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

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Key words

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*;

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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.

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 Hanson (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 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; Houbracken et al., 2011). We also recovered isolates provisionally assigned to *P. glabrum*, traditionally placed in subgenus *Aspergilloides* section *Aspergilloides*, or to *P. parahequei* 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.

Table 1. Fungal isolates used in this study

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

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 µl 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 to 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 MgCl₂, 50 pmol of each primer, and 1 µl of GoTaq® polymerase (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 sec, 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.

2.4 Inoculum preparation, inoculation, incubation and measurement

Isolates were revived from filter paper stocks onto ½V8 agar, grown for 8-14 days at room temperature under diurnal (12 hrs/day) near-ultraviolet/cool white light, washed with sterile water, serially diluted, quantified on a hemocytometer, and adjusted to 1.5×10^5 conidia per ml in sterile 0.2% semi liquid agar. Inoculum was stored at 5 °C for 1-2 days during conduct of a given experiment.

Plant host cloves (garlic) or bulbs were surface-disinfested prior to inoculation for 5 min in 0.5% sodium hypochlorite, rinsed three times for 5 min in sterile water and allowed to dry on sterile Kimwipes (Kimberly-Clark, Roswell, GA) in a biological safety cabinet overnight. Each bulb or clove was wounded on its side with a sterile probe creating a single wound 4 mm deep x

1 mm diam. Thirty five μ l of inoculum, or in the case of controls 35 μ l of sterile semi-liquid agar, was applied to the wound site, and each treated bulb placed into an individual sterile container of appropriate size for a given host, with the lid of each container having a single 4 mm hole. Containers of a given host inoculated with a single fungal isolate, and the associated controls, were randomized within one incubation tray (Nalgene product 6900-0020) including a single 150 ml tub of sterile water, and the incubation tray sealed with plastic wrap to maintain humidity at or above 90% during the incubation period. HOBO data loggers (HOBO[®], Onset Computer Corp., Borne, MA) were placed inside of incubation trays in order to assure uniformity of conditions between experiments. Inoculated materials were incubated at room temperature (23-24 °C), monitored for development of rot and incubated for 21-34 days, at which time the maximum diameter of each lesion developing on the wound site was measured and recorded from all hosts inoculated on a given date. Maximum lesion diameters present at the wound site, or simply the wound site itself in the absence of rot, were measured and recorded using Storm[™] electronic calipers.

Re-isolation of isolates was attempted on two representative symptomatic bulbs or cloves per trial for those trials in which statistically significant decay was observed. Lesion margins were excised, surface disinfested, and plated to ½V8 agar amended with 50 μ g/ml streptomycin sulfate and tetracycline hydrochloride. Recovered single-conidium isolates were characterized by β -tubulin sequence analyses.

2.5 Statistical analysis

Lesion diameters were converted to rates of lesion expansion, (mm/week) and used as the independent variable in T-tests conducted for each host-isolate pair (inoculated materials vs.

controls) in order to assess pathogenicity of an isolate on a given host. Comparative virulence (aggressiveness) of isolates was analyzed as multiple pair-wise comparisons by General Linear Model (GLM). Analyses were conducted in Systat 13.10 (Systat Software, Inc., Chicago, IL), with GLM pair-wise comparisons grouped by treatment using post hoc test of Fisher's least significant difference (LSD) and Error Term = model mean square error (MSE).

3. Results

3.1 Isolates

No isolate strongly diverged from standard morpho-cultural descriptions for species determinations (Pitt 1979, 2000; Frisvad and Samson 2004) assigned largely on the basis of β -tubulin gene sequences specified below, although the reverse of PenVDC36:2013 on YES agar was more red-yellow than brown-yellow or orange.

Isolate Pen VDC36:2013 (GenBank KY172961) shared 100% identity with *P. albocoremium* ex-type CBS 47284 (Houbraken et al., 2016), and 99% with that same isolate plus *P. albocoremium* CBS 109614 and CBS 109582, as sequenced by Samson et al. (2004). The next match was 99% identity with *P. hirsutum* NRRL 999 (Dugan et al., 2014).

Isolate PenYC#1:2011 (GenBank KY172962) shared 99% identity with CBS 101025 and CBS 47184, determined as *P. crustosum* (Samson et al., 2004), each of which, as well as PenYC#1:2011, were 97% homologous with the type for *P. crustosum*, CBS 11503 (Samson et al., 2004). PenYC#1:2011 had 100% identity with *P. crustosum* ATHUM 5080 and DTO-266B3 (Khodaei et al., 2015), 98% with *P. solitum* CBS 14786 (ex-type) and 97% with CBS 14686 (also from Samson et al., 2004).

Isolate Pen347-W:2010 (GenBank 172963) shared 99% identity with *P. expansum* ex-

type CBS 32548 and CBS 48184, and CBS 28197, the latter two also determined as *P. expansum* in Samson et al. (2004). *P. expansum* strains ITEM 7026-RA6, ITEM 7027-RA7, ITEM 7028-RA8, and ITEM 7030-P8 (Sanzani et al., 2016) each shared 100% identity with Pen 347W:2010.

Isolate OPen #15:2011 (GenBank KY172964) was 100% identical with *P. glabrum* CBS 229.28 (Barreto et al., 2011) and *P. frequentans* Westling DTO 053-F2 (Houbraken et al., 2014). The latter researchers re-introduced *P. frequentans* as a distinct species separate from *P. glabrum* with which it was formerly synonymous. Our isolate had 99% identity with other *P. glabrum* isolates from these same two publications.

Isolate Pen 308-N:2010 (GenBank KY172965) was 100% identical with *P. paraherquei* ex-type CBS 430.65 (Houbraken et al., 2011; Park et al., 2014).

Isolate Pen1:2010 (GenBank KY172966) was 100% identical with *P. polonicum* isolates G5314 (Chen et al. 2013) and D116-102 (Guevara-Suarez et al., in press); and shared 99% identity with *P. polonicum* isolates CBS 101479 and CBS 69077 and 98% identity with *P. polonicum* ex-type CBS 22228, all from Samson et al. (2004).

Isolate PenCB#1:2011 (GenBank KY172967) had 99% identity with *P. radicicola* CBS 109554, CBS 109551 and ex-type CBS 112430 (Samson et al., 2004). PenCB#1:2011 had 98% identity with *P. radicicola* CBS 112425, *P. hirsutum* ex-type CBS 13541, *P. hirsutum* strain NRRL 999, and *P. tulipae* CBS 111217 all from Samson et al. (2004).

3.2 Host range and pathogenicity

Summary reactions of hosts to individual isolates of recovered taxa are provided in Table 2 and an example of a positive result in Fig. 1. Means, standard deviations, and P values for paired T-tests and GLM are provided for all isolate-host challenges in Appendix A.

In a small number of instances, quiescent fungi interfered with re-isolation of the inoculated agent, or there was a problem with viability (Appendix A), but re-isolation was always successful in a minimum of one trial and sporulation in lesions induced by inoculation was consistent between initial and repeat trials. Identity of inoculated agent was additionally confirmed by β -tubulin sequences except for the instances just specified (Appendix A) and was accomplished for each host-isolate pair in which the isolate induced lesions statistically differing from controls.

Table 2. Reactions of test bulbs to artificial inoculation in paired T-tests, inoculated vs. controls

Host → <i>Penicillium</i> spp. ↓	<i>A. sativum</i>	<i>A. cepa</i> Gold Pearl	<i>A. cepa</i> Forum	<i>A. stipitatum</i>	<i>Iris hollandica</i>	<i>Tulipa</i> Purple Prince
<i>P. albocoremium</i>	+	+	+	±	+	+
<i>P. crustosum</i>	-	+	+	-	±	-
<i>P. expansum</i>	±	+	-	-	+	+
<i>P. glabrum</i>	+	+	+	-	±	-
<i>P. paraherquei</i>	+	-	+	-	-	-
<i>P. polonicum</i>	+	+	+	-	-	-
<i>P. radicola</i>	+	+	+	-	+	-

+ = $P \leq 0.05$ in both tests (pathogenicity supported)

± = $P \leq 0.05$ in one test and $P \leq 0.10$ in the other (moderate support for pathogenicity)

- = all instances other than those denoted as + or ± (pathogenicity not supported)

Figure 1. *Penicillium albocoremium* PenVDC36:2013 inoculated to tulip: Controls (left three columns), inoculated (right three columns) at 31 days post-inoculation and ambient laboratory temperature, illustrating a result of pathogenicity with moderate virulence.

3.3 Lesion expansion and virulence

Rates of lesion expansion (mm/week, mean of two replicated tests) for *Penicillium* species on the hosts to which they proved pathogenic (Table 2) were as follows: *P. albocoremium* (garlic 10.28; GP onion 7.90; Forum onion 6.57; ornamental onion 2.80; iris 2.20; tulip 2.34), *P. crustosum* (GP onion 0.98; Forum onion 1.00; iris 1.75), *P. expansum* (garlic 2.50; GP onion 0.85; iris 2.33; tulip 2.21), *P. glabrum* (garlic 3.41; GP onion 1.14; Forum onion 0.89; iris 1.68), *P. paraherquei* (garlic 2.52; Forum onion 0.88), *P. polonicum* (garlic 9.76; GP onion 5.87; Forum onion 5.65), *P. radicicola* (garlic 6.15; GP onion 2.92; Forum onion 4.56; iris 1.88).

For species of demonstrated pathogenicity in paired T-tests (Table 2), and differing from each other in virulence (aggressiveness) at $P \leq 0.05$ in multiple pair-wise comparisons (GLM), *P. albocoremium* and *P. polonicum*, followed by *P. radicicola*, were the most virulent on garlic, with *P. albocoremium* most virulent in one test and *P. polonicum* most virulent in the repeat. On

Gold Pearl onion, order of virulence (highest to lowest) was *P. albocoremium*, *P. polonicum* and *P. radicicola*; *P. glabrum* was consistently of intermediate virulence among remaining pathogenic species. On Forum onion, *P. albocoremium* was consistently most virulent. On iris, *P. albocoremium* and *P. expansum* were most virulent, and did not statistically differ, while the next most virulent, *P. radicicola*, consistently differed from *P. albocoremium* only at $P \leq 0.10$. *Penicillium albocoremium* and *P. expansum* did not consistently differ in virulence on tulip. All other differences in virulence were either statistically insignificant or the order of virulence was inconsistent between tests (data not shown).

4. Discussion

4.1 Behavioral differences of isolates

Penicillium albocoremium (*P. hirsutum* var. *albocoremium* Frisvad) differed from *P. radicicola* in host preference (Table 2, Fig. 1) and aggressiveness, even though both are traditionally in series *Corymbifera* and very closely related (Frisvad and Samson 2004). "No significant micromorphological variation exists between *P. albocoremium* [and] *P. radicicola*" but the latter, segregated in 2003 from the former, differs in secondary metabolites and some other characters (Overy and Frisvad 2003). *Penicillium radicicola* was referenced with "Similar species: See *P. albocoremium*" in Frisvad and Samson (2004). Although series *Corymbifera* is not strictly phylogenetic, all members are in section *Viridicata* (*Fasciculata*) and with the exception of *P. hordei* (occurring mostly on barley), are united by a habit of infecting bulb crops. Our findings represent further confirmation that species in this series may differ in host range. Isolates of both species are recorded from *Allium* spp. in Denmark (Frisvad and Samson 2004). To our knowledge, this constitutes the first report of *P. albocoremium* and *P. radicicola* from

bulb crops in North America. *Penicillium albocoremium* was the only agent to induce blue mold on *A. stipitatum* (other species failed to do so, see Dugan et al., 2011, 2014), but support for pathogenicity was only moderate ($\pm$).

4.2 Distributions on bulb crops

Penicillium crustosum is documented from "mouldy onion" in Denmark (Sonjak et al., 2005). Varieties in *P. aurantiogriseum* Dierckx or *P. expansum* have previously been listed as synonyms of *P. crustosum* (Pitt 1979), but *P. crustosum* is distinct from *P. aurantiogriseum* and *P. expansum* under current concepts, and is sister to a clade containing *P. solitum* Westling (Frisvad and Samson 2004). We believe this to be the first report of *P. crustosum* on bulb crops in North America.

Penicillium expansum is reported on onion without geographical attribution (Sumner et al., 2008), from New Zealand and (as *P. glaucum* Link) from China (Farr and Rossman n.d.), from onion in Lithuania (Lugauskas et al., 2005), and from onion and garlic in Korea (Yu et al., 1997). Reports from ornamental bulbs are less frequent; it is reported from unspecified species or cultivars of *Lilium* bulbs from India, but without specifying method of identification (HemaMoorthy and Prakasam 2013). The name has been applied to isolates rotting onion in Washington State (John Fellman, personal communication; Stevens et al., 2006) but to our knowledge this is the first instance from Washington State of identification using either stringent morpho-cultural criteria or molecular-genetic criteria for putative *P. expansum* on any of our hosts, including *A. cepa*. Noteworthy is the difference in varietal susceptibility of onion, with Gold Pearl susceptible, and Forum resistant to our isolate.

Penicillium glabrum is documented as a pathogen of onion, but is usually restricted to outer layers (Frisvad et al., 2007, with literature review). Varga et al. (2008) specified *P. glabrum* on onion. Some specimens (e.g. IMI 374732) have been recovered from ornamental bulbs (*Narcissus*, www.herbimi.info), and *P. frequentans* (often placed in synonymy with *P. glabrum*), identified on morpho-cultural criteria, was isolated repeatedly from commercial tulip bulbs in Poland (Piwani 2007). The closely related (and morphologically similar) *Penicillium spinulosum* Thom has been isolated from onion bulbs in Lithuania (Lugauskas et al., 2005). *Penicillium glabrum* and related species are recently examined by molecular-genetic and morpho-cultural criteria (Barreto et al., 2011; Houbraken et al., 2014). We know of no previous reports of *P. glabrum* on edible or ornamental bulbs from the PNW.

Penicillium paraherquei (as well as *P. brasilianum* Bat.) was previously considered a synonym of *P. simplicissimum* (Oudem.) Thom (Pitt 1979, 2000). Isolates identified as *P. simplicissimum* on the basis of morphological criteria using Pitt (1979) are recorded from onion in Pakistan (Ibatsam et al., 2012). *Penicillium brasilianum* is recorded on onion in Korea, and was distinguished from *P. simplicissimum* by multi-gene topology (Sang et al., 2014). *Penicillium paraherquei* and *P. brasilianum* are sister taxa by multi-gene topology (Oh et al., 2015, reporting on *P. georgiense* S.W. Peterson & B.W. Horn as a pathogen of onion). Mansouri et al. (2013) show *P. paraherquei* and *P. brasilianum* as sister taxa by multi-gene topology, in a clade sister to *P. simplicissimum*. To our knowledge, this is the first report of *P. paraherquei* on edible or ornamental bulbs in North America.

Our assay for *P. polonicum* obtained the same result as in Dugan et al. (2014), except that in 2014 the isolate used (not identical to the isolate here but also from seed of onion cultivar 'Prince') was $\pm$ (not -) on iris. *Penicillium polonicum* is reported from onion in Serbia (Duduk et

al., 2014), Turkey (Çakir and Maden, 2015), and from each of our hosts in Dugan et al. (2014). *Penicillium polonicum* (= *P. aurantiogriseum* var. *polonicum* (K.M. Zaleski) Frisvad & Filt.) was once considered a synonym of *P. aurantiogriseum*, as were *P. cyclopium* Westling, *P. solitum* and several other names (Pitt 1979), but is best regarded as distinct (e.g., Frisvad and Samson 2004). *Penicillium aurantiogriseum* is listed as attacking onion and garlic in Sumner et al. (2008) and is reported from garlic in Brazil (Farr and Rossman n.d.). *Penicillium cyclopium* is regarded as a pathogen of onion in Snowden (1992). One or more of these closely-related *Penicillium* spp. are listed on various plants (*Allium*, *Gladiolus*, *Lilium*, *Tulipa*, etc.) of the lily family (sensu lato) (Farr and Rossman n.d.), but such fungal names in older literature are unlikely to correspond precisely to the same names as applied under current taxonomic criteria. This situation is reminiscent of the name *P. corymbiferum*, applied in the past to a wider variety of taxa than would be admissible under current taxonomic criteria, and sometimes still applied (usually inappropriately) in more recent literature. The members of series *Corymbifera* in this study, *P. albocoremium* and *P. radicola*, appear not to be previously recorded in North America (Farr and Rossman n.d.).

4.3 Management implications

Current commercial practices for onion vary, even for advanced agricultural economies. Typical for the United Kingdom is postharvest curing at 20-28 °C for up to six weeks (Chope et al., 2012), or for the PNW one to three weeks (Matson et al., 1985). After curing, onions are most usually stored under refrigerated conditions, often with modified atmospheres, but onions are also exposed to non-refrigerated conditions for up to several days during conditioning prior to packaging or pallet storage prior to shipping (Matson et al., 1985). Storage of garlic, iris and

tulip bulbs also typically involves significant periods of exposure to non-refrigerated conditions (Gross et al., 2014). Such temperatures and times, approximate to our experimental conditions, could allow formation of lesions from several mm to several cm, depending on the causal agent and host. Impact of blue mold clearly varies with species identity of the causal agent and host, therefore host rotation is a potential management option. Pre-planting fungicides have proven of some efficacy (Dugan et al. 2007), but we have no data indicating that *Penicillium* species causing blue mold of bulbs will strongly differ in response to a given fungicide, although isolates of a given species may develop resistance. *Allium aflatunense* and *A. stipitatum* (ornamental onions, subgenus *Melanocrommyum*, section *Megaloprason*) proved resistant to decay upon inoculation with *Penicillium allii* (Dugan et al. 2011), and *A. stipitatum* displayed discernible resistance here. The mechanism of this resistance is unknown.

4.4 Summary and conclusions

Results obtained herein deepen understanding of *Penicillium* spp. pathogenic in edible and ornamental bulbs. Results must be interpreted with caution because our collections have less depth (often only a single isolate per species from a given bulb crop) than corresponding collections of bulb-rotting isolates in Europe. Also, except for using a wounding tool, we did not attempt to mimic any predisposing factors that might sometimes occur during commercial processing, such as hot water treatment, suboptimal temperature control in drying sheds, wounding during lifting, etc. Moreover, given the world-wide movement of edible and ornamental bulbs, it is often uncertain whether a given taxon is indigenous to a given geographic region, merely endemic, or undetected. Limitations in seasonal availability and other constraints precluded challenge of all pertinent bulb crops, such as *Narcissus*. Nonetheless, the various

Penicillium species inducing blue mold in the PNW clearly differ in host range and aggressiveness. Precise identification of such agents and determination of their host ranges should enable refinement of management strategies, such as crop rotation, postharvest storage and marketing.

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APPENDIX A

Host → <i>Penicillium</i> species and strain ↓	garlic <i>Allium</i> <i>sativum</i>	onion GP <i>A. cepa</i>	onion forum <i>A. cepa</i>	ornamental onion <i>A. stipitatum</i>	iris <i>Iris</i> <i>hollandica</i>	tulip <i>Tulipa</i>
<i>P. albocoremium</i>						
PenVDC-36:2013 sci1						
Initial Test Date	1/14/2016	1/14/2016	7/31/2014	8/13/2015	9/16/2015	9/30/2015
inoculated	11.69 +/- 0.88	8.67 +/- 1.32	6.03 +/- 2.00	2.16 +/- 0.36	2.22 +/- 0.46	2.57 +/- 1.04
control	1.92 +/- 0.30	1.60 +/- 0.26	1.13 +/- 0.08	1.88 +/- 0.25	1.50 +/- 0.30	1.31 +/- 0.17
<i>P</i> value T-test	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.001	<i>P</i> = 0.082	<i>P</i> = 0.001	<i>P</i> = 0.003
<i>P</i> value GLM	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.076	<i>P</i> = 0.000	<i>P</i> = 0.000
Repeated Test Date	5/27/2016	5/27/2016	5/27/2016	09/17/2015	5/27/2016	10/16/2015
inoculated	8.87 +/- 0.72	7.13 +/- 0.52	7.11 +/- 1.58	3.43 +/- 0.87	2.17 +/- 0.40	2.10 +/- 0.71
control	3.14 +/- 2.67	1.83 +/- 1.93	1.56 +/- 1.05	2.22 +/- 0.26	1.18 +/- 0.15	1.31 +/- 0.19
<i>P</i> value T-test	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.001	<i>P</i> = 0.001	<i>P</i> = 0.006
<i>P</i> value GLM	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.001
<i>P. crustosum</i>						
Pen YC#1:2011 sci1						
Initial Test Date	1/28/2015	11/20/2014	12/11/2014	8/13/2015	9/16/2015	9/30/2015
inoculated	1.62 +/- 0.30	1.14 +/- 0.52	1.16 +/- 0.47	2.14 +/- 0.22	1.69 +/- 0.40	1.36 +/- 0.17
control	1.50 +/- 0.36	0.69 +/- 0.06	0.68 +/- 0.12	1.97 +/- 0.43	1.42 +/- 0.15	1.33 +/- 0.16
<i>P</i> value T-test	<i>P</i> = 0.438	<i>P</i> = 0.016	<i>P</i> = 0.008	<i>P</i> = 0.301	<i>P</i> = 0.090	<i>P</i> = 0.775
<i>P</i> value GLM	<i>P</i> = 0.818	<i>P</i> = 0.203	<i>P</i> = 0.331	<i>P</i> = 0.255	<i>P</i> = 0.041	<i>P</i> = 0.898
Repeated Test Date	2/11/2015*	1/14/2015*	1/15/2015	9/17/2015	11/12/2015 [§]	10/16/2015
inoculated	2.02 +/- 0.36	0.82 +/- 0.23	0.84 +/- 0.23	1.70 +/- 0.24	1.80 +/- 0.27	1.17 +/- 0.19
control	1.62 +/- 0.19	0.63 +/- 0.08	0.65 +/- 0.05	1.80 +/- 0.37	1.50 +/- 0.19	1.35 +/- 0.09
<i>P</i> value T-test	<i>P</i> = 0.013	<i>P</i> = 0.028	<i>P</i> = 0.026	<i>P</i> = 0.501	<i>P</i> = 0.001	<i>P</i> = 0.016
<i>P</i> value GLM	<i>P</i> = 0.589	<i>P</i> = 0.618	<i>P</i> = 0.730	<i>P</i> = 0.609	<i>P</i> = 0.044	<i>P</i> = 0.433
<i>P. expansum</i>						
Pen 347-W:2010 sci1						
Initial Test Date	1/28/2015*	11/20/2014	12/11/2014	8/13/2015	9/16/2015	9/30/2015
inoculated	1.70 +/- 0.15	0.99 +/- 0.28	0.95 +/- 0.19	1.87 +/- 0.37	2.13 +/- 0.32	2.11 +/- 0.44
control	1.56 +/- 0.24	0.72 +/- 0.11	0.75 +/- 0.13	2.00 +/- 0.29	1.38 +/- 0.19	1.32 +/- 0.19
<i>P</i> value T-test	<i>P</i> = 0.086	<i>P</i> = 0.020	<i>P</i> = 0.020	<i>P</i> = 0.424	<i>P</i> = 0.000	<i>P</i> = 0.000
<i>P</i> value GLM	<i>P</i> = 0.793	<i>P</i> = 0.442	<i>P</i> = 0.683	<i>P</i> = 0.392	<i>P</i> = 0.000	<i>P</i> = 0.000
Repeated Test Date	2/11/2015	1/14/2015	1/15/2015	9/17/2015	11/12/2015	10/16/2015
inoculated	3.27 +/- 2.28	0.71 +/- 0.10	1.53 +/- 2.13	1.77 +/- 0.42	2.52 +/- 0.64	2.30 +/- 0.64
control	1.39 +/- 0.09	0.62 +/- 0.07	0.64 +/- 0.07	1.51 +/- 0.22	1.70 +/- 0.25	1.32 +/- 0.17
<i>P</i> value T-test	<i>P</i> = 0.008	<i>P</i> = 0.041	<i>P</i> = 0.219	<i>P</i> = 0.132	<i>P</i> = 0.002	<i>P</i> = 0.001
<i>P</i> value GLM	<i>P</i> = 0.010	<i>P</i> = 0.805	<i>P</i> = 0.107	<i>P</i> = 0.193	<i>P</i> = 0.000	<i>P</i> = 0.000

	garlic	onion GP	onion forum	ornamental onion	iris	tulip
<i>P. glabrum</i>						
O. Pen#15:2011 sci2						
Initial Test Date	1/28/2015	11/20/2014	12/11/2014	8/13/2015	9/16/2015	9/30/2015
inoculated	3.83 +/- 2.36	1.19 +/- 0.40	0.88 +/- 0.23	2.04 +/- 0.39	1.60 +/- 0.19	1.33 +/- 0.22
control	1.61 +/- 0.35	0.68 +/- 0.07	0.71 +/- 0.07	1.88 +/- 0.29	1.43 +/- 0.29	1.30 +/- 0.40
<i>P</i> value T-test	<i>P</i> = 0.016	<i>P</i> = 0.002	<i>P</i> = 0.046	<i>P</i> = 0.331	<i>P</i> = 0.013	<i>P</i> = 0.943
<i>P</i> value GLM	<i>P</i> = 0.000	<i>P</i> = 0.156	<i>P</i> = 0.736	<i>P</i> = 0.285	<i>P</i> = 0.066	<i>P</i> = 0.846
Repeated Test Date						
inoculated	2/11/2015	1/14/2015	1/15/2015	9/17/2015	11/12/2015	10/16/2015
control	2.99 +/- 0.35	1.09 +/- 0.35	0.90 +/- 0.16	1.88 +/- 0.58	1.76 +/- 0.28	1.26 +/- 0.19
	1.68 +/- 0.17	0.64 +/- 0.09	0.68 +/- 0.06	1.87 +/- 0.34	1.56 +/- 0.17	1.30 +/- 0.24
<i>P</i> value T-test	<i>P</i> = 0.000	<i>P</i> = 0.001	<i>P</i> = 0.001	<i>P</i> = 0.962	<i>P</i> = 0.093	<i>P</i> = 0.675
<i>P</i> value GLM	<i>P</i> = 0.078	<i>P</i> = 0.224	<i>P</i> = 0.685	<i>P</i> = 0.955	<i>P</i> = 0.177	<i>P</i> = 0.840
<i>P. paraherquei</i>						
Pen 308-N:2010 sci1						
Initial Test Date	1/28/2015	11/20/2014	12/11/2014	8/13/2015	9/16/2015	9/30/2015
inoculated	2.26 +/- 0.60	0.78 +/- 0.24	0.93 +/- 0.27	1.81 +/- 0.33	1.60 +/- 0.25	1.34 +/- 0.18
control	1.44 +/- 0.31	0.74 +/- 0.09	0.63 +/- 0.05	1.78 +/- 0.19	1.27 +/- 0.11	1.29 +/- 0.13
<i>P</i> value T-test	<i>P</i> = 0.004	<i>P</i> = 0.633	<i>P</i> = 0.005	<i>P</i> = 0.784	<i>P</i> = 0.002	<i>P</i> = 0.476
<i>P</i> value GLM	<i>P</i> = 0.145	<i>P</i> = 0.908	<i>P</i> = 0.546	<i>P</i> = 0.830	<i>P</i> = 0.011	<i>P</i> = 0.749
Repeated Test Date						
inoculated	2/11/2015	1/14/2015	1/15/2015	9/17/2015	11/12/2015	10/16/2015
control	2.77 +/- 1.44	0.80 +/- 0.20	0.83 +/- 0.27	1.76 +/- 0.25	1.72 +/- 0.25	1.31 +/- 0.18
	1.58 +/- 0.23	0.65 +/- 0.05	0.63 +/- 0.03	1.69 +/- 0.39	1.64 +/- 0.19	1.40 +/- 0.20
<i>P</i> value T-test	<i>P</i> = 0.026	<i>P</i> = 0.048	<i>P</i> = 0.032	<i>P</i> = 0.665	<i>P</i> = 0.449	<i>P</i> = 0.345
<i>P</i> value GLM	<i>P</i> = 0.099	<i>P</i> = 0.685	<i>P</i> = 0.710	<i>P</i> = 0.715	<i>P</i> = 0.598	<i>P</i> = 0.687
<i>P. polonicum</i>						
Pen1:2010 sci1						
Initial Test Date	1/28/2015	11/20/2014	12/11/2014	8/13/2015	9/16/2015	9/30/2015
inoculated	9.19 +/- 0.89	6.22 +/- 1.84	4.21 +/- 2.65	1.92 +/- 0.38	1.50 +/- 0.20	1.46 +/- 0.17
control	1.73 +/- 0.27	1.04 +/- 0.13	0.75 +/- 0.29	1.96 +/- 0.31	1.46 +/- 0.24	1.38 +/- 0.19
<i>P</i> value T-test	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.844	<i>P</i> = 0.678	<i>P</i> = 0.396
<i>P</i> value GLM	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.830	<i>P</i> = 0.731	<i>P</i> = 0.654
Repeated Test Date						
inoculated	2/11/2015	1/14/2015	1/15/2015	9/17/2015	11/12/2015	10/16/2015
control	10.33 +/- 1.25	5.72 +/- 1.96	7.09 +/- 1.12	1.89 +/- 0.17	2.04 +/- 0.28	1.58 +/- 0.71
	1.98 +/- 0.40	0.67 +/- 0.06	0.76 +/- 0.10	1.98 +/- .038	1.66 +/- 0.26	1.39 +/- 0.52
<i>P</i> value T-test	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.541	<i>P</i> = 0.010	<i>P</i> = 0.528
<i>P</i> value GLM	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.650	<i>P</i> = 0.009	<i>P</i> = 0.393

<i>P. radicicola</i> Pen CB#1:2011 sci2	garlic	onion GP	onion forum	ornamental onion	iris	tulip
Initial Test Date	1/28/2015	11/20/2014	12/11/2014	8/13/2015	9/16/2015	9/30/2015
inoculated	6.08 +/- 3.13	4.33 +/- 1.41	5.33 +/- 2.27	2.01 +/- 0.26	1.84 +/- 0.31	1.64 +/- 0.38
control	1.79 +/- 0.46	0.75 +/- 0.10	0.64 +/- 0.07	1.99 +/- 0.40	1.53 +/- 0.30	1.42 +/- 0.16
<i>P</i> value T-test	<i>P</i> = 0.001	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.893	<i>P</i> = 0.045	<i>P</i> = 0.135
<i>P</i> value GLM	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.000	<i>P</i> = 0.872	<i>P</i> = 0.018	<i>P</i> = 0.202
Repeated Test Date	2/11/2015	1/14/2015	1/15/2015	9/17/2015	11/12/2015	10/16/2015
inoculated	6.21 +/- 3.75	1.50 +/- 0.63	3.79 +/- 2.69	2.06 +/- 0.41	1.91 +/- 0.37	2.72 +/- 1.00
control	1.77 +/- 0.23	0.69 +/- 0.08	0.71 +/- 0.06	2.09 +/- 0.43	1.57 +/- 0.27	1.42 +/- 0.23
<i>P</i> value T-test	<i>P</i> = 0.003	<i>P</i> = 0.002	<i>P</i> = 0.004	<i>P</i> = 0.866	<i>P</i> = 0.039	<i>P</i> = 0.001
<i>P</i> value GLM	<i>P</i> = 0.000	<i>P</i> = 0.033	<i>P</i> = 0.000	<i>P</i> = 0.865	<i>P</i> = 0.021	<i>P</i> = 0.000

§ = Replications as well as treatment were significant in the repeat test.

* = Quiescent fungi or problems with viability interfered with re-isolation of inoculated agent in one of two trials, but symptoms, including sporulation of the causal agent, were apparent in both trials.