Trans. Br. mycol. Soc. **86** (3), 451–456 (1986) *Printed in Great Britain*

A LOCULATE STROMATIC CONIDIOMATAL ISOLATE OF *GLOMERELLA CINGULATA*

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An isolate of *Glomerella cingulata* (anamorph *Colletotrichum gloeosporioides*) which formed loculate stromatic conidiomata was recovered from a single ascospore line from soybeans (*Glycine max*). The loculate stromatic conidomata were formed on the surface of potato-dextrose agar and subepidermally in apple fruits. The loculi were without ostioles and broke open presumably from pressure exerted by developing conidia. Conidiogenesis and conidial morphology are similar to ascospore lines of three other acervular anamorphs of *G. cingulata*.

Glomerella cingulata (Stonem.) Spauld. & Schrenk has a wide host range causing anthracnose of several crop species (Shear, 1913; Simmonds, 1965; Tiffany & Gilman, 1954). The fungus is isolated frequently from soybean plants showing symptoms of anthracnose (Manandhar, Hartman & Sinclair, 1984, 1986; Roy, 1982; Sinclair, 1982). *Glomerella cingulata* has several anamorph species, groups, or strains (Shear, 1913; von Arx, 1957), which are separated by host specificity, cultural characteristics and conidial morphology. The fungus is weakly heterothallic (Vermeulen, Ferranto & Gielink, 1984) showing high fertility when opposite mating types are grown in close proximity. Single ascospore isolates show great variation.

Isolates of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., one of the anamorph strains of *G. cingulata*, vary widely in pathogenicity and culture characteristics, and size of conidia (Burger, 1921; Shear, 1913). Single spore isolates readily mutate in culture (Burger, 1921).

We report the isolation of a loculate stromatic conidiomatal isolate of *G. cingulata* from a single ascospore line recovered from soybeans (*Glycine max* (L.) Merr.).

MATERIALS AND METHOD

A single conidium isolate of *C. gloeosporioides* was isolated from an immature soybean pod (Manandhar *et al.*, 1986). The resulting culture, designated as A-1, produced acervuli and perithecia on potato-dextrose agar (Difco) (PDA) under 12 h alternating cool fluorescent light (800 μEin/m^2/s flux) at 26 °C. Single ascospore isolates were recovered from mature perithecia by either of two methods: (i) the top of a 9-cm diam culture plate containing a sporulating culture of *G. cingulata* was replaced with the bottom of another culture plate containing fresh PDA and held in place with cellophane tape for 24 h; or (ii) by collecting the matrix from five to ten mature perithecia with a sterile loop and diluted to 10^3 ascospores/ml with sterile, deionized water, then streaking a 0·1 ml sample from each suspension separately in PDA culture plates. Regardless of the source of ascospores, approximately half of the resulting colonies produced acervuli and half produced loculate conidiomata. Conidia from acervuli and loculi were measured to confirm identification of the isolates producing ascospores.

Two types of isolates produced loculate conidiomata: isolate P-1, which produced appressed mycelial growth on PDA with numerous small (78–108 μm) loculi; and P-2, which produced fluffy mycelial growth in culture with few large loculi (described later). The two types were deposited in the American Type Culture Collection (ATCC) and designated as ATCC-58221 and ATCC-58222, respectively.

Pairings were made in culture between P-1, P-2, and other isolates that produced either acervular or loculate conidiomata. All combinations of crosses were done according to the method of Vermeulen *et al.* (1984). All mating combinations resulted in production of clumps of glomerate perithecia at the coalescing margins of compatible mating types. Isolate P-2 was selected for further study.

Pathogenicity

Soybean (cv. Corsoy 79) seedlings in the V-1 growth stage (first trifoliolate leaf stage) (Fehr *et al.*, 1971) were inoculated using an atomizer with a conidial suspension of isolate P-2 ($4–5 \times 10^5$ conidia/ml) until runoff. Plants sprayed with sterile

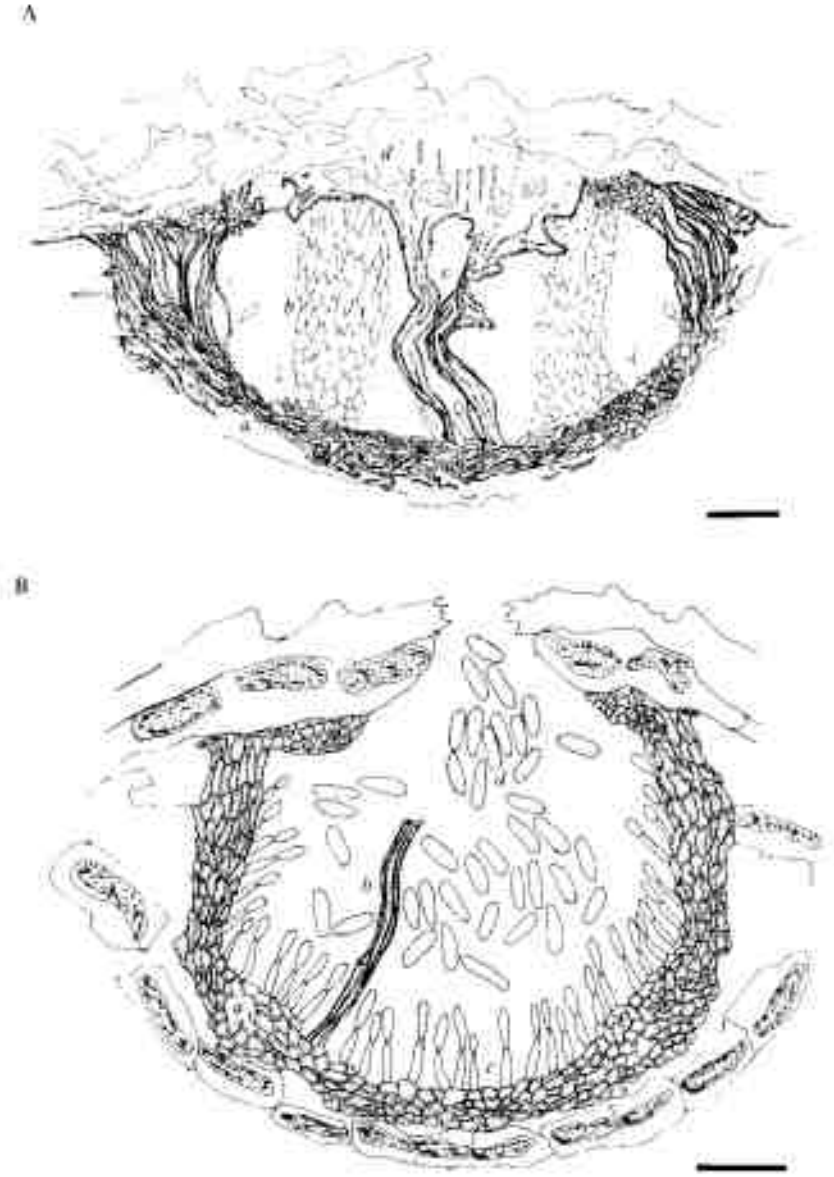


Fig. 1. Camera lucida drawings of vertical sections through loculate stromatic conidiomata of P-2 ascospore line of *Glomerella cingulata*. (A) A vertical section of a developing stromatic locule, a, locular wall, b, pseudoparenchymatous tissue, c, sterile brown hyphal strands, and d, host cuticle and epidermis. Bar = 27 μm. (B) A vertical section of a matured stromatic locule, a, locular wall, b, remains of sterile brown hyphal strands, c, conidiogenous region, and d, conidia. Bar = 27 μm.

deionized water served as controls. There were three plants for each of five pots per treatment. All plants were placed in a mist chamber under alternating 12 h dark and cool fluorescent light (900 μEin/ml^2/s lux) for 72 h at 25 ± 2°. After 72 h all plants were transferred to the greenhouse. After 5 d, the seedlings were harvested and surface sterilized with 0·5 NaOCl (Clorox) for 4 min and washed twice in sterile deionized water. To induce sporulation, plant parts were soaked in an aqueous solution of commercial paraquat (1,1'-dimethyl (-4,4'-bipyridinium dichloride) 28·1% active ingredient) (1:40) (Cerkauskas & Sinclair, 1980). All plant parts were placed on moist cellulose pads (Kimpac) and incubated at near 100% r.h. under continuous light (800 μEin/m^2/s lux) for 5–7 d at 25°.

Histopathology

To study the development of loculate stromatic conidiomata, apple (*Malus sylvestris* Mill., three of each cv. Jonathan and cv. Red Delicious) were inoculated using hyphae from the edge of a 3-day-old colony of isolate P-2 on PDA. A plug of tissue was cut from each fruit using a sterile 5 mm cork borer, then using the same size borer, a fungal plug was cut from the culture and placed in the hole. Uninoculated plugged fruits served as controls. All holes were sealed with cellophane tape and incubated under 12 h alternating dark and cool fluorescent light for 10 d at 26°. Tissue samples were collected from inoculated areas every 24 h and fixed in a formaldehyde:acetic acid:50% ethyl alcohol (5:5:90)(v:v)(FAA) for 48 h, dehydrated in tertiary butyl alcohol series and embedded in paraffin (paraplast containing 0·4 to 1% beeswax and filtered through cotton) (Johansen, 1940). Paraffin blocks were sectioned in slices 9 μm thick, stained first with safranin and then light green and mounted in Canada balsam (Johansen, 1940).

RESULTS

Culture characteristics

After 10 d on PDA isolate P-2 produced fluffy, pallid brownish drab or pale olive-gray colonies (Ridgway, 1912) with 15–25 scattered, large (250–325 μm diam) stromata bearing loculate conidiomata on the agar surface. Colony growth was 60 mm under continuous or alternating 12 h fluorescent light after 5 d at 26°.

On soybean stems superficial loculate stromatic conidiomata were formed in 5 d or more after inoculation. On apple fruits, vinaceous brown to chocolate lesions formed after 5 d.

Development of loculate stromatic conidiomata

Primordia of loculate conidiomata originated as either symphogenous or meristogenous in equal proportion. Primordial cells multiplied rapidly and rounded up until a distinguishable loculate stromatic conidioma was formed whether superficially on PDA and soybean stems, or subepidermally on apple fruits. The locular wall was plectenchymatous, soft, of textura angularis, 2–5 cells thick measuring 3–18 μm (Figs 1A, B, 2C–E). The minute subepidermal loculate stromatic conidiomata on apple fruits (Figs 1A, B, 2A–C, 3A–C) measured 196·3–436·7 × 190·6–328·5 μm.

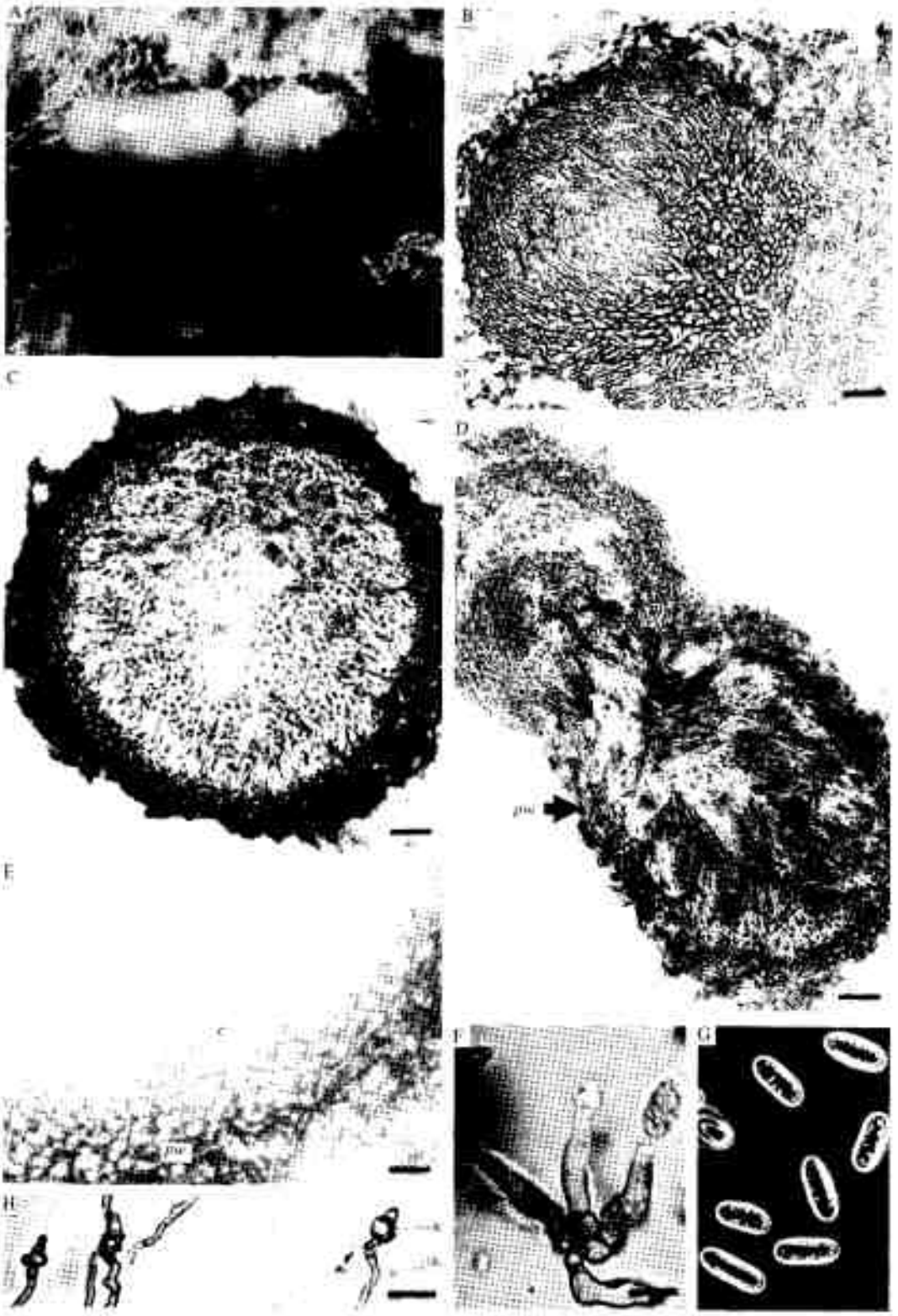
The loculi appeared to be formed schizogenously by rupturing pseudoparenchymatous tissue on PDA and soybean stems, but both schizogenously and lysigenously from subepidermal sterile brown hyphae on apple fruits (Fig. 1A, B).

The conidiogenous region developed over the entire internal surface of the stromatic locule, producing conidia which soon filled the cavity (Figs 1A, 2C). On apple fruits, the conidiogenous regions were restricted to the bottom and sides of the subepidermal loculate stromata. No ostioles were formed, but the loculi ruptured due to mechanical pressure exerted by the conidial mass (Fig. 2D). On apple fruits, the epidermis and cuticle were ruptured by the growth of sterile brown hyphae (Figs 1B, 3D), followed by rupture of the loculi.

Conidiogenous cells of isolate P-2 were hyaline, phialidic, collaretes sometimes present, measuring 14·6–28·4 μm (Fig. 2E, F). Conidia were straight, 14·5–20·3 × 4·4–6·4 (mostly 17·4 × 5·8) μm (Figs 1B, 2G), were not significantly ($P = 0·05$) different from those produced by either isolate P-1, measuring 14·3–18·4 × 5·6–7·2 (mostly 16·6 × 6·2) μm, or from pionnotal acervuli without setae, measuring 14·5–17·4 × 5·2–7 (mostly 14·5 × 5·8) μm. Appressoria were readily formed from vegetative hyphae (Fig. 2H), navicular to ovate, irregularly lobed, dark brown with single germination pore in the middle. Individual appressoria measured 5·8–11·3 × 5·2–8·1 (mostly 8·7 × 5·8) μm which were not significantly ($P = 0·05$) different from those developed from either ascospores or conidia.

DISCUSSION

Locular stromatic conidiomatal types were described by Sutton (1980). Some of our ascospore lines of *G. cingulata* with the same conidiogenesis and conidial morphology produced acervuli, while others, such as isolate P-2, produced stromatic loculi, and others, such as isolate P-1, produced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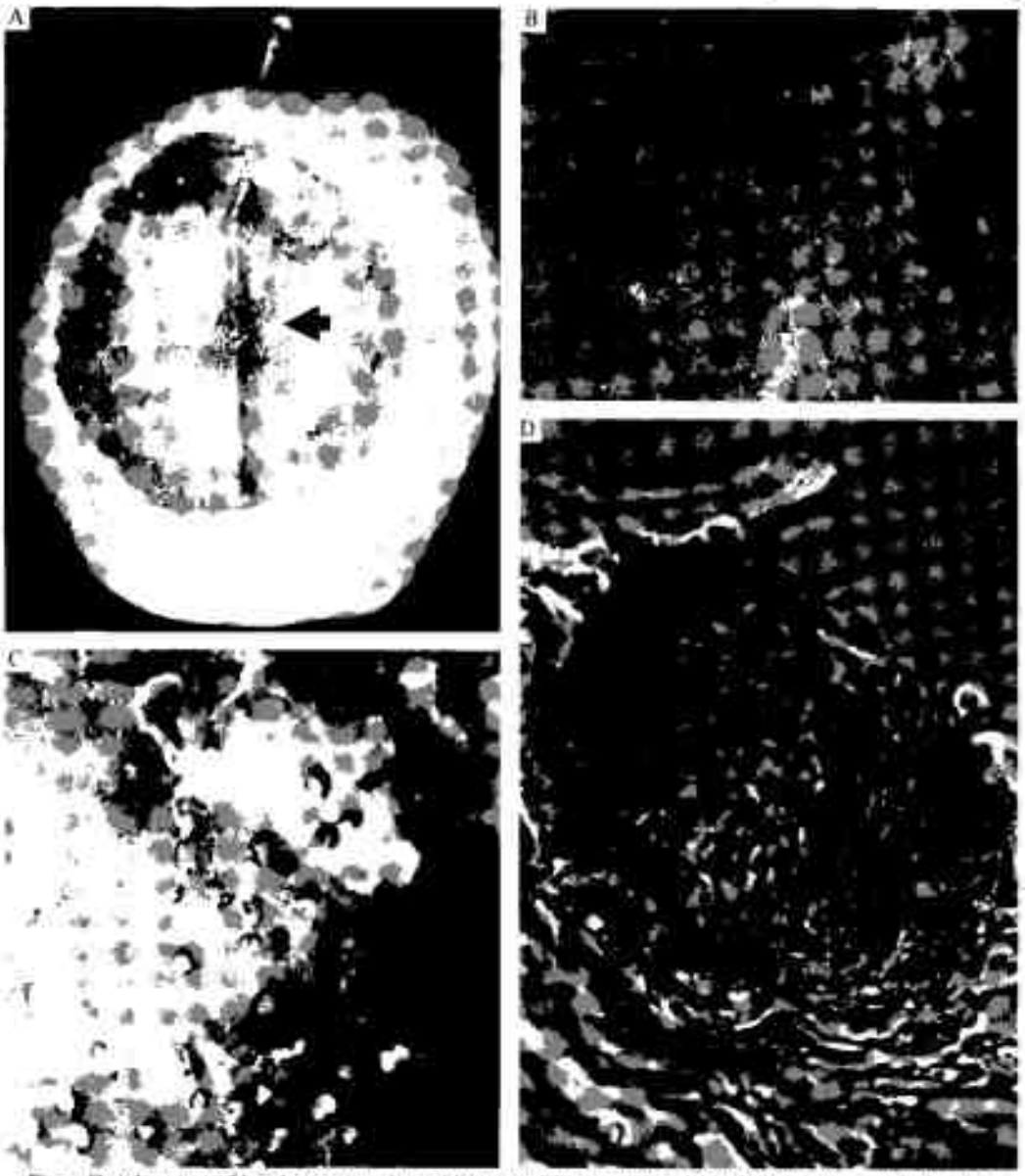

Fig 3. Development of loculate stromatic conidiomata of P-2 ascospore line of *Glomerella cingulata*. (A) A lesion on the inoculated Red Delicious apple fruit (one half of its natural size) showing locule formation (arrow). (B) A magnified view of the developing stromatic locule. Bar = 100 μm. (C) A magnified view of mature stromatic locule showing exposed masses of conidia (arrow). Bar = 50 μm. (D) A vertical section of a stromatic locule through apple tissue. Bar = 25 μm.

Fig. 2. Development of loculate stromatic conidioma on potato-dextrose agar (PDA) and microscopic studies of P-2 ascospore line of *Glomerella cingulata*. (A) Loculate stromata formation on PDA, arrow showing oozing mass of conidia. Bar = 100 μm. (B) A vertical section of a developing locule. Bar = 20 μm. (C) A vertical section of a matured locule prior to exposing its conidia. c, conidia, pc, locular cavity, and pw, locular wall. Bar = 35 μm. (D) A vertical section of a matured stromatic locule, after its conidia have been exposed, c, conidia, and pw, locular wall. Bar = 35 μm. (E) An enlarged view of a piece of a vertical section of a matured stromatic locule, c, conidiogenous region, and pw, locular wall. Bar = 20 μm. (F) Conidiogenous cells (arrow). Bar = 10 μm. (G) Conidia under phase contrast microscopy. Bar = 15 μm. (H) Hyphal appressoria. Bar = 10 μm.

both. *G. cingulata* appears to be pleomorphic (Carmichael, 1979; Savile, 1969; Weresub, 1979). A 'closed acervular' type of *G. cingulata* with viable ascospores compatible with other mating types of *G. cingulata* was mentioned by Vermeulen *et al.* (1984) without giving details on conidiogenesis and conidiomatal ontogeny. Other reported pleomorphic fungi are *Peziza lythri* (Desm.) Shear & Dodge (Dodge, 1930), *Nannizzia gypsea* (Nannizzi) Stockd. and *N. incurvata* Stockd. (El-Ani, 1968).

Conidiomatal ontogeny and conidiogenesis of a fungus has taxonomic implications (Cole, 1981; Nag Raj, 1981; Punithalingam, 1966). The locular conidiomata of isolate P-2 originated both meristogenously and symphogenously, similar to that reported for other fungi that form pycnidia or stromatic loculi (Kempton, 1919; Punithalingam, 1966).

We hesitate to give a name to this new loculate stromatic conidiomatal isolate of *G. cingulata*, since we lack cytological evidence as to whether the loculate conidiomata are produced as a result of chromosomal aberration or as gene function. Additional studies will have to be made.

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(Received for publication 29 July 1985)