Negative Interplot Interference in Field Experiments with Leaf Rust of Wheat

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ABSTRACT

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Negative interplot interference occurred between plots of wheat infected with *Puccinia recondita* f. sp. *tritici* and led to the overestimation of cultivar resistance. Our study showed that negative interference was greater between two large plots (16 m^2) than between two smaller plots (4 m^2). Disease in the large plots was also greater. Individual factor effects, guard area widths (2 m and 4 m) and guard crops (wheat and corn) had no significant effect on the amount of interference that occurred; plots separated by 4 m had greater disease severities than those separated by 2 m. The amount of negative interference was least when plots, regardless of size, were separated by a 4-m wheat guard.

Interplot interference, one of the components of the "representational error" described by Vanderplank (15), can be an important factor in evaluating the effectiveness of cultivars with differing levels of resistance to disease (8,12). Parlevliet and Ommeren (8) concluded that the partial resistance of cultivars in a "mosaic of small adjacent plots" was underestimated compared to the degree of resistance observed in the same cultivars in isolated plots due to interplot interference (8).

When a plot with lower disease severity (partial control) is adjacent to a plot with higher disease severity (no disease control), the former has more disease (positive interference) when compared with the same treatment adjacent to a treatment of similar disease severity. Negative interference resulted in lower disease severity in a plot with partial control, adjacent to a plot in which complete disease control was achieved, compared to a similar plot adjacent to another of the same partial control (4). James et al (4,5) found treatment effects were overestimated in fungicide-treated plots when negative interference occurred and that negative interference was proportionally higher than the corresponding positive interference. Cultivar trials to assess horizontal resistance resemble fungicide trials in having multiple disease levels (15).

Reducing interplot interference is desirable, as is the reduction of statistical errors. Since interplot interference may lead to the overestimation or underestimation of a treatment's effectiveness, its occurrence may lead to the acceptance of new, though not more effective, chemicals and cultivars, or the rejection of useful chemicals and sources of resistance for disease control. When improved cultivars or better treatments are being tested, positive interference may increase Type II errors (ie, the possibility of accepting the null hypothesis that a "new" treatment does not differ from an old one, when the alternative is actually true), and negative interference may increase Type I errors (the possibility of rejecting the null hypothesis when it is true). One suggestion for reducing interference between treatment plots is to maintain a "high standard of guarding" (10). Vanderplank (15) suggested grouping treatments in an experiment so that disease severities within groups were approximately similar. He also recommended the use of large square plots or rectangular plots oriented parallel to the prevailing wind.

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This study was designed to evaluate the amount of negative interference that may occur between plots of two cultivars of wheat (*Triticum aestivum* L.) with different responses to *Puccinia recondita* Rob. ex Desm. f. sp. *tritici*. The negative interference effects of plot size, guard width (the space separating two experimental plots), and the crop in the guard area were studied.

MATERIALS AND METHODS

Field experiments. The basic experimental design was a pair of plots separated by a guard area (Fig. 1). Pairs of plots made up the 2 × 2 × 2 factorial main experiment. The factors were plot size, guard width, and guard crop. The levels of each factor were: plot size—2 m × 2 m (4 m^2) and 4 m × 4 m (16 m^2); guard width—2 m and 4 m; and guard crop—wheat and corn (Table 1).

Each treatment was made up of two pairs of plots replicated at least twice. The paired plots, regardless of treatment, were separated from each other by a minimum of 100 m of crop that was resistant or immune to leaf rust, to avoid intraexperiment interference. In a treatment, all plots were of the same dimensions; one pair of plots (S2,S2) was planted to the same susceptible cultivar of wheat (Thatcher in 1980, Lee in 1981 and 1982). The second pair of plots (S1,R1) in a treatment was planted to a susceptible cultivar (S1) and a resistant cultivar (R1–Chris).

Field experiments consisting of all eight treatments were conducted in 1980 and 1981 at Rosemount, MN. In 1982, treatments 1, 4, 5, and 8 were repeated at Rosemount (Table 1). Wheat plots and guards were planted at the same time. The corn guards were planted within 6 wk of the wheat.

Prior to tillering a light oil suspension of uredospores (about 0.01 gm of spores per square meter) of *P. recondita* was misted onto plants with a controlled-droplet applicator (Mini-Ulva; Micron Corporation, Houston, TX) in all plots to ensure epidemic development. Epidemics observed in inoculated plots were more severe than in nearby susceptible wheat plots until the "dough" stage indicating that contamination of plots by natural inoculum was minimal early in the season. Disease assessments for leaf rust based on a percentage scale (3) were periodically recorded for the three youngest leaves on 8–15 randomly selected plants per plot. Disease observed was distributed uniformly within plots and was similar between plots of the same treatment throughout the growing season. Growth stage (GS) was recorded according to the decimal code developed by Zadoks et al (17). Wheat plots and guards were the same height (±10 cm) throughout each of the growing seasons. Corn guard plants were small and sparse enough to be more analogous to bare soil than a "barrier" between plots. Wheat heads were randomly selected from plots in 1981 and 1982 at maturity and harvested for 1,000-kernel weights.

Analysis of data. Disease severity of a plant was calculated as the average of the disease percentages on the three youngest leaves on that plant. Disease severity of a plot was the average of the disease severities of the sampled plants. The data presented are disease severities of plots. Disease development in the S2 plot (in the same relative position as the S1 of the S1,R1 pair) was compared to disease development in the corresponding S1 plot of each treatment to determine the extent of negative interference (4,5). For example, the single plot of the susceptible cultivar (S1) would be expected to have disease development similar to the corresponding S2 in an S2-S2 pair. If there was an appreciable difference between the S1 and S2 plots, it was evidence of interference by adjacent plots.

Data on average disease severity per plant from any one assessment were combined to test differences (according to Student's t-test) in individual factors, regardless of all other factors.

Three methods (4) were used to estimate interference, as well as 1,000-kernel weight. The first method involved the difference between the areas under the disease progress curves (AUDPCs) from S1 and S2 plots. This difference, expressed as a percentage of the area under the S2 curve, is an estimate of negative interference.

For the second method, differences between disease severities in the S2 plot and the corresponding S1 plot were calculated. The differences were expressed as percentages of disease severity.

In the third method, the apparent infection rates (13) of the disease progress curves were calculated by using the equation:

$$r = [1/(t_2 - t_1)] \left\{ \ln[x_2/(1-x_2)] - \ln[x_1/(1-x_1)] \right\}$$

in which x is disease severity and t is time. Initial severity (x_1) corresponded to the initial assessment and the second disease severity (x_2) was that observed at a later assessment. Infection rates were calculated for the S2 and corresponding S1 disease progress curves, and the difference between the two values of r was used as an estimate of interference.

In the fourth method, the difference in 1,000-kernel weight between the S1 plot and the corresponding S2 plot was calculated. The differences were expressed as percentages, with the 1,000-kernel weight of the S1 plot being set at 100%. When the 1,000-kernel weight from the S1 plot was more than that from the S2 plot, negative interference was said to have occurred.

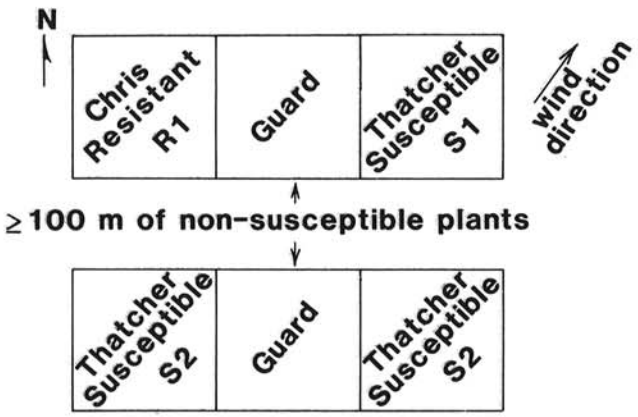

Fig. 1. General design of field experiments for determining negative interplot interference showing plot orientation and prevailing wind direction.

TABLE 1. Treatments of the three-factorial experiment for measuring negative interference in plots of wheat infected with leaf rust

Treatment	Plot size (m)	Guard	
		Width (m)	Crop
1	2 × 2	2	Wheat[a]
2	2 × 2	2	Corn
3	2 × 2	4	Wheat
4	2 × 2	4	Corn
5	4 × 4	2	Wheat
6	4 × 4	2	Corn
7	4 × 4	4	Wheat
8	4 × 4	4	Corn

[a]Cultivar Chris was used in 1980; cultivar Era was used in 1981 and 1982.

RESULTS

Representative disease progress curves from S1 and S2 plots are presented for several treatments in this study. Disease progress curves from the 4-m^2 S1 and S2 plots (treatments 1, 2, 3, and 4 in Table 1) at Rosemount are shown in Fig. 2a–d. These curves show that disease in the 4-m^2 plots stayed at a relatively low level. The

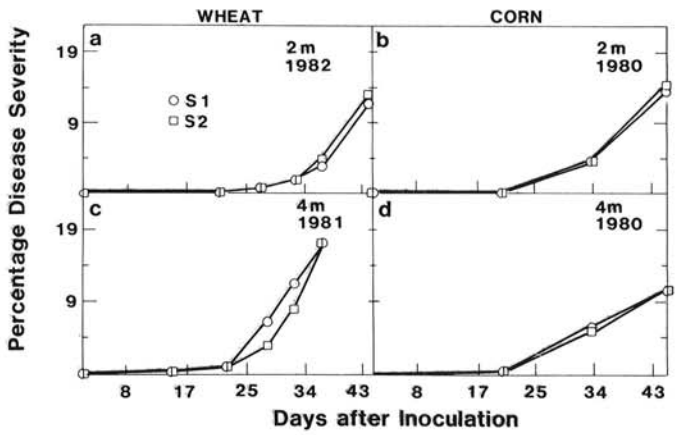

Fig. 2. Disease progress curves for mean wheat leaf rust severity (percent per plant) at Rosemount, MN, in 4-m^2 plots (2 m × 2 m), from a plot of a susceptible cultivar (Thatcher in 1980 and Lee in 1981 and 1982). S1 disease progress curves were from plots adjacent to an identical plot of a resistant cultivar (Chris) and S2 from plots adjacent to a plot of the same susceptible cultivar; plots of the pair separated by: **a,** a 2-m wheat guard in 1982; **b,** a 2-m corn guard in 1980; **c,** a 4-m wheat guard in 1981; and **d,** a 4-m corn guard in 1980.

TABLE 2. Average wheat leaf rust severities for each level of the experimental factors independently for each of three years

Year	GS[a]	Days after inoculation	Disease severity (%)					
			Plot size		Guard strip width		Guard crop	
			4 m^2	16 m^2	2 m	4 m	Wheat	Crop
1980	Watery-ripe	32	4.0	13.2[b]	6.9	9.5[c]	9.5	6.8[d]
1981	Hard dough	35	22.1	18.8	18.0	22.7[c]	19.1	21.4
1982	Late milk	35	4.7	7.1	5.3	6.5[c]	...[e]	...

[a]GS = growth stage.
[b]Significantly ($P = 0.01$) greater disease severity in the 16-m^2 plots than in the 4-m^2 plots.
[c]Significantly ($P = 0.01$) greater disease severity in plots separated by the 4-m guard than by the 2-m guard.
[d]Significantly ($P = 0.01$) greater disease severity in plots separated by wheat than in plots separated by corn.
[e]Guard crop was correlated to guard size—the 2-m guard was wheat and the 4-m guard was corn, which were not evaluated in 1982.

maximum disease severity was 19% in 4-m^2 plots separated by the 4-m wheat guard (treatment 3) in 1981 (Fig. 2c).

Disease progress curves from the 16-m^2 S1 and S2 plots (treatments 5, 6, 7, and 8 in Table 1) at Rosemount are shown in Fig. 3a–f. Generally, disease in these plots progressed at a greater rate and reached a greater severity than in the 4-m^2 plots (compare Figs. 2 and 3). The difference between disease severities in S1 and S2 plots was as much as 18% in 16-m^2 plots separated by the 2-m-wide wheat guard (treatment 5) (Fig. 3a). Disease severity in 16-m^2 plots separated by the 2-m wheat guard was higher than in 16-m^2 plots separated by 2 m of corn (Figs. 3a and 3c versus Figs. 3b and 3d). Decreasing disease in plots, late in the season, was largely due to host senescence and was influenced by drought.

The overall average disease severity in 16-m^2 plots was significantly higher (by Student's t-test; $P = 0.01$) than in the 4-m^2 plots (Table 2). Disease severities in plots separated by the 4-m guard was significantly higher ($P = 0.01$) than in plots separated by the 2-m guard in 1980 and 1981. In 1982, the 2-m guard was resistant wheat and the 4-m guard was corn, but there still was significantly greater disease severity ($P = 0.01$) in plots separated by the 4-m guard.

Analysis of individual factor effects showed that negative interference was occurring (Table 3). Overall, leaf rust was less severe in plots that were adjacent to plots of resistant wheat (S1) than in plots that were adjacent to plots of susceptible wheat (S2). Differences in disease severities resulted from negative interference (leaf rust severity less in S1 than in S2 plots) in the three years in 16-m^2 plots regardless of guard width or guard crop. The 4-m^2 plots showed negative interference only in 1982. Negative interference was a trend in plots separated by either 2-m or 4-m guard strips and regardless of guard crop, although significance was not consistent over the years for other factor effects.

Negative interference (Table 4) was shown by differences in areas under the disease progress curve (AUDPC) between S1 and S2 plots, as well as by differences in percent disease severity in 4-m^2 plots separated by 2 m of corn, and in 16-m^2 plots separated by 2 m of wheat and 4 m of corn, when calculated for the first 32 days after inoculation in 1980; between all plots except 4-m^2 plots separated by a 4-m guard and 16-m^2 plots separated by a 4-m wheat guard, when calculated for the first 31 days after inoculation in 1981 and in all treatments, for the first 35 days after inoculation in 1982. Differences in infection rate indicate that interference had an effect in 4-m^2 plots separated by a 2-m guard and a 4-m wheat guard and 16-m^2 plots separated by a 2-m wheat guard in 1980, all plots except 4-m^2 plots separated by 2 m of corn and 16-m^2 plots separated by 4 m of wheat in 1981 and 4-m^2 plots separated by 2 m of wheat and 4 m of corn in 1982. Negative interference was shown by 1,000-kernel weights of grain samples in the same 4-m^2 plots that showed negative interference by infection rates in 1981 and 1982 and in 16-m^2 plots separated by 4 m of corn in 1982.

TABLE 3. Mean wheat leaf rust severities for treatments showing differences by main factor effects and the adjacent plots

	Disease severity (%)					
	1980		1981		1982	
Factor	S1	S2[a]	S1	S2	S1	S2
Treatments[b]	7.2	− 9.1[c]	19.2	−21.7[c]	5.2	−6.6[c]
Plot size 4 m^2	4.2	3.9	23.2	21.1	4.1	−5.3[c]
Plot size 16 m^2	10.8	−15.6[c]	15.7	−22.3[c]	6.4	−7.8[c]
Guard 2-m wide	5.4	−8.5[c]	16.9	−19.1	4.3	−6.1[c]
Guard 4-m wide	9.2	−9.8	21.3	−24.3	5.9	7.2[c]
Wheat guard	8.1	−10.9[c]	18.3	−19.9	...[d]	...
Corn guard	6.3	−7.3	19.9	−22.8	...	...

[a] S1 and S2 represent similar treatments, but S1 refers to a plot adjacent to a plot of a resistant cultivar; S2 refers to a plot adjacent to a plot of a susceptible cultivar.
[b] Eight treatments.
[c] Significantly ($P = 0.05$) lower disease severity in the S1 plot than in S2. Minuses (−) indicate effect of negative interference—lower disease severity in S1 than in S2.
[d] Not evaluated in 1982.

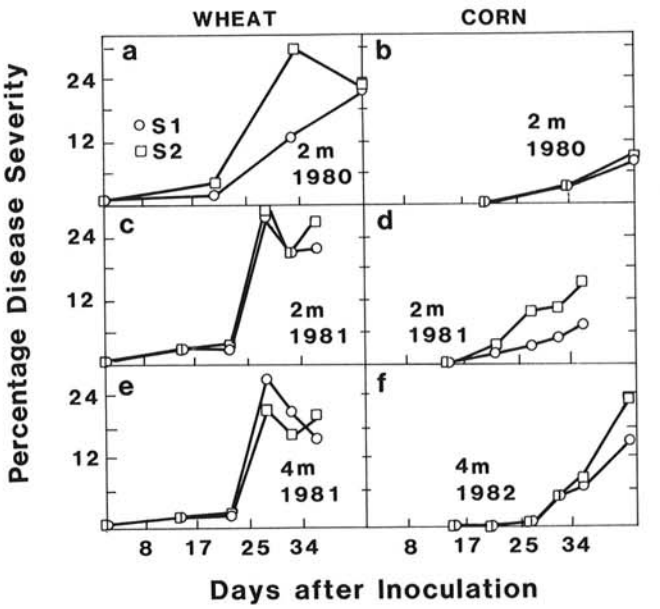

Fig. 3. Disease progress curves for wheat leaf rust in 1980 and 1981 at Rosemount, MN, with 16-m^2 plots. The data designated S1 are for plots of a susceptible cultivar adjacent to plots of a resistant cultivar. The data designated S2 are for a susceptible cultivar whose adjacent plot was the same. Elements **a and c,** a 2-m wheat guard; **b and d,** a 2-m corn guard; **e,** a 4-m wheat guard; and **f,** a 4-m corn guard.

TABLE 4. Estimates of negative interference in field experiments with leaf rust of wheat, presented as differences between a susceptible plot paired with a resistant plot and a susceptible plot paired with another susceptible plot

Plot size, guard strip width, and guard crop	Area under disease progress curve (percent-days)			Disease severity (%)			Apparent infection rate (r)			1,000-kernel weight per year (grams)[a]	
	1980	1981	1982	1980	1981	1982	1980	1981	1982	1981	1982
4 m^2, 2 m, wheat	80.55	−4.83	−12.31	1.44	−1.08	−0.81	−0.145	−0.024	−0.001	−0.567	−0.364
4 m^2, 2 m, corn	−10.39[c]	−6.44	[b]	−0.54	−0.94	[b]	−0.012	0.001	[b]	0.37	[b]
4 m^2, 4 m, wheat	2.05	56.32	[b]	0.08	3.42	[b]	0.011	−0.000	[b]	0.28	[b]
4 m^2, 4 m, corn	16.41	40.48	−0.962	0.55	5.08	−1.79	−0.060	−0.002	−0.048	−0.62	−12.95
16 m^2, 2 m, wheat	−52.81	−8.91	−0.642	−16.73	−0.45	−1.93	−0.033	−0.004	0.017	−5.07	20.34
16 m^2, 2 m, corn	4.41	−55.25	[b]	0.13	−5.00	[b]	0.027	0.017	[b]	−13.54	[b]
16 m^2, 4 m, wheat	2.21	15.85	[b]	0.58	4.22	[b]	0.019	0.031	[b]	1.00	[b]
16 m^2, 4 m, corn	−33.70	−4.58	−10.68	−5.10	−1.50	−0.69	0.102	−0.005	0.017	−2.23	−15.48

[a] 1,000-kernel weights were not taken in 1980.
[b] Treatments not included in experiment in 1982.
[c] Minus (−) indicates negative interference may have been occurring.

In 1980 and 1981, the treatments were compared for relative amounts of negative interference. Negative interference was greatest in the 4-m^2 plots with the 2-m-wide corn guard and the 16-m^2 plots with the 2-m-wide wheat guard and 4-m corn guard (4-m^2 plots separated by 2 m of corn, and 16-m^2 plots separated by 2 m of wheat and 4 m of corn). Negative interference was least evident in treatments with the 4-m guards (except in 16-m^2 plots separated by the 4-m corn guard) in both years.

DISCUSSION

Vanderplank (14) stated that large fields can be expected to lose a "smaller proportion of spores than small fields, because a greater proportion of the spores which are released fall back within the field." He also thought that the retention of spores in large fields did not necessarily influence the multiplication of disease. However, the model by Fleming et al (1) indicated that the production rate of a pathogen is proportional to the area in which it is established. Therefore, a crop disease with easily dispersed inoculum "may be unable to establish itself at all" if fields are small enough. Thus, Fleming et al (1) concluded that the disease severity will be less in smaller plots than in larger plots.

Our data seem to contradict Vanderplank's hypothesis (14,15), and support the conclusions of Fleming et al (1). Greater amounts of disease was observed in the 16-m^2 plots than in the 4-m^2 plots of this study. The negative interference observed was also greater between 16-m^2 plots than between 4-m^2 plots.

The discrepancy between Vanderplank (15) and Fleming et al (1), as well as with our study, could be because Vanderplank did not fully accept the premise that larger plots would have greater multiplication of disease and, therefore, greater absolute amounts of inoculum relative to smaller plots. If, as Fleming et al (1) said, the amount of disease in smaller plots is less than that in larger plots, then it follows that the absolute amount of inoculum moving out of smaller plots is less than that moving out of larger plots. While a greater proportion of inoculum is lost from small plots than from large ones, the absolute amount of inoculum lost from plots influences disease in nearby plots (6,7); therefore, higher disease levels would be expected in larger plots than in smaller ones.

Shoemaker (13) pointed out that plots with low disease severity would probably show the effects of positive interference, whereas negative interference would become evident in plots with high disease severity. Since the 16-m^2 plots of our study had higher levels of disease severity than the 4-m^2 plots, negative interference was easier to discern in the 16-m^2 plots.

The overall disease severities in plots separated by the 4-m guard were greater than in plots separated by the 2-m guard (Table 2). This seems to contradict earlier studies (2,11) that have shown that as the distance between a plot and a spore source increases, the amount of inoculum in the receiving plot decreases. Reasons for this discrepancy are not known. It may be that theoretical models describing spore dispersal deal only with "long distance" dispersal, ie, 4 m or more. The distances under consideration in our study are 4 m or less (ie, the guard width). Another explanation may be air turbulence effect on inoculum moving between plots, over plot canopies and over the guard areas. For example, if eddies, which result in a spore cloud, are strong enough to raise that cloud more than 1 m above the canopy, deposition of spores from that cloud may not occur for several meters along the ground. The effect of negative interference in plots separated by a 2-m guard was not consistently greater than when plots were separated by a 4-m guard (Table 3).

In this study, spore movement was not measured in absolute terms, but the effect of the net inoculum movement was assessed in relative terms by disease differences. James et al (4) stated that negative interference was the result of a net loss of inoculum from a plot because of low inoculum levels in adjacent plots. This differs from the view that negative interference occurs when a large proportion of the inoculum produced within a plot is dispersed outside that plot's boundaries (9).

The type of crop in the guard area had no consistent effects on disease development in the associated plots or on negative interference between plots separated by either the wheat or corn guard.

The estimates for negative interference include several high positive values for differences between S1 and S2. The reasons for these differences are not clear. The paired plots of our study were widely separated (100 m) and different environmental conditions could have affected the interference occurring between the paired plots. These positive values may also have been due to positive interference, which we could not evaluate because of our plot design.

The amount of negative interference seemed to be the least when plots, regardless of size, were separated by a 4-m wheat guard or were in 4-m^2 plots separated by a 4-m corn guard (Table 4). This may indicate that the interaction of guard crop and guard width had more influence than plot size on the amount of interference occurring between plots. This also indicates that increasing or decreasing the crop area size, as recommended by Vanderplank (13) and Waggoner (16), respectively, will not necessarily decrease the interference. In field experiments, in which interference is undesirable and where conditions are similar to those studied here (in terms of plot sizes, crops, and especially, pathogen characteristics), it would be better to increase the guard width than the plot size when space is limited. The interactions of plot size, guard width, and guard crop can be important and merits additional study.

Our study has shown that plot size and guard width affects the amount of negative interference between plots. These effects may be important considerations in future experiments aimed at finding cultivars with horizontal or partial resistance to some disease. However, the results of our study on the effects of plot size, etc, are presently inadequate to apply to actual field situations. More work needs to be done on the effects of different plot sizes, guard widths, and their interaction. Further study is also needed on positive interference in general and the effects of the factors in this study on positive interference.

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