Factors Affecting Saprophytic Colonization of Wheat Straw by Fusarium roseum f. sp. cerealis 'Culmorum'

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ABSTRACT

Saprophytic colonization of clean, bright, untreated wheat straw by *Fusarium roseum* f. sp. *cerealis* 'Culmorum' was severalfold greater than that of weathered straw buried in the same soil under the same conditions. Colonization of straw by Culmorum decreased as the period of exposure of the straw to weathering prior to burial increased.

Weathered straws contained dematiaceous fungi, aspergilli, penicillia, and other airborne saprophytes. When weathered straws were autoclaved prior to burial, saprophytic colonization by Culmorum increased. Apparently, the establishment of saprophytic fungi in straw prior to burial prevents colonization by Culmorum after burial, and this accounts for the lack of saprophytic colonization of wheat straw by Culmorum in the Pacific Northwest.

Effects of soil temperature and water could not explain the limited saprophytic colonization by Culmorum in the field. Colonization was maximal at 20-25 C, but occurred in significant amounts at 4 C. Colonization was maximal at water potentials of −50 to −60 bars (relatively dry soil), but occurred at −0.5 to −1 bar. Colonization was prevented only under extreme conditions (i.e., 1 C or soil nearly air-dry).

Culmorum populations 10^2 and 10^3 propagules/g of soil were the minimum levels to achieve colonization of straws in the laboratory. The limited saprophytic colonization of straw by Culmorum in the field cannot be attributed to insufficient inoculum of the fungus, since soils of several naturally infested fields contained Culmorum populations in excess of 10^3 propagules/g of soil. Unweathered straws buried and incubated in these soils in the laboratory were 10-75% occupied by Culmorum within 1 week. Phytopathology 60:1672-1676.

Additional key words: antagonism, preemption, saprophytic fungi.

The occupancy of wheat straw (*Triticum aestivum* L.) by *Fusarium roseum* (Lk. ex Fr.) emend. Snyd. & Hans. f. sp. *cerealis* (Cke.) Snyd. & Hans. 'Culmorum' under field conditions in the Pacific Northwest results largely from parasitism of the crowns and bases of culms during plant growth, and not from saprophytic colonization of straw residue after harvest (8). Isolations from wheat straws recovered from Culmorum-infested fields 1 year after harvest and tillage revealed the presence of Culmorum in basal sections only. In contrast, those portions of straws not colonized through parasitism were free of the fungus.

The minor role of saprophytic colonization of straw in the epidemiology of Culmorum under Pacific Northwest conditions was further demonstrated by results of dilution-plate counts of propagules of the fungus with soil from random wheat fields (8). Culmorum populations were less than 100 propagules/g of soil in 71 of 80 fields in spite of the fact that all fields tested had been exposed to intensive wheat culture and straw return for decades.

On the other hand, the burial of straw segments in Culmorum-infested soil under laboratory conditions has consistently resulted in high percentages of saprophytic colonization by this fungus (3, 4, 8, 10, 12, 13). Based on this display of saprophytic ability, Park (11) categorized Culmorum among those pathogens whose economic importance results not from "efficiency in parasitic properties, but because their high competitive saprophytic ability causes difficulties in controlling their presence and numbers in soil". High percentages of colonization of straws through saprophytism in specialized laboratory situations have little epidemiological significance if such colonization rarely occurs in nature. The present studies were made to determine why Culmorum is relatively unsuccessful as a saprophytic colonist of wheat straw in Pacific Northwest fields.

Materials and Methods.—In all studies, 25-mm lengths of straw were buried in 140-ml glass jars containing 180 g of a Ritzville silt loam (RSL) or a Palouse silt loam (PSL). Five jars, each containing 10 straws, were used for each treatment. The soils were from naturally infested fields near Ritzville, Adams Co. (RSL), or Pullman, Whitman Co. (PSL), Washington. Soils of similar types from noninfested fields also were artificially infested with chlamydospores of Culmorum (7). In all cases, the Culmorum population was estimated by soil dilution-plate counting prior to burial of the straw.

The straws were clean and bright (hereafter referred to as unweathered) and were from fields of mature Gaines wheat near Pullman. In experiments concerned with effects of straw condition, the straws were collected at different times after harvest and included some exposed to field conditions and weathering for 8 months after harvest (August to April). Straws of the same wheat variety collected in different seasons and different ecological areas within the Pacific Northwest were also compared as substrata for colonization by Culmorum.

Water contents of the respective soil samples were adjusted to 11% and 17% (wt per cent) for the RSL and PSL, respectively. These water percentages corresponded to about −1.5 to −2.0 bars water potential as inferred from vapor pressure measurements by thermocouple psychrometry (5). In tests employing water as the variable, water potentials were adjusted to cover a range from lower than −60 bars to greater than −1 bar. Incubation temp was 20 C except when temp was the variable under study, in which case the range was 1-25 C.

Straws were removed from the jars and tested for the presence of Culmorum at 1, 4, or 10 weeks after burial, using a method of isolation described earlier (8). Each straw was cut transversely into two 12.5-mm lengths and placed on Difco cornmeal agar acidified to pH 4.7. Two bisected straws were placed on each plate. The plates were incubated in the dark at 9 C for 7-10 days, and at 25 C in diffuse daylight for an additional 3-4 days.

The proportion of each 12.5-mm length of straw yielding growth of Culmorum was estimated on a scale (score) of 1-5. Scores of the respective halves of each whole straw were then summed, making a total possible score of 10 for each straw and 100 for the 10 straws of each jar.

RESULTS.—*Influence of Culmorum population and duration of burial.*—Unweathered straws were buried for 1, 4, and 10 weeks in two naturally infested samples of RSL containing 270 and 1,575 Culmorum propagules/g, respectively; four artificially infested samples of a PSL containing 150, 1,900, 10,410, and 126,530 Culmorum propagules/g, respectively; and noninfested PSL. In addition, unweathered straws were buried for 1 week in five artificially infested samples of RSL containing 140, 370, 1,625, 15,110 and 49,770 propagules/g, respectively.

Time had little effect on colonization of straw by Culmorum over the 1-10 week period tested (Table 1). Detection of Culmorum as a straw colonist was max by the first week after burial. The fungus was not as easy to detect in straws buried for 4 and 10 weeks. All subsequent tests were terminated 1 week after burial.

In soils from the two naturally infested fields of RSL, colonization was consistently greater in the one with the greater Culmorum population (Table 1). Similarly in artificially infested RSL and PSL, percentage occupancy of the straws by Culmorum increased as population of the fungus increased (Fig. 1-A). Results with the artificially infested soils indicated that the min population for measurable colonization by Culmorum was between 100 and 1,000 propagules/g.

Influence of temp.—Straws were buried in naturally infested RSL containing 2,360 Culmorum propagules/g and PSL containing 7,900 Culmorum propagules/g and incubated at 5, 9, 15, 20, and 25 C. Also, straws were buried in artificially infested RSL containing 23,000 Culmorum propagules/g and incubated at 1, 4, 9, 15, 20, and 25 C. In all three soils, colonization was max at 20 and 25 C but was not prevented at 4 and 5 C (Table 2). In the naturally infested soils, there was little or no difference in amounts of colonization over the temp range 5, 9, and 15 C. At 4 C, and especially at 1 C, colonization was restricted. The Culmorum detected in straws after burial in soil at 1 C probably resulted from contamination. Some soil inevitably remained on or within the straws after washing. With 23 Culmorum propagules/mg of soil, even trace amounts of soil left as a residue on the occasional straw resulted in some germination and growth of Culmorum from straws of any given treatment.

Influence of soil water.—Naturally infested samples of PSL (9,750 Culmorum propagules/g) and RSL (1,730 Culmorum propagules/g) and two artificially infested samples of RSL (10,000 and 23,000 Culmorum propagules/g, respectively) were used to study the effect of soil water content and soil water potential on colonization of straw by Culmorum. Water contents of the two naturally infested soils were adjusted to five levels within the range available for higher plant growth (Table 3). Six water contents ranging from nearly air-dry to about −1 bar water potential were used in tests with artificially infested soil (Table 4).

Colonization was progressively greater as the soil

TABLE 1. Saprophytic colonization of unweathered, untreated wheat straw by *Fusarium roseum* f. sp. *cerealis* 'Culmorum' following burial of the straws for varying lengths of time in naturally infested Ritzville silt loam (RSL) and artificially infested Palouse silt loam (PSL)[a]

Soil	Culmorum population (prop/g)	% Occupancy by Culmorum per time Weeks 1	4	10
PSL	126,530	63.0[b]	66.0	60.2
	10,410	17.8	9.2	14.5
	1,900	8.0	1.6	2.6
	150	3.4	1.0	1.2
	Undetectable	0.4	0.2	0.8
RSL	1,575	23.4	13.8	15.6
	270	15.6	8.8	9.8

[a] Incubation temp, 20 C.
[b] Each value is the average percentage occupancy based on estimates made from ten 25-mm lengths of straw from each of five jars.

TABLE 2. Influence of temp on colonization of unweathered, untreated wheat straws by *Fusarium roseum* f. sp. *cerealis* 'Culmorum' in artificially and naturally infested soil

Temp	Percentage occupancy by Culmorum 1 week after burial in: Artificially infested RSL[a]	Naturally infested PSL[a]	Naturally infested RSL
C	*23,000 prop/g*	*7,900 prop/g*	*2,360 prop/g*
1	3.6[b]		
4	9.0		
5		21.8	12.2
9	45.4	29.8	9.7
15	45.0	27.8	9.3
20	50.7	66.3	18.1
25	72.0	48.3	16.5

[a] RSL = Ritzville silt loam; PSL = Palouse silt loam.
[b] Each value is the average percentage occupancy based on estimates made from ten 25-mm lengths of straw from each of five jars.

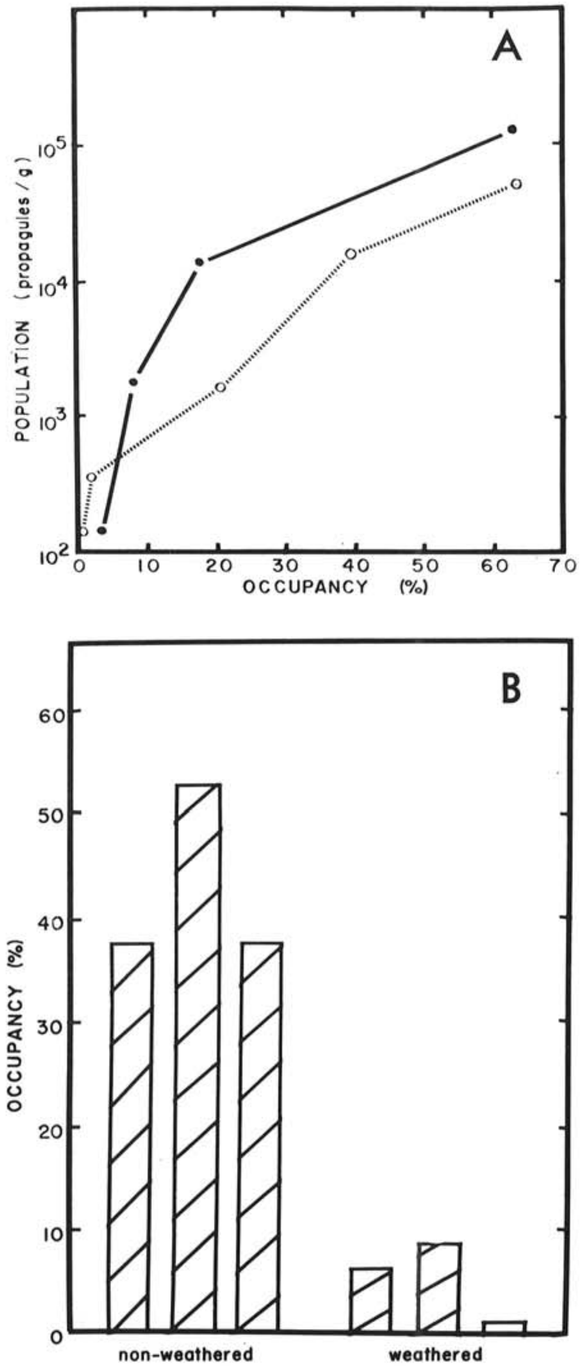

Fig. 1. A) Saprophytic colonization of unweathered, untreated wheat straws by ***Fusarium roseum*** **f. sp.** ***cerealis*** 'Culmorum' in Ritzville (broken line) and Palouse (solid line) silt loams, as affected by population of Culmorum; **B)** saprophytic colonization of weathered versus unweathered wheat straw by Culmorum in Palouse silt loam containing an estimated 10,000 Culmorum propagules/g of soil incubated at 20 C.

water potential was reduced to an opt of between −60 and −90 bars (Table 4). On the other hand, the amount of straw occupied by Culmorum when the experiment ended was high even in the wetter soils (Tables 3, 4). Colonization was limited only in extremely dry soil (Table 4). The three samples where water was limiting were too dry for water potential measurements because the lower limit of the thermocouple psychrometer was −90 to −100 bars.

Influence of weathering and prior-colonization by airborne saprophytic fungi.—Unweathered wheat straws were collected from each of three fields in August 1966 (just after harvest) and stored air-dry in the laboratory for subsequent use. Additional straws were collected in April 1967 from three different wheat fields that had undergone stubble-mulch tillage operations; the straw existed as standing and broken stubble on the soil surface and had been exposed to winter rains, freezing, and thawing. Such straw showed extensive discoloration due to the presence of fungi of the genera, *Alternaria, Stemphylium, Cladosporium, Aspergillus, Penicillium,* and others. Straws from all six fields were buried in PSL naturally infested with 10,000 Culmorum propagules/g of soil and the amount of colonization subsequently determined.

Percentage occupancy of the unweathered straws by Culmorum ranged from 38-53 a week after burial. By contrast, only 10% (and in one case 1%) of the weathered straws were occupied by Culmorum a week after burial (Fig. 1-B). Most of the fungal growth originating from weathered straws after burial was of fungi already present in the straw at time of burial.

In another experiment, stubble straws were collected from a Culmorum-infested field near Ritzville in August and November 1967 and April 1968, and from an adjacent Culmorum-infested field in August and September 1968. Straw segments were cut from the upper ends of the stubbles to insure that none was already occupied by Culmorum. The straw sections were then buried in naturally infested RSL containing 800 Culmorum propagules/g. Percentage occupancy of the straws 1 week after burial was directly proportional to the degree of weathering and discoloration (Table 5). Straws collected after harvest in 1968 showed more weathering for a given period in the field, and this was reflected as less colonization by Culmorum.

Severely discolored straws of a standing wheat crop were collected August 1968 at Puyallup, Wash., where summer rains are more frequent as compared to Pacific Northwest wheat-growing areas east of the Cascade Mountains. Growth of aboveground parasites and saprophytes on wheat is generally profuse at Puyallup. *Fusarium roseum* f. sp. *cerealis* 'Avenaceum' and its *Gibberella* perfect stage (6) were present on some of Puyallup straws collected. One-half the Puyallup straws were autoclaved at 121 C for 30 min and, together with untreated Puyallup straws and untreated, unweathered, Pullman straws, were buried in artificially infested RSL containing 23,000 Culmorum propagules/g.

Occupancy of the clean Pullman straws by Culmorum

TABLE 3. Influence of soil water content and soil water potential on colonization of unweathered, untreated wheat straws by *Fusarium roseum* f. sp. *cerealis* 'Culmorum' in naturally infested Ritzville silt loam containing 1,730 Culmorum propagules/g and Palouse silt loam containing 9,750 Culmorum propagules/g[a]

Ritzville silt loam			Palouse silt loam		
Water content	Water potential[b]	Occupancy[c]	Water content	Water potential	Occupancy
%	*−bars*	*%*	*%*	*−bars*	*%*
15.6	0.9	30.4	23.8	1.3	62.6
13.1	1.0	20.0	21.0	1.5	58.8
11.5	1.2	30.1	16.4	2.0	66.6
8.4	4.0	29.6	14.1	2.6	75.2
6.4	9.0	47.8	10.1	5.5	75.4

[a] Temperature of incubation, 20 C.
[b] Inferred from vapor pressure measurements by thermocouple psychrometry.
[c] Each value is the average percentage occupancy based on estimates made on ten 25-mm lengths of straw from each of five jars.

TABLE 4. Influence of soil water content and soil water potential on colonization of unweathered, untreated wheat straws by *Fusarium roseum* f. sp. *cerealis* 'Culmorum' in two artificially infested samples of Ritzville silt loam containing 10,000 and 23,000 Culmorum propagules/g of soil, respectively[a]

10,000 propagules/g			23,000 propagules/g		
Water content	Water potential[b]	Occupancy	Water content	Water potential	Occupancy
%	*−bars*	*%*	*%*	*−bars*	*%*
14.6	1.5	63.0[c]	17.2	1.0	57.3[c]
7.3	5	63.5	6.3	15	67.7
6.0	11	66.0	4.6	57	88.5
5.0	22	74.5	3.8	<90	28.6
3.9	48	77.0	3.0	<90	4.0
3.3	90	81.5	2.7	<90	3.2

[a] Temperature of incubation, 20 C.
[b] Inferred from vapor pressure measurements by thermocouple psychrometry.
[c] Each value is the average percentage occupancy based on estimates from ten 25-mm lengths of straw from each of five jars.

1 week after burial was estimated at 75%. In contrast, occupancy of untreated, weathered, Puyallup straws was estimated at only 17%. Instead of Culmorum, the latter straws yielded growth of those fungi present on the straw at the time of burial, including Avenaceum. Occupancy by Culmorum in autoclaved Puyallup straw was 43%.

TABLE 5. Influence of straw weathering on subsequent colonization of the straw by *Fusarium roseum* f. sp. *cerealis* 'Culmorum' in a naturally infested Ritzville silt loam containing 800 propagules/g[a]

Straws collected[b]	Condition of straw	% Occupancy by Culmorum
August 1967	Bright	18.6[c]
November 1967	Slightly weathered	12.4
April 1968	Severely weathered	3.2
August 1968	Slightly weathered	14.8
September 1968	Weathered	5.8

[a] Temperature of incubation, 20 C.
[b] Straws collected on the first three dates as well as the soil used for burial were collected from one field. Straws collected on the last two dates were collected from a different but adjacent field.
[c] Each value is the average percentage occupancy based on estimates made from ten 25-mm lengths of straw from each of five jars.

DISCUSSION.—Condition of straw at the time of actual contact with propagules of Culmorum in soil is probably the major factor limiting saprophytic colonization by this fungus in Pacific Northwest fields. On some farms, stubble is left uncultivated until spring of the year following harvest. On other farms, growers practice stubble-mulch tillage for wind erosion control. With this latter practice, much of the straw remains on the soil surface during the fall tillage operations, and is not completely incorporated into soil until spring or early summer of the fallow year. Even with mold-board plowing, straws remain in clumps, partially exposed aboveground or partially exposed in voids among soil clods; blending of straw with soil awaits spring and early summer tillage operations. In all cases, straws are exposed to leaching, weathering, and airborne inoculum of saprophytes for weeks and even months before exposure to Culmorum in the soil. Prior-colonization by other fungi together with a reduced nutrient status apparently prevents or limits colonization of straw by Culmorum.

In contrast, soil temp, water, and the concn of Culmorum inoculum are sufficient for colonization of straw by Culmorum in the Pacific Northwest. Except in extreme situations with frozen or near frozen soil in the winter, extremely dry soil in the summer, or extremely low Culmorum populations, colonization should be possible in the field, as indicated by the fact that colonization occurred experimentally at all temp tested above 4-5 C, at water potentials above −60 bars, and with populations as low as 100 Culmorum propagules/g of soil. Temperature and moisture conditions within this broad range prevail during much of the calendar year in soil in fields with Culmorum populations greater than 1,000 propagules/g; yet saprophytic colonization has not been demonstrated in those fields.

On the other hand, temp, water, and the Culmorum population affect the saprophytic activities of this fungus, given straw not already occupied by other fungi. In general, saprophytic colonization of unweathered straws by Culmorum increased as soil temp increased from 4-20 C, as soil water potential decreased from −1 to −60 bars, and as population of the fungus increased. The fact that amounts of colonization did not increase over the soil temp range, 5-15 C, confirms results of Burgess & Griffin (3) and their suggestion that higher soil temp increase the level of antagonism to Culmorum by the general soil microflora. Similarly, the greater activity of Culmorum in dry soil may relate to restricted growth of competitive microorganisms at low water potentials (9).

The exclusion of Culmorum from straw by other fungi is in accordance with the "prior-colonization-possession" phenomenon reported for several different fungi established as pioneer colonists in wheat straw (2). Barton (1) showed that *Pythium mamillatum* is an effective colonist of wood blocks buried in soil, only if the blocks are free of other colonists at the time of burial. Pioneer colonists, whether present in a substrate because of saprophytic or previous parasitic activities, have an advantage over secondary colonists. This phenomenon can be interpreted as a form of biological control of Culmorum. Without the presence of "prior-colonist", Culmorum presumably could invade and multiply in the dead straw returned to soil and exist in higher populations in Pacific Northwest wheat fields.

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