Chlamydospores of Fusarium roseum 'Graminearum' as Survival Structures

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ABSTRACT

Macroconidia of *Fusarium roseum* f. sp. *cerealis* 'Graminearum' formed chlamydospores when placed in nonautoclaved soil stored at 21-30 C but not in soil stored at 5-11 C. Protoplasm in the terminal and adjacent cells moved into middle cells of macroconidia, which then appeared denser and stained darker with cotton blue than cells in macroconidia that had not undergone movement of protoplasm. When changed macroconidia germinated, the cells containing the dense protoplasm rounded up and the germ tubes appeared to originate from the inner wall of a double-walled cell. After 500 days of burial in nonautoclaved soil, chlamydospores germinated and infected wheat. Phytopathology 60:1175-1177.

Fusarium roseum Lk. (emend Snyd. & Hans.) f. sp. *cerealis* (Cke.) Snyd. & Hans. 'Graminearum' has long been known to cause seedling blight, foot rot, and head blight of wheat (4). Although many *Fusarium* spp. form chlamydospores as survival structures, Graminearum has not been reported to do so. Little is known about the survival of Graminearum from growing season to season, although it can persist in soil in the Pacific Northwest as mycelium growing within crop refuse (3). Graminearum produces the perfect stage *Gibberella zeae* (Schw.) Petch on wheat straw refuse in Minnesota. The overwintering of perithecia constitutes a means of survival from growing season to season with ascospores providing primary inoculum to infect wheat the following growing season.

Chlamydospores as a survival structure of Graminearum have not been investigated, and are reported to seldom form within hyphae or macroconidia in culture (4, 7). Contradictory evidence by Oswald (8), however, showed that Graminearum colonies often undergo morphological changes, including formation of chlamydospores, in culture.

Macroconidia of Graminearum, that had apparently been converted into chlamydosporelike structures, were observed by the author for 2 growing seasons in soil from wheat and corn plots at Rosemount, Minn. With Oswald's report, this suggests the possibility that chlamydospores are survival structures of Graminearum in soil.

My purpose was to determine (i) if Graminearum could form chlamydospores as survival structures in soil, and (ii) whether cultures that grew from chlamydospores could infect wheat.

MATERIALS AND METHODS.—Ten single conidial cultures of Graminearum, isolated from and pathogenic to wheat, were used.

A modified technique by Chinn (2) was used to recover macroconidia from soil. Cultures were grown on 2% water agar (used instead of other media because more macroconidia were produced) for 30 days. Macroconidia from each petri dish (eight petri dishes/culture) were suspended in 2 ml of a 1:5,000 solution of Tween 20 (polyoxyethylene sorbitan monolaurate) in sterile distilled water. Conidial suspensions of each culture were grouped, transferred to 3% water agar kept at 45 C, and adjusted to a concn of 25,000 conidia/ml. Sterile microscope slides were dipped 1 to 2 sec in the agar-conidial suspension, leaving an agar film 2-mm thick containing 100-250 macroconidia/slide, and held vertically until dry.

A sandy loam, half of which was autoclaved at 121 C for 2 hr (control), was passed through a 2-mm mesh sieve and moistened to 40% water holding capacity (dry wt basis). Soil (750 g) was carefully placed in 1-liter glass jars (four microscope slides/jar) to minimize disturbance to the agar films on the slides. Each slide protruded 10 mm above the soil.

Macroconidia, placed in autoclaved and nonautoclaved soils, were stored either (i) outside in June when the maximum daytime temp were 21-30 C and in November when maximum daytime temp were 5-11 C for the first 7 days of the experiment, respectively, or (ii) in the laboratory, half at 24 C and half at 5 C. At the end of each test period, a slide was recovered by removing soil from one side and tilting the slide so that the agar film on the other side was not disturbed. The slide was washed gently in tap water, and agar from one side removed. The whole slide was immersed in dilute cotton blue stain and lactophenol for 10 min. Two slides were examined microscopically (×100) per treatment (50 macroconidia/slide). One washed slide was placed on potato-dextrose agar (pH 4.5-5.0); 100 macroconidia were examined for germination after 6 to 12 hr.

Inoculum of Graminearum (originating from chlamydospores) was increased on 3% cornmeal-sand medium for 30 days. The infested cornmeal-sand medium was mixed (1:30, v/v) with nonautoclaved soil and placed in 15-cm clay pots; five wheat seeds treated with captan were planted. Root and crown rot observations were taken when wheat plants were mature.

Origin of Graminearum cultures isolated from field soil was determined by placing a 1:10,000 soil dilution on solidified PCNB (pentachloronitrobenzene) agar. The petri dishes were examined microscopically (×100) after 6 to 12 hr, the area of the colony was marked, and the type of structure fungal growth originated from was noted.

TABLE 1. Changes in morphology and germination of *Fusarium roseum* f. sp. *cerealis* 'Graminearum' after either 100 days in nonautoclaved soil at 2 temp in the laboratory or after 500 days in nonautoclaved soil outdoors in June and November

Observation	Location[a]	Temp	Days macroconidia stored[b]				
			0	1	7	100	500
		C	%	%	%	%	%
Changed spores	Laboratory	5	0	3	5	1	
		24	0	51	42	57	
	Outdoors	5-11 (Nov.)	0	1	1	9	4
		21-30 (June)	0	43	49	54	51
Germination	Laboratory	5	81	74	43	6	
		24	79	81	80	33	
	Outdoors	5-11 (Nov.)	91	67	7	3	23
		21-30 (June)	84	85	73	66	21

[a] Soil was kept in the laboratory at either 5 or 24 C and outdoors when the maximum daytime temp for the first 7 days of burial were 5-11 C in November and 21-30 C in June.
[b] Percentages based on 100 macroconidia.

RESULTS.—Within 1 day, 51% and 43% (Table 1) of the macroconidia placed in nonautoclaved soil at 24 C in the laboratory and at high temp outside, respectively, had changed morphologically. Usually, protoplasm appeared to have moved from terminal and adjacent cells into middle cells of the changed macroconidia, as there was no evidence of protoplasmic leakage nor increased bacterial activity around macroconidial cells void of protoplasm compared to cells in normal macroconidia (Fig. 1-A). Protoplasm in middle cells of the changed macroconidia appeared denser and stained darker with cotton blue than cells of macroconidia placed in autoclaved soil and without this change (Fig. 1-B). Infrequently, protoplasm of middle cells appeared to have moved into terminal cells.

Although there was no rounding up of cells into which protoplasm had moved or evidence of two cell walls, I considered these changed cells as chlamydospores, because at germination the cells rounded up and germ tubes originated from the inner wall of a double-walled cell (Fig. 1-C). Unchanged macroconidia did not appear to have double-walled cells at germination, but rounding up of cells occurred typical of germinating fungus spores (Fig. 1-D). Most germ tubes originated from the terminal cells of unchanged, but from the middle cells of changed, macroconidia.

These changes occurred most frequently with macroconidia in nonautoclaved soil stored either outside (21-30 C) initially or in the laboratory (24 C), and infrequently with macroconidia placed in nonautoclaved soil stored outside (5-11 C) initially, in the laboratory (5 C), or in autoclaved soil stored at either high or low temp. Percentage of macroconidia that converted to chlamydospores did not vary appreciably at 100 or 500 days from the percentage of macroconidia converted to chlamydospores at 1 day. Most conversion of macroconidia to chlamydospores occurred in 1 day.

After approximately 30 days, most macroconidia stored in autoclaved soil at high temp germinated. Mycelium grew throughout the soil, and secondary macroconidia formed that were subsequently converted into chlamydospores. Percentage of macroconidial germination was closely related to percentage of macroconidia converted into chlamydospores. Germination was least in treatments where few macroconidia were converted to chlamydospores. The relatively high germination of macroconidia stored initially at low temp outside for 500 days possibly was due (i) to secondary macroconidia; (ii) to macroconidia that survived unchanged; or (iii) to chlamydospores not recognized as such. Some chlamydospores present at the end of the experi-

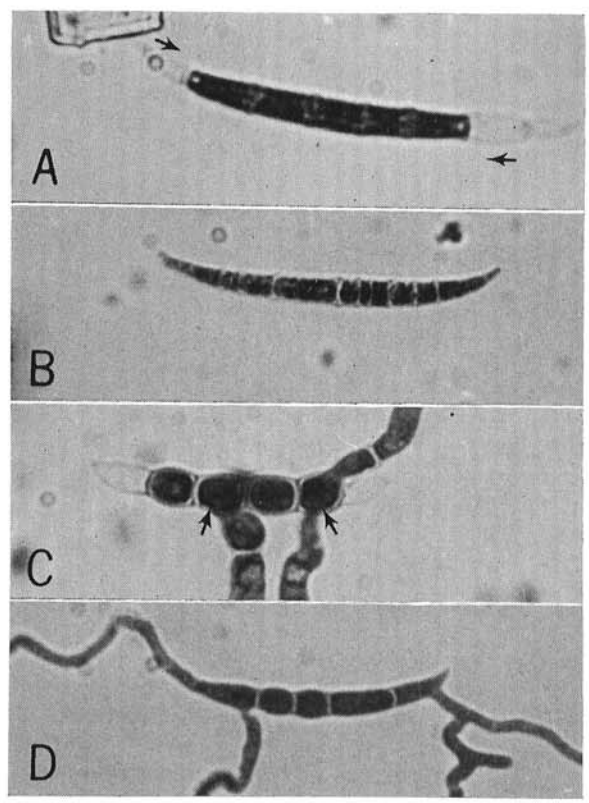

Fig. 1. Morphological changes and germination pattern of macroconidia of Graminearum (×645). **A)** Changed macroconidium, showing protoplasm that has moved from terminal and adjacent cells into middle cells. **B)** Unchanged macroconidia. **C)** Germination of changed macroconidium, germ tubes originating from an inner wall of double-walled cell. **D)** Germination of unchanged macroconidium.

ment originated from secondary macroconidia, but most chlamydospores present were formed within macroconidia initially placed in soil, as there was little germination after macroconidia were added to nonautoclaved soil.

One culture was selected at each test period from each treatment, increased on 3% cornmeal-sand media, and added to nonsterile soil in the greenhouse. All cultures thus treated caused root and crown rot of wheat (three to four tillers/plant) and were reisolated from the infected wheat plant.

Cultures of Graminearum originated from chlamydospores in soil on which corn or wheat was grown the preceding growing season (Table 2). Despite severe agitation of soil prior to pouring of dilution plates, most propagules were not separated from pieces of organic matter; consequently, most growth originated from organic matter. The chlamydospores found in field soil resembled the structures artificially produced in soil.

DISCUSSION.—Perhaps one of the reasons chlamydospores of Graminearum have not been readily detected in culture is that macroconidia convert to chlamydospores only under circumstances related to soil fungistasis. Ko & Lockwood (6), stated that a lack of nutrients account for most fungal spores not germinating in natural soil. Macroconidia of Graminearum germinated immediately when added to autoclaved soil, which contains adequate nutrients for fungal spore germination. When macroconidia were added to nonautoclaved soil, considered to be depleted in nutrients by Ko & Lockwood, protoplasm migrated and condensed within a few cells in a macroconidium. The resulting structure is more resistant to inimical conditions than a macroconidium, and eventually germinates when conditions become favorable.

A chlamydospore-inducing substance was initially characterized by Alexander et al. (1). This substance was found to increase chlamydospore production of *F. solani,* but was not found in all soils sampled. Similar substances were also found in soil by Ford et al. (5). These workers found that clones of *F. solani* f. sp. *phaseoli* responded differentially to each of the chlamydospore-inducing fractions. This latter phenomenon of different clones of *F. solani* responding differentially to chlamydospore formation may also help to explain why Graminearum has previously not been thought to form chlamydospores as a survival mechanism. Certain soils may not contain the particular substances which favor chlamydospore formation by Graminearum, and hence chlamydospore formation by Graminearum is not a universal phenomenon.

Venkata Ram (9) was also able to increase chlamydospore production of *F. solani* by the addition of bacteria from soil to fungus cultures. Bacteria would explain why chlamydospores of Graminearum were formed in natural field soil but not in autoclaved soil.

The conversion of macroconidia to chlamydospores, and the germination of the chlamydospores up to 500 days in nonautoclaved soil, presents an alternate survival mechanism other than growth of hyphae and perithecia for *F. roseum* f. sp. *cerealis* 'Graminearum'.

TABLE 2. Origin of cultures of *Fusarium roseum* f. sp. *cerealis* 'Graminearum' isolated from soil on which wheat or corn was grown the preceding growing season[a]

Field location	No. colonies originating from propagules			
	Chlamydospore	Organic matter	Macroconidia	Mycelium
Wheat	58	158	29	75
Corn	66	227	78	54

[a] Data is the combination of 2 years of observations.

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