Antagonism Between the Cereal Root Rot Pathogens *Fusarium graminearum* and *Bipolaris sorokiniana*

S. C. SCARDACI, Farm Advisor, University of California Cooperative Extension, Davis 95616, and R. K. WEBSTER, Professor, Department of Plant Pathology, University of California

ABSTRACT

Scardaci, S. C., and Webster, R. K. 1981. Antagonism between the cereal root rot pathogens *Fusarium graminearum* and *Bipolaris sorokiniana*. Plant Disease 65:965-967.

Antagonism between *Fusarium graminearum* and *Bipolaris sorokiniana* occurred when both were infested simultaneously on barley, resulting in lower levels of seedling blight and root rot. Both pathogens competed equally well for host substrate when infested together at planting. However, when they were infested in sequence, one 21 days before the other, the pathogen infested first was reisolated most frequently, indicating the importance of prior colonization and possession of substrate.

Soil fungi, bacteria, and actinomycetes have been shown to inhibit the cereal root rot pathogens *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem., *Fusarium graminearum* Schwabe, and *F. culmorum* (W. G. Sm.) Sacc. Several fungi, including *Trichoderma viride* Pers. ex Fr., *Phoma humicola* Gilman & Abbott, and *Epicoccum purpurascens* Ehrenb., are antagonistic to *Helminthosporium sativum* Pamm., King, & Bakke (= *B. sorokiniana*) and cause lysis of its mycelium (4,5). *Trichoderma lignorum* Harz suppresses common root rot disease on wheat when inoculated with *H. sativum* or *F. culmorum* (2). Numerous actinomycetes and *Pseudomonas viscosa* (Frankland & Frankland) Migula were shown to produce antibiotics inhibitory to *H. sativum*, reducing the level of common root rot (10). In addition, more than 50 isolates of bacteria and actinomycetes were shown to inhibit *F. graminearum* and *H. sativum* in culture (7).

Tinline (11) reported studies of single or combination inoculations of wheat with *Cochliobolus sativus, F. culmorum,* and *F. acuminatum.* He found that prepossession of the internode by *C. sativus* does not prevent subsequent invasion by the fusaria, but that prepossession by the fusaria greatly reduces subsequent infection by *C. sativus.*

Ledingham (6) demonstrated antagonism between isolates of *F. culmorum* and *H. sativum* when inoculated simultaneously on wheat. He also showed that germination of *H. sativum* conidia is inhibited by the presence of conidia of *F. culmorum.* These findings appear to support the conclusions of Tinline (11).

Oswald (8,9) isolated *Gibberella*-producing clones of *F. roseum* f. sp. *cerealis* (Cke.) Snyd. & Hans. (= *F. graminearum*) and *H. sativum* (= *B. sorokiniana*) from wheat and barley showing typical root rot symptoms. He reported that these pathogens were frequently isolated from the same field and several times from the same plant.

This paper concerns the antagonistic interaction of single isolates of *F. graminearum* and *B. sorokiniana* on barley.

Accepted for publication 20 March 1981.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

0191-2917/81/12096503/$03.00/0

MATERIALS AND METHODS

Disease interactions of single isolates of *F. graminearum* and *B. sorokiniana* on barley cultivar CM72 were evaluated in the greenhouse either infested alone or together at various time intervals. Plants were grown for 8 wk in 6-in. plastic pots containing pasteurized U. C. mix (1). Inoculum of *F. graminearum* was produced from single conidia grown on CMA+ (17 g of Difco cornmeal agar, 0.1 g of yeast extract, 0.1 g of Casamino Acids, and 1 L of distilled water). *B. sorokiniana* was grown on V-8 juice agar. Both were incubated under cool white fluorescent light at room temperature (24 ± 2 C) for 2–3 wk.

Plates were flooded with tap water and the agar surface scraped with a glass slide. The resulting suspension was strained through two layers of cheesecloth, stirred, and the conidial concentration was determined using a hemacytometer. Inoculum was applied with a 50-cc hypodermic syringe 2–4 cm beneath the U. C. mix surface near the seed or plant at planting or 21 days after planting at rates of 500 or 1,000 propagules per gram of soil within the entire pot. The experiment, with six plants per pot after thinning, consisted of nine treatments with four replicates each.

After 8 wk, blighted seedlings were counted and plants were rated for disease severity on a four-point scale with 0 = no symptoms, plant healthy; 1 = light symptoms, discoloration of the leaf sheath; 2 = moderate symptoms, discoloration of the culm and/or the subcrown internode; 3 = severe symptoms, discoloration and rotting of the crown; 4 = plant killed, postemergence seedling blight. A mean disease rating (MDR) was calculated by multiplying the number of plants in each category by their numerical rating, adding them, and dividing by the total number of plants rated.

Isolations were made from plants that received a rating of 2 or greater. Diseased tissue from each plant was surface-sterilized by submersion in 0.05% sodium hypochlorite for 5 min, cut into five pieces, and placed on acid potato-dextrose agar (pH 4–5). After 5 days of incubation, culture plates were examined for the presence of *B. sorokiniana* and/or *F. graminearum*. Seedling blight data expressed as a percentage were analyzed using the arc sin transformation; MDR data were analyzed without transformation.

RESULTS

Antagonistic interactions between *F. graminearum* and *B. sorokiniana* affected the percentage of blighted seedlings, the MDR, and reisolation percentages. The MDR of plants grown in pasteurized U. C. mix infested at different times and in different combinations with *F. graminearum* and *B. sorokiniana* is shown in Table 1. Table 2 shows the reisolation percentage of these pathogens from different treatments. Percentage of seedling blight, MDR, and reisolation percentages for treatments where *B. sorokiniana* or *F. graminearum* was infested at 1,000 propagules per gram at either planting or 21 days after planting were similar to their counterparts infested at 500 propagules per gram.

When both pathogens were infested together at planting, the percentage of seedling blight and MDR of barley were significantly lower ($P = 0.05$) than for plants grown in pasteurized U. C. mix infested at planting with *F. graminearum* alone. However, percentage of seedling blight and MDR were not lower than for plants grown in pasteurized U. C. mix infested at planting with *B. sorokiniana*. Barley grown in pasteurized U. C. mix infested with *F. graminearum* at planting and *B. sorokiniana* 21 days after planting had a significantly lower percentage of seedling blight ($P = 0.05$) than plants infested at planting with *F. graminearum* alone.

F. graminearum and *B. sorokiniana* were isolated less frequently from the same plant, even though they were infested simultaneously. Usually, one or the other was isolated from individual plants (79 and 94%, respectively). They were isolated from different plants at about the same frequency (42 and 37%, respectively) when both were infested together at planting. However, when one of these pathogens was infested before the other, the one infested first was isolated most frequently. When both were infested together 21 days after planting, *B. sorokiniana* was isolated more frequently (83 vs 11%) than *F. graminearum*. A significantly lower level of disease ($P = 0.01$) was measured on plants grown in pasteurized U. C. mix infested with *F. graminearum* 21 days after planting (MDR of 0.69) when compared with plants infested with *F. graminearum* at planting (MDR of 3.06). This reduction in disease was not apparent when plants were infested with *B. sorokiniana* at different times (MDR of 3.15).

Table 1. Antagonism between *Bipolaris sorokiniana* and *Fusarium graminearum* and its effect on common root rot of barley[a]

Pathogen infested[b]			
At planting	**21 days after planting**	**Seedling blight, %[c]**	**MDR[d]**
Check	Check	0 z[e]	0.00 z
F. graminearum + *B. sorokiniana*		5 z	2.05 y
F. graminearum		63 x	3.06 x
B. sorokiniana		0 z	1.77 y
F. graminearum	*B. sorokiniana*	39 y	3.15 x
B. sorokiniana	*F. graminearum*	0 z	1.66 y
	F. graminearum	0 z	0.69 z
	B. sorokiniana	0 z	1.54 y
	F. graminearum + *B. sorokiniana*	0 z	1.70 y

[a]The barley cultivar CM72 was grown in the greenhouse for 8 wk.
[b]All isolates were infested at 500 propagules per gram of U. C. mix.
[c]Values are means of four replicates.
[d]Mean disease rating (MDR) ranges from 0 (healthy) to 4 (severe).
[e]Means followed by common letters in the same column do not differ significantly ($P = 0.05$).

Table 2. Differential colonization of barley by *Bipolaris sorokiniana* and/or *Fusarium graminearum*[a]

Pathogen infested[b]		Reisolation[c]			
At planting	**21 days after planting**	**No. of plants**	***F. graminearum*, %**	***B. sorokiniana*, %**	***F. graminearum* + *B. sorokiniana*, %**
F. graminearum + *B. sorokiniana*		19	42	37	16
F. graminearum		17	100	0	0
B. sorokiniana		21	0	86	5
F. graminearum	*B. sorokiniana*	21	71	24	5
B. sorokiniana	*F. graminearum*	15	0	100	0
	F. graminearum	6	100	0	0
	B. sorokiniana	16	0	75	0
	F. graminearum + *B. sorokiniana*	18	11	83	6

[a]The barley cultivar CM72 was grown in the greenhouse for 8 wk.
[b]All isolates were infested at 500 propagules per gram of U. C. mix.
[c]Only plants that received a rating of 2 or greater, on a scale of 0 (healthy) to 4 (severe), were included in the isolations.

DISCUSSION

Antagonism occurred between *F. graminearum* and *B. sorokiniana*, reducing common root rot severity and seedling blight of barley when inoculated simultaneously at planting. Ledingham (6) observed antagonism between *F. culmorum* and *H. sativum* (*B. sorokiniana*), although neither was consistently antagonistic to the other. Tinline (11) reported multiple infections of wheat by different isolates of *C. sativus* when inoculated at different times of plant development, and he concluded that "cross protection" by isolates of *C. sativus* was lacking. He further noted a differential antagonism between *C. sativus* and *F. culmorum* or *F. acuminatum* that depends upon which fungus first colonizes the subcrown internode. The fusaria readily invade internodes infected with *C. sativus*, but "prepossession" by fusaria restricts subsequent attack by *C. sativus*. He concluded that *C. sativus* was probably the initial invader when *C. sativus* and fusaria are isolated from the same plant.

Our results indicated that *B. sorokiniana* and *F. graminearum* were capable of precluding the establishment of the other within the host. We found that infection and subsequent colonization of barley by the pathogen infested first inhibited the establishment of the pathogen that followed. This may be similar to the phenomenon described by Bruehl and Lai (3), where prior colonization and possession of substrate are important in the saprophytic colonization and survival of fungi on wheat straw. Our findings suggest that this phenomenon of "prior colonization-possession" is also important for infection and pathogenesis of fungi attacking the same host and may explain why these pathogens were isolated infrequently from the same plant.

F. graminearum and *B. sorokiniana* competed equally well for barley when inoculum was applied together at planting, whereas they did not compete equally well when inoculum was applied together 21 days after planting. In the latter case, *F. graminearum* was less competitive than *B. sorokiniana*.

We emphasize that this study and others (6,11) were carried out using autoclaved soil. This approach is necessary to avoid masking of the interaction of the cereal root rot pathogens by other microflora (2,4,7,10). Tests measuring differences in amount of seedling blight or root rot of barley in autoclaved and unsterile field soil have consistently resulted in significantly less disease development in unsterile soil. Thus, until the interactions of the entire microflora are better known, we should be cautious in accepting suggestions that antagonistic interactions between common root rot pathogens may provide some degree of biological control when fields contain populations of both pathogens.

LITERATURE CITED

1. Baker, K. F. 1957. The U. C. system of producing healthy container-grown plants. Calif. Agric. Exp. Stn. Ext. Serv. Manual 23. 332 pp.
2. Bisby, G. R., James, N., and Timonin, M. 1933. Fungi isolated from Manitoba soil by the plate method. Can. J. Res. 8:253-275.
3. Bruehl, G. W., and Lai, P. 1966. Prior-colonization as a factor in the saprophytic survival of several fungi in wheat straw. Phytopathology 56:766-768.
4. Campbell, W. P. 1956. The influence of associated microorganisms on the pathogenicity of *Helminthosporium sativum*. Can. J. Bot. 34:865-874.
5. Griffin, D. M. 1972. Ecology of Soil Fungi. Syracuse University Press, Syracuse, NY. 193 pp.
6. Ledingham, R. J. 1942. Observations on antagonism in inoculation tests of wheat with *Helminthosporium sativum* P., K., and B., and *Fusarium culmorum* (W. G. Sm.) Sacc. Sci. Agric. 22:688-697.
7. Old, K. 1965. Fungistatic effects of soil bacteria on root-rotting fungi with particular reference to *Helminthosporium sativum*. Phytopathology 55:901-905.
8. Oswald, J. W. 1942. Taxonomy and pathogenicity of fungi associated with root rot of cereals in California, with special reference to the fusaria and their variants. Ph.D. thesis, University of California, Berkeley. 129 pp.
9. Oswald, J. W. 1950. Etiology of cereal root rots. Hilgardia 19:447-462.
10. Sallans, B. J. 1965. Root rots of cereals. III. Bot. Rev. 31:505-536.
11. Tinline, R. D. 1977. Multiple infections of subcrown internodes of wheat (*Triticum aestivum*) by common root rot fungi. Can. J. Bot. 55:30-34.