Ecology and Epidemiology

Persistence of Chlamydospores of *Fusarium culmorum* in Wheat Field Soils of Eastern Washington

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ABSTRACT

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Chlamydospores of *Fusarium culmorum* occurred in wheat field soils of eastern Washington nine times more frequently as endoconidial types than as mycelial types. Endoconidial chlamydospores survived slightly better in Palouse silt loam soil at Pullman, WA, than in Ritzville silt loam soil at Lind, WA, over a 3-yr study. Slope values of the survival curves (percentage of surviving propagules over time) obtained by using the log-probit transformation were −3.41 and −1.41 for Lind and Pullman, respectively. LD_{50} values (time when 50% of the propagules were dead), interpolated from the transformed curves, were 208 and 267 days for Lind and Pullman, respectively. TS_{50} values (time when 50% of the propagules survived), estimated directly from arithmetic plots, were 215 and 330 days for Lind and Pullman, respectively. Despite the ability of the fungus to survive slightly longer in field soil at Pullman than at Lind, field survey data have consistently demonstrated a higher frequency of infested fields in the Lind than in the Pullman area. Plant water stress is required for disease development and is common in the Lind area (25 cm annual precipitation) but is rare near Pullman (53 cm annual precipitation). Because the relatively lower incidence of infested fields in the Pullman area cannot be explained by the inability of the fungus to survive as chlamydospores in the soil, it apparently relates to environmental conditions that are unfavorable for disease development or the production of new chlamydospores.

Additional key words: Fusarium foot rot, soilborne pathogen, *Triticum aestivum*.

In eastern Washington, Fusarium foot rot of wheat (*Triticum aestivum* L.) caused by *Fusarium culmorum* (W. G. Smith) Sacc. occurs commonly in the semiarid areas such as Adams and Lincoln counties (20–40 cm annual precipitation) but rarely in the intermediate- to high-rainfall areas such as Whitman County (40–60 cm annual precipitation). The fungus persists in the soils of both regions as chlamydospores that originate either from macroconidia (endoconidial chlamydospores) or hyphae (mycelial chlamydospores) (5). However, as with the geographical distribution of the disease, wheat fields infested with chlamydospores of *F. culmorum* are more common in the semiarid than in the subhumid (intermediate to highrainfall) areas of the state (7).

Prior observations indicated that chlamydospores of *F. culmorum* may be short-lived in soils in the subhumid areas. Cook (6) reported that wheat in a field near Pullman, Whitman County, was severely damaged by *F. culmorum* in 1965 and the soil contained an estimated 10,000 propagules per gram of soil in the fall after harvest of the crop. Yet the field was essentially free of the pathogen (populations nearly undetectable) 12 mo later. Similarly, a population of *F. culmorum* established artificially at about 5,000 propagules per gram of soil in a plot at Pullman declined steadily to near extinction over a 24-mo period (2). In comparison, fields near Ritzville and Harrington in Adams and adjacent Lincoln counties (where serious outbreaks of Fusarium foot rot occurred in 1964, 1968, and 1974) were infested with this pathogen at one to 3,000 propagules per gram throughout the late 1960s and early 1970s, and some of these fields still contain high inoculum densities. Moreover, surveys of wheat field soils from throughout eastern Washington have, for the past 15 yr, revealed that fields infested with *F. culmorum* occur at frequencies up to 10 times higher in Adams and Lincoln than in Whitman County (R. J. Cook and J. W. Sitton, *unpublished*). Such observations suggest that soilborne propagules of *F. culmorum* may survive better in Adams and Lincoln counties, but a direct comparison of the longevity of the fungus at the two locations has never been made.

Sitton and Cook (14) showed that endoconidial chlamydospores of *F. culmorum* survive better than those of *F. graminearum* Schwabe, yet *F. graminearum* typically is most important in the

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hottest and driest areas of the state, where conditions for survival presumably are most adverse. They concluded that the occurrence of *F. graminearum* was correlated with conditions favoring development of foot rot caused by this fungus rather than conditions favoring survival of *F. graminearum* in the soil.

Chlamydospores of *F. culmorum*, like those of other species of *Fusarium* (13), undergo formation, dormancy, and germination as the major events of their life cycle. If any event in the life cycle fails, the fungus may not establish or persist in the soil. Soils in which chlamydospores are unable to form, germinate, and/or survive have been variously labeled resistant, long-lived, immune, intolerant, antagonistic, or suppressive (2). Cook and Papendick (8) showed that in the fine-textured, high-organic-matter soil of Whitman County (Palouse silt loam), chlamydospores of *F. culmorum* germinate in lower percentages and lyse more rapidly than those in the coarser textured Ritzville silt loams of Adams County that contain less organic matter. However, it is unclear whether the Palouse silt loam has a similar effect on the formation or survival ability of chlamydospores of the fungus. This study was done to determine the relative longevity of chlamydospores of *F. culmorum* in the semiarid versus subhumid areas of eastern Washington.

MATERIALS AND METHODS

Assessment of relative frequency of endoconidial to mycelial chlamydospores in field soils. Chlamydospores obtained directly from soil were prepared for observation and identification by modifying Warcup's technique (15). Two fields were sampled, one in Lincoln County and one in Whitman County, each with about 500 propagules of *F. culmorum* per gram of soil. Twenty 250-g samples from each field were bulked, mixed, screened through a 1-cm-mesh sieve, air-dried, and then pulverized with a Braun Type UA Soil Pulverizer (Braun Institute, San Francisco, CA). Three grams of pulverized soil was added to 100 ml of sterile 0.1% water agar containing a few beads of Calgon Water Softener (Beecham Products, Pittsburg, PA) and shaken for up to 12 hr on a rotary wrist-action shaker. One-tenth milliliter of the soil suspension was placed in a sterile petri dish and suspended in 7 ml of 2% water agar (amended with streptomycin sulfate at 100 ppm and cooled to nearly 45 C before pouring). The plates were gently agitated before the agar solidified to suspend propagules and soil particles in the agar medium. After 16 hr at 27 C in the dark, fungal spores with germ tubes could be observed with a dissecting microscope. Hyphal tips of single germinating spores were transferred to a slant of dilute (half-strength) potato-dextrose agar (0.5-strength PDA) medium (125 g of sliced potatoes, 10 g of dextrose, and 20 g of agar per liter) and incubated at room temperature with a 12-hr alternating light cycle for identification. The corresponding germinated spore was then removed together with a small square of agar to a glass slide, stained with 0.1% acid fuchsin in 50% lactic acid, and observed with a light microscope. Eight representative isolates of *F. culmorum* were deposited as isolates R6564–R6572 in the culture collection of the Fusarium Research Center (Pennsylvania State University, University Park).

Assessment of longevity of endoconidial chlamydospores in uncropped soils. The comparative longevity of endoconidial chlamydospores was assessed in Ritzville silt loam at Lind in Adams County and in Palouse silt loam at Pullman in Whitman County. The Lind site, at the Washington State University Dryland Research Unit, had not been cropped to wheat since the station was established in the early 1920s; the Pullman site, on the Plant Pathology Farm, had not been cropped to wheat for 2 yr. Lind receives 25 cm average annual precipitation and has a mean July temperature of 22 C. Pullman receives 53 cm average annual precipitation and has a mean July temperature of 19 C (40-yr averages from the National Oceanic and Atmosphere Administration, Asheville, NC). Soil pH, cation exchange capacity, and sand, silt, and clay content for the two soils have already been published (8). An isolate of *F. culmorum* (R6564) recovered from a wheat plant with foot rot from Harrington, WA (Lincoln County), was used throughout the study.

Endoconidial chlamydospores were prepared by growing the fungus on plates of 0.5-strength PDA according to the method of Sitton and Cook (14). After 2 wk, macroconidia were scraped from the plates, added to sterile water, and filtered twice through Miracloth (Chicopee Manufacturing Co., Milltown, NJ) to remove mycelial fragments. The respective conidial suspensions were blended into several kilograms each of screened nonsterile Ritzville and Palouse silt loams. Each soil was air-dried for 1 wk to provide time for the conversion of macroconidia to endoconidial chlamydospores (14). The soil was examined microscopically to confirm that endoconidial chlamydospores had formed. The soils were later diluted with additional screened soil by mixing for several hours in a Twin Shell Dry Blender (The Patterson Kelly Co. Inc., East Stroudsburg, PA). The number of propagules of *F. culmorum* per gram of soil was estimated (5) by dilution-plate counting on the *Fusarium*-selective medium of Nash and Snyder (10). Final concentrations were 8 and 10×10^6 propagules per gram of soil for the Palouse and Ritzville silt loams, respectively.

Infested soils were buried in nylon bags 10 cm below the soil surface in September 1978 and sampled until July 1981. The bags were constructed of 100% nylon organdy and sewn with 100% nylon thread. Each bag measured 15×15 cm and contained 100 g of soil. The soil thickness in each bag did not exceed 5 mm so that all soil was within 2.5 mm of surrounding field soil. Three bags were recovered from each site at selected sampling dates (16 times at Pullman and 17 times at Lind), measured for water content with a Moisture Determination Balance (Van Waters and Rogers, Inc., San Francisco, CA), and subsampled by removing three 10-g portions to estimate numbers of surviving propagules by dilution plating.

A duplicate experiment was started 1 yr later at the same two locations. The results of the second experiment were so similar to those of the first experiment that only data from the first experiment are reported.

The percentage of surviving propagules was plotted with time using both arithmetic and semilogarithmic graphs. Because linearity was not apparent on semilogarithmic graphs, half-life values as discussed by Dimond and Horsfall (9) were not calculated. Instead, TS_{90}, TS_{50}, and TS_{10} values (time in days when 90, 50, and 10% of the initial population still survived) were estimated directly from arithmetic plots. The times when the populations first reached 90, 50, and 10% of the initial level were used.

Data on survival were also transformed to probit units and plotted against time on a logarithmic scale. The log-probit transformation has been used previously to analyze survival of fungi with multicelled propagules in soil. The procedure allows sigmoid-shaped curves to be transformed to straight lines; thus, the time required for death of 50% of the inoculum can be interpolated and the slopes of the survival curves can be determined and compared (3). The LD_{10}, LD_{50}, and LD_{90} values (time in days required for death of 10, 50, and 90% of the initial population) were compared with estimated TS values to assess whether the LD values reasonably represented the death rate of the population.

RESULTS

Relative frequency of endoconidial to mycelial chlamydospores in field soil. Endoconidial chlamydospores of *F. culmorum* occurred eight to nine times more frequently than mycelial chlamydospores in the soils sampled. Ninety percent (45) of the chlamydospores of *F. culmorum* recovered from soil at Harrington, Lincoln County (semiarid area), were endoconidial types, whereas only 10% (5) were mycelial types. Eighty-six percent (43) of the chlamydospores recovered from soil at Pullman, Whitman County (subhumid area), also were endoconidial types and only 14% (7) were mycelial types.

Of the endoconidial chlamydospores recovered, most occurred in chains of two, three, or four and represented adjacent intercalary cells of macroconidia. Because the walls of the one-time apical and foot cells of the conidium were still attached to parts of the propagules, the origin of the chlamydospores as macroconidial

was obvious. Little variation in shape between individual endoconidial chlamydospores was observed, and the average size was 6.5 × 6.5 μm. Of the mycelial chlamydospores of *F. culmorum* recovered, most were in chains of four to eight viable cells. The chains were somewhat convoluted and were associated with small bits of organic material. The average size for mycelial chlamydospores was 7.5 × 8.6 μm. Both chlamydospore types were the same as those shown by Cook (5).

Longevity of endoconidial chlamydospores in uncropped soils. In the first year, the populations of endoconidial chlamydospores of *F. culmorum* in soils buried at Pullman and Lind increased slightly at first, fluctuated, then declined steadily. In the second year, the population at Lind remained at about 10% and that at Pullman at about 30% of the initial inoculum density. During the third year, the population at Lind was negligible and finally undetectable, whereas the population at Pullman declined to 3% of the initial level (Fig. 1).

The TS values estimated directly from the arithmetic plots of the survival curves were similar to the LD values obtained by using the log-probit transformation to change the sigmoid curves to straight lines (Table 1). By both methods, the time required for death of 10% of the inoculum was about 3 mo at Lind and 1 mo at Pullman. Death of 50% of the propagules occurred 2–4 mo sooner (7 vs. 9–11) at Lind than at Pullman. Death of 90% of the propagules occurred within about 16–17 mo at Lind but required 33–37 mo at Pullman, depending on whether TS_{10} or LD_{90} values were compared. Slope values of survival curves over time transformed to the log-probit transformation were −3.41 for Lind and −1.41 for Pullman, affirming that the fungus dies out somewhat more quickly at Lind than at Pullman. However the variability of data was such that neither the position of the survival curves nor the slopes were significantly different ($P = 0.05$) by analysis of covariance.

Soil smears made from the infested soils at the end of the study revealed that cell walls from the apical and foot cells of one-time macroconidia were no longer attached to chlamydospores. The remaining chlamydospores at both Lind and Pullman were either single or paired.

DISCUSSION

Endoconidial chlamydospores are the predominant chlamydospore type for *F. culmorum* in wheat field soils of eastern Washington, occurring eight to nine times more frequently than mycelial types. This ratio suggests that chlamydosopores of the fungus formed after sporulation and subsequent washing of macroconidia into the soil are considerably more important as a source of inoculum than chlamydospores formed in the mycelium of colonized crop residue. Soil populations of *F. culmorum* are known to increase significantly when oats are included in a rotation (12) because parasitized oats are especially supportive of high amounts of asexual sporulation by the fungus (7)

The longevity of endoconidial chlamydospores appears to be slightly, but not significantly, greater in soil near Pullman than near Lind. The characteristically lower incidence of infested fields and the generally lower populations of this fungus in Whitman than in Adams and Lincoln counties cannot therefore be explained by an inability of the fungus to survive in soils in the subhumid area. Fusarium foot rot is more serious in the lower rainfall areas of eastern Washington where the low plant water potentials needed for predisposition of the host to this disease are most likely to occur

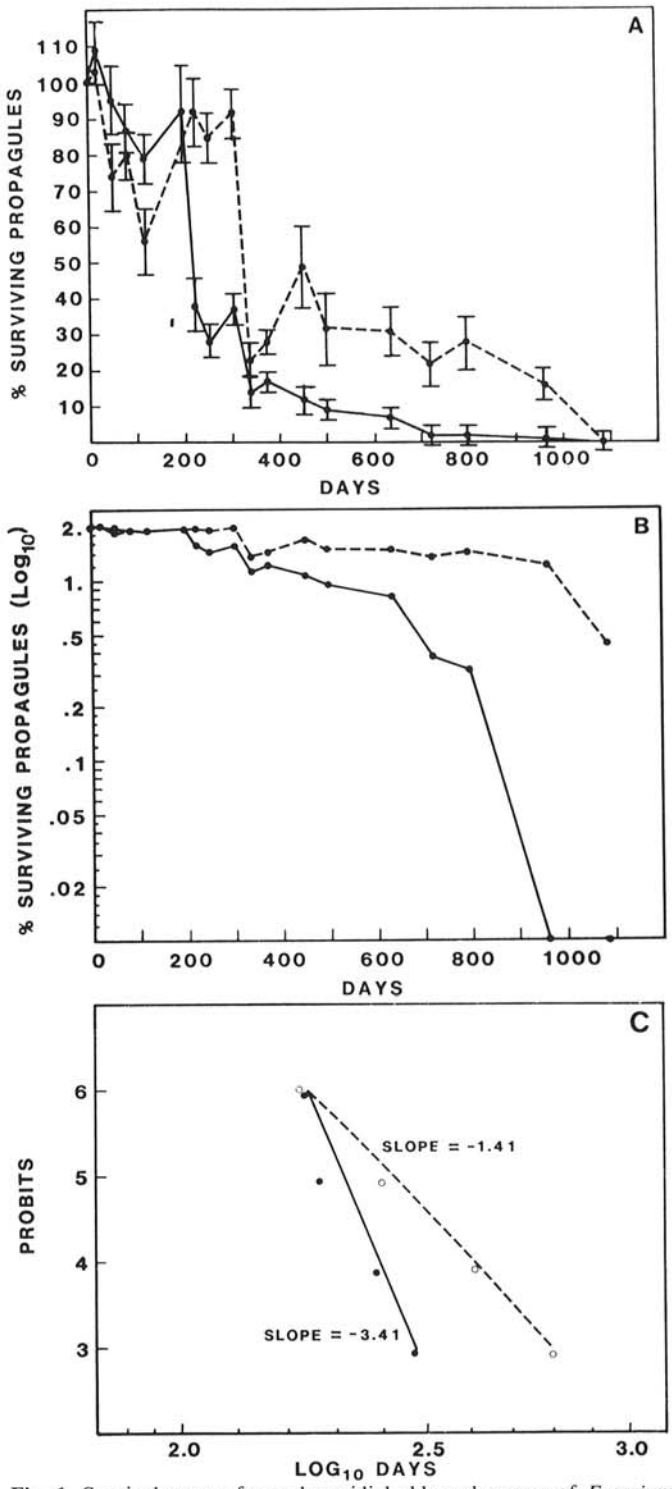

Fig. 1. Survival curves for endoconidial chlamydospores of *Fusarium culmorum* buried 3 August 1978 through 26 July 1981 at Pullman (-----) and Lind (——), WA: **A,** arithmetic plot, error bars = ± 1 standard error of the mean; **B,** semilogarithmic-transformed; and **C,** log-probit-transformed, neither the position of the survival curves nor the slopes were significantly different ($P = 0.05$) by analysis of covariance.

TABLE 1. Survival of endoconidial chlamydospores of *Fusarium culmorum* buried for 3 yr at Pullman and Lind, WA[a]

	Arithemtic plot[b]			Log-probit plot[c]		
Location	TS_{90}	TS_{50}	TS_{10}	LD_{10}	LD_{50}	LD_{90}
Lind	75	215	480	88 (59–114)	208 (172–243)	494 (411–641)
Pullman	25	330	1,015	33 (3–77)	267 (142–450)	2,173 (1,015–17,045)

[a] Values within parentheses indicate 95% of fiducial limits.
[b] TS values = days estimated directly from arthemtic plots of the survival curves at which 90, 50, and 10% of the initial population still survived.
[c] LD values = days calculated by using the log-probit transformation when 10, 50, and 90% of the initial population had died.

(11). The occurrence of more fields infested with *F. culmorum* in the lower rainfall area may relate to environmental conditions more favorable for disease and hence for the production of inoculum by this fungus as concluded by Sitton and Cook (14) for *F. graminearum*. Furthermore, establishment of *F. culmorum* in wheat stems through parasitism is a prerequisite to subsequent inoculum formation (4), whether as macroconidia formed in sporodochia on culms and then washed into soil or as chlamydospores formed in mycelium in the stems left as residue in the soil. Although the fungus, once established, may survive longer in soils near Pullman, the rare occurrence of conditions needed for disease probably limits its opportunity to produce new inoculum.

The death rate of endoconidial chlamydospores of *F. culmorum* at Pullman and Lind during the 3-yr period, as expressed on both arithmetic and semilogarithmic graphs (Fig. 1), is not logarithmic, at least not initially. The number of recoverable units increased during the first few months in the soil. Fragmentation of adjoining chlamydospores into smaller but more numerous propagules rather than formation of new chlamydospores is most likely the reason for this initial increase. Nutrients would have been inadequate in the soils to support chlamydospore germination and subsequent formation of new chlamydospores; no more than 2–5% of the chlamydospores of this fungus germinate in soil in response to wetting and drying (R. J. Cook, *unpublished*). In addition, soil smears made of the propagules towards the end of the study revealed that surviving inoculum consisted of a series of one or two chlamydospores when originally each series consisted of three to four chlamydospores.

Survival of endoconidial chlamydospores of *F. culmorum* at Pullman and Lind generally follows the five phases of survival described by Baker (1) for other multicelled fungal propagules in soil. Phase 1 is the introduction of inoculum into the soil and occurred in autumn, when diseased wheat culms and adhering macroconidia of *F. culmorum* (on lower nodes) are normally incorporated into the soil by washing and tillage (7). Phase 2 is an initial increase in population levels. This phase was most evident for chlamydospores buried at Lind but probably resulted at both locations from fragmentation of chlamydospores in one-time macroconidia into single or paired chlamydospores. Phase 3 is a period of rapid decline, which occurred at both locations by the end of the first year. Log-probit analysis indicated that LD_{50} values were 208 days at Lind and 267 days at Pullman and that calculated death rates were slightly higher but not significantly different at Lind (slope value $= -3.41$) compared with Pullman (slope value $= -1.41$). Phase 4 is long-term survival of a few resistant propagules and was evident in the second and third year for chlamydospores at Lind but was not evident until the end of the third year (and likely into the fourth year) for chlamydospores at Pullman. Phase 5 is when all inoculum is extinct. Total extinction in this study (i.e., no more detection on dilution plates) occurred for chlamydospores buried at Lind but not for those buried at Pullman.

The long-term survival of chlamydospores of *F. culmorum* for 2–4 yr in soil explains why fallowing for 1–2 yr is ineffective in controlling Fusarium foot rot. Periodic recycling of the pathogen in the rhizosphere, even that resulting from a low incidence of disease, combined with long-term persistence of the most resistant chlamydospores, greatly extends the longevity of this fungus.

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