

# Production of *Pseudocercospora herpotrichoides* Spores

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## ABSTRACT

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Incubation on oat kernels in the laboratory for longer than 30 days diminished the ability of *Pseudocercospora herpotrichoides* to produce conidia when these oats were placed outdoors. Heaviest sporulation occurred on oats colonized for fewer than 30 days. *P. herpotrichoides* used easily digestible substrates for spore production on naturally infected wheat stems but apparently did not compete saprophytically for tissue not colonized during the parasitic phase or not easily digested.

Many workers have used oat kernels (*Avena sativa* L.) as a medium for producing inoculum of *Pseudocercospora herpotrichoides* (Fron) Deighton. The oat hull is advantageous during autoclaving because sterilized oats do not clump together; this makes it easy to dry and separate them into individual kernels after incubation. Bruehl and Nelson (2) used oats inoculated with *P. herpotrichoides* incubated 1-2 mo at 20 C. Different inoculum batches differed greatly in spore production. Oat kernels with very little pseudoparenchyma supported greater sporulation than oats dark with heavy pseudoparenchyma.

Deacon (3,4) presented evidence that *P. herpotrichoides* is a poor saprophyte in nature and that sporulation is supported by food within hyphae established in the substrate during pathogenesis. This led us to believe that longer incubation on the oat kernels might produce more spores. The fungus does not sporulate on the oat kernels in the laboratory at 20 C so the hyphae that form within the oats should be extensive

and rich in stored food.

Pseudoparenchyma (scurf) is characteristic of advanced natural lesions on wheat stems, and Deacon (4) proved that pseudoparenchyma is formed by *P. herpotrichoides* when its growth is stopped by exhaustion of nutrients in the medium. He found no evidence of spores produced on the pseudoparenchyma. We compared spore production on oat kernels incubated for different periods of time and on natural lesions of differing severity on wheat stems.

## MATERIALS AND METHODS

**Oat kernel inoculum.** One hundred grams of oat kernels plus 56 ml of deionized water were autoclaved in 1-L Erlenmeyer flasks at 121 C for 45 min, cooled, and inoculated with mycelial fragments of five isolates of *P. herpotrichoides* suspended in 10-ml water blanks in experiments 1 and 2. The oats were shaken and incubated at 20 C for varying periods of days. The oats were shaken two or three more times at intervals during early incubation to facilitate uniform colonization of the kernels. After incubation, the oats were dried in a layer 1-2 cm thick at 20 C for 2-4 wk, weighed to determine weight loss during incubation, and stored dry until testing for spore production.

For spore production, the oats were placed between two layers of plastic screen outdoors on sand beds in late October. They were washed periodically in 200 ml of tap water to harvest spores,

then returned outdoors. The spores in the wash water were counted with a hemacytometer at  $\times 100$ . Conidia of *P. herpotrichoides* are easy to identify by their shape. Variations of the incubation periods were used with two other batches of oats, but the methods were otherwise similar (experiments 1-3).

**Infested straws.** Straws from naturally diseased wheat plants were graded for lesion severity on a scale of 1-4, where 1 = shallow but definite eye-spot lesion, 2 = deeper lesion, 3 = deep lesion with some pseudoparenchyma, and 4 = deep lesion with abundant pseudoparenchyma. Straws with single lesions were used. The lesioned part of the stem was removed for incubation outdoors as was done with oat kernels.

Both oats and naturally infested straw fragments were washed once by submerging in water and stirring for about 2 min. The amount of water used to wash spores from the straws varied from 50 to 125 ml depending on the number of straw pieces in the sample. The water was decanted and this water constituted the spore suspension from which counts were made.

## RESULTS

In experiment 1, oat kernels incubated 18, 50, and 80 days after inoculation in the laboratory averaged 97.5, 88.9, and 84.4 g after incubation, respectively. During incubation, a significant loss from the original 100 g in dry matter occurred. Spores were washed from these oats once on each of the following dates: 3 and 19 March, 5 and 21 April, and 11 May 1981. Oats incubated 18 days yielded  $6.5 \times 10^{10}$  spores, oats incubated 50 days yielded  $4 \times 10^{10}$  spores, and oats incubated 80 days yielded  $2 \times 10^{10}$  spores. The shortest laboratory incubation yielded the greatest number of spores.

Experiment 2 was done the same way with the same five isolates of the fungus, except the oats were washed six times, three times during November and

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December 1982 and three times during January to March 1983. After laboratory incubation, control oats weighed 97.5 g, oats incubated 20 days weighed 93.0 g and yielded  $2 \times 10^{10}$  spores, oats incubated 47 days weighed 82.3 g and yielded  $1 \times 10^{10}$  spores, and oats incubated 74 days weighed 73.2 g and yielded  $0.4 \times 10^{10}$  spores. As in experiment 1, spore production declined as the length of laboratory incubation increased.

In experiment 3, only 58 g of oats were inoculated in 500-ml Erlenmeyer flasks, and the inoculum per flask consisted of 10 disks of young mycelium 4 mm in diameter cut from the margins of six isolates growing on potato-dextrose agar at 20 C. Incubation in the laboratory, with shaking as needed, was for 11, 24, and 37 days. Colonization of the kernels was rapid in the smaller flasks. Oats incubated 11 days were colonized but light in color, those incubated 24 days were dark, and those incubated 37 days were very dark with much pseudoparenchyma. Oats incubated 11 days weighed 57 g and produced  $6 \times 10^8$  spores, oats incubated 24 days weighed 54.9 g and produced  $1.5 \times 10^8$  spores, and oats incubated 37 days weighed 43.9 g and produced  $0.8 \times 10^8$  spores, all in a single washing. Again, heaviest sporulation occurred after the shortest incubation period.

In experiment 4, the number of spores produced per straw with natural lesions (grades 1-4) was measured in three washings. The straws with well-developed lesions (grades 2 and 3) produced the most spores. Those with superficial (grade 1) and very advanced (grade 4) lesions produced the fewest spores (Table 1).

## DISCUSSION

Garrett (5) presented evidence that for a fungus to survive saprophytically, hyphae must extend into fresh substrate and obtain food adequate to sustain growth. Wheat straw is an unbalanced diet for fast-growing fungi, and additional nitrogen lengthens the period of saprophytic survival of *Gaeumannomyces graminis* and *Fusarium culmorum*. Added nitrogen shortens the period of saprophytic survival of fungi that grow more slowly (*Bipolaris sorokiniana* and *Phialophora radiculicola*) by favoring competitors more than it favors the initial occupant. *P. herpotrichoides*, according to Macer (6) and Deacon (3), does not respond to added nitrogen, either positively or negatively. Its survival is unchanged by added nitrogen. Deacon (3) concluded that *P. herpotrichoides*

**Table 1.** Spores of *Pseudocercospora herpotrichoides* produced per straw on Stephens and Viking wheats<sup>a</sup>

Lesion grade	Stephens		Viking		Total spores (both cultivars)
	No. of straws	Spores $\times 10^4$	No. of straws	Spores $\times 10^4$	
1	46	19	59	20	39
2	100	52	100	80	132
3	70	68	100	76	144
4	49	19	35	20	39

<sup>a</sup> Straws with single natural lesions graded on a scale of 1-4, where 1 = shallow but definite eye-spot lesion, 2 = deeper lesion, 3 = deep lesion with some pseudoparenchyma, and 4 = deep lesion with much pseudoparenchyma.

survives primarily as slightly pigmented hyphae in the substrate, that these hyphae support sporulation, and that saprophytic growth in the straw is negligible.

In this study, *P. herpotrichoides* sporulated heavily after minimal establishment in the substrate. Long incubations with heavy production of pseudoparenchyma reduced sporulation. The loss in weight of oat kernels with increased incubation time is evidence that substrate was continually being digested. Highest sporulation occurred on oats in which digestible nutrients were still abundant. When *P. herpotrichoides* is adequately established in the substrate but not incubated to the extent that all easily digested substances have been used, the fungus would obtain nourishment saprophytically as long as these substances exist, as in oat kernels with short incubation periods or in stem lesions graded 2 or 3.

The poor sporulation from stem lesions graded 1 and 4 is evidence that *P. herpotrichoides* does not competitively colonize fresh straw tissue. These lesions were all bordered by unexploited tissue, but the fungus did not profit from it. Since superior sporulation occurred on lesions graded 2 and 3, the tissues invaded during parasitism but not totally digested were important to sporulation.

Our observations confirm the conclusion of Deacon (3) that pseudoparenchyma does not directly or indirectly support sporulation. We tested the oats through two additional spring seasons (autumn to early spring in two additional years) and sporulation was essentially equal on the oats regardless of the laboratory incubation period. The oats continued to support production of a few spores with no evidence that those with heavy scurf sustained sporulation longer than oats with little scurf, i.e., evidence that food was not obtained from the scurf. The fungal pseudoparenchyma apparently has no function.

When Rowe and Powelson (7) measured spore production on naturally infested straws in Oregon, they found

that initial heavy sporulation diminished with time and that after heavy sporulation, the fungus died in straw on the soil surface in July and August. We kept oats air-dry in the laboratory (20 C) during the summers and the fungus survived (at least in some of the kernels), because small numbers of spores continued to be produced for as long as three winter periods after which the experiment was terminated.

In an earlier paper (1), we reported heaviest sporulation on stems graded 4, but these results are not comparable. In the present study, straws with single lesions were used. In the earlier study, most straws graded 4 had multiple lesions.

Thorough establishment of the test organism in the substrate, by giving it complete possession before incubating the infested substrate in natural soil, was thought to be advantageous to the test organism. In reality, the ability of the pathogen to sporulate or survive may be reduced by overly long incubation periods before burial. If the nutrients available to the pathogen are largely spent before burial, results of survival experiments could be misleading.

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