Mycoscience 39: 71–75, 1998

Conidiomatal development of *Pestalotiopsis guepinii* and *P. neglecta* on leaves of *Gardenia jasminoides*

Kyoko Watanabe[1], Yoji Doi[2] and Takao Kobayashi[3]

[1] Faculty of Agriculture, Tamagawa University, 6–1–1, Tamagawa-gakuen, Machida, Tokyo 194–8610, Japan
[2] Faculty of Agriculture, Tokyo University, 1–1–1, Yayoi, Bunkyo, Tokyo 113–0032, Japan
[3] Tokyo University of Agriculture, 1–1–1, Sakuragaoka, Setagaya, Tokyo 156–0054, Japan

Accepted for publication 29 January 1998

This study has clarified the conidiomatal development of *Pestalotiopsis guepinii* and *P. neglecta* on leaves of *Gardenia jasminoides*. Acervuli of *P. guepinii* and *P. neglecta* developed in a similar manner, in two stages. In the first stage, cells aggregated, the central cells of the aggregate gradually disappeared, and the cells of inner layer produced numerous conidia. This conidioma was pycnidium-like in appearance, and in some cases ceased development at this stage. In the second stage, the upper layer of the pycnidium-like structure broke open, forming an acervular conidioma.

Key Words——acervulus development; intermediate structure; *Pestalotiopsis guepinii*; *Pestalotiopsis neglecta*.

Coelomycetous fungi have conidia formed within a cavity lined by fungal or fungal/host tissue (Hawksworth et al., 1995). The conidia-bearing structure (conidioma) is classified into five types according to exterior morphology: pycnidial, pycnothyrial, acervular, cuplate, and eustromatic (Hawksworth et al., 1995). The morphological structure is one of the most important keys in classification of the Coelomycetes (Sutton, 1980). These facts suggest that the morphogenesis of conidioma has taxonomic value.

Many researchers have described pycnidial-type conidiomatal development (Archer, 1926; Chippindale, 1929; Dodge, 1930; Harris, 1935; Maas et al., 1979; Mercer, 1913; Punithalingam, 1966). Development is divided into three stages: primordia, cavity formation, and conidiogenesis, with each pycnidial fungus having a determinate mode in each stage. In describing acervular development, Archer (1926) noted that the pseudo-acervulus in the genus *Pestalotia* (written as *Pestalozzia*) is formed by the breaking open of the pycnidial wall to form a structure similar in appearance to an acervulus. He also noted an other manner of acervulus development in which the upper cells of a cell aggregation proliferate and produce conidia. Generally, acervuli are formed by the breaking open of the wall of a pycnidium-like structure after it has developed as a pycnidium.

This study describes the development ontogeny of *Pestalotiopsis guepinii* (Desm.) Stey. and *Pestalotiopsis neglecta* (Thuemen) Stey., which have pycnidium-like structures that become acervuli. Some of the pycnidium-like structures did not become acervuli but remained similar in shape to pycnidia. This paper describes the acervuli developed from pycnidium-like structures in *P. guepinii* and *P. neglecta*, and offers speculation on the difference between acervuli which develop through pycnidium-like structures and true pycnidia.

Materials and Methods

Fungi *Pestalotiopsis guepinii* (MAFF 410282), *P. neglecta* (MAFF 236024 and one strain derived from Forestry and Forest Products Research Institute, Kyushu Research Center, Forest Pathology Laboratory). These fungi were cultured by the agar-leaf disk method using leaves of *Gardenia jasminoides* Ellis (Kishi, 1994). A piece of each fungal colony was inoculated on the boiled leaf, which was placed in a 6-cm plane agar plate, and kept under 60 W white fluorescent lamps at 23-25°C.

Light microscopy (LM) (a) Acervuli were fixed in 8% formaldehyde at 4°C. Specimens were frozen on stage at −25°C and sectioned at 10 μm with a sliding microtome (MCR802A). (b) Acervuli were fixed in buffered 4% glutaraldehyde for 3 h at 4°C, washed five times in buffer solution, then post-fixed in buffered 1% osmium tetroxide for 3 h at 4°C. The materials were dehydrated though an ethanol series for 15 min, infiltrated with acetone, then embedded in Spurr low-viscosity plastic (Spurr, 1969). Thin sections (0.3–0.5 μm) were cut with an ultra-microtome (JEOL JUM7) using a glass knife. These sections were stained with 1% toluidine blue O (Sigma).

Transmission electron microscopy (TEM) The same specimens as LM (b) were used for TEM. They were sectioned with an ultra-microtome (JEOL JUM7) using a diamond knife. The sections were picked up on mesh sheets, then poststained for 40 min on droplets of 0.5% uranyl acetate followed by 7 min on lead acetate. They were observed with a TEM (JEOL 100S) at 80 kV.

Scanning electron microscopy (SEM) The acervuli were

fixed in phosphate buffered 1% osmium tetroxide for 2 h at 4°C, then dehydrated through an ethanol series. The dehydrated specimens were thrown into liquid nitrogen for instantaneous freezing and cut with a chisel. They were hydrated through an ethanol series, fixed in phosphate-buffered 0.1% osmium tetroxide for 24 h at room temperature, then fixed in phosphate-buffered 1% osmium tetroxide for 1 h. These specimens were dehydrated again through an ethanol series. They were then critical point dried (Eiko DX-1), coated with gold (JEOL JFC 1100), and examined with a SEM (JEOL 5200) at 10–25 kV.

Results

Development of pycnidium-like structures Acervuli of *P. guepinii* and *P. neglecta* formed under or on the epidermis of *G. jasminoides* 3 wk after inoculation of the leaves. Both fungi have the same process of acervulus development.

The acervulus developed in the early stage from an aggregation of cells, consisting of more swollen cells than hyphal cells, below the epidermis of leaves of *G. jasminoides* (Figs. 1, 9, arrows indicate epidermis). These cells divided and produced new cells (Fig. 2). The new cells had higher-density cytoplasm and several large lipid bodies, and appeared very active (Figs. 3, 10a). Conversely, cells in the central portion of the aggregations included large amounts of lipids, large vacuoles containing myelin figures, and low-density cytoplasm with a few ribosomes and a cavity (Figs. 3, 10b). The appearance of the cavity was followed by cytolysis of low-density cells (Figs. 11, 12). The wall inner layer of cells proliferated toward the central cavity and most changed into a conidiophore with a high-density body and lipids, and produced conidia (Figs. 4, 5, 13, 14). The cavity then enlarged peripherally as a result of cell collapse. Many conidia were observed in the cavity (Figs. 6, 7, 14, 15). A round pore (ostiole) formed at the apex of the pycnidium-like structure (Fig. 7). Some pycnidium-like structures did not develop beyond this stage to become acervuli.

Breaking open of wall of pycnidium-like structure At the upper wall of some pycnidium-like structures, the number of cell layers decreased, and the inner layer cells did not form conidia. These layers disappeared, probably due to cytolysis or breakdown, and the acervuli developed (Figs. 8, 16).

Discussion

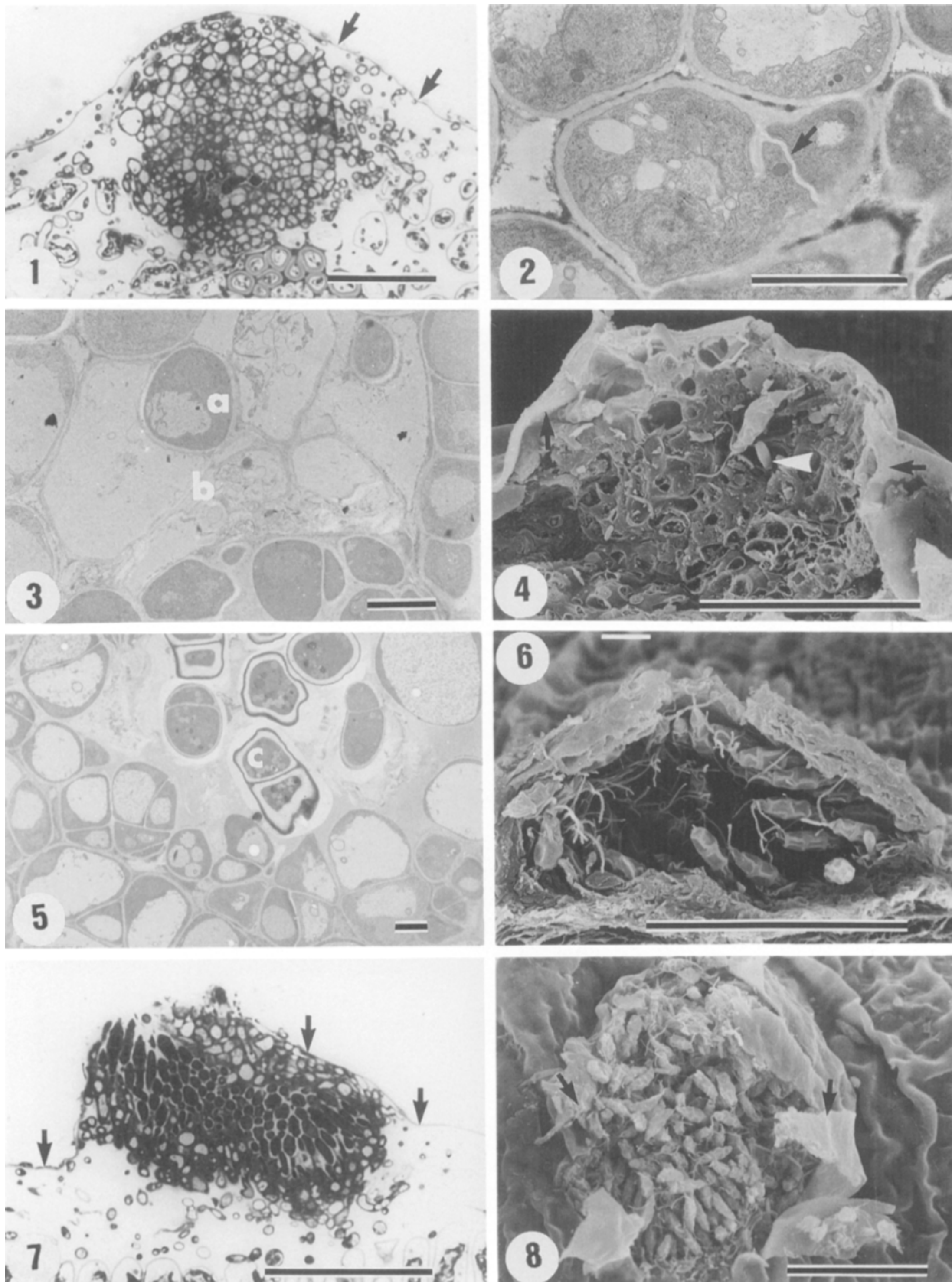
Two types of acervulus development were identified by Archer (1926). One type has the same early morphology as pycnidia, and the initially-closed pycnidia finally become wide open as shown in *Pestalotia guepinii* (as *Pestalozzia guepini*) Desm. In the other type, the cells proliferate by cell aggregation and produce conidia as shown in *Colletotrichum circinans* (Berk.) Vogl. Acervulus development of *P. guepinii* and *P. neglecta* in the present study is the same as the former type.

The development of the pycnidial primordium (the earliest stage of pycnidial development) is systematized as simple meristogenous, compound meristogenous, symphogenous (Kempton, 1919) and hyphal coiling (Maiello and Peterson, 1976). Primordia grow by aggregation of cells due to hyphae accumulating at the primordial surface. We were unable to observe pycnidial primordia. However, the aggregation of swollen cells in our specimens was observed under the leaf tissue or on the leaf surface, indicating that the cell aggregation originated from hyphae accumulating in the same way as in pycnidial primordia.

Cavity formation in pycnidia and stromatic conidioma is schizogenous, lysigenous, or a combination of both (Nag Raj, 1981). Each fungus has a particular type of cavity formation (Harris, 1935). In *P. guepinii* and *P. neglecta*, cavity formation is mainly lysigenous. The origin of the cavity is due to cytolysis of the central cells of the cell aggregation. The TEM photograph of the central portion matches the photographs of the autolysis phase of cells in *Phyllosticta harai* Togashi (Watanabe et al., 1997). As development progresses, the cavity enlarges by autolysis of conidiogenous cells after conidium formation. At this time, conidia are formed on the inner layer at the base, side, and upper portion of the wall of the pycnidium-like structure. The development of this pycnidium-like structure is quite similar to the pycnidial development of common pycnidial fungi such as *Phoma richardiae* (Mercer) Goid. (Mercer, 1913). No difference can be seen between the pycnidium-like structures of *P. guepinii* and *P. neglecta* and true pycnidia, in either exterior morphology or morphogenesis. Furthermore, the observation that some pycnidium-like structures in *P. guepinii* and *P. neglecta* stop developing suggests that both species have pycnidia.

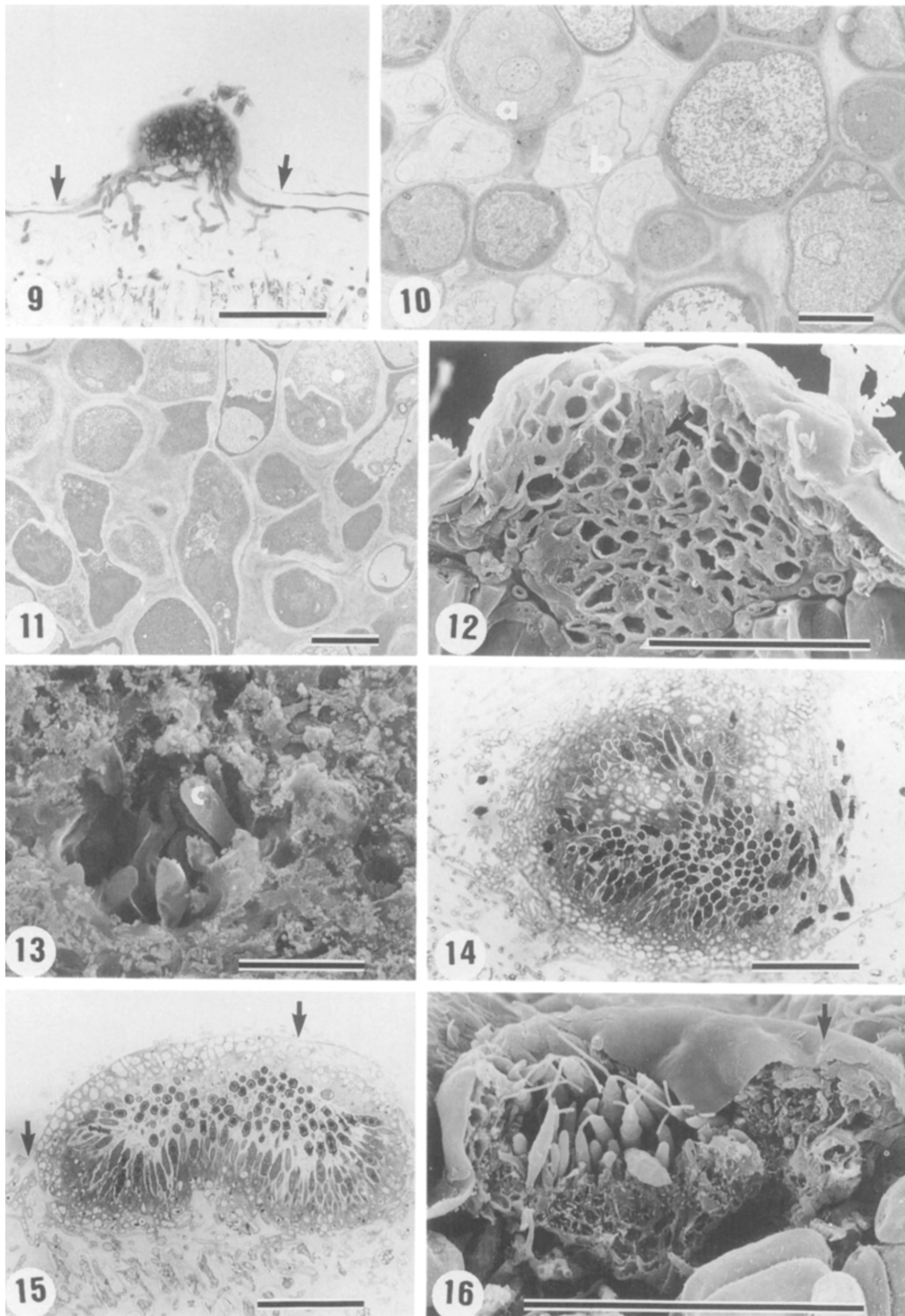
Many pycnidium-like structures developed into acervuli. The inner layer of the upper wall of some pycnidium-like structures stopped conidium formation, gradually became thin and then broke open by cytolysis, completing the acervulus development. This last process is important and necessary in acervuli formation. We speculate that the difference between acervuli and pycnidia in mature conidioma caused by the result of this last stage based on our result.

Acervuli of *P. guepinii* and *P. neglecta* are of a more-developed morphology than true pycnidia. We may argue that the morphologies of the acervuli and pycnidia have evolved by the same process, since one fungus shows both acervuli and pycnidia. Alberch et al. (1979) proposed how heterochronic change in ontogeny relate to phyletic trends. The notion of heterochrony which is difference of timing or speed of morphogenesis leading difference of mature shape on evolutionary sift. However, we cannot discuss heterochrony here, because we do not show whether acervular fungi have a more developed character than pycnidial fungi, or whether pycnidial fungi have an intermediate morphology in the morphogenesis of acervular fungi. The answers to these questions require detailed analysis and molecular data on morphogenesis.



Figs. 1–8. *Pestalotiopsis guepinii*.

1: Aggregation of hyphal cells under epidermis of *Gardenia jasminoides* (arrows), LM. 2: Cell division (arrow) in aggregation of cells, TEM. 3: Active cells (a) and autolytic cells (b) in aggregation of cells, TEM. 4: Conidia (arrowhead) formed at inner cell layer of small cavity, SEM. Arrows indicate epidermis of *G. jasminoides*. 5: Cavity and conidia (c) in aggregation of cells, TEM. 6: Conidia in pycnidium-like structure, SEM. 7: Vertical section of pycnidium-like structure, LM. Arrows indicate epidermis of *G. jasminoides*. 8: Mature acervulus, SEM. Arrows indicate epidermis of *G. jasminoides*. Figs. 1, 4, 6–8: Bars=50 μm; Figs. 2, 3, 5: Bars=3 μm.



Figs. 9–16. *Pestalotiopsis neglecta*.

9: Aggregation of hyphal cells under epidermis of *Gardenia jasminoides* (arrows), LM. 10: Active cells (a) and autolytic cells (b) in aggregation of cells, TEM. 11: Dispersion by autolysis in aggregation of cells, SEM. 12: Early structure of cavity formation aggregation of cells, SEM. Arrow indicates cavity. 13: Conidial formation (c) in small cavity of aggregation of cells, SEM. 14: Transverse section of pycnidium-like structure, LM. 15: Transverse section of pycnidium-like structure, LM. Conidia are formed at basal wall of pycnidium-like structure. Arrows indicate epidermis of *G. jasminoides*. 16: Mature acervulus,SEM. Arrow indicates epidermis of *G. jasminoides*. Figs. 9, 12, 14–16: Bars=50 μm; Figs. 10, 11: Bars=3 μm; Fig. 13: Bar=10 μm.

Literature cited

Alberch, P., Gould, S. J., Oster, G. F. and Wake, D. B. 1979. Size and shape in ontogeny and phylogeny. Paleobiology **5**: 296–317.

Archer, W. A. 1926. Morphological characters of some Sphaeropsidales in culture. Ann. Mycol. **24**: 1–84.

Chippindale, H. G. 1929. The development in culture of *Ascochyta gossypii* Syd. Trans. Br. Mycol. Soc. **14**: 201–215.

Dodge, B. O. 1930. Development of the asexual fructifications of *Chaetomella raphigera* and *Pezizella lythri*. Mycologia **22**: 169–174.

Harris, H. A. 1935. Morphological studies of *Septoria lycopersici*. Phytopathology **25**: 790–799.

Hawksworth, D. L., Kirk, P. M., Sutton, B. C. and Pegler, D. N. 1995. Ainsworth and Bisby's Dictionary of the fungi, 8th ed., pp. 104–105. CAB International, Wallingford, U.K.

Kempton, F. E. 1919. Origin and development of the pycnidium. Bot. Gaz. **68**: 233–261.

Kishi, K. 1994. Production of pycnidia and acervuli by agar-leaf disk method. Ann. Phytopathol. Soc. Japan **60**: 345. (In Japanese.)

Maiello, J. M. and Peterson, J. L. 1976. Pycnidium ontogeny in *Phyllosticta antirrhini*. Mycologia **68**: 1121–1125.

Maas, J. L., Pollack, F. G. and Uecker, F. A. 1979. Morphology and development of *Pilidiella quercicola*. Mycologia **71**: 92–102.

Mercer, W. B. 1913. On the morphology and development of *Phoma richardiae* n. sp. Mycol. Zentralbl. **2**: 244–253.

Nag Raj, T. R. 1981. Coelomycete systematics. In: Biology of conidial fungi, (ed. by Cole, G. T. and Kendrick, B.), pp. 43–84. Academic Press, New York.

Punithalingam, E. 1966. Development of the pycnidium in *Septoria*. Trans. Br. Mycol. Soc. **49**: 19–25.

Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Urtrastr. Res. **26**: 31–43.

Sutton, B. C. 1980. The Coelomycetes, pp. 9–21. CMI, Kew, Surrey, U.K.

Watanabe, K., Doi, Y. and Kobayashi, T. 1997. Pycnidial development of *Phyllosticta harai* and *Sphaeropsis* sp. Mycoscience **38**: 259–265.