

**Comparative Morphology and Survival of Chlamydo­spores of
Fusarium roseum 'Culmorum' and 'Graminearum'**

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ABSTRACT

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Macroconidia of both *Fusarium roseum* 'Culmorum' and 'Graminearum,' added to nonsterile soil, converted after 4-7 days to thick-walled chlamydo­spores. Both fungi formed double-walled chlamydo­spores (as viewed in thin-section by transmission electron microscopy) in macroconidia exposed for 4 days to soil extract solution at 25 C. Although morphologically indistinguishable, the chlamydo­spores of Graminearum, but not those of Culmorum, were killed by rapid air drying, by a 35-sec exposure in moist soil to 20 kW microwave treatment, and by incubation

for 2-4 wk in moist soil at 35 C. Nearly all chlamydo­spores of Culmorum incubated in air-dry soil at 9 C were alive after 8.5 yr, whereas those of Graminearum declined steadily in number and were finally eliminated by natural attrition after 5.5 yr. Greater resistance to adversity may explain why, in the Pacific Northwest dryland wheat area, Culmorum persists in many fields and is consistently recovered by dilution-plating, whereas Graminearum has not yet been recovered by dilution-plating.

Additional key words: soilborne pathogens, wheat, *Triticum aestivum*.

Fusarium roseum Lk. ex. Fr. emend. Snyder and Hansen 'Culmorum' and *F. roseum* 'Graminearum' Group I (8) both cause root and crown rot of wheat (*Triticum aestivum* L.) in the Pacific Northwest (6,18). Symptoms produced by these two fungi are nearly indistinguishable (6). Culmorum survives in Northwest wheat field soils as chlamydo­spores (7), but whether Graminearum also survives in these soils as chlamydo­spores or only as mycelia in host refuse is not known. Of thousands of wheat field samples processed by dilution-plating (author's *unpublished*) over the past 14 yr, we have detected only Culmorum even though both fusaria occur in the Northwest and both grow equally well on the selective medium (14) used. Wearing and Burgess (21) concluded that for conditions in eastern Australia, Graminearum Group I survives mainly as mycelium in host debris. They recovered Graminearum on dilution-plates of soil from some fields, but not from others in

which the fungus obviously occurred as revealed by the presence of diseased wheat plants. The colonies that developed on dilution plates originated mainly from hyphae in organic matter, or occasionally from propagules referred to as modified macroconidia (21).

Many workers (1-6,9,11,22) report the occurrence of double-walled chlamydo­spores in Culmorum, but it is not clear whether double-walled chlamydo­spores occur in Graminearum. Nyvall (15) and Booth (3) both report that Graminearum conidia were transformed to chlamydo­spores when added to soil, but others (9,22) have concluded that chlamydo­spores of Graminearum are rare or absent or are not true chlamydo­spores (2,11,21,22). If Graminearum forms chlamydo­spores under Pacific Northwest conditions, they must be short-lived or we would have detected at least a few propagules by our dilution plating methods.

Nash and Alexander (13) found that although the propagules of *Fusarium solani* f. sp. *cucurbitae* and *F. solani* f. sp. *phaseoli* are morphologically similar, the chlamydo­spores of f. sp. *phaseoli* persist in soil longer and in higher numbers than those of f. sp.

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cucurbitae. No research has been carried out to date on the relative survival of chlamydo spores (if present) of *Graminearum* compared with those of *Culmorum* in soil. The objectives of this work were: to determine whether *Graminearum* forms chlamydo spores like those of *Culmorum* when introduced into soil as conidia; and, if so, to determine the relative survival of chlamydo spores of the two fungi in soil.

MATERIALS AND METHODS

Production of macroconidia for experimental soil infestation. Macroconidia of both *Graminearum* Group I and *Culmorum* were produced in pure culture and subsequently added to soil in water suspensions. Those of *Culmorum* were produced on homemade potato-dextrose agar (PDA). Sporulation of *Culmorum* was best when the plates were seeded uniformly with a spore suspension and incubated at 25 C in diffuse daylight. *Graminearum* did not sporulate well on PDA under the conditions used, but did so on the *Fusarium*-selective medium of Nash and Snyder (14). The medium used to produce macroconidia of *Graminearum* contained the peptone and salts, but not the PCNB or streptomycin. The plates with 8–15 colonies each were incubated at 25 C in diffuse daylight.

The original isolate of *Graminearum* came from a diseased wheat plant from a field near Quincy, WA. The *Culmorum* isolate was recovered by dilution plating from soil from a field (with a long history of disease caused by *Culmorum*) near Harrington, WA. The respective isolates used for the study were culturally indistinguishable from isolates of *Culmorum* and *Graminearum* recovered from Pacific Northwest fields over the past years and thus were considered to be typical of the two pathogens. Two additional isolates of *Culmorum* and one additional isolate of *Graminearum* were used in some experiments, but since we found no detectable difference in survival among the different isolates of a given cultivar, the results reported here are limited to one isolate of each *Fusarium*.

Assessment of spore fate and longevity. The fate of macroconidia in soil was observed directly by using the Warcup soil plate technique (20). This permitted the isolation and observation of individual propagules from soil and also allowed for their germination and culture, and confirmation of their identity. The longevity of the spores was monitored by dilution-plate counting using a *Fusarium*-selective medium (14). The measurements and observations of conidia and chlamydo spores in soil were made for as short as 3 hr in one experiment and as long as 8.5 yr in another.

Induction of chlamydo spore formation by using soil extract solution. Agar blocks with sporodochia of the two fusaria were suspended in a soil extract solution that had been passed through a 1.2- μ m Millipore filter. The soil extract solution was prepared initially by passing 1 L of hot tap water through 1 kg of Palouse silt loam (PSL). The solutions with conidia were then shaken in 250-ml Erlenmeyer flasks on an orbital shaker, with a gentle swirl, for 4 days at 25 C before examination.

After 4 days in flasks on the shaker, the agar blocks with adhering sporodochia were transferred to a primary fixative of 3.5 glutaraldehyde and 4% paraformaldehyde in 0.2 M cacodylate buffer at pH 7.2 (12). The specimens were immersed in 2% nonbuffered osmium tetroxide for 2 hr, dehydrated in an ethanol series, and then infiltrated over a period of 3 days in an ascending series of propylene oxide and Spurr resin (19). The material was sectioned with a Porter-Blum MT-2B ultramicrotome and then stained with uranyl acetate for 1 hr and lead citrate for 15 min (17). The preparations were observed with a Hitachi HU-125 electron microscope.

Tests with known soil water potential-soil temperature combinations. The comparative longevity of chlamydo spores of the two fusaria was determined in nonsterile soil adjusted to different soil water potentials and incubated at different temperatures. Macroconidia of each isolate were added in water suspensions to separate samples of air-dried Ritzville silt loam (RSL) obtained from a virgin grassland site at Lind, WA. The spores were thoroughly mixed into the soil by shaking and by kneading in a polyethylene bag, or by blending in a twin-shell

blender. Water was added with the spores to adjust the soil to 16% water (about -0.3 bar water potential). The infested soils were then incubated moist for 4–7 days in the plastic bags (unless specified otherwise) to allow conversion of the conidia into chlamydo spores.

After the initial incubation period, the soils were subdivided and dried for 1–3 days to different water contents (and hence water potentials) at 25 C on a laboratory table. An exception was where soils were rapidly air-dried in a rotary evaporator at room temperature (25 C) to determine the effect of rapid drying. The variously dried soils were then sealed in either plastic vials with “snap-top” lids (40 cm³ volume; 25–30 g of soil per vial; about two-thirds full) or glass jars covered with Parafilm (140 cm³ volume; 100 g of soil per jar; about two-thirds full). These containers of soil were stored together with moist paper toweling (to minimize evaporative loss) in plastic refrigerator-type “crisper” boxes with lids tightly sealed in incubators at different temperatures. Actual soil water potentials were determined for each treatment by thermocouple psychrometry (16) (unless air dry or wetter than -1 bar) just before placing the soil in the vials or jars.

Differential destruction of chlamydo spores of *Culmorum* and *Graminearum* in soil by microwave treatments. RSL (about 1 kg) infested with conidia of either *Culmorum* or *Graminearum* was exposed wet (17% water by weight, more than -1 bar) or dry (2% water by weight, obtained by drying for 3 days on a laboratory table) for 35 sec to 5 or 20 kW, or no microwave treatment. Survival was subsequently determined by dilution plate counting at 18 hr and 10 days after treatment and the numbers were compared with plate counts made just prior to treatment (0 time). The conidia were conditioned in the soil at 25 C for 4 days at the respective moisture levels before the microwave treatments were imposed.

Survival of macroconidia in the field. To determine the relative survival of chlamydo spores of the two fungi under field conditions, we added conidia of each *Fusarium* to separate samples of RSL and then buried the samples in a field near Lind, WA. Similarly, separate samples of PSL containing conidia of *Culmorum* or *Graminearum* were buried in a field near Pullman, WA. Plastic mesh baskets (22.5-cm long \times 12.5-cm wide \times 7.5-cm deep) were used to contain the soil and facilitate recovery. The test soils were in complete contact with the surrounding soil and presumably were at temperature and moisture equilibrium with the surrounding field soil. The baskets were buried level with the earth surface and the adjacent soil was smoothed and tamped to conform to the original terrain. Populations for each soil at each location were determined five times during a 1-yr period (October 1973 to September 1974), by dilution-plate counting three 1-g samples of soil for each determination.

RESULTS

Morphology of propagules from soil. Examination of the conidia recovered from soil revealed only chlamydo spore-like structures for both *Culmorum* (Fig. 1B) and *Graminearum* (Fig. 1E). In both cases, the centermost cells (one, two, or three cells) contained the cytoplasm and the end cells were empty. Morphologically, the chlamydo spores of the two cultivars did not differ. Although under light microscopy the walls appeared to be thickened (Fig. 1B,E), it was not possible by this method to determine whether the thickening was due to secondary wall formation or a thickened primary wall.

Morphology of chlamydo spores formed in soil extract solutions. Soil extract solutions caused the macroconidia of both *Culmorum* (Fig. 1C) and *Graminearum* (Fig. 1F,G) to convert to chlamydo spores. Macroconidia not exposed to the solution had thin primary walls when examined by either light microscopy (Fig. 1A,D) or electron microscopy. In contrast, macroconidia of both fungi subjected to soil extract solution rounded-up into chlamydo spores with double walls (Fig. 1C,G).

Long-term survival as influenced by temperature, water potential, and rapid drying. A mixture of macroconidia of *Culmorum* and *Graminearum* was blended with RSL with sufficient water to wet the soil to 16.3%. One-third of the soil was immediately dried (without a 4- to 7-day conditioning of the spores

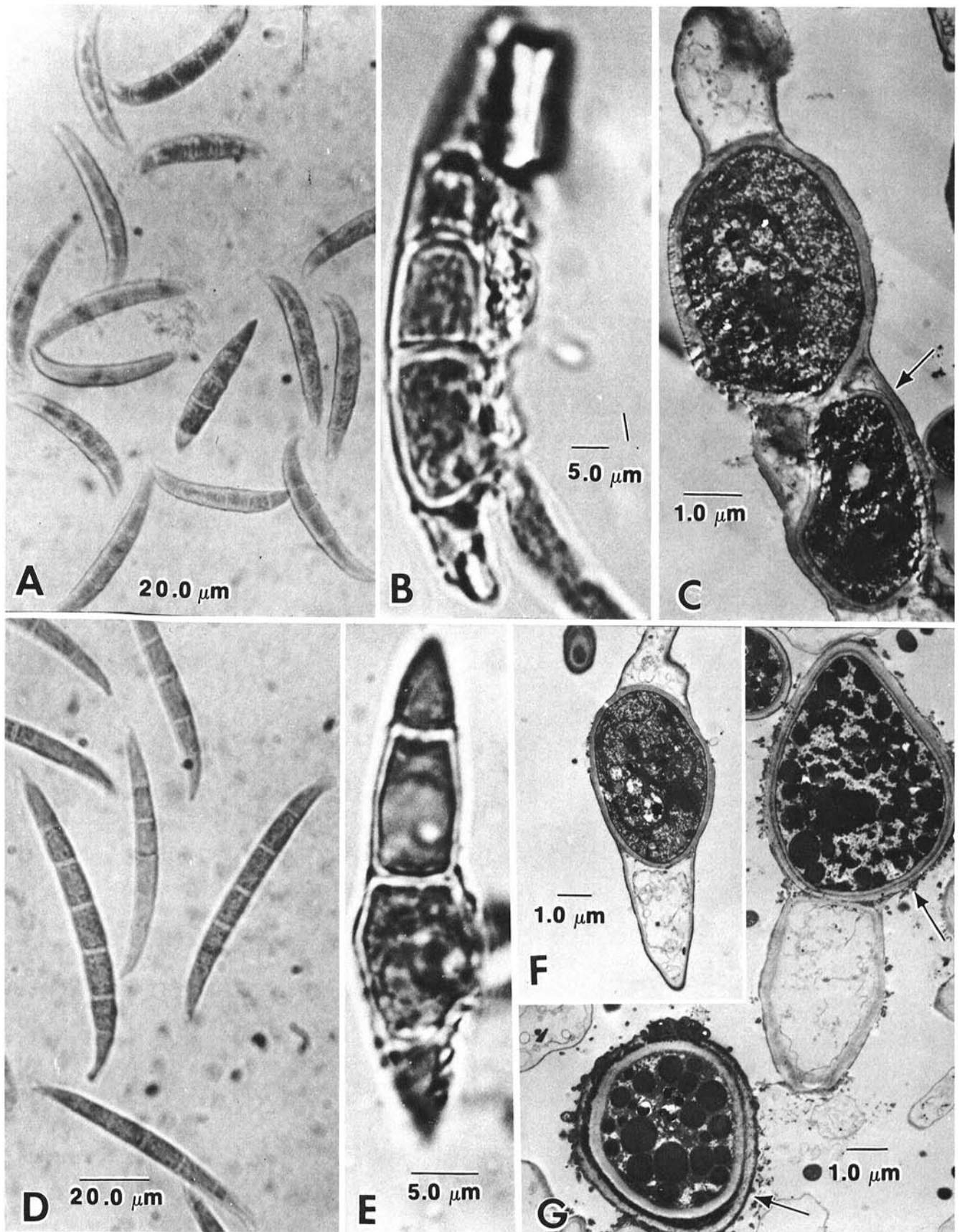


Fig. 1. Macroconidia and chlamydospores of *Fusarium roseum* 'Culmorum' and 'Graminearum'. **A**, Macroconidia of Culmorum freshly harvested from PDA. **B**, Chlamydospore of Culmorum formed in a macroconidium in soil. **C**, Double-walled (arrow) chlamydospore of Culmorum formed in a soil extract solution. **D**, Macroconidia of Graminearum freshly harvested from PCNB medium. **E**, Chlamydospore of Graminearum formed in a macroconidium in soil. **F**, Chlamydospore of Graminearum formed in soil extract solution. **G**, Double-walled (arrow) chlamydospores of Graminearum formed in soil extract solution.

in the soil) to 4.5% (-30 bars water potential) and another one-third was dried to 1.3% water content in a rotary-evaporative dryer. Approximately 4 hr was required to dry the soil to 1.3% water. The remaining one-third was kept at 16.3% water. Each soil sample was then transferred to a glass jar, sealed with Parafilm, and incubated at 9 C. No attempt was made to replenish water lost from the soils by evaporation during the long-term incubation. One 1-g sample of soil was removed from each jar (and the jar then resealed) at 3 hr, 4 days, 1, 3, 5, 11, 17 wk, and 5.5 and 8.5 yr, and dilution-plate-counted on five plates of *Fusarium*-selective medium. By 8.5 yr (probably much earlier), all treatments had dried to approximately the same water content (probably in equilibrium with the relative humidity of the incubator).

Culmorum withstood the rapid drying to 1.3% water content (Table 1). In contrast, Graminearum was virtually eliminated from the soil by this treatment. In soils initially at 16.3 and 4.5% water

TABLE 1. Survival of chlamyospores of *Fusarium roseum* 'Culmorum' and of *F. roseum* 'Graminearum' in soil kept moist at 25 C and in soil dried quickly to 1.3% water (w/w) and then cooled to 9 C

Treatment		Propagules per gram (in thousands) after treatment for				
		0 wk	1 wk	3 wk	5 wk	11 wk
Soil moist	Culmorum	204	206	178	208	202
	Graminearum	72	35	28	64	48
Quick dry	Culmorum	130	146	202	106	156
	Graminearum	0	0	2	0	0

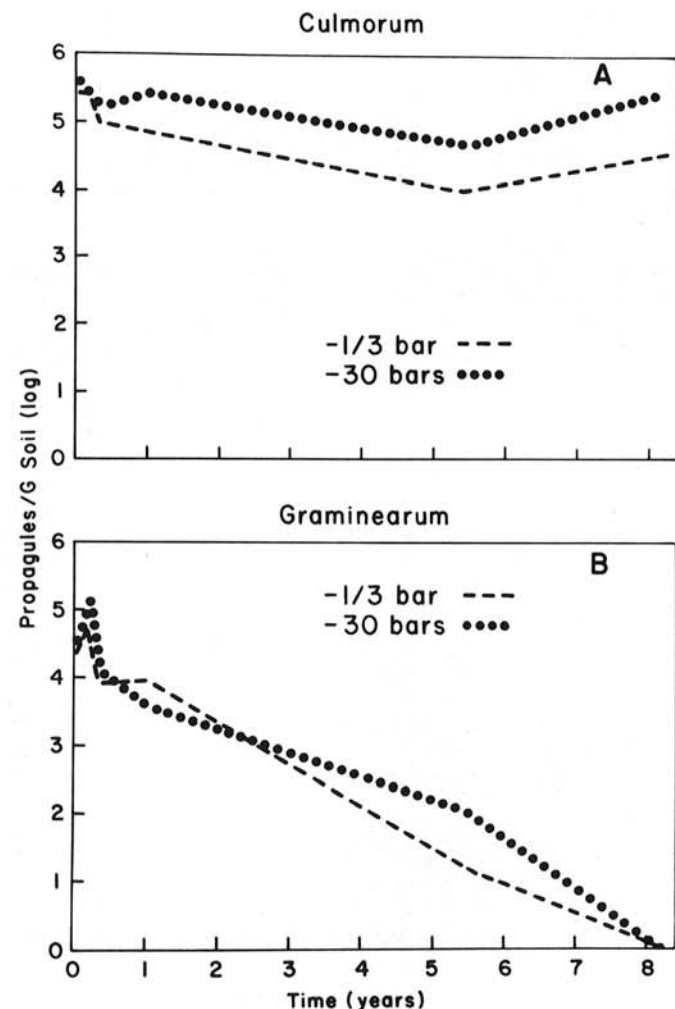


Fig. 2. Populations of *Fusarium roseum* 'Culmorum' and *F. roseum* 'Graminearum' as chlamyospores at different times during incubation at 9 C and initial water potentials of -1/3 bar and -30 bars.

contents, both cultivars survived at 9 C for 5.5 yr, but only Culmorum survived 8.5 yr (Fig. 2).

Influence of water/temperature combinations on longevity of chlamyospores in soil. Culmorum and Graminearum added initially as conidia to separate lots of RSL were incubated at five temperatures (5, 15, 25, 35, and 45 C) and three water contents (1.5% [in equilibrium with room air at less than 25% RH], 5.5% [-15 bars], and 14% [more than -1 bar]). A sufficient number of "snap-top" vials were used to provide for destructive sampling and dilution-plate counting at 0, 1, 2, and 4 wk after the soil had been placed in the vials. One gram of soil was removed, respectively, from the top, middle, and bottom of each vial and dilution plated separately on three plates of the *Fusarium*-selective medium (nine plates per vial).

Both cultivars remained at uniformly high populations throughout the 4-wk experiment, provided the soil was 25 C or cooler (Fig. 3). Moreover, both cultivars remained at high populations at 35 C, provided the soil was air dry (Fig. 3). The differentiation between Culmorum and Graminearum populations occurred in moist soil at 35 C; Culmorum survived these conditions whereas the Graminearum population had dropped markedly by 4 wk. At 45 C, the population of both fungi dropped to zero in 1 wk at 5.5 and 14% water contents, and by 4 wk if the soil was air dry.

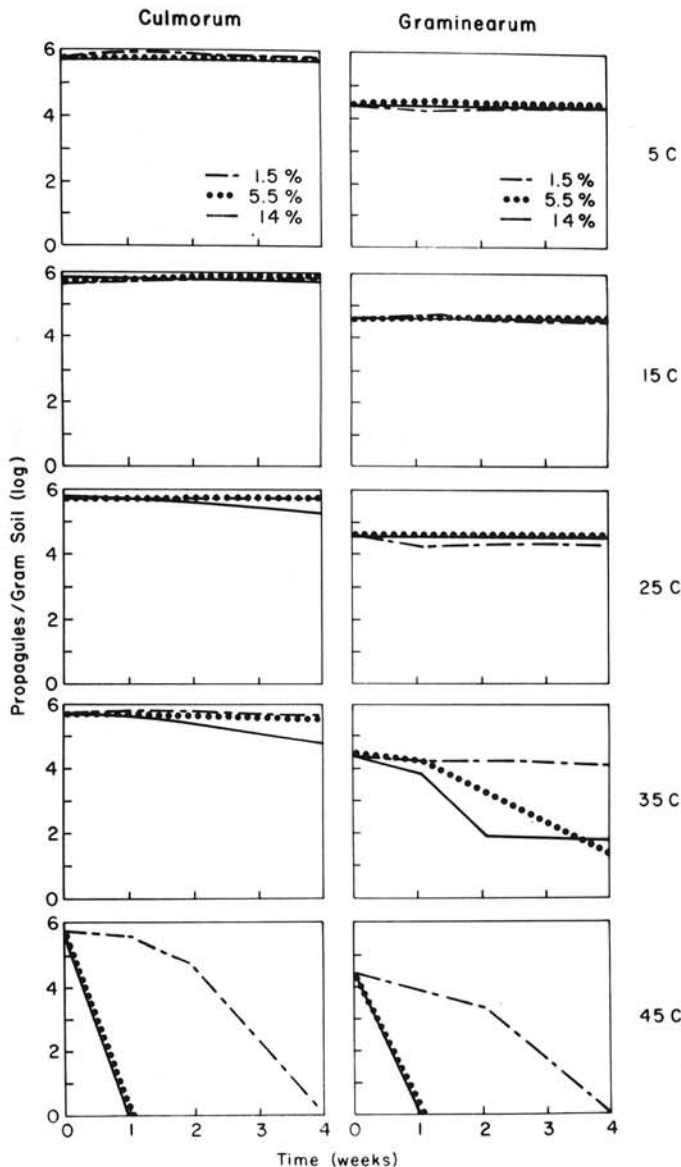


Fig. 3. Populations of *Fusarium roseum* 'Culmorum' and *F. roseum* 'Graminearum' as chlamyospores at different times during incubation at different combinations of soil temperature and soil water content.

TABLE 2. Survival of chlamydo-spores of *Fusarium roseum* 'Culmorum' and *F. roseum* 'Graminearum' at 18 hr and 10 days after exposure at two water contents (2 and 17%) to two levels of microwave radiation

Treatment	Time after treatment	Propagules per gram of soil	
		Culmorum	Graminearum
None, wet	18 hr	493,000	102,000
	10 days	579,000	59,800
None, dry	18 hr	655,000	32,000
	10 days	756,000	53,200
5 kW microwave, wet	18 hr	555,000	88,000
	10 days	577,000	69,600
5 kW microwave, dry	18 hr	573,000	37,000
	10 days	810,000	32,600
20 kW microwave, wet	18 hr	270	0
	10 days	88,000	0
20 kW microwave, dry	18 hr	673,000	43,000
	10 days	630,000	36,900

Differentiation of Culmorum from Graminearum by microwave soil treatment. Neither microwave treatment affected the population of either *Fusarium* when the soil was dry (Table 2). Moreover, the 5-kW level of radiation had no effect on the population of either fungus, whether in dry or wet soil. The two cultivars were differentiated when exposed to 20-kW microwave treatment in wet soil; Graminearum was destroyed (no longer detectable by dilution plating), but about 10% of the Culmorum propagules were detectable at 10 days after exposure.

Survival under field conditions. After 48 wk in the field at Pullman, only 10% of the Graminearum propagules were still detectable by dilution plating, whereas the Culmorum population changed little or not at all (Fig. 4). A similar trend was evident from soil buried at Lind, but unfortunately the baskets at Lind were stolen during the experiment.

DISCUSSION

The isolate of Graminearum used in this study formed chlamydo-spores in conidia in soil and soil extract that were indistinguishable morphologically from those formed by Culmorum under the same conditions. Chlamydo-spores of both fungi had a thickened wall which, when viewed in thin section by electron microscopy, was evident as an inner secondary wall. Such structures fit the description of a chlamydo-spore (2).

On the other hand, survival of the conidial chlamydo-spores of the two fusaria in soil was different; those of Culmorum survived better than those of Graminearum when exposed to rapid air drying, to 20-k W microwave treatment for 35 sec in wet soil, or to 35 C for 4 wk in moist soil. Moreover, those of Culmorum survived for 8.5 yr in soil at 9 C whereas those of Graminearum were detectable at 5.5 but not 8.5 yr, and those of Culmorum survived better than those of Graminearum when buried in the field. These results are similar to those of Nash and Alexander (14), who observed no morphological difference between the chlamydo-spores of *F. solani* f. sp. *phaseoli* and *F. solani* f. sp. *cucurbitae*, yet the former survived better than the latter in soil.

In Washington state, foot rot caused in wheat by Graminearum occurs almost exclusively in the drier, warmer counties (Grant, Franklin, and Benton counties). The average high air temperature in July for six locations in these counties, where weather records exist, is 31.0 C. Culmorum causes disease mainly in the northeastern counties of Washington (Adams, Lincoln, Whitman, and Douglas counties) where the average high in July for six locations is 28.7 C. Obviously, the occurrence of *Fusarium* foot rot in Washington cannot be explained on the basis of conditions favorable to survival of the respective pathogens; Graminearum chlamydo-spores are less hardy than those of Culmorum, yet Graminearum is the more important of the two pathogens in the hotter and drier areas. Our failure to detect Graminearum by dilution plate counts of soil from northeastern counties of Washington may relate simply to the fact that this fungus does not

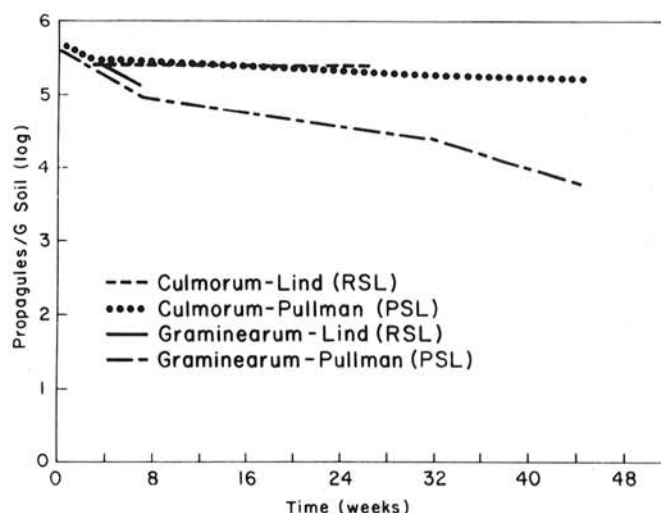


Fig. 4. Populations of *Fusarium roseum* 'Culmorum' or *F. roseum* 'Graminearum' at different times after burial as chlamydo-spores in Palouse silt loam in the field at Pullman, and in Ritzville silt loam in the field at Lind, WA.

produce inoculum in this area, because it does not produce disease. We have observed sporodochia of Graminearum on plants killed by this fungus and presumably conidia from these become mixed with soil and are converted into chlamydo-spores. Our data suggest, however, that even if chlamydo-spores of Graminearum are formed in conidia in soil in areas favorable to disease caused by this fungus, their chances of survival are poor. Presumably, the Graminearum inoculum in these areas is mycelium in residue of past wheat crops, much as is reported for Australian conditions (21). In contrast, the chance for Culmorum to survive as conidial chlamydo-spores in Pacific Northwest soils apparently is excellent, at least in the areas where foot rot caused by this fungus is most likely to occur.

Cook (6) illustrated two kinds of chlamydo-spores for Culmorum recovered from Washington wheat-field soils. One was originally a conidium, with the two center cells converted into chlamydo-spores, and the other was a cluster of thick-walled globose spores associated with a fragment of organic matter (presumably remnant host tissue). The latter were larger and more rounded than chlamydo-spores formed in conidia, and were likely chlamydo-spores formed within mycelium. Ability to form chlamydo-spores in conidia is a mechanism whereby populations of the pathogen can increase severalfold in a single season. Those formed in mycelium in host refuse become distributed in soil more slowly, probably because the crop residue breaks down slowly. Our work was done entirely with chlamydo-spores formed in macroconidia. The relative survival of mycelial chlamydo-spores of these two fusaria, or indeed whether Graminearum forms chlamydo-spores in mycelium in host refuse, is still uncertain.

Earlier *Fusarium* workers observed structures within macroconidia of Graminearum and related *Fusarium* species that they hesitated to call chlamydo-spores. Wollenweber and Reinking (22) described "hyphal swellings" in species now grouped in *F. roseum* Snyder and Hansen, and Bennett (2) referred to "pseudochlamydo-spores" in *F. avenaceum* (= *F. roseum* 'Avenaceum') formed by the centermost cells of the conidium. Griffin and Pass (10) referred to the survival structure of *F. roseum* 'Sambucinum' as "chlamydo-spore" macroconidia. Nyvall (15) described the formation of what he called chlamydo-spores in the center cells of macroconidia of Graminearum, but Wearing and Burgess (21) referred to these structures as "modified" macroconidia. Our work confirms the conclusion of Nyvall (15) that the structures formed in macroconidia of Graminearum in soil are, indeed, chlamydo-spores.

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