

## CHAPTER 2

### LITERATURE REVIEW

#### 1.1 Powdery mildew fungi

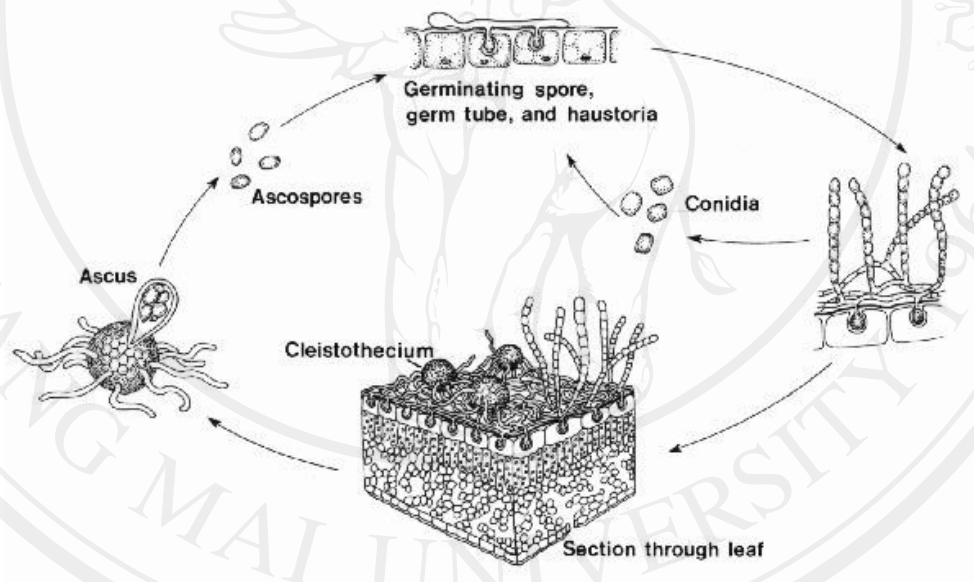
Powdery mildews are cosmopolitan plant pathogenic diseases of the world (Yarwood, 1978; Glawe, 2008). A wide range of angiosperm plants about 10,000 species in all parts of the world are affected severely by powdery mildews (Amino, 1986). The host plant species are mainly distributed in temperate regions of the northern hemisphere. However, the subtropical or tropical areas are also represented by the powdery mildew fungal infection, but mostly occur at the conidial states (Hirata, 1955a; Braun, 1987). These fungal pathogens have been reported in many regions of the world causing plant diseases in several plant species such as cereals, ornamentals, cucurbits, fruits, weeds, shrubs, forest trees (Agrios, 2005). In California, powdery mildew on tomato caused by *Leveillula taurica* has been found in all tomato-growing regions of the state (Correll *et al.*, 1988). Powdery mildew of grapevine is the common disease caused by *Erysiphe necator*. They can affect on stems, fruits and leaves (Gubler, 2012). The fungus *Oidium mangiferae* attacks the mango leaves, which has been reported as a serious disease of mango in many regions of South Africa and Hawaii (Schoeman *et al.*, 1995; Nelson, 2008). In Thailand, the fungus causing powdery mildew in five chilli species has been identified as *Oidiopsis sicula* (*Leveillula taurica* in teleomorph) by using molecular data to clarify morphological taxonomy (Monkhung *et al.*, 2011).

Fungi causing powdery mildews behave as obligate biotrophic parasite and can not be cultured on artificial media. But recently there was a report on the powdery mildew fungus of barley, *Blumeria graminis* f. sp. *hordei*, could grow in a special medium (Agrios, 2005). These plant pathogenic fungi often cause typical symptoms as white to grayish mildew in the areas of infection. They are commonly on leaves, but they also affect buds, pods, young shoots and stems (Agrios, 2005). In compatible interaction between fungi and plant cells, powdery mildews cause plant disease. During the procedure to colonize in plant cells, these parasitic fungi attempt to penetrate the leaf cuticle, cell wall and underlying epidermal cell by forming appressorium. And then, they manipulate in a host epidermal cells to establish a feeding organ as haustorium that is special structure used for obtain nutrients from host. Eventually, they continue their growth by produce conidiophores emerging from the superficial mycelium and release newly formed conidiophores into surrounding area, which may start another infection cycle. Additionally, creation of a metabolic sink ( $\text{Fe}^{3+}$ ) accumulates locally in infected cells (Eichmann and Hückelhoven, 2008).

### 1.1.1 The life cycles

Powdery mildew fungi are obligate parasite and specific to host plant species. They can attack only the proper host plant. The general life cycle of powdery mildew fungi can be divided into two states of asexual (imperfect reproduction) and sexual (perfect reproduction). The fungi grow as white mycelia on the surface of the infected plant parts. The mycelia form the hyphae and develop into conidiophores. Spores will be dispersed from the tip of conidiophores which called “conidia”. With the overwintering, chasmothecia may be formed and adhere on the plant debris e.g. bud, bark. The ascospores are discharged from chasmothecia. Both types of spore (conidia and ascospores) infect the plant cell surface by germinating and producing haustoria to adsorb nutrients from host cells. Spores disseminate by wind, insect and rain (Glawe, 2008). (Fig. 1)

The life cycles may lack either the teleomorph or the anamorph. In the subtropical or tropical areas, most powdery mildew fungi only represent in the anamorph (conidial states) (Braun, 1987). However, the life cycles in the regions with cold climates appear in both of the anamorph and teleomorph. In Pacific Northwest where set in the mild winter areas, the teleomorph can be difficult to find (Glawe, 2006).



**Figure 1** Generalized life cycle of powdery mildews (Schumann, 1991)

## 1.2 Morphological characteristics of value for the taxonomy of powdery mildew

The morphological characteristics of powdery mildews are valuable information for the taxonomy. The characteristics of the anamorphs are the base for the generic taxonomy of Erysiphales and reflect the phylogeny in this fungal group. The teleomorphs are less important for generic taxonomy but provide useful features on the species level (Bélanger *et al.*, 2002).

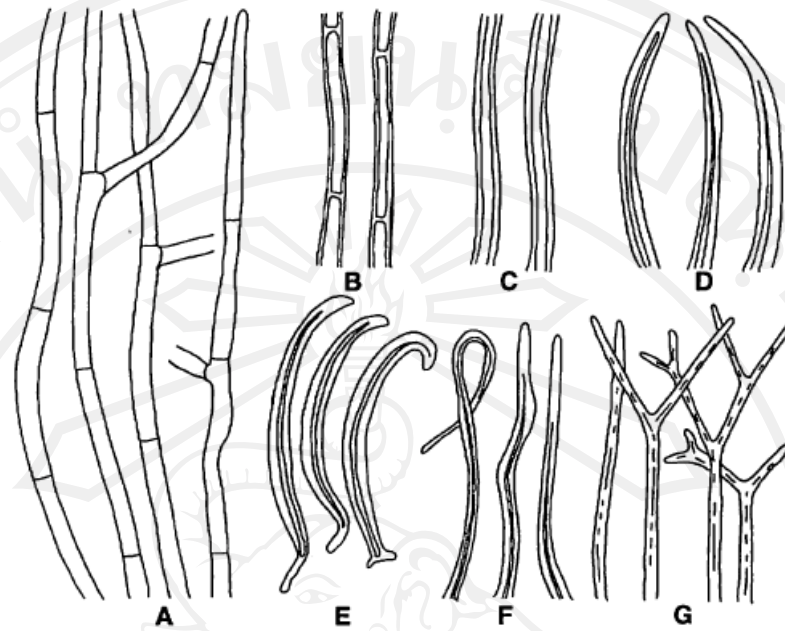
### 1.2.1 Anamorph (Asexual reproduction)

The morphological characteristics in the anamorph of powdery mildew are important features for taxonomy that can be described as follows:

#### 1.2.1.1 Mycelium, appressoria, haustoria

##### **Mycelium**

The mycelium (Fig. 2) is usually epiphytic in all genera that composed of genus *Arthrocladiella*, *Blumeria*, *Cystotheca*, *Erysiphe*, *Golovinomyces*, *Neoerysiphe*, *Podosphaera*, *Sawadaea*, *Sphaerotheca*, Hemiendophyetic mycelia are found in the species of *Phyllactinia* and *Pleochaeta*. The mycelium developed well, superficial hyphae enter the leaves surface through stomata and form internal mycelium. However, one of the genus *Leveillula* species is endophytic. Its mycelium grew in the mesophyll of plant cells and its conidiophore emerged through stomatal opening (Salmon, 1906; Yarwood, 1963; Jarvis, 1964; Braun, 1987).



**Figure 2** Illustration of mycelium types; (A) Primary mycelium (B-E) Secondary mycelium (B) *Podosphaera* (*Sphaerotheca*) *mors-uvae* (C) *Podosphaera* (*Sphaerotheca*) *euphorbiae* (D) *Blumeria graminis* (E) *Cystotheca wrightii* (F) *Cystotheca lanestris* and (G) *Pleochaeta turbinata*. (Drawing by H.D. Shin) (Braun *et al.*, 2002)

The primary mycelium of most Erysiphaceae species is hyaline throughout the season, septate and thin-walled. Shin (2000) mentioned that sometime *Blumeria* and *Podosphaera* in primary mycelium become somewhat grayish or yellowish, more rarely faintly brown. Furthermore, some powdery mildew species may produce thick-walled secondary hyphae. *Cystotheca* species and several species of *Podosphaera* (*P. pannosa*, *mors-uvae*, *euphorbiae*, *fugax*, *fusca*, *alpina*) possess the secondary hyphae. The width of the hyphal cells varies usually from 2 to 10  $\mu\text{m}$  and from 20 to 150  $\mu\text{m}$  in the length; branchings are present. The hyphae are less or more straight to flexuous, geniculate and may be evanescent to more or less persistent. (Braun, 1987)



### Appressoria

The appressoria are lateral outgrowths of the hyphae that forming structures which attach the mycelium to the host surface and initiate the haustoria. This structure also occurs at the ends of conidial germ tubes (Bélanger *et al.* 2002). They are present singly or opposite in pairs or even in sequences on about 20–50% of the hyphal cells (Boesewinkel, 1980; Braun, 1995). Boesewinkel (1980) established five types of appressoria (Fig. 3). Afterward, Braun (1987, 1995) agreed with Boesewinkel's concept and proposed the following appressoria system as described below:

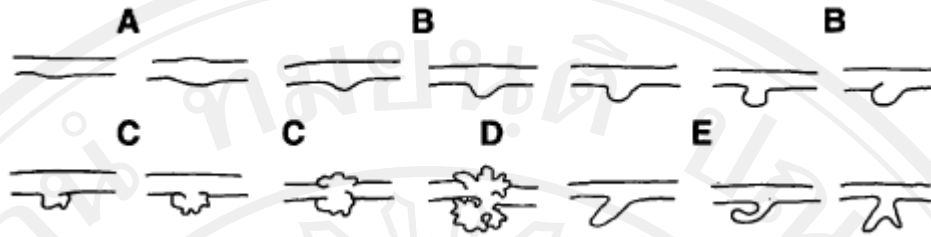
(1) Indistinct: This type characterized by a widening of the hyphae. It is found in various species of *Podosphaera* and *Cystotheca*. De Bary and Woronin (1870) have described this type for the first time in term of “haustoria exappendiculata”.

(2) Nipple-shaped: This type was first described by the brothers Tulasne on *Blumeria graminis* in 1861 and De Bary and Woronin (1870) proposed unlobed appressoria appearance as “haustoria appendiculata”. This type is widely distributed in most species of powdery mildew fungi belonging to the genera *Arthrocladiella*, *Blumeria*, *Golovinomyces*, *Neoerysiphe* (except *N. galeopsidis*), *Podosphaera* and also some *Sawadaea* species.

(3) Lobed: This type, De Bary described as “haustoria lobulata”. The outline of this type is irregular and lobed. This shape is rather variable from slightly lobed to multi-lobed. Lobed appressoria are widely distributed in most species of powdery mildew fungi belonging to the genera *Erysiphe* and *N. galeopsidis* (Braun, 1987).

(4) Hooked or elongated: This type is mostly elongated, hooked, branched or not and found in some species of *Phyllactinia*. Gorter (1988) described a special type of branched appressoria in *Leveillula*, which also can be included in this type.

(5) Coral-shaped: The outline of this type is much more lobed and bigger than those of lobed type. Shin and Zheng (1988) firstly used this term for big appressoria found in some species of *Erysiphe* sect. *Uncinula*.

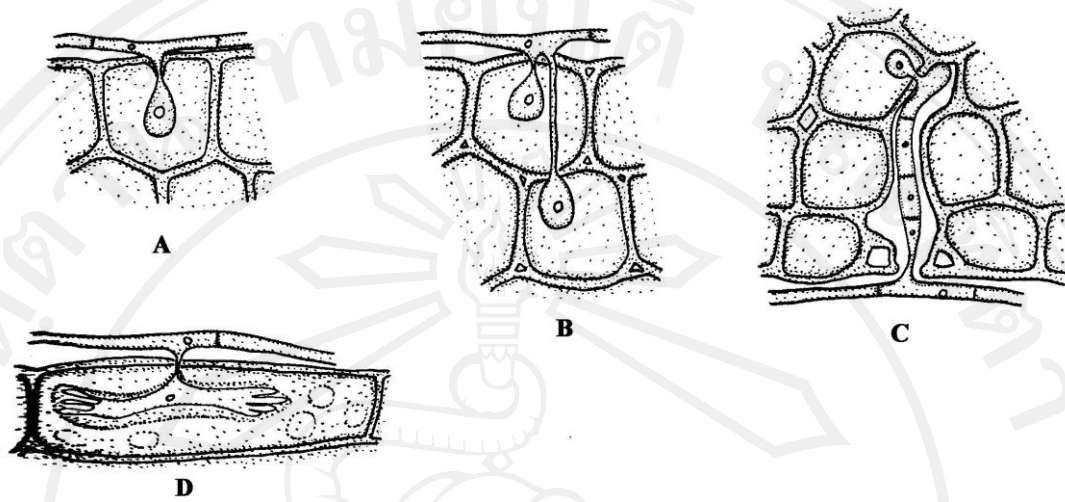


**Figure 3** Appressorial types of powdery mildew fungi; (A) indistinct (B) nipple-shaped (C) lobed (D) coral-like and (E) elongated or hooked. (Drawing by H.D. Shin) (Braun *et al.*, 2002)

Recently, Shin (2000) proposed additional appressorial type in term as “hypha-like” that was first found in *Phyllactinia salmonii*. This type is similar to the short hyphal branches or to the very young conidiophores, it is distinguished from the short hyphal branch by having definite length of less than 60  $\mu\text{m}$  and also from the young conidiophores by having a sharp constriction at the branching point. This feature is also similarly found in *Erysiphe akebiae*.

### Haustoria

The haustoria (Fig. 4) are feeding organs which were first described by de Bary and Woronon (1870). Erysiphaceous haustoria were extensively first studied by Smith (1900). The haustoria of ectophytic powdery mildews species arise from centre of attachment of mycelial appressoria (Blumer, 1967; Webster, 1980). These haustoria originate from below the appressorium and form within the epidermal cells or rarely in deep layers of the host which belonging to the genera *Arthrocladiella*, *Blumeria*, *Cystotheca*, *Erysiphe*, *Golovinomyces*, *Neoerysiphe*, *Podosphaera*, *Sawadaea* (Braun, 1987). In contrast with the endophytic mycelium of the powdery mildew species, the haustoria arise from the internal hyphae and are produced in deeper layers; mesophyll or palisade cell that belongs to genera *Leveillula* and *Phyllactinia* (Blumer, 1967; Braun, 1987).



**Figure 4** Representation of haustorium types in some powdery mildew species; (A) *Erysiphe polygoni* (B) *Erysiphe adunca* (C) *Phyllactinia guttata* and (D) *Blumeria graminis*. (Braun and Cook, 2012)

In most powdery mildew species, the haustoria are globose to pear-shaped structures. The multi-lobed haustoria of *Blumeria graminis* with a digitate appearance represent a special case (Bélanger *et al.* 2002). (Fig 5)



**Figure 5** Haustorium of *Blumeria graminis* with a digitate appearance. (Bar 10  $\mu$ m) (Götz and Boyle, 1998)



### 1.2.1.2 Conidiophores, foot-cells, conidia, germination

#### Conidiophores

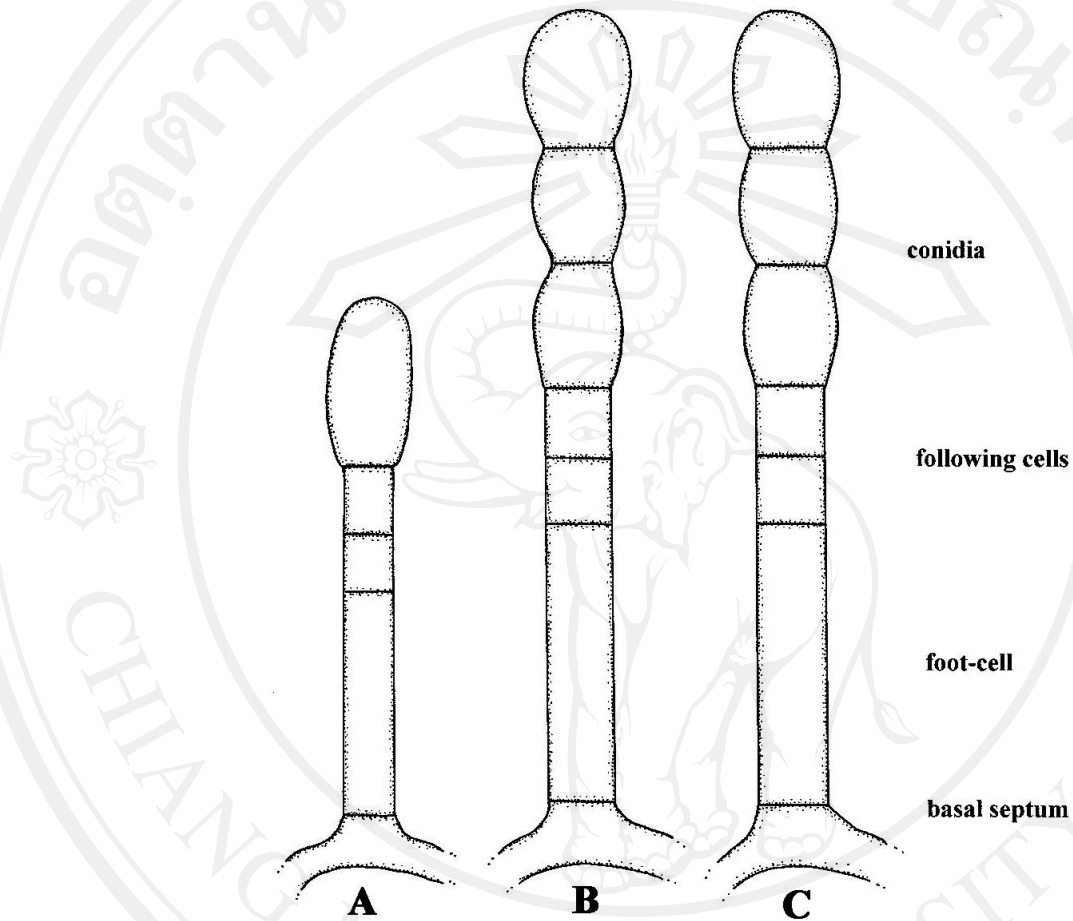
The conidiophores (Fig. 6 and 7) typically arise from the special hyphae that produce conidia. The basal cell is usually called foot-cell. They consist of one to three cylindrical cells (occasionally more than three, or may be lacking), followed by one or several immature conidia which are wider than the lower cells. The length of these following cells is variable and often characteristic for particular species. Most species of powdery mildews produce their conidiophores from the central position of mother cells. Shin (2000) revealed that several species of powdery mildews have their conidiophores on non-central position of mother cells. Shin and La (1993) divided the latter type of conidiophores into two groups according to the edge lines formed by immature conidia as follows:

(1) Conidia singly: This type produces a single conidium on conidiophores. Most species of *Erysiphe*, *Leveillula*, *Phyllactinia*, and *Pleochaeta* are included in this type.

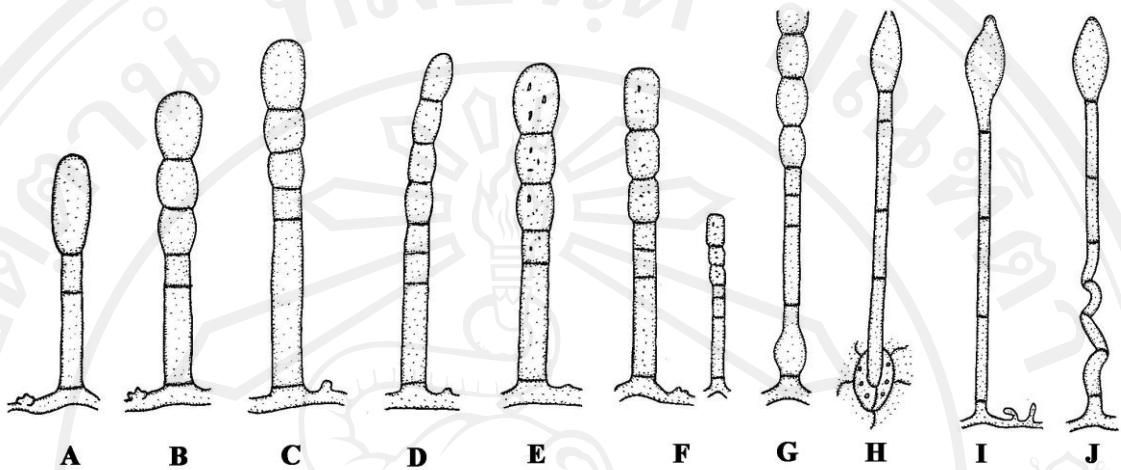
(2) Conidia in chain: This type can be divided into two types according to edge line

2.1 Conidia in chain with crenate edge line: This type produces conidia in chain on conidiophores, and edge line formed by immature conidia is crenate. Most species of *Arthrocladiella*, *Blumeria*, *Podosphaera*, and *Sawadaea* are included.

2.2 Conidia in chain with sinuate edge line: This type produces conidia in chain on conidiophores, and edge line formed by immature conidia is sinuate. Most species of *Cystotheca*, *Golovinomyces* and *Neoerysiphe* are included.



**Figure 6** Conidiophores: (A) Conidia formed singly (*Pseudoidium* type), (B) and (C) Conidia catenulent with edge line sinuous and crenate, respectively. (Braun and Cook, 2012)



**Figure 7** Conidiophore types: (A) Pseudoidium type (B) Euoidium type of *Neoerysiphe* (C) Euoidium type of *Golovinomyces* (D) Euoidium type of *Arthrocladiella* (E) Euoidium type of *Podosphaera*, *Cystotheca* (F) Euoidium type of *Sawadaea* with micro-conidia and micro-conidiophores (G) Oidium type of *Blumeria* (H) Oidiopsis type (I) Ovulariopsis type and (J) Streptopodium type. (Braun and Cook, 2012)

#### Foot-cells in conidiophores

The basal cell is usually called foot-cell. The length and shape of this cell is variable and often characteristic for particular species. The following types are commonly accepted as described below:

(1) Straight: More or less straight cylindrical foot-cells occur in majority of species. Most species of *Arthrocladiella*, *Blumeria*, *Cystotheca*, *Erysiphe*, *Golovinomyces*, *Leveillula*, *Neoerysiphe*, *Phyllactinia*, *Podosphaera*, and *Sawadaea* are included in this type. The base of foot-cells may be somewhat swollen in several species of *Podosphaera* (Braun, 1987; Shin, 2000).

(2) Bulbose: This basal part of foot-cells is markedly swollen (bulbose) in *Blumeria graminis* (Blumer, 1933). This type of foot-cells has not been found in the other species of powdery mildews.

(3) Flexuous: The basal part of foot-cells is more or less flexuous in some species of *Erysiphe* (Jaczewski, 1927).

(4) Coiled or twisted: Spirally or subspirally coiled bases have been known in some species of *Pleochaeta* and *Phyllactinia* (Braun, 1987).

(5) Curved: The conidiophores in some species of powdery mildews are arising from the side of mother cells, and thus the basal parts of foot-cells are curved (L-shape) . This type of conidiophores has been known in *G. sordidus* and some species of *Erysiphe* sect. *Uncinula* (Shin, 1988; Shin and Zheng, 1998). Shin (2000) revealed that some species of powdery mildews, i.e. *N. galii*, *E. carpinicola*, *E. clandestina*, *E. kenjiana*, *E. kusanoi*, *E. togashiana* and *E. zelkowae*.

(6) Thick-walled and rigid: This type of foot-cells has been found only *Ph. rigida* (Boesewinkel, 1980 and Shin, 2000).

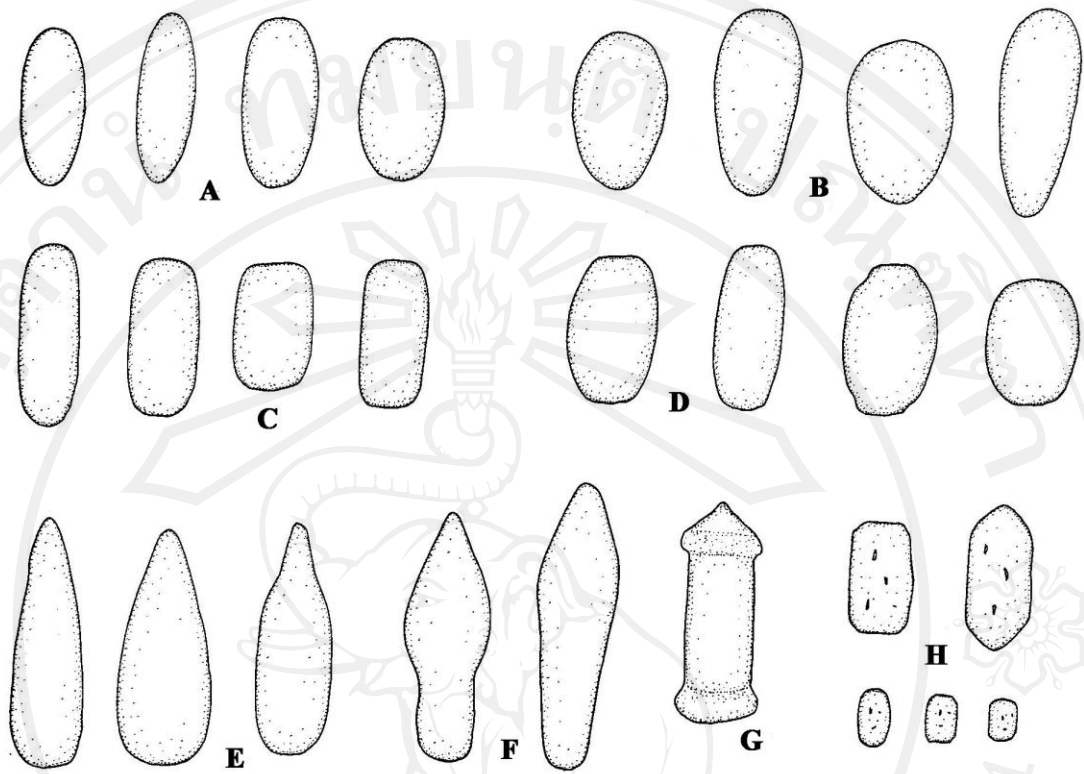
### **Conidia**

The conidia of the Erysiphaceae (Fig. 8) are meristem arthrospores (Hughes, 1953). They are colorless, one-celled, uninucleate, thin-walled, vacuolate and sometimes containing granules and oil-drops. The vacuoles may contain water (Yarwood, 1952), and Brownian movement of particle can occasionally be observed (Yarwood, 1952, 1957). The conidia of some genera (*Cystotheca*, *Podosphaera*, *Sawadaea*) contain conspicuous refractive particles which are generally known as “fibrosin bodies”. Fibrosin bodies are seen only in fresh conidia. Zopf (1887) is the first person who detected. The true nature of these structures is still unclear. The conidia are produced either in chain (Euoidium-type or genus *Oidium*) or singly (Pseudoidium-type of *Oidium* or the genera *Pseudoidium*, *Ovulariopsis*, *Oidiopsis*, *Streptopodium*).

The sizes and shape of conidia are often variable and often very characteristics for species delimitation. The length/width ratio and shape is more constant and more usable for taxonomic purposes. The conidia in most powdery mildew anamorphs vary from cylindrical, ellipsoid or ovoid to doliform or lemon shaped (Bélanger *et al.*, 2002). *Sawadaea* is unique, having dimorphic conidia that consisted of macro- and micro-conidia (Braun, 1987). Additionally, characteristically dimorphic conidia can be known in *Oidiopsis* (teleomorph: *Leveillula*) and *Streptopodium* (teleomorph: *Pleochaeta*). The first conidium (primary conidium) in these genera is more or less lanceolate with apically pointed, where as the subsequently formed ones (secondary conidium) are more or less ellipsoid-cylindric or sometimes oblanceolate.

The fresh conidia do not fully agree with dried herbarium samples in their size and shape. Blumer (1933) revealed constant factors for the conversion of data obtained on the basis of dried specimens described as multiplication of 1.2 for conidial width and 1.15 for the length to arrive at a size equivalent to fresh specimens. Latterly, Shin (2000) confirmed and reevaluated this rule and demonstrated that Blumer's idea is valuable for follower.





**Figure 8** Conidial shapes of the Erysiphaceae; (A) ellipsoid (B) ovoid (C) cylindrical (D) doliform (E) lanceolate (F) clavate, spathulate (G) dumbbell-like (cylindrical with cingulum-like rings at the ends) and (H) macro- and micro-conidia of *Sawadaea*. (Braun and Cook, 2012)

### Conidial germination

The conidial germination was extensively studied and demonstrated the important numerous data by Neger (1902), Hirata (1942, 1955a, b) and Zaracovitis (1964, 1965). On the host cells, a primary germ tube of the conidial germination will be developed into mycelium. The development of the germ tubes depend on environmental factors; e.g. temperature, humidity. The characteristic germination types are valuable features for taxonomy of the Erysiphaceae.

In 1955, Hirata provided the germination patterns may be divided into several types. And then, Braun (1987) proposed four types of the conidial germination. Recently, To-anun *et al.* (2005) demonstrated the differential characteristic of conidial germination amongst the previous conidial germination patterns of powdery mildews and proposed in termed as “**Microidium-type**”. Microidium-type pattern is confine to *Oidium phyllanthi*. Hence, the conidial germination patterns of powdery mildews can be divided into five types as following nomenclatures described below:

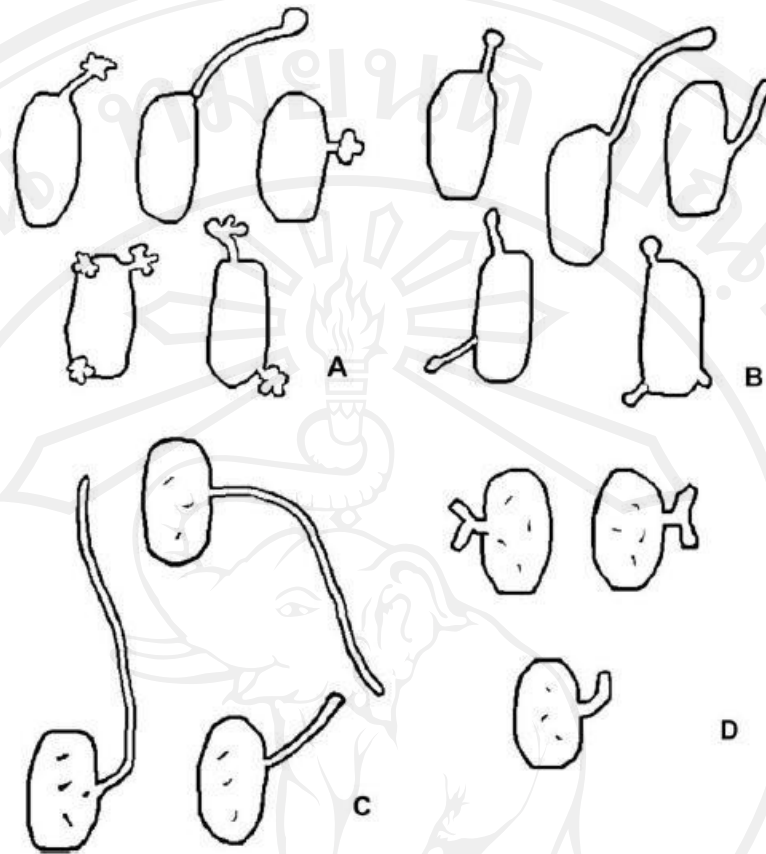
(1) Polygoni-type [syn. Pseudoidium]: Germ tubes terminating in a lobed appressorium, arising from end or side walls, usually short to moderately long, full development rather quick (about 5 hr.). Conidia of this type do not contain any fibrosin bodies.

(2) Cichoracearum-type [syn. Reticuloidium]: Germ tubes terminating in a club-shaped appressorium, arising mostly from end walls, short to long, mostly moderately long, full development about 8-10 hr. Conidia of this type do not contain any fibrosin bodies.

(3) Pannosa-type [syn. Fibroidium]: Germ tubes moderately to very long, without conspicuous appressorium, usually arising more or less laterally from the side walls, full development rather slow (more than 10 hr.). Conidia of this type contain fibrosin bodies.

(4) Fuliginea-type [syn. Magnicellulatae]: Germ tubes always short, without conspicuous appressorium, usually arising laterally from the side walls, often characteristically forked and broad, full development rather slow. Conidia of this type contain fibrosin bodies.

(5) Microidium-type: Germ tube usually swollen along most of its length, e.g. cigar-shaped, bearing lobed or nipple-shaped appressoria. Conidia of this type contain oil-drop particle without conspicuous fibrosin bodies. (Fig. 10)



**Figure 9** Conidial germination types after Braun (1987) and Braun *et al.*, (2002).  
 (A) Pseudoidium (B) Reticuloidium (C) Fibroidium (D) Magnicellulatae.



**Figure 10** Microidium type pattern of conidial germination that found on *Phyllanthus* spp.; (A) *P. acidus*, (B) *P. amarus* and (C) *P. reticulatus*.  
 (X400)

However, Braun (1987) has found that all species might form simple conidial germ tubes without appressoria. The conidial germination patterns can be useful morphological character to classify *Erysiphales* in anamorph subgenera of *Oidium* according to Braun *et al.*, (2002). The patterns of powdery mildews were revised and then proposed the new term of germination patterns as the following nomenclature: *Pseudoidium* (former *polygoni*), *Reticuloidium* (former *chicoracearum* and now *Euoidium*), *Fibroidium* (former *pannosa*) and *Magnicellulatae* (former *fuliginea*) types. Cook and Braun (2009) have comprehensively studied the conidial germination patterns of powdery mildew fungi and used for identification of the *Oidium* anamorphs of powdery mildews and introduced new patterns in term as “*longitubus*” pattern. Categorization of conidial germination types were revised and proposed germination patterns as shown in the following nomenclatures (Fig. 11 and Table. 1).

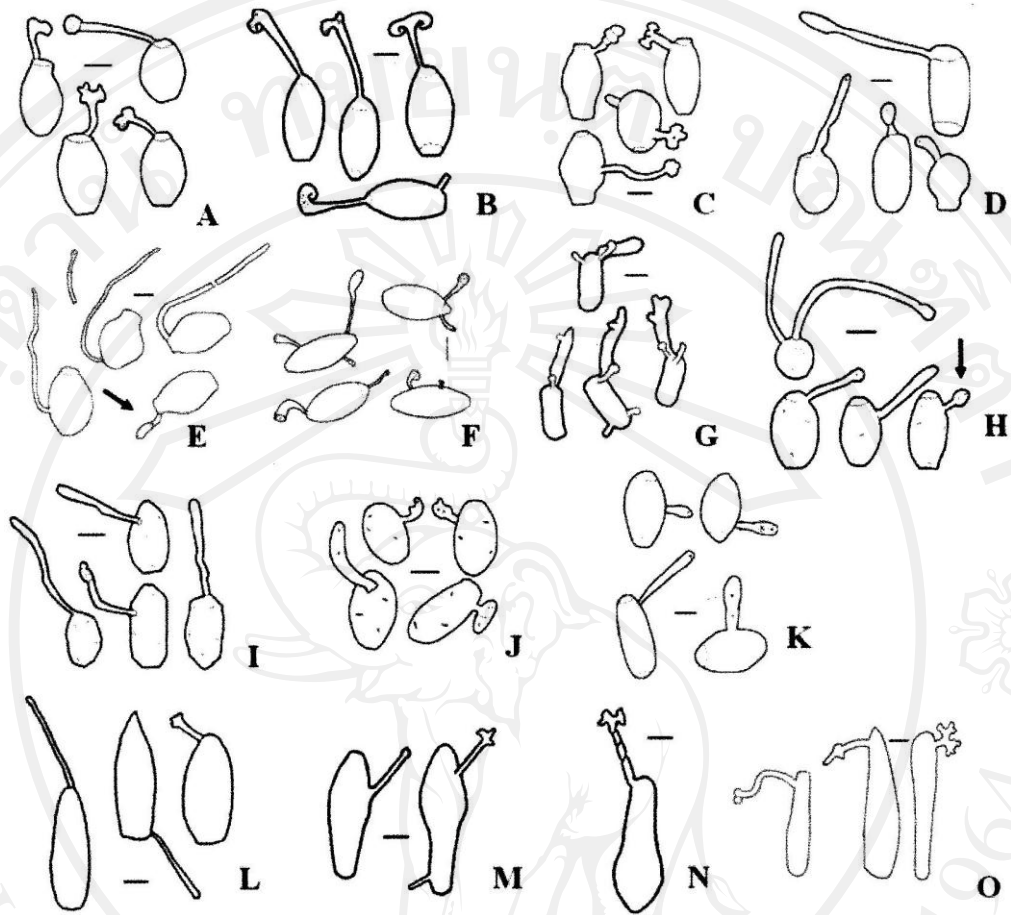
**Table 1** Summary of revised patterns of conidial germination (Cook and Braun, 2009)

<b>Expected Hirata type (1955)</b>	<b>Revised patterns of conidial germination</b>
Polygoni type	<i>Pseudoidium</i> type – <i>Longitubus</i> pattern
–	<i>Striatoidium</i> type
<i>Chicoracearum</i> type	<i>Reticuloidium</i> type – <i>Longitubus</i> pattern
–	<i>Blumeria</i> type
–	<i>Microidium</i> type
<i>Pannosa</i> type	<i>Fibroidium</i> type – subtype <i>orthotubus</i> – subtype <i>brevitubus</i>
<i>Fuliginea</i> type	<i>Magnicellulatae</i>

Nowadays, Braun and Cook (2012) demonstrated that the conidial germination in powdery mildews can be categorized into 7 patterns as described below:

1. *Pseudoidium* type: The type of *Erysiphe emend.* [*Erysiphe*, *Microsphaera*, *Uncinula*] can be divided into 4 patterns as follows:
  - 1.1 *Longitubus* pattern
  - 1.2 *Alobatus* pattern
  - 1.3 *Lobatus* pattern
  - 1.4 *Extensitubus* pattern
2. *Striatoidium* type: This type belongs to genus *Neoëysiphe*.
3. *Ovulariopsis* type: This type is composed of 3 genera, all are proposed to be in tribe *Phyllactineae*. [*Leveillula*, *Phyllactinia*, *Pleochaeta*]
4. *Euoidium* type: The type is *Golovinomyces* and *Arthrocladiella*.
  - 4.1 *Longitubus* pattern
5. *Blumeria* type: The type is composed of *Blumeria graminis*.
6. *Microidium* type: The type is composed of *Microidium phyllanthi*
7. *Fibroidium* type: The type is composed of *Podosphaera* sect. *Podosphaera*, *Podosphaera* sect. *Sphaerotheca*, *Cystotheca*, *Sawadaea* and is divided into 2 patterns of 2 subtypes as indicated below:
  - 7.1 *Orthotubus* subtype
  - 7.2 *Brevitubus* subtype





**Figure 11** Comparison of conidial germination types in powdery mildew fungi: (A), (B) *Pseudoidium* type, (C) *Striatoidium* type, (D) *Euoidium* type, (E) *Longitubus* pattern within *Euoidium* type, (F) *Blumeria* type, (G) *Microidium* type, (H-I) *Orthotubus* subtype within *Fibroidium* type, (J-K) *Brevitubus* subtype within *Fibroidium* type and (L-O) *Ovulariopsis* type, (L) *Leveillula taurica*, (M) *Phyllactinia guttata*, (N) *Ph. thirumlachari*, (O) *Pleochaeta indica*. (Braun and Cook, 2012; Cook and Braun, 2009)

### 1.2.2 Teleomorph (Sexual reproduction)

The fruiting bodies of powdery mildew fungi are more or less spherical or somewhat flattened, closed ascomata. They have been classified as either cleistothecia or perithecia. Braun (1987) discussed this problem to find a correct term for Erysiphaceous ascocarp and preferred to propose a new name as “Erysiphaceous cleistothecium”. Saenz *et al.* (1994) examined sequences of *Blumeria graminis* by molecular analysis (18S rDNA). The results revealed that the Erysiphaceae is not closely allied to either Plectomycetes or Pyrenomycetes. Hence, the term “cleistothecium” and “perithecium” should not be used for powdery mildew fungal fruit bodies. Latter, a special name is proposed in the term as “chasmothecium” (chasm + thecium = slit + fruitbody) for Erysiphaceous ascocarp (Bélanger *et al.*, 2002).

#### 1.2.2.1 Ascomata

The ascomata of Erysiphaceae are closed, non-ostiolate, cleistothecium-like fruit bodies with persistent asci, and by mode of ascus discharge which resemble the situation in perithecia. The wall of ascomata, the peridium consists of two conspicuous layers in most powdery mildew genera. The inner layer forms a loose layer of thin-walled with abundant protoplasm. The cells of inner layer are fairly uniform in size and shape. On the other hand, the outer layer forms mostly irregularly polygonal cells with thick-walled, darkly pigment (Braun, 1987).

#### 1.2.2.2 Appendages

The appendages are outgrowth of the outer pseudoparenchymatous cells of ascomata. These appendages may emerge from all parts of the peridium from base to top. The morphological characteristic of ascomatal appendages is an important one for generic delimitation of the Erysiphaceae. The previously literature study (Braun 1987,

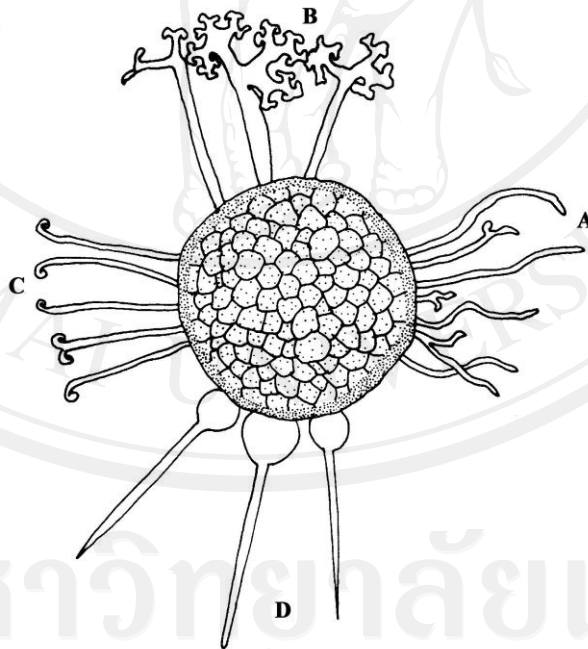
1995; Shin 2000; Bélanger *et al.* 2002) can be summarized that the appendage types are divided into four types (Fig. 12) as the following features:

(1) Mycelioid: This type belongs to the genera *Blumeria*, *Cystotheca*, *Erysiphe*, *Golovinomyces*, *Leveillula*, *Neoerysiphe* and *Podosphaera* sect. *Sphaerotheca*.

(2) Dichotomously branched: This type belongs to the genera *Arthrocladiella*, *Erysiphe* sect. *Microsphaera* and *Podosphaera* sect. *Podosphaera*.

(3) Unicinulate (coiled/circinate): This type belongs to the genera *Sawadaea* and *Erysiphe* sect. *Uncinula*.

(4) Acicular: This type is a unique among powdery mildew fungi which confined to *Phyllactinia* species. The morphological appendage is acicular with bulbous swelling at the base. Furthermore, the gelatinous appendages which second type are called as “penicillate cells”.

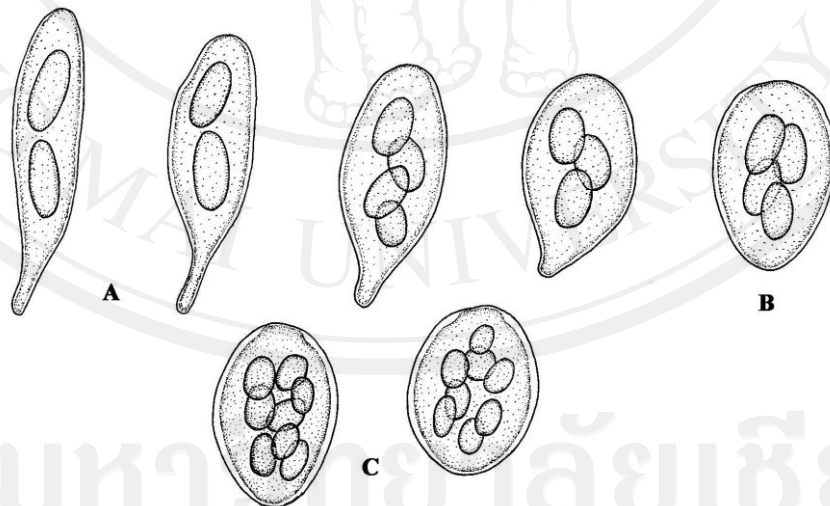


**Figure 12** The illustration of appendage types; (A) mycelioid, (B) Dichotomously branches, (C) circinate and (D) acicular (needle-like).

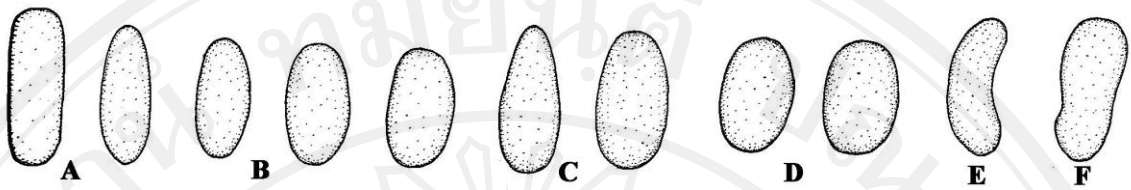
### 1.2.2.3 Asci and Ascospores

The asci of the Erysiphaceae are unitunicate and thin to thick-walled. The feature of asci resembles a sac containing ascospores. The upper part of asci is thinner than the rest of the wall. Their functions perform the place for discharging spore. Mature asci are sessile or shortly stalked (Fig. 13). Their numbers in an ascoma vary in each genus can used for taxonomy study. The ascoma in genera *Cystotheca* and *Podosphaera* contains single ascus and the remaining genera have 2 to 40 asci (Braun, 1987).

The ascus contains 2 to 8 ascospores. The ascospores are one-celled, colorless to yellowish without conspicuous vacuoles, usually subglobose to ellipsoid-ovoid, rarely subcylindric in shape (Fig. 14). The ascospores of the Erysiphaceae are mostly formed during the current season, before wintering (Braun, 1987).



**Figure 13** Ascus types; (A) stalked (B) sessile and (C) asci of *Podosphaera* spp. (Braun and Cook, 2012)



**Figure 14** Shape of ascospores; (A) subcylindric (B) ellipsoid (C) ovoid (D) subglobose (E) curved and (F) irregular. (Braun and Cook, 2012)

### 1.3 Taxonomy and phylogeny of Erysiphales

The scientific classification of powdery mildew fungi based on the morphology associated with the phylogenetic relationship revealed the placement of powdery mildew species as follows (Hibbett *et al.*, 2007):

Kingdom: Fungi

Phylum: Eumycota

Subphylum: Pezizomycotina

Class: Leotiomycetes

Order: Erysiphales

Family: Erysiphaceae

The classification of powdery mildew fungi at “class” level has been confusedly studied in the previous time. Considering on teleomorph state of powdery mildews, the taxonomists have classified as either Plectomycetes or Pyrenomycetes. Erysiphales was placed in “Plectomycetes” as having a close fruiting body without ostiole which is similar to true cleistothcium (Webster, 1980; Braun, 1987). However, Erysiphales produce asci from a basal hymenium and discharge ascospores from



perithecium. These important characters can be classified into “Pyrenomycetes” (Yarwood, 1973).

Saenz *et al.* (1994) have resolved this classification controversy with molecular phylogeny analysis using the nuclear small subunit ribosomal RNA (18 rDNA) of *Blumeria graminis* f. sp. *hordei*. The results rejected the placement of the Erysiphales amongst both the Plectomycetes and Pyrenomycetes but could not reject placement include in Discomycetes (apothecial fungi). However, there is no strongly bootstrap support to group together with the apothecial fungi.

Recently, Wang *et al.* (2006) have studied to classify the Leotiomycetes carried out to phylogenetic relationship based on rDNA regions (SSU + LSU + 5.8S rDNA). The results demonstrated that the Leotiomycetes includes the Cyttariales, Helotiales, Rhytismatales and Erysiphales. This study confirmed the position of Erysiphales to include in Class Leotiomycetes. Nowadays, this studied classification was accepted by the taxonomists all over the worlds.

Modern taxonomy of the powdery mildews (Erysiphaceae) started by Léveillé (1851). He recognized only the morphological characteristics of the teleomorph. Some problematic identification is found in two genera of *Erysiphe* sect. *Uncinula* and *Pleochaeta*. These two genera possess circinate to helicoid appendages, but these genera have different character in the conidial states. Similar problems are connected with *Erysiphe* sect. *Uncinula* and *Sawadaea* or *Erysiphe* sect. *Microsphaera* and *Arthrocladiella* (Braun, 1987). Anamorphic state correlated with teleomorph of powdery mildews were first considered and perceived by the brothers Tulasne (1861). According to the taxonomic systems of powdery mildews are introduced by many taxonomists all the world that demonstrated both of the morphological characteristics anamorph and teleomorph are necessary features for the taxonomy. The characteristics of anamorphs are the base for the generic taxonomy of Erysiphales. And, the teleomorphic characters are important taxonomic features for taxonomy in the species level (Bélanger *et al.* 2002). To observation in anamorphic characteristics, the pattern of conidial germination is value for taxonomy of powdery mildews apart

from the appearance of conidia (Hirata, 1942). Braun (1987) demonstrated that the germination patterns can be divided into four types namely, Cichoracearum, Fuliginea, Pannosa and Polygoni. In 2009, Cook and Braun proposed newly nomenclature of conidial germination patterns as following: Fibroidium (syn. pannosa), Magnicellulatae (syn. fuliginea), Pseudoidium (syn. polygoni) and Reticuloidium (syn. cichoracearum).

Salmon (1900a, b) who published the first monograph of powdery mildews that identified based on the morphological characteristics of the teleomorph, exclusively based on morphological features of the ascomata. His monograph is extensively referred to study the powdery mildews at that time. Afterwards, the comprehensive monographs of powdery mildews have been published by many mycologists in worldwide (Blumer, 1933; Homma, 1937; Braun, 1987; Palti, 1988; Braun, 1995; Bolay, 2005; Paul and Thakur, 2006; Shin, 2000; Liu, 2010). A monograph of Erysiphales that published by Braun (1987) was most extensively studied and generally used in taxonomic system.

The new taxonomical system of the Erysiphales is the result of the scientific progress, i.e., scanning electron microscopy (SEM) examinations and molecular phylogenetic analysis. Cook *et al.* (1997) illustrated features on the septa and on the outer wall of conidia for identification and classification of the powdery mildews using light and scanning electron microscopy and including host range data. The results revealed that taxonomic systems of powdery mildew fungi are proposed three newly tribes as follows: Blumerieae, Sawadaeae and Phyllactinieae. Likewise, they have been constructed keys for the prediction of the anamorphs of all the powdery mildew genera found in the U.K.

Recently, molecular technology is most important advances in plant pathogen diagnostics that provide reliable identification, sensitive detection and accurate quantification of plant pathogens. In addition, these techniques allow detection of unculturable microorganism. Polymerase chain reaction (PCR) are being developed and implemented in horticultural and agricultural practice (Lievens and Thomma,

2005). Increasingly molecular techniques are employed in studies requiring the pathogen identification and also detection of fungi causing disease in plants, animals and humans. Developing direct detection assays enable a deeper understanding of natural microbial communities, especially as many fungi are difficult or impossible to cultivate. This research demonstrated the latest diagnostic techniques that were used to detection of fungi, including fluorescence *in situ* hybridisation (FISH), DNA array technology, multiplex tandem PCR and Padlock probe technology with rolling circle amplification and loop-mediated isothermal amplification (LAMP) (Tsui *et al.*, 2011). For the study of powdery mildews, molecular analysis is a useful tool to clarify the confused identification including study on phylogeny and evolution.

The molecular phylogeny and evolutionary analysis based on the nucleotide sequences of the nuclear ribosomal DNA are used to clarify the taxonomic systems of powdery mildews in the modern age (Mori *et al.*, 2000a, 2000b; Takamatsu, 2004; Takamatsu and Matsuda 2004). Phylogenetic analysis revealed that the Erysiphales is split into five distinct lineages, and the respective lineage is harmoniously support with the morphological studies (Braun, 1987; Cook *et al.*, 1997). Mori *et al.* (2000b) calculated the evolution time of Erysiphales, the first splitting within the Erysiphales occurred 138–92 million years (myr) ago and the radiation of the Erysiphales coincided with the evolution of angiosperm plants. According to the new molecular clock (1.26%/100 myr) by Berbee and Taylor (1993), the first split within the Erysiphales calculated as 76 myr ago and these fungi have been evolved along with the evolution of angiosperm as 100 myr ago. This assumption based on molecular clock of Berbee and Taylor may be more reliable than the previously calculation (Takamatsu, 2004).

The new nomenclature of powdery mildews (Braun and Cook, 2012) is proposed the new understanding of classification carried out the morphological studies consistent with molecular phylogenetic relationships. The generic taxonomy of powdery mildews consisted of 16 genera in a total, of which 12 genera were ectoparasitism and 4 genera were endoparasitism and divided into five tribes as described below (Table. 2):

**Table 2** The current systematics and taxonomy of the powdery mildews.  
(Braun and Cook, 2012)

<b>Tribes</b>	<b>Subtribes</b>	<b>Anamorphic genera</b>	<b>Teleomorphic genera</b>
<i>Erysipheae</i>		<i>Pseudoidium</i>	<i>Erysiphe emend.</i> ( <i>Erysiphe</i> sect. <i>Erysiphe</i> , <i>Erysiphe</i> sect. <i>Microsphaera</i> , <i>Erysiphe</i> sect. <i>Uncinula</i> )
<i>Golovinomy- ceteae</i>	<i>Neoerysiphinae</i> <i>Golovinomyce- tinae</i>	<i>Striatoidium</i>  <i>Euoidium</i>	<i>Neoerysiphe</i> <i>Golovinomyces</i> ( <i>Golovinomyces</i> sect. <i>Golovinimyces</i> , <i>Golovinomyces</i> sect. <i>Depressi</i> )
<i>Cystothecaeae</i>	<i>Cystothecinae</i>	<i>Graciloidium</i> <i>Setoidium</i>  <i>Fibroidium</i>	<i>Arthrocladiella</i> <i>Cystotheca</i> <i>Podosphaera</i> ( <i>Podosphaera</i> sect. <i>Podosphaera</i> , <i>Podosphaera</i> sect. <i>Sphaerotheca</i> subsect. <i>Sphaerotheca</i> , <i>Podosphaera</i> sect. <i>Sphaerotheca</i> subsect. <i>Magnicellulatae</i> )
<i>Phyllactinieae</i>	<i>Sawadaeinae</i>	<i>Octagoidium</i> <i>Oidiopsis</i> <i>Ovulariopsis</i> <i>Ovulariopsis</i>	<i>Sawadaea</i> <i>Leveillula</i> <i>Phyllactinia</i> <i>Pleochaeta</i>
<i>Blumerieae</i>		Unnamed <i>Oidium s. str.</i>	<i>Queirozia</i> <i>Blumeria</i>
<b>Unnamed tribe</b>		<i>Microidium</i>	Unknown

#### 1.4 Study of powdery mildews in tribe Phyllactinieae

(Braun, 1987; Braun and Cook, 2012; Shin, 2000; Takamatsu *et al.* 2008)

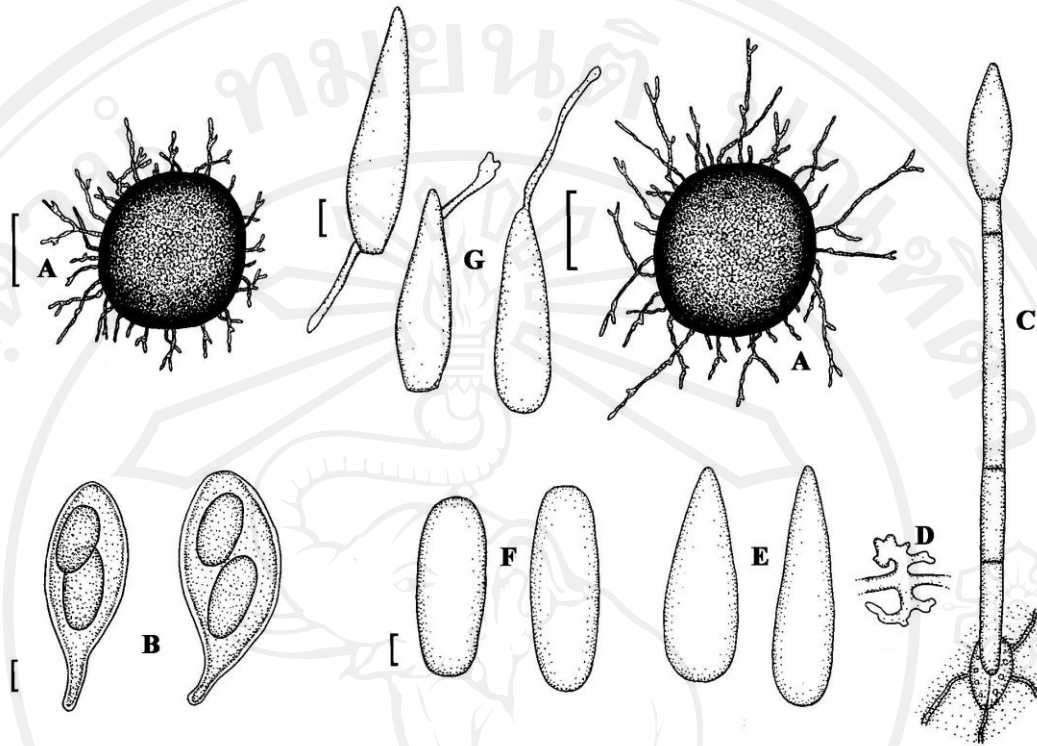
##### Tribe Phyllactinieae

The tribe Phyllactinieae is unique tribe within the Erysiphales, which having mode of parasitism in either the partly or true endo-parasitism. Phyllactinieae consists of four genera, of which three genera are partly endo-parasitism as genera *Phyllactinia* (in anamorph: *Ovulariopsis*), *Pleochaeta* (in anamorph: *Streptopodium*) and *Queirozia*. *Queirozia* is known as a unique genus in the powdery mildews which represent the first dematiaceous anamorphic powdery mildew and morphological features are closely related to *Pleochaeta* (Liberato *et al.*, 2006). The remaining genus, *Leveillula* (in anamorph: *Oidiopsis*) is only genus within the Erysiphaceae having a completely endo-parasitic nature. The host range of Phyllactinieae is confined to deciduous trees to woody plants.

*Leveillula* Arnaud, Ann. Epiphyt. 7:94 (1921)

Anamorphic state belongs to *Oidiopsis*; mycelium endophytic as well as ectophytic, white; appressoria well-developed, variable in shape, nipple-shaped to coral-like; conidiophores usually arise from internal mycelium through stomata, long and slender, producing conidia singly; foot-cells straight; conidia hyaline, dimorphic features, conspicuous fibrosin bodies absent. In teleomorphic state, chasmothecia have myceloid appendages and produce numerous asci which contain two ascospores in ascus (Fig. 15).



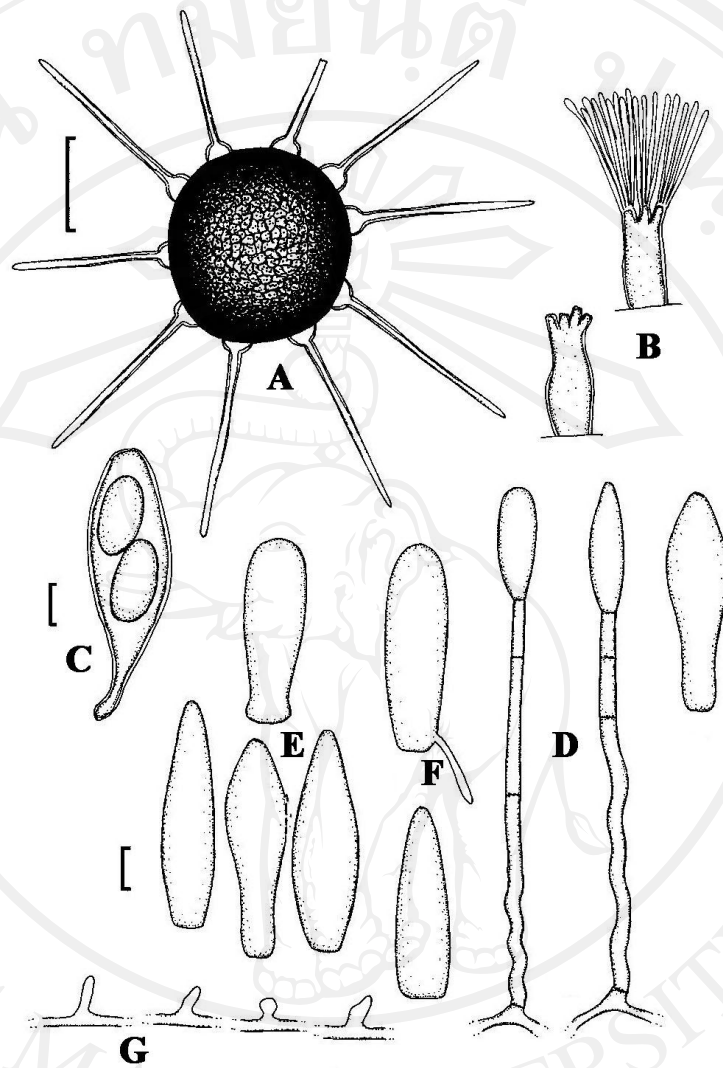


**Figure 15** *Leveillula taurica*.

A: chasmothecia, B: asci, C: conidiophore, D: appressorium, E: primary conidia, F: secondary conidia and G: conidial germination. (Braun and Cook, 2012)

*Phyllactinia* Lév., Ann. Sci. Nat., bot., 3 sér., 15 :144 (1851)

Anamorphic state belongs to *Ovulariopsis*; mycelium ectophytic as well as partly endophytic, white; appressoria well-developed, variable in shape, nipple-shaped to hook-shaped, occasionally coral-like; conidiophores arising from the upper part of the external mycelium, long and slender; foot-cells straight but flexuous or twisted in some species; conidia hyaline, clavate, conspicuous fibrosin bodies absent. This genus is characterized by chasmothecia having appendages two types, of which acicular appendages and special structure named as penicillate cells which adhesive material (Takamatsu, 2004). Chasmothecium produces numerous asci which contain 2(–4) ascospores in ascus (Fig. 16).

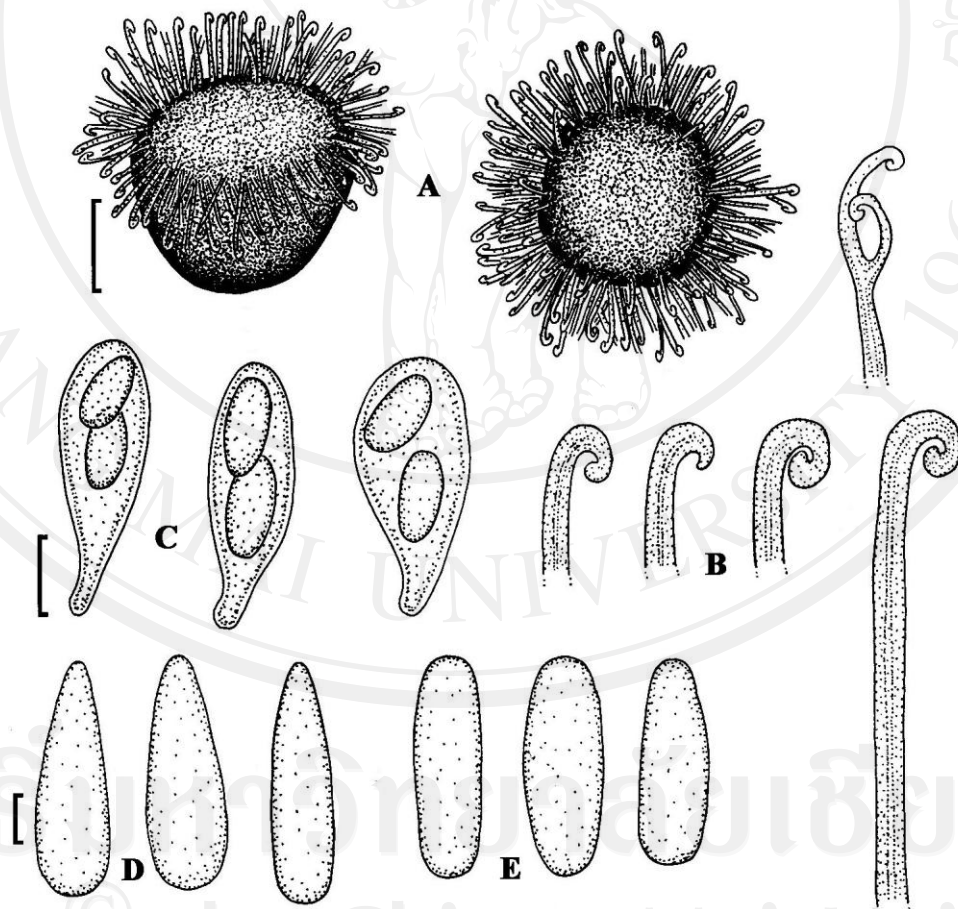


**Figure 16** *Phyllactinia robiniae*.

A: chasmothecia, B: penicillate cells, C: ascus, D: conidiophores, E: conidia, F: conidial germination and G: appressoria. (Braun and Cook, 2012)

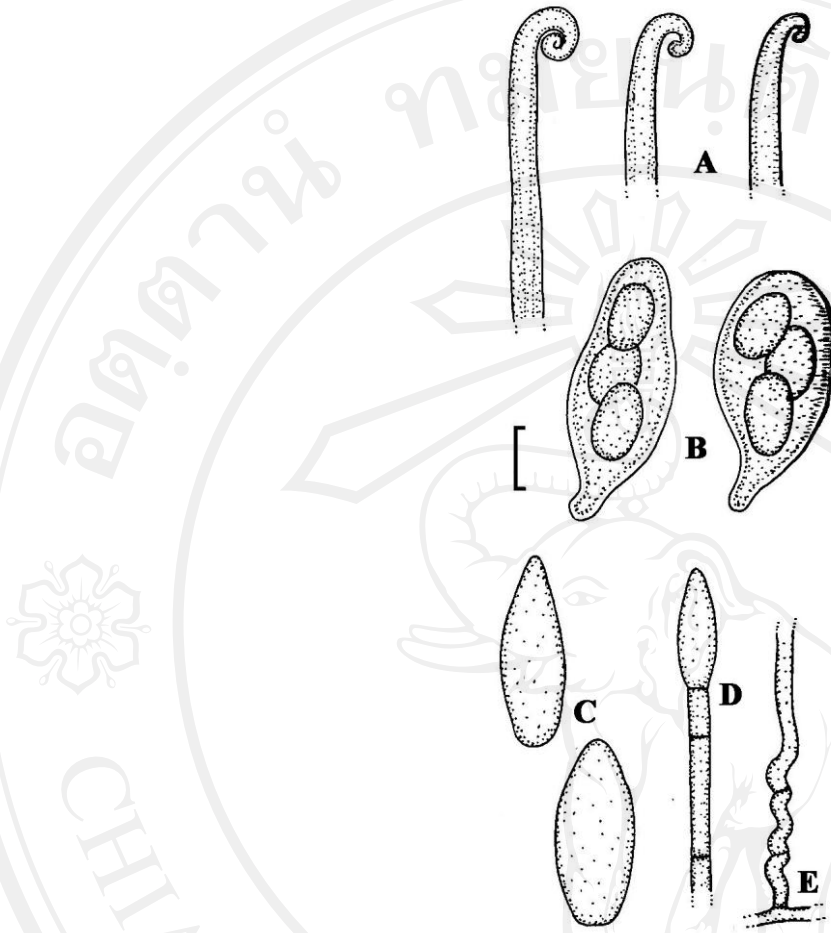
*Pleochaeta* Sacc. & Speg., in Sacc., *Michelia* 2 :373 (1881) emend.  
Kimbrough & Korf (1963)

Anamorphic state belongs to *Streptopodium*; mycelium ectophytic as well as partly endophytic, white; appressoria well-developed, variable in shape, nipple-shaped to hook-shaped; conidiophores arising from the upper part of the external mycelium, long; foot-cells spirally twisted; conidia hyaline, dimorphic features, conspicuous fibrosin bodies absent. In teleomorphic state, chasmothecia have circinate apex appendages and produce numerous asci which contain two ascospores in ascus (Fig. 17 and 18).



**Figure 17** *Pleochaeta robiniae*.

A: chasmothecia, B: appendages, C: asci, D: primary conidia and E: secondary conidia. (Braun and Cook, 2012)



**Figure 18** *Pleochaeta shiraiana*.

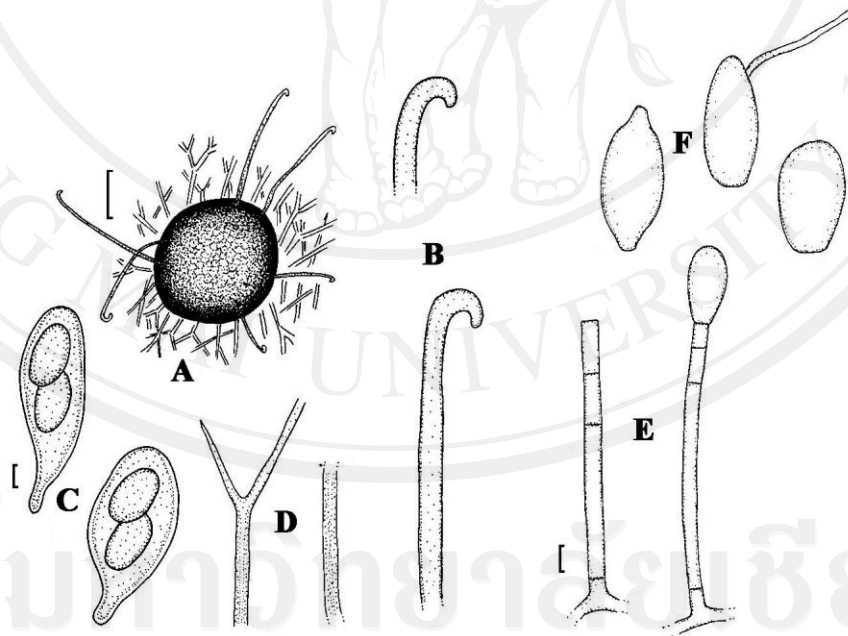
A: appendages, B: asci, C: conidia, D: conidiophore and E: foot cells.

(Braun and Cook, 2012)



*Queirozia* Viégas & Cardoso (1944)

This genus is proposed in the new taxonomic system of powdery mildews according to Braun (2011). Mycelium hypophyllous, hemiendophytic, septate, branched, hyaline to yellowish-brown. Special aerial hyphae rigid, simple or dichotomously branched. Conidiophores originate from the external mycelium, erect, simple, yellowish-brown; foot-cells cylindrical, either straight or having a sinuous base. Conidia produced apically, mostly singly or in short basipetal chains, subclavate or predominantly lemonshaped, aseptate, yellowish-brown. Chasmothecia hypophyllous, large, reddish-brown; peridium cells irregularly shaped; appendages few, hyaline, sinuous, cylindrical, in the upper half of the ascocarp with apex uncinately-circinate; asci hyaline, clavate to cylindrical produced 2(–4) ascospores in ascus, hyaline. This genus represents the first dematiaceous anamorphic powdery mildew (Liberato *et al.* 2006) (Fig. 19).



**Figure 19** *Queirozia turbinate*.

A: chasmothecium, B: appendages, C: asci, D: special aerial hyphae, E: conidiophores and F: conidia. (Braun and Cook, 2012)