# Cephalosporium gramineum Populations in Soil Under Winter Wheat Cultivation

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Michigan Agriculture Experiment Station Journal Series Article No. 7128.
Accepted for publication 29 April 1975.

ABSTRACT

Effects of seasonal variations and cultural practices on the number, distribution, and longevity of *Cephalosporium gramineum* propagules in wheat field soil were monitored using a selective culture medium assay. Monthly assays over a 2-year period in fields under continuous winter wheat cultivation and with a history of Cephalosporium stripe disease, detected distinct periods of high and low soil populations of the causal fungus. Highest soil levels (normally exceeding 100,000 propagules/g fresh weight) were detected between October and February and resulted from the development of the saprophytic, sporodochial stage of the fungus, *Hymenula cerealis,* on infested host residue on the surface and in the upper 7.6 cm (3 inches) of soil. Residue deterioration, short conidial longevity, and limited saprophytic activity at higher temperatures markedly reduced soil populations during early spring so that typically <5,000 propagules/g were present from May through July. Removing straw or plowing it under, as opposed to disking it into the soil prior to planting, reduced fungus levels and the incidence of Cephalosporium stripe disease. Infested straw beneath the soil surface supported the multiplication of *H. cerealis* during the first autumn but was degraded and nonsupportive by the second. However, straw undisturbed on the soil surface decayed slowly and supported the saprophyte during fall and winter for three consecutive years. Propagules in moist field soil screened of residue and held at 23 C and 7 C, respectively, had a half life of 1 and 26 weeks. Such propagules had a capacity to multiply at 7 C, but not at 23 C or in dry soil.

Phytopathology 65:1129-1133

*Cephalosporium gramineum* Nisikado & Ikata is the fungus which causes Cephalosporium stripe disease in wheat, *Triticum aestivum* L. (1). The fungus survives as a systemic vascular parasite in living gramineous host plants (1, 6) and as a saprophyte, *Hymenula cerealis* Ell. & Ev., on infested host residue (2). The fungus is easily isolated from its living hosts, but until the recent development of a selective culture medium (5, 7) it was impossible to qualitatively and quantitatively isolate it directly from soil.

This ability to detact and enumerate soil-borne levels of the fungus was used in this study to (i) evaluate the influence of seasonal changes and cultural practices on the quantity, distribution, and longevity of *C. gramineum* propagules in wheat field soil, and (ii) determine the relationship of the level and distribution of the fungus to the incidence of Cephalosporium stripe in wheat.

MATERIALS AND METHODS.—A field at East Lansing, Michigan, maintained under continuous winter wheat (*Triticum aestivum* L., 'Ionia') cultivation was used for this study. Unless otherwise indicated, after harvest, all crop residue was turned under by plowing prior to planting the subsequent crop in late September. The field had a history of *C. gramineum* infestation and each spring a minimum of 10% of the developing wheat plants bore Cephalosporium stripe symptoms. To detect seasonal variations in soil levels of the *C. gramineum* fungus, soil samples were collected and assayed monthly over a 2-year period.

A portion of the field described above was used to determine the influence of crop residue on subsequent soil populations of the fungus and amounts of Cephalosporium stripe disease. After harvest in mid July, an area approximately 0.1 hectare (1/4 acre) in size was mechanically cleared of straw and then burned over with a Hudson Flame Sprayer in early August. The cleared area and an adjoining uncleared area of the same size were further equally divided by deep plowing or light disking prior to planting in late September. Soil samples from each area were collected and assayed monthly thereafter, for a period of one year. The percentage of tillers which exhibited Cephalosporium stripe symptoms was calculated from random counts of a minimum of 400 tillers in each treatment area during the following spring (May).

TABLE 1. Vertical distribution of *Cephalosporium gramineum* propagules in soil at three stages of winter wheat cultivation

| Depth range (cm) | Propagules/g soil | | |
|---|---|---|---|
| | June[a] | August[b] | October[c] |
| 0 to 7.6 | 300 | 300 | 139,000 |
| 7.6 to 15.2 | 0 | 800 | 0 |
| 15.2 to 22.9 | 0 | 300 | 0 |
| 22.9 to 30.5 | 0 | 2,200 | 0 |

[a]Wheat headed, showing Cephalosporium stripe symptoms and beginning to mature.
[b]After plowing down infested residue from harvested crop.
[c]After emergence of fall-sown wheat.

Soil samples for these studies were ten randomly-collected 2.5-cm diameter cores of soil taken at 7.6-cm (3-inch) increments to a maximum depth of 30.5 cm. Cores of a given sample were composited by depth and thoroughly mixed.

To study the persistence of the fungus in the field, sufficient field soil with approximately 980 propagules of *C. gramineum* per gram was collected in August to fill three 35.6 × 50.8 × 10.2-cm (14 × 20 × 4-inch) deep metal flats. Infested straw (approximately 200 grams) was mixed into the soil in one flat and layered on the soil surface in another. The remaining flat of soil received no straw. The flats which had perforated bottoms, were buried even with the soil line in a fallow area of the field. The soil in each flat remained undisturbed except for sampling and supported no plant growth for over 2 years. At intervals during this period, three cores of soil were removed from each flat, composited and mixed for assay.

To test propagule longevity approximately 2 kg of wheat field soil with 15% moisture was collected in October, mixed, passed through a 0.71-mm (pore size) ASTM No. 25 sieve and either placed in plastic bags or permitted to air dry at 23 C or 7 C in the dark. At points during storage small amounts of soil were removed for assay.

All soil sampling in the field included careful cleaning of the soil probe between samples and, where surface host residue was visible, only the soil beneath it was collected.

Five-gram subsamples of soil were used for assays. Each subsample was transferred to 500 ml of distilled water, agitated for 5 minutes and 10 ml of the resultant suspension was pipetted into 90 ml of distilled water. One ml of this $10^{-3}$ soil dilution was layered onto each of five plates of green wheat agar (GWA). The GWA selective culture medium was prepared and *C. gramineum* colonies were counted as previously described (7). Moisture in the soil samples ranged from 10-40%. All data herein was adjusted for expression as propagules per gram of soil at 20% moisture.

RESULTS.—Each year of wheat cultivation included a distinct high and low period of soil infestation by *C. gramineum* propagules (Fig. 1). After harvest in July, a minor and temporary increase occurred, and autumn (September) marked the beginning of a dramatic increase in the fungus. Propagule numbers peaked during mid-winter at levels exceeding $1 \times 10^5$/g and subsided thereafter, so by early spring, $< 5 \times 10^3$/g were detected. Counts were consistently lowest and the fungus often not detected in soil during the summer months of June and July.

In both years of this test the fungus was typically recovered only from the surface 7.6 cm of soil. A distinct exception, however, was the period of approximately 30 days immediately after fall plowing when the fungus was distributed to the full 30.5-cm depth (Table 1).

Modifications in handling infested residue from the previous crop had dramatic effects on subsequent populations of the fungus and the frequency of Cephalosporium stripe disease (Fig. 2). Thorough removal of such residue prior to planting nearly eliminated both the fungus and the disease. Where residue was allowed to remain, levels of the fungus and disease were related to the amount of residue in and on the

uppermost 7.6 cm (3 inches) of soil. In this regard, residue disked into the soil prior to planting supported four times as many propagules, and twice the incidence of disease as when it was turned under by deep plowing.

The importance of crop residue on the survival of the pathogen was further demonstrated when residue-amended soil was confined to flats in the field. Multiplication of the pathogen was again dependent on the availability of host residue (Fig. 3). Furthermore, the activity of the fungus was inversely related to the rate of residue decay. When straw was mixed into the soil, the fungus multiplied during the first autumn, but in subsequent years existed only at the sparse levels that appeared each year in the nonamended soil. Within the first year, the integrity of all such residue was destroyed and its decay was nearly complete. On the other hand, straw on the soil surface decayed more slowly, remained visible and supported *H. cerealis* multiplication into the third growing season.

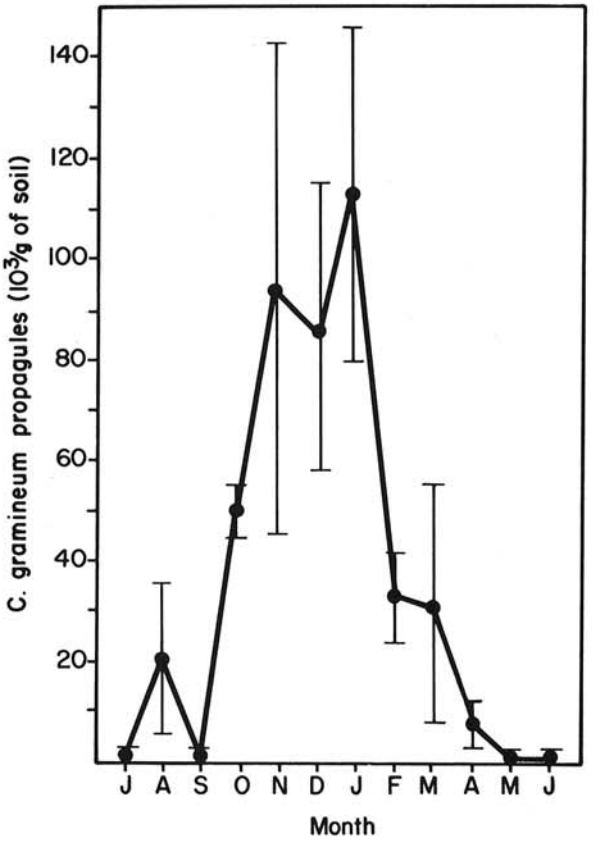


**Fig. 1.** Levels of *Cephalosporium gramineum* propagules in the uppermost 7.6 cm (3 inches) of wheat field soil. Average of 2 years' data.

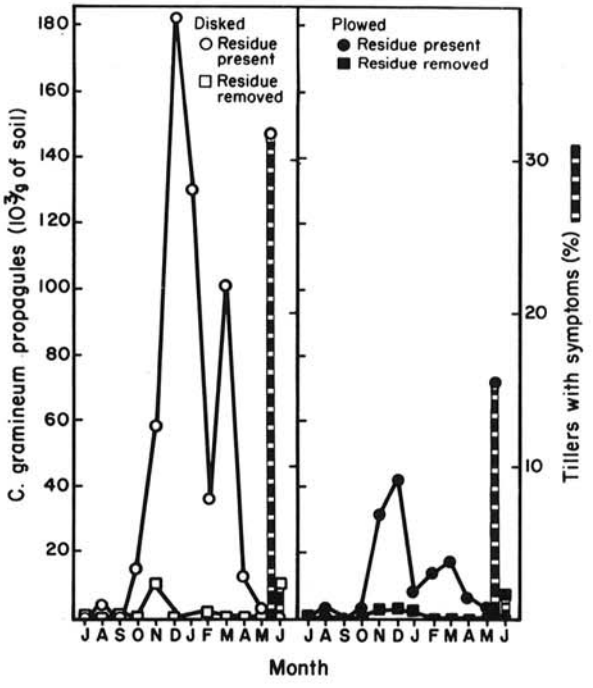


**Fig. 2.** Levels of *Cephalosporium gramineum* propagules and Cephalosporium stripe disease as affected by preplant disking or plowing field areas with and without residue from the previous winter wheat crop.

In the laboratory, as in the field, the fungus showed a limited capacity to survive without host residue as a substrate for its saprophytic stage. Propagules in autumn-collected field soil had a half-life of 0.5-2.5 weeks at 23 C whether the soil was moist or dry. The fungus survived longer in soil held at 7 C and, at this temperature, soil moisture had a greater influence on its activity (Fig. 4). At 7 C, *C. gramineum* propagules increased from $6 \times 10^4$ to $4.2 \times 10^5$/g of soil during the initial 6 weeks then declined to $2.5 \times 10^4$/g of soil by 26 weeks. The soil allowed to dry at 7 C also showed an initial increase in propagules before reaching moisture equilibrium; thereafter, the propagules had a half life of approximately 17 weeks.

DISCUSSION.—This research provides the first quantitative measurements of the activity of the *C. gramineum* fungus in soil and, additionally, supports earlier reports on its survival as a pathogen and saprophyte (2, 3, 4).

The systemic distribution of the parasite in the vascular tissues of wheat (6) provides the potential for large amounts of infested tissue to return to the soil. Favored by wet, cool weather, sporodochia of *H. cerealis* proliferate on such infested host residue, and apparently the spores therefrom are the primary source of inoculum for infection of fall-sown wheat. We have detected no specialized resistant or resting propagules in soil. The propagules we have identified thus far are conidia (5, 7) which decline rapidly in response to warm temperatures and/or the depletion of their residue substrate (Fig. 1, 2). Infection of the host prior to the advent of warmer temperatures and residue depletion in the summer season,

however, makes the marked depletion of the saprophyte from soil at that time of little or no consequence to the current wheat crop. When levels of the fungus in soil are lowest, the organism is multiplying in plant parts above ground.

The greatest period of activity for the fungus in soil began in autumn and coincided with the profuse development and sporulation of the saprophytic, *H. cerealis* stage-(2). The minor increase in soil propagules recorded in late summer after harvest (Fig. 1) is likely an expression of the fungus within, or released from, infested host residue which was returned to the soil during harvest operations. Some germination and/or multiplication of the fungus in such residue may have occurred prior to its conversion to, and development as, a soil-borne saprophyte. The germination and multiplication of free conidia in moist soil has been observed under other circumstances (Fig. 4 and Ravenscroft, *unpublished*).

Cephalosporium stripe symptoms were more abundant when residue and fungus levels in the soil were high (Fig. 2). However, in this study and in more recent ones (Ravenscroft, *unpublished*), propagule numbers could not be more closely associated with disease incidence. The sparse levels of propagules in soil prior to fall planting (Fig. 1, 2, and Table 1) were not indicative of subsequent disease incidence. Infection of wheat seedlings in the field is presumed to take place during periods of root breakage in fall and winter. If the natural infection period could be more specifically defined, the accompanying fungus level might be indicative of subsequent disease levels.

The limited longevity of the fungus apart from the living host or host residue is partially offset by its remarkable rate of multiplication when substrate and environmental conditions are not limiting (Fig. 1, 2, 3, 4). The fungus also has the capacity to multiply in soil apart from the development of *H. cerealis* sporodochia (Fig. 4). Nonetheless, the fungus and the Cephalosporium stripe disease can be controlled by employing cultural practices that increase the decomposition of, or eliminate, infested host residue at or near the soil surface. Wheat in 2- and preferably 3-year rotations with nonhost crops like corn or beans in Michigan shows only infrequent symptoms of Cephalosporium stripe disease compared to wheat following wheat or other gramineous host crops. However, where host residue remains undisturbed the fungus can survive saprophytically and produce infectious conidia for at least three growing seasons.

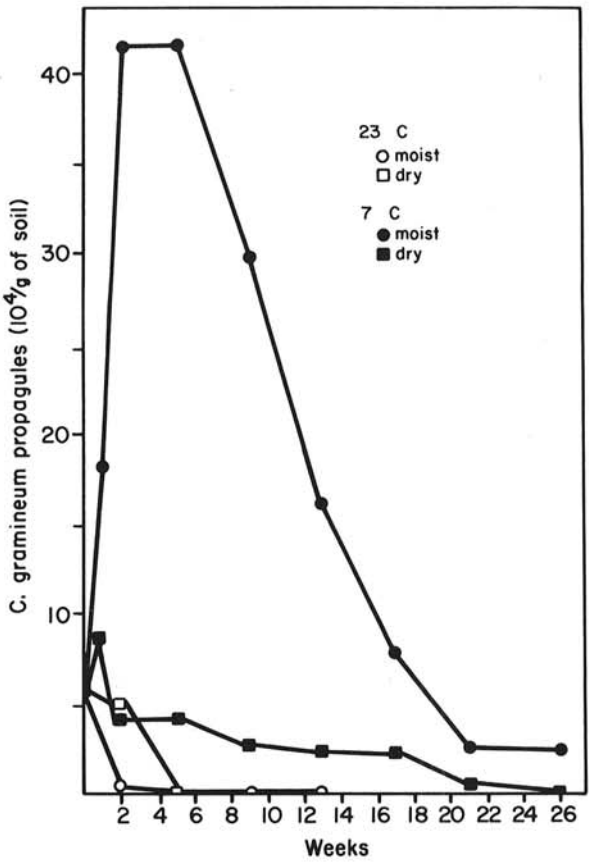


**Fig. 4.** Influence of temperature and moisture on the longevity of *Cephalosporium gramineum* propagules in naturally-infested field soil.

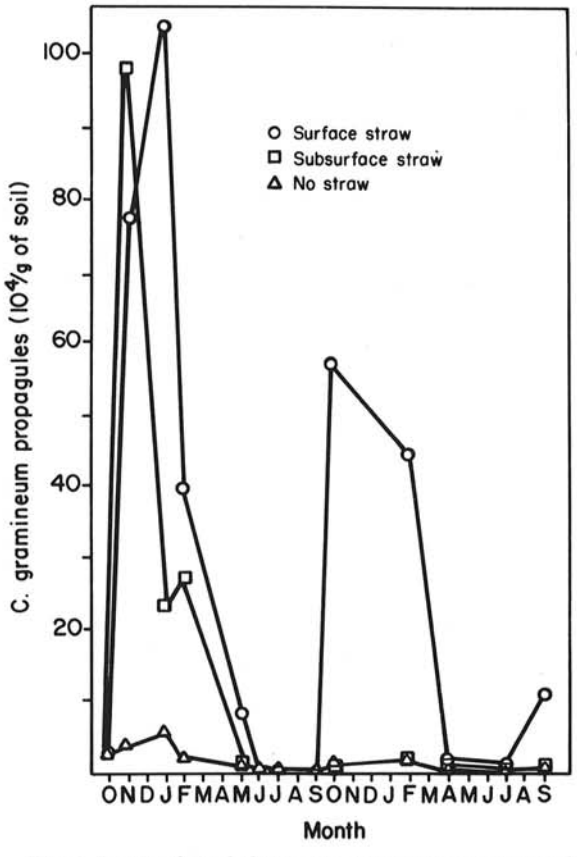


**Fig. 3.** Levels of *Cephalosporium gramineum* propagules in field soil over a 24-month period as affected by surface and subsurface straw.

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