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FULL PAPER

Development of appressoria on conidial germ tubes of *Erysiphe* species

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Abstract Modes of branching of appressoria on conidial germ tubes of 36 *Erysiphe* spp. were studied. Only unlobed appressoria, termed *alobatus* pattern, were seen in *E. lonicerae*, *E. magnifica* and *E. symphoricarpi*. Viewed from above with light or scanning electron microscopes, other species had ± irregular lobing, but from below in the plane of contact with the substrate successive dichotomous branchings at 120° were seen to produce a five-lobed appressorium within 6 h. Each division produced a temporarily dormant outward-facing lobe and an inward limb that continued growth and division to form the axis of curved, hooked, single- or double-headed symmetrical or asymmetrical structures in a helicoid cyme-like pattern. Outlines of extracellular material after removal of germinated conidia confirmed this manner of branching. After 36 h some lobes re-divided forming botryose or jigsaw patterns even extending with extra appressoria to form candelabra-like structures. Conidia developed only one true germ tube; rarely secondary unswollen tubes emerged from spare shoulders or ends. The same true germ tubes developed initially on host surfaces, where secondary tubes and/or extensions from appressorial lobes grew into colony-forming hyphae. Lobed appressoria of *Neoerysphe* and *Phyllactinia* also branched at 120°. *Podosphaera xanthii* exhibited a simpler branching pattern.

Keywords Dichotomous branching · Helicoid cyme · Identification · Longitubus · *Pseudoidium*

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Introduction

In a review of the conidial germination types in powdery mildews, the patterns were shown to be relatively uniform with most having germ tubes ending in simple, unlobed appressoria (Cook and Braun 2009). The inside of a petri dish lid with its hydrophobic surface being more favourable than a glass surface for appressorial development revealed distinctly lobed appressoria in the holomorphic taxa *Erysiphe* emend (Fig. 1a–c), *Neoerysiphe* (Fig. 1d), *Podosphaera xanthii* (Fig. 1e) and in the tribe Phyllactinieae (Fig. 1f–h). Of these, the species within *Erysiphe* showed the greatest variation in pattern. Its anamorph *Pseudoidium*, re-described by Cook et al. (1997), is well characterised by conidia maturing singly rather than in a developing chain on the conidiophore. *Erysiphe* emend. now encompasses the previous genera, *Erysiphe s. str.*, *Microsphaera*, *Uncinula* and several smaller segregated genera (Braun and Takamatsu 2000; Braun et al. 2002). The whole taxon contains the highest number of species of powdery mildew, i.e., 259 out of the 516 fully recognised by Braun (1987). Although these species exhibit chasmothecia with a range of appendage types previously used to define the old genera, this morphology was shown by rDNA ITS sequencing not to be of fundamental significance in phylogeny (Takamatsu et al. 1998; Saenz and Taylor 1999; Mori et al. 2000). Still useful at a subgeneric level, the old names are retained to define morphological,

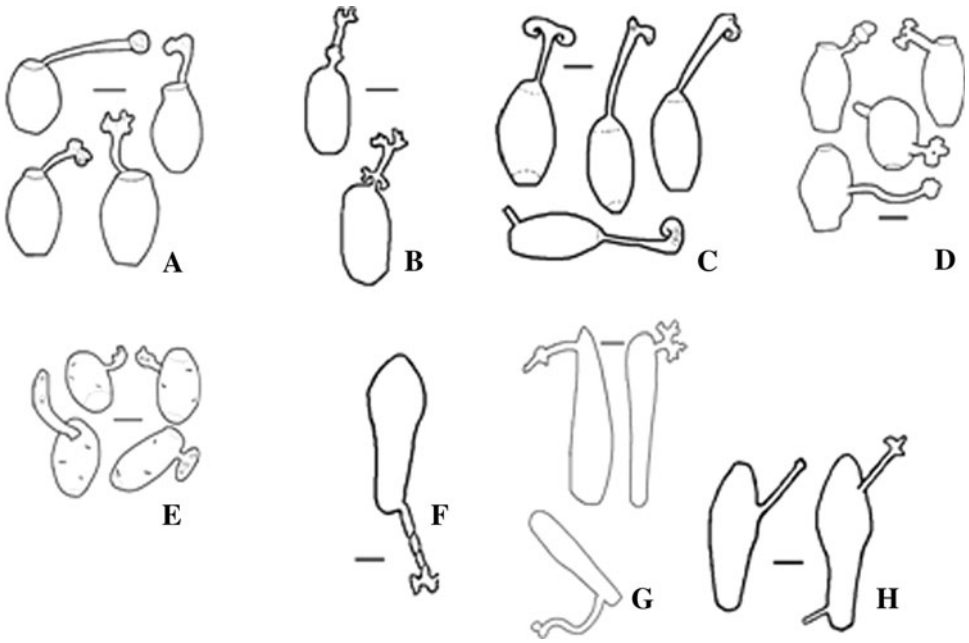

Fig. 1 Conidial germination patterns: **a** *Erysiphe howeana*, **b** *E. carpinicola*, **c** *E. trifoliorum*, **d** *Neoerysiphe galeopsidis*, **e** *Podosphaera xanthii* (syn. *P. fusca*), **f** *Leveillula taurica*, **g** *Pleochaeta indica*, **h** *Phyllactinia guttata*. *Bars* 10 µm (after Cook and Braun 2009)

non-phylogenetic sections within *Erysiphe* emend. So far, it has not been possible to distinguish sections or other groupings within *Pseudoidium*, and so any variation in patterns of conidial germination could be very helpful in identification. Confirmation of this requires an extensive survey of patterns in very many species and specimens, and as a first step, this study investigated the ontogeny and morphology of lobing of germ tube tips in *Erysiphe* emend.

Materials and methods

Over three seasons from June 2008 to August 2010, a standard germination test (Cook and Braun 2009) designed to detect the typical germination type was used on 82 specimens of 36 *Erysiphe* spp. from 49 hosts and 10 locations plus 9 specimens of *Neoerysiphe* spp., *Phyllactinia guttata* and *Podosphaera xanthii* from four locations. The sources, dates of collection and herbarium accession numbers of key species mentioned in the text are listed in Table 1. The standard test involves lightly tapping infected shoots to remove older conidia before placing their bases in water in still air overnight. A leaf surface bearing fresh sporulation, held ca. 2 cm above the inner surface of a plastic petri dish lid (PDL), was tapped sharply to release conidia. The lid was placed over its base containing paper tissue moistened with a saturated aqueous solution of potassium sulphate, giving a relative humidity of ca. 97% to reduce condensation and prevalence of the longitubus pattern. After incubation for 24–48 h in the dark at room temperature (20–25°C), the inner surface of the lid was examined directly under the compound microscope. If germination was very low, it was sometimes improved by placing shoot bases 48 h in flower preserver (sugar 20 g, spirit vinegar 20 ml, sodium hypochlorite bleach as domestic product 2.5 ml, all in 2 l water). Where possible at least 50 germ tubes per specimen were examined, and the mode of branching, shape, size and number of lobes on each tube were recorded with freehand drawings and photographs from a hand-held digital camera (Samsung Digimax S600).

In August 2010, germinating conidia of *Erysiphe heraclei* and *E. trifoliorum* (*E. trifolii*) were compared on the PDL and host surfaces, respectively detached leaves of *Heracleum sphondylium* and *Trifolium dubium* collected in the field around York with no evidence of mildew. After rinsing with water, leaflets of the *Trifolium* and pieces, ca. 2 cm^2, from leaves of *Heracleum* were spread adaxial surfaces uppermost onto a folded sheet of damp paper towel covering one half of the inside of the PDL. The lids were exposed a few minutes in a ventilated but dust-protected container until surfaces dried. Inoculum was then tapped equally over both halves of the lid. Leaf pieces of *Brassica oleracea* var. *italica* (broccoli), a non-host plant, were inoculated in the same way with both pathogens. The petri dish bases containing taped pieces of paper towel dampened with a saturated solution of potassium sulphate were inverted over the lids and incubated at ambient daylight and room temperature. After 36 h, germinated conidia were removed from the leaf surfaces with transparent adhesive tape, stained with cotton blue in lactophenol and

Table 1 Sources of powdery mildews[a] mentioned in text

Species of *Erysiphe*	Host	Locality, Date, Accession number
adunca	*Salix caprea*	Llan Caer, Wales, 3 Oct 2009 K(M)166499
akebiae	*Akebia quinata*	Harlow Carr, Harrogate, North Yorkshire, 13 Sep 2009 K(M)166496
alphitoides	*Quercus* robur	Guernsey, Channel Isles, 10 Jun 2008
		RHS Garden Wisley, Surrey, 10 Jun 2009 K(M)166495
		Heworth Without, York, Oct 2009
	Q. petraea	Le Luc, Var, France, 8 Oct 2010[b] K(M)167379
alphitoides, as *Oidium bixae*	*Bixa orellana*	Eden Project, St Austell, Cornwall, Apr 2010 K(M)167376
aquilegiae var. aquilegiae	*Aquilegia vulgaris*	Heworth Without, York, 10 Sep 2009 K(M)166494
		Wisley, 19 Aug 2010
	Caltha palustris	Heworth Without, York, 12 Jul 2010
arcuata	*Carpinus betulus*	Wisley, 26 May 2009 K(M)166492; 20 Sep 2009
		Cuiseaux, Saône-et- Loire, France, 8 Oct 2010[b] K(M)167378
azaleae (anamorph) (teleomorph)	*Rhododendron luteum*	Wisley, 12 Jun 2009 K(M)167403
		Wisley, Oct 2009[b] K(M)167404
berberidis	*Berberis thunbergii* var. *atropurpurea*	Harpenden, Herts, 10 Jul 2009[b] K(M)167380
	Mahonia aquifolium	Heworth, York, 10 Sep 2009 K(M)167387
betae	*Beta vulgaris*	Heworth Without, York, 12 Oct 2008; 10 Sep 2009 K(M)166493
catalpae	*Catalpa bignonioides* var. *aurea*	Heworth Without, York, 20 Aug 2010 K(M)167391
circaeae	*Circaea lutescens*	Llan Caer, Wales, 2 Oct 2009
convolvuli	*Convolvulus arvensis*	Heworth, York, 18 Sep 2007; 14 Sep 2009; 17 Oct 2010 K(M)167388
elevata	*Catalpa bignonioides*	Wisley, Sep 2004 K(M)129494
flexuosa	*Aesculus hippocastanum*	Harlow Carr, Harrogate, 13 Sep 2009[b] K(M)167374
		St Albans, 17 Jul 2010 K(M)167383
heraclei	*Anthriscus sylvestris*	Heworth, York, 14 Oct 2008; 17 May 2009; 6 Aug 2010 K(M)167386
	Heracleum sphondylium	Guernsey, 10 Jun 2008
		Heworth, York, 14 Oct 2008; 15 Sep 2009 K(M)167389
		St Albans, 17 Jul 2010 K(M)167382
howeana	*Oenothera biennis*	Heworth Without, York, 20 Sep 2007; 14 Oct 2008 K(M)166491; 17 May 2009
Pseudoidium anamorph, cf. *E. lonicerae*	*Lonicera periclymenum*	Guernsey, 10 Jun 2008
		Heworth, York, 1 Nov 2008; 26 May 2009; 16 Jul 2009 K(M)166490
		Wisley, 19 Aug 2010 K(M)167402
	L. tragophylla	Wisley, 19 Aug 2010 K(M)167401
magnifica	*Magnolia* × *soulangiana* Alba	Wisley, 20 Sep 2009 K(M)167400
	Magnolia × cv. Judy	Wisley, 19 Aug 2010 K(M)167399
	cv. Susan	Wisley, 19 Aug 2010 K(M)167398
pisi	*Pisum sativum* Mangetout	Heworth Without, York, 9 Sep 2009 K(M)167392
	Lathyrus pratensis	Heworth, York, 14 Oct 2009[b] K(M)166489
platani	*Platanus* × *acerifolia*	Fréjus, Var, France, 15 Aug 2009; 15 Sep 2010[b] K(M)167377
polygoni	*Polygonum arenastrum*	Heworth Without, York, 10 Sep 2009[b] K(M)167394
		Harpenden, Herts, 17 Jul 2010 K(M)167381

Table 1 continued

Species of *Erysiphe*	Host	Locality, Date, Accession number
russellii	*Oxalis europaea* (*stricta*)	York, 21 Aug 2009 K(M)167385
symphoricarpi	*Symphoricarpos albus*	Heworth, York, 12 Oct 2008 K(M)166498; 2 Nov 2008; 2 Sep 2009; 8 Jul 2009
trifoliorum	*Trifolium dubium*	Heworth Without, York, Sep 2008; 9 Jun 2009; 17 Sep 2009; 6 Aug 2010 K(M)166501
	T. hybridum,	Heworth, Oct 2009
	Melilotus officinalis	York, 3 Oct 2008; 10 Sep 2009 K(M)167384
Other genera		
Neoerysiphe galeopsidis	*Catalpa bignonioides* var. *aurea*	Wisley, 20 Oct 2004 K(M)166497; 18 Jun 2009
	Acanthus mollis	Laleham, Staines Middx, 19 Aug 2010 K(M)166397
	A. spinosus	Wisley, 19 Aug 2010 K(M)167397
N. geranii	*Geranium pratense* cult.	Firswood, Manchester, 7 Sep 2009[b] K(M)167375
	G. magnificum cult.	Wisley, 20 Sep 2009[b] K(M)167395
	ex *Geranium* sp.	Wisley, 19 Aug 2010 K(M)167396
Striatoidium anamorph, cf. *N. nevoi* var. *nevoi*	*Lapsana communis*	Heworth, York, 23 Oct 2008 K(M)167390; 8 Jul 2009
Phyllactinia guttata	*Salix caprea*	Tintern Station, Monmouth, 4 Oct 2009
Podosphaera xanthii	*Taraxacum officinalis*	Heworth Without, York, 17 Jul 2008 K(M)166500; 12 Oct 2010
		Heworth, 4 Nov 2010

[a] All collections made by first author except *N. galeopsidis* on *A. mollis* collected by M. Parslow

[b] Chasmothecia present

viewed conventionally under the light microscope for comparison with germination on the uncovered portion of the PDL. Numbers of lobes per appressorium and lengths of germ tubes (expressed as a proportion of conidial width in steps of one quarter) were assessed on up to 50 germinated conidia per test. These data were arranged in numerical order of categories, i.e. lobe number per appressorium and number of quarter conidial widths per germ tube, so that the middle value in each series represented the median value. The dishes were incubated for a further 21 days to allow powdery mildew colonies to develop on the leaf material. The experiment was repeated with inoculations of *E. aquilegiae* var. *aquilegiae*, *E. lonicerae* and *E. magnifica* on their respective host surfaces, namely leaflets of *Aquilegia vulgaris*, whole leaves of *Lonicera periclymenum* and leaf pieces of *Magnolia soulangiana*, all from non-infected plants growing in a garden in York. The *Lonicera* and *Magnolia* powdery mildews each came from two sources, *E. lonicerae* inoculum from *L. periclymenum* and *L. tragophylla* and *E. magnifica* inocula from *Magnolia* hybrids Susan and Judy, all from Wisley. After 48 h incubation, they were examined as above. In this test broccoli was not inoculated, but non-inoculated pieces of the *Magnolia* leaves were incubated and examined in the same way, as a test for contamination.

To determine whether ascospores could germinate on a dry plastic surface, in March 2010 portions of overwintered leaves of *Quercus robur* seedlings bearing chasmothecia of *E. alphitoides* were placed over two 15-mm-diameter rings in a PDL so that chasmothecia were suspended 5 mm above the surface. The leaf material was kept moist by covering it with a small ball of water-soaked cotton wool. Some ascospores were also liberated by gently crushing detached chasmothecia in water drops on the inside of another PDL. Both dishes were incubated as above and examined microscopically at 2-day intervals for evidence of ascospore germination.

Conidial germination in *E. heraclei* and *E. howeana* was examined using a scanning electron microscope (SEM). The host leaf surface and aluminium foil (having a surface as smooth as plastic) were used in place of the PDL, which is not ideal for use under the SEM. Therefore, conidia were tapped over detached leaves of the hosts (respectively *Anthriscus sylvestris* and *Oenothera biennis*) placed in a damp chamber and also over a PDL lined with a sheet of aluminium foil. After incubation for 7 or 24 h at 20°C, samples, ca. 1 cm^2, were sputter coated with gold and viewed directly at 5 kV accelerating voltage using a Philips XL-20 SEM fitted with a lanthanum hexaboride emitter as described by Cook et al. (1997). Prior to the SEM examination, confirmation of germination on both surfaces was

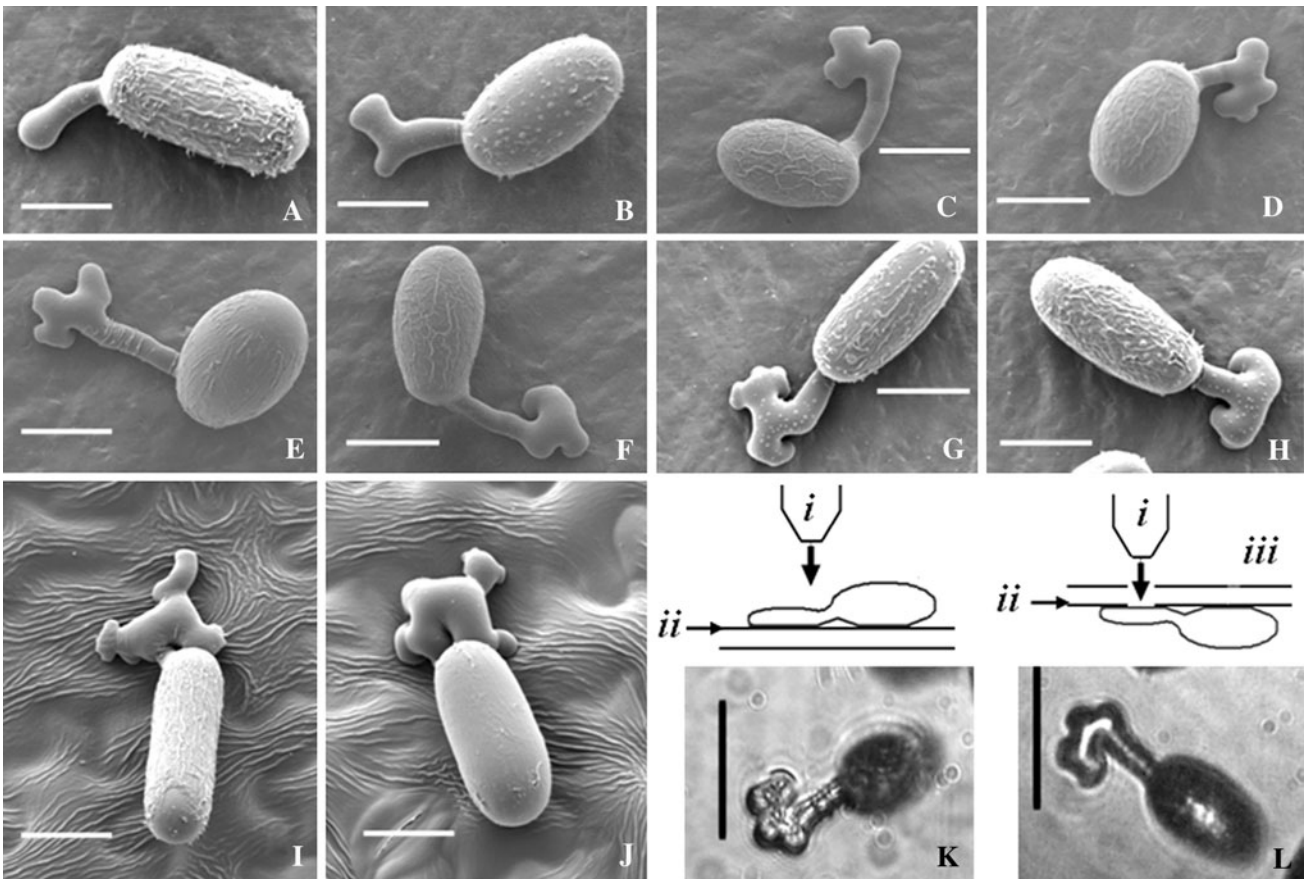

Fig. 2 Scanning electron micrographs of germinated conidia after 24 h on aluminium foil: **a**–**d** *Erysiphe howeana*, **e**–**h** *E. heraclei*, **i**, **j** *E. heraclei* on host leaf (*Anthriscus sylvestris*). **k**, **l** Light microscope images of the same conidium of *E. akebiae* (**k** viewed from above, **l** from below, *i* objective, *ii* inner surface of Petri dish lid, *iii* lid reversed). *Bars* **a**–**j** 10 μm; **k**, **l** 20 μm

obtained by removing conidia with a strip of transparent adhesive tape and viewing them under the light microscope.

Results

In all *Erysiphe* spp. examined, conidial germ tubes were typical of the pseudoidium type of *Erysiphe* emend., i.e. they were terminal or subterminal, very rarely lateral, sessile to long, length mostly 0.25–2 times the width of the conidium (Fig. 1a–c, after Cook and Braun 2009). In most species, outlines of appressoria ranged from simple unswollen or club shapes to highly multilobed, one-sided or symmetrical (two-sided) structures (Fig. 1a). When viewed conventionally from above, lobes appeared as blobs (Fig. 2k). They appeared similar on aluminium foil under the SEM, with *E. howeana* and *E. heraclei* (Fig. 2a–h); on the host surface, branching was even more irregular (Fig. 2i, j). The SEM study showed that the basic framework of branching formed within 7 h, but the actual manner in which lobes developed was still not clear. However, branching within appressoria could be clearly seen by reversing the PDL and focusing down through its thickness onto the exact plane of contact with the appressorium. The same germinating conidium of *E. akebiae* was viewed conventionally from above (Fig. 2k) and then from below (Fig. 2l), when lobes were seen to result from a series of successive dichotomous branchings diverging at angles of ca. 120°.

Amongst 36 *Erysiphe* spp. examined, lobed appressoria were very rare in only three species, namely *E. lonicerae*, *E. magnifica* and *E. symphoricarpi*. Moreover, these species showed hardly any evidence of branching within usually simple, somewhat swollen or club-shaped appressoria (Fig. 3a), even though they were sometimes irregularly bent, even hooked (Fig. 3b). Rarely were two short, irregular lobes formed by a non-dichotomous fork at the tip. These tubes were distinguished from the aerial, unlobed longitubus pattern exhibited to varying extents by most species (Cook and Braun 2009), since they were shorter and firmly attached to the substrate with distinct swollen

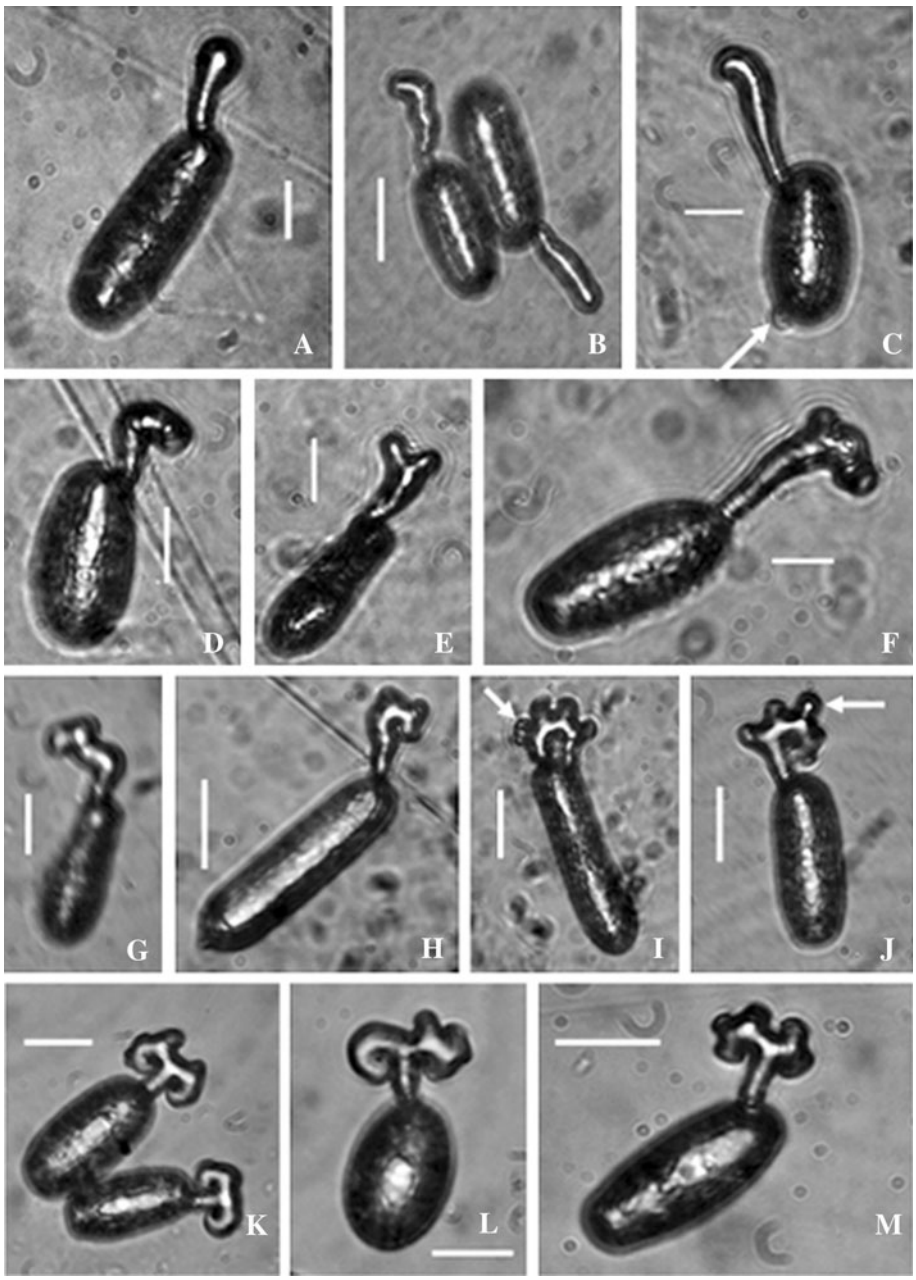

Fig. 3 Light micrographs of germinating conidia viewed from below after 24 h: **a–c** alobate patterns [**a** *Erysiphe symphoricarpi*, **b** *E. lonicerae*, **c** *E. trifoliorum* (*arrow* marks initial colony-forming hypha)]. **d–m** Multilobed patterns (**d–f** *E. trifoliorum*, **g** *E. polygoni*, **h** *E. azaleae*, **i** *E. elevata*, **j** *E. betae*, **k**, **l** *E. heraclei* with branchings at >120°, **k** ram's horn, **l** asymmetric patterns, **m** *E. circaeae*). *Bars* 10 μm

tips. We hereby propose the term alobate for this pattern. *Erysiphe trifoliorum* was intermediate in having mainly alobate tubes, which were often curved (Fig. 3c) or re-curved (Fig. 3d), but also some had clear dichotomous 120° branching (Fig. 3e, f). Several species also produced a high proportion of alobate tubes, e.g. *E. pisi* (sect. *Erysiphe*), and *E. berberidis* and *E. russellii* (both sect. *Microsphaera*). However, most produced three or more lobes with branching mostly two-dimensional (planar) and at least initially at an angle of 120°. Three divisions formed a simple pike-staff-like pattern (Fig. 3f) and four either a zigzag pattern (Fig. 3g), or more often a hook shape (Fig. 3h). When the germ tube was particularly short, the distal end of the hook could re-engage the conidium to form a staple-like structure (Fig. 3i). When viewed from above, it was then difficult to distinguish the base of the tube from the tip of the appressorium. With five divisions the axis usually described a regular hexagon, often with well-developed outward-facing lobes (Fig. 3j). In one-sided appressoria equal numbers turned either left or right.

The ontogeny of lobe formation was first revealed when conidia of *E. howeana* were examined periodically from beneath. The first dichotomy of a germ tube tip seen after 4 h was monitored for 30 h (Fig. 4). The left-hand branch remained dormant whilst the right-hand branch divided twice within 6 h (Fig. 4a–c). This could have produced

three outward-facing lobes, but by this time the first lobe had retracted into the first point of division, forming an apparent bend. Enlargement of the second lobe and the beginnings of a fourth lobe occurred within 13.5 h (Fig. 4d). Unfortunately observing and drawing caused intermittent periods of lower humidity, which interrupted development and caused some shrivelling. Nevertheless, by 30 h the second lobe had enlarged prior to proliferation (Fig. 4e). Interference with development was reduced by photographing a conidium of *E. akebiae* at 15-min intervals over 6 h. Here a germ tube emerged after 2 h (Fig. 5a) and completed its extension by 3 h (Fig. 5b). By 3.5 h both sides of the tip swelled (Fig. 5c), presaging the first dichotomous division that formed by 3.75 h an aborted lobe and an extended limb at an angle of 120° to each other (Fig. 5d). Within 4 h the tip of the extended limb started to produce a second lobe and limb (Fig. 5e), and by 4.25 h a swelling at the tip presaged a third division (Fig. 5f) to form a third lobe and a further extension of the limb. By 4.5 h a fourth division had just formed a fourth lobe (Fig. 5g), and by 6 h a fifth successive dichotomous division was forming a total of five lobes, i.e. four outward and a final inward-facing lobe (Fig. 5h). Thus, this mode of branching generally produced outward-facing lobes that were temporarily dormant and an inward limb that continued growth and division to form the main axis of a structure that we describe as helicoid-cymoid. This is similar to the flowering pattern in angiosperms known as a helicoid cyme as listed by Crozier (1892), but is not identical since, in plants, the apical shoot terminates in a flower bud from which the main axis diverges at ca. 60°.

Whilst the branching pattern of *E. akebiae* was typical of most *Erysiphe* spp., occasionally in some species the first dichotomous division of the germ tube tip produced two equal limbs at a 120° angle to each other, and further divisions led to usually ± symmetrical (Fig. 3k, m), but

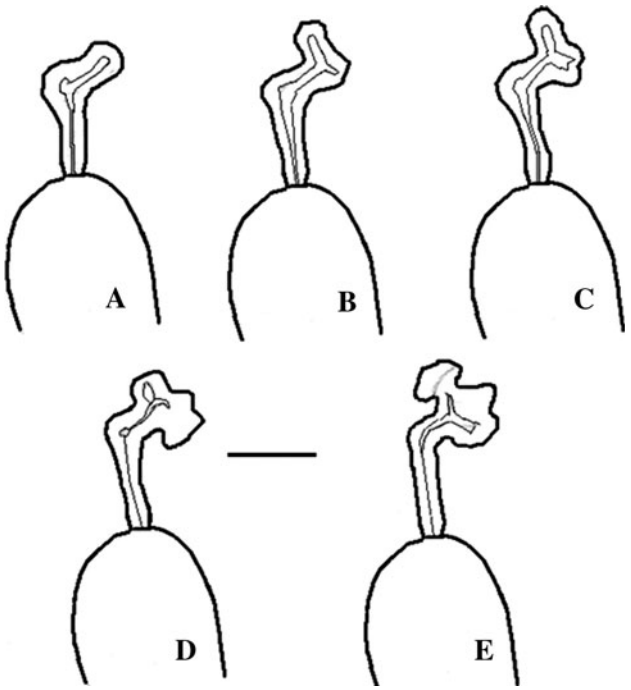

Fig. 4 Development of an appressorium of *Erysiphe howeana* after: **a** 4 h, **b** 4.5 h, **c** 6 h, **d** 13.5 h, **e** 30 h (shrivelling due to lower humidity when drawing). *Bar* 10 μm

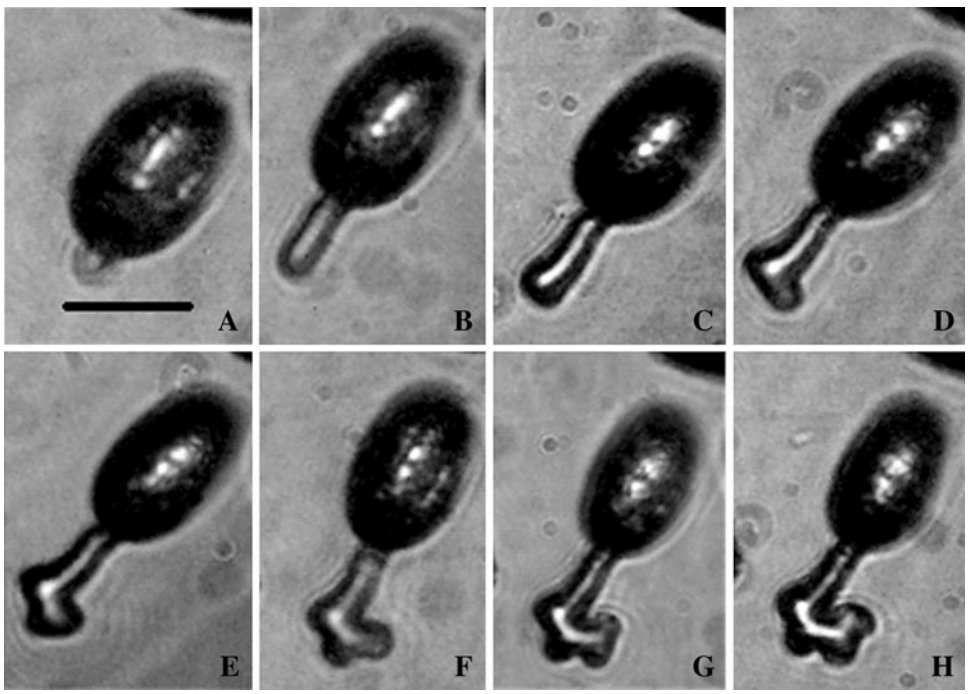

Fig. 5 Development of an appressorium of *Erysiphe akebiae* photographed after: **a** 2 h, **b** 3 h, **c** 3.5 h, **d** 3.75 h, **e** 4 h, **f** 4.25 h, **g** 4.5 h, **h** 6 h. *Bar* 20 μm

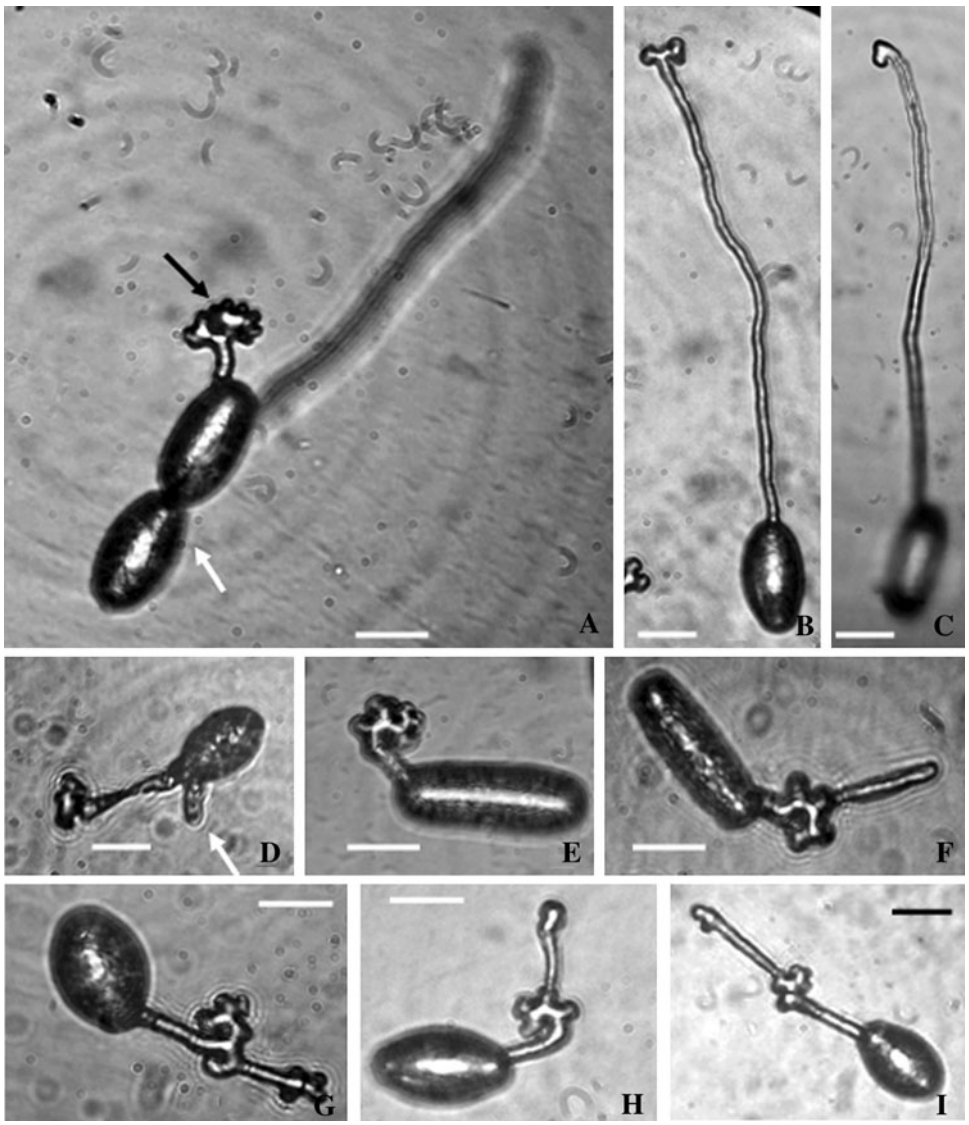

Fig. 6 Germinated conidia after 36 h. **a** *Erysiphe arcuata* with proliferating lobes (*black arrow*) and aerial germ tube (lower conidium, *white arrow*). **b**, **c** Longitubus patterns (**b** *Oidium bixae* tube appressed to surface, **c** *E. pisi* aerial tube on glass slide). **d** *E. catalpae* potential colony-forming hypha (*arrowed*). **f** *E. convolvuli* with proliferating lobes (*arrowed*). **g** *E. flexuosa* appressorial extension. **h**, **i** Extensions with second appressoria (**h** *E. alphitoides*, **i** *O. bixae*). *Bars* 10 μm

sometimes startlingly asymmetrical structures (Fig. 3l). Branching angles often appeared greater than 120°, even up to 180°, often associated with reflexing of the main axes to form almost smooth curves, resembling a ram's horn (Fig. 3k, l), or with more pronounced outward-facing lobes, a toasting-fork-like pattern (Fig. 3m). The lobes did not usually remain dormant; by 24 h some proliferated to form secondary lobes in a jigsaw like pattern (Fig. 3i) and by 36 h a complex botryose structure (Fig. 6a). Frequently one or two primary or secondary lobes grew out to form an extension within 24 h (Fig. 3j), and these extended considerably by 36 h (Fig. 6f). The tip could develop another appressorium to form two tiers (Fig. 6g–i). Often extensions continued in the same direction as the original germ tube, undoubtedly explaining the strange candelabra shapes illustrated by Braun (1987). Here, hypothetical branching patterns have been superimposed on drawings pertaining to *E. carpinicola* and *E. miyabei* (Fig. 7a, b).

Rarely a second germ tube producing a typically lobed appressorium developed from another potential germination site on a conidium, i.e. near its shoulders (subterminal) or end (terminal). More often a second tube, seen as an initial after 24 h (Fig. 3c), remained short and undifferentiated (Fig. 6d).

As demonstrated for *E. pisi* by Green et al. (2002), conidial appressoria are bound tightly to the substrate by extracellular material, seen under an SEM as a shadow on the host surface after removal of a germinated conidium. Similarly, ghost-like outlines of appressoria were seen in our tests with *E. platani* on a PDL examined after an infestation of culture mites had removed conidia, leaving outlines of extracellular material (Fig. 8a) and their droppings as evidence of their presence (Fig. 8b). These outlines confirmed the branching patterns illustrated above.

Exceptionally long tubes (longitubus pattern) often predominating in *E. trifoliorum* and *Golovinomyces* sect.

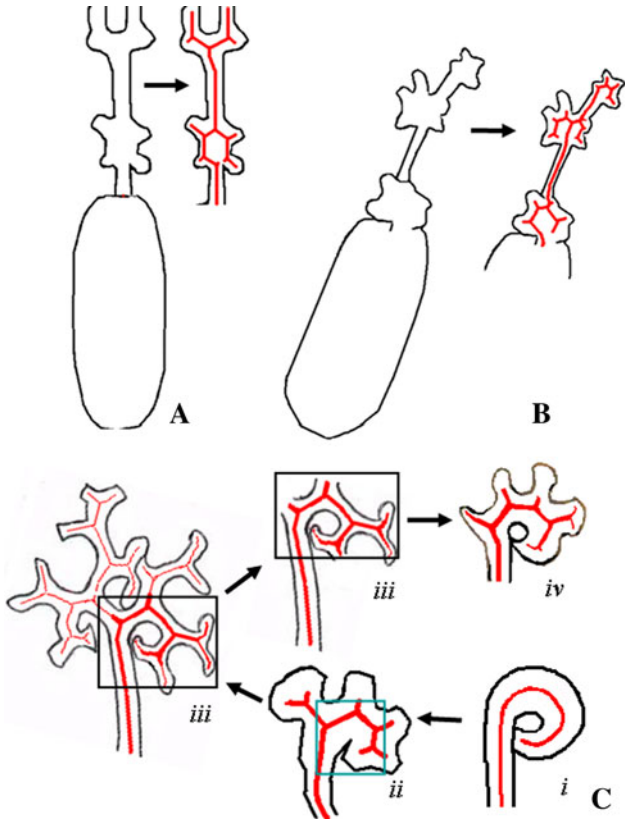

Fig. 7 Suggested branching patterns in tiered germ tubes. **a** *Erysiphe carpinicola*, **b** *E. miyabei* (after Braun 1987). **c** Hypothetical relationships amongst structures: *i*, *ii E. adunca*; *iii*, *iv E. elevata*; *i*, *iii* chasmothecial appendages; *ii*, *iv* conidial appressoria

Depressus on glass in 100% RH (Cook and Braun 2009) occurred to varying degrees in most species even on plastic at 97% RH when the germ tube failed to contact the surface, often, but not invariably, associated with the whole or part of a conidium being held away from it, e.g. when resting on a clump, on an end wall or the tube was deflected over another conidium (Fig. 6a). By 36 h many of these tubes produced small appressoria often with some branching whether in contact with the surface (Fig. 6b) or not (Fig. 6c).

The study on the host surface, non-host surface (broccoli leaf) and PDL gave a more complete picture of conidial germination. The initial patterns on these surfaces closely matched those on the PDL. With the five pathogens tested, the median length of germ tubes remained relatively constant on all surfaces, i.e. one times the conidial width with *E. trifoliorum*, *E. heraclei* and the two isolates of *E. lonicerae,* and 1–1.5 times with the two isolates of *E. magnifica*. There was interesting variation in the numbers of lobes per appressorium, the median values tending to be higher on plant surfaces than on the PDL. For instance with *E. trifoliorum*, they were increased from one lobe per appressorium on the PDL to two on the host and broccoli surfaces (a value of 1 represents an alobate pattern since the first division of the tube creates two lobes). With *E. heraclei* they increased from four on PDL to six on broccoli, although they remained four on its own host. In contrast, lobes on *E. aquilegiae* var. *aquilegiae* increased from two on the PDL to four on its host (see "Discussion" for an explanation of these differences). However, both isolates of the two fully alobate species tested, *E. lonicerae* and *E. magnifica*, remained alobate on their hosts. Despite increased lobing with *E. heraclei* on broccoli no further development occurred, appressoria apparently degenerating into blobs (Fig. 9c). Only on a host surface did very long hyphae extend from appressorial lobes and/or alternative germination points on the conidium and produce hyphal appressoria before branching into superficial mycelium of a young colony. These secondary tubes we hereby term colony-forming hyphae and are illustrated with *E. heraclei* where two emerge as extension tubes from a lobed appressorium and two from the shoulders at the opposite end of the conidium (Fig. 9a). These were all potential sites of tube emergence seen on the PDL. *Erysiphe aquilegiae* var. *aquilegiae* showed similar patterns. Some irregular tips on tubes of the longitubus pattern appeared also capable of infection (not illustrated). With alobate species, the pattern was similar except that the appressorium produced only one extension tube, or rarely a second tube from a non-dichotomous side branch, as predicted from studies on the PDL. The illustration for *E. lonicerae* shows a single extension tube coming from the alobate appressorium and a secondary tube from the neighbouring shoulder (Fig. 9b). A very similar pattern was observed in October 2008 on the host of the third truly alobate species, *E. symphoricarpi*. With *E. magnoliae*, development was more limited on the host although a few conidia produced an appressorial extension and a colony-forming hypha (Fig. 9d). The exception was *E. trifoliorum* where, despite the increased lobing on the host, there was no further growth, and conidia shrivelled (Fig. 9e). Interestingly, the poor development of *E. magnoliae* on its host was followed by very limited non-sporulating growth after 21 days, and the lack of further development of *E. trifoliorum* was reflected in the absence of any visible infection. Apparently by late summer these hosts were resistant. In contrast, abundantly sporulating colonies developed with all the other species and isolates. The thorough examination of ca. 2 cm^2 of incubated, but not inoculated, *Magnolia* leaves revealed seven clearly shrivelled powdery mildew conidia. Of these, two had produced lobed appressoria, one of them even bearing a 10-µm extension tube. The relevance of this finding is discussed below. Over all the species tested on PDL, there were considerable differences in numbers of lobes per appressorium and germ tube lengths. However, tube lengths were relatively

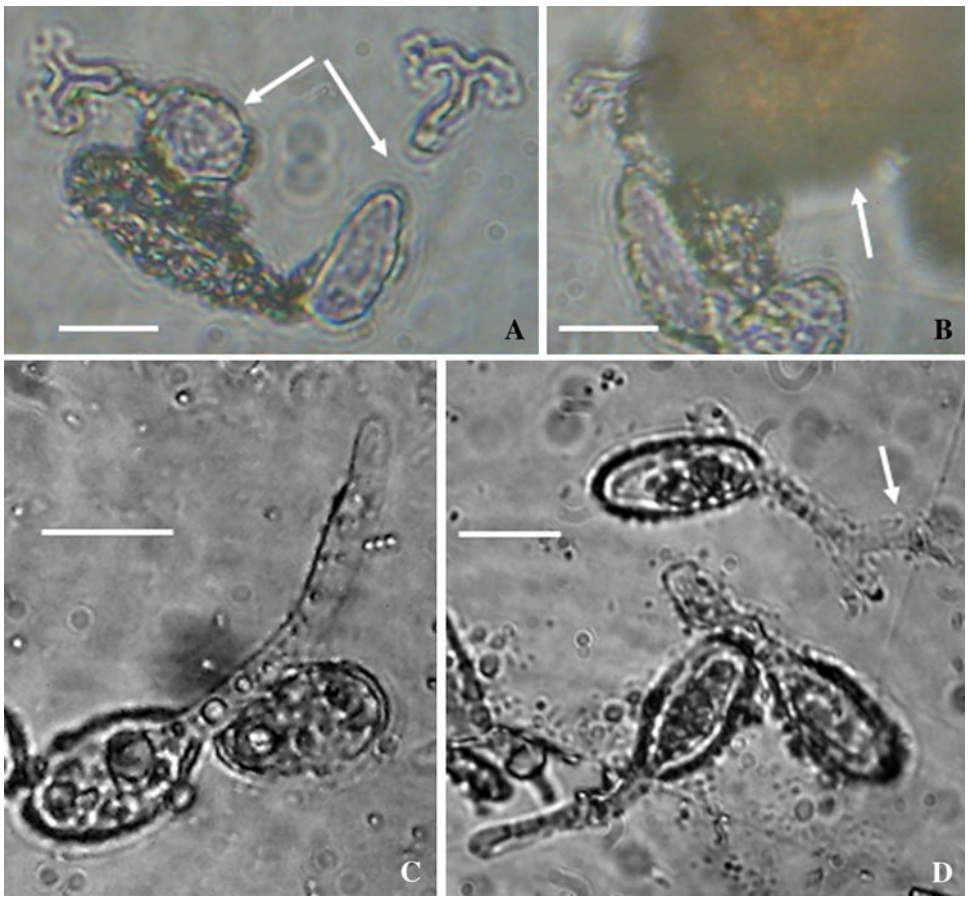

Fig. 8 *Erysiphe alphitoides*. **a** Outlines (*arrowed*) of conidial appressoria after their removal by mites. **b** Infestation proved by mite excreta (*arrowed*). **c**, **d** Germ tubes of ascospores after 48 h in water on petri dish lid, **c** unbranched, **d** non-dichotomously branched (*arrowed*). *Bars* 10 μm

consistent amongst isolates of the same species compared to the more variable numbers of lobes (data not shown).

When viewed from above, branching patterns were also impossible to determine in species other than *Erysiphe* that produce lobed appressoria. In the striatoidium germination type of *Neoerysiphe* apparent 180° branching often made its strongly re-curved lobes resemble a winding key (cf. Fig. 1d, after Cook and Braun 2009), but from beneath, distinct 120° branching was evident, e.g. in *Neoerysiphe galeopsidis* (Fig. 10a) as well as a zigzag pattern in *N. geranii* (Fig. 10b). Similar patterns were observed in the present collections from the UK of a powdery mildew from *Lapsana communis*, the appressoria having very clear 120° cymoid branching (Fig. 10c–e) and often a helicoid-cymoid pattern (Fig. 10e). However, a *Neoerysiphe* has not been reported on *L. communis*, which is only known as a host of *P. erigerontis-canadensis* (as *Sphaerotheca fusca*) and *Golovinomyces cichoracearum* (as *Erysiphe cichoracearum*) (Braun 1987). A careful examination of these specimens revealed superficial mycelium with multilobed hyphal appressoria and conidia developing in chains (catenescent) without fibrosin bodies, but with very fine longitudinal striations on their surfaces. So this conformed fully to the *Striatoidium* anamorph of *Neoerysiphe* as described by Braun et al. (2002) and could not belong to *Podosphaera* or *Golovinomyces*.

Although mostly very short and alobate, appressoria of the fibroidium brevitubus type of *Podosphaera xanthii* (syn. *Sphaerotheca fusca*) ex *Taraxacum* often exhibited similar branching to *Neoerysiphe* when viewed from above, even occasionally forming winding key shapes (cf. Fig. 1e, after Cook and Braun 2009). However, when viewed from beneath, distinct bending and/or branching was seen. Bending could be slight (Fig. 10f, right hand partially shrivelled conidium), but more unusual shapes were due to a very sharp bend (Fig. 10f, left-hand conidium) or to branching often at more than 160° (Fig. 10g) even up to 180° (Fig. 10h). Any branching was limited to one, rarely two, divisions, and a clear helicoid-cymoid pattern was not observed.

On a larger scale, distinct 120° branching was confirmed in *Phyllactinia guttata* (Fig. 10i), even within its coralloid appressorial structures (Fig. 10j). Lobing of the microidium type of *Oidium phyllanthi* (To-anun et al. 2005) is, however, unique in being irregular and not forming a cymoid pattern.

The attempt to obtain germination of ascospores of *E. alphitoides* on a dry plastic surface even under

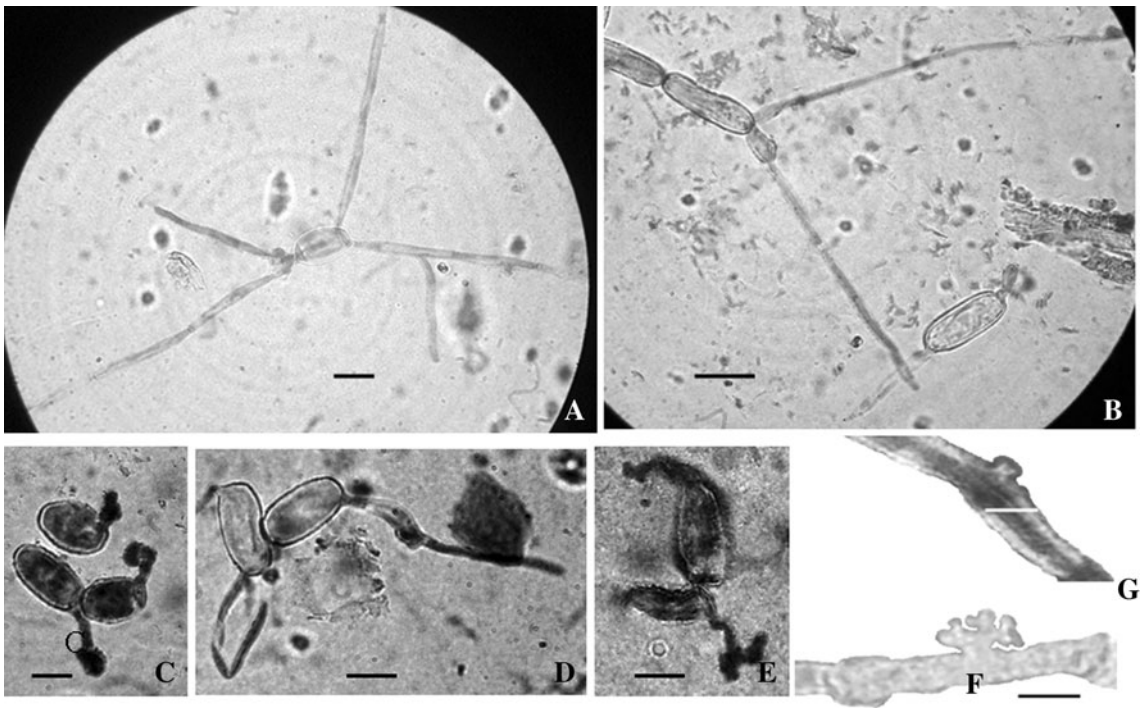

Fig. 9 **a**–**e** Conidial germination after 36 h on host leaf surfaces (plus non-host broccoli): **a** *Erysiphe heraclei* on *Heracleum sphondylium*; **b** *E.* sp., cf. *E. lonicerae*, on *Lonicera periclymenum*; **c** *E. heraclei* on broccoli; **d** *E. magnifica* on *Magnolia* × *soulangiana*; **e** *E. trifoliorum* on *Trifolium dubium* (failed infection). **f**, **g** Hyphal appressoria on mycelium: **f** *E. aquilegiae* var. *aquilegiae* ex *Aquilegia*, **g** *E. catalpae* ex *Catalpa*. *Bars* **a**, **b** 20 μm; **c**–**e** 10 μm; **f**, **g** 5 μm

conditions close to 100% RH was unsuccessful. The few ascospores that were released shrivelled and failed to germinate, contrasting with ascospores liberated into water on the PDL where many produced short to long mainly unbranched germ tubes (Fig. 8c); those that branched had no multiple lobing (Fig. 8d).

Discussion

Whilst fulfilling its principal aim to elucidate the ontogeny and structure of germ tube tips, this study also highlighted considerable variation in conidial germination patterns within *Erysiphe* emend. Previously considered simply lobed or unlobed, we now know appressoria range from simple, bent or curved tubes to limbs with clear-cut 120° branching in a hexagonal cymoid arrangement. After the first division of the tip, both limbs are more or less equally outward facing; hence formation of two-sided structures is understandable. After 36 h on the PDL the basic structure can be modified by two further processes. Temporarily dormant lobes can renew dichotomous branching to produce very short secondary lobes forming jigsaw-like or botryose effects (lobe proliferation). Then, a primary or secondary lobe may extend, develop secondary appressoria and with further extensions form a candelabra effect. We contend that these extensions as well as the undifferentiated second tubes on some conidia seen on the PDL are hyphae that form the colonies we have observed on host surfaces. Whilst several such hyphae can emerge from lobes on the appressorium and from potential germination sites on the conidium itself, there is nearly always only one tube capable of immediately forming a large lobed or unlobed appressorium clearly responsible for the first act of penetration. We contend that the term, germ tube, should be reserved for this; other tubes should be referred to colony-forming hyphae rather than primary and secondary germ tubes, the terms used by previous workers (e.g. Braun 1987, 1995; Braun et al. 2002; Shin 2000). We never observed production of conidiophores directly from potential germination sites (microconidial conidiogenesis) as reported by Kiss et al. (2010), probably because our observations on host surfaces were much more limited.

Structures having 120° branching in *Erysiphe* may be more universal than generally supposed. Appressoria on the superficial hyphae (hyphal appressoria) usually show an irregular or indefinable shape, but sometimes resemble branching on germ tubes (Fig. 9f). Careful focussing down into the structure can sometimes reveal an internal pattern of 120° branching (Fig. 9g). Also in *E.* sect. *Microsphaera* chasmothecial appendages most frequently branch at this

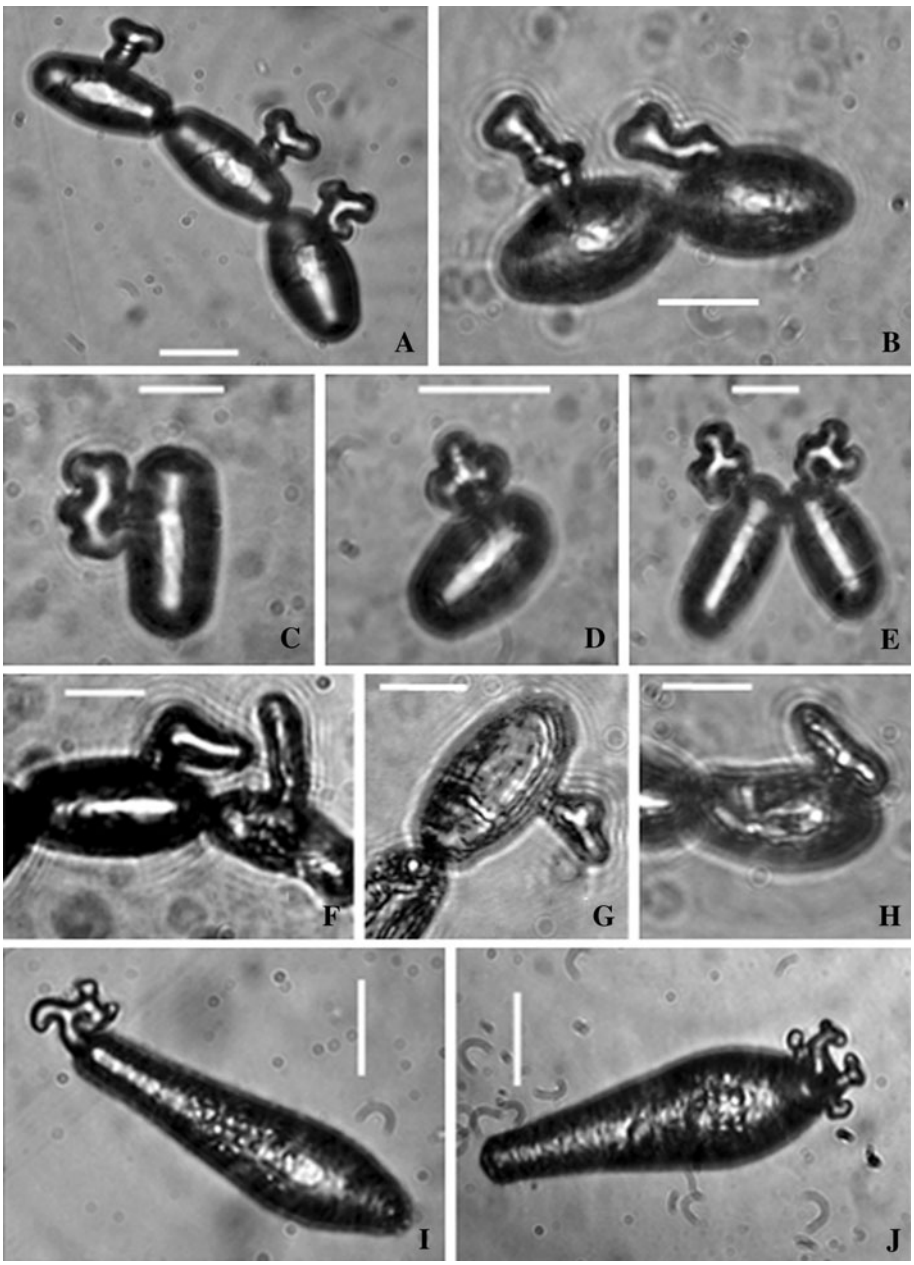

Fig. 10 Conidial germination in genera other than *Erysiphe*: **a** *Neoerysiphe galeopsidis* ex *Catalpa bignonioides* var. *aurea*, **b** *N. geranii* ex *Geranium* cult, **c–e** *N.* sp. cf. *N. nevoi* var. *nevoi* ex *Lapsana communis*, **f–h** *Podosphaera xanthii* ex *Taraxacum officinalis*, **i**, **j** *Phyllactinia guttata* ex *Salix caprea*. *Bars* 10 μm

angle (Fig. 7c, *iii*). Indeed, the shape of an appressorial germ tube can be envisaged within the structure of a chasmothecial appendage as illustrated in the appressorium drawn from life in *E. elevata* (Fig. 7c, *iv*). Although basically symmetrical, the basal inward-facing limbs in appendages of most *E.* sect. *Microsphaera* species are usually the most strongly developed and re-curved. The main difference is that, with conidial appressoria, the branching is at least initially confined to one or two axes of the primary branches, and secondary lobing (i.e. lobe proliferation) is very much condensed, whereas the main limbs of chasmothecial appendages divide six or more times with ± equal dichotomies. The tendency for germ tubes to curl at their tips, either smoothly as in *E. trifoliorum* (Fig. 1c) or in a hexagonal cymoid structure as in *E. adunca* (Fig. 7c, *ii*), is similar to tips of chasmothecial appendages in *E.* sect. *Uncinula* (Fig. 7c, *i*). Since *Uncinula*-like appendages are believed to be ancestral in the phylogeny of the Erysiphales (Takamatsu et al. 2005), it is not surprising that a coiled pattern is in some way reflected in appressoria of all three *Erysiphe* sections. As outlined in Fig. 7c, we propose a hypothetical progression from the uncinulate appendage of sect. *Uncinula* through its helicoid-cymoid appressorium to the branched appendage of sect. *Microsphaera* and also to typical conidial appressoria of all three *Erysiphe* sections.

Not to forget the function of an appressorium, we should consider its penetration peg. This organ penetrates the host cell to form a haustorium that then absorbs nutrients from the host. When Green et al. (2002) examined the extracellular material on the host surface after removal of a germinated conidium of *E. pisi*, they revealed the site of the peg in the centre of the distal and largest appressorial lobe. However, in our tests a potential penetration site was not visible in this position within extracellular material left behind on the PDL. We now know the distal lobe would have been the last formed, and this fits well with a recent finding that germ tubes of *O. neolycopersici* on a susceptible tomato leaf remain unlobed if they immediately succeed in penetrating the epidermal cell wall and form a haustorium, but if for any reason there is no penetration or if the peg is blocked by a host papilla and the haustorium fails to develop, then lobes are produced successively until a haustorium is produced (Nonomura et al. 2010). This agrees with our finding that multilobed species will sometimes produce more lobes on the host surface than on the PDL. There was no need for increased lobing in the test on *Heracleum* because this host was very susceptible, witness its support of rapid and abundant colony growth and sporulation. Lobing was, however, increased on the incompatible host, broccoli, probably due to continued, but unsuccessful, attempts at infection. *Erysiphe trifoliorum* produced more lobing on its host, probably because repeated attempts failed to infect now resistant leaves. Lobed appressoria of *E. magnifica* have been reported on the host (Shin 2000). However, in our careful search of a *Magnolia soulangiana* leaf taken directly from the garden and not inoculated, two germinated, albeit shrivelled, conidia-bearing lobed appressoria were observed, and one of them had formed an extension tube, although there was no powdery mildew on this plant or on the leaves following incubation for 21 days. So it is likely these conidia originated from another host, since viable conidia can go through the early stages of germination on an incompatible host as illustrated with our inoculation of broccoli with *E. heraclei*. Therefore, observations of conidia germinating on field specimens should be viewed with caution. At least they should be checked for colony-forming hyphae as evidence of true infection. Clearly further research is needed to clarify this point.

Species with a high incidence of longitubus pattern are termed 'dimorphic', e.g. *E. trifoliorum* (Cook and Braun 2009) and *E. palczewskii* (Schmidt and Scholler 2002). However, because this pattern occurs to varying degrees, in most species, we propose that the term is now redundant and misleading in this context. Our definition of the longitubus structure as a pattern rather than a germination type should highlight the need to carefully distinguish these initially aerial and undifferentiated germ tubes from those of the alobate species, *E. symphoricarpi*, *E. magnifica* and *E. lonicerae*, where appressoria are unlobed, but soon differentiated, appressed and often hooked, and both these patterns from those of *Golovinomyces* and *Podosphaera* (respectively reticuloidium and fibroidium orthotubus types) where germ tube tips are appressed, simple, usually slightly swollen or clubbed, but rarely hooked (Cook and Braun 2009).

In addition to germ tubes in *Phyllactinia* having a similar pattern of branching to *Erysiphe*, the same must apply to other genera within the tribe Phyllactinieae, e.g. *Leveillula* and *Pleochaeta* (cf. Fig. 1f, g) based on illustrations in Braun et al. (2002), Kiss et al. (2006), and Cook and Braun (2009).

This study has thrown light on two *Neoerysiphe* species in the UK. The one on *Lapsana communis* almost certainly belongs to *N. nevoi* var. *nevoi* recently identified on *Crepis* and other hosts belonging to the same tribe, Cichorieae, of the family Asteraceae in the Ukraine and Israel (Heluta et al. 2010). This is undoubtedly not a new record for the UK, because Zaracovitis (1965) described a mildew on *Lapsana* (and *Crepis*) as being exceptional in having catenescent conidia that produced lobate appressoria. Braun (1980) described an *Oidium* sp. on *Crepis biennis* from Germany with catenescent conidia and distinctly lobed hyphal appressoria, and supposed that it possibly belonged to "*Erysiphe galeopsidis*". Therefore, examinations of further specimens on these two hosts are needed to confirm that *N. nevoi* is present in both Germany and the UK. In contrast *N. geranii* must be a new introduction into the UK. It has not been reported in Germany, although it was reported in the Ukraine in 2001, apparently introduced from Japan or New Zealand (Heluta et al. 2010).

At first sight, branching associated with the fibroidium brevitubus type exemplified by *Podosphaera xanthii* could be confused with the striatoidium type of *Neoerysiphe*. However, the branching of the former is rarer and less extensive and, of course, its conidia are easily distinguished by the presence of fibrosin bodies.

Whilst the presence of the teleomorph is still essential to identify the section as well as the individual species within *Erysiphe* emend., the information gained in this study on differences in appressorial lobing and germ tube length may assist identification of a *Pseudoidium* anamorph. Since it represents over 50% of the Erysiphales species worldwide, this information would be useful when/if a *Pseudoidium* attacks a new host species or enters a new geographic region. In these circumstances the teleomorph is often absent and may not appear until many years later, if at all, as discussed by Inman et al. (2000) in connection with the introduction of rhododendron powdery mildew into Europe. Knowledge of the appressoria can also help to detect mixed infections on hosts infected by more than one

powdery mildew. By this means *Catalpa* was found simultaneously infected by *E. elevata* and *Neoerysiphe galeopsidis* (Cook et al. 2006). Even when only one powdery mildew actually infects a host, experiments involving host/parasite interactions can be jeopardised by contaminating conidia from another host. In a study on tomato powdery mildew caused by *Oidium neolycopersici* (a *Pseudoidium*), Matsuda et al. (2005) had attempted to distinguish germinating conidia of this pathogen from those of *E. trifoliorum*, which had also germinated on the tomato plants. Many conidia had extra long germ tubes, although unknown to them at the time, this could indicate the presence of *E. trifoliorum* with its prevalent longitubus pattern. A reliable recognition of germination patterns could also help identify powdery mildew conidia on spore traps. The trapping of *Pseudoidium* conidia has often been reported (e.g. Celio and Hausbeck 1998; Byrne et al. 2000; Willocquet and Clerjeau 2007). It would be easy to provide a plastic trapping surface suitable for germination and thus help confirm the identity of the target mildew.

At present it is not clear which criterion of germination is most useful for identification of *Erysiphe* species. Whilst germ tube length appeared less variable than degree of lobing amongst isolates of a species, it may show less interspecific variation. Possibly a combination of the two criteria is needed. Whilst the prevalence of the longitubus pattern is very variable amongst isolates, it is not known whether this criterion has any biological significance or diagnostic value. Thus, this work has paved the way for further assessments of conidial germination patterns to determine whether there are consistent and therefore useful differences amongst species or groups of species that will aid identification.

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References

Braun U (1980) Morphological studies in the genus *Oidium*. Flora 170:77–90

Braun U (1987) A monograph of the Erysiphales (powdery mildews). Beih Nova Hedwig 89:1–700

Braun U (1995) The powdery mildews (Erysiphales) of Europe. Fischer Verlag, Jena

Braun U, Takamatsu S (2000) Phylogeny of Erysiphe, Microsphaera, Uncinula (Erysipheae) and Cystotheca, Podosphaera, Sphaerotheca (Cystotheceae) inferred from rDNA ITS sequences—some taxonomic consequences. Schlechtendalia 4:1–33

Braun U, Cook RTA, Inman A, Shin H-D (2002) The taxonomy of the powdery mildew fungi. In: Bélanger RR, Bushnell WR, Dik AJ, Carver TLW (eds) The powdery mildews: a comprehensive treatise. American Phytopathological Society Press, St Paul, MN, pp 13–55

Byrne JM, Hausbeck MK, Shaw BD (2000) Factors affecting concentrations of airborne conidia of *Oidium* sp. among poinsettias in a greenhouse. Plant Dis 84:1089–1095

Celio GJ, Hausbeck MK (1998) Conidial germination infection structure formation and early colony development of powdery mildew on poinsettia. Phytopathology 88:105–113

Cook RTA, Braun U (2009) Conidial germination patterns in powdery mildews. Mycol Res 113:616–636

Cook RTA, Inman AJ, Billings C (1997) Identification of anamorphs of powdery mildews using morphological and host range data. Mycol Res 101:975–1002

Cook RTA, Henricot B, Henrici A, Beales P (2006) Morphological and phylogenetic comparisons amongst powdery mildews on *Catalpa* in the UK. Mycol Res 110:672–685

Crozier AA (1892) A dictionary of botanical terms. Henry Holt and Company, New York (Digitised by Microsoft for University of Toronto, Internet Archive 2008). http://www.archive.org/details/dictionaryofbota00crozuoft. Accessed 12 Oct 2010

Green JR, Carver TLW, Gurr SJ (2002) The formation and function of infection and feeding structures. In: Bélanger RR, Bushnell WR, Dik AJ, Carver TLW (eds) The powdery mildews: a comprehensive treatise. American Phytopathological Society Press, St Paul, MN, pp 66–82

Heluta V, Takamatsu S, Harada M, Voytyuk S (2010) Molecular phylogeny and taxonomy of Eurasian *Neoerysiphe* species infecting Asteraceae and *Geranium*. Persoonia 24:81–92

Inman AJ, Cook RTA, Beales PA (2000) A contribution to the identity of rhododendron powdery mildew in Europe. J Phytopathol 148:17–27

Kiss L, Khosla K, Jankovics T, Seiko N, Braun U, Takamatsu S (2006) A morphologically ill-founded powdery mildew species, *Pleochaeta indica*, is recognized as a phylogenetic species based on the analysis of the nuclear ribosomal DNA sequences. Mycol Res 110:1301–1308

Kiss L, Pintye A, Zséli Gy, Jankovics T, Sventiványi O, Hafez YM, Cook RTA (2010) Microcyclic conidiogenesis in powdery mildews and its association with intracellular parasitism by *Ampelomyces*. Eur J Plant Pathol 126:445–451

Matsuda Y, Sameshima T, Moriura N, Inoue K, Nonomura T, Kakutani K, Nishimura H, Kusakari S, Takamatsu S, Toyoda H (2005) Identification of individual powdery mildew fungi infecting leaves and direct detection of gene expression by single conidium polymerase chain reaction. Phytopathology 95:1137–1143

Mori Y, Sato Y, Takamatsu S (2000) Evolutionary analysis of the powdery mildew fungi using nucleotide sequences of the nuclear ribosomal DNA. Mycologia 92:74–93

Nonomura T, Nishitomi A, Matsuda Y, Soma C, Xu L, Kakutani K, Takikawa Y, Toyoda H (2010) Polymorphic change of appressoria by the tomato powdery mildew *Oidium neolycopersici* on host tomato leaves reflects multiple unsuccessful penetration attempts. Fungal Biol 114(11–12):917–928

Saenz GS, Taylor JW (1999) Phylogeny of the Erysiphales (powdery mildews) inferred from internal transcribed spacer ribosomal DNA sequences. Can J Bot 77:150–169

Schmidt A, Scholler M (2002) Studies in Erysiphales anamorphs (II): *Colutea arborescens*, a new host for *Erysiphe palczewskii*. Feddes Repert 113:107–111

Shin H-D (2000) Erysiphaceae of Korea. Plant Pathogens of Korea 1. National Institute of Agricultural Science and Technology, Suwon

Takamatsu S, Hirata T, Sato Y (1998) Phylogenetic analysis and predicted secondary structures of the rDNA internal transcribed spacers of the powdery mildew fungi (Erysiphaceae). Mycoscience 39:441–453

Takamatsu S, Braun U, Limkaisang S (2005) Phylogenetic relationships and generic affinity of *Uncinula septata* inferred from nuclear rDNA sequences. Mycoscience 46:9–16

To-anun C, Kom-un S, Sunawan A, Sato Y, Takamatsu S (2005) A new subgenus, *Microidium*, of *Oidium* (Erysiphaceae) on *Phyllanthus*. Mycoscience 46:1–8

Willocquet L, Clerjeau M (2007) An analysis of the effects of environmental factors on conidial dispersal of *Uncinula necator* (grape powdery mildew) in vineyards. Plant Pathol 47:227–233

Zaracovitis C (1965) Attempts to identify powdery mildew fungi by conidial characters. Trans Br Mycol Soc 48:553–558