

## Grain Yield Responses of Winter Wheat Coinoculated with *Cephalosporium gramineum* and *Gaeumannomyces graminis* var. *tritici*

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### ABSTRACT

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Winter wheat (*Triticum aestivum*) was coinoculated at planting with one of four levels of inoculum of *Cephalosporium gramineum* and/or *Gaeumannomyces graminis* var. *tritici* in all possible combinations during each of 5 yr in the field. Response-surface models of the data were generated that described the effect of the interaction of the two pathogens on grain yields. There was a linear effect ( $P \leq 0.001$ ) of *C. gramineum* inoculum on yield loss in all 5 yr, and a quadratic effect ( $P \leq 0.01$ ) in 2 of the 5 yr. For inoculum of *G. g. tritici*, there were linear and quadratic effects ( $P \leq 0.001$ ) all 5 yr. Additionally, a negative interaction ( $P \leq 0.01$ ) occurred between inoculum levels of the two fungi in all 5 yr. These data indicate that, when the two organisms occur simultaneously in a field, they can compete slightly with each other as pathogens of the wheat host.

Additional keywords: Cephalosporium stripe, pathogen interactions, take-all

Wheat (*Triticum aestivum* L.) is affected by approximately 85 diseases caused by various infectious agents (26). Of these diseases, 14 have been important recently on winter wheat in Kansas and collectively have caused an estimated annual loss of 15.3% (22). These losses occurred in spite of the numerous control procedures practiced by producers.

Because different wheat diseases frequently occur together in the same field (22), interactions among different pathogens and the host are common and have the potential to affect the damage to the plant. Three types of resultant damage from such interactions are possible: synergism, addition, and antagonism.

Two of the pathogens that cause important diseases of winter wheat in Kansas occupy similar habitats. *Cephalosporium gramineum* Nisikado & Ikata in Nisikado et al causes Cephalosporium stripe, and *Gaeumannomyces graminis* (Sacc.) Arx & D. Olivier var. *tritici* J. Walker causes take-all. Both of these fungi are soilborne, inhabit wheat residue (14,27), infect the roots (6,13), and are favored by continuous wheat production (5,9), reduced tillage (4,17), high soil moisture (1,10), and early planting

(11,21). They both tend to occur in the same geographical area of Kansas (22), and the potential exists for interactions between them.

The hypothesis of this research is that a significant antagonistic effect occurs between the two pathogens, because they occupy similar ecological niches. Successful infection by *C. gramineum* requires damage to wheat roots, usually from low soil pH (1) or root freezing and breakage from soil heaving that occurs from late fall through early spring (2,18). Once inside the root, the fungus moves and multiplies entirely within the vascular system of the wheat plant (25). Before or during the infection period for *C. gramineum*, *G. g. tritici* produces root lesions that progress to the stele and occlude the vascular system (3,8,12). These take-all lesions could block host colonization by *C. gramineum* as it moves through the xylem, limiting the expression of Cephalosporium stripe. Previous research on these diseases involved investigations with the pathogens alone; to our knowledge, there are no reports of how these fungi interact. Therefore, the objective of this research was to quantify the effects of coinoculations with various levels of these two pathogens on subsequent grain yields of winter wheat in the field.

### MATERIALS AND METHODS

Experiments were established during 5 yr from the 1985–86 season through the 1990–91 season at the Rocky Ford Research Farm near Manhattan, Kan-

sas. The soil type was a Chase silty clay loam (pH 6.0 in 2:1 water:soil), but experimental sites were different each year. Each site was cropped to winter wheat in at least one previous year; however, no evidence of naturally occurring Cephalosporium stripe or take-all was seen at any site.

Because microplots were suggested for studying associations between pathogens (20), the following experimental design was used: single-row plots, 4.6 m long and spaced 1.0 m apart, were arranged in a randomized complete-block design with four (1985–86 and 1990–91) or five (remaining 3 yr) replications. The winter wheat cultivar Sturdy, which is susceptible to both pathogens, was used in all experiments. Seeding (67.2 kg/ha) occurred between 22 September and 3 October, and grain was harvested between 18 and 30 June with a small-plot combine. Standard fertilizer recommendations for the area were followed based on a bulked soil test for the site (24). This usually involved a spring application of  $\text{NH}_4\text{NO}_3$  (34 kg N/ha) when leaf sheaths were strongly erected. Weeds were controlled by hand hoeing.

Each experiment included 16 treatments consisting of four levels of inoculum of each of the two pathogens in all possible combinations. Colonized, air-dried oat grains were used as the carrier for inoculum of between four and six isolates of each of the fungi. This type of artificial inoculum simulates natural inoculum of the pathogens (9,16). Based on past experience with the fungi, inoculum rates were chosen so that the highest rate of one pathogen would produce about 75% yield loss in a conducive year. During 1985–86, inoculum rates were 0, 3.0, 8.0, or 20.0 g of colonized oat kernels per 4.6-m row for *C. gramineum* and 0, 1.0, 2.0, and 6.0 g per 4.6 m for *G. g. tritici*. In subsequent years, inoculum rates were 0, 2.0, 5.0, and 10.0 g for *C. gramineum* and 0, 0.5, 1.5, and 3.0 g per 4.6 m for *G. g. tritici*. Inoculum in the various combinations was introduced (5 cm deep) with the seed at planting.

**Statistical analysis.** Percentage yield losses were calculated by comparing the grain yields of individual plots with the yield of the noninoculated check within that replication. Yield losses due to *C.*

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*gramineum* and/or *G. g. tritici* were partitioned into linear, quadratic, and interaction terms using stepwise multiple regression analysis. Year  $\times$  inoculum interactions were examined using the General Linear Models (GLM) Procedure of SAS (SAS Institute, Cary, NC), and years were combined where appropriate.

## RESULTS

Grain yield potential at the sites was at or above the normal Kansas average (2,300 kg/ha). Yields for the noninoculated treatment were: 3,373, 3,769, 3,322, 2,224, and 4,603 kg/ha for 1985–86, 1986–87, 1987–88, 1988–89, and 1990–91, respectively.

Severe take-all developed during all 5 yr; 59–91% yield loss occurred with the high inoculum rate of *G. g. tritici* alone (Figs. 1 and 2). Conversely, the environment was highly conducive to development of *Cephalosporium* stripe during

only 2 of the 5 yr (Fig. 1). In the other 3 yr (Fig. 2), yield loss was less than 27% with the high inoculum rate of *C. gramineum* alone. The 1988–89 and 1990–91 seasons had no significant year  $\times$  treatment interactions for inoculum of either pathogen by itself or for the interaction effect of inoculum of both pathogens; therefore, those years were combined for analysis (Fig. 2B). All of the other comparisons between years had significant interactions, and these data were not combined.

The data were described by the general equation  $Y = (\beta_1 * Cg) + (\beta_2 * Cg^2) + (\beta_3 * Ggt) + (\beta_4 * Ggt^2) + (\beta_5 * Cg * Ggt)$ , where  $Y$  = yield loss;  $Cg$  = level of inoculum of *C. gramineum* (in grams of oat-kernel inoculum per 4.6 m of row length);  $Ggt$  = level of inoculum of *G. g. tritici*; and  $\beta_1, \beta_2, \beta_3, \beta_4,$  and  $\beta_5$  are parameter estimates. Because the noninoculated treatment was considered 0%

yield loss, the intercept ( $\beta_0$ ) equals zero. Using these equations, surface-response models had high  $R^2$  values for all 5 yr (Table 1). For the effect of level of *C. gramineum* inoculum, linear parameter estimates ( $\beta_1$ ) were significant ( $P \leq 0.001$ ) in all 5 yr (Table 1). The quadratic estimates ( $\beta_2$ ) were significant ( $P \leq 0.01$ ) in only 2 yr; however, during 1986–87, the quadratic estimate was significant at  $P \leq 0.10$ . For the effect of the level of *G. g. tritici* inoculum, parameter estimates for both the linear ( $\beta_3$ ) and the quadratic ( $\beta_4$ ) components of the equation were significant ( $P \leq 0.001$ ) in all 5 yr. Additionally, a significant ( $P \leq 0.01$ ) negative interaction (5) occurred between inoculum levels of *C. gramineum* and *G. g. tritici* in all 5 yr. This was true even during 1986–87, 1988–89, and 1990–91, when total yield loss from the high rate of both pathogens together was relatively low compared with the

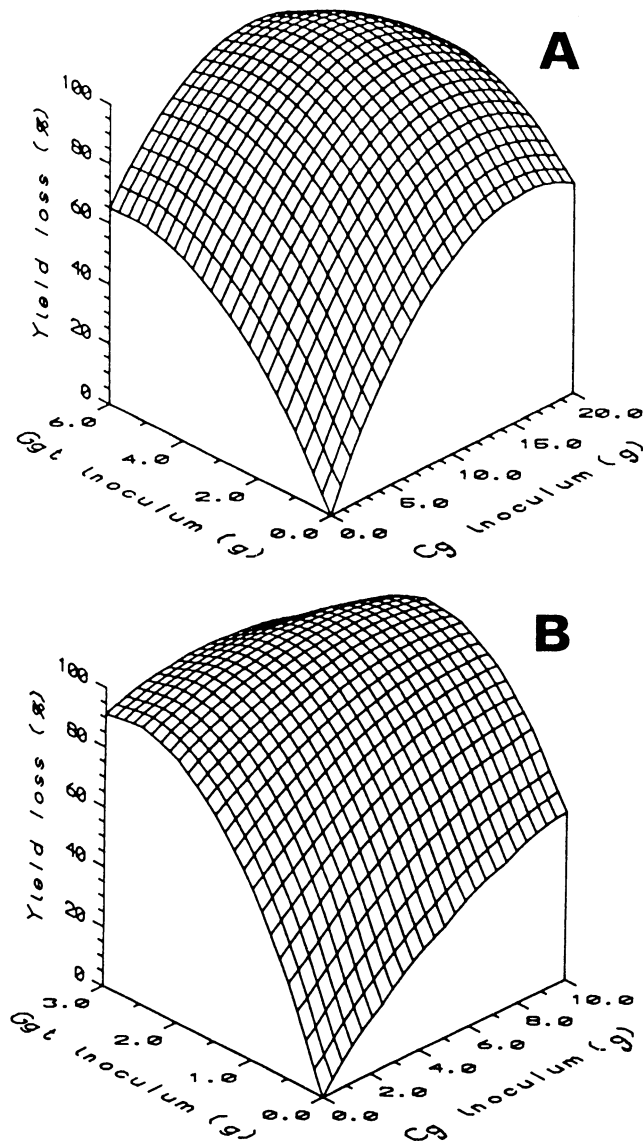


Fig. 1. Response-surface curves for effect of level of inoculum (g/4.6-m row) of *Gaeumannomyces graminis* var. *tritici* (Ggt) and/or *Cephalosporium gramineum* (Cg) on winter wheat grain yield loss during the (A) 1985–86 season and (B) the 1987–88 season.

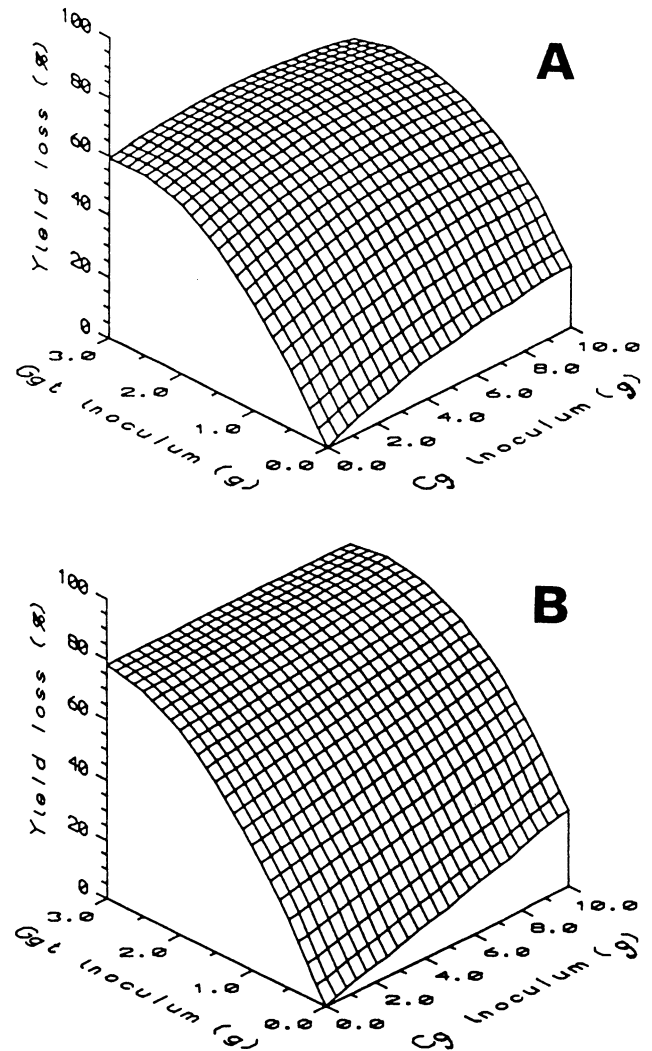


Fig. 2. Response-surface curves for effect of level of inoculum (g/4.6-m row) of *Gaeumannomyces graminis* var. *tritici* (Ggt) and/or *Cephalosporium gramineum* (Cg) on winter wheat grain yield loss during (A) the 1986–87 season and (B) the combined seasons of 1988–89 and 1990–91.

other 2 yr and did not exceed 66, 81, and 82%, respectively (Fig. 2).

## DISCUSSION

Relatively simple response-surface models were adequate to describe the effect of inoculum levels of both pathogens occurring together on loss of grain yield. These models incorporated terms for the linear and quadratic effects of inoculum level of each pathogen and a term for the interaction of inoculum of both pathogens. The apparent slight increases in yield (reductions in yield loss) observed in the upper quadrants of the surfaces for two of the years (Fig. 1) are artifacts of the models. The actual response-surface data flatten off rather than descend.

Although data from two of the years were combined, significant year  $\times$  treatment interactions occurred among the other years, and no single model was able to describe the response for all 5 yr. This was evident from the examination of losses that occurred in different years from a single inoculum rate of one of the pathogens by itself. Based on estimates from the models generated, the 10-g rate of inoculum of *C. gramineum* caused between 26 and 65% loss, depending on the year. Similarly, the 3-g rate of *G. g. tritici* resulted in 52–91% loss. Environmental differences among years probably resulted in the different losses from equivalent amounts of inoculum and, thus, the significant year  $\times$  treatment interactions. Therefore, inoculum level by itself is not an accurate predictor of yield loss.

In addition to the variable environment, the inoculum potential of oat-kernel inoculum was probably slightly different among years. The nutritional status and degree of fungal colonization of the oat kernels would be variable from year to year. However, differences in inoculum potential were probably small and could not account for the variability in yield loss among years.

Relatively little *Cephalosporium* stripe developed during 3 of the 5 yr of this study (Fig. 2). These years had mild winters, and there was probably little root damage from soil heaving due to freezing, resulting in fewer infection courts (2,18). Thus, the terms incorporated into the equations for these 3 yr were different from those for the remaining 2 yr. In the 3 yr when yield loss from the high rate of inoculum of *C. gramineum* was low (Fig. 2), the quadratic effect of inoculum level was not significant (Table 1). Conversely, when yield loss was high (Fig. 1), the quadratic effect was significant. This follows typical yield loss/inoculum level curves for other pathogens (7). The addition of a given amount of inoculum has less effect on disease when a relatively high percentage of plants is infected than when a low percentage is infected. Intra-

pathogen competition for photosynthate, space, and other parameters, or an asymptote for maximum loss (minimum yield) produces the quadratic effect (7). Our data showed significant intrapathogen competition for *C. gramineum* when yield loss for the high rate (no *G. g. tritici*) was 60–70% (Fig. 1), but no competition when loss was less than 27% (Fig. 2).

Unlike results for *C. gramineum*, significant quadratic effects occurred for inoculum of *G. g. tritici* in all 5 yr. This was true even in 1985–86 and 1986–87, when yield loss from the high rate (with no *C. gramineum*) was relatively low (60 vs. 80–90%). This provides evidence for intrapathogen competition with this fungus when yield loss is less than 60%. However, because no years showed very low losses (10–50%) from the high rate, we do not know at what level the intrapathogen competition begins for *G. g. tritici*. Analysis of data after the removal of values from the high inoculum rates in an attempt to address this question was not done, because it would not have left enough inoculum variables for an accurate analysis (7).

Many synergistic reactions have been reported between pathogens (20). As an example, coinoculation of corn with maize chlorotic mottle and wheat streak mosaic viruses causes a severe necrotic response, whereas each virus by itself produces only mild symptoms (19). Similarly, infection by the powdery mildew fungus increases the susceptibility of barley to leaf rust (23). Such an interaction could be hypothesized for *C. gramineum* with *G. g. tritici*. *C. gramineum* requires root wounding for infection (2,18), and *G. g. tritici* produces

necrotic root lesions during the fall and winter (3), corresponding to the time for infection by *C. gramineum*. However, our experiments showed no evidence for a synergistic interaction between *C. gramineum* and *G. g. tritici*. Apparently, the type of root injury produced by *G. g. tritici* does not increase infection of the host by *C. gramineum*.

Our data show significant negative interactions between *C. gramineum* and *G. g. tritici*. Much of this antagonism could be explained by the fact that yield loss was approaching the 100% asymptote. However, a significant interaction occurred even in years when the high rates of both pathogens together produced only 66–82% loss. The negative interactions must have begun well below these values; therefore, we believe that a small but significant antagonism exists between these two fungi. We do not know if the antagonism is the result of a phenomenon similar to the systemic immunization that occurs between *Colletotrichum lagenarium* and at least 13 other pathogens (15). A more likely possibility is that, as pathogens occupying similar ecological niches, they may compete with each other. As an example, part of our original hypothesis was that occlusion of the stele by the take-all fungus (3,8,12) may limit systemic movement in the root vascular tissue by *C. gramineum*. Further research is necessary to see if this is one of the factors contributing to the observed negative interaction. The antagonism between *C. gramineum* and *G. g. tritici* demonstrated here is a small effect and probably of little or no importance to the epidemiology or management of the diseases in producers' fields.

**Table 1.** Parameter estimates and *F* values from wheat yield loss equations for *Cephalosporium gramineum* coinoculated with *Gaeumannomyces graminis* var. *tritici* during 5 yr

Variable	Year			
	1986	1987	1988	1989, 1991 <sup>a</sup>
Parameter estimates				
<i>C. gramineum</i> <sup>b</sup>				
Linear ( $\beta_1$ )	9.50	3.82	9.43	2.63
Quadratic ( $\beta_2$ )	-0.30	-0.18	-0.38	...
<i>G. g. tritici</i> <sup>b</sup>				
Linear ( $\beta_3$ )	23.83	43.50	71.10	50.99
Quadratic ( $\beta_4$ )	-2.17	-7.92	-13.57	-8.32
Interaction ( $\beta_5$ )	-0.43	-0.67	-2.05	-0.89
<i>R</i> <sup>2</sup>	0.98	0.96	0.97	0.96
<i>F</i> values <sup>c</sup>				
<i>C. gramineum</i>				
Linear	264.6***	17.1***	62.4***	91.2***
Quadratic	107.1***	3.4	9.5**	NS
<i>G. g. tritici</i>				
Linear	136.6***	185.4***	297.0***	351.9***
Quadratic	40.7***	52.0***	91.6***	75.7***
Interaction	39.9***	8.5**	47.6***	20.8***

<sup>a</sup> No year  $\times$  treatment interactions occurred between years for 1988–89 and 1990–91, and these years were combined.

<sup>b</sup> Parameters for the equation  $Y = (\beta_1 * Cg) + (\beta_2 * Cg^2) + (\beta_3 * Ggt) + (\beta_4 * Ggt^2) + (\beta_5 * Cg * Ggt)$  where: *Y* is yield loss;  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$ ,  $\beta_4$ , and  $\beta_5$  are parameter estimates; and *Cg* and *Ggt* are levels of oat-kernel inoculum (g/4.6-m row) for *C. gramineum* and *G. g. tritici*, respectively.

<sup>c</sup> *F* values followed by asterisks denote significance at  $P \leq 0.05$  (\*),  $P \leq 0.01$  (\*\*), and  $P \leq 0.001$  (\*\*\*). Significance at  $P < 0.10$  is unmarked, and NS is not significant.

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