Ecology and Epidemiology

Interactions Between *Cochliobolus sativus* and *Pyrenophora tritici-repentis* on Wheat Leaves

W. C. da Luz and G. C. Bergstrom

Former graduate student and assistant professor, Department of Plant Pathology, Cornell University, Ithaca, NY 14853. The first author is currently a research plant pathologist at Centro Nacional de Pesquisa de Trigo, Passo Fundo RS, 99100, Brazil.

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ABSTRACT

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Following mixed inoculation with *Cochliobolus sativus*, incitant of spot blotch, and *Pyrenophora tritici-repentis*, incitant of tan spot, wheat leaves developed less necrosis than the average produced by the two pathogens alone at inoculum concentrations equal to those used in the mixed inocula. Spot blotch predominated over tan spot following simultaneous inoculations or sequential inoculations where *P. tritici-repentis* preceded *C. sativus* by up to 6 hr. Antagonism occurred even when inocula contained only 20% *C. sativus*. Inoculation with *C. sativus* resulted in reduced conidial germination, slowed germ tube development, and reduced appressorium formation in *P. tritici-repentis*. Tan spot development may be suppressed in the field where environmental conditions favor spot blotch. *C. sativus* or its metabolites could potentially be manipulated to produce an effective biological control for tan spot of wheat.

Additional key words: *Bipolaris sorokiniana*, *Drechslera tritici-repentis*.

Fungal foliar diseases of wheat commonly occur together in the same field and frequently on the same plant (5,7). During pathogenesis of plant leaves, fungi may interact synergistically, antagonistically, or exhibit neutralism toward each other.

Synergistic interactions between pathogens of aerial wheat organs are well documented (2,13,15,23). Antagonistic interactions between fungal pathogens of aerial wheat organs have also been reported (3,11,19,20). The latter studies involved either none or only one leaf spot fungus in the interaction.

Interactions among components of the wheat leaf spot syndrome are poorly documented. Jenkins and Jones (12) found no significant evidence for synergistic or antagonistic interaction between *Phaeosphaeria nodorum* (Muller) Hedja. (anam. = *Stagonospora nodorum* (Berk) Cast. & Germ.) incitant of Septoria nodorum spot, and *Mycosphaerella graminicola* (Fckl.) Sand., incitant of *Septoria tritici* spot on wheat plants. da Luz (6) observed neutralism between *Pyrenophora tritici-repentis* (Died.) Drechs. (anam. = *Drechslera tritici-repentis* (Died.) Shoem.), incitant of tan spot, and *P. nodorum*, and either neutralism or antagonism between *Cochliobolus sativus* (Ito and Kurib. in Kurib.) Drechs. ex Dastur (anam. = *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoemaker), incitant of spot blotch, and *Phaeosphaeria nodorum*, depending on the wheat cultivar used.

Ghazanfari-Heramabadi (8) observed that *C. sativus* inhibited pseudothecial formation and growth of *P. tritici-repentis* in culture. No information is available on interactions between *C. sativus* and *P. tritici-repentis* on wheat plants.

P. tritici-repentis and *C. sativus* are commonly involved in the wheat leaf spot syndrome (5,7,10,21). Therefore, interactions between these pathogens may influence significantly the development of foliar disease. This study was done to determine if interactions between these fungi occur on wheat leaves and, if they do, to partially characterize the interactions.

MATERIALS AND METHODS

Germ plasm and growth conditions. Seeds of hard red spring wheat (*Triticum aestivum* L.) cultivars BH1146, BR8, Candiota (obtained from the Centro Nacional de Pesquisa de Trigo

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[EMBRAPA], Passo Fundo, RS, Brazil), and Max (obtained from Cornell University) were sown under glasshouse conditions, and new seeds were produced for experimental studies. Apparently healthy plants of each cultivar were grown in autoclaved sandy loam soil in 11-cm-diameter clay pots, five plants per pot, in a controlled environment chamber. The chamber was programmed to give 14 hr of light at 23 C and 10 hr of darkness at 19 C. Plants were watered adequately and uniformly.

Maintenance of fungal isolates. The fungal isolates used were a monoconidial isolate of *C. sativus* (Cs030B82) originally collected in 1982 from lesions on spring wheat grown in Brazilia DF, Brazil, and a monoconidial isolate of *P. tritici-repentis* (Ptr004NY84) collected in 1984 from naturally infected, susceptible spring wheat cultivar Max, grown in Steuben County, New York. Pure cultures of these isolates were maintained at 4 C on V-8 juice agar and on potato-dextrose agar (PDA), respectively. Each medium was supplemented with 100 ppm of streptomycin.

Inoculum production. Inoculum of *C. sativus* was produced by growing the fungus for 7 days on V-8 juice agar with 100 ppm of streptomycin in petri plates at 21 ± 1 C, under near ultraviolet (UV) light from four Sylvania F40-BLB lamps and a 12-hr photoperiod. Conidial suspensions were prepared by flooding the cultures with distilled water and then rubbing the culture surfaces with a glass slide to dislodge spores.

Inoculum of *P. tritici-repentis* was produced following the method of Khan (14) as modified by Platt et al (18), Odvody and Boosalis (17), Alcorn (1), and da Luz (6). Disks were cut from the PDA and streptomycin stock cultures with a flamed 0.2-cm-diameter cork borer and were transferred to the center of a petri plate containing V-8 juice agar. The cultures were grown at 21 ± 1 C under a 12 hr-diurnal cycle of near-UV light for 5 or 6 days. After this period, all but the outer 1-cm-wide ring of the culture was discarded. Aerial fungal structures were removed from the remaining mycelium by scraping the culture surface with a flamed and cooled end of a microscope slide. The scraped cultures were maintained for 24 hr under near-UV light, and conidiophores grew abundantly. The petri dishes were then incubated in the dark at 21 ± 1 C for 20–24 hr, and profuse sporulation occurred. Conidia were dislodged from the conidiophores on the 1-cm-wide ring by cutting the ring into pieces and shaking them in an Erlenmeyer flask containing distilled water.

The conidial suspension of *C. sativus* was filtered through two layers of cheesecloth; however, the conidial suspension of *P. tritici-repentis* was not filtered. The suspensions were blended for 30 sec with 200 ml of distilled water in a Waring blendor, and then were adjusted to 10^4 conidia per milliliter. The conidial counts were made using a hemacytometer for *C. sativus* and a volume of 50 μl in a watch glass for *P. tritici-repentis*. Thirty microliters of Tween 20 (polyoxyethylene sorbitan monolaurate) per 200 ml of suspension was added to ensure uniform wetting of the leaves.

Artificial inoculations. Plants at growth stage 23 (five leaves unfolded, main shoot, and three tillers) (24) were sprayed until runoff with a suspension of inoculum using a hand-operated sprayer at a pressure of about 0.5 kg/cm^2. A uniform distribution of spores was obtained.

Immediately after inoculation, plants were placed in a mist chamber with cold water for 30 hr of continuous leaf wetness. The temperature regime in the mist chamber was 22 C during a 14-hr photoperiod and 18 C during a 10-hr dark period. The pots were then transferred to a controlled environment chamber maintained at the same temperature regime and at 80% relative humidity. This chamber was programmed to give 14 hr of light every day (16 incandescent Sylvania bulbs, 50W each, and 16 fluorescent General Electric tubes, 1,500W total) with an intensity of 16,000 lx at 40 cm above the soil line followed by 10 hr of darkness. Uninoculated controls, sprayed with a Tween 20 solution were maintained similarly.

Mixed inoculation experiments. Four cultivars were used in the study. There were six treatments per cultivar including an uninoculated check. Inoculations were done with each fungus individually (100% of the original inoculum concentration) and with both *P. tritici-repentis* and *C. sativus* at the same time by mixing inocula in the proportions 50:50, 20:80, and 80:20 of the conidial concentrations described previously.

Each treated plot consisted of one pot with five plants per pot. Each plot was replicated four times. Individual experiments were performed with a single cultivar. Treatments were arranged randomly within a single growth chamber for each experiment. Each experiment was repeated two times.

Leaf spot severity was recorded as the estimated percentage of total leaf area of each leaf that was necrotic or chlorotic 7 days after inoculation. An average percentage of leaf area visibly diseased was calculated for each plot.

Microorganisms were recovered from individual lesions on the third leaf of each plant by plating surface-disinfested leaf pieces on V-8 juice agar plus streptomycin. Leaf pieces were surface disinfested by dipping them into 70% ethyl alcohol for 15 sec, then into a 1% solution of sodium hypochlorite for 15 sec, and finally into sterile distilled water. The pieces were maintained for 4 days at 21 ± 1 C under a 12-hr diurnal cycle of near-UV light. Two hundred lesions from plants in each treatment replicate were examined. Identification of the pathogens recovered was based on fungal morphology, and the results were expressed as the percentage recovery of each species.

The incubation period (inoculation to first visible symptom) was determined by using the second leaves of plants of each cultivar.

Germination, germ tube growth, and appressorium formation were evaluated on 20 leaf pieces (about 0.5 cm long) cut from the first leaf of each treatment replicate, 18 hr after inoculation. The first leaf was also removed from the uninoculated control. The leaf pieces were transferred to a fixing-clearing solution containing 3:1 (absolute ethyl alcohol:acetic acid) for 24 hr. The pieces were transferred to 0.1% acid fuchsin in lactophenol for 2 min and then to lactophenol for 10 sec. Leaf pieces were removed from the lactophenol, mounted in 50% glycerin on microscope slides, and examined microscopically. Germination of conidia and formation of appressoria were determined for each fungus by examining 200 randomly selected conidia per treatment replicate (about 150 from slides of leaf pieces and about 50 disassociated conidia from the previously used solutions). Germ tube elongation was evaluated by microscopic measurements of 100 germinated conidia from each replicate.

Grain was harvested manually from plants of cultivar Candiota, and the yield was expressed as grams per plot.

TABLE 1. Percentage of leaf area showing symptoms following two successive inoculations with *Cochliobolus sativus* and *Pyrenophora tritici-repentis*

	Inoculum and sequence							
	C.s. + C.s.[a]		P.t-r. + P.t-r.		P.t-r. + C.s.		C.s. + P.t-r.	
Cultivars	7 days	14 days	7 days	14 days	7 days	14 days	7 days	14 days
Candiota	66.5[b]	91.0[b]	36.5	76.2	34.0	80.2	68.3	72.3
BR8	45.0	75.1	24.8	55.9	24.2	73.4	46.7	50.2
Max	69.0	86.4	42.5	91.0	44.8	92.1	68.7	84.2
BH1146	50.2	78.0	13.8	46.4	14.1	72.6	48.7	54.0

[a] C.s. = *C. sativus*, P.t-r. = *P. tritici-repentis*. The second inoculation was done 7 days after the first. Each inoculation was done singly with 50% of the original (10^4 conidia per milliliter) inoculum concentration of each pathogen.

[b] Mean of four replicates. Fisher's "protected" LSD values were: Candiota (7 days = 8.9, 14 days = 7.8), BR8 (7 days = 6.1, 14 days = 10.5), Max (7 days = 6.8, 14 days = 10.1), BH1146 (7 days = 7.2, 14 days = 11.2).

Sequential inoculations experiments. Interactions were evaluated at various time intervals of inoculation in two experiments. In the first set of sequential experiments, inocula of the two species were added 7 days after the first inoculation with the following treatments for each cultivar: *C. sativus* and *C. sativus*, *C. sativus* and *P. tritici-repentis*, *P. tritici-repentis* and *P. tritici-repentis*, *P. tritici-repentis* and *C. sativus*. Fifty percent of the original concentration of conidia was applied at each inoculation.

Disease severity was evaluated at 7 days after each inoculation. The pathogens were reisolated from lesions taken from each replicate of each treatment. The results were expressed as percentage of recovery of either organism or their combination.

In the second set of sequential experiments, only the cultivar Candiota was used. The treatments were inocula combinations of conidia of *C. sativus* and *P. tritici-repentis* applied simultaneously, and *P. tritici-repentis* inoculated first, followed by inoculations with *C. sativus* after 6, 12, 24, 72, and 168 hr. Disease severity was rated 14 days after inoculation. Fungal reisolations from lesions were made on the third leaf 4 days after the second inoculation time. All other procedures were similar to previous experiments.

Statistical analyses. All plot means data were transformed to arc sin and were statistically analyzed by analysis of variance. Mean differences were indicated by Fisher's "protected" LSD test.

RESULTS

Mixed inoculations. Disease severity in response to single or mixed inoculations of *C. sativus* and *P. tritici-repentis* is presented in Figure 1. Disease severity was greatest on all four wheat cultivars in the 100% *C. sativus* treatment, while the corresponding *P. tritici-repentis* treatment induced a significantly lower level of disease (Fig. 1). Of the wheat cultivars tested, Max and Candiota were the most susceptible to both pathogens, and BH1146 and BR8 were the two cultivars least susceptible.

The results indicated a definitive antagonistic interaction between *C. sativus* and *P. tritici-repentis* in causing decrease of leaf spot when both organisms were applied together (Fig. 1). Leaf spot severity on plants inoculated with *C. sativus* 50% + *P. tritici-repentis* 50% was less than the sum of the severities obtained on plants inoculated with each of the fungi alone (100%) divided by two (Fig. 1), or by the average of the severities obtained by 50% of the inoculum concentration of each pathogen as indicated in Table 1. Similar results were obtained with other ratios of pathogen mixture.

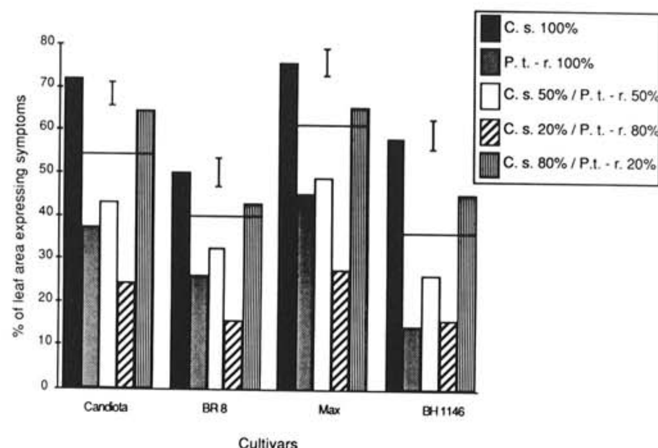

Fig. 1. Percentage of leaf area showing symptoms after inoculation with *Pyrenophora tritici-repentis* and *Cochliobolus sativus* alone or in simultaneous inoculations 7 days after inoculation. Mean values are from four replicates. Vertical bars for each cultivar indicate values of the Fisher's "protected" least significant differences between means at $P = 0.05$. Horizontal bars represent half the sum of the disease scores induced by both pathogens alone.

Lesions induced on plants inoculated simultaneously with conidial mixtures of both pathogens in any ratio yielded significantly more *C. sativus* than *P. tritici-repentis* (Table 2). Recovery of *P. tritici-repentis* leaf spots induced by mixed inocula was markedly reduced when compared to recovery from leaf spots induced by *P. tritici-repentis* alone (Table 2).

Sequential inoculation effect on disease severity. Leaf spot severity on plants inoculated twice consecutively with *C. sativus* was significantly greater than on plants inoculated twice consecutively with *P. tritici-repentis* in all cultivars except Max (Table 1). This was probably due to Max being more susceptible. Disease severity on all cultivars except Max was also greater with sequential inoculation treatments of *P. tritici-repentis* + *C. sativus* than with sequential inoculation treatments of *C. sativus* + *P. tritici-repentis*.

The results of another sequential experiment in which only the cultivar Candiota was studied are presented in Table 3. Leaf spot severity decreased markedly when *C. sativus* was inoculated together with or up to 6 hr after *P. tritici-repentis* in comparison with longer inoculation intervals.

Disease severity increased progressively in plants inoculated first with *P. tritici-repentis* and then inoculated 12–168 hr (7 days) later with *C. sativus*.

Reisolations from sequentially inoculated plants. Reisolations of the fungi in this experiment with cultivar Candiota are shown in Table 4. Only *C. sativus* or *P. tritici-repentis* was recovered from leaf spots induced by those fungi in double inoculations.

After inoculation of one fungus 7 days before the other, the one inoculated first was recovered in higher percentages than the one inoculated second; however, the percentage of isolation of each pathogen was lower than that when each was inoculated twice consecutively.

TABLE 2. Percentage of lesions in the wheat cultivar Candiota from which *Cochliobolus sativus* and *Pyrenophora tritici-repentis* were isolated singly and together 7 days after inoculation with these fungi, either individually or simultaneously in combinations at the indicated percentages

Inoculum[b]	Recovery (%)[a]		
	C.s.	P.t-r.	C.s. and P.t-r.
C.s. (100%)	89 ± 5	0	0
P.t-r. (100%)	0	84 ± 5	0
C.s. (50%) + P.t-r. (50%)	82 ± 8	3 ± 1	4 ± 1
C.s. (20%) + P.t-r. (80%)	75 ± 6	6 ± 3	4 ± 2
C.s. (80%) + P.t-r. (20%)	86 ± 4	2 ± 1	0

[a] Mean value and standard deviation of four replicates of 200 lesions per treatment.

[b] C.s. = *C. sativus*, P.t-r, = *P. tritici-repentis*. Original (100%) suspensions contained 10^4 conidia per milliliter.

TABLE 3. Percentage of leaf area showing symptoms and percentage of fungal recovery, after inoculation of wheat plants with *Pyrenophora tritici-repentis* followed by *Cochliobolus sativus* at different intervals

Inoculation intervals (hr)[b]	Disease severity (%)[c]	Fungal reisolations (%)[a]		
		C.s.	P.t-r.	C.s. + P.t-r.
0	47.1	79 ± 7	4 ± 1	6 ± 1
6	49.0	42 ± 6	18 ± 3	31 ± 4
12	55.2	26 ± 3	32 ± 4	29 ± 5
24	64.9	24 ± 4	38 ± 3	28 ± 6
72	72.0	17 ± 4	52 ± 6	25 ± 4
168	83.7	10 ± 2	73 ± 8	16 ± 2

[a] Mean and standard deviation of four replicates of 200 leaves per treatment. C.s. = *C. sativus*, P.t-r. = *P. tritici-repentis*.

[b] The numbers followed by hour indicate the time elapsed between inoculations with *P. tritici-repentis* and *C. sativus*.

[c] Disease severity was rated as percentage of leaf area showing symptoms at 14 days after the first inoculation. Means of four replicates. Fisher's "protected" LSD value = 5.3 at $P = 0.05$.

In the second sequential inoculation experiment (Table 3), recovery of *P. tritici-repentis* from leaf lesions was significantly reduced when this fungus was inoculated simultaneously with or 6 hr before inoculations with *C. sativus*. Recovery of *P. tritici-repentis* increased considerably after sequential inoculation with *P. tritici-repentis* and *C. sativus* where *C. sativus* was inoculated 12–168 hr after *P. tritici-repentis*.

When *P. tritici-repentis* was inoculated 12 hr or longer before *C. sativus*, the former was isolated more frequently than the latter.

Incubation period in mixed inoculation experiments. The incubation period of *P. tritici-repentis* was significantly increased by mixed inocula with *C. sativus* (Fig. 2).

Yields in mixed inoculation experiments. Inoculations with both pathogens alone or in combination (50:50) were evaluated for yield reduction in cultivar Candiota. All inoculation treatments caused a marked reduction in grain yield compared with uninoculated plants (Table 5). The maximum yield reduction (40%) was detected in plants inoculated with *C. sativus*; the smallest reduction was observed in the plants inoculated with *P. tritici-repentis*.

The yield reduction induced by double inoculation was smaller than half the calculated sum of reductions induced by each fungus separately. These results clearly confirmed the antagonistic association between *C. sativus* and *P. tritici-repentis* observed when disease severity was evaluated (Fig. 1).

Germination, germ tube elongation, and appressorium formation in mixed inoculation experiments. Conidium germination of *C. sativus* from the basal cell produced generally an initial germ tube growth in the direction of the long axis of the conidium. Bipolar or unipolar germ tubes emerged from 87% of the spores. Predominantly (62%) polar germination occurred by a single germ tube on cultivar Candiota determined 18 hr after inoculation. The percentage of condia of *C. sativus* with bipolar germination increased with an increase in time after inoculation. The orientation of germ tube growth was random, but appressorium formation usually occurred at the juncture of two epidermal cells.

For *P. tritici-repentis*, 83% conidium germination was observed on the wheat cultivar Candiota. Germination was amphigenous, unipolar, or bipolar. Germ tubes from the basal (proximal) cell of the conidium were usually lateral, midway between the basal septum and the hilum. Amphigenous germination occurred in 49% of the conidia. The growth of basal germ tubes was rarely in the direction of the long axis of the conidium. Most of the germ tubes arose from the terminal cells during the first 12 hr after inoculation, but the percentage of conidia with amphigenous germination increased with increase in time after inoculation. Occasionally two germ tubes originated from a single cell. Appressoria generally formed over the juncture of epidermal cells. A minority of conidia formed appressoria over the stomatal area.

The cultivars on which the conidia germinated did not significantly affect conidium germination. Usually, germ tube elongation in *C. sativus* was much greater than in *P. tritici-repentis* (Table 6).

C. sativus decreased conidium germination and reduced germ tube growth and appressorium formation of *P. tritici-repentis* on the wheat leaf surface when the two organisms were used simultaneously as inocula. For example, on cultivar Candiota, conidium germination was only 67% in mixed conidial inoculum as compared with 83% in inoculum composed of conidia of *P. tritici-repentis* only (Table 6).

TABLE 4. Reisolations of *Pyrenophora tritici-repentis* and *Cochliobolus sativus* from plants of wheat cultivar Candiota sequentially inoculated with these pathogens

Inoculation sequence[b]		Fungi isolated at 14 days (%)[a]		
1st	2nd	C.s.	P.t-r.	P.t-r. + C.s.
C.s.	C.s.	87 ± 2	0	0
P.t-r.	P.t-r.	0	88 ± 5	0
P.t-r.	C.s.	12 ± 3	69 ± 4	14 ± 3
C.s.	P.t-r.	85 ± 6	2 ± 1	2 ± 1

[a] Mean and standard deviation of four replicates of 200 lesions per treatment.

[b] C.s. = *C. sativus*, P.t.-r. = *P. tritici-repentis*. Each inoculation was done with 50% of the original (10^4 conidia per milliliter) inoculum concentration of each pathogen. The second inoculation was done 7 days after the first.

TABLE 5. Effects of single and simultaneously mixed inocula of *Cochliobolus sativus* and *Pyrenophora tritici-repentis* on grain yield of wheat cultivar Candiota

Treatments[a]	Yield (g)[b]	Yield reduction (%)
Uninoculated	81.6	0
C.s. 100%	48.9	40.0
P.t-r. 100%	65.1	20.2
C.s. (50%) + P.t-r. (50%)	62.7	23.1

[a] C.s. = *C. sativus*, P.t-r. = *P. tritici-repentis*. Original (100%) suspensions were 10^4 conidia per milliliter.

[b] Mean of four replicates. Fisher's "protected" LSD = 10.4. Mean differences greater than the "protected" LSD differ significantly at $P = 0.05$.

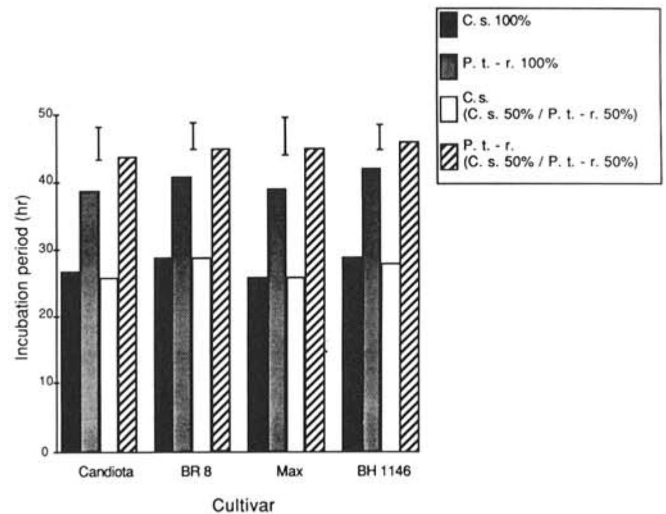

Fig. 2. Effects of single and simultaneously mixed inocula of *Cochliobolus sativus* and *Pyrenophora tritici-repentis* on incubation period on four spring wheat cultivars. Mean values are from four replicates. Vertical bars for each cultivar indicate values of the Fisher's "protected" least significant differences between means at $P = 0.05$.

TABLE 6. Germination, germ tube growth, and appressorium formation 18 hr after inoculation of wheat cultivars with individual or mixed (50:50) inocula of *Pyrenophora tritici-repentis* and *Cochliobolus sativus*

Pathogens[a] and cultivars	Conidium germination (%)		Germ tube length (mm)		Appressorium formation (%)	
	C.s.	P.t-r.	C.s.	P.t-r.	C.s.	P.t-r.
C.s. 100%						
Candiota	87[b] ± 4	...	241 ± 14	...	45 ± 4	...
BR8	83 ± 4	...	236 ± 12	...	43 ± 4	...
BH1146	85 ± 3	...	240 ± 11	...	45 ± 3	...
Max	86 ± 6	...	243 ± 9	...	42 ± 6	...
P.t-r. 100%						
Candiota	...	83 ± 4	...	71 ± 6	...	42 ± 5
BR8	...	81 ± 3	...	66 ± 5	...	43 ± 6
BH1146	...	80 ± 5	...	69 ± 4	...	40 ± 5
Max	...	84 ± 6	...	72 ± 6	...	43 ± 2
P.t-r. 50% + C.s. 50%						
Candiota	83 ± 5	67 ± 3	244 ± 8	53 ± 5	44 ± 7	31 ± 3
BR8	86 ± 3	64 ± 5	238 ± 11	50 ± 4	43 ± 4	30 ± 4
BH1146	84 ± 4	66 ± 6	241 ± 10	49 ± 6	46 ± 5	29 ± 2
Max	85 ± 5	69 ± 3	240 ± 9	54 ± 3	44 ± 2	33 ± 3

[a] C.s. = *C. sativus*, P.t-r. = *P. tritici-repentis*. Original (100%) inoculum concentration was 10^4 conidia per milliliter.

[b] Mean and standard deviation of four replicates of 200 conidia per treatment.

The percentage of germination, appressorium formation, germ tube length, and incubation period were also evaluated on cultivar Candiota inoculated with 50% of the original conidium concentration of each pathogen alone (Table 7). No differences were observed in any of these parameters when compared to the original (100%) spore concentrations shown in Table 6.

DISCUSSION

Microorganisms commonly occur in mixed populations on plant leaf surfaces. During pathogenesis, the relationship between pathogenic microorganisms may be antagonistic, synergistic, or neutralistic; each type of interaction has been reported between fungal foliar pathogens on wheat (3,6,11,12,15,19). However, the present results demonstrated antagonism but not synergism or neutralism between *C. sativus* and *P. tritici-repentis*. Neutralism and antagonism have been reported in interactions between *C. sativus* and *Phaeosphaeria nodorum*, and neutralism was observed between *P. tritici-repentis* and *Phaeosphaeria nodorum* (6). The absence of synergism or antagonism between *Mycosphaerella graminicola* and *Phaeosphaeria nodorum* has also been reported elsewhere (12).

Because of their similar modes of pathogenesis, one might have expected the necrotrophic fungi, *C. sativus*, *Phaeosphaeria nodorum*, and *P. tritici-repentis*, to interact synergistically on wheat leaves. However, an antagonism of *C. sativus* towards *P. tritici-repentis* was observed, both when the organisms were inoculated simultaneously or when *C. sativus* was inoculated up to 6 hr after *P. tritici-repentis*. The antagonism of *C. sativus* towards *P. tritici-repentis* affected initial phases of fungus development by suppressing conidium germination, appressorium formation, and germ tube elongation on the leaf surface. Presumably, these effects resulted in the decreased disease severity observed. Ghazanfari-Heramabadi (8) observed previously that *C. sativus* inhibited the perithecial formation and growth of *P. tritici-repentis* in culture.

We found that the incubation period of *P. tritici-repentis* increased in the presence of *C. sativus*. Environmental conditions were probably not responsible for the interaction since *P. tritici-repentis* developed well when inoculated alone. An increase in plant resistance to *P. tritici-repentis* induced by *C. sativus* could also account for the decrease in tan spot symptoms. However, the observed inhibition of the initial phases of pathogenesis suggests that the direct effect of *C. sativus* on *P. tritici-repentis* was significant. Competition also may have been involved in the interaction, not only because *C. sativus* has a shorter incubation period than *P. tritici-repentis*, but also because of the greater number of reisolations of *C. sativus* resulting from simultaneous inoculation or subsequent inoculation up to 6 hr after *P. tritici-repentis*. *P. tritici-repentis* was recovered in higher percentages when inoculated 12–168 hr before *C. sativus*. This seemed to be enough time for *P. tritici-repentis* to become established and to compete for plant nutrients and space. The significantly greater number of reisolations of *C. sativus* resulting from mixed inoculations also suggested that this fungus exerted an inhibitory effect on *P. tritici-repentis* during the associative phases of pathogenesis (i.e., penetration and postpenetration events).

Our results also indicated an antagonistic effect of *C. sativus* on grain yield reductions induced by *P. tritici-repentis*. The yield reduction in plants inoculated with *C. sativus* (50%) + *P. tritici-repentis* (50%) was smaller than half the sum of the reductions obtained in plants inoculated with each of the fungi (100%) independently. In the absence of antagonism or synergism, such reductions should be similar.

The inhibitory action of *C. sativus* against *P. tritici-repentis* may be significant in the field. Where *C. sativus* is an important foliar wheat pathogen, *P. tritici-repentis* may be inhibited, resulting in a reduction of tan spot. This may be the case in Brazil where *C. sativus* is an extremely important wheat pathogen (5). In addition, the antagonistic activity of *C. sativus* against *P. tritici-repentis*, even at low inoculum concentrations of *C. sativus*, suggests that, in fields where *P. tritici-repentis* is an important pathogen, little or no *C. sativus* may be present on the wheat leaf surface. This may be the case in New York (7), North Dakota (10), and Western Canada (21). Because both wheat pathogens are favored by long periods of leaf wetness (4,9) and both require similar temperatures for optimal development (6), the potential for antagonism of *C. sativus* against *P. tritici-repentis* is apparently great, and could result in natural biological control. The study described was done with only one isolate of each pathogen. Further studies including other isolates are worthy of investigation. Other fungi in addition to foliar cereal pathogens have been shown to be inhibited by *C. sativus* (6,16,20,22). *C. sativus* or its metabolites potentially could provide effective biological protection against tan spot of wheat. The prospects for a practical biological control would be enhanced if naturally occurring or genetically engineered isolates of *C. sativus* nonpathogenic to cultivated cereals or grasses proved to have suppressive activity against *P. tritici-repentis* and other cereal pathogens.

TABLE 7. Germination, germ tube length, appressorium formation, and incubation period after single fungal inoculation of wheat cultivar Candiota with 50% of the original inoculum (10^4 conidia per milliliter) of *Pyrenophora tritici-repentis* and *Cochliobolus sativus*

Pathogen[a]	Germination (%)	Germ tube length (μm)	Appressorium formation (%)[b]	Incubation period (hr)
C.s	84 ± 5	244 ± 9	42 ± 4	28 ± 2
P.t-r.	83 ± 6	75 ± 6	41 ± 6	38 ± 3

[a] C.s. = *C. sativus*, P.t-r. = *P. tritici-repentis*.
[b] Mean and standard deviation of four replicates of 200 conidia per treatment.

LITERATURE CITED

1. Alcorn, J. L. 1983. Generic concepts in *Drechslera*, *Bipolaris* and *Exserohilum*. Mycotaxon 17:1-8.
2. Brokenshire, T. 1974. Predisposition of wheat to *Septoria tritici* infection following attack by *Erysiphe*. Trans. Br. Mycol. Soc. 63:393-397.
3. Chester, K. S. 1944. Low incidence of wheat leaf rust associated with unfavorable late winter weather and antagonism of *Septoria tritici*. Plant Dis. Rep. 28:280-287.
4. da Luz, W. C. 1982. Influêncía do período de umidificacção pós-inoculacção na reacção de cultivares de trigo à mancha foliar (*Cochliobolus sativus*). Fitopatol. Bras. 7:111-115.
5. da Luz, W. C. 1984. Yield losses caused by fungal foliar wheat pathogens in Brazil. Phytopathology 74:1403-1407.
6. da Luz, W. C. 1986. Development of the wheat leaf spot syndrome as influenced by temperature, interactions among fungal pathogens and triadimenol seed treatment. Ph.D. thesis. Cornell University. 118 pp.
7. da Luz, W. C., and Bergstrom, G. C. 1986. Distribution, prevalence, and severity of fungal foliar diseases of spring wheat in New York in 1984 and 1985. Plant Dis. 70:842-847.
8. Ghazanfari-Heramabadi, J. A. 1983. Interactions of the tan spot fungus, *Pyrenophora trichostoma*, and selected microorganisms. Ph. D. thesis. Oklahoma State University. 54 pp.
9. Hosford, R. M., Jr. 1971. A form of *Pyrenophora trichostoma* pathogenic to wheat and other grasses. Phytopathology 61:28-32.
10. Hosford, R. M., Jr. 1976. Fungal leaf spot diseases of wheat in North Dakota. N. D. Agric. Exp. Stn. Bull. 500. 12 pp.
11. Hyde, P. M. 1978. A study of the effects on wheat of inoculation with *Puccinia recondita* and *Leptosphaeria nodorum*, with respect to possible interactions. Phytopathol. Z. 92:12-24.
12. Jenkins, P. D., and Jones, D. G. 1981. The effects of dual inoculation of wheat cultivars with *Septoria tritici* and *Septoria nodorum*. Phytopathol. Z. 101:210-221.
13. Johnston, C. O. 1934. The effect of mildew infection on the response of wheat leaf tissues normally resistant to leaf rust. Phytopathology 24:1045-1046.
14. Khan, T. N. 1971. Effect of light on sporulation in *Drechslera tritici-repentis*. Trans. Br. Mycol. Soc. 56:309-311.
15. Lang, W. 1917. Uber die beeinflussung der Wertpfanze durch *Tilletia tritici*. Z. Pflanzenkr. Pflanzenshutz 27:81-99.
16. Morton, D. J., and Peterson, G. A. 1960. An inverse relationship between the severities of Helminthosporium leaf blade and Septoria leaf sheath symptoms on barley. Plant Dis. Rep. 44:23-24.
17. Odvody, G. N., and Boosalis, M. G. 1978. A rapid technique to study

sporulation requirements of *Pyrenophora trichostoma*. Phytopathol. News 12:212-213.
18. Platt, H. W., Morral, R. A. A., and Green, H. E. 1977. The effects of substrate, temperature and photoperiod on conidiation of *Pyrenophora tritici-repentis*. Can. J. Bot. 55:254-259.
19. Sempio, C. 1938. On an experimental case of distinct antagonism in vivo (*Tilletia caries*–*Erysiphe graminis* on "Mentana"). Riv. Patol. Veg. 28:377-384.
20. Stewart, D. M., and Hill, J. H. 1965. Toxicity of *Helminthosporium sorokinianum* and other fungi to *Puccinia graminis* var. *tritici*. Plant Dis. Rep. 49:371-374.
21. Tekauz, A. 1976. Distribution, severity and relative importance of leaf spot diseases of wheat in Western Canada in 1974. Can. Plant Dis. Surv. 56:36-40.
22. Tyner, L. E. 1966. Associative effects of fungi on *Cochliobolus sativus*. Phytopathology 56:776-780.
23. van der Wall, A. F., Shearer, B. L., and Zadoks, J. C. 1970. Interactions between *Puccinia recondita* f. sp. *triticina* and *Septoria nodorum* on wheat, and its effects on yield. Neth. J. Plant Pathol. 76:261-263.
24. Zadoks, J. C., Chang, T. T., and Konzak, C. F. 1974. A decimal code for growth stages of cereals. Weed Res. 14:415-421.