Interplot Interference in Field Experiments with Late Blight of Potato (Phytophthora infestans)

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ABSTRACT

Results from 10 field experiments with potatoes (*Solanum tuberosum*) infected with late blight disease, caused by *Phytophthora infestans*, were used to measure interplot interference. The merits of the experimental design, and four methods of measuring interference, are discussed. Measuring interference in terms of percentage loss in tuber yield, rather than percentage disease, was considered the most relevant. If interference in a treatment caused an increase in disease, interference was said to be positive. Conversely, negative interference was said to be present in a treatment, if disease was decreased. The presence of an induced epidemic in an experiment exerted a positive interference on the adjacent unsprayed plots. The results were not conclusive as to whether various fungicides spray schedules could suppress positive interference. The "representational error" that may arise in the results of variety trials for assessing horizontal resistance to blight, when interference is present is discussed.

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Comparisons of results from field experiments are usually made assuming that the treatments do not interfere with one another (3). Agronomists usually try to satisfy this requirement by using guard rows to prevent interplot interference, but for entomologists and pathologists experimenting with organisms that can travel kilometers, the requirement is more difficult to satisfy. The interplot effect in entomological experiments has been measured by Joyce (8), Joyce & Roberts (9), Roberts (14) and others, but the problem has received less attention from phytopathologists.

Christ (2) demonstrated that the interplot effect was important in field experiments with *Alternaria solani* and *Xanthomonas vesicatoria* on tomatoes. He showed that the presence of an unsprayed plot in an experiment increased disease severity from 8 to 28% in a plot sprayed with fungicide. Van der Plank (16,

18) has noted the existence of interplot interference in experiments involving foliage diseases (10, 12, 13, 15) and comprehensively discussed the "representational error" when these results are applied to agricultural practice. For example, the efficacy of a fungicide is underestimated when there is interference between two treatments (unsprayed and sprayed) in an experiment designed to test the efficacy of a fungicide under farming conditions. Large numbers of spores may move from the unsprayed to the sprayed plots in the experiment but, in contrast, a farmer's sprayed fields will not be subject to the same influx of spores. An additional error arises because the unsprayed plots lose a larger proportion of spores (exodus) than the large unsprayed fields (17). Therefore, at any given time, unsprayed plots will probably have less diseases (due to lower inoculum) than unsprayed fields.

Before rational control measures can be developed, the phenomenon of interplot interference must be understood to allow proper interpretation of experimental results. The existence of interplot interference is equally as important in cultivar trials for assessing resistance to disease, particularly horizontal resistance. The objective of the present work was to study and measure the amount of interplot interference under different conditions in field experiments with the late blight disease of potato caused by *Phytophthora infestans* (Mont.) de By.

METHODS.—*Field experiments.*—Ten field experiments of potatoes (*Solanum tuberosum* L.) were conducted in 1972 at three locations, Ottawa, Fredericton, and Charlottetown. At each location, the distance between any two experiments was 100 to 300 m in an attempt to minimize interexperiment interference. Each experiment had two treatments with either four or six replicates in a randomized block design. Each plot consisted of four rows approximately 15 m (50 ft) long. The treatments and cultivars used in the experiments are given in Table 1. The disease development resulting from each treatment has been designated by a letter.

A water suspension containing spores of *P. infestans* was applied to treatments A, C, and E in Ottawa in an attempt to initiate three epidemics with different characteristics. At Fredericton and Charlottetown no inoculum was applied and Dithane M-45 (coordination product of zinc ion and maneb 80% WP) was used in different treatments at the rate of 2.24 kg in 1,348 liters of water per ha (2.0 lb in 120 gal/acre) in an attempt to achieve partial or complete control of blight. Disease assessments for blight, using the British Mycological Society Key (1), were periodically recorded (Fig. 1-8) for the center two rows. Other experimental details were similar to those described by James et al. (5).

The treatments at Fredericton and Charlottetown were similar, except for the partial control treatments. In Charlottetown, the first spray was applied on 13 July (29 days from planting), before the disease was observed in the partial control plots; sprays were repeated at 40, 48, and 56 days from planting. In Fredericton, the first spray was not applied until the disease was observed in the partial control plots, and the interval between sprays was

TABLE 1. *Phytophthora infestans* inoculum and fungicide treatments applied to two cultivars in potato field experiments at three locations in Eastern Canada 1972

Location:	Ottawa		Fredericton[a]		Charlottetown[b]	
Cultivar:	Katahdin		Katahdin		Green Mountain	
Experiment I						
Treatment 1	Inoculum applied early	A	No treatment	A	No treatment	A
Treatment 2	No treatment	B	Partial control by fungicide	B	Partial control by fungicide	B
Experiment II						
Treatment 1	Inoculum applied mid-season	C	Partial control by fungicide	C_1 ┐C	Partial control by fungicide	C_1 ┐C
Treatment 2	No treatment	D	Partial control by fungicide	C_2 ┘	Partial control by fungicide	C_2 ┘
Experiment III						
Treatment 1	Inoculum applied late season	E	Partial control by fungicide	D	Partial control by fungicide	D
Treatment 2	No treatment	F	Complete control by fungicide	E	Complete control by fungicide	E
Experiment IV						
Treatment 1	No treatment	G_1 ┐G				
Treatment 2	No treatment	G_2 ┘				

[a] In the partial control plots the first spray was applied after disease was observed in the B, C, and D plots at 77 days from planting and sprays repeated at 97 and 104 days from planting. In the complete control plots, E, eight sprays applied at approximately weekly intervals starting 18 July.

[b] In the partial control plots the first spray was applied before disease was observed in the B, C, and D plots at 29 days from planting and sprays repeated at 40, 48, and 56 days from planting. In the complete control plots, E, eight sprays applied at approximately weekly intervals starting 13 July.

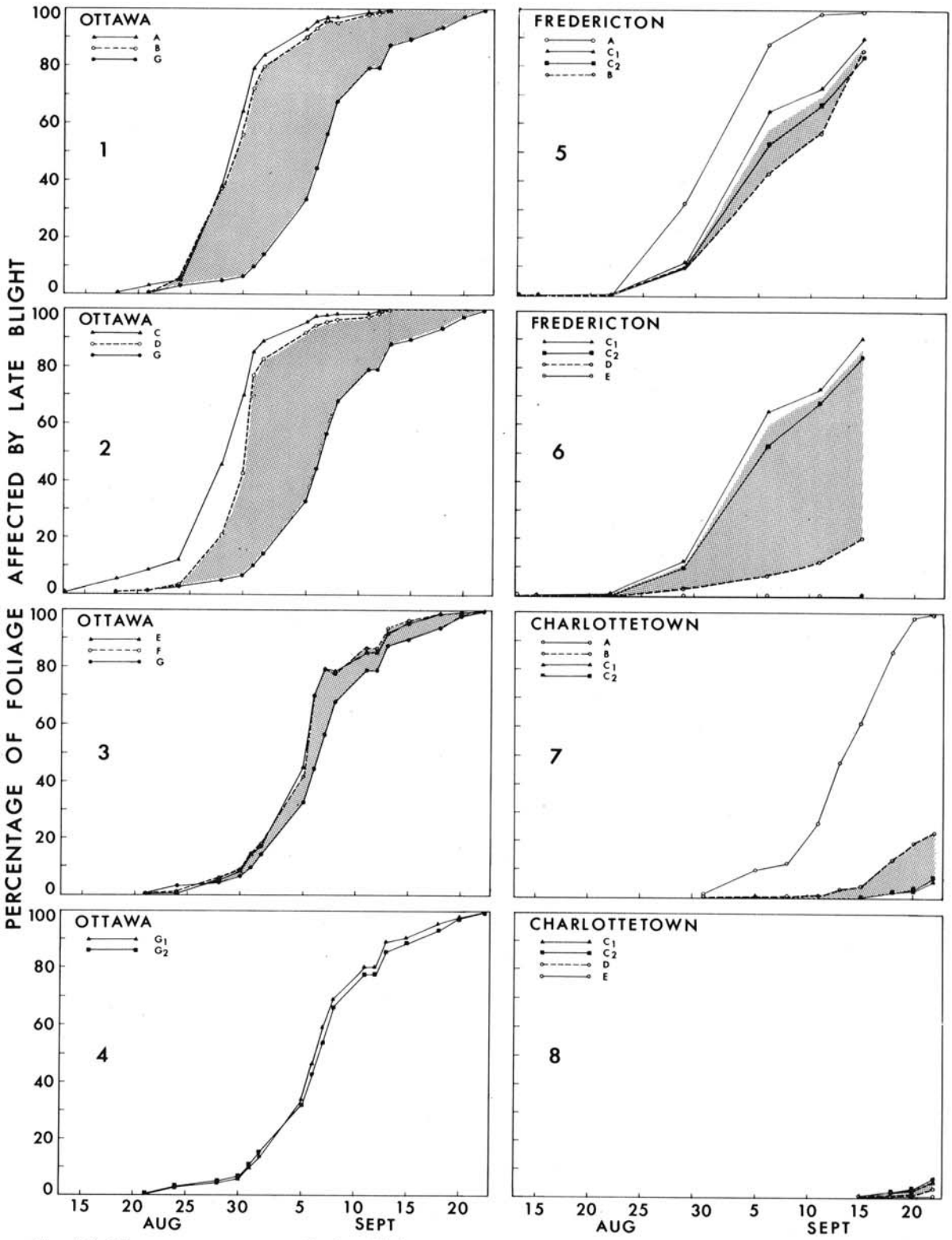

Fig. 1-8. Disease progress curves for late blight on potatoes in 10 field experiments featuring two treatments per experiment and including induced or natural epidemics in unsprayed plots, and also various fungicide spray schedules to give partial or complete control of the disease. Shaded areas represent graphical expression of interplot interference due to late blight. **1-4)** Results from four experiments at Ottawa with treatments; A, B; C, D; E, F; G_1, G_2 : epidemics A, C, and E were induced, whereas B, D, F, and G were not, and developed in unsprayed plots. **5-8)** Results from three experiments at both Fredericton and Charlottetown with treatment A, B; C_1, C_2 ; D, E: epidemic A developed naturally in unsprayed plots whereas epidemics B, C_1, C_2, and D were partially controlled natural epidemics, and almost complete control was achieved in E.

longer than at Charlottetown (see Table 1). This difference in spray schedule for the partial control plots led to a relatively better control of the disease at Charlottetown than at Fredericton.

Analysis of data.—Disease developments for the treatments are plotted in Fig. 1-8. Since the two treatments of experiment IV at Ottawa were identical, the difference between the two epidemics G_1 and G_2 can be regarded as natural variation in disease development. The average, denoted by G, is therefore used to represent the disease development in experiment IV. Similarly, at Fredericton and Charlottetown C is used to represent the disease development in experiment II.

Disease developments for various treatments (B, D, F, and G at Ottawa; and B, C, and D at Fredericton and Charlottetown) were compared to determine the extent of interplot interference; using the G curve at Ottawa and the C curves at Fredericton and Charlottetown as references for the comparisons. For example, the unsprayed plots (B) in experiment I at Ottawa normally would have been subject to a natural infection, and would be expected to have a disease development similar to G. If the disease development, B, for the unsprayed plots differed appreciably from G, the plots can be considered to have been interfered with by the adjacent inoculated plots. We shall say, for convenience, that there was interference in B, and the extent of interference can be estimated by the difference between the two disease progress curves; i.e., B–G. The expressions for the interferences investigated are listed in Table 2.

There are two types of interplot interference. The interference is said to be positive (+) if the disease progress curve in question is above the reference curve, and negative (–) if below. The four methods used to estimate interplot interference are described.

In Method 1 the difference between the areas under a disease progress curve and its corresponding reference curve is calculated (equivalent to the stippled areas in Fig. 1-8); the difference, expressed as a percentage of the area under the reference curve, is the interference. For example, the stippled areas in Fig. 1 and 6 are +52% and −83% of the areas under curves G and C, respectively.

Method 2 is similar to that used by Lapwood (11) for comparing the severity of disease progress curves, which was based on the number of days for 50% of the foliage to be destroyed by blight. In our method, the number of days (from planting) for 25, 50, and 75% of foliage to be destroyed by blight was calculated for each disease progress curve. The difference (in days) between a progress curve and the corresponding reference curve gives a measure of the interference. For example, the interference for B −G at Ottawa is +10 for the 75% defoliation level (Table 2).

In Method 3, the disease progress curves were described by the equation:

$$\log_{10} \frac{x}{1-x} = a + rt$$

where x = defoliation level and t = time. The infection rate r (18) is equivalent to the slope of the lines, and was calculated for the disease progress curve, and for its corresponding reference curve. The difference between the two r values, calculated for

TABLE 2. Interplot interference in field experiments with late blight of potato, estimated by four different methods[a]

		Method 1	Method 2			Method 3	Method 4
		Percentage of area under disease progress curve	Number of days, from planting to destruction of given % of foliage			Infection rate, r	Loss in tuber yield, relative to potential yield
Location	Interference		25%	50%	75%		(%)
Ottawa	Induced first epidemic on natural epidemic; interference in B: B–G	+52	+8	+8	+10	+ .049	+10.8
Ottawa	Induced second epidemic on natural epidemic; interference in D: D–G	+48	+6	+8	+10	+ .046	+ 6.4
Ottawa	Induced third epidemic on natural epidemic; interference in F: F–G	+12	+1	+1	+ 3	+ .029	0.0
Fredericton	Natural epidemic on partially controlled epidemic; interference in B: B–C	−18	−1	−4	− 1	+ .002	− 2.9
Fredericton	Absence of an epidemic on partially controlled epidemic; interference in D: D–C	−83				− .029	−12.4
Charlottetown	Natural epidemic on partially controlled epidemic; interference in B: B–C	+579				− .090	+ 2.3
Charlottetown	Absence of an epidemic on partially controlled epidemic; interference in D: D–C	−47				− .048	− 0.6

[a] Interplot interference is calculated as the difference between the disease progress curve and its respective reference curve.

the two curves, is a measure of interference. For example, the interference in B (B–G) at Ottawa is estimated as (0.184–0.135) = +0.049.

Method 4 calculates interference in terms of % tuber yield loss, relative to potential yield, using the equation described by James et al. (6, 7). The percentage loss, relative to potential yield, is calculated for each curve. The difference between the loss corresponding to a progress curve and the loss corresponding to the reference curve is a measure of interference. For example, the interference in B (B–G) at Ottawa is estimated as (28.4 – 17.6) = +10.8% yield loss (Table 2).

RESULTS.—The three epidemics (A, C, and E) at Ottawa were intended to represent epidemics with different characteristics. However, variable weather during the experiment resulted in the first (A) and second (C) epidemics being more similar than intended, but both were earlier than the third (E) epidemic. The natural epidemics (A) at both Charlottetown and Fredericton were less severe than usual and corresponded with the low blight levels found in commercial crops in Prince Edward Island in 1972, and the very late infections reported for New Brunswick.

Using the four methods described above, interplot interference was estimated for the disease progress curves in Fig. 1-8; the results are shown in Table 2. Irrespective of the method used, the Ottawa experiments showed that there was positive interference when an early epidemic developed in a plot next to an unsprayed plot; the earlier the epidemic, the greater the interference; e.g., 10.8 and 6.4% loss for early epidemics (Table 2). Using Method 4, positive interference (B–C) was also recorded in Charlottetown when a natural epidemic (A) was allowed to develop in plots adjacent to plots (B) where disease was partially controlled by a fungicide spray program; negative interference (B–C) was recorded for the corresponding situation in Fredericton. At both Charlottetown and Fredericton, negative interference (D–C) was recorded due to the absence of an epidemic (E) in plots adjacent to plots where the epidemic (D) had been partially controlled.

DISCUSSION.—Gregory (4) reported that the dispersal of sporangia of *Phytophthora infestans* conforms with his equation for dispersal in air with low turbulence, which predicts a steep gradient of disease away from a point source, followed by a flattening of the gradient at approximately 100 m. The gradient flattens nearer the source of infection when the source develops into an area of infection rather than a point source (18, 19). Consequently, interplot interference is always present in field experiments with *Phytophthora infestans* where there are two or more treatments with different levels of disease.

In field experiments, treatments to be compared are generally applied to neighboring plots, so that they may be studied under conditions as uniform as possible. For two reasons, both a consequence of the high mobility of spores, this principle was not taken into consideration in the study of interplot interference in our field experiments with late blight of potato. Firstly, the reference treatment, against which other treatments are compared, must be placed at some distance from the other treatments so that it will be reasonably free from the influx of spores produced in plots more severely infected by the disease. Secondly, no more than two treatments should be placed together or the interference resulting from the exchange of spores among more than two treatments will be confounded and the results difficult to interpret. In the current study, it was for these two reasons that the treatments at each location were allocated to separate experiments placed at some distance from one another.

Within the confines of the experimental area at each location, soil heterogeneity existed which made it unreliable to compare yield data from different experiments. Weather conditions were less variable; which was confirmed in our experiments by the concurrent initiation of disease in the unsprayed plots in different experiments at Fredericton. Therefore, disease progress curves, rather than yield data, were used in the evaluation of interplot interference. Our experiments within each location were subject to essentially the same weather conditions, but since the distance between experiments ranged from 100-300 m, interexperiment interference was limited to a minimal acceptable level.

The first method used to estimate interplot interference does not allow comparisons of data from different locations and may also exaggerate the importance of positive interference; e.g., interference in B at Charlottetown. The second method is not applicable to all disease progress curves, and the choice of defoliation level (25, 50, and 75%) is arbitrary. Method 3 is not suitable for situations where the disease in the reference treatment develops late in the season, resulting in a higher r value for the reference curve than for the other disease progress curve (B and C curves at Charlottetown). This would lead to the complication of a negative difference in r values representing a positive interference; e.g., interference in B is .164 – .254 = –0.090. Furthermore, these three methods estimate interference in terms of disease development, and are only meaningful to agricultural practice if there are corresponding effects on tuber yield. Two disease progress curves of different shape may result in similar percentages of tuber loss (7) if the difference in disease occurs late in the season when most of the yield has been accumulated (5). This phenomenon explains why Methods 1, 2, and 3 showed apparent positive interference in treatment F at Ottawa, whereas Method 4 showed no interference. Since diseases are primarily studied because of the losses they cause, the measurement of interference, in terms of percentage loss using Method 4, is considered more relevant than the other methods for our purposes, and is the method referred to in the remainder of this discussion.

The importance of the two components of interference, termed "influx" (movement of spores into the plot) and "exodus" (movement of spores out

of the plot), were not measured in absolute terms in this study, but their relative importance was evaluated. The interference resulted from a situation where either influx or exodus was the principal component of interference. In the Ottawa experiments and experiment I at Charlottetown, the principal component was influx and the positive interference was due to the high influx of spores from adjacent plots. In experiment III at Charlottetown and Fredericton, the principal component was exodus and the interference was negative because of a low influx of spores from the adjacent plots, where little or no blight was present. No simple explanation can be tendered for the negative interference in experiment I at Fredericton.

Interference has been studied under two general conditions: at Ottawa, no fungicides were applied and the epidemics were induced in an attempt to quantify interference in a more empirical manner; whereas, at Fredericton and Charlottetown, the modifying effect of fungicide sprays was used in conjunction with natural epidemics.

The results of the Ottawa experiments show that there was substantial interference (10.8%) when the epidemic A was induced in plots next to the unsprayed plots G. This finding can be applied to a cultivar trial screening for horizontal resistance, where the most susceptible cultivar and the least susceptible cultivar can be equated to the induced epidemic and unsprayed plot, respectively; this would underestimate the commercial yielding capacity of the least susceptible cultivar. These findings should direct attention to improving the design of experiments for screening for horizontal resistance to blight, and should include the effect of plot size and shape, and guard or buffer areas of varying size. Van der Plank (18) suggested that only cultivars of approximately equal susceptibility should be incorporated in a field trial with large square plots arranged in one straight line.

The Ottawa results also show that interference increases with the earliness of the epidemics. The three epidemics at Ottawa would not be considered early, relative to recorded severe early epidemics in the past; therefore, 10.8% may be rather a low estimate of the interference possible under field conditions. For example, if interference was calculated for the same disease progress curves displaced 1 week earlier, the corresponding level of interplot interference would be 38.8 rather than 10.8% yield loss. It is highly probable that positive interference will increase under all conditions where disease severity increases. Accordingly, interference will probably be more important for early epidemics, for highly susceptible cultivars, for unsprayed or poorly sprayed plots.

It is noteworthy that the presence of a sprayed plot with no disease can result in a substantial negative interference and is the converse of the interference reported by Christ (2). Consequently, a "representational error" may arise in an experiment designed to compare the efficacy of a fungicide, where the two treatments are complete and partial control. The results of such an experiment would overestimate the efficacy of the partial control treatment as opposed to an experiment with unsprayed and partial control treatments which would underestimate the effect of the partial control treatment. Experimental techniques can also lead to an inaccurate estimate of interference; e.g., negative interference is overestimated when fungicide spray drifts from complete control to partial control plots. The interplot interference observed in the current experiments can be used as an indication of the magnitude of representational errors, with the proviso that a greater proportion of spores have been lost from the small experimental plots compared with larger fields receiving the same treatment. Circumstantial evidence from other published works (5, 12, 13, 18) suggests that, if an unsprayed plot is included in the experiment, interplot interference is present even when a fungicide is applied every 7 days to control late blight. The evidence from the current experiments at Fredericton and Charlottetown is inconclusive as to whether the fungicide schedules employed can completely suppress positive interference. If further study demonstrates that interplot interference in fungicide trials is important, this could radically affect the development of control measures in the future.

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