SELECTING BIOLOGICAL CONTROL AGENTS TO LIMIT THE SAPROPHYTIC ABILITY OF *PYRENOPHORA TRITICI-REPENTIS*

by

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INTRODUCTION

Tan spot, caused by the fungus Pyrenophora tritici-repentis (Died.) Drechs., is an important foliar pathogen of wheat and other Gramineae throughout the world (4,8,11,13,20). Yellow leaf spot, eyespot or yellow leaf blotch are also descriptive names for this disease, which produces a characteristic yellow halo around a brown necrotic center. Reported losses from severe tan spot epidemics range from 20 to 50% of the grain yield (15,18). Under Kansas conditions losses can be 25 to 30% of the yield (14). Since tan spot was first reported in Kansas in 1947, its severity has increased due to changing crop management practices and low levels of resistance found in cultivars grown in the state (14).

Pyrenophora tritici-repentis is carried over from year to year in the saprophytic stage of the life cycle residing on the culms and leaf sheaths of wheat straw. Pseudothecia initials form on the wheat straw during late summer to early winter and mature to form ascospores the following spring. When sufficient moisture is present, the ascospores are forcibly ejected onto the wheat leaves and produce lesions, thus serving as primary inoculum. As the lesions enlarge, conidia are produced in the necrotic areas and are airborne throughout the crop canopy causing secondary spread. Severity of the secondary infections

depends to a high degree on environmental conditions; temperature and leaf wetness being most important (6,10). Currently there are few acceptable tan spot control measures available to growers in Kansas (14). While sources of resistant germplasm have been identified (9,10,14,8), they are cultivars which are agronomically unacceptable under Kansas growing conditions. The costs involved and market price of wheat make fungicide use uneconomical in most years and other controls such as burning or incorporating wheat refuse into the soil are practiced less today because of fuel costs and soil erosion concerns. Crop rotation is also an effective control measure for tan spot, but there are few acceptable alternative crops for Kansas conditions. Another possibility is to develop a biological control system for tan spot (5,12).

Approximately nine months or 75% of the life cycle of P. tritici-repentis is spent in the saprophytic stage surviving in wheat refuse left on the soil surface. During this period of time the straw is exposed to an array of microorganisms which can reduce its ability to survive and/or reproduce on the straw. If the straw is buried or partially buried under the soil, microbes are able to act on the straw and reduce or eliminate P. tritici-repentis from the straw. Observations indicate that no pseudothecia are produced on buried straw. Thus, isolation of organisms from soil and plant refuse that are antagonistic to P. tritici-repentis, and enhancement of their populations on the surface straw could result in reduced numbers of pseudothecia and reduced primary inoculum. The work reported here involved isolations of microorganisms antagonistic to P.

tritici-repentis, development of laboratory and field techniques for testing antagonism, and quantifying fitness characters of P. tritici-repentis related to its ability to compete with antagonists.

MATERIALS AND METHODS

Isolation of antagonists. Microbes to be tested for their ability to inhibit P. tritici-repentis were isolated from a variety of sources. Samples from these sources were chopped or blended with water, diluted and plated out on one quarter strength potato dextrose agar (1/4 PDA) and beef lactose agar. Sources of potential antagonists included plant roots (12 seperate sources), soil dilutions (14 sources), decaying plant material (8 sources), wheat straw (4 sources), and P. tritici-repentis pseudothecia (20 sources). Colonies obtained on the isolation plates were selected based on unique visual characteristics to obtain as many different isolates as possible. Wheat residue collected from the field after harvest was the main source of fungus isolates. These were obtained by periodic isolations made from the residue kept in moist chambers over a ten month period, during which time the chambers dried and were remoistened numerous times. A total of 273 bacterial and 34 fungal isolates were collected and stored on agar slants for further study.

Inhibition on agar plates. The bacteria isolates were tested for their ability to inhibit P. tritici-repentis on both 1/4 PDA and nutrient agar plus yeast extract NBY. A 5-mm hyphal plug was taken from near the advancing edge of P. tritici-repentis mycelium growing on 1/4 PDA and placed in the center of a test plate. Three bacteria isolates were spotted on the agar at equal distances from the plug and each other

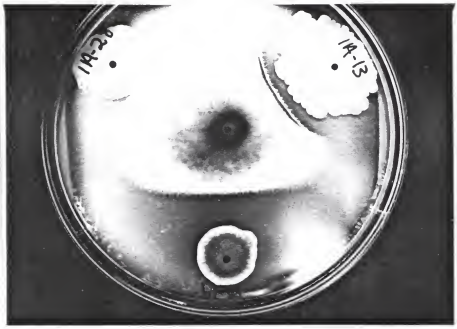

Fig. 1. Inhibition of Pyrenophora tritici-repentis mycelium by three bacterial isolates on agar media.

(Fig. 1). After six to seven days growth at room temperature, the inhibition zone between the isolate and the fungus colony was measured.

Straw bioassay. All of the fungi but only 153 of the 273 bacterial isolates which demonstrated inhibition on agar plates were tested against P. tritici-repentis on sterile wheat straw. In this experiment the number of pseudothecia produced by P. tritici-repentis on straw was used as the indicator of inhibition. Straw used in the bioassay was collected from wheat grown under low humidity in the greenhouse and appeared unweathered and relatively free of saprophytic fungal colonists. One-to eight-cm segments were cut, soaked in distilled water for a minimum of two hours, drained and then autoclaved for 45 min . The straw segments were then placed in eight to ten parallel rows in a sterile petri dish (18 to 22 segments per dish). Conidia (3,000-8,000 per ml) of P. tritici-repentis obtained using the method described by Raymond et al (14) were sprayed onto the straw using an atomizer sprayer (about 2 ml/dish). After 48 hr mycelium had formed a network completely covering the straw but pseudothecia initials had not yet formed. The candidate inhibitor isolates were then sprayed onto the straw. Inoculum for the challenge bacteria or fungal isolates was produced on 1/4 PDA or NBY. After sufficient growth of isolates occurred, sterile distilled water was poured onto the plate and the agar surface scraped with a sterile metal spatula to dislodge hyphae and/or spores or cells. All isolate suspensions were filtered through one layer of cheesecloth as they were transferred to the sprayer. After applying turbid suspensions of the inhibitor isolates, the plates were placed in a growth chamber at

12 hr light/12 hr dark, 16C until mature ascospores were observed in pseudothecia from nonchallenged check plates (about 30 days). All procedures were performed in a sterile hood to prevent contamination. All spraying operations involved the use of an atomizer (the DeVilbiss Co. Somerset, PA 15501, model 152) at 0.35 kg/cm^2 pressure (5.0 psi).

Due to the large number of pseudothecia produced per plate, a grid was used to obtain a representitive sampling from each plate. A petri dish lid marked off in a 1-cm-square grid system was placed over the straw and the number of pseudothecia under five randomly selected squares were counted. Ten pseudothecia from each plate were also examined microscopically for ascospore production.

Field studies. Isolates found to be most strongly inhibitory to pseudothecia formation of P. tritici-repentis on straw and/or hyphal growth on agar plates were tested under field conditions. A total of 20 bacteria, 12 fungi, and three fungicides were tested during the course of three years. A randomized, complete block design with 15-20 treatments, and four to five replications was used to test isolates for their ability to reduce the number of pseudothecia of P. tritici-repentis formed on naturally infested straw. Straw of the wheat cultivar TAM 105,which was heavily infected with tan spot the previous season, was collected immediately after harvest and moved to a level, weed-free area and spread evenly over the soil surface. The area was covered with wire mesh fencing to keep the straw in place and then staked off in 1-m-square plots. Straw was placed in the plot area during July for each year and sprayed with the isolates within four

days.

Inoculum of the challenge isolates for the field experiment was obtained by growing them on agar plates and suspending the conidia and hyphae or cells in distilled water plus one drop Triton B 1937 per 100 ml suspension. To prevent sprayer clogging, suspensions were passed through one layer of cheesecloth. Each plot received 60 ml of a turbid suspension at each application date. The spray was applied with a 950 ml plant mister (Continental Mfg. Co., Mexico).

The plots were sprayed with antagonists once the first year, three times the second year and four times the third year. A 1-m-square wooden frame was placed over each plot while spraying to contain drift. Spraying was performed at dusk after briefly watering the plot area to moisten the straw and aid in isolate colonization and survival on the straw.

Weed control was maintained by spraying the plot with a 1% Glyphosate solution whenever necessary (three to four applications per experiment). Pseudothecia were enumerated by placing a petri dish lid similar to the one used in the straw bioassay at three to five random locations within the plot and counting the pseudothecia underneath. The field plot was sampled nine times from August to March the first season and once during May the following two years.

Water potential. The osmotic water potential component of 1.5% water agar was adjusted to cover a range from approximately -0.1 to -9.7 MPa (-1 to -97 bars). NaCl or KCl was added to the water agar to obtain the desired water potential (17). Plates containing the adjusted agar were

inoculated by placing a 5-mm-hyphal plug from the advancing edge of a colony of P. tritici-repentis in the center of the plate. Four P. tritici-repentis isolates obtained from different locations in Kansas were used in this study. Colony radius was measured after seven days growth at 22±3C.

pH requirements. Straw infusion agar was adjusted with HCl or NaOH to produce a pH range from two to ten. To make the straw infusion agar, 25 grams of mature wheat leaves and culms were chopped in a blender, added to one liter distilled water, boiled for 20 min, filtered through three layers of cheesecloth, adjusted to one liter with water, 20 g agar added and autoclaved.

The pH of the media was adjusted after autoclaving when the media reached approximately 65 C. HCl at 1M and 0.1M or NaOH at 4M and 0.4M were added while stirring the liquefied agar and petri dishes poured immediately after adjusting pH. The more concentrated acid or base solutions were used for coarse adjustment of pH while the dilute solutions were used for fine adjustment.

Plates with adjusted agar were inoculated with P. tritici-repentis by placing a hyphal plug in the center of the plate as in the water potential experiment. After six days growth, the radius of the hyphal mat was measured. The pH of the agar was remeasured after incubation to determine if growth of P. tritici-repentis had changed the pH. The pH of noninoculated plates was also measured to determine if the pH had changed due to factors other than fungus growth.

In a seperate study a surface pH probe was used to take the pH of

straw segments from various sources. Straw was obtained from the field or the greenhouse and was either colonized or not colonized by P. tritici-repentis. The straw was placed in distilled water for two min, and then placed in glass petri dishes in such a way that segments did not contact each other. Once the straw had absorbed the water the segments were cut longitudinally and opened up to provide a flat surface on which the pH probe could be placed. Approximately four hours after the straw was placed in water the pH was taken by placing the probe on the flattened straw segments. The weight of the probe was sufficient to form a liquid film covering the probe tip by pressing a small amount of water out of the saturated straw segment.

Temperature requirements. A temperature growth curve for P. tritici-repentis was generated by growing two different isolates on 1/4 PDA at nine temperatures ranging from 5 to 40C. There were five plates for each isolate placed at each temperature and the plates were wrapped in aluminum foil to eliminate light as a factor. The plates were left at the various temperatures until fungal growth was limited by the size of the petri dish or 20 days, whichever occured first. To obtain growth rates the radius of the fungus colony was measured at three locations on each plate every 24 hr during this period.

In a seperate study straw segments, artificially colonized with P. tritici-repentis, were exposed to eight temperatures ranging from 40 to 100C for a period of 24 hr to determine thermal death point. Straw segments were colonized with P. tritici-repentis using the same technique described for the wheat straw bioassay except that the fungus

grew on the straw for five days. Half of the colonized straw was kept moist during the treatment while the other half was air-dried before treatment. After exposure the segments were placed on 1/4 PDA plates to determine percent of the straw segments which had viable fungus.

Important aspects of all experiments were repeated. This included microbial isolates showing inhibition in vitro or on straw and all water potential, pH and temperature studies.

RESULTS

Inhibition on agar plates and straw. Of the 273 bacterial isolates tested 43% were able to inhibit P. tritici-repentis to some degree on 1/4 PDA or NBY (Table 1). Thirty-seven percent of them exhibited a zone of inhibition from 1 to 14mm on NBY. Twenty-three percent of the isolates inhibited P. tritici-repentis on 1/4 PDA with the largest inhibition zone being 18mm. Six percent of the bacterial isolates were able to reduce pseudothecia formation in the straw bioassay over 50%, and three percent showed over 65% reduction (table 2).

Of the 34 fungus isolates tested one reduced pseudothecia number about 50% and another reduced it more than 60%. Another fungus (isolate number F-18) failed to inhibit pseudothecia formation, however, none of the more than 40 pseudothecia examined contained any stage of developing asci. All samples from treatments other than F-18 had various stages of centrum development, from undifferentiated to mature asci. For the bacterial isolates, low pseudothecia maturity (10-20%) was always associated with high reduction in pseudothecia number (55% or above).

The correlation between isolate inhibition on one type of agar media (1/4 PDA) and inhibition on the other (NBY) was low (r=0.57). Furthermore there was no correlation between the ability to inhibit P. tritici-repentis on 1/4 PDA or NBY and ability to reduce the number of pseudothecia in the straw bioassay (r=0.12 and 0.05 respectively).

TABLE 1. Inhibition of Pyrenophora tritici-repentis by bacterial isolates on agar media.

Media	Width of Inhibition Zone			
	<3mm[x]	4-7mm	8-11mm	>11mm
NBY (no. of isolates)[y]	216	35	18	4
NBY (% of isolates)[z]	79	13	7	1
PDA (no. of isolates)	226	16	14	17
PDA (% of isolates)	83	6	5	6

[x]Size of inhibition zone between Pyrenophora tritici-repentis mycelium and bacterial colony on one quarter strength potato dextrose agar (PDA) or nutrient agar plus yeast extract (NBY).

[y]Number of bacterial isolates out of 273 which have inhibition zone indicated.

[z]Percentage of bacterial isolates which have inhibition zone indicated.

TABLE 2. Reduction in number of pseudothecia of Pyrenophora tritici-repentis by bacteria and fungi on wheat straw.

Isolate Type	% Reduction in number of pseudothecia[X]				
	<10%	10-25%	26-50%	51-65%	>65%
Bacteria (no. of isolates)[Y]	45	45	54	6	3
Bacteria (% of isolates)[Z]	29	29	35	4	2
Fungi (no. of isolates)	26	6	1	1	0
Fungi (% of isolates)	76	18	3	3	0

[X]Reduction in pseudothecia formation on straw colonized with Pyrenophora tritici-repentis and sprayed with bacterial or fungus isolates when compared with the untreated check.

[Y]Number of isolates which reduced number of pseudothecia by percent indicated at top of column.

[Z]Percent of total bacterial isolates which reduced number of pseudothecia by percent indicated at top of column.

Three types of inhibition were observed in the lab assays. Inhibition of hyphal growth occurred on the straw bioassay as well as the agar plates (Fig. 1). Microscopic examination of the inhibited hyphae revealed shorter, rounded cells having the appearance of chlamydospores (Fig. 2A). In close association with the stunted hyphae a red crystaline substance often formed in the agar or on the straw (Fig. 2B). Another type of inhibition was a reduction in the number and size of pseudothecia (Fig. 3). This type of inhibition tended to occur in conjunction with, and may have been a result of hyphal inhibition. The third type of inhibition observed was a reduction in the percentage pseudothecia with mature asci. Two fungal isolates produced large reductions in percent mature pseudothecia (90-100%) while not reducing pseudothecia number suggesting that they may produce substances which interfere with normal development of ascogenous hyphae in pseudothecia of P. tritici-repentis.

Most of the bacterial isolates displaying inhibition to P. tritici-repentis on agar or straw were identified as Bacillus spp. or non-florescent Pseudomonas spp. Fungi showing the most reduction in pseudothecia formation on straw incluced species of Alternaria, Penicillium, Bipolaris and Trichoderma. The fungus isolate numbered F-18 is an unidentified basidiomycete which forms small, oval sclerotia with short stalks on wheat straw and agar media. Despite numerous attempts to induce sporulation on straw, agar media and various small grains, no other structures were formed.

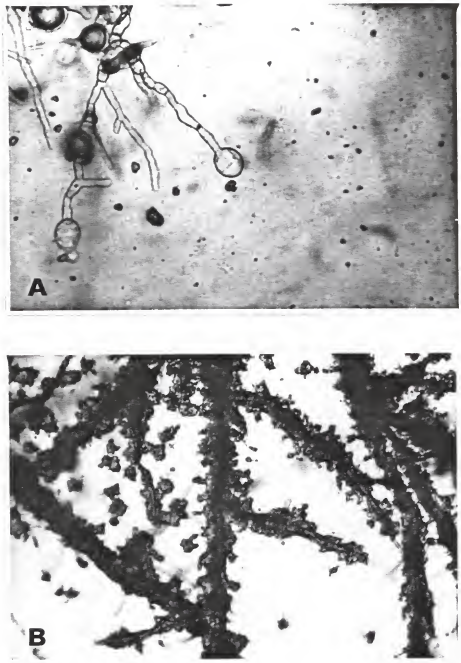

Fig. 2. Inhibition of mycelium of Pyrenophora tritici-repentis by antagonists: Shorter, rounded hyphal cells at the zone of inhibition on agar media (A). Red crystalline substance associated with inhibited hyphae (B).

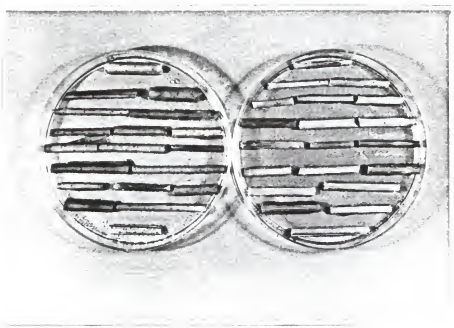

Fig. 3. Reduction in number of pseudothecia of Pyrenophora tritici-repentis formed on straw when sprayed with bacterial cell suspensions (left=nonsprayed plate, right=challenged with an antagonist).

Field studies. None of the treatments used in the field trials significantly reduced the number of pseudothecia formed on the straw (Table 3). During the 1983-84 study treatment B-3 reduced pseudothecia number the most, however, this was not significant statistically and the same isolate did not reduce the number of pseudothecia in the 1982-83 study. Furthermore, F-18 was unable to prevent pseudothecia from forming asci as it did in the straw bioassay. All of the pseudothecia observed from the field displayed normal centrum development when examined under the microscope. Applications of three different fungicides also failed to reduce the number of pseudothecia.

Water potential. Growth of P. tritici-repentis on water agar adjusted to various water potentials is summarized in figure 4. Determinations in a thermocouple psychrometer showed that osmotic water potential of unamended water agar was approximately -0.1 MPa and the adjusted agar was essentially identical with calculated values ranging to approximately -9.7 MPa. Growth showed a 15% increase from -0.1 to -0.4 MPa, it then declined to about 50% percent of maximum at -1.5 MPa and ceased between -8.0 and -9.7 MPa. There was very little variability between isolates or between solutes used to adjust the media.

pH requirements. Growth first occurred between pH 2.5-3.0 and stopped at approximately pH 10 (Fig. 5). Since variation between isolates was slight, the values were pooled. The pH of the agar dropped as much as 2.5 units as it solidified if the agar was adjusted above pH 8, thus, values reported in figure 5 are after solidification. During the course of the experiment the pH changed no more than 0.2 units on the non-inoculated plates.

TABLE 3. Effect of microbial isolates and fungicides on the number of Pyrenophora tritici-repentis pseudothecia formed on wheat straw in field trials.

Year of Study					
1982-83[t]		1983-84[u]		1984-85[v]	
Treatment	Number of Pseudothecia	Treatment	Number of Pseudothecia	Treatment	Number of Pseudothecia
check[w]	27.2 -	B-3	47.7 NS	check	22.5 -
B-6	27 3 NS[y]	B-19	50.7 NS	F-18	22.6 NS
mix[x]	28.0 NS	B-1	55.1 NS	Imazilil	
check	28.4 NS	B-14	56.8 NS	+ TCMTB[z]	24.4 NS
mix	28.4 NS	mix	58.0 NS	B-16	25.9 NS
mix	28.5 NS	F-15	58.2 NS	Vangard[z]	25.1 NS
B-8	29.2 NS	F-10	58.6 NS	B-25	25.5 NS
B-4	29.3 NS	B-11	58.9 NS	B-16	25.9 NS
mix	30.2 NS	B-7	59.9 NS	B-17	28.0 NS
F-1	30.3 NS	check	60.5 -	PCNB[z]	28.1 NS
mix	30.5 NS	B-2	61.6 NS	F-23	29.9 NS
F-41	30.6 NS	B-12	62.8 NS	F-18	31.0 NS
mix	31.1 NS	F-29	63.1 NS	F-18	31.8 NS
B-7	31.2 NS	F-2	64.4 NS	B-18	31.9 NS
B-3	31.8 NS	F-8	64.4 NS	B-20	32.0 NS
B-15	32.3 NS	B-5	66.2 NS	B-16	32.8 NS
B-1	32.5 NS	F-27	66.3 NS		
mix	32.9 NS	mix	67.1 NS		
mix	33.5 NS	F-3	67.2 NS		
		F-28	72.7 NS		

[t]Treatments applied once.

[u]Treatments applied three times.

[v]Treatments applied four times.

[w]B indicates bacterial isolate, F indicates fungus isolate.

[x]Mixture of two to nine isolates.

[y]Values are averages of four to five replications and three to five counts per replicate. NS=not significantly different from the check according to ANOVA (P=0.05).

[z]Fungicides applied at a rate of 0.1 ml or 0.1 g/25 ml water, 60 ml/m^2.

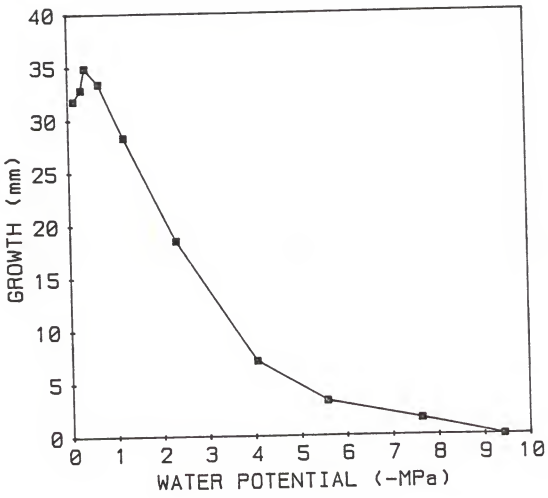

Fig. 4. Radius of mycelial mat of Pyrenophora tritici-repentis after seven days growth on water agar adjusted to various water potentials.

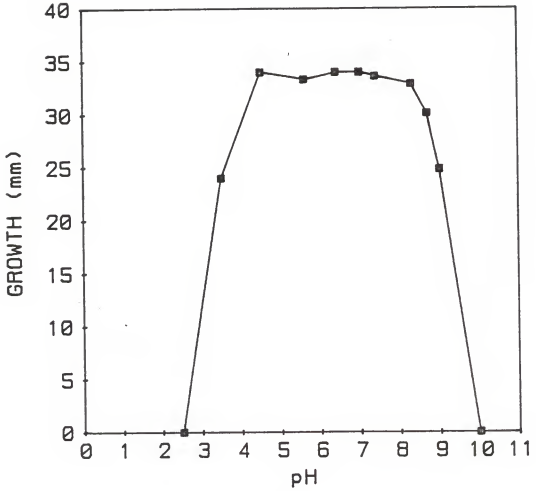

Fig. 5 Radius of mycelial mat of Pyrenophora tritici-repentis after six days growth on wheat straw infusion agar adjusted to various pH values with HCl or NaOH.

Growth of P. tritici-repentis did not change the pH more than 0.6 units on straw agar at pH values of 2.6, 3.6, 7.5, 8.3, 8.7 or 9.0. However, it did raise the pH of the straw agar 2.6, 2.0 1.4 and 0.8 units at pH 4.5, 5.6, 6.4 and 7.0 respectively. The pH values shown on the graph represent those obtained from non-inoculated plates at the conclusion of the experiment.

Straw collected from the field had an average pH of 7.5 ±1.0. Straw from the greenhouse which was not colonized by P. tritici-repentis was 6.2 ±0.9 and straw artificially colonized was 7.3 ±0.5. Autoclaved straw from the greenhouse was 5.3 ±0.3.

Temperature. The temperature growth curve (Fig. 6) illustrates growth requirements for P. tritici-repentis similar to other plant pathogenic fungi (19). The maximum growth rate occurred at approximately 30 C, however, a similar study designed to more accurately resolve the temperature for the maximum growth rate indicated 28 C. Measurable growth occurred at temperatures from 5.0 to 37 C. While growth occurred at temperatures above 30 C the advancing edge of the thallus became uneven and the fungus began to develop tufts of aerial mycelium.

The thermal death point for P. tritici-repentis which had colonized straw was found to be between 95 and 100 C when the straw was dried before heating. However, the fungus was unable to survive 24 hr exposure to temperatures as low as 40 C when the straw was kept moist during treatment. The fungus survived on 100 percent of the dry straw pieces at temperatures from 40 to 80 C. At 90 C, percent survival ranged from 20 to 60 percent, depending upon the experiment, while none of the straw pieces showed viable fungus on them at 100 C.

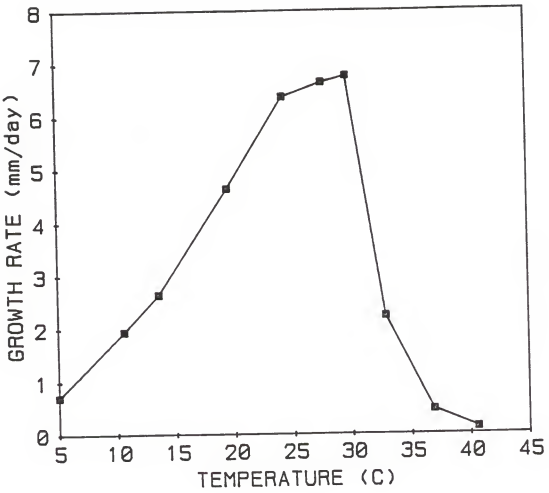

Fig. 6. Mycelial growth rate (mm/day) of Pyrenophora tritici-repentis at various temperatures.

DISCUSSION

Inhibition of microorganisms to each other is easily demonstrated on agar plates and the results are often dramatic (1). Although this method is an obvious first choice for screening potential biological control agents, initial results can be misleading and may cause the investigator to discard useful organisms. Results of this study substantiate this since numerous organisms were found which were capable of inhibiting P. tritici-repentis to some degree on agar media, however, inhibition on agar was not correlated with ability to inhibit the fungus on straw. Thus, we recommend the use of the straw bioassay technique described in this paper as a more reliable indicator of antagonism to P. tritici-repentis since it more closely simulates the natural substrate than does agar media. The wheat straw assay allowed testing of the potential antagonists against a number of P. tritici-repentis isolates on a substrate similar to that encountered in the field. The bioassay also allowed P. tritici-repentis to colonize the straw before the antagonists were introduced, simulating field conditions where an antagonist must exert its influence on a fungus which has already colonized the substrate. The use of straw naturally infested with P. tritici-repentis could be a further refinement of the bioassay, although the high moisture conditions cause naturally-occurring microbes on nonsterile straw to proliferate. This could be beneficial or detrimental depending upon the objectives of the experiment.

There are several possible explanations for the poor correlation

between agar and straw bioassays. Many of the microorganisms may not be able to utilize the nutrients available on the straw or, in the 48 hr period which P. tritici-repentis has to colonize the straw before being challenged, nutrients which the potential antagonist needs to grow may be tied up. Also, even though an antagonist may grow on straw, it may not produce the inhibitory metabolites that it does on artificial media. The isolate may grow too slow on the straw so that P. tritici-repentis is able to form pseudothecia before enough of the inhibitory substance is produced. Physical parameters of the straw habitat may also influence the ability of the potential antagonist to grow and/or produce inhibitory substances.

Despite identifying several microorganisms which could significantly inhibit P. tritici-repentis on straw in the laboratory , no inhibition was observed in the field experiments. While selection in the bioassay for microbes which could compete on the natural substrate was a necessary prelude to obtaining a biological control agent, successful control in the field is also profoundly influenced by environmental factors. Results of this study showed that P. tritici-repentis has several fitness characters which probably allowed it to outcompete the antagonists on surface straw under field conditions.

The pH growth curve (Fig. 5) for P. tritici-repentis shows that it can grow over the broad range of about 3.0 to 9.5. More importantly, growth at one half the maximum can occur over the range of 3.25 to 9.25 and near maximum growth occurs from pH 4.5 to 8.25. Since measurements of straw fell in the 5.0 to 8.5 range, P. tritici-repentis can grow near

to its maximum over the fairly broad range of pH that may be encountered on straw. Potential biocontrol agents would be at a disadvantage if they did not display a similar pH growth curve.

The osmotic water potential growth curve (Fig. 4) also demonstrated the fitness of P. tritici-repentis in the surface straw environment. At least some growth could occur between -0.1 and -8.0 MPa with maximal growth at -0.7 MPa and 37% of maximum at -2.5 MPa. Many bacterial antagonists would be unable to grow below -1.0 MPa and none could grow below -2.5 MPa (2). Furthermore, straw in the field can progress from near 0 MPa immediately after a rain or dew, to -50.0 or even -80.0 MPa in the afternoon of the same day (W. Zhang unpublished). While these latter numbers are too low for growth of P. tritici-repentis, as the straw dries there will be a period of time during the day when it can grow and the bacterial antagonists cannot. Over a period of several months, these short periods of growth could be significant. Survival of antagonists after repeated exposure to cycling of 0 to -80.0 MPa would also be an important factor with regard to their ability to supress P. tritici-repentis..

The temperature growth curve (Fig. 6) was similar to several related fungi (3,17,19) and shows no striking competitive advantage for P. tritici-repentis. The range of temperatures used in this experiment are those routinely encountered in nature and include temperatures at which an antagonist would most likely grow. Related to its ability to grow at various temperatures was the ability to survive at extremely high temperatues. Exposure to temperatures as high as 95 C in dry straw did not thoroughly kill P. tritici-repentis. In mid-summer temperatures

of surface straw could reach as high as 65 C on a hot, sunny day. These high temperatures would not affect P. tritici-repentis since the surface straw would be dry when temperatures are highest, but, the ability of various antagonists to withstand high temperatures on straw could be a key factor in their success.

There are two apparent factors involved in preventing antagonists from displacing P. tritici-repentis from the straw. First is the advantage P. tritici-repentis has by invading host tissue as a parasite, thus being the pioneer colonist (2). The second is the extremely harsh environmental conditions which the colonized straw is exposed to after wheat harvest until the following spring. The dry, hot surface layer of straw does not provide an ideal environment for the establishment of microorganisms. Nevertheless, we have described a bioassay technique which we believe is a more accurate way than agar plates to select biocontrol agents to compete with or displace P. tritici-repentis after it has colonized straw. However, P. tritici-repentis displays several fitness characters which would make it extremely competitive in the straw environment in the field. These include the ability to grow over a broad pH range, or in relatively dry conditions and the ability to survive extremely high temperatures. These parameters can be controlled in the bioassay technique and future selections of potential biocontrol agents should use these pressures, in addition to antagonism, to obtain antagonists which may be more competitive in the field.

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SELECTING BIOLOGICAL CONTROL AGENTS TO LIMIT THE SAPROPHYTIC ABILITY OF *PYRENOPHORA TRITICI-REPENTIS*

by

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ABSTRACT

Microorganisms were tested as potential biocontrol agents of tan spot of wheat using various methods to measure their ability to inhibit the causal fungus P. tritici-repentis . The microorganisms included 273 bacteria and 34 fungi and were isolated from various sources including soil, roots, and plant material. The antagonism of bacteria was tested in vitro by measuring the width of the inhibition zone between the advancing mycelium of P. tritici-repentis and the bacterial colony on 1/4 potato dextrose agar (1/4 PDA), and on nutrient agar plus yeast extract (NBY). Forty-three percent of the bacteria were able to inhibit P. tritici-repentis to some degree on agar media, however, correlation between isolate inhibition on one type of agar media and inhibition on the other was low (r=0.57).

In a separate assay, the number of pseudothecia produced on straw artificially colonized by P. tritici-repentis was counted after spraying the straw with propagules of the candidate antagonist. Twenty-six percent of the fungi and 70 percent of the bacteria tested reduced the number of pseudothecia formed by at least ten percent. However, there was no correlation between the ability of a bacterial isolate to inhibit P. tritici-repentis on 1/4 PDA or NBY and its ability to inhibit the number of pseudothecia on straw (r=0.12 and 0.05 respectively).

Microorganisms showing the most inhibition in the lab assays, were sprayed onto straw naturally infested with P. tritici-repentis in the field. There were no significant differences in pseudothecia number

between the checks and any of the antagonist treatments in the three years of the study.

Agar plates adjusted to various pH or water potential values, or placed at various temperatures, were utilized to determine physical growth reguirements of P. tritici-repentis. The fungus grew at pH values ranging from 3.5 to 9.0, water potentials of -0.1 to -8.0 MPa, and temperatures from below 5 to 40 C. In a seperate study, P. tritici-repentis survived on colonized wheat straw after being placed at temperatures up to 95 C for 24 hrs. These physical growth characteristics indicate how competitive P. tritici-repentis is in the surface straw habitat and potential biocontrol agents should be selected for similar characteristics.