ARTICLE

Colletotrichum: species, ecology and interactions

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Abstract: The presentations of the Special Interest Group meeting *Colletotrichum: species, ecology and interactions*, held on 1 August 2010 during IMC9 in Edinburgh, UK, are outlined. Seven research projects, ranged from systematics and population genetics to host-pathogen interactions and genome projects were presented. The meeting revealed that currently major species complexes in the genus *Colletotrichum* are being revised and the identities of many pathogens clarified on the basis of molecular phylogenies, and that the genomes of four species are sequenced and decoded providing an enormous amount of data that are used to increase our understanding of the biology of *Colletotrichum* species.

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INTRODUCTION

A Special Interest Group meeting *Colletotrichum: species, ecology and interactions*, was held on 1 August 2010 at the 9th International Mycological Congress (IMC9) in Edinburgh, UK. The meeting, organised by Paul Cannon (UK) and Ulrike Damm (The Netherlands), brought together 23 scientists from 12 countries working in different fields of mycology, but with a common interest in the genus *Colletotrichum*. Seven presentations, covering a wide range of topics, ranged from systematics and population genetics to host-pathogen interactions and genome projects. This contribution provides a synopsis of the presentations made at that meeting.

Systematics and identification

The first four presentations dealt with systematics and identification of major *Colletotrichum* species complexes containing various important anthracnose pathogens worldwide. Four of these species are illustrated in Fig. 1. While the identity of many important species still require revision (Hyde *et al.* 2009), molecular techniques improve the delimitation of species that are hard to distinguish based on morphology alone and reveal their phylogenetic relationships (Cai *et al.* 2009, Crouch *et al.* 2009, Damm *et al.* 2009). This will inevitably result in name changes but has implications for everyone working with this genus, especially plant pathologists, and will improve our understanding of the role these species play in nature.

Ulrike Damm gave an overview of her ongoing collaboration with Paul Cannon about the phylogeny of three species complexes. The aim of this project is to delimitate species within these complexes, characterise known and new species and designate epitypes to provide the basis for accurate identifications of *Colletotrichum* species. This goal has so far been achieved for species with curved conidia from herbaceous hosts (Damm *et al.* 2009), which in the past were mostly identified as *C. dematium*. Multi-gene analyses and morphological characterisation revealed several diverse and distantly related species, including four new species. Seven species were epitypified, including *C. dematium* and the type species of the genus, *C. lineola*. A second study confirmed most of the previously recognised groups (Sreenivasaprasad & Talhinhas 2005) within the *C. acutatum* species complex. Most of these could be defined on the basis of type strains or strains suitable for epitypification. Literature reports (Lubbe *et al.* 2004, Johnston *et al.* 2005) and preliminary studies using ITS sequence data indicated that *C. boninense* represents a species complex as well. A multilocus molecular phylogenetic

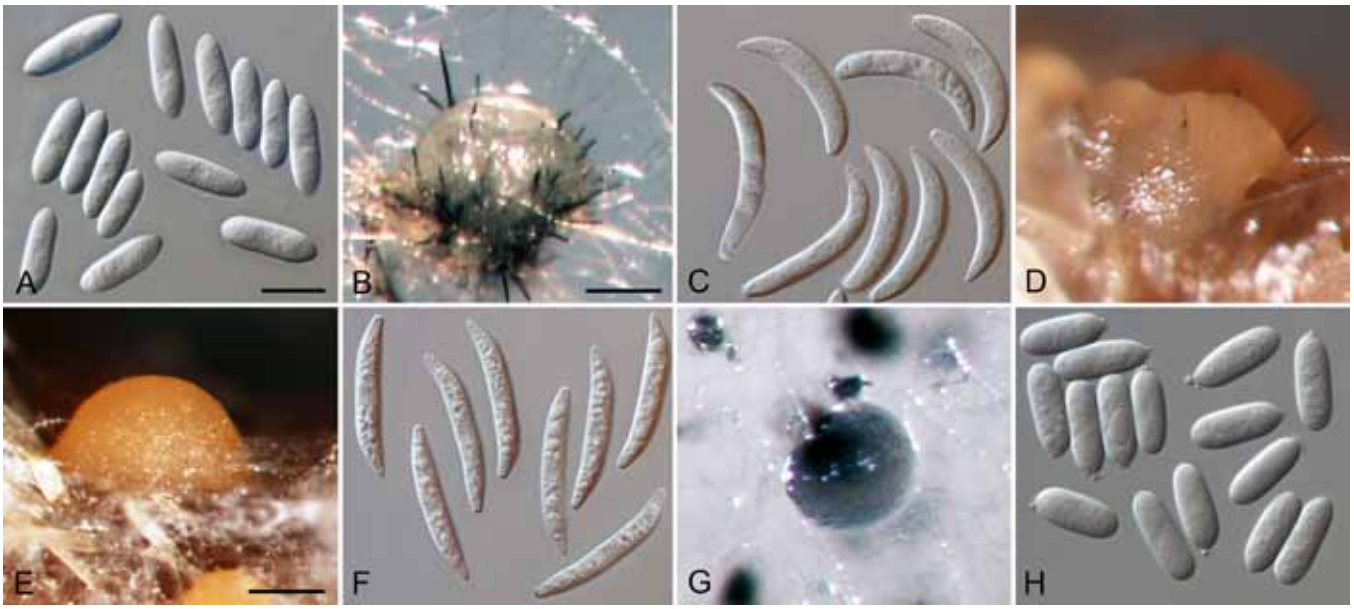

Fig. 1. Conidiomata and conidia of four *Colletotrichum* species. **A, E.** *C. acutatum* (CBS 112996, ex-paratype strain). **B, F.** *C. lineola* (CBS 125337, ex-epitype strain). **C, G.** *C. truncatum* (CBS 151.35, ex-epitype strain). **D, H.** *C. gloeosporioides* (CBS 112999, ex-epitype strain). **A, C, F, H.** Conidia on SNA. **B, G.** Conidiomata on SNA. **D, E.** Conidiomata on *Anthriscus* stem. Scale bars: A = 10 µm, B = 100 µm, E = 200 µm. A applies to A, C, F, H. B applies to B, D, G.

analysis of strains previously identified as *C. boninense* resulted in clades that could be recognised as separate species with differences in host range, distribution and morphology, including *C. boninense sensu stricto*, *Glomerella phyllanthi*, *C. hippeastri* and several presumably new species. Most of the species in the *C. boninense* complex and some in the *C. acutatum* species complex form teleomorph states in culture. Publications on the *C. acutatum* and *C. boninense* species complexes will appear in a 2011 issue of *Studies in Mycology.*

Colletotrichum gloeosporioides sensu lato is a species complex with broad genetic and biological diversity grouped together by similar conidial morphology and ITS sequences. Bevan Weir and Peter Johnston (Landcare Research, Auckland, New Zealand) presented their research on this species complex and possible approaches to species delimitation through the Genealogical Concordance Phylogenetic Species Recognition (GCPSR). It was shown that that the taxa *C. musae*, *C. kahawae*, *C. xanthorrhoeae*, *C. nupharicola*, *C. fragariae,* C. *gloeosporioides* sensu stricto, *C. horii*, *C. theobromicola*, *C. ignotum*, *C. tropicale*, *C. asianum*, *C. siamense*, *C. fructicola* and *C. hymenocallidis*, as well as many putative undescribed species are part of the *C. gloeosporioides sensu lato* complex. They recently characterised and neotypified one of these species, *C. horii* (Weir & Johnston 2010). The GCPSR concept was used to delimit taxa within *C. gloeosporioides sensu lato*. This concept considers that phylogenetic trees of different genes show discordance within a species due to gene flow between individuals. The common node where different gene trees show concordance is considered the speciation point. They applied the GCPSR with eight genes using recently developed Bayesian analysis tool, BUCKy (Ané *et al.* 2007). The GCPSR concept worked well for species delimitation along currently recognised lines, except for *C. kahawae*, which was insufficiently distinct from several genetically similar non-Coffee Berry Disease causing taxa. It was suggested that this may be due to the recent emergence (1920) of *C. kahawae* as a pathogen and that insufficient time had passed for ecological niche specialisation to show as mutations in the genes used. They suggested that *C. kahawae* be recognised at the subspecific rank. A publication on their work on the *C. gloeosporioides sensu lato* species complex will appear in the 2011 Studies in Mycology issue on *Colletotrichum* as well.

Colletotrichum acutatum causes economically significant losses of temperate, subtropical and tropical crops. Globally, *C. acutatum* populations display considerable genotypic and phenotypic diversity. Riccardo Baroncelli (University of Warwick, Wellesbourne, UK) presented his research on evolutionary relationships in *C. acutatum* populations in collaboration with Charles Lane (FERA, Sand Hutton, York, UK) and Prasad Sreenivasaprasad (University of Warwick, Wellesbourne, UK). The overall objective is to understand the evolutionary relationships within the species with particular reference to the pathogen populations associated with the strawberry production systems in the UK. More than 150 *C. acutatum* isolates related to different hosts worldwide have been assembled. Phylogenetic analysis of sequence data from the rDNA block, Mat1-2 and βtubulin-2 genes shows eight distinct genetic groups within *C. acutatum*. The subsets of isolates represented within these genetic groups corresponded to the previously identified groups A1 to A8. Almost all of the homothallic isolates capable of sexual reproduction comprise a single genetic group, A7. Isolates representing populations capable of heterothallic sexual

reproduction belong to two distinct genetic groups A3 and A5. Molecular characterisation of *C. acutatum* populations representing the introduction and spread of the pathogen in the strawberry production systems in the UK showed the presence of three genetic groups (A2, A3 and A4). Their results suggest the existence of *C. acutatum* populations potentially undergoing speciation processes, related to their reproductive behaviour and host association patterns. Further molecular and phenotypic characterisation is in progress.

Lei Cai (Key Laboratory of Systematic Mycology & Lichenology, Institute of Microbiology, Beijing, China) and Kevin Hyde (School of Science, Mae Fah Luang University, Chiang Rai, Thailand) presented their research on *Colletotrichum* species from Asian fruits and leaves. Fruit rots (anthracnose) were previously often attributed to *C. gloeosporioides* and *C. acutatum*. Identifications were, however, based on morphological characters or, if gene sequence data were used, comparisons were often made with wrongly applied names. *Colletotrichum gloeosporioides* was recently epitypified (Cannon *et al.* 2008) so that living cultures and sequence data are, for the first time available for comparison with fresh collections. Analysis of sequence data of 25 isolates (selected from 140 obtained strains based on diversity of host and morphology) from eight tropical fruits are compared with the *C. gloeosporioides* epitype. Contrary to previous assumptions, none of these isolates from tropical fruits was *C. gloeosporioides sensu stricto* (Phoulivong *et al.* 2010). The five gene regions used in this study resolved *C. asianum, C fructicola, C. horii, C. kahawae* and *C. gloeosporioides* in the *C. gloeosporioides* species complex as distinct phylogenetic lineages with high statistical support. Many tested strains could not be assigned to any known taxa in this analysis. They also reported *Colletotrichum* species from *Amaryllidaceae, Orchidaceae, Cordyline fruticosa* and *Jasminum sambac*, with the latter including two new species (Wikee *et al.* 2010), and updated the typifications of *C. coccodes, C. falcatum* and *C. musae*.

Genomic studies

Colletotrichum species provide excellent models for studying fungal-plant interactions (Perfect *et al.* 1999). Several large-scale genome projects are in progress for *Colletotrichum* species aiming to produce high-quality assemblies of the genome sequences to provide resources for comparative genomics and the molecular analysis of fungal pathogenicity, which allows the identification of genes and proteins relevant to each stage of plant infection.

Colletotrichum graminicola is a destructive pathogen of maize, causing stalk rot and leaf blight, while *C. higginsianum* attacks many cultivated forms of *Brassica* as well as *Arabidopsis thaliana*, providing a model pathosystem in which both partners can be genetically manipulated (O'Connell *et al.* 2004). Both pathogens employ a hemibiotrophic infection strategy, but while the biotrophic phase of *C. graminicola* extends into many host cells, that of C. *higginsianum* is confined to single epidermal cells. Richard O'Connell (Max Planck Institute for Plant Breeding Research, Cologne, Germany) gave an overview of the *C. higginsianum* and *C. graminicola* genome research, which he is conducting in collaboration with Lisa Vaillancourt (University of Kentucky, USA), Li-Jun Ma (MIT-Broad Institute, USA) and Mike Thon (CIALE-University of Salamanca, Spain). Comparing the genomes of two species with contrasting pathogenic lifestyles and host specificities will allow them to study lineage-specific expansions and contractions of gene families and identify genes undergoing rapid evolution (diversifying selection), which may be involved in interactions with the host plant, e.g. those encoding secreted effector proteins. The 57.4 Mb genome of *C. graminicola* comprises 13 chromosomes and was sequenced at the Broad Institute (8X Sanger, 11X paired-end 454) giving an assembly of 1,151 contigs in 653 scaffolds (<broadinstitute.org/annotation/genome/colletotrichum_group/MultiHome.html>). The 52.5 Mb genome of *C. higginsianum* comprises 11 chromosomes and was sequenced with a combination of 454 (24X), Illumina paired-end (60X) and Sanger fosmid sequencing (0.2X). These data assembled into 8,301 contigs in 367 scaffolds (<mpiz-koeln.mpg.de/english/research/pmi-dpt/oconnell/index.html>). For genome annotation, 890,000 ESTs were generated from *C. higginsianum* by 454-sequencing cDNA libraries representing appressoria formed *in vitro*, appressoria penetrating leaf epidermis, biotrophic hyphae and necrotrophic mycelium. In addition, ~22,000 Sanger ESTs were obtained from the *in vitro* mycelium of *C. graminicola*. Both genomes have now been annotated by the Broad Institute, with 12,006 protein-coding genes predicted for *C. graminicola* and ~15,900 for *C. higginsianum*.

Colletotrichum orbiculare is an anthracnose fungus which infects *Cucurbitaceae*. Yasuyuki Kubo (Kyoto Prefectural University, Japan) gave an update of the *C. orbiculare* genome project conducted in collaboration with Yoshitaka Takano (Kyoto University, Japan) and Ken Shirasu (RIKEN Plant Science Center, Yokohama, Japan). Pathogenicity, morphology and the hemibiotrophic infection strategy of strain 104-T (=MAFF 240422) are well studied, some of the rationales to select this strain for the genome sequencing project. Moreover, strain 104-T has been very stable for pathogenesis and morphogenesis for many years and a variety of insertional mutants are available. Gene manipulation techniques such as *Agrobacterium tumefaciens*-mediated transformation or protoplast transformation are established (Tsuji *et al.* 2003) and for host parasite interaction studies, a model plant *Nicotiana benthamiana* is being used as a susceptible host. So far, several factors involved in infection related morphogenesis have been identified in *C. orbiculare* 104-T. Signal transduction pathways, such as cAMP dependent pathway and MAP kinase pathway are essential for germination, appressorium development, infection hyphae formation and invasive growth, while the melanin biosynthesis pathway is essential for appressorium function. Several structural and regulatory genes involved in these pathways were identified (Takano *et al.* 1997, Tanaka *et al.* 2009). Peroxisome function is essential for pathogenesis as well; and two peroxisome biogenese gens, PEX6 and PEX13

were functionally analysed (Kimura et al. 2001, Fujihara *et al.* 2010). Recently, it was reported that pexophagy factor ATG26 is essential for appressorium function (Asakura *et al.* 2009). The genome analysis of *C. orbiculare* is now going to be completed, which allows comparisons of genomic data of three *Colletotrichum* species, *C. higginsianum*, *C. graminicola* and *C.orbiculare* that belong to different phylogenetic clades within the genus. These data will provide comprehensive basis for studying the biology of the different *Colletotrichum* species.

A study on host-pathogen interaction between *C. orbiculare* and *N. benthamiana* was presented by Kae Yoshino (Graduate School of Agriculture, Kyoto University, Kyoto, Japan). This project is conducted together with Fumie Sugimoto (Graduate School of Agriculture, Kyoto University, Kyoto, Japan), Hirofumi Yoshioka (Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan), Tetsuro Okuno, and Yoshitaka Takano (both Graduate School of Agriculture, Kyoto University, Kyoto, Japan). By functional screening of *C. orbiculare* cDNAs using *N. benthamiana-A. tumefaciens* in planta expression system they identified a novel effector gene *NIS1* whose product induces cell death in *N. benthamiana*. Deletion of the signal peptide of Nis1 abolished its cell death activity, indicating Nis1 recognition in apoplasts of *N. benthamiana*. The GFP-based localization study together with biochemical analysis revealed that Nis1 with the inducting activity is secreted from *C. orbiculare* cells at fungal invasion phase inside plant tissue. Nis1-mediated cell death was cancelled by virus-induced gene silencing (VIGS) of *SGT1* or *HSP90* in *N. benthamiana*, whereas VIGS of *RAR1* had no effects. These indicate that SGT1 and HSP90, the components of *R*-gene mediated defences, are involved in the Nis1-inducing cell death. Surprisingly, the deletion of *NIS1* has no significant effects on the infection behaviour of *C. orbiculare* on *N. benthamiana*. Overexpression of Nis1 in *Arabidopsis thaliana* caused growth reduction, implying that the *NIS1*-encoded effector has an activity to interfere plant development. These data indicate that *C. orbiculare* secretes the effector that can be recognized by *N. benthamiana*, but this potential effector-triggered immunity might be repressed in this susceptible interaction.

Alan Buddie (CABI Europe-UK, Egham, UK) announced a further genome project for *C. gloeosporioides*. The project is a collaboration between CABI and The Genome Analysis Centre (TGAC), Norwich, UK (<tgac.bbsrc.ac.uk>), to carry out a whole genome sequence of the ex-epitype strain of *C. gloeosporioides* (IMI 356878). The DNA sequencing phase is nearing completion and they are going to start soon with the assembly.

The meeting provided good evidence of the rapidity with which our understanding is improving of *Colletotrichum* genomics, and nicely complemented the outputs of another *Colletotrichum* workshop that was held earlier in 2010, in conjunction with the 10th European Conference on Fungal Genetics in Leeuwenhorst, the Netherlands. It was particularly exciting to witness the increasing power of genomic research tools, and their potential impact on our understanding of fungal systematics and speciation. It provided good opportunities to coordinate research programmes, exchange data and share experiences.

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