A survey of *Penicillium brevicompactum* and *P. bialowiezense* from indoor environments, with commentary on the taxonomy of the *P. brevicompactum* group[1]

James A. Scott, Bess Wong, Richard C. Summerbell, and Wendy A. Untereiner

Abstract: We investigated the diversity of the *Penicillium brevicompactum* Dierckx group in dust from 54 houses in Wallaceburg, Ontario, Canada. Two taxa were predominant, *P. brevicompactum* and *Pencillium bialowiezense* Zaleski, accounting for 88.6% and 5.4% of the sample set, respectively. We further characterized multilocus haplotypes of isolates by characterizing three polymorphic genetic loci, β-tubulin (*benA*), histone 4 (*his4A*), and the internal transcribed spacer regions of ribosomal DNA (nucITS) amplified by PCR amplification and screened using heteroduplex mobility assay (HMA). Eight unique haplotypes were observed in *P. brevicompactum* s. str., and two in *P. bialowiezense*, both with a distribution characteristic of a predominantly clonal reproduction mode. Phylogenetic analysis of the β-tubulin and nucITS loci were carried out for members of the *P. brevicompactum* group, including ex-type material, that revealed three well-supported lineages corresponding to *P. brevicompactum*, *P. bialowiezense* (=*Penicillium biourgeianum* Zaleski), and *Penicillium neocrassum* R. Serra & S.W. Peterson. The mycophilic nature of many isolates of *P. bialowiezense*, and some isolates of *P. brevicompactum*, suggests that observation of members of the *P. brevicompactum* group in indoor environments may predict extensive and longterm fungal colonization. We also address some nomenclatural problems in the group and epitypify *P. bialowiezense*.

Key words: DNA sequence analysis, dust biology, fungal population genetics, indoor air quality, indoor moulds, heteroduplex mobility assay.

Résumé : Les auteurs ont examiné la diversité du groupe *Penicillum brevicompactum* Dierckx dans la poussière de 54 maisons à Wallaceburg en Onrario au Canada. Deux taxons prédominent, le *Penicillum brevicompactum* et le *Penicillum bialowiezense* Zaleski, représentant 88.6 % et 5.4 % de l'ensemble des échantillons, respectivement. Ils ont de plus caractérisé les haplotypes à lieux multiples d'isolats, en déterminant trois lieux génétiques polymorphes, la bêta-tubuline (*benA*), l'histone 4 (*his4A*) et des régions de l'espaceur interne transcrit de l'ADN ribosomal (nucITS) amplifiées par amplification PCR et tamisées à l'aide du test de mobilité hétéroduplex (HMA). On reconnaît huit haplotypes uniques chez le *P. brevicompactum* s. str., et deux chez le *P. bialowiezense*, les deux montrant une distribution caractéristique d'un mode de reproduction clonale. Les auteurs ont conduit l'analyse phylogénétique des lieux de la bêta-tubuline et du nucITS chez des membres du groupe *P. brevicompactum*, incluant du matériel ex-type qui révèle trois lignées bien supportées, correspondant aux *P. brevicompactum*, *P. bialowiezense* (=*Penicillium biourgeianum* Zaleski) et *Penicillium neocrassum* R. Serra & S.W. Peterson. La nature mycophile de plusieurs isolats du *P. bialowiezense* et de quelques isolats du *P. brevicompactum* suggère que l'observation des membres du groupe *P. brevicompactum* dans les environnements fermés pourrait prédire la colonisation fongique extensive et de longue durée. Les auteurs discutent également quelques problèmes de nomenclature et font du *P. bialowiezense* un épitype.

Mots-clés : analyse des séquences ADN, biologie des poussières, génétique des populations fongiques, qualité de l'air des édifices, test de mobilité hétéroduplex.

[Traduit par la Rédaction]

Received 23 July 2007. Published on the NRC Research Press Web site at botany.nrc.ca on 23 June 2008.

J.A. Scott,[2] B. Wong, and R.C. Summerbell. Dalla Lana School of Public Health, University of Toronto, Toronto, ON M5T 1R4, Canada; Sporometrics Inc., 219 Dufferin Street, Suite 20C, Toronto, ON M6K 1Y9, Canada.
W.A. Untereiner. Biology Department, Brandon University, Brandon, MB R7A 6A9, Canada.

[1]This paper is one of a selection of papers published in the Special Issue on Systematics Research.
[2]Corresponding author (e-mail: james.scott@utoronto.ca).

Introduction

Many moulds, notably in the genera *Aspergillus*, *Penicillium*, and *Stachybotrys*, grow in building interiors in the presence of superfluous moisture (Scott 2001; Flannigan and Miller 2001; Koster et al. 2003). Certain of these fungi are primarily known from indoor materials: *Stachybotrys chartarum* (Ehrenb. ex Link) Hughes, for example, was originally described by Ehrenberg (1818) (as *Stilbospora chartarum* Ehrenb.) from mouldy household packing paper (the species epithet "*chartarum*" being derived from the Latin word for paper), and the genus *Stachybotrys* was later

 doi:10.1139/B08-060

introduced by Corda (1837), based on a specimen from the walls of a house. Other taxa, like most members of the genera *Aspergillus* and *Penicillium*, were originally described from foods or soil, and are largely known from those substrates. In general, relatively little is known about the ecology and biodiversity of indoor fungi, with most studies of these taxa having been based on isolates from food materials and soil.

Despite these knowledge gaps, many species known from food and soil habitats are important health-relevant indoor contaminants. *Penicillium chrysogenum* Thom, the most common indoor species of *Penicillium*, formerly thought to be relatively unimportant as an indoor contaminant (Dillon et al. 1996; Nielsen and Gravesen 1999), can induce allergic inflammation and asthma in mouse models (Schwab et al. 2003, 2004; Chung et al. 2005), with pathophysiologic changes and biochemical features similar to those seen in human allergic asthma (Rochlitzer et al. 2006; Chung et al. 2007). Although much of this work has relied on whole cells or antigen extracts, there is a suggestion that the mycotoxin fraction alone may in part be responsible for specific cytotoxic and inflammatory responses (Rand et al. 2005). However, extrapolation of laboratory studies to realistic exposure scenarios remains difficult, because virtually all that is known about the mycotoxin profiles of many common indoor fungal taxa comes from studies of food and soil isolates, which may not accurately reflect their indoor biodiversity. As a case in point, de la Campa and co-workers (2007) recently examined a large set of phylogenetically diverse isolates of *P. chrysogenum* that were primarily of indoor origin (Scott et al. 2004), and found remarkable variability in mycotoxin profiles not previously observed in studies of this species. A better understanding of the phylogenetic biodiversity of indoor isolates may facilitate comparative assessments of mycotoxin production, and thus provide a basis for well-defined toxicology studies with relevance to indoor environmental exposures (de la Campa et al. 2007).

The present study was intended, in part, to address the need for phylogenetic understanding of indoor isolates in the *Penicillium brevicompactum* Dierckx group. This objective was pursued through the investigation of a large set of indoor isolates compared with representative strains from a variety of substrates and geographic localities. The biodiversity of *P. brevicompactum* group isolates from indoor origin is unexplored. Furthermore, despite recent studies (Frisvad and Samson 2004; Peterson 2004; Serra and Peterson 2007), the taxonomy of the group remains poorly understood, relative to other terverticillate penicillia. Also, the relation, if any, of phylogenetic subgroups to particular types of indoor habitats has not been investigated. In the present study we distinguished (*i*) urban from rural houses, and (*ii*) those with manifest mould growth on interior surfaces from those without, in an attempt to discern the influence of these factors on the composition of indoor fungal communities.

Materials and methods

Samples of floor dust were collected during the winter of 1993 from 369 houses in Wallaceburg, Ontario, Canada, a town with a municipal area of approximately 9 km^2 and an approximate population of 11 800 (Statistics Canada 1991). Fifty (13.6%) of the sampled houses were rural. Dust was collected from the entire main living area floor for 10 min using a Euroclean HEPA-filtered UZ 930 vacuum outfitted with DACI cotton filter collection thimbles (Johns Hopkins University Dermatology, Allergy, and Clinical Immunology, Baltimore, Md.). The collection thimbles were attached at the nozzle of the vacuum to avoid cross-contamination of samples, and vacuum attachments were decontaminated between homes. At the time of sample collection, the houses were also inspected for the presence of mould damage on indoor surfaces. Further details of the inspection method are given by Dales et al. (1997).

Isolation and identification of strains

One-, 10-, and 1000-fold dilutions in 2% peptone broth were made from each of two 50 mg dust samples from the same home. Each dilution series was plated in duplicate on Rose Bengal agar (Malloch 1981) with and without 25% glycerol to favour the isolation of both mildly xerophilic and hydrophilic species, respectively (Scott 2001), and plates were incubated for 14 d at room temperature. Species of *Penicillium* were grown on the diagnostic media described by Pitt (1980) and the modified formula of creatine–sucrose agar medium (CSA) given by Frisvad (1993). Identifications were made using the monographs of Pitt (1980), and Frisvad and Samson (2004). Each isolate obtained was assigned a unique strain number consisting of an arbitrary "house number" combined with an incremental accession number for each isolate of the group obtained from that house. Additional isolates including ex-type strains and geographically diverse voucher isolates were included in phylogenetic studies (Table 1). Culture collection acronyms conform to the World Federation of Culture Collections on-line listing (wdcm.nig.ac.jp/hpcc.html), and herbarium acronyms conform to those given by the on-line listing of Index Herbariorum (sciweb.nybg.org/science2/IndexHerbariorum.asp) (both databases were accessed 25 March 2008).

Air sampling was conducted at 18 outdoor locations distributed evenly throughout the study site during the late summer, using a standard RCS Sampler (Biotest, Dreieich, Germany) with Rose Bengal agar (Malloch 1981) and a sampling volume of 80 L per sample. Samples were incubated and analyzed as above.

DNA isolation and characterization

Fungal high molecular weight DNA was isolated and purified using the method of Scott et al. (2004). Three polymorphic loci were investigated, consisting of partial regions spanning introns in the gene encoding β-tubulin (*benA*) (using primers Bt2a and Bt2b in Glass and Donaldson 1995), partial histone 4 (using primers H41a and H41b in *his4A*) (Glass and Donaldson 1995), and the region spanning the internal transcribed spacer regions of the nuclear ribosomal DNA genes (nucITS) (using primer ITS5 in White et al. 1990, and primer WNL1 in Untereiner et al. 1995). PCR and heteroduplexing conditions follow Scott et al. (2004).

PCR templates were purified using a QIAquick PCR purification kit (QIAGEN, Inc., Valencia, Calif.) and sequenced using a *Taq* DyeDeoxy cycle sequencing kit (Applied Biosystems, Inc. (ABI), Foster City, Calif.), using the same

Table 1. Strains examined in this study.

Taxon	Strain No.	Status	Substratum, locality	GenBank reference	
				nucITS	Bt2
Penicillium astrolabium	NRRL 35611	*P. astrolabium* **T**	Wine grapes, Portugal	DQ645804	DQ645793
P. bialowiezense	CBS 227.28	*P. bialowiezense* **T**	Soil under conifers, Poland	EU587315	AY674439
P. bialowiezense	CBS 116044	*P. biourgeianum* **T**	Forest soil, Poland	AY484911	EU587342
P. bialowiezense	SCCM 10-I5	(B132.1)	House dust, Wallaceburg, Ontario, Canada (this study)	EU587316	EU587343
P. bialowiezense	SCCM 11-B3	(B65.5)	House dust, Wallaceburg, Ontario, Canada (this study)	EU587317	EU587344
P. bialowiezense	NRRL 2013		Mushroom spawn, Germany	AY484897	DQ645786
P. bialowiezense	NRRL 28149		Agaric on dead logs, USA	AY484900	DQ645790
P. bialowiezense	NRRL 32205		Fresh coconut, Illinois, USA	AY484904	DQ645791
P. bialowiezense	NRRL 32207		Christmas fern, Washington, USA	AY484905	DQ645792
P. brevicompactum	ATCC 10111	*P. stoloniferum* **T**	Decaying bolete, Connecticut, USA	EU587318	EU587345
P. brevicompactum	DAOM 147684		*Collybia dryophila* fruiting body, Ottawa, Ontario, Canada	EU587319	EU587346
P. brevicompactum	DAOM 191327		*Cytospora* sp. on *Prunus persica*, Harrow, Ontario Canada	EU587320	EU587347
P. brevicompactum	DAOM 192262		Urea formaldehyde foam insulation, Ottawa Ontario Canada	EU587321	EU587348
P. brevicompactum	DAOM 193712	*P. brevicompactum* **T**	*Substr. incertum*, ?Belgium	EU587322	EU587349
P. brevicompactum	DAOM 193713	*P. stoloniferum* **T**	Decaying bolete, Connecticut, USA	EU587323	EU587350
P. brevicompactum	DAOM 214776		Decaying mushroom, Denmark	EU587324	EU587351
P. brevicompactum	DAOM 215331		Spruce lumber, Quebec, Canada	EU587325	EU587352
P. brevicompactum	DAOM 215332		On *Picea*, Quebec, Canada	EU587326	EU587353
P. brevicompactum	DAOM 215335		Spruce lumber, Quebec	EU587327	EU587354
P. brevicompactum	SCCM 11-G1	(ALG 1)	Decaying agaric, Algonquin Prov. Pk., Ontario, Canada (this study)	EU587328	EU587355
P. brevicompactum	SCCM 11-G2	(ALG 2)	Decaying agaric, Algonquin Prov. Pk., Ontario, Canada (this study)	EU587329	EU587356
P. brevicompactum	SCCM 10-A1	(B65.4)	House dust, Wallaceburg, Ontario, Canada (this study)	EU587330	EU587357
P. brevicompactum	SCCM 10-A6	(B65.6)	House dust, Wallaceburg, Ontario, Canada (this study)	EU587331	EU587358
P. brevicompactum	SCCM 10-B8	(B99)	House dust, Wallaceburg, Ontario, Canada (this study)	EU587332	EU587359
P. brevicompactum	SCCM 10-C2	(B114)	House dust, Wallaceburg, Ontario, Canada (this study)	EU587333	EU587360
P. brevicompactum	SCCM 10-E4	(B240)	House dust, Wallaceburg, Ontario, Canada (this study)	EU587334	EU587361
P. brevicompactum	SCCM 10-E6	(B306.2)	House dust, Wallaceburg, Ontario, Canada (this study)	EU587335	EU587362
P. brevicompactum	SCCM 10-G1	(B117)	House dust, Wallaceburg, Ontario, Canada (this study)	EU587336	EU587363
P. brevicompactum	SCCM 10-G2	(B251)	House dust, Wallaceburg, Ontario, Canada (this study)	EU587337	EU587364
P. brevicompactum	SCCM 10-H7	(B75.3)	House dust, Wallaceburg, Ontario, Canada (this study)	EU587338	EU587365
P. brevicompactum	SCCM 10-I3	(B112)	House dust, Wallaceburg, Ontario, Canada (this study)	EU587339	EU587366
P. brevicompactum	SCCM 10-I6	(B244)	House dust, Wallaceburg, Ontario, Canada (this study)	EU587340	EU587367
P. brevicompactum	NRRL 2012		Paprika, locality unknown	AY484913	DQ645785
P. brevicompactum	NRRL 28120		Wood decay fungus sporocarp, USA	AY484916	DQ645789
P. brevicompactum	NRRL 28139		Stroma of wood decay fungus, Illinois, USA	AY484917	DQ645795
P. neocrassum	NRRL 35639	*P. neocrassum* **T**	Wine grapes, Portugal	DQ645805	DQ645794
P. neocrassum	NRRL 35648		Wine grapes, Portugal	DQ645806	DQ645802
P. olsonii	CBS 232.60	*P. olsonii* **T**	*Picea abies* root, Austria, Pitztal, alt. 1980 m	EU587341	AY674445

Note: T denotes ex-type material.

primers employed for amplification. Extension products were run on an ABI50 fluorescent automated sequencer.

Phylogenetic analysis

Alignment of the sequences was performed using ClustalX version 1.82 (Thompson et al. 1997) and adjusted by visual inspection. Phylogenetic relationships were inferred from aligned sequences using the maximum parsimony (MP) method in PAUP* version 4.0b10 (Swofford 2002). All searches were performed using tree bisection-reconstruction (TBR) branch swapping with MulTrees and steepest descent options activated. *Penicillium olsonii* Bainier & Sartory CBS 232.60 and *Penicillium astrolabium* R. Serra & S.W. Peterson NRRL 35611 were used as outgroups. Bootstrap support (BS) for branches was evaluated from 1000 full heuristic searches. Congruence between the *benA* and ITS datasets was measured based on 1000 heuristic searches employing the partition-homogeneity test (Farris et al. 1995) included in PAUP*.

Results

Isolates of the *P. brevicompactum* group were recovered from 54 of 369 houses (15%). Forty-seven of these houses (92%) were located within town limits, and 6 (8%) were rural (i.e., outside the town limits). Seventy-two isolates were retained for further study. Three quarters of these were from urban locations; the remainder were from rural locations. Fourteen percent of isolates represented the *Penicillium bialowiezense* Zaleski clade (see Fig. 1). Although only 8% of the houses sampled were rural, these houses contributed over two thirds of *P. bialowiezense* isolates recovered. Members of the *P. brevicompactum* group were not observed in the 18 outdoor air samples taken throughout the study site. The levels of *P. brevicompactum* from dust ranged from approximately 100–44 000 CFU·g^{-1}, with a geometric mean of 1696 CFU·g^{-1} originating from 49 houses (Table 2). For *P. bialowiezense*, the dust concentration ranged from approximately 500–19 000 CFU·g^{-1}, with a geometric mean of 3591 CFU·g^{-1} originating from seven houses (Table 2). We did not observe a correlation between the levels of either taxon with the mouldiness condition of the house as determined by visual inspection by a field investigator.

Haplotype characterization

The haplotypes are summarized in Table 3. The lowest degree of allelic variation was observed in the histone 4 locus (2 alleles, correlating to *P. brevicompactum* s. str. and *P. bialowiezense*), whereas the highest variation (6 alleles) was seen in the β-tubulin locus. For *P. brevicompactum* s. str., the two most commonly observed haplotypes accounted for 66% and 19% of isolates. The remaining 15% of isolates comprised six minor haplotypes. Two haplotypes were observed for *P. bialowiezense*, accounting for 90% and 10% of isolates. No isolates of *Penicillium neocrassum* R. Serra & S.W. Peterson were recovered.

Mixed populations of two or more haplotypes were observed in two thirds of houses where multiple isolates of the *P. brevicompactum* group were obtained, suggesting the stable coexistence of multiple haplotypes within individual houses.

Phylogenetic analysis

DNA sequences were obtained for representatives of each allele for the *benA* and ITS loci. Difficulties were encountered in obtaining unambiguous sequence for the *his4A* locus in house dust isolates and voucher strains. Further attempts to resolve these difficulties were not made owing to the low allelic variation in this locus, and it was excluded from sequence analyses.

MP analysis of the combined dataset (*benA* 442 bp, ITS 509 bp) for 38 taxa yielded seven most parsimonious trees (MPTs). Results of 10 000 heuristic searches implementing the partition-homogeneity test ($P = 0.45$) demonstrated that sequences from the two different loci were not incongruent.

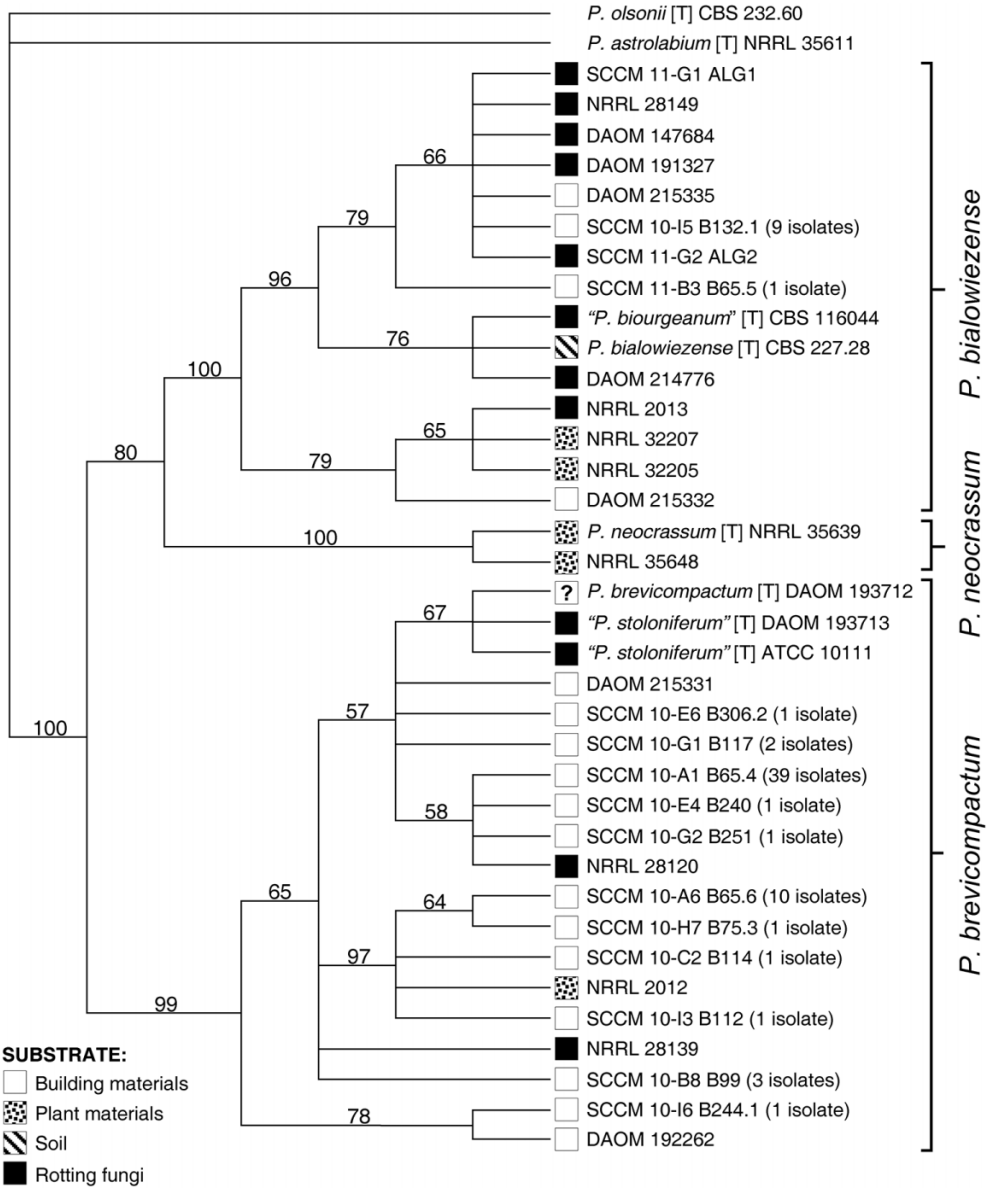
Penicillium bialowiezense and *P. neocrassum* comprised strongly supported clades (100% and 99% BS, respectively) in the combined ITS-partial β-tubulin tree (Fig. 1). A moderate amount of clade substructure was observed in *P. bialowiezense*. One well-supported subclade (96% BS) within this lineage contained strains predominantly isolated from fungal fruit bodies. The types of *P. bialowiezense* (CBS 227.28) and its later synonym *Penicillium biourgeianum* Zaleski (CBS 116044) were situated in this group. Several strains in this subclade originated from house dust, but the houses from which these strains were obtained were predominantly rural. A second, less supported subclade (79% BS) contained a mix of isolates from mycophilic habitats and plant materials, including lumber.

The clade comprising strains of *P. brevicompactum* s. str. contained two sister subclades. The first of these subclades (65% BS) contained some substructure. One well-supported group (97% BS) included four strains that represented a single haplotype we observed in 21% of newly-analyzed indoor isolates (SCCM 10-A6, SCCM 10-H7, SCCM 10-C2, and SCCM 10-I3), and a sequence from a voucher strain from paprika (NRRL 2012), but no clearly mycophilic (fungal associated) strains (Fig. 1). A second, weakly supported group (57% BS) contained 71% of the indoor dust isolates originating from this study. Although substrate affinities in this group tended to be predominantly from indoor materials, two isolates from mushroom fruiting bodies, both from the USA (*Penicillium stoloniferum* Thom DAOM 193713 T and *P. brevicompactum* DAOM 193712 T), were also clustered here. The placement of a representative isolate of three newly analyzed indoor strains (SCCM 10-B8) and a single patently mycophilic isolate (NRRL 28139) remained unresolved. The second subclade in *P. brevicompactum* (78% BS) contained 2% of indoor isolates (SCCM 10-I6 and a voucher isolate from urea formaldehyde foam insulation from Canada, DAOM 192262). No isolates from obviously fungal substrates strains were observed in this clade.

Discussion

The array of isolates obtained in the present study shed considerable light on the infraspecific genetic structure of *P. brevicompactum* and *P. bialowiezense*. The combined ITS – β-tubulin tree of Serra and Peterson (2007). Of all

Fig. 1. Phylogenetic relationships of members of the *Pencillium brevicompactum* group inferred from nucITS and partial β-tubulin gene sequences. This strict consensus is based on parsimony analysis of the combined dataset (*benA* 442 bp, nucITS 509 bp) for 38 taxa which yielded 7 MPTs (236 steps, CI = 0.864, RI = 0.969). Bootstrap values greater than 50% calculated from 1000 replicates are indicated above the branches. The outgroup taxa are *P. olsonii* CBS 232.60 and *P. astrolabium* NRRL 35611. The collection substrate is noted beside each isolate (see caption).



the isolates of *P. brevicompactum* and *P. bialowiezense* that were newly analysed in the present study, only two novel *P. brevicompactum* haplotypes were observed that were supported by more than one isolate (see DAOM 192262 and SCCM 10-A6, Fig. 1). No novel haplotypes supported by multiple isolates were observed for *P. bialowiezense*. Since the habitats and geography covered by the present study and the Peterson (2004) studies encompass a wide range of temperate and indoor, as well as some tropical habitats, it would appear that the basic population structure of *P. brevicompactum* and *P. bialowiezense*, at least in temperate areas, is well on the way to being mapped in terms of its common, major clades. The isolates studied here elevated the bootstrap value attributable to one *P. brevicompactum* subclade containing isolate NRRL 2012 (from paprika), to 97%. It is not clear what, if anything, should be done to attribute taxonomic status to such well genetically marked, but otherwise (as yet) undistinguished subclades; at the moment, it seems sufficient to note them and thus anchor a search for habitat regularities and stable phenetic characters differing from those found in other subclades.

The present study, in its inclusion of diverse Canadian reference isolates not included in previous studies, has also strongly reinforced the impression that most or all of the subclades of *P. brevicompactum* and *P. bialowiezense* share at least a partial association with mycophily and (or) mycoparasitism. Unambiguous mycophilic isolates, recovered

Table 2:. *Penicillium brevicompactum* and *P. bialowiezense* in house dust.

House No.	Average concentration (CFU·g^{-1}) *Penicillium brevicompactum*	*Penicillium bialowiezense*
42	9 764	—
45	—	9 328
62	19 629*	—
65	512	2 980
67	14 598	18 868
70	5 419	—
72	489	—
74	943*	—
75	3 889	—
79	984	—
81	477	—
87	946	—
91	4 621	—
98	44 269*	—
99	3 275*	—
100	4 361	—
109	473	—
112	1 107*	—
114	480*	—
117	92*	—
119	9 765	—
132	—	4 779
136	470	—
166	3 770	—
170	1 320	—
183	4 911	—
185	191	—
189	498	—
192	2 440*	—
201	491	—
204	489*	—
217	1 480*	—
228	24 557*	—
233	961	961
240	3 401	—
244	3 652	—
245	3 353*	—
251	475	—
259	950*	—
263	763	—
264	486	—
266	4 752*	—
273	470	—
274	489	—
280	957	—
306	959	—
319	2 896	—
322	566	—
325	6 377	—
340	—	477*
353	—	6 701
373	4 725*	—
374	5 494	—

*RBA without 25% glycerol.

from reproductive structures or mycelium of both basidiomycetous and ascomycetous fungi, occur in nearly every subclade of *P. brevicompactum* and *P. bialowiezense* (Fig. 1). Even though *P. brevicompactum* is stated by Frisvad and Samson (2004) and other references to be regularly isolated from food spoilage including spoiled fruit juices and other definitely nonfungal materials, a surprising proportion of reference isolates included in collections appears to be from fungal or potentially fungal habitats, as with *P. bialowiezense*. Of the *P. brevicompactum* isolates of known origin, other than house dust, considered in the present study, 50% (3 of 6) are from fungal substrates, while in *P. bialowiezense* half the isolates of known origin (5 of 10) are confirmed mycophiles. It is unlikely that selective collecting is involved, since the mycophilic isolates included are from widely separated sampling times and geographic localities, and diverse fungal substrata, going back to 1910. Many of the isolates of *P. brevicompactum* and *P. bialowiezense* we examined that were not overtly derived from fungal substrata may still be suspected of mycophily. For example, there were numerous isolates from lumber and related woody substrates. As Seifert (1983) and others have noted, fungi common on wood tend to be divided into ecologically competent wood degraders (e.g., *Dacrymyces* spp. in the Seifert (1983) study), and co-occurring mycophiles and mycoparasites (e.g., *Tremella* spp.) that historically were routinely misinterpreted as additional wood degraders. Mycophily must always be suspected of isolates reported from woody materials, unless isolation techniques or other circumstances specifically exclude this possibility.

Penicillium species are among the most common microfungi on well-decomposed wood (Crawford et al. 1990), although they are not known to be aggressive agents of wood decay (Seifert and Frisvad 2000). *Penicillium brevicompactum* is able to degrade cellulose in vitro (Domsch et al. 1980) and has been recorded from soft rot of timber (Seehan et al. 1975). As such, the principal ecological role of these fungi on woody materials may well be mycophily. Forest soil is another substrate not connoting mycophily to the inexperienced interpreter, but well known to fungal ecologists as being a rich habitat for mycophilic organisms. Figure 1 contains *P. bialowiezense* isolates from soils of forests consisting of ectomycorrhizal trees such as pine; these soils tend to be heavily and conspicuously invested with mycelia of mycorrhizal and mycorrhizosphere fungi (Ogawa 1977; Summerbell 2005). The clade containing the ex-type isolates of *P. bialowiezense* and *P. biourgeianum* from Polish forest soils also contains a Danish isolate from a mushroom fruiting body.

Like soil, house dust has a deceptively complex ecology. The recovery of fungi from house dust by dilution plating does not itself demonstrate that the dry indoor dust sampled is a bona fide habitat for the parent mycelium. While this can certainly be true for some highly osmophilic fungi, in many cases house dust serves as a reservoir where colony-forming elements formed elsewhere deposit and, over time, accumulate. Over the longer term, species of *Aspergillus* and *Penicillium* may appear to become predominant through comparatively long cell viability (Sussman 1968) or less effective removal by cleaning (Scott et al. 2000, 2004;

Table 3. Haplotype frequencies of *Penicillium brevicompactum* group from dust.

	Locus			
Representative isolate	Bt2	H4A	ITS	Haplotype frequency
P. bialowiezense				
SCCM 10-I5 [B132.1]	G	B	D	9/10 (0.900)
SCCM 11-B3 [B65.5]	H	B	D	1/10 (0.100)
P. brevicompactum				
SCCM 10-A1 [B65.4]	A	A	A	41/62 (0.661)
SCCM 10-B8 [B99]	B	A	A	3/62 (0.048)
SCCM 10-G1 [B117]	C	A	A	2/62 (0.032)
SCCM 10-G2 [B251]	A	A	B	1/62 (0.016)
SCCM 10-E6 [B306.2]	D	A	B	1/62 (0.016)
SCCM 10-I6 [B244]	E	A	B	1/62 (0.016)
SCCM 10-A6 [B65.6]	F	A	B	12/62 (0.194)
SCCM 10-H7 [B75.3]	F	A	C	1/62 (0.016)

Buttner et al. 2002). *Penicillium brevicompactum* s.l. has frequently been reported from house-dust isolations (Mallea et al. 1982; Miller et al. 1988; Hoekstra et al. 1994; Verhoeff et al. 1994; Horner et al. 2004). Because this species is known to degrade fine cellulose fibres (Marsh et al. 1949), house dust and other cellulose-rich indoor materials may represent legitimate primary habitats for it under suitable moisture conditions. Indeed, numerous indoor sites supporting fungal growth have yielded *P. brevicompactum*, including: broadloom (Beguin and Nolard 1999), painted plaster walls (Mallea et al. 1982), gypsum wallboard paper (Scott et al. 2000; Scott 2001), potted plants (Summerbell et al. 1992), and urea formaldehyde foam insulation (Bissett 1987). However, like soil and decaying wood, indoor materials subject to superfluous moisture support fungal communities that are predominantly polymycotic. As indoor fungal communities emerge and mature, generally over a period of several weeks to a few months, depending on moisture conditions, they also become host to organisms dependent on previous fungal growth: the bulk of existing data relate to fungivorous arthropods (van Bronswijk 1981: 148, 176). The suitability of house dust fungi for arthropod feeding is variable. It was noted by van de Lustgraff (1978) that the growth of *P. brevicompactum* in the rearing medium of the dust mite *Dermatophagoides pteronyssinus* somewhat negatively affected the development of the mite. Moreover, *P. olsonii* and other penicillia have been noted both as food sources for fungivorous mites and as post mortem colonists of the mites themselves (Scott 2001). These observations collectively imply a complex but poorly known suite of trophic relationships between fungi and arthropods in house dust. Mycophilic fungi have been reported commonly from indoor environments, including mycoparasitic species of *Acremonium*, *Clonostachys*, *Sporothrix*, *Trichoderma*, and *Verticillium* (Ostrowski 1999; Scott 2001). Based on mycophilic tendency of members of the *P. brevicompactum* group, the presence of these taxa in houses should be investigated as a possible indicator of extensive and longterm fungal colonization.

Besides potentially broadening our understanding of the ecology of members of the *P. brevicompactum* group, our study also has permitted a close examination of some members of a group of penicillia which have seldom been subject to discussion in the taxonomic literature. Some relevant observations are summarized below.

Penicillium bialowiezense

Thom (1930: 304) received Zaleski's type culture of *P. bialowiezense* from the Centraalbureau voor Schimmelcultures (CBS), Baarn, which he accessioned as "Thom 5010.4". Peterson (2004) listed NRRL 863 as derived from Thom 5010.4, implying that the former represented the ex-type culture of *P. bialowiezense* in the NRRL collection. He also correctly noted that Pitt (1980: 373–374) mistakenly listed NRRL 863 as a subculture of 'Biourge 42', the ex-type of *P. brevicompactum*, although Raper and Thom (1949: 410) appear to have initiated it. This error was perpetuated most recently by Samson et al. (2004: 177). Pitt (1980: 375) correctly indicated that Thom 5010.4 was equivalent to CBS 227.28; therefore, CBS 227.28 and NRRL 863 should represent equivalent isolates. However, Peterson's (2004) sequence of partial nucITS-LSU referred NRRL 863 instead to the phylogenetically distant *Penicillium polonicum* Zaleski, and on that basis, he proposed *P. bialowiezense* as a later synonym of *P. polonicum* (these taxa were described on pp 450 and 445, respectively, of Zaleski's (1927) monograph). Conversely, our nucITS and β-tubulin sequences, as well as those of Frisvad and Samson (2004) derived from the CBS version of the ex-type of *P. bialowiezense*, CBS 227.28, clustered this isolate within the *P. brevicompactum* group, identical in sequence to the ex-type strain of *P. biourgeianum* (CBS 116044), over which it has nomenclatural priority. Frisvad and Samson (2004) made several typographical errors in their discussion of this matter, incorrectly citing the ex-type culture of *P. bialowiezense* as "CBS 227.38", rather than CBS 227.28 (2004: 14, 15), and referring to the protologue for this taxon as "Zaleski 1927: 462", rather than Zaleski 1927: 450 (2004: 14, 62).

The databases of NRRL and CBS culture collections cross-reference NRRL 863 with CBS 116043: the CBS database assigns these strains to *Penicillium cyclopium* Westling [accessed 25 March 2008], whereas they are unlisted in the ARS database [accessed 25 March 2008]. We are in agree-

ment with Frisvad and Samson (2004) that NRRL 863 is a specious isolate not representative of *P. bialowiezense*.

The poor condition or questionable authenticity of ex-type strains of *P. bialowiezense* noted by several authors (Pitt 1980; Peterson 2004) warrants the stabilization of this taxon. Thus, we designate DAOM 239766 (derived from CBS 227.28) as an epitype of *P. bialowiezense*, in accordance with Art. 9.7 of the International Code of Botanical Nomenclature (McNeill et al. 2006).

Penicillium biourgeianum

Thom received Zaleski's type strain of *P. biourgeianum* in July 1928 from CBS, and he accessioned it as "Thom 5010.5" (Thom 1930). Thom initially accepted *P. biourgeianum* (Thom 1930), but later reduced it to synonymy with *Penicillium stoloniferum* Thom (Raper and Thom 1949). The direct parent strain was lost from the CBS collection; however, the strain remains in the ARS collection as NRRL 865. Frisvad deposited a subculture of NRRL 865 to the CBS collection as CBS 116044. Although the CBS database acknowledges equivalency of this isolate with NRRL 865 and Thom 5010.5, the collection data fails to note that it is an ex-type strain of *P. biourgeianum* [CBS database accessed online, 25 March 2008]. Frisvad and Samson (2004) noted that *P. biourgeianum* and *P. bialowiezense* were conspecific, but provided no data to support this, and failed to include the type strain of *P. biourgeianum* in their phylogenetic study of *Penicillium* subgenus *Penicillium* sect. *Coronata*. Based on our sequence data, this isolate is conspecific with the ex-type strain of *P. bialowiezense*, CBS 227.28, and the name *P. bialowiezense* has priority over *P. biourgeianum*.

Penicillium brevicompactum

Penicillium brevicompactum, originally, although inadequately described by Dierckx (1901), was included in Biourge's (1923) monograph, and later neotypified by Pitt (1980) with a specimen derived ultimately from isolate "Biourge 42". The robust conidiophores, characteristic apically swollen metulae, and aspergilloid habit of this taxon has long been regarded as highly distinctive, and the species has been recognized conventionally since Thom (1930). It is one of the few species of *Penicillium* that is generally considered to be easily recognizable. The possibility of multiple embedded lineages within *P. brevicompactum* was noted by Seifert and Frisvad (2000). In their study on *Penicillium* species on solid wood products, these authors reported two discrete micromorphologies for isolates of *P. brevicompactum*, in which certain isolates exhibited predominantly biverticillate branching and strongly apically inflated metulae in fresh cultures, while others showed a more characteristic terverticillate penicillus morphology (Seifert and Frisvad 2000). While the former group tended to converge on the more typical morphology after several serial transfers, the authors suggested that the initially biverticillate variants may represent a distinct taxon. We have examined the complete set of *P. brevicompactum* group isolates deposited by Seifert and Frisvad (2000) in DAOM in conjunction with their study. Although tentative, our results suggest that *P. brevicompactum* may comprise three distinct lineages that likely correspond to phylogenetic species in the sense of Taylor et al. (2000). However, comparison of Seifert and Frisvad's (2000) observations against our phylogeny is impossible because their morphological observations were not referenced to individual strains, and the distinctions they saw are no longer clear. With a larger DNA sequence data set and a more systematic search for stable phenetic traits, formal recognition of these lineages as segregate species may be justifiable.

Penicillium stoloniferum

Thom (1910) described *P. stoloniferum* from a decaying mushroom in Connecticut. This species was reduced to synonymy with *P. brevicompactum* by Thom (1930), but was later included in the *P. brevicompactum* series by Raper and Thom (1949). This series included *P. brevicompactum*, *P. stoloniferum*, and *Penicillium paxilli* Bainier. Pitt (1980) suggested that isolates of *P. stoloniferum* and *P. brevicompactum* showed a continuum of variation, and he once again reduced *P. stoloniferum* to synonymy with *P. brevicompactum*. This synonymy is supported by the present study.

Acknowledgements

We are grateful to Carolyn Babcock, (Canadian Collection of Fungal Cultures (CCFC), Agriculture and Agri-Food Canada, Ottawa, Ont.) and Stephen W. Peterson (Agricultural Research Service (ARS) Culture Collection, USDA, Peoria Il.) for providing isolates used in this study. We thank the guest editor, Sean Graham, and two anonymous reviewers for improving this paper through their helpful comments. Funding for this work was kindly provided by the University of Toronto, Faculty of Medicine Dean's Fund (J.A.S.), Sporometrics Inc., and Natural Sciences and Engineering Research Council of Canada (W.A.U.).

References

Beguin, H., and Nolard, N. 1999. Relationship between mycobiota in wall-to-wall carpet dust and age of carpet. Aerobiologia, **15**: 299–306. doi:10.1023/A:1007637330436.

Biourge, P. 1923. Les moisissures du groupe *Penicillium* Link. Etude monographique. Cellule (Louvain), **33**: 1–322.

Bissett, J. 1987. Fungi associated with urea-formaldehyde foam insulation in Canada. Mycopathologia, **99**: 47–56. doi:10.1007/BF00436681.

Buttner, M.P., Cruz-Perez, P., Stetzenbach, L.D., Garrett, P.J., and Luedtke, A.E. 2002. Measurement of airborne fungal spore dispersal from three types of flooring materials. Aerobiologia, **18**: 1–11. doi:10.1023/A:1014977900352.

Chung, Y.J., Coates, N.H., Viana, M.E., Copeland, L., Vesper, S.J., Selgrade, M.K., and Ward, M.D.W. 2005. Dose-dependent allergic responses to an extract of *Penicillium chrysogenum* in BALB/c mice. Toxicology, **209**: 77–89. doi:10.1016/j.tox.2004.12.010. PMID:15725516.

Chung, Y.J., Farraj, A., Coates, N.H., Gavett, S.H., and Ward, M.D.W. 2007. Increased neurotrophin production in a *Penicillium chrysogenum*-induced allergic asthma model in mice. J. Toxicol. Environ. Health (Part A), **70**: 1020–1026. doi:10.1080/15287390601172023. PMID:17497413.

Corda, A.C.I. 1837. Icones Fungorum, **1**: 21.

Crawford, R.H., Carpenter, S.E., and Harmon, M.E. 1990. Communities of filamentous fungi and yeast in decomposing logs of

Pseudotsuga menziesii. Mycologia, **82**: 759–765. doi:10.2307/3760163.

Dales, R.E., Miller, J.D., and McMullen, E. 1997. Indoor air quality and health: validity and determinants of reported home dampness and moulds. Int. J. Epidemiol. **26**: 120–125. doi:10.1093/ije/26.1.120. PMID:9126511.

de la Campa, R., Seifert, K.A., and Miller, J.D. 2007. Toxins from strains of *Penicillium chrysogenum* isolated from buildings and other sources. Mycopathologia, **163**: 161–168. doi:10.1007/s11046-007-0091-8. PMID:17380432.

Dierckx, F. 1901. Essai de revision du genre *Penicillium* Link. Note préliminaire. Ann. Soc. Sci. Brux. **25**: 83–88.

Dillon, H.K., Heinsohn, P.A., and Miller, J.D. 1996. Field guide for the determination of biological contaminants in environmental samples. American Industrial Hygiene Association, Fairfax, Va.

Domsch, K.W., Gams, W., and Anderson, T.-H. 1980. Compendium of soil fungi. Academic Press, New York, N.Y.

Ehrenberg, C.G. 1818. Sylvae Mycologicae Berolinensis. T. Bruschcke, Berolini, Germany.

Farris, J.S., Källersjö, M., Kluge, A.G., and Bult, C. 1995. Constructing a significance test for incongruence. Syst. Biol. **44**: 570–572. doi:10.2307/2413663.

Flannigan, B., and Miller, J.D. 2001. Microbial growth in indoor environments. *In* Microorganisms in home and work environments. *Edited by* B. Flannigan, R.A. Samson, and J.D. Miller. Taylor & Francis, London, UK. pp. 35–67.

Frisvad, J.C. 1993. Modifications on media based on creatine for use in *Penicillium* and *Aspergillus* taxonomy. Lett. Appl. Microbiol. **16**: 154–157. doi:10.1111/j.1472-765X.1993.tb01382.x.

Frisvad, J.C., and Samson, R.A. 2004. Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*: a guide to identification of food and air-borne terverticillate Penicillia and their mycotoxins. Stud. Mycol. **49**: 1–173.

Glass, N.L., and Donaldson, G.C. 1995. Development of primer sets designed for use with the pcr to amplify conserved genes from filamentous ascomycetes. Appl. Environ. Microbiol. **61**: 1323–1330. PMID:7747954.

Hoekstra, E.S., Samson, R.A., and Verhoeff, A.P. 1994. Fungal propagules in house dust: a qualitative analysis. *In* Health implications of fungi in indoor environments. Air Quality Monographs 2. *Edited by* R.A. Samson, B. Flannigan, M.E. Flannigan, A.P. Verhoeff, O.C.G. Adan, and E.S. Hoekstra. Elsevier, Amsterdam, Netherlands. pp. 169–177.

Horner, W.E., Worthan, A.G., and Morey, P.R. 2004. Air- and dustborne mycoflora in houses free of water damage and fungal growth. Appl. Environ. Microbiol. **70**: 6394–6400. doi:10.1128/AEM.70.11.6394-6400.2004. PMID:15528497.

Koster, B., Scott, J.A., Wong, B., Malloch, D., and Straus, N.A. 2003. A geographically diverse set of isolates indicates two phylogenetic lineages within *Stachybotrys chartarum.* Can. J. Bot. **81**: 633–643. doi:10.1139/b03-056.

Mallea, M., Renard, M., and Charpin, J. 1982. La flore fongique des habitations. Rev. Fr. Malad. Resp. **10**: 121–130.

Malloch, D.W. 1981. Moulds: their isolation, cultivation and identification. University of Toronto Press, Toronto, Ont.

Marsh, P.B., Bollenbacher, K., Butler, M.L., and Raper, K.B. 1949. The fungi concerned in fiber deterioration. 2. Their ability to decompose cellulose. Text. Res. J. **19**: 462–484. doi:10.1177/004051754901900803.

McNeill, J., Barrie, F.R., Burdet, H.M., Demoulin, V., Hawksworth, D.L., Marhold, K., Nicolson, D.H., Prado, J., Silva, P.C., Skog, J.E., Wiersema, J.H., and Turland, N.J. (*Editors*). 2006. International code of botanical nomenclature (Vienna Code) adopted by the 17th International Botanical Congress, Vienna, Austria, July 2005. Gantner Verlag, Ruggell, Liechtenstein.

Miller, J.D., Laflamme, A.M., Sobol, Y., Lafontaine, P., and Greenhalgh, R. 1988. Fungi and fungal products in some Canadian houses. Int. Biodeterior. **24**: 103–120. doi:10.1016/0265-3036(88)90053-X.

Nielsen, K.F., and Gravesen, S. 1999. Production of mycotoxins on water damaged building materials. *In* Bioaerosols, fungi and mycotoxins. Proceedings of the 3rd International Conference on Fungi, Mycotoxins and Bioaerosols. Saratoga Springs, New York. 23 September – 25 September 1998. *Edited by* E. Johanning. Eastern New York Occupational and Environmental Health Center, Albany, New York, N.Y. pp. 423–431.

Ogawa, M. 1977. Microbial ecology of the "shiro" in *Tricholoma matsutake* (S. Ito et Imai) Sing., and its allied species. III. Fungal florae in shiro soil and on the mycorrhiza. Bull. Gov. For. Exp. Stn. **293**: 105–170.

Ostrowski, R. 1999. Exposure assessment of moulds in indoor environments in relation to chronic respiratory diseases. Ph.D. thesis, Aachen Technical University, Aachen, Germany.

Peterson, S.W. 2004. Multilocus DNA sequence analysis shows that *Penicillium biourgeianum* is a distinct species closely related to *P. brevicompactum* and *P. olsonii.* Mycol. Res. **108**: 434–440. doi:10.1017/S0953756204009761. PMID:15209283.

Pitt, J.I. 1980. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic Press, New York, N.Y.

Rand, T.G., Giles, S., Flemming, J., Miller, J.D., and Puniani, E. 2005. Inflammatory and cytotoxic responses in mouse lungs exposed to purified toxins from building isolated *Penicillium brevicompactum* Dierckx and *P. chrysogenum* Thom. Toxicol. Sci. **87**: 213–222. doi:10.1093/toxsci/kfi223. PMID:15958659.

Raper, K.B., and Thom, C. 1949. A manual of the Penicillia. Williams and Wilkins, Baltimore, Md.

Rochlitzer, S., Nassenstein, C., and Braun, A. 2006. The contribution of neurotrophins to the pathogenesis of allergic asthma. Biochem. Soc. Trans. **34**: 594–599. doi:10.1042/BST0340594. PMID:16856870.

Samson, R.A., Seifert, K.A., Kuijpers, A.F.A., Houbraken, J.A.M.P., and Frisvad, J.C. 2004. Phylogenetic analysis of *Penicillium* subgenus *Penicillium* using partial β-tubulin sequences. Stud. Mycol. **49**: 175–200.

Schwab, C.J., Cooley, J.D., Brasel, T., Jumper, C.A., Graham, S.C., and Straus, D.C. 2003. Characterization of exposure to low levels of viable *Penicillium chrysogenum* conidia and allergic sensitization induced by a protease allergen extract from viable *P. chrysogenum* conidia in mice. Int. Arch. Allergy Immunol. **130**: 200–208. doi:10.1159/000069519. PMID:12660424.

Schwab, C.J., Cooley, J.D., Jumper, C.J., Graham, S.C., and Straus, D.C. 2004. Allergic inflammation induced by a *Penicillium chrysogenum* conidia-associated allergen extract in a murine model. Allergy, **59**: 758–765. doi:10.1111/j.1398-9995.2004.00481.x. PMID:15180764.

Scott, J.A. 2001. Studies on indoor fungi. Ph.D. thesis, Department of Botany, University of Toronto, Toronto, Ont.

Scott, J.A., Malloch, D., Wong, B., Sawa, T., and Straus, N.A. 2000. DNA heteroduplex fingerprinting in *Penicillium. In* Integration of modern taxonomic methods for *Penicillium* and *Aspergillus* classification. *Edited by* R.A. Samson and J.I. Pitt. Harwood Academic Publishers, Amsterdam, Netherlands. pp. 225–236.

Scott, J.A., Untereiner, W.A., Wong, B., Straus, N.A., and Malloch, D. 2004. Genotypic variation in *Penicillium chysogenum* from indoor environments. Mycologia, **96**: 1095–1105. doi:10.2307/3762092.

Seehan, F., Liese, W., and Kesa, B. 1975. List of fungi in soft-rot tests. International Working group on Wood Preservation, Document No. 1RG/WP/105.

Seifert, K.A. 1983. Decay of wood by the Dacrymycetales. Mycologia, **75**: 1011–1018. doi:10.2307/3792657.

Seifert, K.A., and Frisvad, J.C. 2000. *Penicillium* on solid wood products. *In* Integration of modern taxonomic methods for *Penicillium* and *Aspergillus* classification. *Edited by* R.A. Samson and J.I. Pitt. Harwood Academic Publishers, Amsterdam. pp. 285–298.

Serra, R., and Peterson, S.W. 2007. *Penicillium astrolabium* and *Penicillium neocrassum*, two new species isolated from grapes and their phylogenetic placement in the *P. olsonii* and *P. brevicompactum* clade. Mycologia, **99**: 78–87. PMID:17663125.

Statistics Canada. 1991. Census subdivisions showing population, MIZ category and north/south location, 1991 Census, Table 2. Version updated 26 September 2002 [online]. Available from www.statcan.ca/english/research/92F0138MIE/00001/t35csd96.csv [accessed 21 July 2007].

Summerbell, R.C. 2005. From Lamarckian fertilizers to fungal castles: recapturing the pre-1985 literature on endophytic and saprotrophic fungi associated with ectomycorrhizal root systems. Stud. Mycol. **53**: 191–256.

Summerbell, R.C., Staib, F., Dales, R., Nolard, N., Kane, J., Zwanenburg, H., Burnett, R., Krajden, S., Fung, D., and Leong, D. 1992. Ecology of fungi in human dwellings. J. Med. Vet. Mycol. **30**(Suppl. 1): 279–285. doi:10.1080/02681219280000971. PMID:1474453.

Sussman, A.S. 1968. Longevity and survivability of fungi. *In* The fungi: an advanced treatise. Vol. 3. *Edited by* G.C. Ainsworth and A.S. Sussman. Academic Press, New York, N.Y. pp. 447–486.

Swofford, D.L. 2002. PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Sunderland, Mass.

Taylor, J.W., Jacobson, D.J., Kroken, S., Kasuga, T., Geiser, D.M., Hibbett, D.S., and Fisher, M.C. 2000. Phylogenetic species recognition and species concepts in fungi. Fungal Genet. Biol. **31**: 21–32. doi:10.1006/fgbi.2000.1228. PMID:11118132.

Thom, C. 1910. Cultural studies of species of *Penicillium*. Bureau of Animal Industry, Bulletin No. 118. USDA, Washington, D.C.

Thom, C. 1930. The Penicillia. Williams & Wilkins, Baltimore, Md.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **25**: 4876–4882. doi:10.1093/nar/25.24.4876.

Untereiner, W.A., Straus, N.A., and Malloch, D. 1995. A molecular-morphotaxonomic approach to the systematics of the Herpotrichiellaceae and allied black yeasts. Mycol. Res. **99**: 897–913.

van Bronswijk, J.E.M.H. 1981. House dust biology for allergists, acarologists and mycologists. J.E.M.H van Bronswijk, Zoelmond, the Netherlands.

van de Lustgraff, B. 1978. Ecological relationships between xerophilic fungi and house-dust mites (Acarida: Pyroglyphidae). Oecologia, **33**: 351–359. doi:10.1007/BF00348118.

Verhoeff, A.P., van Reenen-Hoekstra, E.S., Samson, R.A., Brunekreef, B., and van Wijnen, J.H. 1994. Fungal propagules in house dust. I. Comparison of analytic methods and their value as estimators of potential exposure. Allergy, **49**: 533–539. doi:10.1111/j.1398-9995.1994.tb01125.x. PMID:7825720.

White, T.J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* PCR protocols. A guide to methods and applications. *Edited by* M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White. Academic Press, San Diego, Calif. pp. 315–324.

Zaleski, K. 1927. Über die in Polen gefundenen Arter der Gruppe *Penicillium* Link. I, II und III Teil. Bull. Acad. Pol. Sci. Ser. Sci. Math. Nat. Ser. B. Sci. Nat. **1927**: 417–563.