

Suppression of Ascocarp Formation in *Pyrenophora tritici-repentis* by *Limonomyces roseipellis*, a Basidiomycete from Reduced-Tillage Wheat Straw

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ABSTRACT

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A fast-growing basidiomycete, previously isolated from microbial communities of reduced-tillage wheat straw in which *Pyrenophora tritici-repentis* appeared to be declining, was tested in the laboratory for its ability to suppress sexual reproduction of *Pyrenophora*. Nonsterile straw pieces, which had been colonized by *P. tritici-repentis* during parasitic growth, were inoculated with the basidiomycete and incubated under various conditions of temperature and moisture. The basidiomycete reduced ascocarp and ascospore production significantly. The degree of suppression varied from 50–99%, depending on test conditions, but was

especially effective in straw sheath tissue (vs. culm tissue) that was wetted daily and incubated under warm, low-humidity conditions. The basidiomycete was identified as *Limonomyces roseipellis*, which causes pink patch, a mild disease of turfgrass. The mechanism of antagonism is unknown but may involve mycoparasitism, as this fungus has chitinolytic ability. Although competition for nutrients also may be involved, this does not appear to be the sole mechanism, because *Trichoderma koningii*, an aggressive colonist, did not significantly suppress sexual reproduction of *P. tritici-repentis* under any conditions tested.

Conservation tillage, in which crop residues are left on the soil surface between cropping seasons to reduce soil and water loss, is becoming increasingly common. However, such reduced tillage can increase the incidence of certain plant diseases caused by pathogens that survive well in surface-borne crop residues (1). In Kansas and several other Great Plains states, the most important residue-associated pathogen of wheat is *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph: *Drechslera tritici-repentis* (Died.) Shoem.), which causes the foliar disease tan spot (4).

In Kansas, where winter wheat (*Triticum aestivum* L. em Thell) is grown, the period between crops is from July through September. During this time, *P. tritici-repentis* begins to produce ascocarps on infested straw. Additional ascocarps are formed on the straw after the next wheat crop is planted in the late fall. The ascospores, which require a cold period for maturation, are released in early spring and initiate infection on wheat as it emerges from winter dormancy. Secondary infection cycles are caused by the *Drechslera* stage of the fungus.

Because tan spot is clearly associated with residue-borne inoculum, one reasonable approach toward control of this disease is biological control of the pathogen in its survival stage, during which ascocarps are formed and ascospores mature. This approach would require selection of one or more antagonists capable of displacing the pathogen from plant tissue it had already colonized during the parasitic phase of the disease cycle. In a previous study of straw-colonizing microorganisms (7), we noted that if straw remains above the soil surface (within a mulch layer), *P. tritici-repentis* survives well in association with a number of other parasites and primary saprophytes, whereas in soil-borne straw it is displaced by a community composed of actinomycetes and soil-borne fungi. Between these extremes of microbial community types are several in which *P. tritici-repentis* is present at much reduced levels in the presence of secondary saprophytes. These latter organisms were considered to be candidates for biocontrol, as they were capable of colonizing surface-borne residue and were possibly active in displacing *P. tritici-repentis* from the straw. One particular fungus, designated "unidentified basidiomycete" in the earlier study (7), was of interest, because it was one of the earliest secondary saprophytes to invade the surface-borne straw and it displayed chitinolytic activity in vitro. In addition, it occasionally

was isolated from ascocarps of *P. tritici-repentis* collected in the field (W. F. Pfender, unpublished).

In the present study, the antagonistic ability of this basidiomycete was tested in the laboratory. Because antagonism tests conducted on agar plates may be poor predictors of interactions in the more complex natural situation, a test was developed to assess the interaction in nonsterile, natural substrate. We tested the ability of the basidiomycete to suppress ascocarp formation of *P. tritici-repentis* in the laboratory by using nonsterile straw precolonized during normal parasitic growth of the pathogen.

MATERIALS AND METHODS

Fungal cultures. Isolate 6R180 of *P. tritici-repentis* was obtained from infected wheat plants in Kansas. It was maintained on half-strength V-8 agar (100 ml of clarified V-8 juice per liter) (12) at 4 C and renewed at 6-mo intervals by obtaining reisolates from greenhouse-grown plants inoculated with the culture. The antagonistic basidiomycete (later identified as *Limonomyces roseipellis* Stalpers & Loerakker) was isolated from minimum-till wheat straw in Kansas (7). Two isolates, designated 3T163 and 6T207, were used. The activity of the basidiomycete was compared with that of *Trichoderma*, a fungus that repeatedly has been demonstrated to have biological control capabilities (6). For this purpose, an isolate of *T. koningii* Rifai obtained from soil-borne wheat straw was used. These fungi also were maintained on half-strength V-8 juice agar at 4 C.

Test material. Antagonism tests were conducted with nonsterile straw infested with *Pyrenophora*. Winter wheat (cv. TAM 105) was vernalized and grown to maturity in the greenhouse. At flowering, the plants were inoculated with a spore suspension (about 2,500 conidia per milliliter) of *D. tritici-repentis*, placed in a mist chamber under 14-hr daylength at about 24 C for 48 hr (9), and then returned to the greenhouse for disease development. Six wk later, after plants had senesced and dried, the infested straw was harvested, and the top two internodes were selected and cut into 1.5-cm pieces.

For antagonism tests, the sheath was removed and discarded from some pieces before use; those pieces were designated "culm." Other pieces, designated "combination," were used intact, that is, with the sheath enveloping the culm.

Inoculum of *L. roseipellis* or *T. koningii* consisted of small bits

of culm tissue (2 mm × 2 mm) that were autoclaved and then placed on agar-plate cultures of the respective fungus. These straw bits were colonized for 3 days (*L. roseipellis*) or 1 day (*T. koningii*) before removing them for use as inoculum.

Test conditions. The dry pieces of straw infested with *Pyrenophora* (culm or combination) were soaked in water for 30 min, then placed in petri dishes on autoclaved, moistened vermiculite (60 cc of vermiculite per 100 mm × 20 mm dish). Three culm and three combination straw pieces were placed randomly in each dish. In check treatments, straws infested with *Pyrenophora* were not inoculated with an antagonist. For inoculated treatments, two inoculum bits of either *L. roseipellis* or *T. koningii* were placed on the straw piece, one at either end. For *L. roseipellis*, inoculum of isolate 3T163 was placed at one end, 6T207 at the other. All six straws in each dish were inoculated with either *L. roseipellis* or *T. koningii*, or were uninoculated; thus, each dish contained culm and combination pieces exposed to a single inoculation treatment.

Dishes were incubated under 12-hr daylength (cool-white fluorescent tubes 20 cm above dishes) in three different environments. Some were placed on the laboratory bench at room temperature (about 26 C) and 50 ± 10% relative humidity (RH). Others were placed in an incubator at 16 C and 93 ± 2% RH, or in an incubator at 6 C and 95 ± 2% RH. There were two different moisture treatments, designated moist and wet/moist, applied to the dishes in each environment. In the moist treatment, dishes were left uncovered at all times and watered when the vermiculite became dry. In the wet/moist treatment, dishes were covered every night to permit condensation on the straw and uncovered every day. These dishes also were watered when the vermiculite became dry. The material at 26 C dried more quickly and thereby experienced more rapid wetting/drying cycles than that at 16 C. The slowest drying occurred at 6 C. Thus, a number of treatments differing in temperature as well as moisture cycles was used. Measurement of straw water potential by means of a thermocouple psychrometer (Decagon, Pullman, WA) indicated that straws were at about -5 to -10 bars when wettest, although in the moist treatments only the lower surface of the straw pieces achieved such a high water potential. Indeed, in the moist treatments at 26 C, upper surfaces of straws rarely got above -50 bars. In moist treatments, straws dried to about -250 to -500 bars before being watered. In wet/moist treatments, straws at 26 C also became very dry before being watered, but those at 6 and 16 C often fell only to about -20 bars on their lower surfaces before being watered.

Previous work with several isolates of *Trichoderma* spp. showed they grew very poorly at low temperatures (7). Therefore, *T. koningii* was not included as a treatment at 6 C, as it could not be expected to interact with *P. tritici-repentis* under conditions that precluded its growth.

After 4 wk under the above conditions to permit ascocarp development, all dishes were moved to the 16 C incubator for an additional 4 wk to stimulate ascospore production, which requires cool temperatures (5).

Experimental design. A split-split-plot design was used for the experiment. The whole plots were the incubators (or laboratory bench), and whole-plot treatments were the temperature/relative humidity environments. Each whole plot occurred in two trials, and the trials were two separate times at which the experiment was performed. The subplots were the petri dishes containing straw pieces, and subplot treatments were factorial combinations of inoculum (uninoculated, *L. roseipellis*, or *T. koningii*) and water application (moist or wet/moist), e.g., "uninoculated moist." In the 6 C/95% whole plots, there were no subplots containing *T. koningii* treatments. The subplots were replicated two times (two dishes per inoculum × water treatment at each environment) in the first trial, and three times in the second trial. Subsubplots were straw pieces, and the subsubplot treatments were culm or combination (sheath + culm). There were three repeated measures of subsubplot treatment per dish (i.e., three culm pieces and three combination pieces per dish); data from each set of three straw pieces were averaged to provide the datum for each replicate dish subsubplot.

Data collection and analysis. At the end of the 8-wk test period,

each straw piece was examined with a stereomicroscope to determine the number and size of ascocarps. A sample of ascocarps (5-10) from each of two size classes ($\leq 200 \mu\text{m}$ $\geq 300 \mu\text{m}$) was taken from each piece and examined with a compound microscope at 100×. A previous study (8) showed that ascocarps smaller than 200 μm contain few, if any, ascospores. The proportion of ascocarps with ascospores, and the number of ascospore-bearing asci per ascocarp, was determined. The number of ascospores produced on a straw piece was estimated from this information.

The data were examined by analysis of variance (ANOVA). To test the effect of *L. roseipellis* on ascocarp and ascospore production by *P. tritici-repentis*, the split-split-plot analysis was performed (Table 1) after deleting data from *T. koningii* treatments. Because there were significant interaction terms for tissue type and several other effects, and for ease of interpreting comparisons among uninoculated, *L. roseipellis*, and *T. koningii*, the data were next separated into individual data sets for each environment × tissue type (Tables 2 and 3). One-way ANOVAs were performed on these data, with trials described as above, and treatments were the inoculum × water combinations.

Identification of the basidiomycete. This fungus was compared with cultures of known basidiomycetes of similar appearance, viz., *Limonomyces* and *Laetisaria*. Colony morphology, number of nuclei per cell, and pathogenicity characteristics were compared. To stain the nuclei, the fungi were first grown onto the bare surface of a plastic petri dish from an agar plug. Hyphae were pretreated with 95% ethanol, which was allowed to nearly dry before staining with a mithramycin solution. The solution, prepared according to Franklin et al (3), contained 62 $\mu\text{g/ml}$ of mithramycin (Sigma Co., St. Louis, MO) in 15 mM MgCl_2 at pH 6.8, and was diluted 3:1 (v:v) with 95% ethanol. Nuclei were counted after 30 min by viewing with epifluorescence microscopy (Zeiss microscope fitted with a 450-550 BP excitation filter and LP 520 barrier filter).

Pathogenicity tests were conducted on wheat and perennial ryegrass (*Lolium perenne* L.) in the greenhouse. Plants were grown in pots (10-cm diameter) of steamed soil (1:1:1 loam:sand:peat). Fungi were grown for 1 wk on an autoclaved 1:1 mixture of wheat bran and fescue seed, then air-dried (N. R. O'Neill, personal communication). Approximately 5 cc (1.3 g) of this inoculum was sprinkled on the soil surface of newly-seeded pots or onto the turf of older plantings. Freshly seeded wheat (cv. TAM 105) was

TABLE 1. Analysis of variance for split-split plot experiment comparing *Pyrenophora tritici-repentis* reproduction on uninoculated straws and straws inoculated with *Limonomyces roseipellis*

Source	df	Mean squares for		
		Total ascocarps	Large ascocarps ^a	Ascospores ($\times 10^3$)
Trial	1	6,369** ^b	4,301**	1,515
Environment (E)	2	1,804*	1,508**	2,568
Whole-plot error	2	41	3	486
Inoculum (I)	1	12,212**	13,704**	7,936**
Water (W)	1	51	189	1,950
W × I	1	248	482	1,502
W × E	2	4	4	1,056
E × I	2	80	176	3,138
E × W × I	2	11	7	862
Subplot error	9	264	279	523
Tissue type (T)	1	12,266**	9,675**	3,873**
T × E	2	714*	641*	1,962**
T × W	1	40	10	692
T × I	1	4,703**	4,627**	3,069**
T × W × I	1	21	23	540
T × W × E	2	117	164	218
T × I × E	2	172	174	737*
T × W × I × E	2	3	2	162
Residual error	84	204	188	217
	119			

^a Ascocarps > 300 μm in diameter.

^b Mean squares significant at $P = 0.01$ (**) or $P = 0.05$ (*).

inoculated 3, 5, or 14 days after planting. A clipped turf of perennial ryegrass (cv. York) was inoculated at 8 wk of age. After inoculation, the pots were placed in a greenhouse at about 24 C on a mist bench receiving 10 sec of mist every 10 min. There were two pots per treatment (inoculum × host age or species) arranged as a completely randomized design. Plants were examined periodically during 3 wk for visible evidence of pathogenicity to leaves. The experiment was repeated once.

RESULTS

Identification of the basidiomycete. The antagonistic fungus was initially examined by E. B. Dorworth (USDA Forest Products Lab, Madison, WI), who determined that it belongs to a group of pink basidiomycetes that includes species of *Laeticorticium*, *Limonomyces*, and *Laetisaria*. These fungi are differentiated on the basis of clamp connections, number of nuclei in vegetative cells, growth rate, and pathogenicity (11). The antagonist most closely resembles *Limonomyces roseipellis* Stalpers & Loerakker in that it has clamp connections at most septa, binucleate vegetative cells, and a fast growth rate. *L. roseipellis* is a pathogen of perennial ryegrass, and, in greenhouse tests, the isolates from straw showed the same degree of aggressiveness on ryegrass as did several known *L. roseipellis* isolates (supplied by E. B. Dorworth and N. R. O'Neill). Isolate 3T163 was confirmed by the Centraalbureau voor Schimmelcultures (Baarn, the Netherlands) to be *L. roseipellis*; 6T207 could not be identified definitively, but closely resembled *L. roseipellis*.

Antagonism tests. The split-split-plot analysis of data for *L. roseipellis* and uninoculated check (Table 1) showed that the main effect of environment (temperature/RH) was significant for ascocarp production, but not for ascospore development. Also, inoculation with *L. roseipellis* significantly reduced ascocarp and ascospore production, but water treatment and its interactions with inoculation and environment were not significant. The tissue type (culm or combination) had a significant effect on reproduction by *P. tritici-repentis*, i.e., more ascocarps were produced on combination pieces (almost exclusively in the sheath tissue) than on culm pieces, but there was a significant interaction

effect of tissue type with inoculum treatment. Because there were several interaction terms that were significant, and to include comparisons of *T. koningii* with *L. roseipellis* as inoculants, one-way analyses (Tables 2 and 3) were performed as described above.

The respective check and *T. koningii* treatments were similar in all cases but one: within a given temperature × moisture treatment, *T. koningii* did not significantly suppress ascocarp or ascospore formation by *P. tritici-repentis* (Tables 2 and 3). Although the 26 C/50% RH and 16 C/93% RH environments generally were similar in their effects on ascocarp production in check treatments and treatments with *Trichoderma*, production of spores was clearly reduced in the 26 C/50% RH environment. As indicated previously, the straws in this environment were not only warmer, but also experienced the greatest and most frequent moisture fluctuations.

In all treatments, the number of ascocarps produced in the presence of *L. roseipellis* was less than that in the respective check treatments and treatments with *T. koningii*; the reduction was statistically significant for all wet/moist treatments (straw wetted daily) (Table 2). In the moist treatments, ascocarp production was significantly reduced for combination tissue types at 16 and 26 C, but not at 6 C, nor for any temperature in culm tissue. In most cases, ascocarps produced on straws inoculated with *L. roseipellis* were observed in the central part of the straw, i.e., farthest from the inoculation points at the ends (Fig. 1).

Ascospore production (Table 3) followed the general pattern of ascocarp production, but some of the treatment differences were accentuated by reduced fertility of ascocarps in certain treatments. Fertility of ascocarps was particularly reduced in drier treatments (moist vs. wet/moist). There was very low ascospore production in the 26 C treatments, which were drier than the other treatments. As with ascocarp numbers, *L. roseipellis* caused a significant suppression of ascospore production in wet conditions and, in some cases, also in moist treatments. Ascocarps that formed in the presence of the basidiomycete were usually somewhat smaller (Fig. 1) and contained fewer fertile asci than those formed on uninoculated straw or straw inoculated with *T. koningii* (data not shown). Overall, the basidiomycete reduced total spore production more than ascocarp numbers; mean spore production in the

TABLE 2. Number of ascocarps (> 300 μm) of *Pyrenophora tritici-repentis* produced on straw culms or combinations (sheath + culm) inoculated with *Limonomyces roseipellis*, *Trichoderma koningii*, or uninoculated, then incubated under various environmental conditions

Inoculation	Moisture	Incubation environment					
		6 C, 95% RH		16 C, 93% RH		26 C, 50% RH	
		Culm	Combination	Culm	Combination	Culm	Combination
Uninoculated	Wet/moist	20 a ^x	53 a	16 ab	57 a	13 a	36 a
Uninoculated	Moist	11 ab	49 a	11 abc	43 a	9 ab	27 a
<i>T. koningii</i>	Wet/moist	ND ^y	ND	21 a	64 a	13 a	24 a
<i>T. koningii</i>	Moist	ND	ND	6 bc	41 a	7 ab	18 ab
<i>L. roseipellis</i>	Wet/moist	7 b	17 b	0.2 c	5 b	0 b	0 b
<i>L. roseipellis</i>	Moist	5 b	26 ab	5 bc	3 b	2 b	0.1 b

^x Within each column, values followed by the same letter do not differ ($P = 0.05$) as determined by Duncan's multiple-range test.

^y ND = not determined.

TABLE 3. Number of ascospores ($\times 10^3$) of *Pyrenophora tritici-repentis* produced on straw culms or combinations (sheath + culm) inoculated with *Limonomyces roseipellis*, *Trichoderma koningii*, or uninoculated, then incubated under various environmental conditions

Inoculation	Moisture	Incubation environment					
		6 C, 95% RH		16 C, 93% RH		26 C, 50% RH	
		Culm	Combination	Culm	Combination	Culm	Combination
Uninoculated	Wet/moist	6.5 a ^x	20.3 a	5.7 a	20.7 a	0.5 a	0.7 a
Uninoculated	Moist	2.3 b	9.8 b	1.4 b	5.6 b	0.6 a	0.5 abc
<i>T. koningii</i>	Wet/moist	ND ^y	ND	9.2 a	22.4 a	0.6 a	0.1 bc
<i>T. koningii</i>	Moist	ND	ND	0.6 b	2.3 b	0.6 a	0.6 ab
<i>L. roseipellis</i>	Wet/moist	1.3 b	2.6 b	0.4 b	0.7 b	0.0 b	0.0 c
<i>L. roseipellis</i>	Moist	0.4 b	1.6 b	0.7 b	0.2 b	0.1 b	0.0 c

^x Within each column, values followed by the same letter do not differ ($P = 0.05$) as determined by Duncan's multiple-range test.

^y ND = not determined.

presence of the basidiomycete was 12% of that in the check, whereas ascocarp production was 20% of that in the check.

Pathogenicity of *L. roseipellis* to wheat. The straw isolates of *L. roseipellis* and those from perennial ryegrass showed very weak pathogenicity to wheat, causing occasional infection of senescent leaves when inoculated plants were incubated under intermittent mist for several weeks. Even when wheat seedlings had to emerge through a mycelial mat of these fungi, no infection was noted on growing tissue.

DISCUSSION

Limonomyces roseipellis, a basidiomycete chosen from a community of secondary colonizers in wheat straw, suppressed formation of ascocarps and ascospores of *P. tritici-repentis* under a range of conditions imposed on nonsterile, infested straw in the laboratory. It is noteworthy that the challenge was successful even though *P. tritici-repentis* was firmly established as a primary colonist of straw tissue. Although suppression of ascocarp and ascospore formation occurred over a range of environmental conditions, the degree of suppression was affected by environment. Suppression usually was most effective in straw that was wet daily and in straw incubated at warm temperatures. It also was more effective in sheath tissue than in culm tissue. These observations indicate that differential effects of environment and substrate relationships on *P. tritici-repentis* and *L. roseipellis* may be an important force influencing the outcome of their interaction.

These tests were designed to assess the ability of a potential antagonist to invade a substrate already colonized by *P. tritici-repentis* and other organisms. This ability would be required of any biocontrol agent to be applied to postharvest straw for the control of *Pyrenophora*. *L. roseipellis* clearly was capable of acting as an aggressive secondary colonizer, as predicted from its position in the succession of fungi we previously observed on straw (7). It appeared early in the season in straw that directly contacted the soil (where survival of *Pyrenophora* was very poor); later in the season it was recovered in significant amounts from straws resting several centimeters above the soil surface (upon other straws), an environment where *P. tritici-repentis* appears to survive quite well. Thus, *L. roseipellis* is able to grow on straw in microenvironments where *P. tritici-repentis* typically produces its primary inoculum, but may normally get there too late in the season to suppress the pathogen. Biological control might result if *L. roseipellis* were

placed into this microenvironment earlier in the disease cycle of *P. tritici-repentis*. For such an approach to be effective, the antagonist would have to be applied quite thoroughly to the straw; in our laboratory tests, inhibition of ascocarp formation sometimes extended only about 5 mm from the point of inoculation with the antagonist. Field studies are in progress to test its efficacy when deliberately placed on the above-soil straw.

The mechanism by which *L. roseipellis* reduces ascocarp and ascospore production by *P. tritici-repentis* has not been investigated. A nonspecific effect due simply to aggressive growth through the substrate does not seem indicated, because *T. koningii*, itself an aggressive colonizer, did not significantly reduce ascocarp production. The involvement of some degree of nutrient competition by the basidiomycete is, however, suggested by the fact that ascocarps produced in its presence are smaller and contain fewer ascospores than those formed on unchallenged straw. In a study of nutritional effects on ascocarp production by *P. tritici-repentis* (8), ascocarps produced under conditions of limiting nitrogen were smaller and contained fewer spores than those formed under adequate nutritional conditions. There is also circumstantial evidence that mycoparasitism may be involved. The straw isolates of *L. roseipellis* produce chitinase (7), giving this antagonist the potential to directly attack fungal mycelia. Indeed, it was distinctive among the chitinolytic organisms we recovered from straw in being the earliest chitinolytic organism to appear in the succession on straw (7). Besides this indication of potential mycoparasitism, *L. roseipellis* has been observed to coil around hyphae of *P. tritici-repentis* in agar-plate cultures, and occasionally has been isolated from its ascocarps on straws collected in the field (W. F. Pfender, personal observations).

Because *L. roseipellis* is a turfgrass pathogen, its eventual use as a biocontrol agent is questionable. However, the pink patch disease it causes is not considered to be a major problem, because the pathogen spreads slowly, damages only a small number of leaves in the affected patch, and does not significantly discolor the turf or slow its growth (10). The disease generally is controlled by routine mowing. Further, it may be possible to select or create by mutagenesis a nonparasitic strain of this fungus. *L. roseipellis* is categorized by Smiley (10) as a weak facultative parasite. Indeed, its ability to colonize senescent grass leaves may be advantageous in allowing it to survive in the field and to interact with *P. tritici-repentis* early in its saprophytic phase on wheat.

Perhaps more important than possibilities and problems with the direct application of this fungus as a biocontrol agent is the demonstration that, through a descriptive study of fungal communities in straw (7), a potential antagonist to *P. tritici-repentis* could be chosen. This basidiomycete was selected for study because it appeared to be an aggressive secondary colonizer whose chitinolytic ability suggested an "interactive" life strategy, in the terminology of Frankland (2), i.e., a fungus that is adapted for growth in the presence of other active colonizers of a substrate. Its occurrence in communities from which *P. tritici-repentis* apparently is displaced, and its ability eventually to grow into habitats that are favorable to survival of *P. tritici-repentis*, also demonstrate its capability to occupy the required habitats for interaction with the pathogen. By choosing such an organism from the straw microbial community and studying its response to environmental variables, it may be possible to develop a combined approach to control of residue-borne plant pathogens whereby biocontrol agents are applied to residue that is managed (e.g., by modified tillage) to optimize the environment for the antagonist's activity.

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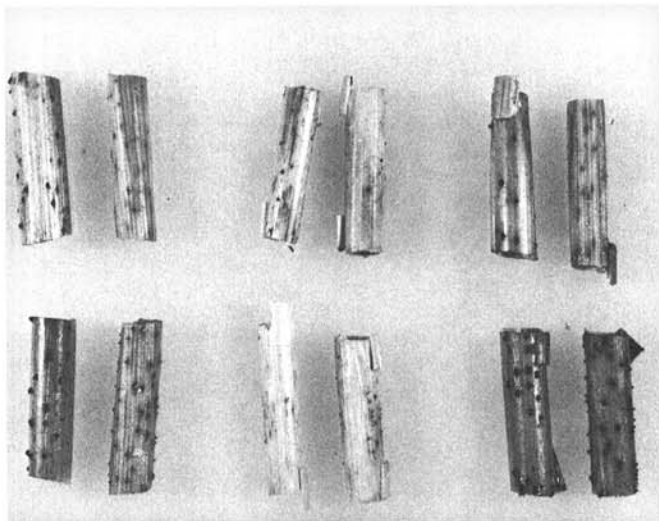


Fig. 1. Straw previously infected with *Pyrenophora tritici-repentis*, then incubated for 2 mo at 16 C/93% relative humidity. The straws are shown in pairs, with the culm piece to the left of the combination piece in each pair. Upper row, moist treatment; lower row, wet/moist treatment. Straw pairs inoculated with: check, left; *Limonomyces roseipellis*, center; *Trichoderma koningii*, right. Note that ascocarps present in the pieces inoculated with *Limonomyces* generally are smaller and are located at the center of the pieces (farthest from the inoculation points).

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