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A revision of the history of the *Colletotrichum acutatum* species complex in the Nordic countries based on herbarium specimens

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One sentence summary: Sequencing parts of the fungal ITS region of tissues with antrachnose symptoms from herbarium specimens changes the history of the *Colletotrichum acutatum* species complex in the Nordic countries.

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ABSTRACT

Herbaria collections containing plants with disease symptoms are highly valuable, and they are often the only way to investigate outbreaks and epidemics from the past as the number of viable isolates in culture collections is often limited. Species belonging to the *Colletotrichum acutatum* complex infect a range of important crops. As members of the *C. acutatum* complex are easily confused with other *Colletotrichum* species, molecular methods are central for the correct identification. We performed molecular analyses on 21 herbaria specimens, displaying anthracnose symptoms, collected in Norway and Denmark before the first confirmed findings of *C. acutatum* complex members in this region. Sequencing parts of the fungal ITS regions showed that members of the species complex were present in 13 of the 21 specimens collected in different parts of Norway and Denmark between 1948 and 1991, representing seven plant hosts (three cherry species, apple, raspberry and rhododendron). This is the first time herbarium specimens have been used to study these pathogens under Nordic conditions. Differences in the ITS sequences suggest the presence of different genotypes within the complex, indicating a well-established population.

Keywords: herbarium; PCR; *Malus*; *Prunus*; *Rhododendron*

INTRODUCTION

Biologists have for centuries stored dried plant material in herbaria and thereby making valuable collections for the future. Originally, herbaria were used for morphological investigations but molecular methods have opened completely new possibil-

ities. PCR followed by sequencing is particularly popular (e.g. Bruns, Fogel and Taylor 1990; O'Gorman *et al.* 2008; Brock, Döring and Bidartondo 2009; Uematsu *et al.* 2012). Recently, Lavoie (2013) showed in a comprehensive literature review that the number of studies using herbaria is rapidly growing but only 5% of the hitherto published herbaria papers were studying plant pathogens.

A very prominent example of the use of herbaria samples is the identification of the causal agent of the Irish Potato Famine in the 1840s as *Phytophthora infestans* haplotype Ia (Ristaino, Groves and Parra 2001; May and Ristaino 2004). A follow-up study investigating potato samples from the Rothamsted archives showed that haplotype Ia was still present in the UK 30 years after the outbreak and that it was probably the only haplotype present (Ristaino, Hu and Fitt 2013). *Colletotrichum acutatum* was first described as a disease-causing organism on papaya, strawberry and tomato in Australia (Simmonds 1965) and dried cultures were deposited, but no type specimen was selected. Three years later Simmonds designated a holotype and six paratypes from three different hosts (*Carica papaya*, *Capsicum frutescens* and *Delphinium ajacis*; Simmonds 1968). The type specimens were selected by Simmonds to represent the variability of *C. acutatum*, but the selection of seven types from different hosts might have been the beginning of the broad species concept of *C. acutatum* (Than *et al.* 2008). *Colletotrichum acutatum* has since the first description been recorded worldwide (Peres *et al.* 2005; Damm *et al.* 2012). As seen for a number of species within the *Colletotrichum* genus, the species definition of *C. acutatum* is very broad, and it should therefore be regarded a species complex (also referred to as *C. acutatum* sensu lato; Sutton 1992; Du *et al.* 2005; Damm *et al.* 2012). However, the broad species definition has made the correct identification of members of the *C. acutatum* species complex difficult as they have overlapping morphological characteristics, overlapping host range and cause similar symptoms as other *Colletotrichum* species, (Freeman, Shabi and Katan 2000; Cannon, Buddie and Bridge 2008; Damm *et al.* 2012). The identification of the bitter rot pathogen on cherry in Norway is an example of this confusion as the pathogen was first identified as *C. gloeosporioides* (Børve and Stensvand 2004). However, PCR-based methods later showed that the disease-causing fungus was in fact a member of the *C. acutatum* species complex (Stensvand *et al.* 2006).

As a consequence of the lack of morphological characters suitable for identification, characterization of populations within the *C. acutatum* species complex has often been based on molecular methods (e.g. Johnston and Jones 1997; Lardner *et al.* 1999; Sreenivasaprasad and Talhinhas 2005). The molecular groups (A1–A8) made on the basis of internal transcribed spacer (ITS) sequences by Sreenivasaprasad and Talhinhas (2005) have gained broad acceptance and more recently, Damm *et al.* (2012) proposed a complete revision of the *C. acutatum* complex with a split into 31 species based on both morphological and molecular data. In Damm's revision, *C. acutatum* (*sensu stricto*) only covers isolates referred to group A5 by Sreenivasaprasad and Talhinhas (2005). Damm *et al.* (2012) used 331 fungal strains for their investigation including a number of strains originally isolated in Northern Europe and some of them were collected in the first part of the 20th century. However, members of the *C. acutatum* species complex were first identified as plant pathogens in the Nordic countries in the late 1990s and only a very limited number of isolates collected before this period can be found in culture collections (Damm *et al.* 2012). However, the Norwegian Institute for Agricultural and Environmental Research and the University of Copenhagen in Denmark house herbaria collections of infected plants. The aim of the present study was therefore to investigate whether members of the *C. acutatum* species complex were present in herbarium specimens with anthracnose symptoms, thereby confirming the presence or absence of members of this complex in Norway and Denmark long before the first official reports.

MATERIALS AND METHODS

Plant material

The herbaria specimens used in this study were from the plant pathogenic herbaria at the Norwegian Institute for Agricultural and Environmental Research and Copenhagen University. These two collections were searched for specimens collected in Northern Europe clearly displaying symptoms characteristic for members of the *C. acutatum* species complex (Fig. 1). In total, 21 specimens collected during 1948–91 and representing nine different plant species were selected from the herbaria in Norway and Denmark for further examination (Table 1). The disease-causing pathogens of the specimens were originally recorded as *Gloeosporium rhododendri*, *Glomerella cingulata*, *C. gloeosporioides* or *Colletotrichum* sp.

DNA extraction

All experiments were carried out in laboratories with no history of work with members of the *C. acutatum* species complex and with new pipettes, kits and reagents in order to avoid contamination during DNA extraction, PCR amplification and ligation. Furthermore, filter tips were used throughout the experiments.

A lesion was exited from each herbarium specimen and ground to a fine powder under liquid nitrogen using a mortar and pestle. DNA extractions were carried out according to a protocol from Doyle and Doyle (1987), but with modifications. Approximately 20 mg ground tissue was added to 720 μl CTAB extraction buffer (2% hexadecyltrimethylammoniumbromide (CTAB), 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl (pH = 8) and 0.2% mercaptoethanol). Samples were incubated at 60°C for 30 min. with gentle mixing every 10 min. This solution was extracted once with phenol/chloroform (1:1) followed by one extraction with chloroform/isoamylalcohol (1:24). Finally, DNA was precipitated with isopropanol, and pellets were washed with washing buffer (76% ethanol and 10 mM ammoniumacetate). DNA was resuspended in 50 μl TE buffer and visualized on an ethidium bromide-stained 0.8% agarose gel run in TBE buffer.

PCR, sequencing and bioinformatics

PCR mixtures contained 1× *Taq* PCR standard buffer including 1.5 mM MgCl$_2$ (Ampliqon, Odense, Denmark), 0.15 mM dNTP, 0.5 μM of each forward and reverse primer and 2.5 U *Taq* polymerase (Ampliqon). To each reaction, 0.5 or 1 μl of either undiluted or 10× diluted genomic DNA was added. The PCR reactions were made in a total volume of 20 μl, and the PCR amplification was performed using a Mastercycler Personal (Eppendorf, Hamburg, Germany). An initial denaturation at 95°C for 5 min was followed by incubation at 35 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 30 s. The PCR was completed with a final extension step at 72°C for 5 min. The PCR products were visualized on an ethidium bromide-stained 1.5% agarose gel run in TBE buffer.

PCR amplification was carried out using the primers acut1 and col2 (Martinez-Culebras *et al.* 2003) specifically targeting members of the *C. acutatum* species complex. In cases where this primer set did not amplify a PCR product, the primers FITS-Fw1 and FITS-R1 (Louarn *et al.* 2013) were used. These primers were designed to specifically amplify the ITS2 region of the ribosomal DNA in ascomycetes. Cloning and sequencing was carried out as described by Sundelin, Collinge and Lübeck (2009), and the DNA sequences of acut1/col2 amplified PCR products were deposited
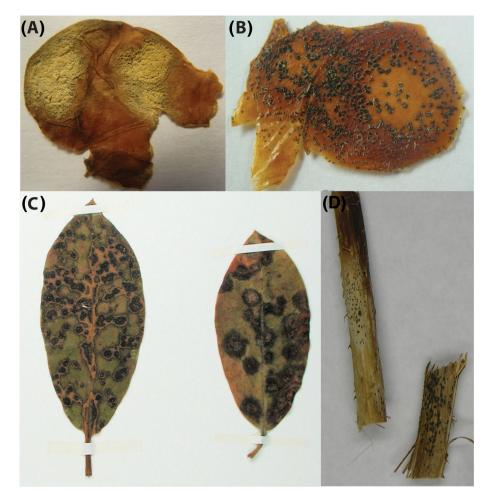
Figure 1. Pictures of selected herbarium specimens where fungi within the *C. acutatum* species complex were detected in this study. (**A**) *Prunus cerasus* specimen N7, (**B**) *Malus domestica* specimen N76, (**C**) *Rhododendron* sp. specimen DK1 and (**D**) *Rubus* sp. specimen N109.

in GenBank. GenBank (www.ncbi.nlm.nih.gov) and the UNITE database (Nilsson *et al.* 2014; http://unite.ut.ee) were searched using BLAST to identify the organism of origin of the resulting ITS sequences. Sequences that turned out to be members of the *C. actuatum* complex were aligned with selected sequences from the complex representing each of the groups A1–A8 (Sreenivas-aprasad and Talhinhas 2005) using ClustalW incorporated in the CLC Main Workbench software (version 6.5). DNA from herbaria specimens that tested positive for *C. acutatum* complex members was also used in PCR reactions using the primer sets TBCA/TB5 targeting the β-tubulin (tub2) gene (Talhinhas *et al.* 2002, 2005), GDF1/GDR1 targeting a 200-bp intron in the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene (Guerber *et al.* 2003), CHS-354R/CHS-79F targeting the chitin synthase 1 gene (Carbone and Kohn 1999), ACT-512F/ACT-783R targeting the actin gene (Carbone and Kohn 1999) and CYLH3F/CYLH3R targeting the histone 3 gene (Crous *et al.* 2004). These primer sets were selected as they were used for species identification in Damm *et al.* (2012).

RESULTS

DNA isolated from the herbaria specimens was very fragmented as it appeared as a smear with a size below 500 bp on the gel, and there was no correlation between the age of the herbarium specimen and the level of fragmentation of the extracted DNA. In 14 out of the 21 samples, the PCR products amplified with either the acut1/col2 or the FITS-Fw1/FITS-R1 primers, originated from fungi being able to cause the symptoms displayed on herbarium specimens (Table 1). *Colletotrichum acutatum* complex members were found in 13 samples, in which 10 were identified by the acut1/col2 primers and 3 by the FITS-Fw1/FITS-R1 primers. The host plants of these specimens were sweet cherry (*Prunus avium*), sour cherry (*P. cerasus*), hybrid cherry (*P. effusa*), apple (*Malus domestica*), raspberry (*Rubus ideaus*) and rhododendron (*Rhododendron* sp.). In one of these samples (N109, *Rubus* sp.), *Didymella applanata* was also present, and this fungus may also have been responsible for the displayed symptoms. The specimen DK3 (beech, *Fagus sylvatica*) was the only specimen where a possible disease-causing organism, the beech anthracnose pathogen *Apiognomonia errabunda*, was found without the presence of *C. acutatum* species complex members. The PCR reactions using the other primers than acut1/col2 or FITS-Fw1/FITS-R1 did not amplify a product for any of the herbarium specimens.

The PCR fragments amplified with the primers acut1/col2 were 270 bp long, and the BLAST search in GenBank and the UNITE database revealed that these primers only amplified DNA from members of the *C. acutatum* complex. The sequences of the acut1/col2 amplified products were deposited in

Table 1 Herbarium specimens examined in this study. For each specimen the host species, locality for the collection as well as the collection date are stated. For each primer set used, the plant pathogenic fungi that could possibly be associated with the observed symptom are stated, together with the GenBank accession number. The DNA sequences of the FITS-Fw1 /FITS-R1 amplified PCR-products are presented in Table S1 (Supporting Information).

ID number	Pathogen stated on the specimen	Host	Locality (country, county)	Collection date (dd/mm year)	Identified pathogen using primer acut1/col2	Accession number	Identified plant pathogen using primer FITS-Fw1/FITS-R1
N1	Glomerella cingulata	Prunus avium	NO, Hordaland	23/8 1952	C. acutatum	JQ775540	n.t.
N5	G. cingulata	P. avium	NO, Hordaland	9/8 1991	C. acutatum	JQ775541	n.t.
N6	G. cingulata	P. cerasus	NO, Hordaland	31/7 1948	n.d		C. acutatum[a]
N7	G. cingulata	P. cerasus	NO, Hordaland	5/8 1948	n.d		C. acutatum
N8	G. cingulata	P. cerasus	NO, Hordaland	15/8 1951	C. acutatum	JQ775542	n.t.
N17	G. cingulata	P. cerasus	NO, Hordaland	9/8 1954	C. acutatum	JQ775543	n.t.
N42	G. cingulata	P. cerasus	NO, Hordaland,	18/8 1954	C. acutatum	JQ775544	n.t.
N47	G. cingulata	P. cerasus	NO, Hordaland	24/8 1954	n.d.		n.d.[b]
N58	G. cingulata	P. cerasus	NO, Buskerud	15/9 1960	n.d.		n.d.[c]
N60	G. cingulata	P. cerasus	NO, Akershus	25/8 1981	C. acutatum	JQ775545	n.t.
N62	G. cingulata	P. effusa	NO, Hordaland	12/8 1954	n.d		C. acutatum[d] Monilinia laxa
N64	G. cingulata	P. pumila	NO, Hordaland	18/7 1962	n.d		M. laxa
N74	G. cingulata	Malus domestica	NO, Sogn og Fjordane	1947	n.d		n.d.
N76	G. cingulata	M. domestica	NO, Hordaland	6/2 1957	C. acutatum	JQ775546	n.d.[e]
N80	G. cingulata	M. domestica	NO, Akershus	22/1 1964	n.d.		n.d.[f]
N84	G. cingulata	M. domestica	NO, Buskerud	2/9 1957	C. acutatum	JQ775547	n.t.
N101	G. cingulata	Sorbus aucuparia	NO, Telemark	9/10 1964	n.d.		n.d.
N109	Colletotrichum sp.	Rubus idaeus	NO, Akershus	25/8 1979	C. acutatum	JQ775548	n.t.
DK1	G. cingulata	Rhododendron sp.	DK, Nordjylland	6/5 1988	C. acutatum	JQ775549	n.t.
DK2	G. rhododendri	R. catawbiense	DK, Midtjylland	29/6 1971	n.d		n.d.
DK3	C. gloeosporioides	Fagus sylvatica	DK, Nordsjaelland	29/4 1990	n.d		Apiognomonia errabunda

The following fungi have been found in addition to the above mentioned: [a]N6: *Exserohilum rostratum*, [b]N47: *E. rostratum, Ampelomyces* sp., [c]N58: *Aureobasidium pullulans, Neofabraea malicorticis*, [d]N62: *Phoma eupyrena, N. malicorticis, E. rostratum, A. pullulans*, [e]N76: *Davidiella tassiana*, [f]N80: *Epicoccum nigrum*. NO: Norway. DK: Denmark. n.d.: no pathogen detected. n.t.: not tested.

GenBank under the accession numbers JQ775540–JQ775549. The PCR products amplified using FITS-Fw1/FITS-R1 were approximately 160 bp long, and these sequences are included in Table S1 (Supporting Information). In addition to members of the *C. acutatum* complex, a number of saprotrophic fungi were identified in the samples amplified with fungal primers FITS-Fw1/FITS-R1 (Table 1).

The alignment of the sequences from the herbaria specimens (Supporting Information, Fig. S1) showed that they were highly similar, both mutually and with the sequences retrieved from GenBank. Two herbaria specimens (N6 and N7) proved to contain two different genotypes each named N6-1, N6-2, N7-1 and N7-2, respectively (Supporting Information, Fig. S1). The genotypes N6-1 and N6-2 differed in one nucleotide, whereas N7-1 and N7-2 differed in four nucleotides. None of the herbaria specimens could uniquely be ascribed to one of the species described in Damm *et al.* (2012) using GenBank or the UNITE database searches or to a specific phylogenetic group A1–A8 described by Sreenivasaprasad and Talhinhas (2005).

DISCUSSION

For the first time, the presence of members of the *C. acutatum* species complex has been shown in old herbarium collections, collected in the Nordic countries, adding a new historical angle to the study of this fungus. Herbaria are valuable scientific collections, and the molecular methods used here and in other studies (Ristaino, Hu and Fitt 2013) could not have been foreseen at the time of collections. These herbarium specimens are snapshots and are a rich and unique source of information with significance to studies of e.g. molecular phylogenetics, fungal biodiversity or fungicide resistance.

We used PCR to examine samples collected from 1948 to 1991, and proved that the pathogen was present in Norway in 1948 and in Denmark in 1988. Investigations of the distribution of isolates within the *C. acutatum* species complex were primarily initiated in Europe after serious outbreaks of strawberry black spot in the 1990s, and the Nordic countries were no exception (Stensvand *et al.* 2001; Sundelin *et al.* 2005). At that time it was believed to be a newly introduced pathogen but with the results presented here, this does not seem to be the case. This therefore leads to the implication that the introduction either happened earlier or that the complex is in fact indigenous to the Nordic countries. The discovery of members of the *C. acutatum* species complex on seven different plant hosts (three cherry species, apple, raspberry and rhododendron), all displaying clear symptoms and being collected in different regions of Norway and in Denmark could confirm the latter assumption. Several isolates from the great revision of the *C. acutatum* species complex by Damm *et al.* (2012) originated from plants in Northern Europe, e.g. *C. godetiae* (from *Clarkia hybrida*, Denmark, 1943), *C. kinghornii* (from *Phormium tenax*, UK, 1935) and *C. salicis* (from *Solanum lycopersicum*, Germany, 1914 and from *Salix* sp., UK, 1927). This also points in the direction that members of the complex have a long history in the Nordic countries. Furthermore, Damm *et al.* (2012) have suggested that the morphological description of *Phyllachora amenti* collected in Dovre, Norway by Rostrup (1891) was as an early description of *C. salicis*, thereby making it very likely that at least some members of the *C. acutatum* species complex are native to the Nordic countries.

Even though some historical isolates of the *C. acutatum* species complex are stored in culture collections, the herbaria investigated in the present study have allowed us to make the most comprehensive investigation of historical samples from the Nordic countries to date. Although the obtained *C. acutatum* complex member sequences were highly similar, different genotypes seemed to occur (alignment of the sequences is presented in Fig. S1, Supporting Information). However, because of the small size of the PCR products, and the fact that we were only able to amplify parts of the ribosomal ITS region, we were not able to place them in the new phylogeny made by Damm *et al.* (2012). Thus, the results from the UNITE database search showed that several of the isolates could be both *C. godetiae* and *C. fioriniae*, whereas others could be *C. lupini*, *C. tamarilloi* or *C. sloanei* (data not shown). Basically, the ITS region can be used to distinguish species complexes within the genus *Colletotrichum*, while more genes are needed to identify single species within the complexes (Hyde *et al.* 2014).

Previous investigations in Norway revealed the teleomorph of one of the *C. acutatum* species complex members on naturally infected highbush blueberry fruits (Talgø *et al.* 2007) and in culture from isolates originally infecting apple and other hosts (Stensvand *et al.* 2008), suggesting that the genetic diversity within the populations in Norway could be due to sexual reproduction. The genetic diversity of *C. acutatum* species complex members within the UK has been linked to multiple introductions (Calleja *et al.* 2013), but with our results in mind, the presence of a long existing population on various host plants and the possible sexual recombination could also explain the diversity.

The extracted DNA in this study was highly degraded, which is common when working with herbarium specimens (Bruns, Fogel and Taylor 1990; Lambertini *et al.* 2008). However, our data clearly show the importance of using different PCR primer sets amplifying fragments of different lengths in studies of samples with limited DNA. The successful primer sets used in the present study represents a targeted (acut/col2) and a broader, unbiased approach (FITS-Fw1/FITS-R1). The ITS region has recently been proposed as the universal genetic barcode for fungi (Schoch *et al.* 2012), and the region is present in a large number of copies per cell (Vilgalys and Gonzalez 1990). Thus, small fragments of this region are an appealing target for sequencing samples with low amounts of DNA.

In conclusion, the data we are presenting dates back the first record of *C. acutatum* complex members in the Nordic countries with more than 50 years, and it is the most comprehensive study of historical samples of this fungus to date. Since *C. acutatum* complex members were identified on seven different host species and in different parts of Norway and in Denmark it could be speculated that they are in fact indigenous to this part of the world or they could have been introduced much earlier than previously believed.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSLE online.

REFERENCES

Børve J, Stensvand A. Non-abscised aborted sweet cherry fruits are vulnerable to fruit decaying fungi and may be sources of infections for healthy fruits. *Acta Agr Scand B-S P* 2004;**54**: 31–7.

Brock PM, Döring H, Bidartondo MI. How to know unknown fungi: the role of a herbarium. *New Phytol* 2009;**181**:719–24.

Bruns TD, Fogel R, Taylor JW. Amplification and sequencing of DNA from fungal herbarium specimens. *Mycologia* 1990;**82**:175–84.

Calleja EJ, Ilbery B, Spence NJ, *et al*. The effectiveness of phytosanitary controls in preventing the entry of *Colletotrichum acutatum* in the UK strawberry sector. *Plant Pathol* 2013;**62**:266–78.

Cannon PF, Buddie AG, Bridge PD. The typification of *Colletotrichum gloeosporioides*. *Mycotaxon* 2008;**104**:189–204.

Carbone I, Kohn LM. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 1999;**91**:553–6.

Crous PW, Groenewald JZ, Risède J-M, *et al*. *Calonectria* species and their *Cylindrocladium* anamorphs: species with sphaeropedunculate vesicles. *Stud Mycol* 2004;**50**:415–30.

Damm U, Cannon PF, Woudenberg JHC, *et al*. The *Colletotrichum acutatum* species complex. *Stud Mycol* 2012;**73**:37–113.

Doyle JJ, Doyle EE. Preservation of plant samples for DNA restriction endonuclease analysis. *Taxon* 1987;**36**:715–22.

Du M, Schardl CL, Nuckles EM, *et al*. Using mating-type gene sequences from improved phylogenetic resolution of *Colletotrichum* species complexes. *Mycologia* 2005;**97**:641–58.

Freeman S, Shabi E, Katan T. Characterization of *Colletotrichum acutatum* causing anthracnose of anemone (*Anemone coronaria* L.). *Appl Environ Micob* 2000;**66**:5267–72.

Guerber JC, Liu B, Corell JC, *et al*. Characterization of diversity in *Colletotrichum acutatum sensu lato* by sequencing analysis of two gene introns, mtDNA and intron RFLPs and mating compatibility. *Mycologia* 2003;**95**:872–95.

Hyde KD, Nilsson RH, Alias SA, *et al*. One stop shop: backbones trees for important phytopathogenic genera: I (2014). *Fungal Divers* 2014;**67**:21–125.

Johnston PR, Jones D. Relationships among *Colletotrichum* isolates from fruit-rots assessed using rDNA sequences. *Mycologia* 1997;**89**:420–30.

Lambertini C, Frydenberg J, Gustafsson MHG, *et al*. Herbarium specimens as a source of DNA for AFLP fingerprinting of *Phragmites* (Poaceae): possibilities and limitations. *Plant Syst Evol* 2008;**272**:223–31.

Lardner R, Johnston PR, Plummer KM, *et al*. Morphological and molecular analysis of *Colletotrichum acutatum sensu lato*. *Mycol Res* 1999;**103**:275–85.

Lavoie C. Biological collections in an ever changing world: Herbaria as tools for biogeographical and environmental studies. *Perspect Plant Ecol* 2013;**15**:68–76.

Louarn S, Nawrocki A, Thorup-Kristensen K, *et al*. Proteomic changes and endophytic micromycota during storage of organically and conventionally grown carrots. *Postharvest Biol Tec* 2013;**76**:26–33.

Martinez-Culebras PV, Querol A, Suarez-Fernandez MB, *et al*. Phylogenetic relationships among *Colletotrichum* pathogens of strawberry and design of PCR primers for their identification. *J Phytopathol* 2003;**151**:135–43.

May KJ, Ristaino JB. Identity of the mtDNA haplotype(s) of *Phytophthora infestans* in historical specimens from the Irish Potato Famine. *Mycol Res* 2004;**108**:471–9.

Nilsson RH, Hyde KD, Pawlowska J, *et al*. Improving ITS sequence data for identification of plant pathogenic fungi. *Fungal Divers* 2014;**67**:11–9

O'Gorman DT, Sholberg PL, Stokes SC, *et al*. DNA sequence analysis of herbarium specimens facilitates the revival of *Botrytis mali*, a postharvest pathogen of apple. *Mycologia* 2008;**100**:227–35.

Peres NA, Timmer LW, Adaskaveg JE, *et al*. Lifestyles of *Colletotrichum acutatum*. *Plant Dis* 2005;**89**:784–96.

Ristaino JB, Groves CT, Parra GR. PCR amplification of the Irish potato famine pathogen from historic specimens. *Nature* 2001;**411**:695–7.

Ristaino JB, Hu CH, Fitt BDL. Evidence for the presence of the founder Ia mtDNA haplotype of *Phytophthora infestans* in the 19th century potato tubers from the Rothamsted archives. *Plant Pathol* 2013;**62**:492–500.

Rostrup FGE. Bidrag til kundskaben om Norges soparter. II. Ascomycetes fra Dovre. *Kristiania Videnskabs-Selskabs Forhandlinger* 1891;**9**:1–14.

Schoch CL, Seifert KA, Huhndorf S, *et al*. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *P Natl Acad Sci USA* 2012;**109**: 6241–6.

Simmonds JH. A study of the species of *Colletotrichum* causing ripe fruit rots in Queensland. *Queensl J Agric Anim Sci* 1965;**22**:437–59.

Simmonds JH. Type specimens of *Colletotrichum gloeosporioides* var. *minor* and *Colletotrichum acutatum*. *Queensl J Agric Anim Sci* 1968;**25**:178A.

Sreenivasaprasad S, Talhinhas P. Genotypic and phenotypic diversity in *Colletotrichum acutatum*, a cosmopolitan pathogen causing anthracnose on a wide range of hosts. *Mol Plant Pathol* 2005;**6**:361–78.

Stensvand A, Aamot HU, Strømeng GM, *et al*. *Colletotrichum acutatum* from Norway frequently develops perithecia in culture. *J Plant Path* 2008;**90**(2 Suppl):93.

Stensvand A, Strømeng GM, Langnes R, *et al*. First report of *Colletotrichum acutatum* in strawberry in Norway. *Plant Dis* 2001;**85**:558.

Stensvand A, Talgø V, Strømeng GM, *et al*. *Colletotrichum acutatum* in Norwegian strawberry production and sources of potential inoculum in and around strawberry fields. *IOBC-WPRS Bull* 2006;**29**:87–91.

Sundelin T, Collinge DB, Lübeck M. A cultivation independent, PCR-based protocol for the direct identification of plant pathogens in infected plant material. *Eur J Plant Pathol* 2009;**123**:473–6.

Sundelin T, Schiller M, Lübeck M, *et al*. First report of anthracnose fruit rot caused by *Colletotrichum acutatum* on strawberry in Denmark. *Plant Dis* 2005;**89**: 432.

Sutton BC. The genus *Glomerella* and its anamorph *Colletotrichum*. In: Bailey JA, Jeger MJ (eds). *Colletotrichum Biology, Pathology and Control*. Wallingford: CABI, 1992, 1–26.

Talgø V, Aamot HU, Strømeng GM, *et al*. *Glomerella acutata* on highbush blueberry (*Vaccinium corymbosum* L.) in Norway. Online. *Plant Health Progress* 2007, DOI: 10.1094 /PHP-2007-0509-01-RS.

Talhinhas P, Sreenivasaprasad S, Neves-Martins J, *et al*. Genetic and morphological characterization of *Colletotrichum acutatum* causing anthracnose of lupins. *Phytopathology* 2002;**92**:986–96.

Talhinhas P, Sreenivasaprasad S, Neves-Martins J, *et al*. Molecular and phenotypic analysis reveal association of diverse *Colletotrichum acutatum* groups and a low level of *C. gloeosporioides* with olive anthracnose. *Appl Environ Microb* 2005;**71**:2987–98.

Than PP, Shivas RG, Jeewon R, *et al*. Epitypification and phylogeny of *Colletotrichum acutatum* J.H. Simmonds. *Fungal Divers* 2008:**28**:97–108.

Uematsu S, Kageyama K, Moriwaki J, *et al*. *Colletotrichum cartami* comb. Nov., an anthracnose pathogen of safflower, garland chrysanthemum and pot marigold, revived by molecular phylogeny with authentic herbarium specimens. *J Gen Plant Pathol* 2012;**78**:316–30.

Vilgalys R, Gonzalez D. Organisation of ribosomal DNA in the basidiomycete *Thanatephorus praticola Curr Genet* 1990;**18**:277–80.