Effect of Wetting-Period Duration on Ascocarp Suppression by Selected Antagonistic Fungi in Wheat Straw Infested with *Pyrenophora tritici-repentis*

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ABSTRACT

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Wheat straw axenically or naturally infested with *Pyrenophora tritici-repentis* was inoculated with one of several antagonistic fungi and exposed to various treatments of alternate wetting and drying, and ascocarp production by *P. tritici-repentis* on straw was measured. Wetting treatments were repeated wet periods of 6, 12, 24, or 48 h separated by drying periods. In the absence of suppressive fungi, ascocarp formation by *P. tritici-repentis* was observed in both axenically colonized straw and naturally infested field straw in all wetting treatments, and the effect of wetting duration on ascocarp formation was not obvious. Suppression of ascocarp formation in both field and axenic straw by selected antagonistic fungi was affected by the duration of intermittent wetting. In field straw, the number of large ascocarps produced by *P. tritici-repentis* was significantly reduced by *Laetisaria arvalis*, Sterile II, and *Limonomyces roseipellis* under 24- and 48-h wetting periods but not under 6- and 12-h wetting periods compared to the uninoculated control. Compared to field straw, suppression of ascocarp formation in axenic straw by the test fungi was much less. *L. arvalis* reduced ascocarp formation in the 24- and 12-h wetting treatments compared to the uninoculated control. Under the 48-h wetting treatment, Sterile II inhibited ascocarp formation in one experiment but increased ascocarp formation in the other. *Limonomyces roseipellis* did not inhibit ascocarp formation in axenic straw. Thus, ascocarp suppression in wheat straw by selected antagonistic fungi occurred only under relatively long (>12 h) wetting periods.

Tan spot, caused by *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph: *Drechslera tritici-repentis* (Died.) Shoemaker), is a foliar disease of wheat (*Triticum aestivum* L.) that occurs in the central plains of North America (5,6). This disease has recently become recognized as one of the most important and widespread diseases of wheat. Losses due to tan spot epidemics have been as high as 50% of a grain yield (6).

P. tritici-repentis produces ascocarps in surface-borne residue in the field. Later during the saprophytic stage of this fungus, ascospores (primary inoculum for tan spot epidemics) are produced and released from ascocarps under favorable moisture conditions. Levels of primary inoculum have been correlated with tan spot epidemic development in the field (1,17).

Primary inoculum production of tan spot depends on successful survival and saprophytic growth of *P. tritici-repentis* in the residue. These processes can be affected by many physical and biological factors in the microenvironment, as suggested by laboratory observations (9,10,13,15). Substrate moisture conditions have been shown to affect greatly the saprophytic growth of the tan spot pathogen. Mycelial growth on an artificial medium (15) or in dead wheat leaves (11) declines with decreasing water potential, and the growth in dead leaves is completely eliminated at a water potential lower than −10 MPa. Similarly, high-moisture conditions favor ascocarp formation (11), at least in the absence of antagonistic microflora.

In addition to its direct effects, straw moisture may influence the activity of *P. tritici-repentis* indirectly through effects on other microorganisms in the residue. In the field, a gradual change in the composition of the straw-associated microbial community occurs over time during saprophytic survival of the pathogen in residue (14). The straw, initially occupied by primary colonizers such as *P. tritici-repentis*, is taken over gradually by other saprophytes with weak parasitic ability and then by secondary saprophytes and soil inhabitants in the later stages of the succession. These secondary colonizers have a great impact on the survival and growth of the pathogen in residue by competing for nutrients in the substrate. Some of them also may be antagonistic to the pathogen. If the secondary organisms are stimulated by moist conditions, high water potential could indirectly suppress *P. tritici-repentis*.

Several observations support the possible importance of moisture to microbial interactions with *P. tritici-repentis*. A laboratory study (16) showed that the tan spot fungus survives poorly in wheat straw mixed with soil under warm, moist conditions. Pfender and Wootke (14) also found that the pathogen does not survive well in straw in close contact with soil in the field, and they suggested that straw-moisture conditions may be an important factor in microbial activity and competitive interactions occurring in wheat residue. Our recent study (19) showed that cumulative straw moisture occurring in wetness durations longer than 12 h is negatively correlated with ascocarp production in wheat residue in the field. In a laboratory study, suppression of *P. tritici-repentis* by *Limonomyces roseipellis* Stalpers & Loerakker was affected by straw moisture because it was more effective in straw that remained wet for extended time periods (10). Similarly, although ascocarp formation by *P. tritici-repentis* is favored by high water potential in sterilized leaves, it is relatively low in nonsterile leaves at the same water potential (11).

In wheat fields, the moisture level of wheat residue is likely to undergo frequent fluctuations as weather conditions change. Straw-moisture conditions that can influence the growth and development of *P. tritici-repentis* are determined not only by how wet the straw becomes but also by how long the straw remains wet. Thus, straw-wetness duration may be a more useful moisture parameter than water potential in demonstrating the influence of straw moisture on growth and ascocarp formation by *P. tritici-repentis*. The objectives of this study were to examine the effect of wetting duration 1) on ascocarp production by *P. tritici-repentis* in wheat straw and 2) on ascocarp suppression by several potential fungal antagonists.

MATERIALS AND METHODS

Fungal cultures. Isolate 6R180 of *P. tritici-repentis* was obtained from infected wheat plants in Kansas and used to prepare

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axenically infested straw. The four fungi used in this study to determine the effect of straw-wetness duration on their suppression of ascocarp formation by *P. tritici-repentis* were *Epicoccum nigrum* Link, *Limonomyces roseipellis*, *Laetisaria arvalis* Burdsall, and an agonomycete isolate designated Sterile II. *Limonomyces roseipellis*, *L. arvalis*, and Sterile II have been shown previously to be potentially effective antagonists of *P. tritici-repentis* in dead wheat leaves and straw, whereas *E. nigrum* was not antagonistic (10,12). *Limonomyces roseipellis*, Sterile II, and *E. nigrum* were all isolated from reduced tillage wheat residue in Kansas (10,14). *L. arvalis* was obtained from L. Herr of Ohio State University, Columbus (8). These fungal cultures were maintained on one-fourth strength potato-dextrose agar (1/4 PDA) (6 g of Difco potato-dextrose broth and 15 g of agar per liter of H_2O) at 4 C.

Preparation of straw infested with *P. tritici-repentis*. Field straw was collected after wheat harvest from a field of winter wheat (cv. TAM 105) naturally infested with *P. tritici-repentis*. Straw was then air-dried and stored at room temperature (24 ± 2 C) in the laboratory. The top two internodes of the field straw were selected and cut into 2-cm pieces before use in experiments.

Axenic straw was prepared using wheat straw harvested from wheat plants grown in the greenhouse. Winter wheat (cv. TAM 105) was vernalized and grown to maturity in the greenhouse. After plants had senesced and dried, straw was harvested, and the top two internodes were selected and cut into 2-cm pieces. These pieces were moistened on filter paper in a petri dish and autoclaved twice on successive days at 121 C for 20 min. After sterilization, the moist straw was placed onto a platform (7 × 9 cm) made of two semirigid polypropylene screens (plastic needlework canvas) stacked and held together by several plastic clips. Twenty slots (0.5 × 2.5 cm) were cut in the upper screen to hold one piece of straw per slot, preventing each straw from contacting others. Immediately after placement, each straw was inoculated at one end with a small piece (2 × 2 mm) of straw culm colonized by *P. tritici-repentis*. The culmborne inoculum was prepared by incubating sterilized culm tissues (approximately 2 × 2 mm) along colony edges of *P. tritici-repentis* on petri plates of 1/4 PDA for 3–4 days at room temperature (24 ± 2 C). Those colonies of *P. tritici-repentis* had grown for 3–4 days at room temperature. (Throughout the study, the experiments were performed under ambient light conditions, and the intensity was not controlled.) The infested culm tissues were removed and immediately used in inoculation. After applying the inoculum of *P. tritici-repentis*, straws on the screens were incubated for 24 h in a closed dish at 100% relative humidity (RH) and room temperature, permitting initial establishment of *P. tritici-repentis*. The screens and straws were placed in humidity chambers to control the growth of *P. tritici-repentis* at a water potential that would permit mycelial growth but not ascocarp formation (11). Straw water potential was maintained at approximately −3.5 MPa by isopiestic equilibration with an atmosphere of 95% RH. Humidity chambers were constructed from canning jars fitted with gas-permeable caps, described previously (11). An aqueous glycerol solution of 95% water activity was used to maintain the RH in the chamber. Jars were placed in open-top boxes constructed of thick polystyrene, covered with a clear plastic top, and placed on a laboratory bench at 24 ± 2 C. After 7 days of incubation, straw was removed from humidity chambers and air-dried for several days. Before the colonized straw was used in experiments, culm bits carrying *P. tritici-repentis* inoculum were removed from the ends of the straws.

Inoculum of test fungi. Inoculum was prepared by growing each of the four fungi on wheat bran. Fresh wheat bran (15 ml) was combined with an equal volume of water in a glass petri dish and autoclaved twice on successive days at 121 C for 20 min. After sterilization, the moistened bran in each plate was inoculated with four agar plugs of one of the four fungal cultures from 1/4 PDA plates and incubated at room temperature (24 ± 2 C). After 5–10 days of fungal growth in bran, the colonized bran was air-dried overnight and ground in a sterile mortar with a pestle.

Inoculation of field and axenic straw by potential antagonists. Field and axenic straw infested with *P. tritici-repentis* was inoculated with bran inoculum of one of the four fungi on one end of the straw. For axenic straw, the end opposite that which had been inoculated with *P. tritici-repentis* was inoculated with one of the test fungi. (A preliminary study showed that ascocarp production in axenic straw was not significantly affected by the placement of culm- or branborne inoculum relative to the site of prior inoculation with *P. tritici-repentis*.) A drop of autoclaved 1.3% methylcellulose solution was applied first in a narrow band (3 mm wide) around one end of the straw to serve as an adhesive for the infested bran. A small amount of bran inoculum (0.02–0.05 g) was immediately applied to the ring of methylcellulose on the end of the straw. The inoculated field or axenic straws were placed on a thin layer (about 1 cm deep) of quartz gravel in a square plastic pot (6.5 × 6.5 × 5.5 cm). A plastic screen, resting on the ridge 1.5 cm below the top of the plastic pot was used to support quartz gravel.

Wetting treatments. The pots were placed on a mist bench in the greenhouse, and the inoculated straw was exposed to alternating wet and dry treatments to simulate rain and drying events under field conditions. The mist system was equipped with Flora-Mist nozzles (A. H. Hummert Seed Co., St. Louis, MO) capable of supplying 0.053 m^3 of water per hour under 2.4–2.8 × 10^5 Pa of pressure.

Four wetting treatments were used in this study; the respective intermittent-wetting duration was 6, 12, 24, or 48 h, modified by temperature and sunlight conditions in the greenhouse. These wetting treatments were achieved by applying intermittent mist for 1–2, 6–8, 18–20, or 38–40 h, respectively, and scheduling the misting periods to end near midday, when the straw would dry in several hours. Misting was intermittent during each wetting event, with a misting frequency of 6 s/10 min during the day and/or 3 s/10 min during the night. In preliminary experiments, the water potential of straw samples was measured with a thermocouple psychrometer (Decagon, Pullman, WA) during the time the straw was drying, and it was determined that the stated misting treatments would produce straw moisture wetter than −10 MPa for the desired durations (6, 12, 24, or 48 ± 2 h).

The experiment was stopped after the total number of wetness hours for each wetting treatment reached an equivalent of 14 wetting periods of 48 h (i.e., 672 cumulative hours). The straw was then removed from the pots and examined under a dissecting microscope at 25× to determine the number of total (>100 μm) and large (>300 μm) ascocarps on each straw. Ascocarps smaller than 300 μm are predominantly immature (infertile) (13). Data analysis showed little difference between the numbers of large and total ascocarps produced by *P. tritici-repentis* in field or axenic straw. Therefore, only the analysis for large-ascocarp formation is presented.

Experimental design and data analysis. The influence of the length of wetting periods on ascocarp formation of *P. tritici-repentis*, in the absence of antagonistic fungi, was examined by periodically sampling and counting ascocarps on field and axenic straw during each experiment. After several 48-h wetting periods or the equivalent for each wetting treatment, straw samples (axenic or field straw) were removed from the mist bench, and the number of ascocarps in each straw was counted. Each sampling contained four replicates for each wetting treatment, with two 2-cm axenic or six 2-cm field straws per replicate. The data presented are the number of ascocarps per straw averaged over four replicates and sampled during a total number of 14 or fewer 48-h wetting periods or the equivalent. The experiment was performed twice. Data sampled at the same number of 48-h wetting periods or the equivalent were analyzed as a randomized complete block design for two or more wetting treatments. The LSD value at $\alpha = 0.10$ was computed and used to compare the wetting treatments.

The influence of the length of wetting periods on ascocarp suppression by the test fungi was investigated by determining the number of large ascocarps produced in field or axenic straw at the end of the experiments. There were four fungal treatments

plus an uninoculated control and a bran control (sterile bran applied to straw) in each experiment, with four replicates for each treatment. Each replicate (pot) contained two 2-cm axenic or six 2-cm field straws. The experiment was performed twice. The two repeat runs of the experiment were analyzed separately as a split-plot design with wetting treatments as main plots and fungal treatments as subplots. The LSD value for subplot mean comparisons within each main plot was computed and used to compare fungal treatments. Data analysis was done separately for field and axenic straw.

RESULTS

Wetting-period duration and ascocarp formation. In all wetting-period treatments, *P. tritici-repentis* formed ascocarps in field straw. The development of ascocarps appeared to be slower in the 6-h wetting treatment than in the other wetting treatments in the early stage of the experiments. No appreciable number of large ascocarps developed in field straw until approximately 192 cumulative hours (equivalent to four 48-h wetting periods) of the shorter (6-h) wetting treatment (Fig. 1A and B). A significant difference in the number of ascocarps was found among some of the wetting treatments in the latter stage of the experiments. The number of ascocarps in field straw sampled at the fourth and tenth 48-h wetting periods was significantly smaller ($\alpha = 0.10$) in the 6-h treatment than in the 24-h treatment in the second experiment (Fig. 1B). The number of ascocarps in the 6-h treatment also was lower than that in the 48-h treatment at the tenth wetting period (Fig. 1B). Similarly, ascocarp formation in the 12-h treatment was significantly lower ($\alpha = 0.10$) than that in the 24-h treatment in both experiments after up to 12 48-h wetting periods (Fig. 1A and B). The maximum number of large (>300 μm) ascocarps produced per straw was 8, 8, 17, and 14 (averaged over the two experiments) for 6-, 12-, 24-, and 48-h wetting treatments, respectively (Fig. 1A and B).

Ascocarp formation in axenically prepared straw is shown in Figure 2. In axenic straw, *P. tritici-repentis* produced ascocarps under all wetting treatments. There was no difference in the number of ascocarps among all three wetting treatments during the early stage of the two experiments (Fig. 2A and B), although the number of large ascocarps appeared to be lower in the 6-h wetting treatment than in the other wetting treatments. Ascocarp formation in the 6-h wetting treatment was significantly lower ($\alpha = 0.10$) than that in the 48-h treatment of the first experiment (Fig. 2A) and that in the 12- and 24-h treatments of the second experiment (Fig. 2B) at the eighth wetting period. At the end of the first experiment, the number of ascocarps in the 12-h wetting treatment was higher than that of the 24-h treatment (Fig. 2A). The greatest number of large (>300 μm) ascocarps produced in the straw for 6-, 12-, 24-, and 48-h wetting treatments was 35, 48, 40, and 39 ascocarps per 2-cm axenic straw (averaged over the two experiments), respectively.

Wetting-period duration and ascocarp suppression by antagonistic fungi. The overall analysis of the two experiments showed that the influence of fungal treatment on ascocarp formation in field straw (averaged across all wetting-treatment levels) was significant. Reduced ascocarp formation was observed in field straw treated with bran inoculum of *L. arvalis* or *Limonomyces roseipellis* in both experiments, whereas this was only true in one experiment for Sterile II-treated straw. In one of the experiments, addition of sterile bran increased ascocarp formation in field straw. In field straw, interactions of fungal and wetting treatments had a significant effect on ascocarp formation, indicated by the occurrence of ascocarp suppression only in long-period wetting treatments. In field straw (Table 1), the number of large (>300 μm) ascocarps produced by *P. tritici-repentis* was

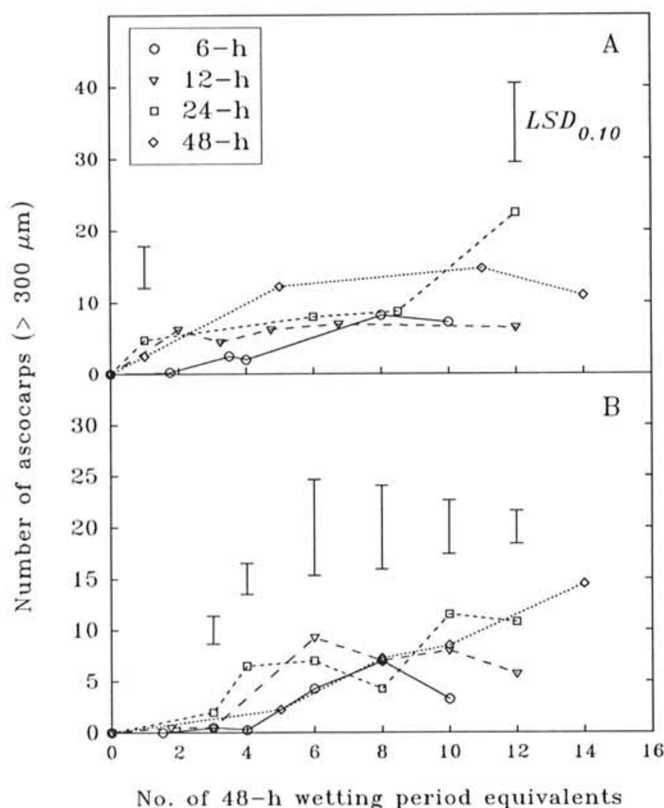

Fig. 1. Effect of wetting-period duration on ascocarp formation by *Pyrenophora tritici-repentis* in naturally infested field straw. The number of large (>300 μm) ascocarps per 2-cm field straw in 6-, 12-, 24-, and 48-h wetting treatments for the **A,** first and **B,** second experiments were measured. Data from two or more wetting treatments at the same 48-h wetting-period equivalent were used to calculate each LSD value at the 0.10 level of probability.

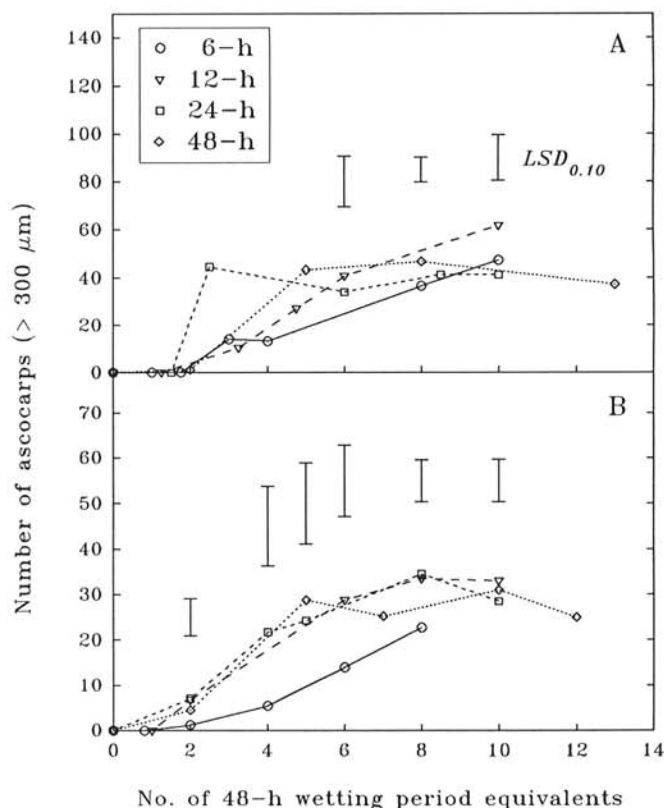

Fig. 2. Effect of wetting-period duration on ascocarp formation by *Pyrenophora tritici-repentis* in axenically infested straw. The number of large (>300 μm) ascocarps per 2-cm field straw in 6-, 12-, 24-, and 48-h wetting treatments for the **A,** first and **B,** second experiments were measured. Data from two or more wetting treatments at the same 48-h wetting-period equivalent were used to calculate each LSD value at the 0.10 level of probability.

significantly reduced by *L. arvalis* and *Limonomyces roseipellis* under 24- and 48-h wetting periods as compared to the uninoculated control. The number of ascocarps also was reduced by Sterile II in field straw under the 24- and 48-h wetting treatments; however, the reduction in the second experiment was not statistically significant. *E. nigrum*, which was not known to be antagonistic to *P. tritici-repentis*, reduced the number of large ascocarps in field straw under 24- and 48-h wetting periods in one of the two experiments but increased ascocarp production under the 12-h treatment. None of the test fungi significantly reduced ascocarp formation under 6- and 12-h wetting periods. Under the 48-h wetting treatment, field straw treated with sterile bran had increased ascocarp formation as compared to the uninoculated control in the first experiment.

In axenic straw, although fungal treatments also significantly affected ascocarp formation, the effect of the test fungi on suppression of ascocarp formation was much less compared to that in field straw. Across all wetting-treatment levels, the only fungal treatment that reduced ascocarp formation in axenic straw was *L. arvalis*, whereas *Limonomyces roseipellis* and Sterile II had little effect on ascocarp suppression or even increased ascocarp formation. In both experiments, *E. nigrum* and sterile bran increased the number of ascocarps in axenic straw. Significant effects of interactions between fungal and wetting treatments on ascocarp suppression also were observed in axenic straw. Under the 12- and 24-h wetting treatments, *L. arvalis* significantly reduced the number of large ascocarps in the first experiment (Table 2). The number of ascocarps (averaged over the two experiments) in straw inoculated with *L. arvalis* in the 48-h treatment also was lower, only 38% of that in the uninoculated control. *Limonomyces roseipellis* showed no inhibition of ascocarp formation under all wetting treatments. In axenic straw inoculated with Sterile II, reduced ascocarp formation was found in the 48-h wetting treatment in one experiment, whereas in the other experiment the fungal treatment increased ascocarp formation. Increased ascocarp formation due to inoculation of Sterile II also was observed under 24-h wetting periods. Under all wetting treatments, sterile bran significantly increased the number of large ascocarps in axenic straw as compared to the uninoculated control in at least one of the two experiments. The number of ascocarps in axenic straw inoculated with *E. nigrum* also was significantly higher than that of the uninoculated control under 12-, 24-, and 48-h wetting periods in at least one of the two experiments.

TABLE 1. Effect of length of wetting periods on suppression of ascocarp formation of *Pyrenophora tritici-repentis* by antagonists in naturally infested field straw

	Number of ascocarps[a]							
	6 h[b]		12 h		24 h		48 h	
Antagonist	I[c]	II	I	II	I	II	I	II
Control								
Untreated	7	4	6	6	22	11	11	15
Sterile bran	3	3	12	10	26	6	23**	9
Epicoccum nigrum	4	3	11	13**	11**	14	12	6**
Laetisaria arvalis	7	4	4	2	1***	3**	3*	5***
Sterile II	5	4	5	8	7***	7	2*	7**
Limonomyces roseipellis	4	4	4	1	5***	2**	6	1***

[a]Each value is an average number of large (>300 μm) ascocarps per straw in four replications, with six 2-cm field straws in each replicate.
[b]Each wetting period was followed by a drying period of at least 24 h. Experiments were terminated after eight or more 48-h wettings or the equivalent total wetting hours for shorter wetting periods.
[c]Data were analyzed as a split-plot design with wetting treatments as main plots and fungal treatments as subplots. Experiments I and II were data for repeat runs of the experiment and were analyzed separately. Values in the same column followed by *, **, or *** are significantly different from the respective uninoculated control at the 0.10, 0.05, and 0.01 levels of probability, respectively.

DISCUSSION

P. tritici-repentis was capable of producing ascocarps under long as well as short cycles of straw moisture in both axenically prepared straw and naturally infested field straw. The number of ascocarps formed was affected by the duration of wetting periods because interruption of microbial growth due to substrate desiccation was more frequent in short-period wetting treatments than in long-period wetting treatments. The growth and development of *P. tritici-repentis* in straw would be less sustained in short-period wetting treatments than in long-period wetting treatments. This assumption was supported by the lower numbers of ascocarps observed in several samplings of field and axenic straw under the 6-h wetting treatment compared to those under longer wetting treatments (Fig. 1A and B). In the present study, whether ascocarp formation in the 6-h wetting treatment would eventually reach the same level as the longer wetting treatments (given enough wetting periods of intermittent misting) could not be determined because of the limited number of wetting periods applied. However, the number of ascocarps in the 6-h treatment of uninoculated axenic straw after eight or 10 48-h wetting periods or the equivalent was close to or even numerically higher than that of the longer wetting treatments.

In long-period (24 and 48 h) wetting treatments, all three antagonistic fungi reduced ascocarp formation (compared with untreated check straw) in field straw. None of the three antagonists known to be suppressive to *P. tritici-repentis* (10,12) reduced ascocarp production in the 6- and 12-h wetting treatments (Tables 1 and 2). Thus, moisture clearly had direct or indirect effects on ascocarp production as demonstrated by the enhanced ascocarp suppression by antagonistic fungi in the long-period wetting treatments.

TABLE 2. Effect of length of wetting periods on suppression of ascocarp formation of *Pyrenophora tritici-repentis* by antagonists in axenically prepared straw

	Number of ascocarps[a]							
	6 h[b]		12 h		24 h		48 h	
Antagonist	I[c]	II	I	II	I	II	I	II
Control								
Untreated	47	22	61	33	41	28	38	25
Sterile bran	39	59***	82	106***	90***	149***	82***	136***
Epicoccum nigrum	31	37	86*	72***	63	75***	61	134***
Laetisaria arvalis	42	43	7***	15	17*	22	18	6
Sterile II	30	40	49	45	65*	65***	11*	50*
Limonomyces roseipellis	31	38	65	42	52	33	45	37

[a]Each value is an average number of large (>300 μm) ascocarps per straw in four replications, with two 2-cm axenic straws in each replicate.
[b]Each wetting period was followed by a drying period of at least 24 h. Experiments were terminated after eight or more 48-h wetting periods or the equivalent total wetting hours for shorter wetting periods.
[c]Data were analyzed as a split-plot design with wetting treatments as main plots and fungal treatments as subplots. Experiments I and II were data for repeat runs of the experiment and were analyzed separately. Values in the same column followed by *, **, or *** are significantly different from the respective uninoculated control at the 0.10, 0.05, and 0.01 levels of probability, respectively.

The "relative competitive advantage" of one organism over another, a concept introduced by Baker and Cook (2), is determined by their respective growth rates at one water potential compared with their respective growth rates at another potential. Studies (3,4,7) of *Fusarium roseum* and certain species of *Aspergillus* and *Penicillium* interacting with other fungi have shown them to be more competitive under conditions closer to the limits of their water-activity tolerance than at the optimum level for their growth, because inhibitory fungi are unable to grow well at low water potentials. This theory can be used to interpret the effect of wetting-period duration on ascocarp suppression by the selected antagonistic fungi. The growth of *P. tritici-repentis* and the test fungi depend on the number of wetting hours within which they can grow, which in this study was affected by their ability to grow under water stress. *P. tritici-repentis*, ecologically adapted to a drier habitat than the other fungi used in this study (12), is capable of growing at a water potential as low as −8.5 MPa (11), whereas the growth of the antagonists is greatly reduced or terminated at a much higher water potential (−0.5 MPa) (12). Thus, if the fungi are exposed to periods of intermediate water potential, *P. tritici-repentis* will have a competitive advantage. Conditions of intermediate water potential occur during initial wetting and during drying of the straw, i.e., before full moisture is achieved and before straw is fully dried during the wet/dry cycle. In short-period wetness treatments, these intermediate moisture conditions represent a larger proportion of cumulative wetness hours than in the long-period wetness treatments (e.g., in 96 h of cumulative wetness, there are only two cycles [and thus four occurrences of intermediate moisture] for the 48-h treatment but 16 cycles [32 occurrences of intermediate moisture] for the 6-h treatment). Therefore, under short wetting-cycle treatments, *P. tritici-repentis* should experience a competitive advantage over the sensitive antagonistic fungi, and the long-period wetting-treatment conditions should favor the antagonists. In this study, the growth of the test fungi in wheat straw was not determined. Therefore, direct evidence is needed to prove that the failure of these test fungi to suppress ascocarp formation under short wetting-duration moisture conditions was due to their limited growth.

Much higher ascocarp production was observed in axenic straw than in field straw (Figs. 1 and 2) and could be attributed to several nutritional and biological factors. Axenic straw was prepared by recolonizing autoclaved greenhouse-grown wheat straw by *P. tritici-repentis*. Thus, the axenic straw constitutes a substrate with a relatively noncompetitive environment and probably a higher level of nutrients (because greenhouse-grown plants were not leached or colonized) than field straw. Initial colonization of axenic straw by *P. tritici-repentis* could also be much more extensive than that of naturally infested field straw, allowing the pathogen to sequester a higher level of nutrients in its thallus before the straw is exposed to conditions that permit growth (and competition) by other fungi. Therefore, the difference in ascocarp formation between the two types of straw was probably partially due to the difference in the amount of initial colonization by the pathogen and also could be affected by the nutritional conditions of the straw and the abundance of other microorganisms. The importance of nutrition and its possible interaction with indigenous microorganisms was demonstrated by the dramatic increases in ascocarp formation in axenic straw after addition of sterile bran and a similar level of ascocarp formation in field straw between sterile-bran treatment and the uninoculated control. The nutrition and microbial factors as well as the possible effect of interrupted growth of *P. tritici-repentis* under short wetting periods also could be explanations for the increased ascocarp formation in axenic straw treated with sterile bran or some of the test fungi under long-period wetting treatments compared to that under short-period wetting treatments.

Suppression of ascocarp formation by selected antagonists in axenic straw was much less than in field straw. *L. arvalis* was consistently antagonistic to *P. tritici-repentis* in both types of straw (Tables 1 and 2). In axenic straw, although ascocarp formation in straw treated with *L. arvalis* was only 38% (average of the two experiments) of that in the uninoculated control in the 48-h wetting treatment, the decrease in the number of ascocarps was not statistically significant because of the variability among individual straws. Both Sterile II and *Limonomyces roseipellis* were ineffective against *P. tritici-repentis* in axenic straw. *Limonomyces roseipellis* did not inhibit ascocarp formation in axenic straw in any of the wetting treatments (Table 2), although it effectively reduced the number of ascocarps in field straw (Table 1). A similar result was obtained in an earlier study (18). Interestingly, *E. nigrum*, not known to be antagonistic to *P. tritici-repentis*, reduced the number of ascocarps produced in field straw in one of the experiments in both 24- and 48-h wetting treatments. Involvement of nonspecific competition for nutrients by *E. nigrum* is a plausible explanation. Because of results reported here, researchers should be aware of differences in performance of antagonists between field and axenic straw.

Extrapolation of results from this greenhouse mist bench test to a natural setting should be made with care. The test fungi used in this study were only a small sample of the microorganisms that occur in crop residue. The inoculation procedure allowed us to apply a single fungus to one straw. In the field, however, displacement of *P. tritici-repentis* in residue involves possible interactions of the whole microbial community, and the duration of straw wetness required for significant ascocarp reduction may not be the same as that in the greenhouse. Nevertheless, the results from this study support our finding from an earlier field study (19) in which the number of straw-wetness hours occurring in wetting periods longer than 12-h was negatively correlated with ascocarp production in wheat residue in the field. The present greenhouse study clearly demonstrated that ascocarp production in wheat straw is directly and indirectly affected by straw-wetness duration through induced antagonism by antagonistic fungi during prolonged periods of high-moisture conditions. Under field conditions, the reduced ascocarp formation in wheat straw in close contact with soil (14) is associated with microbial succession from a microbial community dominated by primary colonizers early in the season to another one composed mainly of secondary colonizers. The change in microbial composition in wheat residue can be enhanced by prolonged straw-moisture conditions that favor the growth of many microorganisms and could result in more intensive microbial interactions detrimental to *P. tritici-repentis*. Therefore, any cultural practices (i.e., irrigation and modified tillage) that prolong high-moisture conditions in wheat residue in the field will ultimately enhance this natural biocontrol process. However, the total number of long-period wetness hours required for suppression of ascocarp formation and possible interactions between straw wetness and other environmental factors were not examined in this study. Therefore, more studies are needed to understand fully the manner in which straw-wetness duration mediates microbial effects on ascocarp production by *P. tritici-repentis* in wheat residue.

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