in Central Washington State in 2010, from ornamental bulbs surveyed in Western Washington State (Chastagner), from bulbs obtained in local retail outlets in north-central Idaho (Dugan, Lupien), or solicited from extant collections of colleagues working with commercial onion seed. Accession number incorporates year of collection (Table 1). Species name reflects final determination (Results). All isolates are deposited with the USDA-ARS (NRRL) Culture Collection in Peoria, IL.

Fungal isolates were assessed for conformity with standard descriptions (Pitt, 1979, 2000; Frisvad and Samson, 2004) by inoculation of single-conidium strains to Czapeck yeast agar at 25 °C and 30 °C, malt extract agar, creatine sucrose agar, and yeast extract sucrose agar at 25 °C, and recording of microscopic characters. Identity was confirmed on the basis of β-tubulin sequences (Houbraken et al., 2016; Khodaei et al., 2015; Samson et al., 2004; Sanzani et al., 2016). Isolates were grown on ½ V8 agar, conidia washed from the agar surface and pelleted by centrifugation, washed twice with sterile water, and the pellet lyophilized, and stored at room temperature until DNA extraction. Lyophilized conidial pellets were disrupted in the presence of 3-mm glass beads in a Fast Prep™ 120 cell disruptor (speed 6 for 30 s). Genomic DNA was isolated immediately following tissue disruption using Qiagen DNeasy® Plant Mini Kit (Qiagen, Valencia, CA) following the manufacturer's instructions. DNA was eluted from the column with 100 ml of sterile water. Amplification of the β-tubulin partial gene sequence was accomplished using primers Bt2a-F and Bt2b-R (Glass and Donaldson, 1995). PCR was conducted in 100 µl reaction mixtures containing 2–10 µl of genomic DNA extract, 20 µl of GoTaq 5X reaction buffer, 2 µl of 10 mm dNTP mix, 3 µl of 25 mM $MgCl_2$, 50 pmol of each primer, and 1 µl of GoTaq® polymerse (Promega, Madison, WI). Amplifications were performed in a Veriti® 96-well thermal cycler under 9700 max mode ramping with an initial denaturation at 95 °C for 4 min followed by 5 cycles of denaturation at 94 °C for 1 min, step down annealing 1 °C per cycle (68 °C to 64 °C), and extension at 72 °C for 2 min followed by 30 cycles of 94 °C for 1 min, 64 °C for 90 s, and 72 °C for 2 min, plus a final elongation step of 10 min at 72 °C. Amplified DNA was purified with Qiagen QIAquick® spin columns and sequenced on both strands using primers Bt2a and Bt2b (Glass and Donaldson, 1995). Additional primers were synthesized to obtain full sequence of the PCR amplicon in both directions. Sequencing reactions and primer synthesis were performed by Eurofins MWG/Operon (Huntsville, AL). Sequence data was assembled and aligned using Sequencher™ 5.4 software (Gene Codes Corp. Ann Arbor, MI). Assembled partial gene sequences were aligned with β-tubulin sequences in GenBank, including authentic and type strains of *Penicillium* subgenus Penicillium (Samson et al., 2004) using BLASTN 2.2.21 software (Zhang et al., 2000).

2.2. Inoculation hosts

The following were experimental inoculation hosts: *Allium sativum* (garlic, Rose du Var W6 35682) from USDA-ARS-WRPIS (Pullman, WA); *A. cepa* (onion, Gold Pearl) from Melissa's Produce (Los Angeles, CA); *A. cepa* (onion, Forum) from Bejo Seeds (Oceano, CA); *A. stipitatum* (ornamental onion, PI 576941), WRPIS; *Iris hollandica*, Purple Sensation, originally from Dutch Grown (Glendale, CA) and subsequently produced in a WRPIS green house; *Tulipa* sp. Purple Prince from ADR Bulbs (Chester, NY) and subsequently produced in a WRPIS green house.

2.3. Experimental design

Each of the six hosts was inoculated with the seven fungal isolates (Table 1). Each treatment consisted of three replications, with each replication containing three bulbs or cloves. Controls for each treatment also contained three replications, each with a sample size of three bulbs or cloves. Each experiment was repeated once.

Table 1
Fungal isolates used in this study.

Penicillium species	Accession numbers	Isolation host	Isolation locale
P. albocoremium (Frisvad) Frisvad	PenVDC36:2013 NRRL 66387	tulip 'Verandi D' tulip petal	Washington State
P. crustosum Thom	PenYC#1:2011 NRRL 66388	'Yellow Cheer' daffodil bulb	Washington State
P. expansum Link	Pen347-W:2010 NRRL 66389	*Allium cepa* bulb	Washington State
P. glabrum (Wehmer) Westling	OPen#15:2011 NRRL 66390	*A. cepa* 'Gold Pearl' bulb	Idaho
P. paraherquei S. Abe ex G. Sm.	Pen308-N:2010 NRRL 66391	*A. cepa* 'Ranchero' bulb	Washington State
P. polonicum K.M. Zalesky	Pen1:2010 NRRL 66385	*A. cepa* 'Prince' seed	Washington State
P. radicicola Overy & Frisvad	PenCB#1:2011 NRRL 66386	'Casablanca' iris bulb	Washington State

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