Representational Errors Due to Interplot Interference in Field Experiments with Late Blight of Potato

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ABSTRACT

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In field experiments the yield loss associated with fungicide treatments may be overestimated (positive interference) if the adjacent plots have more disease, or underestimated (negative interference) if they have less disease. Results from 14 field experiments with potatoes (*Solanum tuberosum*) infected with *Phytophthora infestans*, were used to study the relative importance of positive and negative interference in plots sprayed with Dithane M-45 (coordination product of zinc ion and manganese ethylene bisdithiocarbamate). An epidemic in some plots resulted in positive interference in fungicide sprayed plots in the same experiment despite the application of five protectant sprays. Negative interference occurred in sprayed plots adjacent to plots with no disease, and the magnitude of negative interference was greater than the positive interference. The representational errors between treatments due to interference were equal to or greater than the corresponding experimental errors.

Additional key words: epidemiology, experimental design.

Field experiments are usually conducted to compare treatments so that results can be applied to agricultural practice. Van der Plank (14) noted that if treated plots are not representative of similarly treated farmer's fields, then this constitutes a "representational error". He cautioned that this error should not be confused with the experimental error which is used to compare treatments and is usually expressed in terms of standard error or least significant difference (LSD). He further noted that the representational error was often greater than the LSD at $P = 0.05$.

When treatments in a field experiment have varying levels of disease caused by air-dispersed pathogens, the treatments interfere with each other (4, 5), causing representational errors when these experimental results are applied to agricultural practice (14). James et al. (9) demonstrated that the interplot interference on potatoes due to late blight, can result in either an increase or decrease (either positive or negative interference, respectively) in disease in a particular plot, and they discussed the relative merits of different methods of measurement of interference. Although they demonstrated that the presence of an induced epidemic of late blight in plots of one treatment resulted in a positive interference in the unsprayed plots, evidence was inconclusive as to whether varying fungicide schedules could be employed to suppress, either partially or completely, positive interference (9). However, Christ (3) demonstrated that positive interference was present in plots sprayed with fungicide to control diseases of tomatoes caused by *Alternaria solani* and *Xanthomonas vesicatoria*.

The current experiments were designed firstly, to detect and measure interference in plots sprayed with fungicide to control late blight of potato; secondly, to investigate the relative importance of positive and negative interference in representational errors; and thirdly, to compare the relative magnitudes of experimental error and corresponding interference levels associated with disease assessments at different times.

MATERIALS AND METHODS

Fourteen field experiments with potatoes (*Solanum tuberosum* L.) were conducted in 1973; five at Ottawa, four at Fredericton, and five at Charlottetown. At each of the three locations, all experiments were isolated from one another by at least 100 m to minimize interexperiment interference. Each experiment included two treatments with six replicates in a randomized block design and four row plots about 15 m long. The experimental design was the same at all three locations, except that at Charlottetown a buffer row separated any two adjacent plots. The treatments at Ottawa and Charlottetown were similar, but differed from those at Fredericton (Table 1).

The induced epidemics at Charlottetown were initiated by applying a spore suspension of *Phytophthora infestans* (Mont.) de By. to the buffer rows thereby ensuring that both the unsprayed and sprayed plots were subject to the same influx of spores. At Fredericton and Ottawa, a spore suspension was applied to the sprayed plots as well as the unsprayed, but with the former receiving a Dithane M-45 (coordination product of zinc ion and manganese ethylene bisdithiocarbamate) spray 1 day prior to inoculation. For convenience in reporting and discussing results, and to be consistent with previously published

work (9), letters have been used to designate each treatment (Table 1).

Experiments III and V at all locations and experiment IV at Fredericton each contain identical treatments that are an attempt to obtain an estimate of disease progress in the absence of interplot interference. These treatments are termed reference treatments and are used to estimate the true treatment effect. The remaining eight experiments attempt to measure the degree of over or under estimation of the true treatment effect when it is estimated in the presence of other treatments. Thus, for example, treatments K and N (average of N_1 and N_2 reference treatments) at Fredericton are similar and therefore any difference between them is attributed to the presence of treatment J in experiment I (Table 1 and Fig. 7).

The plots in all the experiments were assessed for late blight using the British Mycological Society Key (1). For the disease assessments made on each date, the difference of the mean disease level of a treatment and that of its reference treatment, and the corresponding one-sided 5% LSD, were calculated. For example for treatments B & E at Ottawa on 31 August, the difference of mean disease levels is 9% (83-74), and the LSD is 1.5. A total of 69 pairs of mean differences and LSD's were obtained and a scatter diagram plotted (Fig. 9).

RESULTS

Figures 1-8 show the disease progress curves for each treatment constructed from disease assessments made periodically; the stippled and lined areas graphically represent positive and negative interference, respectively. The estimates of interference in terms of percentage loss in tuber yield are tabulated in Table 2 using the method suitable for late blight (9). The method uses the equation

TABLE 1. *Phytophthora infestans* inoculum and fungicide treatments applied to two cultivars in potato field experiments at three locations in eastern Canada, 1973

	Inoculum[a] and fungicides[b] treatments (designated by letter) applied at:			
	Ottawa (cv. Katahdin), Charlottetown (cv. Green Mountain)		Fredericton (cv. Katahdin)	
Exp. I				
Treatment	Inoculum applied early to induce early epidemic	A	Inoculum applied early to induce early epidemic	J
Treatment	Inoculum applied early; fungicide applied to partially control early epidemic. Partial Control Schedule 1	B	Inoculum applied early; fungicide applied to partially control early epidemic. Partial Control Schedule 1	K
Exp. II				
Treatment	Inoculum applied late to induce late epidemic	C	Inoculum applied early to induce early epidemic	L
Treatment	Inoculum applied late; fungicide applied to partially control late epidemic. Partial Control Schedule 2	D	Inoculum applied early; fungicide applied to give better control than K above. Partial Control Schedule 2	M
Exp. III[c]				
Treatment	Inoculum applied early. Partial Control Schedule 1 (as B above)	E_1	Inoculum applied early. Partial Control Schedule 1 (as K above)	N_1
Treatment	Inoculum applied early. Partial Control Schedule 1 (as B above)	E_2	Inoculum applied early. Partial Control Schedule 1 (as K above)	N_2
		E		N
Exp. IV				
Treatment	Inoculum applied early. Partial Control Schedule 1 (as B above)	F	Inoculum applied early. Partial Control Schedule 2 (as M above)	O_1
Treatment	Inoculum applied early; fungicide applied weekly to attempt complete control of early epidemic	G	Inoculum applied early. Partial Control Schedule 2 (as M above)	O_2
				O
Exp. V[c]				
Treatment	Inoculum applied late. Partial Control Schedule 2 (as D above)	H_1		
	Inoculum applied late. Partial Control Schedule 2 (as D above)	H_2		
		H		

[a]Inoculum consisting of water suspension of *P. infestans* applied to total area of experiment at Ottawa and Fredericton, but to buffer rows only at Charlottetown; early inoculation, Ottawa 20 July, Fredericton 10 July, Charlottetown 19 and 20 July; late inoculation, Ottawa 14 August, Charlottetown 13 August.

[b]Dithane M-45 at the rate of 2.24 kg. in 1,348 liters of water/hectare (2.0 lb in 120 gal/acre) used for all applications. At Charlottetown partial control schedules 1 and 2 were similar with sprays applied on 19 and 20 July, 16 and 26 July, 15 and 23 August: At Fredericton, partial control schedule 1, sprays applied on 11 and 23 July, 22 and 30 August, and 5 September; partial control schedule 2 had one additional spray on 8 August: At Ottawa partial control schedule 1 sprays applied on 16 and 20 July, 28 August; partial control schedule 2 sprays on 16 and 20 July, 20 August, 5 and 13 September.

[c]Both treatments are identical and they are superscripted for identification only (e.g., E_1 and E_2); the average E represents an independent estimate of treatment effect (tested in the absence of other treatments) and is termed reference treatment in the text.

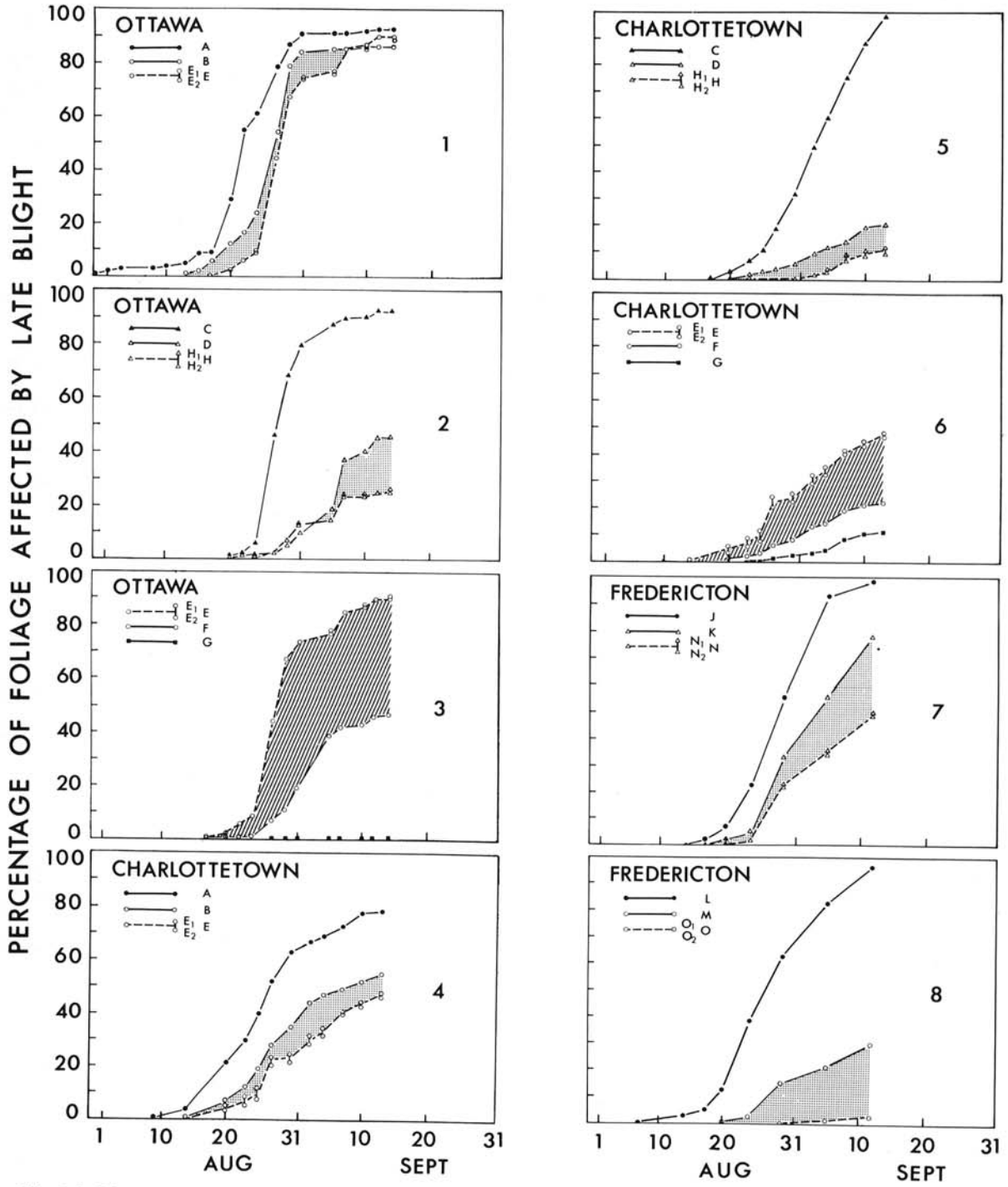

Fig. 1-8. Disease progress curves estimate treatment effects in a total of 14, two-treatment, field experiments at Ottawa, Charlottetown, and Fredericton, in 1973. The broken line (e.g., E in Fig. 1) in each figure represents an estimate of treatment effect when two identical treatments (e.g., E_1 E_2) were tested together in the absence of any other treatments; defined as a reference curve or treatment in the text. Each figure is a graphical representation of the over- or underestimation (stippled and lined areas, respectively) of the same treatment effect (e.g., B) when tested in the presence of a different treatment (e.g., A). Six experiments featured two identical treatments and eight had two different treatments. **1-6)** Results from five similar experiments at Ottawa and Charlottetown. Treatments A, and C were induced early and late epidemics, respectively; B and F represent a level of partial disease control by fungicide with E as a reference treatment, D represents a different level of partial control with H as a corresponding reference treatment, and G was an attempt to achieve complete control. **7-8)** Results from four experiments at Fredericton with treatments J and L representing induced early epidemics. Treatments K and M were two different levels of partial disease control with N and O as corresponding reference treatments, respectively.

described by James et al. (8, 10) to calculate the difference in % loss between the reference curve and its corresponding progress curve; e.g., interference in K at Fredericton, K − N, was estimated as 20.6 − 12.3 = 8.3 (8% yield loss).

At all locations the presence of an induced epidemic in a treatment resulted in positive interference in the adjacent sprayed plots in the same experiment (Fig. 1, 2, 4, 5, 7, 8). This positive interference was observed irrespective of whether the epidemic was initiated by infecting buffer rows (Charlottetown) or applying the inoculum to all the plants in the unsprayed and sprayed plots (Ottawa and Fredericton). The earlier the epidemic the greater the interference tends to be, cf. 9% and 4% at Ottawa and 8% and 4% at Charlottetown (Table 2); this confirms earlier findings (9). The spray applications for partial control schedule 1 and 2 at Charlottetown were identical, therefore, the difference between disease progress curve E and H (Fig. 4-5) is due to the earlier application of inoculum to E compared with H. A measure of the natural variation in disease development is indicated by the difference between two identical treatments (E_1E_2, H_1H_2, N_1N_2, and O_1O_2) in the same experiment; reference to Fig. 1, 2, 4, 5, 7, and 8 will indicate this to be minimal.

The experiments at Ottawa and Charlottetown clearly demonstrate that positive interference is present in sprayed plots and the Fredericton results showed that varying the spray schedule did not result in a decrease in positive interference. Partial control schedule 1 at Fredericton (treatments K and N) differed from partial control schedule 2 (treatments M and O) by only one important spray which was applied to the latter on 8 August. Although this extra spray substantially affected the epidemics (compare N and O, Fig. 7 and 8), the interference in K and H was the same (Table 2).

At both Ottawa and Charlottetown, a negative interference was recorded in the sprayed plots when they were next to plots where the disease had been completely, or nearly completely, controlled (Fig. 3 and 6). At both locations the negative interference was greater than the corresponding positive interference–compare 9% and 22% at Ottawa and 8% and 10% at Charlottetown.

The scatter diagram (Fig. 9) attempts to characterize the relationship between experimental error and the biological error due to interplot interference. Of the 69 differences plotted in the scatter diagram only 10 are not greater than their corresponding LSD; four of these are differences of means of low disease levels, and three are for differences of means of disease levels near 90%. It is well known that comparing a mean difference with its corresponding LSD is equivalent to performing a *t*-test for the difference of two means. Fig. 9, therefore, shows the results of 69 *t*-tests. It should be noted that these tests are not independent for two reasons. First for Ottawa and Charlottetown, both treatments B and F are compared

TABLE 2. Interplot interference in field experiments with late blight of potato

Location	Interference[a]	Loss in tuber yield[b] relative to potential yield (%)
Ottawa	Interference in partial control Schedule 1 Exp. I; interference in B: B-E	+ 9
Ottawa	Interference in partial control Schedule 2 Exp. II; interference in D: D-H	+ 4
Ottawa	Interference in partial control Schedule 1 Exp. IV; interference in F: F-E	−22
Charlottetown	Interference in partial control Schedule 1 Exp. I; interference in B: B-E	+ 8
Charlottetown	Interference in partial control Schedule 2 Exp. II; interference in D: D-H	+ 4
Charlottetown	Interference in partial control Schedule 1 Exp. IV; interference in F: F-E	−10
Fredericton	Interference in partial control Schedule 1 Exp. I; interference in K: K-N	+ 8
Fredericton	Interference in partial control Schedule 2 Exp. II; interference in M: M-O	+ 8

[a]Interplot interference occurs in a treatment if the disease level in a plot is affected by the disease level of adjacent plots. Positive interference occurs if the disease level in a plot is increased due to the higher disease level in the adjacent plots. Negative interference is the reverse of this relationship.

[b]The loss in yield associated with each disease progress curve is calculated using the multiple regression equation $Y\ (\%\ \text{loss}) = b_1 x_1 + b_2 x_2 \ldots$ where x_1 and x_2 are the disease increments for the first and second week, respectively. The loss associated with a disease progress curve resulting from a particular treatment (when tested in the presence of another treatment) is compared with the loss for the corresponding reference curve. The difference is termed positive interference (e.g., 22% in Exp. IV, Ottawa).

with the same reference treatment E; and secondly, all the tests for a pair of treatments (e.g., the 10 tests for B-E at Ottawa) are not independent because all the data were obtained from the same plots. However, the scatter diagram serves the purpose of demonstrating the general relationship between the mean differences and the magnitudes of variability associated with these differences.

DISCUSSION

The work reported in this paper demonstrates that when a potato late blight epidemic develops in an unsprayed check plot, it results in positive interference in fungicide sprayed plots in the same experiment. Even when five sprays of a protectant fungicide were applied at recommended rates, the positive interference in the sprayed plots was not suppressed. In fungicide trials positive interference would lead to an underestimation of treatment effect (9); the findings have practical significance because Shoemaker (12) recently reported that an unsprayed check plot was included in 93% of the 571 fungicide field tests reported in the 1971 and 1972 Fungicide and Nematicide Tests (15, 16).

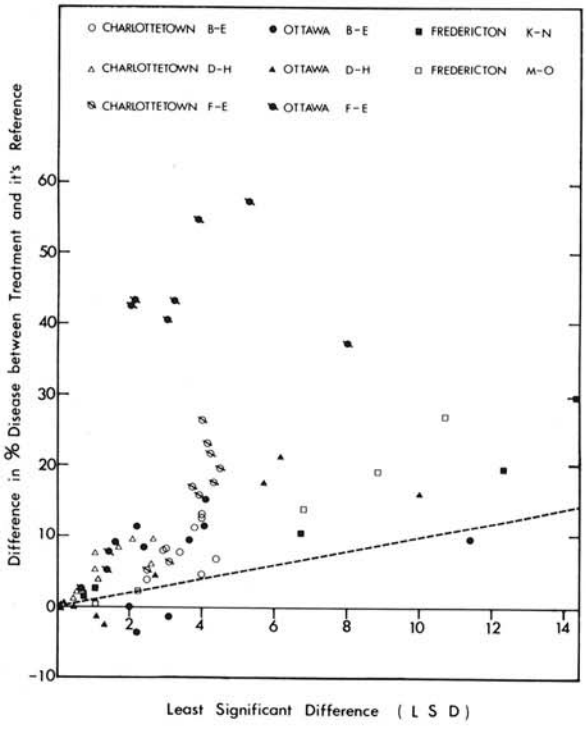

Fig. 9. Data obtained from field experiments to study the validity of obtaining independent estimates of treatment effects (fungicide control of potato late blight) when different treatments were evaluated in the same experiment. Scatter diagram showing relationship between difference in treatment effects (when estimated in the presence of different treatments) and corresponding reference curve (independent estimate when estimated in the absence of other treatments) and the LSD ($P = 0.05$) of this difference. The difference between a treatment effect and its corresponding independent estimate is given by B-E, D-M, F-E, K-N, and M-O, respectively. The dotted line represents equal values on both horizontal and vertical axes.

The results in this paper show that negative interference can be substantial. In practice, it can give rise to representational errors which are equally important to those resulting from positive interference. Therefore the presence of a disease free plot in a fungicide efficacy trial will overestimate the efficacy of a treatment. Technique limitations may explain why previous published work (3, 5, 6, 11, 14) on interplot interference has tended to emphasize the representational errors resulting from positive rather than negative interference. The current work employs a standard design, which is desirable, because it allows interference to be studied in an experimental layout with physical specifications similar to fungicide trials. However, it assumes that the siting of experiments within any location will have a minimal effect on disease development. The consistent results on interference in the current and published work (3, 9) support this assumption. Other workers (5, 6, 7, 11) have attempted to study the problem of interplot interference by studying dispersal gradients of spores and infection. Jenkyn and Bainbridge (11) reported a decrease in spore load and infection on isolated, but similarly treated, fungicide plots at increasing distances from a crop of barley infected with powdery mildew. Although their results can be used to study positive interference, they cannot be used to determine the presence of negative interference.

The experimental designs used in the current work have been restricted to two treatments to facilitate the measurement of interference, whereas in practice multiple treatment experiments (2, 13) are used to test the treatment effect (12) of a particular fungicide. In such multiple treatment experiments, positive interference will probably be present in treatments with low levels of disease and negative interference in treatments with high levels of disease. Positive interference will affect the more efficient treatments, which are necessarily the ones that require careful consideration. The relative importance of the two types of interference in the experiment will probably be governed by the relative number of high- and low-level disease plots and the differences between treatments in level of disease.

It can be reasoned that two sources contribute to the representational error of using plots to represent farmer's fields. One source of error can be measured by using reference plots within the experiment to measure the positive and negative interferences referred to above. The second error arises if the reference plots do not accurately represent the farmer's fields. In this context, since experimental plots are much smaller than farmer's fields, negative interferences may be present in all plots, irrespective of disease level, because the spore population is diluted due to a greater exodus of spores than in a large field (14). It may prove more difficult to find experimental designs to decrease negative interference, rather than positive interference, because not only does the disease-free crop contribute to negative interference, but any area with a lower level of disease also contributes, including bare soil and crops not attacked by the disease under study.

One may question how representative reference plots are of fields when the plot size is only 15 m × 3 m. However, the actual reference used is equivalent to the size of the whole experiment; i.e., 45 m × 15 m, because all

12 plots received the same treatment. Interpreting van der Plank's calculations, the reference experiment would lose approximately 10% of its spores, and this would have a relatively small effect on the infection rate (14). To reduce substantially the effect of a representational error due to negative interference in the reference plots, the experiment would have to be increased to such a size that it could not be accomodated in a normal sized experimental area. If multiple treatment experiments are restricted to treatments with similar levels of disease, as van der Plank has suggested, negative interference within the experiment should also be at a practical minimal level.

Fungicide trials have been used here to demonstrate the practical significance of representational errors. However, a cultivar trial for screening horizontal resistance is equally prone, because the most- and least-susceptible cultivars are analogous to the unsprayed and sprayed plots, respectively. It is noteworthy that pathologists have recognized the importance of errors due to variability in field experiments and have accordingly utilized statistical techniques to measure the error before interpreting and applying experimental results to agricultural practice. However, they have neglected to give equal recognition and consideration to the representational error discussed here. Although the comparison of errors arising from interference and variability was not a strict one (the errors are different in character), it served to demonstrate that the magnitude of the representational error was equal to or greater than the experimental error, and on this basis it should be given due consideration (14). Further work is necessary to study and characterize the representational error, and to develop methods for the evaluation and interpretation of experimental results affected by representational error.

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